Microbial and Biochemical Changes in Fermented Tuna Viscera (*Dayok*)*

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Presentation Outline

- Rationale
- Objectives
- Background information
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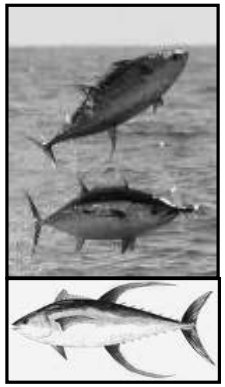


Why the study?

- Food safety concerns (Histamine poisoning)
- Histamine (or scombroid) fish poisoning is a foodborne chemical intoxication caused by eating spoiled or bacterially contaminated fish (Lehane and Olley, 2000).
- Histamine is a biogenic amine formed by bacterial decomposition of free histidine (Rawles *et al.,* 1996).
- Tuna species are most often implicated with histamine poisoning due to its high levels of histidine (Taylor, 1986).

- *Dayok*, a fermented fish product from tuna viscera, is a popular native *bagoong* in Mindanao used as a condiment
- Fish fermentation technique has been found to contain high contents of histamine such as in fish sauce and on fish paste (Tsai *et al.,* 2006)
- However, due to increasing numbers of consumers exposed to this product, it is important to evaluate microbiological and safety risks that *dayok* might pose to its consumers.

Objectives

- To determine the level of histamine in commercial *dayok*;
- To monitor the microbial, chemical and biochemical changes during tuna viscera fermentation; and
- To investigate the factors influencing histamine formation in *dayok*;

Background information

- Fermentation
  - A preservation technique which transforms organic material into simpler compounds either by the action of enzymes or microorganisms (Murano, 2003)
  - Common fish preservation method due to its simplicity of technology and low equipment cost

Biochemistry of Fish Fermentation

- Proteolysis
  - Degradation of proteins into polypeptides and amino acids
  - Caused by enzymes and beneficial bacteria
  - Halophilic and proteolytic bacteria might contribute to the hydrolysis of protein and flavor development (Dissaraphong *et al.*, 2006)

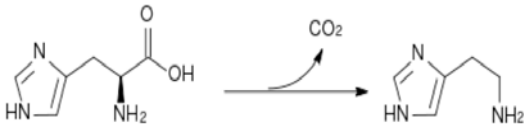
Histamine Poisoning

- Symptoms:
  - Rash
  - Nausea
  - Vomiting
  - Diarrhea
  - Flushing and tingling
  - Itching of the skin
- Onset: ten minutes
- Symptoms last for approximately four to six hours and rarely exceed one to two days
- Scombroid poisoning can be easily confused with allergy symptoms

Pre-requisites for elevation of postmortem histamine concentration in fish:

- High content of histidine
- Presence of bacterial histidine decarboxylase
- Favorable environmental conditions (pH, salt concentration, and temp.) (Zaman *et al.,* 2008)

Conversion of histidine to histamine by histidine decarboxylase

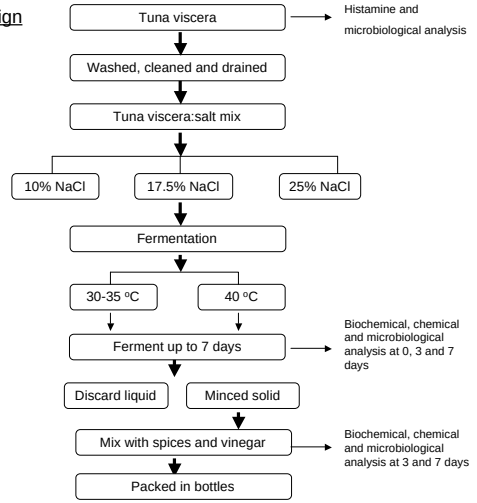


Histidine Histamine

Source: Chen *et al.* (2008); Lehninger (1975)

Methodology

Experimental design



Parameters measured:

- pH
  - Influence amino decarboxylase activity (Santos, 1996)
- Total titratable acidity (% Lactic Acid)
  - Inhibits growth of microorganisms
- Salt content
- Total plate count
- LAB count

Biochemical Parameters

- Amino nitrogen (mg%)
  - Measures degree of protein degradation (peptide and amino acids)
- Total Volatile Base Nitrogen (TVB-N)
  - Index of spoilage used in fresh and fermented fish products caused by spoilage bacteria and autolytic enzymes (Yongjin *et al.,* 2007)
  - ≥30 mg N/100 g (generally unfit)
  - 100 to 200 mg N/100g (salted and dried fish)
- Histamine
  - ≥50 ppm or ≥5 mg/100g (FDA, 2001)

Statistical Analysis

- 2 x 3 factorial experiment by CRD with three replications
- ANOVA
- Pearson correlation and multiple linear regression analysis to determine degree of relationship and influence between histamine to fermentation parameters

Results and Discussion

Commercial Dayok	Histamine (ppm)
1	42.49
2	35.36
3	25.81
Fermented (3 days)	15.41

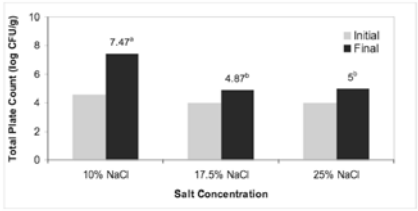
1- Davao City, best before Jan. 2012
2- Davao City, best before Feb. 28, 2011
3- Sta. Cruz, Davao del Sur, no data

Dayok are fermented fish product obtained without heating and therefore histamine content are subject to change during its storage.

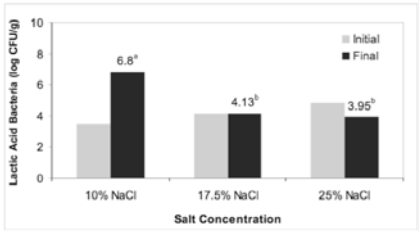
Microbial and Biochemical Changes During *Dayok* Fermentation

Objective 2

Microbiological Changes: TPC and LAB ct

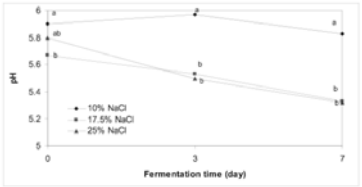


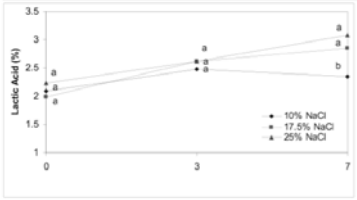
Microbial counts (TPC and LAB) decrease as salt concentration increases



* - significant (p<0.05)

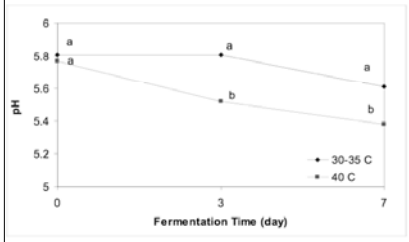
Biochemical Changes: pH and titratable acidity

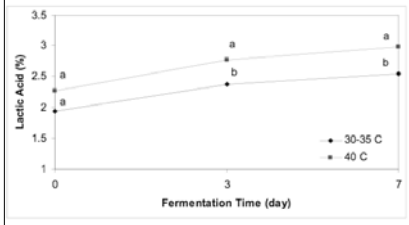




- At a given salt concentration, pH of the samples decreases (more acidic) as salt concentration increases

* - significant (p<0.05)

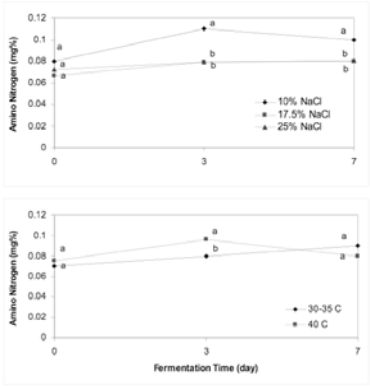




- At a given temperature, samples fermented at 40 C are more acidic than at room temp

* - significant (p<0.05)

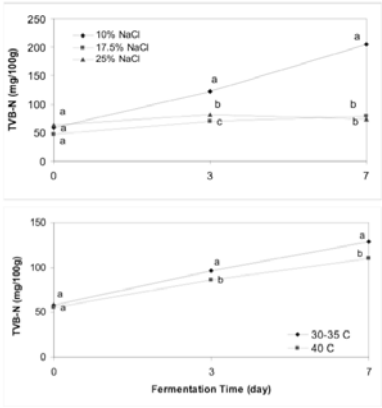
Amino nitrogen



- More amino nitrogen formed at 10% NaCl than at 17.5% and 25% NaCl
- More at 40 C than at ambient temp (day 3) but only to some extent

* - significant (p<0.05)

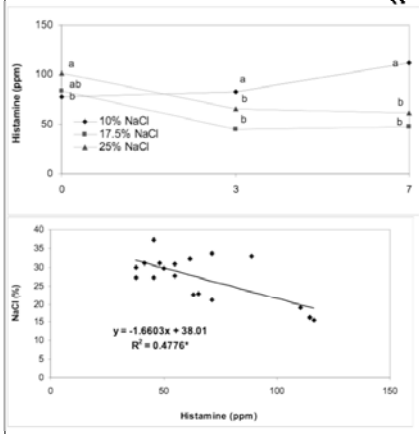
Total Volatile Base Nitrogen



- At a given salt concentration, TVB-N increases as salt concentration decreases
- At a given temperature, TVB-N increases as temperature decreases

* - significant (p<0.05)

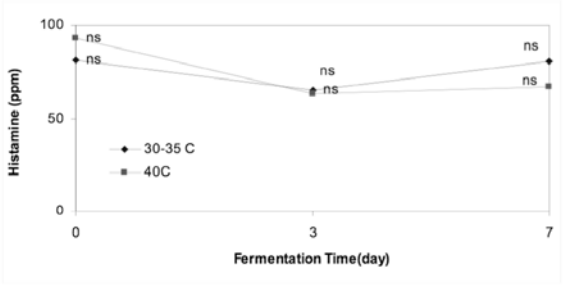
Histamine (ppm)



- At a given salt concentration, histamine increase as salt content decreases
- Higher histamine content at day 3 than at day 7 (25% NaCl)
- Histamine content is influenced by salt concentration

* - significant (p<0.05)

- Fermentation temperature (no influence)



Formation of high levels of histamine on both temperature but they are not significantly different from each other thus fermentation temperature did not significantly influence histamine formation.

Microbial and Biochemical Factors Influencing Histamine Formation During *Dayok* Processing

Objective 3

Raw Material

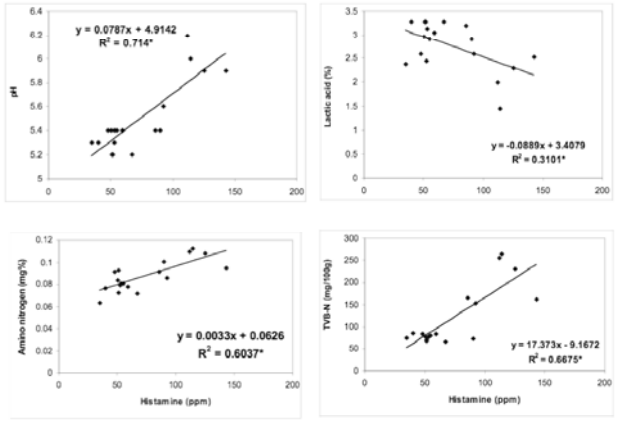
Histamine	TPC (cfu/g)	correlation coefficient
49.6 ppm	10^4 ns	0.0524ns

ns – not significant (p>0.05)

The high level of histamine in raw material does not necessarily correlate with the bacteria present in the raw material since the bacteria responsible for histamine production were already inactivated upon the application of ice to the raw material upon transport.

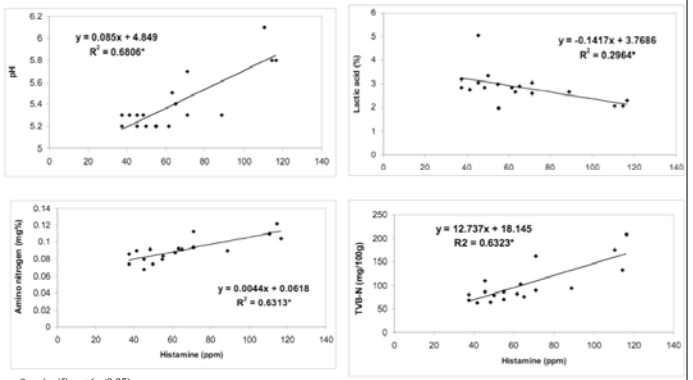
Result agree with previous studies reported by Bjornsdottir (2009)

Fermentation



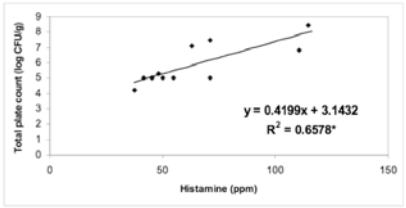
* - significant (p<0.05)

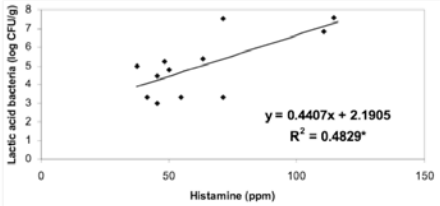
Addition of Spices



* - significant (p<0.05)

Influence of microbial growth to histamine formation (after fermentation)





* - significant (p<0.05)

- An increase in histamine content is affected by an increase in pH, amino nitrogen, TVB-N, total bacterial count, LAB count and a decrease in salt content and lactic acid
- At low salt concentrations, more histamine are formed as compared to those fermented at salt concentrations higher than 10%

Values Influencing Histamine Level (above 50 ppm)

- Salt concentration at 10% NaCl
- pH values between 5.23 to 6
  - Confirmed previous study (Afilal *et al.*, 2006)
- Total plate count of 10^6 to 10^7 cfu/g
  - Confirmed previous study (Bjonsdottir, 2009)

These results fall within the reported values for pH and total bacterial count that enhances the formation of histamine, higher than the food safety limit (50 ppm)

Summary

- Lactic acid(%), amino nitrogen (mg%), total volatile base nitrogen (mg/100g) and microbial count (cfu/g) generally increases while pH decreases during fermentation.
- Microbial count, pH, lactic acid, amino nitrogen, TVB-N and salt content significantly influenced histamine formation.
- Higher values of amino nitrogen, TVB-N and histamine were observed at lower salt concentrations
- High values of histamine are formed at 10% NaCl fermented at 30-35 C than at 40 C. This implies that microorganisms responsible for histamine formation are mesophilic and moderately halophilic bacteria which can be inhibited by salt concentrations of $\geq$17.5%.

Conclusion

- Histamine formation is influenced by salt concentration
- Tuna viscera fermented for only 3 days have higher histamine content than at 7 days.

Recommendation/Implication

- The amount of salt used can be lowered from 25% to 17.5% and fermented for 7 days instead of 3 days to lower histamine formation.
- Immediate icing of raw material and maintenance of low temperature storage to prevent histamine formation
- Pasteurization of tuna viscera after fermentation to inactivate microbial enzymes and enzymes inherent in the material

Thank you!