MICROBIAL ANALYSIS OF SOME DAIRY PRODUCTS (MILK AND YOGHURT)

BY

Victory Aziegbemem EHIOSUN

LSC1505098

UNIVERSITY OF BENIN

BENIN CITY

JULY, 2021

MICROBIAL ANALYSIS OF SOME DAIRY PRODUCTS (MILK AND YOGHURT)

BY

Victory Aziegbemem EHIOSUN

LSC1505098

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc) IN SCIENCE LABORATORY TECHNOLOGY

JULY, 2021

CERTIFICATION

This is to certify that this project work was carried out by Victory Aziegbemem EHIOSUN with matriculation number LSC1505098 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

Mrs. E. S. AGHEDO	**Date**
(Project Supervisor)	
Prof. A. B. O. OGEDEGBE	**Date**
(Project Coordinator)	
Associate Prof. E. O. OSHOMOH	**Date**
(Head of Department)	

EXTERNAL EXAMINER

DEDICATION

This project work is dedicated to God Almighty for his protection, love, guidance and provision.

ACKNOWLEDGEMENT

I am grateful to God almighty for His infinite love and grace towards me, for enabling me to accomplish and finish this project work successfully.

I solely give my heartfelt appreciation to my distinguish supervisor Mrs. E. S. Aghedo whose patience and careful supervision and enlightenment made this project work a great success. I also want to appreciate the H.O.D of Science Laboratory Technology, Dr. E. O. Oshomoh, and to other lecturers and staff of the department for their love and guidance.

I wish to thank my beloved parents, Mr & Mrs Ehiosun for their love and support spiritually, financially and morally throughout my study. I want to also appreciate my most beloved siblings for their love, encouragement and support both financially and morally throughout my study. I want to also appreciate my dear friends Manifest, Benedicta, Precious, Elvis, Russell, Thelma, Rhoda and others who I may have failed to mention, for their love and encouragement during the

course of my study. I want to say a big thank you to my entire class, I Love you all and God bless you.

TABLE OF CONTENTS

LIST OF FIGURE

ABSTRACT

Milk and dairy products are an essential food for human beings and it also acts as a good medium for microorganism's growth. Milk and its products with high biological potential, enriched nutritional values and without health risks and hazards are generally demanded for nutritional purposes. A total of 8 dairy product samples were processed for microbial and biochemical analysis, collected from different locations in Benin City. Microbial count were performed for the detection of the number of bacteria and fungi present in the various samples. The identification of fungi was carried out by the use of mycological Atlas. Analysis was done

on media, while for confirmation of bacteria, various biochemical tests were performed. Both nonpathogenic and pathogenic bacteria and fungi were identified. The highest percentage prevalence of fungi contaminant was seen in CM with a percentage of 83.3% with the least bacteria prevalence of 16.67%. The highest prevalence contaminant of bacterial was observed in PY with 60% prevalence. Milk and its product should not be exposed to atmosphere as it can also get contaminated by photogenic microorganisms. More standard research should be made on pure fermentation of milk and dairy products without any pathogenic contaminant.

INTRODUCTION

1.0 BACKGROUND STUDY

Since ancient times, milk and milk products form a major part of human food and play a prominent role in the diet (Pal, 2014). Milk and dairy products contain many nutrients, such as protein, vitamins, calcium, phosphorus, magnesium, zinc, etc. , which are necessary for healthful living of humans of all age groups and both sex. A product prepared from milk is called as 'dairy product', which preserves the nutritive values of milk, and makes it easily acceptable to consumers (Bernardeau*et al.,* 2006). Milk and milk products constitute a vital component of human diet in many regions of the world. A variety of dairy products are manufactured primarily from cow's milk. However, the milk from other animal species is also used to prepare dairy products in some regions of the world. Several dairy products such as butter, cheese, dried milk

powder, ice cream, and yoghurt are available worldwide (Berger *et al.,* 2007). Food-borne infections have been identified as an important public health and economic problem in developed as well as developing nations. Hence, microbial food safety has emerged as a significant global issue for the consumer, industry, researcher, and regulatory bodies. The microbial contamination is one of the leading causes of food spoilage worldwide (Pal, 2014). The contamination of food with microbes can occur at any stage of the food chain. Spoilage of food involves any change, which renders food unacceptable for human consumption Microbiological safety of food during storage is related to many factors. Ready-toeat food products are consumed without any treatment between final production step and consumption (Vrdoljak*et al*., 2016). The highly nutritious nature of dairy products makes them especially good media for the growth of microorganisms (Ledenbach and Marshall, 2009). Spoilage occurs when microorganisms degrade the carbohydrates, proteins, fats of milk, and produce deleterious end products (Das *et al*., 2015). Dairy products can harbor a variety of organisms, including many zoonotic bacteria such as Brucella abortus, B. melitensis, Campylobacter jejuni, Escherchia coli, Listeria monocytogense, Mycobacterium bovis, M .tuberculosis, Salmonella, Staphylococcus aureus, and Yersinia enterocolitica ,which can cause serious disease, especially in children, pregnant women, elderly, and compromised individuals (Pal, 2014). During the production of various dairy products, it is impossible to avoid contamination of milk with microbes; therefore, the microbial content of milk is a major feature in determining its quality from safety point of view (Singh *et al*., 2011). It is important to mention that pasteurized milk can also harbor the pathogenic bacteria such as *E. coli*, *L. monocytogenes*, *Salmonella spp*., *S. aureus*, and *Yersinia enterocolitica* (Pal, 2014). Hence, it is suggested that microbiological, immunological, and molecular techniques should be employed to detect the pathogens in milk and dairy products in order to maintain the safety of the products for human consumption. A major cause of failure of

processing and packaging systems is the development of biofilms on equipment surfaces. These communities of microorganisms develop when nutrients and water remain on surfaces between times of cleaning and reuse. Bacteria in biofilms are more resistant to chemical sanitizers than are the same bacteria in suspension (Ledenbach and Marshall, 2009). The Gram-positive organisms can be present in raw milk, but they also may enter milk products at various points during production and processing (Singh *et al*., 2011). Reviewed literature indicated poor microbiological quality of raw milk due to bacterial contamination, inadequate packaging system, and improper temperature control, which favour microbial growth and metabolism and brings in undesirable changes (Sarkar, 2015). The sanitation is an essential prerequisite for the successful implementation of a hazard analysis and critical control point (HACCP) system in the food industry. A good sanitation programme has been found to minimize many of the potential hazards in a food operation. The comprehensive information on the importance of sanitation can be gathered from the book on "Sanitation in Food Establishments" authored by Pal and Mahendra in 2015. The present communication is attempted to delineate an overview on the contamination of various dairy products due to bacteria.

Yogurt is most common among the dairy products consumed around the world, and its sensory attributes, have a large effect on consumer acceptability (Al-Hamidi, 2004). Yogurt is perhaps the oldest fermented milk product known and consumed by large segments of our population either as a part of diet or as a refreshing beverage (Bohm and Kruis, 2006). It is nutritiously balanced food containing almost all the nutrients present in milk but in a more assailable form (Chamber and Irlinger, 2009). It is believed that yogurt has valuable therapeutic properties and helps curing gastrointestinal disorders (Chamber and Irlinger, 2009). Whole or skim milk is used for making Yoghurt or dahi which is very popular and nutritious dish in Bangladesh (Chamber

and Irlinger, 2009).Yogurt, is produced when milk or milk products coagulates, causing the lactic acid contained in it to coagulate, via the action of bacterial enzymes lactase provided by the bacteria Streptococcus thermophilus, Lactobacillus bulgaricus breaks down the sugar compound glucose and galactose that the lactose is composed of, under anaerobic conditions (Farnworth et al., 2007). But at the same time yoghurt is highly vulnerable to bacterial contamination and hence it is easily perishable (Gatti*et al.,* 2004). Therefore, to create public health concern, microbiological assessments are necessary for yoghurt. Microbiological methods can reduce economic losses by the early detection of inadequate processing, packaging or refrigeration (Gatti*et al.,* 2004). This can achieve by monitoring the microbiological quality of raw milk supplies, bulk milk and finish milk products immediately after production and during storage. Microbiological parameters are generally used to verify these conditions, especially by yeasts & mold, coliforms and total viable bacteria enumeration. Coliforms are responsible for the development of objectionable taints in milk and milk products rendering them of inferior quality or even unmarketable (Gatti*et al.,* 2004). Yeasts & molds are a major cause of spoilage of yoghurts in which the low pH provides a selective environment for their growth (Fleet and Mian, 1987). Research in the field of quality evaluation of yogurt is the basic need to create awareness among common people the existing situation and protect the consumer's health and rights. Therefore, the objective of this study was aimed to evaluate the microbiological quality of yoghurts from different selected areas of Bangladesh, considering the levels of contamination by coliforms, yeast, molds and total viable bacterial counts.

1.1 STATEMENT OF PROBLEMS

Diary product or milk products is the lacteal secretion obtained by the complete milking of mammalian animals and the products obtained from it via processing. Due to their high

nutritional value for human beings, there are significant foods of nutrition of immense population on earth. When temperature is suitable for growth of microorganisms, the milk appears as an excellent medium for their growth. The milk is contaminated very easily if it is handled carelessly and produced un-hygienically results in its early spoilage (Hayes *et al.,* 2002). Milk serves for the growth of bacterial population. Mostly food-borne diseases are among main public health disquiet throughout the world. Raw and pasteurized milk are daily consumed by millions of people. As a result, infected milk either during milk processing or from infected cow results in different zoonotic diseases to many of them. These diseases include brucellosis, typhoid fever and salmonella food poisoning, tuberculosis, gastroenteritis, Q-fever, dysentery, diphtheria and staphylococcal intoxications (Hayes *et al.,* 2002).

1.2 RESEARCH QUESTIONS

- Is there a high beneficiary microorganisms present in dairy products?
- Which diary products have the highest population of beneficial microbes?
- Which dairy products have the highest pathogenic microbes?

1.3 JUSTIFICATION OF STUDY

Food borne infections have been identified as an important public health and economic problem in developed as well as developing nations. Hence, microbial food safety has emerged as a significant global issue for the consumer, industry, researcher, and regulatory bodies. The microbial contamination is one of the leading causes of food spoilage worldwide (Pal, 2014). The contamination of food with microbes can occur at any stage of the food chain. Spoilage of food involves any change, which renders food unacceptable for human consumption

Microbiological safety of food during storage is related to many factors. Ready-toeat food products are consumed without any treatment between final production step and consumption (Vrdoljak et al., 2016). The highly nutritious nature of dairy products makes them especially good media for the growth of microorganisms (Ledenbach and Marshall, 2009). Spoilage occurs when microorganisms degrade the carbohydrates, proteins, fats of milk, and produce deleterious end products (Hayes *et al.,* 2002). Dairy products can harbor a variety of organisms, including many zoonotic bacteria such as Brucella abortus, B. melitensis, Campylobacter jejuni, Escherchia coli, Listeria monocytogense, Mycobacterium bovis, M .tuberculosis, Salmonella, Staphylococcus aureus, and Yersinia enterocolitica ,which can cause serious disease, especially in children, pregnant women, elderly, and compromised individuals (Pal, 2014). During the production of various dairy products, it is impossible to avoid contamination of milk with microbes; therefore, the microbial content of milk is a major feature in determining its quality from safety point of view (Singh *et al*., 2011). Hence the need for this study to evaluate the microbial population present in some diary product.

1.4 **Aim and Objective of study**

This study aims at analyzing the microbia population in some diary products

Specific objectives

To determine the benefitial microbes present in diaries product

To determine the pathogenic microbes persend in diaries product

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DAIRY PRODUCT

Dairy product, milk and any of the foods made from milk, including butter, cheese, ice cream, yogurt, and condensed and dried milk.

Milk has been used by humans since the beginning of recorded time to provide both fresh and storable nutritious foods. In some countries almost half the milk produced is consumed as fresh pasteurized whole, low-fat, or skim milk. However, most milk is manufactured into more stable dairy products of worldwide commerce, such as butter, cheese, dried milks, ice cream, and condensed milk. Cow's milk (bovine species) is by far the principal type used throughout the

world. Other animals utilized for their milk production include buffalo (in India, China, Egypt, and the Philippines), goats (in the Mediterranean countries), reindeer (in northern Europe), and sheep (in southern Europe). This section focuses on the processing of cow's milk and milk products unless otherwise noted. In general, the processing technology described for cow's milk can be successfully applied to milk obtained from other species (Pal, 2014).

In the early 1800s the average dairy cow produced less than 1,500 litres of milk annually. With advances in animal nutrition and selective breeding, one cow now produces an average of 6,500 litres of milk a year, with some cows producing up to 10,000 litres. The Holstein-Friesian cow produces the greatest volume, but other breeds such as Ayrshire, Brown Swiss, Guernsey, and Jersey, while producing less milk, are known for supplying milk that contains higher fat, protein, and total solids.

2.2 PROPERTIES OF MILK

2.2.1 Nutrient composition

Although milk is a liquid and most often considered a drink, it contains between 12 and 13 percent total solids and perhaps should be regarded as a food. In contrast, many "solid" foods, such as tomatoes, carrots, and lettuce, contain as little as 6 percent solids. Many factors influence the composition of milk, including breed, genetic constitution of the individual cow, age of the cow, stage of lactation, interval between milkings, and certain disease conditions. Since the last milk drawn at each milking is richest in fat, the completeness of milking also influences a sample. In general, the type of feed only slightly affects the composition of milk, but feed of poor quality or insufficient quantity causes both a low yield and a low percentage of total solids. Current

feeding programs utilize computer technology to achieve the greatest efficiency from each animal (Pal, 2014).

The composition of milk varies among mammals, primarily to meet growth rates of the individual species. The proteins contained within the mother's milk are the major components contributing to the growth rate of the young animals. Human milk is relatively low in both proteins and minerals compared with that of cows and goats. Goat milk has about the same nutrient composition as cow's milk, but it differs in several characteristics. Goat milk is completely white in colour because all the beta-carotene (ingested from feed) is converted to vitamin A. The fat globules are smaller and therefore remain suspended, so the cream does not rise and mechanical homogenization is unnecessary. Goat milk curd forms into small, light flakes and is more easily digested, much like the curd formed from human milk. It is often prescribed for persons who are allergic to the proteins in cow's milk and for some patients afflicted with stomach ulcers (Pal, 2014).

Sheep milk is rich in nutrients, having 18 percent total solids (5.8 percent protein and 6.5 percent fat). Reindeer milk has the highest level of nutrients, with 36.7 percent total solids (10.3 percent protein and 22 percent fat). These high-fat, high-protein milks are excellent ingredients for cheese and other manufactured dairy products.

The major components of milk are water, fat, protein, carbohydrate (lactose), and minerals (ash). However, there are numerous other highly important micronutrients such as vitamins, essential amino acids, and trace minerals. Indeed, more than 250 chemical compounds have been identified in milk. The table shows the composition of fresh fluid milk and other dairy products (Singh *et al*., 2011).

2.2.2 Fat

The fat in milk is secreted by specialized cells in the mammary glands of mammals. It is released as tiny fat globules or droplets, which are stabilized by a phospholipid and protein coat derived from the plasma membrane of the secreting cell. Milk fat is composed mainly of triglycerides—three fatty acid chains attached to a single molecule of glycerol. It contains 65 percent saturated, 32 percent monounsaturated, and 3 percent polyunsaturated fatty acids. The fat droplets carry most of the cholesterol and vitamin A. Therefore, skim milk, which has more than 99.5 percent of the milk fat removed, is significantly lower in cholesterol than whole milk (2 milligrams per 100 grams of milk, compared with 14 milligrams for whole milk) and must be fortified with vitamin A (Hayes *et al.,* 2002).

2.2.3 Protein

Milk contains a number of different types of proteins, depending on what is required for sustaining the young of the particular species. These proteins increase the nutritional value of milk and other dairy products and provide certain characteristics utilized for many of the processing methods. A major milk protein is casein, which actually exists as a multisubunit protein complex dispersed throughout the fluid phase of milk. Under certain conditions the casein complexes are disrupted, causing curdling of the milk. Curdling results in the separation of milk proteins into two distinct phases, a solid phase (the curds) and a liquid phase (the whey) (Kagkli*et al.,* 2007).

2.2.4 Lactose

Lactose is the principal carbohydrate found in milk. It is a disaccharide composed of one molecule each of the monosaccharides (simple sugars) glucose and galactose. Lactose is an important food source for several types of fermenting bacteria. The bacteria convert the lactose into lactic acid, and this process is the basis for several types of dairy products (Pal, 2014).

In the diet lactose is broken down into its component glucose and galactose subunits by the enzyme lactase. The glucose and galactose can then be absorbed from the digestive tract for use by the body. Individuals deficient in lactase cannot metabolize lactose, a condition called lactose intolerance. The unmetabolized lactose cannot be absorbed from the digestive tract and therefore builds up, leading to intestinal distress(Ledenbach and Marshall, 2009).

2.2.5 Vitamins and minerals

Milk is a good source of many vitamins. However, its vitamin C (ascorbic acid) content is easily destroyed by heating during pasteurization. Vitamin D is formed naturally in milk fat by ultraviolet irradiation but not in sufficient quantities to meet human nutritional needs. Beverage milk is commonly fortified with the fat-soluble vitamins A and D. In the United States the fortification of skim milk and low-fat milk with vitamin A (in water-soluble emulsified preparations) is required by law (Larvol*et al.,* 2010).

Milk also provides many of the B vitamins. It is an excellent source of riboflavin (B_2) and provides lesser amounts of thiamine (B_1) and niacin. Other B vitamins found in trace amounts are pantothenic acid, folic acid, biotin, pyridoxine (B_6), and vitamin B_{12}. (Larvol*et al.,* 2010)

Milk is also rich in minerals and is an excellent source of calcium and phosphorus. It also contains trace amounts of potassium, chloride, sodium, magnesium, sulfur, copper, iodine, and iron. A lack of adequate iron is said to keep milk from being a complete food.

2.2.6 Physical and biochemical properties

Milk contains many natural enzymes, and other enzymes are produced in milk as a result of bacterial growth. Enzymes are biological catalysts capable of producing chemical changes in organic substances. Enzyme action in milk systems is extremely important for its effect on the flavour and body of different milk products. Lipases (fat-splitting enzymes), oxidases, proteases (protein-splitting enzymes), and amylases (starch-splitting enzymes) are among the more important enzymes that occur naturally in milk. These classes of enzymes are also produced in milk by microbiological action. In addition, the proteolytic enzyme (i.e., protease) rennin, produced in calves' stomachs to coagulate milk protein and aid in nutrient absorption, is used to coagulate milk for manufacturing cheese(Singh *et al*., 2011).

The coagulation of milk is an irreversible change of its protein from a soluble or dispersed state to an agglomerated or precipitated condition. Its appearance may be associated with spoilage, but coagulation is a necessary step in many processing procedures. Milk may be coagulated by rennin or other enzymes, usually in conjunction with heat. Left unrefrigerated, milk may naturally sour or coagulate by the action of lactic acid, which is produced by lactose-fermenting bacteria. This principle is utilized in the manufacture of cottage cheese. When milk is pasteurized and continuously refrigerated for two or three weeks, it may eventually coagulate or spoil owing to the action of psychrophilic or proteolytic organisms that are normally present or result from postpasteurization contamination (Larvol*et al.,* 2010).

Milk fat is present in milk as an emulsion in a water phase. Finely dispersed fat globules in this emulsion are stabilized by a milk protein membrane, which permits the fat to clump and rise. The rising action is called creaming and is expected in all unhomogenized milk. In the United States, when paper cartons supplanted glass bottles, consumers stopped the practice of skimming cream from the top. Processors then introduced homogenization, a method of preventing gravity separation by forcing milk through very small openings under pressure, thus reducing fat globules to one-tenth their original size. Homogenization is practiced in many dairy processes in order to improve the physical properties of products (*see below* Processing) (Szajewska*et al.,* 2006).

Milk and other dairy products are very susceptible to developing off-flavours. Some flavours, given such names as "feed," "barny," or "unclean," are absorbed from the food ingested by the cow and from the odours in its surroundings. Others develop through microbial action due to growth of bacteria in large numbers. (Szajewska*et al.,* 2006). Chemical changes can also take place through enzyme action, contact with metals (such as copper), or exposure to sunlight or strong fluorescent light. Quality-control directors are constantly striving to avoid off-flavours in milk and other dairy foods. (Nakano, 2010).

2.3 FRESH FLUID MILK

Fresh fluid milk requires the highest-quality raw milk and is generally designated as Grade "A." This grade requires a higher level of sanitation and inspection on the farm than is necessary for "manufacturing grade" milk. (Nakano, 2010).

2.3.1 Quality concerns

Raw milk is a potentially dangerous food that must be processed and protected to assure its safety for humans. While most bovine diseases, such as brucellosis and tuberculosis, have been eliminated, many potential human pathogens inhabit the dairy farm environment. Therefore, it is essential that all milk be either pasteurized or (in the case of cheese) held for at least 60 days if made from raw milk. While milk from healthy cows is often totally bacteria-free, that condition quickly changes when milk is exposed to the farm environment (Singh *et al*., 2011).

Milk received at the processing plant is tested before being unloaded from either farm-based tank trucks or over-the-road tankers. The milk is checked for odour, appearance, proper temperature, acidity, bacteria, and the presence of drug residues. These tests take no longer than 10 to 15 minutes. If the tank load passes these tests, the milk is pumped into the plant's refrigerated storage tanks. The milk is then stored for the shortest possible time (Ledenbach and Marshall, 2009).

2.3.2 Processing

Essential steps in the processing of fluid milk into various dairy products are shown in the figure.

2.3.3 Pasteurization

Pasteurization is most important in all dairy processing. It is the biological safeguard which ensures that all potential pathogens are destroyed. Extensive studies have determined that heating milk to 63 °C (145 °F) for 30 minutes or 72 °C (161 °F) for 15 seconds kills the most resistant harmful bacteria. In actual practice these temperatures and times are exceeded, thereby not only ensuring safety but also extending shelf life (Vancannert*et al.,* 2006).

Most milk today is pasteurized by the continuous high-temperature short-time (HTST) method (72 °C or 161 °F for 15 seconds or above). The HTST method is conducted in a series of stainless steel plates and tubes, with the hot pasteurized milk on one side of the plate being cooled by the incoming raw milk on the other side. (Vancannert*et al.,* 2006). This "regeneration" can be more than 90 percent efficient and greatly reduces the cost of heating and cooling. There are many fail-safe controls on an approved pasteurizer system to ensure that all milk is completely heated for the full time and temperature requirement. If the monitoring instruments detect that something is wrong, an automatic flow diversion valve will prevent the milk from moving on to the next processing stage. Higher temperatures and sometimes longer holding times are required for the pasteurization of milk or cream with a high fat or sugar content. Pasteurized milk is not sterile and is expected to contain small numbers of harmless bacteria. (Berger *et al.,* 2007). Therefore, the milk must be immediately cooled to below 4.4 °C (40 °F) and protected from any outside contamination. The shelf life for high-quality pasteurized milk is about 14 days when properly refrigerated (Vancannert*et al.,* 2006).

Extended shelf life can be achieved through ultra-pasteurization. In this case, milk is heated to 138 °C (280 °F) for two seconds and aseptically placed in sterile conventional milk containers. Ultrapasteurized milk and cream must be refrigerated and will last at least 45 days. This process does minimal damage to the flavour and extends the shelf life of slow-selling products such as cream, eggnog, and lactose-reduced milks. (Vancannert*et al.,* 2006)

Ultrahigh-temperature (UHT) pasteurization is the same heating process as ultrapasteurization (138 °C or 280 °F for two seconds), but the milk then goes into a more substantial container—either a sterile five-layer laminated "box" or a metal can. This milk can be stored without

refrigeration and has a shelf life of six months to a year. Products handled in this manner do not taste as fresh, but they are useful as an emergency supply or when refrigeration is not available.

2.3.4 Separation

Most modern plants use a separator to control the fat content of various products. A separator is a high-speed centrifuge that acts on the principle that cream or butterfat is lighter than other components in milk. (The specific gravity of skim milk is 1.0358, specific gravity of heavy cream 1.0083.) The heart of the separator is an airtight bowl with funnellike stainless steel disks. The bowl is spun at a high speed (about 6,000 revolutions per minute), producing centrifugal forces of 4,000 to 5,000 times the force of gravity. Centrifugation causes the skim, which is denser than cream, to collect at the outer wall of the bowl. The lighter part (cream) is forced to the centre and piped off for appropriate use (Kagkli*et al,* 2007).

An additional benefit of the separator is that it also acts as a clarifier. Particles even heavier than the skim, such as sediment, somatic cells, and some bacteria, are thrown to the outside and collected in pockets on the side of the separator. This material, known as “separator sludge,” is discharged periodically and sometimes automatically when buildup is sensed (Ledenbach and Marshall, 2009).

Most separators are controlled by computers and can produce milk of almost any fat content. Current standards generally set whole milk at 3.25 percent fat, low-fat at 1 or 2 percent, and skim at less than 0.5 percent. (Most skim milk is actually less than 0.01 percent fat.

2.3.5 Homogenization

Milk is homogenized to prevent fat globules from floating to the top and forming a cream layer or cream plug. Homogenizers are simply heavy-duty, high-pressure pumps equipped with a special valve at the discharge end. They are designed to break up fat globules from their normal size of up to 18 micrometres to less than 2 micrometres in diameter (a micrometre is one-millionth of a metre). Hot milk (with the fat in liquid state) is pumped through the valve under high pressure, resulting in a uniform and stable distribution of fat throughout the milk (Kagkli*et al,* 2007).

Two-stage homogenization is sometimes practiced, during which the milk is forced through a second homogenizer valve or a breaker ring. The purpose is to break up fat clusters or clumps and thus produce a more uniform product with a slightly reduced viscosity. (Gatti*et al.,* 2004).

Homogenization is considered successful when there is no visible separation of cream and the fat content in the top 100 millilitres of milk in a one-litre bottle does not differ by more than 10 percent from the bottom portion after standing 48 hours (Vrdoljak*et al*., 2016).

In addition to avoiding a cream layer, other benefits of homogenized milk include a whiter appearance, richer flavour, more uniform viscosity, better “whitening” in coffee, and softer curd tension (making the milk more digestible for humans). Homogenization is also essential for providing improved body and texture in ice cream, as well as numerous other products such as half-and-half, cream cheese, and evaporated milk. (Kagkli*et al,* 2007).

2.3.6 Packaging

Until the mid 1880s milk was dipped from large cans into the consumer's own containers. The glass milk bottle was invented in 1884 and became the main container of retail distribution until World War II, when wax-coated paper containers were introduced. Plastic-coated paper followed and became the predominate container. Today more than 75 percent of retail sales are in translucent plastic jugs. Glass bottles make up less than 0.5 percent of the business and are used mostly at dairy stores and for home delivery. (Hayes *et al.,*2002).

Modern packaging machines are self-cleaning and provide an aseptic environment for milk packaging. Their improved design has allowed milk to remain fresh for at least 14 days and has made it possible for use with ultrapasteurizing equipment for extended shelf-life applications. (Kagkli*et al,* 2007).

2.3.7 Specialty milks

Many specialty milks are now available (even in remote areas) as a result of the 45-day refrigerated shelf life of ultrapasteurized milk. One of the most useful products, lactose-reduced milk, is available in both nonfat and low-fat composition as well as in many flavoured versions. The lactose (milk sugar) is reduced by 70 to 100 percent, making it possible for lactose-intolerant individuals to enjoy the benefits of milk in their diets. Lactose reduction is accomplished by subjecting the appropriate milk to the action of the enzyme lactase in a refrigerated tank for approximately 24 hours. (Kagkli*et al,* 2007). The enzyme breaks down the lactose to more readily digestible glucose and galactose. The reaction is halted when the lactose is consumed or when the milk is heat-treated. The resulting beverage is sweeter than regular milk but acceptable for most uses (Pal, 2007).

Other specialty milks include calcium-fortified, special and seasonal flavours (e.g., eggnog), and high-volume flavoured milk shakes (frequently served in schools).

2.4 CONDENSED AND DRIED MILK

2.4.1 Condensed and evaporated milk

Whole, low-fat, and skim milks, as well as whey and other dairy liquids, can be efficiently concentrated by the removal of water, using heat under vacuum. Since reducing atmospheric pressure lowers the temperature at which liquids boil, the water in milk is evaporated without imparting a cooked flavour. Water can also be removed by ultrafiltration and reverse osmosis, but this membrane technology is more expensive. (Kagkli*et al,* 2007). Usually about 60 percent of the water is removed, which reduces storage space and shipping costs. Whole milk, when concentrated, usually contains 7.5 percent milk fat and 25.5 percent total milk solids. Skim milk can be condensed to approximately 20 to 40 percent solids, depending on the buyer's needs (Pal, 2014).

Condensed milk is often sold in refrigerated tank-truck loads to manufacturers of candy, bakery goods, ice cream, cheese, and other foods. When preserved by heat in individual cans, it is usually called "evaporated milk." In this process the concentrated milk is homogenized, fortified with vitamin D (A and D in evaporated skim milk), and sealed in a can sized for the consumer. A stabilizer, such as disodium phosphate or carrageenan, is also added to keep the product from separating during processing and storage. The sealed can is then sterilized at 118 °C (244 °F) for 15 minutes, cooled, and labeled. Evaporated milk keeps indefinitely, although staling and browning may occur after a year. (Nornberg*et al.,* 2010).

New ultrahigh-temperature (UHT) processing and aseptic filling of foil-lined cardboard or metal cans is also practiced. Although this process is more costly, the scorched flavour is not as pronounced as with conventionally processed evaporated milk. (Ong *et al.,* 2006).

Sweetened condensed milk is also made by partially removing the water (as in evaporated milk) and adding sugar. The final product contains about 8.5 percent milk fat and at least 28 percent total milk solids. Sugar is added in sufficient amount to prevent bacterial action and subsequent spoilage. Usually, at least 60 percent sugar in the water phase is required to provide sufficient osmotic pressure for prevention of bacterial growth. Because sweetened condensed milk (or skim milk) is preserved by sugar, the milk merely needs to be pasteurized before being placed in a sanitary container (usually a metal can) (Ledenbach and Marshall, 2009). Dry milk products

Milk and by-products of milk production are often dried to reduce weight, to aid in shipping, to extend shelf life, and to provide a more useful form as an ingredient for other foods. In addition to skim and whole milk, a variety of useful dairy products are dried, including buttermilk, malted milk, instant breakfast, sweet cream, sour cream, butter powder, ice cream mix, cheese whey, coffee creamer, dehydrated cheese products, lactose, and caseinates. Many drying plants are built in conjunction with a butter-churning plant. These plants utilize the skim milk generated from the separated cream and the buttermilk produced from churning the butter. Most products are dried to less than 4 percent moisture to prevent bacterial growth and spoilage. However, products containing fat lose their freshness rather quickly owing to the oxidation of fatty acids, leading to rancidity (Ong *et al.,* 2006).).

Two types of dryers are used in the production of dried milk products—drum dryers and spray dryers. Each dryer has certain advantages.

2.4.2 Drum dryers

The simplest and least expensive is the drum, or roller, dryer. It consists of two large steel cylinders that turn toward each other and are heated from the inside by steam. The concentrated product is applied to the hot drum in a thin sheet that dries during less than one revolution and is scraped from the drum by a steel blade. The flakelike powder dissolves poorly in water but is often preferred in certain bakery products. Drum dryers are also used to manufacture animal feed where texture, flavour, and solubility are not a major consideration. (Ong *et al.,* 2006).

2.4.3 Spray dryers

Spray dryers are more commonly used since they do less heat damage and produce more soluble products. Concentrated liquid dairy product is sprayed in a finely atomized form into a stream of hot air. The air may be heated by steam-heated "radiators" or directly by sulfur-free natural gas. (Ong *et al.,* 2006). The drying chamber may be rectangular (the size of a living room), conical, or silo-shaped (up to five stories high). The powder passes from the drying chamber through a series of cyclone collectors and is usually placed in plastic-lined, heavy-duty paper bags. (Otigos*et al.,* 2005). Spray-dried milk is also difficult to reconstitute or mix with water. Therefore, a process called agglomeration was developed to "instantize" the powder, or make it more soluble. This process involves rewetting the fine, spray-dried powder with water to approximately 8 to 15 percent moisture and following up with a second drying cycle. The powder is now granular and dissolves very well in water. Virtually all retail packages of nonfat dry milk powder are instantized in this manner. (Mohammed and Abdullahi, 2015).

2.5 BUTTER

2.5.1 Composition

Butter is one of the most highly concentrated forms of fluid milk. Twenty litres of whole milk are needed to produce one kilogram of butter. This process leaves approximately 18 litres of skim milk and buttermilk, which at one time were disposed of as animal feed or waste. Today the skim portion has greatly increased in value and is fully utilized in other products. (Moysiadi, 2004).

Commercial butter is 80–82 percent milk fat, 16–17 percent water, and 1–2 percent milk solids other than fat (sometimes referred to as curd). It may contain salt, added directly to the butter in concentrations of 1 to 2 percent. Unsalted butter is often referred to as "sweet" butter. This should not be confused with "sweet cream" butter, which may or may not be salted. Reduced-fat, or "light," butter usually contains about 40 percent milk fat(Ledenbach and Marshall, 2009).

Before World War II much of the butter produced in the United States was made from gathered cream. Farmers separated milk on the farm and shipped cans of cream to a butter factory, sometimes once or twice a week. The cream was often sour and needed to be neutralized (with sodium hydroxide) before churning. When transportation and the value of the skim portion improved, whole milk was shipped to the creamery, providing a supply of "sweet cream" (i.e., cream that had not soured) for butter making. With these improvements came the advent of higher-quality butter and the demise of naturally soured buttermilk. Virtually all butter in the United States today is sweet cream butter. A notable exception is butter made from whey cream salvaged in the cheese-making process. The quality of fresh whey cream butter is indistinguishable from sweet cream butter.

2.5.2 Production

Butter is produced when the cream emulsion in unhomogenized milk is destabilized by agitation, or churning. Breaking the emulsion produces butterfat granules the size of rice grains. The granules mat together and separate from the water phase or serum, which is known as buttermilk. (This milky liquid is drained away and is either concentrated or dried, later to become an ingredient in ice cream, candy, or other foods.) The butterfat is then washed with clean water and "worked" (kneaded) until more buttermilk separates and is removed. Ultimately, only about 16 percent of the water and milk solids present in the original milk remains trapped in the butter. (Ong *et al.,* 2006).

The churning process can take 40 to 60 minutes to complete in a traditional churn, but butter is more commonly made by high-speed continuous "churns" in factories. Although the basic principle is the same, in the continuous churn cream is pumped into a cylinder and mixed by high-speed blades, forming butter granules in seconds. The butter granules are forced through perforated plates while the buttermilk is drained from the system. A salt solution may be added if salted butter is desired. The butter is then worked in a twin screw extruder and emerges ready to be packaged. (Ong *et al.,* 2006).

2.5.3 Quality concerns

The quality of butter is based on its body, texture, flavour, and appearance. In the United States the Department of Agriculture (USDA) assigns quality grades to butter based on its score on a standard quality point scale. Grade AA is the highest possible grade; Grade AA butter must achieve a numerical score of 93 out of 100 points based on its aroma, flavour, and texture. Salt

(if present) must be completely dissolved and thoroughly distributed. Grade A butter is almost as good, with a score of 92 out of 100 points. Grade B butter is based on a score of 90 points, and it usually is used only for cooking or manufacturing. The flavour of Grade B is not as fresh and sweet, and its body may be crumbly, watery, or sticky. (Ong *et al.,* 2006).

2.5.4 Additions and treatment

The addition of salt to butter contributes to its flavour and also acts as a preservative. Added in concentrations of approximately 2 percent, all the salt goes into solution in the water phase. Since the water content of butter is less than 16 percent of the total volume, each water droplet can contain more than 10 percent salt. Such a concentration in the water phase limits bacterial growth overall, since the fat portion of butter is generally safe from microbial degradation.

Butter may contain added colouring. Butter from cows that are eating dry, stored feed during the winter may not contain enough beta-carotene for proper colouring, as it does when cows are pasture-fed. In such cases small amounts of a yellow vegetable colouring from the seed of the annatto tree may be added to enhance the colour. (Moysiadi, 2004).

Because butter is so firm when first removed from the refrigerator, it is sometimes whipped to improve spreadability. Generally, volume is increased by 50 percent by whipping in air—or, better still, nitrogen or an inert gas in order to prevent oxidation of the fat. Whipped butter, both salted and sweet, is sold in small plastic-coated tubs. (Moysiadi, 2004).

Ice cream evolved from flavoured ices that were popular with the Roman nobility in the 4th century BCE. The emperor Nero is known to have imported snow from the mountains and

topped it with fruit juices and honey. In the 13th century Marco Polo was reported to have returned from China with recipes for making water and milk ices (Ledenbach and Marshall, 2009).

The discovery that salt would lower the freezing point of cracked ice led to the first practical method of making ice cream. Making ice cream in the home was greatly simplified by the invention of the wooden bucket freezer with rotary paddles. In 1851 the first wholesale ice cream was manufactured in Baltimore. (Moysiadi, 2004). With the development of mechanical refrigeration, widespread distribution of ice cream became possible. Ice cream parlours and drugstore soda counters flourished. With refrigerator-freezers now a standard domestic appliance, more than half of all frozen desserts are consumed at home. (Pal *et al.,* 2012).

2.6 ICE CREAM AND OTHER FROZEN DESSERTS

Ice cream evolved from flavoured ices that were popular with the Roman nobility in the 4th century BCE. The emperor Nero is known to have imported snow from the mountains and topped it with fruit juices and honey. In the 13th century Marco Polo was reported to have returned from China with recipes for making water and milk ices (Moysiadi, 2004).

The discovery that salt would lower the freezing point of cracked ice led to the first practical method of making ice cream. Making ice cream in the home was greatly simplified by the invention of the wooden bucket freezer with rotary paddles. In 1851 the first wholesale ice cream was manufactured in Baltimore. With the development of mechanical refrigeration, widespread distribution of ice cream became possible. Ice cream parlours and drugstore soda counters

flourished. With refrigerator-freezers now a standard domestic appliance, more than half of all frozen desserts are consumed at home. (Pal, 2007).

2.6.1 Composition of frozen desserts

Standards for ice cream and most frozen desserts are closely regulated. In the United States, for example, ice cream must contain at least 10 percent fat and 20 percent total milk solids. In freezing, the volume may be doubled by the inclusion of air (known as overrun), but the increase in volume is limited to 100 percent by the requirement that the finished product weigh at least 4.5 pounds per gallon. Total food solids must weigh 1.6 pounds per gallon, thus limiting the water content. Regulations also require all ingredients to be listed, with some additives (such as stabilizers) limited to very small amounts. (Moysiadi, 2004).

The principal frozen desserts are ice cream, frozen custard, ice milk, frozen yogurt, sherbet, and water ices. Ice cream has the highest fat content, ranging from 10 to 20 percent. Frozen custard, or French ice cream, is basically the same formula as ice cream but contains added eggs or egg solids (usually 1.4 percent by weight) (Ledenbach and Marshall, 2009). Ice milk may be more commonly called “light” or “reduced-fat” ice cream. It contains between 2 and 7 percent fat and at least 11 percent total milk solids. Frozen yogurt is a cultured frozen product containing the same ingredients as ice cream. It must contain at least 3.25 percent milk fat and 8.25 percent milk solids other than fat and must weigh at least five pounds per gallon. Low-fat frozen yogurt

contains between 0.5 and 2 percent milk fat. Nonfat frozen yogurt is limited to less than 0.5 percent milk fat. Frozen yogurts should always contain live cultures of bacteria (*see under* Yogurt). The target overrun for ice cream, ice milk, and frozen yogurt is 65 to 100 percent. Premium ice creams may be as low as 20 percent overrun, while soft ice creams are in the 30 to 50 percent range (Pal, 2007).

Sherbets contain relatively small quantities of milk products. Most standards require between 1 and 2 percent milk fat and between 2 and 5 percent total milk solids. Sherbet contains considerably more sugar and less air than ice cream (the target overrun is 30 to 40 percent), and therefore it is heavier and often contains more calories per serving. Water ices are similar to sherbet, but they contain no milk solids and have a target overrun of 20 to 30 percent. (Verres *et al.,* 2015).

Imitation ice cream, known as mellorine, is made in some parts of the United States and other countries. It is made with less expensive vegetable oils instead of butterfat but utilizes dairy ingredients for the milk protein part. Mellorines are intended to compete with ice cream in places where butterfat prices are high. (Moysiadi, 2004).

2.6.2 Ice cream manufacture

The essential ingredients in ice cream are milk, cream, sugar, flavouring, and stabilizer. Cheaper ingredients such as dry whey, corn syrup, and artificial flavourings may be substituted to create a lower-cost product. (Vrdoljak*et al.,* 2016).

The first step in ice cream making is formulating a suitable mix. The mix is composed of a combination of dairy ingredients, such as fresh milk and cream, frozen cream, condensed or

dried skim, buttermilk, dairy whey, or whey protein concentrate. Sugars may include sucrose, corn syrup, honey, and other syrups. Stabilizers and emulsifiers are added in small amounts to help prevent formation of ice crystals, particularly during temperature fluctuations in storage (Ledenbach and Marshall, 2009).

The ice cream mix is pasteurized at no less than 79 °C (175 °F) for 25 seconds. The heated mix is typically homogenized in order to assure a smoother body and texture. Homogenizing also prevents churning (i.e., separating out of fat granules) of the mix in the freezer and increases the viscosity. (Since smaller fat globules have more surface area, the associated milk protein can hydrate more water and produce a more viscous fluid.) (Verraes*et al.,* 2015).

After homogenization, the hot mix is quickly cooled to 4.4 °C (40 °F). The mix must age at this temperature for at least four hours to allow the fat to solidify and fat globules to clump. This aging process results in quicker freezing and a smoother product. (Verraes*et al.,* 2015).

The next step, freezing the mix, is accomplished by one of two methods: continuous freezing, which uses a steady flow of mix, or batch freezing, which makes a single quantity at a time. For both methods, the objective is to freeze the product partially and, at the same time, incorporate air. The freezing process is carried out in a cylindrical barrel that is cooled by a refrigerant, either ammonia or Freon (trademark) (Ledenbach and Marshall, 2009). The barrel is equipped with stainless steel blades, called dasher blades, which scrape the frozen mixture from the sides of the freezing cylinder and incorporate or whip air into the product. The amount of air incorporated during freezing is controlled by a pump or the dasher speed. Depending on individual conditions, freezing can be instantaneous in the continuous freezer or require approximately 10 minutes in the batch freezer (Verraes*et al.,* 2015).

Semifrozen ice cream leaves the freezer at a temperature between −9 and −5 °C (16 and 23 °F). It is placed in a suitable container and conveyed to a blast freezer, where temperatures are in the range of −29 to −34 °C (−20 to −30 °F). While in this room, the ice cream continues to freeze without agitation. Rapid freezing at this stage prevents the formation of large ice crystals and favours a smooth body and texture. The length of time in the hardening room depends on the size of the package, but usually 6 to 12 hours are required to bring the internal ice cream temperature to −18 °C (0 °F) or below. In larger manufacturing plants, final freezing takes place in a hardening tunnel, where packages are automatically conveyed on a continuous belt to the final storage area. (Verraes*et al.,* 2015).

Much of the appeal of ice cream comes from the variety of standard and holiday flavours available throughout the year. Most ice cream manufacturers make a standard mix consisting of milk, cream, sugars, and stabilizers, to which flavours may be added just prior to freezing. High-volume flavours such as vanilla, chocolate, and strawberry may be blended in their own large batch tanks. For flavours with large particles, such as fruit, nuts, cookies, or candy parts, a “feeder” on the outlet of the freezer is used to inject the material. Ingredients such as fruits and nuts are carefully selected and specially treated to avoid introducing unwanted bacteria into the already pasteurized mix (Ledenbach and Marshall*,* 2009).

Ice cream and other frozen desserts require no preservatives and have long shelf lives if they are kept below −23 °C (−10 °F) and are protected from temperature fluctuations. Airtight packaging materials have made it possible to consider frozen storage of six months or longer without loss of flavour or body and texture. When ice cream is finally dipped, composition and overrun will determine ideal scooping temperature. This can vary from −16 to −9 °C (3 to 15 °F), with lower temperatures resulting in less dipping loss but more effort on the part of the server.

Ice cream can also be freeze-dried by the removal of 99 percent of the water. Freeze-drying eliminates the need for refrigeration and provides a high-energy food for hikers and campers and a "filling" centre for candy and other confections.

2.6.3 Cultured dairy foods

With the development of microbiological and nutritional sciences in the late 19th century came the technology necessary to produce cultured dairy products on an industrial or commercial basis. Fermented milks had been made since early times, when warm raw milk from cows, sheep, goats, camels, or horses was naturally preserved by common strains of *Streptococcus* and *Lactobacillus* bacteria. (The "cultures" were obtained by including a small portion from the previous batch.) These harmless lactic acid producers were effective in suppressing spoilage and pathogenic organisms, making it possible to preserve fresh milk for several days or weeks without refrigeration. Cultured products eventually became ethnic favourites and were introduced around the world as people migrated (Ledenbach and Marshall, 2009).

Central to the production of cultured milk is the initial fermentation process, which involves the partial conversion of lactose (milk sugar) to lactic acid. Lactose conversion is accomplished by lactic-acid–producing *Streptococcus* and *Lactobacillus* bacteria. At temperatures of approximately 32 °C (90 °F), these bacteria reproduce very rapidly, perhaps doubling their population every 20 minutes. Many minute by-products that result from their metabolic processes assist in further ripening and flavouring of the cultured product. Subsequent or secondary fermentations can result in the production of other compounds, such as diacetyl (a

flavour compound found in buttermilk) and alcohol (from yeasts in kefir), as well as butyric acid (which causes bitter or rancid flavours).

Cultured buttermilk, sour cream, and yogurt are among the most common fermented dairy products in the Western world. Other, lesser-known products include kefir, koumiss, acidophilus milk, and new yogurts containing *Bifidobacteria*. Cultured dairy foods provide numerous potential health benefits to the human diet. These foods are excellent sources of calcium and protein. In addition, they may help to establish and maintain beneficial intestinal bacterial flora and reduce lactose intolerance. (Verraes*et al.,* 2015).

2.7 BUTTERMILK

Because of its name, most people assume buttermilk is high in fat. Actually, the name refers to the fact that buttermilk was once the watery end-product of butter making. Modern buttermilk is made from low-fat or skim milk and has less than 2 percent fat and sometimes none. Its correct name in many jurisdictions is “cultured low-fat milk” or “cultured nonfat milk.” (Verraes*et al.,* 2015).

The starting ingredient for buttermilk is skim or low-fat milk. The milk is pasteurized at 82 to 88 °C (180 to 190 °F) for 30 minutes, or at 90 °C (195 °F) for two to three minutes. This heating process is done to destroy all naturally occurring bacteria and to denature the protein in order to minimize wheying off (separation of liquid from solids). (Pal, 2014).

The milk is then cooled to 22 °C (72 °F), and starter cultures of desirable bacteria, such as *Streptococcus lactis, S. cremoris, Leuconostoc citrovorum*, and *L. dextranicum*, are added to

develop buttermilk's acidity and unique flavour. These organisms may be used singly or in combination to obtain the desired flavour (Gatti*et al.,* 2004).

The ripening process takes about 12 to 14 hours (overnight). At the correct stage of acid and flavour, the product is gently stirred to break the curd, and it is cooled to 7.2 °C (45 °F) in order to halt fermentation. It is then packaged and refrigerated. (Fernandes, 2008)

2.8 SOUR CREAM

Sour cream is made according to the same temperature and culture methods as used for buttermilk. The main difference is the starting material—sour cream starts with light 18 percent cream (Ledenbach and Marshall*,* 2009).

2.8.1 Yogurt

Yogurt is made in a similar fashion to buttermilk and sour cream, but it requires different bacteria and temperatures. Whole, low-fat, or skim milk is fortified with nonfat dry milk or fresh condensed skim milk, in order to raise the total solids to 14 to 16 percent. The mixture is heat-treated as for buttermilk and then cooled to 45.6 to 46.7 °C (114 to 116 °F). At this point a culture of equal parts *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is added to the warm milk, followed by one of two processing methods. For set, or sundae-style, yogurt (fruit on the bottom), the cultured mixture is poured into cups containing the fruit, held in a warm room until the milk coagulates (usually about four hours), and then moved to a refrigerated room. For blended (Swiss- or French-style) yogurt, the milk is allowed to incubate in large heated tanks.

After coagulation occurs, the mixture is cooled, fruit or other flavours are added, and the product is placed in containers and immediately made ready for sale. (Fernandes, 2008).

Many yogurt manufacturers have added *Lactobacillus acidophilus* to their bacterial cultures. *L. acidophilus* has possible health benefits in easing yeast infections and restoring normal bacterial balance to the intestinal tract of humans after antibiotic treatment. (Fernandes, 2008).

2.8.2 Cheese

Primitive forms of cheese have been made since humans started domesticating animals. No one knows exactly who made the first cheese, but, according to one ancient legend, it was made accidentally by an Arabian merchant crossing the desert. The merchant put his drinking milk in a bag made from a sheep's stomach. The natural rennin in the lining of the pouch, along with the heat from the sun, caused the milk to coagulate and then separate into curds and whey. At nightfall, the whey satisfied the man's thirst, and the curd (cheese) had a delightful flavour and satisfied his hunger. (Sarkar, 2015).

From its birthplace in the Middle East, cheese making spread as far as England with the expansion of the Roman Empire. During the Middle Ages, monks and merchants of Europe made cheese an established food of that area. In 1620, cheese and cows were part of the ship's stores carried to North America by the Pilgrims on the *Mayflower*. Until the middle of the 19th century, cheese was a local farm product. Few, if any, distinct varieties of cheese were developed deliberately. Rather, cheese makers in each locality made a cheese that, when ripened under

specific conditions of air temperature and humidity, mold, and milk source, acquired certain characteristics of its own. Different varieties appeared largely as a result of accidental changes or modifications in one or more steps of the cheese-making process. Because there was little understanding of the bacteriology and chemistry involved, these changes were little understood and difficult to duplicate. Cheese making was an art, and the process was a closely guarded secret that was passed down from one generation to the next (Fernandes, 2008).

With increasing scientific knowledge came a greater understanding of the bacteriological and chemical changes that are necessary to produce many types of cheese. Thus, it has become possible to control more precisely each step in the cheese-making process and to manufacture a more uniform product. Cheese making is now a science as well as an art. (Al-Hamidi, 2004)

2.9 SPOILAGE OF FLUID DAIRY PRODUCTS

The experience has shown that fluid dairy products get easily contaminated with microbes than the dried dry milk products. such as *Pseudomonas spp., Staphylococcus spp*., *Streptococcus spp., micrococci*, and lactic acid bacteria (LAB) are most frequently found in milk drawn aseptically from the udder of dairy cows (Fernandes, 2008). Raw milk held at refrigerator temperatures for several days invariably shows the presence of several bacteria of the genera such as Bacill*us, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Microbacterium, Micrococcus Propionibacterium, Proteus, Pseudomonas, Streptococcus, coliforms,* and others (Ledenbach and Marshall, 2009).

2.10 CONTAMINANTS IN VARIOUS DAIRY PRODUCTS

1. **Contaminants in Butter**

Butter is a dairy product prepared by churning fresh or fermented cream and milk and contains fat, protein, and water (Pal et al., 2014a). Modern hygienic manufacturing methods have minimized the bacterial spoilage of butter. However, defects caused by microbes do occasionally occur. Surface taints may develop as a result of growth of *Flavobacterium spp*., *Pseudomonas putrefaciens*, and *Shewanellaputrefaciens*. The fruity odors, rancidity, and proteolytic activity may be caused by the growth of *Pseudomonas fragi*and occasionally, Pseudomonas fluorescens. Black discoloration of butter is reported to be caused by Pseudomonas mephitica and *P. nigrificans* are responsible for a skunk-like odor and black discoloration, respectively (Fernandes, 2008). The main microbiological hazards of butter prepared from raw milk are *L. monocytogenes, verocytotoxin*-producing Escherichia coli (VTEC), and *S. aureus* because these pathogens have been detected in butter. All the three pathogens are known to cause food-borne illness in human beings (Pal, 2007). However, the risk of infection after consumption of raw milk butter is estimated to be relatively lower in comparison with other milk products (Verraes*et al*., 2015).

2. **Contaminants in cheese**

Cheese is a stabilized curd of milk solids produced by casein coagulation and entrapment of milk fat in the coagulum (Fernandes, 2008). It is a ready- to- eat dairy product, which is a rich source of protein, vitamins, calcium, and phosphorus. Microbial contamination of cheese can occur from various sources including handler, packaging material and environment (Pal et al., 2014b). Cheeses as ready-toeat food should be considered as a potential source of foodborne pathogens, primarily *L. monocytogenes*. It is the most important food-borne pathogen in cheese mainly in the postprocessing phase (Vrdoljak et al., 2016). Cracks in cheeses can occur when excess gas is produced by certain strains of *Lactobacillus helveticus*, and *Streptococcus*

thermophilus, and that form carbon dioxide and 4-aminobutyric acid by *decarboxylation* of glutamic acid. *Lactococcus lactis* subspecies lactis can produce small amounts of gas in cheeses. The washed curd types of cheeses are especially susceptible to growth of coliforms (Ledenbach and Marshall, 2009). During the production of hard cheeses, some bacteria such as L. *monocytogenes,* S. aureus, and Salmonella can survive without subsequent growth. The number of E. coli can increase during the cheese production, which is due to a combination of a concentration effect during the formation of the curd as well as due to real growth of the pathogen. In hard cheeses, several pathogens can be inactivated during storage. *Escherichia coli, L. monocytogenes*, and *Salmonella* may decrease in numbers, but can still be detected after long ripening periods. During the production of semi-hard cheeses, some bacteria such as *E. coli, Salmonella* and *S. aureus* can grow. During the ripening and storage of semi-hard cheeses, most bacteria will decrease in numbers, but *Salmonella, E. coli* and *S. aureus* can still be detected. *Brucella spp*., *E. coli*, and *L. monocytogenes* can survive the ripening and storage of cheese. During the production of soft cheeses, *Brucella spp*., E. *coli, and L. monocytogenes* can grow (Verraes et al., 2015). In this context, Pal and others (2014b) mentioned many bacteria of public health significance in the cheese. These organisms are *Campylobacter jejuni, E. coli, L. monocytogenes, Salmonella spp., S.aureus,* and*Y.enterocolitica*. Hence, the microbiological safety plays a very significant role in the quality of cheese and other dairy products.

3. **Contaminants in Cream**

The spoilage of cream is generally similar to that of liquid milk products. Aerobic spore-forming bacteria survive pasteurization, and psychrotrophic strains of Bacillus cereus may cause sweet curdling and bitty cream. Other more heatresistant species, such as *Bacillus coagulans, Bacillus licheniformis, andBacillus subtilis,* may survive sterilization and even ultra high temperature

processes, and may cause bitterness, and thinning in sterilized creams. *Bacillus pumilus* and B. *sporothermophilus*are now recognized as potential contaminants in cream primarily carried over from raw milk. Post-pasteurization contamination of cream could be a potentially serious problem. The post-process hygiene precautions should be applied for cream to check the bacterial contamination (Fernandes, 2008).

4. **Contaminants in Ice Cream**

Ice cream is a frozen dairy product with delicious taste, and has contains a variety of ingredients (Pal et al.,2012b).The main microbiological hazards observed in ice cream made from raw milk, are estimated to be *L. monocytogenes, S. aureus and VerotoxicE.coli*(Verraes et al., 2015). Salmonellae are not able to survive the typical minimum pasteurization processes. Therefore, their presence indicates that the process has not been carried out effectively or that post-process contamination has occurred. They are able to survive for very long periods in ice cream although, they will not survive adequate pasteurization, post process contamination or the use of raw eggs and failure to pasteurize the ice cream mix is a serious risk (Fernandes, 2008). Similarly, the presence of *coliforms* in ice cream is an indication of post-pasteurization contamination (Pal et al., 2012b). Therefore, sanitation is critical for ensuring that dairy products do not get re-contaminated (Pal et al., 2012b). 5. Contaminants in Yogurt Yogurt is a fermented dairy product, which is a good source of calcium, phosphorus, magnesium, potassium, riboflavin, vitamina A, and protein (Pal et al., 2015). The bacteria used to make yogurt are known as "Yogurt Cultures". The fermentation of lactose by these bacteria produce lactic acid, which act

on milk protein to give yogurt its texture and its characteristic taste. It is an excellent growth medium for many kinds of microorganisms, as it provides rich nutrients for microbes. The exposure of yogurt to the potential for microbial contamination during processing, storage and transportation without basic sanitary practices in place and control temperature handling will quickly spoil the product and hence, become unacceptable for human consumption (Mohammed and Abdullahi, 2015).

2.11 TYPES OF SPOILAGE MICROBES

The microbes are living ubiquitous organisms, which are present everywhere, including air, water, and soil. Microbes can reach the milk and dairy products from various sources, and can cause spoilage (P Therefore, the contamination of milk and milk products is a challenge to the producer. It is largely due to human factor and unhygienic conditions (Singh et al., 2011). Post-pasteurization contaminations of milk products are mainly due to the filling machines and gaskets with biofilms (Dogan and Boor, 2003). Biofilm formation on milk post-pasteurization contact surfaces and isolation of Bacillus cereus from the post-pasteurization equipment surfaces of a dairy processing unit indicated that the equipment surfaces can act as reservoirs for milk recontamination, thereby reducing the efficiency of pasteurization and sanitation treatments (Malek et al., 2012; Pal et al.,2013). Biofilms are matrix-enclosed bacterial population adherent to each other and/or to surfaces or interfaces and may have a bacterial count up to 108 CFU cmG2. They are difficult to eradicate employing conventional cleaning and disinfection regimens due to their resistant phenotype and disinfectants do not penetrate the biofilm matrix.

Amongst different sanitizers, chlorine and ozone were effective for inactivating biofilm microflora (Sarkar, 2015).

1. **Psychrotrophs**

Psychrotrophic microbes are cold loving organisms as they prefer to grow at low temperature, and represent a substantial percentage of the bacteria in raw milk. Important characteristics of *pseudomonads* are their abilities to grow at low temperatures 3-7oC and to hydrolyze and use large molecules of proteins and lipids for growth. For cottage cheese, the typical pH is marginally favorable for the growth of Gram-negative psychrotrophic bacteria with the pH of cottage cheese curd ranging from 4.5 - 4.7. The usual salt content of cottage cheese is insufficient to limit the growth of contaminating bacteria; therefore, psychrotrophs are the bacteria that normally limit the shelf life of cottage cheese (Ledenbach and Marshall, 2009). The psychrotrophic bacteria such as *Achromobacter spp., Alcaligenes spp., Flavobacterium spp*. and *Pseudomonas spp*. are of concern in cheese (Fernandes, 2008).

2. **Listeria Monocytogenes**

Listeria monocytogenes is an emerging foodborne zoonotic pathogen of public health significance.Very recently, Pal and Awel (2014) has published a review on the public health significance of *L.monocytogenes* in milk and milk products. It is mentioned in European report that noncompliance of *L. monocytogenes* primarily occurred in soft and semi-soft cheeses made from raw or low heat-treated cow's milk (Verraes et al., 2015). There has been some discussion regarding the potential for *L. monocytogenes* in milk to survive pasteurization. The organism has been shown to be capable of significantly more rapid growth in pasteurized milk than in raw milk at 7°C and is also capable of growth at 4°C in pasteurized milk. It has been shown to grow

slowly in butter made from contaminated cream at 4 or 13°C, and to survive for several months in frozen butter without any appreciable decrease in numbers (Fernandes, 2008).

3. **Coliforms**

Coliforms are Gram negative, facultative anaerobic, rod shaped bacteria, which belong to the family *Enterobacteriacea.* This include *E.coli, Enterobacter aerogenes*, besides *Citrobacter* and *Klebsiella* (Pal and Mahendra,2015).In cheese production, slow lactic acid production by starter cultures favors the growth and production of gas by coliform bacteria, with coliforms having short generation times under such conditions. In soft, moldripened cheeses, the pH increases during ripening, which increase the growth potential of coliform bacteria (Ledenbach and Marshall, 2009).

4. **Spore-Forming Bacteria**

Raw milk is the usual source of spore-forming bacteria in finished dairy products. Their numbers before pasteurization seldom exceed 5,000/ml. They can also contaminate milk after processing. The most common spore-forming bacteria found in dairy products are *Bacillus cereus, B.licheniformis, B. megaterium, B. mycoides* and *B. subtilis* (Ledenbach and Marshall, 2009). Bacterial spores resistant to heat treatment that was present in the raw milk. The improper packaging of milk after heat treatment allowing the entrance of microbes recontamination after heat treatment whereas the spores of B. cereus can form biofilm (Vidal et al., 2016).

2.12 PREVENTION

Milk and dairy products play a key role in healthy human nutrition and development throughout life (FAO, 2013). Dairy products such as pasteurized milk, butter, cheese, cream, ice cream, and yoghurt are all susceptible to microbial spoilage because of their chemical composition (Singh et al., 2011). Ready-to-eat food products including cheeses are intended for consumption without any treatment between final production and consumption (Vrdoljak et al., 2016). Rapid cooling and quick use of raw milk are accepted as best practices, and can affect the spoilage ability of Pseudomonas spp. present in milk. As the quality of raw milk improved, pasteurized milk the pathogenic bacteria likely to be of significance in milk as well as most of the spoilage bacteria will be killed. The addition of carbon dioxide to milk and milk products reduces the rates of growth of many bacteria (Ledenbach and Marshall, 2009). Maintenance of the proper hygienic conditions during the processing of milk can reduce the prevalence of bacteria, which spoil the milk product (Singh et al., 2011). Good hygiene practices (GHP) during milking and subsequent handling of milk are essential to reduce the risk of contamination on the farm and in the milk processing plant (Sarkar, 2015). Worldwide standardized pasteurization practices would be an effective first step in eliminating or reducing the levels of many spoilage microorganisms (Ledenbach and Marshall, 2009). In order to minimize the health risks of milk and dairy products at the point of consumption, all food-chain operators, including the dairy farmer, processor, distributor, retailer and consumer, need to take necessary actions to maintain food safety (FAO, 2013). The education on the principles of food hygiene should be imparted to all who form a part of food chain programme (Pal, 2014). Furthermore, the application of hazard analysis and critical control point system (HACCP) seems imperative in all food processing industries from safety point of view (*Berger et al, 2007*). It is recommended that microbiological monitoring of the milk and dairy products should be conducted to keep the products safe to the consumers.

CHAPTER THREE

3.0 Material and method

3.1 Source of diary samples

The dairy products which includes Hollandia yoghurt, peak milk, 3 crown and peak yoghurt. Used in this study was purchase from stores around university of Benin ugbowo. All samples were transported and stored in the refrigerator at 4^0C ready for use. Prior to serial dilution and ciulturing, all the dairy products samples were exposed to air at room temperature for 48hours.

3.2 Materials

3.2.1 Glassware/Equipment

All glassware used in this study were thoroughly washed with detergent (omo), dried and sterilized in the oven at 121^0C for 15mintes. They were allowed to cool before use. Inoculating loops, needles and forceps were sterilized by flaming until red hot. Equipment used include,

autoclave, incubator, microscope, weighing balance, Disinfectant/ cotton wool, Bunsen burner and water bathe.

3.2.2 Media/ reagent

The reagent and media include,

¼ strength ringer solution, Nutrient broth, neutrent agar, sterile distilled water, pepetone water, crystal violet, Acetone, safranine, iodine, hydrogen peroxide, immersion oil.

Methods

3.2.2.0 Media preparation

The media used for this practical work were oxoid production

3.2.2.1 ¼ RINGER SOLUTION

1 tablet was dissolved in 500ml of distilled water and was kept for some minute for the tablet to properly dissolve. Using a pipette, 9ml was dispensed into a universal bottle with cover and was sterilized at 121^0C for 15minutes.

3.2.2.2 Nutrient Broth

Nutrient broth (10g) was weighted out into a conical flask. Distilled water (769ml) was added. The conical flask was corked with cotton wool, covered with aluminum foil and dissolved by

bringing to boil in an autoclave. It was then sterilized in the autoclave at 121^0C for 15 minutes. The broth was cooled to about 40^0C and then dispensed into sterile Maccartney bottles.

3.2.2.3 Nutrient Agar

Powdered Nutrient agar (28g) was mixed in 1L of distilled water in a conical flask. The conical flasks were corked with cotton wool covered with aluminium foil and bring to boil in an autoclave in order to dissolve. Using a pipette, 20ml was delivered into a clean universal bottle and then sterilized by autoclaving at 121^0c for 15 minutes at pressure of 1.02kg/cm^2.

The autoclaving mixture was cooled at 40^0c and poured into a clean dispensable petridishes at the rate of about 20ml per plate. The plates were allowed to solidify.

3.2.3.4 Potato Dextrose Agar

Powdered Potato dextrose agar (39g) were weighed and 1itre of deionized water was used to dissolved it. Then, Soaked for 10 swirl to Mix and later sterilized by autoclaving at 115^0c for 10minutes. It was then cooled to 55^0c and mixed before processing begins**.**

3.2.3.5 Man Rogosa Sharpe Medium

A suspension of 64.3g of powdered MRS was dissolved on 1litre of distilled water. Heat with frequent agitation and boiled to completely dissolve the powder before being distributed into flasks and sterilize by autoclaving at 121^0c for 15minutes.

3.2.3 Serial Dilution

Directly from the stock sample, a series of dilutions were made as follow, 1ml of exposed diary product sample to 9ml of ¼ strength ringer solution (1:10)-----i

1ml of solution i to 9ml of ¼ strength ringer solution (1:100) ---ii

1ml of solution ii to 9ml of ¼ strength ringer solution (1:1000)---iii

1ml of solution iii to 9ml of ¼ strength ringer solution (1:10000)---iv

1ml of solution iv to 9ml of ¼ strength ringer solution (1:100000)---v

Using the drop plate pipette method, an automatic pipette with a sterile teeth was used to deliver 0.02ml each from the serial diluent, 10^4, 10^3, 10^2 and 10 onto each of the labelled petridishes containing solidified nutrient agar, divided into four parts. The 5plates were left on the bench for the drops to completely absorb into the nutrient agar and then the plates were incubated in an inverted position at 37^0c for 24 hours. After 24 hours, plate count where carried out. The PDA plates were incubated for 48 hours at 25^0c.

3.2.4 Total Plate Count Technique

The total plate count technique was used to estimate the number of viable colonies of bacterial present in the samples.

3.2.4.1 Isolation of pure culture

After 24hours incubation, disimilar colonies were observed from the medium. With the aid of a sterilized wire loop the suspect were subculture. The organism were streaked on the agar plates several times and incubated in an inverted position for 24 hours. Same were done with the PDA plates.

3.2.4.2 Identification of Isolate

Bacteria isolate were identified based on the following: cultural characteristics such as size, surface appearance, and colour on agar plate, colonial morphorlogy of the isolate, staining reaction of each isolayes and the biochemical test such as catalase, oxidase, citrate and indole according to international commission on microbiological specification for foods (ICMSF 1996), and Bergey's Manual of systematic bacteriology (Smeath *et al*., 1986).

Cultural characteristics

Pure culture of isolate on nutrient agar were studied to determine the surface of colonies, elevation, optical characteristics and colour.

3.2.5 MORPHOLOGICAL TECHNIQUE

Morphology of bacterial isolates was determined by carrying out the following techniques.

3.2.5.1 Staining Techniques

This is a differential staining procedure used to divide bacterial into two distinct categories. Those showing a positive result and those showing a negative result.

PROCEDURE

A thin smear of the isolates were made on a clean glass slide and heat fixed.

Crystal violet stain was applied for 30 seconds (which serves as the primary stain, its purpose is to impact colour to all organisms being studied). After which the dye is poured off and the slide rinsed with water.

The slide were covered with lugol's iodine for 60 seconds before the solution was rinsed off with water. The iodine acts as a mordant (a substance which strengthens the affinity of a dye for its substrate).

The slides were flooded with acetone for 1-10 seconds and was rinsed off immediately with water. The acetone acts as a decolorizer removing the primary stain from the type of cells.

The slide were counter-stained with safraninine for 1minute. And then was rinsed with water, allow to dry and viewed under the light microscope using x 100 objective lens with oil immersion. The gram positive cells appeared purple and the gram negative cells appeared red under the microscope.

3.2.6 BIOCHEMICAL TEST

3.2.6.1 Catalase test

This was performed to test the ability of the isolates to produce an enzyme catalase. This enzymes, catalase, catalyzes the breakdown of hydrogen peroxide (H_2O_2) to release free oxygen gas. This test is used to differentiate *streptococcus* (catalase negative) from staphylococcus, which is positive. This test was carried out by using a sterilized wire loop to pick a colony and place it in a drop of hydrogen peroxide on a slide. The production of gas bubbles indicates it's a

catalase producer which confirm the test to be positive. In the absence of gas bubbles, it means the organism is negative.

3.2.6.2 Oxidase test

The oxidase test is used to assist in the identification of pseudomonas, Neisseria, and Vibrios, etc, all of which produce oxidase enzymes.

A piece of filter paper was soaked with a few drops of oxidase reagent. A colony of the test organism was then smeared on the filter paper. Oxidase positive organism turn the paper deep purple colour and oxidase negative organism are non-reactive.

3.2.6.3 Citrate Utilization Test

This test is used to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen. The test organism was inoculated in a medium which contains Koser's citrate, using a sterile straight wire loop, the organism was inoculated into the broth and was incubated at 35-77^{0}C for 24-48hrs. The medium initially green, turns blue in colour showing positive results and a negative results, there was no colour change.

3.2.6.4 Indole Production

This was performed to test the ability of the isolates to enzymatically degrade tryptophan into indole which is not degradable by most organism. Tryptone broth medium was used. 2.5g of tryptone broth powder was dissolved in 250ml of distilled water in water bathe. It was then dispensed into tube and sterilized in the autoclave at 1.02Kg/cm^2 pressure for 15 minutes. This isolates were inoculated into the tube. Tubes were incubated at 37^{0}C for 24hours. After

incubation, few drops of kovac's reagent was added to each tube. Tryptophan breakdown and production of indole was indicated by a red ring colour. For negative results there was no red ring coloration.

CHAPTER FOUR

4.0 RESULT

It has been demonstrated that when normal plating protocols are used to estimate the variety of microorganisms present in a diary product, only a percentage of the true microbial population is enumerated due to partial microbe recovery (Witthuhn *et al*., 2005). Three different media were utilized, each promoting the development of a specific group of microorganisms, to guarantee that a broad spectrum of bacteria was enumerated after plating.

Table 4.1 showing the microbial enumeration of unexposed dairy products

Dairy products	Fungi (PDA) cfu.ml^{-1}	Lactobacillus (MRS) cfu.ml^{-1}	Bacteria (NA) cfu.ml^{-1}
Hollandia yoghurt	**1x10^{2}**	**-**	**-**
Peak milk	**-**	**-**	**-**
Three crown	**-**	**-**	**4x10^{4}**
Peak yoghurt	**-**	**-**	**-**

The following abbreviations were used: PDA = potatoes dextrose agar; MRS = deMan, Rogosa and Sharpe-medium; NA = Nutrient agar.

Table 4.1 shows that no microorganisms were found or counted in the unexposed peak milk and peak yoghurt, whereas 1x102 cfu.ml-1 fungi were found in potatoes dextrose agar (PDA) cultured Hollandia yoghurt and 4x104 cfu.ml-1 bacteria were found in Nutrient agar (NA) cultured plates, these are three crowns.

Table 4.2: Showing the bacteria count of all the exposed dairy products

Dairy products	Exposed samples		P value ($p<0.05$)	Unexposed samples
	Sample 1 cfu.ml^{-1}	**Sample 2 cfu.ml^{-1}**		**Control cfu.ml^{-1}**
Hollandia yoghurt	**2.9 x 10^{7}**	**3.75 x 10^{7}**	**0.0874**	**1 x 10^{2}**

Peak milk	**5.5 x 10^7**	**3.5 x 10^7**	**0.1056**	**NC**
Three crown	**4.25 x 10^7**	**2.8 x 10^7**	**0.0258***	**4 x 10^4**
Peak yoghurt	**3.0 x 10^7**	**3.05 x 10^7**	**0.8310**	**NC**

*Significant at $p<0.005$ NC = No microbial count

Average enumeration value obtained from duplicate media plates of different dairy products.

P1 value < 0.05 indicates that at least one of the mean values differs significantly from another (using one t test).

From table 4.2 a significant difference ($p<0.05$) was observed between all the exposed dairy product when compared to the unexposed dairy product (control) the highest significant difference was found between exposed peak milk and unexposed peak milk at $p<0.05$, while the least significant difference was seen between exposed three crown and unexposed three crown at $p<0.05$.

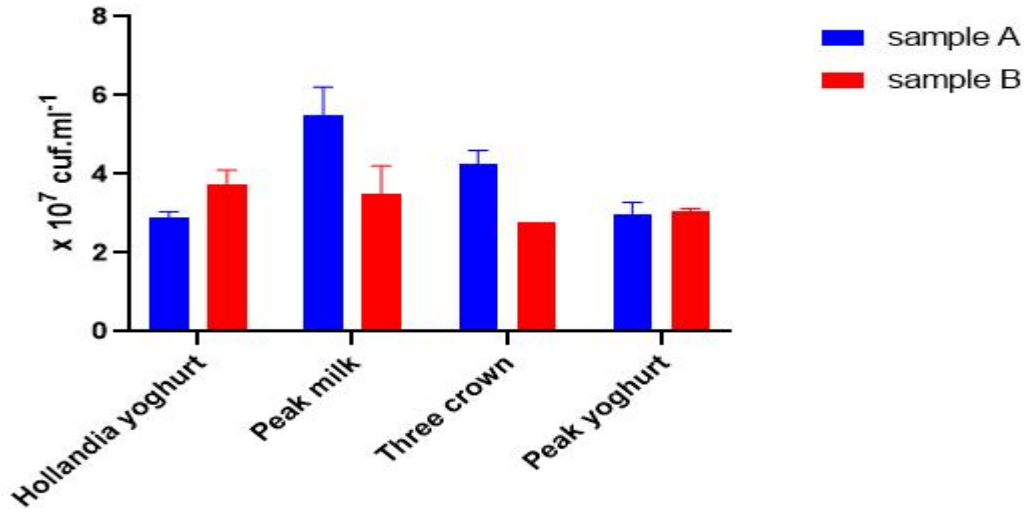

Fig 4.1: showing the microbial count variation of the various dairy products sample

From figure 4.1 a variation of microbial count was observed with peak milk showing the highest microbial count followed by Three crown and Hollandia yoghurt with the least count on peak yoghurt. Also a significant variation was also observed within the various samples of different dairy product.

Table 4.3: Enumeration of lactic acid bacterial for the exposed samples

Dairy products	Sample 1 $cfu.ml^{-1}$	Sample 2 $cfu.ml^{-1}$
Hollandia yoghurt	**$2 \times 10^4 \pm 3.000$**	**$4 \times 10^4 \pm 3.000$**
Peak milk	**NC**	**NC**
Three crown	**NC**	**NC**
Peak yoghurt	**$7 \times 10^4 \pm 0.000$**	**$5 \times 10^4 \pm 0.000$**

*NC= No microbial count

From table 4.3, It was observed that peak yoghurt possesses the highest lactic acid bacterial count with and average value of (6×10^4 $cfu.ml^{-1}$) followed by Hollandia yoghurt with an average value of (3×10^4 $cfu.ml^{-1}$). There was no lactic acid bacterial observed in Three crown and peak milk.

Table 4.4: Enumeration of fungi in the exposed dairy product sample

Dairy products	Sample 1 $cfu.ml^{-1}$	Sample 2 $cfu.ml^{-1}$
Hollandia yoghurt	$2.5 \times 10^4 \pm 3.000$	$3 \times 10^4 \pm 4.000$
Peak milk	$4 \times 10^3 \pm 2.000$	$6 \times 10^3 \pm 0.000$

Three crown	$5 \times 10^3 \pm 1.000$	$5 \times 10^3 \pm 2.000$
Peak yoghurt	$1.5 \times 10^4 \pm 3.000$	2.4×10^4 2.000

* ± SEM

From table 4.4 showing the number of fungi count in the various exposed dairy product it was observed that Hollandia yoghurt possess the highest number of fungi with an average value of ($2.75 \times 10^4 \pm 4.000$) followed by Peak yoghurt ($1.95 \times 10^4 \pm 2.000$). Three crown and peak milk showed the lowest fungi count of ($5 \times 10^3 \pm 2.000$).

Table 4.5: Identification of fungi isolates using introducing mycology by examples atlas pictures

Dairy products	Sample 1 $cfu.ml^{-1}$	Sample 2 $cfu.ml^{-1}$
Hollandia yoghurt	Aspergillus Niger and Penicillium spp.	Aspergillus Niger
Peak milk	Mucor spp., Penicillium Notatum	Aspergillus Niger
Three crown	Aspergillus Niger, Penicillium spp., Yeast	Aspergillus Niger, Mucor spp.

Peak yoghurt	Aspergillus Niger	Aspergillus Niger and Penicillium spp.

*spp = species

From table 4.5 showing the various species of fungi present in the various exposed dairy products. It was observed that more specie of fungi tends to contaminate Three crown namely *Aspergillus Nigar, Penicillium spp., Yeast and Mucor spp*. followed by peak milk. Namely Aspergillus Nigar, Penicillium spp., and Mucor spp. With the least being Hollandia yoghurt and peak yoghurt.

Figure 4.2: Showing the percentage prevalence of fungi contaminant in dairy product

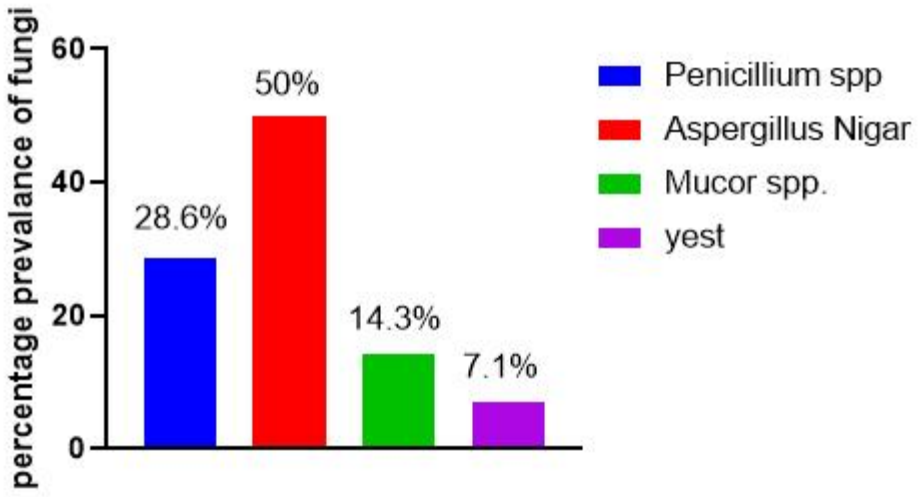

Figure 4.1 Shows the percentage prevalence of fungi contaminant in dairy products. It was observed that *Aspergillus Nigar* has the highest prevalence of fungi contaminant (50%), followed by *Penicillium spp* (28.6%). With yeast showing the least prevalence contamination in the various dairy products (7.1%).

Table 4.7: Biochemical test showing results of various bacterial isolated

Samples	Gram stain	Catalase	Oxidase	Citrate	Indole	Urease	Coagulase	Identified bacterial
PY 1	**+ve**	**-ve**	**+ve**	**-ve**	**ND**	**ND**	**ND**	Streptococcus spp., Bacillus cereus
PY 2	**+ve**	**+ve**	**-ve**	**+ve**	**ND**	**ND**	**ND**	Lactobacillus spp., Bacillus cereus
CM 1	**+ve**	**+ve**	**+ve**	**+ve**	**ND**	**ND**	**ND**	Bacillus subtilis
CM 2	**+ve**	**+ve**	**+ve**	**+ve**	**ND**	**ND**	**ND**	Bacillus subtilis
PCM 1	**+ve**	**+ve**	**-ve**	**+ve**	**ND**	**ND**	**ND**	Bacillus cereus
PCM 2	**+ve** **- ve**	**+ve** **+ve**	**-ve** **+ve**	**+ve** **+ve**	**ND** **ND**	**ND** **ND**	**ND** **ND**	Bacillus cereus, Pseudomonas aeruginosa
HY 1	**+ve**	**-ve**	**-ve**	**-ve**	**ND**	**ND**	**ND**	Lactobacillus spp.
HY 2	**+ve**	**-ve**	**-ve**	**-ve**	**ND**	**ND**	**ND**	Lactobacillus spp., Streptococcus spp.

Figure 4.2: Showing the percentage prevalence of bacteria contaminant in dairy product

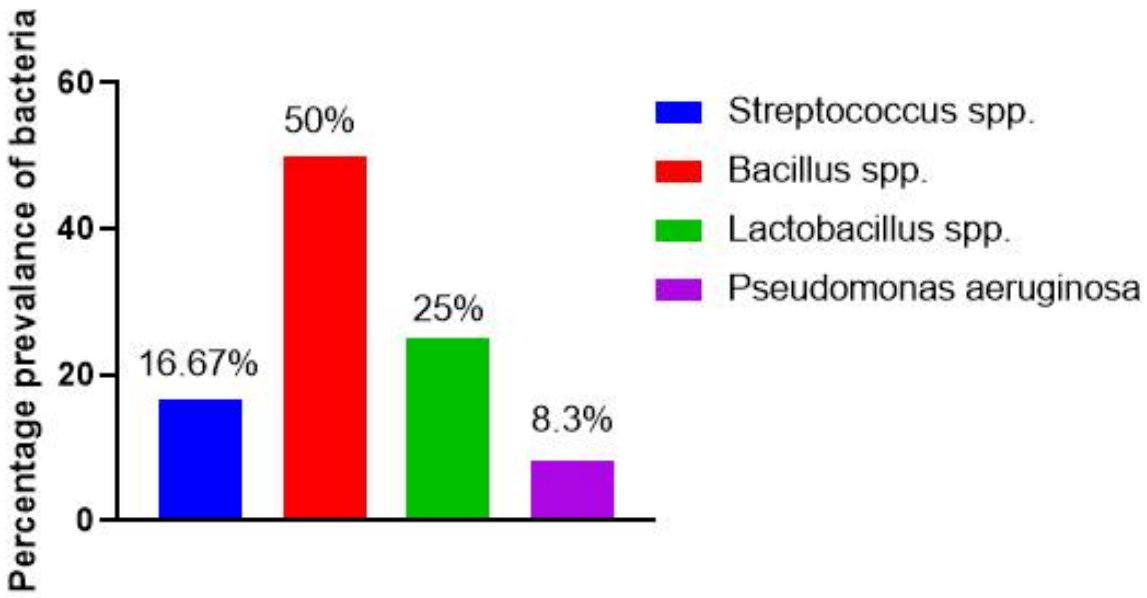

Figure 4.2: reveals that the percentage prevalence of bacteria contaminant in diary product. This figure shows that Bacillus spp. Shows the highest level of percentage prevalence of 50% followed by Lactobacillus spp. The least percentage prevalence of bacteria contaminant was seen in Pseudomonas aerugunosa (8.3%).

Table 4.6: showing the percentage distribution of microbial contaminant on various dairy products

Dairy products	% Bacterial contamination	% Fungi contamination
Hollandia yoghurt	**40%**	**60%**
Peak milk	**40%**	**60%**
Three crown	**16.67%**	**83.33%**
Peak yoghurt	**60%**	**40%**

Table 4.6 shows the percentage distribution of microbial contaminant on the various dairy products. Three crown was seen to have the highest percentage of fungi contaminant (83.33%) and the least percentage of bacterial contaminant followed by Hollandia yoghurt and Peak milk. Similarly, peak yoghurt shows the highest percentage bacterial contaminant and the least percentage fungi contaminant followed by Hollandia yoghurt and peak milk.

CHAPTER FIVE

5.0 DISCUSSION

When typical plating protocols are used to determine the diversity of microorganisms present in a dairy product, only a part of the true microbial population is enumerated due to incomplete microbe recovery (Witthuhn *et al*., 2005). Three distinct media, as indicated in table 4.1, were utilized to ensure that a diverse range of microbes were counted after plating, each favouring the growth of a certain bacterium. This is comparable to Kebede and colleagues' work (2007).

The microbiological count in the unexposed peak milk and peak yoghurt was zero, indicating that the dairy products were manufactured aseptically using a tight quality control technique, as shown in table 4. 1. A fungal count of $1x10^2$ cfu.ml-1 was found in potatoes dextrose agar (PDA) cultured unexposed Hollandia yoghurt, whereas a bacteria count of 4x104 cfu.ml-1 was found in Nutrient agar (NA) cultured unexposed Three crown. The presence of microorganism in these unexposed dairy products could be the result of contamination during the diary product's production as a result of a poor quality control procedure or inappropriate sample handling during the testing approach. This is in line with the findings of Kim and Chun's research (2005).

Table 4.2 shows the enumeration values (cfu.ml-1) obtained from the four (4) dairy product samples on the total of the three (3) selective media. The highest microbial counts for Hollandia yoghurt were obtained from sample 2 (3.75 x 107 cfu.ml-1), the highest enumeration values for Peak milk were obtained from sample 1 (5.5 x 107 cfu.ml-1), the highest enumeration values for Three crown were obtained from sample 1 (4.25 x 107 cfu.ml-1), and Peak yoghurt showed close values of (3.0x107 Peak milk had the highest enumeration values among all three exposed dairy product samples, with a mean microbiological count of 4.5 x 107 cfu.ml^{-1}. Peak yoghurt had the lowest enumeration values, with a mean microbiological count of 3.025 x 107 cfu.ml-1. Similar differences were seen in Nah and Chau's (2010) research of microbial accumulation on several exposed milk products.

Figure 4.1 shows a microbial count range, with peak milk having the highest count (4.5 x 107 cfu.ml-1), Three Crown and Hollandia yoghurt having the second and third highest counts (3.525 x 107 cfu.ml-1 and 3.325 x 107 cfu.ml-1, respectively), and peak yoghurt having the lowest count (3.325 x 107 cfu.ml-1) (3.025 x 107 cfu.ml-1). There was a lot of variety among the two (2) samples of various dairy items. Peak yoghurt's high microbial count could be related to the dairy product's pH value and nutritional content, which allow for more microbe development and survival than peak yoghurt, which has the lowest microbial count. This is consistent with Okonkwo's (2011) study on the influence of pH on the microbial count of different dairy products. Delorme's (2008) work on the diversity of microbial counts on identical dairy products from different batches and farmers is also worth mentioning.

Milk and its products are important human foods, but they also serve as a good source of growth for a variety of microorganisms. Microorganisms that can grow easily in milk include Lactobacillus, Streptococcus, Bacillus spp. and Pseudomona spp. Many microorganisms present in milk, may endanger the consumer’s health (Fonseca and Santos, 2000). Various bacterial species like Salmonella are the major and important pathogenic bacteria found in milk (Brito and Dias, 1997).

There was no microbiological count in the unexposed peak milk and peak yoghurt, indicating that the dairy products were manufactured aseptically with a rigorous quality control technique, as shown in table 4.1. In potatoes dextrose agar (PDA) cultured unexposed Hollandia yoghurt, $1x10^2$ cfu.ml-1 fungal count was seen, while 4x104 $cfu.ml^{-1}$ bacteria count was observed in Nutrient agar (NA) cultured unexposed Three crown. The presence of microorganism in these unexposed diary products, might be due to contamination during production of the diary product as a result of poor quality control procedure or as a result of wrong handling of the sample

products during the experimental procedure. This is in accordance with the study carried out by Kim and Chun, (2005)

The enumeration values (cfu.ml^{-1}) obtained from the four (4) samples of dairy products on the sum of the three (3) media are presented in Table 4.2. For Hollandia yoghurt, the highest microbial counts was obtained from sample 2 (3.75 x 10^7 cfu.ml^{-1}), the highest enumeration values for Peak milk was observed from sample 1 (5.5 x 10^7 cfu.ml^{-1}), the highest enumeration values for Three crown was observed on sample 1 (4.25 x 10^7 cfu.ml^{-1}) while Peak yoghurt show close values of (3.0x10^7 and 3.05x10^7) for sample 1 and 2 respectively. In all three exposed samples of dairy products, the highest enumeration values were observed on peak milk with a mean microbial count of 4.5 x 10^7 cfu.ml^{-1}. While the least enumeration values were observed on peak yoghurt with a mean microbial count of 3.025 x 10^7 cfu.ml^{-1}. Similar variation was seen in the study carried out by Nah and Chau, (2010) on the microbial accumulation on some exposed milk products.

Figure 4.1 shows a range in microbial count, with peak milk having the greatest count (4.5 x 107 cfu.ml-1), followed by Three Crown and Hollandia yoghurt (3.525 x 107 cfu.ml-1 and 3.325 x 107 cfu.ml-1, respectively), and peak yoghurt having the lowest count (3.325 x 107 cfu.ml-1) (3.025 x 107 cfu.ml-1). Within the two (2) samples of the various dairy products, there was also a large diversity. The high microbial count reported in peak yoghurt could be due to the dairy product's pH value and nutritional content, which enable the growth and survival of many microorganisms greater than peak yoghurt, which has the lowest microbial count. This is in line with the report of Okonkwo, (2011) on the effect of pH on the microbial count of some dairy products. And also the work of Delorme, (2008) on the diversity of microbial count on similar diary product from different batch and producers.

From table 4.3, It was observed that peak yoghurt possesses the highest lactic acid bacterial count with and average value of (6×10^4 cfu.ml^{-1}) followed by Hollandia yoghurt with an average value of (3×10^4 cfu.ml^{-1}). There was no lactic acid bacterial observed in Three crown and peak milk. This was also similar to the findings of Pal, (2014), which says the amount of lactic bacterial present in a dairy product depends largely on the type of dairy product and the production process.

Fungi test were positive for all the contaminated samples. The detection of fungi and pathogenic bacteria from the milk and the other dairy product showed that milk contains useful nutrient on which these microorganism can grow (Bonfoh *et al*., 2003). Aspergillus Nigar were present in all the dairy samples. It is a nonpathogenic fungi microbe of great industrial importance.this mold is used extensively in the production of citric acid and in the production of several enzymes such as amylases, pectinases and proteases (Das *et al*., 2015).

Penicillium sp was detected in 4 (28.6%) samples. These results are in agreement with those of Fook *et al*, (2004) who reported that 33.5% samples (312 samples out of 930) were contaminated with *Penicillium sp Penicillium sp* are one of the main leading and important fungi contaminating dairy product. (Hassan *et al*., 2009). Milk and milk products can be very easily contaminated with *Penicillium sp* and mucor spp. fungi and their presence in the milk is a sign of contamination of milk and milk products. Contrary to the presence of Aspergillus Nigar and yeast which were detected in 7 samples (87.5%) and 1 (7.1) samples respectively of the total fungi isolated but it is not pathogenic in nature (Wells *et al*., 1999).

Bacillus spp. was detected in 6 (75%) samples. The presence of *Bacillus spp.* and some other types of nonpathogenic fermenting bacteria such as Lactobacillus in milk and its product has

been essential for the production of many harmless products especially for those persons who consume a lot of milk and its products (Ryser, 1998).

Pseudomonas aeruginosa a pathogenic bacterial was found in the exposed peak and also *streptococcous spp* shows that the exposure of milk and its product can attract pathogenic bacterial to grow which in turn is harmful to humans on consumption. This is in line with the findings of Vrdoljak *et al*. (2016). On the contaminant of milk on normal exposure.

Figure 4.2: reveals that the percentage prevalence of bacteria contaminant in diary product. This figure shows that Bacillus spp. have the highest level of percentage prevalence of 50% followed by Lactobacillus spp. The least percentage prevalence of bacteria contaminant was seen in Pseudomonas aerugunosa (8.3%). From the result it is inferred that on exposure normal atmospheric air, nonpathogenic fermenting bacteria predominately contaminant dairy products due to the presence of lactose. As seen in our study 75% of the contaminant were nonpathogenic fermenting bacteria. Although as seen in our study some pathogenic bacteria harmful to humans are were observed making up the 25% contaminant. Our study was in line with the report of Fernandes, (2008). The exposure of milk to atmospheric air should be avoided due to pathogenic contaminant and also due to spoilage by fermenting microorganism (Verraes *et al*., 2015).

Table 4.6 shows the percentage distribution of microbial contaminant on the various dairy products. Three crown was seen to have the highest percentage of fungi contaminant (83.33%) and the least percentage of bacterial contaminant followed by Hollandia yoghurt and Peak milk. Alternatively, peak yoghurt shows the highest percentage bacterial contaminant and the least percentage fungi contaminant followed by Hollandia yoghurt and peak milk. This implies that three crown has nutrient which support the growth of fungi more than bacteria leading to high level of fungi contamination. While peak yoghurt possesses nutrient which enriches the growth

of bacterial more than fumgi. This is similar with the work done by Pal *et al*. (2012). On the identification of possible diary contaminant.

5.1 CONCLUSION

In the present study eight samples of dairy products were randomly collected from different locations in Benin city. The present study revealed that exposing milk and other dairy product to atmospheric contamination is unhygienic due to microbial and fungi contamination, which might cause health risk or spoilage. An important source of microbial contamination of the milk and other dairy products is the atmospheric microbial pollution probably. Programs like ‘good hygiene practices’ and ‘good production practices’ should be adopted at every step in milk and dairy products handling and processing. Moreover, milk and its product should not be exposed to atmosphere as it can get contaminated by photogenic. More standard research should be made on pure fermentation of milk and dairy products without any pathogenic contaminant.

REFERENCES

Al-Hamidi, A. A. (2004). Properties and applications of proteinaceous antibiotics produced by lactic acid bacteria: a review. *Journal of Saudi Chemical Society.* **8:** 35–46.

Andoh, A. and Fujiyama, Y.(2006). Therapeutic approaches targeting intestinal microflora in inflammatory bowel disease. *World Journal of Gastroenterology.* **12 (28):** 4452–4460.

Bergamini, C. V.; Hynes, E. R.; and Zalazar, C. A. 2006. Influence of probiotic bacteria on the proteolysis profile of a semi-hard cheese. *International Dairy Journal.* **16:** 856–866.

Berger, B.; Pridmore, R. D.; Barretto, C.; Delmas-Julien, F.; Schreiber, K.; Arigoni, F.; and Brussow, H. (2007). Similarity and differences in the Lactobacillus acidophilus group identified by polyphasic analysis and comparative genomics. *Journal of Bacteriology.* **189:** 1311–1321.

Bernardeau, M.; Gueguen, M.; and Vernoux, J. P. (2006). Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. *FEMS Microbiology Reviews.* **30 (4):** 487–513.

Bishop, J. R.; and White. C. H. (1982). Assessment of dairy product quality and shelf life. *Journal ofFood Protection*. **49:** 739-753.

Bohm, S. K.; and Kruis, W. (2006). Probiotics: do they help to control intestinal inflammation? *Annals of the New York Academy of Sciences* **1072:** 339–350.

Cempírková R., M. Mikulová. (2009). Incidence of PsychrotrophicLipolytic Bacteria in Cow's Raw Milk. *Czech Journal of Animal Science*. **54(2):** 65-73.

Chamba, J. F. andIrlinger, F. (2004). Secondary and adjunct cultures. In: Fox, P. F., McSweeney, P. L. H.; Cogan, T. M.; Guinee, T. P. (Eds.), Cheese, Chemistry, Physics and Microbiology, General Aspects, *vol. 1. Elsevier Academic Press, London*. 191–206.

Champagne, C. P.; Laing, R. R.; Roy, D.; and Mafu, A. A. (1994). Psychrotrophs in Dairy Products: Their effects and their control. *Critical Reviews in Food Sci*. **34(1):** 1-30.

Danielsen, M., Wind, A., 2003. Susceptibility of Lactobacillus spp. to antimicrobial agents. *International Journal of Food Microbiology* **82:** 1–11.

Das, S.; Hasan, A. and Parveen, S. (2015). Evaluation of microbial load and quality of milk and milk based dairy products. *Octa Journal of Biosciences* **3:**1-4.

Davidson, R. H.; Duncan, S. E.; Hackney, C. R.; Eigel, W. N.; and Boling, J. W. (2000). Probiotic culture survival and implications in fermented frozen yogurt characteristics. *Journal of Dairy Science.* **83:** 666–673.

FAO. (2013). Milk and Dairy Products in Human Nutrition. Food and Agricultural Organization of the United Nations, Rome, Italy.

Fernandes, R. (2008). Microbiology Handbook of Dairy Products. Leatherhead Publishing and Royal Society of Chemistry, UK.

Food and Drug Administration, Grade "A" Pasteurized Milk Ordinance. (2009). Grade "A" Pasteurized Milk Ordinance (Grade "A" PMO). (2007) revision; 1-26. Fromm, H. I.; Boor, K. J. (2004). Characterization of pasteurized fluid milk shelf-life attributes. J. Food Microbiology and Safety. **69:** 207-214.

Gasser, F. (1994). Safety of lactic-acid bacteria and their occurrence in human clinical infections. Bulletin de L'Institut Pasteur **92:** 45–67.

Gatti, M.; Trivisano, C.; Fabrizi, E.; Neviani, E.; and Gardini, F. (2004). Biodiversity among Lactobacillus helveticus strains isolated from different natural whey starter cultures as revealed by classification trees. *Journal of Applied and Environmental Microbiology,* **70 (1):** 182–190.

Giraffa, G.; Andrighetto, C.; Antonella, C.; Gatti, M.; Lazzi, C.; Marcazzan, G.; Lombardi, A.; and Neviani, E. (2004). Genotypic and phenotypic diversity of Lactobacillus delbrueckii subsp. lactis strains of dairy origin. *International Journal of Food Microbiology,* **91:** 129–139.

Gruetzmacher, T. J. and Bradley Jr. (1998). Identification and control of processing variables that affect the quality and safety of fluid milk. *Journal of Food Protection*. **62:** 625-631.

Jorgensen, J. H.; and Hindler, J. F.; (2007). New consensus guidelines from the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing of infrequently isolated or fastidious bacteria. *Clinical Infectious Diseases.* **44 (2):** 280–286.

Kagkli, D. M.; Vancanneyt, M.; Hill, C.; Vandamme, P.; and Cogan, T. M.; (2007). Enterococcus and Lactobacillus contamination of raw milk in a farm dairy environment. *International Journal of Food Microbiology.* **114:** 243–251.

Kahn P.; and Firstenberg-Eden, R. (1993) Prediction of shelf-life of pasteurized milk and other fluid dairy products in 48 h. *Journal of Diary Science.* **70:** 1544-1550.

Kanawjia, S. K.; Nageswara Rao, K.; Singh, S.; and Sabikhi, L. (1993). Role of Lactobacilli in cheese. *Indian Journal of Dairy Science* **46:** 187–197.

Lawson, P. A.; Papademas, P.; Wacher, C.; Falsen, E.; Robinson, R.; and Collins, M. D. (2001). Lactobacillus cypricasei sp. nov., isolated from Halloumi cheese. *International Journal of Systematic and Evolutionary Microbiology.* **51 (1):** 45–49.

Ledenbach, L. H.; and Marshall, R. T. (2009). Microbiological spoilage of dairy products. Springer Science Business Media. 1-28.

Ledford, R., 1998. Raw milk and fluid milk products. In: Marth, E., Steele, J. (Eds.), Applied Dairy Microbiology. *Marcel Dekker, Inc, New York*: USA. 55–64.

Lee, S. Y.; Chang, C. T.; Lee, M. H.; and Wu, M. S. (2004). Lactobacillus peritonitis: a rare cause of peritonitis in peritoneal dial patients. Renal Failure. **26:** 419–423.

Lewis, G. (2006). Probiotics: a better way to treat infections during pregnancy. *Midwifery Today with International Midwife.* **79:** 30–31.

Malek, F.; Moussa-Boudjemaa, B.; Khaouani-Yousfi, F.; Kalai, A. and Kihal, M. (2012). Microflora of biofilm on algerian dairy processing lines: An approach to improve microbial quality of pasteurized milk. *African Journal of Microbiology Research,* **6:** 3836-3844.

Martin, D. (2005). Results from raw milk microbiological tests do not predict the shelf-life performance of commercially pasteurized fluid milk. *Journal of Diary Science.* 94: 1211.

Moore, J. E.; McIlhatton, B.; Shaw, A.; Murphy, P. G.; Elborn, J.S. (2013) Occurrence of Burkholderiacepacia in Foods and Waters: Clinical Implications for Patients with Cystic Fibrosis. *Journal of Food Protection.* **64(7):** 1076-1078.

Moysiadi, T. (2004). Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low fat pasteurized milk: chemical and sensorial aspects. *Journal of Dairy.* **15(5):** 429-436.

Nakano, Y. (2010). Prediction of plausible bacterial composition based on terminal restriction fragment length polymorphisms using a Monte Carlo method. Microb. Ecol. **60:** 364-372.

Nörnberg, M. F.; Friedrich, R. S. C.; Weiss, E.; Tondo, A.; and Brandelli, W. (2010). Proteolytic activity among psychrotrophic bacteria isolated from refrigerated raw milk. National Agricultural Statistics Service. 2012. Milk Cows and Milk Production. *United States Department of Agriculture.* **10 (11):** 3

O'Mahony, M. (1992). Understanding discrimination tests: A user-friendly treatment of response bias, rating and ranking R-index tests and their relationship to signal detection. *Journal of Sensory Studies.* **7:** 1-47

Ong, L.; Henriksson, A.; and Shah, N. P. (2006). Development of probiotic Cheddar cheese containing Lactobacillus acidophilus, Lb. casei, Lb. paracasei and Bifidobacterium spp. and the influence of these bacteria on proteolytic patterns and production of organic acid. *International Dairy Journal,* **16:** 446–456.

Ortigos, M.; Arizcun, C.; Torre, and P.; Izco, J. M. (2005). Use of wild Lactobacillus strains in an adjunct culture for a Roncal-type cheese. *Journal of Dairy Research.* **72 (2):** 168–178.

Pal, M.; and Jadhav, V. J. (2013). Microbial contamination of various Indian milk products. *Journal of Beverage and Food World.* **40:** 43-44.

Pal, M.; and Mahendra, R. (2015).Sanitation in Food Establishments.1st Ed. LAMBERT *Academic Publishing,* Saarbruchen, Germany.

Pal, M.; Bekele, T.; and Feleke, A. (2012). Public health significance of pasteurized milk. *Beverage and Food World.* **39:** 55-56.

Pal, M.; Deressa, A., Feleke, A.; and Demissie, K. (2014) Hygienic and microbial quality of butter. *Beverage and Food World.* **41:** 37-38.

Sarkar, S. (2015). Microbiological considerations: Pasteurized milk. *International Journal of Dairy Science.* **10:** 206-218.

Singh, V.; Kaushal, S.; Tyagi, A.; and Sharma, P. (2011). Screening of bacteria responsible for the spoilage of milk. *Journal of Chemical and Pharmaceutical Research.* **3:** 348-350.

Szajewska, H.; Ruszczynski, M.; and Radzikowski, A. (2006). Probiotics in the prevention of antibiotic-associated diarrhoea in children: a meta-analysis of randomized controlled trials. *The Journal of Pediatrics.* **149 (3):** 367–372.

Vancanneyt, M.; Naser, S.M.; Engelbeen, K.; De Wachter, M.; Van der Meulen, R.; Cleenwerck, I.; Hoste, B.; De Vuyst, L.; and Swings, J. (2006). Reclassification of Lactobacillus brevis strains LMG 11494 and LMG 11984 as Lactobacillus parabrevisspnov. *International Journal of Systematic and Evolutionary Microbiology.* **56 (7):** 1553–1557.

Verraes, C.; Valemynck, G.; Weyenberg, S. V.; De Zutter, L.; Daube, G.; Sindic, M.; Uyttendaele, M.; and Herman, L. (2015). A review of the microbiological hazards of dairy products made from raw milk. *International Dairy Journal.* **50:** 32-44.

Vidal, A. M. C.; Junior, O. D. R.; Abreu, I. L.; Bürger, K. P.; Cardoso, M. V.; Gonçalves, A. C. A.; Rossi, G. A. M.; and D'Abreu, L. F. (2016). Detection of Bacillus cereus isolated during ultra high temperature milk production flowchart through random amplified polymorphic DNA polymerase chain reaction. *Journal of Ciencia Rural Santa Maria.* **46:** 289-292.