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Research Article

EFFECT OF DIFFERENT STORAGE CONDITIONS TOWARDS THE FORMATION OF HISTAMINE PRODUCING BACTERIA IN CANNED TUNA (*THUNNUS SPP.*)

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ABSTRACT
Canned tuna (*Thunnus spp*.) is among of seafood-based products which is popular among Malaysia consumer. Deterioration in canned products is due to enzymatic reactions, chemical changes and spoilage through the microbial activity. Microbes can metabolize these amino acids producing ammonia, biogenic amines such as putrescine, histamine and cadaverine, organic acids, ketones and sulphur compounds. Several species of bacteria which are able to convert histidine to histamine can cause food poisoning if consumed in large amount. Improper storage conditions of canned products are among of the factor that influence the growth of bacteria associated with the production of histamine. Hence, this study was aimed to identify the presence of histamine-producing bacteria in canned tuna for 1 week storage at room temperature, and 2 to 3 weeks storage in refrigeration and to determine the characteristic of isolated bacteria based on morphology and biochemical properties. The canned tuna sample stored for 1 week at room temperature demonstrates the highest number of bacterial colonies. Throughout this study, a total of 89 bacterial isolates were identified from selective media of Niven's agar. Most of the isolates were identified as Gram negative bacteria with coccobacilli morphology. The bacteria that capable to produce amino acid decarboxylases are *Enterobacter, Clostridium, Hafnia, Klebsiella, Lactobacillus, Photobacterium, Proteus, Pseudomonas and Vibrio spp*. It is recommended that rapid chilling of raw materials and good hygienic practices may reduce the risk of contamination by histamine producing bacteria.

Keywords: *Canned Tuna, Histamine Producing Bacteria, Biogenic Amines*

INTRODUCTION
Canned fish products are nowadays becoming the most popular product among of the consumer needs because it is an economical alternative in the lifestyle. Canned tuna (*Thunnus spp*.) is frequently and largely produced and most popular among Malaysia consumer. The contemporary global of canned tuna processing industry was developed in the mid1950s, in combination with the development of industrial-scale tropical tuna fisheries (Miyake *et al.*, 2010). At first, the productions of canned tuna are mainly by the US mainland, EU and Japan, who collectively accounted for more than two thirds of total global production until the mid-1980s. This was followed by Philippines and Thailand in the early 1980s and later. The global canned tuna production exceeds 1.7 million metric tonnes annually (Miyake *et al.*, 2010).

Basically, canned fish processing involves heat treatment of fish followed by the packaging of treated fish in tightly sealed containers made from tin plates, aluminium, cans or glass. This canned product is fully sterilized to prevent from contamination by pathogenic organisms and stored them in a virtually airtight package. Less information is available for long-term quality changes in canned tuna products. Unofficial information from the canning industry indicated that high histamine levels might be part of problem regarding such long stored products (Iwan, 2006).

Histamine poisoning is known as scombrotoxism or scrombroid poisoning since the symptoms occurs as same result of ingesting spoiled fish of scombridae or scombresocidae. Scombroid fish such as tuna, mahi mahi, bluefish, bonito, skipjack, saury, mackerel, and others (CESFP, 1991; FDA, 2001b; Desphande, 2002). The accumulation of histamine in food requires the presence of microorganisms either gram

negative or gram positive bacteria that react with amino acid decarboxylases and favourable conditions for their growth and decarboxylation activity. Histidine decarboxylase and histaminase are the enzymes that responsible for the conversion of histidine to histamine (Taylor, 1986; Middlebrooks *et al.*, 1988; Flick *et al.*, 2001). The most common symptoms of histamine poisoning including rash, flushing, sweating, burning of the mouth, nausea, vomiting, diarrhea, stomach ache and allergy reaction. The content of biogenic amines in fish products will depend on the type of fish which contain different amount of free amino acids, the way the fish is handled which potential growth of bacteria and the duration, conditions and temperature of storage of the fish (Yoshinaga and Frank, 1982). All this factors can drive to highly variable levels of contamination within individual lots of fish and or within individual fish. This study was aimed to determine and identify the presence of histamine producing bacteria in the canned tuna products kept at different storage conditions for 3 weeks time.

MATERIALS AND METHODS

Sample Collection

Canned tuna used in this study were bought from TESCO hypermarket and Speedmart store. Table 1 shows the six types of canned tuna used and analyzed in this experiment. The manufacturing date of the canned tuna is between July 2011 until July 2013. The expiry date of the canned tuna is between July 2014 until July 2016. All the canned tuna samples were undergone storage treatment of 1 week storage at room temperature and 2 to 3 weeks storage in refrigerator (4ºC).

Table 1: Manufacturing Date and Expiry Date for each Sample of Canned Tuna

Sample	Manufacturing Date	Expiry Date
1	30 November 2012	30 November 2015
2	20 March 2013	20 March 2016
3	05 July 2013	05 July 2016
4	25 July 2011	25 July 2014
5	30 November 2012	30 November 2015
6	20 June 2013	20 June 2016

Preparation of Sample for Bacteriological Examination

The canned tuna samples were analyzed for the presence of bacteria at three different storage conditions: 1 week storage at room temperature, 2 weeks storage in refrigerator (4ºC) and 3 weeks storage in refrigerator (4ºC). The canned tuna was sterilized with 70% ethanol. One gram of tuna sample was taken and homogenized with the addition of potassium phosphate buffer. One ml of the sample was transferred into liquid broth and incubated for 24 hours. A serial dilution of samples was prepared and the samples of dilution 10^{-6} until 10^{-10} were spread onto the Niven's agar. All plates were incubated at 37ºC for 24 hours. The presence of colonies was observed, counted and calculated by using Colony Forming Unit (CFU). After 24 hours, the colonies were picked from each plate and transferred onto trypticase soy agar (TSA). The plates were incubated at 37ºC for 24 hours. All of the positive isolates on the Niven's agar were then cultured on TSA to obtain a pure culture. Biochemical tests such as catalase, oxidase and Gram stain were conducted to identify the morphology and general properties of the isolated bacteria.

RESULTS AND DISCUSSION

The present study was aimed to screen and identify the presence of histamine producing bacteria in canned tuna stored for 1 week at room temperature, 2 weeks in refrigerator (4ºC) and 3 weeks in refrigerator (4ºC). Histamine producing bacteria are able to grow in a wide range of temperature either at high temperature or freezing conditions.

Appearance of Canned Tuna

Table 1 indicates the six canned tuna with the manufacturing and expiry date used in this study. After the can are opened, all the canned tuna were stored in a refrigerator (4ºC) before proceed with the next

treatment. After 2 weeks of storage, the appearance of tuna became moisture due to the formation of droplets of waters from the exterior of the container except for sample 5 and 6 which still in dried condition. As a result, the surface of cans became corrosion. According to Hole (2003), the quality is limited by undesirable changes such as physical, microbiological, chemical and enzymatic possesses and followed by the taste and flavor change. Accumulation of histamine is a result of time and/or temperature abuse, which cause to spoilage during storage and processing (Iwan, 2006). The formation of histamine in canned tuna may occur/associated with the presence of epoxy resins which to protect product from direct contact with the metal surfaces. In the long term, these protective compounds will be migrating from coating into tuna products. As a result, metal ions will react to the products (Catalá *et al.*, 1998; Simal-Gándara *et al.*, 1998; Theobald *et al.*, 2000; Petersen *et al.*, 2003; Munguía-Lopez *et al.*, 2005). According to Iwan, (2006), it is recommended a shelf-life of the products is not longer than three years.

Screening and Isolation of Bacteria

The presence of histamine producing bacteria can be recognized by the formation of purple halo colonies on Niven's agar (Figure 1A). All six types of canned tuna samples were analyzed for the present of bacteria at three different storage conditions: 1 week storage at room temperature, 2 weeks storage in refrigerator (4ºC) and 3 weeks storage in refrigerator (4ºC). Throughout this study, sample of 1 week storage at room temperature have shown a greatly highest number of bacteria load which indicates histamine producer compared with sample of 2 and 3 weeks storage in refrigerator (4ºC). Samples of 2 weeks storage in the refrigerator have shown a high number of bacteria compared with sample of 3 weeks storage.

Table 2: Comparison of Bacterial Colonies Isolated from Canned Tuna Samples Stored at Three Different Storage Conditions

Sample of 1 Week Storage at Room Temperature					
Samples No.	**Diluents**				
	10^{-6}	**10^{-7}**	**10^{-8}**	**10^{-9}**	**10^{-10}**
1	TNTC	1.7×10^{9}	6×10^{9}	8.8×10^{10}	8.3×10^{11}
2	TNTC	TNTC	1.7×10^{10}	1.1×10^{11}	1.2×10^{12}
3	TNTC	TNTC	1.4×10^{10}	1.7×10^{11}	1.4×10^{12}
4	TNTC	1.8×10^{9}	5.6×10^{9}	1.1×10^{11}	7.6×10^{11}
5	TNTC	9.3×10^{8}	8×10^{8}	3×10^{9}	0
6	TNTC	TNTC	TNTC	TNTC	TNTC
Sample of 2 Weeks Storage in Refrigerator (4ºC)					
1	6.7×10^{7}	4×10^{8}	2.8×10^{9}	1.6×10^{10}	1.1×10^{11}
2	TNTC	TNTC	3×10^{8}	6.8×10^{10}	TNTC
3	TNTC	TNTC	1×10^{8}	2.5×10^{10}	9×10^{10}
4	TNTC	6.4×10^{8}	7×10^{8}	1.3×10^{11}	1.4×10^{11}
5	TNTC	TNTC	TNTC	8.2×10^{10}	1.1×10^{12}
6	1.9×10^{8}	1×10^{8}	2.5×10^{9}	9.4×10^{10}	3×10^{11}
Sample of 3 Weeks Storage in Refrigerator (4ºC)					
1	TNTC	TNTC	TNTC	TNTC	TNTC
2	TNTC	1.06×10^{9}	5.1×10^{9}	3.7×10^{10}	4.5×10^{11}
3	TNTC	TNTC	4.7×10^{9}	1.2×10^{11}	2.2×10^{11}
4	1.9×10^{7}	5.4×10^{8}	1.1×10^{9}	4×10^{9}	1×10^{10}
5	TNTC	3.4×10^{8}	3×10^{9}	1.4×10^{10}	7×10^{10}
6	2.1×10^{7}	5×10^{7}	3×10^{8}	2×10^{9}	1×10^{10}

TNTC = too numerous to count

Table 2 shows the comparison of bacterial counts isolated from canned tuna samples stored at three different storage conditions. Based on the Table 2, the number of colonies for each diluent become reduced and can be counted in 3 weeks. The present study demonstrated that the histamine producing bacteria are able to grow not only at room temperature but also in cold temperature. A total of 89 bacterial isolates from selective media of Niven's agar were identified. Based on the observation on analysis of bacterial count, it was found that the growth of bacteria can be reduced when the sample kept in cold or frozen (Allen, 2004). Freezing may inactivate the enzyme that produced by bacteria.

Morphological Identification

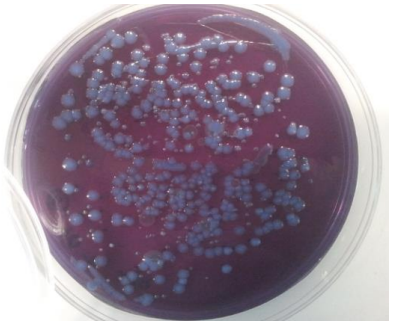
The morphological characteristics were further analyzed by Gram staining. Both Gram positive and negative bacteria can produce histidine decarboxylase but the formation of the enzymes is differ (Bjornsdottir-Butler *et al.*, 2010; EFSA, 2011).

Gram staining analyses indicated that most of the isolated bacteria are Gram negative with coccobacilli morphology (Figure 1B). Histamine-producing bacteria can arise from different part of fish such as gill, guts or muscle. Based on Shalaby (1996), there are three factors which influence the production of bacteria of biogenic amines: 1) the availability of free amino acid substrate, 2) the occurrence of microorganism that able to produce decarboxylate the amino acid and 3) favorable conditions for growth and enzyme production. Once fish was death, the microorganisms may start to grow because the defense mechanisms of the fish are inactive (Visciano *et al.,* 2014).

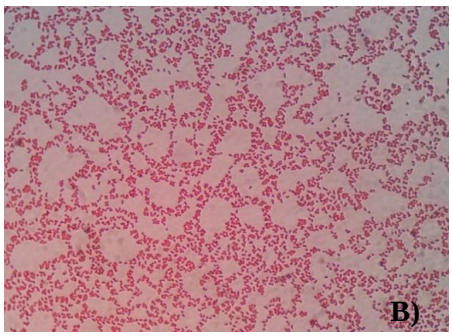
The bacteria that capable to produce amino acid decarboxylases are *Enterobacter, Clostridium, Hafnia, Klebsiella, Lactobacillus, Photobacterium, Proteus, Pseudomonas* and *Vibrio spp*. (Taylor, 1986; Niven, Jr. *et al.,* 1981; Eitenmiller *et al.,* 1982; Yoshinaga and Frank, 1982; Taylor, 1986; Ababouch, 1991; Lopez – Sabater *et al.,* 1996; Ben – Gigirey *et al.,* 1999b; Ben – Gigirey *et al.,* 2000; Flick *et al.,* 2001; Kim *et al.,* 2001; Kim *et al.,* 2002b; Takashi *et al.,* 2003). There are numerous species of bacteria that produce histamines and many belong to the *Enterobacteriaceae* and *Bacillaceae* families (Allen, 2004). The *Enterobacteriaceae* family includes genera of *Escherichia, Shilgella, Salmonella, Enterobacter, Klebsiella, Serratia* and *Proteus*. Based on Joshi and Vishal (2011), production of histamine also has been identified in Staphylococcus. Moreover, *Morganella morganii* has been reported as short bacilli Gram-negative (Kim *et al.,* 2001) which bacterium that frequently found in tuna and tuna products and associated with the presence of histamine.

Biochemical Tests

Biochemical properties of each isolates were further verified by oxidase and catalase tests. Through these tests, most of the isolates showed negative results for oxidase and positive result for catalase. The negative results for oxidase test indicates that the bacteria does not contain cytochrome c oxidase and unable to utilize oxygen for energy production. Meanwhile negative result indicates that the bacteria are from *Enterobacteriaceae*. For catalase test, most isolates showed positive result. This indicates that the bacteria able to break down the hydrogen peroxide. Members of *Enterobacteriaceae* family are catalase positive.



A)



B)

Figure 1: A) Formation of Purple Halo Colonies on Niven's Agar Plate; B) Morphology of Gram Negative Coccobacilli

Histamine producing bacteria is able to grow in wide range of temperature either high temperature or low temperature. From the observation on analysis of bacterial count, it was found that the growth of bacteria can be reduced when the sample kept in cold or frozen temperature. Meanwhile, histamine is heat stable which it cannot be reduce or eliminate by cooking, canning or freezing. For recommendation, the production of histamine can be controlled by rapid chilling of the raw material and stored at frozen temperature.

Moreover, good hygienic practices and proper cooling of tuna after catching and during transportation may reduce the risk of contamination by histamine producing bacteria. Although understanding of histamine producing bacteria has improved over the year, but much remains unknown. Further studies can be suggested which include: 1) identification of the specific bacteria that responsible for histamine production and histamine poisoning, 2) determination of the specific condition and the origin of histamine production in canned products and 3) determination of approach used to control or eliminate toxin production by histamine producing bacteria in canned products.

Conclusion

In the present study, a total of 89 bacterial isolates were identified as histamine producing bacteria from the selective media of Niven's agar. Most of the bacteria were isolated from canned tuna samples stored for 1 week at room temperature in comparison with other samples stored for 2 weeks in refrigerator (4ºC) and 3 weeks in refrigerator (4ºC). Majority of the isolated bacteria were identified as Gram-negative with coccobacilli morphology. Further identification of the bacterial isolates can be conducted including PCR amplification of *hdc* gene for confirmation of bacteria with the properties as histamine producer and identification of the bacterial species based on the sequence of 16S rRNA gene.

REFERENCES

Ababouch L (1991). Histamine food poisoning: an update. *Fish Technology News* **11** 3 – 5, 9.

Allen DGJr (2004). *Regulatory Control of Histamine Production in North Carolina Havested Mahi-Mahi (Coryphaena Hippurus) and Yellowfin Tuna (Thunnus Albacares): A HACCP – Based Industry Survey.* Published thesis. North Carolina State University, United State.

Ben-Gigirey B, de Sousa JMVB, Villa TG and Barros-Velazquez J (1999b). Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (Thunnus alalunga). *Journal Food Protection* **62**(8) 20 – 24.

Ben-Gigirey B, de Sousa JMVB and Villa TG (2000). Characterization of biogenic amine-producing Stenotrophomonas maltophilia strains isolated from white muscle of fresh and frozen albacore tuna. *International Journal of Food Microbiology* **57** 19 – 31.

Bjornsdottir-Butler K *et al.* (2010). Development of molecular-based methods for determination of high histamine producing bacteria in fish. *International Journal of Food Microbiology* **139** 161-167.

Catalá R, Cabañes JM and Bastidas JM (1998). An impedance study on the corrosion properties of lacquered timplate cans in contact with tuna and mussels in pickled sauce. *Corrosion Science* **40**(9) 1455 -1467.

European Food Safety Authority (EFSA) (2011). Scientific Opinion on risk based control of biogenic amine formation in fermented foods. *European Food Safety Authority Journal* **9** 2393 10.2903/j.efsa.2011.2393

Eitenmiller RR, Orr JH and Wallis WW (1982). Histamine formation in fish: microbiological and biochemical conditions. *Chemistry and Biochemistry of Marine Food Products*, edited by Martin RE, Flick GJ, Hebard CE and Ward DR (AVI Publishing Company, Westport, Connecticut, USA) 39 – 50

Food and Drug Administration (FDA) (2001b). *Fish and Fisheries Products Hazards and Controls Guidance* 3rd edition. (U. S. FDA Center for Food Safety and Applied Nutrition, Maryland, USA).

Flick GJ, Oria MP and Douglas L (2001). Potential hazards in cold-smoked fish: biogenic amines. *Journal of Food Science* **66** 1088 – 1099.

Hole M (2003). Storage stability. In: *Encyclopedia of Food Science and Nutrition* **9**, edited by Caballero B Trugo LC and Finglas PM (Academic Press, Oxford, England) 5604 – 5624.

Iwan SK (2006). *Factors Affecting Histamine Level in Indonesian Canned Albacore Tuna (Thunnus Alalunga).* (Department of Marine Biotechnology Norwegian College of Fishery Science, University of Tromso, Norway).

Joshi PA and Vishal Bhoir S (2011). Study of Histamine Forming Bacteria in Commercial fish samples of Kalyan city. *International Journal of Current Scientific Research* **1**(2) 39 – 42.

Kim SH, Field KG, Morrissey MT, Price RJ, Wei CI and An H (2001). Source and identification of histamine-producing bacteria from fresh and temperature-abuse albacore. *Journal of Food Protection* **64**(7) 1035 – 1044.

Kim SH, Field KG, Morrissey MT, Price RJ, Wei CI and An H (2002b). Occurrence of histamine-forming bacteria in albacore and histamine accumulation in muscle at ambient temperature. *Journal of Food Science* **67**(4) 1515 – 1521.

Lopez-Sabater EI, Rodriguez-Jerez JJ, Hernández-Herrero MM and Mora-Ventura MT (1996). Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. *International Journal of Food Microbiology* **28**(3) 411 – 418.

Middlebrooks BL, Toom PM, Douglas WL, Harrison RE and McDowell S (1988). Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (Scomberomorus maculatus). *Journal of Food Science* **53** 1024 -1029.

Miyake *et al.* (2010). Canned tuna production originally dates back as far as the 1860s in Europe and the early 1900s in the US. *Global Tuna Market* 85-87.

Munguía-López EM, Gerardo-Lugo S, Peralta E, Bolumen S and Soto-Valdez H (2005). Migration of bisphenol A (BPA) from can coatings into a fatty-food simulant and tuna fish. *Food Additives & Contaminants* **22**(9) 892 – 898.

Niven Jr.CF, Jeffrey MB and Corlett Jr.DA (1981). Differential plating medium for quantitative detection of histamine-producing bacteria from scombroid fish and mahi mahi. *Applied and Environmental Microbiology* **41**(1) 321 – 322.

Petersen H, Schaefer A, Buckow CA, Simat TJ and Steinhart H (2003). Determination of bisphenol A diglycidyl ether (BADGE) and its derivatives in food: identification and quantification by internal standard. *European Food Research and Technology* **216**(4) 355 – 364.

Shalaby AR (1996). Significance of biogenic amines to food safety and human health. *Food Research International* **29**(7) 675 – 690.

Simal-Gándara J, Paz-Abuín S and Ahrné L (1998). A critical review of the quality and safety of BADGE-based epoxy coatings for cans: implications for legislation on epoxy coatings for food contact. *Critical Reviews in Food Science and Nutrition* **38**(8) 675 – 688.

Takashi H, Kimura B, Yoshikawa M and Fujii T (2003). Cloning and sequencing of the histidine decarboxylase genes of gram-negative, histamine-producing bacteria and their application in detection and identification of these organisms in fish. *Applied and Environmental Microbiology* **69**(5) 2568 -2579.

Taylor SL (1986). Histamie food poisoning: toxicology and clinical aspects. *CRC Critical Reviews in Toxicology* **17**(2) 91 – 128.

Theobald A, Simoneau C, Hannaert P, Roncari P, Roncari A, Rudolph T and Anklam E (2000). Occurrence of bisphenol-F-diglycidyl ether (BFDGE) in fish canned oil. *Food Additives & Contaminants* **17**(10) 881 – 887.

Visciano P, Schirone M Tofalo R and Suzzi G (2014). Histamine poisoning and control measures in fish and fishery products. *Front Microbiology* **5** 500.

Yoshinaga DH and Frank HA (1982). Histamine-producing bacteria in decomposing skipjack tuna (Katsuwonus pelamis). *Applied and Environmental Microbiology* **44**(2) 447 – 452.