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Influence of Salt Concentration on Histamine Formation in Fermented Tuna Viscera (*Dayok*)

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ABSTRACT

The formation of histamine in fermented tuna (Thunnus albacares) viscera (*dayok*) at different salt concentrations (10%, 17.5% and 25%) for 7 days at ambient temperature was investigated. Effect on chemical and microbiological changes on tuna viscera were monitored. Results demonstrated that the levels of pH, lactic acid, amino nitrogen and total volatile base nitrogen (TVB-N) increased as time of *dayok* fermentation progressed. The total plate count and lactic acid bacteria count decreased with increasing salt concentration. Histamine levels decreased during fermentation as salt concentration increased. Histamine levels above FDA standard limit of 50 ppm are formed at low salt concentration (10%) with a total plate count of 10^7 cfu/g. Results revealed that application of salt concentration greater than 17% can minimize the formation of histamine.

Keywords: Histamine; Tuna Viscera; Fermentation

1. Introduction

Dayok, an indigenous fermented food, is a popular native *bagoong* (fish paste) in Mindanao areas in the Philippines and is widely consumed as a condiment/sauce. Fish fermentation as a preservation technique has been found to contain high contents of histamine such as in fish paste and fish sauce [1]. Histamine (or scombroid) fish poisoning is a foodborne chemical intoxication caused by eating spoiled or bacterially contaminated fish [2]. Ingestion of food containing small amount of histamine when taken in large amounts (>50 mg/100g) can cause scombroid fish poisoning [3] and tuna species are most often implicated with histamine poisoning due to its high levels of histidine which is a precursor for histamine formation [4]. Histamine is formed from the action of microbial histidine decarboxylase on histidine in fish by spoilage bacteria [1].

To this effect, several studies have been conducted on fermented fishery products regarding its safety and quality parameters, e.g. studies on histamine and other biogenic amines [1,5-9]. However, only limited information is available on the use of tuna viscera in fish paste processing. Fermentation process provides suitable conditions conducive to the production of histamine due to the presence of bacterial enzyme histidine decarboxylase and free amino acid histidine coupled with favorable environmental growth conditions on histamine forming bacteria.

The purpose of this study was to monitor the microbiological and biochemical changes of tuna viscera during fermentation and to investigate the factors influencing histamine formation in *dayok* fermentation.

2. Materials and Methods

2.1. Tuna Viscera (*Dayok*) Preparation

Yellowfin tuna (*Thunnus albacares*) viscera excluding the bile sac and heart was used in the study. Fresh tuna viscera were obtained from eviscerated yellowfin tuna and placed in an 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 varying salt concentrations (10%, 17.5% and 25%). The salted samples were placed in sterilized sealed bottles and were allowed to ferment for 7 days at ambient temperature. Samples for chemical and biochemical analysis were blended, then heated to 95˚C for 5 minutes and cooled to room temperature before analysis. Simultaneous microbial and chemical analyses were conducted.

2.2. Microbiological Analysis

Dayok samples (5 g) were transferred aseptically and

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were homogenized in 45 ml of sterilized peptone (0.5% peptone) saline solution (depending on the initial salt concentration of the sample). The homogenates were serially diluted in the same diluent. One ml (1 ml) of the appropriate dilution were pipetted out and poured in Petri dishes with molten plate count agar (PCA) containing initial salt concentration of the sample for total viable count and MRS agar medium +1% $CaCO_3$, for lactic acid bacteria (LAB) count. Total bacterial colonies 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. Chemical Analysis

2.3.1. Determination of PH

The pH of homogenized tuna viscera samples were measured using a pH pen. The pH pen was calibrated with pH 4.0 and pH 7.0 standard buffers prior to its use.

2.3.2. Determination of Salt Content

Samples (5 g) were diluted 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 on it. The solution was titrated with 1/50 N silver nitrate solution to a light orange endpoint. The percentage of salt was then calculated using the formula:

$$\mathrm{NaCl}(\%) = \mathrm{A} \times 0.00117 \times \mathrm{F} \times \mathrm{C} \times \mathrm{DF}$$

where A = titration value, F = factor of 1/50 silver nitrate, C = correction constant and DF = dilution factor.

2.3.3. Determination of Total Titratable Acidity (Expressed as Percent Lactic Acid)

Five (5) mL of blended fish paste sample was diluted 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. Percent lactic acid was then computed as follows:

$$\text{Lactic Acid}(\%) = \frac{\text{mL NaOH} \times \mathrm{N}_{\mathrm{NaOH}} \times 0.09 \times 100}{\text{Volume of sample}(\mathrm{mL}) \times \mathrm{DF}}$$

2.3.4. Determination of Amino Nitrogen Content

Amino nitrogen contents were 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 decreased upon addition of formaldehyde). The mixture was titrated with standard 0.1 N NaOH to pH 8.2. Amino nitrogen was expressed as mg% using the formula:

$$\text{Amino nitrogen}(\mathrm{mg}\%) = 0.0014 \times \text{mL NaOH} \times \mathrm{F}_{\mathrm{NaOH}} \times 100/10$$

where F = 0.1 N NaOH/standardized NaOH.

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

The TVB-N contents of the samples were determined by the Conway micro-diffusion method [10] while histamine was analyzed by the standard fluorometric method [11].

2.4. Statistical Analyses

Data were analyzed using Analysis of Variance (ANOVA) with three replications following the Complete Randomized Design. The Duncan's multiple range test was further used to determine the differences of means. In addition, pearson correlation and multiple linear regression analysis were carried out to determine the relationship and degree of influence between histamine values to other parameters such as salt content, temperature, pH, amino nitrogen, TVB-N and microbial counts in fermented tuna viscera. Statistical analysis was performed using the Statistical Analysis System (SAS) program.

3. Results and Discussion

3.1. Effect of Varying Salt Concentration on Microbiological Changes of Tuna Viscera during Fermentation

The changes in microbial flora of tuna viscera during fermentation are shown in **Table 1**. The total plate count (TPC) in all samples increased during the fermentation period however counts decreased with increasing salt concentration. Salt concentration affects the microbial count of microorganisms 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

Table 1. Microbial count (log CFU/g) of fermented tuna viscera samples at varying temperature and salt concentration.

Salt concentration	TPC		LAB	
	Initial	Final	Initial	Final
10%	4.54	7.47a	3.48	6.80a
17.5%	4.00	4.87b	4.17	4.13b
25%	4.00	5.00b	4.84	3.95b

Means in the same column with different letter designation are significantly different from each other ($p < 0.05$).

for total plate count of 10^5 cfu/g ($p < 0.05$) [12] 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. According to Sanchez (2008) bacteria isolated from the intestines of marine fishes are halophilic and these microorganisms can also be introduced in the addition of salt. The principal halophilic species isolated from solar salt are *Bacillus* and *Micrococcus*, and *Micrococcus* in rock salt [13].

In addition, lactic acid bacteria count in all samples decreases during fermentation. Salt concentration significantly affects LAB count. 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.

Generally, microbial counts decreased as salt concentration increased. The preservative action of salt is through osmotic pressure, it withdraws water from the tissue or microbial cells leading to microbial death.

3.2. Effect of Varying Salt Concentration on Biochemical Changes of Tuna Viscera during Fermentation

Salt content. Salt content of tuna viscera increased during fermentation. Salt content of the fermented products with initial concentration of 10%, 17.5% and 25% increased to 19.20%, 29.76% and 31.39%, respectively. Salt content values for samples fermented at 17.5% and 25% falls within the range from 17.5% to 35.4% as reported by Tsai *et al.* (2006) for commercial fish paste.

Sodium chloride plays an important role in microbial growth and therefore influences the activity of their amino acids decarboxylase [14]. High salt concentration also affects the growth of lactic acid bacteria since these type of bacteria are generally tolerant of moderate salt concentrations in the range of 10% to 18% [13].

pH and titratable acidity. Changes in pH values and titratable acidity during fermentation of tuna viscera are shown in **Figure 1**. The pH values of fermented tuna viscera with different salt concentrations slowly but continously decreased throughout the fermentation period with a corresponding increase in titratable acidity or lactic acid. At the end of the fermentation period, the initial pH of raw tuna viscera decreased from 6.13 to 5.61. The pH values of the samples drop within the acceptable range of 5.4 - 6.2 on fermented fish products [13].

The pH of the samples decreases as salt concentration increases during the fermentation period. However, fermented tuna viscera at 17.5% and 25% NaCl have comparable pH values (5.33 and 5.32) and are significantly more acidic (2.84% and 3.08% lactic acid) than at 10% NaCl (pH 5.83 and 2.34 % lactic acid). At increasing salt concentration, pH decreases or becomes acidic [15]. However, the final LAB count of samples at 17.5% and 25% NaCl are lower than 10% NaCl even though they are more acidic. This could be attributed to the formation of other acids (not lactic acid) during fermentation which affected the obtained values. Slow but gradual production of acid was observed throughout the fermentation period. This could be due to the presence of very little fermentable carbohydrate in the fish and the presence of a few numbers of lactic acid bacteria in fresh/raw material and in the fermenting fish during the early stages of fermentation.

Amino nitrogen. The amino nitrogen of *dayok* during fermentation at varying salt concentration ranged from 0.07% to 0.11%. These values are within the reported data on fish sauce ranging from 0.07% to 1.43% [13]. An increase in amino nitrogen concentration is related to the degradation of the protein to amino acids released during hydrolysis. The quantity of amino acids formed went up with increasing time and salt concentration as shown in **Figure 2**.

Higher amino nitrogen are formed at 10% NaCl compared to 17.5% and 25% NaCl of which the latter values are relatively similar ($p < 0.05$). This indicates that microorganisms present in the mixture are able to degrade protein at 10% NaCl which can be classified as moderately halophilic bacteria. According to Dissapharong *et al.*

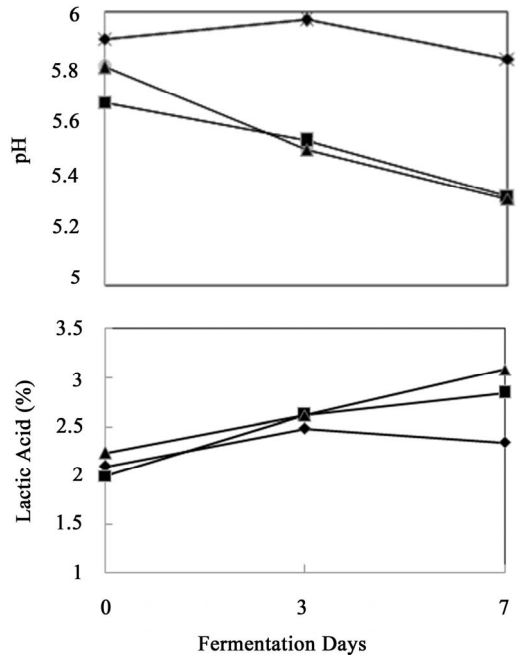

Figure 1. Changes in pH and lactic acid (%) during fermentation of tuna viscera at (♦) 10%, (■) 17.5% and (▲) 25% salt concentration.

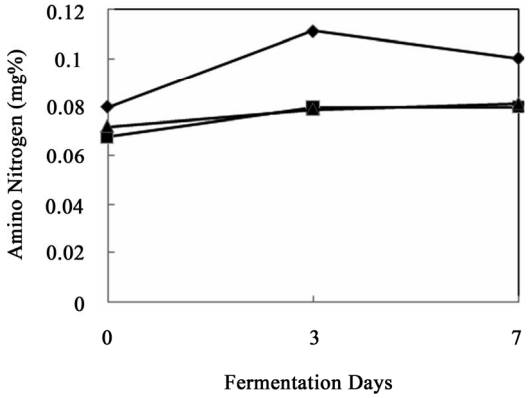

Figure 2. Changes in amino-nitrogen (mg%) during fermentation of tuna viscera at (♦) 10% , (■) 17.5% and (▲) 25% salt concentration.

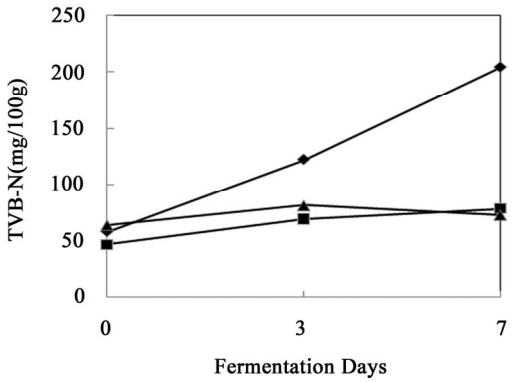

Figure 3. Changes in TVB-N (mg/100g) during fermentation of tuna viscera at (♦) 10%, (■) 17.5% and (▲) 25% salt concentration.

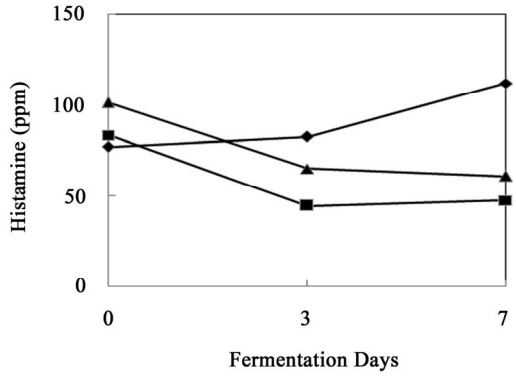

Figure 4. Changes in histamine (ppm) level during *dayok* fermentation at 10% (♦), 17.5% (■) and 25% (▲) salt concentration.

(2006) the increase in nitrogenous basic compounds are caused by microbial proteolytic enzymes and consequently the utilization of amino acids by fermenting microorganisms. During storage, the protein breakdown products, peptides and amino acids, represent precursors for amine formation used by spoilage microorganisms.

Total volatile base nitrogen (TVB-N). Changes in TVB-N during fermentation of tuna viscera are shown in **Figure 3**. The measurement of TVB-N indicates the degree of proteolysis of samples caused by spoilage bacteria, autolytic enzymes, deamination of amino acids and nucleotide catabolites [3]. The TVB-N values of tuna viscera at 10%, 17.5% and 25% NaCl are 226.80, 81.60 and 76.41 mg/100g, respectively. The TVB-N content of all samples except those fermented with 10% NaCl (226.80 mg/100g) are within the limits set for dried and salted fish at 100 to 200 mg/100 g fish [16].

TVB-N content increases as salt concentration decreases ($p < 0.05$). The increase in TVB-N was due to the presence of higher number of microorganisms present in 10% NaCl as compared to 17.5% and 25% NaCl. At salt concentrations higher than 10%, TVB-N values are lower which could be attributed to the preservative action of salt. Similar results was observed by Tsai *et al*. (2006) who reported that the increase in TVB-N value in fermented fish products during fermentation was due to bacterial and enzymatic actions, particularly of the halophilic bacteria.

Generally, TVB-N values increases during fermentation of tuna viscera. The increase in TVB-N content over the fermentation period reflects the deterioration of the samples which is significantly affected by salt concentration.

3.3. Effect of Varying Salt Concentration on Histamine Formation during Tuna Viscera Fermentation

Salt concentration significantly influences histamine formation due to its effect on the activity of histidine decarboxylase which is responsible for the conversion of histidine to histamine ($p < 0.05$). At a given salt concentration, histamine formation increases as salt content decreases (**Figure 4**). This indicates that high concentration of salt $\geq$ 17.5% - 25% retards microbial histidine decarboxylase activity. This phenomenon can be attributed to reduce microbial cell activity due to the presence of high sodium chloride concentration causing withdrawal of water and other soluble contents from the cell through osmosis thus retarding or inhibiting their growth [14].

3.4. Influence of Fermentation Parameters to Histamine Formation during Tuna Viscera Fermentation

Results revealed that histamine content in fresh raw tuna viscera (49.60 ppm) was not significantly correlated to its initial total plate count. This was attributed to the immediate washing and icing of the raw material during transport which might inhibit or control the growth of histamine producing bacteria even though the histamine level is close to the FDA standard limit of safety (50 ppm).

However, as fermentation progresses, histamine formation in tuna viscera is significantly affected by pH, salt content, amino nitrogen, TVB-N and bacterial count (TPC and LAB). Significant linear relationship was observed among samples at different salt concentration and fermentation parameters on the formation of histamine in fermented tuna viscera. An increase in histamine content is affected by an increase in pH, amino nitrogen, TVB-N, total plate count, LAB count and a decrease in salt content and lactic acid.

At low salt concentration, more histamine are formed compared to those fermented at salt concentrations higher than 10% with corresponding higher values in total plate count, LAB count, amino nitrogen, TVB-N at higher pH (less acidic environment).

Kimura *et al.* (2001) and Satomi *et al.* (2008) were able to observe histidine decarboxylase enzyme in halophilic lactic acid bacteria *Tetragenococcus muriaticus* [17] and *Tetragenococcus halophilus* [18] isolated from fish sauce. Furthermore, the histamine level of samples fermented at 17.5% and 25% NaCl with values of 44.2 and 62.0 ppm, respectively showed no significant difference on pH, TPC and LAB count.

Tuna viscera fermented at 10% and 25% salt concentrations have higher histamine values higher than the standard limit of safety at 50 ppm. On the other hand, histamine content of samples fermented at 17.5% salt concentration was below the food safety limit. The findings of the study suggest that tuna viscera fermented at low salt concentrations (10%) have higher histamine formation in tuna viscera than at higher salt concentration of 17.5%.

4. Conclusion

The formation of histamine is significantly influenced by salt concentration. As salt content decreases histamine content increases. Low salt concentration at 10% NaCl boasted histamine levels higher than the FDA safety limit. Therefore, the amount of salt to be added for *dayok* fermentation can be lowered from 25% (usual practice) to 17% to reduce histamine formation.

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