CHAPTER ONE

1. INTRODUCTION

1.1 Background of Study

Yoghurt is a fermented milk product from an anaerobic fermentation of milk and milk products by the lactic acid fermentation through the action of majorly *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* Other microorganisms like *Lactobacillus acidophilus, Lactobacillus subsp. casei, and bifidobacteria* are also used in yoghurt fermentation (FAO/WHO,1977; Priyanka et al., 2012).Yoghurt is formed when milk is coagulated, or form curds by the work of lactic acid or its degree present or introduced into milk enough to coagulate it (Priyanka *et al*., 2012).

According to Priyanka *et al.,* (2012), yoghurt is known to be a functional food because it contains probiotics and helps in the improvement of specific functions in human health. Functional food are foods that includes probiotics, prebiotics and symbiotics, and are consumed for specific health benefits or functions. They could be in forms of drug supplements or food. Probiotics are known to be "live microbial feed supplements that benefit the host by enhancing the host's gut microbial balance". Prebiotics are "non-digestible foods and food ingredients that benefit the host by encouraging the growth and the activity of one or a small number of useful bacteria in the colon". Symbiotics are a mix of probiotics and prebiotics that "beneficially effects the host by increasing the survival and implantation of live health-promoting microbial organisms in the gastro-intestinal tract by selectively stimulating the development and/or activating the metabolism of one or a limited number of these health-promoting bacteria" (Priyanka *et al.,*2012).

Weerathilake *et al.,* (2014)mentioned that "The nutritional composition of yoghurt depends on the fermentation time, type of milk used (animal milk or imitation milk) and the strain of starter culture". The nutritional composition of yoghurt is generally like that of milk. Yoghurt is said to be a dense food and is rich in protein, carbohydrates, amino acids, minerals (Calcium and Phosphorus) and vitamins (thiamin-B1, riboflavin-B2, niacin-B3, folate-B9, cobalamin-B12, and vitamin C), but is lacking in iron. The fat content of yoghurt depends on the fat content of the mixture and the type of milk used (Weerathilake *et al.,* 2014). According to the NDBsr26, a 100g

serving of plain low-fat yoghurt contains 183 milligrams of calcium, 17 milligrams of magnesium, 234 milligrams of potassium, 144 milligrams of phosphorus, and 0.9 milligrams of zinc(El-Abbadi *et al.*, 2014).

Zhang and Mahoney (1989) mention that, animal milk products are commonly consumed all over the world, with high or sufficient proportions of proteins, vitamins, and minerals except iron. The lack of iron in dairy products decreases the iron density of diets because dairy products are consumed daily. The fortification of dairy product with staple available products is essential for iron improvement daily by diet diversification.

The terms iron deficiency (ID) and iron deficiency anaemia are not interchangeable (IDA). Iron deficiency is the major cause of anaemia in the world and is also a major health problem mainly in underdeveloped countries (Llanos *et al.,* 2016). Because iron is a component of haemoglobin and cytochromes, it is extremely vital for blood production. The pathophysiological anaemia causes can be grouped into different categories namely, blood loss, increased destruction of red blood cells and decreased production of functional red blood cells. (Dicato *et al.,* 2010). Debasmita and Binata, (2017) mentioned that "in most developing countries, majority of anaemia cases are due to inadequate supply of nutrients like iron, folic acid and vitamin B12, proteins, amino acids, vitamins A, C, and other vitamins of B-complex group i.e., niacin and pantothenic acid which are also involved in the maintenance of haemoglobin levels in the blood". To reduce and prevent the growing rate of anaemia, dietary improvement, supplementation, and food fortification or enrichment are helpful and beneficial ways for whole population or certain group in a population.

1.2 Statement of the Problem

Yoghurt is a good source of protein, carbohydrate, fat, and vitamins. It is also rich in calcium and phosphorus. Yoghurt is observed to be lacking in iron because iron bioavailability in milk is extremely below the daily dietary recommendation (Hadi *et al.,* 2015).

Fortification and enrichment of yoghurt has previously been achieved by using iron-chelated protein isolates, salt solutions like ferrous sulphate and ferrous fumarate etc. (El-Kholy *et al.,* 2011, Nayak and De, 2017) which is known to have effects such as off-flavours, oxidized flavour

and metallic flavour, oxidation of fat which reduces the absorption of this element in the fortified milk. Nutrition scientists have cited that fortification/enrichment of food products using natural resources like fruits, cereal, vegetables etc. is one of the best ways to improve the overall nutrient intake of food with minimal fallouts. Hence, fermented milk products are gaining high interest as of recent, it is thereby a highly-consumed food in the world. Yoghurts are now used to deliver nutritional components into human diet. Furthermore, fortification/enrichment is one of the good ways to improve nutrient intake in daily food products (Hadi et al., 2015).

Iron enrichment and fortification from plant based/sources is important for not only the improvement of the texture and consistency are particularly important characteristics that affects its quality such as appearance, mouth feel and over all acceptability, but also the improvement of essential minerals like Iron for a healthy blood and circulatory system and to reach the daily diet recommendation of iron.

1.3 Aim and Objectives of the Study

The purpose of this study is to improve the nutritional quality of cow milk yogurt by enrichment with malted *Pennisetum glaucum* (pearl millet), *Telfairia occidentalis* (fluted pumpkin leaves) and *Glycine max* (soybeans). The objectives of this study are:

1. to produce yoghurt enriched with malted Pennisetum glaucum (pearl millet), Telfairia occidentalis (fluted pumpkin leaves) and Glycine max (soybeans).
2. to determine the nutritional quality, physicochemical and microbial characteristics of the enriched yoghurt.
3. to evaluate the consumer acceptability of the enriched yoghurt.

1.4 Scope of the Study

This study work centers on the enrichment of cow milk yoghurt with malted *Pennisetum glaucum* (pearl millet), *Telfairia occidentalis* (fluted pumpkin leaves) and *Glycine max* (soybeans) to improve the nutritional quality and consumer acceptability of the yoghurt.

1.5 Significance of the Study

According to Hadi *et al.,* (2015), Yoghurt is observed to be lacking iron because iron bioavailability in milk is extremely below the daily dietary recommendation. The significance of this study is to improve the bioavailability of iron in yoghurt by enriching it with known plant-sources of iron.

1.6 Definition of Terms

1.6.1 Yoghurt

Yoghurt is a milk product from fermentation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus*. It is formed when milk is coagulated, or form curds by the work of lactic acid or its degree present or introduced into milk enough to coagulate it.

1.6.2 Anaemia

Anaemia is defined as the condition that results from the inability of the erythropoietic tissues to maintain a normal haemoglobin concentration on account of inadequate supply of one or more essential nutrients leading to reduction in the total circulating haemoglobin.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Yoghurt

The term "yoghurt" comes from the Turkish word *"jugurt,"* which was used to designate acidic fermented dairy dishes and drinks (Priyanka *et al.,* 2012).*Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus* ferment milk to make yogurt. It contains some digested lactose, and is richer in some essential nutrients like protein, calcium, phosphorus, riboflavin, thiamin, vitaminB12, folate, niacin, magnesium and zinc compared to milk (Hadi *et al.,* 2015, Ademosun *et al.,* 2019). Yoghurt is formed when milk is coagulated, or form curds by the work of lactic acid or its degree present or introduced into milk enough to coagulate it (Priyanka *et al.,* 2012).

Reeta *et al.,* (2015) mentioned that the nutritional composition of yoghurt varies depending on the strains of starter culture used in the fermentation, the type of milk used (whole, semi skimmed, or skimmed milk), the species from which the milk is obtained (bovine, goat, or sheep), the type of milk solids, solid non-fat, sweeteners, and fruits added before fermentation, and the length of the fermentation process.

2.1.1 History and origin of yoghurt

Fermentation is a food processing technique that has been used for thousands of years to preserve food. Acidifying bacteria are beneficial microorganism that helps in milk preservation and in the improvement of the shelf life of milk by preventing the growth of undesirable microorganisms (Françoise, 2017).

According to Weerathilake *et al.,* (2014), yoghurt over the centuries has been recognized as the most popular fermented food product and it has a wide range acceptance worldwide. It is known to have tremendous nutritional and health benefits. Yoghurt origin is dated back to the 6000 B.C. in central Asia when the Neolitic people began food producers by milking their cows and storing them in sheep-skin. This accidentally led to the discovery of fermented milk products which includes yoghurt. Over centuries, yoghurt has evolved into a commercial making/production

which has further improved into the production and availability of varieties with a range of flavours, forms, and textures (Weerathilake *et al.*, 2014).

Yoghurt was found by nomadic peoples in the Middle East approximately 5,000 B.C(Francoise, 2017). It has been eaten by various cultures for thousands of years. Yoghurt gets its name from the Turkish term yogurmak, which implies thickening, coagulating, or curdling (Moreno *et al.*, 2013). Yogurt is also known as katyk (Armenia), dahi (India), zabadi (Egypt), mast (Iran), lebenraib (Saudi Arabia), laban (Iraq and Lebanon), roba (Sudan), iogurte (Brazil), cuajada (Spain), coalhada (Portugal), dovga (Azerbaijan), and matsoni in many cultures and nations (Georgia, Russia, and Japan) (Ramandeep *et al.*, 2017, Fisberg and Machado 2015).

In early France, yogurt was known about the time 1542. King Francis I was healed of chronic diarrhea by consuming yogurt (Fisberg and Machado, 2015). In 1905, Stamen Grigorov, a Bulgarian medical student studying in Geneva, Switzerland, was the first to describe a spherical and rod-shaped lactic acid bacterium that is found in Bulgarian yogurt; the species was named Bacillus bulgaricus. Around the 20th century, Russian Nobel laureate Elie Metchnikoff, a scientist at the Pasteur Institute in Paris, hypothesized that Bulgarians lived long lives based on the theory that they regularly consumed yogurt; his research helped make yogurt popular in Europe and served as the foundation for the field of probiotics consumption. Danone is a private company that started in the 1960's, they helped in the commercialization of yoghurt and the food was industrialized and spread round Europe (Francoise, 2017).

In recent times, yogurt is typically milk that has been fermented and acidified with viable and well-defined bacteria, creating a thickened, often flavoured, product with an extended shelf life. It contains essential nutrients and is a medium for fortification (were other health improving and nutrient modifying probiotics, fibers, vitamins, and minerals are added). Yoghurts also represent functional food and can be modified with sweeteners, fruits, and flavours to affect the nutritional and health benefit, consistency, and aroma. Yoghurt is recently produced from other animal milk like goat, sheep, buffalo, camel, etc. and plant sources like rice, soy, and nuts (Fisberg and Machado, 2015).

2.1.2 Types of yoghurt

Weerathilake *et al.*,(2014)) and Ramandeep *et al.*,((2017)) mentioned that different types of yoghurt are available in different varieties and forms based on the various factors associated with their production. The numerous categories of yoghurt are:

A. Based on the chemical composition of the milk: Milk is the major ingredient in yoghurt production, the different variety of nutrient composition is based on the nutrient of the milk used. Due to diet diversification, milk type production and dairy diet preference, yoghurt production can come in forms of regular yogurt or full-fat, low-fat yogurt and non-fat yogurt. Low-fat yogurt and non-fat yogurt are produced from low-fat milk or partially-skim milk, and skim milk respectively.
B. Based on the physical nature of the product:
    1. Set yoghurt: Set yoghurt is also known as solid yoghurt. It is majorly characterized as incubated and cooled in final packaging during production.
    2. Stirred yoghurt: Stirred yoghurt are known as semi-solid yoghurt. During production the mixture is incubated, after fermentation breaking is done by stirring before cooling and packaging.
    3. Drinking yoghurt: Drinking yogurt are in fluid state. In production it usually undergoes the process of homogenization to reduce the particle size which assured the hydro colloidal distribution and stabilization of protein suspension.
C. Based on the flavour of the product:
    1. Plain/Natural yoghurt: It is made to be unsweetened and is a naturally fermented milk product containing no added colour or any other additives. It is closer to the nutritional value of milk, provides the nutritional benefits associated with fermentation and is low in calories. Plain/natural yoghurt has the richest calcium content amongst other yogurt products.
    2. Flavoured yoghurt: Yoghurt comes in different flavours due to different consumer preference, needs and demands. Flavours are added during production stage based on the need for a wide array of tastes and to increase the sweetness of the product.

D. Based on the manufacturing processes:

1. Pasteurized and UHT yoghurt: Pasteurized yogurt are prepared after fermentation by heat treatment with different time-temperature combinations in order to prolong the shelf life and to reduce the natural tartness of the yogurt.
2. Frozen yoghurt: The Pennsylvania Code defines frozen yogurt as a food which is prepared by freezing while stirring a pasteurized mix. It is inoculated and incubated to get the fermented yoghurt product before it is frozen. It is produced to have the same consistency as ice-cream.
3. Dried yoghurt/yoghurt powder: It is produced by fermenting a non-fat milk with standard starter culture/microorganism to the right pH and consistency, the freeze drying it to get yoghurt powder. Yoghurt powders ae used in the production of confectioneries and in baked products.
4. Concentrated yoghurt: After fermentation, the coagulum is broken down then the yogurt is concentrated by boiling off some water under vacuum conditions. Heating of low pH Yogurt leads to denaturation of protein which produces a rough and gritty texture.

E. Based on their production method or Origin:

1. Balkan-style Yogurt: Balkan-style yogurt is also known as set-style yogurt which is produced to have a characteristic thick texture and made in small and individual batches. It is incubated for over 12 hours or more until the desired thickness, flavour and creaminess is attained. It can be used as a substitute for sour cream, salad dressings or topping. It can also be consumed regularly, either unsweetened, sweetened or with addition of fruits, cereals or anything of choice.
2. Greek-style Yogurt: It is also known as Mediterranean-style yogurt, it is manufactured with partially condensed milk or by staining whey from plain yogurt to make it thicker and creamier. It is available in full-fat and low-fat yogurt.
3. European-style Yogurt/Stirred Curd Yogurt: The European-style yogurt is a type of stirred yogurt with a characteristic creamy and smooth texture. It is manufactured by fermenting the yogurt mixture in a large vat instead of individual cups, then cooling and stirring in order to obtain the creamy texture. It is mostly

produced with added fruits (like blueberries, strawberries, mango, and peach) and flavours.

4. French-style Yogurt: This style of yogurt is also known as custard-style yogurt. It is made by direct culturing in a pot according to a French culture, its final product is in a pudding-like texture. Sometimes French-style yogurts are flavoured with fruit pieces which is stirred into the mixture. It is known to be a good source of iron, protein and vitamin A.
5. Fruit Yogurt: Fruit yoghurt can either be produced by setting the fruits at the bottom of the packaging (sundae-style yogurt) or the uniform distribution of fruit within the yogurt itself (Swiss-style yogurt). Fruit pieces or pulp are added at production stage, it produces variety of tastes and increases the consumer appeal and sweetness of yoghurt.
6. Herbal Yogurt: this is produced by the addition of herbal substances, spices and seeds like cinnamon, fenugreek, Moringa, Ugu etc. during the production process before fermentation or after fermentation of the yoghurt.

2.1.3 Production of yoghurt

Yogurt is made with a variety of ingredients including milk, sweeteners, stabilizers, fruits, flavours, and bacterial cultures (Weerathilake *et al.*,2014, Corrieu and Be'al, 2016). Production processes of yoghurt are as follow (Figure 2.1):

1. Milk Standardization: In the production of yoghurt, milk standardization is important for the mixing of solid fat (SF) and solid not fat (SNF) (Ramandeep *et al.*, 2017). According the Codex Alimentarius Commission, yogurt should have a minimum protein content of 2.7% and a maximum fat content of 15% in order to achieve this, the FAO/WHO standards stipulate that milk should be standardized with the minimum SNF and milk fat content of 8.2% and 3%. The average composition of bovine milk comprised of 4.5% lactose, 3.3% protein, 3.5% of fat and 0.7% mineral matter, to attain the desired SNF content the milk mixture is fortified with milk powder. (Weerathilake *et al.*, 2014) Stabilizers such as pectin and gelatin are added to the yogurt mix in order to attain the characteristic properties of yogurt namely, texture, mouth feel, appearance, viscosity and

to inhibit the whey separation, the use of thickeners and stabilizers (gelatin, pectin, xanthan gum, carrageenan, starch, etc.) at concentrations varying from 5% to 10%is allowed by FAO/WHO to improve the yogurt texture (Corrieu and Be'al2016).

2. Homogenization: Homogenization is a size reduction process; it involves the breaking down of fat globules into smaller size in order to get uniformity and a size of 1µm throughout the yoghurt product (Ramandeep *et al.,* 2017) Homogenization is a very important stage and process in yoghurt production because it prevents the separation of fat from whey and also make the creaminess of milk uniform in order to attain a good end product. Homogenizers and Viscolizer are used in this process. Milk homogenization is accomplished by forcing the liquid milk through a small opening at a high speed to break down the fat globules with shearing force (Weerathilake *et al.,* 2014)

   Homogenization pressures often range between 10 and 20 MPa for 10 -17 minutes. Since the efficiency of homogenization is much better when the fat phase is in a liquid state, the process is usually carried out at high temperatures (55^{o}C to 80^{o}C) (Lange *et al.,* 2020).Serra *et al.,* (2009) identified that recently, ultra-high-pressure homogenization are introduced into commercial yogurt production. This has brought the increase in yogurt firmness and water holding capacity in comparism to the conventional homogenization process (Ramandeep *et al.,* 2017).

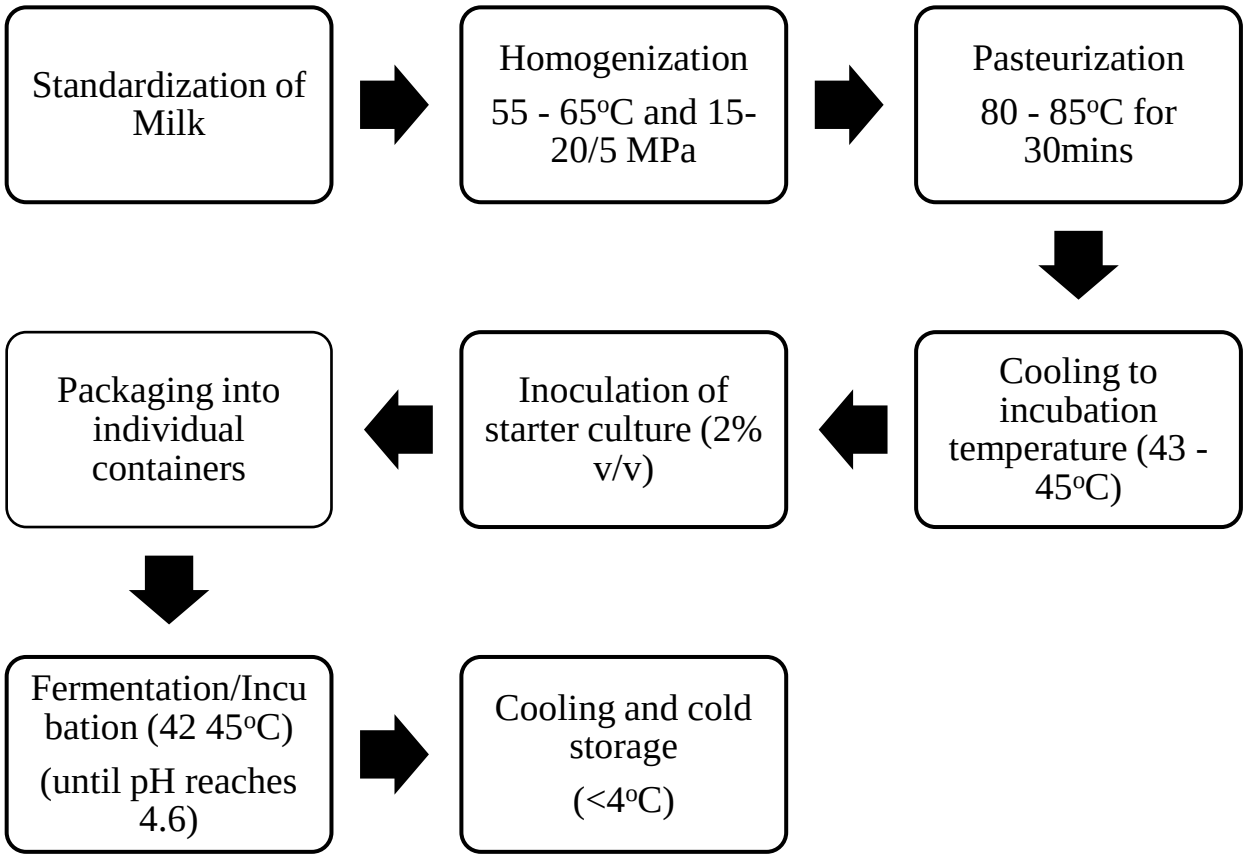
3. Pasteurization (Heat treatment): The aim of pasteurization in yoghurt production is to rid the milk of all pathogen, to substantially reduce the total bacterial count for improved quality and to destroy lipase and other milk enzymes. Pasteurization of milk is done using plate heat exchanger at industrial yogurt manufacturing. The mix is heated at 90 or 95 C for 3–7 min (5min) before cooling down to fermentation temperature. The heat exchanger can either be for batch process (Holding method or low temperature long time (LTLT) method) or a continuous process (High temperature short time (HTST) method) (Corrieu and Be'al, 2016).

4. Inoculation and Fermentation: After pasteurization, the temperature of the mix is allowed to reduce to 43-46^{o}C. 2% (v/v) of starter culture is added in ratio with the yogurt mix (Weerathilake *et al.,* 2014).A typical standard starter culture consists of *Staphylococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus* in 1:1 ratio. In bacterial fermentation, lactose is converted to lactic acid which reduces the pH of the milk from

6.7 to ≤ 4.6 which causes the formation of a gel/coagulation of the protein casein. This process is known as milk acidification (Lange *et al.,* 2020). During milk acidification, volatile compounds are produced which gives the yoghurt a characteristic flavour and aroma. (Ramandeep *et al.,* 2017)

5. Cooling and Packaging: packaging and cooing of yoghurt is based on their physical nature, whether it is a stirred type or a set type of yoghurt. A stirred type yoghurt is produced fermenting the yoghurt mix in a tank followed by breaking and stirring prior to packaging and cooling. While a set type yoghurt is produced by filling the mix into the packaging material and fermenting/incubating in the packaging material, then cooling. According to the USDA Specifications, after the final steps in manufacturing and/or packaging, the yogurt should be cooled and maintained at temperatures less than 7.2 ºC (Weerathilake *et al.,* 2014).

Figure 2.1: Production steps for Yoghurt



Sources: Lange *et al.*, 2020; Weerathilake *et al.*,2014

2.1.4 Nutritional and Health Benefits of yoghurt

Aryan and Olson, (2017) identified in history during the 1500s, that King Francis I of France was cured of a severe diarrhea by consuming prescribed yoghurt. Yoghurt is known to contain the same nutritional profile as milk. Consumption of yoghurt may lead to enhancement in bone and muscle health of both developing children and young adults and also the elderly. Calcium, protein and vitamin D are essential for good muscle and bone growth, development and maintenance. Due to the rich micro flora (probiotics) in yoghurt which are viable cells, consumption of yoghurt may lead to the enhancement of immune response and reduction in risk of infectious diseases associated with gut, stomach and the small intestine. Studies shows that deficiency of vitamins and minerals leads to immune impairment, micronutrients like zinc and vitamin B6, beneficial bacteria and protein are essential for the improvement of human and animal immunity (El-Abbadi *et al.,* 2014).

Weerathilake *et al.,* (2014) stated that the consumption of probiotics are beneficial in maintaining excellent health and restoring bodily vitality. They also discovered that probiotics in yoghurt are therapeutic and important in the treatment of intestinal disorders, as well as the prevention of urogenital infection, constipation relief, diarrhea prevention, infantic diarrhea prevention, hypercholesterolemia prevention, colon/bladder cancer prevention, and osteoporosis prevention. Yogurt consumption is also reported to be effective in cytokine production, T-cell function and natural killer-cell activity, and thereby result an overall immunological enhancement, also provides preventive effects on the relapse of ulcerative colitis, maintenance of normal intestinal flora, enhancement of the immune system, reduction of the lactose-intolerance, serum cholesterol levels, and the enhance anticarcinogenic activity.

The nutritional composition of yoghurt is said to be similar to that of milk. Yogurt contains protein, amino acids, carbohydrates, calcium, phosphorus, vitamins and minerals (Table 2.1). The nutritional composition of yogurt varies according to the variety or type of Yogurt. He also identified that yogurt is a rich source of riboflavin (Vitamin B2), thiamin (Vitamin B1), vitamin B12, folate, niacin, magnesium and zinc. The carbohydrate present in yoghurt is lactose. In total, raw milk contains about 4.6% lactose content, the amount of lactose is normally lowered by 20-30% during the fermentation process by the conversion of lactose into a simpler form of glucose and galactose due to the metabolic activity of lactic acid bacteria (Ramandeep *et al.,* 2017).

Table 2.1 Nutritional composition of different varieties of yoghurt (per 100 g)

Component	*Whole milk yoghurt*	*Low fat yoghurt*	*Non-fat yoghurt*	*Greek style yoghurt*	*Drinking yoghurt*
Energy (kcal)	79	56	54	133	62
Carbohydrate (g)	7.8	7.4	8.2	4.8	13.1
Fat (g)	3.0	1.0	0.2	10.2	Trace
Thiamin(mg)	0.06	0.12	0.04	0.12	0.03
Riboflavin(mg)	0.27	0.22	0.29	0.13	0.16
Niacin (mg)	0.2	0.1	0.1	0.1	0.1
Vitamin B6(mg)	0.10	0.01	0.07	0.01	0.05
Vitamin B12(mg)	0.2	0.3	0.2	0.2	0.2
Folate (µg)	18	18	8	6	12
Carotene (µg)	21	Trace	Trace	Trace	Trace
Vitamin D	0	0.1	Trace	0.1	Trace
Potassium(mg)	280	228	247	184	130
Calcium (mg)	200	162	160	126	100
Phosphorus (mg)	170	143	151	138	81

Source: Weerathilake *et al.*,(2014)

2.2 Pearl Millet

Pearl millet is botanically known as Pennisetum glaucum. It is one of the four most important staple cereals (rice, maize, sorghum and millets) cultivated and consumed in tropical semi-arid regions of the world principally in Africa and Asia. It is one of the majorly common drought resistant crops. Pearl millet is rich in trace minerals like iron and zinc that its deficiency can lead to hidden hunger and other health defects; it contains a high number of antioxidants and these nutrients along with the antioxidants may be beneficial for the overall health and wellbeing (Nambiar *et al.,* 2011).

Di Stefano *et al.,* (2017) identified pearl millet to be a small seeded grain in the sub-family *Panicoideae,* of the grass family of *Poaceae* and it is majorly reported to have harvest of 46%, followed by foxtail, proso, and finger millet specie in the world. It is the staple diet for rural households in the underdeveloped countries of the world, it as a grain and its stover is a valuable livestock feed in India and Africa. Amongst the low-income population preponderantly in Northern Nigeria and some parts of West Africa countries such as Niger, Mali, and Burkina Faso, pearl millet is consumed almost daily (Ibidapo *et al.,* 2019).

Pearl millet has different species which include *Pennisetum typhoideum, Pennisetum glaucum, Pennisetum americanum,* and *Pennisetum spicatum* and other millet species include *Setariaitalica* (foxtail millet), *Panicum miliaceum* (hog millet), *Eleusine coracana*(finger millet) (Taylor 2004;Florence andAsna2011; Ibidapo *et al.,* 2019).Florence and Asna (2011) stated that GOI (2008) mentioned pearl millet to be ranked third after wheat (*Triticum aestivum*) and rice (*Oryza sativa*) in terms of its production in the world. Florence and Asna (2011) described pearl millet as a commercially (from local farmers) available crop with lower shelf life compared to those produced from various agricultural institutes. One of the reasons for its low shelf life could be longer periods in storage time due to transportation factors and some amount of processing and chemical storage before they reach the market.

In the consumption and preparation of pearl millet as food, commonly used industrial and traditional processing techniques used includes decortication, malting, fermentation, roasting, flaking, and grinding to improve their edibility, nutrient bioavailability, nutritional and sensory properties and acceptability (Ahmed *et al.,* 2013; Pelembe 2002).

In most Africa and Asian countries, millet is mostly used as weaning food, breakfast cereals, children and infant food, in porridge, soups, sprouts, bread and stuffing's, in the production of beverages and traditional alcoholic drinks and in beer production (Krishnan and Meera 2018; Pelembe 2002).

Millet's porridge is a traditional food in Russian, German and Chinese cuisines. Millet is estimated to account for about 35% of total cereal food consumption in Burkina Faso, Chad and Gambia. In Mali and Senegal, millets constitute roughly 40% of total cereal food consumption per capital, while Niger and Arid Namibia it is over 65%, other countries in Africa where millets are common food source include Ethiopia, Nigeria and Uganda (Odusola *et al.,* 2013).

2.2.1 Nutritional and Health Benefits of Pearl Millet

Approximately, Millets contain 7-11% proteins, 60-70% carbohydrates, 2-7% crude fibre and 2-5% fat (Adeoti *et al.,* 2020).

Pearl millet nutritionally is a rich energy source, fiber, B-vitamin and some micronutrients such as potassium, phosphorus, copper, magnesium, zinc, iron and manganese, folic acid and b-carotene. Additionally, it contains rare amino acids like methionine, proteins, carbohydrates, fats, crude fibre and several physiological functional components such as phytochemicals, which include dietary fiber and polyphenol compounds and antioxidants required for human health (Ibidapo *et al.,* 2019; Krishnan and Meera2018; Owheruo *et al.,* 2018).

Due to the high antinutritional factors of pearl millet such as phytic acid, tannin, polyphenols, and oxalic acid, it hinders the protein digestibility, solubility of starch and in vitro and bio-accessibility of minerals in the body during consumption (Odusola *et al.,* 2013; Owheruo *et al.,* 2018)

In order to increase the digestibility, solubility, bio-accessibility of nutrients and sensory property, processes such as germination, malting, fermentation, thermal and mechanical treatments of grains aid the reduction their antinutrient content (Kindiki *et al.,* 2015; Ahmed *et al.,* 2013).

Pearl millet contains 8mg/100g of Iron and3.1mg/100g of zinc which may help to increase the haemoglobin levels, it also contains a high level of antioxidants like phenolic compounds which may have anticancer properties. Phenolic compound especially flavonoids, have been found to inhibit tumor development (Huang and Ferraro 1992).

Omega-3 fatty acids is said to be present in pearl millet as compared to any other cereal grain, omega-3 fatty acid has a role in the prevention and treatment of cardiovascular diseases, diabetes, arthritis and certain types of cancer. Researchers found that certain n−3 fatty acids are also converted into eicosanoids, they are studied to cause a reduction in the concentration of triglycerides in the blood, improve immune response, brain and eye function, and in infant development (Kinsella *et al.,* 1990).

2.2.2 Effects of Steeping, Malting (germination) and Fermentation on the nutrients in Pearl Millets

I. Steeping: Steeping which is also known as soaking is a food processing technique used for the reduction of antinutritional compounds such as phytic acid, tannin and polyphenol to improve bioavailability of proteins and minerals (Ahmed *et al.,* 2013). Generically, soaking provides moist conditions for grains during germination which make them soft and also activate an endogenous enzyme like phytase to enhance ease of further processing and is associated with the reductions in levels of enzyme inhibitors as well as other anti-nutrients in order to increase the digestibility and nutritional value. It is also an important requirement for fermentation in food production. Antinutritional factors are often water soluble in nature, which helps in their removal from foods through leaching (Samtiya *et al.*, 2020)

II. Malting/Germination: Nkhata *et al.,* (2018) noted that germination is considered as a highly suitable method for reducing the anti-nutrient components of plant-based foods. Germination of seeds generally activates the enzyme phytase, which degrades phytate and leads to decreased phytic acid concentration in grains. (Samtiya *et al.,* 2020). Some of these anti-nutrients are essential and are embedded in seeds of grains and legumes for germination and growth. During malting, the seed uses these anti-nutrients and produces malt flavour, and it improves the quality of seed for further processing. Archana and

Kawatra (2001) reported that germination appreciably improved the in vitro protein (14% to 26%) and starch (86% to 112%) digestibility in pearl millet, Arora *et al.,* 2011 also reports germination and probiotic fermentation to be significant factor in the improvement and accessibility in the contents of thiamine, niacin, total lysine, protein fractions, sugars, soluble dietary fiber, and in vitro availability of Ca, Fe, and Zn of food blends. (Ahmed *et al.,* 2013)

III. Fermentation: Ahmed *et al.,* (2013) stated that the chemical compositions of millet grains and their food products were observed to be altered by fermentation. This is one of the most important process by which millet grains are used to produce different kinds of traditional fermented foods and drinks in developing countries in Africa and Asia.

In grain like pearl millet, phytic acid normally forms complexes with the metal cations including iron, zinc, calcium and proteins. These complexes are generally degraded by enzymes, which require an optimum pH maintained by fermentation processes. Thus, this kind of degradation decreases the phytic acid content and liberates soluble iron, zinc and calcium, which enhance the nutritional level of food grains (Gibson *et al*., 2010). Fermentation of millet grains by lactic acid bacteria (LAB) has been observed to increase free amino acids and their derivatives by proteolysis and by metabolic synthesis. Fermented grains show improvement their nutritional value by increasing the content of essential amino acids such as lysine, methionine and tryptophan (Mohapatra *et al*., 2019).

2.3 Soybean

Mercy *et al.,* (2017), reported soybean (*Glycine max L.*) a leguminous plant originating from the native to Eastern Asia and later introduced into Nigeria in 1908. The production of soybean spread across United States (36%), Brazil (36%), Argentina (18%), China (5%) and India (4%) to be the world popular producers.

Soybean (*Glycine max)* belongs to the pea vegetable family *Leguminosae*, it is one of the oldest cultivated crops of the tropics, sub-tropical and temperate regions, and one of the world's most important sources of protein and oil. Soybean is specially unequalled to other legumes for a couple reason; it is thereby classified as a highly valuable economical agricultural commodity; it is known to possess agronomic characteristics which is its ability to adapt to a wide range of soil

and climate; and its nitrogen fixing ability. This makes it to be a good rotational crop for use with high nitrogen consuming crops such as corn and rice (Adelakun *et al.,* 2013,Mercy *et al.,* 2017).

Glycine max seed has the richest food value amongst most seed plants foods consumed in the world. It is often used in the production of composite flour, and by leading infant food manufacturers all over the world because of its high nutritional value. Soya bean is also processed into soy milk for consumption and production of ice-cream, yoghurt and in soy drink beverages; soy cuds such as awara (northern food), tempeh, tofu and cheese; soy flour for further production of cookies, biscuits and bread. Its oil is used in the production of edible oils, salad dressing, and is used in local paint, cosmetics, and cloth print inks and in soap making industries. The meal and soybean proteins are used in the manufacture of soy-chelating agents, synthetic fibre (artificial wool) adhesives and textile it is also used in livestock feed production (Ruth *et al.,* 2018; Adelakun *et al.,* 2013)

2.3.1 Nutritional and Health Benefit of Soybean

Soybean is a commonly used, inexpensive and nutritional source of dietary protein all over the world and most especially underdeveloped countries. Its protein content which is 40%, is higher and more economical than that of beef 19%, chicken20%, fish 18% and groundnut 23% (Ruth *et al.,* 2018; Mercy *et al.,* 2017)

Glycine max does not only contain 40% protein of high biological value but also essential amino acids particularly glycine, tryptophane and lysine. It also possesses 23% carbohydrate, 20% fat and oil and reasonable amount of minerals, vitamins and dietary fiber, and is high in antioxidants, omega-3 fatty acids and other beneficial compounds like phytosterols, lecithin and phenolic acids. It is commonly known to possess some anti-nutrients that inhibit the accessibility and digestibility of its protein, dietary fibre and minerals (Baranwal*etal*2013; Ruth *et al.,* 2018).

Soybean flour lately has been incorporated as an ingredient in foods, beverages, and condiments production in order to promote higher optimal health benefit (Ruth *et al.,* 2018).

Mercy *et al.,* (2017), reported Modern researchers have taken concern in soy protein health benefits and have concluded that, Soy protein has the ability to lower LDL levels and decrease the risk of coronary heart disease (CHD). It also provides health benefits to diabetics' patients, by helping in the maintenance of sugar levels. Soy-oil is discovered to be very rich in essential fatty acids like linoleic and linolenic acids, which is very important for human health in the regulating of blood pressure and facilitate the absorption of vital nutrients. Soybean contains isoflavones that minimizes the risk of developing certain cancers, particularly, genistein—an isoflavone that has extensive antioxidant properties. Soybeans serves as a good source of calcium, magnesium, lecithin, riboflavin, thiamin, fiber, folate (folic acid) and iron which are essential in combating malnutrition, hidden hunger, and maintaining a healthy balance in blood haemoglobin levels. Soybean benefits the ecosystem agriculturally by improving soil fertility during nitrogen fixation from the atmosphere. Soybean seed, like other legumes, is known to trypsin inhibitors, phytate and oxalates which are anti-nutritional factors are usually removed or greatly reduced by steaming/cooking, soaking, fermentation, roasting and hydrothermal treatment during processing.

2.4 Fluted Pumpkin

Fluted pumpkin (*Telfairia occidentalis* Hook. F.) is a vegetable plant, belonging to the genus *Cucurbita* and the family *Cucurbitaceae*. *Cucurbitaceae* consists of 90 genera and 750 species, it is an herbaceous family of vines which are known as gourd plants and it include: cucumber, melon, squash and pumpkin. Some of its species include *Cucurbita pepo, Cucurbita moshata,* and *Cucurbita maxima*(Akintayo *et al.,* 2018; Onovo *et al.,* 2009; Odiaka *et al.,* 2008; Olorunfemi *et al.,* 2014).

Fluted pumpkin is a tropical vine commonly grown in some West and Central Africa, particularly in Nigeria, Cameroon, Ghana, Sierra Leone and Benin Republic, for its edible seeds and leaves (Gbadamosi *et al.,* 2018). It is cultivated in nearly all the agroecological zones of Nigeria (Adeyemo and Tijani2018).

Fluted pumpkin shares various features with plants that have large leaves, creeping, or climbing systems usually with tendrils, fleshy fruits with many seeds and more or less fibrous root system (Abdussamad *et al.,* 2015; Akintayo *et al.,* 2018).

In Nigeria, Telfairia occidentalis Hook. F. is popularly known as Ugu. The leaves are mostly consumed as food in soups, stews, sauces and in healthy local drinks and smoothies, in pottages and porridges; they are also used for medicinal purposes. The seeds are consumed roasted or boiled. It is also used as condiments, or in paste form as food thickeners, in oils extraction and processing, and as a propagating material (Orole *et al.,* 2020;Odiaka*et al.,* 2008; Usunobun and Egharebva 2014).

2.4.1 Nutritional and Health Benefits of Fluted Pumpkin

Gbadamosi *et al.,* (2018) recorded fluted pumpkin to be a good source of carbohydrate, protein, fibre, minerals, vitamins like carotenoids (vitamin A), Tocopherol (vitamin E), B-complex (ThiamineB1, RiboflavinB3 and NicotinamideB5) and Ascorbic acid (Vitamin C), antioxidants and pigments (chlorophyll). Fluted pumpkin contains antioxidant properties like carotenoids (vitamin A) and tocopherols (Vitamin E) that helps in skin health to reduce skin damages from sun rays and it also acts as an anti-inflammatory agent.

Carotenoids like Lutein and Zeaxanthin which are found in green leafy vegetables are important factors for human vision improvement. Generally, carotenoids and chlorophylls perform key roles in the prevention of illnesses like cancer, cardiovascular diseases and other chronic diseases associated with oxidative stress.

Consumption of fluted pumpkin leaves may also be considered to reduce blood pressure, stimulate digestion and improve healthy gut and metabolism. They are known to be beneficial in the management of liver problems, hypercholesterolemia, hypertension, diabetes, meningitidis, threatening and in weakened immune defense system and curing heart diseases.

Telfairia occidentalis Hook. F. has been medically acknowledged to perform other in terms medicinal functions like anti-anaemia, antidiabetic, antioxidant and antimicrobial activities. It has also been observed to act as blood purifiers.

2.5 Anaemia and Iron Deficiency

Anaemia is one of the worlds -wide problem that is common among young children, pregnant woman, and adolescent girl. Nutritional anaemia may be defined as the condition that results from the inability of the erythropoietic tissues to maintain a normal haemoglobin concentration on account of inadequate supply of one or more essential nutrients leading to reduction in the total circulating haemoglobin (Debasmita and Dr. Binata 2017, Liberal *et al.*,2020). Iron deficiency anemia occurs when there is a decrease in the production of red blood cell or low iron stored up in the body reserve. Some linked sources to the causes of anemia are inadequate intake of iron, reduced iron absorption, increased iron demand in the blood, and drastic increase in blood loss or iron loss in the blood (Short and Domagalski 2013). According to Dicato *et al.,* (2010), Anaemia is defined as a haemoglobin level <14 g/dl for men and <12 g/dl for women. It has been subdivided into mild (10 g/dl—normal), moderate (8–10 g/dl), severe (6.5–8 g/dl) and life threatening (<6.5 g/dl or unstable patient) anaemia.

Yoghurt is a good source of protein, carbohydrate, fat, and vitamins. It is also rich in calcium and phosphorus. Yoghurt is observed to be lacking iron because iron bioavailability in milk is extremely below the daily dietary recommendation. Fortification and enrichment of yoghurt has previously been achieved by using iron-chelated protein isolates, salt solutions like ferrous sulfate and ferrous fumarate etc. (El-Kholy *et al.,* 2011). Iron-fortified yogurt has a relatively high iron bioavailability, yet it is observed to have other effects such as off-flavours, oxidized flavour and metallic flavour, which are due to the catalytic role of iron and the presence of iron salts. Oxidation of fat occurred in yogurt and milk which were fortified with ferrous sulfate, ammonium and ferric reduces the absorption of this element in the fortified milk (Dr. Nayak and De, 2017). Nutrition scientists have cited that fortification/enrichment of food products using natural resources like fruits, cereal, vegetables etc. is one of the best ways to improve the overall nutrient intake of food with minimal fallouts. Hence, fermented milk products are gaining high interest as of recent, it is thereby a highly-consumed food in the world. Yoghurts are now used to deliver nutritional components into human diet. Furthermore, fortification/enrichment is one of the good ways to improve nutrient intake in daily food products (Hadi *et al.,* 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Location

The experiment was carried out in the Laboratory of Food Science and Technology in Mountain Top University, Km 12 Lagos-Ibadan expressway, behind MFM Prayer City Ibafo, Ogun State**,** Nigeria**.**

3.2 Raw Materials and Equipment

3.2.1 Source of raw materials

The cow milk (Peak brand) powder was obtained from Mountain Top University mini-mart. The ugu leaves, soybean and millet seeds were obtained from Ibafo market-Ogun state. The starter culture was obtained from Payless Baker Centre No.14 Olabisi Street, Ojota Lagos.

3.2.2 Equipment and Instruments

Air oven dryer, attrition milling machine, refrigerator, blender, weighing balance, stainless steel trays, pot, cooking stove, gas cylinder, bucket, colander sieve, roasting pan, metal sieve, nylon bags, jute sack bag, bowl, paper tape, cooking spoon, plastic bottles, plastic bowl plates, fume cupboard, digestion box, Khejal distillation machine, measuring cylinders, beakers, conical flask, burets, separating funnel, retort stand, muffle furnace, Muffle furnace (Vulcan 3-550), funnels, reagent bottles, distilled water bottles, Analytical balance, Potato Dextrose Agar (PDA) [Microxpress, A division of Tulip Diagnostics (p) Ltd], de Man, Rogosa and Sharpe (MRS) Agar [Titan Biotech Ltd], Plastic sterile petri-dishes, Durham bottles, Bunsen burner, spirit lamp, McCartney bottles, micro pipette, Eppendorf pipette, water bath, Autoclave, Colony counter[UNISCOPE Colony Counter; SURGIFRIEND MEDICALS, ENGLAND], Microscope, Porcelain crucibles, Volumetric flasks (2000ml), 50ml polyethylene centrifuge tube, Precision balance (0.0001g accuracy) [Denver], Vortex mixer [Genius 3], Weighing paper, Centrifuge [5810R machine], Atomic Absorption Spectrophotometer [Buck 211], Inductively Coupled Plasma –Optical Emission Spectrophotometer (ICP/OES) [Perkin Elmer].

3.2.3 Chemicals and reagents

The chemicals and reagents were of analytical grade and was obtained from the Laboratory of Food Science and Technology, Mountain Top University, Km 12 Lagos-Ibadan expressway, behind MFM Prayer City Ibafo, Ogun State**,** Nigeria**.**

They include: Khejal tablets, Conc. H_2SO_4, 40% NaOH, 40% Boric acid (Bromocresol green, ethanol, 0.1g Methyl red), 0.1000 HCl, 0.1N NaOH, Petroleum ether, Ethanol, Diethyl ether, Nitric acid (HNO3) [Redistilled, min 69%, GFS chemical], Hydrochloric Acid (HCl 37%) [Merck no. 1.00317], Aqua Regia and distilled water.

3.3 Sample Preparation

3.3.1 Production of Soybean flour

The soybean seeds were thoroughly sorted and cleaned with water to eliminate damaged seeds, metals, stones, chaff, and other debris. It was then boiled for 10 minutes and steeped in cool water for 30 minutes. The tenderized seeds were subjected to dehulling by hand rubbing/washing. The dehulled soybean seed was then cleaned in water and dried in the air oven drier (memmert air oven model UN 55, (SCHWBACH, GERMANY) and (UNISCOPE SM9053 laboratory Oven, (SURGIFRIEND MEDICALS, ENGLAND). The dried seeds were milled using a Local Attrition Mill and sieved with a wire mesh, it was then packaged in a polyethylene bag at room temperature prior to use, as shown in a flow chat in Figure 3.1 below (Ome *et al.*,2018).

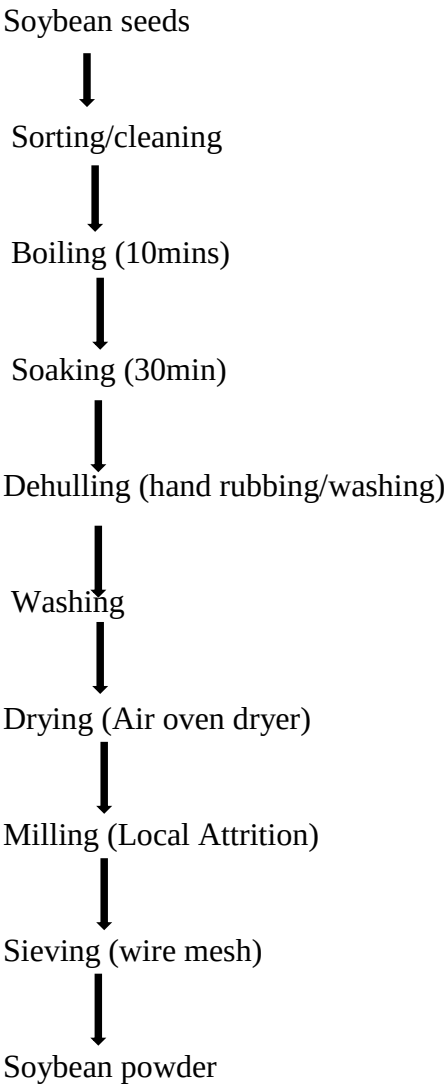


Figure 3.1 Flowchart for soya bean flour production (Source: Ome A.P., *et al.*,2018)

3.3.2 Production of Millet flour

The millet grains were sorted and stones and other kinds of grains were removed, it was then washed and steeped in clean water for 7hrs. The water was decanted and the millet grains were placed in a colander to drain out the remaining water. The grains were left to germinate for 96hrs and sprouting occurred. The sprouted grains was then dried in an air oven drier at 50^{o}C (memmert air oven model UN 55, (SCHWBACH, GERMANY) and (UNISCOPE SM9053 laboratory Oven, (SURGIFRIEND MEDICALS, ENGLAND) for 15hrs. The dried sprouts was then detached from the grains and winnowed out from the malted millet. The malted millet was then milled into powder with a local attrition mill, sieved with a wire mesh and packaged in a polyethylene bag prior to use. The steps are shown in a flow chart below in figure 3.2 (Badau *et al.,* 2006, Suma and Urooj 2011, Owheruo *et al.,*2018).

3.3.3 Production of Ugu Paste

The fluted pumpkin leaves were destalked and washed under running water to eliminate the sand, insects and other contaminants. The cleaned leaves were blanched for 3min, left to cool under room temperature and was frozen over-night. The frozen leaves was thawed and then blended into paste. The ugu paste was then packaged in a plastic container and kept frozen at 4^{O}C prior to use. The flow chart is shown in figure 3.3 below. (Korshima *et al.,* 2019).

3.3.4 Production of Yoghurt samples

The milk and enrichment sources were prepared and then thoroughly mixed for homogeneity. The milk was pasteurized in a cooking pot for 30min at 85-90^{o}C. This was done to achieve the following:

Produce sterile and conducive environment for the starter culture.

Denature and coagulate whey proteins to enhance the viscosity and texture.

The pasteurized milk was allowed to cool to a temperature of 43-45^{o}C before it was inoculated with the starter culture for 7hours at 40-45^{o}C. The fermented milk produced was harvested and broken down into stirred/drinkable yoghurt. The flow chart for yoghurt production is shown below in figure 3.4:

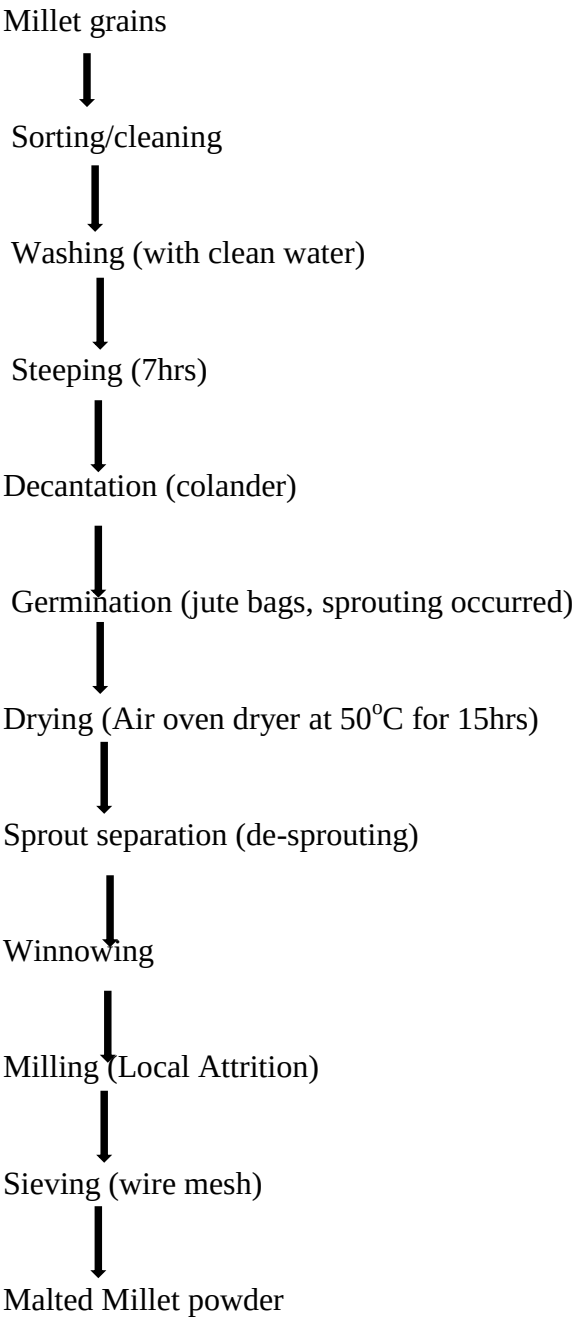


Figure 3.2 Flowchart for malted millet flour production

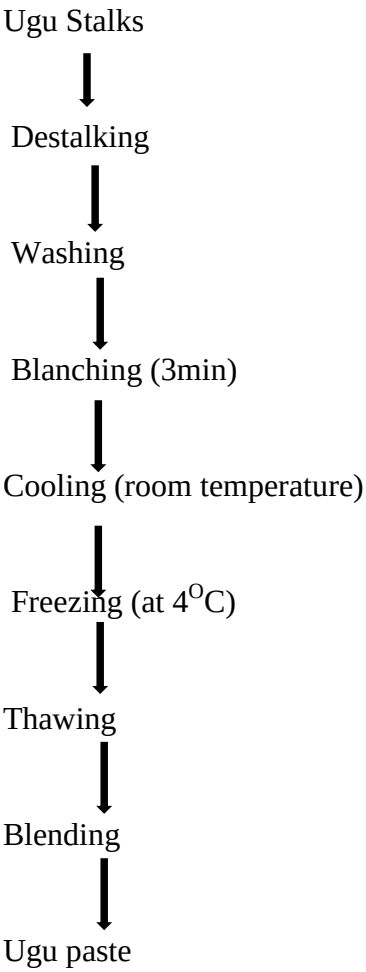


Figure 3.3 Flowchart for ugu paste production

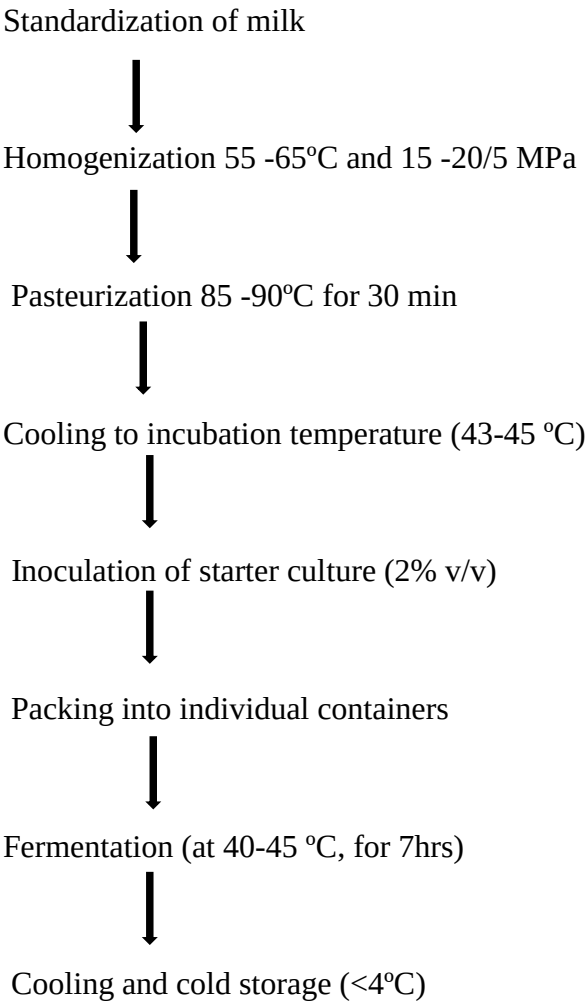


Figure 3.4 Flowchart for yoghurt production(Reeta *et al*., 2015)

3.3.5 Yoghurt samples

3.3.5.1 SY1: 70%cow milk and 30% soybeans yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 140g of milk powder and 60g of soybeans powder were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.3.5.2 SY2: 60%cow milk and 40% soybeans yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 120g of milk powder and 80g of soybeans powder were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.3.5.3 SY3: 50%cow milk and 50% soybeans yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 100g of milk powder and 100g of soybeans powder were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

Table 3.1: yoghurt samples proportion

Samples	Cow milk (%)	Enrichment sources (%)
		Soybeans
SYI	70	30
SY2	60	40
SY3	50	50
		Malted Millet
MY1	70	30
MY2	60	40
MY3	50	50
		Ugu paste
UY1	95	5
UY2	90	10
UY3	85	15
CRL	100	0

SY= soybeans yoghurt, MY= malted millet yoghurt, UY= ugu yoghurt, CRL= control

3.3.5.4 MY1: 70%cow milk and 30% malted pearl millet yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 140g of milk powder and 60g of millet powder were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.3.5.5 MY2: 60%cow milk and 40% malted pearl millet yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 120g of milk powder and 80g of millet powder were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.3.5.6 MY3: 50%cow milk and 50% malted pearl millet yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 100g of milk powder and 100g of millet powder were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.3.5.7 UY1: 95%cow milk and 5% Ugu yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 190g of milk powder and 10g of ugu paste were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.3.5.8 UY2: 90%cow milk and 10% Ugu yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 180g of milk powder and 20g of ugu paste were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.3.5.9 UY3: 85%cow milk and 15% Ugu yoghurt

200g of milk was dissolved in 1Litre of water to make the control sample, to make the percentage ratio, 170g of milk powder and 30g of ugu paste were dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.3.5.10 CRL: 100%cow milk yoghurt

200g of milk was dissolved in 1Litre of water and was pasteurized to 90^{o}C. The sample was inoculated with starter culture (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*) after it was cooled to 43^{o}C and incubated at 45^{0}C for 7hours. After fermentation, the yogurt was broken down with agitation to produce stirred/drinkable yoghurt. It was then transferred to refrigerator at 4ºC.

The yoghurt samples proximate, physiochemical and microbial component was analyzed and sensory evaluation done, replicated for each treatment.

3.4 Proximate Analysis

Proximate analysis was determined according to the official method of analysis described by the Association of Official Analytical Chemist (AOAC 2010, AOAC 2012).

3.4.1 Determination of protein content

The protein content of the yoghurt was determined according to AOAC, (2012). 10g of samples was weighed into a digestion flask and 1 kjeldahl catalyst tablet was added, 12ml of conc.H2SO4 was added and digested for 4 hours in a (baker) fume hood with (reactor 1001 digester) until a clear solution was obtained. The digest was cooled, 30ml of 4% boric acid was dispensed into a conical flask with 0.132g of methyl red indicator and 0.198g bromocresol green plus in a 200 ml alcohol.

The digest was diluted with 75ml distill water was dispensed into Kjeldahl distillation flask, the conical and the distillation flask was fixed in place and 50ml of 4% NaOH was added through the glass funnel into the digest, in the kjeldahl auto distillation unit. The steam exit was closed and it was stopped when the solution of the boric acid and indicator reached 150ml total volume. The distillate was titrated with 0.1 NHCl until an end point was reached (violet colour).

$$\% \text{ Total Nitrogen (gN/L)} = \frac{(T-B)\times N\times 14.007}{volume\ of\ sample\ (ml)}$$

T = Titration volume of sample (ml)

B = Titration volume of blank

N = Normality of acid to 4 place decimals

Therefore, the crude protein content was determined by multiplying percentage Nitrogen by a constant factor of 6.38i.e.

% Crude protein = % N × 6.38

3.4.2 Determination of moisture content

10g of each sample was weighed using analytical balance (Denver instrument company, TR-2102) into an evaporating dish. The weighed samples were put into the pre-set oven (memmert air oven model UN 55, (SCHWBACH, GERMANY) at 105℃ for 3hours. The samples were removed and cool in a desiccator to room temperature and the weight was noted, they were then returned to the oven at 105℃ for 1 hour, this was repeated until a constant weight was obtained for each sample. The differences in weight between sample before drying and sample after drying is the moisture loss (AOAC,2012).

$$\% \text{ Moisture content} = \frac{(\text{weight of sample before drying} - \text{weight of sample after drying})}{\text{weight of sample}} \times 100$$

3.4.3 Determination of Ash content

Ash content was determined using the AOAC (2012) method. 10g of the yoghurt samples was weighed in dried ceramic crucibles till all the moisture was evaporated. The samples were then incinerated to ash in a muffle furnace for 5 hours at 550℃. The crucibles were then removed, cooled in desiccator and the samples were weighed. The ash was and the percentage of ash was calculated as;

$$\% \text{ Ash content} = \frac{\text{weight of ash}}{\text{original weight of sample}} \times 100$$

3.4.4 Determination of fat content

This was determined by using the Rose Gottlieb method described by AOAC, (2012). 10gof yoghurt sample was weighed into a separating funnel, 1mL of ammonia solution and 10 mL of 95% ethanol and mixed thoroughly. 25ml of perioxide-free-diethyl-ether was added and shake for 1 minute. This was then followed by addition of 25 mL of petroleum ether and shaken vigorously to mix well. The mixture was then left to stand for an hour to allow aqueous and organic phase to separate. The fat extract (organic phase) was collected and the solvent was removed by distillation. The fat in the flask was dried in the oven at 100℃ for 30 minutes and the solvent was removed completely. The flasks were then cooled in a desiccator and were weighed for their mass of fat. The percentage fat was calculated by the following formula.

$$\% \text{ Fat} = \frac{weight\ of\ extracted\ fat\ (g)}{\text{original weight of sample used (g)}} \times 100$$

3.4.5 Determination of carbohydrate content.

The determination of carbohydrate in the samples was determined by a difference method. That is the values or percentages of moisture, ash, protein, fat and fibre was summed up and then the results was subtracted from hundred which gives the carbohydrate content (AOAC, 2012)

CHO = 100 - % (ash + protein + fat + crude fibre + moisture)

3.4.6Mineral Analysis

3.4.6.1 Preparation of Aqua Regia:

In a 2 liters volumetric flask, add about 1.2 liters distilled water. Carefully add 400ml concentrated Hydrochloric acid and 133ml of 69% nitric acid and diluted to 2 liters.

3.4.6.2 Procedure for mineral analysis:

0.50 - 0.52ml of the sample was measured into a clean porcelain crucible. The weight was recorded to the nearest (+0.001g).Each batch of the samples should contain five internal control samples and one external reference sample and two blanks. They were placed in a cool muffle furnace and ashed at 500^{o}C over a period of 4 hours. It was then allowed to cool down. The samples were removed in a breeze free or air flowing free environment. The ashed samples where poured into a well labeled 50ml centrifuge tubes. The crucible was rinsed with 5ml of distilled water into the centrifuge tube and rinsed again with 5ml of aqua regia. It was rinsed two or more times to make a total volume of aqua regia 20ml.the sample was vortexed the sample for proper mixing. The sample was centrifuged for 10 minutes at 3000 rpm, the supernatant was decanted into a clean vial for macro and micronutrient determination (this procedure can be used for the analysis of P, Ca, Mg, K, Na, Zn, Cu, Mn, Fe and B. it cannot be used for N and S) using the atomic absorption spectrophotometer or inductively coupled plasma. (Hunter *et al.*, 1984;Benton and Vernon 1990).

3.5 Physico-chemical Analysis

The physico-chemical analysis were determined according to the official method of analysis described by the Association of Official Analytical Chemist (AOAC 2010, AOAC 2012).

3.5.1 Determination of pH

The pH was done using a pH meter (JENWAY 3505) as described by AOAC (2010). The electrode was dipped in already weighed 5 ml of the yoghurt and the pH was recorded.

3.5.2 Determination of total solid content

This was determined by the method described by AOAC, 2010.10g of the sample was dried to constant weight in a hot air oven (memmert air oven model UN 55, (SCHWBACH, GERMANY) at 105^{o}C. The total solid content will be obtained as:

$$\% \text{ total solid} = \frac{\text{weight of dried sample}}{\text{original weight of sample}} \text{x } 100$$

3.5.2 Solid-Non-Fat:

Solids Non- Fat (S.N.F) content was determined from the following equation:

SNF (%) = % T.S% – Fat% (AOAC, 2012).

3.5.3 Determination of titratable acidity

The titratable acidity was determined by the method described by AOAC (2010). 10g of the sample was weighed and 30ml of warm water was added. 1ml of phenolphthalein was added and titrated with 0.1 N alkali (NaOH)until a change in colour to pink is observed. The titration will be repeated to get the average value.

$$\%\text{Titratable acid as lactic acid} = \frac{9 \times A \times N}{W}$$

A = Volume of NaOH used

N = Normality of NaOH solution

W = Weight of sample used

3.6 Microbial Analysis

After the production of the yoghurt, samples were collected using sterile McCartney bottles, and Analytical study was done in the Microbiology laboratory of Mountain Top University, Km 12 Lagos-Ibadan expressway, behind MFM Prayer City Ibafo, Ogun State**,** Nigeria for lactic acid bacteria count and Fungi count and identification.

3.6.1 Preparation of media

The media selected for isolation were; Potato Dextrose Agar (PDA) for fungi count and identification and de Man, Rogosa and Sharpe (MRS) Agar for Lactic Acid Bacteria count. The

Durham bottles, McCartney bottles were sterilized using the dry heat sterilization method (oven) at 160^{o}C for 1hr. the media were prepared by 23.4g of PDA (for cultivation of enumeration of yeast and mould from dairy and other food products) was weighed in two places and poured into 2 Durham bottles and 600 ml was added into each of them. 40.29g of MRS (for isolation and cultivation of lactobacillus species) was weighed in two places and poured into 2 Durham bottles and 600 ml was added into each of them. These were stared respectively and kept in the water bath for 10min to homogenize, it was then transferred into the autoclave to sterilize at 120mmHg. After sterilization, it was transferred to the water bath so as to maintain their temperature and prevent them from solidifying.

3.6.2 Serial dilution

9ml of distilled water was measured into the McCartney bottles, the micro pipette, Eppendorf pipette were filled into the pipette rack and they were sterilized with using the auto clave at 120mmHg. 1ml of each yoghurt sample was aseptically withdrawn using the Eppendorf pipette and transferred into the McCartney bottles containing 9ml of sterile distilled water for the stock solution. Serial dilution of the sample was carried from the stock using a six-fold dilution, from 10^{-1} to 10^{-6} decimal dilution from the solution by serially adding 1ml into the preceding concentration to 9ml of the diluent.

3.6.3Isolation of Microorganisms

The petri-dishes were labeled with the sample names and the dilution factor from 10^{-1} to 10^{-6} respectively for the 10 samples, a control petri dish was also labelled. An aliquot of 0.1ml from each diluent was measured into the petri-dishes for each sample from 10^{-1} to 10^{-6} respectively using a micropipette, 20ml of agar was aseptically poured into the sterilized pipette, the plates were then rock-mixed clock wise and anticlockwise to allow uniform mixing of the inoculum. The plates were then allowed to set and incubated at 37^{o}C for 24 - 48 hours for bacteria and 28^{o}C for 3-5 days for fungi. Microbial growth was observed in all media at the end of the incubation periods as described by Afolabi *et al.*, (2017).

3.6.4Counting and identification of organisms

The total bacteria count was achieved by counting the bacteria on the plates with a colony counter [UNISCOPE Colony Counter; SURGIFRIEND MEDICALS, ENGLAND]. The fungal isolates was counted and they were identified and characterized based on their colonial morphology and microscopic appearance when compared with those of known taxa as described by Afolabi L.O *et al.,* (2017).

3.7 Sensory Evaluation

Samples of the product were evaluated using hedonic method and overall acceptability by 13 panelists drawn from student of in Mountain Top University, Km 12 Lagos-Ibadan expressway, behind MFM Prayer City Ibafo, Ogun State**,** Nigeria**.** They were served coded samples of the product and were asked to compare for appearance/colour, taste, texture, aroma/smell, mouthfeel, after taste, and overall acceptability using a 9-point hedonic scale (9 – like extremely to 1-dislike extremely) (Appendix) described by Omola *et al.,* (2014).

3.8 Statistical Analysis

All the data reported in this study was carried out in triplicate. In each case, a mean value and standard error will be calculated. The data was analyzed using SPSS version 26 statistical software. Statistical parameters will be estimated with analysis of variance(ANOVA).Differences between means will be evaluated by the Duncan multiple range test and significance will be accepted (p=0.05)**.**

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 PROXIMATE COMPOSITION

The proximate composition of the yoghurt samples were presented in Table 4.1.Sample UY3 enriched with 15 % fluted pumpkin (ugu) had the highest moisture content (86.56%) and CRL had the lowest moisture content (80.74%).According to Dr.Nayak and De (2017), the moisture content of the samples fortified with ammonium ferrous sulfate in different concentration of 20mg, 30mg, 40mg iron/kg respectively was ranged from 87.4 to 88% on the fresh yoghurt samples. These results showed that yoghurt fortified with iron-salts have more moisture content than yoghurt enriched plant-based iron sources due to the type of cow milk used, and the addition of solid/paste plant materials to the cow milk yoghurt. There was no significant difference between SY1 which contain 30% soybeans and CRL($p<0.05$). There was no significant difference between SY2 (40% soybeans) and SY3 (50% soybeans) ($p<0.05$). Sample UY3 and MY3 had no significant difference($p<0.05$).

The protein content of the samples ranged from 3.39 to 8.79% as MY3 which was enriched with 50% malted pearl millet having the lowest protein content and SY3 which was enriched with 50% soybeans having the highest protein content. The protein content of the control sample was more than the samples enriched with malted pearl millet and fluted pumpkin, this could be because soybeans are known to be used as a protein enrichment source than mallet pearl millet and fluted pumpkin which is more in carbohydrate and fibre. Ziena and Nasser (2019) reported protein content values which ranged from 3.30 to 3.60% between samples fortified with Iron amino acid chelate, Ferrous sulfate, Ferrous fumarate and Ferric hydroxide poly maltose on the fresh yoghurt produced. Amove *et al.*,(2019) stated the protein content of the yoghurt milk enriched

Table 4.1 proximate composition of the different samples of yoghurt

Samples	Moisture (g/100g)	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Carbohydrate (g/100g)
SY1	81.69±0.16^{f}	5.95±0.13^{c}	3.00±0.00^{de}	0.98±0.20^{a}	8.39±0.18^{b}
SY2	84.38±0.06^{de}	8.45±0.03^{b}	2.90±0.10^{de}	0.88±0.09^{ab}	3.40±0.01^{e}
SY3	84.49±0.02^{de}	8.79±0.05^{a}	3.35±0.05^{b}	0.78±0.01^{bc}	2.60±0.10^{f}
MY1	85.29±0.03^{cd}	4.04±0.23^{e}	2.80±0.20^{e}	0.79±0.01^{bc}	7.09±0.05^{c}
MY2	84.15±0.10^{e}	3.66±0.11^{f}	2.50±0.20^{f}	0.59±0.10^{c}	9.11±0.09^{b}
MY3	85.52±0.05^{bc}	3.39±0.07^{g}	2.20±0.10^{g}	0.59±0.00^{c}	8.30±0.03^{b}
UY1	86.50±1.67^{ab}	4.14±0.00^{e}	3.20±0.10^{bc}	0.68±0.19^{bc}	5.49±1.38^{d}
UY2	85.57±0.06^{bc}	4.18±0.16^{e}	3.15±0.05^{bc}	0.72±0.15^{bc}	6.39±0.31^{c}
UY3	86.56±0.50^{a}	4.02±0.14^{e}	3.05±0.05^{cd}	0.99±0.01^{a}	5.39±0.42^{d}
CRL	80.74±0.07^{f}	4.64±0.13^{d}	3.60±0.10^{a}	0.14±0.05^{d}	10.88±0.01^{a}

Mean values with different superscript in the same column are significantly different ($p<0.05$).

**SY= soybeans yoghurt, MY= malted pearl millet yoghurt, UY= ugu (fluted pumpkin) yoghurt, CRL= control

Key:

CRL=100, SY1=70:30, SY2=60:40, SY3=50:50, MY1=70:30, MY2=60:40, MY3=50:50, UY1=95:5, UY2=90:10, UY3=85:15

Where ratio represents = cow milk: enrichment sources

with 10% to 40% whole soybean flour ranged from 4.88 to 9.23%. These results showed that the yoghurt samples enriched with plant-based iron sources has more protein content than the iron-salt fortified yoghurt due to the protein content in the plant-based enrichment sources that contribute to the enhancement of protein of the yoghurt. There was no significant difference ($p<0.05$) between the three samples enriched with 5, 10 and 15% fluted pumpkin (UY1, UY2 and UY3) andMY1 which was enriched with 30% malted pearl millet. There was a significant difference ($p>0.05$) among SY1, SY2, SY3, MY2, MY3, and CRL in their protein content percentages.

The fat content of the samples SY1, SY2, SY3, MY1, MY2, MY3, UY1, UY2, UY3 and CRL are 3.00, 2.90, 3.35, 2.80, 2.50, 2.20, 3.20, 3.15, 3.05, and 3.60% respectively as CRL had the highest fat content and MY3 had the lowest fat content. There was a decrease in fat content as the proportion of malted pearl millet and fluted pumpkin increased. Ziena and Nasser (2019) reported fat content values which ranged from 3.30 to 3.50% between samples fortified with Iron amino acid chelate, Ferrous sulfate, Ferrous fumarate and Ferric hydroxide poly maltose on the freshly produced yoghurt samples. Antonella *et al.*,(2019), reported 3.2 to 4.5% fat content in yogurts supplemented with quinoa flour. From the above results, there is no much difference in the fat content with the samples enriched with plant-based iron sources and the yoghurt samples fortified with iron-salt and chelated iron sources. There was no significant difference ($p<0.05$) among SY1 and SY2, UY1 and UY2 in fat content. There was a significant difference ($p>0.05$) among SY3, MY1, MY2, MY3, UY3 and CRL in fat content percentage.

Table 4.1 shows that UY3 enriched with 15% fluted pumpkin had the highest ash content (0.99%) and the CRL had the lowest ash content (0.14%). The ash content of all the enriched samples is higher than the ash content of the control sample. There was a decrease in ash content as proportions of soybeans and malted pearl millet increased, and there was an increase in ash content as the proportions of fluted pumpkin increased. This could be due to the fibrous properties of fluted pumpkin leaves. Ziena and Nasser (2019) reported ash content values which ranged from 0.82 to 0.85% between samples fortified with Iron amino acid chelate, Ferrous sulfate, Ferrous fumarate and Ferric hydroxide poly maltose on the freshly produced yoghurt samples. Kibui *et al.*,(2018), worked on the proximate composition and nutritional characterization of chia enriched yoghurt, and reported the ash content in yoghurt samples

ranged from 0.52%to 1.10%. According to the results above, the ash content of the different plant-based enriched yoghurt sources had higher ash than the iron-salt fortified yoghurt, which indicated that plant-based enrichment sources are better and higher sources of ash. SY1 and UY3 had no significant difference ($p<0.05$) between each other in ash. SY3, MY1, UY1 and UY2 had no significant difference ($p<0.05$) among each other in ash composition. MY2 and MY3 had no significant difference ($p<0.05$) between each other in ash content. CRL had a significant difference ($p>0.05$) from the other samples in fat composition.

The carbohydrates content of enriched sources is shown to be lower than the carbohydrate content of the control sample. The carbohydrate content ranged from 2.60 to 10.88% with CRL had the highest and SY3 had the lowest. There is a decreasing trend in the carbohydrate content of the sample enriched with soybeans this may be due to the high protein content in soybeans. Ezeonu *et al.,* (2016) conducted analysis on coconut, tiger nut and fresh cow milk yoghurt, and reported the carbohydrate content of the yoghurt samples to had ranged from 3.38 to 7.89%.Kibui*et al.,*(2018), worked on the proximate composition and nutritional characterization of chia enriched yoghurt and observed the carbohydrate content of the yoghurt samples to range from 1.16% to 1.85%. The reduction in the carbohydrate content of plant-based enriched yoghurt reduced due to the higher availability of other proximate parameters like protein, ash and moisture and the available lactose in the yoghurt had been converted to lactic acid. There was no significant difference ($p<0.05$) among SY1 (30% soybeans), MY2 (40% malted pearl millet) and MY3 (50% malted pearl millet) in carbohydrate composition. There was no significant difference ($p<0.05$) between MY1 (30% malted pearl millet) and UY2 (10% fluted pumpkin) in their carbohydrate content. There was no significant difference ($p<0.05$) between UY1 (5% fluted pumpkin) and UY3 (15% fluted pumpkin) in their carbohydrate content. Also, there was a significant difference ($p>0.05$) among SY2 (40% soybeans), SY3 (50% soybeans) and CRL (100% cow milk) in their carbohydrate composition.

4.2 MINERAL COMPOSITION

The result obtained from the mineral analyses in Table 4.2showed that the control sample was high in P, Ca, K, and Na than the enriched samples. The enriched samples contained 0.08% P

and the control sample contained 0.1% P. The Ca content of the samples was recorded as SY 0.12%, MY 0.13%, UY 0.19% and CRL 0.23% respectively. The K content ranged from 0.19 to 0.24% with CRL as the highest and SY as the lowest. The Mg content of SY, MY and CRL was recorded as 0.02 and UY recorded as 0.1. This result shows fluted pumpkin (ugu) is higher in Mg than malted pearl millet and soybeans.

The Na content of CRL was more than the enriched samples, CRL, UY, MY, and SY containing 22.01, 19.74, 19.09, and 17.15ppm were reduced respectively, which is beneficial as high sodium in the human blood leads to high blood pressure, stroke, heart disease, kidney disease and other harmful cardiovascular related illnesses(Cappuccio *et al.,* 2013).Antonela *et al.*,(2019) recorded Na content in yogurts supplemented with quinoa flour to had ranged from 76 to 78 ppm (mg/ml) which indicated that yoghurt enriched with soybeans, malted pearl millet and fluted pumpkin had lower Na content which is more beneficial as high Na intake can be harmful to the human health.

The Mn content of the samples ranged from 0.35 to 3.91ppm. The Mn content of SY, MY, UY and CRL was recorded as 3.91, 0.77, 0.52 and 0.35ppm respectively. The Cu content of the samples ranged from 0.23 to 1.18ppm. The Cu content of SY, MY, UY and CRL was recorded as 1.18, 0.23, 0.34 and 0.26ppm respectively. The Zn content of the samples ranged from 3.73 to 6.04ppm. The Zn content of SY, MY, UY and CRL was recorded as 6.04, 4.81, 3.73 and 4.64ppm respectively. Ponka *et al.*, (2013) documented Zn, Cu and Mn which ranged from 1.03 to 4.21ppm, 0.02 to 0.06ppm and 0.01 to 0.05ppm respectively in the composition of raw cow milk.

Table 4.2 Mineral composition of the different samples of yoghurt

Minerals/Samples	SY	MY	UY	CRL
P (%)	0.08	0.08	0.08	0.1
Ca (%)	0.12	0.13	0.19	0.23
Mg (%)	0.02	0.02	0.01	0.02
K (%)	0.19	0.22	0.23	0.24
Na (ppm)	17.15	19.09	19.74	22.01
Mn (ppm)	3.91	0.77	0.52	0.35
Fe (ppm)	7.79	6.09	2.38	1.5
Cu (ppm)	1.18	0.23	0.34	0.26
Zn (ppm)	6.04	4.81	3.73	4.64

**SY= soybeans yoghurt (50% cow milk: 50% soybeans), MY= malted pearl millet yoghurt (70% cow milk: 30% malted pearl millet), UY= ugu (fluted pumpkin) (85% cow milk: 15% fluted pumpkin) yoghurt, CRL= control (100% cow milk) yoghurt.

yoghurt collected in Maroua (Cameroon). This may be due to the mineral component of the cow milk in that location.

The Fe (iron) content of the enriched sources was higher than the control samples. SY (soybeans enriched yoghurt) had the highest Fe content (7.79ppm) and CRL had the lowest Fe content (1.5ppm). MY had Fe content of 6.09ppm and UY had Fe content of 2.38ppm.

Iron deficiency anaemia occurs when there is a decrease in the production of red blood cell or low iron stored up in the body reserve. Iron deficiency anaemia is the most common nutritional illness round the world. Some linked sources to the causes of anaemia are inadequate intake of iron, reduced iron absorption, increased iron demand in the blood, and drastic increase in blood loss or iron loss in the blood. Discovering and emphasizing on the cause and administering the right treatments is very essential in iron deficiency management (Short and Domagalski 2013).

Iron deficiency illnesses or conditions is mostly prevalent among growing children, menstruating females, pregnant women and elderly people (Liberal *et al.*,2020).Iron fortification and enrichment continues to stay as an anchor aimed at the treatment or prevention of iron deficiency anaemia (Miller,2013).

According to Gera *et al.*,(2012), which conducted a study on the effect of iron-fortified foods on hematologic and biological outcomes: systematic review of randomized controlled trials, iron food fortification resulted in the increase in haemoglobin, serum ferritin, and other biomarkers of iron nutriture and a reduced risk of anaemia and iron deficiency.

Food plant and animal sources is recognized as one of the main iron-based dietary sources in its bioavailability and it is dependent on the chemical arrangement, nutritional features and concentrations of the food source (Liberal *et al.*,2020).

Dr.Nayak and De (2019)fortified yoghurt with iron-salts of ammonium ferrous sulfate in different concentration of 20mg, 30mg, 40mg iron/kg milk, and reported iron to range from 19.5 to 43.5µg/ml (ppm) which is higher than plant-based enriched sources.

Minerals are very essential as they perform several beneficial functions in the metabolic pathway, respiratory function, blood transport, oxygen movement, DNA activities etc. in the human body. Lack of mineral in their right proportions in the body can lead to health challenging conditions

4.3 PHYSICOCHEMICAL COMPOSITION

Table 4.3 records the total titratable acidity of the samples which ranged from 0.98% to 1.45%. The samples enriched with soybeans increased in total titratable acidity as the proportions of soybeans increased. The total titratable acidity of the samples enriched with malted pearl millet decreased as the malted pearl millet proportions increased. There was no significant difference ($p<0.05$) between SY2 and SY3 in total titratable acidity. There was no significant difference ($p<0.05$) between the titratable acidity of UY2 and CRL. There was a significant difference ($p>0.05$) among the titratable acidity levels of SY1, MY1, MY2, MY3, UY1 and UY3 samples. Ziena and Nasser (2019) reported titratable acidity to range from 0.86 to 1.04% for fresh samples of yoghurt fortified with iron-salts. DrNayak and De (2019)fortified yoghurt with iron-salts and reported total titrated acid on fresh samples to range from 0.27 to 0.28%. Ezeonu *et al.,* (2016) conducted analysis on coconut, tiger nut and fresh cow milk yoghurt, and reported the titratable acid of the yoghurt samples to had ranged from 0.60 to 0.81%. Mbaeyi-Nwaoha*et al.*,(2017) analyzed flavoured yoghurt enriched African bush mango and recorded titratable acid 0.76 to 0.88%. The data analyzed above showed that yoghurt samples enriched with plant-based sources had more titratable acid than yoghurt fortified with iron-salt or iron-chelated sources. This could be due to the acid content of the individual plant products.

The total solid content of sample SY2, SY3 and MY2 has no significant difference ($p<0.05$). MY1, MY3 and UY1 showed that there is no significant difference ($p<0.05$) among the total solid content of the samples. UY2 and UY3 had no significant difference among the total solid content of the samples. From table 4.3, SY1 and CRL showed that there was a significant difference ($p>0.05$) between the total solid content of the samples. The sample CRL has the highest total solid content (19.25%) and UY3 has the lowest total solid content (13.43%). The

Table 4.3 Physicochemical composition of the different samples of yoghurt

Samples	Total titratable acidity (g/100g)	Total solid (g/100g)	Solid-Not-Fat (g/100g)	pH (g/100g)
SY1	0.98±0.01^{h}	18.31±0.16^{b}	15.31±0.16^{a}	4.39±0.00^{c}
SY2	1.01±0.00^{g}	15.62±0.05^{c}	12.72±0.04^{c}	4.41±0.00^{b}
SY3	1.02±0.00^{g}	15.50±0.02^{c}	12.15±0.03^{d}	4.32±0.00^{e}
MY1	1.45±0.00^{a}	14.70±0.03^{d}	11.90±0.17^{d}	4.36±0.01^{d}
MY2	1.38±0.02^{b}	15.84±0.10^{c}	13.34±0.10^{b}	4.36±0.00^{d}
MY3	1.15±0.00^{c}	14.47±0.05^{d}	12.27±0.05^{cd}	4.43±0.00^{a}
UY1	1.07±0.01^{e}	14.48±0.69^{d}	11.28±0.59^{e}	4.28±0.00^{g}
UY2	1.11±0.00^{d}	13.92±0.44^{e}	10.77±0.49^{ef}	4.25±0.01^{h}
UY3	1.04±0.00^{f}	13.43±0.50^{e}	10.38±0.55^{f}	4.25±0.01^{h}
CRL	1.12±0.00^{d}	19.25±0.07^{a}	15.65±0.17^{a}	4.30±0.00^{f}

Mean values with different superscript in the same column are significantly different ($p<0.05$).

**SY= soybeans yoghurt, MY= malted pearl millet yoghurt, UY= ugu (fluted pumpkin) yoghurt, CRL= control

Key:

CRL=100, SY1=70:30, SY2=60:40, SY3=50:50, MY1=70:30, MY2=60:40, MY3=50:50, UY1=95:5, UY2=90:10, UY3=85:15

Where ratio represents = cow milk: enrichment sources

samples enriched with soybeans and fluted pumpkin (ugu) showed a decreasing total solid content as their proportions increased. Kemelo *et al.*,(2019) analyzed yoghurt from different samples in Maseru, Lesotho and reported the total solid to range from 19.93 to 23.56%.Mbaeyi-Nwaoha*et al.*,(2017) analyzed flavoured yoghurt enriched African bush mango and recorded the total solid which ranged from 13.00 to 13.85%. DrNayak and De (2019) fortified yoghurt with iron-salts and reported the total solid on fresh samples to range from 12.0 to 12.6%. The total solid recorded for the different yoghurt samples might be dependent on the fortification/enrichment source, the conditions (of and) during production, and the topography and type of cow milk purchased due to the above results.

Solid-Not-Fat (SNF) was obtained by subtracting the fat content from the Total solid content. The samples enriched with fluted pumpkin (ugu) and soybeans showed a decreasing pattern in the SNF content as the proportions increased. The CRL has the highest SNF content (15.65%) and UY3 (10.38%) has the lowest SNF content. There was no significant difference ($p<0.05$) between SYI and CRL in their SNF composition. There was no significant difference ($p<0.05$) between SY3 and MY1 in their SNF content. There was a significant difference ($p>0.05$) among the SNF composition of SY2, MY2, MY3, UY1, UY2 and UY3 respectively. Kemelo *et al.*,(2019) analyzed yoghurt from different samples in Maseru, Lesotho mentioned that the SNF of the samples ranged from 17.03 to 21.68% which is higher than the enrichment sources that was produced.

The pH content of the samples SY1, SY2, SY3, MY1, MY2, MY3, UY1, UY2, UY3 and CRL was recorded as 4.39, 4.41, 4.32, 4.36, 4.36, 4.43, 4.28, 4.25, 4.25 and 4.30 respectively. The pH of the samples enriched with soybeans and malted pearl millet was recorded to be higher than the control sample. There was an increasing pH in the samples enriched with malted pearl millet as the proportions increased, while there was a decreasing pH in the samples enriched with Ugu as the proportions increased. There was no significant difference ($p<0.05$) between MYI and MY2 in their pH levels. There was no significant difference ($p<0.05$) between UY2 and UY3 in their pH levels. There was a significant difference ($p>0.05$) among the pH levels ofSY1, SY2, SY3, MY3, UY1 and CRL respectively. Ademosun *et al.*,(2019) enriched yoghurt with tomato juice and reported pH level which ranged 4.14 to 4.25. Ezeonu *et al.,* (2016) conducted analysis on coconut, tiger nut and fresh cow milk yoghurt, and was reported the pH to range from 4.21 to

4.52. %. Mbaeyi-Nwaoha*et al.*,(2017) analyzed flavoured yoghurt enriched African bush mango and recorded the pH to range from 4.69 to 5.01. The result of the pH levels recorded above shows that the pH of plant-based yoghurt enrichments are along the same pH levels of acidity.

4.4 MICROBIAL ANALYSIS

The Bacteria analysis was conducted on *Lactobacillus* using an MRS agar. Serial dilution of 10^{-2} was used to calculate the CFU/ml (10^{-2}) and was recorded. The sample that had the highest *lactobacillus* count was SY2 (3.56×10^{3} CFU/ml) and the sample with the lowest *lactobacillus* count was MY2 (1.00×10^{2} CFU/ml).

Ziena and Nasser (2019) reported *lactobacillus* count of yoghurt fortified with iron amino acid chelate, Ferrous sulfate, Ferrous fumarate and Ferric hydroxide poly maltose which contained 12×10^{3} CFU/ml to 65×10^{3} CFU/ml which is higher in concentration than the plant-based enriched yoghurt.

Table 4.4 Microbial Analysis of the different samples of yoghurt

Samples	Bacteria CFU/ml (10^{-2})	Mould CFU/ml (10)	Yeast CFU/ml (10)	Coliform CFU/ml (10)
SY1	2.32×10^{3}	ND	ND	ND
SY2	3.56×10^{3}	ND	ND	ND
SY3	1.36×10^{3}	ND	ND	ND
MY1	4.17×10^{3}	ND	ND	ND
MY2	1.00×10^{2}	ND	ND	ND
MY3	4.50×10^{2}	ND	ND	ND
UY1	2.20×10^{3}	ND	ND	ND
UY2	6.70×10^{2}	ND	ND	ND
UY3	9.30×10^{2}	ND	ND	ND
CRL	1.43×10^{3}	ND	ND	ND

**SY= soybeans yoghurt, MY= malted pearl millet yoghurt, UY= ugu (fluted pumpkin) yoghurt, CRL= control, and ND= not detected.

Key:

CRL=100, SY1=70:30, SY2=60:40, SY3=50:50, MY1=70:30, MY2=60:40, MY3=50:50, UY1=95:5, UY2=90:10, UY3=85:15

Where ratio represents = cow milk: enrichment sources

4.5SENSORY EVALUATION

The sample that had the highest appearance acceptability was CRL (8.31) and the sample with the lowest appearance acceptability was MY3 (4.23) (Table 4.5). There was no significant difference ($p<0.05$) among the appearance in SY1, UY1 and UY2 samples. There was no significant difference ($p<0.05$) among SY2, SY3 and UY3 samples in appearance. The samples enriched with malted pearl millet had no significant difference ($p<0.05$) in appearance from each other. The sample CRL had a significant difference($p>0.05$) in appearance from the other samples. The samples enriched with fluted pumpkin(ugu) had the best appearance acceptability than the other enrichment sources.

The sample enriched with ugu has the most acceptable taste than those enriched with other enrichment sources. CRL had the highest taste quality (8.08) and MY1 had the least (4.62) taste quality. There was no significant difference ($p<0.05$) between the value of SY1 and UY2 samples in taste. There was no significant difference ($p<0.05$) between SY2 and SY3 samples in taste. The was no significant difference ($p<0.05$) in taste between the sample MY2 and MY3 in taste. The samples MY1, UY1, UY3 and CRL had no significant difference ($p<0.05$) between each other in their taste values.

CRL had the highest texture parameter value (7.85) and MY1 had the lowest (4.62). There was no significant difference ($p<0.05$) between SY1 and SY2 samples. There was no significant difference ($p<0.05$) between SY3 and MY1, MY2 and MY3 samples. The samples enriched with fluted pumpkin had no significant difference ($p<0.05$) in appearance from each other. The sample CRL had a significant difference ($p>0.05$) in texture from the other samples.

Table 4.5 Sensory evaluation of the different samples of yoghurt

Samples	Appearance/Colour	Taste	Texture	Smell/Aroma	Mouthfeel	Aftertaste	Overall/General acceptability
SY1	7.00 ± 1.47^{ab}	6.08 ± 1.93^{bcd}	6.00 ± 1.96^{bc}	5.69 ± 2.02^{abc}	5.62 ± 2.10^{ab}	5.23 ± 2.28^{bcd}	5.69 ± 2.06^{ab}
SY2	6.23 ± 2.24^{b}	5.15 ± 2.61^{cde}	5.69 ± 1.80^{bc}	4.92 ± 2.50^{bcd}	4.85 ± 1.82^{cd}	4.85 ± 2.03^{cd}	5.69 ± 1.93^{ab}
SY3	6.00 ± 2.20^{b}	5.00 ± 2.77^{cde}	4.92 ± 2.14^{c}	4.54 ± 2.26^{bcd}	4.23 ± 1.92^{cd}	4.31 ± 2.21^{de}	4.92 ± 2.02^{ab}
MY1	4.62 ± 1.66^{c}	4.62 ± 2.10^{de}	4.62 ± 1.76^{c}	4.31 ± 2.02^{cd}	4.08 ± 2.50^{cd}	3.15 ± 1.95^{e}	4.08 ± 1.55^{b}
MY2	4.46 ± 2.03^{c}	4.08 ± 2.22^{e}	5.08 ± 1.75^{c}	3.69 ± 1.65^{d}	4.08 ± 2.22^{cd}	3.00 ± 1.83^{e}	4.54 ± 3.99^{a}
MY3	4.23 ± 2.01^{c}	4.08 ± 1.93^{e}	4.77 ± 1.59^{c}	4.00 ± 1.68^{cd}	3.54 ± 1.76^{d}	3.23 ± 1.69^{e}	4.23 ± 1.36^{b}
UY1	7.38 ± 1.33^{ab}	7.54 ± 1.13^{ab}	6.77 ± 1.17^{ab}	7.23 ± 2.13^{a}	7.08 ± 1.19^{ab}	6.92 ± 1.32^{a}	7.54 ± 0.88^{ab}
UY2	7.08 ± 1.04^{ab}	6.31 ± 1.60^{bcd}	6.85 ± 1.41^{ab}	6.85 ± 2.08^{a}	6.92 ± 2.06^{ab}	6.15 ± 1.77^{abc}	7.23 ± 1.90^{ab}
UY3	6.38 ± 1.33^{b}	6.69 ± 1.60^{abc}	6.85 ± 0.99^{ab}	6.15 ± 2.23^{ab}	6.62 ± 1.85^{ab}	6.69 ± 1.55^{ab}	6.92 ± 1.19^{ab}
CRL	8.31 ± 0.63^{a}	8.08 ± 1.04^{a}	7.85 ± 0.90^{a}	6.92 ± 2.10^{a}	7.62 ± 1.04^{a}	7.46 ± 1.39^{a}	8.15 ± 1.07^{ab}

Mean values with different superscript in the same column are significantly different ($p<0.05$).

**SY= soybeans yoghurt, MY= malted pearl millet yoghurt, UY= ugu (fluted pumpkin) yoghurt, CRL= control

Key:

CRL=100, SY1=70:30, SY2=60:40, SY3=50:50, MY1=70:30, MY2=60:40, MY3=50:50, UY1=95:5, UY2=90:10, UY3=85:15

Where ratio represents = cow milk: enrichment sources

MY2 had the lowest aroma property value (3.69) and UY1 had the highest (7.23). There was no significant difference ($p<0.05$) between SY2 and SY3 samples in aroma. There was no significant difference ($p<0.05$) between MY1 and MY3 samples in smell/aroma. There was no significant difference ($p<0.05$) among the aroma values of UY1, UY2, and CRL. The sample SYI, MY2 and UY3 had a significant difference($p>0.05$) in texture from each other. The samples enriched with millet had the least aroma acceptability and fluted pumpkin having the best among the enrichment sources.

MY3 had the lowest mouthfeel value (3.54) and CRL had the highest (7.62). There was no significant difference ($p<0.05$) among SY1 and the samples enriched with fluted pumpkin (ugu) in mouthfeel. There was no significant difference ($p<0.05$) among SY2, SY3, MY1 and MY2 samples in mouthfeel. The sample MY3 and CRL had a significant difference ($p>0.05$) in mouthfeel from each other.

CRL had the highest aftertaste value (7.46) and MY2 had the lowest (3.00). There was no significant difference ($p<0.05$) between UY1 and CRL samples in aftertaste. There was no significant difference ($p<0.05$) among the samples enriched with malted pearl millet in aftertaste. The sample SY1, SY2, SY3, UY2 and UY3 had a significant difference ($p>0.05$) in aftertaste from each other.

The sample with the highest overall/general acceptability was recorded as CRL (8.15) and MY1 had the lowest (4.08). the sample enriched with fluted pumpkin (ugu) had the highest overall/general acceptability than the other enrichment sources. There was no significant difference ($p<0.05$) between MY1 and MY3 samples in general acceptability. The samples enriched with soybeans, fluted pumpkin and CRL had no significant difference ($p<0.05$) in the overall/general acceptability from each other. The sample MY2 had a significant difference($p>0.05$) in the overall/general acceptability from the other samples.

Among the enriched yoghurt samples, fluted pumpkin was the most preferred and pearl millet was the least preferred in all the sensory parameter on the hedonic scale.

Ziena and Nasser (2019) fortified with iron amino acid chelate; ferrous sulfate, ferrous fumarate and ferric hydroxide poly maltose were observed to obtain sensory attributes of 7.75 to 8.75 in colour, 8.00 to 8.50 in texture, 8.25 to 8.50 for the overall acceptability within the first day of

production. 7.25 for all the samples in colour, 7.75 to 8.00 in texture, 8.00 to 8.50 for the overall acceptability with 3 days. Lastly 7.00 to 7.50 in colour, 7.25 to 7.50 in texture and overall acceptability within 7days of storage. This shows a decreasing trend in sensory properties as the storage days increased.

From the results shown above, iron-salt fortified yoghurts have a higher sensory acceptability in colour, texture and overall acceptability than the plant-based enriched yoghurt.

Plate 1: Ugu enriched yoghurt

Plate 2: Soybeans enriched yoghurt

Plate3: Millet enriched yoghurt

Plate 4: Control sample yoghurt

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The yoghurt samples enriched with soybeans improved nutritionally particularly in terms of the protein content. The fat content of SY3, UY1 and UY2 were comparable to the cow milk yoghurt. Fat binds with some essential vitamins like A, D, E and K to ensure its availability and functionality in the body. Plant based enriched yoghurt had an improved ash content compared to plain cow milk yoghurt, indicating a relatively better mineral profile.

The sodium (Na) content of the soybeans, millet and ugu enriched yoghurts were lower than the plain cow milk yoghurt. This is a beneficial outcome as high sodium in the human blood could lead to high blood pressure, stroke, heart disease, kidney disease and other harmful cardiovascular related illnesses. Zn content improved in the millet and soybeans enriched yoghurt respectively while Cu content improved in the soybeans and fluted pumpkin enriched yoghurt. The iron (Fe) content of the ugu, millet and soybeans enriched yoghurt improved. This is highly beneficial for improved haemoglobin levels in the blood and to ameliorate anaemic conditions.

Lactobacillus is beneficial and important in the fermentation of yoghurt; it is a probiotic which provides essential health benefits in the stomach and Gastrointestinal Tracts of humans and aids bowel moments. It also converts lactose sugar to lactic acid in milk which brings about curd/yoghurt formation in milk. Mould, yeast and coliform were not detected in the yoghurt samples. This suggests that the yoghurt would be safe for consumption.

The trend of preference for the enriched yoghurt samples was the fluted pumpkin, soybean and pearl millet respectively in all the sensory parameters. Fluted pumpkin enriched yoghurt had a better sensory acceptability while the soybeans enriched yoghurt had the best nutritional profile. Enrichment of yoghurt with plant-based sources showed potential benefits for the improvement of the nutritional quality of yoghurt and health improvement.

5.2 RECOMMENDATIONS

I recommend that further studies may be done on how to extract iron ions (Fe-ions) substances from plant-based sources so as to improve the sensory acceptability and appearance of fortified/enriched yoghurts.

I recommend that further studies maybe done on the development of new food products with soybeans, pearl millet and fluted pumpkin to improve the availability of essential minerals like iron and to reduce hidden hunger.

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APPENDIX

Name: ……

9 – Like extremely

8 – Like very much

7 – Like moderately

6 – Like slightly

5 – Neither like or dislike

4 – Dislike slightly

3 – Dislike moderately

2 – Dislike very much

1 – Dislike extremely

Sample	SY1	SY2	SY3	MY1	MY2	MY3	UY1	UY2	UY3	CRL
Appearance										
Taste										
Texture										
Smell										
Mouthfeel										
After taste										
Overall/General acceptability										

Comments: ……
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