Histamine profile of dried-salted fish sold in local supermarkets of Samar, Philippines

Ruel H. Amascual,[1]
Harold O. Panganoron,[1]
Elmer A. Irene,[2] Noeme D. Pajarillo[1]

[1]College of Fisheries and Marine Sciences, [2]College of Education, Samar State University, Catbalogan City, Philippines

Abstract

The Food Safety Act of the Philippines strengthens the welfare of consumer's health by protecting the public from foodborne illnesses such as scombroid fish poisoning due to unusually high levels of histamine. The present study investigated the formation of histamine substance in dried-salted fish products that were sold in different local supermarkets of Samar. A total of fifty samples of dried-salted fish samples were used to analyze the histamine substance. Results of the analysis revealed that 81.3% are detected beyond the regulatory limits of 200.0mg/kg by the Bureau of Food and Drugs-Philippine National Standard. Based on the Box-whisper plot, the species with the highest concentration of histamine are *Bolinao (Stolephorus sp.)*, *Hasa-hasa (Rastrelliger sp.)*, *Lambiao (Selar boops)*, and *Tamban (Sardinella sp)*. In the non-linear regression model, a low r^2 found out that histamine concentration in the products generally increased through time as sold in the local supermarkets.

Introduction

Dried-salted fish is an important value-added fishery product in the Philippines and consumed widely locally and abroad. However, despite its popularity, this prime commodity just like other fry-to-eat foods is often prepared in unhygienic condition (Sumitha, Sundar & Halady Shetty, 2014; Adesetan, Mabekoje & Bello, 2017; Bukar, Uba & Oyeyi, 2010). Most fishermen from this region rely on indigenous knowledge and traditional practices in dealing with their products and on interpreting natural events (Irene & Abadiano, 2017). Hence, most processing method is traditional and done in the backyard and sold to local consumers or dealers in wholesale. The said dealers will eventually sell their products in the local supermarkets.

In Samar, the primary business opportunity is in the trade and processing of marine resources. According to Conference on Business and Cooperatives in Samar (CBCS) in 2006, Samar is currently a major supplier of processed dried, salted fish and other fermented products to other provinces in the region and the rest of the country. In 2016, the volume of dried fish that were exported to the other country totaled to 4,289 MT with a FOB value of 18.92 million US dollar (Philippine Fisheries Profile, 2017). However, due to the high demand and the rapid increase of production of this commodity, the quality of dried fish products also decreases with some contributory factors such as poor sanitary environment, improper handling and storing of raw materials and product contamination due to histamine substance in the processed fishery products.

Histamine is a biogenic amine that can be found in fish and formed when histidine, a naturally occurring compound in fish, is converted into histamine by certain bacteria (Lin *et al*., 2012). Histamine fish poisoning (or scombroid poisoning) is considered the most frequent foodborne intoxication involving biogenic amines and associated to various incidents (Hungerford, 2010) and has been used as an indicator of quality and safety in fish and fishery products (Prester, 2011). According to Noche R. *et al*. (1998) , seven male employees of the Ninoy Aquino International Airport were examined at the Airport Medical Clinic for allergy-like signs and symptoms after eating "tambakol" (*Thunnus albacores*). The affected persons developed dizziness, palpitations, flushing, dyspnea, headache, perioral numbness, urticarial rashes, pruritus, and paresthesias of the hands. They were initially given oral antihistamines and were subsequently referred to a nearby hospital for further treatment. The leftover fish tested by Bureau of Fisheries and Aquatic Resources (BFAR) contained 30 mg histamine/100g flesh (standard limit: 200.0mg/kg flesh), confirming the diagnosis of scombroid fish poisoning.

The toxic effects of histamine are related to its typical physiological actions in the body. In particular, the dilation of the peripheral blood vessels results in hypotension, flushing, and headache, while the increased capillary permeability causes urticaria, hemoconcentration, and eyelids edema; the symptoms affecting the gastrointestinal system are due to the contraction of smooth muscles leading to abdominal cramps, diarrhea, nausea, and vomiting. Histamine also exerts a stimulatory action on the heart by increasing its contractility and exhibiting palpitations and tachycardia, while it is a potent stimulant of sensory and motor neurons producing pain and itching associated with the rash [Food and Agriculture Organization/World Health Organization (FAO/WHO), 2012].

In the Philippines, histamine has regulatory limit of 200.0 mg/kg for salted-dried fish products by the Bureau of Food and Drugs (BFAD-PNS, 2006). Republic Act 10611 otherwise known as Food Safety Act of 2013 strengthen the food safety regulatory system in the country by protecting the public from food-borne and water-borne illnesses and unsanitary, unwholesome, misbranded or adulterated foods (Joint DA-DOH Administrative Order No. 2015-0007). It is therefore imperative to provide a profile on histamine producing products sold in the local supermarkets and be used

Correspondence: Ruel H. Amascual, College of Fisheries and Marine Sciences, Samar State University, Mercedes Campus, Brgy. Mercedes, Catbalogan Samar, 6700 Philippines.
E-mail: ruel.amascual@ssu.edu.ph

Key words: Histamine, dried fish, scombroid poisoning, supermarkets, Samar, Philippines.

Acknowledgements: The authors would like to thank Samar State University for the fund support and the facilities used in the study.

Contributions: The authors contributed equally.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: The research was funded by Samar State University – Research and Development Office.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate: This research was conducted in accordance with all relevant guidelines and procedures.

Consent for publication: The manuscript does not contain any individual person's data in any form.

Received for publication: 1 June 2019.
Revision received: 16 September 2019.
Accepted for publication: 28 November 2019.

Italian Journal of Food Safety 2020; 9:8322
doi:10.4081/ijfs.2020.8322

as a baseline to improve the technology of drying process in Samar and the rest of the country.

This study aimed to investigate the histamine formation and level of concentration in dried-salted fish obtained from local supermarkets in Samar, Philippines and correlate the histamine content based on variety of fish and market location.

Materials and Methods

Collection of samples

Fifty samples of dried-salted fish products were purchased from supermarkets across the province of Samar. Supermarket vendors resell these commodities at a bigger price. Samples were stored and maintained its temperature as the same from supermarkets at 30-32°C until transported to BFAR Fisheries Analytical Laboratory for histamine analysis.

Histamine content analysis

Histamine content was analyzed using Fluorometric method (AOAC, 2005). A 5 grams from each sample were chopped into small pieces followed by homogenization by adding 50.0mL analytical grade methanol (CH_3OH, Sigma, USA) for 2 minutes. The sample were placed in a 100 mL volumetric flask, and the homogenizer was rinsed thoroughly with methanol and the rinse off was added into the flask. The flask containing the homogenized samples were placed in a hot water bath at a temperature of 60.0°C for 15 minutes. The samples were allowed to cool to 25.0°C and diluted with methanol. The sample solution was shaken and filtered using Whatman filter no. 1, and the filtrate was received into a new container and covered.

Histamine was extracted from the sample using methanol as solvent interfering compounds such as histidine and other polyamines and then removed by ion-exchange chromatography. Histamine were purified by elution of sample filtrates. The column bed of resin eluted with 4-5 mL distilled and discarded the eluate. The 50 mL volumetric flask containing 5.0 mL of 1.0N hydrochloric acid (HCl, Sigma, USA) was placed at the column outlet. About 1.0 mL filtrate sample was pipetted onto the column and added with 4-5 mL water. Eluate was collected using the 50 mL volumetric flask containing the 5.0 mL of 1.0N HCl. Flowrate was maintained at approximately 1-2 drops using the prescribed column US Contess brand. When liquid level is approximately 2mm above the resin, another 5.0 mL water was added and let it flow through the resin. The addition of water was repeated by gradually increasing the volume increments until the eluate reaches about 40 mL in the volumetric flasks and volume to 50 mL with water. The eluate was refrigerated until further for fluorescence measurement.

In fluorophore formation, a separate 50 mL Erlenmeyer flasks, 5.0 mL of blank eluate were pipetted, each working as standard (total to 4 of each sample eluate). Ten (10) mL of 0.1N HCl and 3.0 mL of 1.0N Sodium hydroxide (NaOH Sigma, USA) were added into each flask and thoroughly mixed. After 5 minutes, the solution was added with O-Phthaldehyde (OPT, Sigma, USA) reagent. After 4 minutes, 3.0 mL of 3.57N Phosphoric acid (H_3PO_4, Sigma, USA) was added and mixed immediately and let it stand for 15-20 minutes and the formation of fluorophore in the samples occurred. For blank and control samples a 1.0 mL of CH_3OH were passed through the column resin bed and the eluate was collected as though it were a fish extract samples. Passed 1.0 mL of 10.0µg/mL histamine ($C_5H_9N_3$.2HCl, Sigma, USA) control sample solution through the same column resin bed every after elution of blank sample and every end of a set of eluate samples. The resins in the column were added with 10mL water before every elution of another filtrate sample. The fluorescence of fluorophores present in the sample was recorded by measuring the fluorescence intensity after 1.5h at an excitation wavelength of 450 nm using the Jenway model 6280 Fluorimeter (Keison, UK) and by Linear Equation/ Regression Calculation. The sensitivity or gain setting on the fluorimeter, which will give approximately 80.0% full-scale reading with 0.3 µg/mL $C_5H_9N_3$.2HCl standards was used.

Statistical analysis

Statistical analysis was performed using Box-whisper plot to summarize the set of data measured in an interval scale and Kruskal-Wallis Test to assess for significant differences on a continuous dependent variable by a categorical independent variable. A fitted nonlinear regression model was used to determine the difference between the mean and every point of data in the set.

Results

Comparison of histamine content of dried fish in different local supermarkets

Histamine content analysis showed an average of 75% non-compliance, with 82% of the dried-salted fish samples exceeding the maximum requirement of 200 mg/kg. A comparison of the percent compliance for each sampling areas, as presented in Figure 1 showed uniformly non-compliance due to improper storage of the products from the supermarkets.

Figure 1 presents a box-whiskers plot summarizing the results of histamine analysis. The symbol X represents the mean while the lines within the box is the median. Medians that are located above the mean means that most of the values are located above the mean histamine level for a specific location and vice-versa. Significant observations from the Figure 1 show that samples collected from the local supermarkets of Zumarraga and Daram showed the highest concentration of histamine substance based on the Box-whisker plot, the median for Zumarraga and Daram lies near the upper quartile and higher than the mean indicating that samples are near the maxi-

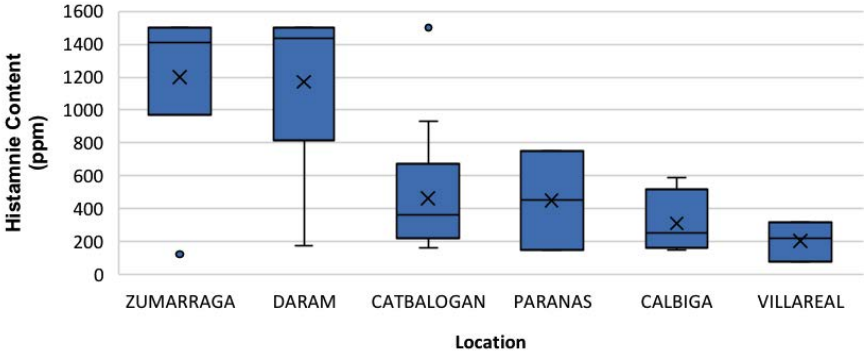

Figure 1. Box-whisker plot for the histamine content in different local supermarkets in Samar.

mum detectable limit in Fluorometer (Table 1). Using the Kruskal-Wallis test at 95.0% confidence level results showed an $H_{calculated}$ (21.76), which is higher than the $H_{critical}$ (11.07). This means that at least one distribution is different among sampling areas. A post-hoc Dunn's test joint analysis also showed that using calculated standard errors and number of samples for each area, Daram and Zumarraga had statistically significantly higher histamine content against all other municipal supermarkets. The same conclusion holds for Zumarraga against other areas except for Daram. While, Catbalogan, Paranas, Calbiga, and Villareal have no significant difference in histamine substance level in a pairwise comparative test. Comparison of histamine content of different varieties of dried-salted fishes *(for the common and scientific names of fishes, refer to Appendix).* For the percentage of non-compliance based on the BFAD-PNS regulatory limit, about 81.3% proven non-compliant, as shown in Figure 2. Similarly, X represents the mean histamine content in the dried-salted fish samples, the line within the boxes are the median while the red-dashed line is the BFAD-PNS regulatory limit (200 mg/kg). Based on the data, the variety of fishes that were determined to be completely non-compliant are *balanak, baysa, bolinao, dinorado, galunggong, lambiao, lusod*, and *tamban* while *sap-sap* was only 50% non-compliant.

As presented in Figure 3, the least common dried fish samples were the *baysa*, *bokaw*, *dinorado*, *galunggong*, and *lusod*. Box-whisker plots showed the lesser spread in the box-plots while *assorted fish*, *bolinao*, *hasahasa*, *lambiao*, and *tamban* have a higher spread of values based on the interquartile range (Table 2).

Histamine concentration versus storage time

A nonlinear regression model was fitted to determine the relationship between the number of days in storage and the histamine level of dried fish samples. Measurements which exceeded the maximum detectable histamine content in Flourimeter (>1500 ppm), as well as outliers in the data set.

A graphical representation of the modeled equation is presented in Figure 4, where h is the histamine concentration (ppm), and t is the storage time (days). Results showed a trend similar to other studies on histamine production against the storage time (Kung, Huang, Lin, Liaw, Lee and Tsai, 2015; Zou and Hou, 2016).

A low r^2 was attributed to the different fish types, processing methods, sampling locations, handling practices, and storage conditions used for each sample. More accurate representation of histamine production over time may be derived if the same type of fish was used, prepared, and studied in a controlled environment. The graph showed that histamine concentration of dried fish products that were sold in the local supermarkets directly increased every three days. It presents a problem for increasing the penetration of these goods in the local and national market when stricter quality monitoring is not imposed.

Discussion

The supermarkets of Zumarraga and Daram in Samar has considerably consistent high histamine content in all dried fish samples. The dried-salted fish such as *balanak*, *baysa*, *bolinao*, *dinorado*, *galunggong*, *lambiao*, *lusod*, and *tamban* was determined as 100% non-compliance while *Sap-sap* reaches only for about 50% non-compliance. Post-hoc Dunn's test showed

Table 1. Summary of results for histamine analysis of dried-salted fish in different local supermarkets in Samar.

Location	Samples	Histamine (ppm)		
		Minimum	Median	Maximum
Zumarraga	6	122.72	1327.68	>1500
Daram	8	171.33	1439.6	>1500
Catbalogan	27	162.25	361.38	>1500
Paranas	2	147.5	449.875	752.25
Calbiga	4	147.5	250.75	590
Villareal	3	73.27	221.25	313.59

Table 2. Summary of results for histamine analysis of different varieties of dried-salted fish.

Fish Used	Samples	Histamine (ppm)			
		Minimum	Median	Maximum	IQR
Assorted fish	3	221.25	309.75	1327.68	553.215
Balanak	4	221.25	405.625	671.13	278.408
Baysa	1	-	1379.2	-	-
Bokaw	1	-	191.75	-	-
Bolinao	8	221.25	1126.125	>1500	945.03
Danggit	4	191.75	271.11	361.38	106.128
Dinorado	1	-	376.13	-	-
Galunggong	1	-	730.13	-	-
Hasahasa	3	177	737.5	>1500	661.5
Lahing	3	162.25	202.25	590	213.875
Lambiao	4	295	560.5	>1500	699.5
Lusod	1	-	693.25	-	-
Sapsap	6	73.27	188.915	724.41	71.6275
Tamban	10	147.5	759.625	>1500	881.56

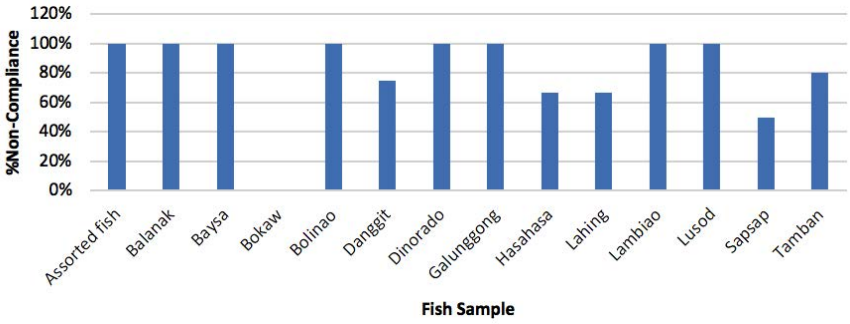

Figure 2. Percent non-compliance of histamine of each variety of dried-salted fish samples.

that there is a significant difference in the histamine content of dried *bolinao* with *danggit*, *lahing*, and *sapsap*, implying a significantly higher concentration in said variety. There is also a significant difference in the histamine content of dried *sapsap* with *baysa*, *lambiao*, and *tamban*, which, on the other hand, indicates a significantly lower histamine concentration. In a study of Simora and Peralta (2018), it was found that 76.2% of traditional dried fishes in the Philippines namely, sardines, mackerel and anchovies had exceeded United States Food and Drug Administration guideline of 5 $mg.100g^{-1}$ which is consistent with the results of the present study.

Analysis of histamine levels within the market chain for *Sardinella spp.* from Simora *et al*. (2016), indicates histamine levels were low from fresh samples from fishermen while levels significantly higher from samples obtained from processors, trailers and retailers. The same type of market flow is present in Samar though verification is necessary to determine if the same trend applied. In addition, Simora et. al. (2016), and Simora and Peralta (2018) has reported that low salt levels in the dried fish samples are important considerations in regulating the histamine levels of dried fish in the market. Salt inhibits bacterial growth which are precursors to the conversion of histidine to histamine in dried through the histidine decarboxylase enzyme containing bacteria or hs (US FDA, 2011). Such enzymes are present in bacteria of Enterobacteriaceae family (Huang *et al*., 2010). Factors that can considered which yield high histamine level are improper evisceration, use of a contaminated or dirty knife, improper cooling and storage, and mixing with contaminated goods. This scenario presents a lack of knowledge or compliance on fundamental good manufacturing practices (GMP) of the local manufacturers in Samar. Further, processing procedures that are non-regulated and non-monitored showed the highly varied histamine levels for each area and some fish species. Measurement of factors such as pH and salt levels are necessary to provide a more complete understanding of the progression of histamine levels within the supply chain.

The high non-compliance of histamine content over the maximum allowable level is a serious health risk to consumers, particularly for scombroid poisoning in Samar. Though often consumers experienced mild symptoms, severity is dependent on the ingested amount and sensitivity to histamine. Analysis of some dried fish products from different markets in Samar resulted in values that were about eight times than the regulatory limit which increases the probability of experiencing more severe symptoms. However, since no researches have delved into scombroid poisoning incidents in Samar, assessment of the frequency and severity is difficult.

The current scenario necessitates local agencies and academe to assist local producers. Fulfillment of the required BFAD recommended standards would ensure consumers receive safe to consume products and likewise improve market penetrability when implemented monitoring procedures are stricter.

Conclusions

Histamine level in dried fish samples exceeded the PNS/BFAD regulatory limit of 200.0 mg/kg. Among the samples that were analyzed, the supermarkets of Zumarraga and Daram produced the highest histamine concentration. The most common varieties of fishes showed a significant concentration of histamine concentration are the *balanak, baysa, bolinao, dinorado, galunggong, lambiao, lusod*, and *tamban*. Though a low r^2 (0.3777) was determined between the storage time and histamine levels, the graphical representation presents an increasing trend until the Day 9. Difference in processing methods and initial histidine content in the different fish types are seen to be contributory to the low correlation coefficient complete understanding of the factors affecting histamine concentration within the market chain and for each fish species is necessary that will provide a foundation and mitigation to prevent the formation of histamine substance in dried-salted fish products in the supermarkets. This also includes measurement of processing factors such as salt content, pH and the bacteria present.

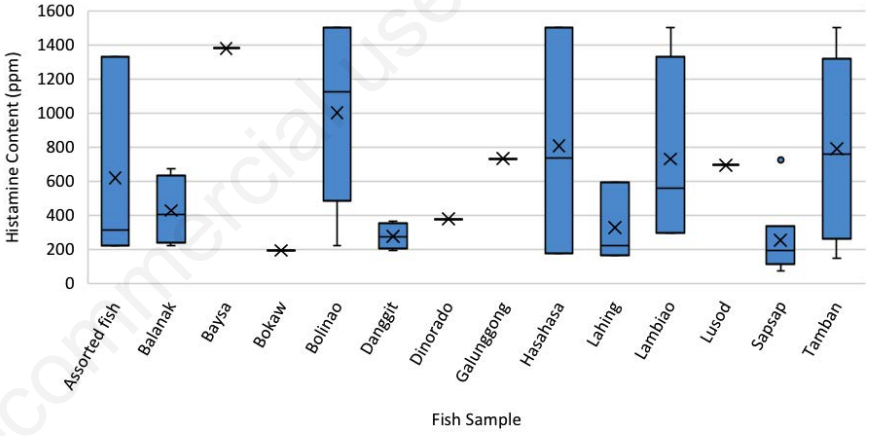

Figure 3. Box-whisker plot for the histamine concentration of each variety of fish

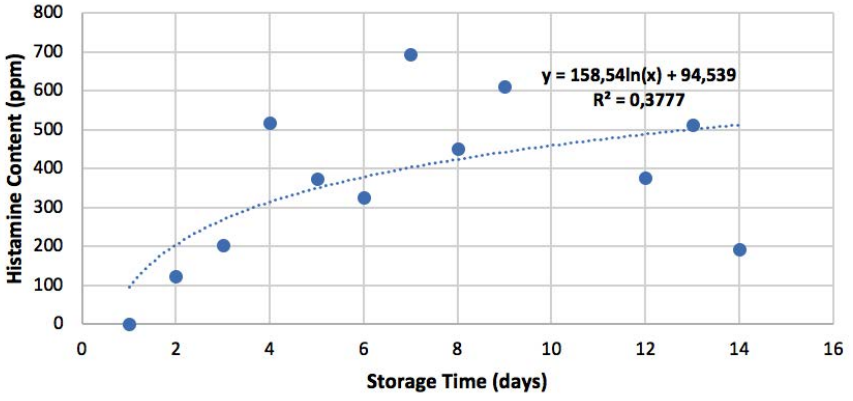

Figure 4. Fitted nonlinear regression model for histamine in dried fish against storage time. The regression model has an r^2 = 0.3777 and a regression equation.

References

Adesetan TO, Mabekoje OO, Bello OO, 2017. Bacteriological quality of street vended ready-to -eat foods in Ago-Iwoye, Nigeria: A study of University environment. Int J Microbiol Res Rev 6:215-29.

AOAC. 2005. Fish and other marine products. In Official Methods of Analysis of AOAC International Chapter 35 (P.A. Cunniff, ed) pp. 16-17, AOAC International, Virginia. R.

BFAR – Philippine Fisheries Profile (BFAR-PFP). 2017. Exports of Fish and Fishery Products by Kind, Quantity, and Value, 2016.

Bukar A, Uba A, Oyeyi Tl, 2010. Occurrence of Some Enteropathogenic Bacteria in Some Minimally and Fully Processed ready-to-eat foods in Kano Metropolis, Nigeria. Afr J Food Sci 4:32-6.

Bureau of Fisheries and Aquatic Resources (BFAR). Fish Quality Control Laboratory. Histamine Determination. Standard Methods by the Association of Analytical Chemists (AOAC). Doc. No. BFAR XI FQCL LQAM 2006

Bureau of Food and Drug-Philippine National Standard (BFAD-PNS). 2006. Ethnic food products – Dried, salted fish – Specifications. Manila: BFAD-PNS.

Connell J, 1980. Control of Fish Quality. 2nd ed. Fishing News Ltd., Surrey, 137 p.

Department of Trade and Industries. Conference on Business and Cooperatives in Samar. Catbalogan, Samar, Philippines. 2006.

Department of Trade and Industries. Philippine National Standard, PNS/BFAD 04:2006

Food and Agriculture Organization (FAO). Studies on the histamine content of fermented fishery products. 382-385. In: Reilly A. (ed.), Spoilage of Tropical Fish and Product Development, vol. FAO Fisheries Report 317. FAO, Rome, Italy.

Food and Agriculture Organization/World Health Organization (FAO/WHO). 2012. Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products. Joint FAO/WHO expert meeting report. Rome: FAO Headquarters 1–111.

Food and Drug Authority. 2012. Joint FAO/WHO expert meeting on the public health risks of histamine and other biogenic amines from fish and fisheries products. 23-27 July 2012, Rome, Italy.

Food and Drug Authority. 2001. Food compliance program: Domestic fish and fishery products. Washington DC: Dep. Health Serv. Food Drug Adm.-Cent. Food Safety Appl. Nutr.-Off. Seafood.

JM, 2010. Scombroid poisoning: A review. Toxicon 56:231-43.

Huang YR, Liu KJ, Hsieh HS, Hsieh CH, Hwang DF, Tsai YH, 2010. Histamine level and histamine-forming bacteria in dried fish products sold in Penghu Island of Taiwan. Food Control 21:1234–9.

Irene EA, Abadiano M, 2017. Exploring Indigenous Knowledge, Community Resilience and Belief Systems in Typhoon-prone Areas of Samar, Philippines. J Acad Res 2:1-15.

Peralta EM, Serrano Jr. AE, 2015. Histamine formation and microbiological quality of deboned milkfish (Chanos chanos) during ambient storage. ELBA Bioflux 7:1-9.

Prester L, 2011. Biogenic amines in fish, fish products and shellfish: a review. Food Addit Contam 28:1547-60.

Simora RC, 2016. Histamine content and quality assessment of dried-salted sardines (Sardinella spp.) along the supply chain. Phil J Nat Sci 21-22:31-9.

Simora RC, Peralta EM, 2018. Occurrence of Histamine and Histamine-forming Bacteria in Philippine Traditional Dried-salted Fish Products. Asian Fish Sci 311:73-88.

Sumitha D, Sundark K, Haladyshetty P, 2014. Occurrence of Histamine Forming Bacteria and Histamine in Dried Fish. Adv J Food Sci Tech 6:428-32.

Taylor SL. Histamine Poisoning Associated with Fish, Cheese, and other Foods. Food Research Institute. University of Wisconsin, 1925 Willow Drive, Wisconsin USA. VPH/FOS/85.1

Vijayan PK, Gopamur KK, Balachandran KK, 1991. Formation of histamine in Indian mackerel (Rastrelliger kanagurta). Paper presented at FAO/IPTC WP Fish Tech Marketing.

Visciano P, Schirone M, Tofalo R, Suzzi G, 2012. Biogenic amines in raw and processed seafood. Front Microbiol 3:1-10.