Reduction of biogenic amines in fermented fish sauces by using Lactic acid bacteria

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Abstract

Fish sauce is a fermented product which is used mainly in south Asian countries. Fish sauce contains many vitamins and minerals, and all essential amino acids and is especially high in lysine. Despite the positive nutritional characteristic, these products contain biogenic amines as unhealthy compounds. Biogenic amines are secondary metabolites which formed by microbial decarboxylation of respective amino acids or by amino acid transaminase activity on aldehydes and ketones, and categorized as bases with low molecular weight. These compounds are one of the prevalent issues regard to consumption of sea-foods, especially the cured and fermented products. Among the various compounds of biogenic amines, histamine, tyramine, putrescine, and cadaverine, are used for computing quality and biogenic amine indices for raw/processed sea-foods. Biogenic amines are a major causative agent of food borne diseases hence, their identification, and applying procedures corresponding to their decrement are significantly important in food industry. Many of the bacterial species are able to decrease biogenic amines in fish sauces. The application of bacteria possessing the biogenic amines degrading enzymes or starter cultures are novel techniques explored for reducing biogenic amines concentration in fermented fish products.

Keywords: Biogenic amines, Fish, Sauces, Bacteria, Fermentation

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Introduction

Fish sauce, a salty brown liquid with mild fishy flavor, is a fermented product which consumed since ancient times such as" Garum " made by romans from viscera and blood of mackerel." Garos " prepared in Greece made from the liver of Scomber colias, and also "Botargue" and "Ootarides" which produced in Italy and Southern Greece (Boziaris, 2014). Now a days in Southeast Asia especially in Thailand fish sauce production has annually extended deeper in to international markets. More over these kinds of products are also popular in Malaysia (Budu), Philippines (Patis), Indonesia (Ketjap-ikan), Japan (Ishiru), and China (Yeesu), respectively. As a general recipe for preparing this product is to store salted whole small fish in underground earthenware for 9 to 12 months in order to complete the fermentation and hydrolysis procedure. Fermented fish sauce can be consumed as condiment or in some cases as a main dish regard to its nutritional value, having significant proportion of amino acids, especially high in lysine, being a good source for obtaining vitamins, various especially B12 and also minerals such as sodium (Na), calcium (Mg),iron magnesium (Ca), (Fe), manganese (Mn), and phosphorus (P). On the other hand fish sauce has some consumption limitations because of salt concentration, biogenic amines which produced during the fermentation period and also the bacterial flora.

Biogenic amines are basic nitrogenous composition producing in meat and fish

products, mostly due to the amino acid decarboxylation activities of some microbes (Boziaris. 2014). Consumption of these products could lead to scombroid poisoning consumers (Tsai et al., 2006). Eight major amines i.e. histamine, tyramine, putrescine, cadaverine, tryptamine, phenylethylamine, spermine and spermidine were recognized in fish sauce, and paste products. However histamine, putrescine and cadaverine are the most abundant compounds, respectively (Zaman et al., 2010). Using cold storage can be used as an inhibitory process for histamine formation during fermentation period (FDA, 2011). It is assumed that the source of histamine in most fish sauces, was from Gram-negative bacteria being prevailing within fish during the early stages of processing; by the presence of free amino acids, decarboxylase activity of Gram-negative bacteria (Petaja et al., 2000). Lactic acid bacteria are being applied in many fish sauces; however studies (Munoz-Atienza et al., 2011) showed that lactic acid bacteria do not produce histamine or other biogenic amines. Moreover many authors revealed the regulation potential of histamine synthesis in fermented products by addition of halophilic lactic acid bacteria, as starter culture (Pierina et al., 2012). This article reviews the potential of Lactic acid bacteria in reducing the content of biogenic amines in fermented fish sauce.

Biogenic amines in processed sea food

Formation of biogenic amines is possible during processes such as

brining, salting. smoking. drving. pickling until the fermenting. and product is fully shelf-stable. Using refrigeration can be prevented histamine formation during these Processing operations (FDA, 2011). Samples of fermented fish products (fish sauce, fish paste, and shrimp paste) were analyzed for histamine content (Tsai et al., 2006), which was 394, 263, and 382mg/kg, respectively. More than 500mg/kg of histamine in three fish sauces, two fish pastes, and two shrimp paste products are reported. Moreover 7.4% of the tested samples contained >1000mg/kg. The mean amount of diverse BAs in target samples was reported less than 90mg/kg. Rihaakuru (one kind of fish paste), which is an important flavouring in the Maldives, have high amount of BAs, was originated from raw tuna. being exposed temperature abuse. to Histamine was identified at the high concentration in twenty-eight samples of Rihaakuru were analyzed for some BAs (5487mg/kg) (Naila et al., 2011). Tryptamine was not detected in most of the samples (only three samples contained <5mg/kg) and phenylethylamine only occurred at low levels (<25mg/kg). The authors supposed that the histamine found in Rihaakuru samples was most likely to have originated from Gram-negative bacteria growing in the fish before processing or within the fish during the early steps of manufacture. There are other processed seafood which have been investigated for BAs content. In southern China, three fish products are widely consumed: salted and fermented fish, canned fish, and packaged fish. Zhai et al. (2012) examined 49 fish products from the China market. The maximum total BAs content of lightly cured horse mackerel was 484.42mg/kg compared to 167.86mg/kg or less for the other salted and fermented fish products. In the Spanish mackerel sample, histamine was detected within range of 15.74-28.70mg/kg, the whereas the maximum histamine level was 26.95mg/kg in canned anchovies, 22.38mg/kg in canned sardines and less than 10mg/kg in all other canned samples tested (Zhai et al., 2012). Mah et al. (2002) found high levels of (155-579 mg/kg)histamine in fermented fish products made from anchovies, whereas Hwang et al. (2010) reported large amounts of histamine in dried fish products (63.1-479.0mg/kg). Anchovies prepared from fish of the Engraulis encrasicholus species, and are one of the traditional fish sauces in some Mediterranean countries. Pons-Sánchez-Cascado et al. (2005a)reported in salt-ripened anchovies tyramine has the most amount of biogenic amines with values up to 90mg/kg, whereas histamine did not exceed 20mg/kg. Then, the same authors analyzed samples of vinegaranchovies marinated and reported values for higher tyramine than (7.81)histamine and 0.54mg/kg, respectively) in 14 days of refrigerated storage (Pons-Sánchez-Cascado et al., 2005b). The condition for formation

BAs may be match with fermentation process condition like accessibility of free amino acids, the attendance of decarboxylase-positive microorganisms conditions causing bacterial and growth, decarboxylase synthesis, and decarboxylase activity (Petäjä et al., 2000). The result cleared certain fish products. especially that sauce produced from sardine and mackerel, mostly have large amount of histamine, almost 1000mg/l or greater (Tsai et al., 2006; Kuda and Miyawaki, 2010), as a result of the histidine decarboxylase of Tetragenococcus spp., a halophilic lactic acid bacterium. However, most studies (Thapa et al., 2006; Muñoz-Atienza et al., 2011) showed that in fermented fish products LAB produced no histamine or other BAs. Kuda et al. (2012) said that addition of some halophilic LAB, like a starter culture, can regulate histamine accumulation in salted and fermented fish products of nukazuke (salted and fermented fish with rice bran). 13 strains of 200 isolates from nukazuke fish, have ability for producing histamine at levels more than 200µg/ml but130 isolates produced no histamine. Moreover, 22 of the tested strains cleared to suppress histamine production (Kuda et al., 2012).

Microorganisms related to biogenic amines production

A lot of bacterial species are capable to convert histidine to histamine.

Enterobacteriaceae are generally considered as a primary cause of histamine production in scombroid fish. Morganella morganii, Klebsiella pneumoniae and Hafnia alvei are the strongest histamine producers (EFSA, 2011). Other bacterial species able to producing histamine include Morganella psychrotolerans. **Photobacterium** phosphoreum, Photobacterium psychrotolerans Ozogul, 2006). (Ozogul and Clostridium spp, Vibrio alginolyticus, Acinetobacter lowffi, Plesiomonas shigelloides, Pseudomonas putida. Pseudomonas fluorescens, Aeromonas *spp.* (Yatsunami and Echigo1993; Hwang et al., 2010). Some intestinal bacterial species able to producing histamine include Proteus vulgaris, Proteus mirabilis. Enterobacter Enterobacter cloacae. aerogenes, Serratia fonticola, Serratia liquefaciens and Citrobacter freundii (Tsai et al., 2005; al.. 2009). Kung et Staphylococcus spp., Vibrio spp, and Pseudomonas spp were found as histamine producing bacteria in fermented fish by Yatsunami and Echigo (1993). Fifteen histamine producing bacteria were isolated from tuna dumpling products by Kung et al. (2010).These are Raoultella. ornithinolytica (four strains). Enterobacter. aerogenes (two strains), prolific Abaylyi (one strain) for histamine formers and Pantoeasp (one strain). Serratia. marcescens (one starin). Bacillus. megaterium (one starin), Klebsiella. oxytoca (two strains)

and Klebsiella. Pneumoniae (onestrain). Kung et al. (2009)recognize Hafnia.alvei as a weak histamine Raoultella. planticola former. and Raoultella.ornithinolytica as prolific histamine formers with the ability to produce >500 ppm histamine in tuna sandwiches. Hwang et al. (2010) introduced **Staphylococcus** piscifermentans, Bacillus spp, and Bacillus subtilis as week histamine producer in tuna candy products. Bacillus, Citrobacter, Clostridium, Klebsiella, Escherichia, Proteus, Pseudomonas, Salmonella, Schigella, Photobacterium. Lactobacillus. Pediococcus, Streptococcus are capable of decarboxylating one or more amino acids (Brink et al., 1990). Lee et al. (2015a)identified ten histamine forming bacterial strains from milk fish having an ability to produce 373-1261 ppm of histamine in trypticase soy broth supplemented with 1% Lhistidine. The microorganisms identified were Enterobacteraerogenes (4 strains), Ecloacae (1strain), Morganellamorganii (2strains). Serratia marcescens (1 strain), Hafnia alvei (1 strain) and Raoultella orithinolytica (1 strain). Kung et al. (2015) cleared that Staphylococcus xylosusas, a halotolerant bacterium of dried frying fish products had a consistent ability to form more than 300 ppm of histamine at 3% sodium chloride concentration. In fermented seafood. Staphylococcus and Tetragenococcus spp produce histamine (Yatsunami and Echigo, 1993). Bacillus coagulans and Bacillus megaterium were identified as week histamine producer in fermented fish products by Tsai et al. (2006).The main phenylethylamine producers are tyrosine decarboxylating bacteria and its production is related to tyramine formation. According to Hu et al. (2012), the growth of mesophilic and psychrophilic bacteria in blue cod and octopus positively associated with the formation of amines such as putrescine, cadaverine, histamine and tyramine during chill storage.

Biogenic Amines intolerance

Histamine intolerance (HIT) due to high sensitivity to histamine is a metabolic disorder which varies between individuals because of the level of diamine oxidase activity, reduction of histamine catabolism rate and metabolic disorders (Maintz and Hungerford Novak 2007; 2010). Moreover some sort of therapeutics i.e. antidepressants and tuberculostatics act as diamine oxidase inhibitors (Prester, 2011); and ulcerative colitis, Crohn's disease, colorectal neoplasm (Petersen et al., 2002), atopic eczema (Maintz et al.. 2006)are effective on hypersensitivity of patients to histamine. Although the symptoms could be controlled through keeping on a free- histamine diet or by substitution of DAO (Prester, 2011).

The same problem is detected for consuming tyramine rich food stuffs in sensitive patients due to genetic or tyramine catabolism disorders; which maybe sever in those cases who take antidepressant and anti-parkinsonian drugs (McCabe-Sellers *et al.*, 2006).

As these food sensitivities become more common over the last decades, therefore producers especially fermented and fisheries industry try to control the level of biogenic amines in products.

Measuring Biogenic amines in foods

Regard to the importance of controlling the levels of biogenic amines in ingredients and processed foods. various analytical methods have been introduced as a part of food control processes. Generally these methods are classified into biological and analytical methods. In biological method which was the first method of AOAC for determining the histamine. it is measured as the amount of histamine that induces response in histamine sensitive organ like guinea ileum (Biji et al., 2016).

Chromatographic methods are generally used for quantification measurements of biogenic amines especially in seafood; such as gas chromatography, thin layer chromatography, high performance chromatography liquid and paper electrophoresis (Biji et al., 2016). As a convenient method test kits based on selective antibody and immunoassay reactions also used for histamine determination (Hungerford and Wu, 2012)

Biogenic amines degradation by microorganism

Many bacteria have been shown to primary amines convert via an oxidative deamination step into products that can be utilized either as a source of carbon and/or energy and as a of nitrogen. or both source (Hacisalihoglu et al., 1997; Levering et al., 1981). The potential role of microorganisms in food fermentation with amino acids oxidases and dehydrogenases activity has been investigated to prevent or reduce the accumulation of biogenic amines in foods. These findings are useful for the application of microbial degradation of biogenic amines (Dapkevicius et al., 2000; Siddiqui et al., 2000; Bozkurt and Erkmen, 2002; Gardini et al., 2002). Kongkiattikajorn (2015) reported that biogenic amines formation (cadaverine, putrescine. histamine. tryptamine, phenylethylamine, and tyramine) was significantly lower in som-fug (Thai traditional fermented fish sausage) fermented with starter culture (Table 1). Accumulation of these biogenic amines in som-fug could reduce significantly by the incubation with of L. sakei KM5474 and L. plantarum KM1450. Starter culture L. plantarum KM1450 had the highest ability in decreasing biogenic amines accumulation in somfug compared with starter culture L. sakei KM5474.

Type of product	Type of microorganisms	Researchs and date of publishing
som-fug	<i>L. sakei</i> KM5474 and <i>L. plantarum</i> KM1450	Kongkiattikajorn, 2015
sausage	Lactobacillus sakei CTC494 and L. curvatus CTC371	Bover-Cid et al(2000
fish pastes	Lactic acid bacteria species	Maria et al., 2000

Table 1: Microorganisms reduce biogenic amines in marine products.

Moreover mixed starter cultures of L. sakei KM5474 and L. plantarum KM1450 indicated the highest potential in reducing the amount of biogenic amines. Bover-Cid et al. (2000) cleared that the amount of biogenic amines fermentation during sausage and storage at 4 and 19°C reduced by the effect of Lactobacillus sakei CTC494, a negative amino acid-decarboxylase starter culture. The addition of starter culture resulted in a decrease on the biogenic amines formation, depending on the strain inoculated. A great reduction in tyramine content was achieved when L. sakei CTC494 was inoculated. whereas L. curvatus *CTC371* only attenuated tyramine accumulation compared with the control batch. The production of and cadaverine putrescine were significantly limited by both starter cultures, and they had inhibition effect on tryptamine and phenylethylamine formation by the wild microbial flora. Maria et al. (2000) showed that some bacteria, among which some Lactic acid bacteria species, are able to degrade biogenic amines by means of amino oxidases. This could be of interest for fish silage production, to control biogenic amines build-up in this product. Seventy-seven Lactic acid bacteria cultures isolated from fish pastes submitted to natural fermentation at two temperatures (15 and 22°C) and selected combinations of these isolates were examined for histamine, tyramine, cadaverine and putrescine production. Degradation occurred all at temperatures tested (15, 22 and 30°C), but not at pH 4.5. The application of bacteria possessing the biogenic amines degrading enzymes or starter cultures are novel techniques explored for reducing biogenic amines concentration in fermented fish products. Using a proper selected starter culture in sausages production was advised to produce safer product with low contents of biogenic amines.

Conclusion

Seafood products are important from nutritional and economical points of view, however formation of secondary metabolites, biogenic amines, may limited their consumption regard to be a of causative agents illness and physiological disorders. Many regulatory standards are set as the limitation levels of biogenic amines presence in food products. In this regard, prevention methods such as

applying microorganism starter culture are used to keep the level of biogenic amines lower than the announced acceptable standards.

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