



Effects of Fermentation on Physical Properties and Lactic Acid Bacteria Growth in *Ikan Pekasam* (Fermented Fish)

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ABSTRACT

Fermentation of fish is a food processing method that enables the storage of fish for extended periods. *Pekasam* is an example of freshwater fermented fish from Malaysia. Fermented fish or seafood causes the presence of lactic acid bacteria (LAB) such as *Lactobacillus spp.* to aid in the fermentation process. However, the fermentation process can alter the physical properties of the fish, which directly affect consumer acceptability and product quality. Therefore, there is a critical need to evaluate the physical changes occurring in freshwater fermented fish throughout the fermentation period. The goals of this study were to determine the physical properties of different freshwater fish before and after fermentation and to evaluate the colony of LAB during the production of fermented fish. Three species of freshwater fish, silver barb, three-spot gourami, and climbing perch were prepared and fermented for 7 days. The water activity (a_w), salt content, and protein composition were measured using the AOAC method. The LAB growth was measured using the total plate count method (TPC). The a_w for all fishes, silver barb, climbing perch, and three-spot gourami significantly decreased after 7 days of fermentation from 0.96% to 0.77% due to the presence of salt in fermented fish. Silver barb recorded the highest salt content (2.70%) after 7 days of fermentation, followed by three-spot gourami (2.10%) and climbing perch (1.60%). The crude protein content for all fish species decreased significantly from a range of 78.07% to 59.82% before fermentation and 57.76% to 37.83% after fermentation due to the protein hydrolysis process. The result of the viability of the LAB growth showed that the three-spot gourami had the highest log CFU/mL, which was 7.46. This corresponds with its highest protein content among the three fish species.

Keywords: Freshwater Fish, Protein, Water Activity, Fermentation

INTRODUCTION

Fermented fish or “*pekasam*” is one of the oldest Malaysian delicacies that uses freshwater fish as the raw material (Huda, 2012). Fermented fish production is concentrated in areas such as Perlis, Kedah, Perak, and Kelantan (Huda, 2012). In Indonesia, fermented fish known as *bekasam*, is a product prepared from common carp (*Cyprinus caprio*) from the Indonesian island of Maluku (Mahulette & Kurnia, 2020), *pekasam ale-ale* from the Province of Kalimantan Barat, Indonesia (Nofiani et al., 2025), *Ngapi-gaung* from Myanmar, and *Bagoon* from the Philippines (Boziaris, 2014). The popular fishes for fermented fish manufacturing are Tilapia (*Oreochromis mossambica*), spotted gourami (*Trichogaster trichopterus*), catfish (*Clarias batracus*), java barb (*Puntius javanicus*), and snake head (*Channa striatus*). Freshwater fish is a great source of high-quality animal protein. The protein level of mostly freshwater fishes ranges between 15 and 20% of wet weight (Ravichandran et al., 2011), a very low-fat content of 1–5%, calcium, phosphate, iron, and vitamin B, specifically riboflavin and niacin (Babji, 2015). The crude protein content for Talapia was reported to be 19.90 % (Mahyudin et al., 2015), three-spotted gourami (22.45%) (Rahmawati & Aisyah, 2018), silver barb 18.43% (Subandiyono et al., 2018) and climbing perch 15.46% (Varghese & Mathew, 2020).

Drying or adding salt to food during fermentation preserves the food by lowering its a_w , inducing selective bacterial growth, and contributing to the desired physical changes. The fish is allowed to ferment for at least one week and up to one month (Mahyudin et al., 2015). The high salt content (20-30%) ensures that spoilage due to bacteria is prevented, and the number of bacteria present drops as quickly during fermentation (Panda et al, 2011). Thai fermented fish (*pla-ra, pla-som, pla-chao, som-fak*) fermented with salt (2.3–11.0 % w/w) (Boziaris, 2014), *pekasam ale-le* fermented with 40% (w/w) of salt (Nofiani et al., 2025), and *pekasam* from freshwater fish, black tilapia (*Oreochromis mossambicus*) and Javanese carp (*Puntius gonionotus*) with 30 % (w/w) of salt (Ezzat et al, 2021). A major factor that determines the microbial, chemical, and enzymatic stability of foods is the water activity (a_w). Rice provides a carbohydrate supply for the LAB involved in fermentation, which imparts a distinct flavour to the products (Boziaris, 2014). Only ground- roasted rice is used as a carbohydrate source during the natural fermentation of fermented fish. However, in addition to rice, other common food acidifiers, such as tamarind pulp or dried slices of *Garcinia atroviridis*, are used to speed up the fermentation process, which is known as acid-assisted fermentation (Ezzat et al., 2021).

Lactic acid bacteria (LAB) are the most common microorganisms isolated in food preservation processes such as fermentation. LAB has the ability to break down carbohydrate sources in order to produce lactic acid (Rhee et al., 2011). The result showed that the iridescent shark (*Pangasianodon hypophthalmus*) has the highest number of *Lactobacillus spp.* (6.21 log CFU/mL) followed by tilapia (*Oreochromis niloticus*) (6.11 log CFU/mL), Japanese threadfin bream (*Nemipterus japonicus*) (6.08 log CFU/mL), and skipjack tuna (*Katsuwonus pelamis*) (6.05 log CFU/mL) (Mahyudin et al., 2015). Interestingly, the growth of LAB in *pekasam* is highly dependent on the amount of protein present in the substrate used (Khudair et al., 2023). In a study by Mahyudin et al. (2015), they found that the highest protein-fish (patin) showed a significantly higher *Lactobacillus* growth compared to the other freshwater fish species.

The main objective of this study is to evaluate the effects of fermentation on physical changes in freshwater fish (silver barb, three-spot gourami, and climbing perch) and the colony of LAB growth during the production of fermented fish.

MATERIALS AND METHODS

Materials

Three types of fish were used namely silver barb (*Barbonymus gonionotus*), three-spot gourami (*Trichopodus trichopterus*) and climbing perch (*Anabas testudineus*). These fishes were taken from a pond at a breeder's house in Pasir Mas, Kelantan.

Fermented fish preparation

The fish was cut and cleaned thoroughly. It was then patted dry with a kitchen towel and left to rest for approximately 15 minutes to remove excess moisture. Next, 25% (w/w) of salt was coated onto the fish and left overnight. The following day, excess water was discarded, and 30% (w/w) of ground-sautéed rice was coated onto the fish. After that, the fish was sealed in a container and stored at ambient temperature (28°C) for 7 days.

Determination of Water Activity (a_w)

The a_w of the fermented fish was measured using a water activity meter (Aqua Lab, Malaysia). 0.75 g sample was placed in a sample cup before being placed in the water activity meter. Analysis was made in triplicate for each sample before and after fermentation.

Determination of Salt Content

The salinity of the samples was determined using a handheld refractometer salinity meter. The refractometer was calibrated with diluted water before the analysis started. About 1 gram of fermented fish was dissolved in 10 mL of distilled water to make a solution. After that, a few drops of the solution were placed on the prism. The readings of % salinity were carried out before and after the fermentation process. Analysis was made in triplicate for each sample before and after fermentation.

Analysis of Protein using the Kjeldahl Method

Protein analysis was carried out before and after the fermentation process was conducted. The protein analysis was carried out by using the standard Kjeldahl method (AOAC, 2000). 1 gram of appropriately homogenised fish sample was digested, and the protein value was calculated using the conversion factor of 6.25. The analysis was conducted in triplicate. The percentage of protein present in the sample was calculated using the following formula:

$$\% N = \frac{A \times (T - B) \times 14.007 \times 100}{\text{weight of sample used (g)} \times 100}$$

$$\% \text{ Crude protein} = \% N \times F$$

Where:

- T = Volume acid for the sample
- B = Volume acid for blank
- A = Normality of HCl
- F = Protein factor, 6.25

Microbiology Test

Fish sample (10g) was homogenized with 90ml of sterile normal saline solution in a stomacher (Seward, UK) for serial dilution and labelled as 10^{-1} . The tubes were labelled with dilution from 10^{-2} to 10^{-4} . Then, 0.1 ml of the appropriate dilution was pipetted onto each plate. These steps were repeated for other dilutions and lastly, the plates were inverted and incubated at 37°C for 48 hours. Microbiology tests for LAB growth during fermented fish production were taken in CFU/mL on day 7. The colony-forming unit (CFU/mL) for each sample was calculated using the following formula:

$$N \text{ (CFU per mL)} = \frac{C}{\text{vd} (n1 + 0.1 n2)}$$

Where:

- C = sum of colonies on all plates counted v = volume applied to each plate (0.1 ml)
- n1 = number of plates counted as the first dilution
- n2 = number of plates counted at the second dilution
- d = dilution from which the first count was obtained

Statistical analysis

All data were performed using Microsoft Excel 2021 and the SPSS Statistics program (version 20). The data were analysed by one-way Analysis of Variance (ANOVA) and paired t-Test for before and after fermentation data. The comparison of means was carried out by Duncan's multiple range tests. Duncan's Multiple Range Test was employed to determine the significance of the difference among treatments. For each treatment, triplicate measurements were taken. Values were considered to be significantly different when $p < 0.05$.

RESULTS AND DISCUSSION

Water activity of freshwater fishes before and after fermentation

The a_w of the three species of freshwater fishes is shown in **Table 1**. For all fishes, silver barb, climbing perch and three-spot gourami before fermentation, the a_w was 0.96 a_w . After 7 days of fermentation, the a_w for the three samples were decreased to 0.77 a_w significantly. These values of a_w are relatively low and insufficient to support enzymatic activity and microbial proliferation including food poisoning bacteria during storage (Anihouvi et al., 2006). According to Anihouvi et al., (2006), most of the salt fermented fish had sufficiently low water activity, 0.65-0.87 a_w to prevent the growth of putrefactive and hazardous microorganisms.

Table 1. Water Activity (a_w) of Freshwater Fishes Before and After Fermentation

Samples	Water Activity (a_w)	
	Before Fermentation	After Fermentation
Silver Barb	0.96 ± 0.00 ^{aA}	0.77 ± 0.01 ^{aB}
Climbing Perch	0.96 ± 0.00 ^{aA}	0.77 ± 0.02 ^{aB}
Three-Spot Gourami	0.96 ± 0.00 ^{aA}	0.77 ± 0.01 ^{aB}

Different small letters (^{a, b}) in the same column are significantly different ($P < 0.05$). Different capital letters (^{A, B}) in the same row are significantly different ($P < 0.05$).

Presented data are the mean value of triplicate ± standard deviation.

Salt content of freshwater fishes before and after fermentation

Table 2 shows the salt content of freshwater fishes before and after fermentation for 7 days. There was a significant difference ($p < 0.05$) in salt content between naturally fermented fish of silver barb, climbing perch, and three-spot gourami. Salt content for all types of raw fish was 0.00, before fermentation. Then, after 1 week of fermentation, the silver barb recorded the highest salt content (2.70%), followed by the three-spot gourami (2.10%) and climbing perch (1.60%). The fat content of the fish, the thickness of the flesh, freshness, temperature, the chemical purity of the salt, and other factors all influence salt uptake and water loss (Basak et al., 2023; Jiang et al., 2019). Lowering of a_w due to the osmotic action of salt, as well as the effect of salt on spoilage bacteria, are presumed to be the reasons behind this salt fermentation technique. The diffusion of salt into the fish and elimination of water through the process of osmosis. The role of salt is highly significant to

guarantee the quality and stability of the finished products in this category. The preservative action of salt is associated with the reduction of a system's a_w , which makes the environment less favourable for microbial life. This explained the preservative action of salt that exerts a poisonous action, makes moisture unavailable for the microorganisms, prevents bacterial growth by dehydrating the cells by plasmolysis, and destroys bacterial protoplasm (Majumdar & Basu, 2010).

Table 2. Salt Content of Freshwater Fishes Before and After Fermentation

Samples	Salt Content (%)	
	Before Fermentation	After Fermentation
Silver Barb	0.0 ± 0.0 ^{aB}	2.70 ± 0.17 ^{cA}
Climbing Perch	0.0 ± 0.0 ^{aB}	1.60 ± 0.10 ^{bA}
Three-Spot Gourami	0.0 ± 0.0 ^{aB}	2.10 ± 0.17 ^{aA}

Different small letters (^{a, b}) in the same column are significantly different ($P < 0.05$). Different capital letters (^{A, B}) in the same row are significantly different ($P < 0.05$).

The presented data are the mean value of triplicate ± standard deviation.

Crude Protein of freshwater fishes before and after fermentation

Table 3 shows the crude protein content (%) of the samples, which are silver barb, three-spot gourami, and climbing perch. Three-spot gourami was found to have significantly the highest in protein content (78.07%), followed by climbing perch (68.67%), and silver barb (59.82%) before fermentation. After 7 days of going through fermentation, the crude protein three-spot gourami, climbing perch, and silver barb were 57.76%, 40.99%, and 37.83% respectively. It was the result of the hydrolyses process, the protein in fresh fish has been changed into amino acid, and the acid was used by bacteria in the fermented process of growth (Udomthawee et al., 2012). Microorganisms differ in their ability to use nitrogenous compounds as a source of nitrogen for growth. Some of the LAB grow best with polypeptides as nitrogen food. The presence of fermentable carbohydrates as substrate was mainly from ground sautéed rice, resulting in acid fermentation and suppression of proteolytic bacteria, which is termed as ‘sparing’ action on the nitrogen compounds (Mahyudin et al., 2015).

Table 3. Crude Protein of Freshwater Fishes Before and After Fermentation.

Samples	Crude Protein (%)	
	Before Fermentation	After Fermentation
Silver barb	59.82 ± 2.98 ^{bA}	40.99 ± 5.14 ^{bB}
Climbing perch	68.67 ± 8.72 ^{abA}	37.83 ± 5.94 ^{abB}
Three-spot gourami	78.07 ± 0.72 ^{aA}	57.76 ± 1.74 ^{aB}

Different small letters (^{a, b}) in the same column are significantly different ($P < 0.05$). Different capital letters (^{A, B}) in the same row are significantly different ($P < 0.05$).

The presented data are the mean value of triplicate ± standard deviation.

Lactic Acid Bacteria Growth

Table 4 shows the number of LAB growth in the fermented fish samples after seven days of fermentation. The result showed that the three-spot gourami has the highest CFU/ml, which was 7.464 log CFU/mL, followed by climbing perch (6.79 log CFU/mL), and silver barb (5.30 log CFU/mL). Three-spot gourami fish contains

the highest protein content, which allows considerable growth and acid production by LAB in the processing of fermented fish (Mahyudin et al., 2015). Bacteria use proteins for many purposes, such as structure, enzymes, or transport. The microflora of salted–naturally fermented fish consisted of various species of microorganisms such as aerobic, halophile, and staphylococcal bacteria, yeasts, and moulds (Gassem, 2019). During the fermentation process, many lactic acid-producing bacteria grow and produce organic acids, in particular lactic acid, which lowers the pH and preserves the product. The presence of acid also contributed to the flavour of the product. The formation of acid, together with the presence of salt, prevented the growth of putrefactive bacteria.

Table 4. Viability of Lactic Acid Bacteria Growth in Fermented Fish.

Samples	Log Colony Forming Unit/ milliliter (cfu/ml)
Silver Barb	5.30 ± 0.68 ^c
Climbing Perch	6.79 ± 0.21 ^b
Three-Spot Gourami	7.46 ± 0.32 ^a

Different small letters ^(a, b) in the same column are significantly different (P<0.05).

CONCLUSION

In conclusion, a_w for all fish species decreases from 0.96 ± 0.00 before fermentation to 0.77 ± 0.01 significantly after 7 days of fermentation. The crude protein content of all species declined significantly over the one-week fermentation period, with the highest value observed in three-spot gourami. The result of the viability of the LAB growth shows that the three-spot gourami has the highest CFU/mL. This corresponds with its highest protein content among the three fish species.

These results suggest the need for further studies on proximate analysis for raw and fermented fish. The analysis of food proximate composition includes moisture, ash, lipid, protein, and carbohydrate content. These food components could be useful in the food industry for product development, quality control, or regulatory purposes.

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