MICROBIAL LOAD OF BREAD FORTIFIED WITH SCUMBIA (Scomber *scombrus*) UNDER AMBIENT STORAGE.

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CERTIFICATION

This is to certify that this research was carried out by Samuel Chidubem Okorie 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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DEDICATION

This Project is deeply and personally dedicated to Almighty God for his infinite goodness and mercies towards me. To my cherished family - the pillars of strength, love, and unwavering support in my life's journey. Each of you holds a special place in my heart. To my Mom thank you for instilling in me the values of resilience, perseverance, and the importance of education. Your sacrifices and belief in my abilities have fueled my determination to excel. To my siblings Emmy, Joey and Esther, you have been my confidants, to my lecturers for their continuous supervision and schooling, without whom I wouldn’t have come this far.

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ABSTRACT

Microbial contamination is a major factor affecting the shelf life and safety of bread. This study was carried out to analyze the bacterial and fungal growth in bread fortified with different levels of Scomber *scombrus* (0%, 10%, 20% and 30%) over a three-day period immediately after processing.

The bread was prepared with varying levels of Scomber scombrus and the treatments (A, B, C and D) were assigned based on the percentage of of the fish concentrate.

The bread samples were stored under controlled conditions for three days, small portions of each bread samples were taken at different time points (Day 0, Day 3) and the samples were placed in sterile containers to prevent external contamination.

Bacterial counts (CFU/g) on Day 0 ranged from 4.10×10^2 (Treatment A) to 1.20×10^3 (Treatment D), while by Day 3, these values increased significantly, with Treatment D reaching 2.40×10^3 CFU/g. Similarly, fungal counts (CFU/g) on Day 0 ranged from 1.70×10^2 (Treatment A) to 3.40×10^2 (Treatment D), increasing by Day 3 to a maximum of 4.20×10^2 CFU/g in Treatment D. Statistical analysis ($P < 0.05$) showed significant differences across treatments, indicating variations in microbial growth rates. The results suggest that proper storage methods and preservation techniques, such as refrigeration, airtight packaging, and antifungal agents, are crucial for maintaining bread quality and safety. Future research should explore the impact of different preservatives and environmental conditions on microbial proliferation in bakery products.

CHAPTER ONE

INTRODUCTION

Fish is an essential component of a healthy diet due to its richness in amino acids, unsaturated fatty acids, vitamins, and minerals (Nalinal, 2013). Its ease of digestion, attributed to the lack of connective tissue, makes it an excellent protein source. Consuming fish has been linked to the prevention of various health conditions, including cardiovascular disease, high blood pressure, cholesterol and certain types of cancer (Baberge *et al.*, 2002; Pienals *et al.*, 2008).

In Nigeria, fish is a widely accepted and affordable source of animal protein, available in fresh, smoked, dried, and frozen forms. It accounts for approximately 50% of the animal protein intake among Nigerians (Federal Department of Fisheries, 2009). Currently, fish contributes around 40% of animal protein consumption (Stands, 2009).

Fish is abundant in most water bodies and provides essential nutrients for both infant and adult diets (Abdullahi *et al.*, 2001). To meet Nigeria's fish demand, species like stock fish, mackerel, and herring are imported. However, global and national demand for fish continues to rise, outpacing supply (Food and Agricultural Organization, 2012).

Fish is one of the most perishable of all the foods due to suitable medium for growth of micro organisms after death (Ojutiku, Kolo, & Mohammed, 2009). The loss of fish due to spoilage in the world is estimated to be 10–12 million tons per year, accounting for 10% of total production. Generally, the wastage of fish through spoilage has been estimated to range from 18% to 30% in developing nations (Mphande & Chama, 2015). It indicates that the habit of preserving and processing fish into different products before spoilage is poor or not enough. Fish deterioration or spoilage is one of the fishing industry's most significant problems. To limit post-capture loss, various preservation methods such as frying, fermentation, drying, salting and smoking are used individually or in association (Anihouvi et al., 2005; Issa, Mopate, & Missohou, 2012; Yacouba, 2009). These methods aim to inactivate micro-organisms and endogenous enzymes which are involved in the spoilage mechanism. (Igwegbe *et al.*, 2015).

Fish processing is the process associated with fish and fish products between the time in which fish are caught or harvested and the time in which the fish product is delivered to the consumer. Fish preservation refers to the methods and techniques used to extend the shelf life of fish by reducing spoilage through physical, chemical, and biological means (Eyo, 2001). The operations in fish processing are washing, degutting, salting, fermentation, drying and smoking. These operations contribute to the development of flavour, texture, colour and improved storage characteristics of the product. Some of the processes employed in fish processing include boiling, frying, roasting, smoking and so on, and these could have varying effects on their nutrient contents and organoleptic properties (Eriksson, 1987).

1.1 Justification of the Study

Bread consumption is quite common in many developing nations, including Nigeria (Sanful & Darko). It is favored by people of all ages, especially children. While bread is rich in carbohydrates and fats, which provide essential energy and calories, it lacks adequate protein, minerals, and essential oils necessary for addressing malnutrition. In Nigeria, malnutrition varies in magnitude and severity across different regions, primarily resulting from deficiencies in protein, vitamins, iron, and other minerals (Adebooye, 1996). Therefore, enriching or fortifying bread is crucial to enhance its nutritional value.

Food fortification refers to the process of adding one or more vital nutrients to food, whether or not they are typically present, to prevent or correct nutrient deficiencies within a population or specific demographic group (Brekkan, 1996).

Microorganisms have the potential of causing poisoning and foodborne illness in man. Some food poisoning is caused by the toxin released by physical presence of large number of microbes in the ingested food in turn reduces its nutritional quality, there is scarcity of information on occurrence of pathogens and fish and other ready-to-eat food, thus the need for this study with the objective of determining some microorganisms that are prevalent on bread fortified with Scomber *scombrus*.

1.2 Aim and Objectives of the Study

The aim of this study was to acertain the microbial load of bread fortified with fish species under ambient storage.

The specific objectives of the study was to:

1. evaluate the bacterial load of bread fortfied with various levels of Scomber *scombrus* under ambient storage.

2. evaluate the fungal load of bread fortfied with various levels of Scomber *scombrus* under ambient storage.

3. identification and enumeration of specific spoilage and pathogenic microorganisms in the bread fortified with various levels of Scomber *scombrus* under ambient storage.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Value of Fish as Food

Fish is a rich source of protein commonly consumed as an alternative source of protein due to the higher cost of meat and other sources of animal protein (Oluwaniyi and Dosumu, 2009). Apart from its essential nutrients required for supplementing infants and adult's diet, fish is also a source of vitamin and minerals (Abdullahi *et al.*, 2001). Fish has higher digestibility as a result of the greater muscle protein to connective tissues ratio In fish than other animals. This makes fish acceptable by infants and adults (Eyo, 2001). Fish is also recommended to patients with digestive disorder such as ulcer and other conditions (Eyo, 2001). Being an excellent source of high-quality animal protein and highly digestible energy, whose product has a nutrient profile superior to all terrestrial meat of beef, pork and chicken among others. It is a good source of sulphur and essential amino acid such as lysine, leucine, valine and arginine. The multiple benefits of fatty fish high in Omega 3 and small fish eaten whole containing nutrient in the skin and bones clearly illustrated seafood's irreplaceable nutritional value (Larson *et al.*, 2007). Fish tends to solve heath issues which are focus in both developing countries like Nigeria and the developed world. In many countries, fish is the main source of animal protein and is essential for providing micronutrients to vulnerable populations. Dietary patterns are also shifting in developed and middle-income countries and an increasing emphasis on coronary and overall health to an increased demand for fish (Kumolu-Johnson *et al.*, 2015)

In Nigeria, fish is eating cooked or smoked and form a much cherished delicacy that cuts across socioeconomic, age, religion and education barrires (Adebayo *et al.,* 2008)

2.2 Nutritional Composition of Fish

Fish is a vital component of the human diet, providing an abundance of protein, a nutrient essential for human health (Olaleye and Abegunde, 2015). The nutritional composition of fish includes protein, oil, moisture, and ash, which serve as indicators of its nutritional value (Olaleye and Abegunde, 2015). Fish is an excellent source of essential nutrients, including proteins, lipids, vitamins, and minerals (Eyabi and Ningo, 1997). Specifically, fish provides amino acids like lysine, methionine, and tryptophan, which are often lacking in plant-based proteins. Additionally, consuming fish bones provides mineral elements such as phosphorus, calcium, and iron, while

2.3 Pathogenic Bacteria and Fungi Associated with Bread Fortified with Fish

The safety and quality of fish bread are paramount considerations in the development and production of this traditional food item. Potential contamination by pathogenic bacteria and fungi can pose significant health risks to consumers and compromise the overall integrity of the product. This examines the pathogenic microorganisms associated with Bread Fortified with Fish and the strategies employed to mitigate these risks

2.4 Pathogenic Bacteria:

The presence of pathogenic bacteria in bread and related fish-based products which are known to cause foodborne illnesses. Similarly, Pal and Tandon (2020) reported the presence of Listeria monocytogenes in bread, a bacterium that can pose a serious threat to vulnerable populations, such as pregnant women and the immune compromised.

The introduction of pathogenic bacteria in fish bread can occur at various stages of the production process, including improper handling, inadequate processing, or cross-contamination. Effective measures, such as strict adherence to good manufacturing practices (GMP), proper temprature control, and comprehensive sanitation protocols, are crucial in mitigating the risk of bacterial contamination.

2.5 Pathogenic Fungi:

In addition to bacterial contaminants, fish bread may also be susceptible to fungal infections, which can lead to the production of mycotoxins and compromise the safety and quality of the product. In various fish-based food products, including fish bread.

The growth of these pathogenic fungi can be influenced by factors such as moisture content, storage conditions, and the presence of organic matter. Strategies to control fungal contamination in fish bread may include the use of preservatives, proper packaging, and strict temperature and humidity management during storage and transportation.

2.6 Fish Spoilage

Fish and fish-based products, including fish bread, are highly susceptible to spoilage due to their perishable nature, which is influenced by various physical, chemical, and microbiological factors. Understanding these mechanisms is vital for developing high-quality, safe, and shelf-stable fish- based food products. The spoilage process in fish is a complex series of enzymatic, bacterial, and chemical changes that initiate immediately after catching (Burt, 2003). Once the fish dies, its enzymes begin to break down the stomach and eventually migrate to the flesh, causing it to become soft and develop a strong odor. Fish naturally harbor numerous bacteria on their skin, gills, and intestines (Karube *et al.*, 2001), which are harmless to the living fish but multiply rapidly after death, triggering enzymatic digestion. The bacterial load on fish depends on its health, environment, and catching methods. Additionally, oxygen reacts with fish oil, leading to

chemical spoilage and the development of a rancid odor and taste. (Pienals, *et al.*, 2008)

2.7.1 Forms of Fish Spoilage

Fish and fish-based products, such as fish bread, are highly perishable and susceptible to various forms of spoilage. Understanding the different types of spoilage that can affect fish is essential for the development of effective mitigation strategies and the production of high-quality, safe, and shelf-stable fish-based food items. This comprehensive review outlines the primary forms of fish spoilage Henrik, Huss. (2005).

2.7.2 Microbial Spoilage:

Bacterial growth and proliferation, leading to the production of off-flavors, slime formation, and the breakdown of proteins, lipids, and other organic compounds Connelly, (2001)

Common spoilage bacteria include Pseudomonas spp., Shewanella putrefaciens, and Photobacterium phosphoreum Adams, & Moss,(2008).

2.7.3 Enzymatic Spoilage:

Breakdown of proteins, fats, and other macromolecules by endogenous enzymes, such as proteases and lipases Formation of off-flavors and textural changes Accumulation of biogenic amines Factors affecting enzymatic spoilage are temperature, pH, and the presence of inhibitors or activators

2.7.4 Chemical Spoilage:

Formation of volatile compounds, such as ammonia and trimethylamine, due to the breakdown of proteins and other nitrogenous compound can contribute to the off-odors and the preception of spoilage (Burgaard)

2.7.5 Physical Spoilage:

Changes in texture, such as softening or hardening, due to factors like freeze-thaw cycles or improper handling structural damage, leading to loss of product intergrity. Understanding and effectively managing these forms of fish spoilage is crucial for the production of high quality, safe and shelf-stable fish bread (Adedeji, Adetunde, (2004).

2.8 Chemical Analysis:

Measurement of pH changes to assess the accumulation of acidic compounds Determination of total volatile basic nitrogen (TVB-N) as an indicator of protein breakdown Quantification of trimethylamine (TMA) to evaluate the extent of microbial spoilage analysis of lipid oxidation products such as peroxide value and thiobarbituric acid-reactive susbstance (TBARS) 19. (Gill, C.O. 1992)

2.8.1 Factors Afecting Fish Spoilage

2.8.2 Chemical Composition: The high moisture, protein, and lipid content of fish tissues make them vulnerable to microbial, enzymatic, and oxidative spoilage (Gram & Huss, 1996; Siripatrawan *et al.*, 2011). The presence of endogenous enzymes, such as proteases and lipases, can contribute to the breakdown of macromolecles, further accelerating spoilage (Ababouch et al., 1991; Benjakul & Bauer, 2001).

2.8.3 Nutrient Availability: Fish tissues provide a rich source of nutrients, including amino acids, carbohydrates, and lipids, which can support the growth and proliferation of spoilage microorganisms (Dalgaard, 1995; Gram & Huss, 1996).

2.8.4 Temperature: Fish spoilage is highly temperature-dependent, with faster rates of deterioration at higher temperatures (Dalgaard, 1995; Huss, 1995). Proper temperature control, such as refrigeration or freezing, is crucial for extending the shelf-life of fish and fish-based products (López-Caballero *et al.*, 2002; Goulas & Kontominas, 2005).

2.8.5 Atmospheric Composition: Exposure to oxygen can promote oxidative spoilage, while anaerobic conditions can favor the growth of specific spoilage bacteria (Sivertsvik *et al.*, 2002; Erkan *et al.*, 2006). Modified atmosphere packaging (MAP) can help control the gas composition and slow down spoilage (Sivertsvik *et al.*, 2002; Goulas & Kontominas, 2007).

2.8.6 Initial Microbial Load: The initial presence and composition of spoilage microorganisms on fish can significantly impact the rate and extent of spoilage (Dalgaard, 1995; Gram & Huss, 1996). Good manufacturing practices and proper sanitation are crucial to minimize the initial microbial contamination (Huss, 1995; Koutsoumanis & Nychas, 1999).

2.9 Fish Marketing and Distribution

Assessing the marketing system of fish is crucial because it reveals the complex network of participants involved in transporting the commodity from producers to end consumers (Olukosi *et al.*, 2004). Unlike other products, fish marketing rarely follows a direct fisherman-to-consumer model (Lawal *et al.*, 2007). Instead, fish marketing ensures widespread availability by facilitating the flow of fish from fishermen to consumers through various business activities (Akanni *et al.*, 2009). Effective fish marketing relies on the establishment of functional markets, where buyers and sellers can interact, agree on prices, and complete transactions, ultimately making fish marketing successful.

2.9.1 Concept of Market

American Marketing Association (AMA) defines marketing as the performance of business activities that direct the flow of goods and services from the producers to user. It includes surprisingly wide range of activities. According to (Crammer *et al.*, 2001) marketing can be defined as all process involved from the production of all commodities until it gets to the final consumer.

2.9.2 Prospects of Marketing fish Products

The prospect of fish marketing are:

i. Provision of special incentive like insulated tracks. This will help enrage the transportof fish product to long distance without becoming deteriorated.

ii. Provision of adequate processing equipment and landing site

iii. Provision of sheds at processing and landing site; this will help to protect the fish from direct sunlight which might cause loss to the taste of freshness

iv. Proviso of adequate storage facilities Martin Adams (2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

This study was carried out in Benin City, Edo State. It lies within latitude 6.3^0N and longitude 5.6^0E. The Binis are known for their beads, body mark as well as their raffia work and subsistence farming. The Bini people have four market days; Ekioba, Ekenaka, Agbado and Eken. Benin City is predominantly urban society and agriculture in Benin City supports more than 70% of the population (FAO, 2014). It is characterized by minor industrialization activities and it is epicentre of business and other activities in Edo State (Tafemi and Idolor, 2008). It is the four-largest in Nigeria with a population of 1,782,000 as of 2021 (Wikipedia, 2021).

3.2 Collection of Samples

The commercial fish species (*Clarias gariepinus*,) were obtained from a reputable fish farm in Benin City, Edo State. A total of 20 table size fish were obtained and used. The fish samples was collected using a plastic bowl with clean tap water sealed with jute bags to prevent contamination.

3.3 Preparation of Materials

The working area were properly cleaned and the equipments were thoroughly washed.

3.4 Processing Apparatus

This study was carried out at the Department of Aquaculture and Fisheries Management processing unit. Some of the apparatus that were used during the study includes; pot, gas oven, knife, mixer among others.

3.5 Processing Methods

3.5.1 Production of Fish Concentrate

The fish were washed thoroughly with clean water so as to remove all extraneous matter. The fish were killed and eviscerated. The flesh of the fish were separated from the bones using a sharp knife. The fish were washed again in order to remove blood and other intestinal waste from the fish. The eviscerated fish was then 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. Thereafter, it was drained and dried. The dried sample were milled to powdery form and was stored in an air-tight polyethylene container for use.

Fish

↓

Washing

↓

Killing

↓

Evisceration

↓

Removal of internal bone

↓

Washing

↓

Size reduction

↓

Soaking in sodium chloride (10min)

↓

Draining

↓

Drying

↓

Milling

↓

Packaging

Fig 1: Flow chart for fish concentrate production (Horner, 1997).

3.5.2 Production of Bread Fortified with Fish Concentrate

All the ingredients which comprises flour, salt, sugar, fish concentrate, butter, yeast, shortening agent were weighed using a precision metter balance. The ingredients was added to the mixer and was thoroughly mixed. The mixed dough were placed in a large container or trough to allow fermentation. During fermentation, the mass of dough were kneaded several times to allow escape of air which was continuously produced during fermentation. After fermentation, the dough was then divided into smaller sizes which eventually make up the finished loaves. The loaves was moulded, shaped and dropped into baking pans that have been cleaned and rubbed with oil. And was taken in to the oven to be bakeedn. The bread was baked at 180^0C for about 25 minutes. The baked loaves was cooled and thereafter packaged and stored for laboratory analysis.

Flour + other ingredients

↓

Mixing

↓

Fermentation

↓

Kneading

↓

Cutting into different sizes

↓

Addition of fish concentrates

↓

Moulding

↓

Panning

↓

Baking (180^{0}C for 25 minutes)

↓

Cooling

↓

Packaging

Fig 2: Flow chart for bread production (Cauvain, 2007**)**.

3.6 Microbial Analysis

For the microbial analysis of the sample, the method that was adopted is that described by Ezeama (2007). Bacteria and fungi were isolated for confirmation, Nutrient agar and Potato dextrose agar were then prepared using the manufacturer's instructions.

3.6.1 Nutrient agar

Seven grammes (7g) of nutritious agar were mixed into 250 millimeters (250ml) of distilled water, which was then stirred to achieve thorough mixing. The mixture was autoclaved at 121^0C for 15 minutes and allowed to cool to 45^0C. The media was poured onto an already-inoculated disposable petri dish. The plates were infected with a pasture pipette incubated at 37^0C for 24 hours, solidified, and inverted. During incubation, nutrient agar plates are inverted to avoid contamination from water or condensation, which can fuse colony forming units. A piece of the growth would be subcultured and gram-stained for microscopic analysis, with the slides inspected under a microscope (x 100) (Ezeama, 2007). Bacteria were isolated using nutrient agar.

3.6.2 Potato dextrose agar

Seventeen grammes (17g) of PDA were measured and dissolved in 250ml of distilled water before they were autoclaved at 121^0C for 15 minutes to inhibit bacterial growth. 40ml of streptomycin was then added. It was then removed and allowed to cool before the pour plate procedure was used aseptically. The gelled plate was then incubated at room temperature for 5 days. Potato dextrose agar was used for the isolation of fungi according to Ezeama (2007).

3.6.3 Preparation of sample and serial dilution

The microbial analysis was conducted for both bacteria and fungi, sample preparation will be made using the method described by Ezeama (2007). The test tubes were washed properly, 10ml of distilled water was measured into the test tubes representing the stock and 9ml in the other test tubes and covered at the top with a stopper made of cotton wool and aluminum foil.1g of bread sample was sliced with a sterilised blade, pounded with a mortar and pestle, weighed and poured into the first test tube (stock), it was properly mixed by shaking. Then 1ml of the stock solution was transferred to the 2 test tube and shaken, which was dilution 1 and it was mixed properly, 1ml of dilution 1 was transferred into the next test tube which was dilution 2, serial dilution was continued until dilution 7. Dilution 5 and dilution 6 test tube were used as dilution factor and 0.1 ml was transferred from the stock test tube into a sterilized petri dish in duplicates. All plates were incubated upside down at 37^0C for 24 hours after which colonies formed were counted and expressed as colony forming units per gram.

3.6.4 Inoculation of media plates

The pour-plate approach was used to inoculate all media plates. A loopful of each stock solution was selected with a well-flamed wire loop and streaked across the surface of each media plate to inoculate them. The inoculums were dispersed evenly by gently spinning the plate. The plates were then incubated for 24 hours at 37^0C before being read. To obtain pure cultures, each separate colony was selected and streaked on fresh medium plates. All plates and test tubes were correctly labelled based on the dilution, agar utilised, and the treatment from which it was obtained.

3.6.5 Isolation of bacteria

Bacterial colonies isolated were purified by replanting on sterile nutrient agar and incubated for 24 hours at room temperature (28 ± 2^0C).

3.7 Statistical Analysis

Data will be subjected to two way analysis of variance (ANOVA) using computer software (Genstat Version 8.1, 2005). Significant differences between means will be determined with Duncan's multiple range tests at $P < 0.05$.

CHAPTER FOUR

RESULTS

Results of the Bacterial Counts (cfu/g) of Bread fortified with different level of Scomber *scombrus* concentrate.

Table 1 provides a clear picture of how bacterial populations increase in bread fortified with different level of Scomber *scombrus* concentrate at (0%, 10%, 20% and 30%) over a three-day period under different treatments, immediately after processing there was no significant difference in the bacterial count of the treatment. Over time on day 0, the bacterial counts in the samples were relatively low, ranging from 4.10×10^2 CFU/g in Treatment A to 1.20×10^3 CFU/g in Treatment D. By day 3, the bacterial populations in all samples had increased, with significant differences between treatments. Treatment D had the highest bacterial load, while Treatment A had the lowest.

Table 1: Bacterial Counts (cfu/g) of Bread fortified with different level of Scomber *scombrus* concentrate.

	TREATMENT				S.E.D
TIME_SPAN	**A**	**B**	**C**	**D**	
DAY_0	$4.10 * 10^{2E}$	$7.30 * 10^{2D}$	$1.10 * 10^{3C}$	$1.20 * 10^{3C}$	
DAY_3	$1.10 * 10^{3C}$	$1.90 * 10^{3B}$	$2.00 * 10^{3B}$	$2.40 * 10^{3A}$	72.5

Values with the same superscripts across the table 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 Fungal Counts (cfu/g) of Bread fortified with different level of Scomber *scombrus* concentrate.

Table 2 shows us the fungal Counts (CFU/g) of bread fortified with different level of Scomber *scombrus* concentrate at (0%, 10%, 20% and 30%) over a 3 days period under different treatment. There was no significant difference in the fungal load of the treatment over the storage period.

At Day 0, fungal counts were relatively low, with Treatment D (3.40×10^2 CFU/g) having the highest contamination and treatment A (1.70×10^2 CFU/g) having the least contamination. By Day 3, fungal levels had increased across all treatments. Treatment D showed the highest fungal count (4.20×10^2 CFU/g), while Treatment A remained the lowest (3.00×10^2 CFU/g).

Table 2: Fungal Counts (cfu/g) of Bread fortified with different level of Scomber *scombrus* concentrate

	TREATMENT				S.E.D
TIME_SPAN	**A**	**B**	**C**	**D**	
DAY_0	$1.70 * 10^{2F}$	$2.70 * 10^{2E}$	$3.30 * 10^{2C}$	$3.40 * 10^{2C}$	8.45
DAY_3	$3.00 * 10^{2D}$	$3.40 * 10^{2C}$	$3.80 * 10^{2B}$	$4.20 * 10^{2A}$	

Values with the same superscripts across the columns 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

FREQUENCY OF OCCURRENCE OF MICROBIAL ISOLATES IN BREAD SAMPLES (Day 0)

Possible bacterial isolates	F(%)	A	B	C	D
Enterobacteraerogenes	1(5.26)	-	-	-	+
Bacillus subtilis	4(21.05)	+	+	+	+
Pseudomonas sp	4(21.05)	+	+	+	+
Klebsiellasp	1(5.26)	-	-	+	-
Bacterial frequency	10	2	2	3	3
Bacterial % frequency	52.63	20.00	20.00	30.00	30.00
Possible Fungal isolates	F(%)	A	B	C	D
*Aspergillus*sp	3(15.79)	-	+	+	+
*Penicillium*sp.	4(21.05)	+	+	+	+
Saccharomyces sp	2(10.53)	-	+	-	+
fungal frequency	9	1	3	2	3
fungal % frequency	47.37	11.11	33.33	22.22	33.33
microbial frequency	19	3	5	5	6
microbial frequency	100	15.79	26.32	26.32	31.58

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

FREQUENCY OF OCCURRENCE OF MICROBIAL ISOLATES IN BREAD SAMPLES (Day 3)

Possible bacterial isolates	F(%)	A	B	C	D
Enterobacteraerogenes	4(11.43)	+	+	+	+
Bacillus subtilis	4(11.43)	+	+	+	+
Pseudomonas sp	4(11.43)	+	+	+	+
Klebsiellasp	1(2.86)	-	-	+	-
Proteus sp.	3(8.57)	+	+	-	+
Micrococcus sp.	2(5.71)	-	+	+	-
Streptococcus sp	2(5.71)	-	-	+	+
Bacterial frequency	20	4	5	6	5
Bacterial % frequency	57.14	20.00	25.00	30.00	25.00
Possible Fungal isolates	F(%)	A	B	C	D
*Aspergillus*sp.	4(11.43)	+	+	+	+
*Penicillium*sp.	4(11.43)	+	+	+	+
*Rhizopus*sp.	2(5.71)	-	+	+	-
Saccharomyces sp	2(5.71)	-	-	+	+
Mucorpiriformis	3(8.57)	+	+	-	+
fungal frequency	15	3	4	4	4
fungal % frequency	42.86	20.00	26.67	26.67	26.67
microbial frequency	35	7	9	10	9
microbial % frequency	100	20.00	25.71	28.57	25.71

CHAPTER FIVE

DISCUSSION

5.1 Bacterial Growth in Bread Fortified with different level Scomber scombrus Concentrate.

Immediately after processing there was on significant difference the bacterial counts were fairly low across all samples, which is expected since the bread was freshly baked. Treatment A (0% fish concentrate) had the lowest count at 4.10×10^2 CFU/g, while Treatment D (30% fish concentrate) already had a higher starting count of 1.20×10^3 CFU/g

By Day 3, the Bacterial levels had more than doubled across most samples, with Treatment D reaching 2.40×10^3 CFU/g, the highest of all. This increase follows a predictable pattern—given time and the right conditions, bacteria multiply. Since the bread wasn't refrigerated (assuming normal room temperature storage), this growth was inevitable.

on Day 0, Treatments C (20% fish concentrate) and D (30% fish concentrate) had nearly identical bacterial counts. This shows that the difference between 20% and 30% fish concentrate wasn't significant in terms of bacterial presence. However, by Day 3, Treatment D showed noticeably higher bacterial growth. This indicates that while small differences in fish concentrate levels may not have an immediate impact, over time, higher fish content does lead to faster microbial growth.

for fungi to multiply, while others saw only modest increases. These variations could be influenced by factors like moisture levels, preservatives, and even how the bread was stored (Ray & Bhunia, 2013). On Day 0, fungal counts ranged from 170 in Sample A to 340 in Sample D. By Day 3, all samples had experienced some growth, but at different rates. Sample A saw the most significant increase, jumping by 76.47% to reach 300 fungal units. Sample B followed with a

25.93% increase, while Sample C showed a more modest growth of 15.15%. Sample D also had a relatively low increase of 23.53%.

The sharp rise in fungal growth in Sample A suggests that conditions in this sample were especially suitable for fungal proliferation perhaps due to higher moisture levels or the absence of effective preservatives (Jay, Loessner, & Golden, 2005). Meanwhile, Samples C and D, which showed the lowest percentage increases, may have contained preservatives or had lower moisture content, making it harder for fungi to thrive (Davidson, Sofos, & Branen, 2005).

These findings highlight the role that environmental conditions and ingredients play in fungal growth. Fungi, like bacteria, need moisture, warmth, and nutrients to multiply (Montville & Matthews, 2013). The slower growth in some samples suggests that preservatives or storage conditions might have been effective in slowing down fungal activity. Common preservatives like sorbates and propionates are known to inhibit fungal growth in baked goods, which could explain the differences in growth rates (Smith & Simpson, 2020).

5.2 Fungal Growth in Bread fortified with different level of Scomber *scombrus* concentrate.

After processing fungal growth progressed in bread with varying levels of Scomber scombrus concentrate (0%, 10%, 20%, and 30%). As expected, fungal counts increased over time, which aligns with what we generally know about food spoilage. Fungi thrive in warm, moist environments where they can feed on available nutrients. Bread, being rich in starch and often stored at room temperature, offers an ideal setting for fungal growth.

On Day 0, fungal contamination was present but relatively low. The control sample (Treatment A, with 0% fish concentrate) had the lowest fungal count at 1.70×10^2 CFU/g, while the sample with the highest fish concentrate (Treatment D, with 30%) had the highest count at 3.40×10^2 CFU/g. This suggests that the fish concentrate may have played a role in the initial fungal presence, possibly due to naturally occurring spores in the fish concentrate or the additional moisture it introduced.

By Day 3, fungal levels had increased across all treatments. Treatment D had the highest fungal count at 4.20×10^2 CFU/g, while Treatment A remained the lowest at 3.00×10^2 CFU/g. This trend is consistent with previous studies showing that fungi multiply rapidly in favorable conditions such as high humidity and ample nutrient availability (Smith & Jones, 2021).

On Day 0, Treatments C and D had similar fungal counts. However, by Day 3, Treatment D had the highest fungal growth, indicating that higher fish concentrate leads to faster spoilage. The Standard Error of the Differences of Means (S.E.D.) was 8.45, suggesting some variability, likely due to storage conditions, ingredient differences, or handling inconsistencies

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1. Conclusion

This study clearly shows that adding Scomber *scombrus* concentrate to bread affects how quickly bacteria and fungi grow. As expected, microbial growth increased over time, but the key takeaway is that the more fish concentrate the bread contained, the faster this happened.

On the first day, all samples had relatively low bacterial and fungal counts, with the control sample (0% fish concentrate) having the least. However, by the third day, the numbers had more than doubled across the board, with the highest levels found in bread with the most fish concentrate (30%). This suggests that while the fish concentrate adds nutritional value, it also brings extra moisture and possibly some natural microbes that speed up spoilage.

Interestingly, at first, the difference between 20% and 30% fish concentrate wasn't very noticeable, but by day three, the higher fish content led to significantly more microbial growth. This confirms that even small changes in fish concentration can impact shelf life over time.

While fortifying bread with fish concentrate can be a great way to boost its protein content, these findings highlight a major challenge: keeping the bread fresh for longer.

6.2. Recommendations

Based on the findings from this study, the following recommendations are proposed:

1. Better Storage Methods Since higher fish content leads to faster spoilage, proper storage is crucial. Refrigeration or vacuum-sealing the bread could help slow down microbial growth and extend freshness.

2. Natural Preservatives adding natural antimicrobial ingredients, such as vinegar, citrus extracts, or certain spices, might help keep bacteria and fungi in check without affecting the bread's nutritional value.

3. Finding the Right Balance while higher fish concentrate adds more nutrients, it also shortens shelf life. A moderate level (perhaps 10-20%) could be a good compromise between nutrition and storage stability.

4. Strict Hygiene Practices since microbes likely come from the fish concentrate itself, ensuring that it is handled and processed in the cleanest possible conditions could reduce contamination from the start.

5. Further Research More studies should be done to explore ways to make fish-fortified bread last longer, such as testing different packaging, drying techniques, or alternative preservative options.

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