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# Protein content and microbial quality of fish sauce in selected provinces of the Philippines

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ABSTRACT

Fish sauce, a traditional Asian condiment, is derived from the prolonged hydrolysis of salted small fishes, resulting in a mixture of amino acids and proteins. While fermented products are generally considered safe for consumption, ensuring the safety of public consumers remains paramount. Despite this concern, limited research exists in the Philippines on the protein content and microbial quality of fish sauce. Therefore, this study aims to assess the protein content, total halophilic count, and the presence of *Salmonella* spp. and *Staphylococcus aureus* in fish sauce produced by unregistered manufacturers in specific provinces of the Philippines. The Kjehldal method was employed to determine the crude protein content, and the organoleptic characteristics, including color, odor, flavor, and aftertaste liking, were evaluated by a panel of 10 sensory experts using a descriptive sensory score sheet. Furthermore, the acceptability of the samples was assessed through a 9-point hedonic scale. The results indicated that the protein content of the five fish sauce samples ranged from 1.00% to 2.06%. According to the required minimum standard set by the Department of Health (DoH) (4.5-2.5%), all fish sauce samples were classified as "fish sauce below standard." Regarding microbiological quality, the Philippines currently lacks standardized limits for fish sauce products. The halophilic count in the samples ranged from 308 to 930 cfu·g-1, while all samples yielded a Staphylococcal count of <10 MPN·g-1 and were negative for Salmonella spp. Furthermore, based on the 9-point hedonic scale, the acceptance score of the five fish sauces was found to be 5.0. This research sheds light on the protein content, microbial safety, and consumer perception of fish sauce produced by unregistered manufacturers in select provinces of the Philippines, emphasizing the need for establishing quality standards to safeguard public health.



## 1. Introduction

The interrelation between food and microorganisms has been a subject of profound interest and intrigue, dating back to prehistoric times long before written records were established (Espejo-Hermes, 1998). Consumed by consumers as a source of essential nutrients, foods serve as an excellent substrate for microbial growth. The outcome of this interaction is contingent upon the types of microorganisms involved, leading either to food spoilage or preservation through fermentation processes (Espejo-Hermes, 1998). Fermentation is scientifically characterized as the enzymatically facilitated decomposition of organic substances into more elementary constituents, primarily achieved through the activity of microorganisms (Lapsongphon et al., 2013). Fundamentally, fermentation plays a pivotal role in prolonging the shelf-life of food while concurrently augmenting its flavor and nutritional attributes (Visessanguan et al., 2004). Microorganisms are harnessed in the transformation of raw materials into fermented products (Tamang et al., 2016). However, it is essential to acknowledge that food items can also serve as potential reservoirs for disease transmission, underscoring the significance of detecting and controlling pathogens and spoilage organisms.

Pathogens rarely multiply at high salt concentrations. Fish sauce is among those classified as high-salt food (Ibrahim, 2010). As it is usually

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prepared by mixing salt with small fish, such as anchovies (*Stolephorus* spp.), sardines (*Sardinella* spp.), or round scad (*Decapterus macrosoma*) and allowing them to solubilize in closed containers or tanks at tropical temperatures for not less than six months (FAO/WHO, 2018). The important amino acid lysine is found in fish sauce, which is regarded as a rich source of protein. Fish sauce has a wide variety of vitamins and minerals, and it is particularly high in salt, calcium, magnesium, iron, manganese, and phosphorus. Fish proteins are reduced to tiny peptides during fermentation under the action of microbial enzymes (Nazari et al., 2021).

Fish sauce, a liquid seasoning commonly utilized in numerous Southeast Asian countries, including the Philippines, is produced via enzymatic fermentation (Sanceda et al., 2003; Sim et al., 2015; Hakimi et al., 2022). The bacteria *Staphylococcus aureus* can survive for many weeks in salted products. It is also known that many organisms survive in saturated salt solutions. For instance, *Salmonella* is found to thrive in a 10% salt solution for one to three months (Espejo-Hermes, 1998). In the Philippines, fish sauce or locally known as *patis* has been one of the popular fish products sold in the country (Gildberg et al., 1984; Park et al., 2001). While some commercially available fish sauces are manufactured by registered producers, the majority of locally available varieties are crafted by unregistered processors. The production of fish sauce (*patis*) by these unregistered manufacturers raises significant concerns, as their operations lack proper monitoring and regulation by relevant government agencies. Consequently, these products pose potential health risks to consumers.

The presence of halophilic bacteria and halotolerant pathogens at levels deemed unacceptable and unsafe not only compromises the quality of the product but also poses a threat to public health. Presently, the focus is on ensuring the overall quality of food, which entails not only nutritional balance but also microbiological safety. Accordingly, this study endeavors to ascertain the total halophilic count and the presence of *Salmonella* and *Staphylococcus aureus* in *patis* produced by unregistered manufacturers in Maasim Sarangani Province and General Santos City.

## 2. Materials and methods

### 2.1. Sample collection

A total of 250 ml samples of *patis* from five distinct manufacturers were procured and acquired from the public markets of General Santos City and Maasim Sarangani Province. These manufacturers were determined to be unregistered or unlicensed based on records from the Business Permit and Licensing Office of General Santos City and Sarangani Province. The collected samples, designated as Samples A, B, C, D, and E, were handled with utmost care and transferred into tightly sealed, sterile containers. Subsequently, they were transported to the Analytical Solutions and Technical Services (ASTS) laboratory facility in General Santos City. Upon arrival at the laboratory, the samples underwent immediate analysis.

### 2.2. Determination of protein content

The protein content of the samples (0.5-1.0 g) was analyzed using the Kjeldahl Method (AOAC, 2000). The amount of protein was computed as follows:

$$\text{Protein (\%)} = \frac{(A-B)(N)(14.007)(6.25)}{W}(100) \quad (1)$$

where, A=volume (ml) of 0.2 N HCl used sample titration; B=volume (ml) 0f 0.2 N HCl used of blank titration; N=Normality of HCl; W=weight (g) of sample; 14.007=atomic weight of nitrogen; 6.25=the protein-nitrogen conversation factor for fish and its by products.

### 2.3. Microbiological analysis

For microbiological analysis, samples of fish sauce weighing 10 g were homogenized with 90 ml of 0.1% peptone water utilizing a Stomacher® Lab Blender 400 (Seward Medical, London, U.K) for 30 seconds at a standard speed. Decimal dilutions were prepared using a sterile 0.1% peptone water solution. From the resulting homogenate, 1 ml was extracted for microorganism determination. Enumeration was performed using the pour plate and spread techniques as outlined in the Compendium of Methods for Microbiological Examination of Foods (APHA, 2001).

Halophilic bacterial counts were determined using Plate Count Agar medium (Oxoid-PCA) supplemented with 10% NaCl. Subsequently, the plates were incubated at 37°C for 3 days, after which the colonies were counted, and the colony-forming units (CFU) were computed using the following formula.

CFU/gram=*average of colonies per plate x dilution factor*

where, the dilution factor (df) for the pour plate method is calculated using the following formula:

*Pour plate method: df=10 for* $10^{-1}$

*Staphylococcus aureus* was enumerated using Baird Parker Agar (Oxoid CM 275) supplemented with egg yolk tellurite and incubated at 37°C for 24–48 h. After 24-28 hours of incubation, the plates were examined for the presence of *Staphylococcus aureus* growth.

To detect *Salmonella* spp., a 10 ml fish sauce sample was mixed with 90 ml of Lactose broth and homogenized for 1 minute, followed by incubation at 35°C for 24 hours. One milliliter of the pre-enrichment broth was inoculated into 10 ml of tetrathionate broth (TTB) and selenite and incubated for 24 hours at 35°C. After incubation in

TTB and selenite, each culture was streaked onto Xylose Lysine Deoxycholate (XLD) and Bovine Serum Albumin (BSA) plates to obtain isolated colonies, which were then incubated at 35°C for 24 hours. Subsequently, the plates were examined for suspected colonies of Salmonella.

### 2.4. Sensory evaluation

A sensory evaluation was conducted to assess the acceptability of the product with regard to appearance, odor, flavor, saltiness, and overall liking. The sensory panel comprised ten fourth-year BS-Fish Processing students from the College of Fisheries, Mindanao State University in General Santos City, all of whom had received training in Sensory Evaluation. They were tasked with evaluating the organoleptic properties of the fish sauce samples, focusing on color, odor, flavor, saltiness, and general acceptability. The assessment was performed using a nine-point hedonic scale, with the acceptance threshold set at a score of 5.0 for the evaluation (Sorio et al., 2020).

### 2.5. Statistical analysis

The sensory evaluation was carried out utilizing a Complete Randomized Design, and the data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test to ascertain any significant differences ($P<0.05$). All statistical analyses were conducted using IBM SPSS version 25. Additionally, the analyses were performed in triplicate to ensure the robustness and reliability of the results.

## 3. Results and discussion

### 3.1. Protein content

Table 1 shows the mean protein content of the five fish sauce samples. The mean protein content of samples A, B, and C were 1.19, 2.06, and 1.50 g·L$^{-1}$, respectively, while samples D and E had 1.00 g·L$^{-1}$. Fish sauce (*patis*) can be an important source of protein. Fish sauce with protein content below the minimum requirement of 4.5% but not below 2.5% shall be labeled as "fish sauce below standard" or "fish sauce flavor" (DoH, 1977). The protein content of the five fish sauce samples ranged from 1.00% to 2.06%. According to Espejo-Hermes (1998), fish sauce with a protein content below the minimum requirement of 4.5% but not below 2.5% shall be labeled as "*patis* below standard" or "*patis* flavor." Any liquid seasoning or flavoring with similar characteristics but whose protein content is less than 2.5% shall not be considered fish sauce and shall not be allowed to be sold under the name "*patis* flavor." Therefore, all samples from unregistered fish sauce manufacturers should not be considered fish sauce of commercial grade according to the fish sauce Standard of Identity and Quality (Espejo-Hermes, 1998).

**Table 1:** Mean crude protein content of fish sauce collected from five unregistered manufacturers in Sarangani province and General Santos City

| Sample code | %Crude protein (g·L$^{-1}$) |
|---|---|
| A | 1.19±0.01 |
| B | 2.06±0.03 |
| C | 1.50±0.25 |
| D | 1.00±0.10 |
| E | 1.00±0.10 |

Values are expressed as mean±SEM

### 3.2. Microbiological analysis

Table 2 shows the halophilic count of fish sauce samples from the five different unregistered fish sauce manufacturers. Sample E yielded the highest halophilic count of 930 cfu·g$^{-1}$. Sample A (308 cfu·g$^{-1}$) and sample B (436 cfu·g$^{-1}$) were observed to have lower halophilic count than Sample C, D, and E (880, 590, and 930 cfu·g$^{-1}$, respectively). For the presence of *Staphylococcus aureus and Salmonella* spp. in fish sauce samples, the results showed that all the unregistered fish sauce samples were found to be negative for the presence of *Staphylococcus aureus and Salmonella* spp. (<10 MPN·g$^{-1}$).

**Table 2:** Mean of Halophilic count, *Staphylococcus* and *Salmonella,* of fish sauce collected from five unregistered manufacturers in Sarangani province and General Santos City

| Sample code | Halophilic count (cfu·g$^{-1}$) | *Staphylococcus* (cfu·g$^{-1}$) | *Salmonella* (per 25 g) |
|---|---|---|---|
| A | 308±1.52 | <10 | Absent |
| B | 436±3.05 | <10 | Absent |
| C | 880±24.43 | <10 | Absent |
| D | 590±10.50 | <10 | Absent |
| E | 930±34.64 | <10 | Absent |

Values are expressed as mean±SEM

Presently, fermented fish and fishery products in the Philippines has no standard for microbiological limit (Sorio et al., 2020). The results of the halophilic counts in this study showed that Sample E yielded the highest halophilic count of 930 cfu·g$^{-1}$. The high microbial load could be due to the salt quality and raw material (microbiological and chemical) used in processing and the contamination brought about by poor postharvest handling practices. However, ocular inspection during the sample collection at the working area where Sample E was collected revealed that the processors were found to observe good manufacturing practices (GMP). Samples A and B were observed to have lower halophilic count (308 and 436 cfu·g$^{-1}$, respectively) than samples C, D, and E (880, 590, and 930 cfu·g$^{-1}$, respectively). This could be due to the different way of processing techniques used in fish sauce making and the raw material used. For instance, a high amount of salt was used since the application of high concentrations of salt can minimize the formation of bacteria (Jesebel and Erlinda, 2012). Halophilic bacteria are inherently adapted to saline environments, including those found in salted and fermented fish products.

Numerous studies have consistently detected the presence of halophilic bacteria in such fermented fish products. Gassem (2019) and Ibrahim (2010) enumerated a total of 4.32 log cfu·g$^{-1}$ and 2.0 log cfu ml$^{-1}$ halophilic bacteria in salted-fermented fish products, respectively.

*Staphylococcus aureus*, a halotolerant pathogen known to thrive in both human and animal environments, has the capacity for respiration or fermentation. It can potentially contaminate food during handling and preparation by a food handler, leading to growth and the production of enterotoxins (Gaudy et al., 1963). Thus, the presence of this bacterium or its enterotoxins in processed foods or on food processing equipment generally signifies poor sanitation practices.

In this study, all the fish sauce samples obtained from unregistered manufacturers were subjected to analysis and were found to be free from the presence of *Staphylococcus aureus*, with counts below the detection limit (<10 MPN·g-1). Similarly, the results of Salmonella detection in fish sauces from the five unregistered manufacturers revealed that all samples tested were negative for Salmonella spp. These findings indicate that the five unregistered fish sauce manufacturers implement effective sanitation procedures and adhere to good manufacturing practices, ensuring the production of fish sauce that complies with food safety standards and is available in public markets of Maasim, Sarangani Province, and General Santos City.

It is important to note that individuals who recover from infections caused by these pathogens may become carriers and continue to shed the organisms in their feces (Gaudy et al., 1963). While gastrointestinal disease due to foodborne *Salmonella* is sometimes referred to as food poisoning, it is more accurately termed *Salmonella* food infection, as symptoms only manifest after the pathogen grows in the intestine. Therefore, symptoms can be experienced several days after consuming contaminated food.

The absence of *Salmonella* contamination in all fish sauce samples analyzed in this study signifies that these products are safe for consumption and do not pose health risks to the general public.

### 3.3. Sensory evaluation

The sensory evaluation test was conducted on five fish sauce samples collected from unregistered manufacturers in Sarangani Province and General Santos City. The results revealed that the acceptance score, based on the 9-point hedonic scale, for all samples was 5.0. Fig. 1 illustrates the sensory attributes of the collected fish sauce.

Regarding the color attribute, Sample D obtained the highest score of 7.6±1.01, while Sample C received the lowest score of 4.8±0.67, with adjectival ratings of "like, very much" and "neither like nor dislike," respectively. The mean scores for the color attribute in Samples A, B, D, and E were above the limit score, indicating that these unregistered fish sauces were acceptable. However, the score for Sample C was significantly lower, and the panelists did not accept it. No significant difference (P>0.05) was observed in the color of the unregistered fish sauce samples.

For the odor attribute, Sample D received the highest mean score of 7.5±0.94, with an adjectival rating of "like, very much," but it showed no significant difference (P>0.05) compared to Samples A, B, C, and E.

Regarding the flavor attribute, Sample D obtained the highest mean score of 7.1±0.93, with an adjectival rating of "like, moderately." In contrast, Sample C had the lowest score of 4.6±0.53, with an adjectival rating of "neither like nor dislike." There was a significant difference (P<0.05) among the scores of all fish sauce samples concerning flavor.

For the aftertaste liking mean scores, Samples D (6.9±1.17) and E (6.0±0.87) scored above the limit of 6.0, with adjectival ratings of "like, slightly" and "like, moderately," respectively. Samples A (5.0±0.87), B (5.7±0.71), and C (4.5±0.50) scored below the limit of 6.0, with adjectival ratings of "neither like nor dislike," "like, slightly," and "neither like nor dislike," respectively. No significant difference (P>0.05) was observed among the scores of all fish sauce samples for aftertaste liking.

In terms of overall liking, Sample D obtained the highest mean score of 7.6±0.73, with an adjectival rating of "like, very much," followed by Sample B with a mean score of 6.6±0.88 and an adjectival rating of "like, moderately." Conversely, Sample C had the lowest overall liking mean score of 4.9±0.60, with an adjectival rating of "neither like nor dislike." Additionally, the 10 panelists selected for the evaluation noted the presence of sediment in Sample C.

Fish sauce typically exhibits a color ranging from yellowish to brownish (Lopetcharat et al., 2001). In this study, the color of all the samples was identified as amber-brown. Based on the results of the sensory test, all fish sauce samples were deemed acceptable, with the exception of Sample C (4.8±0.67). The acceptable limit in this study was set at 6.50, with an adjectival rating of 'like, moderately.' Panelists noted that Sample C had a cloudy appearance and the presence of sediment, rendering it unacceptable as a condiment.

Regarding the odor attribute, all fish sauce samples were considered acceptable, except for Sample C (5.1±0.60), which received an adjectival rating of "Neither like nor dislike." Panelists commented that Sample C had a strong and unacceptable odor as a condiment. On the other hand, Sample D obtained the highest average score of 7.5±0.94, with an adjectival rating of "like, very much." Panelists mentioned that it had a normal fishy odor and was suitable as a condiment. It is worth noting that fermented fish sauce can exhibit aromas ranging from ammoniacal and meaty to cheesy (Dougan and Howard, 1975; Yimdee and Wang, 2016). The aroma of fermented fish sauce is influenced by various elements, and microorganisms

may contribute to its creation (Bel-Rhlid et al., 2018; Xu et al., 2021).

Regarding flavor quality, Samples A, B, D, and E were rated higher than Sample C. Panelists observed that Sample C had an "extremely rancid flavor" compared to the other samples. The aftertaste liking for fish sauce Samples D and E was highly acceptable as a condiment, with all unregistered fish sauce manufacturers receiving the same aftertaste liking quality, according to the 10 panelists.

In terms of overall liking, Samples A, B, D, and E were considered acceptable, while Sample C was rated as unacceptable by the sensory panelists. Among the samples tested, Sample D was the most preferred, as panelists opined that it resembled commercially manufactured fish sauce.

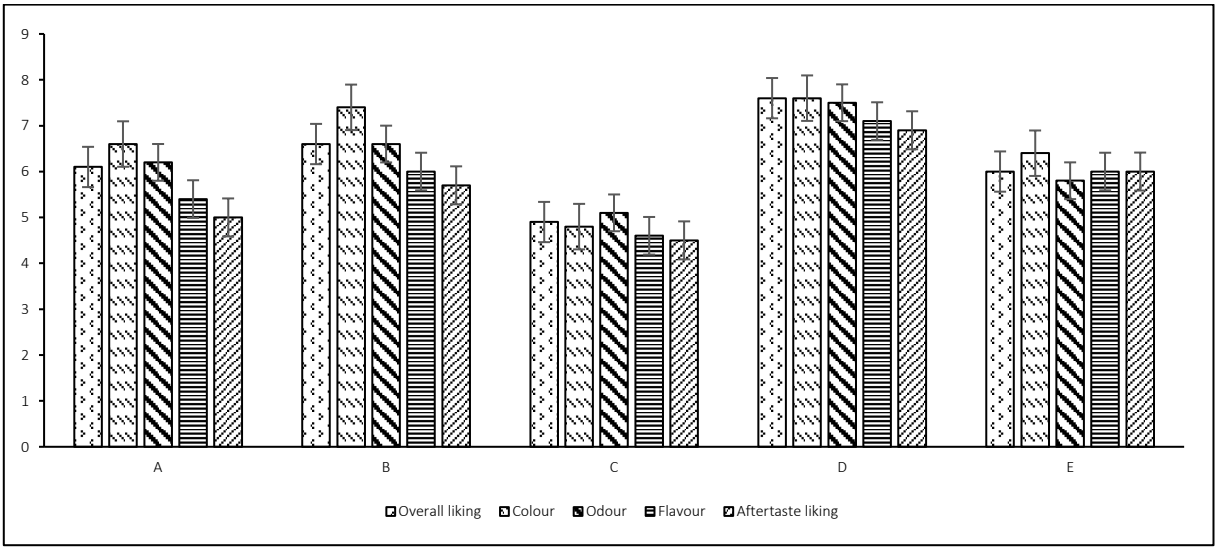


**Fig. 1:** Mean score of the overall liking, color, odor, flavor, and aftertaste from the acceptability test of the different samples collected from the unregistered manufacturers of fish sauce in Sarangani province and General Santos City; Values are expressed as mean±SEM

## 4. Conclusion

Inadequate adherence to hygiene and sanitation principles, along with contamination by human or environmental pathogens during food production, processing, and preparation, can render food microbiologically hazardous to consumers. In this study, all fish sauce samples from various unregistered manufacturers were found to be free of *Salmonella* and *Staphylococcus aureus*, while the halophilic counts were below the acceptable limit. Consequently, the fish sauce samples were deemed biologically safe for human consumption. However, it is noteworthy that the crude protein content in all fish sauce samples fell below the minimum standard requirement set by the Department of Health, indicating a significantly low protein content in fish sauce produced by unregistered establishments. Furthermore, based on the study's findings, fish sauce samples did not meet the minimum nutritional requirements mandated by government regulatory agencies. As a result, effective monitoring measures must be implemented to oversee local fish sauce production and ensure that the sauces being sold are nutritionally adequate.

To gain a more comprehensive understanding, it is recommended that further research be conducted on shelf life, total solids, proximate and nutritive value, and salt content analysis of fish sauce samples. Additionally, considering the observation of a biting taste in some fish sauce samples, histamine analysis is also suggested. These additional investigations will contribute to a thorough evaluation of fish sauce quality and safety, fostering enhanced consumer protection and promoting overall food security.

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## Compliance with ethical standards

## Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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