CSIR COLLEGE OF SCIENCE AND TECHNOLOGY

DEVELOPMENT OF STARTER CULTURE FOR THE PRODUCTION OF DEGUE, A WEST AFRICAN TRADITIONAL FERMENTED MILK PRODUCT

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Thesis submitted to the Department of Agro-processing Technology and Food Bio-sciences of the CSIR College of Science and Technology, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Food Science and Technology

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DECLARATION

I herby declared that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature…….....Date……

Name: Leonardo Abormegah

Supervisor's Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the CSIR College of Science and Technology.

Principal Supervisor's Signature……Date……

Name: Prof. Wisdom Kofi Amoa-Awua

Co-Supervisor's

Signature…….....Date……

Name: Dr. Margaret Owusu

ABSTRACT

Burkina is a milk/millet product which has suddenly become popular in Ghana in the last few years. The product is thought to have originated from Burkina Faso but Burkina is unknown in that country. Rather Degue, a Fulani fermented milk product containing millet is well known in West African Sahelian countries including Burkina Faso. This work was carried out to compare the burkina and Degue processes, and develop a starter culture for the production of Burkina/ Degue in Ghana. A survey was carried out on Burkina producers and consumers in Accra, and fermenting milk samples collect on separate occasions from two original degue producers in Accra. Burkina was found to be the same as Degue based on the processing procedure except that producers in Ghana used reconstituted powdered milk whilst Degue producers in Sahelian countries use fresh cow milk. The spontaneous fermentation which occurs during Degue production was found to be a lactic acid fermentation and the population of LAB increased by 4 log units to about 8 log 10 CFU/ml during 24 h of fermentation. Percentage titratable acidity increased from 0.14 to 0.72% during fermentation whilst the pH values dropped from about 6.5 to 4.12. Lactic acid bacteria species identified in the spontaneous milk fermentation were *Lactobacillus plantarum* (43.75%), *Lactobacillus delbrueckii* ssp. *lactic* (31.25%), *Lactobacillus brevis* (18.75%), and *Lactobacillus buchneri* (6.25%). The LAB were identified by their pattern of carbohydrate fermentation and also by MALDI-TOF Mass Spectrometry which is based on the analysis of protein spectrum from bacterial ribosome. Both methods gave the same species of LAB. For the development of starter culture,

isolates of the lactic acid bacteria were used to inoculate pasteurized milk either singly or in combinations and the rate of acidification determined. Antimicrobial activity of the isolates against *Staphylococcus aureus, Escherichia coli* 0157; H7, *Salmonella typhimurium* and *Listeria monocytogenes* were also determined as well as survival of the pathogens in milk inoculated with the isolates. Production of amylase and exopolysaccharides by the isolates were also determined. In sensory testing, Degue produced using the different isolates were evaluated by a sensory panel. For overall acceptability in the consumer preference test, the panellists ranked Degue fermented with *Lb. delbrueckii ssp lactis* and also Degue fermented with *Lb. brevis* first with an average score of 7.35 each which corresponded with the products being liked moderately, followed by *Lb. plantarum* (6.83), *Lb. buchneri* (6.45), spontaneously fermented Degue (5.98), and lastly Degue fermented with a combination of *Lb. delbrueckii ssp lactis* and *Lb. plantarum* (5.90). However, the frequency with which *Lb. delbrueckii*- Degue was picked as the best product by panellists was 16 hence *Lb. delbrueckii ssp lactis* was selected as the starter culture for producing Degue in Ghana.

KEY WORDS

Degue

Fermentation

Lactic Acid Bacteria

Starter Culture

Technological Properties

Traditional Femented Foods

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DEDICATION

To my family and friends

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CHAPTER ONE

INTRODUCTION

Background to the Study

Fermentation of food has been a low cost economic method which has been used for processing and preserving food by humans for thousands of years. Fermentation of milk by both traditional and commercial/industrial methods are practiced worldwide. Fermentation of milk is carried out in many parts of Africa and is one of the oldest methods used to extend the shelf-life of milk. Fermentation involves harnessing microorganisms and their enzymes to produce food with distinct quality attributes quite different from their original raw materials. Fermentation therefore depends on the ability of microorganisms to produce a range of metabolites which suppress the growth of undesirable microbes in foodstuffs.

Generally, fermentation is carried out in order to bring diversity into the kinds of food and beverages available; making otherwise inedible foods edible; flavour dishes; enhance the nutritional values; decrease toxicity; preserve food and decrease cooking times and energy requirements (Steinkraus, 2018). In Africa, indigenous processed foods are mostly food-grade liquids comprising of single or mixed cereals/legumes, animal milk, and various plant parts. Simple food processing techniques are employed out of which fermentation tops the list (Chibundu, Kolawole, Misihairabgwi, Yinka, Chibuzor-Onyema, Oyedele, Abia, Sulyok, Gordon, & Krska, 2018).

Fermentation helps to conserve perishable foods and also improves their nutritional and organoleptic qualities.

Characteristics of fermented products vary from region to region depending on the local indigenous microflora, which in turn reflects the climatic conditions of the area (Yu, Wang, Menghe, Jiri, Wang, Liu, Bao, Lu, Zhang, & Wang, 2011). Thus traditionally fermented milk products from regions with a cold climate contain mainly mesophilic bacteria such as *Leuconostoc spp*. and *Lactococcus* whiles in the hot, subtropical or tropical climate, thermophilic bacteria mainly *Lactobacillus* and *Streptococcus* species prevail (Akabanda, Owusu-Kwarteng, Glover, & Tano-Debrah, 2010).

In Ghana two traditional fermented milk products are produced: Nunu and Nyarmie but are not produced nationwide. On the other hand, yoghurt a European product, is widely known, very popular, produced locally by SMEs and large companies and available throughout the country. It could be said that it is because negligible quantities of fresh milk are produced locally hence traditional milk products are hardly known. Perhaps the traditional milk product which is more widely known is Wagashie, but this is traditional soft cheese and is not fermented.

In the last two or three years a local fermented milk product has become very popular and is produced and sold by street vendors in the major towns. The product is called Burkina and does not seem to have any historical origin in

Ghana. It bears close semblance to Degue, an indigenous fermented milk product of the Fulani popular in some West African countries including Burkina Faso, from where the Ghanaian producers may have acquired the technology, hence the name Burkina.

Statement of the Problem

Milk is a good growth medium for many microorganisms because of its high water content, near neutral pH, and variety of available nutrients. These components consist of lactose, fat, protein, minerals and various non-protein nitrogenous compounds. Many microorganisms cannot utilize lactose and therefore must rely on proteolysis or lipolysis to obtain carbon and energy. In addition, freshly collected raw milk contains various growth inhibitors which decrease in effectiveness with storage (Breidt & Fleming, 1997). Despite these facts milk supports the growth of many pathogens which poses a risk to consumers of milk products hence milk processing demands high standards of hygiene. Procedures used in milk processing must assure the safety of the products, which unfortunately is not the case in the artisanal/traditional processing of milk and other products in Ghana. Due to the, popularity and wide patronage of Burkina in Ghana, it is important that the process and product are subjected to scientific study. This will enable the process and product quality to be standardized and upgraded for SME and industrial production, the safety of the product assured and the shelf life of the product extended.

Rationale of the Study

The rational of the study is to study the procedure for the production of Burkina and compare it to that of Degue to confirm whether they are the same product. It is also to conduct a study of the fermentation of milk during Burkina/Degue production in order to identify the microbial species responsible for the fermentation and the roles they play during fermentation. The technological properties of the fermentative microbial species will also be evaluated and the results used to develop a starter culture for the fermentation of milk during Burkina/Degue production. The antimicrobial properties of the fermentative microorganism will also be studied and used in selecting the suitable microorganism for starter culture to help assure the safety of the product. The information generated in the study can be used to upgrade the procedure used for Burkina production at the SME/industrial level.

General Objectives

The main objective is to develop a starter culture for the production of Burkina/Degue in Ghana with respect to upgrading the traditional procedure.

Specific Objectives

1. To assess the similarities between Ghanaian Burkina and indigenous West African Degue fermented milk products based on the production methods.

2. To identify the microorganisms responsible for the fermentation of milk during Burkina/Degue production in Ghana and their antimicrobial interactions.
3. To determine the chemical changes which occurs during Burkina/ Degue fermentation.
4. To develop a starter culture for Burkina/Degue production based on the technological properties of the dominant fermentative microorganisms.

Delimitation

Although yeasts can also be used as starter cultures in improving the safety and sensory qualities of fermented products, the study focused on the lactic acid bacteria in the fementing milk and their technological properties.for the starter culture development.

Limitations

Eventhough the field study was carried out using thirty (30) known Burkina processors from six (6) muslim communities in Greater Accra Region, only two indigenous Degue processors were identified and used for the study. This is as a result of some processors unwillingness to release the needed information on the Degue processing process whiles other just learn the processing methods from friends and decided to go into production in order to earn income; hence, the need to increase the study area probably into other regions for results generalization.

Organization of the Study

This thesis is arranged in six chapters. Chapter one contained background to the study, problem statement, rational of the study, main objectives, specific objectives, delimitations of the study, limitations of the study and the organization of the study. Chapter two includes an extensive review of relevant literature subjected to the study as well as possible methodologies that may be useful for the study. Chapter three provided methodologies used in arriving at the study objectives. Resuts are presented in chapter four. Discussion in chapter five and summary, conclusion and recommendations made based on the results obtained in chapter six.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

This chapter encompasses review of related works done by other intellectuals in the fied of traditional femented foods.

African Traditional Fermented Food Beverages

Indigenous food processing plays an important role in contributing to curtailment of post-harvest loses (Agoda, Atanda, Usanga, Ikotun, & Isong, 2011). African indigenous beverages are generally produced by women and children as a home art and if commercialised at the local setting, they become a means of economic empowerment to them (Abawari, 2013).

Traditional food beverages in Africa offer its consumers nutritional and therapeutic values especially the nonalcoholic grades (Aka, Konan, Fokou, Dje, & Bonfoh, 2014; Onuoha, Haruna, Yelmi, Samuel, Uhiara, & Ngwu, 2014). These beverages are full of vitamins and minerals. They have easily utilizable carbohydrates (sugars) due to the mixtures of grains used and the fermentation process they undergo (Amadou, Gbadamosi, & Le, 2011; Aka *et al.*, 2014). Beverages such as Kunu Gyada, a variety of Kunu from Nigeria are supplemented with nuts, tubers, and spices which further boost up their protein and amino acid level and at the same time their antioxidant properties (Ugwuanyi, Abubakar, & Onah, 2015).

Diversity of Traditionally Processed Food Beverages in Africa

Food beverages produced across Africa are usually unique to particular ethnic or cultural groups and the raw materials, origin, and processing techniques employed also defined to some extent, the socioeconomic class and tribe of the consumers (Kubo, Funakawa, Araki, & Kitabatake, 2014; Tafere, 2015). The processing techniques used include malting, boiling, pasteurization, fermentation, distillation and so on; which plays important role in flavour addition, complex compound digestion, anti-nutrient degradation, toxin biotransformation or elimination, and overall product quality improvement of the various beverages Egwaikhide, Malu, Lawal, Adelagun, & Andrew, 2014; Kubo *et al.,* 2014). Some traditional beverages produced in and across Africa run into millions of liters per annum, yet generally consumption per capita data are lacking (Kanyana, Ouma, & Van, 2013).

The following are some of the traditional food beverages produced in Africa:

Borde; this is a non-alcoholic drink produced from Barley, maize and wheat by malting, roasting, fermentation and boiling. This drink is produced in Ethiopia (Aka *et al.,* 2014). Mahewu, non-alcoholic drink from South Africa produced from maize through boiling and fermentation (Aka *et al.,* 2014). Oshikundu, non-alcoholic drink produced in Namibia from millet or sorghum through hot water treatment, back-slopping and fermentation Mu-Ashekele, Embashu, & Cheikhyoussef, 2012).

Malwa, a non-alcoholic drink obtained from finger, millet germination, roasting, boiling and fermentation. This drink is produced in Uganda (Aka *et al*., 2014). Bushera; non-alcoholic drink produced from sorghum/millet through germination, malting and fermentation. It is also produced in Uganda (Muyanja, Narvhus, & Langsrud, 2012). Fura da Nono, *is a n*on-alcoholic drink produced from fresh milk through pasteurization, back-sloping and fermentation in Nigeria (Egwaikhide *et al.,* 2014) Kunu or Kunu-zak, a non-alcoholic drink produced from sorghum or millet or maize through steeping, milling, boiling, fermentation and filtration. This traditional drink is produced in Nigeria (Amusa & Odunbaku 2009).

Mangisi, a non-alcoholic drink produced in Zimbabwe from millet malting, boiling, filtration and fermentation (Aka *et al.,* 2014) Zob, is another non-alcoholic drink produced from Roselle flowers through boiling and filtration in Nigeria (Onuoha *et al.,* 2014) Sour sop juice is a non-alcoholic drink produced from sour sop through extraction, fermentation and boiling. It is produced in Nigeria (Vwioko, Osemwegie, & Akawe, 2013). Palm wine a non-alcoholic drink produce in Ghana, Nigeria and Cameroon from palm sap through fermentation (Obahiagbon, 2009). Koko, from maize or millet from Ghana and Nigeria (Tulashie, Appiah, Torku, Darko, & Wiredu, 2017).

All these indigenous processed beverages are mostly consumed food-grade liquids, from single or mixed cereals, legumes, animal milk, and various plant

parts. They are produce using simple food processing techniques (Chibundu *et al.*, 2018) which are dated back to the prehistoric era and has consistently been a home-made art (Amadou, Gbadamosi, & Le, 2011).

Millet as a Cereal Grain

According to Amadou *et al.,* (2013), millet grain is a primary human food source in many regions of Pakistan, India, Asia and Africa. This cereal grain is believed to have its origin from North Africa and has been consumed since pre-historic times. The grains according to (Yang *et al*., 2012) have four main varieties which include; Pearl millet (*Pennisetum glaucum*), Finger millet (*Eleusine coracana*), Proso or white millet (*Panicum miliaceum*), and Foxtail millet (*Setaria italica*). FAO, (2009) indicated that, millet produce globally is about 32 million tonnes of which India produces the highest quantity (10,610,000) followed by Nigeria (7,700,000), Niger (2,781,928), China (2,101,000), Burkina Faso (1,104,010), Mali (1,074,440), Sudan (792,000), Uganda (732,000), Chad (550,000) and Ethiopia (500,000).

Nutritional and Health Benefits of Millet

According to Odusola, Ilesanmi, and Akinloye, (2013), millet posses a number of nutrients giving it advantages over other cereals used as food source. It possesses higher phenolic content with moderate reducing ability of high free radical because of its scavenging activity and therefore can serve as a source of antioxidants in diets.

Research according to Amadou *et al.,* (2013) had shown that, millet is very rich in resistant starch, insoluble and soluble dietary fibers, minerals, and antioxidants. Devi, Vijayabharathi, Sathyabama, Malleshi, and Priyadarisini, (2014) also indicated that pearl millet contained approximately, 7.8% crude fiber, 92.5% dry matter, 63.2% starch, 2.1% ash, 2.8% crude fat and 13.6% crude protein. Finger millet on the other hand contain 65-76% of carbohydrate, protein 5-8%, dietary fiber 15-20% and mineral 2.5-3.5%. A study carried out in Memorial University of Newfoundland in Canada, indicated a high antioxidant activity in both soluble and bound fractions of millet (Chandrasekara & Shahidi, 2010).

According to Devi, Vijayabharathi, Sathyabama, Malleshi, and Priyadarisini, (2011), millet has an excellent amino acid profile which is higher than that of corn and sorghum. The protein level of millet according to Odusola, *et al.,* (2013) consists of all varieties of essential amino acids including leucine a very good source of Tryptophan, an amino acid which is capable of raising serotonin level by so doing, helps in stress reduction. The presence of these nutrients makes millet a suitable source for baby's food, snack foods and dietary supplements (Liu, Tang, Zhang, & Zhao, 2012).

Millet Food Drink Products in Africa

Millet food drinks vary across Africa, from light to stiff or thick porridges. In Nigeria and Niger, the light porridge Fourra is very common whiles Senegal is noted for Soungouf, Sankhal and Araw (Obilana, 2014).

The Sahelian countries are noted for their stiff or thick porridges which includes Tuwo (Obilana, 2014). In Ghana, the people living in the Northern, Upper East and Upper West utilize millet as their traditional staple cereal. It is used in preparing foods such as Tuo, Hausa Koko and so on.

Milk

Milk according to Barnabas, Ayodele, Gana, Jiya and Igheghe, (2014), is an opaque liquid produced by the mammary glands of female animal including human which provides all the primary source of nutrition for newborn mammals before they are able to digest other types of food. Milk naturally according to Turck, (2013) is a rich source of energy, fat-soluble vitamins and essential fatty acids. Milk components may differ from species to species but in totality, it contains a significant amount of protein, calcium, saturated fat, B vitamins, B2 (riboflavin), B12, minerals, iodine, potassium and phosphorus (Barnabas *et al.*, 2014). Other components include conjugated linoleic acid, sphingolipids and carbohydrate mainly lactose of which cow milk contains about 4.5g per 100g (Tawiah, 2015).

Milk products forms the key food groups in many national dietary guidelines of which they play a part in dietary quality (Martin, Ling & Blackburn, 2016). Raw milk which is termed as unpasteurized or unhomogenized milk's consumption by humans was dated back to the period before the industrial revolution and was recognized as an important element of the pastoral civilization in Africa not only

for economic value, but also a social and cultural value (Mattiello *et al*., Zecchini, 2017). Indigenous groups like the Borani, Maasai, Tuareg and the Fulani's have a strong historic dairy tradition. They share many practice and regard milk as a product of harmony that is freely offered to relatives, friends and visitors (Ndambi, 2008). FAOSTAT, (2016) recorded that, the total milk production in Africa in the year 2014 was 46,907,955 million of tonnes which amount to only 6% of world average production and out of which cow milk was the majority that is 74.05% of the total production followed by goat 8.74%, buffalo 6.23%, camel 5.76% and sheep 5.23%. Out of this 6%, 50% was produced by six countries with Sudan producing the highest tonnes of 4,391,000 followed by Egypt 5,598,477 tonnes, Kenya 4,925,692 tonnes, Ethiopia 3,699,373 tonnes, South Africa 3,337,018 tonnes and Algeria 4,241,414 tonnes.

A research conducted by Ndambi, (2008) indicated that, only about 15% of the total milk produced in Africa is processed to standard products such as cheese, yoghurt, butter, and so on. The remaining that is, more than 80% of total milk produced goes through informal markets or is consumed on the farm (Tankoano, *et al.,* 2016).

Traditional milk food products

Intake of raw untreated milk is still in used by certain ethnic groups especially the Fulani's. Also, a large number of farm families and workers who also due to the growing segment of the general population although believed that intake of raw

untreated milk is unsafe, bought into the idea that, pasteurization affect the imparts and health benefit provided by the raw unpasteurized milk Tawiah, 2015).

In some countries, attempts are ongoing in order to valorize the local dairy products. For instant in South Africa a Slow Food Presidium has been established to promote high-quality. South African raw milk cheeses, such as ficksburger, ganzvlei vastrap, karoo crumble and huguenot, produced by small-scale local farmers using only raw milk by adopting environmentally and welfare friendly techniques, respecting local culture and traditions (Mattiello *et al.*, 2017). The following are some of the milk food products produced in Africa as shown in table 1

Table 1- *Some Milk Food Products Produce in Africa*

Product's Name	Nature of Product	Country	Reference
Ayib	fresh cheese produced from cow milk	Ethiopia	Geremew, (2014)
Gibna Bayda	Fresh cheese processed from cow milk	Sudan	Salih *et al.*, (2011)
Kariesh	fresh cheese produced from Buffalo or cow or the mixture	Egyptians	Todaro *et al.*, (2013)

Table 1- *Continued*

Product's Name	Nature of Product	Country	Reference
Bouhezza	ripened cheese produced from goat, sheep or cow milk	Algeria	Zitoun *et al.*, (2012)
Mish	ripened cheese produced from cow milk	Sudan and Egypt	Salih *et al.*, (2011)
Amabere amaruranu	fermented cow milk	Kenya	Nyambane *et al.*, (2014)
Amasi	fermented cow milk	Lesotho, Zimbabwe	Schutte, (2013)
Biruni	fermented cow milk	Sudan	Salih *et al.*, (2011)
Ititu	fermented cow milk	Ethiopia	Geremew, 2014)
Kwerionik	fermented cow milk	Uganda	Schutte, (2013)
Lben	fermented milk Africa goat, sheep or cow	Algerian	Benkerroum,(2013)
Madila	fermented cow or goat milk	Botswana	Schutte, (2013)
Masse	fermented cow milk	Mozambique	Schutte, (2013)
Nono	fermented cow milk	Nigerian	Ogbonna, (2011)
Nunu	fermented cow milk	Ghana	Akabanda *et al.*, (2014)

Mattiello *et al.*, (2017)

Degue

Degue is an indigenous fermented milk food drink born out of ethno-knowledge believed to originate from the Fulanis'.

The food drink from its origin is produced from fermented cow milk and pelleted steamed millet flour. It is a widely consumed food drink sold in local markets along the lanes, public places in Burkina Faso, Mali, Benin, and beyond West Africa (Tchekessi, *et al*, 2014). According to Angelov, Petrova, Angelov, Stefanova, Bokossa, Tchekessi, Marco and Gotcheva, (2017), Degue is produced by varying methods and its consumption has been increasing in the past few years. The production generates job for thousands of people especially women, whose education and skills in food processing are often limited.

Processing of Degue

The procedure for the production of Degue is shown in the flow diagram in Fig 1 Degue is produced from whisk and sieved spontaneously fermented cow milk of which the fermentation is carried out in calabashes, gourds, clay pots or plastic container at room temperature for twenty-four hours.

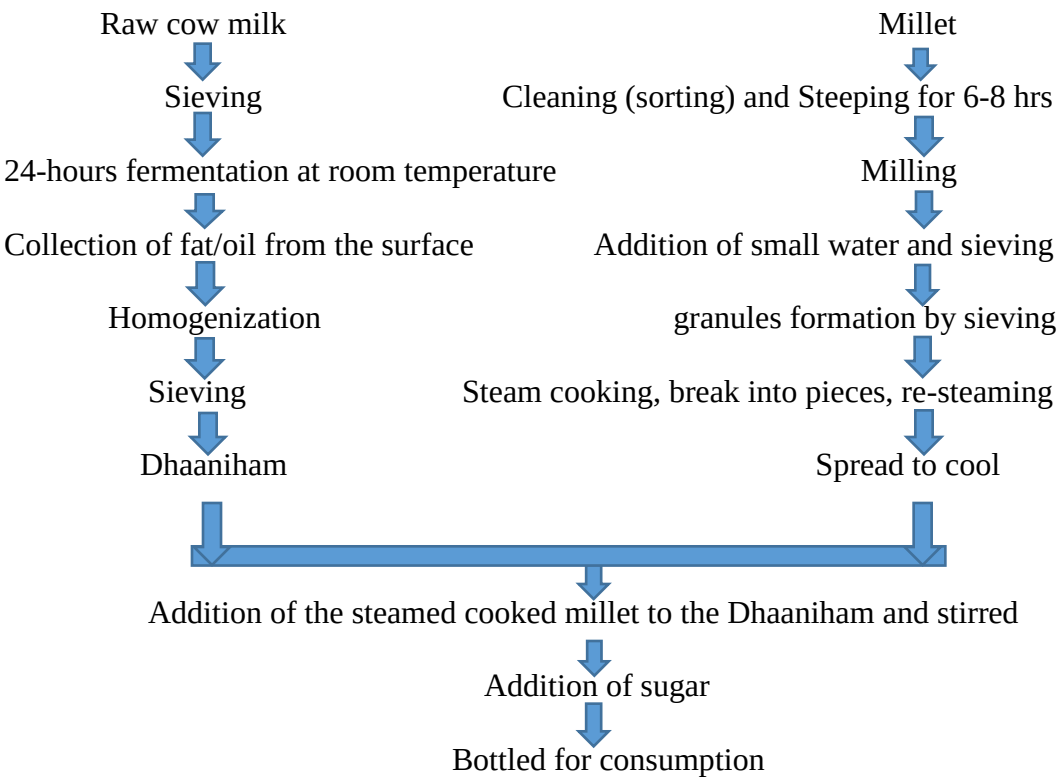

Figure 1: Degue production flow diagram.

During the fermentation, the raw cow milk is allowed to stay undisturbed at room temperature for about twenty-four hours after which the fat/oil in the milk settled at the surface which is then collected; the coagulated milk is then homogenized by whisking using stick stirrer rub in-between the palm and then sieved. The fermented sieved milk at this stage is known in the Fulani language as Dhaanidham. The millet on the other hand is prepared by sorting of the grains, steeped for six to eight hours and milled into flour. The flour is then mixed with a small amount of water and press through a sieve with about 4mm holes to obtain small granules. The granules are then steam in colander for about one and half hour. The steamed millet granules which normally get stuck together is

transferred into a bowl and a small quantity of water is added and the lumps broken into pieces whiles still hot. The granules are then re-steamed for about thirty minutes, transferred into a bowl and allow to cool. The cooled prepared granulated millet is then mix with the already prepared Dhaaniham couple with addition of sugar and then package.

Fermentation

Fermentation according to Smid and Hugenholtz, (2010), is an ancient biotechnology used in food processing which results in desirable properties of the food. During the fermentation process, carbohydrates are converted into alcohol and carbon oxide or organic acids using bacteria or yeast under anaerobic conditions (William & Dennis, 2011).

The food substrates according to Steinkraus, (2018) are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids to nontoxic products with flavors, aromas and textures, pleasant and attractive to the human consumer. The enzymes produced by these edible microorganisms during the fermentation process, helps in the extension of the shelf-life of the food by forming inhibitory metabolites such as organic acid, lactic acetyl, reutrin, and bacteriocins, often with decreased of pH (Gaggia, Di-Gioia, Baffoni, & Biavati, 2011). The safety of the food is also secured since the process helps in pathogens inhibition

(Steinkraus, 2018). The nutritional value of the food is improved as well as its organoleptic properties (Van *et al*., 2010; Sicard & Legras, 2011).

Fermentation processes mostly depend on the climatic condition of the area as well as the type of lactic acid bacteria, yeasts or the mixture of the two groups used as the functional microorganisms. Traditional fermented milk in regions with a cold climate interms of temperature contain mesophilic bacteria such as *Lactococcus* and *Leuconostoc* spp., whilst thermophilic bacteria, which include mostly *Lactobacillus* and *Streptococcus*, prevailed in regions with a hot, subtropical or tropical climate (Akabanda, Owusu-Kwarteng, Glover & Tano-Debrah, 2010; Yu, *et al*., 2011).

Food Borne Pathogens and their Survival in Fermented Milk

Milk is known as one of the natural habitats of lactic acid bacteria which are normally regarded as safe but can also be contaminated by other milk spoilage organisms found in the wash water, equipment used during the milking process, the environment or on the personnel at the point of the milking and handling process (Sansanwal, Ahlawat, & Dhanker, 2017). Fermented milk are considered to be safer than that of raw milk, however, *Escherichia coli 0157:H7, Staphylococcus aureus* were reported as the most common foodborne pathogens that are present in many foods and able to survive in milk and fermented milk products during the past decades (Kivanc & Yapici, 2019). This could be as a result of inadequate pasteurization. Pathogen such as *Listeria monocytogenes,*

Salmonella typhimurium, Escherichia coli O157:H7, Vibrio cholerae and Staphylococcus aureus may grow and multiply in milk since milking in Africa is mostly done by hand in open areas, especially in the country side under very poor hygienic conditions. This contamination can come from several sources including diseased animals, milk handlers, and contaminated equipment (Heredia & García, 2018; Soliman & Ahmed, 2019). The ability of foodborne pathogens to survive the exposure of the acidic conditions (pH 3) encountered in the stomach of their respective host for up to 2 hours before passage to the intestinal tract is the key determinant of their infectious dose (Heredia & García, 2018)

Acidity plays an important role in bacterial control; therefore, the ability of foodborne pathogens to attach to the mucosa of their host, invade the tissues and produce toxins and overcome their host's defense mechanisms determines their virulence nature.

Traditional Milk Fermentation

Dairy products are very important food for many people especially, those engaged in stock farming as a result, milk has been preserved since early times by fermentation yet the exact origin of milk fermentation is difficult to be established but rather safe to assume that it could be date more than 7,000 years ago as the way of life of humans as they changed from food gathering to food producing (Hill *et al.*, (2017).

Fermented milk products are therefore produced traditionally through out the world; that is, in Eastern and Northern Europe, Middle East, Asia, and Africa (Akabanda *et al.,* 2010; Sansanwal, *et al.*, 2017; Parker *et al*., 2018). Studies conducted according to Akabanda *et al.,* (2010) indicated that, the microbiological characteristics of several fermented milk have been studied in Indonesia, Zimbabwe, South Africa, Morocco and Tanzania showing that, some of the major fermentation processes are based on the use of lactic acid bacteria, which produced organic acids.

The presence of these fermentative lactic acid bacteria is indispensable as far as the intrinsic properties of the fermented milk food products are concerned (Hill *et al.*, 2017); hence, Fulanis still ferment their milk in calabashes, gourds and clay pots since such milk products still enjoy loyal following in rural communities (Akabanda *et al.,* 2010). The containers are seeded with natural fresh milk microbial inoculums before used for the production of the fermented milk. The containers are filled with milk, covered and placed in the house. The fermented milk coagulates and the whey and proteins homogenized (Akabanda *et al.,* 2010).

Microflora involved in milk fermentation

Milk is known as one of the natural habitats of lactic acid bacteria (Delavenne, Mounier, Déniel, Barbier & Le-Blay, 2012) and they are the most extensively studied microorganisms for milk fermentation (Perez, Zendo, & Sonomoto, 2014). Yeast on the other hand is known to be the most important contaminants in

milk and a major cause of spoilage in milk products (Pelagia Research Library, 2015) because; the low pH offers a selective environment for their growth.

Leben according to Bensalah, Delorme and Renault, (2009) is Algerian fermented milk product prodced from cow, ewe, goat and camel. Howevere, the predominamt LABs isolated from Leben were *Lactococcus lactis* and *Streptococcus thermophilus*. El-Baradei, Delacroix-Buchet, and Ogier, (2008) also recorded isolating *Streptococcus thermophiles*, *Lactococcus lactis* and *Lactobacillus delbrueckii subsp. Bulgaricus* as predominant LABs from Egyptians fermented milk known as Zabady produced from fermented cow and Buffalo milk.

Lben a fermented milk product produced in Morocco from fermented cow or goat milk also recorded predominant LAB such as *Lactococcus lactis* according to Mangia, Garau, Murgia, Bennani and Deiana, (2014) *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were recorded by Nyambane, Thari, Wangoh, and Njage, (2014) as predominant LABs isolated from Amabere Ama Ruranu a fermented cow milk produced in Kanya. Suusac, a fermented camel milk produced in Kenya and Somalia according to Njage *et al*., (2011) and Jans, Bugnard, Njage, Lacroix, and Meile, (2012) also recorded *Streptococcus thermophilus* and *Weissella confuse* as predominant LAB isolates. Nyarmie, a fermented cow milk produced in Ghana according to Obodai and Dodd as cited by Jans *et al*., (2017) recorded *Leuconostoc mesenteroides subsp*. *Mesenteroides*

as one of the predominant LABs. *Lactobacillus plantarum* and *Lactobacillus fermentaum* were also isolated and identified by Akabanda *et al.*, (2013) as predominant LABs associated with Nunu a fermented cow milk produced in Ghana.

The initial facts about the microbiological composition of fermented milk products came to light at the end of the 19th century when diverse microorganisms were reported in the early investigations made by Grigoroff in the year 1905 and Metchnikoff in the year 1907. Metchnikoff (1907) first isolated *Bacillus bulgaricus*, cocci and yeasts from yoghurt after Grigoroff isolated rod-shaped bacteria called *Bacillus* A from Bulgarican milk in the year 1905 (Lakofa, 2009). Based on his findings, Metchnikoff suggested that man should consume milk fermented with *lactobacilli* to prolong life as cited by Sansanwal, *et al.*, (2017).

The presence of lactic acid bacteria in the milk during the time of fermentation can either be spontaneous or by the used of starter cultures. Spontaneous fermentation method is the traditional method used over the years. It is the fermentation process in which starter inoculums are not used. The contaminated organisms in the milk are allowed to undergo competitive activities and those best adapted to the milk substrate eventually dominate the process. In numerous traditional fermentation processes, the 'back-sloping' method is applied. That is,

the material from previous successful batches is added to facilitate the initiation of a new process (Tamang, Watanabe, & Holzapfel, 2016).

Starter Culture

Sulieman, (2017) defined a starter culture as microbial preparation of large number of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. On the other hand, it can also be defind as a mixture of viable microorganisms that may be added intentionally to a food substrate in order to accelerate its fermentation process with the intention of providing desirerable changes in the end product. The addition of the selected starter culture directly to the raw material according to SKLM, DFG Senate Commission on Food Safety, (2010) has been a breakthrough in the processing of fermented foods resulting in a high degree of control over the fermentation process and standardization of the end product.

Starter cultures are originally maintained by daily propagation but are currently available in frozen concentrates, dried or lyophilised preparations produced in an industrial scale with some allowing direct vat inoculation (SKLM, 2010). Continuous revolving of starter cultures through continuous propagation can lead to the loss of their uniqueness; this is because, the organism used can loss its metabolic traits that is plasmid-encoded or genetic material due to adaptation to

the food matrix (Sulieman, 2017). The microorganisms used in starter culture industry include bacteria, yeasts and moulds.

LAB Starter Cultures and their Products

LABs are intentionally introduced in to the milk in a form of starter culture which is intended to bring about unique qualities of the milk with regard to preservation, nutritional value, wholesomeness, texture, taste and flavour. They are also used as probiotics; that is, their presence in the milk product if ingested in sufficient amount benefits the host by improving the microflora in their gut (Yerlikaka, 2014). *Lactococcus lactis subsp. lactis* according to Sulieman, (2017) is being used in Gouda and Edam; many cheeses, butter, butter milk, sour cream and lactic butter. Gemechu, (2015) also recorded that, *Lactococcus lactis subsp. lactis* is used as mesophilic starter for many cheese types, butter and butter milk. *Streptococcus thermophilus* on the other hand is used as thermophilic starter for yogurt and many cheese types particularly hard and semi hard high-cook cheeses (Ravyts, Vuyst, and Leroy, 2012). *Lactobacillus plantarum* according to Gemechu, (2015) is also in used for probiotic milk and cheese ripening adjunct culture.

Lactic Acid Bacteria

Lactic acid bacteria according to Sulieman, (2017) and Bintsis, (2018) is a broad term used for a group of non-motile, non-spore forming, rods or cocci, catalase-negative and Gram-positive bacteria. They are fastidious organisms which require

a complex growth medium. They are able to adapt to both anaerobic and aerobic conditions distinguishing them as important microbs for industrial applications. They attack carbohydrates by fermentation (in the absence of oxygen) through metabolic pathways; that is, either homo or hetero pathways (Embden-Meyerhof or Phosphoketolase pathways) to produce lactic acid as their major end product (Ravyts *et al.*, 2012).

The homofermentative lactic acid bacteria produce only lactic acid whiles the heterofermentatives produce 50% lactic acid, 25% acetic acid and ethyl alcohol and 25% carbon dioxide (Adams & Moss, 2008). All lactic acid bacteria make used of the glycolytic pathway except group III *Lactobacilli, Oeno cocci, Weissellas* and *Leuconostocs (*Adams & Moss, 2008). The type of metabolic pathway used by the lactic acid bacteria during the fermentation process is used accordingly in grouping them. The important members of this group are the following genera; *Bacillus, Lactococcus, Leuconostoc, Streptococcus* and *Pediococcus.* Generally, most of these lactic acid bacteria have complex nutritional requirements since they lack many biosynthetic capabilities hence termed heterotrophic organisms (Sulieman, 2017).

LAB Identification

Rapid and accurate bacterial identification has been in search over the years. New techniques are being search resulting in evolution from the first generational methods (Conventional method) which includes the identification based on

growth in different pH, growth in different temperatures, air, colony examination, microscopy, biochemical test up to the use of API bioMérieux. The second generational method (Automated phenotypictest) which involves the use of Microscan-Walkway, Phoenix®BD and Vitek:2®bioMérieux had given way to the third generational method which involves the used of molecular techniques such as PCR, and 16s rDNA sequencing. The fourth generational method (MALDI-TOF SM) is the latest method used in the bacteria identification. It involves the use of Axima Assurance Vitek MS® Shimadzu bioMérieux and MALDI Biotyper ® Microflex LT Bruker BD (Santos, Cayô, Schandert and Gales, 2013)

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF Mass Spectrometry emerged as a new technology for bacteria identification. It can be used to analyze the protein composition of a bacterial cell, measuring the exact sizes of peptides and small proteins, which are assumed to be characteristic for each bacterial species (Nacef, Chevalier, Chollet, Drider & Flahaut, 2017). The used of MALDI-TOF MS has proven to be a recognized bacterial chemotaxonomic method. This was noted when compared with conventional phenotypic and PCR-based identification. It shows rapid turned around time, low sample volume requirements and modest reagent costs (Cherkaoui *et al.,* 2010). It is very fast in terms of organism identification and can determine the species within a few minutes if the analysis starts with whole cells, cell lysates, or crude bacterial extracts (Nacef *et al.,* 2017).

How MALDI-TOF MS Works

MALDI-TOF MS bacterial identification is based on the analysis of protein spectrum from bacterial ribosome, which is closely related to the analysis of gene 16S rDNA sequence. The identification is based on the assessment of ribosomal proteins in the cell since they are part of the cell translational apparatus and can be found in all living cells. The matrix however, permits these proteins which are non-volatile and thermally labile compounds to be ionized and form intact ions in the gaseous phase; with infrared or ultraviolet pulsed (a laser beam) serving as ionization or desorption source. The matrix then absorbs the laser energy and induces partial vaporization of the sample which is immediately transferred electrostatically to a mass spectrometer vacuum chamber as soon as the vaporization and ionization of molecules occurred. The migration of these ionized particles formed is proportional to the ratio charge mass; that is, the minor ion reaching TOF detector before larger ions. Identification therefore is based on the score value of the ions released by the equipment (Santos *et al*., 2013).

MALDI-TOF MS runs on BioTyper database containing references. The identification is done by matching between the experimental bacteria isolates and the reference. The results are expressed by BioTyper according to a Log (Score) and an associated-colour code (green, yellow and red). A BioTyper Log (score) exceeding 2.3 (green colour) indicates a highly probable identification at the species level. Log (score) between 2.0 and 2.3 means highly probable identification at the genus level (green colour). A Log (score) between 1.7 and 2.0

(yellow colour) implies only probable genus identification, while score value under 1.7 (red colour) means no significant similarity between the unknown profile and any of those of the database (Nacef *et al.,* 2017).

MALDI-TOF MS is currently the new technology for identifying lactic acid bacteria cultures. A research conducted by Ozaslan, Kilic, Bayil-Oguzkan, Kurt and Erdogan, (2017) indicated that, MALDI-TOF MS can be used for the rapid identification of lactic acid bacteria associated with traditionally fermented food products.

Factors to Consider in Selecting Lactic Acid Bacteria as Starter Cultures for Milk Fermentation

In selecting lactic acid bacteria for starter culture development for milk fermentation, there are a number of functional properties that need to be measured since the desired characteristics of the final product largely depend on them (Soro-Yao, Brou, Amani, Thonart, & Djè, 2014).

Bio-preservative properties of lactic acid bacteria

Milk being a highly perishable product cannot stand at a point in time therefore the need to prolong its shelf-life and by so doing, preserve its nutritive components. As stated early on by Delavenne *et al.,* (2012). The LAB's presence in the food brings about the production of different metabolites and organic acids by the utilization of metabolize sugars. These metabolites and organic acids are

found to be antimicrobial compounds that serve as preservatives for the fermented food or the milk. These compounds include; organic acid (lactic acid, acetic acid, formic acid, phenylactic acid, caproic acid), bacteriocins, ethanol, hydrogen perioxide acetaldehydes, diacetyl, carbon dioxide and reuterin (Özogul & Hamed, 2018).

Bacteriocins production

In order to extent the shelf-life of the milk, Adams and Moss, (2008) indicated that, lactic acid bacterial present in the milk produce a product called Bacteriocins, a bacteriocidal compound which is active against other organisms. They are of protein structure either proteins or polypeptides that possesses antimicrobial properties and are produce at the primary phase of the bacteria growth. They can be grouped into three classes according to Yantyati and Andi, (2014). These are:

1. Lantibiotics; a small (<5kDa), heat stable peptide substance which contain polycyclic thioether amino acids that is lanthionine or methyllanthionine. It also contains dehydroalanine and 2-aminoisobutyric acid.
2. Non- Lantibiotics; a little bigger in size (<10kDa) which is also relatively heat stable. It contained active membrane peptides.
3. Bacteriocins; they are generally larger with a molecular weight of (>30kDa) and are found to be heat labile proteins.

Nisin a bacteriocins produced by *Lactococcus lactis* according to Balciunas *et al.*, (2013) is the only bacteriocins officially approved worldwide (Yantyati *et al.*, 2014).

Fast acid production

Lactic acid bacteria exert antimicrobial effect around themselves by producing organic acids such as acetic acid, lactic acid and propionic acids which lower the pH of their environment making it unsafe for other spoilage organism to cope (Gemechu, 2015). Acidification during fermentation influence product quality such as the food safety improvement and organoleptic qualities improvement (SKLM, 2010).

The ability of the LAB to produce sufficient quantities of organic acids to reduce pH to or below 4.0 within 24 hours during the fermentation is an essential requirement for lactic acid bacteria used for the production of the starter culture. Moon *et al.*, (2012) reported that, a newly identified *Lactobacillus paracasei subsp. paracasei* CHB2121 was used to produce 192 g/L lactic acid from medium containing 200 g/L of glucose showing that the organic acid production by LAB is largely influenced by the type of media used. This was proven by a study conducted using 10 strains of *Lactobacillus* according to Yantyati *et al.*, (2014). *Lb. fermentum* was also noted for its role in acidification by lowering pH, exhibiting faster rate of acidification during spontaneous fermentation of many cereals. This was also reported by Mukisa, Ntaate, and Byakika, (2017). A study

conducted according to Yantyati *et al.,* (2014) shows strongly the inhibition capacity of *Lactococcus lactis* strain that is *Lb. lactis* subsp. *lactis* biovar. *diacetylactis* to the pathogenic *Escherichia coli*and *Salmonella enteritidis* as a result of its fast acid production resulting in rapid pH reduction making it recommendable for use as a starter culture.

Dominating the population among the indigenous microbiota

Lactic Acid Bacteria dominates the indigenous microbiota during milk fermentation has been related to its ability to grow under fermentation conditions. Also its ability to produce antagonistic substances, such as bacteriocins, lactic acid, acetic acid, ethanol, hydrogen perioxide acetaldehydes, diacetyl, carbon dioxide and reuterin (Özogul & Hamed, 2018) creating unfavourable condition for other competitive microbiota.

Good probiotic effects

Most LABs involved in fermented milk products are considered as possible probiotics. They interact intensively with the milk matrix and their environment by exchanging components of the medium for metabolic products (Burgain, et al., 2014). Probiotic LABs hydrolyzed milk proteins to produce essential amino acids and milk lipids to produce short-chain fatty acids (Motta & Gomes, 2015). They also take care of lactose intolerance people by stimulating the secretion of enzyme β-galactosidase in their intestine since luck of this enzyme makes it difficult for them to make use of lactose efficiently (Kim *et al.,* 2016).

Lactic acid bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* when used as probiotic bacteria simultaneously induced production of these enzymes which hydrolyses lactose reducing its content by 20-30 % or more making digestibility of dairy products possible and their nutrients readily accessible to their host (Wedajo, 2015; Bintsis, 2018). They regulate γ-interferon production by human peripheral blood lymphocytes invitro which exhibits antiviral and anti-proliferative effects. They also activate macrophages and lymphocytes and Ig A by enabling them to protect their host against infection cause by enteric pathogens (Soccol *et al.*, 2010).

LABs expected to be used as probiotics must be able to trigger antagonism in the intestine of their host and must be able to adhere to the intestinal mucusaof the host organism, colonized the lumen of the tract in order to stabilize the intestinal microbiota. They must also be able to produce antimicrobial substances that will counteract the action of pathogenic microorganisms and stimulate host immune response (Soccol *et al*., 2010). LAB used as probiotics prevent the growth of pathogenic microorganisms through competition leading to the production of antimicrobial compounds including organic acid which eliminates other microbiota. They also survive passage through the upper gastrointestinal tract due to their tolerance to organic acid (Soro-Yao *et al.*, 2014).

Organoleptic and Sensory Properties of Lactic Acid Bacteria

Fermented milk products are known for their desired texture, taste, aroma and flavour. To maintain these desired qualities in pasteurized milk products, LABs with these organoleptic functional properties are considered for the starter cultures. This is implemented by carefully selecting LAB strains that will express the desired properties, maintain a perfect natural and healthy product (Ravyts *et al.*, 2012; Bintsis, 2018). Ravyts *et al.*, (2012) recorded that several lactobacilli and streptococci possesses these functional properties. They contribute in the development of the organoleptic properties due to their carbohydrate metabolism.

Texture and taste improvement

Fermented foods provide the consumer with some desirable qualities. Fermentation process leads to the acidification of the food, resulting in tangy lactic acid taste (Motta & Gomes, 2015). The desire to improve upon the mouthfeel, texture and viscosity of fermented food products has created a lot of attention leading to exploration of Expolysacharides (EPSs) (Nwodo, Green and Okoh, 2012).

Expolysacharides are sugar monomers composed of one type monomers (homopolysaccharides) or multiples of monomers (heteropolysaccharides) and are presented in a form of loose slime or capsule tightly attached to the cell wall of LABs according to Nwodo *et al*., (2012) and Mishra and Jha (2013). The EPs enhances the viscosity, taste and also stabilizes the food matrix by directly

defusing into the matrix of the food to enhance water binding capacity of the food metrix. The LABs such as *Lb. delbruekii* subsp *bulgaricus, Lb. lactis ssp. lactis* and *S. thermophilus*are known to be Expolysacharides producing Starter with a specification of Heteropolysaccharides whiles *Leu. Mesenteroides* is known for homopolysaccharides production (Behare, Singh, Kumar, Prajapati & Singh, 2009; Mishra & Jha, 2013).

Production of aroma and flavour

The quality of the dairy product in terms of aroma and flavour largely depends on the LAB type, microbial load and spectrum of the raw milk. The proteolytic and lipolytic activities of LAB in milk fermentation offer the milk product some desirable aroma and flavour (Motta & Gomes, 2015).

The production of these desirable aromatic compounds is very important since they improve the products quality and increases its consumer acceptability. The most important factors that give fermented milk its specific identity are the flavour and aroma compounds. These compounds include acetoin, diacetyl, acetylaldehyde, and alcohol in some cases. One of the most flavouring compounds produced by lactic acid bacteria during milk fermentation is acetylaldehyde, which happen to be a very important aroma compound in yoghurt and other related fermented milks products; and is required at 10-15 mg/L to provide the yogurt and other fermented milk product their typical and fresh aroma they required (Yıldız-Akgül, Yetişemiyen, Şenel, & Yıldırım, 2018).

Diacetyl on the other hand when produced in yoghurt at a very small quantity enhances the principal aroma compound contributing largely to the pleasant and delicate flavour of the fermented milk product (Ao *et al*., 2012). The metabolism of lactose, lactate and citrate, the lypolysis of fatty acids and the proteolysis casein and amino acid catabolism by LAB during fermentation brings about flavour and aroma development (Motta & Gomes, 2015).

Flavour and aroma formation in fermented milk products by LAB largely depend on high biosynthetic capacity they posses; however, Motta and Gomes, (2015) reported that, available data indicating that, majority of the intensive flavour associated with traditionally fermented products is a proceed from non-starter lactic acid bacteria (NSLAB) which developed through mutation as a secondary flora within the period of fermentation.

Sensory Evaluation

Sensory tests have been part of human beings since memorial when man began to evaluate goodness or badness of consumables including food and water. Sensory evaluation of traditional milk products has been based on the determination of off-flavours or defects and the quality scorecard for defect identification was first proposed by the Federal Dairy Division in the early 1900s according to Schiano, Harwood and Drake, (2017). For today's consumers, so long as food smells good, tasty and appealing to the eye, consumers are willing to pay for it without thinking of its safety and nutritional qualities.

The critical process in this food choice decision making by the consumers can be linked to the cognitive, affective, conative and economic considerations of the consumers (Singh-Ackbarali & Maharaj, 2014). According to Schiano *et al*., (2017), to develop a new product that will find its place, remain in the market and survive the market's competition, it is essential to produced it in accordance with the needs of the targeted market and its end-users; that is, understanding consumer perception, especially as they applied to food. Also, to obtain a successful business in terms of new product, strategic quality management is an important activity (Hansen & Hamilton, 2011).

Sensory evaluation was defined by Lawless and Heymann (2010) as a scientific discipline used to evoke, measure, analyse and interpret responses to products as perceived through the senses of sight, smell, touch, taste and hearing. This means in evaluating sensory qualities of food products, sensory receptors such as olfactory, taste, and visuals are employed (Singh-Ackbarali & Maharaj, 2014); hence, companies in food industries today are integrating sensory science and methodology in their marketing departments in order to conduct consumer tests and preference on their products in order to maintain the product's quality and also acceptability of innovated products (Camargo and Henson, 2015).

Sensory evaluation methods

Sensory methods used in evaluating food and beverage involves the expertise of one, two or more trained personnel who assign quality scores to the appearance,

taste, texture and flavour of the products. The scores are however based on the presence or absence of predetermined defects which traditionaly have several shortcomings. They can not really predict consumer acceptance since the quality assessments employed are mostly subjective (Lawless, & Heymann, 2010). The testing and scoring are determined by individual decisions based on the use of senses without the use of any mechanical device. The tests are of three types. This includes Discrimination or Difference Tests, Descriptive Analysis and Affective or Preference Hedonic Tests. Difference testing is designed to determine whether there are any detectable differences existing between the products. Preference testing on the other hand is used to determine the acceptability or preference between products. Whiles descriptive testing is the testing which provides information on selected characteristics of food samples.

In assessing food quality in terms of appearance the panels take into consideration the colour, size, shape, transparency, dullness, gloss, grainy, foamy, greasy, shiny, stringy, crystalline etc. nature of the food. Also when considering the tasty nature of the food, the food is being examine on the bases of mouth feel, sweet, salty, sour, bitter, cool, zesty, hot, tangy, sharp etc. whiles flavour and aroma, is analyzed based on fragrant, rotten, acrid, musty, scented, pungent etc.

CHAPTER THREE

MATERIALS AND METHODS

This chapter includes the study design and the methods employed to achieve the set objectives.

Study Design

The study involved a survey to gather information on the traditional Degue and Burkina production for the purpose of identifying traditional Degue producers for sample collection for laboratory microbiological and chemical analysis to select isolates for fermentation trial, product starter culture development, production of Degue using the developed starter culture and sensory analysis on the starter culture developed degue. The survey was carried out using open-ended semi-structured questionnaire in exploring the knowledge level of thirty (30) Burkina producers on the bases of steps involve in Degue and Burkina production, the differences between Degue and Burkina, the origin of Degue, the materials used and the origin of Degue and Burkina, origin of the producer's production techniques. Bases on the survey, only two women were identified to have indegeneous knowledge in Degue production and were selected for the study; one a Fulani and the other lived in Burkina Faso for a long time and was familiar with the production of traditional Degue.

Study Area

The survey was carried out rondomly in the following six Muslim communities in Greater Accra known for Burkina production: Ashiyie, Fulani, Animal Research, Madina, Nima and Mamobi. These areas were selected because according to Tawiah, (2015), Burkina is produced mostly in the Muslim communities and also most venders indicated that most of the burkina they have been selling were from these areas.

Sample Collection and Preparation

Each processor produced Degue for analysis on two separate occasions.

The fermenting milk samples were collected from the two processors at 0 h, 12 h and 24 h of the fermentation for chemical and microbiological analysis. Steeped millet water was also collected at 0 h and 8 h of steeping. The samples were collected aseptically using steril zip lock bags and transported on ice in a clean sterile ice chest to Council for Scientific and Industrial Research - Food Research Laboratory where the were kept in a refregirator after which microbiological and chemical analysis were carried out on them.

Chemical Analysis

Determination of pH

The pH of the fermenting milk and the steeped water were determined directly using a pH meter (Radiometer pH M 92. Radiometer Analytical A/S, Bagsvaerd, Denmark) after calibration using standard buffers.

Determination of titratable acidity

For each sample 10 ml or 10 g was titrated against 0.1 N NaOH using 0.5 % freshly prepared phenolphthalein as indicator as described by AOAC(2000).

Microbiological Analysis

Enumeration of aerobic mesophiles, lactic acid bacteria, yeast and moulds

To enumerate aerobic mesophiles, lactic acid bacteria, yeast and moulds, one hundred milliliters (100 ml) of the fermenting samples was homogenized in a stomacher (Lad Blender, Model 4001, & Seward Medical). One milliliter (1ml) of the homogenized sample was added to 9 mls of pepton water [0.1 % peptone water (Oxiod CM0009; Oxoid Ltd., Basingstoke, Hampshire, UK)] diluted to form the 10^{-1} dilution. Similarly, other ten-fold dilutions were prepared. From the appropriate ten-fold dilutions, enumeration of Aerobic Mesophiles was carried out on plate count agar [Oxiod CM325; Oxoid Ltd., Basingstoke, Hampshire, UK] in accordance with (ISO 4833-1, 2003). The plates were incubated aerobically and anaerobically at 30 ^{0}C for 24 to 48hours.

Lactic acid bacteria were enumerated by the pour plate techniques using deMan, Rogosa, Sharpe medium (MRS, Oxoid CM361) agar with pH 6.2 and 0.1 % cycloheximide added to suppress yeast growth. The plates were incubated anaerobically in an anaerobic jar at 30 °C for 48 hours using CampyGenTM 2.5L (Mitsubishi Gas Chemical Company inc. Japan) to create anaerobic conditions during the incubation (De Man, J. C., Rogosa, R. & Sharpe, 1960).

Yeasts and Moulds were enumerated by the plate count techniques on Chloramphenicol supplemented Dichloran Rose-Bengal Choramphenical Agar (Oxoid CM727; Oxoid Ltd., Basingstoke, Hampshire, UK) to inhibit bacteria growth. The pH was adjusted to 7.0 and incubated at 25 °C for 72 h in accordance with ISO 21527-1, (2008).

Isolation of lactic acid bacteria

About 16 colonies of lactic acid bacteria were selected from a segment of the highest dilution on suitable MRS agar plate. Each colony was sub-cultured in MRS broth (Oxoid CM359; Oxoid Ltd., Basingstoke, Hampshire, UK) and purified on MRS agar (MRS, Oxoid CM361) by repeated streaking.

Determination of LAB species population in the fermenting milk

This was carried out by giving code to individual LAB isolates in a segment of highest dilution on suitable MRS agar plate before counting. The count allocated for each coded individual isolate was recorded. The coded colonies were individually fransferred into respective coded tubes containing 5 ml steril MRS broth (Oxoid CM359; Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 30 ^{0}C for 48 h and purified on MRS agar (MRS, Oxoid CM361) by repeated streaking.

Based on their phenotypic characteristics, the individual coded isolates were grouped and identified using API 50CHL and MALDI-TOF. Based on the code given to individual isolates on the enumerated plates, and the count allocated to

each isolate, the total count of each LAB species was determined in terms of colony forming unit per milliliter (CFU/ml).

Isolation of yeasts

About 12 yeast colonies were selected from a segment of the highest dilution and suitable plate on Chloramphenicol supplemented Dichloran Rose-Bengal Choramphenical Agar (DRBC) (Oxoid CM727; Oxoid Ltd., Basingstoke, Hampshire, UK). Each colony was sub-cultured into Malt Extract Broth (Oxoid CM0057; Oxoid Ltd., Basingstoke, Hampshire, UK) pH 5.4 and purified by streaking repeatedly on to Malt Extract Agar (MEA) (Oxoid CM0059).

Determination of yeast species population in the fermenting milk

This was also carried out by giving code to individual yeast isolates in a segment of highest dilution on suitable DRBC agar plate before counting. The count allocated for each coded individual isolate was recorded. The coded colonies were individually fransferred into respective coded tubes containing 5 ml steril Malt Extract Broth (Oxoid CM0057; Oxoid Ltd., Basingstoke, Hampshire, UK) pH 5.4 and incubated at 25 ^{0}C for 48 h. and purified by streaking repeatedly on to Malt Extract Agar (MEA) (Oxoid CM0059). Based on their phenotypic characteristics, the individual isolates were grouped and identified using API 50CHL and MALDI-TOF. Based on the code given to each individual isolate on the enumerated plates, and the count allocated to each isolate, the total count of each

yeast species was determined in terms of colony forming unit per milliliter (CFU/ml).

Characterisation of Lactic Acid Bacteria

Gram stain

Portion of the purified LAB colony was emulsified in SBS on microscopic slides using inoculating loop, heat fixed and stain using Gram staining techniques. The slides were flooded with Crystal violet stain for forty seconds and washed with running clean water. This was followed by application of mordant which was Logus iodine for 35 seconds. The slides were washed using clean running water and a de-colourizer (acetone) was applied for a few seconds and washed. This was followed by the application of the counter stain; 0.1% Neutral Red also for 40 seconds and washed). The slides were air dried and examined using compound microscope magnified at x 100 with oil immersion for the gram reaction and cellular morphology.

Catalase reaction

For catalase reaction, a drop of 3% freshly prepared hydrogen peroxide solution was placed on a clean glass slide and a single colony of the pure culture picked and emulsified. This was then observed for bubbles or effervescence resulting from the liberation of free oxygen as gas bubbles. This was an indication that the organism produced the catalase enzyme in the culture medium.

Microscopic examination

Cell shape and arrangements were determined using compound microscope and a gram staining technique. The air dried Gram stained bacteria cells were examined under a compound microscope magnified at x 100 with oil immersion for the cellular morphology.

Growth at different temperatures

Three pure discret colonies from each of the representative pure LAB group isolates were used to inoculate three tubes each containing 5ml steril MRS broth culture (Oxoid CM359; Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 5 °C, 10 °C, 45 °C or 50 °C for 24-48 h and examined for growth by visual turbidity.

Growth at different pH

Three tubes each containing 5ml steril MRS broth culture (Oxoid CM359; Oxoid Ltd., Basingstoke, Hampshire, UK) with pH adjusted to 3.0, 3.5, 4.0, 4.4, 7.0 and 9.6 were inoculated using three pure discret colonies from each of the representative pure LAB group isolates and incubated at 30 °C for 24-48 h and examined for growth by visual turbidity.

Salt tolerance test

Three tubes each containing 5ml steril MRS broth culture (Oxoid CM359; Oxoid Ltd., Basingstoke, Hampshire, UK) containing 3 %, 6.5 % or 18 % (w/v) NaCl

were innoculate using three pure discret colonies from each of the representative pure LAB group isolates and incubated at 30 °C for 24-48 h and examined for growth by visual turbidity.

Grouping of LAB isolates

All the LAB isolates were grouped based on the result obtained from their phenotypic characteristics that is, cell morphology, growth at different temperatures, growth at different pH salt tolerance etc. Two representatives isolate from each group was identified by their carbohydrate fermentation patterns using API 50 CHL (BioMérieux, Marcy-l 'Etoile, France) and results compared to the API database.

Identification of LAB by MALDI-TOF

Representative isolates of each LAB group was identified by MALDI-TOF Mass Spectrometry. The isolate was cultured on MRS agar for 18-24 hrs at 30 ^{0}C. A pure colony was applied directly on MALDI sample target using a sterile loop. The sample was allowed to air dry for 5min after which it was overlaid with 1µl of 5-chloro-2-mercaptobenzothiazole (3µgml^{-1}) saturated solution in water, methanol and acetonitrile, (1:1:1), 0.1 % formic acid and 0.01M 18-crown-6-ether. The prepared target was air dried for 1 hour and analysed using Bruker MALDI Biotyper and the spectra produced compared MALDI Biotyper data base for identification.

Grouping of yeast isolates

Yeast Colonies on MEA media were grouped based by their colony morphology (colour, surface, size, form, margin, and elevation). Identification of yeast by carbohydrate fermentation and assimiliation patterns was done using two representative isolates from each group in the API ID 32 C gallaries (BioMérieux, Marcy-l'Etoile, France).

Identification of Yeast by MALDI-TOF

The representative isolates of the yeast groups were also identified by MALDI-TOF Mass Spectrometry. A pure colony was applied directly on MALDI sample target using a sterile loop. The sample was allowed to air dry for 5min after which it was overlaid with 1µl of 5-chloro-2-mercaptobenzothiazole (3 µgml-1) saturated solution in water, methanol and acetonitrile, (1:1:1), 0.1 % formic acid and 0.01M 18-crown-6-ether. The prepared target was air dried for 1 hour and analysed using Bruker MALDI Biotyper and the spectra produced compared MALDI Biotyper data base for identification.

Technological Properties of LAB

Antimicrobial property

Agar Well Diffusion method described by Schillinger and Lücke (1989) and Olsen, Halm and Jakobsen (1995) was used to investigate the inhibitory potential of the lactic acid bacteria isolates against five enteric pathogens cultures.

A representative isolate from each of the five groups was used. A 24 hrs broth culture of the LAB isolate was vortex and 0.1ml transferred into a circular well on MRS agar plate which had been made using a sterile 5 millimeter cork borer.The plate were incubated for 4 h to allow the broth to diffused into the media after which the wells were over laid with about 10 ml of nutrient 0.7% soft agar (Nutrient broth Oxiod CM0067 NO. 2 plus Agar Bacteriological NO. 1, Oxoid LP0011; Oxoid Ltd., Basingstoke, Hampshire, UK) containing 0.25 m1 of 10^{-1} dilution overnight culture of a pathogen. The procedure was repeated for six different pathogens. *Escherichia coli* (0157; H7*), Vibrio cholerae, Staphylococcus aureus, Listeria monocytogens* and *Salmonella typhimurium*.

The plates were incubated at 30 ^{0}C for 18 to 24 hrs. Inhibition of the growth of the pathogen by the LAB isolate was indicated by a clear zone of no growth around the wall in which the LAB isolates were placed and the clear zones were measured using ruler. This was carried out in triplicates.

Rate of acidification of raw milk by the LAB

The rate of acidification trial was deployed using representative of each five dominant LAB group cultures identified early on during the spontaneous fermentation as described by Rönkä *et al.*, (2003). The raw milk was pasteurised at 110 ^{0}C for 10 min. and allowed to cool. 100 ml of the milk was inoculated with 1% supplement test LAB strain prepared from 16-hour culture incubated anaerobically at 30 ºC of which 0.1 ml of the culture was transferred into sterile

SPS and diluted to a concentration of 10^7 cfu/ml. This was carried out in triplicate and allowed to fermente at ambient temperature for 24 h. pH and Titratable acidity of the culture was determined at every 6 hours. One batch of the milk was not inoculated and was used as control (spontaneous fermentation).

Amylase secretion tests for the LAB isolates

Ten isolates each from each of the five LAB groups were streaked on Nutrient Agar (Oxiod CM3; Oxoid Ltd., Basingstoke, Hampshire, UK) to which 2 % soluble starch had been added (with pH adjusted to 7.2). The plates were incubated in an anaerobic jar containing Campy GenTM2.5L (Mitsubishi Gas Chemical Company inc. Japan) to create anaerobic conditions and incubated at 30 °C for 3 days. The plates were then flooded with iodine solution. Amylase secretion by the isolate was indicated by a clear zone around the LAB colonies. This was carried out using ten representative isolates from each of the five LAB groups.

Exopolysaccharides (EPS) production by LAB isolates

Screening of isolates for EPSs production was carried out according to Ludbrook, Russell and Greig, (1997). Five milliliter 5 ml liquid EPS selecting medium (90 g skim milk, 3.5 g yeast extract, 3.5 g peptone and 50 g /L glucose) was inoculated with 16 h Isolates cultured on MRS agar and incubated anaerobically at 30 ºC for 48 h. The “ropiness” of the culture medium after 48 h of incubation was determined by its resistance to flow easily through graduated pipette (Vedamuthus

and Neville, 1986). This was carried out using ten representative isolates from each of the five LAB groups.

Development of Starter Culture

Milk pasteurization

Five liters of fresh raw milk was purchased from CSIR-Animal Research Institute and transported in an ice chest to the laboratory. The milk was pasturised at 110 ^{0}C for 10 min.

Starter cultures

Based on the results of the technological properties of the LAB isolates, five isolates were selected for the starter culture trials. The cultures which had been preserved in 50 % glycerol at -80 ^{0}C were revived and subcultured twice in MRS broth. The isolates were *Lb. brevis* (8AL16), *Lb. plantarum* (4RL2), *Lb. delbrueckii ssp. Lactis* (4AL1), *Lb. buchneri* (8RL15) and *Lb. plantarum* (8AL10).

Fermentation with single starter culture

One hundred mls of pasteurised milk was innoculated with 1 % of 10^6 to 10^7 cfu/ml of the test culture [*Lb. brevis* (8AL16), *Lb. plantarum* (4RL2), *Lb. delbrueckii ssp. Lactis* (4AL1) *Lb. buchneri* (8RL15) and *Lb. plantarum* (8AL10)] and allowed to ferment at ambient temperature for 24 h. Samples were taken at 6 hours and analyzed for pH, titratable acidity and microbial count. For

each of experiment, all five isolates were tested in addition to a non inoculated sample as a control. The experiment was carried out in triplicate.

Fermentation with combined culture

One hundred mls of pasteurised milk was innoculated with 1 % each of two isolates of LAB as a combined starter culture as follows: (i) *Lb. brevis* (8AL16) + *Lb. plantarum* (4RL2), (ii) *Lb. brevis* (8AL16) + *Lb. delbrueckii ssp. Lactis* (4AL1), (iii) *Lb. brevis* (8AL16) + *Lb. buchneri* (8RL15), (iv) *Lb. brevis* (8AL16) + *Lb. plantarum* (8AL10), (v) *Lb. plantarum* (4RL2)+ *Lb. delbrueckii ssp. Lactis* (4AL1), (vi) *Lb. plantarum* (4RL2), + *Lb. buchneri* (8RL15), (vii) *Lb. plantarum* (4RL2)+ *Lb. plantarum* (8AL10), (viii) *Lb. delbrueckii ssp. Lactis* (4AL1) + *Lb. buchneri* (8RL15), (ix) *Lb. delbrueckii ssp. Lactis* (4AL1) + *Lb. plantarum* (8AL10), (x) *Lb. buchneri* (8RL15) + *Lb. plantarum* (8AL10). The milk was allowed to ferment at ambient temperature for 24hours. This treatment was made in duplicates making a total of 20 batches. Similarly, samples were taken at 0 h, 6 h, 12 h 18 h and 24 h of fermentation for determination of pH, titratable acidity and microbial count on MRS agar.

The ability of different enteric pathogens to survive the fermentation process

The ability of the selected enteric pathogens to survive the fermenting milk was studied by the method described by Mante, Sakyi-Dawson and Amoa-Awua, (2003) with a little modification. The enteric pathogens used were *Listeria monocytogenes, Vibrio cholerae, Salmonella typhimurium, Escherichia coli*

(0157; H7) and *Staphylococcus aureus*. All used pathogens were reference culture obtained from CSIR - Food Research Institute Microbiology Laboratory. About 18 to 24 h old pure isolates of each pathogen was inoculated into 5ml nutrient broth (Oxiod CM0067; Oxoid Ltd., Basingstoke, Hampshire, UK) which was introduced into the milk containing the starter cultures about to start fermenting at a diluted concentration of 10^6 to 10^7cfu/ml. The surviving gpathogens were enumerated by pulling 1ml of the fermenting milk at every 6 hours' intervals and plating them on Plate Count Agar (Oxiod CM325; Oxoid Ltd., Basingstoke, Hampshire, UK) using plate count techniques. The plates were incubated aerobically at 37 ^{0}C for 24 hours.

Sensory Analysis

The millet was prepared by sorting of the grains, steeping for seven hours and milled into flour. One gram (1kg) of flour was mixed with one litter of water and press through a sieve with about 4 mm holes to obtain small granules. The granules were steamed in colander for about 1.5 h. The steamed millet granules which stuck together was transferred into a bowl, five hunhred milliliter (500ml) of water added and the lumps broken into pieces whiles still hot.

The granules were re-steamed for about thirty minutes, transferred into a bowl and allowed to cool. Two litters of fresh pasturised milk was poured into a clean plastic container and inoculated with 1 % of starter culture. This was done for each of the following starter cultures or combination of cultures: [*Lb. brevis*

(8AL16), *Lb. plantarum* (4RL2*), Lb. delbrueckii ssp. Lactis* (4AL1) *Lb. buchneri* (8RL15) and *Lb. plantarum* (8AL10)]. Two litters of fresh unpasturised milk was also fermented spontaneously for 24 h representing the traditional Degue process. The fat/oil was then collected from the surface of the fermented milk which had coagulated and was homogenized by whisking using stick rub in-between the palm and then sieved. The millet granules were added to the milk stirred and sugar added to taste. The Degue was packed in 500 mls plastic bottles.

Sensory evaluation of the different Degue samples

The six different Degue samples that is five produced with different starter cultures and the spontaneously fermented sample were assesd by a 40-member panel by preference test using a 9-point hedonic scale. The attributes assessed were aroma, colour, taste and overall acceptability. The 40 panelists were selected from the CSIR-Food Research Institute (FRI) and were all familiar with Burkina

The samples were given to the panelists in booths in the sensory laboratory of the CSIR-FRI. The panelists rinsed their mouths with water after tasting each of the six products and scored (1 - ‘dislike extremely’, 2 - ‘dislike very much’, 3 - ‘dislike moderately’, 4 - ‘dislike slightly’, 5 - ‘neither like nor dislike’, 6 - ‘like slightly’, 7 - ‘like moderately’, 8 - ‘like very much’, 9 - ‘like extremely’).

Data Analysis

All data was statistically analyzed using Statistical Package for Social Sciences (SPSS) version 16 Software. Significant differences between means were

calculated using one-way Analysis of Variance (ANOVA). Microsoft Excel 2010 was used to generate means, standard deviations and randomizes three digit codes for the sensory samples. Xlstat (2014) was used in generating randomized order of sample presentation for sensory panel.

CHAPTER FOUR

RESULTS

This chapter covers all the results obtained from the survey and the tests carried out in the study.

The Differences in the Production Process of Degue and Burkina

The survey carried out indicated that there is no difference between Degue and Burkina. The product is known as Burkina in Ghana but in Burkina Faso and other countries where it is produced, it is known as Degue. Below is a process flow diagram for Degue and Burkina as shown in Fagure 2 and 3.

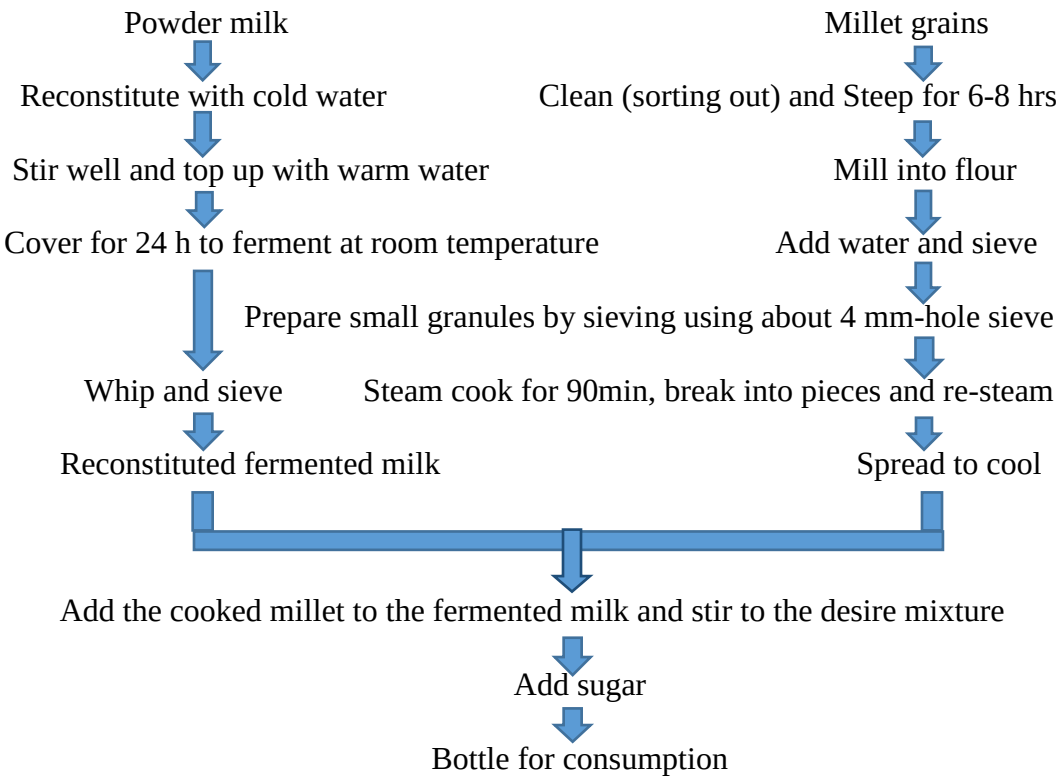

Figure 2: Burkina process flow diagram

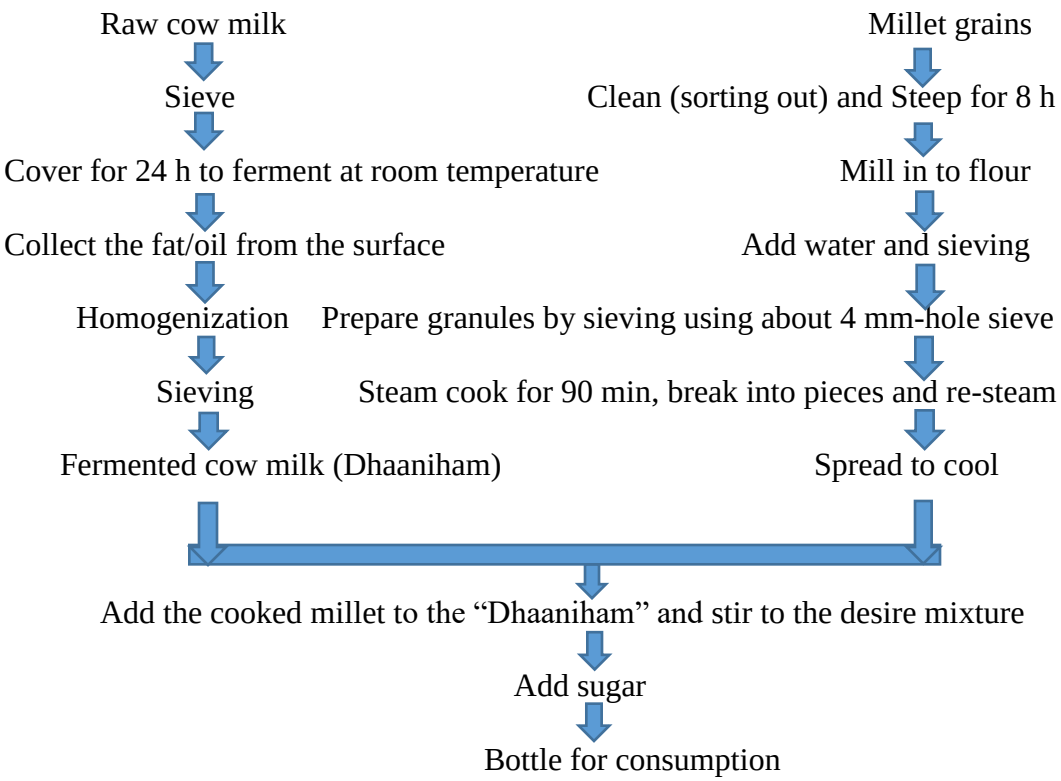

Figure 3: Degue process flow diagram

Changes in Microbial Population of Spontaneous Fermented Milk during Degue Production

Aerobic mesophiles

The population of the aerobic mesophiles in Degue samples from two different processors were both made up of both Gram positive and Gram negative bacteria, also both catalase-positive and catalase-negative bacteria, rods and cocci. At the start of the fermentation, the mean aerobic mesophilic population was 6.38 log10 CFU/ml; increased to 8.32 log10 CFU/ml at 12 h and dropped to 7.28 log10 CFU/ml at 24h. The same pattern and similar counts were recorded at production site 2 (Figure 4).

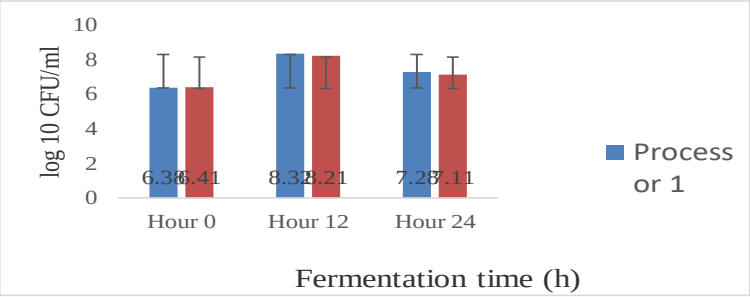

Figure 4: Changes in the population of aerobic mesophiles during the spontaneous fermentation of milk at two Degue production sites in Accra

Lactic acid bacteria

The population of LAB which was enumerated on MRS increased from a mean count of 4.23 log10 CFU/ml at 0h to 7.29 log10 CFU/ml at 12 h and 8.28 log10 CFU/ml at 24 h at production site 1. At production site 2 similar values were also recorded as shown in Figure 5.

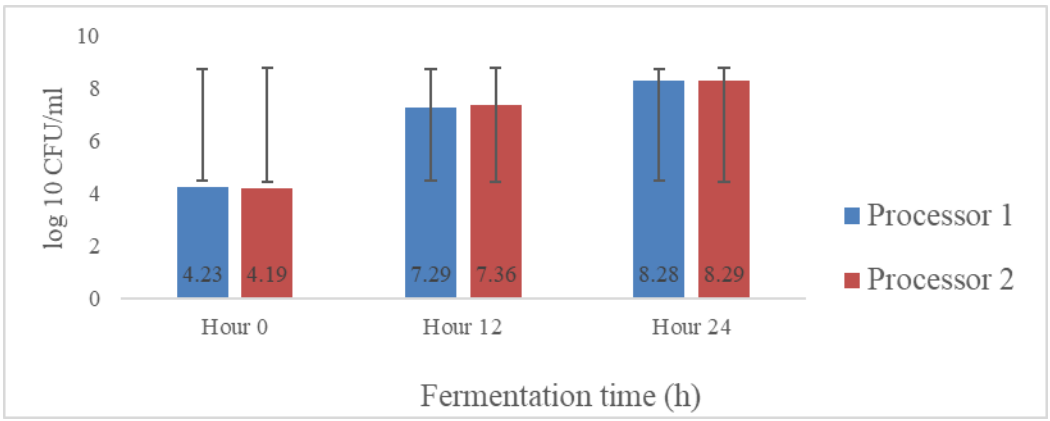

Figure 5: Population of LAB during spontaneous fermentation of milk in Degue production at two production sites in Accra

Yeasts population during spontaneous fermentation

The yeast population enumerated on DRBC increased from a mean count of 4.23 log10 CFU/ml at 0 h to 7.67 log10 CFU/ml at 12 h and 8.29 log10 CFU/ml at 24 h at production site 1. At production site 2, similar values were also recorded as shown in Figure 6.

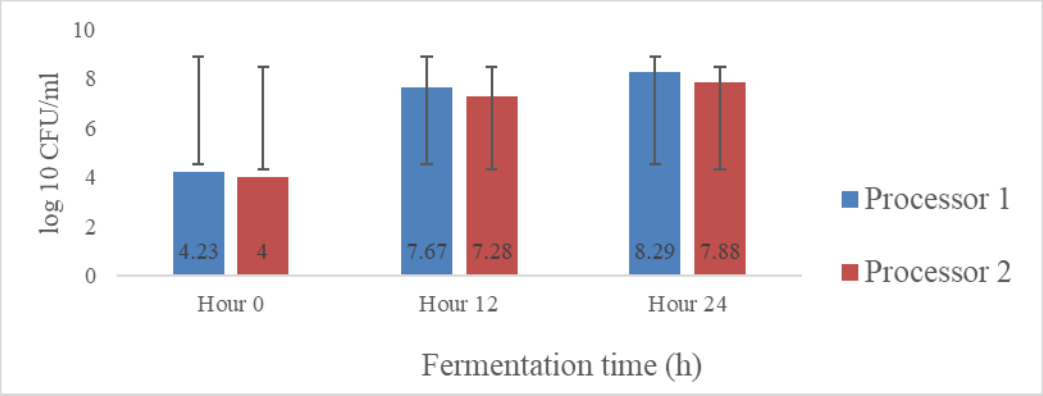

Figure 6: Population of yeast during spontaneous fermentation of milk in Degue production at two production sites in Accra

Change in pH during spontaneous fermentation of milk in Degue production

The pH of the milk during the spontaneous fermentation decreased from 6.65 at 0 h to 4.14 at the 24 h at the production site 1. Production site 2 also recorded pH of 6.66 at 0 h and 4.17 at the 24 h as shown in Figure 7

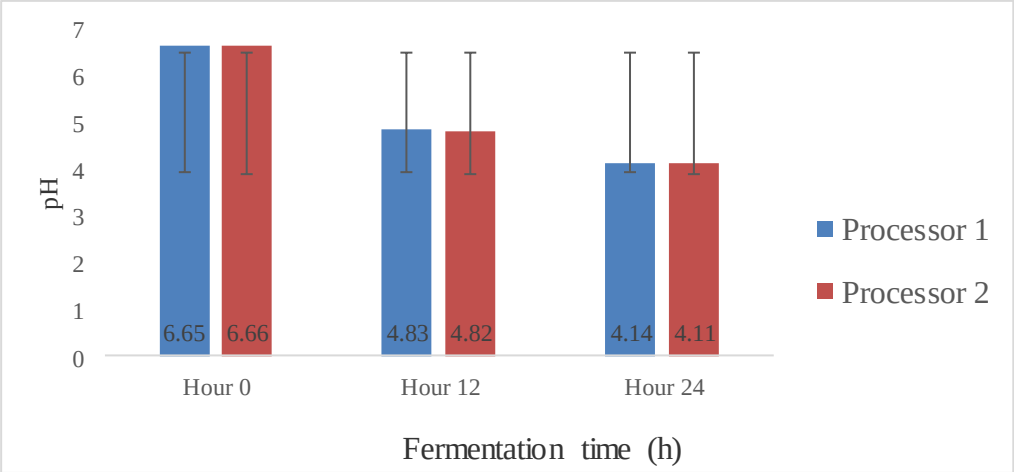

Figure 7: Change in pH during spontaneous fermentation of milk in Degue production

Change in tritratable acidity during spontaneous fermentation of milk in Degue production

The percentage titratable acidity (% TTA) of the milk during the spontaneous fermentation increased from 0.14 % at 0h to 0.72 % at the 24 h at the production site 1. At production site 2 similar values were also recorded as shown in Figure 8.

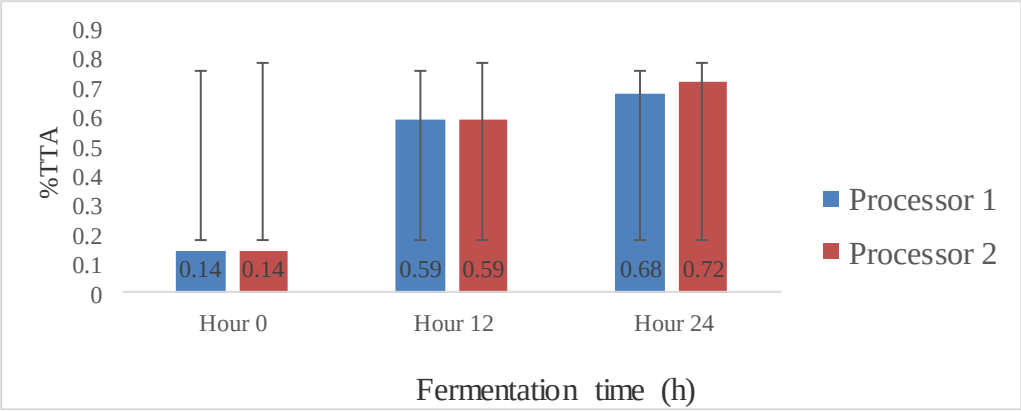

Figure 8: Change in percentage titratable acidity during spontaneous fermentation of milk in degue production

Changes in Microbial Population during Steeping of Millet Grains in Degue Production

Aerobic mesophiles

The population of the aerobic mesophiles which was made up of both Gram positive and Gram negative, catalase-positives and catalase-negatives, rods and cocci bacteria at both production sites increased from 6 log10 CFU/ml to 8 log10 CFU/ml during 8 h of steeping as shown in Table 2.

Table 2 - *Population of Aerobic Mesophiles during Steeping of Millet Grains in Degue Production*

Sample	Mean Mesophiles Counts (log cfu/ml)	
Steep water	Processer 1	Processer 2
0 h	6.38 ± 0.02	6.41 ± 0.01
8 h	8.32 ± 0.04	8.21 ± 0.03

Lactic acid bacteria

The population of LAB increasd by 2 log units from 6 log10 CFU/ml to 8 log10 CFU/ml at both production sites as shown in Table 3.

Table 3 - *Population of LAB during Steeping of Millet Grains in Degue Production*

Sample	Mean LAB Counts (log CFU/ml)	
Steep water	Processer 1	Processer 2
0 h	6.38 ± 0.04	6.41 ± 0.05
8 h	8.32 ± 0.04	8.21 ± 0.08

Yeasts Population during Steeping of Millet

The population of the yeasts also increased by 4 log units from about 3.0 log10 CFU/ml to 7.0 log10 CFU/ml during steeping at both production sites as shown in Table 4

Table 4 - *Population of Yeasts during Steeping of Millet in Degue Production*

Sample	Mean Yeast Counts (log cfu/ml)	
Steep water	Processer 1	Processer 2
0 h	3.11 ± 0.03	3.41 ± 0.02
8 h	7.20 ± 0.03	7.51 ± 0.08

Changes in pH during steeping of millet grains in Degue production

The change in pH during the steeping of millet in Degue production at the two production sites are shown in table 5. In 8h of steeping, the mean pH of the steep water decreased from 6.58 to 5.46 at production site 1 and from 6.56 to 5.45 at the production site 2.

Table 5 - *pH of Steep Water*

Sample	Mean pH for Steep Water	
Steep water	Processer 1	Processer 2
0 h	6.58 ± 0.02	6.56 ± 0.01
8 h	5.46 ± 0.01	5.45 ± 0.02

Changes in percentage titratable acidity during steeping of millet in Degue production

The changes in percent titratable acidity during the steeping of millet in Degue production sites are shown in Table 6. As expexted, the mean %TTA increased from 0.15 % to 0.45 % at production site 1 and from 0.16 % to 0.44 % at production site 2.

Table 6 - *Titratable Acidity of Steep Water*

Sample	Mean %TTA for Steep Water	
Steep water	Processer 1	Processer 2
0 h	0.15 ± 0.03	0.16 ± 0.01
8 h	0.40 ± 0.01	0.44 ± 0.02

Grouping and identification of lactic acid bacteria involved in Degue milk fermentation

The results of the charactarisation of the LAB isolates are shown in table 4.6. The table shows the results of cell morphology, Gram test, catalase test, growth at pH 3.0, 3.5, 4.0 and 7.0, growth in 3.0 % NaCl, 6.5 % NaCl, growth at temperature

10 ^{0}C, 45 ^{0}C and 50 ^{0}C. All the isolates were rods and also Gram positive and catalase negative been isolated on MRS so were assumed to be LAB. Based on all of these results, the isolates were grouped into 5groups and the characteristics of each group are given in Table 7.

Table 7 - *Phenotypic Characterisation of Isolated LAB Groups*

Phenotypic Characterisation	Isolate Groups				
	LABG1	LABG2	LABG3	LABG4	LABG5
Shape	Rod	Rod	Rod	Rod	Rod
Gram stain	+	+	+	+	+
Catalase	-	-	-	-	-
Growth at pH					
3.0	-	W	-	-	W
3.5	+	+	-	+	+
4.0	+	+	+	+	+
7.0	+	+	+	+	+
Growth NaCl					
3.0%	+	+	+	+	+
6.5%	-	+	-	+	+
Growth at temp.					
10 ^{0}C	+	+	-	+	+
45 ^{0}C	-	W	+	W	W
50 ^{0}C	-	-	-	-	-
% isolate	18.75 %	18.75 %	31.25 %	6.25 %	25 %

+ : Positive, - : Negative, w : - Weakly positive

Identification of LAB Isolates by Biochemical Test

Two isolates were taken from each of the five groups and their species tentatively identified by determining the pattern of carbohydrate fermentation in API 50 CHL galleries. The most frequently occurring isolates, LAB group 5 [LABG5 (25 %)] occurred as single or paired rods which grew at 10 ^{0}C and 45 ^{0}C, at pH between 3.0 and 7.0, in Sodium Chloride (NaCl) concentration range from 3.0 to 6.5%. They fermented Ribose, Galactose, D-Glucose, D-fructose, D-mannose, L-arabinose, N acethyl glucosamide, Amygdalin, Arbutin, Esculin, Salicin, Cellobiose, Maltose, Lactose, melibiose, Saccharose, Melezitose, D-raffinose, β gentiobiose, D- turanose, D-arabitol and Gluconate in API 50 CHL galleries. They were tentatively identified as *Lactobacillus plantarum* 1 using the API databased. The isolates from LAB group 2 [LABG2 (18.75 %)] also produced similar results and were identified as *Lactobacillus plantarum* 2 in the API test.

The next most dominant LAB strains [LABG3 (31.25 %)] were single and paired rods which could not grow below pH of 4.0. They grew in 3.0 % salt but not in 6.5 % salt. They fermented D-Glucose, D-fructose, D-mannose, N acethyl glucosamide, Maltose and Lactose in API 50 CHL galleries. They were tentatively identified as *Lactobacillus delbrueckii ssp lactis*. The isolates in group 1 [LABG1 (18.75 %)] were short rods which did not grow below pH of 3.5 and temperature of 45 ^{0}C. They could grow in 3.0 % but not in 6.5 % salt. In the API test, they fermented Ribose, Galactose, D-Glucose, D-fructose, D-mannose, L-arabinose, N acethyl glucosamide, Amygdalin, Arbutin, Esculin, Salicin,

Cellobiose, Maltose, Lactose, Saccharose, β gentiobiose, and Gluconate and were tentatively identified as *Lactobacillus brevis.*

The last LAB group [LABG4 (6.25 %)] grew in a salt concentration of 3.0 % and 6.5 %, at the pH 3.5 and 7.0 and a temperature range of 10 ^{0}C and 45 ^{0}C. They fermented L-arabinose, Ribose, D-xylose, Galactose, D-Glucose, D-fructose, α methyl glucosamide, Melibiose, Saccharose, Melezitose, D- raffinose, Gluconate and 5 cetoglunate in the API galleries and were identified as *Lactobacillus buchneri.*

Identification of LAB Isolates by Bruker MALDI-TOF MS

The species of one of the two representative isolates of the LAB groups identified by the biochemical test that is API was identified using Bruker MALDI-TOF Mass spectrometry since identification of microbial species by biochemical methods is not considered reliable. The MALDI-TOF Mass spectrometry gave the same results obtained from the biochemical or classical tests as seen in table 4.7. Thus, LAB 8AL16 was confirmed to be *Lactobacullus brevis*, LAB 4RL2 and LAB 8AL10 confirmed as *Lactobacillus plantarum*, LAB 4AL1 as *Lactobacillus delbrueckii ssp lactis* and LAB 8RL15 as *Lactobacillus buchneri.*

Population of Lactic Acid Bacteria Species in the Spontaneous Fermented Milk

Table 8 shows the population of LAB species in the fermented milk stating from the 0 h to 24 h at both production sites.

Table 8 - *Population of LAB Species in the Fermenting Milk*

		Bacteria population (CFU/ml)	
Fermentation time (h)	LABs	Production site 1	Production site 2
0	*Lb. plantarum*	1.0×10^4	9.0×10^3
	Lb. delbrueckii ssp. lactis	8.0×10^3	7.0×10^3
	Lb. brevis	3.0×10^3	2.0×10^3
	Lb. buchneri	1.0×10^3	1.0×10^3
12	*Lb. plantarum*	7.0×10^6	1.6×10^7
	Lb. delbrueckii ssp. lactis	7.0×10^6	9.0×10^6
	Lb. brevis	3.0×10^6	7.0×10^6
	Lb. buchneri	2.0×10^6	4.0×10^6
24	*Lb. plantarum*	1.2×10^8	1.2×10^8
	Lb. delbrueckii ssp. lactis	8.0×10^7	7.0×10^7
	Lb. brevis	4.0×10^7	6.0×10^7
	Lb. buchneri	4.0×10^7	4.0×10^7

LAB: - lactic acid bacteria

Lb: - Lactobacillus.

Identification of Yeast by Biochemical Test

The yeast isolates were examined and placed into 3 different groups based on their colony characteristics and cell morphology. Three isolates from each group were examined in API ID 32C galleries for their pattern of carbohydrate fermentation and assimilation. The most dominant yeasts [YG2 (40 %)] utilized galactose, glucose, lactose, and were identified as *Candida kefir*. The second dominant yeast [YG3 (37 %)] utilized glucose, galactose, maltose, saccharose and was identified as *Candida tropicalis.* The third yeasts isolates [YG1 (23 %)] utilized glucose, saccharose, lactose, galactose and trehalose and was identified in API ID 32C galleries as *Candida famata.*

Identification of Yeasts by Bruker MALDI-TOF Mass Spectromatry

A representative isolate of the three groups of yeast were also identified using Bruker MALDI-TOF Mass Spectrometry. The same yeasts were identified except (8AY10) which were identified as *Candida orthopsilosis.*

Population of Yeasts Species in the Fermenting Milk

The population of yeast species in the fermented milk stating from the 0 h to 24 h at both production sites are shown in Table 9.

Table 9 - *The Population of Yeast Species in the Fermenting Milk*

Fermentation time (h)	Yeast	Yeast population (CFU/ml) Production site 1	Production site 2
0	*Candida kefyr*	9.0×10^3	2.0×10^4
	Candida tropicalis	8.0×10^3	1.0×10^4
	Candida orthopsilosis	5.0×10^3	1.0×10^4
12	*Candida kefyr*	3.0×10^7	1.0×10^7
	Candida tropicalis	2.0×10^7	1.0×10^7
	Candida orthopsilosis	7.0×10^7	8.0×10^6
24	*Candida kefyr*	1.0×10^8	5.0×10^7
	Candida tropicalis	1.0×10^8	3.0×10^7
	Candida orthopsilosis	8.0×10^6	8.0×10^6

Development of Starter Culture

Technological properties of the identified LAB

Rate of acidification

The rate of acidification of milk by the representative isolate of each of the given original group that is [*Lb. brevis* (8AL16), *Lb. plantarum* (4RL2), *Lb. delbrueckii ssp. Lactis* (4AL1), *Lb. buchneri* (8RL15) and *Lb. plantarum* (8AL10)] change in pH and % TTA of milk samples inoculated with each of the isolates as well as a control which was spontaneously fermented as shown in Figure 9 and 10.

The Figure 9 shows that acidification of the milk sample was faster in all the samples which were fermented with a strain of LAB compared to the spontaneous fermented sample. It also shows that the rate of acidification of milk by *Lb.*

delbrueckii ssp. Lactis (4AL1) was faster than all the other isolates. All the other isolates had a fairly similar rate of acidification. At the end of 24 h, the pH of the control was 6.53, *Lb. delbrueckii ssp. Lactis* (4AL1) was 4.6, and that of the other isolates were between 4.9 and 5.09. For the titratable acidity also, a similar trend was observed as expected.

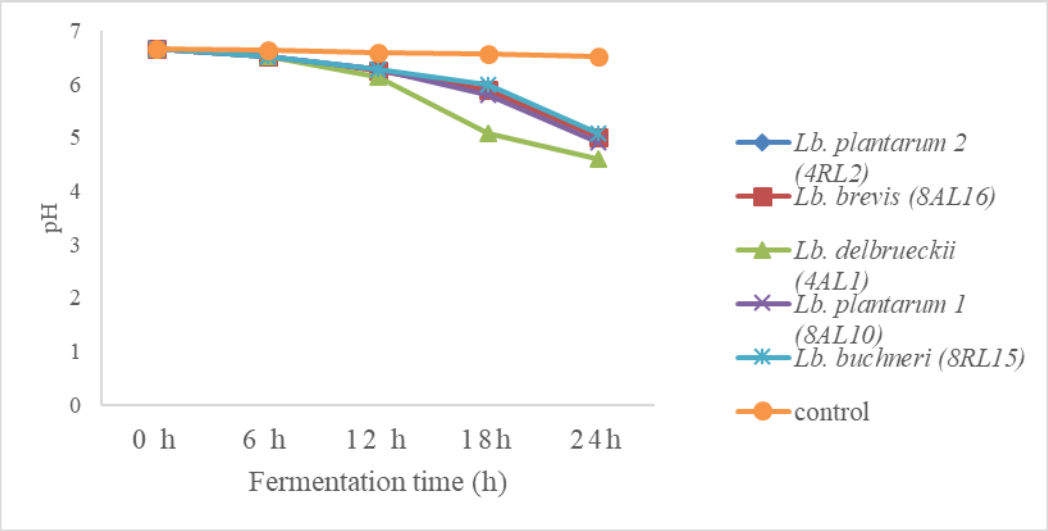

Figure 9: Change in pH during 24 h milk fermentation using the LAB isolates

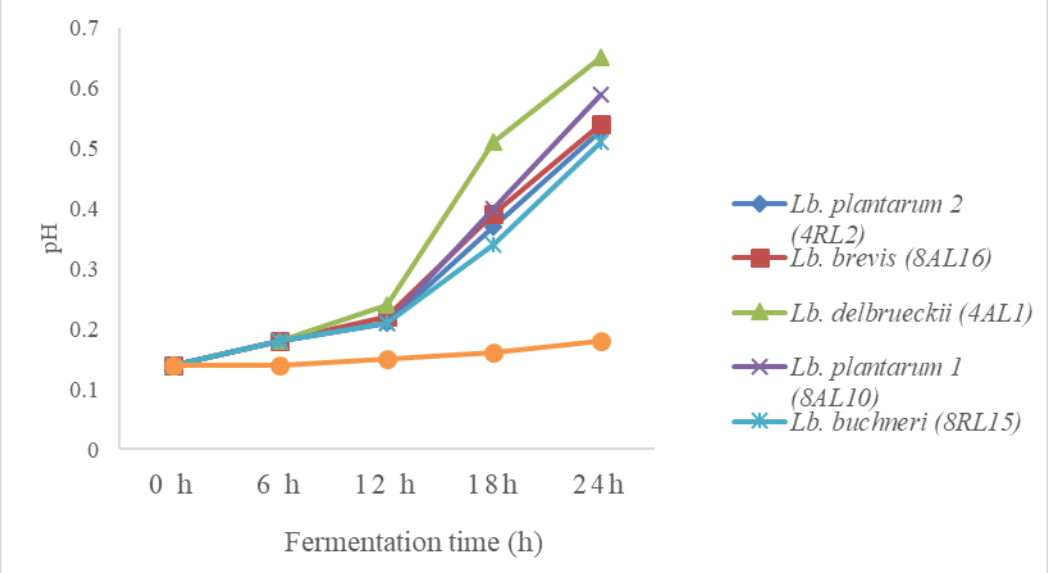

Figure 10: Change in percentage titratable acidity during 24 h milk fermentation using the LAB isolates

Exopolysaccharide production and amylase secretion

For exopolysaccharide production, all the ten representative isolates from each group showed a degree of "ropiness" indicating their exopolysaccharide producing potentials. *Lb. plantarum* (8AL10) and *Lb. plantarum* (4RL2) emerges as the highest producers followed by *Lb. delbrueckii ssp lactic* (4AL1), *Lb. brevis* (8AL16) and *Lb. buchneri* (8RL15). For the ability of the isolates to secrete amylase, few isolates displayed a degree of clear zone around each colony when flooded with iodine solution after three days' anaerobic incubation as shown in Table 10.

Table 10 - *Exopolysaccharide Production and Amylase Secretion of LAB Isolates*

		% Isolates			
Isolates	Test	ND	+	++	+++
Lb. brevis	EPS production	0.00	30.00	20.00	50.00
(n=10)	Amylase secretion	80.00	20.00	0.00	0.00
Lb. plantarum 2	EPS production	0.00	10.00	10.00	80.00
(n =10)	Amylase secretion	0.00	80.00	20.00	0.00
Lb. delbrueckii	EPS production	0.00	20.00	60.00	20.00
(n =10)	Amylase secretion	70.00	30.00	0.00	0.00
Lb. Buchneri	EPS production	0.00	70.00	20.00	10.00
(n =10)	Amylase secretion	80.00	20.00	0.00	0.00
Lb. Plantarum 1	EPS production	0.00	10.00	20.00	70.00
(n =10)	Amylase secretion	40.00	50.00	10.00	0.00

For Amylase secretion, ND: no clearing zone; +: 1-2mm clearing zone; ++: 3-4mm; +++: 5mm clearing zone.

For exopolysaccharide production, ND: no ropiness; +: 1-2mm length of ropiness; ++: 3-4mm length of ropiness; +++: 5mm length of ropiness

Antimicrobial activities of LAB isolates against some foodborn pathogens

All five isolates tested [*Lb. brevis* (8AL16), *Lb. plantarum* (4RL2), *Lb. delbrueckii ssp. Lactis* (4AL1), *Lb. buchneri* (8RL15) and *Lb. plantarum* (8AL10)] showed antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* (0157; H7) which were demonstrated as inhibition zones of 1-2 mm diameter around the walls. (Table 11). Apart from *Lb. buchneri*, inhibition of the isolates against *Vibrio cholera* were stronger, 3-4 mm inhibition zones and strongerst for *Lb delbrueckii ssp lactic*, 5 mm. Against *Salmonella typhimurium*, the inhibitions were weak, 1-2mm except for *Lb. delbrueckii ssp lactic* and *Lb. plantarum* (8AL10), 3-4 mm. There was no antimicrobial interaction between the different species of the LAB isolates during the fermentation of the milk. There was also no antimicrobial interaction between the LAB and the yeasts isolates.

Table 11 - *Antimicrobial Activities of LAB Isolates against Foodborn Pathogen*

LAB isolates	Pathogens				
	Sal. typhinurium	*Staph. aureus*	*L. monocytogenes*	*E. coli*	*V. cholerae*
Lb. brevis	+	+	+	+	++
Lb. plantarum (4RL2)	+	+	+	+	++
Lb. delbrueckii	++	+	+	+	+++
Lb. Buchneri	+	++	+	+	+

Table 11 - *Continued*

LAB isolates	Pathogens				
	Sal. typhinurium	*Staph. aureus*	*L. monocytogenes*	*E. coli*	*V. cholerae*
Lb. plantarum (8AL10)	++	+	+	+	++

LAB isolates -: no inhibition; +: 1-2mm inhibition zone; ++: 3-4mm inhibition zone; +++: 5mm inhibition zone.

Starter Culture Trial

Rate of acidification of milk using single starter cultures

The study of rate of acidification using starter culture was evaluated using the pH and % TTA. The rate of acidification of the milk in the fermentation trial with single isolates recorded changes in pH and % TTA as shown in figure 11 and 12. At the start of fermentation, the pH value was 6.60 for all isolates including that of spontaneous fermenting milk (control). The pH of the control dropped from 6.60 to 6.53, whiles that of the starter isolates dropped to between 5.09 and 4.6 after 24 h fermentation. *Lb. delbrueckii ssp. lactis* (4AL1) recorded the fasterst rate of acidificateion of pH 4.6 followed by *Lb. plantarum* (8AL10) and *Lb. plantarum* (4RL2). *Lb. brevis* (8AL16) and *Lb. buchneri* (8RL15) recording pH between 5.0 and 4.9. The % TTA values also recorded correpending increased.

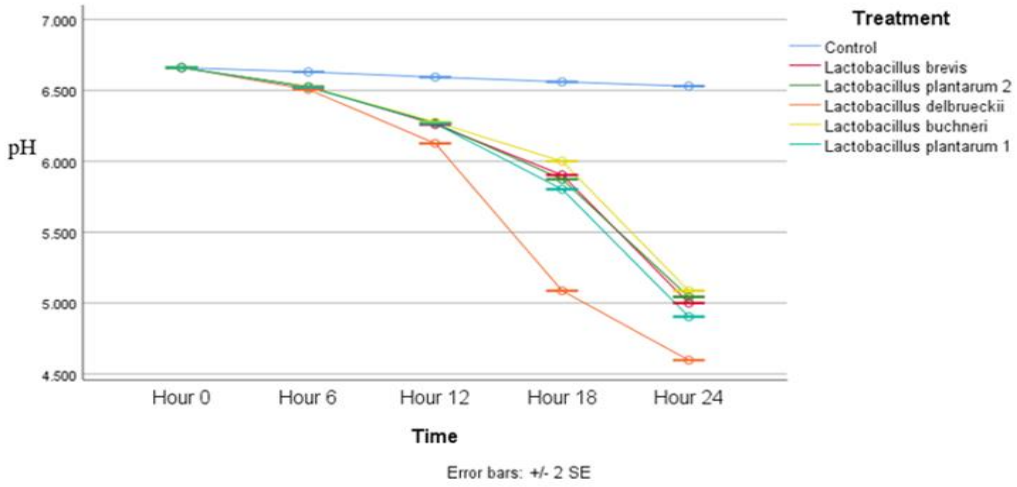

Figure 11: Change in pH of milk during acidification using single cultures

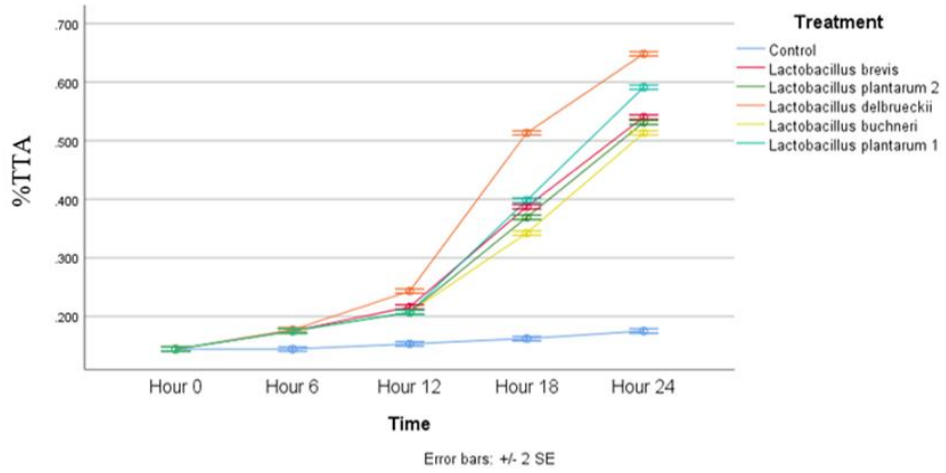

Figure 12: Change in percentage titratable acidity of milk during rate of acidification using single cultures

Rate of acidification of milk using combined starter cultures

The rate of acidification of the milk by the combination of representative isolates are displayed in figure 13 and 14. The changes in pH of the milk sample inoculated with the combined isolates as well as % TTA shown faster acidification compared to spontaneously fermented milk (control). At the start of the fermentation, the control recorded pH of 6.66 dropped to 6.50 after 24 h

fermentation. The pH values for the combined starter culture at the start was the same as that of the control but dropped within the range of 5.09 and 4.65 at 24 h of fermentation. *Lb. brevis* (8AL16) + *Lb. delbrueckii ssp. Lactis* (4AL1) combination produced higher acidification, pH 4.65. This was followed by *Lb. delbrueckii ssp. Lactis* (4AL1) + *Lb. plantarum* (8AL10) combination pH 4.70. The pH of the remaning combinations at 24 h fermentation was in the range of 5.09 and 4.72. There was a corresponding increase in Titratable acidity in all the fermentations. The % TTA of the control ranged from 0.14 to 0.16 whiles that of the combined starter was between 0.14 and 0.62 after the 24 h fermentation as shown in Figure 13 and 14.

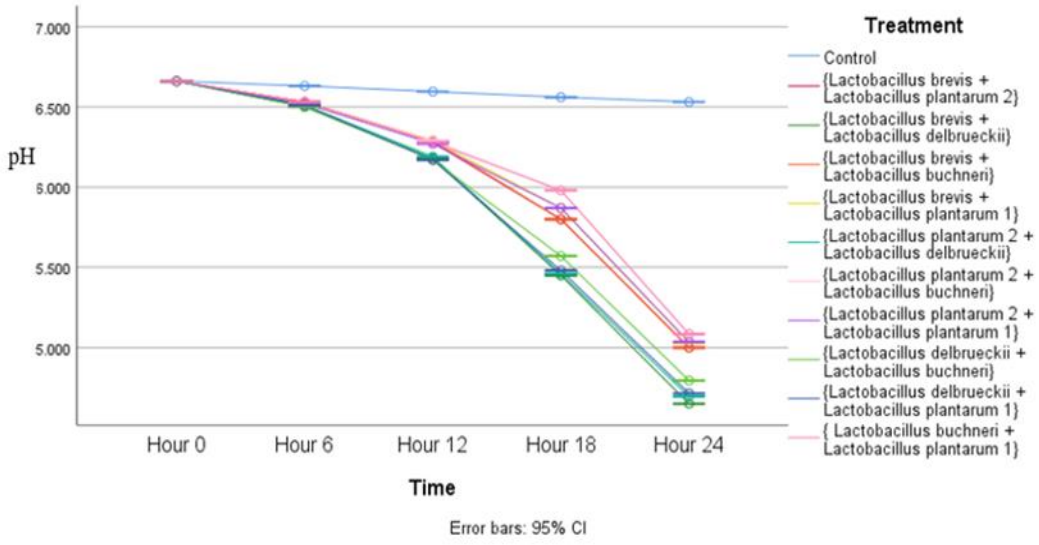

Figure 13: Changes in pH of Milk using combination of two LAB isolates

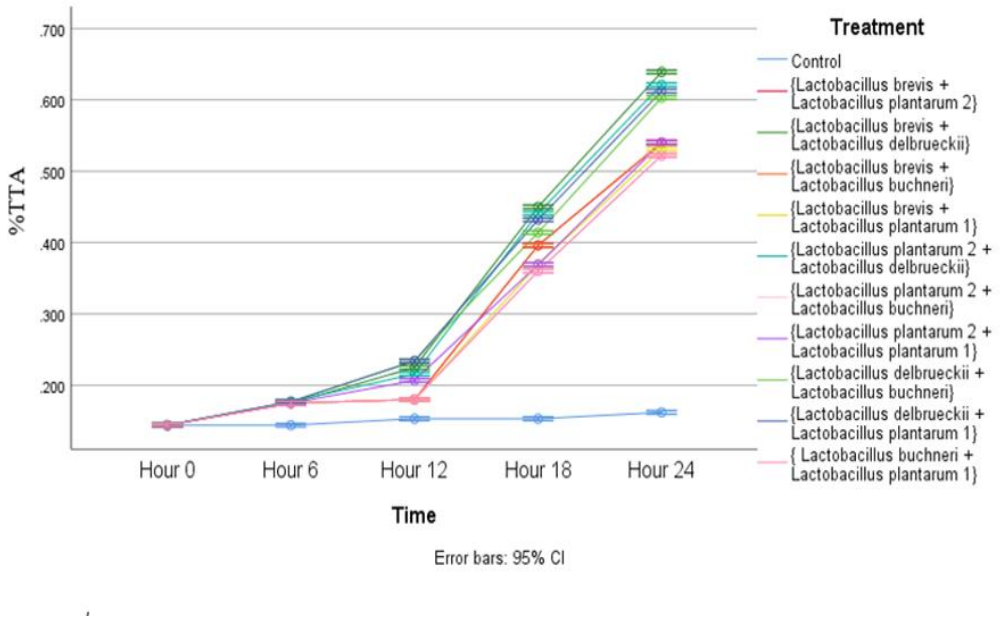

Figure 14: Changes in titratable acidity of milk using combinations of two LAB isolates

Survival of foodborne pathogens in fermenting milk inoculated with starter culture

The survival of tested foodborne pathogens in the starter culture inoculated milk are shown in Figure 15, 16, 17, 18, and 19. The pathogens were inoculated at the beginning of milk fermentation at a concentration between 10^6 to 10^7cfu/ml. Generally, the population of all pathogens in the various fermenting milk increased in the first six hours of the fermentation and later declined as the milk increases in acidity due to the fermentation.

The population of *Vibrio cholerae* increased by 4log cfu/ml at the first 6 hours of the fermentation but later began to declind at the 12th hour of the fermentation and finally disappeared completely at the 24th hour of fermentation. Similarly,

Salmonella typhinurium at the first 6 hours of the fermentation but later decreased and disappeared completely except in the *Lb. plantarum* (4RL2) fermenting milk where it recorded 0.15log cfu/ml. *Escherichia coli* also indicated an increased at the first six hours of the fermentation but later suffered antagonistic effect from the fermenting LABs resulting in drastic decrease in the fermenting medium. *Listeria monocytogenes* and *Staphylococcus aureus* also followed similar parttern although they were not completely eliminated at the end of the 24h fermentation.

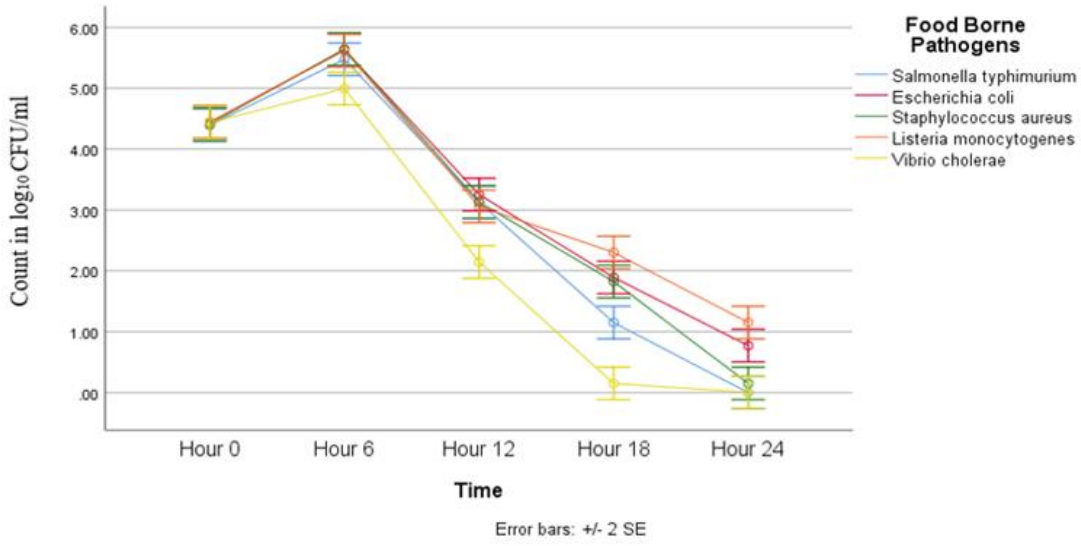

Figure 15: Survival of pathogens in fermenting milk inoculated with *Lb. brevis* (8AL16)

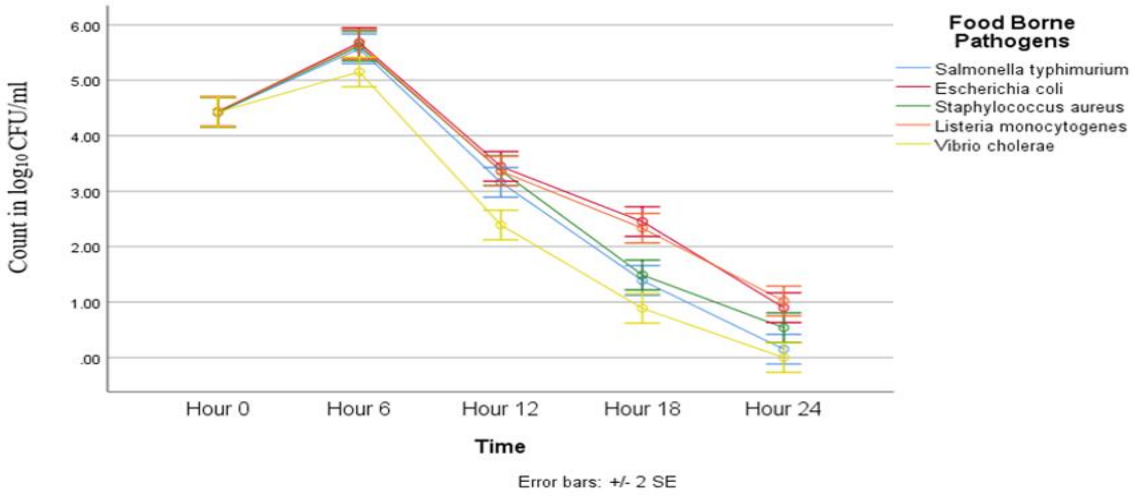

Figure 16: Survival of pathogens in fermenting milk inoculated with *Lb. plantarum* (4RL2)

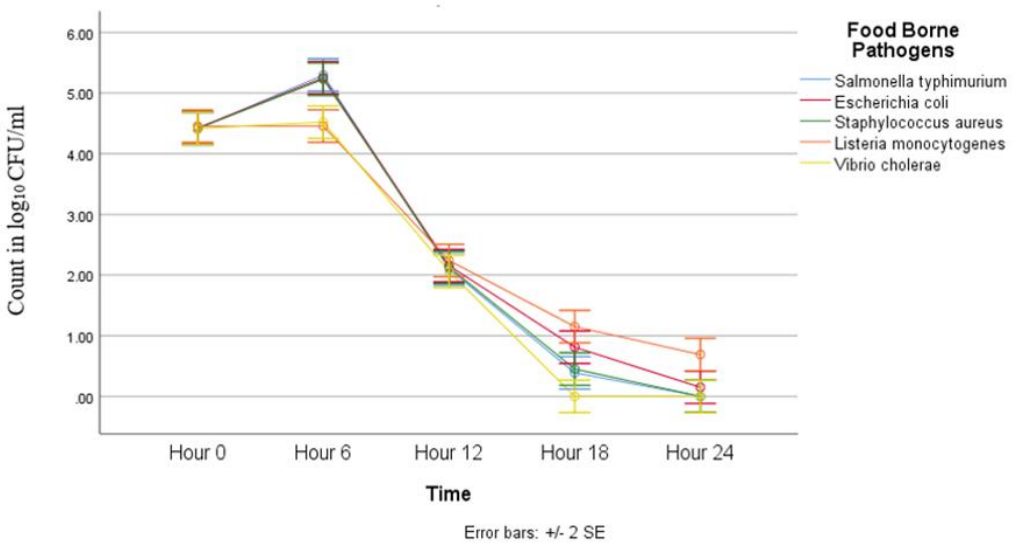

Figure 17: Survival of pathogens in fermenting milk inoculated with *Lb. delbrueckii ssp lactis* (4AL1)

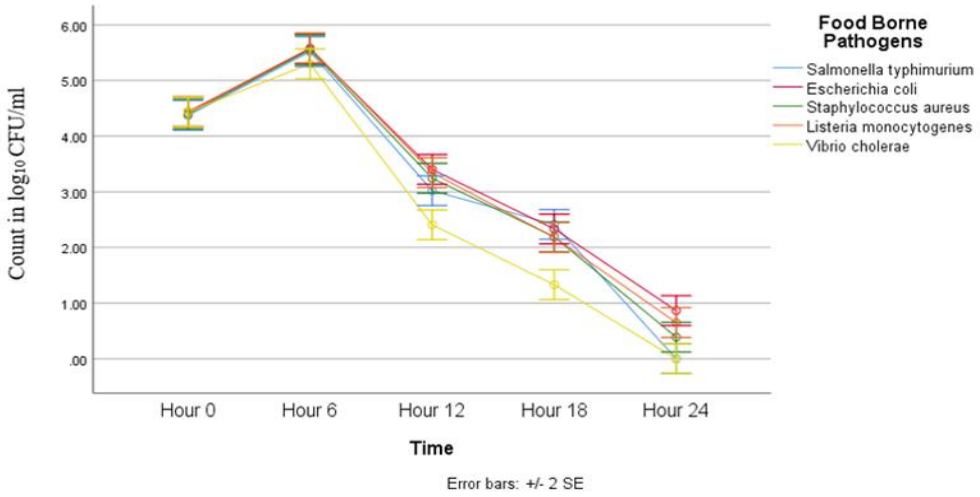

Figure 18: Survival of pathogens in fermenting milk inoculated with *Lb. buchneri* (8RL15)

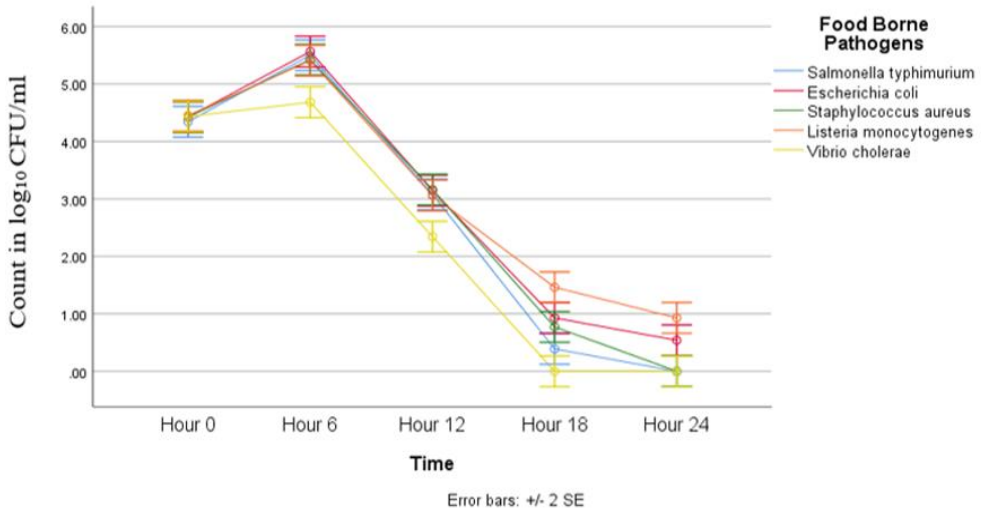

Figure 19: Survival of pathogens in fermenting milk inoculated with *Lb. plantarum* (8AL10)

Sensory Analysis of Degue

The sensory panel found the spontaneously fermented Degue, as well as all five samples of Degue fermented with starter cultures developed in the present work acceptable. With respect to overall acceptability, the highest ranked sample was

the Degue fermented with *Lb. delbrueckii ssp. lactis* (4AL1) followed by that of *Lb. brevis* (8AL16), *Lb. plantarum* (8AL10), *Lb. buchneri* (8RL15), the spontaneously fermented one and lastly the one with the *Lb. delbrueckii ssp. Lactis* (4AL1) and *Lb. plantarum* (4RL2) combination. For taste, the highest rranked sample was *Lb. brevis* (8AL16) fermented Degue; aroma and colour, *Lb. delbrueckii ssp. Lactis* (4AL1) fermented Degue was rated as the highest by the panel as shown in Figure 20.

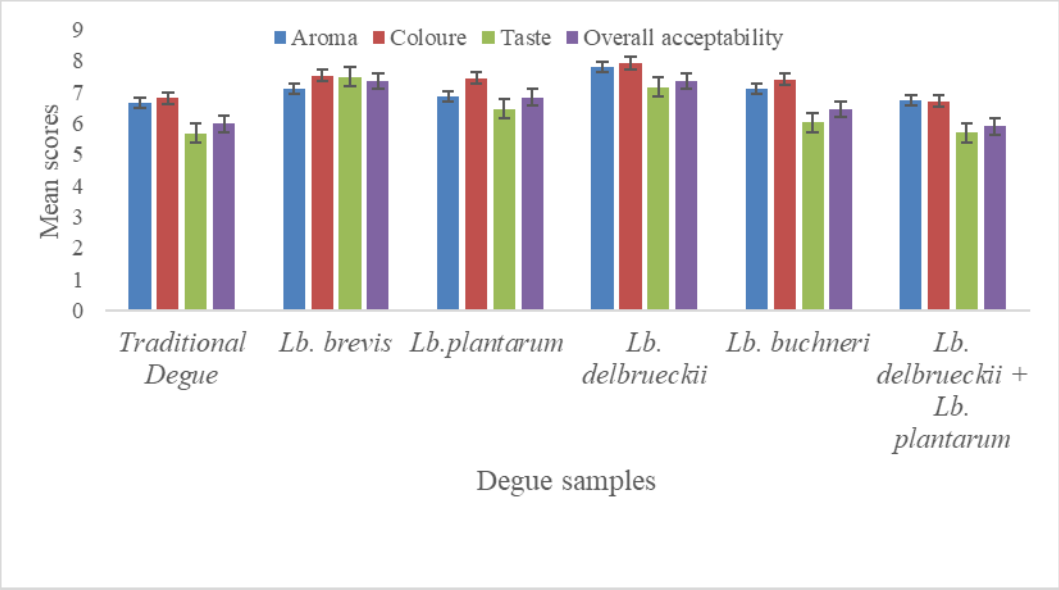

Figure 20: Mean score values for sensory attributes of five starters culture-developed degue samples and traditionally produced degue

Figure 21 also shows that Degue fermented with *Lb. delbrueckii* starter culture was the most frequently selected by the panelists as the best sample followed by that of *Lb brevis, Lb. plantarum, Lb. buchneri,* the spontaneously fermented one and lastly the one with the *Lb. delbrueckii ssp lactis* and *Lb. plantarum* combination.

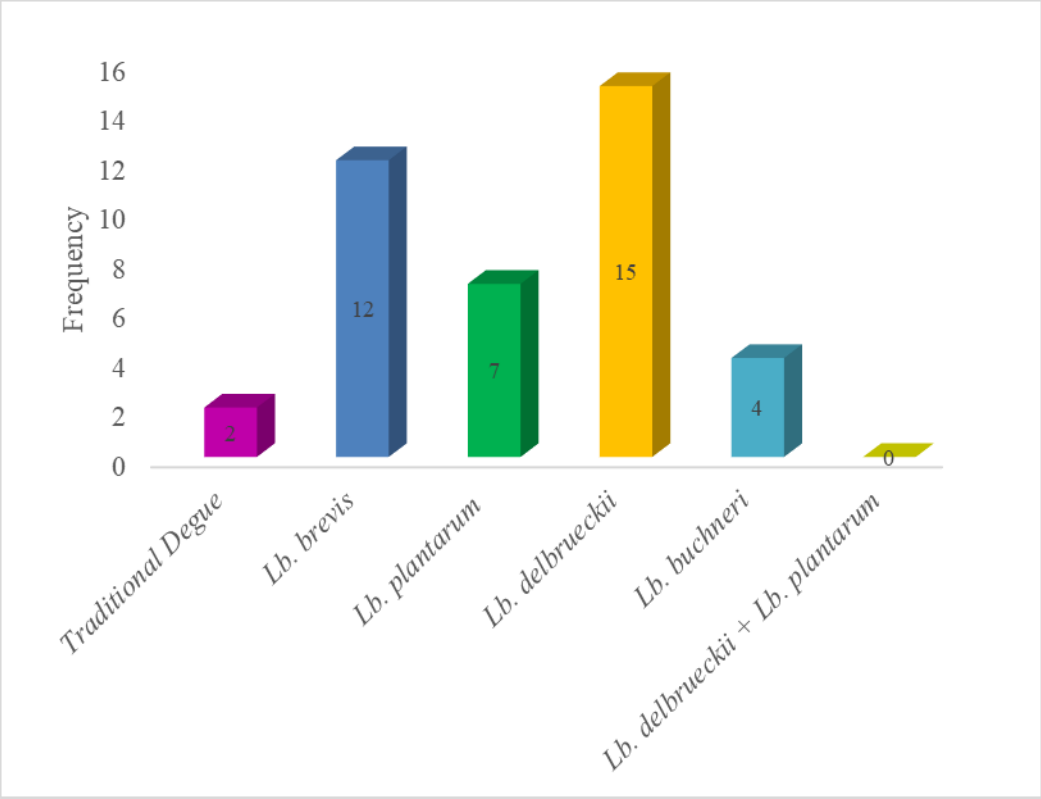

Figure 21: Frequency of a Degue sample being selected as the best by panelist among the five starter culture-developed samples and the traditionally produced degue

CHAPTER FIVE

DISCUSSION

The Role of Lactic Acid Bacteria in the Fermentation of Milk during Degue Production

The main purpose for fermenting milk is to produce a sour product and which also has a longer shelf -life. In this study on the spontaneous fermentation of milk during Degue production, fermenting samples obtained from two different production sites on two separate occasions, showed a steady increase in the population of lactic acid bacteria during fermentation. The lactic acid bacteria were enumerated as Gram-positive catalase-negative rods which grew on MRS media.

The mean population of lactic acid bacteria from triplicate samples from the two different production sites was about 1.0 x 10^4 CFU/ml at the start of fermentation. This increased by 3 log units within the first 12 hours of fermentation to about 1 x 10^7 CFU/ml, and further by another log unit to a population of about 1.0 x 10^8 CFU/ml at the end of fermentation at 24 h. The consequence of the growth of the lactic acid bacteria in the milk during fermentation was the souring of the milk. The mean percentage of titratable acidity in the fermenting samples increased from 0.14 to 0.59 % at 12 h and further to 0.68-0.72 % at 24 h. The mean pH values dropped from 0.65 to 4.83 at 12 h and then to 4.12 at 24 h when the fermentation was stopped. These results indicate clearly that milk used in Degue production is allowed to undergo a spontaneous lactic acid fermentation which gives the product a

desired sour taste associated with several traditional and non-traditional milk products.

The souring of food resulting from the production of organic acids by lactic acid bacteria is a common practice in many West African countries including Ghana. The sour aromatic flavours acquired during fermentation are very well appreciated and may form a natural image of the bulk of foods consumed. During the fermentation, homofermentative lactic acid bacteria convert glucose to lactic acid whilst heterofermentative lactic acid bacteria convert glucose to lactic acid, CO_2, ethanol and / or acetic acid (Sharpe, 1979; Axellsson, 1993).

The primary role of such lactic acid bacteria in food fermentation used to be to effect preservation by converting sugars to organic acids mainly lactic acid thus causing a reduction in pH, by removing carbohydrates as nutrient sources and by producing antimicrobial compounds like hydrogen peroxide, bacteriocins, diacetyl and secondary reaction products. They may also often exhibit probiotic tendencies (Johansson, 1995). Presently the more important role of lactic acid bacteria in food fermentation is to provide diversity in food supply by altering flavour, texture and appearance of raw commodities in a desirable way. The sour aromatic flavours imparted by lactic acid fermentation are desirable traits in fermented products and give a natural image to the product. In addition to lactic acid, which has a preservative effect, various important flavours, texturing and nutritional compounds are produced through the activity of lactic acid bacteria enzymes, both during

fermentation and product maturation (De Vos & Hugenholtz, 2004). According to Ehrmann, Kurzak, Bauerr and Vogel, (2002) the presence of fermentative lactic acid bacteria is indispensable as far as the intrinsic properties of fermented milk food products are concerned. In Ghana, products which are fermented by lactic acid bacteria include Ga-Kenkey, Fante-Kenkey, Nsiho, Gari, Agbelima, Akyeke, Hausa Koko, Maasa (Olsen *et al*., 1995; Amoa-Awua, Appoh, & Jakobsen, 1996; Lei, 2006; Annan, Obodai, Anyebuno, Tano-Debrah & Amoa-Awua, 2015).

In this study *Lactobacillus plantarum* was identified as the dominant species of lactic acid bacteria responsible for the fermentation of milk during Degue production. At both production sites, the concentration of *Lb. plantarum* at the start of the spontaneous milk fermentation was between 1.0 x 10^3 to 9.0 x 10^3 CFU/ml. At 12 h of fermentation the population of *Lb. plantarum* had increased to between 1.0 x 10^6 to 1.6 x 10^7 CFU/ml at both production sites. At the end of fermentation at 24 h the population of *Lb. plantarum* had increased further to 1.2 x 10^8 CFU/ml at both production sites. The other species of lactic acid bacteria which were identified in Degue fermentation at both production sites were *Lactobacillus delbrueckii* ssp. *lactis, Lactobacillus brevis* and *Lactobacillus buchneri.* The concentration of these species also increased steadily during Degue fermentation at both production sites. The population of *Lactobacillus delbrueckii* ssp. *lactis* at both production sites was between 2.0 – 3.0 x 10^3 CFU/ml at 0 h, between 7.0 – 9.0 x 10^6 CFU/ml at 12 h and between 7.0 – 8.0 x 10^7 CFU/ml at the end of fermentation at 24 h. The population of the other two lactic acid bacteria species, *Lactobacillus brevis* and *Lactobacillus*

buchneri, occurred at the same levels as *Lactobacillus delbrueckii* ssp. *Lactis* at the different stages of the Degue milk fermentation.

In this study two different methods were used to identify the lactic acid bacteria isolated from Degue fermentation. After grouping of the isolates based on their morphological and biochemical characteristics, representative isolates were first identified by determination of their pattern of carbohydrate fermentation using the API kit. Identification of microbial species using classical or biochemical methods are however considered unreliable so the isolates were also identified by MALDI-TOF Mass Spectrometry.identification of microorganisms. MALDI-TOF Mass Spectrometry is based on the analysis of protein spectrum from bacterial ribosome, which is closely related to the analysis of gene 16S rDNA sequence. The identification is based on the assessment of ribosomal proteins in the cell since they are part of the cell translational apparatus and can be found in all living cells.

The matrix however, permits these proteins which are non-volatile and thermally labile compounds to be ionized and form intact ions in the gaseous phase; with infrared or ultraviolet pulsed (a laser beam) serving as ionization or desorption source. The matrix then absorbs the laser energy and induces partial vaporization of the sample which is immediately transferred electrostatically to a mass spectrometer vacuum chamber as soon as the vaporization and ionization of molecules occurred. The migration of these ionized particles formed is proportional to the ratio charge mass; that is, the minor ion reaching TOF detector before larger ions. Identification

therefore is based on the score value of the ions released by the equipment (Santos, *et al.,* 2013). The species of all the isolates of LAB identified by the MALDI-TOF Mass Spectrometry agreed completely with the results of the pattern of carbohydrate fermentation determined in the API galleries.

The most dominant lactic acid bacteria species which was found in Degue fermentation in the present work was *Lactobacillus plantarum* 43.75 %, followed by *Lactobacillus delbrueckii* ssp *lactic* 31.25 %, *Lactobacillus brevis* 18.75 %, and *Lactobacillus buchneri* 6.25 %. In Nunu, another fermented milk product in Ghana, Akabanda, Owusu-Kwarteng, Tano-Debrah, Glover, Nielsen and Jespersen, (2013) identified *Lb. fermentum, Lb. plantarum, Lb. helveticus, and Leu. mesenteroides* as the dominant species responsible for the fermentation of the milk.

Obodai and Dodd, (2006) on the other hand identified *Leuconostoc mesenteroides* ssp. *mesenteroides*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* ssp. *lactis* and *Lactococcus lactis* in fermenting samples of Nyarmie also a traditional milk product produced in Ghana. In the case of Nyarmie, the milk is sieved in an aluminium strainer, pasteurized at 65–75 ^{o}C) for 30–45 min, cooled, the fat accumulated on the surface collected before the milk is allowed to undergo spontaneous fermentation.

The involvement of *Lb. plantarum* in the fermentation of other cow milk products in other parts of Africa have been reported including Leben in Algeria (Bensalah *et al.,* 2009), Amabere Ama ruranu and Kule naoto in Kenya (Nyambane *et al.,* 2014; Mathara *et al.,* 2004) and Amasi in South Africa (Osvik, Sperstad, Breines, Hareide, Godfroid, Zhou, Ren, Geoghegan, Holzapfel and Ringø, 2013). El-Baradei *et al.,* (2008) isolated *Lactobacillus delbrueckii* subsp. *bulgaricus* from fermented milk Zabady in Egypt. *Lactobacillus plantarum* has also been reported to be the dominant bacteria in the fermentation of several Ghanaian fermented foods.

In the fermentation of cassava dough into agbelima, Amoa-Awua, Appoh and Jakobsen (1996) and also Mante, Sakyi-Dawson and Amoa-Awua (2003) reported *L. plantarum* to be the dominant lactic acid bacteria accounting for 51 % of the lactic acid bacteria population. In the fermentation of cassava dough during akyeke production, *L. plantarum* accounted for 59.3 % of the lactic acid bacteria (Obilie, Tano-Debrah & Amoa-Awua, 2003), *Lactobacillus plantarum* and *Leuconostoc mesenteriodes* were the dominant lactic acid bacteria responsible for the souring of palm wine which occurs concurrently with the alcoholic fermentation (Amoa-Awua, Sampson & Tano-Debrah, 2007).

Though *Lb. fermentum* is extensively reported as the dominant lactic acid bacteria responsible for the fermentation of maize dough during Kenkey production, some authors have reported the presence of *Lb. plantarum* in Kenkey especially towards

the end of the fermentation (Olasupo, Olukaya & Odunfa, 1997). In fermented nixtamalized maize, Sefa-Dedeh, Cornelius, Amoa-Awua, Sakyi-Dawson and Afoakwa, (2004) reported *Lb. plantarum* to be the dominant lactic acid bacteria in nixtamalized corn prepared with 0.5% lime. In Nsiho i.e Kenkey prepared from dehulled maize meal, Annan *et al., (*2015) reported the dominant LAB as *Lactobacillus fermentum* (47.1 %), *Lactobacillus brevis*, (25 %), *Lactobacillus plantarum* (14.42 %). In the souring of the alcoholic beverage Burukutu, Atter, Obiri-Danso and Amoa-Awua (2014) reported the dominant LAB as *Lb. fermentum* (33.3 %) and *Lb. plantarum* (25 %).

In the present study the population of aerobic mesophiles was also monitored during Degue fermentation. Though the population of aerobic mesophiles increased during the first 12 h of fermentation, a decline in numbers in all samples was observed after 12 hours. Their initial population was at a level of 1.0×10^6 CFU/ml at the start of fermentation and increased to about 1.0×10^8 CFU/ml at 12 h. The population of aerobic mesophiles then decreased by one log unit to about 1.0×10^7 CFU/ml at the end of the 24 h of fermentation. The decline in the population of aerobic mesophiles after 12 hours could be attributed to increase in the population of lactic acid bacteria. Lactic acid bacteria often exhibit antimicrobial activity towards other microorganisms primarily due to the acid they produced resulting in the lowering of pH. At low pH several microbial species are unable to survive. Other antimicrobial compounds produced by lactic acid bacteria include hydrogen peroxide, bacteriocins, diacetyl and secondary reaction products. They may also often exhibit

probiotic tendencies i.e live microorganisms which when administered in adequate amounts confer health benefits to the host (Johansson, 1995). Johansson (1995) was able to demonstrate that *Lactobacillus plantarum* strains isolated from Nigerian Ogi are able to adhere to human intestinal mucosa. Colonisation of the human gut by the lactobaccilli is expected to lead to beneficial effects since they often have antagonistic effects against other bacteria.

Contribution of Lactic Acid Bacteria to the Safety of Degue

According to Özogul and Hamed (2018), lactic acid bacteria dominate the indigenous microbiota during milk fermentation due to its ability to grow under fermentation conditions. Also its ability to produce antagonistic substances, such as bacteriocins, lactic acid, acetic acid, ethanol, hydrogen perioxide acetaldehydes, diacetyl, carbon dioxide and reuterin creating unfavourable conditions for other competitive microbiota.

Several studies have shown that lactic acid bacteria often provide microbial safety in indigenous African fermented foods. Nout, Beernink, Bonants-van and Laarhoven, (1987) showed that *Salmonella typhimurium* was unable to survive in lactic acid fermented sorghum based porridges during storage at 30 ^{o}C for 24 hr. The study of Mensah, Tomkins, Drasar and Harisson, (1990) showed that lactic acid fermentation significantly contributes to the microbial safety of Ghanaian maize porridge if the pH of the ready-to-eat porridge is less than 4.5. The work of Mbugua and Njenga, (1991) also showed that the numbers of *Escherichia coli, Staphylococcus aureus,*

Salmonella typhimurium and *Shigella dysenteriae* decreased when inoculated into fermenting uji, Kenyan fermented cereal porridge during fermentation and storage. Lorri and Svanberg (1994) found a growth inhibition rate of 10^3 for the pathogens *Escherichia coli* (ETEC) and *Campylobacter jejuni* after 3 hr and *Shigella* and *Salmonella* after 7 hr, when the pathogens were inoculated into fermented cereal gruels in Tanzania.

In the present study the LAB isolates demonstrated varying degrees of antimicrobial activity against *Salmonella typhimurium, Staphylococcus aureus, Listeria monocytogenes, Vibrio cholera and Escherichia coli* 0157:H7. *Lb. delbrueckii* demonstrated the greatest inhibition potential against the pathogens whilst *Vibrio cholera* was the most susceptible of the pathogens to the antimicrobial activity of the LAB. In the fermenting Degue inoculated with the different pathogens *Vibrio cholera* could not be detected at 18 h of fermentation and *Salmonella typhimurium* at the end of fermentation at 24 hours. A reduction of one log unit was recorded in the population of *Listeria monocytogenes* during fermentation. *Escherichia coli* exhibited the greatest resistance during the fermentation. The ability of *Escherichia coli* 0157: H7 to survive in pH ranging between 4.5 and 9.0 has been reported by Reinders, Biiesterveld and Bijker, (2001). The results of the well assay in this study were in fairly good agreement with the results of the survival of the pathogens in fermenting milk. Generally, *Lb. delbrueckii* exhibited the widest inhibition zone against the pathogens, followed by *Lb. plantarum, Lb. brevis* and *Lb. buchneri*.

Growth of Yeasts during the Fermentation of Milk in Degue Production

Unlike aerobic mesophiles, the population of yeasts increased throughout the 24 hours of Degue fermentation. The increase in yeast population was by 4 log units in the 24 h fermentation. According to Rohm, Eliskasses-Lechner and Bra¨uer, (1992) and Gadaga, Mutukumira and Narvhus, (2000) yeast is known to be the most important contaminants in milk and a major cause of spoilage in fermented milk because the low pH offers a selective environment for their growth.

According to Jespersen, Halm, Kpodo and Jacobsen, (1994) and Omemu, Oyewole, and Bankole, (2007) acidification of the substrate offers a selective environment for the yeasts to undergo budding due to the co-existence and symbiotic association between lactic acid bacteria and yeasts which have been reported by many traditional African fermented foods. In Ghana, lactic acid fermented foods in which increases in yeast population is reported throughout fermentation include Kenkey (Halm, Lillie, Sørensen & Jakobsen, 1993), Agbelima (Amoa-Awua *et al.*, 1997), palm wine (Amoa-Awua, Sampson & Tano-Debrah, 2007), Burukutu (Atter, Obiri-Danso & Amoa-Awua, 2014).

The yeasts species which were identified in Degue fermentation in this study were *Candida kefir* (40 %), *Candida tropicalis* (37 %) and *Candida orthopsilosis* (23 %). *Candida kefyr* and *Candida tropicalis* were identified both by the MALDI-TOF Mass spectrometry and and the pattern of carbohyudrate fermentation and asymylation, but *Candida orthopsilosis* could only be identified by MALDI-TOF

Mass spectrometry. Of the three yeast species *Candida tropicalis* has also been isolated from the fermentation of cassava into agbelima in Ghana (Amoa-Awua *et al.*, 1997).

Development of Starter Culture for Degue Production

Factors which were taken into consideration in selecting a starter culture for the fermentation of milk during Degue production were rate of acidification of the milk in order to reduce fermentation time, production of exopolysaccharides which enhance viscosity and improves the texture of the milk, antimicrobial activity against common enteric pathogens for safety of the product, secretion of amylase for improvement of aroma and organoleptic quality of the product and its acceptability by consumers. Based on these considerations *Lb. delbrueckii* (isolate no 4AL1) was selected as the starter culture for the production of Degue.

Lb. delbrueckii was able to reduce the pH of the fermenting milk from about 6.7 to 4.1 within the 24 hours of fermentation with an increase in percentage titratable acidity to about 0.72 %. It produced amylase enzyme, exopolysaccharide and also exhibited antimicrobial activity against *Salmonella typhimurium, Staphylococcus aureus, Listeria monocytogenes, Vibrio cholera* and *Escherichia coli*. In the consumer preference test it was ranked highest for colour, aroma and overall acceptability in comparison to the use of *Lb. brevis, Lb. plantatrum, Lb. buchneri* as starter culture, and also the spontaneous fermentation. *Lb. brevis* was however ranked highest for taste ahead of *Lb. delbrueckii*. The score for overall

acceptability for *Lb. delbrueckii* Degue was 7.35 which indicated that the product was liked moderately in comparison to the traditional spontaneously fermented Degue which had a score of 5.98 corresponding to like slightly. In analysis showing the frequency with which each of the different samples was picked as the best product by the sensory panelists, the *Lb. delbrueckii* fermented Degue had the highest frequency of 16, *Lb. brevis* fermented Degue 12, *Lb. plantatrum* fermented Degue 7, *Lb. buchneri* fermented Degue 4, spontaneously fermented Degue 1, and Degue fermented with a combination of *Lb. delbrueckii* and *Lb. plantatrum* 0. Based on all the considerations above *Lb. delbrueckii* were selected as the starter culture for the fermentation of milk during the production of Degue.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The purpose of this study was to explore the similarities between Ghanaian Burkina and indigenous West African Degue based on the production method and to develop a starter culture for Degue production. The starter culture was expected to be use in improving the traditional processing method of Degue on the bases of assuring safety and consistent sensory quality of the product; this is because, instead of a single or defined culture, a wide variety of microorganisms in addition to the main fermentative microorganisms are present whose activities affect the sensory quality of the product.

The fermentative LABs species isolates were study and grouped based on their phenotypic characteristics. A reprensentative isolates from each group was identified using API 50 CH (BioMérieux, Marcy-l'Etoile, France) and Bruker MALDI-TOF Mass spectrometry. Technological properties of the identified LAB specied were also studied and starter cultures developed and used in fermenting pasturized milk which was used in developing five different types of Degue in addition to the spontaneous femented Degue. Ranked by the panel, the overall acceptable Degue was the one produced using *Lb. delbrueckii ssp lactis* as starter culture followed by that of *Lb. brevis, Lb. plantarum, Lb. buchneri,* Spontaneous fermented Degue. Lastly, *Lactobacillus delbrueckii* ssp. *lactis* was selected as a single starter culture for Degue production in Ghana.

Conclusions

The milk/millet product produced and sold in Ghana as Burkina is the same as Degue, a traditional fermented milk product of Fulani origin sold in several Sahelian West African countries. Whereas Degue is made by spontaneously fermented fresh cow milk, Burkina is made from reconstituted milk powder.

The lactic acid bacteria population responsible for the spontaneous milk fermentation in Degue production in Ghana was identified to be composed of *Lactobacillus plantarum* 43.75%, *Lactobacillus delbrueckii* ssp *lactic* 31.25%, *Lactobacillus brevis* 18.75%, and *Lactobacillus buchneri* 6.25%.

All of these isolates exhibited varying degrees of antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli* 0157; H7, *Salmonella typhimurium*, *Listeria monocytogenes* and *Vibrio cholera* which happens to be the most susceptible pathogen to the antimicrobial activity of the LAB.

All the LAB isolates produced amylase and exopolysaccharides and when used as single cultures to produce Degue, a sensory panel ranked the products in terms of overall acceptability as *Lb. delbrueckii* ssp. *lactis*, *Lb. brevis*, *Lb. plantarum*, the spontaneous fermented Degue and lastly *Lb. buchneri*. *Lactobacillus delbrueckii* ssp. *lactis* was therefore selected as a single culture for the fermentation of pasteurized milk during the production of Degue in Ghana.

Recommendations

It is recommended that packaging and storage of Degue should be studied in order to extend the shelf life of the product.

Preservation and distribution of the starter culture in a suitable form should also be studied in order to make the starter culture available to SMEs for Degue production.

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APPENDICES

Appendix A - A Survey on Production of Burkina in some Surburbs of Accra, Ghana Producer Documentation

Background information

Name of the investigator : ……....

Questionnaire Number : ……....

Date of survey……./……./……

Place of survey: ……..……...

- Suburb: ……..……
- District : ……

Person to be interviewed

Surname: ……..…….

First name: ……..……...

Age range: 20 – 25 .□, 26 – 30 □, 31 – 35 □, 36 – 40 □, 40 and above .□

Gender:…….....…

Ethnic /socio-cultural group:……..……

Educational level:……..….

Marital status:……..…...

Number of persons in the household:…….…...

Religion:……..…...

Apart from processing of Burkina which other activity are you engaged in?.....

……

1. Do you have any particular reason why you produce Burkina?

   Family trade □ Profitable □ Employment □ Others

   specify……

2. How did you learn how to produce Burkina?

   Family □ training □ Friends □ Others

   specify.....

3. What materials do you use to make Burkina?

   Powder milk □ Cow milk □ Millet □, Maize □, Sorghum □,

   Others

   specify……..……..

4. Do you know any other product similar to Burkina? Yes □ No □

If yes, please specify.....

5. What is the origin of the Burkina process? Please specify.....

6. Where did it come from? Please specify.....

7. Why do you produce these types? Family trade □ Profitable venture □

   Employment □, Easy to prepare □, Others specify….....

8. Do you steep the cereal before milling? Yes □ No □

9. If yes, how long? 7-8 hours □ 12 hours □ 24 hours □ 36 hours □

   48 hours □ Others specify…….....

10. Do you ferment the milk? Yes □ No □.

11. If yes, how long? 4-6 hours □ 12 hours □ 24 hours □ 36 hours □

    48 hours □ Others (specify).....

12. How do you ferment the milk? Spontaneous fermentation □ Addition of fresh cow milk □ Addition of fermented cow milk □ Addition of yogurt starter □ Addition of yogurt □

13. How do you determine that the milk is fermented enough and can be used to make Burkina? Texture □ Taste □ Smell □ Colour Change □

14. What type of milk do you prefer using for the production of Burkina? .....Why?.....

15. Do you use Spices? Yes □ No □ If yes, specify……..…...

16. What quantity of Burkina do you produce per batch?.....

17. How many workers are involved? 1-2 workers □ 3-5 workers □ above 5 workers □.

18. Are you related to these workers? Yes □ No □.

19. If yes, how closed? Children □ Siblings □ Cousins □ Nieces □.

20. Do you find Burkina producing profitable? Yes □ No □.

21. Averagely, how much of Burkina do you produce in a week? GH C100-500 □ GH C 500-1000 □ GH C 1000-2000 □ GH C Above 2000 □

22. Where do you produce the Burkina? At home □ Factory/location outside home □ Others specify.....

23. What do you do with the Burkina which you are unable to sell? Store it in a refrigerator □ Keep unrefrigerated □ Other specify.....

24. How long can you keep Degue/Burkina and still sell it? 1-3days □, 4-6days □, 7-14days □, Others specify…….....

25. What procedure do you use to produce Burkina?

……..……..

……..……..……..

……..……..……

……..……..

……..……..……

26. In your opinion, which activities if not carried out well will affect the quality of Burkina produced?

Activity	How does it affect quality

27. Have you heard of the product Degue before? Yes □No □. If yes where is it produced?.....

Which ethic group/people produce it?.....

Thank you for your cooperation.

Appendix B - Degue Production Process

Pate: 1 Sieving fresh cow milk

Plate: 2 Whisking of fermented cow Milk

Plate: 3 Steeping of millet grains

Plate: 4 Steeped millet gains

Plate: 5 Millet flour from steeped grains

Pate: 6 Processor making millet granules

Plate: 7 Cooking of millet granules

Plate: 8 Cooked millet granules

Plate: 9 Mixing fermented milk with millet

Plate: 10 Adding sugar to Degue

Plate: 11 Mixing Degue before bottling

Plate: 12 Bottling of Degue

Plate: 13 Degue sold by venders on the street

Appendix C

Consumer Preference for Differently Fermented Degue (Burkina)

Participant Code Number…….

Consent

You are about to taste and evaluate Degue (Burkina) which has been produced under different fermentation conditions. Do you consent to take part in this exercise which is for research purposes only? (Please indicate with a tick)

Yes ☐ No ☐

If you ticked 'Yes' above, please proceed

A:

You have been provided with six (6) samples of Degue (Burkina). Please complete the table by evaluating the samples from left to right and indicate your level of likeness for aroma, colour, taste and overall acceptability of each sample, using the scale provided. Please rinse your mouth with water before tasting each sample.

Scale	Sample code					
	1st Sample	2nd Sample	3rd Sample	4th Sample	5th Sample	6th Sample
9. Like extremely						
8. Like very much						
7. Like moderately						
6. Like slightly						
5. Neither like nor Dislike						

Scale	Sample code					
5. Neither like nor Dislike						
4. Dislike slightly						
3. Dislike moderately						
2. Dislike very much						
1. Dislike extremely						

B:

I. Is this your first time drinking Degue? (Please underline) Yes / No

II. Which of the samples do you like best? Please indicate code……

III. Why? (Please give reasons for your choice above)

……

……

……

……..

Thank you for your participation

Appendix D

Table 1: *Carbohydrates Fermentation Profile of the LAB*

Carbohydrate	Isolates				
	LABG1	LABG2	LABG3	LABG4	LABG5
Glycerol	-	-	-	-	-
Erythritol	-	-	-	-	-
D-arabinose	-	-	-	-	-
L-arabinose	+	-	-	+	-
Ribose	+	+	-	+	+
D-xylose	+	-	-	+	-
L-xylose	-	-	-	-	-
Adonitol	-	-	-	-	-
β methyl-xyloside	-	-	-	-	-
Galactose	+	+	-	+	+
D-Glucose	+	+	+	+	+
D-fructose	+	+	+	+	+
D-mannose	+	+	+	-	+
L-sorbose	-	-	-	-	-
Rhamnose	-	-	-	-	-
Dulcitol	-	-	-	-	-
Inositol	-	-	-	-	-
Mannitol	-	+	-	-	+
Sorbitol	-	+	-	-	-
α methyl-D-mannose	-	-	-	-	-
α methyl-D-glucoside	-	-	-	+	-
N acethyl glucosamide	+	+	+	-	+
Amygdaline	+	+	-	-	+
Arbutin	+	+	-	-	+
Esculin	+	+	-	-	+

Table 1 *Continued*

Carbohydrate	**Isolates**				
	LABG1	LABG2	LABG3	LABG4	LABG5
Salicin	+	+	-	-	+
Cellobiose	+	+	-	-	+
Maltose	+	+	+	+	+
Lactose	-	+	+	-	+
Melibiose	-	+	-	+	-
Saccharose	+	+	+	+	-
Trehalose	-	+	+	-	-
Inulin	-	-	-	-	-
Melezitose	-	+	-	+	-
D-raffinose	-	+	-	+	-
Amidon	-	-	-	-	-
Glycogen	-	-	-	-	-
Xylitol	-	-	-	-	-
β gentiobiose	+	+	-	-	+
D-turanose	-	+	-	-	-
D-lyxose	-	-	-	-	-
D-tagatose	-	-	-	-	-
D-fucose	-	-	-	-	-
L-fucose	-	-	-	-	-
D-arabitol	-	+	-	-	-
L-arabitol	-	-	-	-	-
Gluconate	+	+	-	+	-
2ceto-gluconate	-	-	-	-	-
5 cetoglunate	-	-	-	+	-
LAB Identified	*Lb. brevis*	*Lb. plantar-um* 2	*Lb. delbrueckii-ssp lactis*	*Lb. buch-neri*	*Lb. plantar-um* 1

Appendix E

Table 1 - *Mean Population of Aerobic Mesophiles from the Spontaneous Fermenting Milk (CFU/ml*)

Time	Processer 1	Processer 2
0 h	6.38 ± 0.04	6.41 ± 0.05
12 h	8.32 ± 0.04	8.21 ± 0.08
24 h	7.28 ± 0.02	7.11 ± 0.19

Table 2 - *Mean Population of LAB from Spontaneous Fermenting Milk (log CFU/ml)*

Time	Processer 1	Processer 2
0 h	4.23 ± 0.08	4.19 ± 0.17
12 h	7.29 ± 0.03	7.36 ± 0.07
24 h	8.28 ± 0.04	8.29 ± 0.03

Table 3 - *Mean Population of Yeast from Spontaneous Fermenting Milk (log CFU/ml)*

Time	Processer 1	Processer 2
0 h	4.23 ± 0.13	4.4 ± 0.04
12 h	7.67 ± 0.53	7.28 ± 0.06
24 h	8.29 ± 0.03	7.88 ± 0.51

Table 4 - *Mean Percentage Titratable Acidity from the Spontaneous Fermenting Milk*

Time	Processer 1	Processer 2
0 h	0.14 ± 0.00	0.14 ± 0.00
12 h	0.59 ± 0.00	0.59 ± 0.00
24 h	0.68 ± 0.00	0.72 ± 0.00

Table 6 - *Mean pH from the Spontaneous Fermenting Milk*

Time	Processer 1	Processer 2
0 h	6.65±0.00	6.66±0.00
12 h	4.83±0.01	4.82±0.00
24 h	4.14±0.00	4.11±0.01

Appendix F

Table 7 - *Rate of Acidification of Milk using Single (one) Starter Cultures*

LAB isolates	0 h		6 h		12 h	
	pH	%TTA	pH	%TTA	pH	%TTA
Control	6.66 ± 0.03	0.14 ± 0.01	6.63 ± 0.02	0.14 ± 0.03	6.59 ± 0.01	0.15 ± 0.01
8AL16	6.66 ± 0.01	0.14 ± 0.02	6.53 ± 0.01	0.18 ± 0.02	6.26 ± 0.03	0.22 ± 0.01
4RL2	6.66 ± 0.03	0.14 ± 0.01	6.52 ± 0.01	0.18 ± 0.02	6.27 ± 0.03	0.21 ± 0.02
4AL1	6.66 ± 0.02	0.14 ± 0.04	6.51 ± 0.01	0.18 ± 0.03	6.13 ± 0.01	0.24 ± 0.02
8RL15	6.66 ± 0.01	0.14 ± 0.02	6.53 ± 0.01	0.18 ± 0.03	6.28 ± 0.01	0.21 ± 0.04
8AL10	6.66 ± 0.03	0.14 ± 0.03	6.52 ± 0.01	0.18 ± 0.02	6.27 ± 0.01	0.21 ± 0.03

Table 7- Continued

LAB isolates	18 h		24 h	
	pH	%TTA	pH	%TTA
Control	6.66 ± 0.03	0.14 ± 0.01	6.63 ± 0.02	0.14 ± 0.03
8AL16	6.66 ± 0.01	0.14 ± 0.02	6.53 ± 0.01	0.18 ± 0.02
4RL2	6.66 ± 0.03	0.14 ± 0.01	6.52 ± 0.01	0.18 ± 0.02
4AL1	6.66 ± 0.02	0.14 ± 0.04	6.51 ± 0.01	0.18 ± 0.03
8RL15	6.66 ± 0.01	0.14 ± 0.02	6.53 ± 0.01	0.18 ± 0.03
8AL10	6.66 ± 0.03	0.14 ± 0.03	6.52 ± 0.01	0.18 ± 0.02

Table 8 - *Rate of Acidification of Milk using Combined (two) Starter Cultures*

LAB isolates	0 h		6 h		12 h	
	pH	%TTA	pH	%TTA	pH	%TTA
Control	6.66 ± 0.02	0.14 ± 0.03	6.63 ± 0.02	0.14 ± 0.03	6.6 ± 0.01	0.15 ± 0.02
8AL16 + 4RL2	6.66 ± 0.01	0.14 ± 0.01	6.53 ± 0.01	0.18 ± 0.04	6.28 ± 0.04	0.18 ± 0.03
8AL16 + 4AL1	6.66 ± 0.02	0.14 ± 0.02	6.50 ± 0.03	0.18 ± 0.04	6.18 ± 0.03	0.23 ± 0.01
8AL16 + 8RL15	6.66 ± 0.01	0.14 ± 0.02	6.53 ± 0.01	0.18 ± 0.01	6.29 ± 0.03	0.18 ± 0.04
8AL16 + 8AL10	6.66 ± 0.01	0.14 ± 0.02	6.52 ± 0.04	0.18 ± 0.01	6.29 ± 0.01	0.18 ± 0.02
4RL2 + 4AL1	6.66 ± 0.03	0.14 ± 0.01	6.51 ± 0.03	0.18 ± 0.04	6.19 ± 0.04	0.22 ± 0.04
4RL2 + 8RL15	6.66 ± 0.02	0.14 ± 0.03	6.53 ± 0.01	0.18 ± 0.03	6.29 ± 0.03	0.18 ± 0.04
4RL2 + 8AL10	6.66 ± 0.01	0.14 ± 0.01	6.52 ± 0.02	0.18 ± 0.03	6.27 ± 0.01	0.21 ± 0.01
4AL1 + 8RL15	6.66 ± 0.01	0.14 ± 0.04	6.50 ± 0.02	0.18 ± 0.02	6.17 ± 0.02	0.23 ± 0.03
4AL1 + 8AL10	6.66 ± 0.02	0.14 ± 0.00	6.51 ± 0.05	0.18 ± 0.03	6.17 ± 0.03	0.23 ± 0.04
8RL15 + 8AL10	6.66 ± 0.03	0.14 ± 0.04	6.53 ± 0.02	0.18 ± 0.03	6.28 ± 0.01	0.18 ± 0.04

Table 8 - *Continued*

LAB isolates	18 h		24h	
	pH	%TTA	pH	%TTA
Control	6.56 ± 0.02	0.15 ± 0.04	6.50 ± 0.02	0.16 ± 0.01
8AL16 + 4RL2	5.80 ± 0.04	0.40 ± 0.03	5.00 ± 0.01	0.54 ± 0.02
8AL16 + 4AL1	5.45 ± 0.03	0.45 ± 0.02	4.65 ± 0.03	0.64 ± 0.04
8AL16 + 8RL15	5.80 ± 0.04	0.40 ± 0.04	5.00 ± 0.04	0.54 ± 0.04
8AL16 + 8AL10	5.87 ± 0.01	0.37 ± 0.02	5.03 ± 0.03	0.53 ± 0.03
4RL2 + 4AL1	5.46 ± 0.04	0.44 ± 0.03	4.70 ± 0.01	0.62 ± 0.02
4RL2 + 8RL15	5.98 ± 0.02	0.36 ± 0.03	5.08 ± 0.03	0.52 ± 0.05
4RL2 + 8AL10	5.87 ± 0.03	0.37 ± 0.03	5.04 ± 0.01	0.54 ± 0.01
4AL1 + 8RL15	5.57 ± 0.02	0.41 ± 0.04	4.80 ± 0.01	0.60 ± 0.02
4AL1 + 8AL10	5.48 ± 0.02	0.43 ± 0.04	4.72 ± 0.01	0.61 ± 0.02
8RL15 + 8AL10	5.98 ± 0.02	0.36 ± 0.20	5.09 ± 0.01	0.52 ± 0.03

Table 9 - *Antimicrobial Activities of LAB Isolates against some Foodborne Pathogens*

Foodborne Pathogens	Time (h)	Controls	LAB isolates				
			8AL16	4RL2	4AL1	8RL15	8AL10
Salmonella typhimurium	0	0.00	4.39 ± 0.01	4.41 ± 0.02	4.41 ± 0.01	4.37 ± 0.01	4.34 ± 0.03
	6	0.00	5.48 ± 0.04	5.57 ± 0.02	5.30 ± 0.06	5.52 ± 0.02	5.50 ± 0.01
	12	0.00	3.13 ± 0.07	3.16 ± 0.06	2.10 ± 0.14	3.02 ± 0.03	3.08 ± 0.05
	18	0.00	1.15 ± 0.21	1.30 ± 0.12	0.39 ± 0.12	2.41 ± 0.13	0.39 ± 0.12
	24	0.00	0.00	0.15 ± 0.21	0.00	0.00	0.00
Escherichia coli	0	0.00	4.44 ± 0.01	4.43 ± 0.02	4.42 ± 0.01	4.43 ± 0.02	4.42 ± 0.06
	6	0.00	5.63 ± 0.07	5.68 ± 0.01	5.25 ± 0.15	5.57 ± 0.02	5.56 ± 0.01
	12	0.00	3.25 ± 0.03	3.45 ± 0.02	2.15 ± 0.11	3.40 ± 0.28	3.15 ± 0.04
	18	0.00	1.89 ± 0.83	2.45 ± 0.21	0.81 ± 0.05	2.33 ± 0.01	0.93 ± 0.04
	24	0.00	0.77 ± 0.1	0.90 ± 0.08	0.15 ± 0.21	0.87 ± 0.12	0.54 ± 0.09

Table 9 *Continued*

Foodborne Pathogens	Time (h)	Controls	LAB isolates				
			8AL16	4RL2	4AL1	8RL15	8AL10
Staphylococcus aureus	0	0.00	4.41 ± 0.01	4.42 ± 0.01	4.41 ± 0.02	4.40 ± 0.02	4.42 ± 0.01
	6	0.00	5.64 ± 0.03	5.62 ± 0.07	5.23 ± 0.16	5.55 ± 0.03	5.42 ± 0.01
	12	0.00	3.13 ± 0.02	3.37 ± 0.01	2.13 ± 0.02	3.24 ± 0.14	3.16 ± 0.02
	18	0.00	1.82 ± 0.68	1.49 ± 1.49	0.45 ± 0.21	2.10 ± 0.16	0.77 ± 0.11
	24	0.00	0.15 ± 0.21	0.54 ± 0.09	0.00	0.39 ± 0.12	0.00
Listeria monocytogenes	0	0.00	4.44 ± 0.01	4.44 ± 0.01	4.45 ± 0.01	4.44 ± 0.01	4.45 ± 0.04
	6	0.00	5.62 ± 0.01	5.65 ± 0.01	4.45 ± 0.01	5.59 ± 0.01	5.41 ± 0.01
	12	0.00	3.06 ± 0.03	3.36 ± 0.03	2.20 ± 0.34	3.30 ± 0.12	3.06 ± 0.03
	18	0.00	2.30 ± 0.04	2.33 ± 0.01	1.15 ± 0.21	2.18 ± 0.2	1.46 ± 0.04
	24	0.00	1.15 ± 0.21	1.02 ± 0.03	0.69 ± 0.12	0.65 ± 0.49	0.93 ± 0.04

Appendix G

ANOVA Table: *Population of Aerobic Mesophiles from the Spontaneous Fermentation of Milk sample (log CFU/ml).*

Tests of Between-Subjects Effects

Dependent Variable: Population of Aerobic Mesophiles from the Spontaneous Fermentation of Milk sample (log CFU/ml).

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	10.579[b]	5	2.116	266.347	.000
Intercept	955.391	1	955.391	120267.021	.000
Time	10.515	2	5.257	661.822	.001
Processer	.033	1	.033	4.104	.066
Time * Processer	.032	2	.016	1.994	.179
Error	.095	12	.008		
Total	966.065	18			
Corrected Total	10.675	17			

a. Variable = Population of Aerobic Mesophiles from the Spontaneous Fermentation of Milk sample (log CFU/ml)

b. R Squared = .991 (Adjusted R Squared = .987)

ANOVA Table: *Population of LAB from Spontaneous Fermentation of the Milk sample (log CFU/ml).*

Tests of Between-Subjects Effect

Dependent Variable: Population of LAB from the Spontaneous Fermentation of Milk sample (log CFU/ml)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	54.296[b]	5	10.859	1526.185	.000
Intercept	785.682	1	785.682	110422.581	.000
Time	54.284	2	27.142	3814.618	.000
Processer	.001	1	.001	.151	.705
Time* Processer	.011	2	.005	.770	.485
Error	.085	12	.007		
Total	840.064	18			
Corrected Total	54.381	17			

a. Variable = Population of LAB from Spontaneous Fermentation (CFU/ml)

b. R Squared = .998 (Adjusted R Squared = .998)

ANOVA Table: *Population of Yeast from Spontaneous Fermentation of the Milk sample (log CFU/ml).*

Tests of Between-Subjects Effects

Dependent Variable: Population of Yeast from Spontaneous Fermentation of the Milk sample (log CFU/ml).

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	49.687[b]	5	9.937	105.487	.001
Intercept	789.881	1	789.881	8384.747	.001
Time	49.157	2	24.578	260.906	.001
Processer	.197	1	.197	2.088	.174
Time * Processer	.333	2	.167	1.768	.212
Error	1.130	12	.094		
Total	840.699	18			
Corrected Total	50.817	17			

a. Variable = Population of Yeast from Spontaneous fermentation (CFU/ml)

b. R Squared = .978 (Adjusted R Squared = .968)

ANOVA Table*: pH from the Spontaneous Fermentation of the Milk sample.*

Tests of Between-Subjects Effects

Dependent Variable: pH from the Spontaneous Fermentation of the Milk sample.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	20.467[b]	5	4.093	368404.500	.001
Intercept	487.032	1	487.032	43832884.500	.001
Time	20.466	2	10.233	920953.500	.001
Processer	.000	1	.000	24.500	.001
Time * Processer	.001	2	.001	45.500	.001
Error	.000	12	1.111E-5		
Total	507.499	18			
Corrected Total	20.467	17			

a. Variable = pH from the Spontaneous fermentation

b. R Squared = 1.000 (Adjusted R Squared = 1.000)

ANOVA Table: *Rate of Acidification Trial using One LAB strains for the inoculation if the Milk*

Tests of Between-Subjects Effects

Dependent Variable: Rate of Acidification of Milk using One LAB Isolate.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1571.264[a]	59	26.632	2691570.325	.000
Intercept	1836.748	1	1836.748	185634278.541	.000
Time	7.174	4	1.793	181260.853	.000
Treatment	1.477	5	.295	29856.199	.000
Type	1532.820	1	1532.820	154917193.914	.000
Time * Treatment	1.601	20	.080	8091.069	.000
Time * Type	20.022	4	5.006	505899.022	.000
Treatment * Type	3.972	5	.794	80297.016	.000
Time * Treatment * Type	4.198	20	.210	21211.415	.000
Error	.001	120	9.894E-6		
Total	3408.013	180			
Corrected Total	1571.265	179			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

ANOVA Table: *Rate of Acidification Trial using Two LAB strains for the inoculation of the Milk*

Tests of Between-Subjects Effects

Dependent Variable: Rate of Acidification of Milk using combination of Two LAB Isolates.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1876.004[a]	109	17.211	5409184.850	.000
Intercept	2140.694	1	2140.694	672789696.572	.000
Time	22.280	4	5.570	1750583.329	.000
Treatment	2.857	10	.286	89780.797	.000
Type	1768.530	1	1768.530	555823625.143	.000
Time * Treatment	18.803	40	.470	147737.934	.000
Time * Type	46.912	4	11.728	3685967.900	.000
Treatment * Type	1.546	10	.155	48593.802	.000
Time * Treatment * Type	15.076	40	.377	118451.382	.000
Error	.000	110	3.182E-6		
Total	4016.698	220			
Corrected Total	1876.004	219			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

ANOVA Table*: Antimicrobial Activities of LAB isolates against Foodborne Pathogens (CFU/ml).*

Tests of Between-Subjects Effects

Dependent Variable: Antimicrobial Activities of LAB isolates against Foodborne Pathogens (CFU/ml)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1265.102[a]	149	8.491	237.258	.000
Intercept	1701.779	1	1701.779	47553.795	.000
Pathogen	12.991	4	3.248	90.755	.000
Group	354.838	5	70.968	1983.088	.000
Time	722.498	4	180.625	5047.296	.000
Pathogen * Time	7.932	16	.496	13.853	.000
Pathogen * Group	4.088	20	.204	5.712	.000
Time * Group	156.882	20	7.844	219.193	.000
Pathogen * Time * Group	5.872	80	.073	2.051	.000
Error	5.368	150	.036		
Total	2972.249	300			
Corrected Total	1270.470	299			

a. R Squared = .996 (Adjusted R Squared = .992)