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A Review: Microbiological, Physicochemical and Health Impact of High Level of Biogenic Amines in Fish Sauce

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Abstract: Problem statement: Biogenic amines are basic nitrogenous compounds present in a wide variety of foods and beverages. Their formations were mainly due to the amino acids decarboxylase activity of certain microorganisms. Excessive intake of biogenic amines could induce many undesirable physiological effects determined by their psychoactive and vasoactive action. Fish sauce which is considered as a good source of dietary protein, amino acids, vitamins and minerals was a popular condiment in Southeast Asian countries. However, it has also been reported that fish sauce contain high amount of amines. Hence, attention should be given to ensure the safety of this product. **Approach:** A review study was conducted to deliver an overview on the presence of biogenic amines in fish sauce and to discuss the important factors affecting their accumulation. Impact of amines on human health and efforts to reduce their accumulation in fish sauce were also discussed to give a comprehensive view. **Results:** Histamine, putrescine and cadaverine is the most abundant amines in fish sauce with maximum reported value of 1220, 1257 and 1429 ppm, respectively. Tyramine present in a lesser amount with maximum reported value of 1178 ppm. Other amines such as tryptamine, phenylethylamine, spermine and spermidine were considered as minor amines. However, different profiles of amines were reported in different type of products. This was depended on microbial flora, availability of precursors and physicochemical factors such as temperature, pH, salt, oxygen and sugar concentration. In synergistically supporting physicochemical factors, several microorganisms such as *Enterobacteriaceae*, *Micrococci* and *Lactobacilli* were responsible for biogenic amines formation in fish sauce. **Conclusion:** Since the formation of amines in fish sauce was a result of many factors, it was almost virtually impossible to control each factor during fermentation. Addition of amines degrading bacteria into fish sauce fermentation might be useful to prevent amines accumulation. Concomitantly, a good and hygienic manufacturing procedure will enhance the safety of fish sauce.

Key words: Histamine, fish products, amines forming bacteria, amines toxicity

INTRODUCTION

Biogenic amines are basic nitrogenous compounds with low molecular weight formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones[1,2]. This compounds are ubiquitous and play an important role in human and animal physiological function[1,3]. Low level of biogenic amines in food is considered no significance health risk for consumption[4]. However, many toxicological effects such as headache, rash, diarrhea, respiratory distress, heart palpitation, hypertension or hypotension may occur if these amines are ingested in excessive amounts or when the natural mechanism for their catabolism are inhibited or genetically deficient[5]. The most notorious foodborne intoxication by biogenic amines are related to histamine[2,5,6]. The so called “scromboid poisoning” and “cheese reaction” are typical phenomenon usually caused by ingestion of food containing high level of histamine and tyramine, respectively.

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Biogenic amines formation in food products is either a result of endogenous amino acids decarboxylase activity in raw food material or the growth of decarboxylase positive microorganisms under conditions favorable for enzyme activity. Therefore, prerequisites for biogenic amines formation in foods including the availability of free amino acids, the presence of decarboxylase active microorganism and environmental condition enabling microorganism growth and their enzymatic activity[1,2,5,6]. Biogenic amines formation was also possibly altered by the presence of proteolytic enzymes as they play an important role in the release of free amino acids from protein tissues which offer the substrates for decarboxylation activity.

Fish sauce is a popular condiment in Southeast Asian countries. Thailand is the largest fish sauce producer with the annual production of more than 400 million liters[7]. The demand for this fermented fish product has been increased for over few years. To meet this demand, several manufacturing countries also increased their production. However, the quality of this product as well as its safety was not concerned yet. This was evidenced by the high biogenic amines contents in several fish sauce products from different countries[8-13]. Efforts should be conducted to ensure the safety of fish sauce as it was a good source of protein and amino acids. In order to effectively control and minimize biogenic amines formation, the cause of their formation must be identified. Hence, the objective of this review is to deliver an overview on the biogenic amines in fish sauce and the important factors influencing their accumulation. Attention is also given to their impact on human health and efforts to reduce their accumulation in fish sauce.

Characteristics of fish sauce: Fish sauce is a clear amber to reddish brown liquid obtained by hydrolysis of fish with salt. It is mainly produced from marine fishes such as anchovies (*Stolephorus* spp.), sardine (*Sardinella gibbosa*), mackerel (*Rastrelliger* spp.), herring (*Clupea* spp.) and capelin (*Mallotus villosus*)[14]. Although not popular, fresh water fish such as silver carp (*Hypophthalmichthys molitrix*) can also be used instead of marine fish[15]. Fish sauce is traditionally prepared by mixing fish with salt at the ratio varies from 2:1-6:1 and let to ferment at ambient temperature for 4-12 months[14]. The end of fermentation period is determined from the color, aroma, flavor and clarity which are typical quality of fish sauce[16]. The aroma of fish sauce can be differentiated into three distinctive groups: (1) the amoniacal which is attributable to ammonia, trimethylamine and several amines; (2) a cheesy aroma which is associated to the low molecular weight fatty acids (volatile fatty acids) with ethanoic and n-butanoic acids predominating; and (3) a meaty aroma due to a large number of volatile compound[17].

Fish sauce is considered as an important source of dietary protein and amino acids. It contains about 20 gL^{-1} of nitrogen of which 80% is in the form of amino acids[18]. Total amino acids and their profile in fish sauce were vary depending on the type of fish used as raw material and the method of fermentation. However, amino acids profiles in fish sauce was generally dominated by glutamic acid, lysine, aspartic acid, alanin, valin and histidine[19-21]. Water soluble vitamins including tiamin, riboflavin, niacin and vitamin B_6 and B_{12} were also found in fish sauce[14]. Many researchers had been detected a considerable amount of vitamin B_{12} in fish sauce[22-24]. It was suggested that consumption of 25 mL of fish sauce would result in 0.08-1.46 μg of vitamin B_{12} intake, which is sufficient to prevent megaloblastic anemia[16]. Moreover, consumption of fish sauce was also considered as an effective effort to reduce iron deficiency and prevalence of anemia in Vietnam[25,26].

Fish sauce contains high level of salt which is generally about 15-22%[19,21]. Therefore, its microbial flora isolated from fish sauce predominated by halophilic or halotolerant species such as *Tetragenococcus muriaticus*, *Halobacillus* sp., *Lentibacillus halophilus*, *Lentibacillus salicampi*, *Lentibacillus juripiscarius*, *Halobacterium halobium*, *Halococcus thailandensis* and *Bacillus vietnamensis*[27-33]. Bacteria involved in fish sauce can be classified into two major groups: (1) Bacteria that produce proteolytic enzymes such as *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Staphylococcus* sp., *Halococcus* sp. and *Halobacterium salinarium*; (2) Bacteria that relate to flavor and aroma development such as *Staphylococcus* sp., *Pediococcus halophilus*[14]. Bacteria responsible for flavor improvement during fish sauce fermentation can be originated from starter culture added. *Staphylococcus nepalensis* which improve fish sauce odor in Japanese fish sauce was originated from the fish sauce starter malt[34].

Biogenic amines content in fish sauce: Biogenic amines content of fish sauce are predominated by histamine, putrescine, cadaverine and tyramine[9,11-13,35]. Typtamine was present in lower level and occasionally found in considerable level in some samples[9,11,12]. Other amines such as phenylethylamine, spermine, spermidine and agmatine were considered as trace amine in fish sauce[10,12,13]. Moreover, phenylethylamine, spermine and spermidine are not the end products of bacterial decomposition in fishery products[6].

Table 1: Biogenic amines content of fish sauce from different origins

Fish sauce origin	N[a]	Mean (range value) of biogenic amines (ppm) HIS[b]	PUT	CAD	TYR	TRY	PHE	SPM	SPD	AGM	Ref.
Korea (anchovy)	8	624.5 (352.5-1127.6)	86.5 (33.8-182.1)	181.1 (81.6-263.6)	330.1 (93.9-611.3)	123.3 (60.1-296.8)	31.3 (9.3-54.1)	4.9 (1.9-12.2)	11.7 (4.7-27.1)	nd[c]	[9]
Korea (Pacific sand lance)	8	584.2 (215.4-1124.1)	84.9 (1.6-311.6)	165.3 (52.1-314.8)	342.7 (142.7-583.0)	117.1 (62.0-187.2)	30.4 (10.4-51.7)	4.5 (2.2-12.8)	9.7 (4.0-23.4)	nd	[9]
Malaysia	8	187.7 (99.0-372.9)	38.1 (28.4-64.9)	nd	174.7 (58.0-852.6)	82.7 (0-317.6)	Nd	nd	5.1 (3.0-7.0)	nd	[11]
Southeast asian countries	45	273.53 (0-729)	183.18 (0-1257)	269.91) (0-1429	207.89 (0-1178)	91.71 (0-588)	27.49 (0-251)	nd	nd	nd	[12]
Taiwan	12	394 (45-1220)	24 (2.0-243)	89 (0-243	9.4 (0-42)	87 (0-177)	3.8 (0-42)	52 (0-121)	9.0 (0-98)	18 (0-85)	[13]

[a]: Number of samples examined; [b]: HIS (histamine), PUT (putrescine), CAD (cadaverine), TYR (tyramine), TRY (tryptamine), PHE (phenylethylamine), SPM (spermine), SPD (spermidine), AGM (agmatine), [c]: Not determined

Many researchers had confirmed the high histamine content in fish sauce. However, a slight variability of other amines was also noted in fish sauce from different producing countries which have different fish species as raw material and method of fermentation (Table 1). The value of histamine can reach to 1220 ppm in Taiwan fish sauce[13] and 1380 ppm in Korean anchovy sauce[18]. The majority content of histamine in 549 commercial fish sauces in Thailand was in the range of 200-600 ppm[8]. Putrescine and cadaverine which are potentiators of histamine toxicity also the abundant amines in some commercial fish sauces. Putrescine and cadaverine content were reached up to 1257 ppm and 1429 ppm, respectively[12]. On the other hands, tyramine, tryptamine and phenylethylamine contents were 1178, 588 and 251 ppm, respectively[12]. Spermine, spermidine and agmatine were present in small amount in fish sauce. The highest spermine and spermidine content determined in fish sauce was 127 and 27.1 ppm, respectively13]. Agmatine which is a non routinely determined compound, probably due to its minimal adverse effect on human, could be considered as the trace amine in fish sauces.

Biogenic amines producing microorganism from fish: The type of amines present in food products was characterized by different type of microorganisms present because they have different decarboxylase activity. Table 2 shows that several microorganisms have been attributed to the formation of biogenic amines in fish sauce and its related products. Since histamine is the most abundant amines in fish sauce and mostly related to the poisoning incidence, its producers are of particular interest. Many *Enterobacteriaceae*, *Micrococceae* and *Lactobacilli* are most active in the formation of histamine in fish sauce[1,30,38,43]. Moreover, several species of those bacteria also produce other amines such as putrescine, cadaverine, tyramine, spermine and spermidine.

Table 2: Bacteria responsible for biogenic amines formation in fish sauce and related products

Biogenic amines	Microorganisms	Ref.
Histamine	*Tetragenococcus muriaticus*	[30]
	Tetragenococcus halophilus	[43]
	Enterobacter cloacae, *Pantoea* sp., *Pantoea agglomerans*	[38]
	Bacillus coagulans, *Bacillus megaterium*	[13]
	Morganella morganii, *Klebsiella pneumoniae*, *Hafnia alvei* *Morganella psychrotolerans*	[1]
	Lactobacillus sp., *Lactobacillus sakei*,	[39]
	Lactobacillus mesenteroides	[44]
	Staphylococcus epidermidis, *Staphylococcus capitis*	[37]
Putrescine	*Enterobacter cloacae*, *Pantoea agglomerans*	[38]
	Bacillus megaterium	[13]
Cadaverine	*Enterobacter cloacae*, *Pantoea agglomerans*	[38]
	Bacillus megaterium	[13]
Tyramine	*Paenibacillus tyramiegenes*	[40]
	Lactobacillus brevis	[46]
Spermine	*Pantoaea* sp., *Pantoea agglomerans*	[38]
Spermidine	*Pantoaea* sp., *Pantoea agglomerans*	[38]
	Bacillus coagulans	[13]

Enterobacteriaceae: *Enterobacteriaceae* are generally reported as dominant biogenic amines producers in fish and its products, particularly histamine, putrescine and cadaverine. *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* have been isolated from fish incriminated in scombroid poisoning[1]. Histamine forming enzyme was found in *Proteus mitabilis*[36], *Klebsiella oxytoca*[12], *Enterobacter cloacae*, *Pantoea* sp., *Pantoea agglomerans*[38] and *Morganella psychrotolerans* sp. nov. [39]. Moreover, Tsai *et al.* [38] revealed that *Enterobacter cloacae* and *Pantoea agglomerans* were able to produce putrescine and cadaverine. In addition to those biogenic amines, *Enterobacteriaceae* can also produce other types of amines. *Paenibacillus tyramigenes* isolated from salted and fermented anchovy was a strong tyramine producer[40]. *Pantoea* sp and *Pantoea agglomerans* can produce in a low level of both spermine and spermidine[38].

Micrococcaceae: Amino acid decarboxylase activity was found in some species belonging to genera of *Micrococcus* and *Staphylococcus*. Some species of the both genus isolated from fish sauce are exhibited histamine, putrescine and cadaverine producing enzymes (Unpublished data). It was observed that halotolerant *Staphylococcus epidermidis* and *Staphylococcus capitis* are strong histamine formers from salted anchovy[37]. *Staphylococcus carnosus* isolated from salted mullet roe products was also found to have histamine forming enzyme[41]. *Micrococcus luteus* was reported as dominant histamine former during salting of sardine[42].

Lactic acid bacteria: Lactic acid bacteria are generally recognized as non toxinogenic, although some species isolated from fish and its products can produce biogenic amines. Histidine decarboxylase enzyme was observed in halophilic *Tetragenococcus muriaticus* and *Tetragenococcus halophilus* isolated from fish sauce[30,43]. Some strains of *Lactobacillus* sp., *Lactobacillus sakei* and *Leuconostoc mesenteroides* isolated from fish silage also exhibit ability to produce histamine[4].

Other microorganisms: Biogenic amines forming enzyme are widely distributed in many bacterial species. Histamine forming bacteria belong to the genus of *Bacillus* isolated from fish including *Bacillus coagulans*, *Bacillus megaterium* and *Bacillus pumilus*[13,37]. *Bacillus megaterium* can also produce putrescine and cadaverine, while *Bacillus coagulans* can produce spermidine[13]. Some *Pseudomonas* species isolated from fish sauce and fish paste were found to have histidine decarboxylase enzymes (Unpublished data). Another histamine forming bacteria are including *Pseudomonas cepaceae*[37], *Clostridium perfringens* and *Vibrio alginolyticus*[36], *Photobacterium phosphoreum*[45].

Physicochemical factors influencing biogenic amines formation:

pH: The pH level is an important factor influencing amino acids decarboxylase activity. Since many decades ago, biogenic amines formation by bacteria was considered as a physiological mechanism to counteract acid environment[1,47]. Hence, activity of bacterial amino acids decarboxylase are generally optimum in low pH[30,47,48]. Kimura *et al.* [30] demonsrated that *Tetragenococcus muriaticus* produced higher histamine at pH 5.2 (668.6 ppm) than at pH 7.1 (15.8 ppm). Formation of high level of tyramine in a model system by *Carnobacterium divergens* occurred at initial pH less than 5.0[48]. The highest formation of spermidine by *Oenococcus oeni* T65 *in vitro* was also detected at pH 3, however, tyramine formation by this strain was reduced with the decrease of pH[49].

Temperature: Several authors reported that biogenic amines formation were temperature dependent in fish and its products. The production rate increase generally with the increase of temperature and time[1,50,51]. *Enterobacter cloacae* can produce putrescine at 20°C but not at 10°C. Cadaverine formation by *Klebsiella pneumonia* was also detected more extensively at 20°C than at 10°C[1]. Histamine formation was optimum at 37.8°C and was very dependent upon microbial activity[5]. The rate of histamine formation was slowed at 10°C and almost terminated at 5°C due to the slow growth of histamine forming bacteria[1]. Furthermore, higher temperature might favor proteolytic activity which provide substrate for decarboxylase enzyme resulting in increased amine level. However, the psychrotolerant *Photobacterium phosphoreum* and *Morganella morganii* can produced high concentration of histamine in chilled tuna and garfish[45,50]. The formation of histamine, putrescine and cadaverine in rainbow trout was also detected in high rate during ice storage[52]. It was confirmed that during the ice storage of fish and shrimp, some amine forming bacteria including genera of *Photobacterium*, *Aeromonas* and *Micrococcus* could survive and proliferate rapidly between 9 and 12 days and contribute to the amine formation[53].

Sodium chloride: Sodium chloride plays an important role in microbial growth and therefore influences the activity of their amino acids decarboxylase. The rate of histamine production by *Tetragenococcus muriaticus* was considerably reduced when salt concentration increased from 5-20%[30]. Histidine decarboxylase activity of *Staphylococcus capitis*, *Enterobacter cloacae* and *Pantoea agglomerans* were also retarded by the high concentration of salt[38,54]. This phenomenon can be attributed to reduced cell yield obtained in the presence of high sodium chloride concentration and to a progressive disturb of the membrane located microbial decarboxylase enzymes[55]. In contrast, a study[37] reported that sodium chloride enhanced activity of histidine decarboxylase of halotolerant *Stappylococcus* spp. isolated from salted anchovies. Hence, it can be assumed that the effect of sodium chloride either inhibiting and stimulating biogenic amines production is strains specific.

Oxygen: Oxygen appears to have a marked effect on the formation of amines which is dependent on the producing species. *Enterobacter cloacae* produce about twice putrescine quantity in aerobic compared to anaerob condition, while *Klebsiella pneumonia* produce considerably less cadaverine but acquired the ability to produce putrescine under anaerobic condition[1]. Modified Atmosphere Packaging (MAP) with 40 CO_2/60% O_2 can reduce histamine formation by *Photobacterium phosphoreum* during chilled storage of tuna for 28 days[50]. Biogenic amines content of sardine were highest in sardine stored in air, followed by vacuum packaging and modified atmosphere packaging[56]. In contrast, production of histamine by *Tetragenococcus muriaticus* was higher in oxygen limiting than aerobic condition although the growth rate was similar under both condition[30].

Other factors: The presence of fermentable carbohydrate such as glucose can increase both growth and amino acids decarboxylase of bacteria[1]. *Tetragenococcus muriaticus* produced histamine for only 82.1 ppm in a medium without glucose, but addition of 1-3% (w/v) glucose in the medium significantly increase histamine level (410.6-773.0 ppm)[30]. Halasz *et al.*[1] reviewed that optimum glucose concentration for biogenic amines formation was in the range of 0.5-2.0% (w/v), while level in excess of 3% inhibited decarboxylase activity. Furthermore, the presence of arabinose was also influences the formation of tyramine and spermidine by *Oenococcus oeni*, values able to maximize the production of tyramine minimized spermidine production. The formation of biogenic amines by *Oenococcus oeni* were influenced by the presence of ethanol in the medium. The highest production of tyramine was obtained in the presence of 8% ethanol, whereas spermidine at 12% ethanol level. Moreover, the presence of SO_2 and pyridoxal 5-phosphate also influence production of amines by this bacteria[49]. Repression of histidine decarboxylase activity has been known when the amount of histamine is accumulated in the medium[1]. Several phenolic compounds may also influence biogenic amines formation. Putrescine formation from agmatine by *Lactobacillus hilgardii* X_1B was diminished in the presence of protocatechuic, vanillic and caffeic acids and the flavonoid catechin and rutin[57] This indicated that beside their already beneficial properties to human health, phenolic compounds seem to be potential to diminish biogenic amines formation. Raw material was also influence to the accumulation of biogenic amines in fermented food products. The correlation between histamine level in raw material and final product had been evidenced in fish sauce[58]. Temperature abused fish used as raw material will possibly give a higher content of amine than fresh fish in fish sauce products[35].

Toxicity of biogenic amines: Biogenic amines are needed for many physiological function in human and animals, however consumption of food containing high concentration of biogenic amines can lead toxicological effects[4,59,60]. Histamine which is a common amine found in fish products is also known as a mediator of allergic disorder. Figure 1 shows that the release of histamine either as a response of allergic reaction by mast cell degranulation or consuming histamine containing food (food intolerance) can induce the same effects to the nervous and vascular systems. Therefore, misdiagnoses by physicians are occasionally occurred while investigating histamine poisoning incidence. However, histamine poisoning can be distinguished from allergy based on: (1) the previous history of allergic reaction to the incriminated food; (2) high attack rate in group of outbreaks and (3) the presence of high level histamine in the incriminated food[6]. Histamine poisoning occur throughout the world and probably the common form of toxicity caused by ingestion of food. However, good statistic about the incidence do not exist yet because the incidence often unreported due to the mild illness, lacking adequate system for reporting the incidence and ignorance by medical personnel who misdiagnose histamine poisoning as a food allergy[61,62].

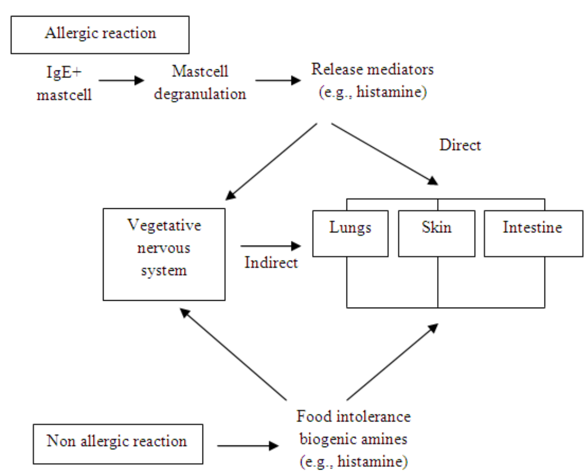

Fig. 1: Histamine release in response to allergic reaction and consumption of histamine containing food[6]

Based on the mode of action, biogenic amines can be differentiated into vasoactive and psychoactive amines[63]. Psychoactive amines influence neural transmitters in the central neural system, while vasoactive amines act either directly or indirectly on the vascular system. Histamine, putrescine and cadaverine are psychoactive amines, while tyramine, tryptamine and phenylethylamine are vasoactive amines. Histamine exerts its toxic by interacting with receptors (H_1, H_2 and H_3) on cellular membranes which are found in the cardiovascular system and in various secretory glands[5,63]. Histamine causes dilatation of peripheral blood vessels, capillaries and arteries, resulting in hypotension, urticaria, flushing and headache. Histamine also induced contraction of intestinal smooth muscle, mediated by H_1 receptor, causes abdominal cramp, diarrhea, nausea and vomiting. Moreover, stimulation of sensory and motor neuron by histamine causes pain and itching[61]. The action of vasoactive presser tyramine, tryptamine and phenylethylamine can cause hypertensive crisis in individuals on MAOI drugs therapy. The physiological effect of tyramine include peripheral vasoconstriction, increases the cardiac output, increases respiration, elevated blood sugar, releases noradrenaline and causes migraine[5,63,64]. Tryptamine may lead into depression and hepatic encephalophaty[64]. Phenylthylamine can cause migraine, dizzines and increase the blood pressure[65]. Putrescine and cadaverine can cause hypotention and potentiate toxicity of other amines, particularly histamine[5,66]. Several biogenic amines are also precursor of carcinogenic compounds[1,5]. Putrescine and cadaverine can be converted into pyrrolidine and piperidine, respectively, from which carcinogenic nitrosopyrrolidine and nitrosopiperidine are formed by heating[6,13]. Agmatine, spermine and spermidine can also react with the added nitrite in some fish, meat and vegetables products to from nitrosamines[1,67].

The toxicological effect of biogenic amines can only occur when they are ingested in excessive amounts or when the natural mechanisms for their catabolism are inhibited or genetically deficient. Human intestinal tract normally detoxifies these compounds in food by enzyme monoamine oxidase (MAO) and diamine oxidase (DAO). Detoxification efficiency varies considerably among individual and was affected by several factors. Dietary intake of some MAO inhibitors is the main factor suppressed detoxification of biogenic amines[6]. Unfortunately, MAO inhibitors are still remains an important class of drugs for a variety of psychiatric conditions, including depressive illnesses, anxiety and eating disorders[68]. It has also been reported that drugs used for antidepressants were nonselective irreversible inhibitor of MAO, while drugs used for anti parkinsonism were selective reversible inhibitor[63]. Therefore, those who are taking this kind of medicinal therapy should avoid consume food products with high potential concentration of biogenic amines. Consumption of alcoholic beverages also decreases the efficiency of detoxification system[6]. In addition, detoxification of one particular amine was inhibited by the presence of other amines. Putrescine and cadaverine hamper the detoxification of histamine and tyramine, tryptamine inhibit DAO and phenylethylamine inhibit HMT (histamine N-methyltransferase)[2,69,70]. These findings highlighted biogenic amines intoxication incident was resulted from interaction of many factors.

The oral toxicity of histamine in fish and its products are considered as slight at 80-400 ppm, moderate at over 400 ppm and severe at over 1000 ppm. Based on many fish poisoning episodes, guidelines for histamine content of fish are < 50 ppm (safe for consumption), 50-200 ppm (possibly toxic), 200-1000 ppm (probably toxic) and >1000 ppm (toxic and unsafe for consumption)[5]. However, the exact toxicity threshold of biogenic amines is not precisely determined because it depending on many factors such as individual acceptability and the presence of other components as toxicity potentiators[1,2,6,61]. Many countries have set regulation for acceptable level of biogenic amines in foods. The acceptable content of histamine set as a manufacturing standard in the United Kingdom in 1992 was 100 ppm. For control of histamine in fish belonging to the Scombridae and Clupeidae families, European Union has established regulation that nine independent samples from each batch should correspond to: (1) an average of histamine concentration lower than 100 ppm; (2) no more than two samples out of the nine with the concentration of histamine between 100 and 200 ppm; (3) no sample with histamine content higher than 200 ppm[61,71]. Australia New Zealand Food Authority (ANZFA) regulated that histamine content must not exceed 200 mg kg^{-1} in a composite sample of fish and fish products, other than crustaceans and mollusks[61]. The US Food and Drug Administration set value of 500 ppm histamine as the toxicity level and 50 ppm as the defect action level[61]. The Canadian Fish Inspection Agency set up the maximum limit for histamine content in fish sauce at 200 ppm[8]. Brink *et al*.[6] reported a legal upper limit for histamine in food (100 ppm) and alcoholic beverages (30 ppm). Furthermore, level of 100-800 ppm for tyramine and 30 ppm for phenylethylamine are considered potentially hazardous to human health[1,6]. However, the threshold levels for other amines are not established yet.

The determination of biogenic amines is important not only from the view point of their toxicity, but also because they can be used as spoilage indicators of food, in particular fish, meat and their products[6,72]. The concentration of histamine, putrescine and cadaverine are generally increases during spoilage of food, whereas concentration of spermine and spermidine are decrease during this process. The relationship between these amines was defined as Biogenic Amine Index (BAI):

$$BAI = \frac{histamine + putrescine + cadaverine}{1 + spermine + spermidine}$$

The concentration of amines are expressed as ppm. Fish or meat with BAI value below 1 is considered as the first quality, whereas BAI value above 10 indicate a very poor microbiological quality. The index score based on the formula above compared favorably to organoleptic values score[6]. Positive correlation between this two score have been reported both for fish[51,56] and meat[72].

Biogenic amine degradation: Biogenic amines are physiologically degraded by oxidative deamination process catalyzed by amine oxidases with the production of aldehydes, ammonia and hydrogen peroxide. A typical reaction of amines degradation is follow to the equivalence: $R\text{-}CH_2\text{-}NH\text{-}R' + O_2 + H_2O \rightarrow R\text{-}CHO + H_2N\text{-}R' + H_2O_2$[73-75]. These enzymes are ubiquitous and plays an important role in the metabolism of biogenic amines in human, plant and animal cells. Furthermore, amine oxidase has also been described for some bacterial strains[44,73-80]. Despite amine oxidase, some bacterial strains of *Pseudomonas aeruginosa*, *Pseudomonas putida* and the methylotroph *Paracoccus versutus* used amine dehydrogenase to oxidase amines[81]. Parrot *et al*.[79] revealed that the growth of bacteria can be supported by acids and ammonia which are produced during the sequential action of an amine oxidase and an aldehyde dehydrogenase in the presence of electron acceptor such as oxygen. The utilization rate of primary amines as a carbon and energy source was varied among microorganisms[81].

Biogenic amines degrading bacteria might also be present during fish sauce fermentation[82]. Several species of *Staphylococcus* isolated from fish sauce were found to have ability to degrade histamine, putrescine and cadaverine (unpublished data). Leuschner *et al*.[76,77] have tested potential amines degrading bacteria isolated from foods, particularly are strains belonging to the genera *Lactobacillus*, *Micrococcus* and *Arthrobacter* as well as to the species *Pediococcus acidilactici*, *Brevibacterium linens*, *Geotrichum candidum* and *Staphylococcus carnosus*. Some strains were reported to have histamine and tyramine oxidase at different activity level. This enzyme activity was affected by temperature (optimum at 37-40°C), pH (optimum at 7-8) and sodium chloride as well as glucose and hydralazine concentration. Dapkevicius *et al*.[44] reported that histamine degradation by diamine oxidase was also affected by temperature. Although the degradation rate of this amine was still considerable at 15 and 22°C, but the highest rate was observed at 37°C. They also reported that five strains of *Lactobacillus sakei* could degrade histamine as much as 20-56% in a model system within 30 hours. Several strains of *Staphylococcus xylosus* isolated from sausages were also showed the ability to degrade histamine and tyramine *in vitro*[78]. They found that *Staphylococcus xylosus* S81 exibited a remarkably potential of histamine degradation (100%), although it can only degrade 11.0% of tyramine under the same condition. However they also found that *Staphylococcus xylosus* S142 could degrade tyramine by 63% and histamine by 47% from their initial concentration after incubation for 48 h at 37°C.

Prevention and control of biogenic amines accumulation: Biogenic amines accumulation in fish sauce should be prevented to ensure its safety. Once amines are formed, there is no treatment capable to remove them. Heat treatment such as autoclaving can not even destroy biogenic amines present in fish[71,83]. Since there are many factors influencing amines formation, it is difficult to control each factor during fermentation. Therefore, eliminating one or more factors are likely worthy to control amines formation. Preparation of raw materials in fish sauce fermentation is suggested as critical phase determined amines content in the end product[35,58]. Fishes are rich in free amino acid content in their flesh and therefore vulnerable to the bacterial amino acid decarboxylase[5]. Fish sauce prepared from temperature abused anchovies has higher amines content than the same product prepared from fresh anchovies[35]. Therefore, a good and hygienic practice should be applied immediately from fish catching until ready to use in fermentation to control amines formation in fish sauce.

Since the last few years, the potential role of microorganisms with amine oxidase activity had become a particular interest to prevent or reduce biogenic amines accumulation in foods. A considerable degradation have been described in many food products. Addition of *Staphylococcus xylosus* as a protective culture was reported to reduce histamine and tyramine accumulation in salted and fermented anchovy[84]. *Micrococcus varians* strains decreased tyramine amount during ripening of fermented sausages[76]. A great reduction of tyramine content during fuet sausages fermentation was also achieved by inoculation of *Lactobacillus sakei* CTC494[85]. Formation of tyramine, putrescine and cadaverine in sauerkraut was significantly suppressed by inoculation of *Lactobacillus plantarum*[86]. Mixed culture of negative decarboxylase bacteria such as *Lactobacillus plantarum*, *Lactobacillus casei* subsp. casei, *Pediococcus acidilactici* and *Staphylococcus xylosus* were suppressed the accumulation of histamine, putrescine, cadaverine, tyramine and tryptamine in silver carp sausages[87]. In addition, the use of bacteriocin producing bacteria can also decrease biogenic amines content in foods. Histamine was not found in cheese with bacteriocin producing starter compared with 200 ppm in control cheese (without bacteriocin) after 4 months of ripening[88]. Moreover, Halasz *et al*.[1] reported that inoculation of *Lactobacillus curvatus* as a starter culture in the range of 5×10^6 to 2×10^7 CFU g^{-1} of cabbage was resulted in lower concentration of tyramine compared to natural fermentation of sauerkraut without starter culture addition. Since the used of amines degrading bacteria is effective to prevent amines accumulation in other food products, the similar method could be applied to the fish sauce fermentation. However, the safety of starter culture used must be taken into consideration to ensure the safety of fish sauce.

CONCLUSION

Only few studies are conducted on factors influencing biogenic amines formation in fish sauce as well as its degradation. The regulation regarding on their level in fish sauce was not also established yet. However, its may exceeded the unsafe level for related products regulated by many food authorities in different countries. Histamine, putrescine, cadaverine and tyramine are the most common biogenic amines found in fish sauce. However, tryptamine and phenylethylamine may also be found in a lesser amount depending on the product type. Biogenic amines presence in this product is often due to the decarboxylase activities of halotolerant or halophilic *Enterobacteriaceae*, *Micrococcaceae* and lactic acid bacteria. These bacteria are endogenously come from either raw material or environmental contamination. The capacity of their decarboxylase enzyme was likely to be strain dependent rather than species. Moreover, alteration or inhibition of amines production by these bacteria were markedly influenced by physicochemical factors such as pH, temperature, salts, oxygen and sugar concentration. The raw material have also gives different level and type of amines in the product since it provides different composition of precursor. The same raw material can even lead to different amines levels in final product if they are temperature abused. Since the production of biogenic amines is influenced by many interacting factors, efforts to control factor individually is difficult under normal fermentation process. Therefore, the effective ways suggested for preventing biogenic amines accumulation in fish sauce are the used of unspoiled raw material, prevent contamination during preparation and fermentation and if possible introduced the safe amines degrading starter culture into the fermentation system. With those particular efforts, the end product of fish sauce will be less in amines content and should be safe for consumption.

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