2014 International Conference on Food Security and Nutrition
IPCBEE vol.67 (2014) © (2014) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2014. V67. 19

Biochemical Changes of Salt-Fermented Tuna Viscera (*Dayok*) and Its Effect on Histamine Content During Fermentation

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Abstract. *Dayok* is a mixture of tuna viscera, salt and small amount of spices (garlic and ginger) fermented at ambient room temperature for 7 days and has not been subjected for heat treatment. The effect of varying salt concentration (10%, 17.5% and 25%), fermentation temperature (30-35 ^{o}C and 40^{o}C) and fermentation period (3 and 7 days) on microbial, chemical and biochemical changes of tuna viscera during fermentation were investigated. Effect of the different fermentation conditions on microbial, chemical and biochemical changes on tuna viscera were monitored. The results demonstrated that the levels of pH, lactic acid, amino nitrogen and TVB-N increased during fermentation. The formation of histamine during fermentation is affected by pH, lactic acid, amino nitrogen, TVB-N, total plate count and LAB count. An increase in pH with a corresponding decrease in lactic acid, increase in amino nitrogen, TVB-N, total plate count and LAB count produces a corresponding increase in histamine. Furthermore, formation of histamine during *dayok* fermentation was found to be influenced by salt concentration and fermentation period and not by fermentation temperature.

Keywords: tuna viscera, histamine, fermentation

1. Introduction

In recent years, concern about food safety, together with consumers demand for safe and healthier products have promoted studies of compounds with harmful effects on human health. Fish fermentation, an old preservation technique, has been found to contain high contents of histamine such as fish sauce and fish paste [1]. Histamine (or scombroid) fish poisoning is a foodborne chemical intoxication caused by eating spoiled or bacterially contaminated fish [2]. Scombroid fish such as tuna, mackerel, bonito and saury are often implicated in scombroid poisoning incidents when not properly processed and stored [3]. In the Philippines, tuna viscera are widely used as a raw material in the manufacture of fermented fish paste. *Dayok* is a mixture of tuna viscera, salt and small amount of spices (garlic and ginger) fermented at ambient room temperature for 7 days and has not been subjected for heat treatment. Little information is available on the use of tuna viscera in fermented products such as fish paste. With the increasing number of consumers being exposed to this product, it seemed appropriate to evaluate more on the safety risks that *dayok* might pose to the consumers to histamine poisoning. This study was undertaken to investigate the processing conditions affecting histamine formation in salt-fermented tuna viscera in order to find ways to control this hazard at levels below the allowable limits. The effect of varying salt concentration (10%, 17.5% and 25%), fermentation temperature (30-35 ^{o}C and 40^{o}C) and fermentation period (3 and 7 days) on microbial, chemical and biochemical changes of tuna viscera during fermentation were investigated.

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2. Methodology

2.1. Fermented Tuna Viscera Preparation

Yellowfin tuna (*Thunnus albacares*) viscera, excluding the bile sac and heart, was used in the study. Fresh tuna viscera were obtained from eviscerated yellow fin tuna and placed in ice box with a fish to ice ratio of 1:2 (w/w) and were transported to the laboratory. Upon arrival, the tuna viscera was washed, drained and mixed with salt at 10%, 17.5% and 25% concentrations. The samples were placed in sterilized sealed bottles and were allowed to ferment for 7 days at varying temperatures (ambient temperature and 40^{o}C). Samples of salted tuna viscera obtained at 0, 3, and 7 days of fermentation were simultaneously analyzed for microbial and chemical analyses.

2.2. Microbiological and Physical Analysis

Total bacterial plate count was counted on PCA plates while colonies on MRS agar + 1% $CaCO_3$ showing clearing around the colonies was counted as LAB after the incubation period at 30 C for 48 hours. The bacterial count of the fermented product was expressed as $\log_{10}$ colony forming units (CFU)/g.

2.3. Determination of pH, Salt Content, Total Titratable Acidity and Amino Nitrogen

The pH values of the samples were measured using a pH pen (Hannah instruments model HI196107). Five grams (5g) of homogenized tuna viscera samples were added with 10 ml of distilled water and pH values were measured. The pH pen was calibrated with pH 4.0 and pH 7.0 standard buffers prior to its use.

The salt content was determined by diluting samples (5 g) with 100 ml of distilled water. Five (5) ml of the solution was placed in a 125 ml Erlenmeyer flask then 1 ml of 2% potassium chromate solution was added. The solution was titrated with 1/50 N silver nitrate solution to a light orange endpoint.

Total titratable acidity (expressed as percent lactic acid) was determined by diluting five (5) mL sample with 100 mL distilled water. Twenty-five (25) mL of the solution was placed in a 125 mL Erlenmeyer flask and titrated against a standardized 0.1 N NaOH to pH 8.2.

Amino nitrogen content was determined by the Formol titration method. The sample used in determining the total titratable acidity which was previously neutralized with 0.1 N NaOH solution to pH 8.2 was used. The sample was added with 10 mL of neutralized formaldehyde (38%, v/v). (Note that for protein-rich samples, the pH of the sample is expected to decrease upon addition of formaldehyde). The mixture was titrated with standard 0.1 N NaOH to pH 8.2. Amino nitrogen was expressed as mg%.

2.4. Determination of Total Volatile Base Nitrogen (TVB-N) and Histamine Content

The TVB-N content of the samples was measured by the Conway Microdiffusion method while histamine was analyzed following the AOAC Fluorometric method [4].

2.5. Statistical Analysis

Test for significance for the analytical study were done using analysis of variance (ANOVA). Values of $p< 0.05$ was regarded as significant. Pearson correlation and multiple linear regression was carried out to determine the relationship between pH, salt content, total bacterial count, lactic acid bacteria count, pH, amino nitrogen, TVB-N and histamine in fermented tuna viscera. Statistical analysis was performed using the Statistical Analysis System (SAS) program.

3. Results and Discussion

Tuna viscera's (*dayok*) traditional fermentation process involves mixing tuna viscera with 25% salt, fermented at ambient temperature (30-35℃) for 3 days, draining, chopping or mincing, addition of spices (ginger and garlic) and then packed in bottles without heating. In this study, the formation of histamine is assessed by monitoring the histamine content in every processing stage during the production of *dayok* in relation to the different fermentation parameters to histamine content.

3.1. Influence of Salting Concentration on Histamine Content during Fermentation

After tuna viscera was mixed with salt at varying concentrations, the varying levels of salt concentration significantly affect histamine formation. The initial histamine content of raw material (49.60 ppm)

significantly increased upon addition of salt with values of 77.20, 83.60 and 101.50 ppm for 10%, 17.5% and 25% salt concentration, respectively. The histamine content increases with increasing salt concentration. These values are higher than those reported by [1] on fish paste at 26.30 ppm. This could be attributed to the difference in fish species or parts used for processing, fermentation condition aside from the high initial histamine content of the raw material. However, no significant correlation exists between pH, salt content, lactic acid, amino nitrogen, TVB-N and fermentation temperature. During the salting process, histamine formation was not influenced by these factors.

3.2. Influence of Fermentation Time on Histamine Content during Fermentation

As shown in Table 1, histamine formation on tuna viscera is significantly affected by salt concentration and temperature when fermented for three (3) days but only to some extent. High levels of histamine were formed at 10% NaCl fermented at 40^{o}C (101.90 ppm) which is twice higher than the FDA regulatory limit of 50 ppm while lower values were obtained at higher salt concentrations of 17.5% and 25% NaCl. However, no significant differences were observed on tuna viscera at 17.5% NaCl fermented either at ambient temperature (30-35^{o}C) and 40^{o}C with histamine values below the regulatory limit at 46.40 and 43.10 ppm, respectively. On the other hand, samples fermented at 10% and 25% NaCl at ambient temperature have higher histamine content above 50 ppm at 63.60 and 84.80 ppm, respectively. The increase in histamine content at 25% NaCl could be attributed to the very high salt concentration which affects the rate of salt penetration into the viscera caused by its saturation point creating slow diffusion of salt into the samples while at 17.5% salt was completely dissolved in the solution, thus faster rate of penetration. The formation of high histamine levels at 10% NaCl indicates that histamine forming microorganisms are moderately halophilic or salt tolerant and histidine decarboxylase activity is better at 40^{o}C which falls within the optimum growth temperature of enzymes at 37-40^{o}C. In addition, the fermentation temperature of 30-35 ^{o}C falls within the optimal growth temperature between 20 ^{o}C and 37 ^{o}C for most bacteria containing histidine decarboxylase enzymes [5] which also explains the high histamine values of tuna viscera at salt concentrations of 10% and 25% fermented at ambient temperature.

Table 1. Histamine content of tuna viscera salt fermented for three days at varying salt concentration and fermentation temperature.

Salt concentration (%)	Fermentation temperature (^{o}C)	Histamine content (ppm)	
		After salt addition	After fermentation
10	30-35	61.60±10.77	63.60±15.50
	40	92.80±16.23	101.90±24.83
17.5	30-35	88.00±15.39	46.50±11.33
	40	79.20±13.85	43.10±10.50
25	30-35	95.30±16.67	84.80±20.66
	40	107.80±18.85	45.80±11.16

Results are expressed as means ±SD (n=3)
Initial histamine content of tuna viscera prior to salting = 49.60ppm

After seven days of fermentation, only salt concentration significantly affects histamine formation regardless of fermentation temperature (Fig. 1). Results have the same trend with that of tuna viscera fermented for only three days but values increases with prolonged fermentation time. Histamine content of tuna viscera fermented at 10% NaCl increases from 82.70 ppm (3 days) to 112.20 ppm (7 days). Similar trend but significantly lower histamine content compared to 10% NaCl was observed at 17.5% and 25% NaCl with 47.80 and 61.20 ppm, respectively. The results show that tuna viscera fermented for 3 and 7 days at 10% salt concentration have histamine values higher than the standard limit for safety.

The formation of histamine in tuna viscera fermented for 7 days is strongly influenced by pH, lactic acid, amino nitrogen and TVB-N. As pH increases, there is a corresponding decrease in lactic acid and histamine content increases. It should be noted that the pH of tuna viscera fermented for seven days range from 5.23 to 6.03 thus, creating a favorable condition for histamine production. The activity of amino acid decarboxylase

is most active in the acidic solution at pH 3 to 6 [6]. Furthermore, bacteria are encouraged to produce these enzymes as part of their defense mechanism against the acidity [5].

As amino nitrogen and TVB-N increases, histamine content increases. This indicates that there is an increase availability of free amino acid (histidine) necessary for the formation of histamine due to the degradation of protein and polypeptides by proteolytic enzymes. Amino nitrogen and TVB-N are used as indicators of protein decomposition but TVB-N values are most useful as index for spoilage in fresh and fermented fish products [7]. Thus, a significant direct correlation between TVB-N and the formation of histamine content implies that bacterial spoilage contributes to histamine formation. Furthermore, higher TVB-N and amino nitrogen values were observed in tuna viscera fermented at 10% NaCl based on the results of the study. *Bacillus* a predominant bacteria found in fermented fish paste is capable of decarboxylating one or more amino acids [6]. Histamine forming bacteria belonging to the genus of *Bacillus* isolated from fish includes *Bacillus coagulans*, *Bacillus megaterium* and *Bacillus pumilus* [8].

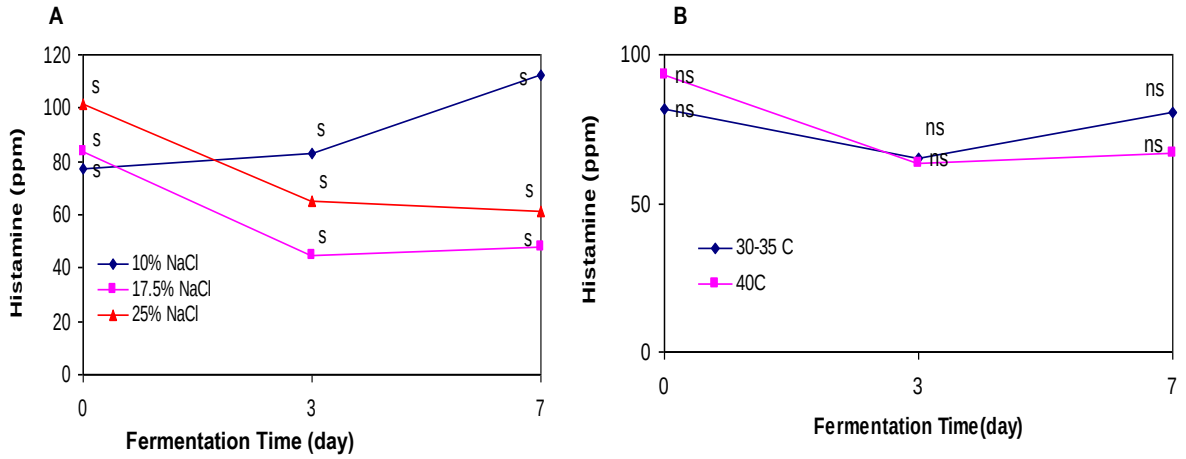

Fig. 1: Changes in histamine (ppm) level during *dayok* fermentation at varying levels of (A) salt concentration and (B) fermentation temperature.

3.3. Influence of the Addition of Spices to Histamine Content during Fermentation

Histamine values of tuna viscera fermented for three and seven days added with spices namely, ginger and garlic, both at the rate of 2% (w/w) per spice. The trend in histamine formation is similar with that of fermented samples before spices were added. Histamine values of sample at 10% NaCl continuously increased until seven days from 76.60 to 90.20 ppm while those fermented at 17.5% and 25% NaCl decreased at seven days from 56.50 to 44.20 ppm and 95.90 to 61.90 ppm, respectively. Tuna viscera fermented at 10% and 25% NaCl have histamine values higher than the standard limit of safety even after the addition of spices. Histamine values of tuna viscera are not significantly different before and after spices were added. This implies that addition of spices at 2% concentration does not significantly lower histamine content.

3.4. Microbiological Changes of Tuna Viscera during Fermentation

The total plate count (TPC) increased while lactic acid bacteria count decreases during the fermentation period. Microbial counts decreased with increasing salt concentration. Only salt concentration affects the microbial count and LAB count in the fermentation of tuna viscera ($p<0.05$). Results further show that *dayok* produced from tuna viscera at 10% salt concentration had total plate count of 10^7 cfu/g higher than the recommended value for total plate count of 10^5 cfu/g ($p<0.05$) and higher than those produced from samples at higher salt concentration with 10^4 and 10^5 cfu/g for 17.5% and 25% salt concentration, respectively. The presence of high viable count of aerobic microorganisms indicates that the product is prone to spoilage especially on tuna viscera fermented at 10% NaCl.

Tuna viscera fermented at 10% NaCl had significantly higher LAB count of 10^6 cfu/g compared to those fermented at 17.5% (10^4 cfu/g) and 25% NaCl (10^3 cfu/g). This indicates that LAB present in the fermented product was able to grow at 10% NaCl as reflected by its high viable count.

Histamine formation is also significantly influenced by total plate count (Fig. 2). The final total plate count of fermented tuna viscera of 10^7cfu/g in 10% NaCl is significantly higher compared to 17.5% and 25% NaCl. It has been reported that histamine production occurs only after aerobic plate counts reach to 7 log CFU/ml [9]. This observation was confirmed in this study.

4. Conclusion

Histamine formation during fermentation is affected by pH and total plate count. It is controlled by salt concentration (≥17.5%) and length of fermentation (7 days).

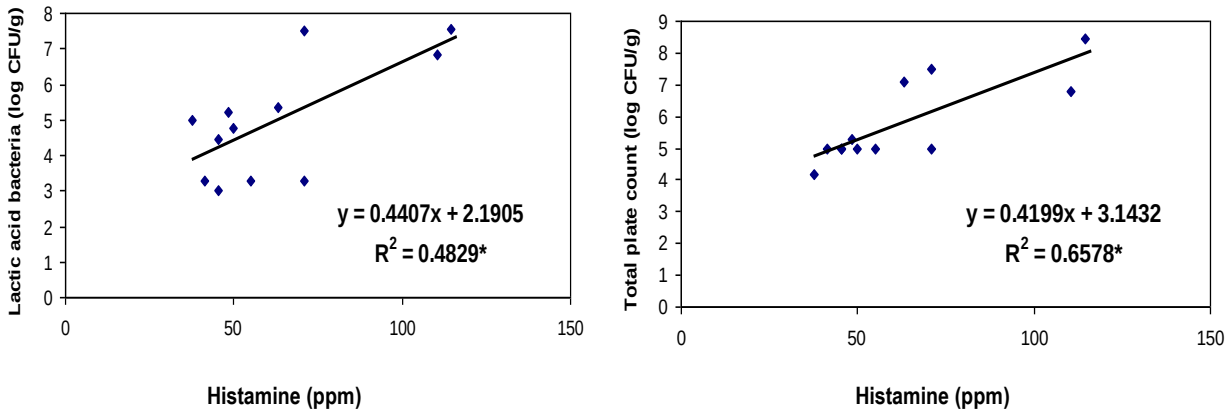

Fig. 2: Relationship of total plate count and LAB count to histamine formation on tuna viscera fermented for 7 days.

5. Acknowledgements

This research was financially supported by the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) and the Commission on Higher Education (CHED) Higher Education Development Program, Philippines.

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