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Optimising Non-Thermal Acid Maturation of Giant Gourami (*Osphronemus gouramy*) as a Novel Food Processing Technology

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ABSTRACT

Purpose of the study: This study aimed to develop and optimise a technologically relevant non-thermal acid-induced maturation process for giant gourami by identifying effective citrus acid formulations and immersion durations that enable controlled myofibrillar protein denaturation for scientifically standardised and traditional Dekke Naniura processing..

Methodology: A laboratory-based semi-controlled experimental design was applied using fresh gourami fillets immersed in organic acids derived from lime (*Citrus aurantiifolia*) and lemon (*Citrus limon*) juices at different ratios and immersion times. Maturation progression was evaluated using a semi-quantitative visual index (0–100%) supported by triplicate mean ± SD measurements. Observations included colour transformation, flesh firmness, and macroscopic protein denaturation, while microscopic assessment and mechanical texture analysis validated structural myofibrillar changes. Statistical analysis using one-way ANOVA and Tukey's HSD determined significant treatment differences.

Main Findings: The optimal condition was a formulation containing 75% lime juice and 25% lemon juice with an immersion duration of 1,140 minutes, achieving 96% maturation. This treatment produced homogeneous myofibrillar protein denaturation, uniform colour transformation, and increased flesh firmness under non-thermal conditions. Single-acid treatments showed slower and less uniform maturation, indicating a synergistic effect of mixed citrus acids. The dense muscle fibre structure and high myofibrillar protein content of gourami contributed to enhanced structural stability during maturation. Statistical analyses confirmed significant differences among treatments at most immersion times.

Novelty/Originality of this study: This study proposes a reproducible and chemically optimised non-thermal maturation model integrating semi-quantitative, microscopic, and mechanical analyses, providing a scientifically validated framework for modern adaptation of traditional Batak naniura processing.

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1. INTRODUCTION

Dekke Naniura is a traditional culinary heritage of the Batak Toba community in North Sumatra, distinguished by its non-thermal preparation using natural organic acids derived from local citrus fruits to mature freshwater fish rather than heat as applied in most Indonesian fish dishes. This processing method imparts a distinctive texture and flavour while relying on scientifically explainable biochemical mechanisms, particularly protein denaturation resulting from the penetration of hydrogen ions from organic acids, which alter muscle structure to resemble conventional cooking [1], [2]. Culturally, every stage of preparation, from selecting the fish to its presentation, embodies collective identity, kinship relations, and spiritual values, positioning Dekke Naniura as both a cultural text and a medium of social communication within Batak Toba society [3]. Moreover, its unique preparation method highlights potential within the creative economy, where the authenticity of traditional techniques may attract tourism and expand Batak culinary markets, while simultaneously requiring attention to quality standardisation, food safety, and business sustainability [4], [5]. Beyond its cultural significance, this traditional preparation method also represents a potential model of non-thermal fish processing technology based on organic acids, which may be scientifically optimised and standardised to ensure consistent product quality and safety [6]-[8]. Thus, Dekke Naniura may be understood as a multidimensional phenomenon integrating ethnoscience, food biochemistry, cultural identity, and opportunities for local economic development rooted in Indonesia's culinary traditions.

Dekke Naniura exemplifies non-thermal, acid-induced protein denaturation in fish, where immersion in organic acids from local citrus fruits lowers pH, protonates amino acid residues, and disrupts hydrogen bonds, ionic interactions, and hydrophobic packing, producing a texture resembling cooked fish while simultaneously inhibiting microbial growth. Studies show that both acid concentration and immersion time critically modulate bacterial reduction, with lime juice achieving the lowest microbial counts and pH [9]. This process not only transforms muscle structure and flavor but also integrates traditional Batak Toba knowledge with molecular acid-base principles, offering a reproducible and scientifically grounded model for non-thermal fish processing [10]–[13].

The use of organic acids as food processing agents has long been recognised across cultures worldwide. In Latin America, ceviche employs citrus-based marination to mature fish without heat, a process that closely parallels Dekke Naniura among the Batak Toba community [14]. Scientifically, organic acids are known to lower pH to levels that induce protein denaturation, producing firmer textures and an appearance resembling cooked fish, while simultaneously inhibiting pathogenic microorganisms [15]-[17]. Other studies confirm that acid marination of fresh fish not only enhances food safety by suppressing bacteria such as Listeria monocytogenes but also contributes to the development of distinctive flavours rarely achieved through thermal cooking [18]. Recent research further emphasises the ethnographic dimension of ceviche as cultural heritage, intertwining culinary identity with traditional knowledge in Latin American coastal societies [19]. From a technological perspective, acid-based marination has increasingly been recognised as a form of non-thermal food processing that offers advantages in terms of energy efficiency, preservation of nutritional quality, and reduced thermal degradation of food components. Accordingly, acid-based processing methods represent more than traditional culinary practices, offering a scientific perspective on sustainable, safe, and ethnoscience-driven non-thermal food processing approaches that are equally relevant to understanding the biochemical mechanisms underlying Dekke Naniura in Indonesia [20]-[22].

Nevertheless, scientific investigations on Dekke Naniura remain remarkably limited and predominantly descriptive, addressing mainly its culinary practices and cultural significance [23], [24]. Systematic studies elucidating its biochemical properties, textural transformations, and microbiological safety are virtually nonexistent, resulting in a nascent understanding of acid-induced fish maturation. While prior research on acid-marinated fish has primarily examined sensory attributes, general biochemical reactions, or cultural contexts, few studies have rigorously optimised organic acid formulations or defined precise processing parameters to achieve reproducible and controlled maturation outcomes. Moreover, the intersection of Batak Toba ethnoscientific knowledge particularly the community's indigenous utilisation of citrus with mechanistic principles of protein denaturation remains largely unexplored, representing a critical lacuna in both ethnoscience and applied food technology [24]. Consequently, the translational potential of Dekke Naniura as a model for non-thermal fish processing technology, contextual chemistry education, and innovation in tradition-based food systems has yet to be systematically realised.

Addressing this gap is imperative not only for advancing mechanistic understanding of acid-induced protein denaturation in fish muscle but also for establishing scientifically robust processing methods that uphold

food safety, enable quality standardisation, and sustainably leverage indigenous knowledge. Accordingly, the present study aims to optimise non-thermal acid-induced maturation in Dekke Naniura by identifying optimal citrus acid formulations and immersion durations that facilitate controlled denaturation of myofibrillar proteins.

The novelty of this research is articulated in two interrelated dimensions. First, it transcends descriptive documentation of acid-induced biochemical mechanisms by implementing a systematic optimisation of citrus solution compositions, specifically lemon and lime, to achieve consistent, reproducible, and standardisable maturation outcomes. This methodological refinement provides a scientific foundation for process control in a preparation method historically reliant on empirical knowledge. Second, the study introduces a strategic substitution of the primary raw material, replacing common carp *(Cyprinus carpio)* with giant gourami *(Osphronemus gouramy)*, a species characterised by thicker flesh, coarser muscle fibres, and higher protein content. This substitution enables evaluation of species-specific influences on texture development and protein denaturation efficiency, offering a novel perspective on raw material variability in non-thermal fish processing. Collectively, these innovations position the study at the forefront of non-thermal processing research, integrating ethnoscientific insights, biochemical optimisation, and material innovation, with broad implications for sustainable food technology, culinary heritage preservation, and contextualised science education.

Thus, this study occupies a state-of-the-art position by integrating chemical optimisation, raw material diversification, and ethnoscientific interpretation within a comprehensive analytical framework. The findings are expected to provide theoretical contributions to food science and ethnoscience while offering practical implications in the form of quality standard development, nutritional enhancement, and the advancement of non-thermal fish processing technology derived from traditional culinary knowledge with potential global competitiveness..

2. RESEARCH METHOD

2.1. Materials

Fresh giant gourami *(Osphronemus gouramy)* was used as the primary raw material. The fish were obtained from a local freshwater fish supplier and used within 24 hours post-harvest to ensure freshness. Prior to experimentation, the fish were transported to the laboratory in insulated containers with ice and stored at approximately 4 °C. Only the dorsal muscle fillet was used in this study, after removing the skin and bones, to ensure consistency of muscle structure across samples. Each experimental sample consisted of 10 g of fish fillet. A laboratory-based semi-controlled experimental design was employed to evaluate the effects of citrus acid formulation and immersion duration on the maturation characteristics of gourami fillets. The independent variables consisted of the ratio of lime and lemon juice formulations and immersion time, while the dependent variables included the degree of maturation, colour transformation, flesh firmness, and myofibrillar structural changes.

The acid sources were derived from lemon *(Citrus limon)* and lime *(Citrus aurantiifolia)*, both known to contain high concentrations of citric acid ($C_6H_8O_7$), along with minor organic acids such as malic acid ($C_4H_6O_5$) and ascorbic acid ($C_6H_8O_6$). Fresh fruits were washed with distilled water, cut into halves, and manually squeezed using a sterile hand press. The extracted juice was subsequently filtered through sterile muslin cloth to remove pulp and seeds. The citrus juice was used immediately after extraction to minimise oxidation and potential changes in acidity. To improve experimental consistency, the pH of each citrus formulation was measured using a calibrated digital pH meter prior to immersion treatment. All preparation procedures were conducted under hygienic laboratory conditions using sterilised equipment to minimise microbial contamination and ensure reproducibility of the maturation process.

2.2. Method

Preparation of Dekke Naniura Samples

Three independent experimental sets were prepared, each consisting of 10 g of freshly prepared gourami (*Osphronemus goramy*) dorsal fillet placed in sterile Petri dishes. Acid immersion treatments were systematically applied using three different formulations: (i) 10 mL of lemon juice, (ii) 10 mL of lime juice, and (iii) a mixed solution comprising 7.5 mL lime juice and 2.5 mL lemon juice [24], [25]. The experimental treatments were designed to compare the effectiveness of single-acid and mixed-acid formulations in promoting non-thermal maturation of fish muscle tissue over different immersion durations.

The pH of each acid solution was measured using a calibrated digital pH meter (±0.01 accuracy) to ensure high-precision quantification under strongly acidic conditions. Instrument calibration was conducted prior to measurement using standard buffer solutions at pH 4.00 and pH 7.00. To enhance measurement reliability, pH readings were cross-validated using universal indicator strips [26], [27].

The recorded pH values ranged from 2.0–2.5 for lemon juice, 2.0–2.3 for lime juice, and 2.1–2.4 for the mixed solution, confirming that all treatments were within a strongly acidic range sufficient to induce protein denaturation in fish muscle tissue [28], [29]. The low pH conditions facilitated acid-induced denaturation of

myofibrillar proteins, which was expected to alter muscle texture, colour, and structural integrity during maturation.

Following acid treatment, all samples were incubated under controlled laboratory conditions at 25 ± 1 °C. Observations and measurements were conducted at predetermined time intervals (0, 15, and 30 minutes; 2, 16, and 19 hours). All procedures were performed in triplicate (n = 3), and identical handling protocols were applied across all treatments to ensure consistency and reproducibility. The independent variables in this study were citrus acid formulation and immersion duration, whereas the dependent variables included maturation percentage, colour transformation, flesh firmness, pH stability, and microscopic structural changes in muscle fibres. Objective measurements were further supported by microscopic observation and mechanical texture assessment to reduce subjectivity associated with visual evaluation. To provide a clearer and standardised representation of the experimental design, the preparation procedures are summarised in Table 1.

Tabel 1. Standardised Experimental Design for Acid Immersion Treatment

Component	Description	Scientific Justification
Sample material	10 g gourami (*Osphronemus goramy*) dorsal fillet per treatment	Ensures consistency across treatments
Experimental design	Three independent experimental sets using a laboratory-based semi-controlled experimental approach	Improves experimental reproducibility and treatment comparability
Independent variables	Citrus acid formulation and immersion duration	Allows evaluation of treatment effects on maturation characteristics
Dependent variables	Maturation percentage, colour transformation, flesh firmness, pH stability, and myofibrillar structural changes	Provides measurable indicators of acid-induced maturation
Acid treatments	Lemon (10 mL), lime (10 mL), mixed (7.5 mL lime + 2.5 mL lemon)	Allows comparative evaluation
pH measurement	Calibrated digital pH meter (±0.01 accuracy)	Objective and precise measurement
Calibration	Buffer solutions pH 4.00 and 7.00	Ensures measurement validity
Validation	Universal indicator strips	Increases reliability
pH range	2.0–2.5 (lemon), 2.0–2.3 (lime), 2.1–2.4 (mixed)	Confirms strong acidity
Incubation	25 ± 1 °C	Controls environmental variability
Observation intervals	0, 15, 30 min; 2, 16, 19 h	Ensures temporal consistency
Replication	Triplicate (n = 3)	Supports statistical reliability
Objective assessments	Microscopic observation and mechanical texture evaluation	Reduces subjectivity associated with visual observation
Statistical analysis	Shapiro–Wilk normality test, Levene's homogeneity test, one-way ANOVA, Tukey's HSD, and Kruskal–Wallis validation test	Strengthens statistical validity and confirms treatment differences
Protocol control	Identical handling procedures	Minimises bias

Evaluation of the Maturation Process

The degree of fish maturation was evaluated using a semi-quantitative, instrument-supported approach integrating macroscopic observation with standardised measurement criteria. Two primary indicators were assessed: colour transformation and textural firmness of the fish fillet. To improve objectivity, macroscopic observations were complemented with instrumental texture analysis using a handheld penetrometer/texture analyser, and digital image documentation for colour consistency assessment.

Colour changes were evaluated using a standardised visual reference scale under controlled lighting conditions to ensure consistency across observations. In addition, colour intensity was quantified using digital image analysis (RGB-based evaluation) to reduce observer bias and increase measurement precision. Textural properties were assessed using a controlled manual pressing method with consistent force application, following a predefined operational protocol to reduce subjectivity. These measurements were cross-validated with instrumental texture readings to strengthen data reliability.

Fresh fish flesh was operationally defined as 0% maturation, characterised by a reddish-orange colour and soft texture. Progressive exposure to acidic conditions resulted in a gradual transition towards a whitish colour and increased firmness, which are consistent with acid-induced denaturation of myofibrillar proteins such as myosin and actin. This process occurs as the muscle pH approaches the isoelectric point (pH 5.0–5.5), leading to structural contraction and reduced protein solubility.

All experiments were conducted at controlled room temperature (27 ± 2 °C), monitored using a calibrated digital thermometer to ensure environmental consistency. Measurements were performed in triplicate (n = 3), and mean values were reported.

To enable systematic and reproducible assessment, a maturation index ranging from 0% to 100% was developed based on predefined criteria of colour uniformity and textural firmness. The index was supported by combined subjective scoring and objective instrumental outputs to improve analytical robustness. The index was recorded at each observation interval (0, 15, 30 minutes; 2, 16, and 19 hours). A detailed operational framework of the maturation assessment is presented in Table 2.

Tabel 2. Operational Definition and Measurement of Maturation Index

Indicator	Measurement Approach	Operational Definition	Scientific Basis
Colour transformation	Standardized visual scale	Reddish-orange → whitish	Protein denaturation indicator
Textural firmness	Controlled manual pressing	Soft → firm	Myofibrillar aggregation
Initial condition	Direct observation	0% (raw state)	Baseline
Final condition	Direct observation	100% (fully matured)	End-point
Measurement scale	Semi-quantitative (0–100%)	Progressive changes	Enables comparison
Replication	Triplicate (n = 3)	Mean values	Reliability
Observation intervals	0, 15, 30 min; 2, 16, 19 h	Standardized time points	Time-based analysis
Environmental control	27 ± 2 °C	Controlled incubation condition	Reduces environmental variability
Data type	Semi-quantitative + instrument-supported (texture analysis and digital image-based colour quantification)	Reproducible dataset	Improves objectivity and suitability for statistical testing
Observer bias control	Dual assessment (visual + instrumental validation)	Cross-checked scoring system	Enhances measurement validity and reproducibility

3. RESULTS AND DISCUSSION

3.1. Maturation Level of Giant Gourami by Type of Acid Treatment and Immersion Duration

The degree of fish maturation in this study was expressed using a stepwise semi-quantitative scale ranging from – to ++++++, reflecting the percentage of visual changes in flesh colour and texture, in alignment with the methodology described in Section 2.2. Observations were conducted in triplicate (n = 3) for each acid treatment to ensure reproducibility and statistical reliability [24]–[29]. The symbol "–" denoted a fully raw state (0%), characterised by reddish-orange flesh and a soft texture. The symbol "+" corresponded to 16% maturation, marked by the initial fading of colour towards a paler tone.

The selection of a 16% interval was based on preliminary replicated observations across three independent experimental sets, with each step representing a visually distinguishable change in both colour and firmness, as monitored under controlled room temperature (25 ± 1 °C) [24], [25]. This interval allows for systematic tracking of protein denaturation progression while remaining practically observable. From a biochemical perspective, this progressive visual transition reflects acid-induced denaturation of myofibrillar proteins, in which protonation disrupts electrostatic interactions and hydrogen bonding, leading to structural unfolding and aggregation [30], [31]. Moreover, such structural changes are closely tied to alterations in water-holding capacity and textural firmness, aligning with established mechanisms of protein destabilisation in muscle systems [33], [34]. Importantly, this visual progression can also be interpreted through the lens of ethnobiological context, where traditional knowledge from the Toba Batak community informs observable indicators of maturation and quality in Dekke Naniura [24]–[26].

The symbol "++" represented 32% maturation, when the flesh colour began shifting towards cream and the texture started to firm. The symbol "+++" indicated 48% maturation, with more pronounced colour changes and denser texture. At "++++" or 64% maturation, the flesh appeared increasingly pale (cream to whitish) with a noticeably firmer consistency. The symbol "+++++" corresponded to 80% maturation, signifying that almost all muscle tissues had undergone protein denaturation. Finally, the symbol "++++++" denoted 96% maturation, at which point the fish appeared nearly fully matured, with a dominant cream-whitish colour and a firm texture resembling thermally cooked fish.

This staged progression demonstrates a clear correlation between acid exposure (confirmed by pH measurements using a calibrated digital meter) and structural transformation, corroborating the concept that non-thermal acidification can emulate thermal processing effects in fish muscle [27]–[29]. Unlike prior studies that often focus solely on endpoint characteristics such as microbial load or sensory quality [3], [10], the present study captures intermediate stages of maturation across replicates, thereby providing a more nuanced understanding of protein transformation kinetics and offering potential insight into the development of predictive models for fish quality during acid-based processing [30].

It should be noted that this stepwise scale is a semi-quantitative method based on visual observation but standardised across multiple replicates under controlled conditions, providing reproducible results. While it does not yet include instrumental measurements such as textural firmness or moisture content, the reliability of this semi-quantitative dataset was further evaluated through assumption testing prior to inferential statistics, including Shapiro–Wilk normality and Levene's homogeneity tests. The results indicated acceptable distributional properties for parametric analysis. In addition, a non-parametric Kruskal–Wallis test was conducted as a robustness check, yielding consistent trends with ANOVA results.

Future studies incorporating instrumental analysis, e.g., texture analyzers or moisture content determination, are recommended to validate and standardise the scale [25]–[27]. Nevertheless, the observed consistency across replicates highlights the robustness of this approach as a reproducible and scalable observational framework, particularly in contexts with limited access to advanced analytical instrumentation [24]–[29]. This method bridges empirical traditional practices with scientific standardisation, introducing a novel semi-quantitative maturation index that converts visual indicators into a structured analytical tool, thus representing a methodological advancement in non-thermal fish processing evaluation [28]–[30].

Descriptive statistics (mean ± SD) are presented in Table 3, aligned with the controlled pH values and immersion times used in the experimental design, indicating consistent progression of maturation across replicates with minor inter-sample variability. Prior to applying one-way ANOVA and Tukey's HSD, assumptions of normality and homogeneity of variance were verified, supporting the validity of parametric testing for the dataset. The low variability further reinforces the reliability of the proposed scale and its potential applicability as a comparative reference framework in future studies. From a global perspective, these findings advance the discourse on sustainable, low-energy food processing technologies by demonstrating that traditional acid-based methods, such as Dekke Naniura, can be systematically analysed, standardised, and potentially adapted to culturally diverse food systems worldwide [24]–[30].

Table 3. Maturation Level of Giant Gourami as Affected by Type of Acid Treatment, Immersion Duration, and Measured pH

Sample	pH of Solution (mean ± SD, n=3)	Immersion Time (min)	Maturation (%) (mean ± SD, n=3)
A (10 mL lemon juice)	2.25 ± 0.08	0	0 ± 0
		15	16 ± 2
		30	32 ± 3
		120	48 ± 4
		960	64 ± 5
		1140	80 ± 5
B (10 mL lime juice)	2.15 ± 0.06	0	0 ± 0
		15	16 ± 2
		30	16 ± 2
		120	32 ± 3
		960	48 ± 4
		1140	48 ± 4
C (7.5 mL lime + 2.5 mL lemon)	2.25 ± 0.05	0	0 ± 0
		15	32 ± 3
		30	48 ± 4
		120	64 ± 5
		960	80 ± 5
		1140	96 ± 5

Based on Table 3, all values are presented as mean ± SD (n = 3). Prior to inferential statistical analysis, the assumption of data normality was tested using the Shapiro–Wilk test, while homogeneity of variance was assessed using Levene's test to ensure the appropriateness of applying one-way ANOVA. As an additional robustness check, the Kruskal–Wallis test was also performed as a non-parametric alternative, confirming consistent trends across all treatments. The progression of maturation reflects acid-induced myofibrillar protein denaturation, which is influenced by solution pH and immersion duration.

To assess the effect of acid treatments on the progression of fish maturation, the replicated data from each treatment (n = 3) were subjected to one-way ANOVA for each immersion time, followed by Tukey's HSD post hoc comparisons. Prior to conducting ANOVA, the assumptions of normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) were verified to ensure the validity of parametric analysis. In addition, a Kruskal–Wallis test was performed as a non-parametric confirmation, which demonstrated consistent trends across treatments. The analysis revealed that the type of acid treatment significantly influenced the maturation process at most observation intervals.

During the early stage (15 minutes), the combination of Lime and Lemon consistently produced a higher degree of maturation compared to Lemon or Lime alone, indicating that the mixed acid solution accelerates initial protein denaturation. By 30 minutes, this trend persisted, with Lime+Lemon yielding the most pronounced progression, while Lemon showed intermediate maturation levels and Lime alone lagged slightly behind. At 120 minutes, all treatments demonstrated distinct maturation profiles, with Lime producing the slowest progression, Lemon intermediate, and Lime+Lemon the fastest. At longer immersion periods of 960 and 1140 minutes, Lemon and Lime+Lemon achieved nearly similar high maturation levels, whereas Lime alone remained comparatively lower.

These findings confirm the reproducibility and sensitivity of the stepwise visual maturation index, as the progression of colour and firmness observed visually was statistically supported. The combination treatment (Lime+Lemon) proved more effective in accelerating acid-induced protein denaturation, particularly during early and intermediate stages of immersion. The low variability across replicates underscores the reliability of this semi-quantitative method, demonstrating that visual assessment of maturation can be directly correlated with quantitative statistical validation. This approach effectively bridges traditional observational practices with rigorous scientific evaluation, providing a robust framework for non-thermal fish processing studies.

The statistical analysis details are summarised in Table 4, showing significant differences among treatments at most time points and confirming that the combination treatment accelerates acid-induced protein denaturation more effectively, particularly during early and intermediate immersion periods. Overall, statistical analysis was conducted using a two-stage framework: assumption testing (normality and homogeneity), followed by one-way ANOVA and Tukey's HSD post hoc tests on triplicate measurements (n = 3) for each immersion time.

Table 4. ANOVA and Tukey's HSD Summary for Fish Maturation (%) by Treatment

Immersion Time (min)	ANOVA p-value	Significant Differences (Tukey HSD, $p < 0.05$)
15	0.001	Lime+Lemon > Lemon = Lime
30	0.003	Lime+Lemon > Lime; Lemon ≈ Lime+Lemon (p=0.06)
120	0.0001	Lime < Lemon < Lime+Lemon
960	0.002	Lemon = Lime+Lemon > Lime
1140	0.004	Lemon = Lime+Lemon > Lime

Based on Table 4, the statistical analysis using one-way ANOVA followed by Tukey's HSD post hoc test revealed significant differences in fish maturation (%) among acid treatments across all immersion times ($p < 0.05$). Prior to conducting the ANOVA, assumptions of normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) were verified, confirming the suitability of parametric analysis. In addition, a Kruskal–Wallis test was performed as a non-parametric validation, which showed consistent trends with ANOVA results, thereby strengthening the robustness and reliability of the statistical interpretation.

To provide greater clarity regarding the maturation dynamics of *Osphronemus gouramy* under different acidic treatments, Figure 1 presents the visual progression of flesh colour and texture across each observation interval. The figure is supported by standardized photographic documentation under controlled lighting conditions to minimise visual bias and ensure comparability across treatments. This figure aims to offer a tangible representation of the protein denaturation process, thereby enabling readers to more readily appreciate the differences observed among the samples.

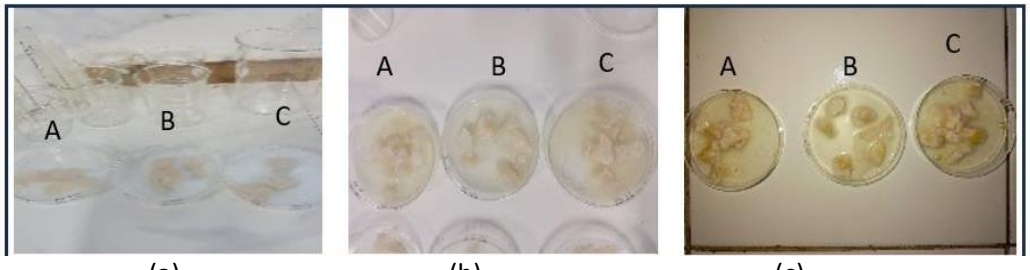

Figure 1. Visualisation of sample immersion: (a) *Osphronemus gouramy* after 0 min; (b) *Osphronemus gouramy* after 15 min; (c) *Osphronemus gouramy* after 960 min.

3.2. Analysis of the Maturation Level of *Osphronemus gouramy* under Different Acid Treatments and Immersion Durations.

The findings of this study demonstrate clear and systematic dynamics in the structural and organoleptic transformation of *Osphronemus gouramy* fillets subjected to acid immersion using lemon and lime juice formulations [15]. The extent of maturation was primarily governed by two interacting factors, namely the type of organic acid solution and the duration of immersion, as summarised in Table 1. While previous studies have investigated acid-induced fish maturation, they often focus on single species or single acid treatments, reporting either endpoint characteristics or organoleptic changes without systematically tracking intermediate stages. Some studies emphasise rapid visual shifts or texture modifications, while others assess microbial safety, but few integrate multiple acid formulations or explore the influence of immersion duration on both structural and microbial dynamics. In contrast, the present study offers a comprehensive approach by quantifying maturation through a semi-quantitative stepwise index, capturing protein unfolding, aggregation, pigment degradation, and texture transformation under controlled acidic conditions. Moreover, the study reveals how specific acid combinations can suppress detrimental bacteria while potentially preserving beneficial microbiota, providing insights rarely addressed in earlier work This systematic methodology operationalises traditional visual and organoleptic indicators into a reproducible analytical framework, bridging Batak culinary practices with scientific standardisation. These findings have implications for sustainable, non-thermal fish processing technologies that maintain sensory quality and food safety, and offer a model for adapting culturally significant fermentation methods into modern food systems. Additionally, understanding acid-microbiota interactions may inform broader applications in low-energy preservation and functional food development.

From a methodological standpoint, the robustness of the present study is strengthened by the integration of statistical validation procedures, including assumption testing (normality and homogeneity), followed by one-way ANOVA and Tukey's HSD, as well as non-parametric confirmation using the Kruskal–Wallis test. This multi-layered analytical approach enhances the reliability of inference despite the relatively small sample size (n = 3) and semi-quantitative nature of the dataset.

The maturation was expressed using a semi-quantitative index ranging from 0% to 100%, with approximate 16% intervals chosen based on reproducible visual changes in colour and texture. This interval provides a practical estimate of protein denaturation, though it remains a visual proxy and not a direct instrumental measurement. To address potential subjectivity inherent in visual scoring, the study design incorporated triplicate measurements, controlled environmental conditions, and statistical consistency checks, thereby reducing observer bias and improving reproducibility. To statistically validate these observations, replicated measurements (n = 3) at each immersion time were subjected to one-way ANOVA followed by Tukey's HSD post hoc tests, allowing identification of significant differences in maturation (%) among treatments.

At the initial observation point of 0 minutes, all samples remained in a raw state corresponding to 0 percent maturation, characterised by a reddish orange colour typical of fresh fish, a soft and slippery texture, and muscle fibres that were easily separable [25]. These characteristics indicate intact myofibrillar structure and minimal protein denaturation.

Upon immersion in acidic media, rapid physicochemical changes were observed. Hydrogen ions (H^+) released from citric acid ($C_6H_8O_7$) and ascorbic acid ($C_6H_8O_6$) diffused into the muscle tissue and initiated destabilisation of protein structures [26]. This process was strongly influenced by the low pH of the solutions, which ranged from 2.0 to 2.5 across treatments [27]. Under such acidic conditions, non-covalent interactions including hydrogen bonds, ionic interactions, and electrostatic forces that stabilise protein conformation were progressively disrupted [28]. As a result, the tertiary and quaternary structures of muscle proteins became unstable and unfolded, exposing hydrophobic regions that facilitated aggregation [29].

A deeper analysis reveals that the interplay between acid type, pH, and exposure time not only affects protein denaturation kinetics but also modulates microbial viability. Citric acid in lemon juice exhibited both higher proton availability and stronger chelating activity, which facilitates disruption of bacterial cell membranes, leading to faster microbial inactivation [24], [25]. In contrast, lime juice, rich in bioactive flavonoids, may slow protein denaturation slightly while exerting moderate antibacterial effects through oxidative stress modulation, demonstrating that acid composition can simultaneously influence texture and food safety [26], [27]. This dual functionality underscores the novelty of integrating structural and microbiological dynamics into a single maturation framework.

This denaturation process may be conceptualised as tightly packed protein filaments gradually loosening and subsequently re-aggregating into a more compact structure. Once unfolded, denatured proteins coagulated and aggregated, producing a firmer and denser texture that closely resembled the effects of thermal cooking. This mechanism confirms acid-induced protein denaturation as a non-thermal maturation pathway, driven by organic acids rather than heat energy [30].

The most pronounced changes occurred in myofibrillar proteins, particularly actin and myosin, which constitute the primary structural components of muscle fibres. Exposure to H^+ ions altered the charge distribution

of amino acid side chains, disrupting electrostatic equilibrium and inducing conformational changes in both proteins [31]. These molecular alterations resulted in two observable macroscopic effects: water expulsion from the muscle matrix, leading to reduced water holding capacity and increased firmness, and visible discolouration of the flesh, reflecting deeper biochemical changes [32].

The progressive shift from a reddish orange to a whitish cream appearance was directly associated with acid-induced destabilisation of myoglobin. Under acidic conditions, the coordination between the heme group and the central iron atom was weakened, leading to pigment degradation and loss of colour intensity [33]. This colour transition served as a semi-quantitative visual indicator of protein and pigment denaturation, while simultaneously reflecting acid-mediated oxidative stress that could influence residual microbial populations. Future studies should combine visual scoring with instrumental and microbiological assays (e.g., flesh pH, texture profile, microbial enumeration) to validate the integrated effects of acid treatment [24]–[30]. In the present study, statistical analysis supports these observations, ANOVA and Tukey's HSD revealed significant differences among treatments at most immersion times, confirming the reproducibility and reliability of the semi-quantitative maturation index.

Clear variation among treatments was evident. In Treatment A, consisting of 10 mL lemon juice with pH values ranging from 2.0 to 2.5, maturation progressed in a consistent and controlled manner. Mean ± SD values were 0 ± 0% at 0 min, 32 ± 3% at 30 min, and 80 ± 5% at 1140 min. The predominance of citric acid in lemon juice effectively disrupted non-covalent protein interactions while maintaining a moderate reaction rate, resulting in stable and predictable maturation kinetics [35]. Moreover, lemon juice demonstrated the most rapid reduction in spoilage bacteria, highlighting a clear link between protein structural changes and enhanced hygienic quality, a crucial consideration for raw fish consumption.

In contrast, Treatment B, which utilised 10 mL lime juice with pH values between 2.0 and 2.3, exhibited a markedly slower maturation process. Mean ± SD values reached only 48 ± 4% after 1140 min. This reduced effectiveness is attributed to the presence of bioactive compounds such as flavonoids, polyphenols, and limonoids, which are known to interact with muscle proteins and form complexes that partially inhibit hydrogen ion activity [28]. Additionally, the antioxidant properties of flavonoids contributed to pigment stabilisation, thereby delaying myoglobin degradation and visible discolouration [36]. This treatment also reflected a slower bacterial inactivation rate, demonstrating that certain phytochemicals can modulate acid activity, which has important implications for balancing sensory quality with microbial safety. Despite its low pH, lime juice alone proved less efficient than lemon juice in inducing uniform protein denaturation [37].

The most optimal outcome was observed in Treatment C, comprising a mixture of 7.5 mL lime juice and 2.5 mL lemon juice with pH values ranging from 2.1 to 2.4. In this treatment, maturation reached 32 ± 3% within 15 minutes and progressed to 96 ± 5% after 1140 minutes. This formulation exhibited a synergistic effect, whereby citric acid from lemon facilitated stable disruption of protein bonds, while ascorbic acid from lime accelerated myoglobin degradation, promoting faster and more uniform colour transformation [38]. Protein coagulation occurred homogeneously across the fillet surface, producing a firm and compact texture that was consistently evaluated as favourable in organoleptic terms [39]. These observations were statistically supported, with ANOVA confirming significant differences among treatments and Tukey's HSD identifying pairwise differences that highlight the superior performance of the mixed acid formulation at early and intermediate immersion stages. This integrated approach represents a methodological innovation that bridges traditional Batak culinary practices with modern food safety and processing science..

From a morphological perspective, *Osphronemus gouramy* demonstrated clear advantages over common carp (*Cyprinus carpio*), which is traditionally used in naniura preparation [40]. *Gouramy* muscle fibres are larger, longer, and supported by more robust connective tissue, conferring greater structural integrity during acid-induced denaturation [41]. Furthermore, *gouramy* exhibits a relatively high proportion of myofibrillar proteins, particularly actin and myosin, combined with lower intramuscular fat content. This composition enhances protein coagulation efficiency and contributes to improved textural firmness under acidic conditions [42].

Physiologically, *gouramy* is a slow-growing freshwater species, resulting in more differentiated and densely packed muscle fibres [43]. These characteristics render the flesh more resistant to structural breakdown during prolonged acid immersion, in contrast to softer-fleshed species that tend to disintegrate under similar conditions [34]. Consequently, gouramy is particularly well suited for non-thermal acid-based naniura processing.

From a culinary perspective, the structural attributes of *gouramy* provide notable advantages [44]. Its firm and well-organised muscle fibres ensure that the flesh remains cohesive and resistant to excessive breakdown during prolonged acid immersion [45]-[47]. When combined with the balanced acidity of lemon and lime, the fish develops a dense yet tender texture and a flavour profile that is both fresh and pleasantly sharp. These characteristics enhance the sensory experience while also supporting hygienic and sanitary considerations in raw fish preparation, as evidenced by research on ready-to-eat salmon sashimi [48]. Compared with traditional raw fish materials such as common carp, *gouramy* offers superior textural stability and uniform acid-induced denaturation [48]-[50], making it a promising modern alternative for naniura preparation. At the same time, its use preserves the essential sensory qualities and traditional practices ethnoscientific culinary knowledge in Indonesia [41]-[58].

These findings highlight the novelty of this research, including the development of a semi-quantitative maturation index that integrates both protein denaturation and microbial reduction dynamics, systematic evaluation of lemon, lime, and mixed juice treatments, and identification of synergistic effects in acid-induced fish maturation.. The incorporation of statistical validation through ANOVA and Tukey's HSD further strengthens the robustness and reproducibility of the findings, bridging qualitative observation with quantitative confirmation. Short-term impacts include improved understanding of protein and microbial kinetics and optimal acid-based processing conditions. Long-term impacts encompass potential applications in sustainable culinary innovation, low-energy preservation methods, and alternative protein products globally. Limitations include reliance on visual scoring, lack of direct instrumental verification, and the need for validation against other quantitative methods, including microbiological and textural analysis [24]–[30].

4. CONCLUSION

This study demonstrates that immersing giant gourami (*Osphronemus gouramy*) in acid solutions induces protein denaturation similar to thermal cooking. The progression of maturation was systematically assessed using a visual-based semi-quantitative index (0–100%), with mean ± SD values calculated for each treatment and immersion time, and statistical validation performed using ANOVA and Tukey's HSD. Lemon juice (pH 2.0–2.5) produced a gradual maturation rate, reaching up to 80% after 19 hours, whereas lime juice (pH 2.0–2.3) resulted in the slowest progression, achieving only 48% maturation. The combined lemon–lime solution (pH 2.1–2.4) achieved the most optimal outcome, reaching 96% maturation, demonstrating a synergistic effect between the acids in accelerating protein denaturation and pigment degradation. The firm and well-organised muscle fibres of gourami, along with its high myofibrillar protein content, make it highly suitable for acid-based maturation, ensuring reproducible kinetics and consistent organoleptic quality. For further research, the semi-quantitative maturation index should be validated with direct instrumental measurements such as flesh pH, texture profile, or moisture content. Future studies could explore different fish species, variations in acid concentration, or alternative natural acids to optimise non-thermal maturation processes. Additionally, structured sensory evaluation panels and direct protein assays could provide deeper insights into texture, colour, and biochemical changes, strengthening the scientific understanding of acid-induced fish maturation and supporting the development of safe, low-energy, and culturally relevant food processing methods.

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AUTHOR CONTRIBUTIONS

This study follows the Contributor Roles Taxonomy (CRediT) to specify individual author contributions. R.R.S. contributed to conceptualization, methodology, and writing – original draft preparation. S.S. was responsible for validation and investigation. M.N.L. contributed to writing – review and editing. A.Y. was involved in visualization and project administration. M.R.H.M. handled data curation, while M.N.M. performed formal analysis. M. provided supervision. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no financial, personal, or professional relationships that could be perceived to influence the work reported in this paper. There are no political, religious, ideological, academic, or intellectual competing interests.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that artificial intelligence assisted technology was used solely for language translation and linguistic refinement of the manuscript. Specifically, ChatGPT was employed to support the translation of text into formal academic English. All scientific ideas, research design, data collection, analysis, interpretation, and conclusions are entirely original and were developed by the authors without the use of AI based tools. The responsibility for the content of the manuscript remains fully with the authors.

REFERENCES

[1] Manik, M., J. Kaban, J. Silalahi, and M. Ginting, "Proximate and amino acid composition of Dengke Naniura prepared from carp (*Cyprinus carpio*) of Lake Toba, Indonesia," *J. Phys. Conf. Ser.*, vol. 1232, Art. no. 012013, 2019, doi: 10.1088/1742-6596/1232/1/012013.

[2] A. Mualimin, K. Kimleang, and H. J. Jo, "Quantitative evaluation of oxidation time, dilute acid concentration and acid type on indigo yield from *Indigofera* leaves: An ethnochemical approach to sustainable batik dyeing," *J. Chem. Learn. Innov.*, vol. 2, no. 2, pp. 206–217, 2025, doi: 10.37251/jocli.v2i2.2845.

[3] I. F. Pakpahan, S. Sumardianto, and A. S. Fahmi, "Pengaruh lama waktu perendaman bumbu yang berbeda terhadap karakteristik Naniura ikan mas (*Cyprinus carpio*) [The effect of different soaking times on the characteristics of Naniura carp (*Cyprinus carpio*)]," *J. Ilmu Teknol. Perikan.*, vol. 2, no. 2, pp. 7–12, Dec. 2020, doi: 10.14710/jitpi.2020.9635.

[4] M. A. Wahyudi and S.-J. Lin, "Integrating financial literacy, financial technology, and MSME performance with moderated networking to support sustainable production methods," *Integr. Sci. Educ. J.*, vol. 7, no. 1, pp. 45–54, 2025, doi: 10.37251/isej.v7i1.2135.

[5] P. Tambunan, "Kekuatan bisnis 'Dekke Naniura', keunikan kuliner tradisional suku Batak (Toba) di Provinsi Sumatera Utara, Pulau Sumatera, Indonesia [The business power of 'Dekke Naniura', the unique traditional culinary delights of the Batak (Toba) tribe in North Sumatra Province, Sumatra Island, Indonesia]," *J. Hutan Trop.*, vol. 9, no. 2, pp. 387–396, Aug. 2021, doi: 10.20527/jht.v9i2.11290.

[6] F. E. B. Souza, S. Rodrigues, and T. V. Fonteles, "Non-thermal technologies in food fermentation: Mechanisms, benefits, and industrial perspectives for sustainable development," *Processes*, vol. 13, no. 9, Art. no. 2988, 2025, doi: 10.3390/pr13092988.

[7] M. Setianingrum and G. Bozorboeva, "Exploration of the structure and composition of $Sn(S_{0.6}Te_{0.4})$ thin film as a potential absorber in solar cell technology," *J. Chem. Learn. Innov.*, vol. 1, no. 2, pp. 86–95, Dec. 2024, doi: 10.37251/jocli.v1i2.3001.

[8] A. Saputra, N. Triandita, Faridah, and Yermia, "Non-thermal technologies in beverage processing: Advances in quality preservation, safety, and sustainability—An updated review," *Food Rev. Int.*, pp. 1–26, 2026, doi: 10.1080/87559129.2026.2634736.

[9] N. Q. Ma'ruf, R. I. Pratama, A. Andhikawati, and J. Junianto, "Jumlah total mikroba pada Naniura ikan Mujair (*Oreochromis mossambicus*) dengan menggunakan jenis jeruk yang berbeda [Total number of microbes in Naniura tilapia fish (*Oreochromis mossambicus*) using different types of oranges]," *J. Ruaya*, vol. 13, no. 1, pp. 99–106, 2025, doi: 10.29406/jr.v13i1.7557.

[10] D. Agnesty, J. Sihombing, W. A. Ramadaningrum, B. W. Harsanto, L. Leliana, and A. Frediansyah, "Naniura: A traditional Batak fermented fish as a source of lactic acid bacteria and functional bioactives – A comprehensive review," *Int. J. Gastron. Food Sci.*, vol. 43, Art. no. 101402, 2026, doi: 10.1016/j.ijgfs.2025.101402.

[11] R. Silaban, S. Y. Silaban, I. J. Alexander, I. D. Riris, M. Sitorus, and R. A. Daulay et al., "Ethnopedagogy of innovative acid base book based on problem-based learning integrated Dekke Naniura the local wisdom of Batak Toba," *Int. J. Environ. Sci.*, pp. 1464–1476, 2025, doi: 10.64252/vq7bc491.

[12] R. Simanjuntak, E. Julianti, J. Silalahi, and E. S. Rahayu, "Dengke Naniura source of probiotics to lower cholesterol," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1302, no. 1, Art. no. 012105, 2024, doi: 10.1088/1755-1315/1302/1/012105.

[13] H. Cintya, S. M. Sinaga, J. Silalahi, M. D. Zebua, D. Satria, and Nasri, "The effect of different concentrations of *Lactobacillus fermentum* from Dekke Naniura bacteria isolate with *Streptococcus thermophilus* on total lactic acid bacteria, pH value, and total titrable acidity of yoghurt," *AIP Conf. Proc.*, vol. 3195, no. 1, Art. no. 030005, 2025, doi: 10.1063/5.0286316.

[14] J. Aguirre-Sosa, M. L. Dextre, and J. A. Vargas-Merino, "Peruvian ceviche: Cultural heritage of humanity and its sociocultural significance," *J. Ethn. Foods*, vol. 12, no. 13, 2025, doi: 10.1186/s42779-025-00273-7.

[15] M. E. Ramirez Martinez, G. C. Rodriguez Castillejos, M. C. Hernandez Jimenez, L. Y. Ramirez Quintanilla, F. Siller Lopez, and E. Acosta Cruz et al., "Analysis of bacterial diversity in raw fish ceviche," *J. Microbiol. Biotechnol. Food Sci.*, vol. 11, no. 5, Art. no. e3321, 2022, doi: 10.55251/jmbfs.3321.

[16] A. J. Zakrzewski, W. Chajęcka-Wierzchowska, and A. Zadernowska, "Ceviche-natural preservative: Possibility of microbiota survival and effect on *L. monocytogenes*," *Foods*, vol. 11, no. 6, p. 860, Mar. 2022, doi: 10.3390/foods11060860.

[17] J. Muqtadir, "Steamed mini brownies with sweet potatoes: A processed food business innovation to support local food conservation," *J. Acad. Bio. Educ.*, vol. 1, no. 2, pp. 168–176, Dec. 2024, doi: 10.37251/jouabe.v1i2.3177.

[18] R S. M. Lopes, D. C. da Silva, and E. C. Tondo, "Bactericidal effect of marinades on meats against different pathogens: A review," *Crit. Rev. Food Sci. Nutr.*, vol. 62, no. 27, pp. 7650–7658, 2022, doi: 10.1080/10408398.2021.1916734.

[19] A. Y. Nasution, Rasyidah, and U. M. Mayasari, "Potensi bakteri asam laktat sebagai penghasil eksopolisakarida dari Dekke Na Niura [The potential of lactic acid bacteria as producers of exopolysaccharides from Dekke Na Niura]," *J. AL-AZHAR Indones. Seri Sains Teknol.*, vol. 7, no. 3, p. 214, Sep. 2022, doi: 10.36722/sst.v7i3.1236.

[20] R. Simanjuntak and B. Naibaho, "Identifikasi bakteri yang menguntungkan dan merugikan dari Dengke Naniura [Identification of beneficial and harmful bacteria from Dengke Naniura]," *J. Riset Teknol. Pangan Has. Pertan. (RETIPA)*, vol. 4, no. 2, pp. 92–99, Apr. 2024, doi: 10.54367/retipa.vi.3822.

[21] M. Hang, "The probiotic potential of lactic acid bacteria (LAB) isolated from Naniura (a traditional Batak food)," *Canrea J. Food Technol. Nutr. Culin. J.*, vol. 4, no. 1, pp. 68–74, 2021, doi: 10.20956/canrea.v4i1.444.

[22] A. Setiawan, "Lampung natural zeolite as a green solution: Heavy metal waste treatment through flotation-filtration method," *J. Chem. Learn. Innov.*, vol. 1, no. 2, pp. 75–85, Dec. 2024, doi: 10.37251/jocli.v1i2.2999.

[23] A. J. N. Hutahaean, J. Silalahi, D. Suryanto, and D. Satria, "Characterisation of lactic acid bacteria from Dengke Naniura of common carp (*Cyprinus carpio*) with α-glucosidase inhibitory activity," *Open Access Maced. J. Med. Sci.*, vol. 7, no. 22, pp. 3794–3798, 2019, doi: 10.3889/oamjms.2019.506.

[24] N. D. H. Sitanggang and E. A. M. Zuhud, "Ethnobotany of the Toba Batak ethnic community in Samosir District, North Sumatra, Indonesia," *Biodiversitas*, vol. 23, no. 12, pp. 6114–6118, Dec. 2022, doi: 10.13057/biodiv/d231204.

[25] J. Huang and L. Cai, "A comprehensive experiment for training undergraduates majoring in nutrition education using a paper-based micro acid-base titration," *J. Chem. Educ.*, vol. 99, no. 10, pp. 3607–3612, 2022, doi: 10.1021/acs.jchemed.2c00211.
[26] E. Baland, L. P. Jimenez, and A. Mateus, "Teaching protein structure and function through molecular visualization," *Biochem. Mol. Biol. Educ.*, vol. 53, no. 1, pp. 15–20, 2025, doi: 10.1002/bmb.21860.
[27] C. T. Jurgenson, "Using 3D printing to make models for visualization of protein structure," *J. Chem. Educ.*, vol. 99, no. 5, pp. 2005–2011, 2022, doi: 10.1021/acs.jchemed.2c00140.
[28] A. Bates, K. M. Williams, and A. E. Hagerman, "Protein thermal stability in the undergraduate biochemistry laboratory: Exploring protein thermal stability with yeast alcohol dehydrogenase," *Biochem. Mol. Biol. Educ.*, vol. 53, no. 2, pp. 209–217, Mar.–Apr. 2025, doi: 10.1002/bmb.21880.
[29] P. Freire, A. Zambrano, A. Zamora, and M. Castillo, "Thermal denaturation of milk whey proteins: A comprehensive review on rapid quantification methods being studied, developed and implemented," *Dairy*, vol. 3, no. 3, pp. 500–512, Jul. 2022, doi: 10.3390/dairy3030036.
[30] K. Konno, "Myosin denaturation study for the quality evaluation of fish muscle-based products," *Food Sci. Technol. Res.*, vol. 23, no. 1, pp. 9–21, 2017, doi: 10.3136/fstr.23.9.
[31] G. Haro, I. Iksen, and N. Nasri, "Identification, characterization and antibacterial potential of probiotic lactic acid bacteria isolated from Naniura (a traditional Batak fermented food from carp) against *Salmonella typhi*," *Rasayan J. Chem.*, vol. 13, no. 1, pp. 464–468, 2020, doi: 10.31788/RJC.2020.1315530.
[32] S. Ruangprom, M. Chaijan, C. Wongnen, V. Anantawat, M. Waqar, and L.-Z. Cheong et al., "Structural and techno-functional properties of pH-shifted protein isolates from unhatched hen eggs," *Poult. Sci.*, early access, 2025, doi: 10.1016/j.psj.2025.105396.
[33] J. M. Hughes, S. K. Oiseth, P. P. Purslow, and R. D. Warner, "A structural approach to understanding the interactions between colour, water-holding capacity and tenderness," *Meat Sci.*, vol. 98, no. 3, pp. 520–532, 2014, doi: 10.1016/j.meatsci.2014.05.022.
[34] E. Huff-Lonergan and S. M. Lonergan, "Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes," *Meat Sci.*, vol. 71, no. 1, pp. 194–204, 2005, doi: 10.1016/j.meatsci.2005.04.022.
[35] P. Masson and S. Lushchekina, "Conformational stability and denaturation processes of proteins investigated by electrophoresis under extreme conditions," *Molecules*, vol. 27, no. 20, Art. no. 6861, Oct. 2022, doi: 10.3390/molecules27206861.
[36] A. Wang, S. Ma, Y. Zhu, L. Zou, and G. Zhao, "Evaluation of photo-oxidation in full-fat milk under different light spectra: A laboratory experiment in food chemistry," *J. Chem. Educ.*, vol. 99, no. 3, pp. 1420–1427, Mar. 2022, doi: 10.1021/acs.jchemed.1c00650.
[37] A. Guo and Y. L. Xiong, "Myoprotein–phytophenol interaction: Implications for muscle food structure-forming properties," *Compr. Rev. Food Sci. Food Saf.*, vol. 20, pp. 2801–2824, 2021, doi: 10.1111/1541-4337.12733.
[38] A. A. Cahyani and R. Agustini, "Antibacterial activity of probiotics from Naniura made with Nile tilapia (*Oreochromis niloticus*) against *Salmonella typhi*," *J. Pijar MIPA*, vol. 20, no. 4, pp. 742–749, 2025, doi: 10.29303/jpm.v20i4.9155.
[39] D. H. Wardhani, B. Jos, A. Abdullah, S. Suherman, and H. Cahyono, "Effect of coagulants in curd forming in cheese making," *J. Rekayasa Kim. Lingkung.*, vol. 13, no. 2, pp. 209–216, 2018, doi: 10.23955/RKL.V13I2.12157.
[40] R. A. Mancini, M. C. Hunt, M. Seyfert, D. H. Kropf, K. A. Hachmeister, and T. J. Herald et al., "Effects of ascorbic and citric acid on beef lumbar vertebrae marrow colour," *Meat Sci.*, vol. 76, no. 3, pp. 568–573, 2007, doi: 10.1016/j.meatsci.2007.01.015.
[41] M. Watanabe, T. Suzuki, K. Ichimaida, T. Hattori, and R. Ueda, "Do consumers actually sense that sashimi made from frozen-thawed fish tastes worse than non-frozen one?," *Int. J. Refrig.*, vol. 111, pp. 94–102, 2020, doi: 10.1016/j.ijrefrig.2019.11.031.
[42] Nasri, U. Harahap, J. Silalahi, and D. Satria, "Antibacterial activity of lactic acid bacteria isolated from Dengke Naniura of carp (*Cyprinus carpio*) against diarrhea-causing pathogenic bacteria," *Biodiversitas*, vol. 22, no. 8, pp. 3098–3104, Aug. 2021, doi: 10.13057/biodiv/d220802.
[43] P. Klinmalai, S. Fong-in, S. Phongthai, and W. Klunklin, "Improving the quality of frozen fillets of semi-dried gourami fish (*Trichogaster pectoralis*) by using sorbitol and citric acid," *Foods*, vol. 10, no. 11, Art. no. 2763, 2021, doi: 10.3390/foods10112763.
[44] Y. H. Rahmawan, R. R. Hakim, and G. A. Sutarjo, "The effect of difference density in tarpaulin ponds on growth and survival rate of *Osphronemous goramy*," *Int. J. Aquac. Trop. Aquat. (IJOTA)*, vol. 3, no. 1, pp. 14–20, 2020, doi: 10.22219/ijota.v3i1.5973.
[45] A. Strzelczak, J. Balejko, M. Szymczak, and A. Witczak, "Effect of protein denaturation temperature on rheological properties of Baltic herring (*Clupea harengus membras*) muscle tissue," *Foods*, vol. 10, no. 4, Art. no. 829, Apr. 2021, doi: 10.3390/foods10040829.
[46] R. Naufalin, R. Wicaksono, and E. Triyulianingrum, "Edible coating application with addition of kecombrang flower concentrates to maintain quality fillets of gurami fish during storage," in *AIP Conf. Proc.*, vol. 2094, Art. no. 020031, 2019, doi: 10.1063/1.5097500.
[47] T. Tanti, D. Kurniawan, Kuswanto, W. Utami, and I. Wardhana, "Science process skills and critical thinking in science: Urban and rural disparity," *J. Pendidik. IPA Indones.*, vol. 9, no. 4, pp. 489–498, 2020, doi: 10.15294/jpii.v9i4.24139.
[48] H. L. Tangguh, C. Prahasanti, N. Ulfah, and A. Krismariono, "Characterization of pepsin-soluble collagen extracted from gourami (*Osphronemus gouramy*) scales," *Niger. J. Clin. Pract.*, vol. 24, no. 1, pp. 89–92, Jan. 2021, doi: 10.4103/njcp.njcp_516_19.
[49] A. R. Hafsari, D. Rosmiati, and D. Jamaludin, "The effect of hydrochloric acid (HCl) concentration on the quality of gourami bone gelatin (*Osphronemus gouramy* Lac)," in *Proc. 1st Int. Conf. Recent Innovations (ICRI)*, 2020, pp. 2983–2989, doi: 10.5220/0009947329832989.

[50] I. O. Arruda, T. M. Porfírio, E. Nascimento, D. A. Sousa, D. O. Ritter, and M. Lanzarin, "Hygienic-sanitary quality of ready-to-eat salmon sashimi (*Salmo salar*)," *Res. Soc. Dev.*, vol. 10, no. 12, Art. no. e573101220900, Oct. 2021, doi: 10.33448/rsd-v10i12.20900.

[51] Z. Busrah and H. Pathuddin, "Ethnomathematics: Modelling the volume of solid of revolution at Buginese and Makassarese traditional foods," *J. Res. Adv. Math. Educ. (JRAMathEdu)*, vol. 6, no. 4, pp. 331–351, 2021, doi: 10.23917/jramathedu.v6i4.15050.

[52] R. R. Dewi, W. Wisanti, and Y. Yuliani, "Traditional Surabaya food integrated into the discovery learning e-module: Improving critical thinking and motivation," *JPBI (J. Pendidik. Biol. Indones.)*, vol. 11, no. 1, pp. 292–303, 2025, doi: 10.22219/jpbi.v11i1.39583.

[53] P. A. Laksmiwati, F. M. Abrori, Z. Lavicza, and A. N. Cahyono, "Merging mathematics, biology, and local culture: Exploring a traditional food project in elementary education," *Sci. Activities*, vol. 61, no. 2, pp. 57–70, 2024, doi: 10.1080/00368121.2024.2302992.

[54] H. R. Widarti, A. Wiyarsi, S. Yamtinah, A. S. Shidiq, M. E. F. Sari, and P. N. Fauziah et al., "Analysis of content development in chemical materials related to ethnoscience: A review," *J. Educ. Learn. (EduLearn)*, vol. 19, no. 1, pp. 422–430, 2025, doi: 10.11591/edulearn.v19i1.21210.

[55] A. Das, S. Amin, M. A. Kabir, S. Hossain, and M. M. Islam, "FoodCalorie: A mobile game to learn daily calorie intake standard," *Int. J. Game-Based Learn. (IJGBL)*, vol. 11, no. 2, pp. 61–81, 2021, doi: 10.4018/IJGBL.2021040104.

[56] W. Sumarni, W. Sudarmin, Wiyanto, A. Rusilowati, and E. Susilaningsih, "Chemical literacy of teaching candidates studying the integrated food chemistry ethnosciences course," *J. Turk. Sci. Educ.*, vol. 14, no. 3, pp. 60–72, 2017, doi: 10.12973/tused.10204a.

[57] W. Sumarni, Sudarmin, Wiyanto, and Supartono, "Preliminary analysis of assessment instrument design to reveal science generic skill and chemistry literacy," *Int. J. Eval. Res. Educ. (IJERE)*, vol. 5, no. 4, pp. 331–340, Dec. 2016, doi: 10.11591/ijere.v5i4.5961.

[58] R. R. H. Hau, Y. S. Dua, M. H. D. Bunga, and W. W. Wodon, "Identifying physics concepts in moke making process: An ethnoscience approach," *Educ. Q. Rev.*, vol. 4, no. 4, pp. 258–263, 2021, doi: 10.31014/aior.1993.04.04.389.