

**SOYBEAN ISOFLAVONES AS A NATURAL PRESERVATIVE THROUGH
ANTI-MICROBIAL ACTION FOR FRESH *Oreochromis niloticus*, Linnaeus 1758
(NILE TILAPIA)**

A RESEARCH PAPER

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of the Requirements for the Degree
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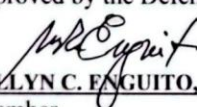
The research paper attached hereto, entitled "SOYBEAN ISOFLAVONES AS A NATURAL PRESERVATIVE THROUGH ANTI-MICROBIAL ACTION FOR FRESH *OREOCHROMIS NILOTICUS*, LINNAEUS 1758 (NILE TILAPIA)" prepared and submitted by GLYCE A. AMACANIN, IAN CRIS DULA, JUN RUSSELL L. PAGLINAWAN, PRINCE CARL D. CIMA FRANCA in partial fulfillment of the requirements for the degree of BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY, is hereby recommended for approval.


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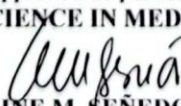
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ABSTRACT

Fresh *Oreochromis niloticus* is highly perishable due to microbial growth, enzymatic activity, and oxidation, making proper preservation essential for maintaining fish quality and safety. This study aimed to evaluate the effectiveness of commercially available soybean oil as a natural preservative for fresh *O. niloticus* by assessing its antimicrobial activity and the physical appearance of the fish. A quantitative experimental design was used, wherein fresh tilapia samples were divided into three groups: soybean oil-treated samples, untreated negative control samples, and refrigerated positive control samples. Swab samples were collected from the gills, body, and gut at 2, 6, 10, and 24 hours, inoculated on Nutrient Agar and MacConkey Agar, and analyzed using colony-forming unit counts per plate (CFU/plate), One-Way ANOVA, and Tukey HSD post hoc test. In Nutrient Agar, significant differences ($p < 0.05$) among treatment groups were observed at all storage periods, with the soybean oil-treated samples showing lower bacterial counts than the negative control at 6, 10, and 24 hours. In MacConkey Agar, significant differences were observed at 2, 10, and 24 hours, while no significant difference ($p < 0.05$) was observed at 6 hours. The soybean oil-treated samples showed lower Gram-negative bacterial counts than the negative control at 10 and 24 hours, suggesting possible delayed and selective antimicrobial activity. However, the treatment did not consistently produce the lowest bacterial count in all time intervals and media. In terms of physical appearance, refrigeration remained the most effective method. At the same time, soybean oil-treated samples exhibited only moderate quality and did not differ significantly from untreated samples in most physical parameters. The study concludes that commercially available soybean oil showed limited, selective, and time-dependent antimicrobial potential, but it cannot fully replace refrigeration. Further studies using controlled soybean oil concentrations, phytochemical screening, bacterial identification, longer observation periods, and combined refrigeration treatment are recommended.

Keywords: *antimicrobial, delayed, preservative, soybean oil, treatment*

DEDICATION

First and foremost, the researchers humbly dedicate this study to Almighty God for granting us wisdom, strength, guidance, and perseverance throughout the course of this study. His blessings and grace gave us hope and courage during every challenge and difficulty we encountered. We also dedicate this study to our beloved families, friends, and relatives for their endless love, understanding, encouragement, and support. Their prayers and motivation inspired us to continue and do our best in achieving this work. We sincerely express our deepest gratitude to our research adviser for the guidance, patience, valuable knowledge, and support shared throughout the conduct of this study. Lastly, we dedicate this research to all individuals who became part of this journey and contributed in one way or another to the success of this study. Your help and encouragement are greatly appreciated and will always be remembered.

Ian, Glyce, Prince, Russelle

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INTRODUCTION

Background of the Study

Fish and fishery products are important contributors to food and nutrition security, income generation, and livelihood activities, especially in the Philippines. Nutrients are obtained from fish, including proteins, peptides, polyunsaturated fatty acids, vitamins, minerals, and other bioactive compounds that contribute to human well-being and food safety (Noreen et al., 2025). In addition, the fisheries industry in the Philippines is a source of income and job creation, providing employment opportunities for people working directly or indirectly in fisheries operations such as fishing, aquaculture, fish processing, packaging, and transport (Southeast Asian Fisheries Development Center [SEAFDEC], 2022).

On the other hand, fish is highly perishable and spoils quickly after harvest, despite its nutritional and economic importance. Some of the factors that lead to fish spoilage include enzymatic autolysis, lipid oxidation, and microbial growth, which influence the chemistry, sensory characteristics, and safety of fish products (Ghaly et al., 2010; Vijayan et al., 2021). In general, fresh fish is more prone to deterioration due to its moisture content, high water activity, and nutrient composition, making it susceptible to microbial infection and biochemical reactions (Tavares et al., 2021).

Mismanagement of fish increases the number of microorganisms in the food product, thereby lowering its quality, posing public health hazards, and causing economic impacts. Fish products are prone to pathogen contamination, enzymatic processes, oxidative changes, and the destruction of natural antioxidants, thereby affecting consumers and the economy (Almughamasi et al., 2026). The careful and effective handling of fish products is therefore important for maintaining their freshness and preventing deterioration.

Preserving fish is important for extending the shelf life of fish products and maintaining their quality, freshness, and sensory properties. Fish preservation techniques generally aim to inhibit and/or slow the growth of microorganisms, enzymes, and oxidation reactions (Nateras-Ramírez et al., 2026). The fish preservation technologies include freezing and chilling, dehydration, salting, smoking, fermentation, modified-atmosphere packaging, canning, and various inhibitory treatments (Ghaly et al., 2010; Nateras-Ramírez et al., 2026). However, fish preservation technologies can also affect the sensory properties of fish, such as appearance, smell, and flavour, influencing consumer acceptability (Nateras-Ramírez et al., 2026).

In recent years, plant-derived preservative agents have gained popularity as an alternative to chemical preservatives in foods. Bioactive compounds extracted from plants have been used to prevent lipid oxidation, inhibit bacterial growth, and improve the sensory quality of fish products (Presenza et al., 2023). The presence of phenolics, terpenoids, aldehydes, ketones, alcohols, and other oxygenated compounds in essential oils and plant extracts contributes to their antioxidant and antibacterial activities (Campolina et al., 2023). These bioactive compounds have been investigated for their potential use as preservatives

to enhance oxidative stability and antimicrobial activity in fish products and seafood (Hassoun & Çoban, 2017).

Soybeans contain isoflavones, which are biologically active phytochemicals that have attracted considerable attention from researchers. Isoflavones are bioactive compounds usually associated with the consumption of soy. There are various benefits associated with the intake of isoflavones. Among these benefits are estrogenic and antiestrogenic properties (Wang et al., 2013). As mentioned earlier, there are health benefits associated with the use of isoflavones and hence the importance of soy products in health studies. In this context, the commercial use of soybean oil, rather than lab-prepared soybean oil, is likely to affect the concentration of the active compound.

The Nile tilapia (*Oreochromis niloticus*) is an important fish in the Philippines. The fish is regarded as the second most important cultured fish in the country due to its widespread acceptance. This is because it adapts well to mass production in backyard and commercial aquaculture (Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development [PCAARRD], 2026). Nile tilapia is widely farmed for its rapid growth, resilience, and adaptability to warm climates (Romana-Eguia et al., 2020). In the Philippines, tilapia is inexpensive, readily available, and an excellent source of animal protein.

While prior studies have focused on the role of natural preservatives, essential oils, plant extracts, and other plant-derived bioactive compounds for enhancing the shelf-life and microbial stability of fish and seafood products, there is little research that has investigated the effectiveness of commercially available soybean oil as a natural

preservative in extending the shelf-life of fresh *O. niloticus*. Prior scientific studies have found the usefulness of plant-derived products in inhibiting lipid oxidation, microbial proliferation, and sensory attributes in fish (Presenza et al., 2023; Hassoun & Çoban, 2017).

Furthermore, soybean isoflavones have also been recognized as bioactive phytochemicals of soybeans (Wang et al., 2013). This study was therefore carried out to determine the efficacy of commercially available soybean oil as an antimicrobial agent for preserving fresh Nile tilapia. The role of soybean oil in reducing bacterial proliferation in *O. niloticus* was compared among soybean oil-treated, untreated, and refrigerated samples. The study sought to address a gap in the literature on the effects of commercially available soybean oil on tilapia preservation.

Objectives of the Study

The general objective of the study was to evaluate the effectiveness of commercially available soybean oil as a natural preservative for fresh *O. niloticus* (Nile tilapia) through antimicrobial assessment and appearance-based consumer acceptance. Specifically, it aimed to:

1. Determine and compare the bacterial load of soybean oil-treated, untreated, and refrigerated *O. niloticus* samples at 2, 6, 10, and 24 hours using Nutrient Agar and MacConkey Agar;
2. Evaluate the antimicrobial effectiveness of commercially available soybean oil based on colony counts expressed as CFU/plate and determine the time interval where bacterial growth reduction was most evident; and

3. Assess the consumer acceptance of soybean oil–treated, untreated, and refrigerated *O. niloticus* samples are based on appearance criteria, including skin coloration, scale condition, eye clarity, gill color, body shape, fin condition, presence of lesions or wounds, slime or mucus layer, and overall appearance.

Significance of the Study

The importance of this research was to evaluate the performance of commercially available soybean oil as a natural preservative for fresh *O. niloticus* (Nile tilapia). From a scientific point of view, the results of this study can be an important source of information on the antibacterial activities of natural oils in food preservation, as evidenced by the analysis of bacterial growth in CFU/plate at specific time points. Environmentally, the research championed the use of natural, eco-friendly ingredients as preservatives, eliminating potential hazards associated with synthetic additives in food products.

From a health-concerns perspective, the study provided useful insights into preserving fish without exposing consumers to hazardous compounds. From an economic standpoint, the use of widely available soybean oil made this procedure economical and practicable for local fish dealers without access to modern cooling equipment. If it works, this preservation process could be a useful tool in maintaining fresh fish.

Scope and Delimitation

The study was designed to determine the antimicrobial potential of commercially prepared soybean oil on fresh *O. niloticus* (Nile tilapia). The experimental groups were treated (application of soybean oil), untreated (control group), and refrigerated (positive control) samples. The refrigeration process was used as the standard for the experiment because it slows microbial activity and enzymatic spoilage. Before sample collection, fresh fish were isolated from other organisms using methylene blue.

Bacterial growth was analyzed at 2, 6, 10, and 24 hours, as microbial growth in food products is time-dependent and requires examination at specific intervals. Samples were taken from the gills, body, and intestines and placed on MacConkey Agar and Nutrient Agar. These locations were chosen because most microorganisms are found in the skin, gills, and intestines. Moreover, the abundance of bacteria in the gills and intestines makes them significant sites of interest for antimicrobial potential (Ju et al., 2022). The number of colonies (CFU/plate) was identified and compared among groups.

The commercial soybean oil product was used in the study instead of manually produced oil due to low yield rates and the lack of specialized equipment. Therefore, the level of antimicrobial substances remained unknown. Only antibacterial activity within 24 hours was tested, without further antifungal and antiviral studies, storage-influence analysis, or bacterial identification.

MATERIALS AND METHODS

Research Design

This study adopted an experimental research design to determine the efficacy of commercially available soybean oil as a natural preservative for fresh *O. niloticus* (Nile tilapia) due to its antimicrobial properties. Three experimental groups were used in the study: tilapia samples treated with soybean oil, tilapia samples refrigerated as a positive control, and tilapia samples without any treatment as a negative control. This helped determine bacterial growth in each sample group relative to the control groups.

Swab samples from the gills, body, and gut of the tilapia were aseptically collected at predetermined time intervals of 2, 6, 10, and 24 hours and plated on Nutrient Agar and MacConkey Agar to measure bacterial growth. Following incubation, the bacterial colonies were counted using the colony-forming unit per milliliter (CFU/plate) method. The bacterial colony counts of each experiment and time were recorded and analyzed. The bacterial colony counts for each experiment and time point were compared to assess the effectiveness of soybean oil as an antimicrobial agent relative to the control samples.

Research Setting

The experiment was conducted at the Natural Arts and Sciences Laboratory, Misamis University, Ozamiz City, 7200 Misamis Occidental (8.1497093°N, 123.8412491°E). Collection of the Tilapia fish (*Oreochromis niloticus*) was done in

Barangay Canicapan, Clarin, 7201 Misamis Occidental (8.2112°N, 123.8288°E). Soybean oil (commercially produced) was purchased from Ria Thrifty Mart, Zamora Street, Ozamiz City, 7200 Misamis Occidental (8.14°N, 123.84°E).

To prevent spoilage of the collected fish and facilitate safe transport, the specimen was placed in an icebox and transported to the Misamis University – Natural Arts and Sciences Laboratory, where the required equipment for the procedure was available. For preparation and bacterial culture, the laboratory at the Natural Arts and Sciences facility was used. The experiment was conducted using sterile conditions to obtain accurate results.

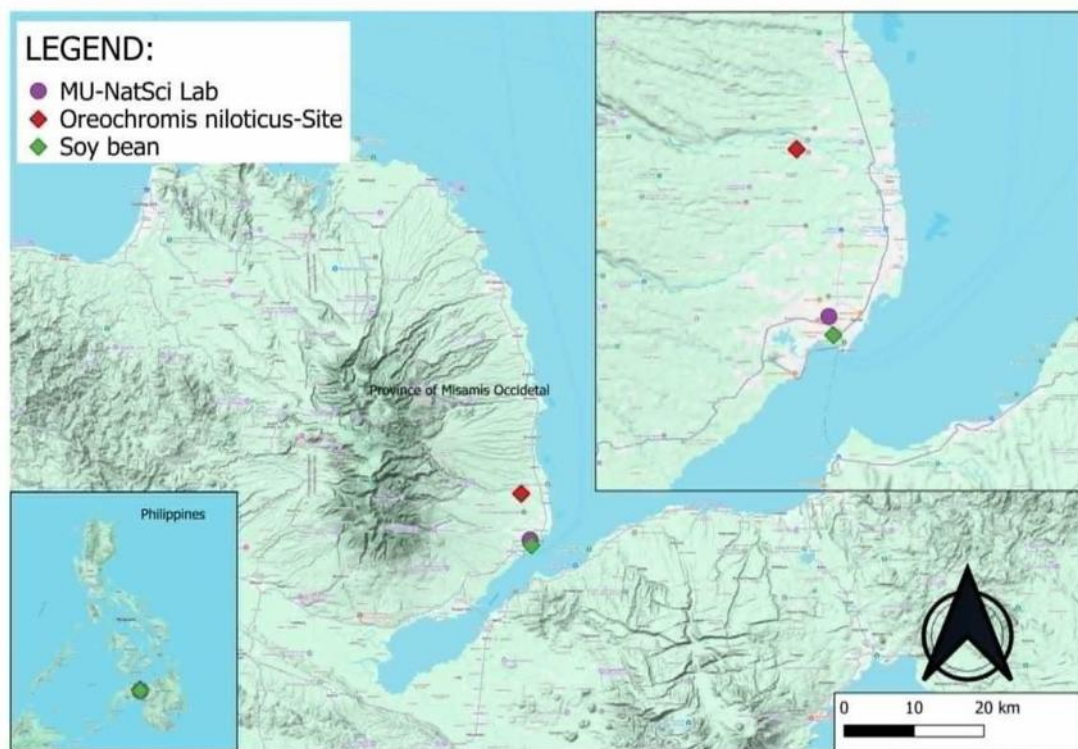


Figure 1. The map pinpointed the specific areas

Conceptual Framework

The conceptual framework of the study outlines the procedure used to determine the antimicrobial efficacy of commercially available soybean oil as a natural preservative for the freshness of *O. niloticus* (Nile tilapia) (Figure 2). The process started with the acquisition and processing of the soybean oil sample. This was followed by the preparation of the fish samples through isolation and selection, after which they were equally distributed into three groups: treated, untreated, and refrigerated. The three groups facilitated a comparison of the results of soybean oil treatment with those obtained using the refrigeration technique, which is known to reduce microbial growth rates (Mafe et al., 2024; Chu et al., 2024).

Application of soybean oil, storage, and observation of the results at 2, 6, 10, and 24 hours were performed, as microbial growth in fish depends on time (Ju et al., 2022). Bacterial growth rate was evaluated microbiologically by collecting swabs from the gills, body, and guts, which were later used to inoculate culture media and subsequently to determine colony-forming units per plate (CFU/plate). This is an internationally recognized parameter for measuring bacterial load in any sample (ISO, 2013).

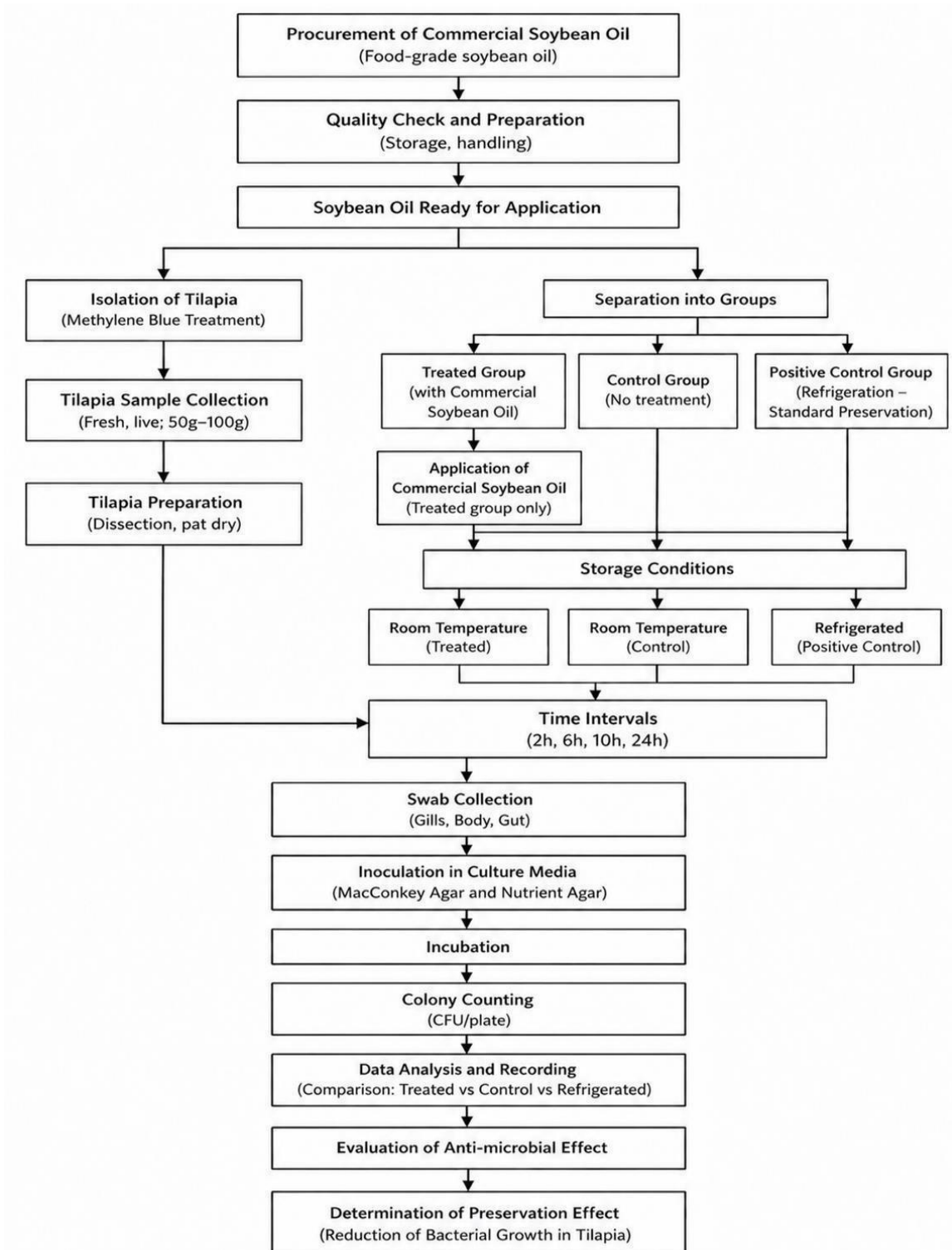


Figure 2. Schematic diagram of the study

Procurement of Commercial Soybean Oil

The test material in the research was commercial soybean oil, available in food-grade quality as Pure Soya Oil, obtained from a local company, Ria Thrifty Mart Store, in Ozamiz City (Figure 3). Soybean oil was selected because soybeans contain bioactive compounds such as phenolics and isoflavones, which have been associated with antioxidant and potential antimicrobial properties (Freitas et al., 2019). The use of commercially available soybean oil was more practical and accessible for experimental applications than laboratory-extracted oil.



Figure 3. Commercially sold soybean oil from the Ria Thrifty Mart Store

Preparation of Soybean Oil

The soybean oil used in the experiment was first examined for basic physical properties, including the presence of impurities or decomposition, before being stored in sterilized bottles. Proper care and preservation of edible oils are important because they

help maintain the product's physical stability and prevent microbial contamination (Choe & Min, 2006; Shahidi & Zhong, 2010).

Fish Sample Collection

Fish specimens were obtained from Canicapan, Clarin, Misamis Occidental. Live, healthy *O. niloticus* weighing 50-100 g were specifically selected to maintain sample consistency throughout the experiment. The use of fresh fish in microbiological studies helps minimize spoilage and allows for a more accurate determination of microbial load (Sarkar et al., 2020). In addition, methylene blue was used to disinfect the fish environment and prevent water contamination of the fish's reservoir (Figure 4). Methylene blue has been reported to possess disinfectant, antifungal, and mild antimicrobial properties in aquaculture, making it useful in reducing microbial contamination and protecting fish from opportunistic pathogens during handling procedures (Nipu et al., 2025; Macsen Laboratories, 2021). Moreover, a study showed methylene blue exposure improved resistance against *Aeromonas hydrophila* infection in fish (Khodadadian Zou et al., 2021).

The fish samples gathered were carefully handled to ensure they were in satisfactory condition before being transported.



Figure 4. Methylene blue obtained from an online shop

The results revealed that microbiological samples should be transferred immediately into an uncontaminated container to preserve their original microbial state before laboratory analysis (Nwiyi et al., 2012). In this study, contamination of containers was avoided by making sure that all sampling containers were pre-cleaned, disinfected, and properly sterilized before use. The containers were made of nonreactive, food-grade materials, rinsed with sterile water, and handled with clean gloves to avoid direct contact with human hands. After collection, they were immediately sealed and only opened during sampling to minimize exposure to environmental contaminants. Furthermore, hygienic handling principles similar to those used in fish transport, such as clean equipment, controlled handling conditions, and avoiding exposure to external contaminants, were also observed to preserve sample integrity (FAO, 1995; Scully RSV, 2022; Wang et al., 2021)

The fish samples were collected alive and kept in well-labelled Styrofoam boxes, which were lined and wrapped with plastic to prevent leakage and contamination during transportation to the laboratory. Transport of the fish samples to the laboratory occurred

immediately after collection to prevent bacterial growth from delayed handling (Sarkar et al., 2020).

The selection of the gill, body surface, and intestine for sampling was justified by microbiological literature indicating that these body parts harbor substantial bacterial flora and are widely used to assess microflora in fish. Conventional methods include the aseptic collection or swabbing of these body parts to measure the bacterial load in the fish (Fatima et al., 2014; Pakingking et al., 2015). A photo of *O. niloticus* from Barangay Canicapan, Clarin is shown in Figure 5)



Figure 5. *Oreochromis niloticus* (Nile tilapia) from Barangay Canicapan, Clarin

Tilapia Preparation and Soybean Oil Application

O. niloticus (Nile Tilapia) fish samples were humanely butchered within 20 minutes of arrival in the laboratory to ensure freshness and prevent changes in the microbial population. It is essential to process the samples immediately after harvesting to prevent bacterial multiplication and ensure that any changes in microorganisms are attributable to experimental conditions rather than to external contamination (Sarkar et al., 2020). Dissection of fish samples was performed under sterile conditions with sterile instruments,

and excess moisture and blood from their surfaces were wiped off with a sterile cloth. Dissection and handling of fish tissue samples under aseptic conditions are common procedures in microbiology to prevent contamination (Nwiyi et al., 2012). The prepared samples were then separated into three groups: soybean oil-treated, untreated negative control, and refrigerated positive control. In the soybean oil-treated group, the fish samples were fully immersed in commercially available soybean oil before the first observation interval. After each observation period, beginning at the 2-hour interval, swab samples were aseptically collected from the fish and inoculated onto the prepared agar plates. The inoculated plates were then incubated to allow bacterial colonies to grow. After inoculation, the same treated samples were re-immersed in soybean oil and kept for the next scheduled time interval; this procedure was repeated for the 6-, 10-, and 24-hour observations. The untreated control group did not receive any treatment, while the positive control group was stored under refrigeration. In accordance with the assigned conditions, all samples were covered with sterilized cheesecloth to prevent contamination during observation. Consistent procedures are crucial in comparative research so that differences among samples can be attributed to the treatment applied (Fatima et al., 2014).

After all necessary steps were completed, the samples were kept under different conditions and observed at 2, 6, 10, and 24 hours. This time-based assessment of microbial growth is very common in food microbiology because bacterial spoilage of fish occurs gradually and requires evaluation at specific time points to assess its effects (Ju et al., 2022).

Microbial Analysis and Colony Counting

For the study of bacteria in treated, untreated, and refrigerated *O. niloticus* (tilapia), conventional microbiological media, including Nutrient Agar (NA) and MacConkey Agar (MAC), were used for bacterial culture. These are commonly used in food microbiology to assess and differentiate bacterial colonies, where NA is used for general-purpose growth and MAC for selective and differential growth of Gram-negative enteric bacteria (Cappuccino & Sherman, 2014; Leboffe & Pierce, 2011).

After using commercial soybean oil and forming experimental groups, inoculating swabs were collected from the gills, body, and gut of the tilapia at 2-hour intervals (2, 6, 10, and 24 hours). Fish spoilage is a complex process driven by three primary mechanisms: enzymatic autolysis, oxidation, and microbial growth. To mitigate these effects, the industry currently relies on low-temperature storage and various chemical techniques to regulate water activity, thereby curbing enzymatic, oxidative, and microbial degradation. (Ghaly et al., 2010) This was chosen because of the large number of microbes in these organs, most of which are typically found in fish gills, skin, and intestine (Ju et al., 2022). After that, all swabs were inoculated onto the corresponding culture media at each time interval to determine the growth rate and antimicrobial activity of the treatment. Time-based inoculations are typical in food microbiology to observe the bacterial growth and determine if a particular treatment is bacteriostatic or bactericidal (Adams & Moss, 2008).

All inoculated plates were properly labelled concerning their respective treatment groups and incubation time. The experiment was repeated multiple times to improve data accuracy. Repeating the procedure several times increases the reliability of microbiological experiments, as replication is required in such research (Cappuccino & Sherman, 2014).

The number of viable bacteria was expressed as colony-forming units per plate (CFU/plate) and determined by counting visible bacterial colonies after incubation. Colony counts were performed after an adequate incubation period to allow the development of distinguishable, visible colonies on the culture medium. The data obtained after incubation were compared to assess the effectiveness of soybean oil as an antimicrobial agent and to determine whether it is bacteriostatic or bactericidal.

Consumer Acceptance through Physical Appearance Evaluation

The physical appearance of the tilapia samples was evaluated using sensory quality indicators adapted from the Food and Agriculture Organization (FAO) guidelines for assessing fresh fish. The parameters assessed included skin coloration, scale condition, eye clarity, gill color, body shape, fin condition, lesions or wounds, slime or mucus layer, and overall appearance. These indicators were used to determine the freshness and visible quality changes of the fish samples under different treatment conditions (FAO, 1989).

Data Processing and Statistical Analysis

Data were collected from each trial to assess the antibacterial activity of commercially available soybean oil against fresh *O. niloticus*. Moreover, the data were tested for normality using the Shapiro-Wilk test. Bacterial colonies were expressed as colony-forming units per plate (CFU/plate), a widely used method for determining bacterial populations in food samples (ISO, 2013).

CFU data for all test groups and time periods were summed to obtain the mean number of bacterial colonies. This allowed for better comparison of the bacterial growth patterns of the soybean oil-treated, control, and chilled samples. The experiments were replicated to increase the reliability of the results and minimize the probable errors (Montgomery, 2017).

$$\text{Mean Bacterial Colonies CFU/plate} = \frac{\sum x}{n}$$

Where: $\sum x$ = total number of colonies counted from all plates, n = number of plates observed

One-Way ANOVA and Tukey's Post Hoc test were employed to determine whether there were significant differences in bacterial counts among the sample groups at different time periods. To assess the impact of the treatment on bacterial growth, soybean oil-treated samples were compared with untreated and refrigerated samples. Changes in bacterial counts over time were also evaluated to determine whether soybean oil slowed bacterial growth or killed germs, depending on the extent of microbial growth inhibition (Adams & Moss, 2008). The results were tabulated to facilitate understanding and interpretation of the bacterial growth patterns.

RESULTS AND DISCUSSION

Mean Bacterial Counts Across Treatments and Time Intervals

Table 1 demonstrates differences in bacterial counts across the culture media, treatment groups, and time intervals. In Nutrient Agar (NA), the soybean oil-treated samples frequently had lower bacterial counts than the negative control at 6, 10, and 24 hours, suggesting that soybean oil may have contributed to reduced bacterial proliferation during prolonged storage. Although the negative control had the least amount at 2 hours, this does not mean that it was better maintained. Since fresh fish naturally have irregular bacterial counts on the skin, gills, and intestines, microbial counts may vary depending on the fish's initial state, handling, and sampling location. Other causes of fish spoilage include microbial growth, enzymatic activity, oxidation, and handling.

Table 1. Mean bacterial count (CFU) of fish under different treatments per observation time

Time Interval	Culture Medium	Positive Control	Negative Control	Treatment
2 hours	Nutrient Agar	138.20 ± 34.39 ^a	48.00 ± 27.50 ^b	122.00 ± 39.86 ^a
	MacConkey Agar	62.60 ± 14.06 ^a	17.20 ± 23.25 ^b	80.20 ± 34.90 ^a
6 hours	Nutrient Agar	136.80 ± 48.46 ^a	55.40 ± 9.34 ^b	66.60 ± 14.99 ^b
	MacConkey Agar	60.60 ± 30.22 ^a	29.20 ± 8.93 ^a	74.20 ± 42.68 ^a
10 hours	Nutrient Agar	74.60 ± 27.41 ^b	157.20 ± 31.64 ^a	56.60 ± 19.27 ^b
	MacConkey Agar	46.80 ± 23.57 ^b	88.60 ± 11.84 ^a	36.40 ± 18.04 ^b
24 hours	Nutrient Agar	40.00 ± 35.11 ^b	214.40 ± 41.09 ^a	51.60 ± 34.67 ^b
	MacConkey Agar	22.20 ± 22.84 ^b	120.80 ± 11.63 ^a	21.60 ± 18.28 ^b

Note: Means within the same row having different superscript letters (e.g., ^a, ^b) are significantly different at $p < 0.05$, while means sharing the same superscript letter are not significantly different based on the Tukey HSD post hoc test.

Bacterial loads can differ even before therapy (Sarkar et al., 2020). Similarly, the number of bacteria in the MAC increased in the soybean oil treatment group compared with the negative

control group at 2 and 6 h, but decreased at 10 and 24 h. This means that soybean oil's action on Gram-negative bacteria was not immediate but could be delayed and selective with time. The observed differences may also be associated with the natural bacterial population of tilapia, which can vary with culture conditions, handling, and storage practices (Bereded et al., 2022; Pakingking et al., 2015). Poor handling may further enhance bacterial contamination in Nile tilapia (Admasu et al., 2023). In general, the results indicate a partial, time-dependent antibacterial effect of soybean oil; however, it was not able to limit bacterial growth across all media and sampling times. The result is in agreement with studies that have demonstrated the utility of plant-based preservatives in extending the shelf life of seafood, but their efficacy depends on the concentration, formulation, storage conditions, and the specific bacteria present (Baptista et al., 2020; Kurek et al., 2024; Presenza et al., 2023). The effects of soy-derived compounds may also vary with bacterial species, chemical structure, and target site (Hummelová et al., 2015; Vázquez et al., 2017). Commercial refining may reduce some bioactive components important for antibacterial activity (Fine et al., 2016).

ANOVA and Post Hoc Results for Nutrient Agar

The statistical analyses discussed are shown in Tables 2 and 3. The One-Way ANOVA results for Nutrient Agar (NA) showed significant differences among the treatment groups at all storage periods: 2 hours, 6 hours, 10 hours, and 24 hours. This means the bacterial counts differed among the positive control, negative control, and soybean oil-treated samples. The increasing F-values from 2 hours to 24 hours also suggest that the differences among the groups became wider as storage time increased. Since NA supports the growth of general culturable bacteria, these results indicate that storage conditions and treatment affected total bacterial growth. This supports the idea that plant-derived compounds may help inhibit microbial proliferation in fish and seafood

products. The possible antimicrobial activity may also be linked to soybean bioactive compounds, since soy isoflavones are known phytochemicals with antimicrobial effects against some pathogenic biofilms. The document also shows that One-Way ANOVA and Tukey HSD were used to compare bacterial counts among soybean oil-treated, untreated, and refrigerated groups across time.

Table 2. ANOVA Results for NA (Across Treatments per Time)

Time	F-value	p-value	Interpretation
2 hrs	9.83	0.0030*	Significant
6 hrs	10.97	0.0020*	Significant
10 hrs	20.33	0.0001*	Significant

Note: *Significant difference at $p < 0.05$

The Tukey HSD results explained which groups were significantly different. At 2 hours, the soybean oil treatment did not differ significantly from the positive control. However, it did differ from the negative control, indicating that the early effect was not yet clearly favorable. At 6 hours, the treatment differed significantly from the positive control but not from the negative control, suggesting a possible early reduction in bacterial growth. At 10 and 24 hours, the negative control differed significantly from both the positive control and the treatment, whereas the positive control and the treatment did not differ significantly from each other. This means the soybean oil-treated samples maintained lower bacterial counts than the untreated group during extended storage and approached the refrigerated group. However, this should be interpreted as a selective, time-dependent antimicrobial response, not as complete bacterial inhibition. The effect may vary because bacterial responses depend on species, chemical structure, and target site (Fine et al., 2016; Vázquez et al., 2017), and commercial refined oils may contain lower levels of minor bioactive compounds that can affect antimicrobial strength.

Table 3. Tukey HSD Post Hoc Comparison for Nutrient Agar (NA)

Time	Comparison	p-value	Interpretation
2 hrs	Positive Control vs. Negative Control	0.0035	Significant
	Positive Control vs. Treatment	0.7411	Not Significant
	Negative Control vs. Treatment	0.0132	Significant
6 hrs	Positive Control vs. Negative Control	0.0026	Significant
	Positive Control vs. Treatment	0.0075	Significant
	Negative Control vs. Treatment	0.8256	Not Significant
10 hrs	Positive Control vs. Negative Control	0.0010	Significant
	Positive Control vs. Treatment	0.5497	Not Significant
	Negative Control vs. Treatment	0.0002	Significant
24 hrs	Positive Control vs. Negative Control	0.000	Significant
	Positive Control vs. Treatment	0.8751	Not Significant
	Negative Control vs. Treatment	0.0000	Significant

Note: Comparisons within the same time point having a significant interpretation indicate statistically different microbial counts at $p < 0.05$, while non-significant interpretations indicate no statistical difference between groups based on the Tukey HSD post hoc test.

ANOVA and Post Hoc Results for MacConkey Agar

Results from the ANOVA and Tukey HSD test for MacConkey Agar showed significant differences (Tables 4 and 5). The ANOVA results for MacConkey Agar (MAC) showed significant differences among the treatment groups at 2, 10, and 24 hours, but not at 6 hours. Since MacConkey Agar is used to culture and differentiate Gram-negative enteric bacteria that may originate from the fish gut, handling processes, or the environment, the results suggest that the treatments affected the growth of Gram-negative bacteria during certain storage periods. At 2 hours, the significant result may be due to an early difference in bacterial load among the samples. However, it should not be interpreted as clear soybean oil inhibition, as the treated group still had a higher bacterial count than the negative control. At 6 hours, the result was not significant,

meaning the bacterial counts among the groups were statistically similar. This may show temporary stabilization or adaptation of bacterial populations.

Table 4. ANOVA Results – MAC (MacConkey Agar)

Time	F-value	p-value	Interpretation
2 hours	8.10	0.0059	Significant
6 hours	2.84	0.0979	Not Significant
10 hours	11.21	0.0018	Significant
24 hours	49.34	0.000002	Significant

Note: p < 0.05 shows a significant difference

The Tukey HSD results showed that at 10 and 24 hours, the negative control significantly differed from both the positive control and the soybean oil-treated group. In contrast, the positive control and the treatment group did not differ significantly. This means that during longer storage, the soybean oil-treated samples had lower Gram-negative bacterial counts than the untreated samples and became closer to the refrigerated group. The most positive result was observed at 24 hours, with the treated group showing a possible delayed suppression of Gram-negative bacteria. However, this should be described as selective suppression and not immediate bactericidal action, because Gram-negative bacteria have an outer membrane that can limit the entry of antimicrobial agents. This is supported by studies showing that flavonoids and plant-derived antibacterial compounds may exert different effects depending on the bacterial type, cell wall structure, and compound properties (Górniak et al., 2019; Shamsudin et al., 2022). Thus, soybean oil showed a possible late-stage antimicrobial effect against MAC, but this effect was not consistent across all time intervals.

Table 5. Tukey HSD Post Hoc Comparison for MacConkey Agar (MAC)

Time	Comparison	p-value	Interpretation
2 hrs	Positive Control vs. Negative Control	0.0388	Significant
	Positive Control vs. Treatment	0.5381	Not Significant
	Negative Control vs. Treatment	0.0055	Significant
6 hrs	Positive Control vs. Negative Control	0.2748	Not Significant
	Positive Control vs. Treatment	0.7669	Not Significant
	Negative Control vs. Treatment	0.0908	Not Significant
10 hrs	Positive Control vs. Negative Control	0.0098	Significant
	Positive Control vs. Treatment	0.6558	Not Significant
	Negative Control vs. Treatment	0.0020	Significant
24 hrs	Positive Control vs. Negative Control	0.0000	Significant
	Positive Control vs. Treatment	0.9985	Not Significant
	Negative Control vs. Treatment	0.0000	Significant

Note: Comparisons within the same time point having a significant interpretation indicate statistically different microbial counts at $p < 0.05$, while non-significant interpretations indicate no statistical difference between groups based on the Tukey HSD post hoc test.

Physical Appearance Evaluation of Tilapia

The physical state of tilapia indicated that chilling was the optimal method for maintaining visual freshness, as shown in Table 6. The chilled positive control had higher marks for skin coloration, scale condition, gill color, body shape, fin condition, slime or mucus layer, and overall look. This is consistent with FAO sensory guidelines, which cite skin brightness, slime condition, eye clarity, gill color, and overall appearance as markers of fish freshness. In addition, in the Quality Index Method for Nile tilapia held in ice, sensory changes are useful indicators of freshness loss. Visual, microbiological, and instrumental indicators can verify fish quality.

Table 6. Physical Condition of Tilapia Based on Mean Scores and Sensory Quality Interpretation

Parameter	Positive Control	Soybean Oil Treated	Negative Control
Skin coloration	4.18 ± 0.90 → Bright, glossy, high freshness (Grade E–A)	2.82 ± 1.19 → Dull, Reduced pigmentation (Grade B)	2.94 ± 1.22 → Slight bleaching, early deterioration (Grade B)
Scale condition	4.12 ± 1.02 → Smooth, intact scales (Grade E–A)	2.97 ± 1.19 → Moderately rough, slight degradation (Grade B)	2.70 ± 1.20 → Roughened surface, reduced integrity (Grade B–Not graded)
Eye clarity	2.72 ± 1.49 → Moderately clear eyes, slight variability (Grade A–B range)	3.01 ± 1.36 → Moderately clear, transitional condition (Grade A–B)	2.94 ± 1.30 → Slight opacity, early freshness loss (Grade B)
Gill color	3.86 ± 1.25 → Red to dark-red gills, fresh condition (Grade E–A)	1.90 ± 1.06 → Pale/bleached gills, stress indicator (Grade B–Not graded)	1.90 ± 1.13 → Very pale gills, advanced deterioration (Not graded)
Body shape	4.22 ± 1.02 → Firm, well-formed body (Grade E–A)	3.53 ± 1.28 → Moderate firmness, slight softening (Grade A–B)	3.04 ± 1.31 → Reduced firmness, early \ deformation (Grade B)
Fins condition	4.18 ± 1.16 → Intact, crisp fins (Grade E–A)	3.67 ± 1.18 → Slight fraying, moderate quality (Grade A–B)	3.36 ± 1.34 → More frayed fins, reduced crispness (Grade B)
Lesions/ Wounds	3.94 ± 1.28 → Low blemishes, good surface integrity (Grade A–E)	3.42 ± 1.52 → Moderate lesions, Mild Stress (Grade B)	3.26 ± 1.40 → More visible lesions, deterioration signs (Grade B)
Slime/ Mucus layer	4.22 ± 0.89 → Transparent, protective mucus (Grade E–A)	2.72 ± 1.20 → Reduced mucus, slight opacity (Grade B)	2.54 ± 1.15 → Low mucus integrity, clotted/uneven (Not graded–B)
Overall appearance	3.98 ± 1.02 → Healthy, fresh appearance (Grade A–E)	2.93 ± 1.16 → Moderately stressed appearance (Grade B)	2.64 ± 0.96 → Dull, Reduced quality appearance (Grade B–Not graded)

Note: Grade E–A → high freshness, optimal fish condition (bright, firm, translucent features)

Grade B → moderate quality, early spoilage indicators (dullness, softening, opacity)

Not graded → advanced deterioration signs (loss of Mucus, pale gills, weak structure)

The physical quality of the soybean oil–treated samples was reasonable, although not as fresh as the chilled positive control. The negative control showed more indicators of deterioration, but the soybean oil-treated group did not differ substantially from the negative control on most metrics. This suggests that soybean oil alone did not significantly improve the physical appearance of tilapia. Studies on edible coatings and plant-based preservation reveal that natural methods can improve fish quality, but they usually require adequate formulation, controlled concentrations, and cold storage to be more effective (Jeon et al., 2002; Karsli et al., 2021; Kurek et al., 2024). Refrigeration remained the best option to keep the fresh appearance of tilapia.

Comparative analysis using Multi-parameter Evaluation and Tukey HSD Post Hoc for Physical Parameters

The comparison analysis and Tukey HSD post hoc results revealed that the treatment condition had a significant effect on the physical appearance of tilapia, as indicated in Table 7. ANOVA demonstrated significant variations in skin color, scale condition, eye clarity, gill color, body shape, fin condition, lesions or wounds, slime or mucus layer, and overall look. Therefore, the three groups differed in their physical state.

Table 7. Comparative Analysis of the Physical Condition of Fish Exposed to Soybean Oil and Control Treatments Using Multi-Parameter Evaluation

Parameter	F-value	df (2,27)	p-value	Decision
Skin coloration	32.41	(2,27)	<0.0001*	Reject H ₀
Scale condition	18.76	(2,27)	<0.0001*	Reject H ₀
Eye clarity	21.93	(2,27)	<0.0001*	Reject H ₀
Gill color	54.12	(2,27)	<0.0001*	Reject H ₀
Body shape	14.58	(2,27)	<0.0001*	Reject H ₀
Fins condition	9.87	(2,27)	0.0006*	Reject H ₀
Lesions/Wounds	11.44	(2,27)	0.0005*	Reject H ₀
Slime/Mucus layer	26.08	(2,27)	<0.0001*	Reject H ₀
Overall appearance	22.67	(2,27)	<0.0001*	Reject H ₀

Note: p <0.05 shows a significant difference

The Tukey HSD results indicated significant differences between the refrigerated positive control and both the soybean oil–treated and untreated groups across all physical measures (Table 8). This reinforces the importance of refrigeration in delaying microbial, biochemical, and sensory decline in fish, as chilling reduces microbial activity, enzymatic breakdown, and oxidative changes (Ghaly et al., 2010; Tavares et al., 2021). The variation in gill color is significant because gills are among the clearest markers of freshness. Fresh fish normally have crimson or brilliant red gills, whereas pale or discolored gills may indicate deterioration (FAO, 1989). However, most physical characteristics in the soybean oil treatment group did not differ substantially from those in the negative control group. This suggests that the soybean oil treatment did not clearly improve the fish's appearance compared with the no-treatment control. Thus, the physical appearance results should be considered separately from the microbiological data. The antibacterial potential

of soybean oil was limited to specified time intervals and did not consistently improve the visual quality of tilapia. This is consistent with research showing that natural preservative systems typically require proper formulation or combination with other preservation methods to maintain sensory quality (Kurek et al., 2024; Presenza et al., 2023; Speranza et al., 2021).

Table 8. Tukey HSD Post Hoc Test Results for Fish Physical Parameters

Parameter	Comparison	Mean Difference	p-value	Decision
Skin coloration	PC vs T	1.36	<0.001*	Reject H ₀
	PC vs NC	1.24	<0.001*	Reject H ₀
	T vs NC	0.12	0.61	Fail to reject H ₀
Scale condition	PC vs T	1.15	<0.001*	Reject H ₀
	PC vs NC	1.32	<0.001*	Reject H ₀
	T vs NC	0.17	0.48	Fail to reject H ₀
Eye clarity	PC vs T	0.29	0.03*	Reject H ₀
	PC vs NC	0.22	0.04*	Reject H ₀
	T vs NC	0.07	0.74	Fail to reject H ₀
Gill color	PC vs T	1.96	<0.0001*	Reject H ₀
	PC vs NC	1.89	<0.0001*	Reject H ₀
	T vs NC	0.07	0.82	Fail to reject H ₀
Body shape	PC vs T	0.68	<0.01*	Reject H ₀
	PC vs NC	0.91	<0.001*	Reject H ₀
	T vs NC	0.23	0.31	Fail to reject H ₀
Fins condition	PC vs T	0.51	0.004*	Reject H ₀
	PC vs NC	0.82	<0.001*	Reject H ₀
	T vs NC	0.31	0.09	Fail to reject H ₀
Lesions/wounds	PC vs T	0.52	0.003*	Reject H ₀
	PC vs NC	0.68	<0.001*	Reject H ₀
	T vs NC	0.16	0.41	Fail to reject H ₀
Slime/Mucus layer	PC vs T	1.50	<0.0001*	Reject H ₀
	PC vs NC	1.68	<0.0001*	Reject H ₀
	T vs NC	0.18	0.55	Fail to reject H ₀
Overall appearance	PC vs T	1.05	<0.0001*	Reject H ₀

Note: p= <0.05 significant difference

PC= POSITIVE CONTROL; NC=NEGATIVE CONTROL; T=TREATED

CONCLUSION AND RECOMMENDATIONS

Commercial soybean oil exhibited limited, time-dependent antimicrobial potential for preserving fresh *Oreochromis niloticus*. The bacterial counts in the treated samples were lower on Nutrient Agar at 6, 10, and 24 hours, whereas the effect was more pronounced on MacConkey Agar at 10 and 24 hours. However, the effect was not uniform across all time intervals, so soybean oil cannot be completely replaced by refrigeration. Refrigeration was still the best way to keep the fish fresh and visually appealing.

Further studies should identify the optimum dose using pure or controlled concentrations of soybean oil. Phytochemical screening is also suggested to confirm the presence of isoflavones, phenolics, and other antimicrobial compounds. Researchers should also identify the bacterial species present, increase the sample size, and extend the observation period beyond 24 hours. It is also recommended to compare soybean oil alone with soybean oil refrigerated better to assess its use as a supplementary natural preservative.

DECLARATION OF THE USE OF AI TOOLS

The authors would like to thank Grammarly for its use in the final phases of writing to assess the work for grammar, spelling, punctuation, sentence structure, coherence, and general clarity. The researchers carefully reviewed and selected the software-generated recommendations to improve the publication's readability, organization, and technical presentation. All changes and conclusions were at the sole discretion and responsibility of the investigators.

The authors also note the use of QuillBot for paraphrasing, phrase improvement, and grammar checking during the preparation of the book. The tool was used only to improve the clarity of language, reinforce the flow of sentences, and enhance the academic tone of selected portions of the document, without altering the original meaning.

The authors also mention the use of ChatGPT (OpenAI) as an additional academic writing and research support tool in the development of this study, entitled Soybean Isoflavones as a Natural Preservative Through Antimicrobial Action for Fresh *Oreochromis niloticus* (Nile Tilapia). I used ChatGPT to help organize ideas, improve the structure of some sections, paraphrase some assertions, enhance the publication's academic presentation, and explain the findings. It also aided in explaining and interpreting several aspects of the Background of the Study, Results and Discussion, Conclusion, and Recommendations sections, especially the bacterial count results, microbiological analysis, treatment comparisons, and the presentation of statistical results.

The types of stimuli utilized in the preparation of the work were:

- “Review the Background of the Study and the Results and Discussion sections, and suggest changes to improve sentence structure, coherence, transitions, interpretation of results, and overall academic clarity.”

- "Paraphrase selected statements for greater clarity, coherence, conciseness, and academic tone, without changing the original meaning."
- "Simplify the Results and Discussion section in layman's terms, but still be scientifically accurate. Interpret bacterial counts, treatment effects, and time intervals."
- "Make recommendations on how to present the results on bacterial growth, differences in microbial count, and the potential antimicrobial effect of soybean oil or soybean isoflavones on fresh Nile tilapia."
- "Assist in improving the wording of the conclusion and recommendations based on the results of the study."
- "Suggest clearer methods of presenting and linking the statistical results, including ANOVA, Tukey post hoc comparisons, and interpretation of significant differences among treatments".

The outputs from these artificial intelligence tools were used solely to provide supplemental assistance with drafting, language polishing, and preliminary structuring of the book. All generated responses were critically evaluated, revised, and validated by the researchers before inclusion in the study. The researchers had full responsibility for the accuracy of the information, originality of the work, interpretation of results to reflect the actual findings of the study, and the principles of academic integrity and responsible research writing.

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APPENDICES

Appendix A – Approved Letters / Forms



Misamis University
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY



CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PAGUCOA)

January 23, 2026

HAYDEE D. VILLANUEVA, PhD.
Dean, College of Arts and Sciences
Misamis University

Subject: **Requesting Approval to use Facility in Natural Sciences Laboratory for Research Purposes**

Dear Dr. Villanueva,

Warm Greetings!

We are the 3rd year BS in Medical Technology students and currently working on our research study entitled "Soybean Isoflavones as a Natural Preservative Through Anti-Microbial Action for Fresh *Oreochromis niloticus* (Tilapia)". This study aims to determine the anti-microbial action of soybean isoflavones to *O. niloticus* (Nile Tilapia). This is important since it will create a plant-based synthetic preservative as an alternative to traditional way of preserving as such will benefit to the community but also for our fellow researchers to further expound the study.

In this connection, we formally request your approval to grant access to the laboratory at the Natural Science Laboratory for research purposes pertaining to our ongoing study. The laboratory will be utilized strictly for experiments necessary for this research, including sample preparation, bacterial culture, and quantitative analysis. All experiments conducted will adhere to established laboratory protocols and safety guidelines to ensure compliance within institutional standards.

Should you require further details, you may contact us through 09686844701 and iancrisdula20@gmail.com.

Thank you very much. Hoping for your positive response on this matter.

Respectfully,

GLYCE AMACANIN

IAN CRIS DULA

PRINCE CIMA FRANCA

JUN RUSSELLE PAGLINAWAN

Noted by:

YUNALYN L. VILLANTES, PhD
Research Adviser

EVANGELINE M. SEÑEDO, RMT, MATMRS
Dean, College of Medical Technology

ARJADE G. ALCABAZA, LPT, RChT
Laboratory In-charge, NatSci

Recommending Approval:

NELFA D. CANINI, Ph.D.
Chair, Department of Natural Sciences

Approved by:

HAYDEE D. VILLANUEVA, PhD.
Dean, College of Arts and Sciences

Appendix A Cont'd



Misamis University

Ozamiz City

MISAMIS UNIVERSITY RESEARCH CENTER

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)



MU-RC-042/13 November 2024

Thesis/Research Data Gathering Approval Form

Date: 01/22/24

Name of Authors: Amorin, Gilda A.; Dela, Jan Cris; Cabañero, Prince Carl D.; Laginawan, Jun Russell
College/Department: College of Medical Technology
Research Title: Soybean Infection as a Natural Ferment through Anti-Microbial Action for Fresh Breadfruit (Tilapia)

Data Gathering Procedure:

a. Type of Research: (Kindly check)

☐ Quantitative	☐ Qualitative	☒ Experimental
☒ Approved Research Title & Problem/Objectives	☒ Approved Data Gathering Procedure	☒ Approved Data Gathering Instrument/Data Sheets

b. Respondents/Participants/Subject/Samples to be Collected: Tilapia, Soybean

c. Procedure:

☐ Survey	☐ Interview	☒ Experimentation	☐ Laboratory Testing
---------------------------------	------------------------------------	---	---

d. Place of Implementation/Study Area: Natural Science Laboratory at Misamis University

If to be conducted outside the school campus, indicate the nature of transportation to be used:

☐ Own private vehicle	☒ public utility vehicle	☐ rental	☐ Others, please indicate _____
--	--	---------------------------------	--

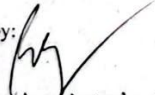
e. Date/s of Implementation/Sampling: February to April 2024

Attachments:

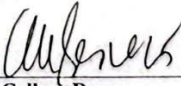
☐ Approved Letter of Consent from the designated authority in the area/place where the study is to be conducted
☒ Signed Parents' Consent

The thesis proposal mentioned above is hereby recommended for the commencement of data gathering implementation.

Recommended by:


Ms. Yunallyn Labajo Villanor
Thesis/Research Adviser


Mr. Huxia D. Canini
Thesis/Research Instructor

Approved by: 
College Dean

CONTROLLED DOCUMENT

Appendix B – Parent's Consent Form



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, GLICE A. MACANINI, to join the DATA GATHERING AND EXPERIMENT (activity) to be held on FEBRUARY to APRIL 2020 at MISAMIS UNIVERSITY under the supervision of MR. YUNALYN LABATO VILLANTES (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

Cristopher Amacanin
Signature over printed name of
Parent/Guardian

01/22/20
Date

GLICE A. MACANINI
Signature over printed name of
Student

01/22/20
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

JAN 22 2020

SUBSCRIBED AND SWORN to before me, this ____ day of ____ at Ozamiz City, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 46
Page No. 25
Book No. 12
Series of 20 W

ATTY. REY B. BINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

Notary Public

Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

Appendix B Cont'd



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Ian Cns Dula, to join the Research Data Collection and Experimentation (activity) to be held on February to April 2024 at Misamis University under the supervision of Ms. Yunallyn Labato Villanueva (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

NIDA S. SANCHEZ
Signature over printed name of
Parent/Guardian

22-1-26
Date

IAN CNS DULA
Signature over printed name of
Student

22-1-26
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this Jan 22 2024 at Ozamiz City, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 473
Page No. 84
Book No. 18
Series of 20 24

ATTY. REY B. BINAS, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLB Compliance No. VIII-0014127; Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

Appendix B Cont'd



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, PRINCE CARL D. CIMA FRANCA, to join the DATA GATHERING AND EXPERIMENT (activity) to be held on FEBRUARY TO APRIL 2026 at MISAMIS UNIVERSITY under the supervision of MS. YUNALYN LABATO VILLANTES (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

Rol Marina Laguer
Signature over printed name of
Parent/Guardian

01/22/26
Date

PRINCE CARL D. CIMA FRANCA
Signature over printed name of
Student

01/22/26
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 22 2026 at Ozamiz City, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 406
Page No. 63
Book No. 14
Series of 20 74

ATTY. FEY B. BINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCE Compliance No. VIII-0014127; Valid until 04/14/2028

Notary Public

Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

Appendix B Cont'd



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Paglinawan Jun Erselle L., to join the Pala gathering and experiment (activity) to be held on February to April 2026 at Misamis University under the supervision of Mrs. Vinalyn Cabala Villantes (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

ROSEL PAGLINAWAN
Signature over printed name of
Parent/Guardian

01 / 22 / 26
Date

JUN ERSILLE PAGLINAWAN
Signature over printed name of
Student

01 / 22 / 26
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 22 2026 at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

ATTY. REY B. BINAG, RMT

Notarial Commission No. 2025-18

Notary Public for Ozamiz City and Clarin

City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City

PTR No. 6007751; 01/05/2026; Ozamiz City

IBP O.R. No. 576165; 12/29/2025; Pasig City

Attorney's Roll No. 70648

MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

Doc. No. 412
Page No. 84
Book No. IX
Series of 20 24

Notary Public

Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

Appendix C – Adviser's Consultation Form



Misamis University

Ozamiz City



MISAMIS UNIVERSITY RESEARCH CENTER

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV

ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

MU-RC-043/19 August 2025

ADVISER'S CONSULTATION FORM

NAME OF THE ADVISER: YUNALYN L. VILLANTES, Ph.D.

TITLE OF THE STUDY: **SOYBEAN ISOFLAVONES AS A NATURAL PRESERVATIVE THROUGH ANTI-MICROBIAL ACTION FOR FRESH *OREOCHROMIS NILOTICUS*, LINNAEUS 1758 (NILE TILAPIA)**

NAME OF PROPONENTS: AMACANIN, GLYCE A., CIMA FRANCA, PRINCE CARL D., DULA, IAN CRIS, PAGLINAWAN, JUN RUSSELLE L.

Date of Consultation	Purpose	Adviser's Comments	Adviser's Signature
01/30/26	Consultation to conduct study	Prepare needed materials: Fish, Soybean, Materials for collection, and experimentation.	
02/12/26	Consultation for methods	Inquire for fish sources, isolation and pond treatment.	
03/02/26	Consultation for facility usage for experimentation	Write a letter an approval letter for conducting experiment at the laboratory.	
03/23/26	Consultation for survey format on fish evaluation	Use a mother journal to refer for physical appearance and refer to FAO for the grading.	
03/30/26	Consultation for Result	Provide the raw data and discuss everything during the experiment; use at least 30 RRL for all the discussion.	
04/24/26	Consultation for results and discussion	Revise the discussions and add more supporting related articles.	

**There must be at least 5 consultations before the proposal defense and at least 5 consultations before final defense.*

CONTROLLED DOCUMENT

Appendix D – Documentations



Extraction of Soybean Oil

Appendix D Cont'd



Extraction of Soybean Oil

Appendix D Cont'd



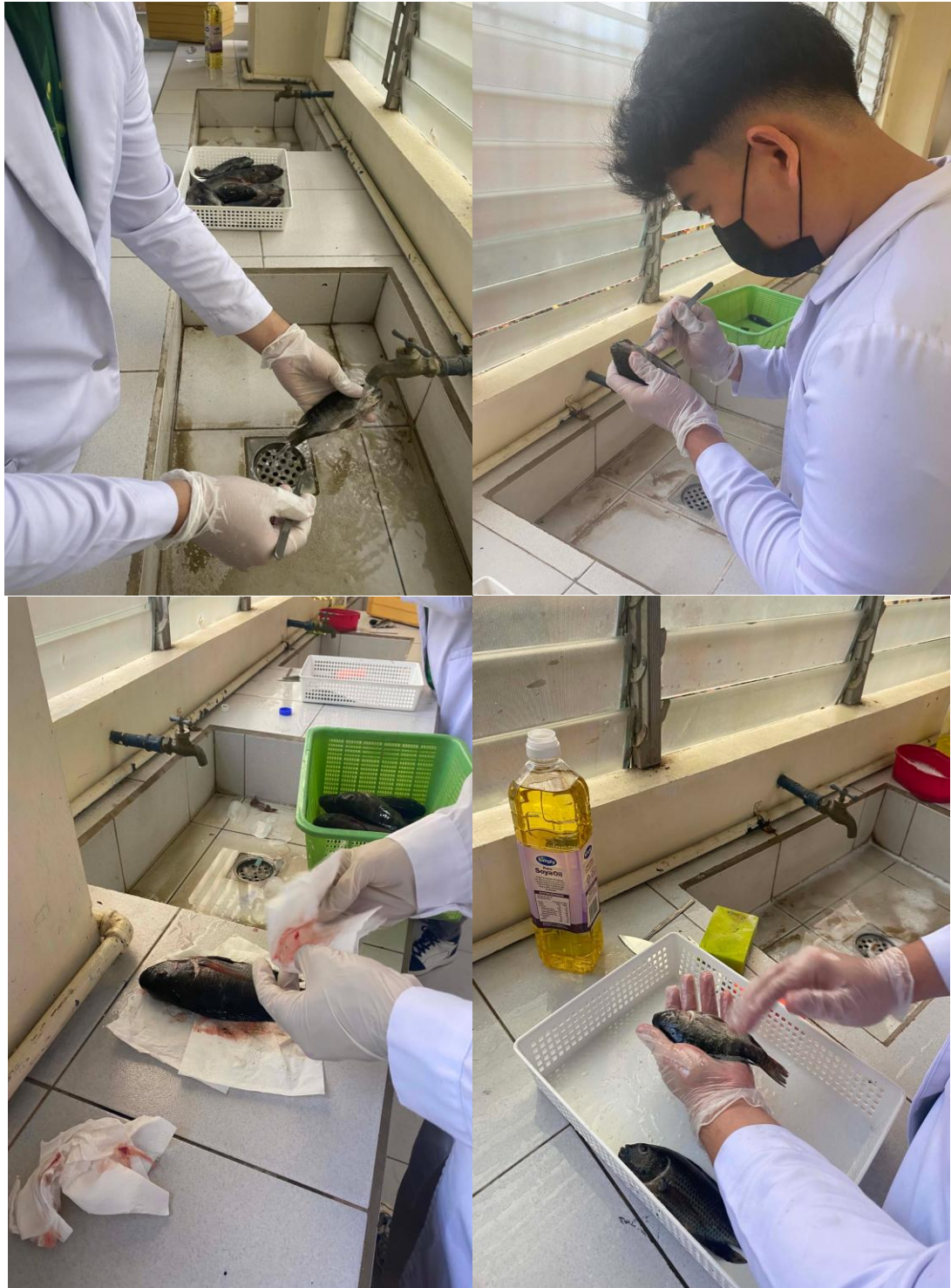
Extraction of Soybean Oil

Appendix D Cont'd



Isolation of Nile Tilapia Fish

Appendix D Cont'd



Treatment of Soybean in Nile Tilapia Fish

Appendix D Cont'd



Preparation For Culturing

Appendix D Cont'd



Preparation of Culture Mediums (NA and MAC)

Appendix D Cont'd



Inoculation of Nile tilapia fish

Appendix D Cont'd



Colony Counting of Cultured Fish Inoculated Samples

Appendix E – Sampling Computations

Table 1. Normality Test (Shapiro–Wilk) for Physical Parameters

Positive Control Group

Parameter	W	p-value	Interpretation
Skin coloration	0.911	0.286	Normal
Scale condition	0.870	0.099	Normal
Eye clarity	0.942	0.574	Normal
Gill color	0.816	0.023	✗ Not normal
Body shape	0.926	0.413	Normal
Fins condition	0.827	0.031	✗ Not normal
Lesions/Wounds	0.880	0.129	Normal
Slime/Mucus layer	0.912	0.294	Normal
Overall appearance	0.855	0.066	Normal (borderline)

Soybean Oil Treated Group

Parameter	W	p-value	Interpretation
Skin coloration	0.890	0.169	Normal
Scale condition	0.940	0.549	Normal
Eye clarity	0.991	0.998	Normal
Gill color	0.835	0.039	✗ Not normal
Body shape	0.870	0.101	Normal
Fins condition	0.959	0.779	Normal

Appendix E Cont'd

Lesions/Wounds	0.864	0.085	Normal
Slime/Mucus layer	0.911	0.288	Normal
Overall appearance	0.925	0.399	Normal

Negative Control Group

Parameter	W	p-value	Interpretation
Skin coloration	0.955	0.722	Normal
Scale condition	0.906	0.251	Normal
Eye clarity	0.925	0.400	Normal
Gill color	0.846	0.053	Normal (borderline)
Body shape	0.883	0.140	Normal
Fins condition	0.965	0.838	Normal
Lesions/Wounds	0.926	0.410	Normal
Slime/Mucus layer	0.952	0.686	Normal
Overall appearance	0.932	0.469	Normal

Legend:

- **W = Shapiro–Wilk statistic**
- **p = significance value**
- **$\alpha = 0.05$**
- **p > 0.05 = Normal distribution**
- **p < 0.05 = Not normally distributed**

Appendix E Cont'd

Statistical Interpretation

- Most parameters across all groups show $p > 0.05$, indicating that the data are approximately normally distributed at the participant level.
- A few exceptions were observed:
 - Gill color (Positive & Treated groups) → significantly non-normal ($p < 0.05$)
 - Fins condition (Positive group) → non-normal distribution
- The Negative control group showed the most consistent normal distribution, suggesting stable scoring patterns among participants.

Appendix F – Grammarly Report

**SOYBEAN ISOFLAVONES AS A NATURAL
PRESERVATIVE THROUGH ANTI-MICROBIAL
ACTION FOR FRESH *Oreochromis niloticus*,
Linnaeus 1758 (NILE TILAPIA)**

by Coify

General metrics

53,321	7,743	756	30 min 58 sec	59 min 33 sec
characters	words	sentences	reading time	speaking time

Score



This text scores better than 99%
of all texts checked by Grammarly

Writing Issues

15		15
Issues left	Critical	Advanced

Plagiarism

This text hasn't been checked for plagiarism

Appendix G – Turnitin Results



Page 2 of 52 - Integrity Overview

Submission ID trn:oid::28447:144466635

16% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

▸ Bibliography

Match Groups

-  **84 Not Cited or Quoted 12%**
Matches with neither in-text citation nor quotation marks
-  **36 Missing Quotations 4%**
Matches that are still very similar to source material
-  **0 Missing Citation 0%**
Matches that have quotation marks, but no in-text citation
-  **0 Cited and Quoted 0%**
Matches with in-text citation present, but no quotation marks

Top Sources

- 9%  Internet sources
- 7%  Publications
- 12%  Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

CURRICULUM VITAE

NAME : Glyce A. Amacanin
BIRTHDATE : March 1, 2005
BIRTHPLACE : Tudela, Misamis Occidental
ADDRESS : Purok 3, Taguima, Tudela, Misamis Occidental
PARENTS : Marelyn A. Amacanin
Cristopher P. Amacanin
CONTACT NUMBER : 09386163536
E-MAIL ADDRESS : amacanin.glyce@gmail.com

II. EDUCATIONAL BACKGROUND

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