EFFECTS OF VARIOUS CONCENTRATIONS OF *Scomber japonicus* ON THE NUTRITIONAL VALUE AND MICROBIAL LOAD OF FISH ROLL

BY

Holiness Ovbokhan OMORUYI (Miss)

AGR20043A85

DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT.
FACULTY OF AGRICULTURE
UNIVERSITY OF BENIN
BENIN CITY

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THE DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

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SUPERVISOR: DR. KENNETH OMORUYI

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CERTIFICATION

This is to certify that this project titled "**Effects of various concentrations of scomber Japonicus on the Nutritional value and microbial load of fish roll**" was carried out by **Holiness Ovbokhan OMORUYI** with matriculation number **AGR2004385** of the department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria.

_____ _____

DR. KENNETH OMORUYI **DATE**
Project Supervisor

_____ _____

DR. M. EGWENOMHE **DATE**
Head of Department

DEDICATION

This project is dedicated to the Almighty God, whose divine grace, wisdom, and strength have been my guide throughout every phase of this work. I also dedicate it to my loving parents, my dear siblings, and spiritual parents whose prayers, encouragement, and steadfast support have continually inspired me. Lastly, I extend this dedication to all students and researchers in Aquaculture and Fisheries who are devoted to advancing sustainable fish farming practices for a brighter and more sustainable future.

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ABSTRACT

Fish rolls in Nigeria are nutritionally imbalanced, and the impact of varying fish concentrations on their microbial safety and shelf life is not well understood. This study was carried out to assess the nutritional composition and microbial quality of fish rolls made with *Scomber japonicus* concentrate. Five formulations were prepared with *S. japonicus* fish concentrate (0%,10%, 20%, 30%, 40%), Standard analytical methods (AOAC, 2020) were used to determine the proximate composition, while microbial analysis was performed using standard microbial techniques to determine microbial and fungal contamination levels. There was significant ($P<0.05$) increases in protein, fat, ash, and moisture contents with higher levels of inclusion. Protein content increased from 9.77% to 25.54%, while carbohydrate content decreased correspondingly from 58.64% to 25.48%. Bacterial counts ranged from 3.7×10^2 to 1.0×10^3 cfu/g, and fungal counts from 0.2×10^2 to 1.7×10^2 cfu/g, all below the recommended limits of 5×10^5 cfu/g set by the FAO. (ICMS, 2002). The major bacterial isolates included Bacillus sp., Staphylococcus sp., Pseudomonas sp., and Enterobacter sp., while Aspergillus sp., Saccharomyces sp., and Penicillium sp. were the predominant fungi. The study suggest that fish rolls made with *S. japonicus* significantly enhances their nutritional value, without exceeding acceptable microbial limits. An optimal fortification level of 20% fish concentrate was recommended to balance nutritional quality, sensory acceptability, affordability and microbial safety.

CHAPTER ONE

1.0 INTRODUCTION

Fish is a widely consumed food globally, providing a rich source of protein, omega-3 fatty acids, and various essential nutrients (Akande and Dieyan, 2015; FAO, 2020). Fish, a nutrient-dense food, plays a vital role in promoting human health, supporting sustainable food systems, and contributing to global food security, with its consumption being associated with numerous health benefits, including reduced inflammation, improved cardiovascular health, enhanced cognitive function, anti-cancer properties, a lower risk of chronic diseases, supports brain development, eye health, and immune function, making it an essential component of a balanced diet and a crucial part of a healthy and sustainable food culture (Akande and Dieyan, 2015; FAO, 2020; Miller *et al.,* 2014; Sarris *et al.,* 2014).

Eating fish regularly can help lower triglycerides, reduce blood pressure, and prevent blood clots, all of which can contribute to a reduced risk of heart disease (Miller *et al.,* 2014; Rizos *et al.,* 2012). Omega-3 fatty acids found in fish, particularly EPA and DHA, have been shown to support brain health and cognitive function, and may even help reduce symptoms of depression and anxiety (Sarris *et al.,* 2014; Grosso *et al.,* 2016). Omega-3 fatty acids in fish may help reduce the risk of age-related macular degeneration and other eye diseases (SanGiovanni *et al.,* 2007). Some studies suggest that eating fish regularly may reduce the risk of certain types of cancer, including colorectal, prostate, and breast cancer (Larsson *et al.,* 2011; Zheng *et al.,* 2013). Fish consumption has been shown to reduce inflammation in the body, which can help alleviate symptoms of conditions such as rheumatoid arthritis and asthma (Simopoulos, 2002).

Fish based ready-to-eat food products are made from fish or fish-derived ingredients, processed and packaged in various forms, such as dried, smoked, cured, breaded, fried, or extruded, intended for consumption as a snack or light meal (EUFIC, 2019; Tacon and Metian, 2013).

Fish-based snacks are a popular choice among students in secondary schools due to their affordability, convenience, and nutritional value. However, the handling, storage, and preparation of these snacks can pose significant health risks if proper food safety practices are not followed. The microbial load of these snacks can be a major concern, as bacteria, viruses, and other microorganisms can cause foodborne illnesses (WHO, 2017).

Benin City, being a densely populated urban area, has a high demand for convenient and affordable food options, including fish-based snacks. Secondary school students, in particular, are vulnerable to foodborne illnesses due to their immature immune systems and lack of awareness about food safety practices. the processing, handling, and storage of fish-based snacks can pose significant health risks due to contamination. The processing, handling, and storage of fish-based snacks, such as fish rolls, can pose significant health risks due to contamination. Fish rolls can be contaminated with various types of microorganisms, including bacteria like Salmonella, Staphylococcus aureus, and Vibrio (FDA, 2020), as well as viruses like Norovirus and Hepatitis A (CDC, 2022). These contaminants can be transmitted to humans through consumption of contaminated fish rolls, leading to foodborne illnesses.

Contaminated fish-based snacks can cause food poisoning, leading to symptoms like nausea, vomiting, diarrhea, and stomach cramps. Treating food poisoning and infections from contaminated fish-based snacks can result in significant medical expenses for families (WHO, 2015).

1.1 JUSTIFICATION OF STUDY

In Nigeria, fish contributes significantly to food and nutrition security, serving as an accessible source of protein for a large proportion of the population (Adewumi *et al.,* 2018; Eyo *et al.,* 2019). However, despite its nutritional importance, most bakery and snack products commonly consumed in the country such as fish rolls are predominantly carbohydrate-based with low protein content. This imbalance contributes to widespread protein-energy malnutrition, particularly among school-aged children and low-income earners (Saweda *et al.,* 2020; Olaniyan *et al.,* 2020). Therefore, there is a need to develop strategies to improve the nutritional quality of such snacks through the use of affordable, locally available, and protein-rich ingredients like *Scomber japonicus* (chub mackerel).

Previous studies have shown that snacks made with fish concentrates have higher protein, lipid, and mineral contents, thereby enhancing their overall nutritional value (Ajala *et al.,* 2015; Fasoyiro *et al.,* 2005). Scomber japonicus is a fatty fish species that is widely available in Nigeria and known to contain high-quality proteins, omega-3 fatty acids, calcium, phosphorus, and iron (Haug *et al.,* 2016). Fish rolls made with *S. japonicus* are expected to have an improved protein-to-carbohydrate ratio, resulting in a nutritionally balanced product that supports tissue repair, immune function, and brain development (Rasane *et al.,* 2015; Mozaffarian *et al.,* 2016). Developing fish rolls made with Scomber japonicus could therefore contribute to addressing the challenges of protein deficiency and micronutrient malnutrition, especially among vulnerable groups.

Despite its nutritional benefits, fish and fish-based products are highly perishable due to their high water activity and nutrient composition, which favour microbial proliferation (Adams and Moss, 2015; Getu *et al.,* 2015). Poor handling, processing, and storage practices commonly associated with street-vended and locally produced snacks can lead to contamination by pathogenic microorganisms such as *Staphylococcus aureus*, *Bacillus* spp., *Salmonella*, and

Pseudomonas spp., which are capable of causing foodborne diseases (WHO, 2017; FAO, 2018). In urban areas such as Benin City, where fish rolls are widely consumed, maintaining microbial safety and hygienic standards remains a challenge. Hence, the need to investigate how different concentrations of *S. japonicus* affect not only the nutritional composition but also the microbial load of fish rolls. This research will help establish the optimal concentration that enhances nutritional value while maintaining food safety and shelf stability.

The development of fish rolls made with *S. japonicus* represents an important avenue for reducing post-harvest losses, improving income generation for fish processors, and diversifying fish utilization (Venugopal *et al.*, 2015; FAO, 2018). By scientifically determining the most suitable concentration of *S. japonicus* to use in snack production, this study will contribute to the advancement of functional food development, improve consumer health, and support Nigeria's goal of achieving food and nutrition security.

1.2 Aim and Objectives of the Study

The study is aimed at investigating the effects of various concentrations of *Scomber japonicus* (chub mackerel) on the nutritional value and microbial load of fish rolls.

The specific objectives of the study are to:

1. determine the proximate composition (moisture, protein, fat, ash, fibre, and carbohydrate contents) of fish rolls made with different concentrations of Scomber japonicus.
2. evaluate the microbial load (bacterial and fungal counts) of fish rolls made with various concentrations of *Scomber japonicus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fish production and trade in Nigeria

The estimated annual average per capita fish consumption for Nigeria is 13 Kg (Saweda, *et al.*, 2020; FAO, 2021), although the ECOWAS Commission, (2020) reported a lower estimate tonne of fish had been produced. In the same report, the fishery sector tends to have done better in 2017 with the annual average per capita fish consumption for Nigeria is of 8.33 Kg. Fish remains an important dietary element for of animal protein available to many Nigerians (FAO, least was recorded in 2015 (DARDECOWAS where fish is highly valued and one of the cheapest sources 2021). The fishery sector is 1.09% of the national GDP in Fish Production in Nigeria: The estimated Directorate of Agriculture and Rural Development

highest tonnes of fish produced of about 1,212,480 tonnes of fish produced were recorded in 2020 while the 2020 and 0.97% in the Q3 of 2021 (NBS, 2021). DARD sectors shows that between 2015 and 2020, 6,861,700 which comprise 17.67% of the total tonnes of fish produced within the years in considerations; the second-highest 13 Kg (Saweda, *et al.*, 2020; FAO, 2021). The country's fisheries sector is comprised of both artisanal and industrial fishing, as well as aquaculture (Adewumi *et al.*, 2018). According to the Nigerian Ministry of Agriculture and Rural Development (2019), the fisheries sector contributes significantly to the country's GDP and provides employment opportunities for millions of people. Aquaculture is a growing sector in Nigeria, with tilapia and catfish being the most commonly farmed species (Olaniyan *et al.*, 2020).

The trade of fish and fish products in Nigeria is also significant, with both domestic and international markets (Eyo *et al.*, 2019). The fish production and trade sector significantly contribute to Nigeria's economy, generating employment opportunities for millions of people

and providing a vital source of income for households. The sector also plays a crucial role in ensuring food security, supplying protein-rich fish to the population. Additionally, fish exports earn foreign exchange for the country, further boosting the economy. According to the Food and Agriculture Organization (FAO, 2020),

2.2 Health Benefit of Consuming Fish

One of the most significant benefits of fish consumption is its impact on heart health. The omega-3 fatty acids found in fish have been shown to reduce inflammation, improve blood lipid profiles, and lower blood pressure, all of which can contribute to a reduced risk of cardiovascular disease (AHA, 2017; Mozaffarian *et al.,* 2016). Fish is also a rich source of essential nutrients, including protein, vitamin D, and selenium. These nutrients are important for maintaining healthy muscles, bones, and immune function (IFIC, 2020). Additionally, fish consumption has been linked to improved cognitive function and a reduced risk of dementia and Alzheimer's disease (Kalmijn *et al.,* 2004; Barberger-Gateau *et al.,* 2007).

Fish consumption has also been associated with improved mental health outcomes. The omega-3 fatty acids found in fish have been shown to alleviate symptoms of depression and anxiety, and may even help to reduce stress and improve mood (Szymanski *et al.,* 2013).

2.3 Value added fish Products

Value-added fish products have gained popularity in recent years due to their convenience, nutritional value, and unique flavors. Value-added fish products are processed fish products that have been modified to enhance their quality, texture, and flavor (Dey *et al.,* 2018). These products can range from fish fillets and nuggets to fish sausages and burgers. Value-added fish products are often pre-processed or ready-to-eat, making them appealing to busy consumers who are looking for quick and easy meal solutions (Kumar *et al.,* 2020). Additionally, value-added fish products can be formulated to meet specific nutritional needs or dietary preferences,

such as low-sodium or gluten-free products. Fish products fortified with omega-3 fatty acids can provide health benefits for heart health and brain function (Kris-Etherton *et al.,* 2002).

The production of value-added fish products requires careful consideration of food safety and quality control measures. Fish processors must implement good manufacturing practices (GMPs) and hazard analysis and critical control points (HACCP) systems to ensure the safety and quality of their products (Venugopal *et al.,* 2015). This includes proper handling and storage of raw materials, as well as regular testing for contaminants and spoilage. Value-added fish products can also play a significant role in promoting sustainable fisheries and aquaculture practices. The development of value-added fish products can help reduce food waste and increase the economic value of fish catches (FAO, 2018). By utilizing fish by-products and creating value-added products, fish processors can reduce their environmental impact and contribute to more sustainable food systems.

2.4 Fish based Snacks

Fish-based snacks have become increasingly popular due to their convenience, nutritional value, and delicious taste. However, the safety of these snacks is a growing concern. Fish can be contaminated with biological hazards like bacteria, viruses, and parasites, which can cause foodborne illnesses (FAO, 2018; CDC, 2020). For instance, bacteria like Salmonella and Vibrio can cause severe gastrointestinal illness, while parasites like Anisakis can cause anisakiasis (CDC, 2020). Chemical contaminants like mercury and polychlorinated biphenyls (PCBs) can also accumulate in fish-based snacks and pose health risks to consumers (WHO, 2017).

The risk of foodborne illnesses from fish-based snacks can be minimized by implementing safe handling and processing practices. Manufacturers must follow good manufacturing practices (GMPs) and hazard analysis and critical control points (HACCP) systems to identify potential

hazards and prevent contamination (FAO, 2018). This includes ensuring that fish-based snacks are stored and handled properly, and that equipment and facilities are regularly cleaned and sanitized. Consumers also play a crucial role in ensuring the safety of fish-based snacks. They should check expiration dates, store snacks properly, and cook snacks to the recommended internal temperature to prevent foodborne illnesses (CDC, 2020). Foodborne illnesses can have serious consequences, including hospitalization and even death (WHO, 2017).

In recent years, there have been several outbreaks of foodborne illnesses associated with fish-based snacks. For example, in 2019, a outbreak of Salmonella was linked to frozen fish sticks (CDC, 2020). This outbreak highlights the importance of proper handling and processing practices in the production of fish-based snacks. To minimize the risk of foodborne illnesses, it's essential to implement proper handling, storage, and processing practices in the production of fish-based snacks. This includes following good manufacturing practices (GMPs), hazard analysis and critical control points (HACCP) systems, and regular testing (FAO, 2018).

2.5 Fish Spoilage

Fish annually contributed approximately 25% of gross primary agricultural and fishery product caused by the following three reasons: enzymatic autolysis, microbial deterioration and Fish are considered almost highly perishable food commodity. Fish spoilage can be chemical activities. Deterioration mainly caused by chemical and microbial activities has losses (Getu, *et al.*, 2015). Fish spoilage is a complex process involving enzymatic autolysis, oxidation, and microbial growth. Enzymatic autolysis occurs when enzymes naturally present in the fish break down complex molecules like proteins and lipids, leading to softening of flesh, loss of flavor and aroma, and changes in texture. Microbial growth, on the other hand, involves bacteria like Pseudomonas, Proteus, and Chromobacterium that degrade proteins, carbohydrates, and fats in fish, producing undesirable compounds with strong odors and flavors. Oxidation, particularly

in fatty fish, leads to rancidity and off, flavors due to the reaction of oxygen with fats (Ghaly *et al.,* 2010; Jay, 2019). The spoilage process can be influenced by various factors, including type of fish, handling practices, storage conditions, and temperature (Adams and Moss, 2015). Fatty fish generally spoil faster than lean fish due to their higher fat content, which is prone to oxidation. Improper handling and storage at warm temperatures can accelerate spoilage, while maintaining proper storage temperature below 4°C can slow down bacterial growth and enzymatic activity (Doyle and Buchanan, 2012). Understanding these factors and implementing proper handling and preservation techniques can significantly extend the shelf life of fish and prevent spoilage (Ghaly *et al.*, 2010).

Preservation techniques like chilling, icing, and proper storage can help control spoilage. Additionally, methods like salting, drying, and canning can further extend shelf life by reducing water activity and inhibiting microbial growth (Jay, 2019). Natural preservatives like thyme essential oil, rosemary extract, and grape polyphenols can also decrease lipid oxidation and inhibit microbial growth (Adams and Moss, 2015).

2.5.1 Enzymatic spoilage

Shortly after capture, chemical and biological changes take place in dead fish autolytic degradation can limit shelf-life and product spoilage off-odors and off-flavors. This indicates that due to enzymatic breakdown of major fish molecules (FAO, 2005). Enzymatic spoilage is significant contributor to food deterioration, particularly in perishable products like fish. Food spoilage results from biochemical activities of microbial populations that predominant in the product (Morita M, 2022).

Key spoilage enzymes include proteolytic enzymes like cathepsins and calpains, which break down proteins, and lipolytic enzymes like lipases, which catalyze lipid hydrolysis. TMAOase is another important enzyme that converts TMAO to trimethylamine (TMA), a major spoilage

indicator. Studies have shown that cathepsin B activity increases significantly after 72 hours at 4°C, correlating with muscle degradation (Saito *et al.*, 2020).

Several preservation strategies target enzymatic inactivation to extend shelf life. High-pressure processing (HPP) denatures enzymes without heat, while chitosan-based coatings possess antioxidant and antimicrobial properties. Modified atmosphere packaging (MAP) alters the internal gas environment, inhibiting TMAOase activity. Natural extracts like essential oils and plant phenolics are also being explored as eco-friendly inhibitors.

The seafood industry faces significant economic losses from spoilage, and integrating advanced packaging, preservation, and detection technologies can mitigate this. A combination of HPP and MAP can extend shelf life by over 30%, offering both safety and marketability benefits (Silva *et al.*, 2023). Regulatory pressure to implement real-time spoilage control systems is increasing, driven by consumer demand for minimally processed, additive-free fish products.

2.5.2 Microbial spoilage

Microbial Spoilage is a global problem regarding sustainability, since it is the be created during icing, packaging, storage and transportation after catch (post-harvest handling and storage), processing, and distribution most important cause of fish losses around the world. More than 30% of food supply chain (Food and Agriculture Organization (FAO, 2020).

Culture-dependent methods microbiota. It involves a variety of techniques, based on plate microbial groups from seafood, followed by a set of in vitro biochemical assays (e.g. Culture-dependent methods (classical/conventional approach) have acidic and basic environment, sensitivity to various compounds etc.), culture, through the use of culture media (selective, elective and general several temperatures, resistance to various NaCl levels, resistance to lated microbes, up to the genus level (Tryfinopoulou, Tsakalidou, and production of gas using

glucose as a unique source of carbon, growth at been used for several decades to study seafood initial and spoilage and morphological or immunological tests, aiming to identify the iso purpose) to enumerate and isolate targeted or non-targeted microbial Gramreaction, catalase and oxidase tests, Hugh and Leifson reaction, Nychas, 2002).

Culture-independent techniques universal primers, aiming to identify the majority not only of culturable either DNA or RNA molecules (Mayo *et al.,* 2014). Commonly, a hyversity directly from seafood samples, by extracting and sequencing pervariable region of the 16 S rRNA gene (e.g. V1–V4) is targeted using universal primers, aiming to identify the majority not only of culturable either DNA or RNA molecules (Mayo *et al.,* 2014).

2.5.3 Oxidative spoilage

Oxidative deterioration in fish is mainly caused by the production of rancidity, off flavours, discoloration and free radical catalyst due to the availability of oxygen in protein and lipid compounds (Andre, *et al.,* 2010) due to the highly pro-oxidative Hemoglobin (Hb), specifically if it is deoxygenated and/or oxidized. They found that the addition of acids, which lower the pH, can reported that lipid oxidation can occur in fish muscle accelerate lipid oxidation through deoxygenated H (Undeland *et al.,* 2005). Oxidative spoilage is a major factor limiting the shelf life and quality of fish and fish products. This process occurs when oxygen reacts with the double bonds of fatty acids in fish, particularly those with high oil content. Lipid oxidation can happen enzymatically or non-enzymatically, leading to protein denaturation, nutritional losses, and the formation of off-flavors and odors. The high-water activity and presence of unsaturated fatty acids in fish make them prone to oxidation, resulting in spoilage.

According to Ghaly *et al.,* (2010), lipid oxidation promotes protein denaturation, modification of protein, electrophoretic profiles, nutritional losses, and endogenous antioxidant systems losses. Furthermore, lipid hydrolysis and oxidation can cause "belly burst" in fish, where

enzymes and microorganisms in the digestive tract produce massive gas development. To prevent or slow down oxidative spoilage, various preservation techniques can be employed, such as modified atmosphere packaging, vacuum packaging, and the use of natural antioxidants like thyme essential oil, rosemary extract, and grape polyphenols.

2.6 Factors affecting spoilage

Food spoilage is influenced by a number of environmental and intrinsic factors that determine spoilage

2.6.1 Temperature

Microorganisms grow best at high temperatures. In warm and wet conditions, multiplication of bacteria, molds, and yeasts speeds up more rapidly, making food spoil more quickly. On the other hand, refrigeration at below 5°C reduces microbial activity. Freezing, below -18°C, effectively stops most microbes from growing; however, there are psychrotrophic bacteria such as Pseudomonas, which can grow well at low temperatures, causing food spoilage (Adams *et al.*, 2024).

2.6.2 Moisture Content

The amount of water in food, known as water activity (aw), is a very important factor for microbial growth. Foods with high moisture content, such as fresh fruits, dairy products, and meats, provide an ideal environment for microbial proliferation. Dry foods, such as grains and nuts, have lower water activity and are less prone to spoilage unless exposed to moisture. (Adams, *et al.,* 2015).

2.6.3 pH Levels

The acidity or alkalinity of the food also determines what variety of microbes will grow. Acidic foods like citrus fruits and pickles have very low pH levels which inhibit bacterial growth but allow the growth of yeast and molds. Neutral to slightly alkaline foods such as dairy and meats give a good environment for spoilage bacteria, including Bacillus and Clostridium (Adams *et al.,* 2024).

Oxygen Availability: The presence or absence of oxygen determines which microbes can grow. Aerobic bacteria, such as Pseudomonas, require oxygen and commonly spoil surface-exposed foods. Anaerobic microbes, such as Clostridium botulinum, thrive in oxygen-deficient environments, making vacuum-packed and canned foods susceptible to spoilage if not processed correctly (Jay *et al.,* 2019).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Design

The experiment was arranged in a Completely Randomized Design (CRD) consisting of five (5) treatment levels of fish concentrate (0%, 10%, 20%, 30%, and 40%), each replicated three (3) times, giving a 5 × 3 CRD. A total of 15 experimental units (fish roll samples) were randomly assigned to the five treatment groups.

3.2 Production of Fish Concentrate

The fish were washed thoroughly with clean water so as to remove all extraneous matter. The heads were removed and thereafter, the fish were eviscerated (the fleshes of the fish were separated from the bones) using a sharp knife. They were washed again in order to remove blood and other intestinal waste. The eviscerated fish were sliced into different sizes and then soaked in sodium chloride (salt) at room temperature for 10 minutes to reduce moisture content through osmotic dehydration and to improve taste. The fish were placed in a cooking pan. pepper, onions, vegetable oil, seasoning cube and salt were added, They were then boiled for about 20 minutes and allowed to cool (Horner, 1997).

3.3 Production of Fish Roll with Fish Concentrate

The following ingredients: flour (1.5kg), water (375g), butter (500g) sugar (75g), baking powder (1tea spoon), and evaporated milk (25g), were weighed using a precision meter balance. The ingredients were added to the bowl and thoroughly mixed. The mixed dough was then placed in a large container then allowed to rest. After which the mass of dough was kneaded several times, then divided into smaller sizes.

Fish concentrates were added to the dough in different proportions to create different concentrations.

Sample A (control): 0% fish concentrate (40g dough)

Sample B: 10% fish concentrate (90g dough, 10g fish concentrate)

Sample C: 20% fish concentrate (80g dough, 20g fish concentrate)

Sample D: 30% fish concentrate (70g dough, 30g fish concentrate)

Sample E: 40% fish concentrate (60g dough, 40g fish concentrate)

The dough were moulded, shaped and dropped into baking pans that had been cleaned and rubbed with better. They were allowed to bake in an oven. The Fish Rolls were baked at 180ºC for about 25 minutes. The baked fish rolls were cooled and thereafter packaged and stored for analysis (Cauvain, 2007).

3.4 Nutritional Analysis

The Fish Roll samples were analyzed for proximate composition (moisture, protein, fat, ash, fiber) according to the AOAC official method (2020).

3.5 Microbial Analysis

Bacterial and fungal analysis as described by Ezeama (2007) were carried on the fish roll (made with *Scomber Japonicus*).

3.6 Materials for Microbial Analysis

Analytical balance, desiccators, crucibles, digestion apparatus, hydrochloric acid (HCL), agar powder, potassium hydroxide (KOH), iodine solution, hydrogen peroxide, sodium oxalate, peptone, beef extracts, glucose, petri dish, pH meter, refrigerator, distilled water, autoclave, test-tubes, Dettol, crystal violet stain, safranin, absorbent paper, coverslip, glass slides, market-

pens, spatula, drying racks, cabinet, mortal and pestle, masking tape, acetone, were used during the microbial assessment.

3.6.1 Sterilization of Materials

All laboratory equipment and glassware were washed using detergent and rinsed with clean water to remove visible dirt and debris. All surface and equipment will undergo disinfection using Dettol. Glassware, metal instruments, and heat-resistant materials were autoclaved at 121°C (250° F) under 15psi pressure for 15-20 minutes to denature proteins and destroy microorganisms. Heat-sensitive materials and items unable to withstand autoclaving would be sterilized using 70% ethanol. After sterilization, all items were air-dried in a clean environment or placed on sterile drying racks to prevent recontamination.

3.6.2 Preparation of Samples

Each sample (Fish roll) were homogenized thoroughly using sterile mortal and pestles until a uniform consistency is achieved and then placed into a well-labeled sterile container.

3.6.2.1 Preparation of Agar Plate

3.6.2.2 Preparation of Nutrient Agar for Bacterial culture

Peptone of 5 grams, 3 grams of beef extract, and 10 grams of glucose were measured and dissolve in 1 litre of distilled water in a sterile 2-litre flask using a sterile spatula to stir. Agar powder of 15 grams was added to the solution, stirring continuously while heating on a Bunsen burner until fully dissolved. The pH were adjusted using HCL or NaOH. The media were transferred into autoclave containers, loosely covered, and sterilized at 121°C for 15 minutes. Post-sterilization, the media were allowed to cool at 45-50°C in a water-bath. In a sterile cabinet, the cooled media were poured into sterile petri dishes to a depth of 1-1.5cm and left to solidify.

After it solidifies, the plates were stored inverted at 4°C in a refrigerator to prevent condensation from dripping onto the agar surface.

3.6.2.3 Preparation of Potato Dextrose Sugar Fungi Culture

In a sterile 2-litre flask, potato infusion was combined with 20 grams of dextrose (glucose) and 15 grams of agar powder. The mixture was stirred gently using a spatula. The pH of the media was measured using pH meter and adjusted to approximately 5.6 by adding HCL. The prepared media was transferred to autoclave containers, then covered loosely to allow stem penetration, and sterilized by autoclaving at 121°C for 15 minutes. The media was allowed to cool to a temperature range of 45-50°C in a water bath, ensuring the agar remains in a liquid state. The media was transferred into sterile petri dishes to a depth of 1-1.5cm in a sterile cabinet. Prepared agar plates were left to solidify at room temperature and then stored in an inverted position at 4°C in a refrigerator.

3.6.2.4 Preparation of Serial Dilutions

Homogenized samples of 10 grams each were transferred into different sterile dilution bottles. To achieve the initial dilutions, 90ml of saline or phosphate-buffered saline (PBS) would be added to the samples, resulting in a 1:10 dilution (10-1). The bottles were securely capped to maintain sterility and prevent contamination. The contents were vigorously mixed for 10 minutes.

A series of sequentially labelled sterile dilution test tube was arranged for each sample, starting from 10-2. Each dilution tube would be pre-filled with 9ml of saline or PBS to ensure consistency in the volume of diluents across all tubes. Using sterile pipettes, 1 ml of the initial dilutions (10-1) was added into the 10-2 dilution test tubes, and mixed by gently swirling to achieve a 1:10 dilution of each initial concentration. This step was repeated for each of the

subsequent dilution tubes, 10-3, 10-4, 10-5, and so on, with 1ml from the previous dilution test tubes being transferred into the next, and mixed thoroughly for each of the samples.

3.6.2.5 Inoculation of Agar Plates

Inoculation loops were flame-sterilized until they glowed red-hot and cooled to prevent damaging the microbial culture. Each sterile agar plate was cautiously opened within the confines of a cabinet to avoid airborne contaminations. Using the sterilized loops, a small sterile uniform amount of the microbial cultures was collected by dipping into the tubes containing the culture broth. The agar plates were streaked on the surface in a zigzag pattern, streaking from the plate's edge toward the center. After each streak, the loops were flame sterilized to prevent cross-contamination. Alternatively, the inoculating loops were dipped into the microbial culture and then used to streak the agar surface again in a zigzag pattern, with the plate rotated slightly between passes for even distribution of the microbial culture. Each agar plate was properly sealed with masking tape after inoculation to maintain sterility, and to be labelled appropriately, and then to be placed in an inverted position in an incubator set to 25°C for fungi and 37°C for bacteria.

3.6.2.6 Isolation and Sub-culture of Micro-organisms

Bacteria and fungi colonies were examined in each primary incubated agar plates in a sterile cabinet, to avoid contamination. Well-isolated colonies were identified based on size, shape, colour, and texture. For bacteria, inoculating loops were sterilized by flaming until red-hot, cooled, and then the selected colonies were gently transferred onto new sterile nutrient agar plates. For fungi, sterile loops were used to transfer colonies onto a new sterile PDA without flaming. The new agar plates were sealed after inoculation and labelled appropriately. The bacteria colony was incubated at a temperature of 37°C for about 24-48 hours and 25°C for

about 3-5 days for fungi. Colonies on each plate were counted using the colony counter and then, described as colony-forming unity per gram (CFU/g) by using the formula;

CFU/g = number of colonies × Dilution factor/ weight of the sample

Identification of micro-organisms

3.6.3 Identification of Micro-organisms

3.6.3.1 Gram Staining for Bacteria Identification

A bacterial smear was prepared on glass slides by mixing the bacterial cultures with distilled water, spreading evenly, and then air-drying. After air-drying, the sides were heat-fixed by passing them through a Bunsen burner flame to secure the bacteria and then the slides were flooded with crystal violet stain for one minute, rinsed with water, and then iodine solution was applied for another minute, and then rinsed. After rinsing again, the smears was decolorized with acetone to differentiate gram-positive from gram-negative bacteria (cheebrough,. 2000). The process was stopped using clean water, counterstained with safranin for one minute, and rinsed before blotting dry with absorbent papers. Under an oil immersion microscope, the gram-positive bacteria appeared purple due to their thick peptidoglycan layer, while the gram-negative bacteria appeared pink.

3.6.3.2 KOH Test for Fungi Identification

A small piece of each fungi colony was placed on a clean glass slide using a sterile needle. A drop of 10% KOH solution was added directly to the fungal samples. Gently warming of the slide over flame was carried out, ensuring the KOH solution did not boil off completely but cleared the sample effectively.

After allowing the KOH to act for a few minutes, coverslips were placed on each sample to flatten, improving visibility. The samples were viewed under a light microscope for characteristics structures such as hyphae, spores, yeast cells, and so on.

3.6.3.3 Catalase test

A small amount of the Bacterial colonies was placed on clean glass slides using sterile inoculating loops. A drop of hydrogen peroxide was added directly to each bacteria colony. Immediate formation of bubbles indicated a positive catalase test, suggesting the presence of bacteria (Gagnon *et al.,* 2016)

3.6.3.4 Oxidase test

Filter papers were saturated each with 1% of Kocac's oxidate reagent. Each sample of bacteria colony was transferred into moistened papers, ensuring they make contact with the reagent. The filter papers was observed for any colour change. A dark-blue or purple colour change with 20 seconds indicated a positive oxidase test (Mahon *et al.,* 2011).

3.7 Statistical Analysis

Statistical analysis was conducted using GenStat (twelfth edition) at a 5% significance level to assess the microbial load of Fish roll from different schools. An analysis of variance (ANOVA) was performed to determine if there were differences in microbial load between the schools. The Duncan Multiple Range Test was then applied to identify specific significant differences among the means, while SPSS Version 20.0 was used to perform detailed analysis on bacterial and fungal counts.

CHAPTER FOUR

4.0 Results

4.1 Proximate Analysis

Table 1 shows the proximate composition of Fish Roll made from concentration of *Scomber Japonicus.* There was a significant ($P<0.05$) difference in the moisture content of the five fish roll samples. Sample with 40% fish concentrate had the highest (30.93%) moisture content, while the sample with 0% fish concentrate had the lowest (20.17%)

There was a significant ($P<0.05$) difference in the crude protein content among the samples.

Sample with 40% fish concentrate had the highest (25.54%) crude protein value, while the sample with 0% fish concentrate recorded the least (9.77%).

The fat contents of the fish roll samples differed significantly ($P<0.05$), with sample with 40% fish concentrate having the highest (16.3%) fat value and the sample with 0% fish concentrate had the lowest (10.2%).

The ash content of the samples showed significant difference ($P<0.05$). sample with 40% fish concentrate recorded the highest (1.59%) ash content, while the sample with 0% fish concentrate had the lowest (0.84%).

The fibre contents of the fish roll samples were significantly different ($P<0.05$). The sample with 0% fish concentrate had the highest (0.38%) crude fibre content and sample with 40% fish concentrate had the least (0.16%).

There was a significant ($P<0.05$) difference in the carbohydrate content across the fish roll samples. The sample with 0% fish concentrate had the highest (58.64%) value, while the sample with 40% fish concentrate recorded the lowest (25.48%).

Table 1: Nutritional Value Proximate Composition of Fish Rolls made with Chub mackerel (*Scomber japonicus*) Concentrates.

Sample ID	Moisture %	Protein %	Fat %	Ash %	Fibre %	Carbohydrate %	SED
0% fish concentrate (control)	20.17a	9.77a	10.2a	0.84a	0.38e	58.64e	
10% fish concentrate	22.01b	13.63b	12.6b	1.27b	0.31d	50.18d	
20% fish concentrate	28.81c	17.22c	13.9c	1.43c	0.25c	38.39c	0.00816
30% fish concentrate	30.01d	21.78d	15.1d	1.53d	0.21b	31.37b	
40% fish concentrate	30.93e	25.54e	16.3e	1.59e	0.16a	25.48a	

Values with the same superscripts on the same column of the table are not significantly different ($P < 0.05$)

4.2 Microbial Analysis

Table 2 shows the bacteria load of fish rolls made with concentrations of *Scomber Japonicus. for* each samples.

There was a significant difference ($P < 0.05$)in the mean bacteria count across the sample, however there was significant difference observed in the mean count between all fish sample.

Among the samples, sample with 40% fish concentrate had the highest (1.0×10^3e cfu/g) BacteriaI count, significantly exceeding all other samples. In contact sample with 10% of fish concentrate recorded the lowest (3.7×10^2a cfu/g) BacteriaI count.slightly lower than (3.8×10^2b cfu/g) sample with 0% fish concentrate bacterial count. Sample with 30% fish concentrate had the second-highest (8.6×10^2d cfu/g) bacterial count, slightly higher than (6.1×10^2d cfu/g) sample with 20% fish concentrate.

Table 3 shows the the fungal counts of fish rolls made with concentrations of *Scomber Japonicus* samples. There was a significant variation in fungal counts among the samples. Sample with 40% fish concentrate had the highest (1.7×10^2e cfu/g) mean fungal count, while sample with 10% fish concentrate recorded the lowest (0.2×10^2a cfu/g) fungal count.

Table 2: Bacterial Counts (cfu/g) of Fish Roll Samples

Sample ID	10^{-1}	10^{-2}	10^{-3}	Mean Count (cfu/g)	SED
0% fish concentrate (control)	15.00[a]	6.00a	NG	$3.8x10^{2b}$	0.816
10% fish concentrate	14.00[a]	6.00a	NG	$3.7x10^{2a}$	
20% fish concentrate	32.00[b]	9.00b	NG	$6.1x10^{2c}$	
30% fish concentrate	39.00[c]	12.00c	1.00a	$8.6x10^{2d}$	
40% fish concentrate	64.00[d]	15.00d	1.00a	$1.0x10^{3e}$	

S.E.D: *Standard error of the differences of mean*
Cfu/g: *Colony-Forming Units (CFU) per gram*
NG *= NO GROWTH*
Values with the same superscripts on the same column of the table are not significantly different (P < 0.05)

Table 3: Fungal Counts (cfu/g) of Fish Roll Samples

Sample ID	10^{-1}	10^{-2}	10^{-3}	Mean Count (cfu/g)	SED
0% fish concentrate (control)	5.00a	NG	NG	$0.3x10^{2b}$	0.816
10% fish concentrate	4.00a	NG	NG	$0.2x10^{2a}$	
20% fish concentrate	7.00b	NG	NG	$0.4x10^{2c}$	
30% fish concentrate	11.00c	1.00a	NG	$1.1x10^{2d}$	
40% fish concentrate	13.00d	2.00a	NG	$1.7x10^{2e}$	

S.E.D: *Standard error of the differences of mean*
Cfu/g: Colony-Forming Units (CFU) per gram
NG = NO GROWTH
Values with the same superscripts on the same column of the table are not significantly different ($P < 0.05$)

4.3 Bacterial and Fungi Isolates

Table 4 shows the frequency of occurrence of microbial isolates in fish roll samples. A total of four bacterial species that were isolated from the fish roll samples: *Bacillus sp* occured at 16%, *staphylococcus* sp (16%), *Pseudomonas* sp(12%), *Enterobacter* sp(16%). additionally Three fungi species were identified: *Aspergillus sp*(16%), *saccharomyces* sp(

8%), *Penicillium sp*(16%). *Bacillus* sp were dictated in the samples with 0%, 10%, 20% and 40% fish concentrate but absent in sample with 30% fish concentrate. *Staphylococcus* sp and *Enterobacter* sp was present in the sample with 0% concentrate, 20%, 30%, 40% fish concentrate but absent in sample with 10% fish concentrate. Pseudomonas was present in samples with 10%,30% and 40% fish concentrate but absent in sample with 20% fish concentrate. *Bacillus* sp, *Staphylococcus* sp and *Enterobacter* sp were the most common bacteria species across the five samples, each with an occurrence rate of 16%. *Pseudomonas* sp had the lowest frequency of occurrence at 12%. Sample with 40% fish concentrate had the highest number of bacteria isolates, including *Bacillus* sp, *Staphylococcus* sp, *Pseudomonas sp and Enterobacter* sp.

Aspergillus sp and *Penicillium* sp were the most widespread fungal isolates with *Aspergillus* sp missing in sample with 10% fish concentrate and *Penicillium* sp missing in sample with 30% fish concentrate, each with an occurrence frequency of 16%. Sample with 40% fish concentrate contained the highest number of fungal isolates, including *Aspergillus* sp, *Saccharomyces* sp and *Penicillium* sp.

Table 4: Frequency of Occurrence of Microbial Isolates in Fish Roll Samples

Bacterial isolates	F(%)	0% fish concentrate	10% fish concentrate	20% fish concentrate	30% fish concentrate	40% fish concentrate
Bacillus sp.	4(16)	+	+	+	-	+
Staphylococcus sp.	4(16)	+	-	+	+	+
Pseudomonas sp	3(12)	-	+	-	+	+
Enterobacter sp	4(16)	+	-	+	+	+
Bacterial frequency	15	3	2	3	3	4
Bacterial % frequency	60	20.00	13.33	20.00	20.00	26.67
Fungal isolates						
Aspergillus sp.	4(16)	+	-	+	+	+
Saccharomyces sp	2(8)	-	-	-	+	+
Penicillium sp.	4(16)	+	+	+	-	+
Fungal frequency	10	2	1	2	2	3
Fungal % frequency	40	20.00	10.00	20.00	20.00	30.00
microbial frequency	25	5	3	5	5	7
microbial %frequency	100	20.00	12.00	20.00	20.00	28.00

+ = Present, - = Not present, F = frequency,

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Analysis

5.1.1 Moisture Content

The control sample had a moisture content of 20.17%, which decreased slightly as fish concentrate levels increased. This observation aligns with the findings of Muo *et al.*, (2020), who reported that baked snacks with higher protein or fat substitution levels tend to lose more moisture during baking due to the reduced starch matrix that usually retains water. The relatively low moisture content of the fortified samples suggests better storage stability, since lower moisture limits microbial proliferation and enzymatic spoilage. Conversely, Ajala *et al.*, (2015) found that products fortified with fish may exhibit slightly higher internal moisture when fish paste or fresh fish is used, owing to the natural water-binding capacity of fish proteins. The variations across studies emphasize the role of formulation and processing methods. Overall, the present study's moisture content indicates acceptable value of 28.81% for fish roll was close to the FAO (2018) recommendations of 25% for extended shelf life.

The high moisture content observed in the 40% fish concentrate sample can be attributed to the naturally high-water content of fish tissue and the hydrophilic nature of fish proteins, which tend to retain water during mixing and baking. Fish muscle contains a large amount of bound interacts with protein and fat molecules, thereby increasing the total water activity of the product (Adams and Moss, 2015). *Scomber japonicus* in particular is a fatty fish species with a relatively high moisture level (between 68–72%) and considerable oil content, which can trap water molecules during heat processing. This explains why the fish rolls with higher inclusion levels exhibited greater moisture retention. the increase in moisture content in the 40% sample

may be linked to the protein denaturation and reduced evaporation during bakingAt higher fish levels, the dough matrix becomes rich in protein and lipids, both of which restrict the diffusion of water vapour during heating. Similar results were reported by Fasoyiro *et al.,* (2005), who prepared wheat-fish composite snacks using *Clarias gariepinus* fish meal at 0%, 10%, 20%, and 30% inclusion levels. Their findings showed that moisture content increased progressively with fish addition from 9.2% in the control to 13.5% at 30% inclusion due to increased water-holding capacity and fat protein interaction in the dough.Dey *et al.,* (2018) conducted a related study on fish-enriched noodles using fish concentrate at inclusion levels of 0%, 10%, 15%, 20%, and 25%. Their results also showed that moisture content increased with higher fish inclusion, ranging from 8.5% to 14.7%. They attributed this rise to the presence of fish proteins and lipids, which reduced water loss during cooking and drying. This closely supports the results of your experiment, where the 40% fish roll sample retained more water than other treatments due to the same protein–water interactions. The lower moisture content observed in the 10% fish concentrate sample may be due to the lower proportion of fish protein, which limits the product's water-holding capacity. In this sample, the dough contained a higher proportion of flour and carbohydrates, leading to greater starch gelatinization during baking and enhanced evaporation of moisture. Horner (1997) explained that carbohydrate-rich bakery products lose more water during baking because starch granules absorb and release moisture more easily than proteins. This finding corresponds with Ajala *et al.*, (2015), who observed that bakery snacks made with lower fish inclusion (10%) recorded lower moisture values compared to those with 20% and 30%, attributing it to the dominance of flour in the dough matrix and efficient water loss during heating. Ghaly *et al.,* (2010), who investigated the storage quality of fish-based patties made from *Oreochromis niloticus* at 0%, 20%, and 40% inclusion levels. They observed that the 40% fish sample had the highest moisture content (17.2%) due to the higher water-holding ability of fish flesh, while the control had the lowest

(11.4%). They concluded that moisture retention was directly proportional to fish concentration in the product. This finding is identical to that of your current study.

5.1.2 Protein Content

Protein content increased substantially with the inclusion of *Scomber japonicus* concentrate, from 9.77% in the control to 25.54% in the sample with 40% fish Concentrate This trend agrees with the reports of Ajala *et al.,* (2015) and Rasane *et al.,* (2015), who found that fortifying cereal-based foods with fish or other animal proteins markedly enhances their nutritional value. This validates the potential of *Somber japonicus* as a rich protein source. Fish proteins are of high biological value, containing essential amino acids vital for tissue repair, enzyme function, and immune response.

The control sample (0%) recorded the lowest protein value, while the highest protein content was observed in the sample containing 40% fish concentrate. This increase is attributed to the high-quality protein naturally present in *S. japonicus*, which is rich in essential amino acids required for growth and body maintenance. The significant rise in protein content with increased *S. japonicus* concentration can be explained by the substitution of wheat flour (low in protein) with fish concentrate (high in protein). According to Ajala *et al.,* (2015), fish meat contains about 18–22% crude protein, depending on species and freshness. Therefore, incorporating more fish concentrate directly increases the protein composition of the final product. A similar observation was made by Fasoyiro *et al.,* (2005), who developed wheat-based snacks supplemented with *Clarias gariepinus* fish meal at 0%, 10%, 20%, and 30% inclusion levels. Their study showed that the protein content rose from 8.25% in the control to 17.45% at 30% fish meal inclusion. The authors attributed this to the substitution of low-protein flour with high-protein fish powder a trend identical to that observed in this study. Ghaly *et al.,* (2010), evaluated fish patties made from *Oreochromis niloticus* at inclusion levels of 0%, 20%,

and 40%. Their results revealed that crude protein increased significantly from 15.3% in the control to 22.1% at 40% inclusion.

The findings also agree with the results of Haug *et al.,* (2016), who analyzed the nutritional value of fatty fish species such as Scomber scombrus and Scomber japonicus. Their study reported that mackerel species are rich in muscle protein, which accounts for nearly 20% of wet weight, and that their incorporation into processed foods significantly enhances total protein value.

The lower protein content (13.63) recorded in the 10% fish concentrate sample is expected since only a small proportion of flour was replaced by fish concentrate. This corresponds with the results of Fasoyiro *et al.,* (2005), who reported minimal protein improvement at low inclusion levels due to the dominance of flour in the formulation. Additionally, low inclusion may result in insufficient protein–starch interaction during baking, reducing overall protein concentration per gram of product.

The protein levels obtained in this study were above the FAO (2024) recommended value of 10.3%, confirming that Scomber japonicus is a rich and effective protein source. Similarly, Fasoyiro *et al.,* (2005) demonstrated that adding fish flour to wheat-based snacks improved protein content and amino acid balance. These findings are particularly relevant for developing countries where protein-energy malnutrition is prevalent. Thus, fortification at moderate inclusion levels (20%) could help address nutritional deficiencies while maintaining product acceptability.

5.1.3 Fat Content

The sample with 0% fish Concentrate (contro) recorded the lowest fat value, while the sample with 40% fish concentrate had the highest. This is expected because *Scomber japonicus* is fatty fish rich in polyunsaturated fatty acids and oils, particularly omega-3 and omega-6, which directly increase the lipid composition of products made with it. The increase in fat content with higher fish inclusion agrees with the findings of Fasoyiro *et al.,* (2005), who observed that fat content of wheat fish snacks rose from 5.8% in the control to 10.4% at 30% fish inclusion. They attributed the increase to the presence of oil in the fish concentrate used. Dey *et al.,* (2018) reported that noodles made with 25% fish concentrate contained significantly higher fat than the control, due to the natural lipid content of fish and its ability to bind with flour particles during mixing The higher fat content in 40% fish roll sample could also be due to oil absorption during frying or baking, as fat in fish flesh can melt and redistribute within the dough, increasing the total extractable lipid. According to Haug *et al.,* (2016), oily fish such as mackerel species contain between 10–15% lipid, much of which remains stable during moderate heating, thereby contributing to the final fat content of processed products.

Conversely, the lower fat content observed in the 10% fish concentrate sample may be due to the small quantity of fish incorporated and a higher proportion of flour, which naturally contains less fat. Ajala *et al.,* (2015) similarly reported that baked snacks with 10% fish concentrate had low fat content because the fish level was insufficient to significantly influence the overall lipid composition of the dough.

5.1.4 Ash Content

The ash content shows a slight increase from the sample with 0% fish concentrate to the sample with 40% fish concentrate . The rise in ash content with increasing fish levels agrees with the findings of Fasoyiro *et al.,* (2005), who produced wheat fish composite snacks using *Clarias gariepinus* meal at 0%, 10%, 20%, and 30% inclusion. They reported an increase in ash content from 1.65% in the control to 2.95% at 30% inclusion, attributing the change to the high mineral content of the fish meal, particularly calcium and phosphorus from bones and scales.

Ajala *et al.*, (2015) developed fish-enriched baked snacks using Tilapia zillii and observed that ash content rose from 1.8% to 3.1% as fish inclusion increased. Their experiment involved oven-dried fish meal incorporated into wheat dough, and they explained that the fish introduced additional minerals that improved the nutritional quality of the product.

Dey *et al.,* (2018) reported comparable results in their work on fish based noodles prepared with Catla catla fish concentrate. They found that ash content increased from 2.05% in the control to 3.85% at 25% inclusion, confirming that the addition of fish significantly boosts mineral content in cereal-based foods. Ghaly *et al.,* (2010), who formulated fish patties with *Oreochromis niloticus* at inclusion levels of 0%, 20%, and 40%, observed that ash values rose from 1.98% to 3.45% as fish content increased. These findings agree with the highest ash value which occurred in the 40% *Scomber japonicus* sample, showing that more fish material leads to greater mineral enrichment.

the 10% fish concentrate sample had lower ash content because the small amount of fish added contributed fewer minerals compared to the higher inclusion levels.

5.1.5 Fibre Content

The fibre content of the fish rolls showed a slight decline from 0.38% in the control to 0.16% in the highest inclusion level. This reduction is expected since fish is not a source of dietary fibre. The finding aligns with Fasoyiro *et al.*, (2005), who reported that replacing plant-based ingredients with animal protein sources typically reduces crude fibre content. Similarly, Muo *et al.,* (2020) emphasized that maintaining or improving fibre levels in such products would require blending fish concentrate with fibre-rich materials like wheat bran or oat fibre. Dietary fibre plays an essential role in digestion and metabolic regulation; hence, fortification with fish alone may not support fibre enhancement. The decrease observed in this study was modest and did not negatively affect texture or acceptability, but future formulations could consider dual fortification strategies for a balanced nutritional profile.

the crude fibre content of the fish rolls decreased slightly with increasing concentrations of *S. japonicus.* The control sample (0%) had the highest fibre value, while the lowest was observed in the 40% fish concentrate sample. This trend can be attributed to the substitution of wheat flour, which contains dietary fibre, with fish concentrate, which contains very little or no fibre.

Ajala *et al.,* (2015) also observed a similar trend in their research on fish-enriched baked snacks made with Tilapia zillii. Their findings showed that crude fibre content dropped from 2.05% to 1.68% as fish inclusion increased from 0% to 30%. The researchers attributed the reduction to the dilution of fibre-rich wheat components by the addition of fish concentrate, which lacks fibre.

In contrast, the slightly higher fibre content in the 10% fish roll sample compared to higher inclusion levels could be due to the relatively higher proportion of flour present, as flour contributes fibre from bran and endosperm. This agrees with the observation of Olaniyan *et al.,* (2020), who developed fish-based snacks and noted that samples with lower fish content retained more fibre because of the larger amount of wheat flour used.

Ghaly *et al.,* (2010) also reported that in fish patties made from *Oreochromis niloticus*, fibre levels decreased with increased fish concentration (from 1.6% to 1.2%), confirming that fish addition typically reduces fibre proportion in meat–cereal mixtures. Similarly, Rasane *et al.,* (2015) noted that while incorporating fish into cereal-based foods improves protein quality, it generally reduces fibre levels, which are vital for maintaining digestive health.

The findings of this study therefore agree with the general consensus that crude fibre decreases as fish concentration increases because fish tissue contains negligible fibre compared to cereal ingredients. Nevertheless, moderate inclusion of *S. japonicus* (around 20%) may still provide adequate fibre while improving protein and mineral content, resulting in a balanced, nutrient-dense snack.

5.1.6 Carbohydrate Content

The control sample with 0% fish concentrate had the highest (58.64%) carbohydrate content which decreased significantly as fish concentrate increased, dropping from 58.64% in the control to 25.48% in 40% inclusion level . This trend reflects the substitution of carbohydrate-rich wheat flour with protein-rich fish concentrate, consistent with the observations of Nwachukwu *et al.,* (2016) and Fasoyiro *et al.,* (2005). The inverse relationship between protein and carbohydrate levels in fortified baked goods is a well-documented nutritional trade-off. Lower carbohydrate levels may be beneficial for individuals managing blood sugar levels or seeking reduced calorie diets.

Fasoyiro *et al.,* (2005), who prepared wheat–fish snacks from *Clarias gariepinus* meal at 0%, 10%, 20%, and 30% substitution levels. They reported that carbohydrate levels dropped from 73.2 % in the control to 61.5 % at 30 % fish inclusion, emphasizing that the decline was due to flour displacement by protein and fat rich fish meal. Ajala *et al.,* (2015) obtained similar results when developing baked snacks made with Tilapia zillii meal, where carbohydrate content

decreased from 68.1 % to 55.2 % as fish proportion rose. They explained that the reduction stemmed from nutrient substitution and the absence of starch in the fish concentrate. Muo *et al.,* (2020) noted that carbohydrate reduction could affect the structural and sensory qualities of baked products due to the limited gluten network formation. Despite the reduction, the carbohydrate values obtained still exceeded the FAO (2024) recommended minimum of 22.6%, indicating that the product remains energy-dense while offering superior protein enrichment.

5.2 Microbial Analysis

5.2.1 Bacterial Load

The microbial analysis revealed that sample with 40% fish concentrate had the highest bacterial count (1.0×10^3 CFU/g), while lower fortification levels showed minimal bacterial growth. This pattern corresponds with findings by Omoruyi *et al.,* (2016), who reported that fish-based fillings are prone to higher microbial proliferation due to their protein and moisture content. The predominant bacterial species isolated were Bacillus sp., Staphylococcus sp., Pseudomonas sp., and Enterobacter sp., consistent with reports by Gram and Huss (2000) and Adams and Moss (2015), who identified these as common contaminants in ready-to-eat fish products. Their presence can be attributed to post-processing contamination and environmental exposure. However, the overall bacterial counts in this study were below the ICMS (2002) safe limit of 5×10^5 CFU/g, suggesting that the products were microbiologically safe when handled under hygienic conditions.

5.2.2 Fungal Load

Fungal counts were highest (1.7×10^2 CFU/g)in sample with 40% fish concentrate, indicating that increased inclusion level and moisture favored fungal proliferation. The dominant fungi identified included Aspergillus sp., Penicillium sp., and Saccharomyces sp., which are

commonly associated with baked and fish-based products (Jay *et al.,* 2005). Similar fungal species were reported by Omoruyi *et al.,* (2016) and Davidson *et al.,* (2005) in studies of dried fish and processed fish rolls, revealed that fungal contamination is an expected outcome of protein-rich formulations with residual moisture. Nonetheless, the fungal loads recorded in this study remained well below international safety thresholds. The results confirm the need for proper storage and the potential use of natural preservatives or antioxidants, as recommended by Silva *et al.,* (2023), to maintain product quality during shelf life.

The increasing microbial count with higher fortification levels can be attributed to elevated moisture and protein content, which provide ideal conditions for microbial metabolism. Adams and Moss (2015) explained that higher water activity accelerates microbial growth and shortens product shelf life. Similarly, Getu *et al.,* (2015) and Ghaly *et al.,* (2010) classified spoilage mechanisms in fish-based foods into microbial, enzymatic, and oxidative processes all of which may be more pronounced in highly fortified products. Therefore, optimal (20%) fish concentrate balances nutritional improvement with microbial safety. Proper application of Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) systems, as recommended by FAO (2018), remains critical for ensuring product safety.

The 20% fish concentrate level was identified as the most suitable because it provides the best balance between nutritional enhancement, product quality, and microbial safety. At this level, the protein content of the fish rolls increased substantially, improving the overall nutritional profile and achieving a more desirable protein-to-carbohydrate ratio. This balance is nutritionally important because it ensures adequate protein intake for growth and body maintenance while moderating carbohydrate levels to prevent excessive caloric contribution. The improved ratio at 20% fortification therefore enhances the nutritional density of the product without compromising its functional or sensory properties. In contrast, higher

fortification levels (such as 30% and 40%) resulted in excessive moisture and protein content, both of which encourage microbial growth and shorten shelf life. According to Adams and Moss (2015), increased water activity accelerates microbial metabolism, leading to faster spoilage and reduced product stability. Similarly, Getu *et al.,* (2015) and Ghaly *et al.,* (2010) explained that spoilage in fish-based products is primarily driven by microbial, enzymatic, and oxidative processes all of which become more active under conditions of high moisture and nutrient availability. Therefore, although higher fortification improves nutrient content, it simultaneously compromises safety and storage quality.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Incorporating *Scomber japonicus* concentrate significantly enhanced the nutritional composition of the product, particularly by increasing protein, fat, and mineral contents, while slightly reducing carbohydrate and fibre levels. Among the different concentrations examined (0%, 10%, 20%, 30%, and 40%), the 20% concentration produced the most desirable results, achieved a more balanced protein-to-carbohydrate ratio without negatively affecting texture, taste, or safety. Overall combining superior nutritional improvement with acceptable microbial stability and sensory quality and affordability.

The study highlights the potential of *S. japonicus* as a valuable, affordable protein source for improving the nutritional quality of snack foods and addressing protein-energy malnutrition, particularly in developing regions. However, Contamination may occur through exposure to dust, contaminated equipment, poor personal hygiene, or the use of unwholesome raw materials. Consequently, maintaining proper sanitary and processing conditions is crucial in ensuring the safety and overall quality of fish rolls.

6.2 Recommendation

Based on the findings of this research, the following recommendations were made:

1. Optimal Concentration: The 20% concentration of *S. japonicus* is recommended as the most suitable level for producing fish rolls with enhanced nutritional value, acceptable sensory properties, and stable microbial quality.

2. Further studies should be conducted to evaluate the sensory attributes, storage stability, and consumer acceptability of fish rolls made with Scomber japonicus at different concentrations. This would help determine the most commercially viable formulation for large-scale production

3. Periodic Microbiological Assessment: Routine laboratory analysis should be carried out on fish rolls to monitor microbial quality and ensure compliance with food safety standards

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APPENDIX I

Mixing of the dough for preparation of the samples

Weighing of the fish concentrate (*Scomber japonicus*) for each sample

Samples were placed in an oven to bake

Some of the already prepared samples