

**MICROBIAL ASSESSMENT OF FISH ROLLS SOLD IN
SOME PRIMARY SCHOOLS IN OREDO LOCAL
GOVERNMENT AREA, BENIN CITY, EDO STATE.**

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FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN,
BENIN CITY**

FEBRUARY, 2025

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**A RESEARCH PROJECT SUBMITTED TO THE
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FEBRUARY, 2025

CERTIFICATION

This is to certify that this research was carried out by Veronica Oremeyi **AMUNEGA** in the Department of Aquaculture and Fisheries Management, under my supervision and in partial fulfillment of the requirements for the award of Bachelor of Agriculture Degree, B. Agric. (Fisheries).

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Date

Dr. M. Egwenomhe
Ag. Head of Department
Department of Aquaculture and Fisheries Management

Date

DEDICATION

This project is dedicated to my Heavenly Father for blessing me with wisdom, knowledge, and understanding to complete this program. It is also dedicated to my wonderful parents, Engr. and Mrs. AMUNEGA, for their support throughout my university journey.

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With deep gratitude, I give thanks to the Lord Almighty for His grace that has brought me this far in my academic journey. His guidance and protection throughout my time at the University of Benin.

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The importance of family cannot be overstated. To my incredible parents, Engr. and Mrs. David Amunega, I am honoured to be your daughter. With joy in my heart and tears in my eyes, I express my heartfelt gratitude for your prayers, love, guidance, and support from my childhood to my university years. Thank you for always being there for me—spiritually, emotionally, physically, and financially. May God bless you abundantly.

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Abstract

*Foodborne diseases, especially among school children who often consume ready-to-eat (RTE) foods, pose a significant health risk. This study was carried out to assess the microbial load of fish roll snacks sold in eight randomly selected primary schools within Oredo Local Government Area, Benin City, Nigeria. Fish roll samples were collected and analyzed using standard microbiological techniques to determine bacterial and fungal contamination levels. The bacterial counts ranged from 5.9×10^2 CFU/g (Sample 5) to 7.6×10^3 CFU/g (Sample 6), while fungal counts varied from 2.9×10^2 CFU/g (Sample 6) to 1.0×10^3 CFU/g (Sample 2). Some of the bacterial species identified were *Bacillus* sp. (4.32%), *Streptococcus* sp. (9.43%), *Pseudomonas* sp. (5.66%), and *Enterobacter aerogenes* (5.66%), while some fungal species such as *Aspergillus oryzae* (9.43%), *Saccharomyces* sp. (9.43%), *Penicillium* sp. (11.32%), and *Mucor piriformis* (9.43%) were also detected. Sample 6, from Adesua Primary School (public sector), had the highest bacterial count (7.6×10^3 CFU/g), while Sample 5 from Ogboe Primary School (public sector) had the lowest bacterial count (5.9×10^2 CFU/g). Sample 2 from Phelim Primary School (private sector) recorded the highest fungal count (1.0×10^3 CFU/g), while Sample 6 from Adesua Primary School had the lowest fungal count (2.9×10^2 CFU/g). The high microbial load observed suggests contamination due to poor hygiene practices, improper food handling, and inadequate storage conditions. However, all values were within the recommended limit of 5×10^5 CFU/g set by the FAO.*

CHAPTER ONE

1.0 INTRODUCTION

Food is essential for sustaining life, serving as fuel that provides energy to the body (Hasselberg *et al.*, 2020). The important components of food include water, carbohydrates, proteins, and fats, with key vitamins and minerals, which are also important for maintaining good health and immunity. These nutrients need to be replaced in the body with new supplies every day. The nutritional requirements in the diet of every individual change with age, physiological demand, and health status (Anuya, 2020).

Food is necessary for various activities, including professional, domestic, and recreational tasks, as it is converted into usable nutrients required for growth and warmth (Ruel *et al.*, 2018). Another vital role of food is in body development. For instance, an infant born at a weight of 2.5-3.0 kg typically grows to 50-60 kg in adulthood, a process reliant on proper nutrition from birth through adulthood (Hörnell *et al.*, 2013). Food helps maintain the body's structure, repair worn-out tissues, and regulate various bodily functions, including heartbeat, temperature control, muscle contraction, water balance, blood clotting, and waste removal. It generally improves the immune system and boosts the body's resistance to diseases (Kord *et al.*, 2021).

Historically, fish and fish-based products have been staple components of diets due to their easy digestibility and high nutritional value (Parvathi *et al.*, 2017). Fish is a significant commodity in trade, largely because of its increasing consumption rates. It accounts for approximately 60% of the global protein supply and provides over 30% of the total protein intake in developing nations each year (F.A.O, 2014).

Globally, fish is one of the most commonly used and low-cost dietary sources of animal protein for humans (Manlu *et al.*, 2020). Processing fish into value-added products improves its market value and acceptability. This innovation offers both taste and convenience, allowing for the consumption of nutritious and delicious food with less time and effort (Varlik *et al.*, 2000). Value addition employs processing techniques, specialized ingredients, and/or innovative packaging to enhance the nutrition,

sensory characteristics, shelf life, and convenience of ready-to-eat (RTE) food products (Pargarkar *et al.*, 2011).

Ready-to-eat foods have been described as the status of foods being prepared for immediate consumption at the point of sale. They could be raw or cooked, served hot or cold, and can be consumed with or without further heat treatment (Tsang, 2002). These foods are typically sold by vendors on streets and other public places such as markets, schools, hospitals, and so on (Goldstein *et al.*, 2001). Examples of RTE include salads, Fish roll, sausage rolls, burgers, dairy products, and so on. (Casersani and Kinston, 2020).

Fish-based foods have been proven to be effective in improving children's nutritional quality. The nutritional status during childhood significantly influences their growth and development (Schönfeldt and Hall, 2012). The school-age period is a critical time in a child's life for building nutrient stores in preparation for rapid growth. Food consumed by children has direct and short-term influences on their physical and mental development, as well as indirect and long-term influences on continued growth and health (Choi *et al.*, 2008).

Fish is one of the most perishable food items, with rapid quality deterioration during handling and storage, thereby limiting its shelf life (Adebayo-Tayo *et al.*, 2012). Fish and fish products undergo undesirable changes during processing and storage, including deterioration, protein denaturation, and lipid oxidation (Siddaiah *et al.*, 2001).

Fish and fish products have been linked to numerous cases of disease outbreaks worldwide, primarily caused by the consumption of contaminated fish, with agents such as bacteria, viruses, parasites, fungi, bio-toxins, and biogenic amines among others (Alerte *et al.*, 2012; Olea *et al.*, 2012). Annually, 220 million children in the world contract diarrheal diseases due to food contamination, resulting in 96,000 deaths (Kirk *et al.*, 2015). Food-borne diseases arise from ingesting toxins produced by microorganisms present in food, with outbreaks often linked to contaminated food that is compromised during harvesting, processing, or storage (Torok *et al.*, 2004). Factors commonly associated with food-borne illness outbreaks include cross-contamination, inadequate processing times or temperatures, contaminated raw materials, the use of non-potable water, unclean containers and equipment, and poor hygiene practices

during food handling (Espinosa *et al.*, 2014; Zúñiga Carrasco *et al.*, 2017).

1.1 Justification of the Study

As the urban lifestyle becomes increasingly hectic, some parents often lack the time to prepare meals at home. Consequently, children are encouraged to change their food preferences and habits (Pikuda and Ileboye, 2009). Due to time as a setback, RTE snacks, which are readily accessible and available, now make up a significant portion of children's diets in both rural and urban areas (Olutay and Akande, 2009). While snacks are appealing and easy to consume, of great taste, and provide quick energy, questions remain about their wholesomeness and safety.

Fish, being a nutritionally important food source, particularly in developing regions, provides essential proteins and nutrients. Ensuring that fish-based snacks are safe for consumption will help retain these nutritional benefits while minimizing health risks.

A significant portion of global outbreaks in educational settings are linked to food-borne illnesses, accounting for approximately 45% of cases (Nagla *et al.*, 2104). In the Republic of Korea, 47% of recorded food-borne disease incidents were associated with schools. Japan reported 11,826 cases and 12 fatalities due to *Escherichia coli* O157 infections in schools during the first seven months in 1996 (Lee and Greig, 2011). In Brazil, 11.6% of food-borne outbreaks in 2005 were traced to school catering services (Niehaus *et al.*, 2011). A 2005 outbreak in South Wales, Great Britain, affected 157 school children with *Escherichia coli*, which was linked to cooked sliced meats served in schools (Cruz, 2020). Similarly, in 2010, France reported 544 adolescents suffering from *Salmonella* poisoning (Ababio *et al.*, 2016). In countries like Egypt, Cambodia, and India, reports have been made on a considerable number of children becoming ill or experiencing food-related health issues after consuming meals provided at schools (Kortei *et al.*, 2020).

The safety of food consumed by children is a critical public health concern, as contaminated food can lead to outbreaks of foodborne illnesses, particularly in schools (Wounang Ngueugang *et al.*, 2021). Children with underdeveloped immune systems, are susceptible to these illnesses. This study assesses if there are microbial risks associated with fish-based snacks sold to children in Oredo LGA schools,

contributing to the prevention of foodborne diseases.

By targeting a high-risk group (school children), this study seeks to protect those who are prone to microbial contamination. The research findings will have direct relevance to primary schools and the wider community in Oredo LGA, potentially influencing food safety policies and practices within schools and vendors. This could lead to improved hygiene standards in the preparation, handling, and storage of RTE snacks.

1.2 Aim and Objectives of the Study

The study was carried out to assess the microbial load of fish-based ready-to-eat snacks (fish-roll) sold at some primary schools in Oredo Local Government Area, Benin City, Edo state, Nigeria.

The specific objectives of the study were to assess the;

1. Bacterial load of fish roll sold in some primary schools in Oredo Local Government Area, Benin-city, Edo state, Nigeria.
2. Fungal load of fish roll sold in some selected primary schools in the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nigerian Fisheries Economy

In Nigeria, like many coastal developing countries, fish is an important source of food for the population, which is currently estimated at 186 million people (World Bank, 2016). A recent study estimated that Nigeria ranks third globally for the number of people dependent on coastal fisheries for food and nutrition security, and the demand for fish is growing, alongside growth in population and incomes (F.A.O., 2018). However, household fish consumption in Nigeria measured at 13.3 kg/capita/year – is low compared with the world's average of 20.3 kg/capita/year (F.A.O., 2018). Increased fish production and consumption may contribute to alleviating food and nutrition insecurity. The majority of households in Nigeria (58%) suffer from chronic or transitory food insecurity (Ogundari 2017).

According to the Food and Agricultural Organization (FAO) of the United Nations (FAO, 2018), the fishery sector is an important source of livelihoods to a lot of households which makes a vital contribution to food and nutrition security globally. Fish remains an important source of nutritious and affordable food for the majority of low-income households worldwide (Bene *et al.*, 2015). Fish accounts for about 17% of animal protein consumed globally and about 88.42% of the total 171 million tonnes of fish produced was consumed by man (F.A.O., 2018).

2.2 Fish and Human Nutrition

A nutrient is a substance providing nourishment to the body and is necessary for both growth and maintenance. Nutrients can fall into seven groups that include carbohydrate, protein, fat, fibre, mineral, vitamin, and water. All groups are essential for the adequate functioning of the body. Macronutrients serve as a source of energy, while micronutrients are important in biochemical reactions.

The population growth, urbanization and growing concerns over healthy eating habits in developed countries as well as the increase in the purchasing power of developing countries have contributed to an increase in the worldwide fish demand (F.A.O., 2012). In developing countries, more than 60 million people rely on fish and their by-products for their income as well as nutritional needs (Mohanty *et al.*,

2019). Fish have a well-balanced amino acid composition in their protein structure (Harnedy and FitzGerald, 2012) and constitute an important source of animal protein in the diet of many populations in the coastal areas. Humans provide a large part of the essential amino acid requirements from fresh, salted, dried and fermented fish products (Majumdar *et al.*, 2016).

In Egypt, fish is the main source of animal protein and is popular among the low- and middle-income class, since it is readily available and affordable (Allam *et al.*, 2020). Globally, around 47 million children are stunted, mainly in low- and middle-income countries (Müller and Krawinkel, 2005). In addition, malnutrition and stunted growth are associated with deficiencies in vitamin A, iron, and iodine, (Ruel *et al.*, 2018), which are characterized by blindness, impaired learning, failure to thrive, increased physical weakness, and mortality. Adequacy of nutrition is an important issue in every country as it closely relates to national development. It involves young children affecting their cognitive development, education, personality traits, and future productivity (Baye *et al.*, 2017). Improper feeding practices during infancy and early childhood lead to nutritional deficiencies with the result that they often suffer from infections and intestinal disorders, which in turn impair optimal growth and development.

The demand for fishing products is increasing due to the increase in the worldwide population. This increase has become more visible in the increasing trade of fish products and better-preserving conditions and industrialization of fish products (Laxe *et al.*, 2018).

Freshwater living inland fishes, comprise most leading food constituents such as proteins, essential fats oils, and micronutrients that can improve human health and have the capacity to fight against malnutrition around the world (Nyboer *et al.*, 2019).

Fish as a rich source of easily digestible protein provides polyunsaturated fatty acids, vitamins, and minerals for human nutrition. Fish has easily digestible high-quality proteins with essential amino acids and also includes fatty acids (omega-3 fatty acids) and essential vitamins and minerals like A, B, D and calcium, selenium, zinc, iodine, and iron respectively (FAO, 2020). Nonetheless, a large proportion of the total landed fish remains unused due to inherent problems related to unattractive colour, flavour, texture,

small size, and high-fat content. Most of these underutilized fish belong to the abundantly available pelagic species, which are by-catch, of some unconventional species (Akande, 1989).

Fish are usually seen as the most cost-effective, high-quality source of animal protein and have a unique position as a high-protein food commodity for human consumption. It is essential for improving nutritional status, food security, cardiovascular health and other health-related disorders (Bezbaruah and Deka, 2021). A fish food, in addition to providing essential nutrients, contains significant amounts of fatty acids, amino acids and some of the most important vitamins and minerals, which serve as a source of energy for healthy living and are sometimes referred to as rich food for poor people (Balami *et al.*, 2019). According to Abdullahi *et al.* (2001), fish is the perfect food for newborns and adults as it is abundant in key nutrients needed during the early stages of development. Nowadays, fish and its products are considered an essential component of the human diet due to their high nutritional content, particularly in terms of protein and omega-3 fatty acids, which are thought to aid in the maintenance of good health like prevention and treatment of cardiovascular, inflammatory and neurological diseases (Jan *et al.*, 2021; Li *et al.*, 2019). Due to the high content of polyunsaturated fatty acids, shreds of evidence suggest that fish flesh helps reduce the serum cholesterol level in humans (Stansby, 2006).

2.3 Nutritional Value of Fish

Fish is a much-diversified food commodity mostly cultured in tropical and subtropical regions. The demand for fish is significantly increasing with the increase in the world population because of their favourable taste, efficient feed conversion and high commercial value (Tavares *et al.*, 2021).

Fish is a valuable and easily available source of food enriched with protein, lipids, vitamins, and minerals (Petricorena, 2015). Considering the good proportion of essential amino acids and degree of digestibility, fish is referred to as a good quality protein source (Salma El and Nizar, 2015, Shaji and Hindumathy, 2013; Tenyang *et al.*, 2014). Adequate consumption of protein is essential for the proper growth and development of children and the energy source of adults (Vijayan *et al.*, 2016). Fish lipid is a rich source of omega-3 fatty acids recommended for human health improvement and disease prevention (Nurnada *et al.*, 2013). Regular intake of a fish meal may keep our heart and brain healthy and also helpful in reducing,

treating, or improving a wide variety of diseases and disorders including, arthritis, Alzheimer's disease, dementia, diabetes, pre-term birth, migraine headaches, inflammatory diseases and many types of cancer (Mustafa *et al.*, 2015). Moreover, fish muscle and bones are good sources of calcium and phosphorus and also essential minerals, such as iron, zinc, iodine, magnesium, and potassium (Ersoy and Ozeren, 2009). Minerals are essential for human health as they play many important body functions such as maintaining acid-base balance, haemoglobin formation, regulating the body's water balance, help bones and teeth formation, and also being involved in enzymatic reactions (Duran and Mendil, 2010). About 65% of fish minerals are deposited in the bone. In our country, small fishes are popular with the people where they take the whole fishes without discarding the bones (Willett *et al.*, 2019). Generally, fish can absorb and assimilate these essential nutrients from the harvested water where they live or from the available diet (Lal and Tibbettes, 2009). The nutritional status of fish varies among different species and also in the same species depending on their habitat, feeding habits, food materials, and surrounding environment (Yeannes and Almandos, 2003).

2.4 Fish Value Addition

Value addition is defined as any activity along the supply chain that increases the usability, culinary attribute, or economic viability of a food item (Fernández *et al.*, 2021). Processing of fish into a wide variety of value-added products is now common with the increase in demand for food products that are ready-to-eat or require little preparation before serving (Pargarkar *et al.*, 2011). Usually, value-added fish products are perceived to be those that have added ingredients such as a coating (breaded/battered) or a sauce, are prepared neatly, or in some way, provide more convenience to the user. It indicates a measure of factors added to the total worth of a product at each stage of production. Value addition ties in with consumer convenience. For example, value addition can be a process for transforming fish fillets into products that are perceived by the customer as having added quality and interest.

Value can be added to fish and fishery products according to the requirements of different markets. These products range from live fish to ready-to-serve convenience products. In general, value-added food products are raw or pre-processed commodities whose value has been increased through the addition of

ingredients or processes that make them more attractive to the buyer and/or more readily usable by the consumer. It is a production/marketing strategy driven by customer needs and perceptions.

2.5 Fish-based Ready-to-eat Snacks

Snacks, referring to the foods and drinks consumed outside of main meals, have become a common part of Westernized eating patterns (Vatanparast *et al.*, 2019).

Snacking has been shown to have a favourable impact on obesity risk and/or overall diet quality in youth (Keast *et al.*, 2010). For instance, Keast *et al.* (2010) found that 12- to 18-year-old — snackers were less likely to be overweight or obese and suffer from abdominal obesity than their non-snacking peers. The greatest increases in snacking were observed amongst young children (Pierns and Popkin, 2010). Childhood is a period of dramatic physical growth and development in addition to cognitive development that requires optimal dietary intake of energy as well as a variety of nutrients (Ogata and Hayes, 2014). Similarly, snacking has been linked to better diet quality among school-aged children as well as older women (Evans *et al.*, 2014; Kong *et al.*, 2011). The process of adding fish as nutritional value to staple foods has been researched by many nutrition scientists and food technologists (ANON 2001). Fish-based foods have been proven to be effective in improving the quality of nutrition in early childhood (Bogard *et al.*, 2015).

2.6 Fish Spoilage

Spoilage is defined as any alteration in food products that leads to unacceptable attributes to the consumers, mainly perceived through sensory evaluation (Rawat, 2015). This includes chemical alterations (change in colour or oxidation), physical damage, and the appearance of off-odors and off-flavors, which could result in the microorganism growth and metabolism in the food products (Gram *et al.*, 2002). Espejo-Hermes (2004) stated that in order to prevent spoilage and subsequent losses from damage, aquatic products must be handled carefully right after harvest.

Globalization has accelerated the exchange processes of fresh and processed food products, as well as changes in food processing and preservation methods, demographic changes, changes in society's eating

habits, meals outside the home, sale of prepared foods and fast foods, (Cruza, 2010; Sotelo *et al.*, 2019). Increased resistance of causative agents (microorganisms) to antimicrobial compounds and acquisition of virulence factors are some of the multiple factors that have contributed to the appearance of diseases due to food consumption (Marin Mendez *et al.*, 2020).

Food is involved in the transmission of diseases because it can be contaminated by physical, chemical, or biological hazards (bacteria, fungi, viruses, and parasites) through elements such as air, water, soil, insects, animals, utensils, and the environment, raw materials, human manipulation during primary production, transportation, processing, storage, manufacturing and distribution (Rosas, 2007; PAHO, 2023). Food by itself can present pathogenic and/or saprophytic microorganisms responsible for food spoilage and consumer illnesses; in this case, the natural micro-biota of a variety of foods of animal origin including fish and fish products may be made up of microorganisms such as *Pseudomonas spp.*, *Moraxella spp.*, *Flavobacterium spp.*, *Bacillus spp.*, *Micrococcus spp.*, *Enterobacter spp.*, *Salmonella spp.*, *Acinetobacter spp.*, *Clostridium botulinum*, *Clostridium perfringens*, *Yersinia spp.*, *Vibrio spp.*, *Escherichia coli*, *Staphylococcus spp.*, viruses and parasites (De Plata, 2003). Thus, fish intended for human consumption are considered a health risk food as they are susceptible to microbial contamination and deterioration, frequently being bacteria that shorten their useful life and contribute to the waste of these foods (Periago *et al.*, 2016), as well as various outbreaks of food-borne diseases globally (Friesema *et al.*, 2022).

Food-borne Diseases are caused by the ingestion of food that contains etiological agents in quantities that affect the health of the consumer (Finger *et al.*, 2019). These diseases present various gastrointestinal symptoms such as nausea, vomiting, diarrhea, abdominal pain, or fever; whereas in serious situations there may be complications, such as meningitis, pediatric sepsis, Guillan Barré syndrome, or death (Soto Varela *et al.*, 2016). The most susceptible populations are the elderly, children, immune-compromised, and people in high levels of poverty and unsanitary conditions (Torrens *et al.*, 2015).

Spoilage and freshness are two factors that affect fish quality. Fresh food is defined as one whose original characteristics remain unchanged. Therefore, spoilage is considered as a postharvest process of fish. This

process may be classified by the changes from fish freshness to limits of customer acceptability (Jiang *et al.*, 2018). This can be associated with physical characteristic changes, such as odour, muscle softness, and colours of eyes and gills.

2.6.1 Mechanism of Fish Spoilage

The spoilage process involves different stages: enzymatic autolysis, microbial and, oxidation. (Ghaly *et al.*, 2013).

2.6.1.1 Enzymatic spoilage

Enzymes are high molecular weight metabolic catalysts, protein in nature and are needed in small quantities. They operate in their native forms and become denatured when conditions become unfavourable. Temperature, acidity, substrate concentration, enzyme activation and synchronization affect enzyme activities. Enzyme spoilage is known as AUTOLYSIS i.e. self-digestion. It is a process whereby enzymes against which the fish is normally protected alive, under optimal conditions for enzymatic activity, post mortem, digest the fish tissues such enzymes are present in the gut, on the skin and in the tissue.

In 2005, FAO outlined that immediately after capture, fish undergoes rapid chemical and biological changes as a result of autolytic enzymes that break down major fish molecules. Research by Hansen *et al.* (2003) indicates that during the initial stages of decay, autolytic enzymes primarily affect the texture of the fish, without generating the typical off-odours and off-flavours associated with spoilage. This suggests that autolytic degradation can decrease shelf-life and compromise product quality, even with low levels of spoilage organisms present.

The degradation of fish muscle and products during storage and processing is predominantly driven by proteolytic enzymes found in the muscle and viscera post-capture. These enzymes contribute to alterations in product characteristics, as highlighted by Engvang and Nielsen (2001). Inappropriate storage conditions for whole fish result in protein degradation and subsequent solubilization through proteolysis (Lin and Park, 2006).

The primary impact of autolytic degradation manifests in textural changes, accompanied by the generation of hypoxanthine and formaldehyde. The digestive enzymes involved in autolysis extensively break down proteins, leading to meat softening, belly wall rupture, and the release of blood water containing both protein and oil.

Autolysis of fish muscle proteins can yield peptides and free amino acids, facilitating fish meat spoilage via microbial proliferation and the production of biogenic amines (Fraser and Sumar, 2008). The rupture of fish bellies is attributed to the leakage of proteolytic enzymes from the pyloric caeca and intestine into the ventral muscle. These proteases exhibit optimal activity within the alkaline to neutral pH range. Martinez and Gildberg (2011) observed a reduction in the degradation rate by proteolytic enzymes when fish were stored at 0°C and a pH of 5.

2.6.1.2 Microbial spoilage

Microbial spoilage is undoubtedly the most widespread cause of spoilage and exhibits itself as conspicuous growth (colonies, slime), textural alterations (polymer degradation), or off- flavours and off-odours (Gram *et al.*, 2002). The vulnerability of aquatic food products to microbial spoilage is due to the presence of high moisture content and nutrient availability (Singh *et al.*, 2021). Generally, aquatic food products are highly susceptible to microbial spoilage compared with meat since meat has lower pH and moisture content. Spoilage types in aquatic food products are greatly dependent on the kind of microorganisms, product composition, muscle type, and storage environment.

The utilization of non-protein nitrogen (NPN) compounds that lead to the formation of ammonia, fatty acids, and volatile compounds are the pre-mature stages of microbial spoilage among fish. The spoilage of fish begins with the release of its body fluids. At the initial stage of spoilage, the development of a sickly-sweet odour can be observed, followed by stale-fish odour caused by trimethylamine, then by ammonia odour, and finally, putrid odours owing to hydrogen sulphide and indole. Fish and fishery products can contain bacterial pathogens initially contaminated from the soil and water, such as *Vibrio*, *Bacillus*, *Escherichia*, *Clostridium*, and *Serratia*. The primary microbial spoilage caused by bacterial flora is Gram- negative facultative anaerobic rods and aerobic rods, as well as coliforms (Erkmen and Bozoglu,

2016).

2.6.1.3 Oxidative spoilage

Lipid oxidation presents a significant issue for the quality and longevity of pelagic fish varieties like Atlantic mackerel (*Scomber scombrus*) and herring (*Clupea harengus*), notable for their high oil/fat content within their flesh (Fraser and Sumar, 2008). This oxidative process operates through a three-phase free radical mechanism: initiation, propagation, and termination (Khayat and Schwall, 2003). The initiation phase sparks the creation of lipid free radicals, catalysed by factors such as heat, metal ions, and irradiation. These radicals then interact with oxygen to produce peroxy radicals. During propagation, peroxy radicals engage with other lipid molecules, generating hydroperoxides and additional free radicals (Hultin, 2004). Termination occurs as a result of an accumulation of these radicals, leading to the formation of non-radical products.

Fish lipids, primarily composed of polyunsaturated fatty acids, are especially susceptible to oxidation due to their double bonds, which readily react with oxygen. The activation of molecular oxygen typically requires transition metals, serving primarily as catalysts (Hultin, 2004). In fish, lipid oxidation may arise enzymatically or non-enzymatically.

Non-enzymatic oxidation is instigated by heme compounds (haemoglobin, myoglobin, and cytochrome), catalysing the formation of hydroperoxides (Fraser and Sumar, 2008). Undeland *et al.*, (2005) demonstrated that lipid oxidation in fish muscle can be attributed to highly pro-oxidative haemoglobin (Hb), particularly when it is deoxygenated or oxidized.

Enzymatic breakdown of fats by lipases, termed lipolysis, yields free fatty acids, contributing to the development of undesirable flavours, rancidity and a decline in oil quality (Huis and Veld, 2006). Lipolytic enzymes may originate from the food product itself or be introduced by psychotropic microorganisms (Huis and Veld, 2006). Lipases found in the skin, blood, and tissues are the primary enzymes involved in fish lipid breakdown (Audley *et al.*, 2008).

2.6.2 Factors Affecting Spoilage

2.6.2.1 Temperature

This is an extremely important factor influencing the growth of micro-organisms, they either remain in a lag phase or die slowly. High tropical ambient temperature favours the growth of microorganisms. Exposure of fish to direct sunlight should be avoided. In artisanal fishery, in the absence of ice, fish should be kept in clean containers and shaded. Most tropical lean fish will remain in good condition for three weeks to four weeks in ice provided the insulated boxes have provision for the exit of melted water to avoid recontamination of the fish. (FAO, 2002).

Temperature affects the growth of microorganisms in many ways. At low temperatures, the chemical reactions in the organism are slowed down leading to suspend growth. High temperature causes the destruction of heat-labile components of the organism such as nucleic acid and protein resulting in death.

2.6.2.2 Moisture content

Water is essential for life and bacteria cells contain 90% moisture. Moisture requirement for spoilage is expressed as water activity (a_w). The water activity is defined as the ratio between the water vapour pressure of a substance and the vapour pressure of water at the same temperature. Microorganisms can grow over a wide range of water activity (0.60 – 0.99) depending on the species of fish. A reduction in the water activity of the flesh will limit the chances of bacteria growing in the fish. Dehydration or evaporation, the presence of solutes such as sugar or ionic material like salts and the formation of crystals during freezing can cause such a reduction. (Eyo, 2001).

2.6.2.3 Initial microbial load

The initial microbial load refers to the population of microorganisms naturally present on the surface and within the tissues of fish at the time of harvest. This microbial load can vary depending on factors such as environmental conditions, handling practices, and the aquatic ecosystem from which the fish originated. A higher initial microbial load increases the likelihood of rapid microbial growth and then, spoilage of fish. Bacteria, yeast, and moulds present in the initial microbial load can proliferate under favourable conditions, such as warm temperatures and high moisture levels, leading to changes in flavour,

texture, and odour of the fish.

2.6.2.4 pH level

The pH level of fish tissue indicates its acidity or alkalinity, typically ranging from 0 to 14, 7 being neutral.

Fish tissue usually exhibits a slightly acidic pH due to specific compounds and metabolic activities.

The pH level significantly impacts microbial growth and enzymatic functions within fish tissue. Fish with higher pH levels are more prone to spoilage, providing an ideal environment for microbial proliferation. Moreover, alterations in pH can influence the activity of enzymes responsible for protein and lipid breakdown, accelerating spoilage processes. Acidification methods, such as incorporating acidic components or marinating fish, can lower pH levels, inhibiting microbial growth and extending the shelf life of fish products.

2.6.2.5 Nutrient composition

The nutrient composition of fish, including its protein, fat, and moisture content, can impact its susceptibility to spoilage. Fish are rich sources of nutrients and are composed of various proteins, lipids, vitamins, and minerals. The nutrient composition of fish provides an ideal substrate for microbial growth and enzymatic activity. High protein and moisture content, combined with favourable temperature and humidity conditions, create an environment conducive to microbial proliferation. Lipids present in fish are particularly susceptible to oxidation, leading to off-flavours and rancidity. Proper handling, storage, and packaging techniques that control temperature, moisture, and oxygen exposure are essential for preserving the nutritional quality and freshness of fish products.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

Oredo Local Government Area is situated in the southern part of Edo state, Nigeria, covering approximately 43 square kilometers. It is one of the 18 LGAs in Edo state. With a population exceeding 500,000 people, Oredo LGA ranks among the most populous LGAs in Edo state. The majority of its residents belong to the Bini ethnic group, and the Bini language is widely spoken throughout the area. Oredo LGA is distinguished by its cosmopolitan character, reflected in its diverse cultural and economic landscape. Benin City boasts of numerous markets, shops, and businesses, driving trade, commerce, and service sectors within the LGA. The LGA accommodates significant government establishments such as the Edo State Government House, the Edo State House of Assembly, and various ministries and agencies. Oredo Local Government Area consists of 207 registered primary schools comprising 121 public primary schools and 86 private primary schools (Ibadin & Ofovwe, 2011). Approximately 4% of the total population of registered primary schools was selected to give a total of eight schools consisting of three registered private schools and five registered public schools that were randomly selected.

3.2 Experimental Design

The experimental design for this study was of two factors: two primary school sectors (Public and Private) × eight randomly selected fish roll vendors in a complete randomized design. Experimental trials were conducted in triplicate.

3.3 Collection of Samples

This study examined Fish roll, a popular ready-to-eat (RTE) fish-based snack in Nigeria. Samples were obtained from eight randomly selected primary schools, including three private schools—Mac-kester Educational Centre, Phelim Schools Nursery and Primary, and Ebenezer Nursery and Preparatory School—and five public schools—Imaguero Nursery/Primary School, Ogboe Primary School, Adesua Primary School, Uwa Primary School, and Ore-oghene Primary School—as this factor may impact vendor activity and snack handling practices. A fish roll sample was purchased from an on-site vendor at each school. During collection, gloves were used, and the samples were placed in clean, labelled polyethylene nylon before being promptly transported to the laboratory for microbial analysis. For identification during the study, the fish roll samples from each school were assigned as follows:

- Sample 1 – Mac-Kester Educational Centre
- Sample 2 – Phelim Schools Nursery and Primary
- Sample 3 – Ebenezer Nursery and Preparatory School
- Sample 4 – Imaguero Nursery/Primary School
- Sample 5 – Ogboe Primary School
- Sample 6 – Adesua Primary School
- Sample 7 – Uwa Primary School
- Sample 8 – Ore-oghene Primary School

3.4 Materials and Methods

3.4.1 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 dishes, incubator, Bunsen burner, inoculating loops, inoculating needles, microscope, pipettes, pH meter, refrigerator, distilled water, autoclave, test-tubes, Dettol, crystal violet stain, safranin, absorbent paper, coverslips, glass slides, marker-pens, spatula, drying racks, cabinet, mortar and pestle, masking tape, acetone, was used during the microbial assessment.

3.4.2 Sterilization of Materials

All laboratory equipment and glassware were washed using detergent and rinsed with clean water to remove visible dirt and debris. All surfaces and equipment will undergo disinfection using Dettol. Glassware, metal instruments, and heat-resistant materials were autoclaved at 121⁰C (250⁰ F) under 15psi pressure for 15-20 minutes to denature proteins and destroy microorganisms. Heat-sensitive materials and items unable to withstand autoclaving were sterilized using 70% ethanol. After sterilization, all items were air-dried in a clean environment or paced on sterile drying racks to prevent recontamination.

3.4.3 Preparation of Samples

Each sample (Fish roll) was homogenized thoroughly using sterile mortar and pestles until a uniform consistency was achieved and then placed into well-labeled sterile containers.

3.4.4 Preparation of agar plates

3.4.4.1 Preparation of nutrient agar for Bacteria culture

Peptone of 5 grams, 3 grams of beef extract, and 10 grams of glucose was measured and dissolved 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 was adjusted to 5.6 using HCl or NaOH. The media was transferred into autoclave containers, loosely covered, and sterilized at 121⁰C for 15 minutes. Post-sterilization, the media was allowed to cool at 45-50⁰C in a water bath. In a sterile cabinet, the cooled media was poured into sterile petri dishes to a depth of 1-1.5cm and left to solidify. Once solidified, the plates were stored inverted at 4⁰C in a refrigerator to prevent condensation from dripping onto the agar surface.

3.4.4.2 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 a pH meter and adjusted to approximately 5.6 by adding HCl. The prepared media was transferred to autoclave containers, covered loosely to allow steam penetration, and sterilized by autoclaving at 121⁰C for 15 minutes. The media was allowed to cool to a temperature range of 45-50⁰C 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 4⁰C in a refrigerator.

3.4.5 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) was 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 tubes were arranged for each sample, starting from 10^{-2} . Each dilution tube was 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 gentle 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.

3.4.6 Inoculation of agar plates

Inoculating 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 sterile confines of a cabinet to avoid airborne contaminations. Using the sterilized loops, a small 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 centre. 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 surfaces 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, labelled appropriately, and then placed in an inverted position in an incubator set to 25⁰C for fungi and 37⁰C for bacteria.

3.4.7 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 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 37⁰C for about 24-48 hours and 25⁰C 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;

$$\text{CFU/g} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Weight of the sample}}$$

3.4.8 Identification of micro-organisms

3.4.8.1 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 slides 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 were 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.

3.4.8.2 KOH Test for fungi identification

A small piece of each fungal 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. Gentle 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 characteristic structures such as hyphae, spores, yeast cells, and so on.

3.4.8.3 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 bacterial colony. Immediate formation of bubbles indicated a positive catalase test, suggesting the presence of bacteria (Gagnon *et al.*, 2016).

3.4.8.4 Oxidase test

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

3.5 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 analyses on bacterial and fungal counts.

CHAPTER FOUR

4.0 RESULT

4.1 Bacteria and Fungi Count

The microbial assessment results of fish roll samples collected from selected primary schools within the study area are presented in Tables 1 and 2.

Table 1 shows the bacterial count for each sample collected from primary schools in Oredo L.G.A. There was a significant difference ($P > 0.05$) in the mean bacterial population across the samples. However, no significant difference was observed between Sample 4 (2.0×10^{3C} cfu/g) and Sample 1 (1.9×10^{3C} cfu/g). Among the samples, Sample 6, from Adesua Primary School in the public sector had the highest (7.6×10^{3A} cfu/g) bacterial count, significantly exceeding all other samples. In contrast, Sample 5, obtained from Ogboe Primary School, also in the public sector, recorded the lowest (5.9×10^{2F} cfu/g) bacterial count. Sample 8 (2.4×10^{3B} cfu/g) had the second-highest bacterial count, slightly higher than Sample 4 (2.0×10^{3C} cfu/g).

The bacterial count for Sample 7 (1.5×10^{3D} cfu/g) was slightly higher than that of Sample 2 (1.1×10^{3E} cfu/g). Additionally, there was no significant difference between the mean bacterial counts of Sample 3 (8.1×10^{2F} cfu/g) and Sample 5 (5.9×10^{2F} cfu/g).

Table 1 also shows the fungal count of the fish roll samples. There was a significant variation in fungal counts among the samples. Sample 2, obtained from Phelim Primary School in the private sector, had the highest (1.0×10^{3B} cfu/g) mean fungal count, while Sample 6 obtained from Adesua Primary School in the public sector, recorded the lowest (2.9×10^{2EF} cfu/g) fungal count.

Table 1: Microbial load of Samples

MICROBES	SAMPLES								S.E.D
	1	2	3	4	5	6	7	8	
Bacteria counts (Cfu/g)	1.9 × 10 ^{3C}	1.1 × 10 ^{3E}	8.1 × 10 ^{2F}	2.0 × 10 ^{3C}	5.9 × 10 ^{2F}	7.6 × 10 ^{3A}	1.5 × 10 ^{3D}	2.4 × 10 ^{3B}	72.5
Fungal Counts (Cfu/g)	9.8 × 10 ^{2B}	1.0 × 10 ^{3B}	4.1 × 10 ^{2D}	2.3 × 10 ^{2F}	3.5 × 10 ^{2DE}	2.9 × 10 ^{2EF}	1.2 × 10 ^{3A}	5.2 × 10 ^{2C}	39.7

Values with the same superscripts across the rows are not significantly different ($P < 0.05$)

S.E.D: Standard error of the differences of mean

Cfu/g: Colony-Forming Units (CFU) per gram

4.2 Bacteria and Fungi Isolates

Table 2 shows a total of six bacterial species that were isolated from the fish roll samples: *Bacillus* sp. (11.32%), *Streptococcus* sp. (9.43%), *Pseudomonas* sp. (5.66%), *Enterobacter aerogenes* (5.66%), *Klebsiella* sp. (3.77%), and *Micrococcus* sp. (11.32%). Additionally, six fungal species were identified: *Aspergillus oryzae* (9.43%), *Aspergillus sojae* (5.66%), *Saccharomyces* sp. (9.43%), *Fusarium* sp. (7.55%), *Mucor piriformis* (9.43%), and *Penicillium* sp. (11.32%).

Bacillus sp. and *Pseudomonas* sp. were detected in samples 1, 4, and 8. *Micrococcus* sp. was present in samples 2, 3, 4, 6, and 7, but was absent in samples 1 and 7. *Micrococcus* sp. and *Bacillus* sp. were the most common bacterial species across six of the eight samples, each with an occurrence rate of 11.32%. *Klebsiella* sp. was found only in samples 1 and 7, with the lowest frequency of occurrence at 3.77%. Sample 8 had the highest number of bacterial isolates, including *Bacillus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Enterobacter aerogenes*, and *Micrococcus* sp.

Penicillium sp. was the most widespread fungal isolate, missing only from samples 4 and 8, with an occurrence frequency of 11.32%. Sample 7 contained the highest number of fungal isolates, including *Aspergillus oryzae*, *Saccharomyces* sp., *Fusarium* sp., *Mucor piriformis*, and *Penicillium* sp.

Table 2: Frequency of Occurrence of Microbial Isolates in Fish Roll Samples

Possible Bacterial Isolates	F (%)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
<i>Bacillus sp</i>	6(11.32)	+	+	-	+	-	+	+	+
<i>Streptococcus sp</i>	5(9.43)	+	-	+	-	+	-	+	+
<i>Pseudomonas sp</i>	3(5.66)	+	-	-	+	-	-	-	+
<i>Enterobacter aerogenes</i>	3(5.66)	-	+	-	-	-	-	+	+
<i>Klebsiella sp</i>	2(3.77)	+	-	-	-	-	-	+	-
<i>Micrococcus sp.</i>	6(11.32)	-	+	+	+	+	+	-	+
Bacterial freq	25	4	3	2	3	2	2	4	5
Bacterial % freq	47.17	16.00	12.00	8.00	12.00	8.00	8.00	16.00	20.00
Possible Fungal Isolates	F (%)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
<i>Aspergillus oryzae</i>	5(9.43)	+	+	-	-	+	-	+	+
<i>Aspergillus sojae</i>	3(5.66)	-	-	+	+	-	+	-	-
<i>Saccharomyces sp</i>	5(9.43)	-	+	-	-	+	+	+	+
<i>Fusarium sp</i>	4(7.55)	+	-	+	-	-	-	+	+
<i>Mucor piriformis</i>	5(9.43)	+	-	-	+	+	-	+	+
<i>Penicillium sp.</i>	6(11.32)	+	+	+	-	+	+	+	-
Fungal frequency	28	4	3	3	2	4	3	5	4
Fungal % frequency	52.83	14.29	10.71	10.71	7.14	14.29	10.71	17.86	14.29
Microbial frequency	53	8	6	5	5	6	5	9	9
Microbial frequency	100	15.09	11.32	9.43	9.43	11.32	9.43	16.98	16.98

CHAPTER FIVE

5.0 DISCUSSION

5.1 Microbial Assessment of Fish Roll Samples

This study revealed that the microbial load of the fish roll samples obtained from eight primary schools located within the study area ranged from 5.9×10^{2F} to 7.6×10^{3A} for bacteria count and 2.9×10^{EF} to 1.0×10^{3B} for fungal count. There is a likelihood that appropriate hygienic conditions in terms of personal hygiene, good manufacturing and safe food practices were not followed by the vendors at the schools. Gopal *et al* (2015) noted that the presence of spoilage microorganisms may be linked to several factors such as improper handling and processing conditions, use of contaminated water during processing, cross-contamination from raw materials used during production, use of dirty processing materials like mixing bowls, knives, rolling pin, amongst many other processing materials.

The result indicates that sample 6 had the highest bacteria contamination with a mean count of 7.6×10^{3A} cfu/g, with the least fungi count (2.9×10^{EF}). This could be due to the heavy inner filling wrapped within the dough. The grounded fish used as inner filling is highly perishable, as fish contains a high proportion of proteins which enhances microbial growth. It was noted by Omoruyi *et al* (2016) that bacteria are abundant in the diet and environment of fish, and are impossible not to be present in fish.

Assessment of the collected fish roll samples revealed the presence of several bacteria and fungi species such as *Bacillus sp.*, *Streptococcus sp.*, *Pseudomonas sp.*, *Enterobacter aerogenes*, *Klebsiella sp.*, *Micrococcus sp.*, *Asperigillus oryzae*, *Asperigillus sojae*, *Sacchromyces sp.*, *Fusarium sp.*, *Mucor piriformis*, and *Penicillum sp.*

Bacillus sp. and *Micrococcus sp.* were the most prevalent contaminating bacteria species, both detected in six, out of the eight fish roll samples, with an occurrence level of 11.32%. *Penicillum* had the highest fungi occurrence of 11.32%, isolated also from six out of the eight samples analyzed. The isolation of *Penicillum sp.* may be as a result of the exposure of the fish-rolls during storage to humid environments

The occurrence of *Asperigillus sp.*, *Fusarium sp.*, and *penicillum sp.* in some of the samples could be

as a result of moisture reabsorption from the environment during storage, which supported the growth of these microorganisms, in addition to contamination during processing, handling and display in show glasses. (Christiana *et al.*, 2010).

In a study carried out by Oranusi *et al* (2011), it was noted that the presence of *Asperigillus sp.*, *Penicillium sp.*, and *Mucor sp.* could be attributed to the surrounding environment and packaging materials.

Mucor is a filamentous fungus capable of causing spoilage, which are made present by unsanitary practices by the fish roll handlers, capable of causing a group of infections such as septic arthritis, renal infections, respiratory infections, and gastritis (Sydney and Woribo, 2018). The presence of *Mucor piriforms* in some of the fish roll samples could be as a result of dried ingredients which was not properly cooked before filling the dough.

Asperigillus oryzae and *Asperigillus sojae* were also isolated with occurrence levels of 9.43% and 5.66%, respectively. *Asperigillus species* are known to produce mycotoxins which pose serious health risks such as kidney, liver and gastrointestinal health issues to consumers who consume food contaminated with *Asperigillus spp.* (Yiannikouris and Jonanyi, 2002).

Pseudomonas sp. is known to be environmental contaminants and opportunistic pathogens. *Pseudomonas spp.* have been reported by Borch *et al* (2009) as dominant fish product spoilage microorganisms.

Pseudomonas biofilm can grow on abiotic surfaces of different equipment and processing surfaces. It is also reported that when organisms like *pseudomonas* form biofilm, they became more resistant to chemicals and antibiotics. Such a biofilm is a potential source of contamination of foods that may lead to spoilage, foodborne diseases and transmission of foodborne pathogens (Gunduz and Tuncel, 2006). The isolation of *Pseudomonas* in some of the fish roll samples is an indication of possible production contamination. This corresponds to the findings of Wilderjans *et al* (2013).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Ready-to-eat foods have become increasingly popular across various regions in Nigeria, largely due to their affordability, convenience, and easy accessibility. These foods are sold in diverse locations such as schools, hospitals, streets, and highways. Fish roll, in particular, are consumed by people of all ages, with a notable preference among school children, making it essential to ensure they remain as free from contamination as possible. The analysis of fish roll samples revealed microbial density levels within the acceptable limit ($\leq 10^3$) established by the International Commission of Microbiological Specification in Food for ready-to-eat foods, as well as the satisfactory level of 10^4 recommended by the Centre for Food Safety (2014). However, the presence of microbes with public health significance, including opportunistic pathogens, suggests environmental contamination. Pathogenic and spoilage bacteria can adhere to food contact surfaces and persist despite cleaning and disinfection (Pal, 2010). The findings from this study indicate a high likelihood of cross-contamination in Fish roll, originating from vendors, contact surfaces, and storage practices. Contributing factors may include improper food handling, inadequate personal hygiene, unclean packaging materials, and vendors' lack of commitment to food safety. The identification of some microorganisms such as *Enterobacter aerogenes*, *Bacillus* sp., *Streptococcus* sp., *Micrococcus* sp., *Penicillium* sp., and *Aspergillus* sp. highlights the potential risk of foodborne illness or toxicity. Hence, there is need to improve on food handling practices to reduce the health hazards posed by microbial contamination in Fish roll sold in schools within the study area.

6.2 Recommendation

The control of foodborne pathogens is an essential measure in preventing the appearance and spread of foodborne diseases to school-going children. Contaminated ready-to-eat foods, such as fish roll sold in primary schools, pose a significant health risk if not handled and prepared under strict hygienic conditions. To reduce these risks, several preventive measures should be implemented to ensure food

safety and protect consumers from foodborne illnesses.

1. Regular microbiological testing of fish-based snacks sold in schools, strict enforcement of food safety standards, and appropriate sanctions for non-compliance should be implemented
2. Food vendors should undergo continuous training on personal hygiene, proper handwashing, and safe food handling techniques to reduce contamination risks.
3. Integration of Hazard Analysis and Critical Control Point (HACCP) measures into the food preparation process to identify and eliminate potential hazards at critical points.
4. Conducting targeted awareness programs for food handlers, processors, vendors, and the general public on the importance of food safety, hygiene, and the proper use of potable water in food processing.
5. Regular disinfection of food preparation areas, utensils, and equipment, proper waste disposal, and the use of clean, food-grade packaging to prevent post-production contamination.

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APPENDIX



Figure 2: Fish rolls stored in a show glass



Figure 3: Fish rolls stored in a transparent plastic bucket



Figure 4: Fish rolls being handled by the vendor using a transparent polyethylene nylon