IDENTIFICATION AND ISOLATION OF TOXIGENIC FUNGI IN TRADITIONAL YOGHURTS SOLD IN LAGOS STATE

BY

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DECLARATION

I, MPAMA-IBEKWE SAMUEL TOOCHUKWU, do hereby declare that this project is entirely my work and composition. The work embodied in this project has not been submitted in candidature for any degree and is not concurrently being submitted for any other degree. All references made to works of other persons have been duly acknowledged.

…… ……

SIGNATURE DATE

CERTIFICATION

This is to certify that this research project was carried out by MPAMA-IBEKWE SAMUEL TOOCHUKWU, with matric number **18/4892** in the Department of Biological Sciences and Biotechnology, Microbiology, Program, College of Pure and Applied Sciences, Caleb University, Imota, Lagos, Nigeria.

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DEDICATION

This project is dedicated to Almighty God, the giver of knowledge and understanding for His guidance. Who began and oversaw me in the entirety of my endeavors to effectively complete my project. I also dedicate it to my father, Lt. mother, and siblings for their adoration, support, and graciousness towards me during this project.

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ABSTRACT

This study aims to Isolate fungi found in traditionally produced yogurts Sold in local government in Yaba, Lagos state. Seven unbranded yogurts samples (A – H) and seven branded samples of yoghurts (1 – 7) were acquired within the same Yaba Local Government Area, Lagos State. Using culture growth medium (Potato dextrose Agar) and the pour plate method, the fungi and molds species present were analyzed. Six species of fungi were isolated from both branded and unbranded yoghurt. The isolates were identified and characterized based on some Pathogenicity tests and Beta Hemolysis for clear zone inhibition. The fungi species are identified and their percentages are as follows *Aspergillus flavus* (30.8%), *Acrymonum* spp.(15.4%), *Aspergillus* spp. (38.5%), *Fusarium* spp. (7.7%) *and Aspergillus niger* (7.7%). The isolates produced a different volume of aflatoxin which varies from 0.06072 e^{-5} to 28.58637 e^{-5}. The pH and the titre values of yoghurt samples ranged from 4.31 – 4.72 and 11.41ml - 22.09ml. The unbranded yoghurt for both room and fridge temperature have the highest colony counting (12.4 X 10^5) while the lowest colony counting was recorded in the branded yoghurts (2.2 X 10^5). The yoghurt samples contain viable fungi cells amongst which are pathogenic strains capable of causing various health complications. This indicates a lack of good manufacturing practice (GMP) or inadequate storage. There is, therefore, the need for proper monitoring and quality control amongst local producers and health workers to ensure that correct guidelines and GMP for yoghurt are maintained. There is also the need to address storage problems to minimize the risk of food-borne infections and intoxication through yoghurt consumption.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

In the world at large, a broad variety of fermented food products are manufactured and consumed. Fermentation preserves raw foods and expands the range of food products available (Ross *et al.,* 2002). Raw foods that are fermented all over the world include cereals, oilseeds, milk, seafood, meat, and vegetables (Lee*,* 1997). Fermented foods, as part of a balanced human diet, can help maintain a healthy intestinal tract and boost the acceptability of dairy products for lactose intolerant people (Brown-Esters *et al.,* 2012). Food fermentation is particularly beneficial in Africa for preventing infant malnutrition and detoxifying raw foods such as cassava, which contain toxic compounds (Holzapfel*,* 2002). The proliferation and fermentation process of the Lactic Acid Bacteria (LAB) used as starter cultures cause majority of chemical and physical changes that occur during the fermentation of products. For milk fermentation, LAB cultures are used as starter cultures. (Panesar*,* 2011).

Yogurt can be described as a product of milk that has undergone fermenting process by bacteria. Lactic acid is made when milk sugar, also known as lactose, is fermented. Lactic acid is the ingredient that gives yogurt its unique tactile and sensory qualities and acts on milk protein (Arican and Andic*,* 2011). Low acidity is also a good environment for yeasts to live in. Yeasts are also present in fermented foods. They have had a favorable effect on bacteria by changing the pH and releasing biological chemicals (Yam *et al.,* 2015). If caution is not exercised, they may be the source of deterioration in fermented products.

Yogurt includes significant amounts of LAB, and the smaller yeast can promote lactose tolerance, balance intestinal microflora, function as an antibiotic, activate the immune system, generate anticancer effects, and induce anti-cholesterolemic effects. Yeast may produce fragrant components and interact with primer starting cultures, such as LAB. LAB, or starter culture, is stabilized by yeast growth (Kavas *et al.,* 2006).

Industrial production of yogurt is a properly controlled fermentation process involving the use of sugar, flavoring, milk, fruit, emulsifiers, coloring, and certain microbe cultures. (Yam *et al.,* 2015). The base of the yogurt is virtually free from microbes because it is heated to around 90°C before inoculation with lactic acid bacteria at a concentration of more than 107 CFU g^{-1} to start the fermentation process. The microbiological aspect of yogurts is essentially restricted to two species of bacteria and they include *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp, which are commonly found in a 2:1 ratio. The presence of any additional microorganisms should be seen as contamination. Filamentous fungi and yeasts frequently play a crucial role in the spoilage of yogurts, even though bacteria can be spoiling organisms (Fleet*,* 1992). Off-flavors, texture caused by the production of gas, and packaging shrinkage and swelling are all caused by them (Foschino *et al.,* 1993). At the time of manufacturing, yogurt should include lower than 10 yeast cells g^{-1}, and values higher than this will most likely result in spoilage before the standard (refrigerated) expiry of 30 days. Flavor fruit and color are usually put into yogurt following fermentation. Yeast contamination is typically linked to poor hygiene practices during the process of packaging and adding fruits, (Fleet, 1992). Lactose-fermenting yeasts have a long history of being associated with dairy products. Yogurts with more than 106 cells g^{-1} have been documented (Silvia *et al.,* 2001). Green and Ibe (1987) reported that up to 60% of samples from retail locations in Portugal, Australia, and Nigeria had counts over 104 cells g^{-1}. In Egypt, molds and yeast values of 104 to 105 cells, g^{-1} have been found (Haridy, 1993). Up to 30% of samples in the UK and Canada had more than 103 cells g^{-1}. In Spain, 95 percent of yogurts had cell counts of higher than 102 cell g^{-1}. Contamination was slightly lower in the United States and the Netherlands (Silvia *et al.,* 2001). Yogurt consumption increased exponentially in Brazil over the previous decade, although it still lags behind that of wealthy countries. In Brazil, yeast and mold contamination of yogurts was generally low, according to data from a recent study (Hoffmann*,* 1996), with 11 of 17 yogurt samples containing about 10 or fewer unknown yeasts or fungi per gram and just two samples containing high levels (104 – 105 cells g^{-1}). This indicated that

better hygienic standards were being used. The study aims at firstly isolating and identifying the contaminating yeasts and molds, as well as to characterize some of the physiological parameters that contribute to their proliferation in yogurts. Preliminary research found that two international-brand manufacturers had the highest standards, with yeast counts consistently less than 10 cells g^{-1}. As a result, time and effort were spent examining smaller-scale, local producers where it was assumed that hygienic standards would not be up to par.

1.2 Aim and Objectives

This study aims to isolate and identify fungi in traditional yogurts sold in supermarkets and roadside sellers across Lagos state.

The main objective of the study is:

1. To isolate and identify toxigenic fungi in traditional yoghurt samples collected in Lagos state.
2. To test for the pathogenicity of toxigenic fungi in traditional yoghurt samples collected in Lagos state.
3. To characterize and identify the isolates using morphological and microscopy techniques
4. To extract toxins from the pure fungi cell isolates and carry out Molecular identification of the isolates using GC-MS

CHAPTER TWO

LITERATURE REVIEW

2.1 Yogurt

2.1.1 Definition and History of Yogurt

Streptococcus thermophilus and *Lactobacillus bulgaricus* cause the production of lactic acid through fermentation of milk-sugar lactose to produce yogurt, a coagulated milk product (Adolfsson *et al.,* 2004). To make a unique yogurt recipe, more lactic acid bacteria (LAB) can be added. The LAB used to make yogurt is required to be living and present in significant levels at the time of eating for it to be called a nutritious food.

The first yogurt was manufactured by the Bulgars in the 2nd century by natural fermentation of milk, and it continued to exist predominantly as food for Western, Central, and South Asia, as well as Central Europe,' South-Eastern Europe. Metchnikoff discovered frequent ingestion of milk products that have undergone fermentation process and containing L. bulgaricus increases health and longevity a little over a century ago, claiming that consuming yogurt lessens the harmful effect of putrefactive bacteria present in the intestine by lowering their viability. (Wollowski *et al.,* 2001). Flavor molecules, such as carbonyl compounds, volatile acids, non-volatile acids, and other chemicals, can be created by lactic acid bacteria during milk fermentation (Serra *et al.,* 2009).

2.1.2 Manufacture of yogurt

The following are the six basic phases in the production of yogurt:

a) Debris removal from milk via filtration,

b) Checking for antibiotics that may interfere with the starting bacteria's action,

c) Milk standardization for high-quality yogurt production

To begin, depending on market demand, the amount of fatty milk in yogurt could range from 0.5-4.5%. As a result, fatty milk can be separated or supplemented to obtain the required final product value.

Second, the nonfat milk solid (NFMS) content increases to the desired level. These values in milk, which range from 8.5- 9%, are made up of 4.5% lactose, 3.3% protein (0.7 percent whey protein and 2.6 % casein), and 0.7 percent mineral salts. For a high-quality product, the NFMS is required. Lactose is the bacteria's energy source, and protein and mineral concentrations influence gel formation. The NFMS value in liquid milk, on the other hand, is insufficient for a firm gel structure, and it should be increased to 13-18 percent by the addition of skimmed milk powder or partially evaporating the liquid under a vacuum.

c) **Milk homogenization**

The size of fat globules is reduced to 1-2 M during homogenization. It reduces fat segregation in the manufacturing process and facilitates the inclusion of other ingredients like skimmed milk powder. The fat globule membrane is damaged during homogenization, lipase enzymes attack the globules that have been damaged. To prevent lipolysis, lipase enzymes should be hindered by subjecting the milk to boiling immediately after homogenization. (Serra *et al.,* 2009).

d) **Application of heat**

The pasteurized milk is passed through a plate heat exchanger for 5-10 minutes at 90-95°C or a processing vessel at 80-85°C for 30 minutes. Heat treatment kills all harmful germs and renders enzymes like lipase inactive. Whey proteins, -lactoglobulin, and -lactalbumin are also denatured. The hydrophilic nature of the casein is increased when -casein and denatured - lactoglobulin combine, and this feature is particularly important for gel strength. Syneresis is reduced and coagulum stability is increased by the combination of -casein and denatured -lactoglobulin. Heating at elevated temperatures diminishes casein micelles' hydrophilicity, thus 85°C is the optimal temperature (Sodini *et al.,* 2004).

f) **Inoculation and incubation**

The milk is chilled at 43°C after the heat treatment before being inoculated alongside the starting culture. The ratio of cocci to bacilli in yogurt starting culture is 1:1 (Pikul and Sokolinska 2004). The

inoculation dose can range from 0.5 to 5% (percent), with 2% being the standard. The starting cells are stirred into the milk. The milk is then distributed into its retail containers or bottles and incubated for 3-4 hours at 42-43°C till the conclusion of the incubation duration and is set at a pH of 4.5 industrially.

g) **Cooling and storage**

The first option for finishing incubation is to chill the yogurt as fast as possible at an acidity of 1.2-1.4 percent to avoid whey syneresis on the surface (pH 4.3), over-acidification, and shrinking of the protein gel. Secondly, the cooling approach is regulated into two-stage chilling, wherein the temperature is initially lowered to roughly 37°C with pH 4. 6, then chilled to 10°C with acidification slowed to pH 4.3. The viscosity of yogurt increases after being refrigerated at 4°C for 1-2 days. When gels are moistened, they get stiffer while casein micelles continue to harden (Tamime and Robinson, 1999).

2.1.3 Factors Influencing the Fermented Milk's Physical Properties

Though fermented milk has a similar moisture content to milk, they act like solids. Milk that has been fermented is shaped like a gel, with several of casein encasing fat and milk serum. Fat quantity, type or amount of protein, total solids, parameters for processing such as thermal treatment, amount and type of starter culture used, pH, the addition of a stabilizer, amount and type of exopolysaccharides, and the temperature of incubation and subsequent storage are the major factors influencing milk gel characteristics (EPS). For example, yogurt manufactured from UHT-treated milk showed higher viscosity and hardness than yogurt manufactured from unheated milk pasteurized at 90°C for 10 minutes (Mottar *et al*., 1989). The yogurts gel strength produced from unheated milk was also shown to be lower than yogurt made from warm milk (Tamime*, et al*., 2007). Yogurt manufactured with homogenized milk has a firmer texture and less syneresis than yogurt made with unhomogenized milk (Tamime *et al.,* 2007). In the production of yogurt, stabilizers like starch, gelatin, carrageenan, pectin, and locust bean gum, are utilized. Stabilizers generally stabilize and enhance the casein network while also raising the viscosity of the continuous phase, lowering syneresis, and boosting yogurt viscosity

(Everett and McLeod, 2005). It is possible to alter the physical properties of yogurt by increasing milk solids for the former and greater storage modulus yogurts with less fermentation time (higher inoculation rate) for the latter, as indicated by better body and texture and reduced syneresis (Lee and Lucey, 2004).

2.1.4 Types of Yogurts

Yogurts are grouped based on their chemical composition (reduced fat, low fat, full-fat yogurt), method of manufacturing (stirred and set-type), post-incubation process, flavor (Shah, 2003). Stirred yogurt (such as Ayran) undergoes fermentation in tanks, with the gel broken before cooling and packaging by stirring, leading to low viscosity stirred yogurt whereas, set yogurt is cultured in a retail container. Yogurt can be divided into various subcategories based on flavor. Fruits can be added to plain yogurt, or they can be flavored with sweetness and coloring components.

2.1.5 Yogurt's nutritional value

Yogurt is high in proteins, B vitamins, and important minerals because it is made from milk (Reid *et al*., 2003). Yogurt is also high in calcium and has the same amount of fat as the milk it is derived from. The milk components, bacteria strains employed during the process of fermentation, source, species, and kind of temperature, fermentation time, and milk solids, are all factors that affect the nutritional content of yogurt (Adolfsson *et al.,* 2004).

Vitamins, antioxidants, probiotics, and other important elements can easily be added to yogurt. Lycopene, docosahexaenoic acid (DHA), CoQ1O, and omega-3 eicosapentaenoic acid (EPA), are some of the most effective antioxidants and are among the nutrients added to yogurt (Gardes and Wegner, 2007). The majority of these elements have positive health benefits (Ozer and Kirmaci, 2010). Yogurt can also be supplemented with probiotics, prebiotics, and phytosterols. The prebiotics, which are primarily polydextrose, act as dietary fibers. Inulin and oligofructose are prebiotics examples (Vasiljevic *et al.,* 2007). Leeks, Artichokes, and garlic contain inulin, consisting of fructose and glucose chains. Oligofructose is generated via enzymatic hydrolysis of inulin or partial enzymatic

hydrolysis of sucrose (transfructosylation by P-fructofuranosidase). Oligofructose and Inulin both have functional and nutritional benefits in foods, supporting probiotics growth like lactobacillus and bifidobacteria, and so enhancing health and digestive efficiency (Shah, Lankaputhra and Bruno, 2002). Sweeteners are required to appeal to particular market segments, especially children. The majority of sweeteners used have no effect on the growth and cultures used, which is beneficial (Ziar and Riazi 2011). Survivability of probiotics and LAB might be as high as 90% and 85% respectively in the samples of yogurt sweetened with sourwood honey. Adding -glucan from barley and oat to yogurt increased the viability and stability of probiotics, according to (Vasiljevic *et al.* 2007). (Bifidobacterium animalis ssp. lactis). Phytosterols are becoming a more common ingredient in yogurt. These are organic sterol substances found in plants and have been used in the treatment of hypercholesterolemia (Monu *et al.,* 2008). Phytosterols are popular in functional meals due to the claimed health advantages linked with their capability to prevent absorption of cholesterol and hence reduce the rate of coronary heart disease. For example, daily consumption of 0.7 percent fat low-fat yogurt with 3 grams of plant sterols reduced LDL cholesterol by 13.7 percent (Noakes *et al.,* 2005).

2.1.6 Yogurt's Health Benefits

Yogurt has a variety of health advantages that are linked to either bacteria found in the yogurt or the byproducts of milk microbiological fermentation. Lactic acid aids those with lactose intolerance in absorbing calcium and digesting a portion of the lactose (Kucukoner and Tarakci, 2004). Yogurt bacteria can produce vitamin B, which the body needs (Adolfsson *et al*., 2004). Yogurt can also help the body absorb calcium, iron, and protein, and by acting as an antibiotic, strengthening the immune system, protecting against gastrointestinal upset, lowering cholesterol in the blood, particularly low-density lipoprotein cholesterol, and lowering the risk of cancer *(*Adolfsson *et al*., 2004*). (2011) (*Andronoiu *et al.)*.

2.1.7 Yogurt's biodefense qualities

The benefits of yogurt to health have been proven in man and animal studies. Among the potential health advantages includes mitigation of constipation, allergies, lactose intolerance, Helicobacter pylori infection, colon cancer, inflammatory bowel disease, and diarrheal diseases (Adolfsson *et al*., 2004). Protective proteins and peptides found in cow's milk yogurt that have specific biological activity have been connected to bio defensive properties in yogurt and fermented milk (Adolfsson *et al*., 2004). Cytokinin's and angiotensin-converting enzyme (ACE) I peptides work by hindering ACE and restricting the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. Milk peptides may be anti-carcinogenic, as proven by their ability to suppress the growth of colon carcinoma and cancer cell precursors due to bioactive cysteine and methionine (Korhonen *et al*., 2000). This is because both amino acids are involved in cellular methylation and DNA stabilization, as well as cellular glutathione production, which is vital in cancer defense mechanisms.

Predigestion of milk by bacteria in yogurt releases free amino acids, which leads to a larger content of amino acids present in yogurt than that in milk (Shahani and Chandan, 1979), validating the argument that enzymes in yogurt are better broken down or digestible than milk proteins.

Yogurts are usually available in fat-free or low-fat forms, demonstrating that lipid hydrolysis does not affect the qualities of yogurt products. Yogurt, on the other hand, contains more CLA, a long-chain bio hydrogenated linoleic acid derivative, than fresh milk from cows. CLA has immune-stimulatory properties and anti-carcinogenic properties (Whigham, Cook, and Atkinson, 2000). Because lactose enhances mineral absorption, the availability of minerals such as zinc, calcium, and magnesium in yogurt may be lowered due to the lower lactose concentration (Pansu and Bronner, 1999). However, since yogurt is acidic (low pH), calcium remains ionic and improves intestinal calcium absorption (Bronner and Pansu, 1999). During the fermentation process, the bacterial cultures used have an impact on the nutrient content of yogurt. Although vitamin B is not necessary for LAB growth, since

certain strains produce them, and hence vitamin loss during processing could be reduced to an extent by proper use of such cultures (Winkler *et al.,* 2005).

2.1.8 Quality of Yogurt

The microbiological, physical, and chemical, aspects of yogurt can all be used to determine its quality. Chemical and microbiological criteria are regulated by each country's food regulation. The Australian Food Standard Code (Standard 2.5.3, 2004), for example, stipulates that "the viable counts of yogurt starter cultures must be at least 106 cfu/g of the product during the storage period". The protein composition of the product must be at least 30 g/kg and must have a pH of less than 4.50. The use of stabilizers is forbidden in various European nations (Degeest *et al.,* 1999). Physical features of a product, on the other hand, are not required by law. Set yogurt texture must be smooth, firm, lumps or grains free, and spoonable without any syneresis on the product's surface (Robinson and Tamime 2007). Physical qualities of yogurts have been assessed using a variety of methodologies, including sensory and instrumental tests.

2.1.9 Rheological properties of Yogurt

Rheology is the study of how matter flows and deforms. In food-related research, the phrase is sometimes interchangeable with texture and this refers to the deformation, disintegration, flow of a sample under stress (Shaker *et al.,* 2000). Viscosity or the ability to withstand flow, refers to liquid foods while texture refers to solid foods, whereas. Some foods, on the other hand, can have both liquid and solid properties, and hence the rheological properties of such foods can be utilized to determine their attributes (Tunick, 2000). Food rheological properties are important for the design of flow processes, quality assurance, storing, and manufacturing, as well as forecasting food texture (Shaker *et al.,* 2000). The texture of food is related to sensory aspects of food since it represents all the rheological and structural features observable through tactile, mechanical, and when appropriate, auditory and visual sensors. Because they provide meaningful information about a product's textural qualities, instrumental methods can be used to objectively examine its evaluation, rheology, and

structure. This feature is quite valuable because instrumental and sensory data are not always easily related (Sodini *et al.,* 2004).

In a dynamic rheological experiment, small amplitude oscillatory testing, sinusoidal oscillating stress or strain with a frequency is applied to the material, and the phase difference between the oscillating stress and strain, as well as the amplitude ratio, are measured (Rao, 2003). As a result of the applied strain, two stress components are created in the viscoelastic material: an elastic component that is in phase with the strain and a viscous component that is out-of-phase (90°). The resulting stress can be expressed as an elastic or storage modulus G' and a viscous modulus V' or loss modulus G" for deformation within the linear viscoelastic range. The loss modulus describes the size of the energy stored in the material or recoverable per cycle of deformation, whereas the storage modulus expresses the size of the energy stored in the material or recoverable (Rao, 2003).

2.1.10 Separation of Whey

Whey or serum separation, as well known as wheying off, is defined as "the emergence of whey on the top of a gel" (Lucey, 2002). It is an everyday fault that takes vicinity at a few levels withinside the gelation and subsequent storage of fermented gadgets inclusive of yogurt. The contraction of a gel without the appearance of external forces (e.g. centrifugation) is known as spontaneous syneresis (Lucey, 2002). Casein gels are by them by nature actively. Whey separation is an idea to be as a result of excessive rearrangements of particles withinside the gel network in advance than and at some point, of gelation. As a result, whey separation is linked to the gel network`s instability, which has an immoderate tendency to undergo further network form rearrangement, resulting withinside the gel`s cap-potential to entrap all of the serum section being lost. High incubation temperature (45 °C), large pre-warmth treatment (> 80°C for 30 min), disruptions at the same time as the gel stays weak, low acid generation (pH 4.9 instead of 4.6), and low total solids content material are all conditions that would result in massive whey separation (Xu *et al., 2008*). The thermal treatment makes yogurt gels stiffer (an essential texture feature), but this doesn't prevent the wheying-off which happens when milk is

kept at extremely high temperatures (Lucey *et al.,* 1998). In acid gels generated from warm milk, there is an increase in loss tangent during gelation, particularly at much greater frequencies, as well as a drop in fracture strain, some of which could aid in whey separation and rearrangements (Lucey, 2001*)*. Al-Kadamany *et al*., (2003) discovered that samples of labneh were preserved at 5 °C syneresis at a smaller degree than those kept at 15 and 25 °C and showed noticeable changes in the degree of syneresis. As a result, the whey separation susceptibility of yogurt gels varies widely and remains a mystery (Al-kadamany *et al.,* 2003).

2.2 Low-fat Yogurt problems

The solids content of milk is generally increased to 18% for yogurt production. Adding dairy products to boost total solids results in a 4-5 percent rise in protein concentration and improved yogurt quality. A significant concentration of milk solids fortification, on the other hand, can cause problems like powdered flavor in yogurt and excess acid production, particularly during storage (Mistry & Hassan, 1992). Additionally, thermal treatment of milk at temperatures above 70 °C before fermentation is common to increase gel stiffness and minimize syneresis.

Heat-induced denaturation of whey proteins causes them to be more sensitive to inter-protein aggregation with other denatured whey proteins or casein micelles, which causes this phenomenon.

2.3 Probiotics and their effects on health

LAB, as well as probiotics in yogurt, have been found to have therapeutic effects in numerous investigations. Two of these impacts are immune activation, particularly from yogurt, and LAB-induced alterations in gastrointestinal microecology (Fiander *et al*., 2005). Probiotic bacteria are defined as "live microorganisms that, when administered at appropriate levels, provide health benefits to the host" (FAO/WHO, 2002). The following parameters must be met by these probiotics (Isolauri *et al*., 2001):

(1) be able to proliferate in the gut of the host;

(2) be able to withstand and survive low pH and bile acids;

(3) be able to stick to the epithelium of the intestine;

(4) not be harmful or toxic;

(5) The host must be able to take advantage of it;

(6) All organisms must be human-specific (except veterinary probiotics);

(7) During storage, it must be steady.

Probiotics can be used to treat digestive issues as well as to prevent and treat other diseases like allergies and immunological disorders (Gill and Guarner, 2004). In actuality, most short-term human research has shown that probiotics such as lactobacilli and bifidobacteria can modulate a host's immune system, making fermented dairy products with probiotics particularly popular due to their health benefits (Shah, 2006). Yogurt or lactic acid bacteria consumption enhances the production of cytokines, which regulate immune processes in a variety of ways. The use of food products that have undergone fermentation and cultured milk products containing living bacteria, for example, has been around for a long time and is now recognized as a valid allergy therapy option.

In the twentieth century, Metchnikoff discovered regular ingestion of sour milk that contains Lactobacillus bulgaricus benefited the health of humans, lifespan despite his claim that consuming yogurt lessens the harmful effect of putrefactive bacteria found in the intestine by inhibiting their growth (Wollowski *et al.,* 2001). Probiotics are effective against food-borne pathogens like E. coli. and Salmonella. Antibacterial benefits of probiotics have been studied extensively, and their influence on gut bacteria cannot be overemphasized. Short-chain fatty acids are produced by some probiotics, which contribute to the colon's low pH, preventing harmful germs from growing while supporting the growth of less virulent microorganisms (Rolfe, 2000). When cultivated in cow's milk, probiotic bacteria and bifidobacteria employ various carbohydrates for metabolism, therefore the health benefits of these probiotics are dose-dependent. (Farnworth *et al.,* 2007). Because strains of probiotics produce various amounts of metabolic products at different fermentation periods, the importance of

fermentation length was underscored in research on the viability and metabolism of certain strains of probiotic bacteria in milk (Ostlie *et al.,* 2003). Antibiotic-associated diarrhea was reduced in adults who drank a probiotic drink containing *Lactobacillus casei*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* (Hickson *et al.,* 2007). Traditional yogurt cultures may be referred to as probiotics in this case since they can help with lactose intolerance symptoms and improve digestion (Guarner *et al.,* 2005).

2.3.1 Interaction between LAB in milk

Probiotic bacteria, in general, are gastrointestinal fauna that develops slowly in milk and frequently perishes when refrigerated. Hydrogen peroxide, pH dissolved oxygen in fermented milk, strains used, interactions between culture condition, species present, the chemical composition of the fermentation medium, nutrient availability, storage temperature, incubation temperature and fermentation time are just a few of the factors that affect their viability (Lucas *et al.,* 2006).

2.3.2 Types of probiotics

2.3.2.a *Lactobacillus delbrueckii* ssp. *bulgaricus*

Lactobacillus is a huge genus with many species, the majority of which have little in common. The % content of guanine and cytosine (G + C) in *Lactobacilli can be used to determine their variety.* Species have a G + C concentration of 32 to 53 %, which is significantly higher than other LAB. Lactobacilli are split into two types depending on final product constituents: homofermentators, which produce >85% lactic acid as their end product from glucos*e* (e.g., *L. delbrueckii* ssp. *bulgaricus*, *L. acidophilus*), and *heterofermentators*, that produce about 50% lactic acid as the end product, with significant amounts of carbon dioxide, acetate, and ethyl acetate (Teixeira, 2000). Even though they all produce lactic acid as a significant end product, the isomeric makeup of the lactic acid generated differs. Just L (+) lactic acid is produced by *L. salivarus* and *L. casei* bacterium. Several fermenters, such as *L. delbrueckii ssp. bulgaricus and L. jensenii*, produce just D (-) lactic acid, but *L. acidophilus* and *L. helveticus* produce both D (+) and L (-). Temperatures between 30 and 40 degrees Celsius are

excellent for their growth. They are also aciduric, with an optimal growth pH of 5.5-5.8, but they can generally thrive at pH values lower than 5.0. (Batt, 2000).

Lactobacillus Bulgaricus and *Lactobacillus delbrueckii ssp.* are Gram-positive bacteria that show in milk as three to four short rods with rounded ends, each measuring 0.5-0.8 2.0-9.0 m. 45 degrees Celsius is the best temperature for growth. It has a homofermentative metabolism, which produces D (-) lactic acid in milk at a concentration of 1.7-2.1 percent. It converts hexoses to lactic acid via the EMP pathway. Although lactic acid is the most typical end product of fermentation, modest quantities of acetone, acetoin, acetaldehyde, and diacetyl can also be produced. *Lactobacillus delbrueckii ssp. Bulgaricus, like S. thermophilus*, can use lactose, fructose, and glucose, and certain strains may also use galactose (Robinson, 2000).

2.3.2.b *Streptococcus thermophilus*

Streptococcus is a genus of Gram-positive, spherical-ovoid, or coccobacillary bacteria. The G + C content of DNA varies between 34 and 46 mol% in this genus' species. Streptococcus sensu stricto is divided into four groups: oral (*S. salivarius, S. mutans, S. mitis, S. thermophilus*); *pyogenic* (*S. pyogenes, S. agalactiae*); and other streptococci (*S. bovis*, S. *equinus*, and *S. alactolyticus*) (Gobbetti and Corsetti, 2000). *S. thermophilus* is non-spore-forming, catalase-negative, and facultatively anaerobic, like other LAB. When cultivated in liquid media with milk, these spherical or ovoid cells (0.7-0.9 Mm diameter) appear in pairs or chains, with long chains of 10-20 cells. It thrives at temperatures between 42 and 45 degrees Celsius. They are heterotrophic and often fussy, needing simple carbohydrates as a source of energy and preformed amino acids as a supply of nitrogen. Lactose is fermented homofermentatively, yielding L (+) lactic acid as the main product (Zirnstein and Hutkins, 2000). Lactose is rapidly distributed all across cellular membranes of *Streptococcus thermophilus* by galactoside permease, a membrane-bound protein. The enzyme -galactosidase hydrolyzes lactose into glucose and galactose inside the cell. The Embden-Meyerhof-Parnas (EMP) route transforms glucose into pyruvate, which is then converted to lactic acid by lactic dehydrogenase.

The galactose and lactic acid produced by most strains of *S. thermophilus* leave the cell and accumulate in the medium, but some strains have a galactokinase that converts the galactose to galactose1-phosphate, which is converted to glucose-1-phosphate via the Leloir pathway and then metabolized via the EMP pathway (Robinson, 2000).

2.3.3 Probiotics in Yogurt

Microorganisms called probiotic bacteria are introduced to fermented dairy products like yogurt. These bacteria belong to the *Lactobacillus* and *Bifidobacterium* genera, which are widespread but non-dominant components of the indigenous microbiota of the human GIT (Vasiljevic and Shah, 2008). Some of the potential health benefits of functional foods containing probiotic bacteria include enhanced nutritional value, enhanced digestibility, antagonistic action against enteric pathogens, improved lactose utilization, colonization in the gut, hypocholesterolemic effect, anticarcinogenic effect, immune modulation, prevention of inflammatory bowel disease, and allergy prevention. (Gomes and Malcata, 1999). *Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium* species *and Lactobacillus paracasei* are the most common bacteria found in yogurt (Holzapfel *et al.,* 2001). Five varieties of *Bifidobacterium* are routinely used by manufacturers of medicinal fermented milk products. (B. *adolescentis, B. bifidum, B. breve, B. infantis* and *B. longum*) (Arunachalam, 1999).

Probiotic strains have different features, thus each one must be investigated separately. Some probiotic bacteria have enough proteolytic activity to develop well in milk, while others require growth boosters. Monosaccharides are required by those who do not ferment lactose. Consumers may not like the texture or flavor of a milk product fermented with a probiotic, or it may be technologically unfeasible. As a result, probiotic bacteria are frequently used in conjunction with regular starter cultures, such as in yogurt (Saxelin *et al.,* 2003). Because they require a small redox potential and peptides derived from the breakdown of casein, a milk protein, to function. Furthermore, when lactobacilli are co-cultured with them, the pH falls, and they become inhibited. (Klaver *et al*., 1993). The survival of probiotics is affected by several parameters which include dietary matrix, strain

characteristics, pH, temperature, and surrounding bacteria (Fondén, 2003). Symbiotic products that combine prebiotic bacteria and probiotic bacteria in just one food can help bifidobacteria survive throughout storage and transit to the digestive tract, as well as lessen competition with microorganisms in the GIT. Commercial probiotic diets frequently include two or more probiotic species since these strains are thought to work together synergistically. As a result, the current trend is to employ yogurt bacteria as the primary starter culture and probiotic bacteria as a backup (Shah, 2006). Various forms of yogurts, cultured buttermilk, LAB drinks such as Yakult, and combinations of probiotic fermented milk and fruit juice are the most common probiotic dairy products globally.

2.3.4 Characteristics of common probiotics

L. acidophilus has experienced various modifications in its metabolic, taxonomic, and functional features since Moro originally isolated it from newborn feces in 1900. It's present in the feces of infants who are breastfed and older adults who eat lactose, high-milk, or dextrin meals (Holzapfel *et al*., 2001). In short chains or pairs, circular end gram-positive rods with (0.5-1 2-10 m) emerge. It's salt-intolerant, non-flagellated, non-motile, and doesn't produce spores. They can thrive at 45°C because of their homofermentative metabolism, it was previously classed as thermobacterium (LAB). Using DNA hybridization tests, *L*. acidophilus was discovered to be a heterogeneous group. *L. amylovorus, L. gasseri, L. crispatus, L. johnsonii*, and *L. gallinarum* are among the six species of *L. acidophilus* that make up the *L. acidophilus* complex. They are related closely and have been tied to some evolutionary group or branch while being designated different species. In static cultures without shaking, Microaerophilic *L. acidophilus* cultures can develop aerobically. They like the “no oxygen” setting and an anaerobic standard gas mixture of 10% hydrogen, 85% nitrogen, 5% carbon dioxide, in broth or agar aids their growth (Klaenhammer and Russell, 2000). *Lactobacillus acidophilus* dietary needs match the bacteria's finicky nature. *L. acidophilus* complex members are currently regarded as obligatory homofermenters. This group uses the EMP pathway to ferment hexoses into lactic acid. Lactic acid is made by all animals, with a yield of 1.8 mol/mol glucose. They also produce hydrogen

peroxide and lactic acid, with a series of antibiotic compounds known as bacteriocins (Malcata and Gomes 1999).

Lactobacillus casei is a type of bacterium found in cheese that may be found in silage, sourdough, cow manure, as well as the human intestine, mouth, and vagina. It is a Gram-positive bacterium that are non-motile, non-sporulating, and catalase-negative, and has a maximum temperature of 30ºC for growth, *casei, pseudoplantarum, rhamnosus, and tolerans* are four subspecies of rod-shaped cells that possess square-like ends and a tendency to be chain formers (Bergey's Manual of Systematic Bacteriology). According to recent classification based on chemical-physiological criteria, L. casei is classified as "facultatively heterofermentative". Pentoses are turned into lactic acid and acetic acid by induced phosphoketolase, hexoses are virtually completely converted to lactic acid by the EMP pathway (Gobbetti *et al.,* 2000).

Till the mid-1980s, when the rising use of *Bifidobacteria* in items marketed as functional foods spurred renewed interest, Individuals in the field of food science and technology were unfamiliar with these bacteria. The genus *Bifidobacterium* produces regular, short, thin rods (1.5-8 m 0.5-1.3) that are slightly bifurcated club-shaped elements in star-like aggregates or grouped in V' or 'palisade' patterns (Hoover, 2000). They are Gram-positive, catalase-negative, non-spore-forming, non-motile, and non-spore-producing cells. They are anaerobic; however, certain species may tolerate a small amount of oxygen. For initial growth, the optimal growing temperature is between 37 and 41°C, with a pH of 6.5-7.0. They solely break down glucose through the fermentation of heterolactic, which produces L (+) lactic acid and acetic acid in a ratio of 2:3 via the fructose-6-phosphate shunt, also known as the bifid shunt. In addition to glucose, all *Bifidobacteria* of human origin may utilize galactose, lactose, and fructose as carbon sources. A proton symport system has been discovered as the lactose transport pathway in *B. bifidum* DSM 20082. (Krzewinski *et al.,* 1996).

2.3.5 Factors affecting the viability of probiotic bacteria

The chosen strains, acidity, interactions between species present, hydrogen peroxide, and pH, produced by the metabolism of bacteria all affect the survival of probiotic organisms throughout the preparation and storage of yogurt (Dave and Shah, 1997). Fermentation time, Acetic and lactic acid concentrations, Storage temperature, oxygen content, growth promoters and inhibitors, inoculation level, limitations of nutrients in milk/soymilk to sustain growth, and post-acidification are all factors that may affect the survival of probiotic organisms in yogurt (Tamime, 2005). Suitable culture selections (Viljeon and Lourens-Hattingh, 2001), microencapsulation (Capela *et al.,* 2006), milk nutritional supplementation (Gilliland and McComas, 2003), and the use of growth enhancers such prebiotics can all help to improve survival and viability (Capela *et al.,* 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and Apparatus Used

The apparatus and equipment used include; glass s0lides, Petri-dishes, Cotton wool, foil paper, disposable nose mask, hand gloves, permanent marker, polythene containers, Microscope, autoclave, MacCartney bottle. masking tape, measuring cylinder, refrigerator, incubator, spirit lamp, wire loop, conical flasks, beaker, spatula, cryovial tube, swab stick, universal bottle, forceps, durham tubes, sanitizer, receptacle, test tube rack, estimating chamber, funnel shaped cup, soul light, widespread container, anti-infection circle, volumetric flasks (100, 250ml), graduated pipette (1, 5, 10)ml, analytical balance, Daul brown sample bottles, 10ml, Pasteur pipettes, pH meter, 5ml plain sample bottles, 5ml needles and syringes, vortex mixer, centrifuge and HPLC 1100 agilent series manual injection with quaternary pump and thermostatic column compact

3.1.2 Media and Reagent Used

Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Blood agar, Lactophenol cotton blue stain, Glycerol broth, Yeast extract broth, Tryptone soya broth, Bovine serum Albumin (BSA), Lauria Bertani broth, Phosphate buffer solution (PSA) and Streptomycin.

3.2 Methods

3.2.1 Sample Collection

In 200 mL plastic containers, 30 yogurt samples [seven branded (A-G) and seven unbranded (1-7)] were obtained within the same Yaba local government Lagos state respectively. The samples were packed in a sterile polythene bag, carefully labeled, and returned to the Caleb university Microbiology laboratory for analysis.

3.2.2 Sample Preparation

After refrigerating the obtained samples, one-fold serial dilutions of each sample were made. 1 ml of each sample was put into a test tube containing 9.0 ml of sterile distilled water, the test tube was shaken, and the test tube was labeled as 10^{-1}. 1.0 mL of the test tube's distilled factors were placed into sterile Petri-dishes containing solidified potato dextrose Agar (PDA). Using the pour plate approach, the diluted samples were utilized to inoculate the prepared media. The inoculated plates were incubated for 7 days at room and refrigerator temperatures.

3.2.3 pH and Titre Value Procedure

Place the pH and ATC probes in the sample and gently agitate it for 15 seconds to eliminate air bubbles, equilibrate the sensor to the sample temperatures, and speed up the electrode response. The temperature and pH value will be presented. Continue measuring for 1 minute at room temperature for yogurt samples, or until pH values are stable (for example, stable to +/- 0.01 pH/min). Make a note of the outcome. Rinse the pH and ATC probes to remove any remaining sample on the sensor and junction. The titratable acidity was determined by titrating 15 ml of yoghurt with 0.1 M sodium hydroxide until the pH hit 8.2, which corresponded to the phenolphthalein end point.

The pH meter was used to take the measurements. The expended NaOH volume was recorded when this value was reached, and the acid percentage of the sample was determined using the formula:

$$\text{Titratable acidity} = \frac{\text{Titre value} \times M \times 90.08 \times 100}{\text{Volume of sample} \times 1000}$$

Where, M = Molar concentration of NaOH = 0.1m

90.08 = Equivalent weight pf Lactic Acid

3.2.4 Preparation of Agar

Some of the media employed in carrying out this project analysis were in solid form; such as MEA, PDA, etc. They were weighed accurately and dissolved in the right amount of distilled water as indicated by the manufacturer's instructions; heated in the water bath until the agar powder was completely dissolved, and the medium was sterilized in autoclave. China, for example, Petri-dishes, test tubes, pipettes, jars, and jugs were sanitized in a hot stove at 170°C for two hours, while refined water was cleaned via autoclaving for 15 min at 121°C.

3.3 Sub-Culture via Pour-Plate Method

The culture plate containing the isolated colony of interest was placed into a laminar airflow. Using a needle, which was sterilized by flaming. The plate was opened very little to prevent the spores from coming out. A loopful of fungal culture was taken and stabbed into a new sterile Potato Dextrose Agar & Malt Extract Agar plate. Then the plate was then closed incubated for 48 -72 hours.

3.4 Microscopy And Characterization Identification Of Fungal Isolates

A drop of lactophenol was placed on a clean slide, then a small portion (an inoculum) of representative fungi mycelium was taken with a sterilized inoculating loop, and teased onto the potassium manganese stain with a sterile needle for microscopy identification. To avoid air bubbles, a clean coverslip was placed gently on the section of the slide with the stain with little pressure. The slide was then mounted on a microscope and it was viewed under the 10x and 40x objective lenses. (Mailafia *et al.,* 2017). The fungi isolates were then photographed for further characterization and comparison with a book on fungi (Sarah *et al.,* 2017) and other representative fungi species photographs.

3.5 Toxigenicity Test (Aflatoxin determination by GC-MS)

Chromatographic Condition: The mobile plate comprised of methanol, water and Acetonitrite (40:50:10) % composition. Column used was ZORBAY SB-C8 EXTENDED 4.6 x 150mm, sum at a flow rate of 0.500ml/mins. The ultraviolet-visible detector was set at 365nm wavelength.

Sample Preparation: 1ml of the sample was taken with the aid of a disposable Pasteur pipette and 3ml of Acetonitrite was added. The solution was shaken and then vortexed for 2 minutes to deprotenize the sample. It was then centrifuged for 5 minutes at 5,000pm. The supernatant was then filtered using micromillipore filter of 0.45Nm particle size. After equilibrating the column for about 50 minutes, 20ml of each sample was individually injected manually and the pink areas recorded and integrated by enhanced integrator. The amount of aflatoxin present in the sample was calculated on the print out.

CHAPTER FOUR

RESULTS

In this study, the the branded yoghurt samples pH ranges between 4.31 and 4.53 while the total titration acidity value ranges between 19.90% and 10.28% (Table 4.1).

Similarly, the average pH of the unbranded yoghurt samples ranges between 4.51 and 4.72 while the total titration acidity value ranges between 19.60% and 16.75% (Table 4.2)

The total colony count at room temperature on Malt Extract Agar ranged between 2.9 x 10^5 CFU/g to 0.06x 10^5CFU/g (Table 4.3)

The total colony counts at fridge temperature on Malt Extract Agar ranges between 2.34 x 10^5 CFU/g and 0.01 x 10^5 CFU/g (Table 4.4).

The total count at room temperature on Potato Dextrose Agar had values ranging between 2.40 x 10^5 CFU/g and 0 x 10^5 CFU/g (Table 4.5).

The total count at fridge temperature on Potato Dextrose Agar had values ranging between 2.02 x 10^5 CFU/g and 0 x 10^5 CFU/g (Table 4.6).

The cellular morphology of the sub-cultured samples (Table 4.7).

The hemolysis and pathogenicity test result for the samples (Table 4.8).

Sample 4 was shown to be the highest total producer of aflatoxin at a value of 28.58637e^{-5} while sample L1 as the lowest total producer of aflatoxin at value 0.06072e^{-5} (Table 4.9).

Table 4.1: pH. Titre value and Total Titration Acidity of the Branded yoghurt samples

Branded	pH	Titre value (ml)	Total Titration Acidity (%)
1	4.31	22.09	19.90
2	4.51	18.01	16.30
3	4.38	13..21	11.90
4	4.46	11.77	10.60
5	4.35	13.43	12.10
6	4.53	11.41	10.28
7	4.36	12.96	11.67

Table 4.2: pH Titre value and Total Titration Acidity of the unbranded yoghurt samples

Unbranded	pH	Titre value (ml)	Total Titration Acidity (%)
A	4.51	17.09	15.39
B	4.63	20.43	18.40
C	4.72	21.76	19.60
D	4.68	19.21	17.30
E	4.53	18.65	16.80
F	4.68	19.30	17.39
G	4.59	19.16	17.26

Table 4.3: Colony count of Yoghurt at room temperature (25°C) on Malt Extract Agar

SAMPLE	YEAST ($x10^5$)	MOLD ($x10^5$)	TOTAL ($x10^5$)
A	2.56	0.37	2.93
B	2.62	0.35	2.96
C	1.97	0.29	2.26
D	0.75	0.10	0.85
E	1.10	0.15	1.25
F	2.76	0.30	3.06
G	2.31	0.33	2.64
1	0.33	0.10	0.43
2	3.06	0.33	3.39
3	0.04	0.02	0.06
4	0.25	0.18	0.43
5	3.46	0.38	3.84
6	0.23	0.12	0.35
7	0.60	0.27	0.87
T1	0	0	0
L1	0	0	0

Table 4.4: Colony count of Yoghurt at fridge temperature (4°C) on Malt Extract Agar

SAMPLE	YEAST ($x10^5$)	MOLD ($x10^5$)	TOTAL ($x10^5$)
A	1.12	0.21	1.33
B	1.03	0.18	1.21
C	0.26	0.05	0.31
D	0.05	0.02	0.07
E	0.67	0.22	0.89
F	2.11	0.23	2.34
G	1.84	0.20	2.04
1	0.03	0.02	0.05
2	0.01	0.01	0.02
3	0.02	0.01	0.03
4	0.02	0.02	0.04
5	0.01	0.02	0.03
6	0	0.01	0.01
7	0.05	0.03	0.08
T1	0	0	0
L1	0	0	0

Table 4.5: Colony count of Yoghurt at room temperature (25°C) on Potato Dextrose Agar

SAMPLE	YEAST ($x10^5$)	MOLD ($x10^5$)	TOTAL ($x10^5$)
A	2.02	0.22	2.24
B	1.12	0.12	1.24
C	1.80	0.20	2.00
D	2.16	0.24	2.40
E	1.73	0.19	1.92
F	0.99	0.13	1.12
G	1.05	0.11	1.16
1	0.55	0.08	0.63
2	0.23	0.15	0.38
3	0.24	0.24	0.48
4	_____	_____	_____
5	0.05	0.03	0.08
6	0.02	0.02	0.04
7	0.30	0.29	0.59
T1	0.21	0.05	0.27
6r	0	0	0

Table 4.6: Colony count of Yoghurt at fridge temperature (4°C) on Potato Dextrose Agar

SAMPLE	YEAST ($x10^5$)	MOLD ($x10^5$)	TOTAL ($x10^5$)
A	0.85	0.30	1.15
B	0.74	0.27	1.01
C	1.58	0.22	1.80
D	1.56	0.08	1.68
E	1.80	0.22	2.02
F	0.88	0.23	1.11
G	0.69	0.16	0.85
1	_____	0.05	0.05
2	0.43	0.10	0.53
3	_____	_____	_____
4	_____	0.01	0.01
5	_____	0.01	0.01
6	_____	0.01	0.01
7	0.19	0.08	0.27
T1	0.26	_____	0.26
6_{room}	0	0	0

Table 4.7: Cellular Morphology of Selected Samples

SAMPLE	COLOUR	TEXTURE	PROBABLE FUNGI
1	Brownish Black	_____	*Aspergillus spp*
4	Greenish Yellow	Slightly Fluffy	*Aspergillus flavus*
5	Brown, Reverse White	_____	*Aspergillus* spp.
6_{room}	Greenish Gray	Slightly Powdery	*Aspergillus flavus*
7	Greenish yellow	Cream reverse	*Aspergillus* spp.
7_{room}	Reverse cream white	_____	*Acremonium* spp.
B	Black mold	_____	*Aspergillus niger*
C	Greenish yellow	Slightly Powdery	*Aspergillus* spp.
D	White to fair peach	_____	*Fusarium* spp.
E	Green	Powdery	*Aspergillus* spp.
G	White	_____	*Acremonium* spp.
A_{room}	Greenish yellow	Slightly Powdery	*Aspergillus* spp.

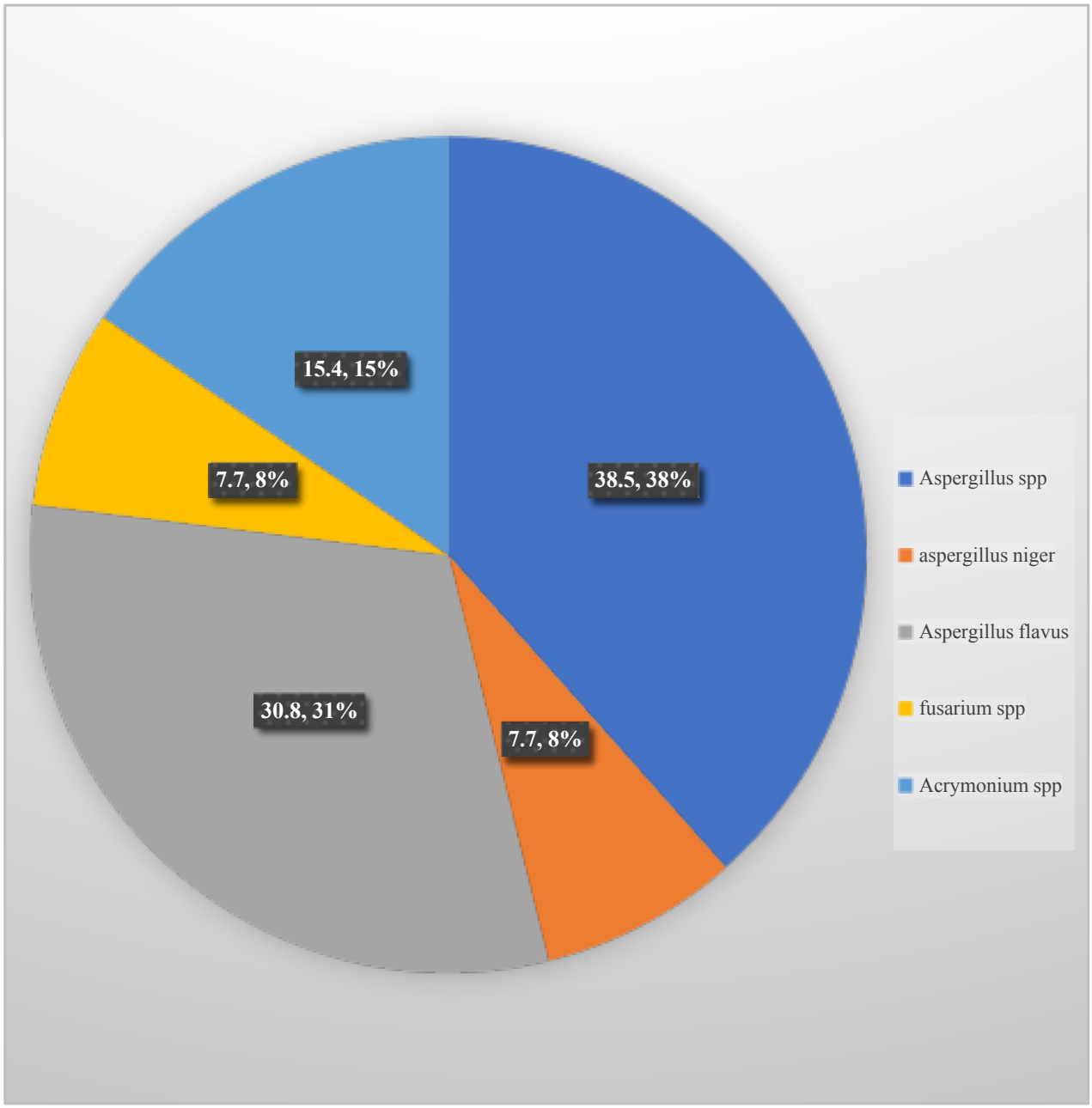

Figure 4.1: A pie-chart showing the frequency distribution of the isolates

Table 4.8: Haemolysis and Pathogenicity test of Selected Samples

SAMPLE	Haemolysis (mm)	Pathogenicity (mm)
1	24.5	29
4	28.5	31
5	23.5	17
6room	20	36
7	28.5	32.5
G	23.5	54
7room	0	0
B	27.5	29.5
C	24	31
D	15.5	29
E	26.5	27
A room	25	28.5

Table 4.9: Toxigenicity test of Selected Samples

SAMPLE	Aflatoxin AfB1	Aflatoxin AfB2	Aflatoxin AfG1	Aflatoxin AfG2	Total Producer
1	_____	_____	$0.46814e^{-4}$	$0.34081e^{-1}$	$0.80895e^{-5}$
4	_____	_____	$22.17223e^{-5}$	$6.41614e^{-5}$	$28.58637e^{-5}$
5	_____	_____	$0.09426e^{-4}$		$0.09426e^{-4}$
6	_____	_____	$2.32921e^{-5}$	$1.25367e^{-5}$	$3.58888e^{-5}$
6 room	_____	_____	$0.03648e^{-5}$	$0.02424\ e^{-5}$	0.06072 e-5
7	$1.02122e^{-5}$	$1.34876e^{-5}$	_____	_____	$2.36998e^{-5}$
7room	_____	$0.11292e^{-2}$	$0.80914e^{-3}$	_____	$0.92206e^{-5}$
B	_____	_____	$1.08249e^{-5}$	_____	$1.08249e^{-5}$
C	$7.17861e^{-5}$	_____	_____	_____	$7.17861e^{-5}$
D	_____	_____	$0.14261e^{-5}$	_____	$0.14261e^{-5}$
E	$16.13871e^{-1}$	$5.12938e^{-3}$	_____	_____	$21.26809e^{-5}$
A room	$13.12295e^{-1}$	$4.11821e^{-3}$	_____	_____	$17.24114e^{-4}$

CHAPTER FIVE

DISCUSSION

The findings of this study revealed that the 30 yogurt samples (ten branded and five unbranded) varied in their reactions to the isolated fungi. These differences included fungus species such as *Aspergillus niger, Aspergillus flavus, Aspergillus* spp*, Acremonium* spp., and *Fusarium* spp. that were collected from each sample product. These fungal isolates were also isolated, identified, and reported in this study (Porter and Dryden, 2005). Isolates of *Aspergillus niger* and *Fusarium* species from Yoghurt samples were previously identified in a similar study (Oyeleke, 2009). The acid content of the Yoghurt may have aided the growth of these fungal species in the various Yoghurt samples examined in this study. This conclusion is backed by a recent study (Porter and Dryden, 2005), which claims that when yeast grows in yogurt, the acidity of the yogurt reduces, allowing other bacteria to thrive. When considering the low degree of sanitary conditions involved in the production, distribution, and handling of these Yoghurts, the fungus species isolated and identified in this investigation are not surprising. The morphological and microscopic characteristics presented here are similar to those of fungus species reported in the findings of Mbajiuka *et al*. (2004). Fungi are known to create aflatoxin, which can be toxic to humans even in little amounts (Zain, 2011). Although some fungal genera, such as *Aspergillus*, *Fusarium*, and *Acremonium* species, create toxic metabolites that might be classified as carcinogenic agents, they can still pose a hazard to human metabolism since they can cause major health problems when taken in large quantities (Uraih and Ogbadu, 2008).

CONCLUSION

The yoghurt samples include viable fungus cells, including pathogenic strains that can cause a variety of health problems. This could be due to a lack of good manufacturing practices (GMP) or insufficient storage. Due to the lack of probiotics cells in the dairy product, many health advantages and protections that the food should supply to the consumer would be lacking, putting the consumer at risk of food borne infection and poisoning. As a result, proper monitoring and quality control among local producers and health workers are required to ensure that suitable norms and GMP for yoghurt are followed. In order to reduce the risk of food-borne illnesses and intoxication from yoghurt consumption, storage issues must be addressed.

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