

**ISOLATION AND IDENTIFICATION OF TOXIGENIC FUNGI FOUND IN YOGURTS
PRODUCED AND SOLD IN LAGOS STATE**

BY

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL
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**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
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ABSTRACT

This study aim to isolate fungi found in locally produced yogurts sold in Ikorodu, Lagos state. Eight unbranded yogurts samples (A – H) were acquired from Trolleys supermarket, Sabo, Yaba, Lagos State while seven branded samples yogurts (1 – 7) were acquired from Oniru market, Oniru, Lagos State. Using culture growth medium (Potato Dextrose Agar) and poured plate method, the fungi and molds species present were analyzed. Six species of fungi were isolated from both branded and unbranded yogurt. The isolates were identified and characterized based on some Pathogenicity test and Beta Hemolysis for clear zone inhibition. The fungi species are identified and their percentages as follow *Aspergillus flavusm* (31%), *Acrymonum sp* (15%), *Aspergillus oryzae* (15%), *Aspergillus terreus* (23%), *Fusarium oxysporum* (8%) and *Aspergillus niger* (8%). The isolates produced different volume of aflatoxin which varies from 0.06072 e^{-5} to 28.58637 e^{-5} . The pH and the titre values of yogurt samples ranged from 4.31 – 4.72 and 11.41ml - 22.09ml. The unbranded yogurt for both room and fridge temperature have the highest colony counting (12.4×10^5) while the lowest colony counting was recorded in the branded yogurts (2.2×10^5). The yoghurt samples contain viable fungi cells amongst which are pathogenic strains capable of causing various health complications. This indicates lack of good manufacturing practice (GMP) or inadequate storage. The paucity of probiotics cells in the dairy product implies that numerous health benefits and protection, which the food should provide to the consumer, will be lacking, with a resultant exposure to high risk of food borne infection and intoxication. 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 is maintained. There is also the need to address storage problems in order to minimize the risk of food borne infections and intoxication through yoghurt consumption.

DECLARATION

I, ADEWUNMI OLUWAWAPELUMI ANUOLUWAPO, with matriculation number 18/4792, hereby declare that this project titled, “ISOLATION AND IDENTIFICATION OF TOXIGENIC FUNGI FROM YOGURT” was carried out by me under the supervision of Dr Bunmi Kotun.

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CERTIFICATION

This is to certify that this work was carried out by ADEWUNMI, OLUWAWAPELUMI ANUOLUWAPO with matriculation number 18/4792, in the department of Biological Sciences and Biotechnology, College of Pure and Applied Sciences, Caleb University, Imota, Imoa Lagos, Nigeria.

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DEDICATION

I dedicate this report to Almighty God who from the beginning has always been there for me. Special dedication to my ever-supportive mother for her unconditional and unending support and to Adewunmi Sikemi and Taiwo-Idowu Kolade for always being there for me. I want to dedicate this thesis to my lecturers for their continual impact of knowledge.

To God be the glory.

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TABLE OF CONTENTS

| | |
|----------------------------|-----|
| FRONT PAGE | i |
| ABSTRACT | ii |
| DECLARATION | iii |
| CERTIFICATION | iv |
| DEDICATION | v |
| ACKNOWLEDGEMENTS | vi |
| TABLE OF CONTENTS | vii |
| CHAPTER ONE | |
| 1.1 INTRODUCTION | 1 |
| 1.2 STATEMENT OF PROBLEM | 4 |
| 1.3 AIM | 4 |
| 1.4 OBJECTIVES | 4 |
| CHAPTER TWO | |
| 2.0 LITERATURE REVIEW | 5 |
| 2.1 YOGURT | 5 |
| 2.2 BIOCHEMISTRY OF YOGURT | 7 |
| 2.3 HISTORY OF YOGURT | 8 |

| | |
|--|----|
| 2.4 DIFFERENT NAMES OF YOGURT | 9 |
| 2. 5 DIFFERENT TYPES OF YOGURTS | 9 |
| 2.6 HEALTH BENEFITS OF YOGURT | 12 |
| 2.7 FUNGI FOUND IN LOCALLY PRODUCED YOGURTS | 14 |
| CHAPTER THREE | |
| MATERIALS AND METHOD | 17 |
| 3.1 MATERIALS | 17 |
| 3.1.1 EQUIPMENT AND APPARATUS USED | 17 |
| 3.1.1 REAGENTS AND MEDIA UTILIZED | 17 |
| 3.2 SAMPLE COLLECTION | 17 |
| 3.3 METHODS | 18 |
| 3.3.1 PREPARATION OF MATERIALS AND MEDIA | 18 |
| 3.3.2 PREPARATION OF SERIAL DILUTIONS | 18 |
| 3.3.3 ENUMERATION OF YEAST AND MOLDS | 18 |
| 3.4 ISOLATION AND IDENTIFICATION OF MICROORGANISMS | 19 |
| 3.5 DETERMINATION OF PH | 19 |
| 3.6 TOTAL OF TITRATABLE ACIDITY | 20 |
| 3.7 AFLATOXIN DETERMINATION BY GC-MS | 20 |

| | |
|---------------------------|----|
| CHAPTER FOUR | |
| RESULTS | 21 |
| CHAPTER FIVE | |
| DISCUSSION AND CONCLUSION | 31 |
| 5.1 DISCUSSION | 31 |
| 5.2 CONCLUSION | 34 |
| REFERENCES | 35 |

CHAPTER ONE

1.0 INTRODUCTION

Matured drinks and dairy items have been around for more than 3500 years (Moubasher *et al.*, 2018), and are remembered to have started with the first neolithic settlements. Aging developed to protect harvests and dairy items as aged food sources by establishing a climate that was less helpful for decay microorganisms. In numerous rustic locales, unconstrained food maturations are as yet the essential procedure of food handling, with back-slopping much of the time used to immunize the new bunch by moving an aliquot from the earlier clump. This method takes into consideration normal determination of strains that flourish in the food framework, just as microbial variation. Past exploration has detailed the seclusion of different yeasts as well as microbes from regular maturations of, for instance, cereal-based dinners (Ogunremi *et al.*, 2015; Kotun, 2017), or milk (Ozoh and Umeaku, 2016), or cheddar (Anyanwu, 2019). The seclusion of microorganisms with positive attributes for use in modern food or feed activities is conceivable on account of examinations of the microbiota related with unconstrained maturations.

Yogurt utilization has expanded significantly in Nigeria after the presentation of privately created yogurt. Yogurt is an especially nutritious eating regimen all by itself (Nwaiwu *et al.*, 2020) for people, all things considered. Since there is no distinct norm for yogurt fabricate, quality varies starting with one creator then onto the next. For maturation, it is typically made in Nigeria with extra "shalom yaourt" or any business brand of yogurt (Camlait or Dolait) (Taiwo *et al.*, 2018). Because of occasions of loose bowels, customers are turning out to be progressively worried about the nature of these matured items. Its high healthy benefit, which is immediately absorbed, makes an optimal living space for microbial pollution, multiplication, and weakening. Microbial pollution

can bring about food contamination flare-ups and inferior items (Khalifa and Nossair, 2016; Kotun, 2017), which is a significant monetary issue all over the planet. Foodborne disease is as yet a central issue in most immature countries, especially in Africa (Motawee and Saleh, 2016). Foodborne microbial ailments and microbial defilement are a significant and developing general wellbeing concern. Indeed, most countries with case-announcing frameworks have seen huge expansions in the commonness of foodborne microbial contaminations throughout the most recent couple of many years (Omola *et al.*, 2014). Milk is a supplement thick food that additionally goes about as an optimal developing substrate for an assortment of microorganisms. Around one-fourth of the world's food supply is squandered because of microbial movement alone (Xu *et al.*, 2015). Gram-negative psychrotrophs, coliforms, lactic corrosive microscopic organisms, yeasts, and molds are among the unfortunate microorganisms that can debase dairy items. Accordingly, the microbiological examination of dairy items ought to get more consideration.

In Africa, food handling issues incorporate hazardous water and poor ecological cleanliness, insufficient foodborne illness reconnaissance, little and medium-scale makers' powerlessness to give safe food, obsolete food guidelines, and deficient law authorization, just as a trouble among partners. Limited scope dairy handling plants in helpless nations should follow the Codex Alimentarius standards as quickly as time permits, as indicated by the World Health Organization (Khalifa and Nossair, 2016). Hardly any African nations have carried out foodborne illness checking frameworks; in Nigeria, regulation administering the sterile control of dairy items have been set up, yet they are seldom authorized, and the milk store network's clean state isn't enough made due (Njomaha, individual correspondence). Inside this family, the Enterobacteriaceae and coliform microorganisms are two of the most normally used pointer creatures in the food area (Obire and Berembo, 2015). If Enterobacteriaceae, a wide and various group of Gram-negative

microscopic organisms, is found in yogurt, it might represent a wellbeing hazard to clients. They're great signs of generally speaking GMP, in spite of the fact that they don't consistently mean waste pollution. The presence of marker life forms in food for the most part flags that there has been a likely issue or disappointment simultaneously, yet their nonattendance in food gives some affirmation that the cleanliness and food handling processes have been followed accurately. Absolute bacterial counts, coliforms, yeasts, and moulds, just as recognizable proof of explicit microorganisms and their poisons, are the most applicable signs of microbiological quality, as per Anyanwu *et al.* (2019). *E. coli*, a coliform, is viewed as regular greenery in human and creature gastrointestinal systems. They've been utilized as bacteriological quality markers for milk and its items (Buehler *et al.*, 2019).

As a general rule, a huge number of coliforms proposes a significant degree of defilement because of unsanitary conditions and deficient creation (Yasmin *et al.*, 2015). Yeasts and filamentous growth are every now and again engaged with the disintegration of yoghurts, in spite of the way that microorganisms may be food waste life forms (Emami *et al.*, 2014). They are an essential driver of yogurt ruining, and the low pH of yogurt supports their expansion (Yasmin *et al.*, 2015). Indeed, even in little numbers, the presence of yeasts and molds in milk and dairy items is unfortunate because of the terrible changes that result, bringing down the item's quality (Anupma and Tamang, 2020). Off-flavors, surface misfortune because of gas creation, and bundling enlarging and shrinkage are totally brought about by them (Ali *et al.*, 2018). Moulds and yeasts that fill in yogurt devour a portion of the corrosive and produce an equivalent drop in corrosiveness, making the food climate more helpless to proteolysis and bacterial rottenness (Samuel and Ifeanyi, 2016).

In many regions of the planet, a great deal of exertion has been done on the sterile nature of yogurt or privately made yogurt (Garnier *et al.*, 2017; Voidarou *et al.*, 2021). When contrasted with business marks, most of them concluded that the privately made ones were of lesser sterile quality. It is basic to do investigate in the subject of market yogurt wellbeing and quality assessment to raise public information about the current circumstance and secure purchasers' wellbeing and privileges.

1.2 STATEMENT OF PROBLEM

Yogurt could be contaminated with different microbial organisms among which the dominant ones are the molds or fungi. Some of these could be pathogenic in nature and some could cause spoilage of the product due to their metabolic activities on the specific products. However, the unhygienic nature of preparation and production method, post production processes, environmental conditions surrounding the production and storage conditions are all necessary for the spoilage of Yoghurt products. Molds and yeast are the primary contaminants in Yoghurts produced in Nigeria and other countries. These microorganisms as they grow in Yoghurt, utilize some of the acid present and cause a corresponding decrease in the acidity of the Yoghurt, which may favor the growth of putrefactive organisms in the product. Therefore, this research study aimed at isolation of fungi found in locally produced Yogurts sold in Ikorodu, Lagos state.

1.3 AIM

The aim of this study is to isolate fungi found in locally produced Yogurts sold in Ikorodu, Lagos state.

1.4 OBJECTIVES

- i. To isolate fungi, present in locally produced yogurt

- ii. To identify the isolated fungi associated with the isolates
- iii. To compare the fungi cells, present in different isolates
- iv. To extract toxins from the pure fungi cell isolates and identify them

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 YOGURT

Yogurt is a matured milk item delivered by lactic corrosive maturation within the sight of *Streptococcus salivarius*, *Lactobacillus thermophilus*, *Lactobacillus delbrueckii*, and *Lactobacillus bulgaricus*. At the point when enough lactic corrosive is made, the milk coagulates, and this coagulated milk is alluded to as yogurt. Maturation of milk brings about fermentation, which is probably the most established method of milk protection (Das *et al.*, 2019).

Yogurt is made by aging milk with lactic corrosive microbes (*Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*) and adding a starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*). *Lactobacillus helveticus* and *Lactobacillus delbrueckii ssp. lactis* are more uncommon microorganisms that are at times included with the starter culture in explicit locales (Chandan, 2017). Despite the fact that aged milk items, for example, yogurts were at first planned exclusively to save the supplements in milk, it was immediately understood that by maturing with various organisms, a wide variety of flavors, surfaces, textures, and, all the more as of late, wellbeing characteristics could be created. Yogurts presently arrive in a wide assortment of flavors to suit all preferences and feast events. Yogurts arrive in an assortment of surfaces (fluid, strong, and mixed curd), fat substance (typical fat, low-fat, and without fat), and tastes (regular, natural product, cereal, chocolate), and can be eaten as a tidbit or as a component of a supper. This adaptability, joined with their all-inclusive acknowledgment as a solid and nutritious food, has brought about their gigantic prominence among every segment bunch (Chandan, 2017).

During the 1940s, yogurt was acquainted with the American eating routine. It had turned into the result of decision for calorie counters and the lunch of decision for youngsters by the 1980s. Yogurt has become one of the quickest developing dairy items because of its utilization as a calcium source, yet it is currently considerably more than that. Yogurt, Kefir, and other aged milk items are en route to becoming key nutraceuticals that can be utilized to treat a wide scope of illnesses (Fernandez *et al.*, 2017). The dietary profile of yogurt is similar to that of the milk from which it is made, yet it might vary marginally if organic product, cereal, or different fixings are added. Yogurt is high in protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1), and Vitamin B12, just as folate, niacin, magnesium, and zinc. Its protein has a high organic worth, and the nutrients and minerals present in milk and dairy food sources, including yogurt, are bioavailable.

Yogurt, particularly low-fat variations, are supplement thick food varieties since they remember a wide scope of fundamental supplements for enormous levels contrasted with their energy and fat substance. Consuming dairy items, like yogurt, works on the general nature of one's eating routine and improves the probability of meeting wholesome rules (Chandan, 2017). Nutrients and minerals are habitually added to things planned for youngsters. Yogurts can be spooned or drank, and they can be utilized as dietary enhancements for babies. Accordingly, they obscure the differentiation between dietary enhancements, clinical food varieties, and conventional food varieties (Fernandez *et al.*, 2017).

The aging of milk with thermophilic starter microbes produces yogurt gels; milk is by and large cooked at high temperatures (e.g., 85°C for 30 minutes) to denaturize whey proteins. On the outer layer of casein micelles, denatured whey proteins associate and cross-connect with - casein. As the

pH of milk brings from 6.6 down to 4.6 during yogurt maturation, casein-casein fascination increments, bringing about gelation as casein moves toward its iso-electric point. Quality and shopper acknowledgment are affected by the actual highlights of three yogurt gels, including whey division. To control the actual highlights of yogurt, you should initially comprehend the gelation cycle during aging (Karnopp *et al.*, 2017).

2.2 BIOCHEMISTRY OF YOGURT

Yogurt is made by maturing milk in an acidic climate. Lactic corrosive is shaped when lactose in milk is changed to lactic corrosive, bringing down the pH. Micelles of caseins, a hydrophobic protein, lose their tertiary design when pH falls under 5, because of protonation of amino corrosive deposits. The denatured protein reassembles by interfacing with other hydrophobic atoms, and this intermolecular casein cooperation brings about the semisolid surface of yogurt (Wang *et al.*, 2020).

The breakdown of lactose into glucose and galactose, which is catalyzed by β -galactosidase, is the initial phase in the development of yogurt. The glucose delivered in this catabolic stage is then changed over to pyruvate in glycolysis. It's been recommended that yogurt microorganisms utilize the Embden-Meyerhof-Parnas glycolysis pathway. Lactate aging, otherwise called homolactic maturation, is the following stage, which makes simply lactic corrosive particles. The formation of ethanol in different kinds of maturation, for example, ethanolic or heterolactic aging, brings about aged food varieties and refreshments like sauerkraut, kimchi, and wine.

Lactic corrosive age is the thing gives yogurt its fundamental construction and surface. Different mixtures, then again, add to the kind of yogurt. Acetaldehyde, an enhancing specialist in yogurt,

and tyrosine, a consequence of proteolytic movement that can inspire harshness at fixations more than 0.5 mg/ml (Chen *et al.*, 2017).

2.3 HISTORY OF YOGURT

Milk maturation is perhaps the earliest strategy for saving milk with a long timeframe of realistic usability utilized by people. The real beginning of milk maturation is obscure; by the by, it seems to have been polished since the beginning of civilization. Early developments like the Samaritans, Babylonians, Pharooses, and Indians were said to have progressed rural and creature cultivation strategies.

As indicated by Persian legend, Abraham's fertility and life span were because of his ordinary utilization of yogurt, and Emperor Francis I of France was said to have been relieved of extreme looseness of the bowels by polishing off goat milk yogurt, provoking the acquaintance of yogurt's medical advantages with the western world in 1542. In 1919, a business called Danone in Barcelona, Spain, started the primary motorized creation of yogurt. Yogurt was at first acquainted with the United States in the mid-20th century as pills made explicitly for those with stomach related issues. Nonetheless, it acquired prevalence in North America after Dannon, a limited scale yogurt maker, started creating yogurt in New York in 1940. Regardless of the way that yogurt has been around for centuries, it had an enormous and dynamic development process in the 20th century, bringing about an assorted scope of items. Natural product yogurts, yogurts with natural product on the base, and mixed yogurts, for instance, were first presented in 1937, 1947, and 1963.

2.4 DIFFERENT NAMES OF YOGURT

Yogurt's advancement seems to have happened in a few regions of the planet after it was first evolved in Central Asia. This could clarify why there are such countless different kinds of yogurts and yogurt-like things with various names, as displayed in Table 1.1

Table 1.1: Yogurt and yogurt-like products originated in different regions of the world

| Region | Country/island or region of origin | Traditional name of the yogurt or yogurt-like product |
|----------------------|---|---|
| Europe | Turkey | Jugurt/eyra/ayran |
| | Balkans | Kisselmleka/naja/yaourt |
| | Balkan mountains | Urgotnic |
| | Greece | Yiaourti |
| | Italy | Cieddu |
| | Sicily | Mezzoradu |
| | Sardinia | Gioddu |
| | Hungary | Tarho/taho |
| | Finland | Viili |
| | Scandinavia | Filmjolk/fillbunke/filbunk/surmelk/taettem |
| | Iceland | Skyr |
| Yugoslavia | Gruzoviz | |
| Portugal | Iogurte | |
| Eurasia | Russia | Donskaya/varenetes/kurugna/ryzhenka/guslyanka |
| | Turkestan | Busa |
| | Transcaucasia (South Caucasian state was once extended across the modern-day countries of Armenia, Azerbaijan, and Georgia) | Katyk |
| | Armenia | Mazun/matsoon,matsun, matsoni, madzoon |
| Middle East and Asia | Lebanon and some Arab countries | Leban/labani |
| | Egypt and Sudan | Zabady/zabade |
| | Iran and Afghanistan | Mast/dough/doogh |
| | Iraq | Roba/rob |
| | India | Dahi/dadhi/dahee |
| | Mongolia | Tarag |
| | Nepal | Shosim/sho/thara |

Note: Adapted and modified from Tamime and Robinson, 1999 [5]

2.5 DIFFERENT TYPES OF YOGURTS

Standard culture yogurt and bio-or probiotic yogurt are the two sorts of yogurts that can be found. Standard yogurt is produced with *Lactobacillus. bulgaricus* and *Streptococcus thermophilus* microorganisms. These microorganisms are thought to not genuinely live in the stomach; however, they can advance the gainful microflora currently present in the stomach, which assists with

keeping up with generally speaking gastrointestinal wellbeing. Bio yogurts, then again, are made by developing accommodating microorganisms that case to have an assortment of medical advantages when consumed, most ordinarily Bifidobacteria and *Lactobacillus acidophilus* probiotic strains. Not at all like standard yogurt societies, these probiotic strains are remembered to give more specific medical advantages and address various sorts of stomach verdure. These yogurts are more well known in light of the fact that they have a gentler, creamier flavor and are lower in corrosiveness. Bioyogurts are likewise professed to further develop processing and advance great wellbeing; in any case, these probiotic strains should stay feasible in adequate amounts to guarantee any medical advantages. Therefore, a term called "Live and Active Cultures" was as of late instituted to allude to the living microorganisms present in yogurt at the hour of production, including customary yogurt societies and probiotic societies.

Beside this arrangement, yogurt items are accessible in a wide scope of tastes, surfaces, and structures to suit a wide scope of palates and feast times. These can be eaten as a tidbit, dessert, or as a fundamental course. This part examines the different kinds of yogurt that can be ordered in view of their physical and substance properties, extra preferences, and post-hatching techniques.

a) Based on the compound structure of the item

Yogurt is partitioned into three sorts in light of its fat substance: standard yogurt, low-fat yogurt, and non-fat yogurt. Customary yogurt is produced using full fat milk that should incorporate basically 3.25 percent milk fat. Low-fat yogurt and non-fat yogurt, then again, are produced using low-fat milk or somewhat skim milk and skim milk, individually.

b) Based on the actual idea of the item

Yogurt can be strong, semi-strong, or liquid in consistency. Set yogurt is a sort of yogurt that has a strong surface (like jam) and is brooded and chilled prior to being bundled. Mixed yogurt and liquid/drinking yogurt, then again, are yogurts that are semi-strong and liquid in nature. Mixed yogurts are made by brooding the combination in a tank, then, at that point, separating it with blending prior to chilling and pressing it. Drinking yogurts normally go through a homogenization methodology to limit molecule size and guarantee hydrocolloid circulation and protein suspension adjustment.

c) Based on the kind of the item

The expansion of flavors would expand client offer while considering a more extensive scope of items. Flavors can be added either preceding or after the homogenization interaction. In light of the kind of the yogurt, it tends to be isolated into three classifications: plain, organic product, and enhanced yogurt. Yogurt (Plain/Natural) this is the most essential and pure type of yogurt, which is created by lactic corrosive bacterial maturation of purified milk to give it its unmistakable surface and flavor. At the end of the day, it's an aged milk item that is unadulterated and unsweetened, with no additional shading or different fixings. Subsequently, it has a healthful substance that is nearer to that of the milk from which it is produced, and it gives every one of the advantages of maturation while giving less calories. Besides, plain yogurt has the most flawless yogurt flavor and the most elevated calcium content of all yogurt items. Organic product (apple, apricot, dark cherry, dark currant, blue berry, lemon, mandarin, raspberry, strawberry, peach), cereal, vegetables, chocolate, vanilla, caramel, ginger, and different flavors are accessible in seasoned yogurts. As a rule, flavors are added to yogurt during the assembling system, and the

option of flavors makes a different scope of flavors, yet additionally supports the item's pleasantness.

d) Yogurt related items

Following the essential hatching process in yogurt creation, contingent upon the assembling methods utilized, like blending in with different combinations, heat treatment, and drying, an assortment of yogurt items, like purified yogurt, UHT yogurt, dried yogurt, etc., may result. Purified and super sanitized yogurt after maturation, heat treatment with different time-temperature mixes is utilized to make these assortments of yogurts. Despite the fact that makers foster such yogurt items to broaden timeframe of realistic usability, heat treatment might kill countless live and dynamic societies, which would be a weakness while assessing the wellbeing benefits of yogurt utilization. Yogurt, Frozen yogurt is characterized by the Pennsylvania Code as a food made by freezing and mixing a sanitized combination of fixings allowed for frozen yogurt that contains at the very least 3.25 percent milk fat, at least 8.25 percent nonfat milk solids, and has a titratable sharpness of at minimum 0.3 percent communicated as lactic corrosive. The low-fat variation, then again, contains more than 0.5 percent however under 2% milk fat and a similar amount of milk strong nonfat.

2.6 HEALTH BENEFITS OF YOGURT

Yogurt is a supplement thick food that gives basic supplements including protein, nutrients, and minerals that are needed for development. Dairy items, for example, yogurt, serve to work on the general nature of the eating routine while likewise helping the conceivable outcomes of satisfying dietary guidelines like RDAs for every supplement consistently. For instance, milk items, like

yogurt, are a high wellspring of calcium in bio-accessible structure, with a serving of 50 g of yogurt giving 41% of a 5-year-suggested old's every day calcium admission (Donovan and Rao, 2019). The wellbeing benefits of aged dairy items, particularly yogurt, seem to have been notable for centuries, as they are referenced in the Bible and antiquated Hindu texts. Yogurt is said to give various medical advantages notwithstanding its rich nourishing profile. Lactose, a disaccharide made out of one particle of glucose and one atom of galactose, is the significant carb present in milk. Lactose is separated into basic sugars by the protein lactase, which works inside the stomach. Lacking emission or obstructions with lactase processing can make undigested lactose stream into the digestive organ, where it is aged by colonic microflora, causing gastrointestinal indications such fart, loose bowels, and stomach torment. Lactose narrow mindedness is the term for this issue (Freitas, 2017).

Lactose narrow mindedness has been connected to decreased calcium admission and low bone mineral thickness, which is probably because of the pointless disposal of milk and dairy items from the eating routine. Thus, it very well may be presumed that yogurt is a proficient way for individuals with lactose bigotry to get every one of the advantages of milk items without encountering the side effects of hypolactasia. It is generally recognized that a sound stomach microflora balance is connected to ideal sustenance and by and large wellbeing. Lactobacilli and Bifidobacteria are additionally known to be the really microbial strains engaged with this harmony. As per accessible investigations, customary utilization of bio-yogurt brings about a positive microbial profile, which has a few restorative impacts. Metchnikoff, a Russian researcher, proposed in 1908 that the Bulgarians' more extended lives were connected to their normal utilization of matured milk items containing lactic corrosive microbes (Fernandez and Marette, 2017).

Yogurt fills in as a probiotic transporter food, simplifying it to ingest probiotics and bringing about high probiotic reasonability. Bio-yogurt is believed to be a phenomenal wellspring of suitable probiotic strains, for example, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, which are the most frequently used probiotics in the dairy area. Nonetheless, it has been noticed that to accomplish the probiotic impact, critical measures of suitable probiotic cells should be consumed consistently, which is known as the restorative least. Accordingly, in excess of 100 g of bio-yogurt containing in excess of 10^6 cfu/mL-1 live cells ought to be consumed (Gouda *et al.*, 2021). Through the restorative and advantageous impacts related with probiotics, they seem to help protect amazing wellbeing, reestablish body strength, and treat digestive issues. Probiotics have been displayed to have remedial impacts, for example, forestalling urogenital contaminations, blockage help, looseness of the bowels avoidance, infantile colic counteraction, hypercholesterolemia anticipation, colon/bladder disease anticipation, and osteoporosis anticipation. Probiotics, then again, are said to give extra advantages, for example, keeping up with ordinary stomach greenery, helping the insusceptible framework, bringing down lactose prejudice and serum cholesterol levels, and expanding anticarcinogenic activity. Some have supported for the utilization of aged milk items to treat gastrointestinal sicknesses; for instance, Tissier has pushed for the utilization of Bifido microbes to treat infantile loose bowels (Das *et al.*, 2019).

2.7 FUNGI FOUND IN LOCALLY PRODUCED YOGURTS

Omola *et al.* (2014) examined the physicochemical, tactile, and microbiological properties of yogurt brands sold in Kano, Nigeria. Utilizing set up conventions, this study assessed the physico-substance, tangible, and microbiological properties of various yogurt brands presented in Kano Metropolis. The Association of Official Analytical Chemists technique was utilized to decide the physico-synthetic characteristics (thickness, explicit gravity, pH, titratable acidity, fat substance)

and tactile properties (shading, flavor, and aroma). The aftereffects of the microbiological assessments depended on Aerobic mesophilic bacterial, Coliform, *Escherichia coli*, and parasitic counts, just as the identification of *Staphylococcus aureus* and *Salmonella sp.* using the Food and Agricultural Organization strategy. *S. aureus* and *Salmonella sp.*, two hurtful microbes, were not found in any of the yogurt tests tried. A few examples, notwithstanding, were positive for mesophilic microbes, Coliform, *Escherichia coli*, and parasite. Substance examination uncovered no measurably critical changes ($p > 0.05$) across yogurt tests. Organoleptically, test YB5 was significantly better than the others as far as shading (7), flavor (9) and generally speaking adequacy ($p < 0.05$). With the exception of yogurt tests (YB3, YB4, YB6, and YB7), the microbial heap of all yogurt tests is underneath satisfactory nearby and global standards.

De *et al.* (2014) assessed the microbiological nature of various brands of packaged yogurt sold in Central Market, Kaduna Metropolis, Kaduna, Nigeria. Standard microbiological conventions were utilized to research the microbiological nature of twenty business tests of ten distinct brands of packaged plain yogurt presented in Kaduna's Central Market. Five of the ten distinct brands were enrolled with NAFDAC, while the other five were not. The pH of the enrolled yogurt tests went from 4.01-4.79, while the pH of the non-enlisted tests was somewhere in the range of 5.28 and 5.63. Absolute bacterial counts (TBC) in enrolled and nonregistered tests went from 3.0×10^3 to 10.5×10^4 and 8.2×10^4 to 28.4×10^5 , separately. Test A21 had the best count of the relative multitude of recorded examples, while test A511 had the most reduced. Test a411 had the best count while test a311 had the most minimal consider as a real part of non-enrolled tests. A factual test ($t = -2.28$ and $F = 9.78$) showed that the state counts of enlisted and non-enrolled tests changed essentially at the 0.05 level. The microorganisms *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which are found in the yogurt starter culture, were found in the examples in general,

as the makers guaranteed on their marking. *Bacillus* sp. was found in each example, but *Staphylococcus aureus* was found in A41, A411, a21, and a211. Every one of the examples investigated contained *Aspergillus* sp. A11, A111, a11, a111, a41, and a411 were utilized to detach *Mucor* sp. A21, A211, A31, A311, a21, a211, a31, and a311 were utilized to detach *Penicillium* sp. A41, A411, a11, and a111 were completely found to have *Acremonium* sp. Since organism like *Aspergillus* and microorganisms like *S. aureus* have been found from some NAFDAC affirmed tests just as a few non-enrolled tests, appropriate consideration should be taken when putting away and taking care of yogurt.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

3.1.1 Equipment and Apparatus Used

The device that was utilized include: Microscope, Incubator, water shower, autoclave, cooler, gauging balance, distilled water, anti-infection circle, forceps, vaccinating circle, swab stick, petri dishes, foil paper, widespread container, needle and needle, cotton fleece, gloves, nose veil, glass slide, matches, soul light, test tube, beaker, stirring glass rod, funnel shaped cup, receptacle, glass slide, cover slip, measuring cylinder, cryovial tube, durham tubes, spatula, estimating chamber, test tube rack, marker, concealing tape, sanitizer, MacCartney bottle, micropipettes and tips of different sizes, Autoclave, weighing balance, incubator, Bunsen burner with gas supply, inoculating loops and needles.

3.1.1 Reagents and Media utilized

Potato Dextrose agar (PDA), Malt Extract agar (MEA), Blood agar, Lacto phenol cotton blue stain, Blood agar, Glycerol broth, Yeast Extract broth, Lauria Bertani broth, Tryptone Soya Broth, Phosphate buffer Solution (PSA), Bovine serum Albumin (BSA), etc.

3.2 Sample Collection

An aggregate of seven marked yogurts and eight unbranded yogurts obtained from various regions in Lagos state was haphazardly bought and was investigated at the Microbiology laboratory, Caleb University Lagos. The unbranded samples (A – G) were acquired from a supermarket at Sabo, Yaba, Lagos State while the branded samples (1 – 7) were acquired from random locations within the same , Lagos State. Two samples of each brand were utilized and the brands were assigned A,

B, C, D, E, F, G, 1, 2, 3, 4, 5, 6, 7 giving an aggregate of 28 yogurt test samples. Two (2) control samples were prepared, labelled T1 and L1 and were added to the previously acquired samples therefore having 30 test samples. One half of the samples were left under room temperature while the other half was refrigerated. After proper labelling, the samples meant to be refrigerated were kept in a fridge at the laboratory while the samples meant to be kept under room temperature were kept on the properly sterilized work bench.

3.3 Methods

3.3.1 Preparation of Materials and Media

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.

3.3.2 Preparation of Serial Dilutions

This was finished by APHA (2004) in which 1.0 ml of yogurt from a homogenous example was sequentially weakened into 9.0 mL of sterile refined water to plan tenfold weakening from 10^{-1} to 10^{-3} . 1ml of weakened examples were spread over pre-arranged dried plates with various media.

3.3.3 Enumeration of Yeast and Molds

Potato Dextrose agar (PDA) (Oxoid) (enhanced with 0.148 g/l streptomycin) was utilized to decide yeast and mold counts. One milliliter (1.0ml) of each sample was seeded on PDA using pour plate

method and after, the pour-plated plates were hatched vigorously at 25°C for 3-5 days, the created settlements (colonies) were assessed and counted. The colonies counted were expressed as log₁₀ colony forming unit per milliliter (log₁₀cfu/ml) of the yogurt sample.

3.4 Isolation and Identification of Microorganisms

The recognized states on the hatched plates were picked and cleansed by continued subculturing done by streaking on the fitting media with a sterile circle (the system comprised of picking 1 settlement to address each noticeably unique morphology on each plate) utilizing the streak technique. Cleaned states were ready in their individual stock: Malt Extract stock for yeast and molds. From these arrangements, 0.5 ml of each was pipetted into 0.5 ml of glycerol and put away in a cooler at -5°C anticipating recognizable proof.

By minuscule perception of each culture following hatching, the immaculateness of disconnects was affirmed and fundamental IDs were finished by Bergey's Manual (Kandler, 1986). Appropriate distinguishing proof to species level was done based on biochemical tests with API 20 C AUX (for the ID of yeast) (bioMerieux, Marcy l'Etoile, France) as indicated by the guidelines of the producer.

The fungi were identified using morphological and cultural characteristics: type of fruiting structure, colony color, shape and size, pigment produced, cellular morphology, cell size/shape were confirmed by microscopical observation and also, by making comparison with mycological atlas.

3.5 Determination of pH

The pH of the sample was determined using a pH meter. The electrode was standardized using a phosphate buffer solution of pH 7.0. The electrode sensor was inserted directly into 20ml sample in a beaker. The value from the recorder was then taken and recorded as the pH value.

3.6 Total of Titratable Acidity

Total titratable acidity was determined according to AOAC method (2009). 10 ml of sample was pipetted into 250ml flask in duplicate and two drops of phenolphthalein solution was added to the sample. The sample was titrated with 0.1m NaOH solution (Sodium hydroxide solution) to phenolphthalein endpoint (pinkish coloration) and the total titratable acidity was calculated according to the formula below as percentage lactic acid.

$$T.A.A = \frac{ml\ NaOH \times N_{NaOH} \times 90.08 \times 100}{10 \times sample\ volume \times 1000}$$

3.7 Aflatoxin determination by GC-MS

Equipment and materials: been Lom, and volumetric flasks (100, 250ml), graduated pipette (1, 5, 10 ml), analytical balance, dark brown sample bottles 10ml, disposable nose mask, hand gloves and pasteur pipette, 5ml plain sample bottles, 5ml needles and syringe, vortex mixer, centurim centrifuge and HPLC1100 Agilent series manual injection with quaternary pump and thermostatic column compact.

Chromatographic condition: the mobile plane comprises of methanol, water. Acetonitrile (40:50:10 %) composition. Column used was ZORBAY SB-C8 EXTENDED 4.6 X 150mm, sum at a flow rate of 0.0500ml/mms⁻¹. The ultraviolet-visible detector was set at 365nm.

Sample preparation: 1ml of the sample was taken with the aid of a disposable Pasteur pipette and 3ml of Acetonitrile was added. The solution shaken and then vortexed for 2 minutes to depolarized the sample. It was centrifuged for 5minutes at 5,000rpm. The supernatant was filtered using micro-Millipore filter of 0.45µm particle seize. After equilibrating the column for about 50minutes, 20ml of each sample was individually injected manually and the peak areas recorded and integrated by enhanced integrator. The amount of aflatoxin present in the sample was calculated on the printout.

CHAPTER FOUR

RESULTS

The result of Colony counting of fungi in yogurt at Room temperature and fridge temperature in PDA plate respectively were showed in **Table 4.1 to 4.4**. The unbranded yogurt for both room and fridge temperature have the highest colony counting while the lowest colony counting was recorded in the branded yogurts.

The result of Colony counting of fungi in yogurt at Room temperature and fridge temperature in MEA plate respectively. The unbranded yogurt for both room and fridge temperature have the highest colony counting while the lowest colony counting was recorded in the branded yogurts (**Table 4.5 and 4.8**).

The pH and the titre values of yogurt samples ranged from 4.31 – 4.72 and 11.41ml - 22.09ml (**Table 4.9 and 4.10**).

Six species of fungi were isolated from both branded and unbranded yogurt. The isolates were identified and characterized based on some Pathogenicity test and Beta Hemolysis for clear zone inhibition. The fungi species are identified as *Aspergillus flavus*, *Acrymonum sp*, *Aspergillus oryzae*, *Aspergillus terreus*, *Fusarium oxysporum* and *Aspergillus niger* (**Table 4.11**).

Frequency of the isolates was represented in the Figure 1 below. *Aspergillus flavus* has the highest frequency followed by *Aspergillus terreus*, while *Fusarium oxysporum* and *Aspergillus niger* has the lowest occurrence.

The isolates produced different volume of aflatoxin which varies from 0.06072 e⁻⁵ to 28.58637 e⁻⁵ are expressed in the table 4.3.

Table 4.1: Colony counting of fungi in unbranded yogurt at Room temperature in PDA plate

| Sample code | Yeast | Mould | Total |
|--------------------|--------------------|--------------------|--------------------|
| A. | 2.02×10^5 | 0.22×10^5 | 2.24×10^5 |
| B. | 1.12×10^5 | 0.12×10^5 | 1.24×10^5 |
| C. | 1.80×10^5 | 0.20×10^5 | 2.00×10^5 |
| D. | 2.16×10^5 | 0.24×10^5 | 2.40×10^5 |
| E. | 1.73×10^5 | 0.19×10^5 | 1.92×10^5 |
| F. | 0.99×10^5 | 0.13×10^5 | 1.12×10^5 |
| G. | 1.05×10^5 | 0.11×10^5 | 1.16×10^5 |

Table 4.2: Colony counting of fungi in branded yogurt at Room temperature in PDA plate

| Sample code | Yeast | Mould | Total |
|--------------------|--------------------|--------------------|--------------------|
| 1. | 0.55×10^5 | 0.08×10^5 | 0.63×10^5 |
| 2. | 0.23×10^5 | 0.15×10^5 | 0.38×10^5 |
| 3. | 0.24×10^5 | 0.24×10^5 | 0.48×10^5 |
| 4. | - | - | - |
| 5. | 0.05×10^5 | 0.03×10^5 | 0.08×10^5 |
| 6. | 0.02×10^5 | 0.02×10^5 | 0.04×10^5 |
| 7. | 0.30×10^5 | 0.29×10^5 | 0.59×10^5 |
| T1. | - | - | - |
| L1 | - | - | - |

Table 4.3: Colony counting of fungi in unbranded yogurt at fridge temperature in PDA plate

| Sample code | Yeast | Mould | Total |
|--------------------|--------------------|--------------------|--------------------|
| A. | 0.88×10^5 | 0.30×10^5 | 1.15×10^5 |
| B. | 0.74×10^5 | 0.27×10^5 | 1.01×10^5 |
| C. | 1.58×10^5 | 0.22×10^5 | 1.80×10^5 |
| D. | 1.56×10^5 | 0.08×10^5 | 1.68×10^5 |
| E. | 0.80×10^5 | 0.22×10^5 | 2.02×10^5 |
| F. | 0.88×10^5 | 0.23×10^5 | 1.11×10^5 |
| G. | 0.69×10^5 | 0.16×10^5 | 0.85×10^5 |

Table 4.4: Colony counting of fungi in branded yogurt at fridge temperature in PDA plate

| Sample code | Yeast | Mould | Total |
|--------------------|------------------------|------------------------|------------------------|
| 1. | - | 0.05 X 10 ⁵ | 0.05 X 10 ⁵ |
| 2. | 0.43 X 10 ⁵ | 0.10X 10 ⁵ | 0.53 X 10 ⁵ |
| 3. | - | - | - |
| 4. | - | 0.01 X 10 ⁵ | 0.01 X 10 ⁵ |
| 5. | - | 0.01 X 10 ⁵ | 0.01 X 10 ⁵ |
| 6. | - | 0.01 X 10 ⁵ | 0.01 X 10 ⁵ |
| 7. | 0.19 X 10 ⁵ | 0.08 X 10 ⁵ | 0.27 X 10 ⁵ |
| T1. | - | - | - |
| L1 | - | - | - |

Table 4.5: Colony counting of fungi in unbranded yogurt at Room temperature on MEA plate

| Sample code | Yeast | Mould | Total |
|--------------------|--------------------|--------------------|--------------------|
| A. | 2.56×10^5 | 0.37×10^5 | 2.93×10^5 |
| B. | 2.61×10^5 | 0.35×10^5 | 2.96×10^5 |
| C. | 1.97×10^5 | 0.29×10^5 | 2.26×10^5 |
| D. | 0.75×10^5 | 0.10×10^5 | 0.85×10^5 |
| E. | 1.10×10^5 | 0.15×10^5 | 1.25×10^5 |
| F. | 2.76×10^5 | 0.30×10^5 | 3.06×10^5 |
| G. | 2.31×10^5 | 0.33×10^5 | 2.64×10^5 |

Table 4.6: Colony counting of fungi in branded yogurt at Room temperature on MEA plate

| Sample code | Yeast | Mould | Total |
|--------------------|------------------------|------------------------|------------------------|
| 1. | 0.33 X 10 ⁵ | 0.10 X 10 ⁵ | 0.43 X 10 ⁵ |
| 2. | 3.06 X 10 ⁵ | 0.33 X 10 ⁵ | 3.39 X 10 ⁵ |
| 3. | 0.04 X 10 ⁵ | 0.02 X 10 ⁵ | 0.06 X 10 ⁵ |
| 4. | 0.25 X 10 ⁵ | 0.18 X 10 ⁵ | 0.43 X 10 ⁵ |
| 5. | 3.46 X 10 ⁵ | 0.38 X 10 ⁵ | 3.84 X 10 ⁵ |
| 6. | 0.23 X 10 ⁵ | 0.12 X 10 ⁵ | 0.35 X 10 ⁵ |
| 7. | 0.60 X 10 ⁵ | 0.27 X 10 ⁵ | 0.87 X 10 ⁵ |
| T1. | - | - | - |
| L1 | - | - | - |

Table 4.7: Colony counting of fungi in unbranded yogurt at fridge temperature in MEA plate

| Sample code | Yeast | Mould | Total |
|--------------------|--------------------|--------------------|--------------------|
| A. | 1.12×10^5 | 0.21×10^5 | 1.13×10^5 |
| B. | 0.03×10^5 | 0.18×10^5 | 1.21×10^5 |
| C. | 0.26×10^5 | 0.05×10^5 | 0.31×10^5 |
| D. | 0.05×10^5 | 0.02×10^5 | 0.07×10^5 |
| E. | 0.67×10^5 | 0.22×10^5 | 0.89×10^5 |
| F. | 2.11×10^5 | 0.23×10^5 | 2.34×10^5 |
| G. | 1.84×10^5 | 0.20×10^5 | 2.04×10^5 |

Table 4.8: Colony counting of fungi in branded yogurt at fridge temperature in MEA plate

| Sample code | Yeast | Mould | Total |
|--------------------|--------------------|--------------------|--------------------|
| 1. | 0.03×10^5 | 0.02×10^5 | 0.05×10^5 |
| 2. | 0.01×10^5 | 0.01×10^5 | 0.02×10^5 |
| 3. | 0.02×10^5 | 0.01×10^5 | 0.03×10^5 |
| 4. | 0.02×10^5 | 0.02×10^5 | 0.04×10^5 |
| 5. | 0.01×10^5 | 0.02×10^5 | 0.03×10^5 |
| 6. | - | 0.01×10^5 | 0.01×10^5 |
| 7. | 0.05×10^5 | 0.03×10^5 | 0.08×10^5 |
| T1. | - | - | - |
| L1 | - | - | - |

Table 4.9: Determination of pH and Titre Values and percentage for unbranded yogurt

| Sample Code | Titre value | pH | (%)TTA |
|--------------------|--------------------|-----------|---------------|
| A. | 17.09ml | 4.51 | 15.39 % |
| B. | 20.43ml | 4.63 | 18.40 % |
| C. | 21.76 ml | 4.72 | 19.60 % |
| D. | 19.21ml | 4.68 | 17.30 % |
| E. | 18.65ml | 4.53 | 16.80 % |
| F. | 19.30ml | 4.68 | 17.39 % |
| G. | 19.16ml | 4.59 | 17.26 % |

Table 4.10: Determination of pH and Titre Values and percentage for branded yogurt

| Sample Code | Titre value | pH | (%)TTA |
|--------------------|--------------------|-----------|---------------|
| 1. | 22.09ml | 4.31 | 19.90 % |
| 2. | 18.10ml | 4.51 | 16.30 % |
| 3. | 13.21ml | 4.38 | 11.90 % |
| 4. | 11.77ml | 4.46 | 10.60 % |
| 5. | 13.43ml | 4.35 | 12.10 % |
| 6. | 11.41ml | 4.53 | 10.28 % |
| 7. | 12.96ml | 4.36 | 11.67 % |

Table 4.11: Pathogenicity test and Beta Hemolysis for clear zone inhibition

| Sample code | Beta Hemolysis | Pathogenicity test | Possible isolate |
|-------------|----------------|--------------------|----------------------------|
| Mold 6r | 26.5mm | 33mm | <i>Aspergillus flavus</i> |
| Mold H | 23.5mm | 54mm | <i>Acrymonum sp</i> |
| Mold 4 | 28.5mm | 31mm | <i>Aspergillus flavus</i> |
| Mold C | 24mm | 31mm | <i>Aspergillus oryzae</i> |
| Mold 6 | 20mm | 36mm | <i>Aspergillus flavus</i> |
| Mold Ar | 25 mm | 28.5mm | <i>Aspergillus flavus</i> |
| Mold 5 | 23.3 mm | 17mm | <i>Aspergillus terreus</i> |
| Mold 1 | 24.5 mm | 29mm | <i>Aspergillus terreus</i> |
| Mold 7 | 28.5 mm | - | <i>Aspergillus oryzae</i> |
| Mold D | 15.5 mm | 29mm | <i>Fusarium oxysporum</i> |
| Mold B | 27.5 mm | 29.5mm | <i>Aspergillus niger</i> |
| Mold 7r | - | 32.5mm | <i>Acrymonum sp</i> |
| Mold E | 26.5mm | 27mm | <i>Aspergillus terreus</i> |

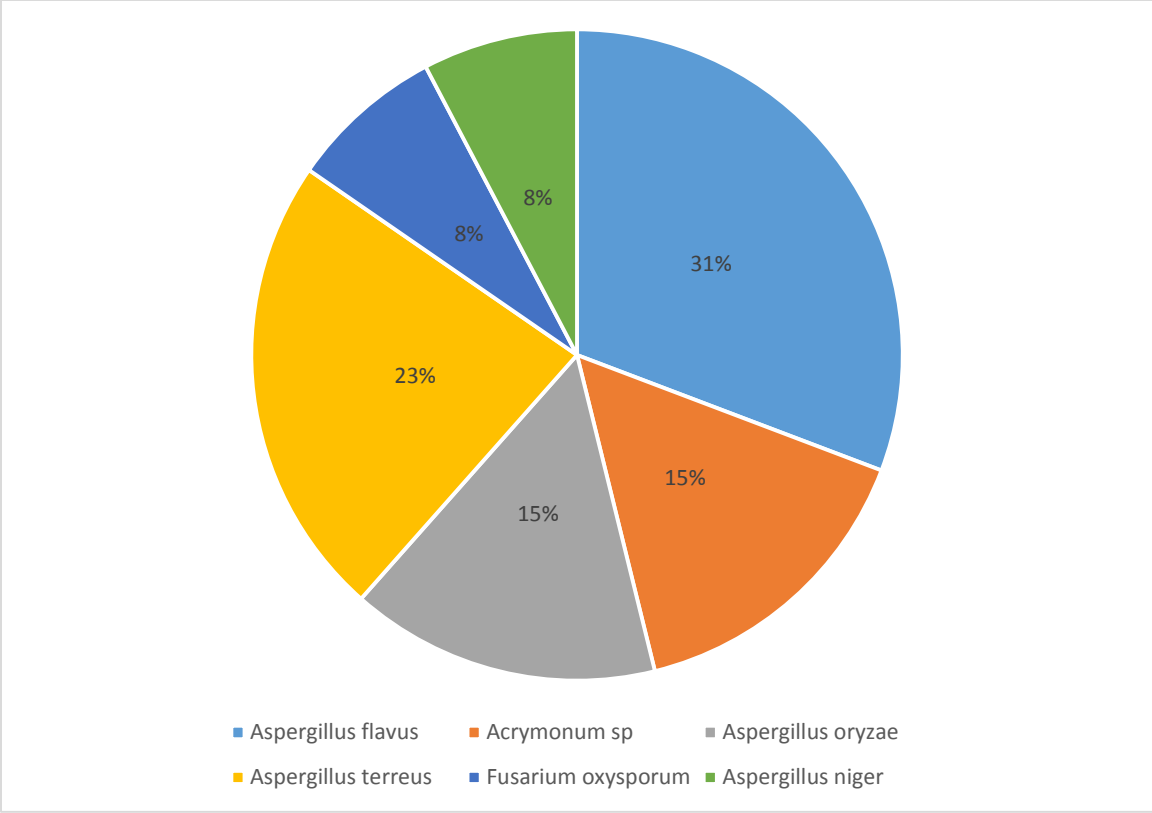


Figure 1: shows Frequency of the isolates.

Table 4.12: shows the Aflatoxin Extraction

| Sample code | Aflatoxin AfB1 | Aflatoxin AfB2 | Aflatoxin AfG1 | Aflatoxin AfG2 | Total produced |
|--------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Mold 6r | — | — | 0.03648e ⁻⁵ | 0.02424 e ⁻⁵ | 0.06072 e ⁻⁵ |
| Mold H | — | — | 0.06874 e ⁻⁵ | | 0.06874 e ⁻⁵ |
| Mold 4 | — | — | 22.17223 e ⁻⁵ | 6.41614 e ⁻⁵ | 28.58637 e ⁻⁵ |
| Mold C | 7.17861 e ⁻⁵ | — | — | — | 7.17861 e ⁻⁵ |
| Mold 6 | — | — | 2.32921 e ⁻⁵ | 1.25967 e ⁻⁵ | 3.58888 e ⁻⁵ |
| Mold Ar | 13.12295 e ⁻¹ | 4.11821 e ⁻³ | — | — | 17.24114 e ⁻⁴ |
| Mold 5 | — | — | 0.09426 e ⁻⁴ | — | 0.09426 e ⁻⁴ |
| Mold 1 | — | — | 0.46814 e ⁻⁴ | 0.34081 e ⁻¹ | 0.80895 e ⁻⁴ |
| Mold 7 | 1.02122 e ⁻⁵ | 1.34876 e ⁻⁵ | — | — | 2.36998 e ⁻⁵ |
| Mold D | — | — | 0.14261 e ⁻⁵ | — | 0.14261 e ⁻⁵ |
| Mold B | — | — | 1.08249 e ⁻⁵ | — | 1.08249 e ⁻⁵ |
| Mold 7r | — | 0.11292 e ⁻² | 0.80914 e ⁻³ | — | 0.92206 e ⁻⁵ |
| Mold E | 16.13871 e ⁻¹ | 5.12938 e ⁻³ | — | — | 21.26809 e ⁻⁵ |

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The results obtained from this current study of Isolation of toxigenic fungi found in yogurts sold in Lagos, showed that the products were grossly contaminated with fungi of public health concern. The high level of fungi contamination in both branded and unbranded yogurt samples could be as a result of low-level hygiene maintained during the processing of the milk products (Lee et al., 2014). It has been reported that the unclean hands of workers, poor quality of milk, unhygienic conditions of the manufacturing unit and water supplied for washing the utensils could be the source for accelerating fungi contamination of milk products beside the post manufacturing contamination (Moubasher et al., 2018). On the other hand, the high numbers of the isolated fungi observed in this study could be due to the fact that yogurt being a good nutritive medium enhanced the growth of fungi contaminant in the yogurt samples studied as stated in International Dairy Federation (De et al., 2014) and Azizi *et al.* (2012). The detection of *Aspergillus spp.* organisms such as *Aspergillus flavus*, *Acrymonum sp*, *Aspergillus oryzae*, *Aspergillus terreus*, *Fusarium oxysporum* and *Aspergillus niger* in the studied yogurt samples, probably indicates possible harvest, storage, and/or transit. Contamination (Nwaiwu et al., 2020) due to poor hygienic practices among handlers of these products since the organisms are saprotrophic and pathogenic fungi. Isolation of similar fungi from milk products has been previously reported (Delavenne et al., 2012; Montaseri et al., 2014). The presence of these fungi in yogurt also suggests contamination from various sources, which may include animal, human, environment, utensils and others (Al-Ruwaili et al., 2018).

The observed pH for both brand and unbranded yoghurts were within the range of data previously reported (Rodrigues et al., 2010, Ifeanyi et al., 2013). The results obtained from this current

study of Isolation of toxigenic fungi found in yogurts sold in Lagos, showed unacceptable levels of fungi, some sp of *Aspegillus* have been implicated in the secretion of aflatoxins, which are carcinogenic to human when consumed, this indicates that most of the yogurt drinks were not good for consumption. Food handling practices by vendors is also a major concern in contaminating what they market, since they don't under go any formal training before embarking on yogurt drinks buying and selling.

According to Table 3, the incidence of AFM1 in tested yogurt samples in the present survey was relatively higher than the results obtained from the other regions of the world (El Khoury et al., 2011; Galvano et al., 2001; Kim et al., 2000), but not very different from the results obtained from other studies in Nigeria (Fallah et al., 2011; Tabari et al., 2012). In the previous similar published research conducted in northern part of Nigeria, Tabari et al. (2012) reported that all tested yogurt samples in were contaminated with AFM1 in range of 4.2- 78.9 ng/kg. All the tested samples had contamination levels below the European regulation (50 ng/kg).

Besides the branded yogurt, production and consumption of unbranded yogurt are common in Nigeria. Branded yogurts are manufactured in dairy industry but unbranded ones are made in ranches or small dairy shops (Fallah et al., 2011) and even in homes. The milk that used for production of unbranded yogurt is mainly supplied by traditional dairy farms; in this type of dairy farms crop residues, weeds, wheat and barley stubble are the sources of animal feed (Tajkarime et al., 2008). Higher level of AFM1 in unbranded yogurt might be due to high level of AFB1 in feedstuff that had been used in the feeding of dairy cattle in traditional husbandry practices. Another possible reason for higher level of AFM1 in unbranded yogurt could be found in the yogurt manufacturing method. It should be indicated that in unbranded yogurt production in Nigeria, the increasing of solid not fat of base milk are gained only by excessive evaporating of

milk; so, this concentration process may led to increasing the level of AFM1 in final product. Lower level of AFM1 in branded yogurt may also be associated with a dilution effect of contaminated milk with non-contaminated milk in dairy industry.

The mean concentration of AFM1 in yogurt samples of this study was relatively lower than the levels of AFM1 in milk samples that observed in some part of Nigeria. Fallah (2010b) showed that the mean concentration of AFM1 in pasteurized and UHT milk in central part of Nigeria were 52.8 and 46.4 ng/l, respectively. In the mentioned study AFM1 content of 26.7% and 17.4% of pasteurized and UHT milk showed to exceed EU regulations. Lower level of AFM1 in yogurt might be attributed to factors such as low pH, formation of organic acids and other fermentation by-products (Govaris et al., 2002).

The current study supports many existing literature to ascertain presence of pathogenic fungi in branded and unbranded yogurt products and also creates awareness for people on the high risk associated with consuming contaminated milk products because of the health complications associated with it.

5.2 Conclusion

The yoghurt samples contain viable fungi cells amongst which are pathogenic strains capable of causing various health complications. This indicates lack of good manufacturing practice (GMP) or inadequate storage. The paucity of probiotics cells in the dairy product implies that numerous health benefits and protection, which the food should provide to the consumer, will be lacking, with a resultant exposure to high risk of food borne infection and intoxication. 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 is maintained. There is also the need to address storage problems in order to minimize the risk of food borne infections and intoxication through yoghurt consumption.

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