ELBA BIOFLUX

Extreme Life, Biospeology & Astrobiology
International Journal of the Bioflux Society

Histamine formation and microbiological quality of deboned milkfish (*Chanos chanos*) during ambient storage

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Abstract. Milkfish has not been implicated in histamine poisoning in the past but it was detected in smoked deboned milkfish collected from a local smoke fish producer. Histamine could have likely formed prior to smoke processing, where the fresh deboned milkfish could have been exposed to temperature abuse. The study evaluated the histamine accumulation and microbiological quality of deboned milkfish exposed to ambient conditions. Results revealed that initial histamine content (1.26±0.50 mg histamine 100 g^{-1} fish flesh) increased to 8.25±1.87 mg 100 g^{-1} on the 6th h, level beyond the allowable maximum limit (5 mg 100 g^{-1}) to be considered safe. Potentially hazardous levels were present (58.8±10.67 to 111.9±11.73 mg 100 g^{-1}) on the 8th-12th h. Total viable count (TVC) and histamine-forming bacteria (HFB) generally increased through time, as influenced by temperature. Initial total viable count (3.94 log cfu/g) significantly increased to 5.53 (log cfu) on the 4th h, the maximum acceptable microbiological limit in food, while the initial HFB count of 1.80±0.68 (log cfu) increased to 5.70±0.11 (log cfu) on the 12th h. The study showed that histamine developed in deboned milkfish beyond the safe levels when exposed to ambient temperature for more than four (4) h and may pose health risks to consumers.
Key Words: histamine, milkfish, HFB, TVC.

Rezumat. Carnea de bangus nu a fost implicată în îmbolnăvirea de scorbut în trecut dar histaminele au fost detectate în bangusul afumat dezosat provenit de la un producător local de peşte afumat. Histaminele, probabil, s-au format înaintea afumării, când carnea proaspătă de bangus dezosată se poate să fi fost expusă la temperaturi excesive. Acest studiu evaluează acumularea histaminelor şi calitatea microbiologică a bangusului dezosat expus la condiţii ambientale. Conform rezultatelor conţinutul iniţial de histamine (1.26±0.50 mg histamine 100 g^{-1} carne de peşte) creşte de la 8.25±1.87 mg 100 g^{-1} în a şasea oră, nivel ce depăşeşte limita maximă admisă (5 mg 100g^{-1}) considerata ca fiind sigură. Nivelele potenţial periculoase au fost prezente de la a 8-a ora la a 12-a. Numărul viabil total şi bacteriile producătoare de histamine în general se înmulţesc în timp, sub influenţa temperaturii. Numărul iniţial viabil (3.94 log cfu/g) a crescut semnificativ la 5.53 (log cfu) în a 4-a oră, limita microbiologică maximă acceptată în alimente, în timp ce numărul bacteriilor producătoare de histamină iniţial 1.80±0.68 (log cfu) a crescut la 5.70±0.11 (log cfu) în a 12 oră. Studiul demonstrează că histaminele s-au format în carnea dezosată de bangus peste nivelele sigure când a fost expusă la temperatură ambientală mai mult de patru (4) ore şi aceasta poate determina riscuri de sănătate pentru consumatori.
Cuvinte cheie: histamine, peste bangus, HFB, TVC.

Introduction. Histamine poisoning is considered the most frequent foodborne intoxication involving biogenic amines and associated to various incidents (Connell 1980; Frank 1985). Histamine is an amine produced by the enzymatic decarboxylation of histidine (Karovicova & Kohajdovic 2005; Lehane & Olley 2000) and ingestion at significant levels may result in intoxication manifested by allergic reactions such as rashes, dizziness, nausea, vomiting, urticarial reactions and diarrhea (Connell 1980; Lehane & Olley 2000).

This type of poisoning has been historically associated with ingestion of spoiled fish of the family *Scombridae* (i.e. tuna, bonito, skipjack, and mackerel) due to unusually high levels of free histidine in the tissues and potential for histamine production (FDA 2001, Sarnianto et al 1985). Spoiled tuna fish generally contain about 10-50 mg histamine to as much as 1000 mg (Frank 1985). However, some non-scrombroid fish species have been involved in histamine poisoning cases such as mahi mahi (*Coryphaena spp*), sardines (*Sardinella spp*), anchovy (*Engraulis spp*) and herring (*Clupea spp*) (FDA 2001, Prester 2011, Visciano et al 2012). Due to the potential hazard pose by histamine poisoning, many countries have accepted a histamine level of 5-10 mg 100 mg^{-1} in fish and fish products as the maximum allowable level (Vijayan et al 1991).

There has been an increasing interest on the histamine development in milkfish in recent years. Milkfish is one of the important food fish in the Philippines where it is extensively farmed for many years. Milkfish contains large amount of free histidine, approximately 441mg 100 g^{-1}, accounting for 80% of the total free amino acids (Chiou et al 1990). Milkfish has not been implicated in histamine poisoning in the past but a food poisoning incident in Taiwan was reported in 2006 following consumption of dried milkfish. The sample was found to contain 61.6 mg 100 g^{-1}, a level higher than the hazard action level of 50 mg 100g^{-1}. Considering the allergy-like symptoms of the victims, it supported the assumption that histamine was the causative agent (Tsai et al 2007). Analysis of 32 dried milkfish products from retail markets in Taiwan revealed that 78.1% of the samples exhibited histamine greater than the FDA allowable limit of 5 mg 100g^{-1} while 14 samples (43.7%) contained above the 50 mg 100 g^{-1} hazard action level (Hsu et al 2009).

Histamine-forming bacteria (HFB) naturally occurs in the marine environment and is commonly found in fish in their gills, skin and intestines (Bjornsdottir et al 2009; Bremer et al 2003; Huss 1995) but most of them seem to be derived from postharvest contamination during handling and processing (Lehane & Olley 2000). Arnold & Brown (1978) have reported that histamine was formed during handling and salting process of dried-salted mackerel but immediately ceased after drying. HFB requires an optimal temperature of 25^{o}C for histamine formation (Kim et al 2002) and their potential to produce toxic levels is enhanced at abusive temperatures (Mozorra-Manzano et al 2000).

The technology of milkfish deboning (Guevarra et al 1973) was developed in the early 1970's to address the safety concern over the numerous spines and bones embedded in the muscle. It has become a pre-processing step in developing value-added products from milkfish. Nowadays, deboned milkfish are sold as fresh displayed at counters in supermarkets and wet market where the commodities in some cases, are not properly iced. The manual deboning exposes the meat to bacterial contamination from the fish or from the surrounding environment and in combination with exposure to abusive temperatures, the overall condition would likely lead to the production of histamine to hazard levels. The presence of histamine in vacuum-packed milkfish which were deboned and smoked sold in supermarkets (Peralta & Olympia 2005) and fresh ones in local wet markets (Espejo 2004) could have developed prior to smoking and packaging. Fletcher et al (1998) has confirmed this observation and has observed that HFB was eliminated during smoking. Despite these observations, incidents of histamine poisoning involving milkfish in the Philippines have not been reported.

The present study investigated the histamine accumulation in deboned milkfish when exposed to ambient conditions and documented the changes in total microbial and histamine-forming bacteria (HFB).

Materials and Method

Sample preparation. Milkfish (*Chanos chanos*), with an average weight of 235.5 $\pm$ 17 g and length of 189.8 $\pm$ 8.6 cm, were purchased from the Iloilo fishing port and transported to Iloilo Central Market for deboning purposes. Deboned milkfish was packed in individual polyethylene plastic bags that were put in icebox and promptly transported to the laboratory. Samples were exposed to ambient conditions (28°-32°C) for 12 h. Triplicate samples were withdrawn every 2 h and subjected to analysis.

Histamine content. For histamine analysis 10 g samples were extracted with methanol at 60°C. Interfering compounds were removed by anion exchange chromatography. The purified histamine was then derivatized with *o*-phtaldialdehyde (OPT) to form flourophore. The intensity was measured by flourometry at 360 nm excitation and 450 emision (AOAC 1990).

Microbiological analyses. Total viable count (TVC) was measured by homogenizing a 30-g sample aseptically mixed in 270 mL sterile peptone broth (0.01%). The homogenate was serially diluted and 1 mL of each decimal dilution was pipetted into sterile plates. Nutrient agar supplemented with 0.5% NaCl was poured, mixed by rotating the plate and allowed to set prior to incubation at 35-37°C for 24-48 h (ICMSF 1978). Histamine-forming bacteria (HFB) count was determined using the above method using histidine decarboxylating bacterial medium (Niven et al 1980) as basal medium. Purple colonies with purple halos on yellow background were considered positive for histamine production. Plates were counted at the end of incubation and reported as colony forming unit per g sample (cfu g^{-1}).

Statistical analysis. TVC, HFB and histamine content were subjected to Analysis of variance (ANOVA). If significance was detected, a post hoc test was done using Duncan's multiple range test (DMRT) at 0.05 alpha level. Pearson correlation test (r) was used to determine relationship between TVC, HFB and histamine.

Results and Discussion. Histamine in milkfish was initially low (1.26-3.00 mg 100 g^{-1}) during the first 4 h of exposure (Figure 1), values that were within the <5mg 100g^{-1} set by FDA guidelines as safe for human consumption (FDA 2011). Content significantly increased ($p<0.05$) to unsafe and potentially hazardous levels (8.25-111.9 mg /100 g) during prolonged storage to 6-12 h.

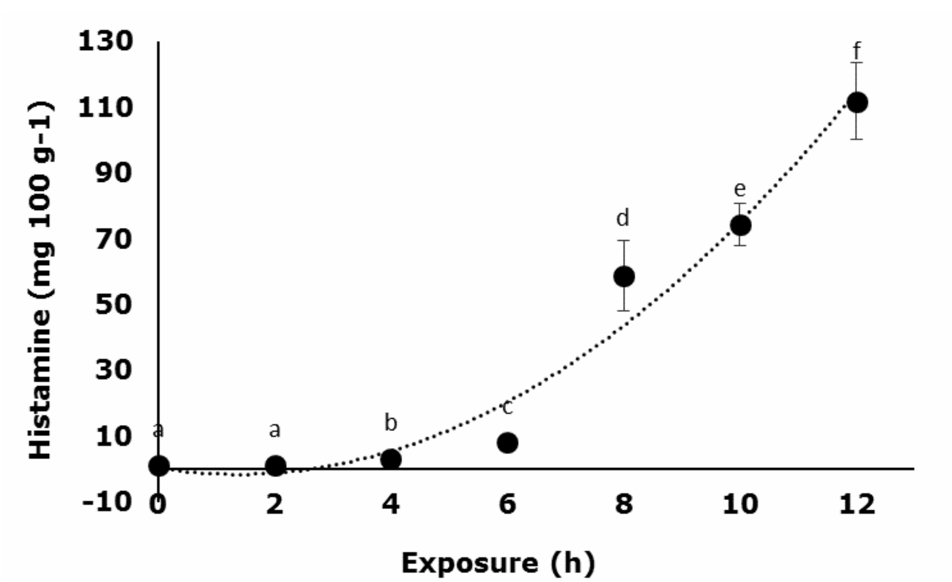

Figure 1. Periodic changes in histamine content in deboned milkfish following exposure at ambient temperature.

Although the level was below the 200 mg kg^{-1}, which is considered hazardous to human health, lower levels like 20 mg 100 g^{-1} and 50 mg kg^{-1} were reported to cause symptoms of scombroid poisoning (FDA 2012; Huss et al 2003; Taylor 1983). Shalaby (1996) considers levels at 5-20 mg 100 g^{-1} as possibly toxic; 20-100 mg 100 g^{-1} as probably toxic and > 100 mg 100 g^{-1} as toxic and unsafe for human consumption.

In the present study, it could be assumed that a considerable level of free histidine was present and, in ambient temperature, histamine formation showed a polynomial increase up to 12 h of exposure. This assumption is in line with the observation that the formation of histamine in fish has been closely associated with elevated storage temperatures, presence of microorganism capable of histidine decarboxylation, and availability of free histidine in the fish muscle (Kose 2010; Zaman et al 2009).

Histidine can be released through bacterial proteolysis during degradation of proteins that contain 2-3% histidine and concentration of 100-200mg 100 g^{-1} fish muscle should at least be present to initiate histidine decarboxylation activity (Ienistea 1973). Histidine levels in fish commonly implicated in histamine poisoning normally range from 210 to >1000 mg (Chen et al 1989). The free histidine content, approximately 441 mg 100 g^{-1} in milkfish (Chiou et al 1990), is adequate to initiate histamine formation.

Abusive temperature conditions greatly affect histamine formation by hastening proteolytic changes, thus increasing availability of free amino acids and favoring microbial growth and activity (Brillantes et al 2002; Koutsounamis et al 2010).

Rodtong et al (2005) reported rapid histamine formation in Indian anchovy (*Stolephorus indicus*) when stored at 35^{o}C for 8 h.

In this study, there was an initial TVC mean log count (cfu g^{-1}) of 3.94 in deboned milkfish and this level significantly increased ($p<0.05$) to 7.15-7.81 (log cfu g^{-1}) after 8-12 h at ambient conditions (Figure 2).

Histamine has been used as an indicator of quality and safety in fish and fishery products where elevated values suggests significant degree of quality degradation caused by bacterial activity (Prester 2011). Total viable count (TVC) has been generally used as an acceptability index in standards, guidelines and specifications to separate the good quality ($\leq 10^5$ cfu g^{-1}) (ICMSF 1978) from unsatisfactory ($> 10^7$ cfu g^{-1}) in food, which has been associated with the point of sensory rejection (Gilbert et al 2000; Olafsdottir et al 1997).

Based on these microbiological criteria, the sample was considered spoiled beyond the 6th h of storage. Bacterial growth was greatly influenced by temperature and could have resulted in limiting the shelf life and acceptability of the product (Huss 1995).

With the increase in bacterial count, histamine contents markedly increased to levels of concerns (Figure 1). Correlation coefficient between bacterial count and histamine in the present study showed positive linear relationship ($r=0.79$) suggesting that bacterial growth greatly influenced histamine formation. Similar observation was reported where significant correlation between histamine and bacterial counts exists in *Feseeikh*, an African fish ferment product (Ahmed et al 2012) and salt-fermented fish *nukazuke* (Kuda et al 2012).

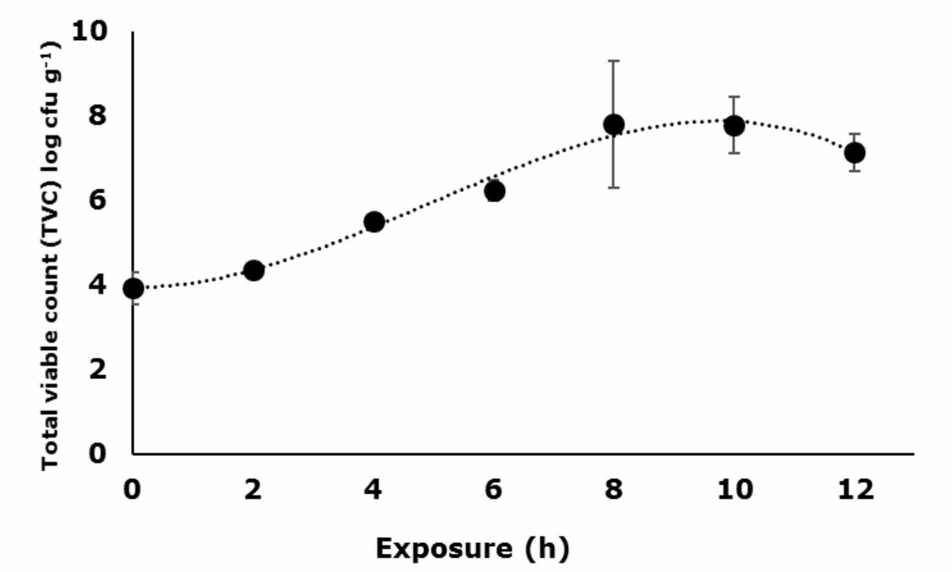

Figure 2. Total viable count (TVC) in deboned milkfish after exposure at ambient temperature.

HFB count was relatively low (1.80-1.95 log cfu g^{-1}) for the first 2h then significantly increased ($p<0.05$) on 4-12h at ambient conditions (Figure 3) in the present study. Histamine may not be directly related to the total number of bacteria present in fish but influenced by the microflora capable of synthesizing histidine decarboxylation (Edmunds & Eitenmiller 1975). HFB are mesophiles that have an optimum growth temperature range of 30-40^{o}C (Huss 1995). It is evident that histamine build-up in the product was the result of enzyme synthesis and activity of HFB under favorable conditions. Most studies agree that the potential of these microorganisms to produce toxic levels is enhanced at abusive temperatures (Brillantes et al 2002; Kim et al 2000; Mozorra-Manzano et al 2000).

The correlation coefficient between HFB and histamine in the present study was somewhat similar ($r=0.83$) with that between TVC and histamine ($r=0.79$). Amino acid decarboxylases are widely distributed among bacterial species that may originate from endogenous and contaminating microbial flora in fish and its environment (Silla-Santos 1996; Tembhurne et al 2013). In the present study, the percentage of HFB to the total bacterial load ranged between 44.7-79.7%, where its proportion to the total bacterial load increased at prolonged exposure. Bacteria from fish gills and guts were reported to have biogenic amine forming abilities (Kose 2010; Visciano et al 2012) and contaminating enterobacters have the ability to produce histamine, putrescine and cadaverine (Zaman et al 2009).

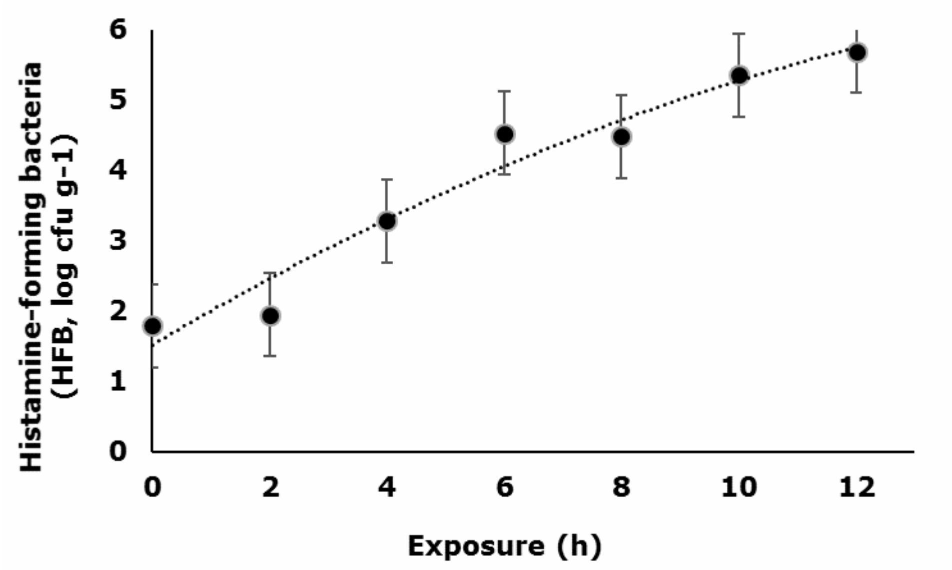

Figure 3. Changes in histamine-forming bacteria (HFB) in deboned milkfish following exposure at ambient temperature.

Conclusions. Histamine content in deboned milkfish was at low levels during the first 5 h of exposure to ambient temperature but significantly increased to unsafe levels during 6-12 h. Total viable count (TVC) of the exposed milkfish meat significantly increased from a low initial value to the level of spoilage after 8-12 h at ambient temperature. Histamine forming bacteria (HFB) count was relatively low for the first 2 h then significantly increased following 4-12 h exposure at ambient conditions providing evidence that histamine build-up in the product was the result of enzyme synthesis and activity of HFB under favorable conditions. The correlation between HFB and histamine were somewhat similar (r=0.83) with that between TVC and histamine (r=0.79) showing that changes in either or both indices could affect changes in the formation of histamine in milkfish meat following exposure to ambient temperature. The present study showed that histamine accumulation in deboned milkfish can reach potentially hazardous levels under improper handling. While there is very little or no recorded information on milkfish products being implicated in histamine poisoning in the Philippines, the unsafe histamine levels that could develop in deboned milkfish can likely cause food poisoning, a likely case similar with the 2006 incident in Taiwan.

Acknowledgements. The authors would like to extend their gratitude to the University of the Philippines Visayas through the Office of the Vice Chancellor for Research and Extension for its financial support and the Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences for the technical assistance.

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Received: 25 January 2015. Accepted: 19 February 2015. Published online: 23 February 2015.
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How to cite this article:
Peralta E. M., Serrano Jr. A. E., 2015 Histamine formation and microbiological quality of deboned milkfish (*Chanos chanos*) during ambient storage. ELBA Bioflux 7(1):1-9.