MICROBIOLOGICAL QUALITY AND PROXIMATE EVALUATION OF LOCALLY FERMENTED MILK (NONO) PRODUCED FROM SELECTED LOCATIONS IN MINNA, NIGER STATE

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MTECH/SLS/2018/8195

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY MINNA, NIGER STATE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS IN TECHNOLOGY (MTECH) DEGREE IN MICROBIOLOGY.

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ABSTRACT

'Nono' is a locally fermented diary product from cow milk which is common in northern Nigeria. This study was designed to determine the microbiological quality and proximate properties of 'nono' produced in three locations in Minna, Niger state. Five samples were collected from different stages of 'nono' production (sample collected directly from the udder, after mixing the milk form several cows, after sieving the milk, after fermentation, after churning) from three different location. The samples were cultured on Nutrient, Macconkey and Sabouraud dextrose agar for total bacterial, total coliform and fungal counts respectively using pour plate method. Results revealed that microbial counts in the different stages of 'nono' production ranged from $1.00x10^6$±0.00 - $10.50x10^6$±0.50 cfu/mL for total viable count, $1.00x10^6$±0.00 - $7.50x10^6$±0.50 cfu/mL for total coliform count and $1.00x10^6$±0.00 - $1.02x10^6$±0.01 cfu/mL for fungal count. A total of 89 isolates were recorded (A=36, B=31 and C=22). The Bacteria isolated include *Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteus, Salmonella enterica, Escherichia coli, Klebsiella aerogenes, Bacillus cereus, Klebsiella oxytoca, Citrobacter freundii, Staphylococcus aureus, Kocuria rosea,* and species *of Lactobacillus, Streptococcus, Proteus, Lactococcus, Vibrio and Shigella. Klebsiella pneumoniae* had the highest frequency 12 (14.63 %) followed by *Bacillus subtilis* 10 (12.19 %) and *pseudomonas aeruginosa* 8 (9.76 %) while the lowest frequency of 1 (1.1 %) was recorded for *Klebsiella oxytoca, Citrobacter freundii, Kocuria rosea* and *Vibrio* sp. The fungal species isolated include *Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Trichophyton verrucossum, Torulopsis dottila* and *Rhizopus arrhizus.* The frequency of occurrence of fungal isolates revealed that *Aspergillus niger* had the highest frequency of (2) 28.60 %. The proximate analysis result showed that the protein values ranged from, 3.35±0.50-6.50±0.21 %, ash content ranged from 0.72±0.02-1.02±0.6 %, moisture content ranged from 81.21±0.1-82.17±0.07 %, total fat ranged from 2.10±0.02-2.58±0.05 %, and carbohydrate content raged from 9.17±0.22-11.32±0.03 %. The result of the physicochemical analysis revealed that pH values ranged from 4.54±0.05-4.87±0.02, titratable acidity ranged from 3.46±0.6-3.90±0.70, and viscosity ranged from 134.50±1.40-143.14±0.25 cP. Consumption of 'nono' produced in the study area pose potential public health threat due to the presence of pathogenic organisms.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

'Nono' is a fermented milk product locally produced by Hausa/ Fulani cattle rearers (Adesokan *et al*., 2011). This product is particularly common in Northern Nigeria, where cattle rearing is a commonplace, and consumption of fresh and locally processed milk has been part of the local diet (Anyanwu, 2019). 'Nono' is nutritious food as it contains essential amino acids, protein, vitamins and phosphorous (Shibdawa *et al*., 2018). It also contains diacetyl, which is an essential property that confers desirable flavor characteristics in fermented products (Adesokan *et al*., 2011).

Milk in the udder of a healthy animal is almost sterile, except for presence of lactic acid bacteria (Banik *et al*., 2014). However, as soon as it comes out of the udder, it is immediately colonized by microorganisms in the teat skin and its epithelial lining (Dafur *et al*., 2018). Microbial contamination of raw milk can be airborne, from animal feed, the soil, dung, milking equipment and grass (Banik *et al*., 2014). The nutrient-dense nature of 'nono' (Rizzoli, 2014; Pfeuffer and Watzl, 2018) makes it an excellent medium for microbial growth, and therefore an agent in the spread of human diseases (Kumbhar *et al*., 2009; Jeppu *et al*., 2015).

'Nono' remains a popular street vended beverage amongst middle and low-income earners in northern Nigeria in spite of a seemingly lack of proper training in safe food processing and handling techniques by vendors (Bello *et al.,* 2020). This has consequently attracted numerous studies into its safety over the years. A number of these studies have established the presence of pathogenic bacteria in retail (Maikai and Madaki, 2018; Uzoaga *et al*., 2020; Esonu *et al*., 2021; Abdulrahman and Sanmi, 2021). The

microbiological quality of ‘nono’ was determined in this study while paying attention to the steps of production that exposes the product to microbial contamination as well as the proximate composition of the final product.

1.2 Statement of Research Problem

Locally produced diary products have become an alternative source of protein due to its accessibility and low cost in the developing countries (Ekumankama *et al*., 2020). However, they are easily perishable and serve as source of foodborne pathogens because they are highly vulnerable to bacterial contamination due to the unwholesome handling and preparation method in the local settings (Ujwal, 2017). The lack of potable water sources also compounds this situation (Alexander *et al*., 2018).

In addition, there is shortage of data on the safety of these products particularly on the intensity and origin of the source of microbial contamination, whether human or animal origin. This pose risk to human health as there has been reports on foodborne outbreak due to contaminated dairy products (Headrick *et al*., 1998; Centre for Disease Control (CDC), 2008; Kousta *et al*., 2010; Langer *et al*., 2012; Paramithiotis *et al*., 2017; Proroga *et al*., 2019; Nicholas *et al*., 2020). This is of great worry because zoonotic diseases contribute to the menace of endemic disease through exposure to animal pathogens especially in the developing world. The present study identified the potential contaminant present at every step of production.

1.3 Aim and Objectives of the Study

The aim of this study was to assess the microbiological quality and proximate composition of locally fermented milk (nono) from selected locations in Minna, Niger state

The objectives of the study were to:

i. enumerate the microbial load at the different stages of 'nono' produced in the study area
ii. isolate and identify microorganisms involved in the preparation of 'nono' collected from the study area
iii. evaluate the proximate composition of 'nono' collected from the study area
iv. determine the physicochemical properties of 'nono' collected from the study area
v. characterize representative isolates from the different stages of 'nono' production

1.4 Justification for the Study

Dairy foods are produced locally in rural areas especially the northern part of the country where populace are unaware of proper hygienic practices that can reduce microbial contamination thereby leading to the production of unsafe products (Dafur *et al*., 2018).

Previous studies (Oladipo *et al.,* 2014; Ezeonu, and Ezeonu, 2017; Hadžić *et al*., 2017; Costanzo *et al*., 2020) focus mainly on the microbiological assessment of these products without paying due attention to the point of entry of contaminating materials, whether at the point of collection, at the point of processing or during storage. In order to identify risks emanating from specific foodborne pathogens and their point of entry, identifying the contaminants at the production steps will help to address these gaps from previous studies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Milk Products

In many nations of the world, the western world in particular, human carry on the consumption of milk past infancy, the usage of animal milk of specifically that of cattle, sheep and goat as food. For ages, cow milk has been processed into dairy merchandise such as skimmed milk, butter, ice cream, condensed milk, yoghurt and the extra durable and without difficulty moveable product, cheese (Anyawu, 2019). Milk and milk products are viewed as an imperative supply of dietry minerals for consumers.

In Nigeria, the wives of pastoralists normally manner clean milk into a range of standard milk merchandise for sale (Abdulkadir and Mugadi, 2012). These comprises sour milk 'Nono', local butter 'Maishanu', sour yoghurt 'Kindirmo', Fulani cheese 'Cuku' and cheese 'Cuku' (Mattiello *et al.,* 2018). These products are normally sold in designated places, such as the livestock markets in some towns or vended from place to place by women (Iyiola-Tunji *et al*., 2020).

These products are frequently accessible within trekking distance of Fulani villages due to their short shelf life and the fact that hawking is done on foot. The women usually walk to sale places such as roadside houses, local markets, and metropolis, carrying these milk products on their heads in gourds and calabashes (Ekumankama *et al*., 2020). For similar reasons, these products are accessible, easily in the northern parts of the country.

2.2 Proximate Composition Analysis

Proximate composition analysis is a method used to determine the values of the macronutrients in food samples. In general, those values are being declared as nutritional facts shown usually on the labels of the final food products, but they are also being determined during the production process. The proximate composition of foods includes moisture, ash, lipid, protein and carbohydrate contents (Thangaraj, 2016).

These food components may be of interest in the food industry for product development, quality control (QC) or regulatory purposes (Thangaraj, 2016). Analyses used may be rapid methods for QC or more accurate but time-consuming official methods. Sample collection and preparation must be considered carefully to ensure analysis of a homogeneous and representative sample, and to obtain accurate results.

It is mandatory for almost all food products to have standardized nutritional labels. This is to ensure that consumers are well aware of the nutritional composition of foods so that they can make informed and knowledgeable decisions about their diet. Additionally, they serve as means to create suitable conditions for fair market competition between food companies (Nielsen, 2006).

2.3 Sources of Contamination of Milk and Milk Products

Possible hazards could be chemical (disinfection and cleaning chemicals and antibiotic residues), physical, and microbiological. Among the microbiological hazards are zoonotic agents (Lehel *et al*., 2021).

Physical contaminants such as hair dirt particles, glass shavings, leaves, rubber, metal particles and pieces of paper, can gain entry in to the milk during milking process. Fragments of dirt from the air, udder or body of the cow, utensils and unsafe water supply can contaminate milk. The hair from the cow's body or the body of the milking person can also fall in the milk. The habits of the milker such as chewing tobacco or beetle leaves can also contribute to some harmful contamination (Kumar *et al*., 2018).

Chemical hazards are problematic in two aspects; environmental pollutants and contaminants mixed with milk that can result in serious ill-health. Numerous environmental pollutants can reduce the worth of milk (Lorenzetti *et al*., 2021). The mammary gland of the animal contributes in the excretion of several xenobiotic substances from residues of veterinary drugs and other chemical residues on the animal feed, grasslands, mud, slurry, feces, soil, and the field crops (Velázquez-Ordoñez *et al*., 2011).

Some toxins (aflatoxins and some mycotoxins) are reported to be carcinogenic, mutagenic, hepatotoxic and tetratogenic in almost humans and animals. After 48 hours of consuming contaminated feed, these toxins can manifest in milk. Others are pesticides and insecticides such as benzene hexachloride, dichlorodiphenyltrichloroethane (DDT), their isomers and other chlorinated chemicals such as aldrin, dieldrin, and heptachlor are common pesticides that may be found in milk (Kumar *et al*., 2018). The most combative residual appear in milk are antimicrobial drugs. The veterinary treatment of dairy livestock includes intra mammary infusion of antibiotics to keep mastitisin check. Various drugs are given to regulate parasites as well as to increasing milk production (Pal and Chakravarty, 2020).

Heavy metals such as mercury, lead, arsenic and cadmium have also been reported in milk and they fine their way into the human body primarily through routes of inhalation and ingestion (Kumar *et al*., 2018). Possible chemical contaminant of milk is the growth hormone or bovine somatotropin; a genetically engineered protein hormone whose main purpose is to improve production of milk in lactating cattle up to 10-15% (Kumar *et al*., 2018). Microbial contamination of milk on the other hand usually emanate from Poor hygienic practices which leads to the spread of pathogens and contamination of the udder which could occur at interval between milking cows by the handlers (Gillespie *et al*., 2009). Another microbial milk contamination may result during long storage period under inappropriate temperature (Lin *et al*., 2016).

Another factor that could be a source of food-borne pathogens in milk is biofilms, which would persist in processed milk products. Food borne pathogens would survive in milk and dairy products that is inadequately pasteurized. (Sultana *et al*., 2021). There has been recent hypothesis on the existence of microbes in the breast (Fusco *et al*., 2020). Bacteria present in milk was once thought to originate from exterior contaminants, the skin of the breast or the mouth of the youngling (Addis *et al*., 2016).

Conversely, numerous research propose that bacteria present in milk do not only originate from exterior contaminants but an autogenous path of its transmission has been presented. Microorganisms present in different locations of the body other than the udder may in some way get into the mammary gland from the intestine by traveling through the mesenteric lymph nodes and possibly through the dendritic cells as hypothesised in the entero-mammary pathway (Addis *et al*., 2016).

The relocation of bacteria found in the intestine to the mammary gland of cattle has been reported by Young *et al*. (2015) who studied the diversity and structure of the micro biota of faeces, white blood cells (WBC) and that of hale and hearty lactating cattle by metagenomics. Young *et al*. (2015) established the common existence of a few bacterial operational taxonomic units (OTUs) that belong to genera and family of bacteria in samples collected from said sections inside the same cow (Young *et al*., 2015; Addis *et al*., 2016). From a microbiological point of view, it is imperative to assess the components and progression of the fresh milk micro biota and the influence they have on components and value of its derived products from the time of milking till its storage, transport, and conversion in dairy products. The stages that permit the conversion of milk in dairy products is paramount (Fusco *et al*., 2020).

2.4 Preparation of 'Nono'

'Nono' is a traditionally fermented fresh cow milk. It is prepared in homes from unpasteurized milk collected in a calabash and naturally fermented for 24 hours (Fagbemigun *et al.,* 2021) by a method involving lactic acid fermentation. The fresh milk obtained straight from the cow's udder into a suitable calabash, then kept open for around two hours in the sun to enable the fat layer to separate (Omola *et al*., 2019). Milk from several cows are usually mixed together with water. It is left at room temperature (37°C) for 24 hours to ferment, then, the fat and whey is removed to give rise to 'nono' (Anyawu, 2019). 'Nono' has smooth, thick and uniform look with a sharp acidic taste like similar to yogurt (Omola *et al*., 2019).

'Nono' can be enjoyed alone or accompanied with millet or maize dumpling called *Fura* (Anyawu, 2019). It is a nutritious food and its consumption crosses the Saharan populations

of West African Sub- region and extends to the people of the Mediterranean county and the Middle East. Some studies claim that fermented milk is more nutritious and promotes health than raw milk (Akabanda and Glover, 2010). The stages of production is shown in Figure 2.

2.5 Microbiology of 'Nono'

Exploration into the microbiology of 'nono' exposed that the fermented product is a culture of *Lactobacillus bulgaricus* growing with *Streptococcus thermophiles* (Chanos *et al.*, 2020). These organisms are symbiotic in their relationship as they are involved in conversion of almost all the sugar to lactic-acid and yielding small amount of bye-product. *S. thermophilus* produce diacetyl while acetaldehyde is produced *L. bulgaricus* (UmaMaheswari *et al*., 2021). They are also known as starter in milk fermentation that should be ample and feasible in the end fermented milk where aside from production of acid, similarly improves the product's flavour significantly (Petrova *et al*., 2021).

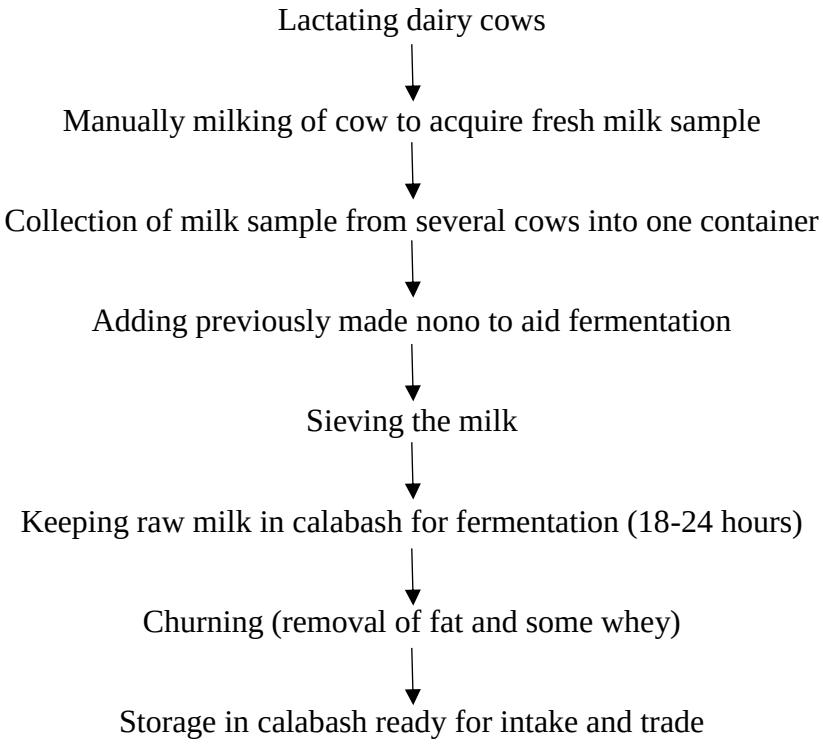


Figure 2: Stages of 'nono' preparation

Source: Adetola *et al*. (2016); Dafur *et al*. (2018)

Species of *Lactobacillus* and *Streptococcus* as well as *Saccharomyces* isolated from 'nono' has been used to develop starter cultures for controlled fermentation (Fagbemigun *et al*., 2021). Other bacteria belonging to the general *Lactococcus*, *Bacillus*, *Leuconostoc*, *Propionibacterium*, *Pediococcus*, and *Bifidobacterium* have been linked with fermented dairy products (Ghosh *et al*., 2019). Species of *Klebsiella, E*nterococcus, *Staphylococcus. Pseudomonas, Citrobacter, Micrococcus, Proteus,* and *Vibrio* (Maikai and Madaki, 2018; Nassarawa and Sulaiman, 2020) has also been isolated. Fungal species that has been isolated include *Aspergillus* sp*, Rhizopus* sp*, Trichophyton* sp and *Mucor* (Bazata *et al*., 2020).

While fermentation process is ongoing by lactic acid bacteria, other bacteria such as *Listeria monocytogenes, Salmonella paratyphi, Brucella melitensis, Clostridium botulinum* and *Escherichia coli* may create possible threat as they produce toxins that cause intoxication of the products (Ezeonu and Ezeonu, 2017). The coliform count may be less in some studies (Yabaya *et al*., 2012; Maikai and Madaki, 2018;) but their presence is an indication of fecal contamination and this can pose a health risk to the consumers.

Enhancements in approaches (culture dependent and culture independent) used to identify microorganisms has greatly improved our knowledge on the micro biota of fresh milk (Pang *et al*., 2018; Wu *et al*., 2019; Fusco *et al*., 2020). The foremost pathogens stirring up in milk and milk products are Shiga-toxin-producing *E. coli*, *Campylobacter* sp., *Salmonella* sp., *Listeria monocytogenes* and coagulase positive *Staphylococcus* species (European Food Safety Authority (EFSA), 2016; EFSA and European Centre for Disease Prevention and Control (ECDC), 2018; Fusco *et al*., 2020).

2.6 Microorganisms Associated with 'Nono'

A major public health menace by pathogenic bacteria in milk have been due to the number of diseases caused by them (Bano *et al*., 2020). Fermented milk (nono) could be predisposed to pre fermentation and post fermentation contaminations. Some cases of milk contamination by pathogenic bacteria as a result of elevated temperature and humidity nature of the African regions, and due to the lack of refrigeration, have been reported (Mattiello *et al*., 2018).

The udder of the cow, its environments, the air, the collection container, and the body and clothes of the milking person may pose as a strong source of pre fermentation infiltration (Ezeonu and Ezeonu 2017). The water, vegetation, and soil are the major sources of psychotropic spoilage organisms, such as *Pseudomonas*, to milk. Psychotropic bacteria can grow and cause significant chemical changes during pre-processing period before pasteurization (Quinto *et al*., 2020). Post-fermentation contamination could also be a consequence of the environment in which fermentation took place as well as the insufficient safety of the product from pests.

The multifaceted organic components, high moisture content and nutritional contents of milk makes it an exceptional medium of growth for both spoilage and pathogenic organisms (Lawan *et al*., 2012) linked with several disease conditions markedly *Salmonellosis, Tuberculosis, Brucellosis, Staphylococcosis*, Cholera, *Shigellosis* and others (Umaru *et al*., 2012).

2.6.1 *Staphylococcus aureus*

Most of the *Staphylococcus aureus* strains that produce catalase, make up the pathogenic strains (Rasigade and Vandenesch, 2014). *Staphylococcus aureus* is a chief root of food intoxication globally (Spanu *et al*., 2012). Adulteration of food by *Staphylococcus aureus* may arise right from infested food-producing animals or due to poor sanitation during stages of production, or the marketing and storing of food (Esonu *et al*., 2021).

A study by Sharma *et al*. (2007), described bovine mastitis as one of the most important health complications of dairy herds as it causes biochemical, bacteriological and physical variations such as watery and flakey appearance in the milk of the animals. Bovine mastitis is evident and easily detected by visible abnormalities, such as red and swollen udder and fever in dairy cow. This causes reduction in values of produced milk with possible public-health complications (Cheng and Han, 2020). When present in milk, the number of *E. coli* O157:H7 are reported to upsurge during the initial phases of manufacture of several cheese types, a sign that pre fermentation adulteration is a serious menace in fermentation of milk products (Ezeonu and Ezeonu, 2017).

2.6.2 *Salmonella* species

Salmonellosis is one of the main zoonotic diseases worldwide with yearly estimate of twenty-two million cases and two hundred thousand deaths due to typhoid-fever and 93.8 million cases of gastroenteritis and 155 000 deaths due to non-typhoidal *Salmonellae* (Majowicz *et al*., 2010). *Salmonella* is a Gram negative, rod-shaped, facultative anaerobe, that belongs to the *Enterobacteriaceae* Family and it is a main pathogenic organism dwelling in the abdominal tract of animals. The genus *Salmonella* encompasses 2 species, *Salmonella bongori* and *Salmonella enterica* (CDC, 2011). *Salmonellosis* is an infection of the digestive tract caused by the bacterium, *Salmonella enterica* which has over 2,000 strains. Fortunately,

cattle are usually clinically infected by less than 10 of these strains. The majority of *Salmonella* that infects cattle are S. *typhimurium, S. montevideo, S. dublin*, or *S. anatum.* This disease has serious economic, animal health and public health implications. In calves, the disease most commonly affects colostrum, and may cause a fever, diarrhea, rapid dehydration and death within 24–48 hours. In adult cattle, Clinical signs include fever, followed by going off feed, depression, and foul-smelling diarrhea with varying amounts of blood, mucus, and shreds of intestinal lining. In milking animals, milk production severely drops. Abortions may occur in infected cattle. Temperatures typically rise 12 to 24 hours before other signs and may drop off again with the onset of diarrhea. Death rates vary depending on the serotype of *Salmonella* involved (Kent *et al*., 2021). Previous studies on the microbiological quality of 'nono', including the work of Omola *et al.* (2020); Esonu *et al.* (2021); Uzoaga *et al*. (2020) and Abdulrahman *et al*. (2021) have shown the repeated occurrence of *Salmonella* and *Staphylococcus* contamination in high numbers.

2.6.3 *Mycobacterium* species

Tuberculosis is a highly infectious disease of the lungs. It is the top killer disease worldwide due to a single airborne infectious pathogen, *Mycobacterium tuberculosis* (Allué-Guardia *et al*., 2021). *Mycobacterium tuberculosis* is a small, rod-shaped, strictly aerobic, acid-fast bacillus. Like other mycobacteria, it is slow growing, resulting in more gradual development of disease when compared with other bacterial infections (Allué-Guardia *et al*., 2021).

When inhaled, *M. tuberculosis* reaches the alveolar space and is bathed in alveolar lining fluid being in intimate contact with soluble components of the lung mucosa before interacting with the cellular compartment such as alveolar macrophages and other immune cells (Allué-Guardia *et al*., 2021). Tuberculosis in cows is a serious medical and veterinary problem

caused by *Mycobacterium bovis*. This latently zooanthropozoic *M. bovis* is excreted in milk without changing its organoleptic parameters while the causative agent is contagious, especially for children (Zimpel *et al*., 2017).

Animal products, and in particular cow's milk, need constant monitoring of biosafety for detection of zooanthropotic pathogens and mycobacterial infection is very difficult to detect quickly and reliably (Alexandrovna, 2022), although Several studies reported the detection of *M. tuberculosis* in milk (Collins *et al*., 2022). A study by Alexandrovna (2022), using guinea pigs as bio indicators for the presence of tuberculosis in cow milk, revealed that *Mycobacterium bovis* was not detected through microbiological processes. However, the guinea pigs developed pathoanatomical tuberculous changes that are of diagnostic value during the biological assays.

2.6.4 *Brucella* species

Brucellosis is the most common bacterial zoonosis globally, causing a debilitating human disease. Human brucellosis is reported worldwide, with at least 500,000 people newly infected annually. Human cases of brucellosis are acquired through contact with infected animals and consumption of contaminated dairy products (Aliyev *et al*., 2022). Five species among the *Brucella* species that infect animals are known to cause disease in humans, with *Brucella abortus*, *Brucella melitensis* and *Brucella suis* being the most important in terms of clinical severity and prevalence (The World Organization for Animal Health (WOAH) 2017). *Brucella melitensis* is considered the most prevalent and virulent *Brucella* species for humans.

The human disease typically presents an acute, nonspecific illness characterized by undulating fever, malaise, myalgia, and weight loss that may resemble other acute febrile diseases. A chronic form of brucellosis lasting longer than 12 months after diagnosis can also occur. *Brucella* infection in women can cause abortions or labor complications and in men, orchitis and epididymitis. *Brucella* infection in livestock causes significant economic losses through the reduction of productivity. The acute form of the disease is characterized by abortion in pregnant females and orchitis and epididymitis in males, whereas the chronic forms is characterized by hygromas, mainly seen in the tarso-metacarpal joints, inducing lameness in infected animals (Aliyev *et al*., 2022).

The main *Brucella* species isolated from livestock worldwide are *B. abortus*, infecting preferentially bovines; When cattle are in contact with infected small ruminants, *B. melitensis* can also infect bovines. In the case of human infection with *B. melitensis*, the source of contamination can be either small ruminants or bovines. (WOAH, 2017; Aliyev *et al*., 2022). Factors such as methods of food preparation and consumption of milk, pasteurization of dairy products, direct contact with infected animals, lack of awareness, and sensitization increase the risk of Brucellosis in communities (Mosiara, 2022).

2.5.5 *Shigella* species

Shigella is a pathogen mainly found in water and faeces causing contamination of animal, human, environment and milk. Members of the *Shigella* genus are classified into four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* and have all been isolated from milk and milk products with *S. dysenteriae* being the most common serogroup (Elkenany *et al* ., 2022).

Dairy products especially raw milk and unpasteurized cheese remain important vehicles for the transmission of Shigella to the rural and urban population (Gould *et al*., 2014).

The risk of *Shigella* is less in fermented milk because it has very effective inhibitory effect such as competing bacterial flora and low pH (4.5) on the growth of the most common enteric pathogens (Elkenany *et al*., 2022; Fagbemigun *et al*., 2021). Infections caused by *Shigella* species are the main causes of bacillary dysentery, which is affiliated with high morbidity and mortality, especially in developing countries. Shigellosis is a universal public health concern; Consumption of contaminated milk with *Shigella* leads to severe diarrhea (Zhu *et al*., 2017).

2.6.6 *Klebsiella pneumoniae*

Klebsiella is a Gram-negative, non-motile, and rod-shaped bacteria. The bacterium has a capsule; it is resistant to the environment and action of disinfectants as well as many antibiotics, which makes it lethal. It has a complex antigenic structure and contains capsular and somatic antigens and endotoxin; some strains can produce exotoxin (Lenchenko *et al*., 2020). *Klebsiella pneumoniae* is a normal flora of the human intestines, where it does not cause disease but if it gets into other areas of the body, it can lead to a range of illnesses,including pneumonia, bloodstream infections, acute intestinal infections, meningitis, and urinary tract infections.

Most cases of *K. pneumoniae* infection are nosocomial. People with compromised immune systems, the sick or injured are more likely to get a *K. pneumoniae* infection than the general population. In the dairy industry, *K. pneumoniae* is one of the known causes of primarily

environment derived *Klebsiella* mastitis and has been the subject of numerous studies (Zadoks *et al*., 2011; Podder *et al*., 2014).

Klebsiella pneumoniae is a common cause of clinical mastitis in dairy cattle although it can be prevalent in the environment without causing significant mastitis problems. Wood products are considered the main source of *Klebsiella* on dairy farms. Environmental hygiene and use of inorganic bedding materials such as sand are recommended to control *Klebsiella* mastitis. However, *Klebsiella* mastitis still occurs on well-managed dairy farms that use sand as bedding material (Munoz *et al*., 2006). Mastitis adversely affects milk production and generally, cows do not regain full production levels post recovery leading to considerable economic losses. It has also been reported that the amount of decrease in milk production depends on the specific pathogen causing the infection (Grohn *et al*., 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted within Minna, the capital of Niger state, North central Nigeria. Minna is situated amid latitude 8°20' N and 11°30' N and flanked by longitude 3°30' E and 7°20' N and sited wholly within the middle belt of Nigeria. It shares borders with Kaduna to the North-east and Federal Capital Territory` to the Southwest. It is elevated at 234 m above the sea level with an overall land-mark of 74344 km^2 (Ojekunle and Owoeye, 2018). The map of Niger state showing the study area is illustrate in Figure 3.1

3.2 Sample Collection and Processing

Samples were collected from three different rural settlements (Maidako, Shatta and Pompo villages) in Niger State. The samples were collected at different stages of production of 'nono' (sample collected directly from the udder, after mixing the milk form several cows, after sieving the milk, after fermentation, after churning) under aseptic conditions into sterile airtight sampling jars (Abdel All and Dadir, 2009). They were further conveyed to Microbiology Department laboratory of Federal University of Technology Minna in an insulated icebox immediately for microbiological analyses. The experimental flow chart for this study is shown in Figure 3.2

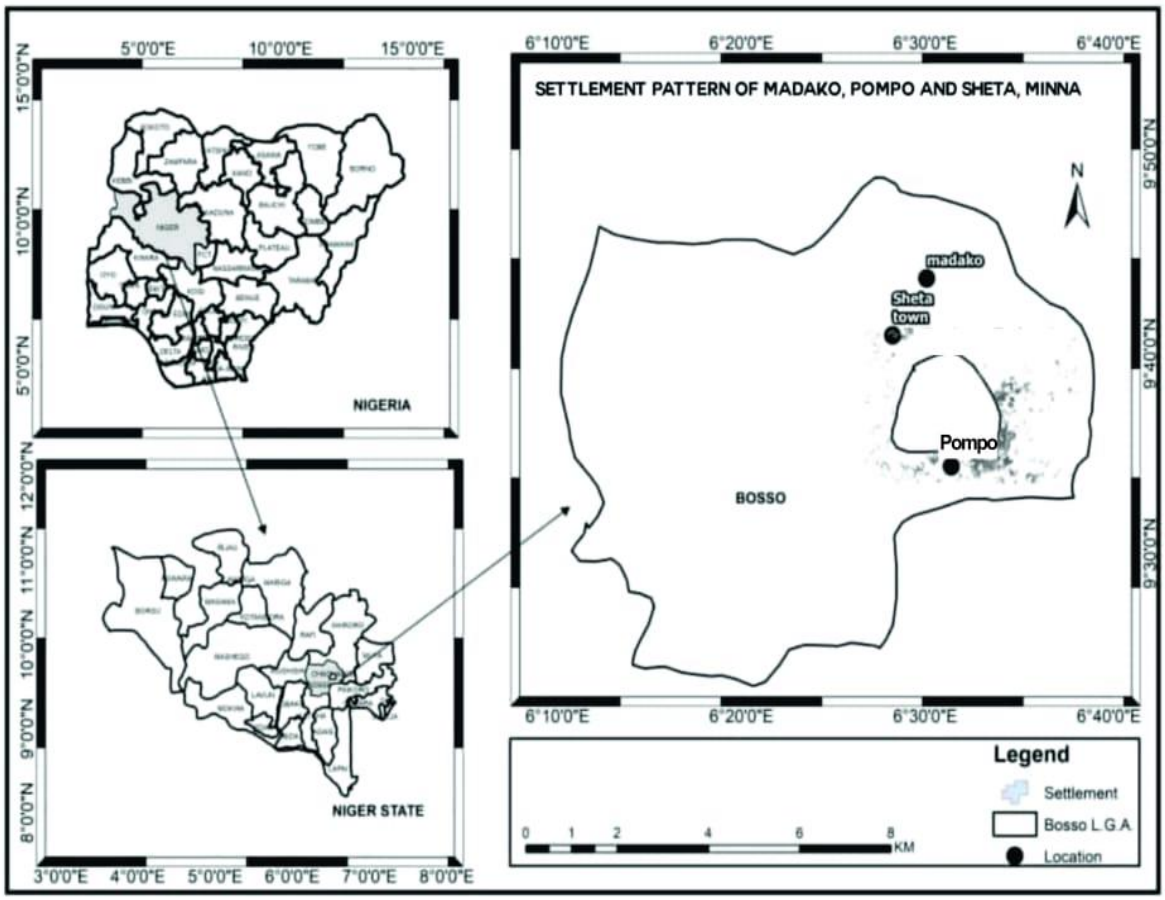


Figure 3.1: Map of Niger State Showing the Study Area.

Source: (Department of Geography, Federal University of Technology Minna).

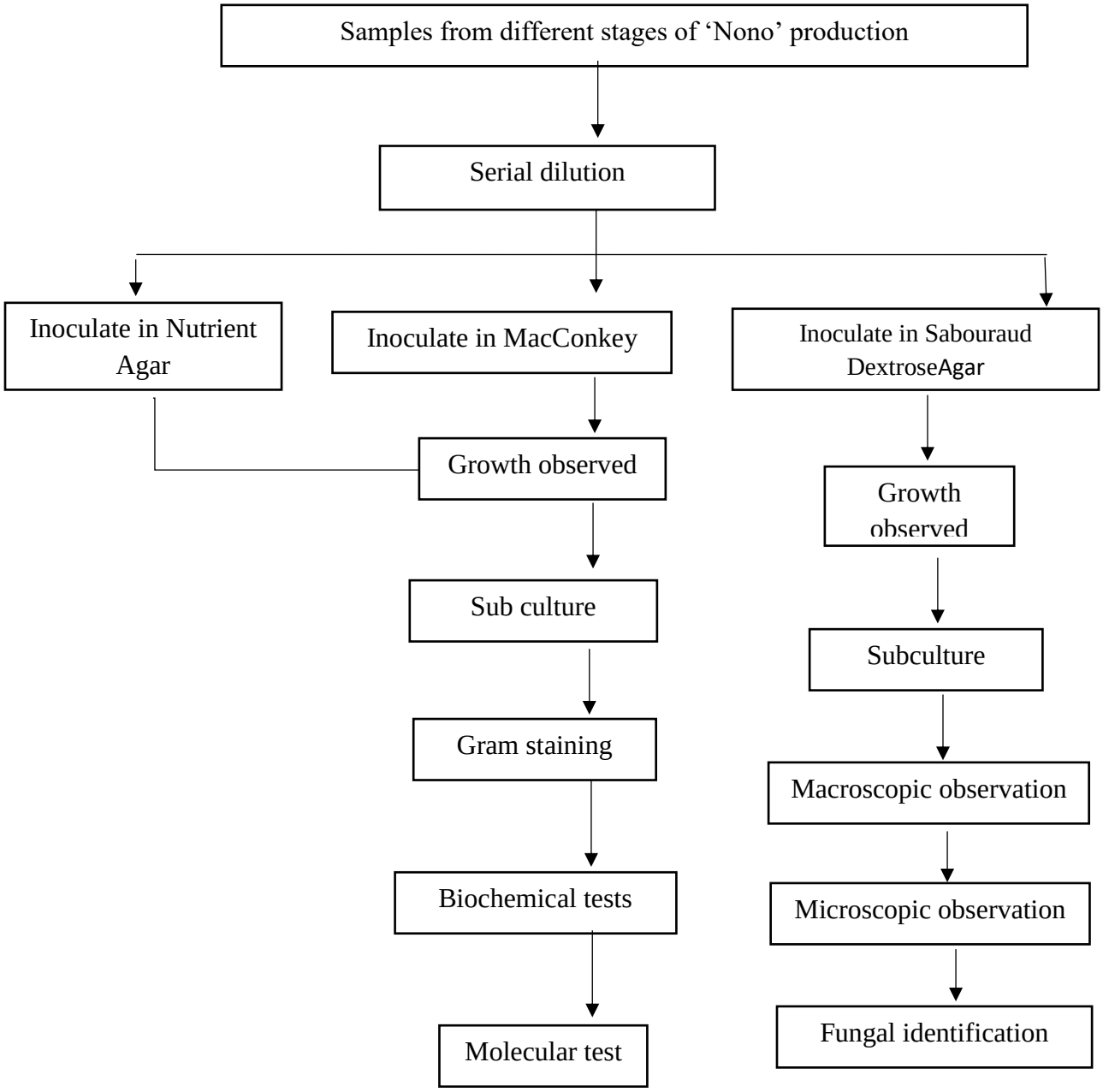


Figure 3.2: Experimental flow chart for this study

3.3 Enumeration and Isolation of Microorganisms from the Stages in 'Nono' Production

The microbial enumeration was done by determining the total viable counts **(**TVC) and total coliform count (TCC) using the techniques of Ogbonna (2011).

3.3.1 Total bacterial count: total viable counts and total coliform counts

One mililitre (1 mL) of the sample was added aseptically to sterilized test tube containing 9 mL of distilled water and mixed thoroughly to achieve serial dilution of 10-folds. The aforementioned dilution was used to make subsequent dilutions.

About 1 mL of the sample was transferred into well-labeled petri dishes using a pipette from the 10^{-5} and 10^{-6} dilution tubes. Twenty mililitre (20 mL) of melted agar was poured into plates and slightly rocked to ensure adequate blending. The petri dishes were incubated at 37 °C for 24 hours after solidification of the agar. The colonies on the plates were then counted and recorded as (cfu/mL) using a colony counter. Each sample was tested in duplicate and the average count was calculated. Total coliform counts were obtained by inoculating samples in 10^{-5} and 10^{-6} dilution tubes on MacConkey agar and incubated overnight.

3.3.2 Isolation of microorganisms from the stages in 'nono' production

Colonies of various colours, shapes and sizes were picked from various plates and sub-cultured repetitively to acquire pure isolated. The pure isolates were stored on agar slant for further characterization and identification.

3.4 Identification of Isolates from Different Stages in 'Nono' Production

Bacterial isolates were identified by cultural, morphological and biochemical methods as described by Mubarack *et al.* (2010) and Ogbonna (2011). Fungal identification was done by macroscopic and microscopic observations (Bala *et al.*, 2017).

3.4.1 Bacterial isolates

Bacterial identification was done using the appearance of colonies on culture media, Gram's reaction, biochemical tests (coagulase, indole, catalase, methyl red, Vogues-proskauer, citrate, carbohydrate fermentation test) and molecular methods (DNA extraction, PCR amplification, gel electrophoresis and sequence analysis) (Mubarack *et al.,* 2010; Ogbonna, 2011).

3.4.2 Cultural identification

Cultural identification was done by observing the appearance of isolates on the culture media and taking notes of the colour, sizes and shapes of colonies (Mubarack *et al.,* 2010; Ogbonna, 2011).

3.4.3 Morphological identification

The morphological identification of bacterial isolates was done using Gram's reaction. A smear of the isolate was made on a clean grease-free slide, dried, and then heat fixed. Crystal violet was poured on the slide and kept for 60 seconds before washing. Iodine was applied for 60 seconds before washing and decolourized with alcohol for 10 seconds. The slide was then washed, stained for 30 seconds using the counter stain (safranin), rinsed again, and left

to dry in the air. Immersion oil was dropped on a slide, which was then observed using the microscope's ×100 objective lens (Mubarack *et al.,* 2010; Ogbonna, 2011).

3.4.4 Biochemical identification

Biochemical tests (catalase, coagulase, methyl red, vogues-proskauer, oxidase, citrate, indole) were carried out to further identify the isolates as described by Mubarack *et al.* (2010) and Ogbonna, (2011).

(i) Catalase test

One drop of H_2O_2 (3 %) was placed on a clean glass-slide and a loop-full of colony is picked and mixed into it using a wooden stick. Rapid bubbling within 5-10 seconds indicates *Staphylococci* (positive results). Absence of bubbling indicates negative result (Mubarack *et al.,* 2010; Ogbonna, 2011).

(ii) Coagulase test

One drop of normal saline was placed on both end of a slide and a loop-full of the isolates was mixed in each drop, resulting in two dense suspensions. To one of the suspensions, a drop of human or rabbit plasma was added and gently mixed. Clumping of the organisms within 10 seconds suggests the presence of coagulase (Mubarack *et al.,* 2010; Ogbonna, 2011).

(iii) Indole test

Peptone water was inoculated with a light inoculum from an 18 to 24 hour pure culture and incubated for 24 hours at 37 $^{\circ}$C. Then, drops of Kovac's reagent was added. Formation of red ring on the surface within 1-2 minutes indicated positive results. Absence of colour change

shows negative results. Formation of orange coloured ring in some cases, indicated the presence of skatole (a precursor of indole formation) (Mubarack *et al.,* 2010; Ogbonna, 2011).

(iv) Citrate utilization test

The medium, simmone citrate agar was used to incubate the organism, which was treated with saline solution at 35°C for 18 - 24 hours. A change of the agar from green colour to blue colour (Mubarack *et al.,* 2010; Ogbonna, 2011).

(v) Methyl red test

The isolates were cultured for 48 hours in glucose phosphate peptone water at 37 °C. A few drops of methyl red reagent were added to the mix. Positive results were indicated by red colour, while presence of yellow colour indicated negative result (Mubarack *et al.,* 2010; Ogbonna, 2011).

(vi) Vogues-Proskauer test

The isolates were inoculated in MR/VP broth, and then incubated for 24 hours at 35 ºC. One milliliter of the broth was added in a washed test-tube, followed by addition 0.6 mL of 5 percent

α-naphthol and 0.2 mL of 40 percent KOH. The test tube was lightly shook to render the medium to ambient oxygen before being left alone for 15 minutes. A positive result was indicated by appearance of red colour within 15 minutes (Mubarack *et al.,* 2010; Ogbonna, 2011).

(vii) Oxidase test

Sterile distilled water was used to moisten a filter paper soaked with tetramethyl-p-phenylenediamine dihydrochloride. The test isolate was smeared onto the filter paper using a wooden loop. Positive result is indicated by change of colour to purple/deep blue within 10 - 30 seconds (Mubarack *et al.,* 2010; Ogbonna, 2011).

(viii) Carbohydrate fermentation test

Bromothymol blue indicator was mixed with prepared peptone water and added into three different conical flasks containing the test sugar (e. g maltose, glucose). The mixture was dispensed into bottles and sterilized for 15 minutes at 121 ºC using an autoclave. The test organism was inoculated into each bottle after cooling and incubated for 24 hours. A change in the colour of the mixture indicated acid production, while bubbles in the container indicated gas production (Mubarack *et al.,* 2010; Ogbonna, 2011).

3.5 Molecular Identification

Molecular identification of representative organisms was done by carrying out DNA extraction, PCR amplification, Gel electrophoresis and sequence analysis.

3.5.1 DNA extraction

Bacterial culture (1.5 mL) was transferred aseptically into a Eppendorf-tube and centrifuged for 5 minutes at 10,000 rpm. The pellet was suspended in 450 µL Tris-EDTA (TE) buffer after the supernatant has been discarded. Forty-five microliters of 10 % SDS and 10 µL RNase A was added. The tube was vortexed and incubated for 1 hour at 37 °C. Proteinase K (20 mg/mL) was added and incubated for 1 hour at 37 °C. Five hundred microliters of Chloroform:Isoamylalcohol (24:1) was added, mixed thoroughly, and centrifuged for 2

minutes at 10,000 rpm. The upper aqueous phase was dispensed in a new tube, and the process was repeated from there.

The upper aqueous phase was redirected to a new tube. 50 µL Sodium acetate and 300 µL isopropanol was added and mixed well. The tube was incubated at -20 °C for 20 minutes and centrifuged at 10,000 rpm for 2 minutes. The supernatant was then washed with 1 mL 70 % cooled ethanol mixed softly by inverting the tube and centrifugation at 10,000 rpm for 5 minutes. The tubes were carefully drained and allowed to air dry. The DNA was suspended in 50 µLTE buffer and stored at 4 °C. Concentration and purity of DNA was measured on Nano photometer (Implen). The PCR amplifications was performed with an Applied Bio-systems Veriti Thermal cycler (Tomar *et al*., 2019).

3.5.2 PCR amplification

Each PCR reaction mixture consisted of 12.5 µL mastermix (2x JENA Ruby hot start mastermix), 1 µL each of forward primer ITS1-TCCGTAGGTGAACCTGCGG and reverse primer ITS4-TCCTCCGCTTATTGATATGC, 1 µL DNA template and 9.5Ml sterile nuclease free water to make up a total reaction volume of 25 µL. PCR amplification was carried out in an Applied Biosystem 2720 thermocycler. The mixture was subjected to an initial denaturation at 95 °C for 5 minutes; followed by 35cycles of 94°C for 45s, 55°C for 45s and 72°C for 45s; and a final extension at 72°C for 5 minutes.

3.5.3 Gel electrophoresis

PCR products were visualized on a 2% agarose gel containing ethidium bromide in 0.5x Tris-borate buffer (pH 8.0) using blue led transilluminator (Plate 1).

3.5.4 Sequence analysis

PCR products were purified and sequenced by Sanger sequencing method using AB1 3730XL sequencer and done by Inqaba biotec, Pretoria, South Africa.

3.6 Fungal Identification

Fungal determination was done using the techniques described by Bala *et al.* (2017). The colony count and identification of fungi was done using Sabouraud dextrose agar (SDA). One mililitre (1 mL) of the sample was added aseptically to sterilized test tube containing 9 mL of distilled water and mixed thoroughly to achieve serial dilution of 10-folds. The aforementioned dilution was used to make subsequent dilutions. About 1 mL of the sample was transferred into well-labeled petri dishes using a pipette from the 10^{-5} and 10^{-6} dilution tubes. Melted SDA (20 mL) was poured into plates and slightly rocked to ensure adequate blending. The petri dishes were incubated at room temperature (25 °C) for 3-5 days after solidification of the agar. The colonies on the plates were then counted and recorded as cfu/mL. Colonies of various colours, shapes and sizes were picked from various plates and sub-cultured repetitively to obtain pure isolates.

Macroscopic examination of the sub cultured plate for colour, shape, and appearance of the colonies was used for preliminary examination (Egwaikhide *et al*., 2014; De *et al*., 2014). A scalpel and pin were used to pick a small part of the fungus culture. It was prepared and stained using lactophenol cotton blue stain, and examined at 10× and 40× objectives. The

structure, shape, spore type, and arrangement of the hyphae were all documented and used to identify the isolates (Obande and Azua, 2013; Bhatia *et al*. 2015). The features of isolates were compared to those of known taxa using Domsch and Gams methods (Domsch and Gams, 1970).

3.7 Proximate Analysis of 'Nono'

Proximate analysis of the fresh product was carried out in duplicates and mean result recorded. The moisture content, protein content, crude fat content, carbohydrate and ash contents was determined by the techniques of Association of Official Analytical Chemists (AOAC, 2000).

3.7.1 Moisture content

The moisture content was evaluated by oven drying. Five milliliter (5 mL) of 'nono' was measured into pre-weighed aluminum dry dishes. The dish and its content were transferred into an electric oven (AstellHeason, England) at 105 °C for 3 hours. The dish was weighed after 30 minutes of cooling in a desiccator. The percentage moisture content was calculated using Equation 3.1:

$$\%\, Moisture = \frac{W1-W2}{W0} \times 100 \qquad (3.1)$$

W_1 = initial Aluminum dish and Sample weight

W_2 = Final Aluminum dish and Sample weight

W_0 = Sample weight

3.7.2 Ash content determination

Five milliliters (5 mL) of 'nono' was precisely measured into a crucible that had been previously fired, chilled, and weigh up. Drops of glycerol were added, thoroughly mixed, then the sample was heated till it burned. The crucible was placed in a muffle furnace and heated to 5500 degrees Celsius until a white grey ash was obtained. After cooling in a desiccator, the crucible was reweighed (AOAC, 2000). The percentage ash was calculated using Equation 3.2:

$$\%\, Total\ ash = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100 \qquad (3.2)$$

3.7.3 Crude protein determination

Two grams (2g) of 'nono' was weighed and placed in a digestive flask. Each flask received an aliquot of kjedhal catalyst (0.8 g) and 15 mL of concentrated H_2SO_4. In the fume cupboard, each flask was cooked for around 30 minutes on a pre-heated digester. This was digested until it was a clear, homogeneous mixture. The flask was then withdrawn from the furnace after digestion, chilled, and the contents were thinned with roughly 50 mL distilled water. The flask was then placed in a micro-kjedahl analyzer, where it was automatically filled with 5 mL of NaOH (40%). The combination was then heated to produce ammonia and was then distilled for about 15 minutes into a conical flask containing 25 mL of boric acid (2%). During this time, the percentage protein was calculated using Equation 3.3:

$$\%\, protein = \frac{\text{A x 0.0014 x 6.25}}{weight\ of\ sample} \times 100 \qquad (3.3)$$

3.7.4 Fat content determination

A soxhlet extractor, a reflux condenser and a distillation flask was used to determine the crude fat content (previously dried and weighed). Two grams (2g) of each sample was measured into a fat-free extraction thimble, which was then stuffed with cotton wool and positioned in the extractor's suitable compartment. The distillation flask was filtered with n-hexane to a two-thirds capacity and cooked on the heating mantle. The distillate was gathered till for 4 hours until the extractor siphoned over. Following that, the n-hexane was gotten back into a clean container, and the remnant in the distillation flask was vaporized in a 700°C oven. After allowing the flask to chill in a desiccator, the ultimate weight of the flask was calculated. The difference between the initial and final the distillation flask weights represent the oil taken out from the sample (AOAC, 2000). The fat content was calculated using Equation 3.4:

$$\%\ crude\ fat = \frac{\text{final weight of the flask} - \text{initial weight of flask}}{weight\ of\ sample} \times 100 \qquad (3.4)$$

3.7.5 Crude fiber content determination

'Nono' (5 mL) was transferred to a 600 mL beaker, along with 700 mL 1.25% H_2SO_4. Every single beaker was heated for about 30 minutes and turned occasionally to prevent solids from adhering to the beakers' sides. Following that, each solution was filtered before being washed with 50 mL boiling water. This was done three times with 50 mL of water each time, then sucked dry. Boiling 1.25% NaOH was added and re-boiled with all residue, then the complete residue was reduced and left in the beaker.

The contents of every beaker was separated and sifted as indicated before. This was rinsed with 25 mL of 1% H_2SO_4, three parts of 50 mL water, and two parts of 25 mL alcohol. This will then be washed with 25 mL of 1% sulphuric acid, three portions of 50 mL water and 25

mL ethanol. The residue was later transferred into ashing dish and desiccated at 103 °C. This was followed by chilling then weighing. The residue was thereafter kindled at 600 °C for 30 minutes in muffle furnace, chilled in the desiccator and re-weighed (AOAC, 2000). The percentage crude fibre was calculated using Equation 3.5:

$$\%\ crude\ fiber = \frac{\text{loss in weight of ignition}}{weight\ of\ sample} \times 100 \qquad (3.5)$$

3.7.6 Carbohydrate content determination

The carbohydrate content of the product was calculated using a difference calculation by subtracting the sum of all the computed proximate values from 100. Equation 3.6:

$$\%\ crude\ fiber = \frac{\text{loss in weight of ignition}}{weight\ of\ sample} \times 100 \qquad (3.6)$$

3.8 Physicochemical Analysis of 'Nono'

Physicochemical analysis of 'nono' was done to determine its pH, titratabe acidity and viscosity.

3.8.1 Determination of pH

The pH of the product (nono) was measured directly using PYEUNICAM Model–292-MK2. The pH meter was homogenized with 4.0, 7.0 and 9.0 buffer solutions. The electrode of the pH meter was immersed in sterile water after two different buffers (4.0 and 7.0) were used, in order to standardize it. The electrode will be used for various samples and readings will be recorded (Jimoh and Kolapo, 2007).

3.8.2 Total titratable acidity

Titratable acidity will be evaluated by pouring 1 mL of product into a 250 mL Erlenmeyer flask, adding 10 mL distilled water, and three (3) drops of phenolphthalein indicator, then titrating against a 0.1 M NaOH solution (AOAC, 2000).

3.9 Data Analysis

Data generated from this research was subjected to one-way analysis of variance (ANOVA) test followed by a post-hoc DUNCAN ALPHA test to determine its significance. The data were evaluated in duplicate and presented as mean $\pm$ standard error of mean, P-values ≤ 0.05 were considered statistically significant. The data were statistically evaluated using the statistical package for social sciences (SPSS) version 26.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Total bacterial counts from the stages of 'nono' production at the different location

The study revealed that bacterial counts in the different steps of 'nono' production ranged from $1.00x10^6 \pm 0.00$ - $9.00x10^6 \pm 0.00$ in A, $2.00x10^6 \pm 1.00$ - $10.50x10^6 \pm 0.50$ in B and $1.00x10^6 \pm 0.00$ - $1.50x10^6 \pm 0.50$ in C (Table 4.1).

Table 4.1: Total bacterial count for the stages of 'nono' production at the different locations

STEPS	A (cfu/mL)	B(cfu/mL)	C (cfu/mL)	SON limit (cfu/mL)
1	$1.00x10^6 \pm 0.00^a$	$6.50x10^6 \pm 0.50^b$	NG	0
2	$3.50x10^6 \pm 0.50^b$	$2.50x10^6 \pm 0.01^b$	NG	0
3	$2.00x10^6 \pm 2.00^a$	$2.00x10^6 \pm 1.00^a$	NG	0
4	$9.00x10^6 \pm 0.00^b$	$10.50x10^6 \pm 0.50^b$	$1.50x10^6 \pm 0.50^a$	0
5	$7.50x10^6 \pm 0.50^b$	$9.00x10^6 \pm 0.00^c$	$1.00x10^6 \pm 0.00^a$	0

Values are in ± mean S.E. (*S. E = Standard error of Mean*). Values bearing the same superscript are not significantly different at the 5% level (P>0.05).

KEY: 1 = samples collected directly from the cow's udder

2 = samples collected after the milk from different cows has been mixed

3 = samples collected after sieving the milk

4 = samples collected before churning

5 = samples collected after churning

NG = No growth, A =Samples of nono from Maidako, B = Samples of nono from Shatta, C = Samples of nono from Pompo, SON =Standard Organization of Nigeria

4.1.2 Total coliform counts for the stages of 'nono' production at the different locations

The study revealed that coliform counts in the different steps of 'nono' production ranged from $1.00x10^6 \pm 0.00$ - $7.50x10^6 \pm 0.50$ cfu/mL in A, $1.00x10^6 \pm 0.00$ - $1.05x10^6 \pm 0.50$ cfu/mL in B and $1.00x10^6 \pm 0.00$ - $2.00x10^6 \pm 0.00$ cfu/mL in C (Table 4.2).

Table 4.2: Total coliform counts for the stages of 'nono' production at the different locations

STEPS	A (cfu/mL)	B (cfu/mL)	C (cfu/mL)	SON limit(cfu/mL)
1	$2.00x10^6 \pm 0.00^a$	NG	$1.00x10^6 \pm 0.00^a$	0
2	$1.08x10^6 \pm 0.05^b$	$1.00x10^6 \pm 0.00^b$	NG	0
3	$1.00x10^6 \pm 1.00$	NG	NG	0
4	$7.50x10^6 \pm 0.50^b$	$1.05x10^6 \pm 0.50^a$	$2.00x10^6 \pm 0.00^a$	0
5	$1.00x10^6 \pm 0.00^a$	NG	$1.50x10^6 \pm 0.05^b$	0

Values are in ± mean S.E. (*S. E = Standard error of Mean*). Values bearing the same superscript are not significantly different at the 5% level (P>0.05).

4.1.3: Fungal counts for the stages of 'nono' production at the different locations

The study revealed that fungal counts in the different steps of 'nono' production ranged from $1.00x10^6 \pm 0.00$ - $1.02x10^6 \pm 0.01$ cfu/mL in A, $1.00x10^6 \pm 0.00$ - $1.00x10^6 \pm 0.01$ cfu/mL in B and $1.00x10^6 \pm 0.00$ - $1.00x10^6 \pm 0.00$ cfu/mL in C (Table 4.3).

Table 4.3: Fungal count for the stages of 'nono' production at the different locations

STEPS	A	B	C	SON limit (cfu/mL)
1	NG	NG	NG	10
2	NG	NG	NG	10
3	$1.02x10^6 \pm 0.01^b$	$1.00x10^6 \pm 0.00^b$	NG	10
4	$1.00x10^6 \pm 0.01^a$	$1.00x10^6 \pm 0.01^a$	$1.00x10^6 \pm 0.00^a$	10
5	$1.00x10^6 \pm 0.01^b$	$1.00x10^6 \pm 0.00^b$	$1.00x10^6 \pm 0.00^a$	10

Values are in ± mean S.E. (*S. E = Standard error of Mean*). Values bearing the same superscript are not significantly different at the 5% level (P>0.05).

Table 4.4 Morphological, cultural and biochemical characteristics of bacterial isolates during the study

GR	CA	OX	CT	UR	IN	MR	VP	M	HAE	SH	COA	GL	FR	LA	SU	RH	ORGANISMS
-R	+	-	+	+	-	-	+	-	-	+		+	+	+	+	+	*Klebsiella pneumoniae*
+R	+	-	+	-	-	-	+	+	+	-		+	+	-	+	-	*Bacillus subtilis*
-R	+	+	+	-	-	-	-	+	+	+		+	+	-	-	-	*Pseudomonas aeruginosa*
+C	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*Micrococcus luteus*
+	-	+	-	-	+	-	+				+	-	-	-	-		*Salmonella enterica*
-R	+	-	-	-	+	+	-	+	-	-	-	+	+	+	+	+	*Escherichia coli*
+R	+	-	+		-	-	+	+	+			+	+	-	+	-	*Bacillus cereus*
-R	+	-	+	-	-	-	+	+				+	+	+	+	+	*Klebsiella aerogenes*
-R	+	-	+	+	+	-	+	-	-	-		+	+	+	+	+	*Klebsiella oxytoca*

-R	+	-	+	-	-	+	-	+	-	-		+	+	+	+	+	*Citrobacter freundii*
+C	+	-	+	+	-	+	+	-	+	-	+	+	+	+	+	-	*Staphylococcus aureus*
+C	+	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	*Kocuria rosea*
+R	-	-	-	-	-	-	-	-		+		+	+	+	+	+	*Lactobacillus* sp.
+C	-	-		-			+	-	+			+	+	+	+	+	*Streptococcus* sp.
-R	+	-	+	+	-	+	-	+				+	+	-	-	-	*Proteus* sp.
+C	-	-	+	-	-	+	+	-	-	-		+	+	+	+	-	*Lactococcus* sp.
-R		+	-	-	+	-	-	+	+			+	+	+	+	-	*Vibrio* sp.
-R	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	*Shigella* sp.

Legend: GR= Gram reaction, CA= catalase test, OX=oxidase test, CT=citrate test, IN=indole test, MR=methyl red test, VP=voges prokauer test, M=motility test, HAE=hemolysis test, SH=starch hydrolysis test, COA=coagulase test, GL=glucose fermentation test, FR=fructose, LA=lactose, SU=sucrose, RH=rhamnose.

4.1.5 Frequency of occurrence of bacterial isolates in 'nono' from the different locations

The results from the microbial analysis revealed eighty-nine (89) isolates which were separated into twenty four (24) genera and species. There were eighteen (18) bacteria isolated and they include *Klebsiella pneumonia, Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteus, Salmonella enterica, Escherichia coli, Klebsiella aerogenes, Bacillus cereus, Klebsiella oxytoca, Citrobacter freundii, Staphylococcus aureus, Kocuria rosea,* and species of *Lactobacillus, Streptococcus, Proteus, Lactococcus, Vibrio* and *Shigella.* The frequency of occurrence for bacterial isolates revealed that *Klebsiella pneumoniae* had the highest frequency of (12) 14.63 % followed by *Bacillus subtilis* (10) 12.19 % and *Pseudomonas aeruginosa* (8) 9.76 % while the lowest frequency recorded was (1) 1.22 % for *Klebsiella oxytoca, Citrobacter freudii, Kocuria rosea* and *Vibrio* sp. (Table 4.4).

4.1.6 Frequency of occurrence of fungal isolates in 'nono' from the different locations

The Fungi isolated during this study were seven (7) and they include *Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Trichophyton verrucossum, Candida glabrata* and *Rhizopus arrhizus* with *Aspergillus niger* having the highest percentage of (2) 28.60 % (Table 4.5).

Table 4.5: Frequency of occurrence of bacterial isolates in 'Nono' from the different locations

S/N	Isolates	Number of isolates from the different locations			Total number of isolates	Percentage (%)
		A	B	C		
1	*Klebsiella pneumoniae*	2	4	6	12	14.63
2	*Bacillus subtilis*	7	3	0	10	12.19
3	*Pseudomonas aeruginosa*	4	2	2	8	9.76
4	*Micrococcus luteus*	3	0	6	6	7.32
5	*Salmonella enterica*	2	2	1	5	6.09
6	*Escherichia coli*	3	2	1	6	7.32
7	*Bacillus cereus*	0	1	3	4	4.88
8	*Klebsiella aerogenes*	0	2	0	2	2.44
9	*Klebsiella oxytoca*	1	0	0	1	1.22
10	*Citrobacter freundii*	1	0	0	1	1.22
11	*Staphylococcus aureus*	3	0	2	5	6.09
12	*Kocuria rosea*	1	0	0	1	1.22
13	*Lactobacillus* sp.	2	4	1	7	8.54
14	*Streptococcus* sp.	1	2	0	3	3.66
15	*Proteus* sp.	0	2	2	4	4.88
16	*Lactococcus* sp.	2	0	1	3	3.66

17	*Vibrio* sp.	0	1	0	1	1.22
18	*Shigella* sp.	2	0	1	3	3.66
	TOTAL	**34**	**28**	**20**	**82**	**100.00**

Key: A= Maidako B= Shatta C= Pompo

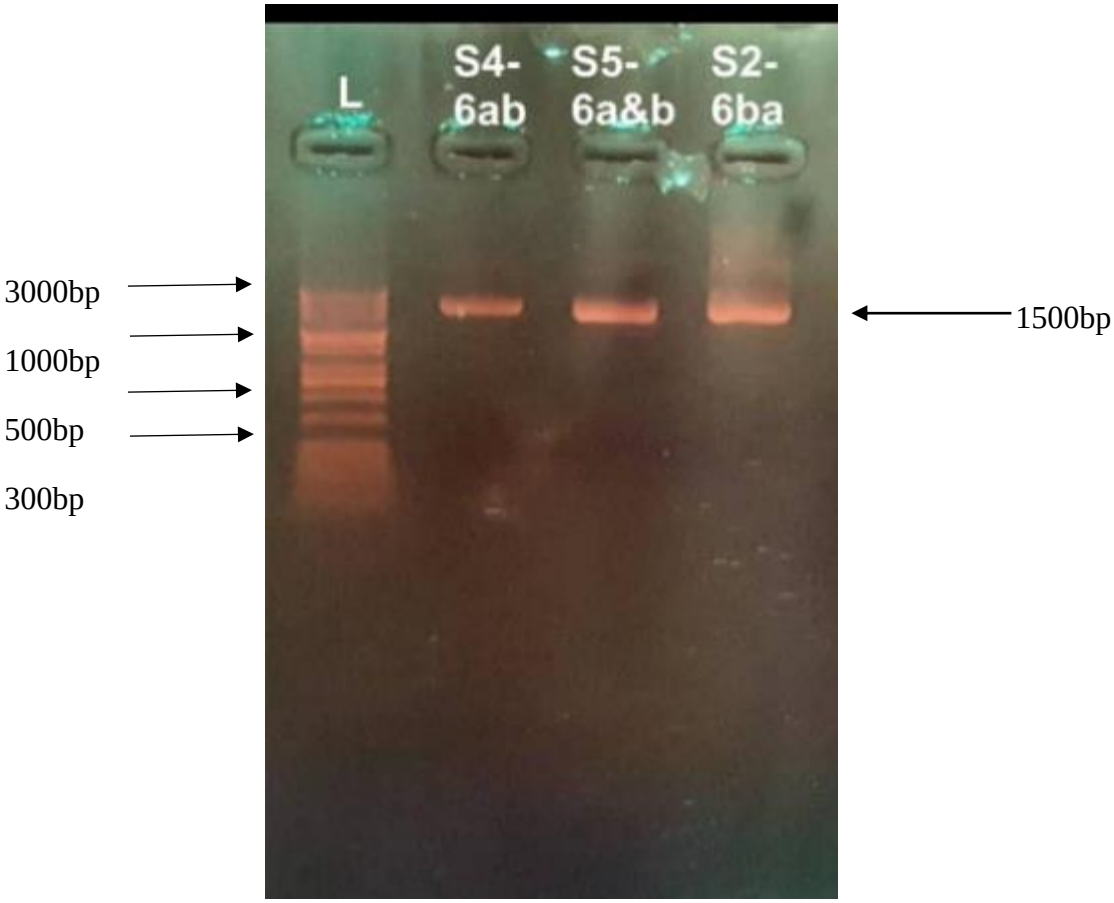
4.1.7 Molecular identity of bacterial isolates.

The three bacterial isolates with the highest frequencies were identified by the 16S rRNA sequence analysis. The result of pulsed gel electrograph with size 1500 bp is shown in plate 1. The sequences obtained were blasted in GenBank of NCBI. BLAST (basic local alignment search tool) result revealed that the test organisms (S4-6ab, S5-6a&b and S2-6ab) were similar to *Klebsiella pneumonia* (NR 117683.1), *Bacillus subtilis*,(NR 112116.2) and *Pseudomonas aeruginosa* (NR 113599.1) at 99.56%, 99.05% and 99.34% similarities respectively (Table 4.7)

Table 4.6: Frequency of occurrence for fungal isolates from the different locations

S/N	Isolates	Number of isolates from the different locations			Total number of isolates	Percentage (%)
		A	B	C		
1	*Aspergillus niger*	2	0	0	2	28.60
2	*Aspergillus terreus*	0	1	0	1	14.28
3	*Aspergillus fumigatus*	0	1	0	1	14.28
4	*Trichophyton verrucossum*	0	1	0	1	14.28
5	*Candida glabrata*	0	0	1	1	14.28
6	*Rhizopus arrhizus*	0	0	1	1	14.28

TOTAL	**2**	**3**	**2**	**7**	**100.00**



L = midrange ladder

Plate I: Gel electrograph of the PCR product of isolate S4-6ab, S5-6a&b and S2-6ba.

Table 4.7: Molecular identity of bacterial isolates.

Sample ID	Scientific name	Max score	E value	Identity	Accession number
S2-6ab	*Pseudomonas aeruginosa*	1642	0.0	99.56%	NR 113599.1
S4-6ab	*Klebsiella pneumoniae*	1700	0.0	99.05%	NR 117683.1
S5-6a&b	*Bacillus subtilis*	1663	0.0	99.34%	NR 112116.2

4.1.8 Proximate composition of 'nono' obtained from the different locations

The proximate analysis result showed that the protein values ranged from, 3.35±0.50-6.50±0.21, ash content ranged from 0.72±0.02-1.02±0.6, moisture content ranged from 81.21±0.1-82.17±0.07, total fat ranged from 2.10±0.02-2.58±0.05, and carbohydrate content raged from 9.17±0.22-11.32±0.03. There was significant difference in all the proximate parameters from the different locations (Table 4.8).

Table 4.8: Proximate composition of 'nono' obtained from the different locations

Sample	Protein (%)	Ash (%)	Moisture content (%)	Total Fat (%)	Carbohydrate (%)
A	6.50±0.21 [c]	1.02±0.6 [b]	81.21±0.1 [a]	2.10±0.02 [a]	9.17±0.22 [a]
B	3.35±0.50 [a]	0.72±0.02 [a]	82.17±0.07 [b]	2.58±0.05 [b]	11.32±0.03 [c]
C	4.83±0.03 [b]	0.93±0.01 [ab]	81.55±0.12 [ab]	2.38±0.08 [b]	10.37±0.01 [b]

Values are in ± mean S.E. (*S. E = Standard error of Mean*). Values bearing the same superscript are not significantly different at the 5% level ($P<0.05$).

Key: A= Maidako B= Shatta C= Pompo

4.1.9 Physicochemical composition of 'nono' obtained from the different locations

The result of the physicochemical analysis as presented in Table 4.6 revealed that pH values ranged from 4.54±0.05-4.87±0.02, titratable acidity ranged from 3.46±0.6-3.90±0.70, and viscosity ranged from 134.50±1.40-143.14±0.25 cP. There was significant difference in all the physicochemical parameters from the different locations. (Table 4.9)

Table 4.9: Physicochemical composition of 'nono' obtained from the different locations

Sample	pH	Titratable Acidity	Viscocity (cP)
A	4.87±0.02 [c]	3.46±0.6 [a]	143.14±0.25 [a]
B	4.54±0.05 [a]	3.90±0.70 [b]	134.50±1.40 [c]
C	4.57±0.10 [b]	3.84±0.15 [ab]	139.15±0.7 [b]

Values are in ± mean S.E. (*S. E = Standard error of Mean*). Values bearing the same superscript are not significantly different at the 5% level (P>0.05).

Key: A= Maidako B= Shatta C= Pompo

4.2 Discussion

4.2.1 Total bacterial counts from the stages of 'nono' production at the different locations (cfu/mL)

The total bacterial counts of $1.00x10^6 \pm 0.00$ -$10.50x10^6 \pm 0.50$ cfu/mL was recorded in this study. There was high microbial count in samples from all locations with the highest counts recorded in shatta at step 4 and 5. The high counts, although lower than the Codex standard of 10^7 cfu/mL, is an indication of contamination, which may have resulted from unhygienic practices such as the use of non-potable water and unsterilized utensils during production.

This result agrees favorably with the work of Abdulrahman and Sanmi (2021) who recorded 1.29 - 7.63 x 10^5 cfu/mL. They reported that the high counts might be as a result of product handling, the handlers sanitary or processing conditions, low level of cleanliness preserved throughout processing of the product (nono), water quality used, and the tools used in production and storage. Other explanations for the high counts detected could be as a result of the length of time in fresh milk stowing used for the making of 'nono' which might permit microbial contaminants to reproduce and increase in number thus prompting the entire viable/bacteria counts and the types of bacteria existing in the bulk fresh milk.

4.2.2: Total coliform counts for the stages of 'nono' production at the different locations

The total coliform counts recorded in this study ranged from $1.00x10^6 \pm 0.00$ - $7.50x10^6 \pm 0.50$ cfu/mL with the highest recorded in Maidako. This result contradicts a higher value of 3.24 x 10^{10} - 5.12 x 10^{10} cfu/mL recorded by Maikai and Madaki, (2018). These values are greater than the codex standard of 10 cfu/mL (FAO/WHO, 2018). Coliforms have been proven and endorsed by public health authorities worldwide as a pointer of post handling contagion in

manufacture (Rojas *et al*., 2020). The occurrence of coliform bacteria in high amounts in 'nono' is a suggestion that the milk has been tainted with faecal materials, inadequate cleaning of milking vessels, unhygienic milking environs, filthy udder and teats of lactating cow's, unclean water and/or cows with sub clinical or clinical mastitis (Owusu-Kwarteng *et al*., 2020). Dafur *et al*. (2018) also reported that some authorities consider coliform count in excess of 100 cfu/mL as indication of dissatisfying production cleanness. Therefore, their occurrence in great amount in dairy products is a sign that the products are possibly harmful to the consumer's health (Frew and Abebe, 2020).

At stage 1 during collection of samples directly from the udder, the presence of coliform in this stage may be due to contamination by fecal matter attached to the teats of the animal or the hand of the milking man. There was decline in coliform counts in stage 2 and stage3. This may mean that some organisms were not picked during culturing. There was an increase significantly in the coliform counts from step 3 where samples were collected after sieving the raw milk, to stage 4 in all locations especially in Maidako. This contamination might have resulted from the utensils used in this step such as the sieve and the sieving container that could have been contaminated before usage. Stage 4 had the highest coliform counts but reduced significantly at stage 5. This could mean that some of the contaminating organisms were removed along with the fat/whey that was removed during the churning process.

4.2.3: Fungal counts for the stages of 'nono' production at the different locations (cfu/mL)

The occurrence of fungi in milk and its products is unwanted even in a small number as they can lead to unpleasant change that reduce the quality of the product. Fungi constitute the major indicator of general essential quality (Huera-Lucero *et al*., 2020). The range of total

fungal count observed in the present study ($1.00x10^6 \pm 0.00$ - $1.02x10^6 \pm 0.01$ cfu/mL) is beyond the standard bound fixed for milk (FAO/WHO, 2018) although it is slightly lower than earlier study by Anyanwu, (2019) who reported mean fungal count that ranged from 2.27×10^5 - 5.34×10^5 cfu/mL in 'nono'. Yeast and mould are key contaminants of fermented milk products. Fungi growing in fermented milk use some of the acid supporting the proliferation of putrefactive bacteria and other disease causing microbes such as *Staphylococcus aureus* and *Listeria monocytogenes* (Banwo, *et al*., 2020). Furthermore, fermented milk is a high acidic product naturally, which is a greatly discerning environment or medium supporting the progression of Fungi as spoilage microbes whose existence in fermented milk is an pointer of poor management practices (Bereda *et al*., 2018).

Fungi can produce poisonous metabolites (mycotoxins) and taint many foods, mycotoxins are not destroyed in the course of food processing (Bereda *et al*., 2018) and could be potentially hazardous to human health. Contamination of milk and its products by fungi could be from air, feed, ineffectively washed milk apparatuses and poor personal cleanliness of milk handlers (Owusu-Kwarteng *et al*., 2020). According to this present study, fungal growth was not observed in stage 1 and 2. The emergence of fungal contaminants in stage 3, 4 and 5 could be as a result of exposure of the milk while transferring from one container to another (Cissé *et al*., 2018), from the air, the milk handlers, or even from the skin of the cow which may have multiplied when the conditions (acidity) became favorable. This is possible since the product did not undergo pasteurization.

4.2.5 Frequency of occurrence of bacterial isolates in 'nono' from the different locations

Fresh milk is a likely source of serious bacterial pathogens. The microorganisms isolated from the different stages of 'nono' production include Gram negative and Gram positive bacteria and fungi. Bacteria such as *Klebsiella pneumoniae, Salmonella enterica, Escherichia coli, Enterobacter aerogenes, Klebsiella oxytoca, Citrobacter freundii, Pseudomonas aeruginosa, Bacillus subtilis, Lactobacillus* sp*, Micrococcus luteus, Staphylococcus aureus, Bacillus cereus, Micrococcus roseus, Streptococcus, Proteus, Lactococcus, Vibrio* and *Shigella* species were isolated. The result corroborates with the report of Maikai and Madaki (2018) who isolated similar organisms such as *Enterobacter, Proteus, Klebsiella* and *Citrobacter* species from 'nono' in Samaru, Kaduna, Nigeria and the work of Bazata *et al*. (2020) who isolated *Staphylococcus aureus, Salmonella* sp, *Lactobacillus plantarum* and *Escherichia coli*.

The presence of *E. coli* (7.32 %) and other enteric bacteria in the samples is a suggestion that they possibly contain other pathogens, as the incidence of *E. coli* in foods is a sign of contamination of faecal source. The improvement of the sample quality is contributed by lactic acid bacteria because these bacteria produce antibacterial materials particularly, organic acids (Mortera *et al*., 2018). The presence of *Salmonella* (6.09 %) and *Shigella* (3.66 %) species agrees with the work of Esonu *et al*., (2021) which could be due to the level of hygienic practices in the area of study and the lack of potable water used in the production processes. The use of water from the streams that are visited by humans and animals may also be a possible reason for this contamination.

The cattle do sit on the same ground where they defecate and their teats are not usually cleaned before milking. This could also be a likely explanation for the presence of these organisms in the samples analyzed. The isolation of *Citrobacter* and *Proteus* species is in concordance with a previous study by Tamba *et al*. (2021). They established that *citrobacter* spp can cause meningitis, septicaemia and pulmonary infections in newborn and young children and has also been found to cause septicaemia in patients with a number of influencing factors. *Proteus* species are opportunistic human disease causing bacteria which can bring about infections in humans with compromised immunity (Tamba *et al*., 2021). Their isolation in this study could be as a result of the presence of house flies and surface run-offs of polluted surfaces into streams and animal houses through rainfalls. The congested poor sanitary practices observed in the research environment messy animal houses and keeping of unhealthy cattle with healthy ones could increase the chance of exposure to disease causing microbes. (Exner *et al*., 2017). They may be a source of complicated urinary tract infections.

The incidence of *Klebsiella pneumoniae* which had the highest frequency of (12) 14.63 % and *Pseudomonas aeruginosa* with frequency (8) 9.76 % in 'nono' is a suggestion of a likely contact with discharge from lacerations and excretory products of either human or cow respectively. This agrees with the study of Wiri *et al*. (2020) who confirmed that *Pseudomonas aeruginosa and Klebsiella* species are connected with wounds and body discharge. *Bacillus* sp with frequency of (10) 12.19 % is known to be pathogenic and resistant to environmental stress due to its spore forming nature and it can cause emetic syndrome (characterized by nausea and vomiting) and food-borne intoxication (Anyanwu, 2019; Noah and Salam, 2020).

4.2.6 Frequency of occurrence of fungal isolates in 'nono' from the different locations

The fungi species isolated include *Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Trichophyton verrucossum, Candida glabrata, Rhizopus arrhizus* The presence of *Aspergillus* and *Rhizopus* species isolated in this study is in concordance with the work of Anyanwu (2019) who reported that molds are the major contaminants of fermented milk in Nigeria. *Aspergillus* is recognized to produce mycotoxin, which makes consumers exposed to food intoxication while *Rhizopus* is an opportunistic disease causing microbe, one of the most common agents that causes invasive mucormycosis. It also causes zygomycosis, in which fungal infection are seen in face and oropharyngeal cavity (Anyanwu, 2016).

The presence of *Trichophyton verrucosum* however, could be from the skin of the animals as *Trichophyton verrucosum* are the main pathogenic fungi causing cattle dermatosis (Guo *et al*., 2020) which may explain their presence in this current study. The use of non-potable water for the preparation of the products were found to be the possible sources of contamination connected with the products. The contaminating microbes could also be through air micro-flora that attaches to apparatuses used in the production processes. In general, these high rates of microbes can be ascribed to the fact that traditional production is neither controlled nor structured, as the majority of producers and distributors are not adequately knowledgeable about sanitary and hazard management processes (Tankoano *et al*., 2016, Cissé *et al*., 2018).

4.2.7 Proximate composition of 'nono' obtained from different locations

The moisture content obtained in this study ranged from 81.21±0.1 - 82.17±0.07 which is similar to moisture content of 74.47±0.04 - 79.81±0.05 reported by Noah and Salam (2020) but differs from the result of Okeke *et al*. (2016) who recorded low values of 6.5 - 12.4. This

may be due to the inadequate regular water supply to the cattle from farms that the researcher studied.

The Crude protein content ranged from 3.35 ± 0.50 - 6.50 ± 0.21 This is in agreement with the work of Noah and Salam (2020) who reported a similar protein content of 3.97 ± 0.01 - 8.46 ± 0.04% in 'Nono' but disagrees with the protein content of 1.50 - 1.61% reported by Abdulrahman and Sanmi (2021). They explained that the low protein detected in 'nono' from the research could be ascribed to the practice of thinning of milk with water after fermentation by the handlers of 'nono*'* to elevate the volume. The higher content of protein in the present study may be due to the fact that, the practice of diluting milk with water is not a rare practice in the study area.

Carbohydrate value recorded in this study ranged from 9.17±0.22 - 11.32±0.03 which is similar to 5.18±0.01 - 10.20±0.01 recorded by Noah and Salam (2020). The values could be ascribed to the process of fermentation, which alters carbohydrate, mainly, lactose to lactic acid. This makes 'nono' an ideal food for individuals who are lactose intolerant (Mohammed and Aliyu 2020). In addition, the results obtained in the ash (1.08±0.03 -1.29±0.03) and fat (4.33±0.02 - 6.02±0.01) content as evaluated in this study corresponds with the study of Okeke *et al*., (2016) on proximate content of milk and milk products. They reported ash and fat content of 'Nono' to range from 1.25±0.8 - 1.77±0.3 and 3.6±0.1 - 3.6±0.0 respectively. The value of ash content is an indication of mineral content, which is necessary for bone and teeth development and other body functions (Trachoo and Mistry, 2011). Fat content in dairy product is controlled by variations in fresh milk value, treatments to lessen fat and adulteration by addition of water to milk. The values for fat content obtained in this study are within the standards of 3.5% fat content for fermented milk (Food and Agriculture

Organization (FAO), 2004; Omola *et al*., 2019) suggesting that the water added during the production process was adequate.

4.2.8 Physicochemical composition of 'nono' obtained from different locations.

The pH range of 4.54±0.05 - 4.87±0.02 of 'nono' samples recorded for this study supports the results of 4.22 - 4.70 obtained by Omola *et al*. (2019) in Kano and 4.56±0.05 - 6.46±0.09 reported by Noah and Salam (2020) in Ogun State. The pH values obtained were within the international standard limits for fermented milk. This low pH prevents the growth of most degeneration and disease causing organisms. The persistence of pathogenic organisms such as *Klebsiella* in stages after fermentation shows that 'Nono' should be pasteurized since they were able to survive at that pH.

Titratable acidity of raw milk has long been used to measure the quality of milk. It gives an indication of freshness of milk and other fermented milk product. The result obtained in this study showed that, titratable acidity ranged from 3.46 ± 0.6 - 3.90 ± 0.70. This is higher than the value of 0.9 ± 0.1 obtained by Okeke *et al*. (2016) on the physicochemical composition of 'nono' and 0.80% – 0.88% and 1.02 – 1.14% for titratable acidity of yoghurt after 6 and 8 hours of fermentation respectively by Choi *et al*. (2016) who explained that the titratable acidity of normally fermented milk ranges between the values of 0.7-1.2%. This showed that the fermentation proceeded in a normal way. The higher acidity reported in the present study as compared to the other studies could be a consequence of extended fermentation time of 24 hours and explains why 'nono' has a sour taste. Therefore, the fermentation is considered to have proceeded in a normal way.

The viscosity of the ‘nono’ samples ranged between 134.50 ± 1.40 - 143.14 ± 0.25 cP. These values are similar to 133.5 - 168.7 cP attained by Omola *et al*. (2019). Largely, viscosity of milk is essential in determining the rate of creaminess, the flow conditions in dairy processes and it also vary with changes in temperature (Janahar *et al*., 2021).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Results from this study indicated the presence of pathogenic microorganisms (*Klebsiella pneumonia* (NR 117683.1), *Bacillus subtilis* (NR 112116.2), *Pseudomonas aeruginosa* (NR 113599.1), and *Aspergillus niger*,) which regards 'nono', produced in the study area a potential public health threat.

The proximate analysis result showed that the protein value, ash content, moisture content, total fat and carbohydrate content ranged from, 3.35±0.50-6.50±0.21, 0.72±0.02-1.02±0.6, 81.21±0.1-82.17±0.07, 2.10±0.02-2.58±0.05 and 9.17±0.22-11.32±0.03 respectively.

The result of the physicochemical analysis revealed that values ranged from 4.54±0.05-4.87±0.02, 3.46±0.6-3.90±0.70 and 134.50±1.40-143.14±0.25 for pH, titratable acidity and viscosity respectively.

5.2 Recommendations

Based on the findings of this study, the following are recommended;

1. Proper sensitization of the 'nono' producers on the proper hygienic practices before, during and after production to minimize the rate of product contamination.
2. 'Nono' producers should ensure the use of potable water during production and pasteurization of the final product.
3. Further studies should be carried out to develop a HACCP plan for 'nono' production.

REFERENCES

Abdel All, A. A. A. & Dadir, H. A. (2009). Hygienic quality of local traditional fermented skimmed milk (Laban Rayb) sold in Egypt. *World Journal of Dairy &Food Science*, 4(2), 205-209.

Abdulkadir, M. & Mugadi, A. G. (2012). Bacteriological examination of fura da nono (fermented milk; cereals mix) sold *in some selected areas of Birnin Kebbi Metropolis. Asian Research Publishing Network Journal of Science and Technology*, 2(special issue), 333-340.

Abdulrahman, F. A. & Sanmi, E. (2021). Physicochemical properties, proximate composition and total viable counts of *Staphylococcus aureus* in 'nono' and yoghurt samples in kaduna, Nigeria. *Turkish Journal of Agriculture-Food Science and Technology*, 9(1), 15-20.

Addis, M. F., Tanca, A., Uzzau, S., Oikonomou, G., Bicalho, R. C. & Moroni, P. (2016). The bovine milk microbiota: insights and perspectives from-omics studies. *Molecular Biosystems*, 12, 2359– 2372.

Adesokan, I. A., Odetoyinbo, B. B., Ekanola, Y. A., Avanrenren, R. E., & Fakorede, S. (2011). Production of Nigerian nono using lactic starter cultures. *Pakistan Journal of nutrition*, *10*(3), 203-207.

Adetola, A. A., Henrietta, A. S. & Adekanmi, A. H. (2016). Effect of pasteurization and selected chemical preservatives on Fura da Nunu during storage. *African Journal of Food Science and Technology*, 7(8), 178-185.

Akabanda, F. & Glover, R. L. K. (2010). Microbiological characteristics of Ghanaian traditional fermented milk product, "nunu". *Nature and Science*, 8(9), 178-187.

Alexander, P., Dahiru, J. N. & Garba, W. H. (2018). Quality assessment of surface and ground water of Hong local government area of Adamawa State, Nigeria. *Asian Journal of Applied Chemistry Research*, 2(2), 1-11.

Alexandrovna, S. A. (2022). Guinea pigs as a bioindicator of *mycobacterium bovis* in cows' milk. In *The 8th International scientific and practical conference "Innovations and prospects of world science" Perfect Publishing, Vancouver, Canada,* 9.

Aliyev, J., Alakbarova, M., Garayusifova, A., Omarov, A., Aliyeva, S., Fretin, D. & Godfroid, J. (2022). Identification and molecular characterization of *Brucella abortus* and *Brucella melitensis* isolated from milk in cattle in Azerbaijan. *BioMed Central Veterinary Research*, *18*(1), 1-9.

Allué-Guardia, A., García, J. I., & Torrelles, J. B. (2021). Evolution of drug-resistant *Mycobacterium tuberculosis* strains and their adaptation to the human lung environment. *Frontiers in Microbiology*, 12, 612675.

Anyanwu, N. C. J. (2019). Microbiological and comparative analysis of indigenous and semi-industrial fermented milk drinks (Fura da Nono and Fura da Yoghurt) sold in Nigeria's capital. *International Journal of Bioassays*, 8, 5716-5723.

Anyanwu, N. C. J., Okonkwo, O. L, Iheanacho, C. N. & Ajide, B. (2016). Microbiological and nutritional qualities of fermented ugba (Pentaclethra macrophylla, Bentham) Sold in Mbaise, Imo State, Nigeria. *Australian Road Research Board*, 9(4), 1-8.

Association of Official Analytical Chemists (AOAC) (2000). *Official Methods of Analysis Association of Official Analytical Chemist*. Washington D. C., U. S. A. 13-23.

Bala J. D., Kuta, F. A., Adabara, N. U., Oyedum, U. M. & Murtala, G. (2017). Microbiological quality assessment of raw salad vegetables sold in Minna metropolis, Nigeria. *Journal of Biological Science*, 25, 1-7.

Banik, S. K., Das, K. K. & Uddin, M. A. (2014). Microbiological quality analysis of raw, pasteurized, UHT milk samples collected from different locations in Bangladesh. *Stanford Journal of Microbiology*, 4(1), 5-8.

Bano, S. A., Hayat, M., Samreen, T., Asif, M., Habiba, U, & Uzair, B. (2020). Detection of pathogenic bacteria *staphylococcus aureus* and *salmonella* sp. from raw milk samples of different cities of pakistan. *Natural Science*, 12(05), 295.

Banwo, K., Ogunremi, O. R. & Sanni, A. I. (2020). Fermentation biotechnology of African traditional foods. In *Functional Foods and Biotechnology* pp101-134. CRC Press.

Bazata, A. Y., Kalgo, Z. M., Gulumbe, B. H., Danlami, B. M., Aliyu, B., Obi, C. & Salihu, A. A. (2020). Assessment of microbiological quality of fura da nono produced in kebbi state, Nigeria. *Equity Journal of Science and Technology*, 7(2), 45-48.

Bello, S., Umoh, V. J., Galadima, M. & Mohammed, S. S. D. (2020). Microbiological quality and HACCP of nono production in a Fulani settlement, Zaria Kaduna state, Nigeria. *Journal of Bioscience and Biotechnology Discovery*, 5(2), 20-31.

Bereda, A., Yilma, Z., Eshetu, M. & Yousuf, M. K. (2018). Hygienic practices, microbial quality and safety of raw cow's milk and traditional fermented milk (Irgo) in selected areas of Ethiopian Central Highlands. *East African Journal of Veterinary and Animal Sciences*, 2(1), 17-26.

Bhatia, A., Gakkhar, N. & Bhojak, N. (2015). Comparative study on physiochemical properties of various milk samples. *International Journal of Recent Scientific Research*, 6(6), 4436-4439.

Centers for Disease Control and Prevention (CDC) (2008). Outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections associated with consumption of unpasteurized Mexican-style aged cheese--Illinois, March 2006-April 2007. *Morbidity and Mortality Weekly Report*, 57(16), 432.

Centers for Diseases Control and Prevention (CDC) (2011). Surveillance System Overview: National *Salmonella* Surveillance, Pp. 1-12.

Chanos, P., Warncke, M. C., Ehrmann, M. A. & Hertel, C. (2020). Application of mild pulsed electric fields on starter culture accelerates yogurt fermentation. *European Food Research and Technology*, 246(3), 621-630.

Cheng, W. N. & Han, S. G. (2020). Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - *A review. Asian-Australasian Journal of Animal Science*, 33(11), 1699-1713.

Choi, Y. J., Jin, H. Y., Yang, H. S., Lee, S. C. & Huh, C. K. (2016). Quality and storage characteristics of yogurt containing *Lacobacillus sakei* ALI033 and cinnamon ethanol extract. *Journal of Animal Science and Technology*, 58(1), 1-7.

Cissé, H., Sawadogo, A., Kagambèga, B., Zongo, C., Traoré, Y. & Savadogo, A. (2018). Milk production and sanitary risk along the food chain in five cities in Burkina Faso. *Urban Science*, 2(3), 57.

Collins, Á. B., Floyd, S., Gordon, S. V. & More, S. J. (2022). Prevalence of *Mycobacterium bovis* in milk on dairy cattle farms: An international systematic literature review and meta-analysis. *Tuberculosis*, 102166.

Costanzo, N., Carlotta, C., Adriano, S., Maria, T. C. & Francesco, C. (2020). Foodborne pathogen assessment in raw milk cheeses. *International Journal of Food Science*, 2020, 1-5.

Dafur, G. S., Iheukwumere, C. C., Azua, E. T. & Dafur, B. S. (2018). Evaluation of the microbial quality of 'nono'sold in Mangu local government area of Plateau State, Nigeria. *South Asian Journal of Research in Microbiology*, 2(2), 1-14.

De, N., Goodluck T. M. & Bobai, M. (2014) Microbiological quality assessment of bottled yoghurt of different brands sold in central market, Kaduna, Kaduna State, Nigeria. *International Journal of Current Microbiology and Applied Science*, 3(2), 20-27.

Domsch, K. H. & Gams W. (1970). *Fungi in agricultural soil*. longman group limited, London, 2(1), 12-15.

Egwaikhide, P. A., Malu, P. S., Lawal, U., Adelagun, R. O. & Andrew, C. (2014) Physicochemical and microbiolgical analysis of fermented cow milk (nono) consumed within Kaduna town, North West Nigeria. *Food Science and Quality Management*, 29, 44-49.

Ekumankama, O., Ezeoha, A. & Uche, C. (2020). The role of multinational corporations in local dairy value chain development: case of Friesland Campina WAMCO (FCW) in Nigeria. *International Food and Agribusiness Management Review*, 23(1), 55-70.

Elkenany, R., Eltaysh, R., Elsayed, M., Abdel-Daim, M. & Shata, R. (2022). Characterization of multi-resistant *Shigella* species isolated from raw cow milk and milk products. *Journal of Veterinary Medical Science*, 84(7), 890-897.

Esonu, D. O., Ismail, S., Ajala, A., Yusuf, S. M. & Otolorin, R. G. (2021). Occurrence and antimicrobial susceptibility patterns of *staphylococcus aureus* and *salmonella* species in fresh milk and milk products sold in Zaria and environs, Kaduna State, Nigeria. *Sahel Journal of Veterinary Sciences*, 18(2), 1-8.

European Food Safety Authority (EFSA). (2016). Multi-country outbreak of Shiga toxin-producing *Escherichia coli* infection associated with haemolytic uraemic syndrome. *European Food Safety Authority Supporting Publications*, 13(4), 1017.

European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA & ECDC). (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *European Food Safety Authority Journal*, 16(12), 5500.

Exner, M., Bhattacharya, S., Christiansen, B., Gebel, J., Goroncy-Bermes, P., Hartemann, P. & Trautmann, M. (2017). Antibiotic resistance: what is so special about multidrug-resistant Gram-negative bacteria. *German Medical Science Hygiene and Infection Control, 12*.

Ezeonu, C. S. & Ezeonu N. C. (2017). biological risks associated with fermented diary products, fruits, vegetables and meat: a critical review. *Advanced Biotechnology and Microbiology*, 2(1), 555577.

Fagbemigun, O., Cho, G. S., Rösch, N., Brinks, E., Schrader, K., Bockelmann, W. & Franz, C. M. (2021). isolation and characterization of potential starter cultures from the nigerian fermented milk product nono. *Microorganisms*, 9(3), 640.

Food and Agricultural Organization (FAO), (2004). *Manuals of food quality control*. food and nutrition food analysis: quality, and adulteration and tests of identity. United Nations, Rome. paper 14, 8.

Food and Agriculture Organization of the United Nations & World Health Organization (FAO/WHO). (2018). General standards for the labelling of packaged Foods-Codex Alimentarius International Food Standards. Pp. 2-12.

Frew, M. & Abebe, K. (2020). Microbial properties of milk and traditional fermented milk products in ethiopia: a review. *Agricultural Reviews*, 41,4.

Fusco, V., Chieffi, D., Fanelli, F., Logrieco, A. F., Cho, G. S., Kabisch, J. & Franz, C. M. (2020). Microbial quality and safety of milk and milk products in the 21st century. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 2013-2049.

Ghosh, T., Beniwal, A., Semwal, A. & Navani, N. K. (2019). Mechanistic insights into probiotic properties of lactic acid bacteria associated with ethnic fermented dairy products. *Frontiers in Microbiology*, 10, 502.

Gillespie, B. E., Headrick, S. I., Boonyayatra, & Oliver, S. P. (2009). Prevalence and persistence of coagulase-negative *Staphylococcus* species in three dairy research herds. *Veterinary Microbiology*, 134, (1-2), 65-72.

Gould, L. H., Mungai, E. & Behravesh, C. B. (2014). Outbreaks attributed to cheese: differences between outbreaks caused by unpasteurized and pasteurized dairy products, United States, 1998–2011. *Foodborne Pathogens and Disease*, 11, 545–551.

Grohn, Y.T., Wilson, D.J., Gonzalez, R.N., Hertl, J.A. & Schulte, H. (2004). Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *Journal of Dairy Science*, 87, 3358–3374.

Guo, Y., Ge, S., Luo, H., Rehman, A., Li, Y. & He, S. (2020). Occurrence of Trichophyton verrucosum in cattle in the Ningxia Hui Autonomous Region, China. *BioMed Central Veterinary Research*, 16(1), 1-9.

Hadžić, V., Bačić, A. & Ahmetović, N. (2017). Microbiological analysis of pasteurized and sterilized milk with emphasis on the impact of storage time and temperature on the growth and development of microorganisms. *Journal of Hygienic Engineering and Design*, 21, 18-22.

Headrick, M. L., Korangy, S., Bean, N. H., Angulo, F. J., Altekruse, S. F., Potter, M. E. & Klontz, K. C. (1998). The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. *American Journal of Public Health*, 88(8), 1219-1221. https://doi.org/10.2105/9780875530024

Huera-Lucero, T., Labrador-Moreno, J., Blanco-Salas, J. & Ruiz-Téllez, T. (2020). A framework to incorporate biological soil quality indicators into assessing the sustainability of territories in the Ecuadorian Amazon. *Sustainability*, 12(7), 3007.

Iyiola-Tunji, A. O., Buba, W., Saleh, I., Mohammed, A. T., Yusuf M. A. & John, P. A. (2020). Cattle milk processing and marketing characteristics among agro-pastoralists in North West Nigeria. *Nigerian Journal of Animal Production*, 46, 1. https://doi.org/10.51791/njap.v46i1.1294

Janahar, J. J., Marciniak, A., Balasubramaniam, V. M., Jimenez-Flores, R. & Ting, E. (2021). Effects of pressure, shear, temperature, and their interactions on selected milk quality attributes. *Journal of Dairy Science*, 104(2), 1531-1547.

Jeppu U., Jayaram N., Satya S. & Purayil A. K. (2015). Microbiological evaluation of ultra-high-temperature (UHT)-treated milk close to expiry date and routine home practices for preservation of milk. *Indian Journal of Community Medicine*, 40, (3), 174-176.

Jimoh, K. O. & Kolapo, A. L. (2007). Effect of different stabilizers on acceptability and shelf-stabilty of soy-yoghurt. *African Journal of Biotechnology*, 6(8), 1000-1003.

Kent, E., Okafor, C., Caldwell, M., Walker, T., Whitlock, B., & Lear, A. (2021). Control of *Salmonella dublin* in a bovine dairy herd. *Journal of Veterinary Internal Medicine*, *35*(4), 2075-2080.

Kousta, M., Mataragas, M., Skandamis, P. & Drosinos, E. H. (2010). Prevalence and sources of cheese contamination with pathogens at farm and processing levels. *Food Control*, 21(6), 805-815.

Kumar, D., Rai, D., Porwal, P., & Kumar, S. (2018). Compositional quality of milk and its contaminants on physical and chemical concern: a review. *International Journal of Current Microbiology and Applied Science, 7*(5), 1125-1132.

Kumbhar, S. B. Ghosh, J. S & Samudre, S. P. (2009) Microbiological analysis of pathogenic organisms in indigenous fermented milk products. *Advanced Journal of Food Science and Technology*, 1(1), 35-38.

Langer, A. J., Ayers, T., Grass, J., Lynch, M., Angulo, F. J., & Mahon, B. E. (2012). Nonpasteurized dairy products, disease outbreaks, and state laws—United States, 1993–2006. *Emerging Infectious Diseases*, 18(3), 385.

Lawan, M. K., Abdulsalawu, F. O., Suleiman, A., Aluwong, T. & Yaqub, L. S. (2012). Microbial assessment of bulk of milk before and after pasteurization in two different dairy farms in Zaria, Nigeria. *International Journal of Dairy Science*, 7(4), 103-108.

Lehel, J., Yaucat-Guendi, R., Darnay, L., Palotás, P., & Laczay, P. (2021). Possible food safety hazards of ready-to-eat raw fish containing product (sushi, sashimi). *Critical Reviews in Food Science and Nutrition*, *61*(5), 867-888.

Lenchenko, E., Blumenkrants, D., Sachivkina, N., Shadrova, N. & Ibragimova, A. (2020). Morphological and adhesive properties of *Klebsiella pneumoniae* biofilms. *Veterinary world*, *13*(1), 197.

Lin H, Shavezipur M, Yousef A, & Maleky F (2016). Prediction of growth of *Pseudomonas fluorescens* in milk during storage under fluctuating temperature. *Journal of Dairy Science*, 99(3):1822-1830.

Lorenzetti, S., Plösch, T., & Teller, I. C. (2021). Antioxidative molecules in human milk and environmental contaminants. *Antioxidants*, 10(4), 550.

Maikai, B. V. & Madaki, P. D. (2018). Enumeration of coliforms in fermented milk product (nono) sold in Samaru, Kaduna State, Nigeria. *Sokoto Journal of Veterinary Sciences*, 16(4), 50-57.

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., & O'Brien, S. J. (2010). International collaboration on enteric disease "burden of illness" studies. the global burden of nontyphoidal salmonella gastroenteritis. *Clinical Infectious Diseases*, 50(6), 882-889.

Mattiello, S., Caroprese, M., Matteo, C. G., Fortina, R., Martini, A., Martini, M & A. S. P. A. Commission "Animal Productions in Development Cooperation Projects". (2018). Typical dairy products in Africa from local animal resources. *Italian Journal of Animal Science*, 17(3), 740-754.

Mohammed, S. S. D. & Aliyu, G. S. (2020). Bacteriological, mycological quality assessment and proximate composition of selected brands of yoghurt sold within Kaduna metropolis, Nigeria. *Annals. Food Science and Technology,* 21(1), 143-159.

Mortera, P., Zuljan, F. A., Magni, C., Bortolato, S. A. & Alarcón, S. H. (2018). Multivariate analysis of organic acids in fermented food from reversed-phase high-performance liquid chromatography data. *Talanta*, 178, 15-23.

Mosiara, S. (2022). Prevalence and risk factors associated with Brucellosis; a critical literature review. *Animal Health Journal*, *3*(1), 16-26.

Mubarack, H. M., Doss, A., Dhanabalan, R., Balachander, S. (2010). Microbial quality of raw milk samples collected from different villages of Coimbatore District, Tamilnadu, South India. *Indian Journal of Science and Technology*, 3(1), 61–63.

Munoz, M. A., Ahlström, C., Rauch, B. J. & Zadoks, R. N. (2006). Fecal shedding *of Klebsiella pneumoniae* by dairy cows. *Journal of Dairy Science, 89*(9), 3425-3430.

Nassarawa, S. S., & Sulaiman, S. A. (2020). Microbiological and chemical evaluation of traditional fermented skimmed milk (nono) in Kano State Nigeria. *International Journal of Food Sciences*, 3(1), 33-46.

Nicholas, M., Conrad, A., Whitlock, L., Stroika, S., Strain, E., Weltman, A. & Williams, I. (2020). Multistate outbreak of *Listeria monocytogenes* infections retrospectively linked to unpasteurized milk using whole-genome sequencing. *Journal of Dairy Science*, 103(1), 176-178.

Nielsen, S. S. (2006). Proximate assays in food analysis. *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*.

Noah, A. A., & Salam, N. O. (2020). Effect of lactic acid bacteria on microbial, proximate and sensory qualities of nono (a nigerian fermented milk product). *International Journal of Microbiology and Application*, 7(1), 1-5.

Obande, G. A. & Azua, E. T. (2013). Extent of microbial contamination of nono, fresh cow milk and yoghurt sold in Makurdi, Benue State, Nigeria. *Journal of Microbiology and Biotechnology Research*, 3(3), 6-14.

Ogbonna, I., O. (2011). Microbiological analyses and safety evaluation of nono: a fermented milk product consumed in most parts of Northern Nigeria. *International Journal of Dairy Science*, 6(3), 181-189.

Ojekunle, J. A. & Owoeye, A. S., (2018) Spatial pattern of household travel in Minna metropolis, Nigeria. *International Journal of Granthalaayah*, 4(5), 291-296.

Okeke, S. K., Abullahi, O. I., Makun, H. A., & Okeke, U. K. (2016). Physico-chemical and nutritional qualities of dairy cattle products. *Global Journal of Life Science and Biological Research*, 2(2), 8-13.

Oladipo, I. C., Atolagbe, O. O., & Adetiba, T. M. (2014). Nutritional evaluation and microbiological analysis of yoghurt produced from full cream milk, tiger-nut milk, skimmed milk and fresh cow milk. *Pensee*, 76(4), 30-38.

Omola, E. M., Kawo, A. H. & Bukar, A. (2019). Microbiological quality of traditionally fermented fresh cow milk (nono) retailed in selected local government areas of Kano State, Nigeria. *Umaru Musa Yar'adua Univerisity Journal of Microbiology Research*, 4(1), 45-52.

Omola, E. M., Kawo, A. H., & Bukar, A. (2020). Evaluation of nutritional quality and mineral composition of fermented milk (nono) sold in Kano, north-western Nigeria. *Bayero Journal of Pure and Applied Sciences*, 12(2), 81-88.

Owusu-Kwarteng, J., Akabanda, F., Agyei, D., & Jespersen, L. (2020). Microbial safety of milk production and fermented dairy products in Africa. *Microorganisms*, 8(5), 752.

Pal, A., & Chakravarty, A. K. (2020). Disease resistance for different livestock species. *Genetics and Breeding for Disease Resistance of Livestock*, 271.

Pang, M., Xie, X., Bao, H., Sun, L., He, T., Zhao, H. & Wang, R. (2018). Insights into the bovine milk microbiota in dairy farms with different incidence rates of subclinical mastitis. *Frontiers in Microbiology*, 9, 2379

Paramithiotis, S., Drosinos, E. H., & Skandamis, P. N. (2017). Food recalls and warnings due to the presence of foodborne pathogens-a focus on fresh fruits, vegetables, dairy and eggs. *Current Opinion in Food Science*, 18, 71-75.

Petrova, P., Ivanov, I., Tsigoriyna, L., Valcheva, N., Vasileva, E., Parvanova-Mancheva, T & Petrov, K. (2021). Traditional bulgarian dairy products: ethnic foods with health benefits. *Microorganisms*, 9(3), 480.

Pfeuffer, M., & Watzl, B. (2018). Nutrition and health aspects of milk and dairy products and their ingredients. *Scientific Research*, 65(2), 22-33.

Podder, M. P., Rogers, L., Daley, P. K., Keefe, G. P., Whitney, H. G. & Tahlan, K. (2014). *Klebsiella* species associated with bovine mastitis in Newfoundland. *Public Library of Science One*, 9(9), 2.

Proroga, Y. T., Capuano, F., Castellano, S., Giordano, A., Mancusi, A., Delibato, E. & Pasquale, V. (2019). Occurrence and toxin gene profile of *Bacillus cereus* in dairy products. *Journal of Microbiology, Biotechnology & Food Sciences*, 9(1), 58-62.

Quinto, E. J., Marín, J. M., Caro, I., Mateo, J. & Schaffner, D. W. (2020). Modelling growth and decline in a two-species model system: pathogenic *Escherichia coli* O157:H7 and psychrotrophic spoilage bacteria in milk. *Foods*, 9(3), 331.

Rasigade, J. P. & Vandenesch, F. (2014). *Staphylococcus aureus*: a pathogen with still unresolved issues. *Infection Genetics and Evolution*, 21, 510-4.

Rizzoli, R. (2014). Dairy products, yogurts, and bone health. *The American Journal of Clinical Nutrition*, 99(5), 1256-1262.

Rojas, A., Murphy, S. I., Wiedmann, M., & Martin, N. H. (2020). Coliform Petrifilm as an alternative method for detecting total gram-negative bacteria in fluid milk. *Journal of dairy science*, 103(6), 5043-5046.

Sharma, N., Maiti, S. K., & Sharma, K. K. (2007). Prevalence, etiology and antibiogram of microorganisms associated with Sub-clinical mastitis in buffaloes in Durg, Chhattisgarh State (India). *International Journal of Dairy Science*, 2, 145-151.

Shibdawa, M. A., Ushie, O., Ogah, E., & Longbap, B. (2018). Physiochemical analysis of local (Fulani) yoghurt syrup sold in Bauchi metropolis. *International Journal of Advanced Research in Chemical Science*, *5*(4), 17-22.

Spanu, V., Spanu, C., Virdis, S., Cossu, F., Scarano, C. & DeSantis, E. (2012). Virulence factors and genetic variability of *Staphylococcus aureus* strains isolated from raw sheep's milk cheese. *International Journal of Food Microbiology*, 153, 53-7.

Sultana, T., Rabbi, M. F., Sarker, B. R., Islam, M. S., Begum, M. I. A., & Hossain, K. M. M. (2021). Prevalence and antibiotic resistance patters of escherichia coli isolated from milk and milk product. *Journal of Bio-Science, 29*(2), 81-91.

Tamba, Z., Ndams, I. S., Oniye, S. J., Kwaga, J. K. P., & Wada, Y. A. (2021). Microbact 24E detection and prevalence of faecal Enterobacteriaceae isolates from lactating cattle in Jos-Plateau and environs, Nigeria. *Dutse Journal of Pure and Applied Sciences*, 7(2), 101-109.

Tankoano, A., Kabore, D., Savadogo, A., Soma, A, Fanou-Fogny N, Compaore-Sereme D, Hounhouigan D. J. & Sawadogo-Lingani, H. (2016). Evaluation of microbiological quality of raw milk, sour milk and artisanal yogurt from Ouagadougou, Burkina Faso. *African Journal of Microbiological Research*, 10(16), 535–541.

Thangaraj, P. (2016). Proximate composition analysis. In *Pharmacological assays of plant-based natural products* (pp. 21-31). Springer, Cham.

The World Organization for Animal Health (WOAH). (2017). Brucellosis (*Brucella abortus*, *B. melitensis* and *B. Sui*) (infection with *B. abortus*, *B. melitensis* and *B. suis*). In: Manual of diagnostic tests and vaccines for terrestrial animals pp. 1–44.

Tomar, P., Arunachalam, K. D., Vinuprakash, K. C. & Thakur, H. (2019). Isolation, characterization and molecular identification of bacteria from commercial source using 16s rRna sequencing for domestic waste water treatment. *International Journal of Innovative Technology and Exploring Engineering*, 8(6), 473.

Trachoo, N., Mistry, V.V. (2011). Application of ultra-filtered sweet butter milk and sweet butter milk powder in yoghurt manufacture of non- fat and low fat yoghurt. *Journal of Dairy science*, 81, 774-788.

Ujwal, D. M. (2017). Basics of HACCP- Dairy and Food Industry. *Journal of Dairy Science and Technology*, 6(3), 30–37.

UmaMaheswari, T., Anbukkarasi, K., Hemalatha, T. & Singh, R. (2021). GTG5 fingerprinting of native *Streptococcus thermophilus* strains and its authentication by principal component analysis–a road to value added commercial yoghurt starter cultures. *International Dairy Journal*, 122, 105161.

Umaru, G.A., Kabir, J., Umoh, V. J., Bello, M. & Kwaga, J. K. P. (2012). Prevalence and antibiogram of coagulase positive *Staphylococci* isolated from fresh and fermented milk In Zaria and Kaduna, *Nigerian Journal of Veterinary and Applied Sciences*, 2, 1-7.

Uzoaga, G. O., Umeokonkwo, C. D., Usman, A. B., Kia, G. S. & Okolocha, E. C. (2020). Bacteriological quality of nono, a milk product sold at retail outlets in Federal Capital Territory, Nigeria. *Journal of Interventional Epidemiology and Public Health*, 3(5).

Velázquez-Ordoñez, V., Valladares-Carranza, B., Gutiérrez-Castillo del, C. A., Talavera Rojas, M., Pescador-salas, N. & Valdés-ramos R. (2011). *Milk production and safety food*. In: Svarc-Gajic, editor. Nutritional Insigthts and Food Safety. New York, NY: Nova Publishers, Inc; pp. 334-357.

Wiri, T. B., Osumenya, I. T. & Gbode, Y. L. (2020). Public health implications of locally fermented milk (*nono*) and antibiotic susceptibility testing of *Pseudomonas aeruginosa* Isolated from the Product in Borokiri, Rivers State Nigeria. *International Journal of Research and Innovation in Applied Science*, 5(3), 165-170.

Wu, H., Nguyen, Q. D., Tran, T. T. M., Tang, M. T., Tsuruta, T. & Nishino, N. (2019). Rumen fluid, feces, milk, water, feed, airborne dust, and bedding microbiota in dairy farms managed by automatic milking systems. *Animal Science Journal*, 90(3), 445– 452.

Yabaya A, Manga S. S., Lucy, M. & Alhassan, H. M. (2012) bacteriological quality of fermented milk sold locally in Samaru and Sabon-gari Market, Zaria-Nigeria. *Continental Journal of Microbiology* 6(1): 14-18.

Young, W., Hine, B. C., Wallace, O. A. M., Callaghan, M. & Bibiloni, R. (2015). Transfer of intestinal bacterial components to mammary secretions in the cow. *Journal of Life and Environmental Sciences*, 3.

Zadoks, R. N., Middleton, J. R., McDougall, S., Katholm, J. & Schukken, Y. H. (2011). Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. *Journal of Mammary Gland Biology and Neoplasia*, 16(4), 357-372.

Zhu, Z., Cao, M., Zhou, X., Li, B. & Zhang, J. (2017). Epidemic characterization and molecular genotyping of *Shigella flexneri* isolated from calves with diarrhea in Northwest China. *Antimicrobial Resistance and Infection Control*, 6, 92.

Zimpel, C. K., Brum, J. S. & de Souza Filho, A. F. (2017). *Mycobacterium bovis* in a European bison (*Bison bonasus*) raises concerns about tuberculosis in brazilian captive wildlive populations: a case report, *BioMed Central Research Notes*. 10(1), 91-106.

APPENDIX I

MAIDAKO (NA)		
S/N	**ISOLATES**	**ORGANISMS**
1	S1-5a	*Lactococcus* sp
2	S1-5b	*Lactococcus* sp
3	S1-6a	*Lactobacillus* sp
4	S1-6b	*Streptococcus* sp
5	S2-5aa	*Lactobacillus* sp
6	S2-5ab	*Micrococcus luteus*
7	S2-5ba	*M. luteus*
8	S2-5bb	*Shigella.* sp *(flexneri)*
9	S2-5bc	*Bacillus subtilis*
10	S2-6aa	*B. subtilis*
11	S2-6ab	*Pseudomonas aeruginosa*
12	S2-6ba	*P. aeruginosa*
13	S2-6bb	*B. subtilis*
14	S2-6bc	*Staphylococcus aureus*
15	S3-5aa	*S. aureus*
16	S3-5ab	*S. enterica*
17	S3-5ac	*Kocuria rosea*
18	S3-5b	*M. luteus*
19	S3-6ba	*B. subtilis*
20	S3-6bb	*M. luteus*
21	S4	*Klebsiella oxytoca*

22	S5-5a	*E. coli*
23	S5-5b/6a	*S. aureus*
24	S5-6b	*B. subtilis*
	(MK)	
25	S1-5	*B. subtilis*
26	S1-6	*Shigella.* sp *(dysentriae)*
27	S2-6aa	*P. aeruginosa*
28	S2-6ab	*P. aeruginosa*
29	S2-6b	*E. coli*
30	S3-5ba	*Citrobacter freundii*
31	S3-6b	*S. enterica*
32	S4	*Klebsiella pneumoniae*
33	S5-5a/b	*E. coli*
34	S5-6a/b	*B. subtilis*

KEY: NA = Nutrient agar, MK= Macconkey agar

APPENDIX II

SHATTA (NA)		
S/N	**ISOLATES**	**ORGANISMS**
1	S1-5aa	*Streptococcus sp*
2	S1-5ba	*Streptococcus sp*
3	S1-5bb	*Lactobacillus sp*
4	S1-5bc	*Lactobacillus sp*
5	S1-6aa	*M. luteus*
6	S1-6bc	*P. aeruginosa*
7	S2-5ba	*Lactobacillus sp*
8	S2-6aa	*B. subtilis*
9	S2-6ab	*M. luteus*
10	S2-6b	*Proteus*
11	S3-5aa	*Vibrio*
12	S3-5ab	*M. luteus*
13	S3-5ac	*Lactobacillus sp*
14	S3-5ba	*B. subtilis*
15	S3-5bb	*B. subtilis*
16	S3-5bc	*B.cereus*
17	S3-6b	*Proteus*
18	S5	*Klebsiella pneumoniae*
	(MK)	
19	S2-5aa	*Klensiella aerogenes*
20	S2-5ab	*Klebsiella aerogenes*
21	S2-5ac	*P. aeruginosa*

22	S2-5b	*S. enterica*
23	S3-5a	*S. enterica*
24	S4-5a	*E. coli*
25	S4-5ba	*Klebsiella pneumoniae*
26	S4-5bb	*Klebsiella pneumoniae*
27	S4-5bc	*Klebsiella pneumoniae*
28	S4-6aa	*E. coli*

KEY: NA = Nutrient agar, MK= Macconkey agar

APPENDIX III

POMPO (NA)		
S/N	**ISOLATES**	**ORGANISMS**
1	S1-5a	*S. aureus*
2	S2-5a	*Lactoccoccus* sp
3	S3-5a	*P. aeruginosa*
4	S3-5b	*B.cereus*
5	S4-5a	*S. aureus*
6	S4-5ba	*B.cereus*
7	S4-6aa	*Klebsiella pneumoniae*
8	S4-6ab	*Klebsiella pneumoniae*
9	S4-6bb	*P. aeruginosa*
10	S5-5a	*E. coli*
11	S5-5b	*S. enterica*
12	S5-6aa	*Lactobacillus* sp
13	S5-6ab	*Klebsiella pneumoniae*
	(MK)	
14	S1-6a	*Proteus*
15	S4-5a	*Klebsiella pneumoniae*
16	S4-5b	*Proteus*
17	S4-6a	*Klebsiella pneumoniae*
18	S4-6ba	*Klebsiella pneumoniae*
19	S4-6bb	*B.cereus*
20	S5-6a	*Shigella* sp *(Sonnei)*

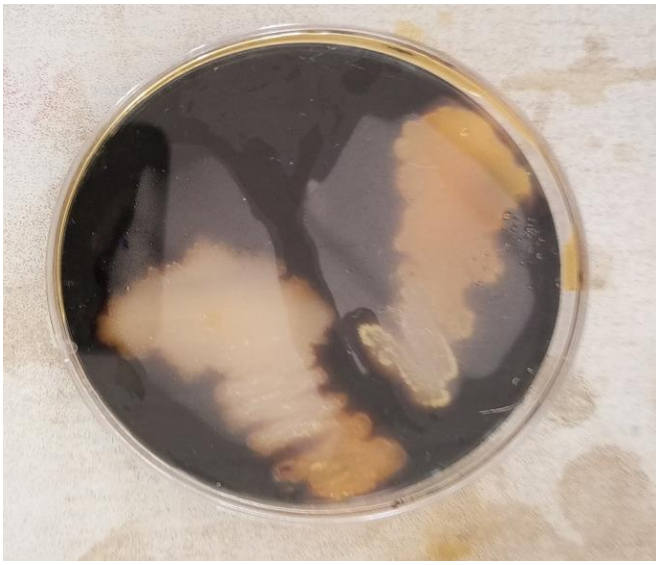
APPENDIX IV

Morphological and Microscopic Characteristics of Fungal Isolates During the study

Morphology on PDA	Microscopic appearance	inference
Black powdery colony with white matt splitting into several columns when ageing	Large globose conidiophore and septate hyphae	*Aspergillus niger*
Grey-green filaments with smooth colony	Branched septate conidiophore	*Aspergillus fumigatus*
Glabrous to lightly downy white or grey on the surface. No characteristic pigment on the reverse	Chlamydoconidia in chains with septate appearing fission flakes and antler hyphae	*Trychophyton verrucossum*
Green-black filaments with fluffy cottony mycelia	Non-septate and branched sporangiophores with globous sporangia	*Rhizopus arrhizus*
Brownish colony with smooth wall, becomes floccose with age achieving hair like soft tufts	Globose shaped, smooth surface biseriate spherical serration with smooth conidia	*Aspergillus terreus*
White to cream Small glossy, covex, smooth colonies.	Small sized spherical cells, less than half the size of a red blood cell. Does not produce hyphae or pseudohyphae	*Candida glabrata*

APPENDIX V

Biochemical tests for bacterial isolates



Starch hydrolysis test

/

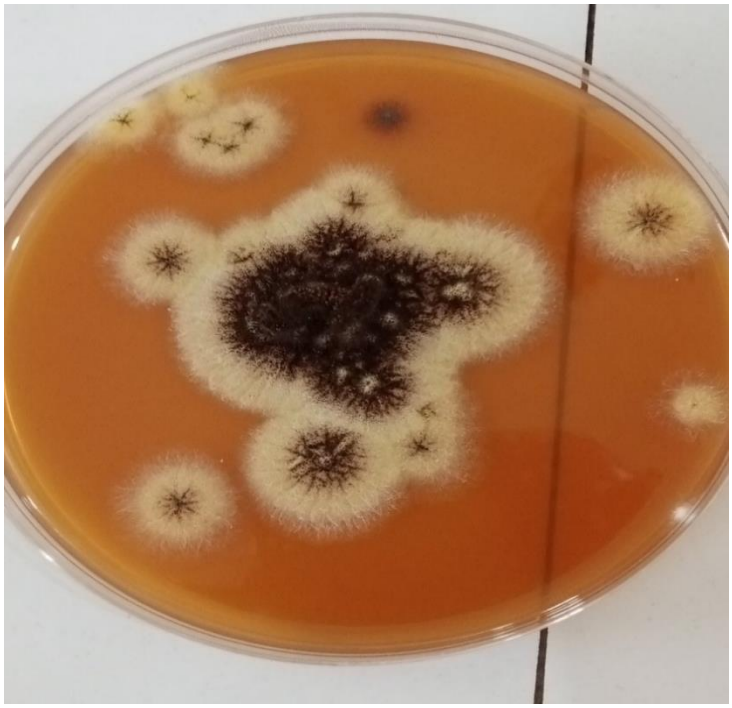
Haemolysis test

Sugar test

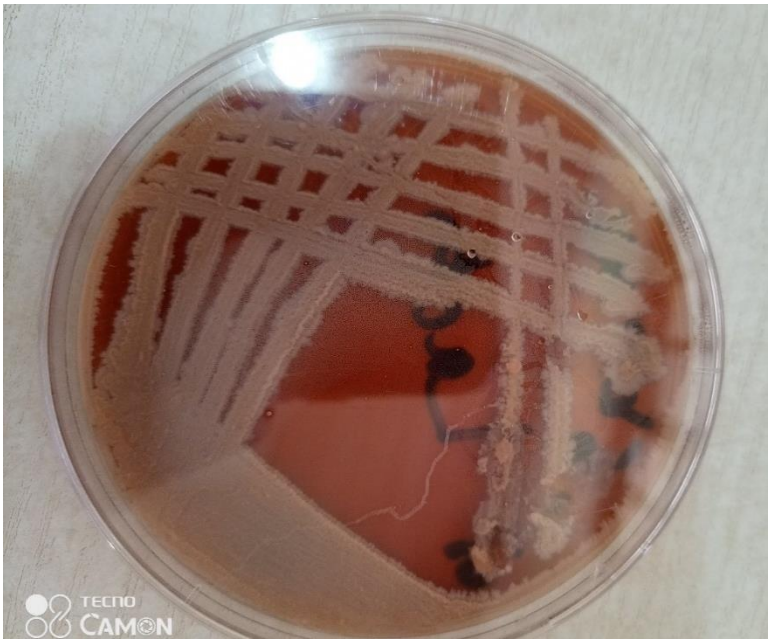
Citrate test

APPENDIX VI

Fungal and bacterial isolates on culture media



Aspergillus niger



Bacillus sp.

APPENDIX VII

Sequence of the representative organisms that underwent molecular identification

S2-6ab

>HWL_184_27f

GCAAGTCGAGCGGACTTAAAAAGCTTGCTTTTTAAGTTAGCGGCGGACGGGTGAGT
AWCACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGGAGCTAAT
ACCGGATAATCCTTTYCTACTCATGTAGGAAAGCTGAAAGACGGTTTACGCTGTCAC
TTACAGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC
GACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC
CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGA
CGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGG
GAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCC
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