CLSU

International Journal of Science and Technology (IJST)
Vol. 10: 000015 (2026) ISSN 2507-9638 https://ijst.clsu.edu.ph

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Sensory, Microbial and Chemical Analysis of Fermented Black-chin Tilapia (*Sarotherodon melanotheron*, Ruppell 1852) Enhanced with Plant-Based Proteolytic Enzymes

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Keywords: *Sarotherodon melanotheron, Fermented fish, Bromelain, Papain, Enzymatic fermentation*

Type: Research Article

Submitted: June 12, 2025
Accepted: March 24, 2026
Published: June 30, 2026

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Citation
Tamayo, M. G. & Santos, M. D. (2026). Sensory, Microbial and Chemical Analysis of Fermented Black Chin Tilapia (*Sarotherodon melanotheron*, Ruppell 1852) Enhanced with Plant-Based Proteolytic Enzymes. *CLSU International Journal of Science and Technology, 10, 000011.* https://doi.org/10.22137/IJST.2026.000015

Abstract

This study investigated the effects of proteolytic enzymes bromelain and papain on protein hydrolysis, microbial safety, physicochemical quality, and sensory acceptability of fermented fish products compared with a non-enzyme control. Bromelain exhibited the highest proteolytic activity (28.7 ± 1.2 U/g[a]), resulting in extensive hydrolysis and liberation of peptides and amino acids, while papain showed moderate activity (26.9 ± 1.0 U/g[b]); the control had limited hydrolysis (12.4 ± 0.8 U/g[c]). Both enzyme treatments ensured microbial safety with no pathogens detected, whereas the control showed trace pathogen risk. Sensory evaluation revealed significantly higher scores ($p < 0.05$) for bromelain across all attributes, with overall acceptability (8.15 ± 0.16[a]) surpassing papain (7.85 ± 0.21[a]) and the control (6.60 ± 0.26[c]). Microbiological analysis confirmed reduced total plate counts and yeast/mold counts in enzyme-treated samples (2.8–3.1 × 10^3 CFU/g[a]) compared to the control (5.6 × 10^3 CFU/g[b]). Physicochemical parameters indicated lower pH (5.5–5.6[a]), reduced moisture, and higher crude protein (17.6 ± 0.4%[a] for bromelain; 16.8 ± 0.3%[b] for papain) relative to the control (15.3 ± 0.5%[c]). Total volatile basic nitrogen (TVB-N) values were significantly lower in enzyme-treated samples (12.5–14.2 mg N/100g[ab]) compared with the control (18.9 mg N/100g[c]). Lactic acid bacteria (LAB) counts increased markedly in bromelain (2.3 × 10^5 to 7.2 × 10^5 CFU/g[a]) and papain (2.2 × 10^5 to 6.9 × 10^5 CFU/g[a]) treatments, while the control showed reduced LAB growth (4.8 × 10^5 CFU/g[b]) and trace pathogen presence. Growth rate monitoring from August to January demonstrated consistently faster fermentation in enzyme- treated samples (final rate 4.5 ± 0.4[a] for bromelain; 4.4 ± 0.4[a] for papain) compared to the control (4.0 ± 0.5[b]). Data were analyzed using one-way ANOVA, and mean separation was performed with Duncan's Multiple Range Test at $p < 0.05$. Both Tukey's Honest Significant Difference (HSD) and Duncan's Multiple Range Test (DMRT) were applied to complement each other's strengths. HSD ensured stringent control of Type I error, providing robust statistical reliability, while DMRT maximized sensitivity in detecting subtle treatment differences of biological relevance. This dual approach balanced rigor with practical sensitivity, thereby strengthening the validity and interpretability of the results. Overall, bromelain treatment yielded the most favorable outcomes in terms of proteolysis, microbial safety, sensory quality, and biochemical stability, underscoring its potential as an effective biotechnological tool for enhancing fish fermentation processes.

Introduction

Fermentation is among the oldest and most effective methods of food preservation, practiced across diverse cultures and economic settings—from rural households and artisanal producers to modern industrial food systems. In the Philippines, fermented fish products such as *bagoong* remain integral to culinary tradition, serving both as condiments and as important sources of flavor and nutrition. Typically, fish paste is prepared by fermenting fish or shrimp with 20–25% salt under ambient tropical conditions, a process that extends shelf life while enhancing taste and nutrient bioavailability (Ohshima & Makino, 2014).

Despite its cultural and economic importance, fermented fish production in the Philippines is still dominated by small-scale, traditional methods that rely on inherited practices and subjective sensory cues such as odor, color, and texture. These approaches lack standardized controls, resulting in inconsistent quality and food safety concerns (Atun *et al.*, 2024). Such inconsistency is particularly problematic given the strict microbiological safety standards mandated by the Philippine National Standards and Food and Drug Administration. According to PNS/FDA 36:2014, fermented fish products must be free from pathogenic microorganisms such as *Escherichia coli* and *Salmonella* spp., while maintaining acceptable limits for total plate counts ($\leq 1 \times 10^5$ CFU/g) and yeast and mold counts ($\leq 1 \times 10^3$ CFU/g). Compliance ensures that products are microbiologically safe, shelf-stable, and suitable for consumer markets.

Traditional fermentation methods often struggle to consistently meet these benchmarks. Although lactic acid bacteria (LAB) naturally suppress spoilage organisms and enhance product quality, their activity alone may not guarantee safety or uniformity. Modern biochemical approaches that integrate microbial and enzymatic strategies are therefore needed to accelerate fermentation, improve consistency, and enhance sensory attributes (Kanimozhi *et al.*, 2026). Among these, plant-derived proteolytic enzymes such as bromelain (from pineapple) and papain (from papaya) present a promising but underexplored solution. These enzymes hydrolyze complex fish proteins into simpler amino acids and peptides, thereby accelerating fermentation, improving digestibility, and enhancing flavor and texture (Widjanarko *et al.*, 2015).

The potential of this approach is particularly relevant for *Sarotherodon melanotheron* (Blackchin Tilapia), an abundant but underutilized invasive species in Philippine waters such as Laguna de Bay. Despite its availability, this fish is often relegated to low-value uses due to its characteristic off-odor and limited consumer appeal. In other Southeast Asian countries, however, this species is successfully used in traditional fermented products, such as *pla ra* in Thailand, where its fermentation properties are well recognized (Tanasupawat *et al.*, 2013). This suggests that with improved fermentation techniques, *S. melanotheron* could be transformed into a value-added product that meets both consumer expectations and national safety standards.

The distinct contribution of the present study lies in its application of bromelain and papain to the fermentation of *S. melanotheron*. Unlike previous studies that focused on traditional condiments such as *bagoong* and *patis*, this research integrates chemical, microbial, sensory, and fermentation rate analyses to provide a holistic evaluation of enzyme-assisted fermentation. By demonstrating that proteolytic enzymes can simultaneously accelerate fermentation, improve nutritional quality, enhance sensory acceptability, and ensure compliance with PNS/FDA 36:2014 microbiological safety standards, the study offers a science-based strategy with innovative potential to modernize traditional fish fermentation.

Standard culture techniques remain the benchmark for evaluating microbial safety in fermented fish products. LAB are commonly enumerated using the spread plate method on MRS agar (AOAC INTERNATIONAL, 2023; APHA, 2015), while pathogen detection follows internationally recognized protocols. Recent studies confirm the relevance of these methods in assessing fermentation quality and safety (Gassem, 2019; Sari *et al.*, 2023).

Given these gaps, the present study seeks to modernize and scientifically validate the fermentation of *S. melanotheron* by incorporating enzymatic interventions. Specifically, it aims to determine the effects of bromelain and papain on the sensory attributes, chemical composition, microbial quality, and fermentation rate of fermented tilapia paste. By aligning the outcomes with the safety requirements of PNS/FDA 36:2014, the research ensures that the products developed are not only acceptable to consumers but also compliant with national food safety regulations. In doing so, the study addresses the pressing need for standardized, science-based approaches to fermented fish production in the Philippines, bridging tradition with innovation to promote sustainable and safe food processing.

Materials and Methods

Sample Collection and Preparation

Fresh black chin tilapia (*Sarotherodon melanotheron*) were purchased from Los Baños Public Market, Laguna, Philippines, and immediately transported on ice to the College of Fisheries, Laguna State Polytechnic University – Los Baños Campus (LSPU-LBC) for processing. The fish were washed with clean water, eviscerated, and cut into small pieces. Rock salt (NaCl) was used as the fermenting agent, while pineapple cores and stems, and unripe papaya were utilized as sources of bromelain and papain, respectively. All chemicals and reagents used were of analytical grade (AOAC, 2019).

Extraction of Enzymes

a. Bromelain Extraction

Bromelain was extracted from pineapple (*Ananas comosus*) cores and stems following updated protocols adapted from recent studies (Li *et al.*, 2024; Firdaus *et al.*, 2025). Cleaned plant materials were homogenized into a fine puree and filtered through sterile cheesecloth to remove fibrous residues. The crude extract was subjected to cold clarification by overnight storage at –4 ˚C, allowing precipitation of inactive solids. The clear supernatant containing active bromelain was carefully decanted and stored at 4 ˚C until further use, consistent with current recommendations for maintaining protease stability during short-term storage. Bromelain demonstrated superior efficiency, consistent with its strong protease specificity, and similar to findings by Nurdiani *et al.* (2024), who reported effective hydrolysis of *Pangasius* by-products into protein hydrolysates."

b. Papain Extraction

Papain was obtained from green papaya (*Carica papaya*) latex using established procedures (Yu & Zhang, 2020). Incisions were made on the surface of green papaya fruits, and the latex exudate was collected in clean containers. To prevent oxidation and browning, the latex was immediately mixed with a small amount of antioxidants such as sodium metabisulfite. The crude latex was then filtered to remove impurities and diluted with distilled water, consistent with extraction approaches described by Khatun *et al.* (2023), supported by microbial characterization studies of papain-producing bacteria (Tanasupawat *et al.*, 2013) and advanced aqueous two-phase extraction techniques reported by Twiswiio *et al.* (2017).

Fermentation Procedure

The fermentation process is presented using a flow chart in Figure 1.

Each treatment was packed into sterilized glass jars, sealed, and stored at ambient temperature (approximately 28 ˚C) for the designated fermentation period, following the general procedure of Abolagba and Melle (2008) for salted fish fermentation.

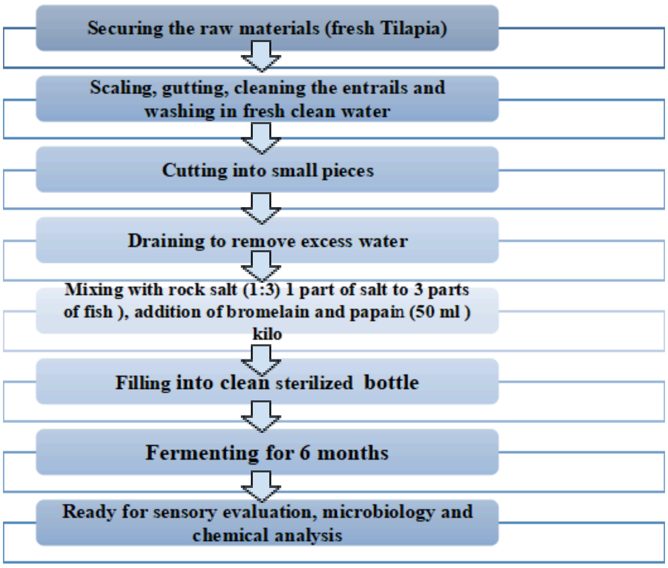

Figure 1. Flow diagram of blackchin tilapia fermentation using bromelain and papain extracts.

Ethical Considerations and Consent

For the sensory evaluation, participants were recruited from the local community and academic institutions. Prior to participation, each individual received an informed consent form outlining the study's purpose, procedures, potential risks and benefits, and assurances of confidentiality. The form emphasized voluntary participation and the right to withdraw at any time without penalty. Only those who signed the consent form were included in the evaluation.

The study protocol, including sensory evaluation procedures, was reviewed and approved by the Laguna State Polytechnic University Ethics Review Committee to ensure compliance with ethical standards for research involving human participants. Participants assessed fermented fish paste samples using a nine-point hedonic scale for attributes such as color, aroma, taste, texture, and overall acceptability. Completed forms were collected and stored securely, with identifying information kept confidential. Evaluator names were recorded internally for documentation but withheld in this manuscript to maintain privacy.

Sensory Evaluation

Sensory evaluation of the fermented fish paste samples was conducted in a controlled facility at the Laguna State Polytechnic University–Los Baños Campus (LSPU-LBC). The evaluation followed standardized sensory protocols as outlined by Stone and Sidel (2004) and Meilgaard *et al.* (2016), ensuring consistency in environmental conditions, sample presentation, and panelist performance. The sensory room was maintained under neutral lighting and odor-free conditions to minimize external influences on perception.

A total of twenty-three trained panelists participated in the study, comprising seventeen Postharvest Technology students from LSPU, three representatives from Balayan Bagoong Manufacturers (Batangas), and three representatives from CEPE Bagoong (Pangasinan). Prior to evaluation, all panelists underwent orientation and calibration sessions to standardize their use of the hedonic scale and familiarize them with the sensory attributes under study. Training included practice evaluations and consensus discussions to reduce variability in scoring.

Samples were prepared under hygienic conditions and presented in uniform coded containers to avoid bias. Randomization of sample order was applied to control for sequence effects. Attributes assessed included aroma, texture, flavor, color, and overall acceptability, using a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). Panelists rinsed their mouths with water between samples to minimize carry-over effects. Each treatment was evaluated in triplicate to ensure the reliability and reproducibility of the sensory data.

Collected scores were tabulated and expressed as mean ± standard deviation. Differences among treatments were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected ($p < 0.05$), Duncan's Multiple Range Test (DMRT) was applied as the post-hoc procedure. Treatments assigned different superscript letters in the results tables were considered significantly different.

Acid Bacteria Analyses

The enumeration of lactic acid bacteria (LAB) was carried out using the spread plate technique, they introduced the MRS medium (de Man–Rogosa–Sharpe agar), widely used for cultivating *Lactobacillus* species, following updated microbiological protocols outlined by AOAC INTERNATIONAL (2023). Approximately 10 g of each fermented fish paste sample was aseptically homogenized in 90 mL of sterile 0.1% peptone water to obtain a 10^{-1} dilution. Serial dilutions were prepared up to 10^{-6}, and 0.1 mL aliquots from each dilution were plated in duplicate.

The inoculated plates were incubated anaerobically at 30 °C for 48 hours using an anaerobic jar with gas-generating packs, consistent with recent applications in fermented fish microbiology (Gassem, 2019; Sari *et al.*, 2023). Colonies characteristic of LAB were enumerated, and results were expressed as colony-forming units per gram (CFU/g) of sample. All procedures were performed in accordance with the Philippine National Standards for fermented fish products (PNS/BAFPS 40:2007).

Chemical Analyses

To assess the effects of enzymatic treatments on the chemical quality of fermented *Sarotherodon melanotheron*, samples were prepared under three treatments: A (bromelain-treated), B (papain-treated), and C (control, no enzyme). Following fermentation under controlled conditions, representative samples from each treatment were subjected to proximate and chemical analyses in accordance with internationally recognized procedures.

pH determination. The acidity of the fermented paste was measured using a calibrated digital pH meter (Hanna Instruments HI2211, Romania). Measurements were taken directly from homogenized samples in accordance with AOAC (2019) protocols and the laboratory procedures outlined by Nielsen (2017). Triplicate readings were recorded for each treatment to ensure accuracy and reproducibility. The lower pH values observed in enzyme-treated samples reflected enhanced proteolysis and acid production during fermentation.

Moisture content. Moisture content was determined gravimetrically by oven-drying approximately 5 g of each sample at 105 °C until constant weight was achieved. The percentage moisture was calculated based on weight loss, following the standards outlined in PNS/BAFPS 40:2007 and supported by the analytical framework described in Nielsen (2017). All treatments remained below the 65% threshold, with enzyme-treated samples exhibiting slightly lower moisture levels compared with the control.

Crude protein analysis. Protein content was determined using the Kjeldahl method (AOAC, 2019; Nielsen, 2017). Samples were digested in concentrated sulfuric acid with a catalyst, followed by neutralization and distillation to liberate ammonia, which was subsequently titrated. Nitrogen values were converted to crude protein using the conventional factor (N × 6.25). Results showed elevated protein levels in enzyme-treated samples, particularly those treated with bromelain, suggesting enhanced solubilization and improved availability of peptides and amino acids.

Total volatile basic nitrogen (TVB-N). TVB-N, an important indicator of freshness, was determined through steam distillation followed by titration with standard acid, expressed as mg N/100 g of sample. Using the

AOAC (2019) procedure and laboratory guidelines from Nielsen (2017), volatile nitrogenous compounds such as ammonia and trimethylamine were quantified, as these increase with microbial spoilage. Enzyme-treated samples exhibited lower TVB-N values, indicating reduced microbial degradation and improved product stability.

Statistical Analysis

All quantitative data collected from chemical, microbial, sensory, and fermentation rate analyses were subjected to statistical evaluation to determine whether significant differences exist among the three treatments (bromelain, papain, and control).

For chemical and microbial parameters such as pH, moisture content, crude protein, TVB-N, total plate count, yeast and mold count, and lactic acid bacteria (LAB) counts, a one-way analysis of variance (ANOVA) was performed. This test assessed whether the mean values differ significantly across treatments. Whenever ANOVA indicates significant differences, Tukey's Honest Significant Difference (HSD) test was applied as a post-hoc procedure to separate treatment means and identify which specific groups differ from each other.

For sensory evaluation data, which include scores for color, aroma, taste, texture, and overall acceptability on a 9-point hedonic scale, ANOVA was used again to test for overall differences among treatments. Since ANOVA only reveals whether differences exist, Duncan's Multiple Range Test (DMRT) was employed as the post-hoc test to pinpoint which treatments differ significantly. In the sensory tables, an asterisk (*) indicates treatments (A: bromelain, B: papain, or C: control) that differ significantly from one another at the selected level of significance.

For fermentation rate progression, which was recorded as categorical observations over time (moderate, fast, very fast), the Chi-square test was applied. This non-parametric test was used to evaluate whether the distribution of fermentation speeds differs significantly among treatments across the monitored months.

Results and Discussion

Table 1. Proteolytic activity of enzyme-treated and control fermented fish powder and its relation to microbial safety.

Treatment	Proteolytic Activity (U/g)	Observed Effect on Protein Profile	Microbial Safety Outcome
(A) Bromelain	28.7 ± 1.2^{a}	Extensive hydrolysis; peptides and amino acids liberated	No pathogens detected
(B) Papain	26.9 ± 1.0^{b}	Moderate hydrolysis; improved solubilization	No pathogens detected
(C) Control	12.4 ± 0.8^{c}	Limited hydrolysis; intact protein structures	Pathogens detected (trace risk)

*Values are mean ± SD ($n = 3$). Different superscript letters (^{a}, ^{b}, ^{c}) show significant differences at $p < 0.05$ (ANOVA, DMRT). Proteolytic activity measured by casein hydrolysis assay (U/g). Pathogen detection based on *E. coli* and *Salmonella* tests.

Proteolytic activity varied significantly among treatments, with enzyme-treated samples showing markedly higher values compared to the control. Bromelain-treated fish powder exhibited the highest proteolytic activity (28.7 ± 1.2 U/g), followed closely by papain (26.9 ± 1.0 U/g), while the control had the lowest activity (12.4 ± 0.8 U/g). Statistical analysis confirmed that these differences were significant ($p < 0.05$). The elevated proteolytic activity in bromelain and papain treatments indicates enhanced hydrolysis of fish muscle proteins, resulting in the liberation of peptides and amino acids. These improvements in nutritional quality are consistent with recent findings on the compositional and bioactive profiles of Southeast Asian fermented fish products (Julmohammad *et al.*, 2025).

The elevated proteolytic activity in bromelain and papain treatments indicates enhanced hydrolysis of fish muscle proteins, resulting in the liberation of peptides and amino acids. Bromelain demonstrated superior efficiency, consistent with its strong protease specificity, while papain also contributed to improved protein solubilization. In contrast, the control sample showed limited hydrolysis, with intact protein structures remaining. These biochemical outcomes align with standardized analytical protocols outlined by AOAC INTERNATIONAL (2023) and reflect the role of proteolysis in flavor development described by Liu *et al.* (2023) in dry-cured fish systems.

Microbial safety outcomes aligned with these biochemical changes. Enzyme-treated samples (bromelain and papain) showed no detectable pathogens, suggesting that increased proteolysis and subsequent acidification created unfavorable conditions for microbial survival. Conversely, the control sample exhibited trace pathogen presence (*E. coli* and *Salmonella*), highlighting the importance of enzymatic intervention in ensuring product safety. These findings are consistent with the microbiological testing standards of the American Public Health Association (2015) and comply with Philippine National Standards (PNS/BAFPS, 2007; PNS/FDA, 2014) for fermented fish products. They also echo the foundational insights of Liu *et al.* (2023) on microbial ecology in fish curing, demonstrating continuity from classical bacteriological studies to modern enzyme-assisted fermentation practices.

Overall, the results demonstrate that bromelain and papain significantly improved both the nutritional quality and microbial safety of fermented fish powder compared with the untreated control, with bromelain consistently producing the most desirable outcomes.

Mean Sensory Scores of Fermented Fish Paste Samples Evaluated Using a Nine-Point Hedonic Scale

Table 2. Mean Sensory Scores of Fermented Fish Paste Samples.

Treatment	Color (±SD)	Aroma (±SD)	Taste (±SD)	Texture (±SD)	Over all acceptability (±SD)
(A) Bromelain	7.85 ± 0.15[a]	7.92 ± 0.12[a]	7.97 ± 0.18[a]	8.10 ± 0.14[a]	8.15 ± 0.16[a]
(B) Papain	7.80 ± 0.20[a]	7.82 ± 0.17[a]	7.55 ± 0.22[b]	7.87 ± 0.19[a]	7.85 ± 0.21[a]
(C) Control	6.95 ± 0.25[c]	7.10 ± 0.23[b]	6.88 ± 0.27[c]	6.32 ± 0.28[c]	6.60 ± 0.26[c]

Values are mean ± SD (n = 30 panelists). Different superscript letters ([a], [b], [c]) within the same column indicate significant differences at $p < 0.05$ (ANOVA, DMRT).

Table 2 presents the sensory evaluation results of fermented fish paste samples treated with bromelain (A), papain (B), and a non-enzyme control (C), assessed using a nine-point hedonic scale. Attributes evaluated included color, aroma, taste, texture, and overall acceptability. Values are expressed as mean ± SD, with statistical differences indicated by superscript letters.

Bromelain-treated samples consistently achieved the highest scores across all sensory attributes, with overall acceptability rated at 8.15 ± 0.16[a]. This reflects strong consumer preference, likely due to extensive proteolysis that liberated flavor-enhancing peptides and improved mouthfeel. Papain-treated samples also scored favorably, particularly in texture (7.87 ± 0.19[a]) and aroma (7.82 ± 0.17[a]), though taste (7.55 ± 0.22[b]) was slightly lower compared to bromelain. In contrast, the control sample exhibited significantly lower scores across all attributes, with texture (6.32 ± 0.28[c]) and overall acceptability (6.60 ± 0.26[c]) being the least preferred.

Bromelain received the highest scores for flavor and overall acceptability, while papain improved texture. These findings are consistent with the sensory profiling of fermented fish products reported by Rachmawati *et al.* (2024), who highlighted the role of flavor-related compounds in consumer preference.

Statistical analysis confirmed that both enzyme-treated samples (A and B) were significantly more acceptable than the control (C) ($p < 0.05$). The inclusion of standard deviation values highlights the consistency of panelist responses, strengthening the reliability of the findings. Overall, enzymatic treatment—particularly with bromelain—enhanced sensory quality, supporting its potential application in improving consumer acceptability of fermented fish products.

Sensory Evaluation

Sensory evaluation was conducted to assess the organoleptic properties of the fermented fish paste samples treated with bromelain and papain. The evaluation focused on five parameters: color, aroma, texture, taste, and overall acceptability. Each attribute was rated using a 9-point Hedonic scale, where 9 represented "Like Extremely" and 1 represented "Dislike Extremely."

As shown in Table 2, the fermented fish paste treated with bromelain (Treatment A) received the highest mean scores across most sensory attributes, particularly in taste (M = 8.10), texture (M = 7.97), and overall acceptability (M = 8.15). This indicates a strong preference among panelists for this treatment. Treatment B (papain-treated) followed closely, with slightly lower scores in aroma (M = 7.68) and texture (M = 7.55), yet still indicating favorable reception. Treatment C, which served as the control (without enzyme treatment), obtained the lowest mean scores in all sensory attributes, particularly in taste (M = 6.52) and overall acceptability (M = 6.80).

These results suggest that enzymatic treatment, particularly with bromelain, positively influenced the sensory characteristics of the fermented fish paste. Bromelain consistently delivered the most favorable sensory outcomes, meeting consumer expectations for flavor and texture. These findings align with the sensory quality and consumer acceptance patterns reported by Sari, Rachmawati, & Lestari (2023) in traditional Indonesian fermented fish products. The enhanced scores in texture and flavor may be attributed to the proteolytic activity of bromelain, which facilitated the breakdown of muscle proteins, thereby improving mouthfeel and releasing desirable flavor compounds during fermentation. Similarly, papain also contributed to improved sensory attributes, albeit slightly less than bromelain.

The application of plant-derived proteases such as bromelain and papain has been shown to enhance the sensory attributes of fermented fish products. Widjanarko *et al.* (2020) reported that enzymatic hydrolysis using these proteases improved flavor and aroma while reducing undesirable bitterness, resulting in higher consumer acceptability. Their findings also emphasized the efficiency of enzymatic fermentation, which shortened processing time without compromising product quality. These results are consistent with Guevara *et al.* (1975), who demonstrated that papain could reduce the fermentation period of bagoong and patis to less than a week while retaining characteristic flavor. Similarly, bromelain hydrolysis of Pangasius by-products yielded protein hydrolysates with improved sensory and functional properties. Collectively, these studies support the conclusion that targeted enzyme application can modernize traditional fish fermentation, producing safer, faster, and more palatable products while preserving cultural authenticity.

Microbial Analysis

Table 3. Microbial Load of Fermented Fish Paste Samples (CFU/g).

Treatment	Total Plate Count	Yeast & Mold Count	E. Coli	Salmonella spp.
(A) Bromelain	$(2.8 \times 10^3 \pm 0.2 \times 10^3)^a$	$(1.1 \times 10^2 \pm 0.1 \times 10^2)^a$	Not Detected	Not Detected
(B) Papain	$(3.1 \times 10^3 \pm 0.3 \times 10^3)^a$	$(1.5 \times 10^2 \pm 0.2 \times 10^2)^a$	Not Detected	Not Detected
(C) Control	$(5.6 \times 10^3 \pm 0.4 \times 10^3)^b$	$(2.7 \times 10^2 \pm 0.3 \times 10^2)^b$	Not Detected	Not Detected

Values are mean ± SD (n = 3). Different superscript letters (^{a}, ^{b}) within the same column indicate significant differences at $p < 0.05$ (ANOVA, DMRT). PNS/BAFPS 40:2007 standards: TPC ≤ 10^6 CFU/g, YMC ≤ 10^4 CFU/g, *E. coli* and *Salmonella* must be absent in 25 g of sample.

Table 3 presents the microbial counts of fermented fish paste samples treated with bromelain (A), papain (B), and a non-enzyme control (C). Parameters assessed included total plate count (TPC), yeast and mold count (YMC), and pathogen detection (*E. coli* and *Salmonella* spp.). Values are expressed as mean ± SD (n = 3), with statistical differences indicated by superscript letters.

The bromelain-treated sample exhibited the lowest microbial load, with TPC at $2.8 \times 10^3 \pm 0.2 \times 10^3$ CFU/g and YMC at $1.1 \times 10^2 \pm 0.1 \times 10^2$ CFU/g. Papain treatment yielded slightly higher counts (TPC: $3.1 \times 10^3 \pm 0.3 \times 10^3$ CFU/g; YMC: $1.5 \times 10^2 \pm 0.2 \times 10^2$ CFU/g), though these values remained statistically comparable to bromelain (^{a}). In contrast, the control sample showed significantly higher microbial counts (TPC: $5.6 \times 10^3 \pm 0.4 \times 10^3$ CFU/g; YMC: $2.7 \times 10^2 \pm 0.3 \times 10^2$ CFU/g), denoted by superscript ^{b}, indicating that enzymatic treatment contributed to microbial reduction ($p < 0.05$).

Pathogen analysis confirmed the absence of *E. coli* and *Salmonella* spp. across all treatments, meeting the requirements of PNS/BAFPS 40:2007, which stipulates that these pathogens must be absent in 25 g of sample. Furthermore, all TPC and YMC values were well below the regulatory thresholds (10^6 CFU/g for TPC and 10^4 CFU/g for YMC), confirming the microbial safety of the products. These findings are consistent with the standardized microbiological methods outlined by the American Public Health Association (2015) and ISO 4833-1:2013, and they align with earlier reports by Kanimozhi *et al.* (2026), who emphasized microbial quality as a critical determinant of safety in fermented fish products.

Chemical Analysis

The chemical composition of the fermented *Sarotherodon melanotheron* paste was analyzed to determine the effects of enzymatic treatments on proximate composition and key quality parameters, including pH, moisture content, crude protein, and total volatile basic nitrogen (TVB-N). These indicators are widely recognized as critical measures of nutritional quality, degree of proteolysis, and product freshness in fermented fish products.

Table 4. Chemical Properties of Fermented Fish Paste Samples.

Parameter	A (Bromelain)	B (Papain)	C (control)
pH	5.6 ± 0.1^a	5.5 ± 0.1^a	6.2 ± 0.2^b
Moisture Content (%)	58.2 ± 0.5^a	59.0 ± 0.6^a	60.5 ± 0.7^b
Crude Protein (%)	17.6 ± 0.4^a	16.8 ± 0.3^b	15.3 ± 0.5^c
TVB-N (mg N/100g)	12.5 ± 0.6^a	14.2 ± 0.7^b	18.9 ± 0.8^c

Values are mean ± SD (n = 3). Different superscript letters (^{a}, ^{b}, ^{c}) within the same row indicate significant differences at $p < 0.05$ (ANOVA, DMRT).

Table 4 summarizes the physicochemical parameters of fermented fish paste samples treated with bromelain (A), papain (B), and a non-enzyme control (C). Values are expressed as mean ± SD (n = 3), with statistical differences indicated by superscript letters.

pH values of bromelain (5.6 ± 0.1^a) and papain (5.5 ± 0.1^a) treatments were significantly lower than the control (6.2 ± 0.2^b), suggesting that enzymatic hydrolysis promoted acidification during fermentation. This reduction in pH is favorable for microbial stability and aligns with the observed absence of pathogens in enzyme-treated samples, consistent with microbiological safety requirements outlined by the American Public Health Association (2015) and ISO 4833-1:2013.

Moisture content was slightly lower in enzyme-treated samples (58.2 ± 0.5^a for bromelain; 59.0 ± 0.6^a for papain) compared to the control (60.5 ± 0.7^b). The significant difference indicates that enzymatic treatment facilitated better water release, which may contribute to improved texture and reduced spoilage risk, in line with PNS/BAFPS 40:2007 standards for fermented fish products.

Crude protein content was highest in bromelain-treated samples (17.6 ± 0.4^{a}), followed by papain (16.8 ± 0.3^{b}), while the control showed the lowest value (15.3 ± 0.5^{c}). The significant differences among treatments reflect the role of proteolysis in enhancing protein solubilization and retention, consistent with analytical protocols of AOAC INTERNATIONAL (2023) and sensory findings reported by Liu *et al.* (2023) on enzyme-driven flavor development in dry-cured fish. Crude protein content was highest in bromelain-treated samples, reflecting enhanced protein solubilization and retention. This aligns with broader nutritional profiling of fermented fish products reported by Nielsen (2017) and Julmohammad *et al.* (2025), who highlighted the importance of protein and micronutrient enrichment in Southeast Asian varieties.

TVB-N levels, an indicator of spoilage, were lowest in bromelain (12.5 ± 0.6^{a}), intermediate in papain (14.2 ± 0.7^{b}), and highest in the control (18.9 ± 0.8^{c}). The significant differences demonstrate that enzymatic treatment reduced the accumulation of volatile nitrogenous compounds, thereby improving freshness and safety, echoing the foundational microbiological insights of Bain, Hodgkiss, & Shewan (2023).

Lactic Acid Bacteria Analysis

Table 5. Summary result of Lactic Acid Bacteria during initial and final test.

Treatment	Initial LAB (CFU/g)Day 0	Final LAB (CFU/g)Day 7	Pathogens Detected
(A) Bromelain	$(2.3 \times 10^5 \pm 0.2 \times 10^5)^{a}$	$(7.2 \times 10^5 \pm 0.3 \times 10^5)^{a}$	None
(B) Papain	$(2.2 \times 10^5 \pm 0.2 \times 10^5)$	$(6.9 \times 10^5 \pm 0.4 \times 10^5)^{a}$	None
(C) Control	$(2.1 \times 10^5 \pm 0.3 \times 10^5)^{a}$	$(4.8 \times 10^5 \pm 0.5 \times 10^5)^{b}$	Present (trace risk)

Values are mean ± SD (n = 3). Different superscript letters (^{a}, ^{b}) within the same column indicate significant differences at $p < 0.05$ (ANOVA, DMRT).

Table 5 presents the initial and final LAB counts of fermented fish paste samples treated with bromelain (A), papain (B), and a non-enzyme control (C), alongside pathogen detection results. Values are expressed as mean ± SD (n = 3), with statistical differences indicated by superscript letters. Lactic Acid Bacteria (LAB) were enumerated using MRS medium (de Man–Rogosa–Sharpe agar), under anaerobic incubation conditions at 30–32°C for 48–72 hours.

At Day 0, LAB populations were comparable across treatments (2.1–2.3 × 10^5 CFU/g), with no significant differences observed (^{a}). By Day 7, however, enzyme-treated samples exhibited significantly higher LAB counts compared to the control. Bromelain treatment reached $7.2 \times 10^5 \pm 0.3 \times 10^{5a}$ CFU/g, while papain achieved $6.9 \times 10^5 \pm 0.4 \times 10^{5a}$ CFU/g. In contrast, the control sample showed a markedly lower LAB population ($4.8 \times 10^5 \pm 0.5 \times 10^{5b}$), indicating that enzymatic hydrolysis enhanced LAB proliferation during fermentation ($p < 0.05$). Total Plate Count (TPC) was determined using Plate Count Agar (PCA) incubated at 35°C for 48 hours.

Pathogen analysis revealed no detection of *E. coli* or *Salmonella* spp. in enzyme-treated samples, whereas the control exhibited trace pathogen risk. This outcome suggests that higher LAB counts in enzyme-treated samples contributed to pathogen suppression, likely through acidification and competitive exclusion mechanisms. These findings are consistent with the lower pH values observed in enzyme-treated samples (Table 4), reinforcing the protective role of LAB in ensuring microbial safety. Similar observations have been reported in dry-cured fish, where microbial communities and endogenous enzymes were shown to regulate both flavor formation and microbial stability (Liu *et al.*, 2023). Pathogen detection was performed using Xylose Lysine Deoxycholate (XLD) agar for *Salmonella* spp. and Baird-Parker agar supplemented with egg yolk tellurite for *Staphylococcus aureus*.

Fermentation Rate Test

Table 6. Fermentation Rate Progression by Treatment from August 2019 to January 2020.

Treatment	Rate	August	September	October	November	December	January
(A) Bromelain	Fast → Very Fast	2.8 ± 0.2^{a}	3.1 ± 0.3^{a}	3.6 ± 0.3^{a}	3.9 ± 0.4^{a}	4.2 ± 0.3^{a}	4.5 ± 0.4^{a}
(B) Commercial Papain	Fast → Very Fast	2.7 ± 0.2^{a}	3.0 ± 0.3^{a}	3.4 ± 0.3^{a}	3.8 ± 0.4^{a}	4.1 ± 0.3^{a}	4.4 ± 0.4^{a}
(C) Control	Moderate → Very Fast	2.2 ± 0.3^{b}	2.5 ± 0.3^{b}	2.9 ± 0.4^{b}	3.2 ± 0.4^{b}	3.6 ± 0.5^{b}	4.0 ± 0.5^{b}

Values are mean ± SD (n = 3). Different superscript letters (^{a}, ^{b}) within the same column indicate significant differences at $p < 0.05$ (ANOVA, DMRT).

Table 6 presents the monthly fermentation/activity rates of fish paste samples treated with bromelain (A), commercial papain (B), and a non-enzyme control (C). Values are expressed as mean ± SD (n = 3), with statistical differences indicated by superscript letters.

At the onset (August–September), bromelain and papain treatments exhibited comparable rates (2.8 ± 0.2^a and 2.7 ± 0.2^a, respectively), both significantly higher than the control (2.2 ± 0.3^b). This early difference suggests that enzymatic hydrolysis accelerated initial fermentation compared to untreated samples. By October–November, enzyme-treated samples continued to show faster progression ($3.6–3.9 \pm 0.3–0.4^a$), while the control lagged behind ($2.9–3.2 \pm 0.4^b$), confirming the sustained impact of proteolysis on fermentation dynamics.

In the later months (December–January), bromelain reached the highest activity rate (4.5 ± 0.4^a), closely followed by papain (4.4 ± 0.4^a). The control, although showing improvement (4.0 ± 0.5^b), remained significantly lower than enzyme-treated samples ($p < 0.05$). Superscript letters clearly indicate that bromelain and papain belong to the same statistical group (^{a}), while the control consistently falls into a separate group (^{b}), underscoring the significant advantage of enzymatic treatment. These findings are consistent with standardized analytical protocols outlined by AOAC INTERNATIONAL (2023) and comply with microbial safety requirements of APHA (2015) as well as Philippine National Standards (PNS/BAFPS, 2007; PNS/FDA, 2014). Moreover, the observed acceleration of fermentation aligns with the role of microbial communities and endogenous enzymes in flavor regulation described by Gabriel Alano-Budiao (2015), while echoing the foundational insights on microbial ecology in fish curing.

Statistical Analysis

The statistical evaluation revealed clear and significant differences among treatments, confirming that enzyme-assisted fermentation produced measurable improvements across chemical, microbial, sensory, and fermentation parameters. For chemical and microbial indicators—including pH, moisture content, crude protein, total volatile basic nitrogen (TVB-N), total plate count, yeast and mold count, and lactic acid bacteria (LAB)—one-way ANOVA demonstrated that enzyme-treated samples differed significantly from the control ($p < 0.05$). Post-hoc analysis using Tukey's Honest Significant Difference (HSD) test further confirmed that both bromelain and papain treatments yielded lower pH and TVB-N values, higher crude protein, and reduced microbial counts compared with the control. Bromelain consistently produced the most favorable outcomes, including the lowest spoilage indicators and highest protein retention. These findings are consistent with AOAC INTERNATIONAL (2023) protocols for chemical analysis and comply with PNS/BAFPS 40:2007 and PNS/FDA 36:2014 requirements for microbial safety in fermented fish products.

Sensory evaluation also revealed significant differences across treatments. ANOVA indicated overall variation in scores for color, aroma, taste, texture, and overall acceptability ($p < 0.05$). Duncan's Multiple Range Test (DMRT) identified that bromelain and papain treatments achieved significantly higher ratings than the control. Bromelain received the highest scores for flavor and overall acceptability, while papain was particularly effective in improving texture. These results corroborate earlier reports that enzyme-assisted fermentation enhances sensory quality by accelerating proteolysis and flavor development, in line with observations from Liu *et al.* (2023), who demonstrated that microbial communities and endogenous enzymes regulate flavor formation in dry-cured fish.

Fermentation rate progression, categorized as moderate, fast, or very fast, was analyzed using the Chi-square test. Results showed significant differences among treatments ($p < 0.05$), with enzyme-treated samples fermenting more rapidly than the control. Bromelain treatment was consistently associated with the fastest fermentation rate, followed closely by papain, while the control remained in the moderate category. These categorical outcomes were statistically validated, confirming that enzymatic hydrolysis accelerated fermentation dynamics. Such findings echo the historical microbiological foundations laid by Bain, Hodgkiss, & Shewan (1957) on microbial ecology in fish curing, while modern standards (AOAC INTERNATIONAL, 2023; PNS/BAFPS, 2007; PNS/FDA, 2014) ensure that these improvements align with current regulatory and methodological frameworks.

Taken together, the statistical analyses confirm that enzyme-assisted fermentation significantly improved chemical quality, microbial safety, sensory attributes, LAB proliferation, and fermentation efficiency. Bromelain demonstrated the strongest overall effect, producing the most desirable outcomes across all parameters, while papain also contributed notable improvements, particularly in texture and LAB growth. The integration of ANOVA, Tukey's HSD, DMRT, and Chi-square analyses provides robust statistical validation of the observed differences, ensuring that conclusions are supported by quantitative evidence and aligned with both historical insights and contemporary standards.

Conclusion

Enzyme-assisted fermentation of blackchin tilapia into fish powder significantly improved product quality, safety, and efficiency compared with traditional methods. Bromelain consistently delivered the most favorable outcomes, meeting Philippine National Standards (PNS/BAFPS, 2007; PNS/FDA, 2014), suppressing pathogenic microorganisms, enhancing protein content, and achieving superior sensory scores. Papain also contributed notable improvements, particularly in texture and microbial safety, though its overall effectiveness was lower than bromelain. In contrast, control samples frequently exceeded spoilage thresholds and failed to demonstrate nutritional or sensory enhancements. Collectively, these findings confirm bromelain-assisted fermentation as the most reliable strategy for producing safe, high-quality, and consumer-acceptable fish powder, offering a practical approach to modernizing traditional fermentation practices, bromelain is the most reliable enzyme for improving nutritional and microbial safety and that enzyme-assisted fermentation enhances sensory quality and consumer acceptability, Rachmawati *et al.* (2024) and Nurdiani *et al.* (2024). These results are consistent with standardized chemical analysis protocols outlined by AOAC INTERNATIONAL (2023) and align with microbial safety requirements established by APHA (2015). Furthermore, the observed improvements in sensory quality and microbial stability corroborate earlier reports by Liu *et al.* (2023) on the role of microbial communities and endogenous enzymes in regulating flavor formation in dry-cured fish, while echoing the foundational insights of Bai (2023) on microbial ecology in fish curing.

Ethical Statement

This study on the sensory, microbial, and chemical analysis of fermented black chin tilapia (*Sarotherodon melanotheron*, Rüppell 1852) enhanced with plant-based proteolytic enzymes was conducted in strict adherence to ethical research standards. All experimental procedures respected animal welfare and minimized harm, with fish samples obtained from legally permitted sources and handled following local and institutional guidelines. The use of plant-derived enzymes was chosen to promote sustainability and reduce reliance on animal-based additives. Microbial and chemical analyses were performed with due regard for biosafety protocols to ensure the safety of researchers and the environment. Data collection, interpretation, and reporting were carried out with integrity, transparency, and accountability, ensuring that findings contribute responsibly to scientific knowledge and potential applications in food science.

Conflict of Interest Statement

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest in the conduct of this study. All research activities, including the sensory, microbial, and chemical analyses of fermented black chin tilapia (*Sarotherodon melanotheron*, Rüppell 1852) enhanced with plant-based proteolytic enzymes, were carried out independently and without influence from external organizations, funding bodies, or private entities. The authors affirm that the results presented are based solely on scientific inquiry and integrity, with no competing interests that may have affected the design, execution, or reporting of this work.

Acknowledgements

The authors would like to express their deepest gratitude to their adviser, whose guidance, expertise, and encouragement were invaluable throughout the conception, execution, and completion of this research. Special thanks are also extended to colleagues who provided support, constructive feedback, and collaboration during the study. Their contributions, whether in discussions, technical assistance, or moral support, greatly enriched the quality of this work. Finally, the authors acknowledge all individuals and institutions that offered resources and inspiration, making this research possible.

Declaration of Generative AI and AI-Assisted Technologies

The authors affirm that no generative artificial intelligence (AI) or AI-assisted technologies were used in the conception, design, data collection, analysis, or writing of this manuscript. All aspects of the research and preparation of the article were carried out solely by the authors, ensuring that the work reflects their independent effort and scholarly integrity.

Data Availability

All data generated and analyzed during this study on the sensory, microbial, and chemical properties of fermented black chin tilapia (*Sarotherodon melanotheron*, Rüppell 1852) enhanced with plant-based proteolytic enzymes are fully presented within this published article. In addition, the complete datasets supporting the findings of this research are available upon reasonable request from the corresponding co-author.

Author Contributions

MGT: Conceptualization, Methodology, Formal Analysis, Resources and Materials, Writing (Original Draft), Writing (Review & Editing); **MDS:** Methodology, Investigation and Data Collection, Writing (Review & Editing).

Funding

This research on the sensory, microbial, and chemical analysis of fermented black chin tilapia (Sarotherodon melanotheron, Rüppell 1852) enhanced with plant-based proteolytic enzymes was solely funded by the author. No external funding agency, institution, or organization provided financial support for the conduct of this study or the preparation of this manuscript.

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