Research Article

The influence of *Etlingera coccinea* on the sensory acceptability and microbial safety of Dusun fermented fish

Amanda Chu Yi Yau[1], Wolyna Pindi[1,2], Rovina Kobun[1,2] and Sylvester Mantihal[1,2]*

[1]Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu, Sabah 88400, Malaysia

[2]Functional Food Research Group, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu, Sabah 88400, Malaysia

*s.mantihal@ums.edu.my

ABSTRACT

This study aimed to identify the best formulation of Dusun traditional fermented fish (Bosou) using sensory evaluation, specifically a 9-point hedonic test. *Etlingera coccinea*, at concentrations ranging from 0% to 8%, was incorporated into fermented fish samples. Results showed that the 2% *Etlingera coccinea* formulation (F1) consistently outperformed others, maintaining the traditional appearance while enhancing aroma, taste, and overall acceptability. F1's superiority was confirmed by a Friedman test ranking, exhibiting the lowest mean rank among alternatives ($p < 0.05$). It struck a delicate balance between enhanced sensory attributes and traditional expectations, making it the best Bosou formulation. Proximate analysis of F1 and control Bosou formulations revealed significant increases in ash, crude fat, and crude protein content for F1, indicating improved nutritional profiles. Shelf-life analysis demonstrated a significant reduction in the total viable count and total yeast and mould counts during later storage periods. Cooking effectively decreased microbial counts, ensuring levels were at safe for consumption (4 $\log_{10}$ CFU/g for total yeast and mould, 6 $\log_{10}$ CFU/g for total viable count). A notable decrease in pH of raw and cooked samples over one month highlighted the recommendation to cook Dusun traditional fermented fish before consumption for safety. In conclusion, *Etlingera coccinea* showed promising sensory characteristics, nutritional content, and microbial quality, offering valuable insights for developing high-quality Bosou formulations.

Received: 3 July 2024
Accepted: 11 August 2024
Published: 27 September 2024
DOI: http//doi.org/10.51200/ijf.v1i2.5211

Keywords: bosou; *Etlingera coccinea*; fermented fish; traditional food

1. Introduction

Traditional foods are those that are inherited over successive generations and carry substantial cultural, social, and historical significance (Ramli *et al.*, 2019). Traditional foods' sensory attributes, preparation techniques, and nutritional profiles are distinctive to specific communities or geographic regions. Fermented foods have held a vital role in human sustenance for centuries, as the fermentation process produces organic acids, enzymes, and other compounds that enhance the flavour, texture, and nutritional value of

the food. This natural process entails the conversion of sugars and carbohydrates into organic acids, gases, and alcohol by bacteria, yeasts, and moulds (Tamang *et al.*, 2016).

Fermented fish has been a dietary staple across diverse cultures all over the world for centuries. The fermentation process involves the conversion of sugars and carbohydrates in the fish into organic acids, alcohol, and gases by microbial agents such as bacteria and yeast. This fermentation process typically entails the deliberate inoculation of fresh fish with specific strains of bacteria or yeasts, leading to the generation of lactic acid and other organic acids that preserve the fish and impart its characteristic tangy flavour profile (Wan-Mohtar *et al.*, 2022). Fermentation improves the flavour, texture, and nutritional value of fish. Fermented fish is an important source of protein and minerals, and it has cultural and culinary importance as it is passed down through generations.

Bosou, also referred to as jeruk ikan in the Malay language, is a fermented fish preparation that is widely consumed across Southeast Asia, particularly in Indonesia and Malaysia. In Sabah, Malaysia, this traditional dish originated from the Kadazan-Dusun ethnic group located in Sabah, a state situated in the northern region of East Malaysia (Yau *et al.*, 2024). This traditional dish is prepared by fermenting small freshwater fish through the addition of rice bran and salt and allowing the mixture to ferment for several days or weeks. Once the fermentation is complete, the fish is rinsed and typically served as a condiment or side dish, often accompanied by salt, cold cooked rice, and pangi fruits. The dish can be consumed either raw or cooked, depending on individual preference (Lajius, 2014).

Etlingera coccinea, a tropical plant indigenous to Southeast Asia, is commonly referred to as "Tuhau" by the Kadazan-Dusun ethnic group in Sabah. This plant is utilized in traditional recipes for fermented fish preparations by the Dusun community, as its inclusion helps to preserve the fish during the fermentation process (Jems *et al.*, 2021). The plant is typically chopped and added to the fish, where its antimicrobial properties help inhibit the growth of spoilage microbes and maintain the fish's quality (Sharma, 2020). *Etlingera coccinea* has been found to possess functional food qualities and traditional medicinal applications. The plant is known to contain bioactive compounds exhibiting antimicrobial, antioxidant, and anti-inflammatory characteristics (Nur Najmi Mohamad Anuar *et al.*, 2018).

Therefore, this study focused on the influence of *Etlingera coccinea* on the sensory acceptability and microbial safety of Dusun fermented fish. Specifically, we investigated the impact of incorporating varying concentrations of *Etlingera coccinea* into the traditional Dusun fermented fish preparation, and evaluated its effects on the product's flavour, texture, and shelf-life. The study aimed to provide insights into the use of this traditional plant ingredient and its potential to enhance the sensory qualities and microbial safety of this culturally significant fermented food.

2. Materials and Methods

2.1 Formulation of Dusun Traditional Fermented Fish

The formulation of Dusun traditional fermented fish was developed through a process of modification and adaptation. This was informed by interview of local experts in Kota Belud, Sabah, who possess extensive experience in preparing this traditional dish. In the development of Dusun traditional fermented fish, the concentrations of *Etlingera coccinea* were selected based on insights from preliminary experiments and traditional knowledge gathered through community interviews. The preliminary tests indicated significant effects on fermentation between 2% and 6% *Etlingera coccinea*, while the community interviews revealed a traditional use of concentrations ranging from 4% to 8%. The formulations, as detailed in Table 1, maintained constant quantities of fresh river fish, salt, *Pangium edule*, and fragrant rice across 1 kg batches for each formulation, including the control sample. The addition of of Etlingera coccinea were added as compliment to the formulation of Bosou. Therefore, the percentage of total formulation presented in Table 1 were exceeding 100%. The sample was stored in a room temperature in a storage cabinet.

Table 1. Formulation of Dusun traditional fermented fish with different concentrations of *Etlingera coccinea*

Formulation	Percentage of fresh river fish (%)	Percentage of salt (%)	Percentage of *Pangium* seed (%)	Percentage of fragrant rice (%)	Percentage of *Etlingera coccinea* (%)
Control	50	5	8	37	0
F1	50	5	8	37	2
F2	50	5	8	37	4
F3	50	5	8	37	6
F4	50	5	8	37	8

2.2 Sensory evaluation

A total of five Dusun traditional fermented fish samples were evaluated by each panellist in this study, which employed a Completely Randomized Design. The panel consisted of 50 semi-trained assessors, comprising students and staff members from the Faculty of Food Science and Nutrition at Universiti Malaysia Sabah. According to Stone *et al.* (2012), the recommended number of panellists for laboratory-type acceptance evaluations is typically between 25 and 50, with a maximum of 75 participants. Each fermented fish sample was prepared by sautéing it with some oil for 6 to 10 minutes at a temperature of 100˚C in a frying pan and then portioned into 10-gram servings. The samples were presented in plastic containers, coded with three-digit numbers, and the order of presentation was randomized from left to right for each panellist. The sensory evaluation utilized a 9-point hedonic scale to assess the panellists' degree of liking for the various attributes, including colour, taste, aroma, texture, and overall acceptability of the fermented fish samples (Koh, 2022). The panellists then conducted a ranking test; they assigned ranks from 1 to 5 for all the samples to determine the most preferred formulation.

2.3 Microbiological Analysis

Approximately 10 g of the traditional fermented fish and 90 mL of peptone water were mixed at 1:10 ratio to create homogenized samples. The mixture was then transferred into a stomacher bag and homogenized in the stomacher bag for approximately 5 minutes. This resulted in a dilution factor of 10^{-1}. Next, 1 mL of the 10^{-1} diluted sample was aseptically transferred into another tube containing 9 mL of sterile saline water to achieve a 10^{-2} dilution (Akhigbemidu *et al.,* 2015). These steps were repeated until a 10^{-3} dilution was obtained. Trial rounds were conducted to determine the dilution factors that yielded countable colonies. All these steps were performed using aseptic techniques to ensure samples were free from contamination.

2.4. Total Yeast and Mould Count

The total yeast and mould count was evaluated using the pour plate method on plate count agar medium within a laminar flow cabinet. A 1 mL aliquot of the sample with the highest dilution was pipetted into an empty petri dish, followed by successively lower dilutions. Approximately 12 – 15 mL of sterile PCA medium were aseptically added to cover the bottom of the petri dish. The plate was rotated in a figure-eight pattern to homogenize the sample and agar. The agar plates were allowed to solidify and then incubated inverted at 37°C for 24 – 36 hours. This process was repeated for dilutions of 10^{-1} and 10^{-2}, with triplicate plates used for each dilution (Febriyanti *et al.,* 2023).

2.5. Total Viable Count

The total yeast and mould count was performed in a laminar airflow using the spread plate method on PDA agar. Approximately 12 – 15 mL of PDA agar medium were aseptically poured onto an empty petri dish

until the bottom surface was fully covered. The agar plates were left to solidify before being cooled in the laminar airflow. Next, 0.1 mL of the sample was pipetted onto the solidified PDA agar, starting with the highest dilution factor of 10^{-7}. An L-shaped glass rod was used to evenly spread the sample across the agar surface. Once again, the agar plates were dried and cooled under the laminar airflow. After that, the petri dishes were incubated inverted at 37°C for 5 to 7 days before the colony counting method. The same process was followed for lower dilutions, and triplicate petri dishes were used for each dilution (Febriyanti *et al.,* 2023).

2.5 Statistical analysis

Statistical analyses were conducted using IBM SPSS Version 29.0. Analytical data were presented as mean ± standard deviation, with statistical significance set at $p<0.05$. For the sensory evaluation, one-way ANOVA was used to assess differences among samples in the 9-point hedonic test, followed by Tukey's HSD test for post-hoc comparisons if significant differences were found. For the ranking test, a non-parametric Friedman test was used, and pairwise post-hoc comparisons were conducted using the Wilcoxon signed-rank test if significant differences were detected. For the shelf-life analyses, one-way ANOVA was employed, with Tukey's HSD test for post-hoc analysis. Two-way ANOVA was used to analyse the shelf-life data.

3. Results and Discussion

3.1. Sensory evaluation

This analysis was conducted to obtain the optimal formulation for Dusun traditional fermented fish through a preliminary sensory evaluation of fermented fish across various concentrations of *Etlingera coccinea* (0%, 2%, 4%, 6%, 8%) as shown in Figure 1. To achieve this, a 9-point hedonic test and a ranking test for sensory evaluation was employed. These methods were used to provide insights into the sensory characteristics and identify the most preferred formulation. The visual appearance of the fermented fish samples from the control to formulation F4 was comparable, as illustrated in Figure 1. This suggests that the incorporation of *Etlingera coccinea* (tuhau) did not significantly alter the appearance and colour of the treated fermented fish samples.

The 9-point hedonic scale is a commonly utilized and effective test method for assessing consumer acceptance and liking of food products (Lawless and Heymann, 2010). The sensory evaluation was carried out in the sensory laboratory of the Faculty of Food Science and Nutrition at Universiti Malaysia Sabah. The results of this assessment are presented in Table 2.

The colour profiles of the different Dusun traditional fermented fish formulations, as shown in Table 2, demonstrates a consistent trend, with mean scores indicating a sensory preference ranging from 'like slightly' to 'like moderately' (6.46±2.26 for F4 to 6.90±1.77 for F1). The consistent colour profiles across different formulations suggests that varying concentrations of *Etlingera coccinea* have minimal impact on the colour. This can be attributed to the persistent use of *Pangium edule* seed, which contributed to the intensity of the colour. According to Anggadhania *et al.* (2023), the fermentation process makes these seeds edible by eliminating cyanide content and enhances their nutritional value and flavour, leading to a transformation in their colour from milky white to brown or black, as well as softening their texture.

The aroma was notably prominent in F1 (Table 2), with a significantly higher mean score of 7.13±1.61, indicative of a preference level of 'like moderately' ($p<0.05$). The data indicates that a 2% concentration of *Etlingera coccinea* appeared to positively impact the aroma profile of the traditional Bosou fermented fish, potentially contributing a distinct and appealing olfactory quality to the product. However, declining scores in F2 (6.23±2.28), F3 (5.58±2.35), and F4 (5.04±1.78) suggest that higher concentrations may not have as positive an impact on the aroma, highlighting the importance of carefully determining the optimal concentration to achieve the desired olfactory profile (Karagul-Yceer *et al.,* 2006). Consistent texture scores (5.98±2.33 to 6.87±2.06) across *Etlingera coccinea*-enriched formulations suggest minimal impact on Bosou's perceived texture. Bosou, a traditional fermented fish product, is often consumed as a stir-fried paste paired with rice. This pairing with rice enhances the acceptability of Bosou, as its favourable texture attributes are well-suited for this traditional consumption method (Sharif *et al.*, 2017). To further validate the consumer preference patterns, a ranking test was conducted.

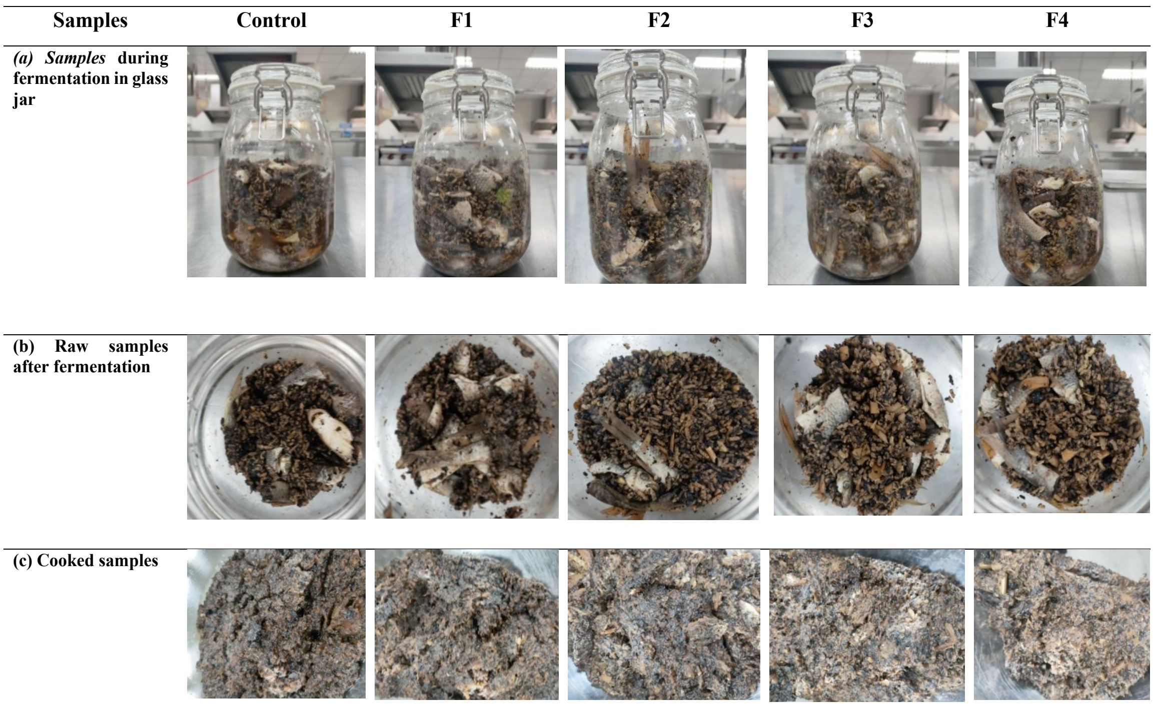

Figure 1. Comparison of samples with different concentrations of *Etlingera coccinea* (a) Samples during fermentation (b) raw samples after fermentation [14 days] (c) cooked samples

Table 2. Sample means scores for colour, aroma, taste, texture, and overall acceptance

Attributes[1]	Control	F1	F2	F3	F4
Colour	6.75±2.06^{a}	6.90±1.77^{a}	6.67±2.02^{a}	6.48±2.08^{a}	6.46±2.26^{a}
Aroma	6.42±2.31^{a}	7.13±1.61^{b}	6.23±2.28^{a}	5.58±2.35^{a}	5.04±1.78^{a}
Taste	6.48±2.31^{a}	6.92±1.28^{b}	6.69±2.03^{a}	5.79±2.29^{a}	4.77±2.14^{a}
Texture	6.87±2.06^{a}	6.75±1.52^{a}	6.60±2.18^{a}	5.98±2.33^{a}	6.06±2.13^{a}
Overall Acceptance	5.79±2.20^{a}	6.98±1.44^{b}	5.71±2.24^{a}	5.46±2.31^{a}	4.36±2.00^{a}

[1]Values are shown in the form of mean ± standard deviation.
$^{a,\,b}$ Different superscripts in the same row indicate a significant difference ($p < 0.05$).

3.1.1. Ranking test

The sensory ranking test, as shown in Table 3, evaluated the different Bosou formulations with varying concentrations of *Etlingera coccinea*. The Friedman test results indicate that the formulation with 2% *Etlingera coccinea*, designated as F1, was ranked the highest, with a mean rank of 2.54. This shows that incorporation of this ingredient effectively enhanced the taste and aroma profiles, resulting in greater consumer satisfaction (Meilgaard *et al.,* 2016).

Table 3. Mean rank scores for formulation of Dusun traditional fermented fish in different concentration of *Etlingera coccinea*

Formulations[1]	**Mean rank scores**
Control	2.88
F1	2.54
F2	2.92
F3	3.17
F4	3.48

[1]Different concentrations of *Etlingera coccinea* were used for formulation of *Bosou* which were Control at 0%; F1 at 2%; F2 at 4%; F3 at 6%; F4 at 8%.

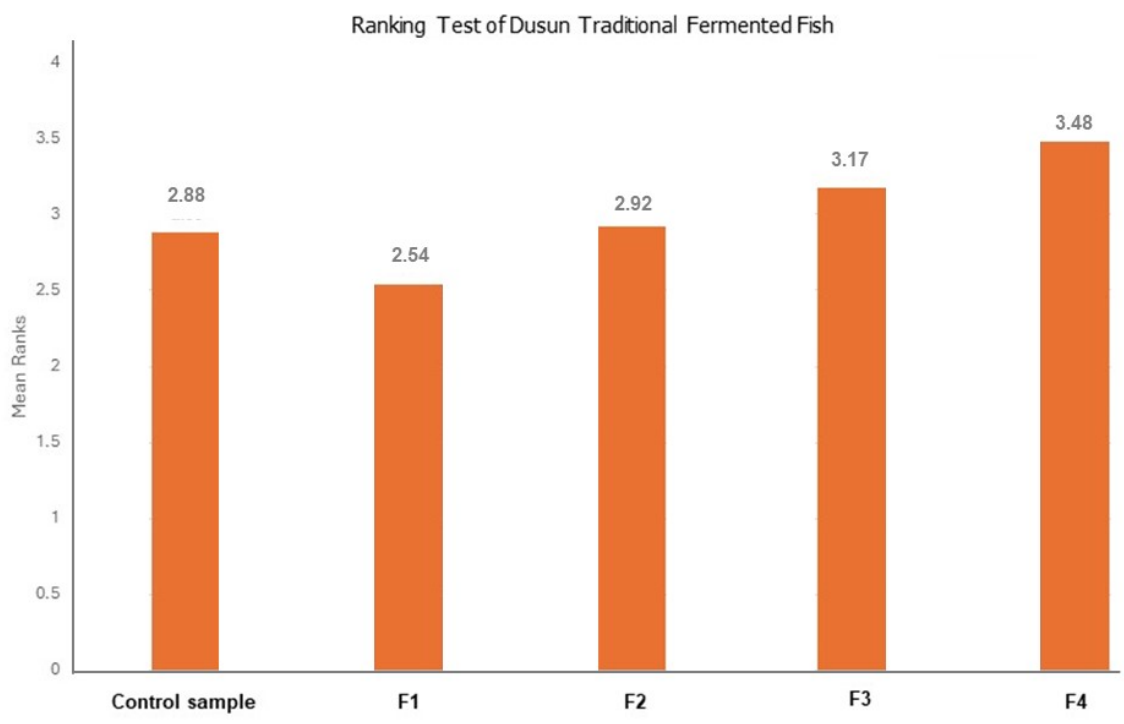

Figure 2. Ranking test of Dusun traditional fermented fish

The findings indicate that the formulation with a 2% concentration of *Etlingera coccinea*, denoted as F1, was identified as the most preferred by the panellists (Figure 2). This is evident from the consistently higher mean scores across various sensory attributes, suggesting this formulation exhibited superior overall sensory characteristics compared to the other formulations. Conversely, the formulation with an 8% *Etlingera coccinea* concentration, F4, received the lowest mean rank of 3.48, indicating diminished consumer satisfaction and making it the least preferred formulation. These tests complement methods such as hedonic rating and intensity scaling and are often utilized to screen products or gain insights from specific consumer populations due to their straightforward and informative nature (Lawless and Heymann, 2010).

3.2. Selection of the best formulation

A comprehensive sensory evaluation approach was employed, incorporating both the 9-point hedonic scale test and subsequent ranking tests (Sharif *et al.,* 2017) to identify the optimal formulation for the Dusun traditional fermented fish. The 9-point hedonic scale test consistently indicated a preference for the F1 formulation, which was enriched with 2% *Etlingera coccinea*. This formulation exhibited the highest mean scores across sensory attributes including colour, aroma, taste, texture, and overall acceptance. F1 was particularly notable for its superior aroma, taste, and overall acceptability, suggesting it was distinctive and widely preferred among the panellists. The subsequent Friedman ranking test further corroborated the superiority of F1, assigning it the lowest mean rank of 2.54. The statistical significance ($p < 0.05$) of these ranking differences supports the conclusion that F1 stands out as the best formulation. The F1 formulation, which incorporates a 2% concentration of *Etlingera coccinea*, represents the optimal formulation for the Bosou fermented fish product. The comprehensive approach, involving both hedonic testing and ranking test, provides a holistic understanding of the sensory profile without redundancy, further supporting the selection of F1 as the preferred formulation.

3.2. Shelf-life analysis of Dusun traditional fermented fish

The shelf-life of Bosou was assessed by conducting a total viable count analysis on raw Bosou samples over the course of storage (Day 0, Day 7, Day 14, Day 21, and Day 28). The results of the total viable count for the control sample and the F1 Dusun traditional fermented fish sample are presented in Table 4.

Table 4. Colony count for total viable count for control and the most preferred *Bosou* sample in different duration of storage

	Total Viable Count ($\log_{10}$ CFU/g)				
Formulation[1]	**Storage Day 0**	**Storage Day 7**	**Storage Day 14**	**Storage Day 21**	**Storage Day 28**
Control	4.48 ± 0.19^{aA}	6.38 ± 1.07^{bA}	7.66 ± 1.55^{cA}	7.53 ± 0.27^{bA}	10.55 ± 1.50^{cB}
F1	3.11 ± 1.92^{aA}	7.22 ± 0.52^{bA}	7.30 ± 2.02^{bA}	7.25 ± 0.72^{bA}	7.47 ± 0.82^{bA}

' Mean±SD; Control, control formulation with 0% of *Etlingera coccinea*; F1, Formulation with 2% of *Etlingera coccinea*

[a, b] Different lowercase superscripts in the same (colomn) denote significant difference ($p<0.05$).

[A,B] Different uppercase superscripts in the same (column) denote significant difference ($p<0.05$).

In the control formulation, characterized by the absence of *Etlingera coccinea*, the TVC exhibits a notable increase from 4.48±0.19 CFU/g on Day 0 to a significantly higher value of 10.55±1.50 CFU/g on Day 28. The observed significant differences ($p<0.05$) indicate a significant microbial proliferation throughout the storage period. This escalation suggests a potential challenge regarding the microbial quality and safety of Bosou as the storage duration extends. Conversely, the F1 formulation, enriched with 2% *Etlingera coccinea*, demonstrates a more controlled trend in TVC. Starting at 3.11±1.92 CFU/g on Day 0, the TVC reaches 7.47±0.82 CFU/g on Day 28. The inclusion of *Etlingera coccinea* in the formulation appears to have a beneficial effect in mitigating the microbial proliferation typically observed during the storage of Bosou, potentially contributing to improved microbial quality and safety (Sikder *et al.,* 2020). As antioxidants are known for their ability to inhibit oxidative processes, they may have exhibited antimicrobial effects (Nur Najmi *et al.*, 2018). These findings emphasize the significance of assessing microbial quality and exploring strategies to extend the shelf-life of the traditional Bosou fermented fish product.

3.3.1 Comparison between raw and cooked sample

Analyses of the total viable count data in Table 4 shows that raw Bosou samples display elevated microbial loads, underscoring the potential need for cooking Bosou prior to consumption. To further assess the shelf-life of Bosou, a total viable count analysis was performed on both raw and cooked samples at storage day 0 and day 14. The results detailing the total viable count in the raw and cooked Dusun traditional fermented fish samples are presented in Table 5 and Table 6.

Table 5. Colony count for total viable count for raw and cooked control *Bosou* sample in different duration of storage

Sample[1]	**Total Viable Count (log_{10} CFU/g)**	
	Storage Day 0	**Storage Day 14**
Control (raw)	4.48 ± 0.19^{b}	7.66±1.55
Control (cooked)	3.21 ± 0.72^{a}	Not detected

[1]Mean±SD; Control (raw), Control (cooked), control formulation with 0% of *Etlingera coccinea*.
[a, b] Different superscripts in the same column denote significant difference ($p<0.05$).

Table 6. Colony count for total viable count for raw and cooked F1 *Bosou* sample in different duration of storage

Sample[1]	**Total Viable Count (log_{10} CFU/g)**	
	Storage Day 0	**Storage Day 14**
F1 (raw)	3.11±1.92	7.30 ± 2.02^{b}
F1 (cooked)	Not detected	2.18 ± 0.32^{a}

[1]Mean±SD; F1 (raw), F1 (cooked), control formulation with 2% of *Etlingera coccinea*.
[a, b] Different superscripts in the same column denote significant difference ($p < 0.05$).

Based on Table 5 (control), the Total Viable Count (TVC) for untreated control Bosou on storage day 0 registers at 4.48±0.19 log_{10} CFU/g, increasing significantly to 7.66±1.55 log_{10} CFU/g by storage day 14. Conversely, the TVC for cooked control Bosou measures 3.21±0.72 log_{10} CFU/g on storage day 0 and becomes 'Not detected' (ND) by storage day 14 (Bolarinwa *et al.,* 2016). In Table 6 (F1), the TVC for raw F1 Bosou on storage day 0 was 3.11±1.92 log_{10} CFU/g, and increased to 7.30±2.02 log_{10} CFU/g by storage day 14. Conversely, for cooked F1 Bosou, microbial counts were 'Not detected' on storage day 0 and increased to 2.18±0.32 log_{10} CFU/g by storage day 14. The 'Not detected' status suggests that cooking effectively has a slight increase in detectable microbial counts in the F1 Bosou by storage day 14 but significantly lower than that of raw sample. According to FAO/WHO expert review of microbiological requirements (CAC/RCP 15, 1976), numbers at or above 6 log_{10} CFU/g are undesirable while values up to 4.7 log_{10} CFU/g of mesophilic aerobic bacteria are safe for human consumption (Ali *et al.,* 2008). Therefore, it can be assumed that to guarantee the highest level of safety quality, the Dusun traditional fermented fish should be only stored for within 14 days at room temperature before being reheated and consumed, or at the least cook with high heat to kills microbes before consumption (Keenleyside, 2019).

3.4 Total Yeast and Mould Counts

In the analysis of the raw sample, total yeast and mould counts were systematically conducted on storage Day 0, Day 7, Day 14, Day 21 and Day 28 for the raw Bosou samples. The obtained results, encompassing the total yeast and mould counts in the examined raw control and F1 Dusun traditional fermented fish samples, are presented in Table 7.

Table 7. Colony count for total yeast and mould count for control and the most preferred Bosou sample in different duration of storage

Formulation[1]	Total Yeast and Mould Count (log_{10} CFU/g)				
	Storage Day 0	**Storage Day 7**	**Storage Day 14**	**Storage Day 21**	**Storage Day 28**
Control	4.53±0.05^{aB}	6.92±0.53^{bA}	7.48±0.83^{bA}	8.19±0.69^{bA}	10.46±0.54^{cA}
F1	3.46±0.15^{aA}	6.10±0.61^{aA}	7.62±0.25^{bA}	8.67±1.08^{cA}	10.80±2.41^{cA}

'Mean±SD; Control, control formulation with 0% of Etlingera Coccinea; F1, Formulation with 2% of Etlingera Coccinea
$^{a,\ b}$ Different lowercase superscripts in the same (colomn) denote significant difference ($p<0.05$).
^{A,B} Different uppercase superscripts in the same (column) denote significant difference ($p<0.05$).

Table 7 shows the colony counts for total yeast and mould in both the control and F1 Bosou samples across various storage durations. The data is expressed in log_{10} CFU/g. In the control formulation, characterized by the absence of *Etlingera coccinea*, there is a noticeable rise in total yeast and mould count from 4.53±0.05 CFU/g on Day 0 to a significantly higher value of 10.46±0.54 CFU/g on Day 28. The notable increase in yeast and mould count over the storage duration suggests a significant proliferation of these microorganisms, which may pose potential challenges to the microbial quality and safety of the Bosou fermented fish product during prolonged storage (Belleggia and Osimani, 2023).

Conversely, in the F1 formulation enriched with 2% *Etlingera coccinea*, there is a more controlled trend in the total yeast and mould count. Starting at 3.46±0.15 CFU/g on Day 0, the count rises to 10.80±2.41 CFU/g on Day 28. The surge in microbial activity may be attributed to the breakdown of the fish product over time. The inhibitory effect on natural flora, facilitated by the elevated salt content and the inherent antibacterial properties of *Etlingera coccinea*, are believed to play a role in this phenomenon (Sim *et al.,* 2015). The incorporation of *Etlingera coccinea* has an impact on the microbial status of Bosou, providing valuable insights into potential avenues for managing yeast and mould proliferation in fermented fish products (Anihouvi *et al.,* 2007).

3.4.1 Comparison of Total Yeast and Mould Count of Raw and Cooked Bosou Sample

Analysis of the total yeast and mould counts shown in Table 7 reveals substantial levels in raw Bosou samples. This emphasizes the critical importance of cooking Bosou before consumption as a necessary step. The results shown in Table 8 provide insights into the total yeast and mould counts observed in raw and cooked control samples of the Dusun traditional fermented fish. Notably, the raw Bosou samples exhibited substantially higher total yeast and mould counts compared to the cooked samples, a trend that was consistent across both the control and F1 formulations.

Table 8. Colony count for total yeast and mould count for raw and cooked control *Bosou* sample in 14 days duration of storage

Sample[1]	Total Yeast and Mould Count ($\log_{10}$ cfu/g)	
	Storage Day 0	**Storage Day 14**
Control (raw)	4.53±0.05^{b}	7.48±0.83
Control (cooked)	1.04±0.25^{a}	Not detected

[1]Mean±SD; Control (raw), Control (cooked), control formulation with 0% of *Etlingera coccinea*.

[a, b] Different superscripts in the same column denote significant difference ($p < 0.05$).

In the raw control sample, the total yeast and mould count was 4.53±0.05 $\log_{10}$ CFU/g on Day 0 and significantly increased to 7.48±0.83 $\log_{10}$ CFU/g on Day 14. In contrast, the cooked control sample exhibits a substantial reduction in total yeast and mould count; it was 1.04±0.25 $\log_{10}$ CFU/g on Day 0 and became 'Not detected' on Day 14. The 'Not detected' result suggests that the cooking process effectively eliminated detectable levels of yeast and mould in the control Bosou by Day 14 of the storage period (Bolarinwa *et al.,* 2016). These results emphasize the crucial significance of cooking in diminishing yeast and mould counts, thereby enhancing the microbial quality and safety of Bosou. The marked differences observed between raw and cooked samples indicate that cooking represents an indispensable step in ensuring safe consumption and prolonging the shelf-life of this traditional fermented fish product.

Conclusion

This study successfully identified the optimal Dusun traditional fermented fish formulation by carefully varying the concentrations of *Etlingera coccinea*, a key ingredient. The 2% *Etlingera coccinea* formulation, designated as F1, consistently outperformed the other tested formulations in a 9-point hedonic evaluation. This F1 formulation achieved a harmonious balance between heightened sensory attributes, such as aroma, taste, and texture, and traditional expectations for the Bosou product. The statistical validation through a Friedman test further confirmed the superior ranking of the F1 formulation among the samples evaluated. Investigating the shelf-life dynamics of the F1 formulation revealed a positive impact on its microbial quality. Compared to the control sample, the F1 formulation exhibited substantial reductions in total viable count, total yeast, and mould counts during the later storage period. These findings emphasize the F1 formulation's contributions to enhancing the safety and extending the shelf-life of the traditional Bosou product. The recommendation to cook Bosou before consumption aligns with the study's overarching commitment to delivering a superior-quality, safe product to consumers. In essence, this comprehensive study has identified the F1 formulation as the optimal choice, excelling in sensory excellence, statistical validation, and enhanced microbial quality, making it the preferred selection for consumers.

Acknowledgment

The corresponding author would like to thank Puan Jaitah from Kampung Tambulion, Kota Belud, for supplying the raw materials to be used in this research.

References

Akhigbemidu, W., Musa, A. & Kuforiji, O. (2015). Assessment of the microbial qualities of noodles and the accompanying seasonings. Nigerian Food Journal, 33(1), 48-53.

Amit, S. K., Uddin, Md. M., Rahman, R., Islam, S. M. R., & Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. Agriculture and Food Security, 6(1).

Anal, A. K., Perpetuini, G., Petchkongkaew, A., Tan, R. P., Avallone, S., Tofalo, R., Van Nguyen, H., Chu Ky, S., Ho, P., Phan, T. G., & Waché, Y. (2020). Food safety risks in traditional fermented food from South-East Asia. Food Control, 109, 106922. DOI: 10.1016/j.foodcont.2019.106922.

Anihouvi, V. B., Sakyi-Dawson, E., Ayernor, G. S. & Hounhouigan, J. D. (2007). Microbiological changes in naturally fermented cassava fish (Pseudotolithus sp.) for lanhouin production. International Journal of Food Microbiology, 116(2), 287-291.

Anjali, K., Mane, B.G., Devesh, T., & Khurana, S.K. (2015). Effect of Incorporation of Lungru (Diplazium esculentum) on the Physico-Chemical, Microbiological, and Sensory Quality of Chicken Patties DGCN-College of Veterinary and Animal Sciences,

CSK HPKV, Palampur-176062 (HP), India. Journal of Meat Science and Technology. 3(2), 28–31.

Belleggia, L., & Osimani, A. (2023). Fermented fish and fermented fishbased products, an ever-growing source of microbial diversity: A literature review. Food Research International, 172, 113112.

Febriyanti, M K., Supomo., Nurhasnawati, H., & Noorcahyati. (2023). The characterization of the simplies and ethanol extracts of limpasu leaf and fruit (Baccaurea\lanceolata (Miq.) Müll. Arg.)

Ilyanie, H. Y., Huda-Faujan, N., Muryany, M. I. & Zuraida, J. (2022). Isolation and characterisation of probiotic lactic acid bacteria from Malaysian fermented fish products budu and bosou. International Food Research Journal, 29(2), 338-348.

Jems, N., Rusdi, A., Mus, A., & Godoong, E. (2021). Chemical Composition of Essential Oil from Etlingera coccinea (Blume) S. Sakai & Nagam in Kadamaian, Kota Belud, Sabah. Journal of Tropical Biology and Conservation, 18, 91–105.

Keenleyside, W. (2019). 14.2 Using Physical Methods to Control Microorganisms. Ecampusontario.pressbooks.pub.

Ketel, E. C., de Wijk, R. A., de Graaf, C., & Stieger, M. (2020). Effect of cross-cultural differences on thickness, firmness, and sweetness sensitivity. Food Research International, 109890.

Koh, W. Y., Matanjun, P., Lim, X. X., & Kobun, R. (2022). Sensory Physicochemical, and Cooking Qualities of Instant Noodles Incorporated with Red Seaweed (Eucheuma denticulatum). Foods, 11(17), 2669.

Lajius, L. (2014). Bosou is the traditional food of the people of Dusun Sabah. University Malaysia Sabah (Utusan Kampus).

Lawless H. T., & Heymann H. (2010). Sensory evaluation of food: principles and practices: Springer Science & Business Media.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2016). Sensory evaluation techniques (5th ed.). CRC Press.

Narzary, Y., Das, S., Goyal, A. K., Lam, S. S., Sarma, H., & Sharma, D. (2021). Fermented fish products in South and Southeast Asian cuisine: indigenous technology processes, nutrient composition, and cultural significance. Journal of Ethnic Foods, 8(1). DOI: 10.1186/s42779-021-00109-0

Nur, Najmi, Mohamad, Anuar., Jamaludin, Mohamed., Erni, Norfardila, Abu Hanipah., Nor, Janna, Yahya., Esther, Mathias, Ajik., & Izatus, Shima, Taib. (2018). The protective effect of Ettlingera coccinea (TUHAU) against autoxidation-induced ox brain homogenate. Jurnal Sains Kesihatan Malaysia, 16 (SI), 35–39.

Ramli, A., Khairunisa, D., Sapawi, A., Salehuddin, M., Zahari, M., & Ramli. (2019). The determinants of food heritage based on age in the Klang Valley Malaysia. Journal of Tourism, Hospitality, and Culinary Arts, 12(1), 396–411.

Sharif, M. K., Butt, M. S., Sharif, H. R., & Nasir, M. (2017). Sensory evaluation and consumer acceptability. Handbook of food science and technology, 361-386.

Sharma, R., Garg, P., Kumar, P., Bhatia, S. K., & Kulshrestha, S. (2020). Microbial fermentation and Its Role in Quality Improvement of Fermented Foods. Fermentation, 6(4), 106.

Sikder, M., Ahmed, I., Rashid, S., & Ramli, A. (2020). Physicochemical Quality Assessment of Salt-Free Fermented Fish Products of Sylhet Region, Bangladesh. International Journal of Engineering Technology and Science (IJETS), ISSN (1), 2462–1269. DOI:10.15282/ijets.7.1.2020.1009.

Sim, K. Y., Chye, F. Y. & Anton, A. (2015). Chemical composition and microbial dynamics of budu fermentation, a traditional Malaysian fish sauce. Acta Alimentaria, 44(2), 185-194.

Swanson, K. M. (2011). Fish and seafood products. In Microorganisms in Foods 8 (pp. 107-133). Springer, Boston, MA.

Tamang, J. P., Shin, D.-H., Jung, S.-J., & Chae, S.-W. (2016). Functional Properties of Microorganisms in Fermented Foods Frontiers in Microbiology, 7. DOI:10.3389/fmicb.2016.00578.

Wan-Mohtar, WAAQI., Ilham, Z., Jamaludin, A.A., David, W., & Mohd, Zaini, N.A. (2022). Fermented foods as alternative functional foods during the post-pandemic in Asia Front. Food. Sci. Technol., 2, 1047970.

Yau, A. C. Y., & Mantihal, S. (2024). Development of Dusun Traditional Fermented Fish (Bosou) Using Different Concentrations of Tuhau (Etlingera coccinea). *Journal of Biochemistry, Microbiology and Biotechnology*, *12*(SP1), 19-21.