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Comparison of Biogenic Amine Concentrations in Fermented Fish with and without Maize Seedling Extract

Jirasak Kongkiattikajorn[1]*

[1]Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok10150, Thailand Corresponding author: jirasakkong@gmail.com*

Abstract

Biogenic amines (BAs) are low molecular weight organic bases with aliphatic, aromatic, and heterocyclic structures. The formation of biogenic amines in food by the microbial decarboxylation of amino acids can result suffering allergic reactions, characterized by difficulty in breathing, itching, rash, vomiting, fever, and hypertension. Amine oxidase activity has become a particular interest to reduce biogenic amines concentration in food products such as Somfug or fermented fish. The aim of this study was to reduce biogenic amines accumulation during Somfug fermentation by maize seedling extract. Amine oxidase activity was measured by coupled reaction with peroxidase and guaiacol with putrescine as substrate at pH 7.5, 30 °C for 30 min. The amine oxidase activity of maize seedling extract was 27.54 U/ g fresh weight. The maize seedling extract of 10-24 ml was added in 100 g Somfug before fermentation. Amine oxidase activity of Somfug with maize seedling extract before fermentation was 5.50 U/ g Somfug. After 7 days of fermentation, total biogenic amines concentration was 83.04 % less in samples added with maize seedling extract, as compared to control samples. These results indicated that maize seedling extract with amines oxidase activity in Somfug fermentation was effective in reducing biogenic amines accumulation.

Keyword : *maize seedling, biogenic amines, fermented fish*

1. Introduction

Biogenic amines are basic nitrogenous compounds produced mainly by bacterial decarboxylation activity toward amino acids in foods (1, 2). Biogenic amines can cause adverse health effect to consumers when ingested at considerable amounts or when the natural mechanisms for their catabolism are inhibited or genetically deficient in the human body. Somfug is a popular fermented fish product and commonly used as a condiment in Thailand. Somfug is prepared by fermenting fish with cooked rice, minced garlic and salt at ambient temperature for 3–7 days. Typically, no starter culture is applied in fish sauce fermentation, since it merely relies on indigenous bacteria from raw materials. Biogenic amines are naturally present in many foods and relatively high contents of some BAs can be present in fermented foods. BAs are organic molecules with low molecular weight. These compounds are usually generated by microbial decarboxylation of amino acids present in foods. The aromatic amines (histamine, tyramine, serotonin, b-phenylethylamine, tryptamine) have been reported as vasoactive or psychoactive amines and they have been associated with food histaminic intoxications, food-induced migraines, and severe hypertensive crisis due to monoamine oxidase inhibitor drug interactions. Moreover, amines such as putrescine and cadaverine could generate carcinogenic nitrosamines in the presence of nitrites (3). Interest in cadaverine, putrescine, tyramine and histamine also lies in their potential as spoilage indicators of food. In addition, they may have unpleasant odours and it was also found that putrescine and cadaverine could inhibit the activity of muscle (4). Amine oxidases catalyze the oxidative deamination of primary amine groups of several biogenic amines in the presence of molecular oxygen, by accepting two electrons from the substrate and transferring them to oxygen, according to Equation 1:

$$\text{R-CH2NH2} + \text{O2} + \text{H2O} \rightarrow \text{R-CHO} + \text{NH3} + \text{H2O2} \quad (1)$$

Amine oxidases are frequently referred as semicarbazide sensitive amine oxidases, due to their characteristic sensitivity to inhibition by this compound. Many reports have demonstrated that the involvement of amine oxidases enzymes in amine catabolism and the products derived from their degradation are involved in a variety of important physiological processes. These include: cell wall maturation and lignification during cell development (5), wound-healing and cell wall reinforcement during pathogen invasion (6) and abiotic stresses, such as osmotic stress, phytohormones and salinity (7).

Amine oxidase activity was found in legumes pea epicotyls, Yiciafubn leaves, Lathyrus satiuus Euphorbiu latex though the enzymes have been in animals bacteria and fungi (8). Amine oxidase was found in cereal seedling, tea leave and maize seedling. Amine oxidase in maize seedling was high activity, comparing to the cereal seedling and tea leave, with the enzyme activities of 151.46 U/g dry wt and 156.75 U/g dry wt, for monoamine oxidase and diamine oxidase, respectively (9).

Monoamine and diamine oxidases had been described from cereal seedlings such as maize, mung bean and soybean and local plants such as green tea leaves and maize seedling monoamine oxidase and diamine oxidase from cereal seedling, green tea leaves and maize seedling. It was found that maize seedling contained high both monoamine oxidase and diamine oxidase (9).

Kalač et al. (10) reported a reduction of biogenic amines concentration in fermented food products by using lactic acid bacteria producing amine oxidase as inoculants and Zaman et al. (11) also reported that application of starter cultures with amines oxidase activity in fish sauce fermentation was found to be effective in reducing biogenic amines accumulation.

Meanwhile, only little information is available on the effect of local plant amine oxidase on biogenic amine reduction in fermented fish. Therefore, the objective of this study was to investigate the effectiveness of maize seedling amine oxidase in inhibiting biogenic amine accumulation during fish fermentation.

2. Materials and methods

2.1 Somfug preparation

Somfug (1 kg) prepared from homogenized fresh fish was mixed with minced garlic (120 g), cooked rice (200 g), and salt (45 g) for 10 min. Minced maize seedling (200 g) was added into the mixture and mixed thoroughly for 10 min. The mixture without minced maize seedling addition was used as the control. The mixture was then stuffed into a polyethylene casing with a diameter of 1.5 inch and the fermentation was conducted for 7 day. From studies of monoamine oxidase activities from maize seedling (9), it was represented catalytic activity in the range of 30-50 °C which the optimum temperature was about 45-50°C. These characteristics were slightly different from the diamine oxidase activities which represented the temperature activity of 30-40°C. The enzyme activities decreased rapidly when incubation in the temperature higher than 40 °C. So in this study, the temperatures in the range of 20-50°C were used for the enzymes to react with biogenic amines during fermentation.

2.2 Sample collection

Somfug samples were incubated in the incubator at 20-50°C for 7 day. During incubation samples were taken every 24 h of fermentation for analyses. In order to prepare the samples for analysis, the casings were removed. Samples were cut up and ground in a meat grinder for 2 min and kept at 4°C for further analysis.

2.3 Sample preparation and extraction

Four grams of sample was mixed with 10 ml of 5% trichloroacetic acid and extracted using homogenizer. The homogenate was centrifuged at 17,212 × g for 10 min at 4°C, the supernatant was collected and the precipitate was extracted again with 10 ml of 5% trichloroacetic acid. After centrifugation, the supernatant was kept at -20°C.

2.4 Derivatization of sample extracts and mixed standards

A 100 µl of 2 N NaOH and 150 of µl saturated $NaHCO_3$ were added to 0.5 ml of the extract, mixed with 1 ml of dansyl chloride (10 mg/kg in acetone) and incubated at 40°C in a water bath for 45 min. To remove residual dansyl chloride, 50 µl of 100% ammonia was added and the solution was centrifuged at 500 ´g for 30 min and the supernatant was filtered through a 0.45 mm filter. Dansyl derivatives of the calibration standards were mixed with the samples as previously described (12).

2.5 Biogenic amine determination

HPLC method with ultraviolet-visible spectrophotometry were performed with a LC 10 AD Shimadzu LC using a 20 µl loop. LC column C18-Hypersil BDS (200 mm. 4.6 mm, 5 µm particle size) was used. The detection wavelength was 254 nm for the dansyl derivatives. Amine standard solutions were prepared in water to a final concentration of 5 mg/kg for each biogenic amine. Putrecine, cadaverine, histamine and tyramine were used. Internal standard solution was prepared by diluting 15 mg of 1, 7-diaminoheptane in 5 ml of water. A gradient elution programme was used with the mobile phase of 100% methanol (solvent A) and nanopure distilled water (solvent B), starting with 55% solvent A and 45% solvent B and finishing with 100% solvent A and 0% solvent B after 45 min. The flow rate was 1.5 ml/min.

2.6 Statistical analysis

Data were expressed as mean ± standard error of mean (SEM) of three-independent experiments.

3. Results and discussions

Putrescine profile of Somfug with maize seedling extract during fermentation was presented in Figure 1 (A). Putrescine concentration slowly decline from 64.1 mg/kg at day 0 to 39.4 mg/kg during 7 days of fermentation in sample at 50°C, respectively. For sample at 30°C, putrescine concentration progressively highly decreased from 64.1 mg/kg at day 0 to 17.5 mg/kg during 7 days of fermentation. In sample at 20, 25, 35, 40 and 45 °C, putrescine concentration also decreased during 7 days of fermentation.

Cadaverine profile of Somfug with maize seedling extract during fermentation was presented in Figure 1 (B). Cadaverine concentration was decrease from 57.4 mg/kg at day 0 to 39.1 mg/kg at day 7 of fermentation in sample at 50°C but cadaverine concentration at 30°C, highly declined to 20.4 mg/kg at day 7 of fermentation. However, cadaverine contents incubated at 20, 25, 35, 40 and 45 °C also slowly declined during fermentation.

Histamine profile of Somfug with maize seedling extract was presented in Figure 1 (C). Sample incubated at 50°C, the concentration of histamine was decrease from 79.2 mg/kg to 63.0 mg/kg during 7 days of fermentation. The sample at 30°C, histamine concentration showed maximal reduction from 79.2 mg/kg to 43.4 mg/kg during fermentation. However, incubation at the other temperatures, histamine concentration slowly decreased.

Tyramine profile of Somfug with maize seedling extract during fermentation was presented in Figure 1 (D). Tyramine concentration was slowest decrease comparing to the temperature 20-45°C. The incubation at 30°C showed tyramine concentration maximum decrease.

Putrescine content in control was higher than that of treated samples throughout fermentation at 30°C. The results showed putrescine concentrations during 7 day of fermentation were increase from 64.1 mg/kg to 161.7 mg/kg, (Figure 2 (A)) in control, while samples treated with maize seedling homogenate, putrescine concentrations decreased to 7.4 mg/kg while putrescine concentration was observed to be higher in control than samples added with maize seedling homogenate during 7 days of fermentation.

Final concentration of putrescine reached 153.6 and 7.2 mg/kg in control and samples with maize seedling, respectively. Cadaverine concentration in control was progressively increased from initially around 57.4 mg/kg to 152.8 mg/kg (Figure 2 (B)) in control during fermentation at 30°C while samples added with maize seedling homogenate, cadaverine concentration decreased to 14.1 mg/kg.

Histamine concentration in control was increased from initially around 79.2 mg/kg to 177.6 mg/kg (Figure 2 (C)) during fermentation at 30°C while samples added with maize seedling homogenate, histamine concentration decreased to 42.8 mg/kg. For tyramine in control, the concentration was increased from 104.0 mg/kg to 249.5 mg/kg (Figure 2 (D)) while samples added with maize seedling homogenate, tyramine concentration decreased to 61.5 mg/kg.

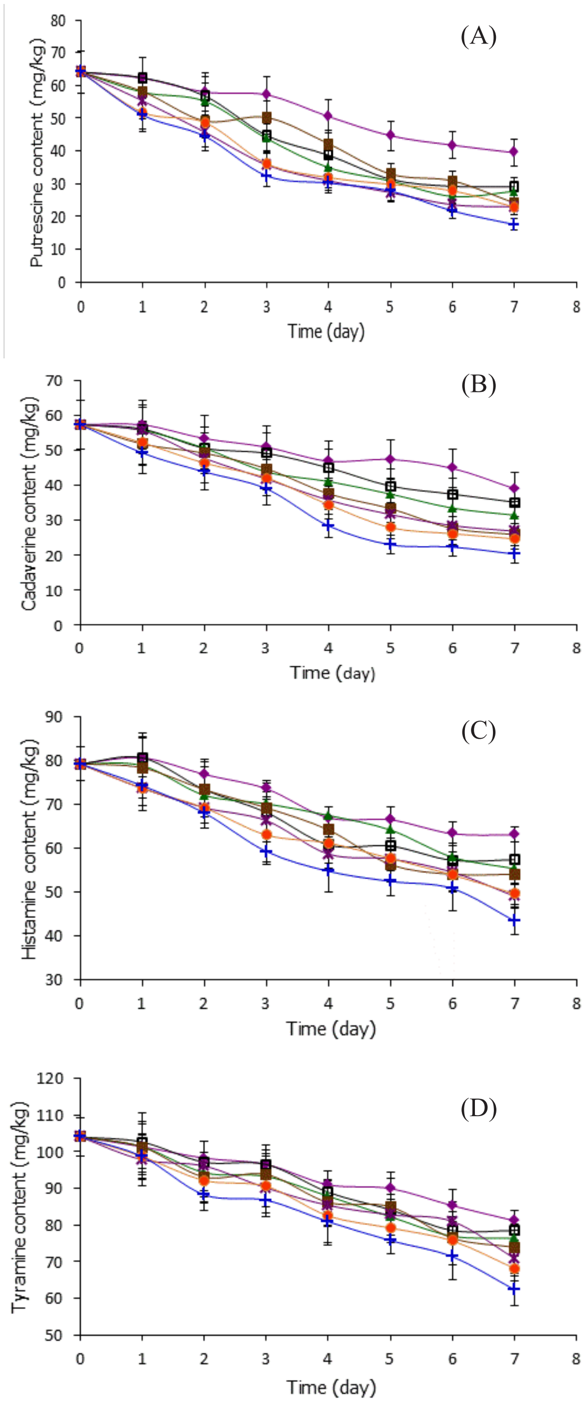

Figure 1. Effect of temperature on biogenic amine during fermentation of fish at pH 6.0 for 7 days. (A): putrescine, (B): cadaverine, (C): histamine and (D): tyramine. ▲: 20°C, ■: 25°C, +: 30°C, ●: 35°C, ✳: 40°C, □: 45°C, ◆: 50°C

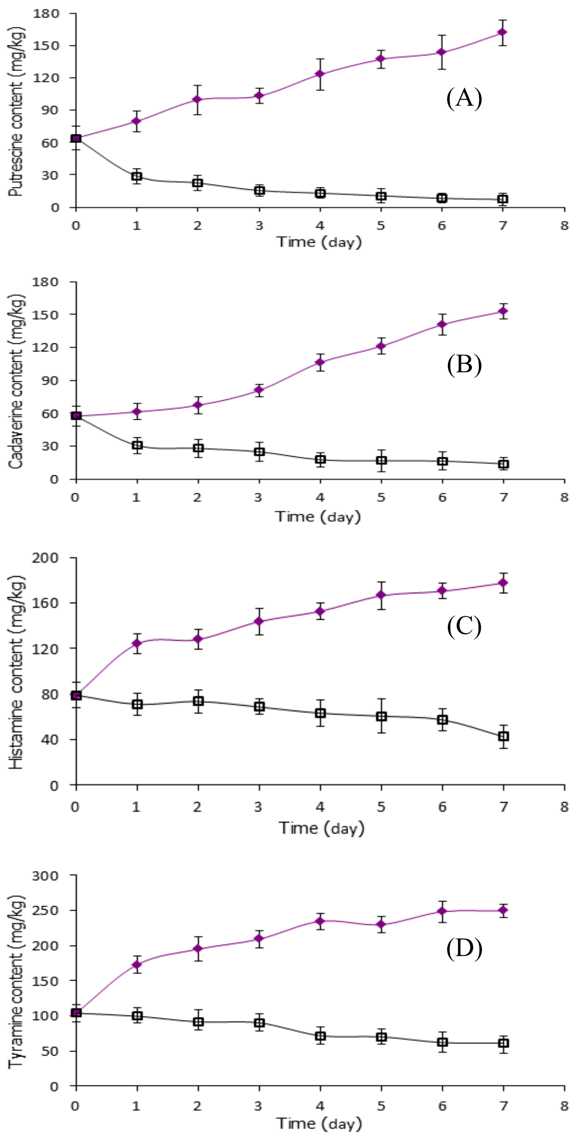

Figure 2. Biogenic amine profiles (n=3) during fermentation of fish at 30 °C for 7 days. (A): putrescine, (B): cadaverine, (C): histamine and (D): tyramine. □: fermented fish added with maize seedling homogenate, ♦: fermented fish without maize seedling homogenate (control)

Seven temperatures were tested for the antibiogenic amine activity of the enzyme: 30-40°C was found to be the optimum temperature of maize seedling amine oxidase according to the report of Yeunyongsuwan and Kongkiattikajorn (9). Whereas high enzyme activity at 30-40 °C was observed, it is plausible to apply maize seedling amine oxidase in high temperature (such as a fish processing fermentation). In this study, 30°C showed highest decrease biogenic accumulation. However, 40°C might be the probable application of the enzyme in fish processing fermentation at incubator during fermentation. Antibiogenic amine activity was determined by measuring the decrease in amine concentration (Figure 2). In contrast to the control group, maize seedling amine oxidase could reduce BAs contents of sample at 20°C -50 °C. However, maize seedling amine oxidase represented a significant reduction in amine content after 1 d at 30 °C in comparison to control group. This is consistent with previous studies (9) and suggests that this temperature is suitable for maize seedling amine oxidase antibiogenic amine activity during fermentation.

The initial putrescine, cadavarine, histamine and tyramine levels were 64.1, 57.4, 79.2 and 104.0 mg/kg, respectively. Putrescine, cadavarine, histamine and tyramine contents of in control were elevated to 161.7, 152.8, 177.6 and 249.5 mg/kg, respectively, after 7 d at 30°C while putrescine, cadavarine, histamine and tyramine contents in Somfug added with maize seedling homogenate were reduced to 7.4, 14.1, 42.8 and 61.5 mg/ kg, respectively. In the report that porcine amine oxidase fitted into an exponential decline model of amine degradation in fermented fish sauce (11), maize seedling amine oxidase also show significance difference in reduction of biogenic amine in fermented fish.

Biogenic amines were regarded as the main factor causing the increase in pH, since their concentration increased in accordance with the increase in pH. It had been considered that formation of biogenic amines by bacteria is a physiological mechanism to counteract the acidic condition (2). Enterobacter cloacae, Pantoea agglomerans and Bacillus megaterium were putrescine and cadaverine producers isolated from fermented fish (13, 14). Paenibacillus tyramigenes was reported as tyramine producing bacteria (15). Lactobacillus brevis is also known as a tyramine producing bacterium (16). Biogenic amines concentration namely histamine, putrescine, cadaverine and tyramine were found to increase throughout the fermentation. The increase was progressive during the 7 days of fermentation. Veciana-Nogues et al. (17) also reported that these four amines have increased during manufacture of semipreserved anchovies. Biogenic amines formation in fish was mainly due to the activity of bacterial amino acid decarboxylation (1). This will consequently lead to the accumulation of biogenic amines during fish fermentation when the environmental condition was suitable for decarboxylase activity. Histamine has been found to be the most active amine; therefore, most of research on biogenic amines has been focused on histamine. It was found that biogenic amines concentration increased markedly during 7 days (Figure 2) of fermentation and therefore resulted in a high rise in the level of histamine within the same period (Figure 2 (C)). Maize seedling which had amine oxidase activity and it was used in this study, indigenous bacteria in fermentation process were deemed responsible for biogenic amine production. So, biogenic amine concentration was higher in control than samples added with maize seedling homogenate throughout the fermentation period.

Fish contains high amount of histamine. In this study, after 7 days of fermentation, histamine concentration was 42.8 mg/kg (Figure 2 (C)) in samples with maize seedling homogenate, as compared to control samples (177.6 mg/kg). This proved that maize seedling could reduce histamine accumulation. It should be noted that histamine is quite resistant to degradation once it is formed in food products. Heat treatment such as autoclaving was found not to be effective to degrade histamine and other amines (18).

In this study, amine oxidase activity in maize seedling homogenate could be estimated to about 27.5 U/g fresh wt according to previous study (9) and the total enzyme activity could be estimated about 5,504 U in 1 kg of minced fish. So, amine oxidase could reduce the biogenic amines in fermented food samples (10, 11). After 7 days of fermentation, the overall biogenic amines concentration (sum of putrescine, cadaverine, histamine and tyramine contents) was 125.8 mg/kg in samples added with maize seedling homogenate, as compared to control samples (741.6 mg/kg). This investigation emphasized that application of local plant with amine oxidase in fish fermentation was effective in inhibiting biogenic amines accumulation. However, further investigation is needed to clarify the factors influencing biogenic amines degradation by the amine oxidase in local plant so that optimal degradation can be obtained. The application of maize seedling amine oxidase in homogenized fish not only prevented the amine raise - more frequent in the homogenized texture of meat through bacterial action-but also reduced the already existent amine to the initial level. Accordingly, it is suggested that the maize seedling homogenate has the feasibility of amine reduction in foods. The antibiogenic amine properties assigned to maize seedling amine oxidase, elicits a novel enzymatic strategy for amine degradation in fermented fish. Its application in fermented fish is more practical because the preparation process is less time and energy consuming.

4. Conclusion

In this study, amount of biogenic amines in Thai fermented fish samples of Somfug made by homogenized fresh fish with and without maize seedling, under laboratory sanitized conditions was determined. It was found that Somfug samples with maize seedling homogenate contained biogenic amines less than Somfug without maize seedling homogenate. Based on the this findings, it can be urged that an approach of using maize seedling homogenate for the production of good quality Somfug could be recommended as a safer alternative for its commercialization. Hence, it is concluded that Somfug samples produced in this study can be recommended for human consumption at commercial level. However, research is in progress on the development of more combinations of beneficial local plants as amine oxidase in order to further improve the quality and safety of Somfug samples in terms of reduced contents of biogenic amines.

5. Acknowledgement

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