**CHAPTER 1**

**1.1 BACKGROUND OF STUDY**

Lactic acid fermentation of cereal and legume has been studied extensively in the past few decades. Yogurt-like products have been produced from various kinds of cereals such as liquefied starch (Shin, 1989), prefermented and extruded rice flour (Lee *et al*., 1992), and cooked maize meal mixture (Zulu *et al*., 1997). A product, so called Risogurt, was produced from mixture of fermented rice and soy protein isolate (Mok *et al*., 1991). Method for producing a highly concentrated lactic product from rice with improved quality by a secondary enzymatic treatment during fermentation was further developed by Mok *et al*., (1993).

There are about nine hundred and twenty nine million hungry people in the world in 2010 with two hundred and thirty nine million from sub-Saharan African. Children and the vulnerable groups are the most victims. Protein calorie malnutrition has been as the deadly form of malnutrition/hunger. This deadly form of malnutrition could be solved if people could have least cost and balanced nutrition on their table. “When people have learnt how to cultivate a suitable flora in the intestine of children as soon as they are weaned from the breast, the normal life may extend to twice my 70 years”. That rather statement by Etie Metchnioff (1921) has arguably never been achieved because there is no report to date that human can normally live up to 140 years. However, the statement emphasizes maintaining balanced intestinal flora to have a healthy life. Over the last few decades the market for fermented food products, especially fermented dairy products such as Yogurt, has increased significantly along with the steadily increasing knowledge of the health benefits for fermented dairy products.

Yoghurt which is semi-fermented milk food is likened and consumed by people all over the world. The food can be used to prevent/control diarrhea due to its therapeutic effect. It can help in modulating the inflammatory response by carcinogen through increasing apoptosis. Yogurt protein is more digestible than cow milk (Belewu *et al*., 2005). Yogurt can be defined simply as a diary product from fermentation of milk. This fermentation produces lactic acid from lactose that, combined with milk protein, gives yogurt its characteristic acidic taste and texture. Yogurt is further defined in the United States as an acidic-gelled product from culturing of cream milk, partially skim milk, or skim milk either alone or in combination by *streptococcus thermophilus* and *Lb. delbrueckii subsp. bulgaricus*. Other ingredients and other additional ingredients, and the micro-organisms used must remain viable in the final product (FSANZ, 2007).

The type and characteristics of micro-organisms used as starter cultures for milk fermentation are two of the most important factors that determine the overall quantity of the fermented products (Bouzar *et al*., 1997). A proper selection and composition of starter culture can improve the product flavor, aroma, stability, and texture. Therefore, it is of great importance to understand the characteristics of the starter bacteria to obtain desirable, quality products.

The microorganisms used in milk fermentation can be categorized into three groups: Mesophilic, Thermophilic, and Artisanal. The optimum temperatures for the first two groups are approximately 26˚C (79˚F) and 42˚C (108˚F) respectively, and they consist of different species of bacteria. The artisanal is consumed yogurt regularly in his native Balkan regions lived longer (Metchikoff 1921, Van de water 2003). This knowledge of health benefits of yogurt has helped to increase yogurt consumption. The trend is also enhanced by the increased palatability of yogurt due to the addition of sweeteners and fruits (Van de water 2003). Yoghurt is usually made through fermentation by thermophilic microorganisms at a relatively high temperature (slightly higher than 45˚C; 113˚F) (Walstra *et al*., 2006) and a shorter fermentation time (5 hours or longer) at which the maximum pH of 4.5 is used as a guide to stop the fermentation. Therefore, this limitation is used to distinguish yogurt from other fermented milk products. The term yogurt is also be used to cover strained products from thermophilic fermentation of milk such as yogurt cheese, greek yogurt and other yogurt with cheese-like textures that retains the acidic characteristics of yogurt.

Milk fermentation culminates in a product with different textures ranging from liquid such as kefir and koumis to semi-solid or solid such as yogurt (Van de water 2003). Milk fermentation with lactic Acid Bacteria (LAB) also results in products with distinctive tastes and aromas. LAB metabolism and interaction between selected strains produces lactic acid and other compounds as well as coagulates milk protein. Fermentation conditions such as temperature, pH, the presence of oxygen, and milk composition also contribute to the characteristics of the final product (Nakazawa and Hosono 1992).

The continued diary milk shortage or absence in the developing countries, therefore had led to the production of milk substitute from vegetable milk. According to Harkins and Sarret (1967), development of milk substitute extracted from cereals and legumes serves as an alternative way of producing an acceptable nutritious food based on vegetable is increasing.

Vegetable milk (analogue milk or plant milk) is a general term for any non-diary cream analogue that is derived from a plant source (<http://www.en.wikipedia/plantmilk,2015>). No formal or legal definitions for vegetable milk exist in most countries. The most common varieties are soy milk, oat milk, coconut milk, but there are also other varieties available which are rice or nut based. There are varieties of reasons for consuming vegetable milk, including severe diseases like PKU, making digestion of animal proteins (especially casein) found in diary milk allergic (approximately 3% of people allergic) (<http://www.foodintol.com/diary.asp>).

Vegetable milk can be used for babies in communities where babies are not given diary milk for ethical reasons and galactosemia (Obizoba and Egbuna 1992). The most common legume and cereal grain, Soybeans and rice will be used alternatively as a milk substitute in production of yogurt. Attention and more research are still being designed to improve the quality of soymilk (Sun-young *et al*., 2000) but rarely on rice milk. Therefore in this study, rice milk will be substituted with soymilk to produce yoghurt in order to improve its nutrient deficiency. Rice milk is cereal grain milk made from rice. It is mostly made from brown rice and commonly unsweetened (<http://www.en.wikipedia/wiki/ricemilk>).

The sweetness in most rice milk varieties is generated by a natural enzymatic process, cleaving the carbohydrates into sugars, especially glucose similar to the Japanese amazake (<http://www.bnet.com/ricemilk>). Some rice milk may nevertheless be sweetened with sugar cane syrup or other sugars. Compared to cow’s milk, rice milk contains more carbohydrate, but does not contain significant amounts of calcium, vitamin B12, vitamin B3 and iron. Rice milk is often consumed by people who are lactose intolerant, allergic to soy or have PKU. It is also used as diary substitute for vegetarians, and rice milk does not contain cholesterol or lactose (<http://www.en.wikipedia/wiki/ricemilk>).

Soymilk is a fine emulsion of soybeans flour or water extract of wet ground soybean. Among the sources of vegetable milk, soybean has received high research attention and more research is still designed to improve the quality of soymilk (Sun-young *et al*. 2000). Though the soybean is classified among the leguminous plant, it differs from other legumes in containing far more proteins and fats than ordinary peas and beans.

Scientific studies have emphasized the fact the soybean contains vitamins in substantial quantities and is a valuable source of B-complex vitamins. Soya beans protein contains essential amino-acids and is therefore a complete protein which satisfies the human requirements for protein metabolism. Soymilk is a complete protein and has about the same protein as cow’s milk; it can replace animal protein and other sources of dietary fiber, vitamins and minerals (Sack *et al*., 2006). Soymilk contains little digestible calcium because calcium is bound to the bean’s pulp, which is indigestible by humans. Unlike cow’s milk, soymilk has little saturated fat and no cholesterol.

Soy products contain sucrose as the basic disaccharide, which breaks down into glucose and fructose. Since soy does not contain galactose, a product of lactose breakdown soy-base formulas can safely replace breast milk in children with galactosemia. Like lactose free cow’s milk, soy milk contains no lactose, which makes it an alternative for those who are lactose-intolerant. The composition of soymilk varies with the varieties of soybean used and the method of production (Wang *et al.,* 1978).

**1.2 STATEMENT OF PROBLEM**

Soyfoods, especially soymilk, are considered a good substitution for dairy products for individuals who have milk intolerance. Prior to the development of such vegetable milk like soymilk which serve as an alternative milk substitute for diary milk, direct milk consumption as a beverage was not common in Nigeria (Iwuoha and Umunnakwe 1997; Onweluzo and Owo 2005). Milk intolerance including cow’s milk protein allergy (CMPA), cow’s milk protein intolerance (CMPI), and lactose intolerance is prevalent in the world, especially in children. CMPA can affect from 2 to 6% of children. Studies have shown that 80% to 90% of these children will resolve CMPA within their fifth year (Host et al., 2002; Wood 2003; Goplan 2011). The important and widely used method used to treat milk intolerance is the exclusion of all forms of animal milk and milk products from the diet. Because of the high plant protein content and similar texture as milk, soymilk is a healthy protein source for those milk intolerant individuals. In addition, cow’s milk contains saturated fats that might increase the risk of cardiovascular diseases (Astrup et al., 2011; Siri-Tario et al., 2010). Soymilk which contains much lower amounts of saturated fat than cow’s milk could be a good choice for individuals who are concerned about heart disease. Diary milk which contains high quality protein and other nutrients is essential for the well-being of an individual, for some reasons not consumed by all, due to health conditions such as inability to digest milk sugar-lactose, milk allergy, and PKU. Casein, a protein found only in milk, contains all of the essential amino acids and accounts for 82% of total proteins in milk are indigestible by some individuals due to this factor, milk from vegetable sources was developed as an alternative means of milk consumption.

Although soymilk and soyfoods are nutritious and their consumption has many health benefits, soymilk has some limitation such as beany flavor, possible allergic reactions and undesirable microbial fermentation after intake by some consumers. Consequently, scientists have tried to resolve these problems by creating some new lines of soybeans for instance, low and ultra-low raffinose family oligosaccharides soybeans, lipoxygenase free soybeans, high oleic soybeans, low P34 allergen soybeans, and high sucrose soybeans. It would be of great interest to know if soybeans could address these problems. This has not been reported in literature (Jing, 2013).

In Nigeria, rice (Oryza spp) is a vital staple food consumed by many people. It is the most important food crop in the world, being the staple food for more than half of the world’s population, predominantly in Asia and Africa where more than 90% of the world’s rice is grown and consumed. It is a very versatile crop and there are many varieties of rice adapted to various environment and cultivation practices (Luc *et al.,* 2010). However, adoption (Utilization) of innovations is a very important tool to measure the effectiveness and efficiency of agricultural product. Its varieties of preparation and use will enhance the appeal for consumption.

**1.3 JUSTIFICATION**

Historically, fermented foods have played an important role in diets of most every society throughout the world. But beyond just the culinary choices and preservation, advantages of fermented foods is the natural phenomenon of fermentation performed by the cells within our bodies that help to keep us healthy. The reasons for fermenting milk are numerous and although the primary function is to extend its shelf-life, other advantages, such as improving the taste of milk, enhancing the digestibility of the product and the manufacture of a wide range of products (i.e. from yogurt to concentrated yogurt to cheese) should not be overlooked. A health aspect attributed to special probiotic micro-organisms used in fermented milks and other dairy products will not be covered in these publications, as they are included in probiotic dairy products within the technical series prepared on behalf of the society of diary technology (SDT) in the UK(Tamime, 2005 )

In lacto-fermentation, lactobacillus bacteria have the ability to convert sugar into acid through a naturally occurring fermentation process. It is a natural preservative that inhibits the growth of harmful or putrefying bacteria. This phenomenon allowed people to preserve foods for extended periods of time before the advent of refrigeration or canning. Lactic acid promotes the growth of healthy bacteria in the intestinal tract. That is why fermented foods are considered probiotic foods (Probiotic means “for life”). Beyond preservation advantages, lacto-fermentation also increases the vitamin and enzyme levels, as well as digestibility, of the fermented food. Several range of micro-organisms have been used in food and beverage preparations for thousands of years by humankind and, according to Tamime (2002), the major functions of microbial starter cultures is either food or other dairy products may be summarized as:

* To bio-preserve the product due to fermentation that results in an extended shelf-life and enhanced safety;
* To produce bacteriocins that may have potential uses as food preservatives;
* To enhance the perceived sensory properties of the product (e.g. due to the production of organic acids, carbonyl compounds and partial hydrolysis of protein and/or fat);
* To improve rheological properties of fermented milk products (i.e. Viscosity and firmness);
* To contribute dietetic/functional/nutraceutical properties of fermented milks, such as occurs with the use of probiotic micro-organisms.

Yogurt is semi-fermented milk consumed by people all over the world. It is classified into different types and variations probably due to the various types of live and active culture used, plain or fruit flavor and most importantly the different sources of milk (animal/plant) (Belewu *et al*., 2005; Belewu, 2006). Rice milk is considered the best hypoallergenic form of milk. The milk enhances immune system and provides resistances to bacteria and viruses invading the body due to high level of antioxidant, selenium and magnesium (<http://www.healthiro.com/diet.food/rice-milk>).

A wide range of fermented milks are manufactured throughout the world and, according to Kurnmann et al., (1992), around 400 generic names are applied to traditional and industrialized products. In UK, for an example, the economic value of yogurt and drinking yogurt sold in 2002 was around £814 million. (Anon, 2003). For those people who cannot or do not want to consume any Rice-Soya yogurt might be an acceptable replacement considering the low protein content in rice milk, rice milk will be complemented with soy milk which contains same amount and quality protein as that in cow’s milk (Sacks et al., 2006) in the production of yogurt. Therefore, properties of rice-soya yogurts made from rice milk and soy milk are tested because texture and mouth feel are the most important characteristics for yogurts.

**1.4 OBJECTIVE OF STUDY**

The main objective of this research is to produce yogurt from locally available raw materials from rice milk and soymilk.

The specific objectives of the research are to:

1. Produce plant yogurt from rice milk and soymilk.
2. Evaluate the physiochemical properties of rice-soy yogurt.
3. Determine mineral contents of the rice-soy yogurt.
4. Evaluate its general acceptability.
5. Carryout microbial analysis on the yogurt samples.

**CHAPTER 2**

**LITERATURE REVIEW**

**2.1 MILK**

Milk is the lacteal secretion of a pale liquid produced by the mammary glands of mammals. It is the primary source of nutrition for infant mammals before they are able to digest other types of food. Early lactation milk contains colostrums, which carries the mother’s antibodies to its young and can reduce the risk of many diseases. It contains many other nutrients (Pehrsson *et al*., 2000) including protein and lactose. Milk is valued because it is an importance source of many of the nutrients essential for the proper development and maintenance of the human body.

As an agricultural product, milk is extracted from mammals during or soon after pregnancy. Dairy farms produced about 730 million tonnes of milk in 2011, (FAO, 2012), from 260 million dairy cows. (FAO, 2014).

**2.1.1 Importance of Milk and Milk Products in Diet**

Fluid milk is not only nature’s food for a new born infant, but also a source for a whole range of dairy products consumed by mankind. Fluid milk is about 87% water and 13% solids. The fat portion of the milk contains fat-vitamins. The solids other than fat include proteins, carbohydrate, water-soluble vitamins and minerals. Milk products contain high quality proteins. The whey proteins constitute about 18% of the protein content of the milk. Casein, a protein found only in milk, contains all of the essential amino acids and accounts for 82% of the total proteins in milk. Milk also contains calcium, phosphorous, magnesium, and potassium. The calcium found in milk is readily absorbed by the body; Vitamin D plays a role in calcium absorption and utilization. Milk is also a significant source of riboflavin (vitamin B2), which helps promote healthy skin and eyes (Diary Fact, 2003). Dairy products such as yogurts, cheeses and ice creams contain nutrients such as proteins, vitamins and minerals.

Consumption of dairy products has been associated with decreased risk of osteoporosis, hypertension, colon cancer, obesity and insulin resistance syndrome (IRS). The main dietary source of calcium and vitamin D are dairy products (Weaver, 2003).

**2.1.2 Fermented Milk Products**

The introduction of fermented milk products such as cheeses and yogurts in to the diet of man is thought to date back to the dawn of the civilization (Mckinley, 2005). Consumption of fermented-milk products is associated with several types of human health benefits partly because of their content of lactic acid bacteria. Several experimental observations have indicated a potential effect of lactic acid bacteria (LAB) against the development of colon tumors (Wollowki *et al*., 2001). Recently, the role of fermented milks containing lactic acid bacteria (LAB), such as *Lactobacillus,Bifidobacterium* and *Streptococcus thermophilus*, been studied (Saikli *et al*., 2004). A wide range of other health benefits, including improved lactose digestion, diarrhea prevention, immune system modulation and serum cholesterol reduction, have been ascribed to fermented milk consumption.

Fermented milk has been used throughout the history of mankind. Nearly every civilization has developed some types of fermented milk product such as buttermilk, labneh, acidophilus milk and yogurt, which were familiar to many people, although the fermentation process was not defined yet. Yogurt, which came from the Turkish term in the eighth century as “Yogurt” (Pashapour and Lou, 2006) and the spelling, changed over time to the present spelling in the eleventh century.

The fermentation processes of dairy products and its bacteria have received great attention over the decades after discovering the importance of viable bacteria in food for health benefits. At the beginning of the last century first scientific work had been done by Metchnikoff to investigate the beneficial effects of fermented milk for human health associated with the consumption of fermented dairy products (Modler *et al*., 1990; Shin *et al*., 2000b).The lactic acid bacteria (yogurt bacteria; *lactobacillus delbrueckii spp. Bulgaricus* and *streptococcous thermophilus*) do not inhibit the human and animal intestinal, nor do survive passing through the digestive system (Klaver *et al*., 1993).

There are now numerous types of fermented milk manufactured in different parts of the world, and recent publications dealing with the major types of yogurt and related products such as drinking-types and concentrated yogurt (e.g. Labneh), include those by IDF (1988), Tamime and Marshall (1997), Tamime and Robinson (1999),Tamime et al., (2001), Robinson et al., (2002), Anon, 2003 and Jaros and Rohin, 2003).

In general, two types of retail product dominate the market for fermented milks. One variant has a firm gel like structure (Set-type), whilst the other has a thick consistency and the apparent background flavor is usually modified by the addition of fruits or flavor and sugar (stirred-type), however, a variant of the stirred product, which is pourable and low in consistency, is the drinking types of fermented milk are broadly similar. Although, many of these fermentations involve specific microfloras. (Marshall and Tamime, 1997). There is considerable degree of similarity in respect of the technological aspects (Tamime and Robinson, 1999), especially when the process is a lactic fermentation. Obviously, the details of manufacture differ from product to product, but certainly many of the processes have much in common.

Epidemiological studies suggest that consumption of fermented milk and the dietary intake of diary proteins are inversely related to risk of hypertension, resulting from a blood pressure lowering effect of released bioactive peptides (Jauhiainen and Korpela, 2003).

**2.2 YOGURT**

Yoghurt is a product of lactic acid fermentation of milk by addition of starter culture produced by bacteria. It is Turkish in origin which refers to tart, or “Jugurt” (thick milk) used to describe any fermented food with an acidic taste (Younus et al., 2002). Historically, yogurt was made by fermenting milk 2with indigenous micro-organisms known as “Yogurt Cultures”. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its textures and characteristics tang. Yogurt having high nutritional and therapeutic is being highly consumed and produce (Karagul et al., 2004). Although fermented milk, was discovered simply as a means of preserving the nutrients in milk, it was soon discovered that, by fermenting with different microorganisms, an opportunity existed to develop a wide range of products with different flavors, textures, consistencies and more recently, health attributes. The market now offers a vast array of yogurts to suit all palates and meal occasions. Yogurts come in a variety of textures (e.g. liquid, set and stirred curd), fat contents (e.g. regular fat, low-fat and fat-free) and flavors (e.g. natural, fruit, cereal, chocolate), can be consumed as a snack or part of a meal, as sweet or savory food. The versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population (Mckinley, 2005). Yogurt is stored at 2-4°C throughout the distribution chain for avoiding risk of spoilage from yeasts (Tamime et al., 2000) and also for preventing further activity by starter culture.

In some countries, less traditional microorganism such as *lactobacillus helviticus* and *lactobacillus delbruekkii* spp are used. Lactis are sometimes mixed with starter culture (McKinley, 2005; Hui, 1992). Cow’s milk is commonly available worldwide, and as such, is the milk most commonly used to make yogurt. Milk from water buffalo, goats, ewes, mares, camels, and yaks is also used make yogurt where available locally. Milk used may be homogenized or not (milk distributed in many parts of the world is homogenized); both types may be used, with rather different results.

Yogurt is produced using a culture of *lactobacillus delbrueckii* subsp. *Bulgaricus* and *lactobacillus delbrueckii* subsp. *bulgaricus* and *streptococcus thermophilus* bacteria. In addition, other *lactobacilli* and *bifidobacteria* are also sometimes added during or after culturing yogurt. Some countries require yogurt to contain a certain number of colony-forming units of microorganisms. Various combinations of starter cultures are selected during manufacturing of yogurt to achieve desirable wide choice of therapeutic benefits. Depending on its activity, manufacturer usually adds 2-4% yogurt culture. Nowadays, there has been an increasing trend to fortify the dairy product with fruits (natural fruit juice, pulp, pulp, dry fruits) (Desai et al., 1994; Ghadage et al., 2008). To produce yogurt, milk is first heated, usually starter to about 85˚C (185˚F) to denature the milk proteins so that they set together then form curds. In some places, such as parts of India and Bangladesh, the milk is boiled. After heating treatment, the milk is allowed to cool to about 40-45˚C (104˚F-113˚F), the optimum temperature for mixed starter culture (Luce, 2004). The starter culture can vary from 0.5% to 6%, depending of type of yogurt and fermentation.

During fermentation, certain physiochemical change takes place to the milk constituents that alter the properties of the yogurt mix and give the characteristics of the final product. These changes include acidity, viscosity and to a lesser extent flavor. The increase in acidity is mainly due to the production of lactic acid, and to a lesser extent, acetic acid from lactose hydrolysis through the homo-fermentative pathway (Cogan 1995, Van de water 2003). Increasing viscosity results from the destabilization of the casein complex, when the casein micelles flocculate at or near their isoelectric point and the colloidal calcium phosphate partially solubilize due to increased acidity (Nauth, 2004). The result of the destabilization is a responsible for the texture of yogurt.

Flavor development also takes place during yogurt manufacture. The flavor compounds in yogurt consist mainly of acetaldehyde and diacetyl. Acetaldehyde is produced from the amino acid, threonine in milk as well as threonine produced by the *lactobacilli* during yogurt fermentation by *streptococcus thermophilus* and, to a lesser extent, Lb. *delbrueckii* ssp. *Bulgaricus*, probably in a manner similar to the mechanism of diacetyl production in soft cheeses such as quark. Because starter culture does not metabolize citric acid, the precursor of the diacetyl i.e., pyruvic acid, is formed during fermentation (Walstra *et al*. 2006).

**2.2.1 History of Yogurt**

Yogurt is one of the most consumed, best known and most popular foodstuffs around the stuffs around the world. It dates back five millennium ago. An historically survey of the origin(s) of fermented milk products shows that they date back to early civilizations around 10000B.C as the way of life of humans changed from food gathering to food producing; this change also include domestication of the cow, sheep, goat, buffalo and Camel (Rasic and Kurmann, 1978; Perderson, 1979).

It is generally agreed among historians that yogurt and other fermented milk products were discovered accidentally as a result of milk being stored by primitive methods in warm climates. Analysis of the *L.delbrueckii* subsp. *bulgaricus* genome indicates that the bacterium may have originated on the surface of a plant. Communities in the Middle Eastand Asia are widely acknowledged as having introduced fermented milks such as yogurt into their diet almost as soon as man began to domesticate animals. Some fermented milks did of course, become popular with local populations in regions like Scandinavia and Russia (Koroleva, 1991); but it was thousands of years later that sections of the general public in Europe and North America began take serious interest in fermented milks. Today, yogurt remains a milk base fermented milk that is presented to the consumer in either a gel form (Set Yogurt) or a viscous fluid (Stirred Yogurt) but figures of consumption have risen, so manufacturers have expanded the market introducing an ever-wide range of fruit flavors and/or changing the image of the product with a luxury image (Robinson, 2000). Nevertheless, despite these and other innovations, the method of manufacture is still based on the system employed by nomadic herdsman many centuries ago. For example, the majority of yogurts consumed worldwide are manufacture with cultures of bacteria with growth optima of 37-45°C, and this characteristic derives from the fact that species in question, namely Lactobacillus delbrueckii Subsp. bulgaricus and streptococcus thermophilus, evolved in the Middle East where the ambient temperature in the summer months is often will in excess of 35°C. Similarly, the universal method of manufacturing a satisfactory yogurt is based on the traditional process, and the principal stages in the production of both set and stirred yogurts. It is well established that the way the milk is handled or prepared, including the processing conditions used in yogurt manufacture greatly influence the gel texture, strength and stability (Lucey and Singh, 1997; Walstra, 1998; Tamime and Robinson, 1999); Jaros and Rohm, 2003).

Milk may have been transferred from the udder of domestic milk producing animals.

In ancient Indian records, the combination of yogurt and honey is called “the foods for the gods”. (Batmangling, 2007). Persian traditions hold that Abraham owed his fecundity and longevity to the regular ingestion of yogurt” (Farnworth, 2008). Until the 1900s, yogurt was a staple in diets of people in the Russian Empire (and especially central Asia and the Caucasus), Western Asia, South Eastern Europe/Balkans, Central Europe, and India.

Stamen Grignorov (1878-1945), a Bulgarian student of medicine in Geneva, first examined the microflora it as consisting of a spherical and a rod-like bacterium was called Bacillus bulgaricus (now *Lactobacillus delbrueckii subsp. Bulgaricus*). The Russian Nobel laureate and biologist IIya IIyich Mechnikov (also known as Etie Metchnikoff) from the institute Pasteur in Paris, was influenced by Grigonov’s work and hypothesized that regular consumption of yogurt was responsible for the unusually long life spans of Bulgarican peasants. Believing Lactobacillus to be essential for good health, Mechnikov worked to popularize yogurt as a foodstuff throughout Europe. Isaac Carraso industrialized the production of yogurt. Although the evolution of the process was strictly intuitive, the production of fermented milk products became an established pattern of preservation and since the early 1900s, defined micro-organisms have been used to prepare these products on a large industrial scale (Marshall and Tamime, 1997; Tamime, 2002). Gradually, other communities learned of this simple preservative treatment for the milk, and defined products like yogurt culture/fermented buttermilk, kefir, kourmiss and/or acidophilus milk gained discrete identities.

**2.2.2 Nutritional Value of Yogurt**

Yogurt’s nutritional profile has a similar composition to the milk from which it is made but will vary somewhat if fruit, cereal or other components are added. Yogurt is an excellent source of protein, calcium, phosphorous, riboflavin (vitamin B2), thiamin (Vitamin B1) and Vitamin B12, and a valuable source of foliate, niacin, magnesium and zinc. The protein it provides is of high biological value and the vitamins and minerals found in milk and dairy foods including the yogurt are bio-available. Yogurt particularly the low-fat varieties, provide an array of important nutrients in significant amounts in relation to their energy and fat content, making them a nutrient dense of food. Eating dairy products such as yogurt helps to improve the overall quality of diet and increases the chances of achieving nutritional recommendation (Mckinley, 2005). Vitamins and minerals maybe added and often are for products given to children. Yogurts may be spoonable and drinkable, and may be considered dietary supplements for infant consumption. So,they cross the line between dietary supplements, medical food and conventionally foods (Katz, 2001).

**2.2.3 Quality and Shelf-life of Yogurt**

Yogurts shelf-life is based whether the products display any of the physical, chemical, microbiological or sensory characteristics that are unacceptable for consumption. Studies of changes in these quality characteristics during the storage would be instrumental in predicting the shelf life of the product (Salvador and Fiszman, 2004).

Salvador and Fiszman (2004) compared yogurts stored at 10, 20 and 30˚C and found that the samples evolved differently at the 3 temperatures studied, their results of physical properties, sensory and microbiological analysis showed that the negative characters such as syneresis, appearance defects, a typical texture/ mouth feel increased with storage time, the results also indicates that, to some extent the changes observed over the storage period could be considered the result of a combination of time and temperature.

**2.3 VEGETABLE MILK**

Vegetable milk has been consumed for centuries in various cultures, both as a regular drink (such as the Spanish horchata) and as a substitute for a diary milk. The most popular varieties are soymilk, almond milk, rice milk and coconut milk. The protein content varies. It contains no lactose or cholesterol and is usually sold with added calcium and vitamins especially B12. Soymilk has high plant protein content and contains polyphenol components, such as isoflavones. Thus, soy foods are classified as a functional food. In addition, some papers indicated that soy foods may decrease the risk of coronary heart disease (Malik et al., 2004).

Vegetable milk is a product of plant origin which presents aspects close to milk of animal origin (Apfelbaum *et al*., 2000). Calcium and some vitamins are added to vegetable milk to adjust their nutritional composition in line with that of cow’s milk. There are several reasons for consuming plant milk, health reasons, including lactose intolerance, milk allergy and PKU, veganism and ovo-vegetarianism.

In the United States soymilk was long the most popular non-diary milk, but starting around 2010 almond milk began to increase in popularity, and in 2013 it surpassed soymilk as the most popular variety. Other popular milk in the US are rice and coconut. In Europe soy and oat milk are popular. There is also halzenut milk and milk from peas and lupin (Victoria, 2008).

Plant milks are used to make ice-cream plant cream, vegan cheese and yogurt for example, soy-yogurt.

**2.3.1 Soybeans and Soymilk**

Soymilk is a fine emulsion of soybean flour or water extract of wet ground soybean. Soymilk looks like diary milk except that it has its own peculiar odor and taste. According to Wang, *et al*., (1978), soymilk originated in the orient by a Chinese. It is an expensive and remarkable versatile high protein food. This has since spread to many parts of the world especially the so-called “third world” although it is still more popular in Asia than any other parts of the world. In view of the continued diary milk shortage or absence in the developing countries, there is a need for increased production of soymilk.

Soymilk is easy and cheap to prepare. The raw material soybean can also grow in many parts of the world all seasons. Cultivation of soybean is relatively easier and cheaper than production of dairy cattle.

Soybean (*Glycine max Merrill)* belongs to the family leguminosae. It is native in china and is one of the oldest world crops. Even though soybean is native in china, it is now widely grown in both tropical and temperate regions of the world. The world leading producer of soybean is United States followed by China (Wang *et al*., 1978).

The total production in Nigeria is very insignificant in the world market. However, with the advent of an increase in soybean production the major areas of soybean production, in Nigeria are Benue, Bauchi, Plateau, Borno and Kaduna states. There are many varieties of soybeans. The shape and size of seeds vary from small round pea to large elongated beans, the color; also vary from yellow, brown, and green to black. The seeds are enclosed in a short hairy pod containing 2-3 seeds attached to the plant.

Soybean is very rich in protein and oil. Soybean is one of the commercial sources of vegetable oil. Soybean on dry weight basis contains about 40% protein, 21% oil, 34% carbohydrate and 4.9% ash. The amino acids distribution of soybean is close to that recommended by Food and Agricultural Organization (FAO) of United Nations for human nutrition in terms of essential and non-essential amino acids (Nwanekezi 1984). However, soybean is deficient in sulphur containing amino acids.

Soybean is also known to contain anti-nutritional and flatus factors, beany flavor, inhibitors, hemaglutinings, and lipozygenease are relatively sensitive to heat. Phytic acid (which interfers with availability of calcium), oligosaccharides including raffinose and stachyose (which are causative factors for flatulence and uncomfortable feeling often experienced upon ingestion of soybean products) are not effectively destroyed by processing heat. The desirable beany flavor of the bitterness of soymilk can be eliminated by cooking in an aqueous sodium bicarbonate at a temperature of about 80˚C followed by extraction of protein and other water-solube components (Nwankezi, 1984). The composition of soymilk varies with the varieties of soybean used and the method of production (Wang *et al*., 1978).

Several processes have been adopted in the production of soymilk. The processing method varies from one place to other. The most common method of soymilk production is the traditional oriental method in which soybean is soaked overnight, crushed wet and sieved to get soymilk.

Johnson and Synder (1978) described two other methods of processing termed Illinios and Cornell methods. These methods were aimed at improving the acceptability of soymilk in terms of odor, and flavor. There are other various methods reported by Omatoye, (1984) and Wang *et al*., (1978). Some of these methods include full fat soy-flour process, protein isolate process, and water emulsion process. Upon heat treatment and some chemical treatments, these undesirable characteristics of soybean are either eliminated or reduced.

Soybeans were first introduced into Nigeria in 1908(Fennel, 1966), but the first successful cultivation was in 1937 with the Malayan variety, which was found suitable for commercial production in Benue State in central Nigeria (Root *et al.,* 1987). Nigeria has been the largest producer of soybeans for food in subscinaran Africa (IITA, 2009).In china, where soybeans are valued as “Vegetable meat”, people call it “da soug” which means “great beans”. In the United States, the soybean has also ben dubbed “the miracle beans”. Worthwhile for this honor, the little round legume is useful and versatile all over the world for it limitless applications. Currently, people are familiar with soy because it is efficient and cheap source of0 high quality protein and seed oil. Soy is utilized industrially for everything from soap to animal feed to environmentally friendly ink. Even Henry Ford once wore suites made of soybeans (Myers, 1993). In 2002, a 3.5billion market for soy foods in the United States indicates that is no longer a niche market any more (SPINS & Soyatech Inc., 2002).

**2.3.2 Soy-Yogurt**

Soymilk based yogurts offer a considerable appeal for growing segment of consumers with certain dietary and health concerns. It has several nutritional advantages over cow’s milk yogurt including reduced level of cholesterol and saturated fats as well as no lactose (Favaro Trindade *et al*., 2001). Additionally, soymilk serves as a base for variety of beverages, contains indigestible oligosaccharides such as raffinose, and stachyose, which are associated with stomach discomfort and flatulence (Rackis *et al*., 1970). The presence of pentanal and n-hexanal also imparts the characteristics undesirable flavor the soymilk. A wide variety of soy products offered on the market include soy-based cheeses, tofu, soy ice-cream, and soy sauce and soy vegetable burgers. Also soybean flour, soy protein concentrates and isolated soy proteins are some of ingredients used in the food industry (Wiseman 1997; Liu 1997). Many improvements have been implemented in the processing conditions and methods of preparations of soymilk to improve functionality and usability of soy proteins in a variety of products, for example, prolonged soaking of soybeans results in increased loss of carbohydrates thus minimizing the amount of fermentable sugars and subsequently avoiding gastrointestinal comfort (Wiseman 1997; Liu 1997).

Fermentation of soymilk offers not only a means of preserving soymilk but also a possibility for modifying and improving its flavor and texture as well as enhancing its beneficial health properties (Liu 1997; Tsangalis *et al*., 2004). It also leads to new types of soy products resembling cultured dairy products (Liu 1997). Soymilk is a good medium for growing Bifodobacterium sp. Due to presence of sucrose, raffinose and stachyose, which are fermented by most of the strain belonging to this genus (Kamaly 1997; Liu 1997; Scalabrini *et al.,* 1998). Although cow milk is a an exceptionally good source of protein because of its excellent assortment of essential amino acid, it is expensive due to rising cost of cow. Thus the development of soy-based milk is a cheap substitute for traditional cow milk yogurt (Pinthong *et al.,* 1980).

Research has shown that soymilk has beneficial effects on health which include; no cholesterol, no sugar, high isoflavone content, high quality protein (Pinthong *et al.,* 1980). In addition, soymilk yogurt improves bone health, reduces menopausal symptoms and risk of heart disease and certain cancers. These immense benefits have stimulated a lot of researchers on incorporating soybean into indigenous diets (Sipos 1988).

**2.3.3 Milk preparation and heat treatment**

Milk is prepared by mixing 12% (w/w) total solids using low heat skim milk powder (SMP), 34% protein (Murray Goulboum Co-operative Co. ltd. Brunswick Australia) in deionised water. For reconstitution, deionised water is heated to 30-40˚C prior to addition of SMP, followed by heating the mix to 50˚C with constant stirring for 30min to dissolve solid particles. By employing a similar procedure, soymilk is prepared by mixing 4% soy protein isolate (SPI) in deionised water. For soy yogurt manufacturing, heat treatment of milk or soymilk is usually carried out at 85˚C for 30min or 50˚C for 10min for milk and 95-100˚C for 30min for soymilk (Liu, 1997). The level of heat treatment is more severe than that used for pasteurization. These are several benefits of using such a high level of heat treatment including:

1. Destruction of all pathogenic and most of spoilage bacteria.
2. Inactivation of most enzymes which may cause undesirable effects to the finished products
3. Expulsion of toxic compounds and a decrease in the oxidation-reduction potential of the medium suitable for the growth of starter cultures by removal of oxygen.
4. Improvement in acid production and in the quality of soymilk as a substrate (Liu 1997).

**2.4 HISTORY AND CONSUMPTION OF SOY FOODS**

Soybeans have a rich and mysterious history, which began over five thousand years ago. Soybeans are valued as a productive and adaptable crop which fits well into the cropping patterns of varying agro-climatic conditions. Soybean is generally considered as a highly versatile grain which has about 365 applications in the formulation of both human and animal foods and other industrial uses (Omotayo *et al.,* 2007). Soybean is a cheap source of quality protein that is superior to all other plant foods because it has good source of quality protein that is superior to all other plant foods because it has a good balance of essential amino-acids. Its seed has a close protein content and fairly close amino-acids with cow milk (Belewu and Belewu, 2007). The fat from the soybean is unsaturated type unlike saturated fats from animal origin and hence is good for heart disease patients (Adegoke *et al*., 2002). Other than the high protein content, it also has good amount of calories and fat. It contains the eight amino acids and is a rich source of polyunsaturated fatty acids (including the good fat-omega 3) and is free of cholesterol (FAO 1999). Soybean contains 43grams of protein per 100grams, which is the highest among the pulses. It contains 19.5grams of fat, 21grams carbohydrate and provides 432 Kcal per 100grams (William and Akiko, 2000). It is one of best vegetarian foods items as far as protein content is concerned, with an average production cycle of 90-110days from planting to harvesting (Fabiyi 2006).

Research has it that one kilogram of soybean contained as much protein as 2kg of boneless meat or 45cups of cow’s milk or 5dozen of eggs (Danshiell 1993). Soybean seed contains about 40% protein, 30% carbohydrate, 20% oil and 10% mineral (Osho and Dashiell, 1998). The beans can be utilized in the liquid, powdery and curd forms for human consumption. The oil could be converted to margarine and salad oil. In most cases, soybean has found wide application in the reduction of malnutrition related problems. Owing to its nutritional value there is a growing demand for soy products such as soymilk, soy oil, soy cake, and soy cheese like soybean curd rich in protein. The medicinal nature of soybean is extremely essential in building body immune system. Soy food has been reported to provide significant, but not total protection against heart diseases, high blood pressure, stroke, ulcer, menopause, diabetes, and cancer (WHO 2004; Fabiyi 2006). Recently, soybean is found to be an industrially important crop used as anti-corrosion agent, core oil, and bio-fuel due to less or no nitrogen element in the oil, and disinfectant, in pesticides, printing inks, plants, adhesives, antibiotics and cosmetics (Ngalamu *et al.,* 2012).

The most important domestic processing forms are dadawa, soymilk, soy ogi and soy cheese (Wara). The soy based products produced by commercial processors are soy oil, soy cake and meal, infant foods, soy flour, soy gum and flax. The infant and instant food industries also utilize the bean in producing, soy flour, baby foods, breakfast foods, snacks and other confectioneries (Omotayo *et al*., 2007).

In tradition soybean growing areas of Nigeria, soybean is most commonly intercropped with cereal crops like maize, sorghum and millet to replenish soil nutrients, soybean entered Nigerian diets in an attempt to improve nutrient intake, especially the protein intake of the low-income populace (Obatolu *et al.,* 2006). Adequate food and proper nutrition are basic requirement for economic development, since underfed nation is an under productive nation. Poverty and malnutrition often afflict the same groups of people, so rates of malnutrition are used as indication of poverty (Adewale, 2005).

**2.4.1 Nutritional Benefits of Soyfoods**

Soy products have been shown to play an important role in health. Soybeans have been prized for their remarkable ability to produce over 35% protein by weight, more than other unprocessed plant or animal food (Shurtleff *et al.,* 1983). Two factors determine soy protein quality-digestibility and the pattern of essential amino acids (Messina *et al.,* 1994). Most commercial soy foods are easy to digest. Tofu, for example, is more than 92% (FAO, WHO and UHU, 1985; Synder 1987; Bressani 1981). Only toasted or steamed whole soybeans are poorly digested. Soybeans contain all of the eight essential amino acids in a configuration readily usable by the human body (Shurtleff *et al*., 1983). Despite its limiting amino acids, methionine, the amino acid pattern of soy protein matches well with human requirements. Therefore, there is actually no essential difference between soy protein and animal protein in eggs, milk and meat.

Soybeans are also rich in nutrients, such as calcium, iron, zinc and many of the B vitamins and Vitamin E (Messina *et al.,* 1994). As an excellent source of fiber (15% by weight), soybeans carry an extremely low ratio of calories to protein (Shurtleff and Akiko, 1983). Containing no lactose, soy foods provide a nutritious and wonderful selection of alternatives to diary foods for people who are lactose intolerant. Inexpensive soy foods have no cholesterol and almost none of the relatively indigestible saturated fats and polyunsaturated fats food in most animal foods. Soy oil only contains monounsaturated fats and polyunsaturated fats that do not increase cholesterol. But the most interesting thing about soy oil is it contains as much as 8% linoleic acid, which is an omega-3 fatty acid that may help reduce the risk of heart disease and may even help to prevent cancer (Dolecek, 1992; Wallingford *et al*., 1991; Isreal *et al*., 1992). In 1999, the US Food Drug and Administration authorized a health claim acknowledging that “Diets low in saturated fat and cholesterol that includes 25grams of soy protein a day may reduce the risk of coronary heart disease (CHD) by lowering blood cholesterol levels”. In addition, soy also naturally contains isoflavones, compounds that have shown to reduce the risk of certain cancers (USDA 2001). The use of soy ingredients in foods is receiving significant attention from the food industry and consumers because of their role as a functional food (Walsh *et al.,* 2010). Yogurt is an ideal food matrix for delivery of beneficial functional ingredients (Baroke, 2008). The functionality of food can be addressed from the perspective of food components (Tapsell, 2009).

In order to improve the organoleptic properties and nutritional profile of any product, fortification with soy milk is very much relied upon by yogurt manufacturers (Tamime and Robison, 2004, Sodini *et al.,* 2005).

**2.5 RICE**

Rice (*Oryza Sativa L.)* which belongs to Porcee gramineae or grass family, is one of the most important staple foods of the world (FAOSTAT, 2014). Rice is a nutritious cereal and one of the cheapest and major sources of food energy and protein for many people in the world. Brown rice contains nutritional components, such as dietary fibers, essential amino acids, minerals, proteins, vitamins and other non-nutrient essential petrochemicals, concentrated in the germ and outer layers of the starchy endosperm (Kohama *et al.,* 1987). The bran layer of rice kernel contains high level of bioactive compounds such as Y-oryzanol, anthocynains and phenolic compounds which may reduce low-density lipoprotein cholesterol, improve lipid profiles and have anti-inflammatory and anti-oxidative activities which help to fight against heart diseases and prevent diabetes (Nontasan *et al.,* 2012).

**2.5.1 Rice milk and Rice Yogurt**

Rice, especially the bran contains dietary fibers which can have beneficial effects against chronic diseases such as cardiovascular diseases, diverticulosis, diabetes and colon cancer (Abdul-Hamid, 2000). Amarakoon *et al*., 2013 demonstrated that cooked rice facilitates the growth and survival of probiotic bacteria such as bifidobacteria in yogurt. The nutritional quality of rice based foods can also be improved through fermentation by amyloytic lactic acid bacteria such as certain strains of lactobacilli and bifido bacteria which may increase the availability of lysine and improve the digestibility of starch in young children (Gobbetti *et al*., 2005; Espiritos-Santo *et al*., 2014). Nowadays, cereal alone or mixed with other ingredients are widely utilized in producing novel food products with enhanced health properties (Blandino *et al.,* 2003; Coda *et al.,* 2012). Nontasan *et al.,* (2012) recently developed a yogurt with good color stability and higher level of photochemical using black waxy rice bran.

There are previous studies focusing on the production of probiotic and fermented dairy products using vegetable based raw materials like oats and soy; however there are limited number of studies on the usage of rice milk in fermented dairy products. In researches, knowing the functional properties of food components, enable us to produce nutritive, healthy and resistant foods with good taste, flavor, and consistency (Faccin et al., 2009; Ramos et al., 2011; Coda et al., 2012). Rice starch is made up of two polysaccharide types called amylase and amylopectin (Fredriksson *et al.,* 1998). During cooking, hot water enters the semi-crystalline regions of raw rice starch, breaks down the intermolecular hydrogen bonds, and binds to the hydroxyl hydrogen bonds, and binds to the hydroxyl hydrogen bonds, and binds to the hydroxyl hydrogen and oxygen hence the chains of starch lose their crystallinity into the amorphous form. This gelatinized starch is not in thermodynamic equilibrium. During cooling or being left at a lower temperature for long periods, the linear molecules, amylase, and the linear parts of amylopectin molecules expel water and rearrange themselves again to a more crystalline structure. This recrystallization is known as retrogradation, resulting in hard and amylase-inaccessible textures with poor sensory quality and low nutritional value in cooked rice (Chung *et al.,* 2006, Okuda et al., 2006; Zhu *et al*., 2013; Li *et al.,* 2014). Therefore, retrogradation process is highly likely to affect the organoleptic and nutritional qualities and reduces the consumer acceptability of rice incorporated yogurts. To the best of our knowledge, very few studies investigated the quality of rice incorporated probiotic bacteria. However, Amarakoon *et al.*, (2013) reported significantly higher hardness development in rice incorporated yogurts compared to plain yogurts refrigerated storage possibly due to retrogradation rice.

**2.5.2 The Nutritional Value of Rice milk**

Rice milk contains more carbohydrates than cow’s milk. However, unlike cow’s milk, rice milk doesn’t contain lactose or cholesterol. It is considered the best hypoallergenic form of milk. It is better to drink rice milk if allergic to soymilk and cow milk. The milk enhances immune system and provides resistance to bacteria and viruses invading the body due to high content of selenium and magnesium (<http://www.healthiro.com/dietfood/ricemilk>).

Rice milk doesn’t contain as much calcium or protein as cow’s milk. Since rice milk does not contain a lot of protein, those who use rice-milk as a milk substitute must plan to include more protein in their diet through other means. Most commercial brands of rice milk, however, are fortified with calcium. This is why a serving of fortified milk provides the same amount of calcium as a serving of cow’s milk. Rice milk is also often fortified with niacin, vitamin B12, vitamin D and iron. One cup of rice milk contains about 140 calories, compound to 185 calories in cup of whole cow’s milk, and 97 calories in a cup of skimmed cow’s milk. (http//www.fitday/fitness-articles/what is rice-milk).

**CHAPTER 3**

**MATERIALS AND METHOD**

**3.1 MATERIALS AND STARTER CULTURES**

The Vegan Yogurt starter culture (Rice maltodextrin and mixed culture of lactic bacteria (*Streptococcus thermophilus, and Lactobacillus delbrueckii ssp. Bulgaricus)* was obtained from Culture for health laboratory, Raleigh, North Caroline USA. The cultures were maintained according to manufacturer’s instructions, at 5˚C until used.

Soybeans and 1kg of Indian gate brown rice were purchased from Market Road, Elekahia Port Harcourt, Rivers state. The samples were prepared in Genesis foods laboratory, Km 17 Aba-Porthacourt express road, Rivers State and the experiment was conducted at NAFDAC laboratory Borokiri, Port Harcourt, Rivers State.

**3.2 PREPARATION OF RICE MILK**

Rice milk was prepared according to the method of Faccin et al., 2009 with some modification. Brown rice (200g) was washed thoroughly and boiled with 2liters of water on a heating mantle on a low temperature of 45˚C with minimum time of 3hours to generate soupy rice pudding. The boiled rice was blended (QASA blender, QBL-20L330, Qlink group, China) with 400ml of water to obtain a smooth content; the mixture was strained through a muslin cloth to obtain the rice milk and pasteurized at 68˚C for 30 minutes

**3.3 PREPARATION OF SOYMILK**

Traditional Chinese method was used in the preparation of the soymilk. Exactly, 200g of soybean was sorted and soaked in 2liters of water for 38hours. It was dehulled and rinsed with tap water. The beans were wet milled (1:4liters of water). The milk was obtained by passing the slurry through muslin cloth followed by pasteurization at 68˚C for 30 minutes (Johnson and Synder, 1978).

Soybean

Rice

Washing

Screening

Cooked until very Soft

Soak in tap water for 28hrs

Blending (200g Rice+400ml Water)

Draining

Sieve using a muslin cloth

Pasteurize at 68°C for 30min Decoat and rinse twice with tap water

Blending (1:4 liters of water)

Rice Milk

Sieve with muslin cloth

Pasteurize at 68°C for 30min

Soymilk

Blends at different ratios

(100%, 80:20, 75:25, 50:50, 25:75)

Add fermentable sugar and inoculate using the bacteria starter culture and incubate for 48hrs

Rice-Soya Yoghurt

**Fig 1: Flowchart for the production of Rice-Soya Yoghurt**

**3.4 RICE-SOYA YOGURT PRODUCTION**

The inoculation of rice-soya yogurt was conducted at the Genesis laboratory, Port Harcourt Rivers State. The Rice-Soya yogurt was prepared by mixing rice milk and soy milk in ratios 80:20, 25:75, 50:50 and 75:25 respectively representing Treatments C, D, E, F. While the Control Treatment was the commercial plain Hollandia yogurt (Y), then treatment B and G represent 100% Soya milk and 100% Rice milk respectively. The various mixtures of rice and soya milk blends were pasteurized at 95ºC for 5 min using electrical thermostatic water bath boiler DK-420 (Medfield equipment & scientific ltd, England). The pasteurized mix was cooled to 40ºC and transferred into the Yogurt-Maker (Yogotherm, Biena, Canada). The Fermentable sugar and Vegan Yogurt starter culture (Rice maltodextrin and mixed culture of lactic bacteria (*Streptococcus thermophilus and Lactobacillus delbrueckii ssp. Bulgaricus*) were inoculated as a direct vat set into the yogurt mix as per the manufactures recommendations. Then the mix was incubated at 45ºC for 48 hrs until curd is formed, then refrigerate for 3 hrs until the rice-soya yogurt is set (Belewu, 2006).

**3.5 DETERMINATION OF PROXIMATE PROPERTIES**

**3.5.1 Moisture Content**

Exactly 2g of the yogurt samples were weighed and the moisture determined with the aid of MA150 Sartorious moisture analyzer (AOAC, 2005), the analyzer expels the moisture at the temperature 105ºC, the analyzer beeps when the sample is completely free from moisture, then the percentage moisture content is ascertained.

**3.5.2 Nitrogen Distillation**

The Markham distillation apparatus was heated for about 15 minutes before using it. 100ml conical flask containing 5ml of boric indicator was placed under the condenser such that the condenser tip is under the liquid. 5ml of the digest was pipette into the body of the apparatus via the small aperture and washed down with distilled water followed by 5ml of 60% NaOH solution. It was steamed through for about 5-7 minutes to collect enough ammonium sulphate.

The receiving flask was removed and the tip of the condenser was washed down into the condensed water. The solution was titrated into the receiving flask using N/100 (0.01N) hydrochloric or sulphuric acid and calculates the nitrogen content and hence the protein content of the food (Onwuka, 2005; AOAC, 2005)

Calculation: the nitrogen content was content was calculated below

% Nitrogen Sample titre x 0.014 x 100

W

Where,

W weight of the sample in grams

% Protein % Nitrogen in protein x 6.25

**3.5.3 Total Ash**

This was done using the furnace incinerator gravimetric method (AOAC, 1990) but the method adopted by Onwuka, (2005). Exactly 5g of the yogurt samples was weighed into a tarred silica or porcelain crucible and pre-ashed on a hot plate.

The pre-ashed sample was charred on a heater or Bunsen flame inside a fume cupboard to drive off most of the smoke. The sample was transferred into pre heated muffle furnace at 550ºC and allowed for 2hours until a white or light gray ash results was obtained. The sample in crucible was carefully removed from the furnace (taking care not to allow air blow the ash away) and cooled in the desiccators and reweighed.

Calculation

% Ash (dry basis)

Weight of sample before drying

W3 –W1

W2- W1

W1

W2

W3

The carbohydrate content was calculated by difference as the nitrogen free extraction (NFE)

%NFE

Where;

a

b

c

d moisture

**3.5.4 Crude Fat**

Rose- Gottleb method of fat extraction was used to determine the fat content of the sample yogurt; 10g of yogurt sample was weighed accurately and transfer into extraction tube. Exactly125ml of ammonia sp.gr 0.8974 was added, mixed and shaked thoroughly. 10ml ethyl alcohol was added and mixed again. 25ml of diethyl ether (peroxide free) stopper was added and shaked vigorously for about a minute. Then 25ml petroleum ether was added (boiling range 40-60 into the solution and shaked again vigorously for about half a minute. It was allowed to stand until the upper ethereal layer separates completely and is clear (alternatively use low r.p.m mojonnier centrifuged). If there is a tendency to form emulsion, a little alcohol may be added to help separation of the layers. The clear ethereal layer is decanted into a suitable vessel (flask, glass bowl, aluminum dish, est.). The delivery end of the extraction tube is washed with a little ether and the washings is added to the flask. T2he extraction of the liquid remaining in the extraction tube is repeated twice using 15ml of each solvent every time. The ethereal extract is added to the same container and evaporated off completely. The flask is dried in an air oven at for two hours, cooled in the dessicator and weighed. The flask is heated again the oven for 30 min. cooled in a dessicator and weighed. The process of heating, cooling and weighing is repeated until the difference between two successive weight does not exceed 1mg. The fat is washed from the flask with petroleum ether carefully leaving any insoluble residue in the flask. The flask is dried in the oven and reweighed. The difference in weight represents the weight of fat extracted from the yogurt samples. (AOAC, 2000).

Calculation:

Fat (%) (w/w) weight100

**3.6 PHYSIOCHEMICAL PROPERTIES**

**3.6.1 pH Measurement**

The pH of the yogurt samples was determined using a digital pHmeter (JENWAY 3505). The pH meter was calibrated with buffer standards of pH4 and pH10 prior to use. 50ml of each yogurt sample was placed in a beaker, the probe electrode of the PH meter was inserted into the yogurt samples to determine each pH value. The probe was rinsed thoroughly with distilled water before use on other samples. (AOAC, 2005).

**3.6.2 Titratable Acidity**

The titratable acidity was measured by titrating 15ml of different yogurt treatments with 0.1M sodium hydroxide (NaOH) until the substances reached a PH value 8.2, corresponding to the end point of the phenolphthalein. The value and the spent NaOH volume were recorded. The acid percentage of the substance (Jacobs, 1999) will be calculated using the formula:

Titratable acidity (%T):

Where, M

**3.6.3 Total Solids**

The weight of the residue obtained from the moisture content analysis was expressed as percentage total solids (AOAC, 2000) using the formula below:

Totalsolids(%)

**3.6.4 Solids-not-Fat**

Solids-not-fat was determined by conducting total solids and fat analyses. Percent fat was subtracted from percent total solid to obtain percent solids-not-fat. (AOAC, 2000).

**3.6.5 Specific Gravity**

A clean dry density bottle (pycnometer) along with the stopper was dried at a temperature of 105 to 110oC, cooled in the desiccator and weighed to the nearest 0.001g (W1), the yogurt sample, was transferred to the density bottle directly from the desiccator in which it was cooled. The bottles and contents together with the stopper were weighed to the nearest 0.001g (W2). Empty the bottle, clean thoroughly and fill the density bottle with distilled water at the same temperature. Insert the stopper in the bottle, wipe dry from the outside and weigh (W3). (AOAC, 2000). The specific gravity was determined using the formula below:

Specific gravity W2  W1

W3W1

i.e.

**3.6.6 Milk Solid Non-Fat (MSNF)**

10ml of the yogurt sample was poured into a conical flask, 1ml or 3 drops of phenolphthalein indicator and 3ml of formalin was added into conical flask and titrated against 0.1N NaOH. The reading was recorded as titre value (Tv) for MSNF (AOAC, 2000). The titre value (Tv) was ascertained by titrating 1ml of phenolphthalein and 3ml of formalin with no sample against 0.1N NaOH.

MSNF value was determined using the formula below:

MSNF Value

**3.6.7 Mineral Elements**

The major, minor and trace elements were determined using Atomic absorption emission spectrophotometer (ASS; SMART MODEL 200A). The trace elements were done using Smartspects, pH meter, and conductivity meter were the 10ml water sample was scanned blank in the smartspects before analysis of the yogurt samples. Each trace element was analyzed by using its various chemical test kits. (AOAC, 2005).

**3.6.8 Refractive Index**

The refractometer (ATAGO RX-7000) was calibrated by adding droplets of water on lens and booting at the temperature of 24. The water droplet was wiped off with a cotton wool to remove particles on the lens. A drop of the yogurt sample was added on lens; the lid was covered and restarted to take the readings. (AOAC, 2005).

**3.6.9 Total Aflatoxin analysis**

The net weight of the sample was measured and 10g of the yogurt sample was weighed into a plastic flask. 50ml of distilled water was added into the flask and place in an orbital shaker at 250rpm for 3mins. 30secs, the mixed sample was allowed to stand and sediment before filtration into the test tubes and analyzed using aflatoxin test kits (consists of conjugates, substrate, standard for calibration, coated antibody wells, mixing vials and reagent boat). The conjugate was poured in the reagent boat, using 100ml pipette inclined with a tip to take up the conjugate which was released into the mixing bars. The former tip was discarded and a new tip inserted to take up conjugate which was released into the yogurt sample. The sample was injected into the mixing bars. The mixture was mixed thoroughly and transferred into the antibody well respectively. The sample mix in the antibody well was allowed to stand for 15mins, then washed with distilled water. The substrates in the reagent boat was pipette into the antibody well, then allow standing for 5mins; washing off and cleaned with cotton wool. The stop solution was pipette into the antibody well. The process was terminated in the presence of a color change. (The deeper the color, they lesser the presence of aflatoxin). Thereafter it was screened with Elisa method. (AOAC, 2005).

**3.6.10 Viscosity**

Viscosity of rice-soya yogurt was measured as described by Ranadheera *et al*. (2012) and Onwuka (2005) with small modifications using a viscometer (BL model Tokimec, Japan). Viscosity was determined based on measuring resistance to a rotating spindle (Spindle no. 4 at 12rpm) at constant temperature (4) for 5 min a yogurt filled 6.36 cm diameter 6cm height cup.

**3.7 MICROBIOLOGICAL ANALYSIS**

Microbial analysis of the yogurt samples were conducted on day 1, 7, 14 and 21 during the storage. One gram of yogurt sample was serially diluted in sterile peptone water and enumeration was done using pour plate technique. The plates were incubated at 37 for 72 hrs in anaerobic jars (Oxoid Ltd, Hamsphire, UK) with an anaerobic environment (<1% O2 and 9-13% CO2), generated using anaerobic AnaeroGen sachets (AN0025A, Oxoid Ltd, Hamsphire, UK). Enumeration of yeast and mold was conducted in Malt Extract Agar (IVD) using spread plate technique. The plates were incubated at 25 for 5days. Enumeration of coliform was conducted in Violet Red Agar using spread plate techniques following aerobic incubation at 35 for 5 days, viable bacteria counts were expressed as log cfu. g-1 of rice-soya yogurt samples. (Speck, 1976; Ezeama, 2007)

**3.8 SENSORY EVALUATION**

All samples were evaluated for organoleptic characteristics (color, aroma, taste, astringency(mouth-feel), beany taste and overall acceptability by 16 panelists that comprised unskilled and skill grade Genesis foods staff members at Port Harcourt Rivers state, Nigeria; using nine point hedonic scale which range from 1(dislike extremely) to 9 (like extremely) described by Iwe (2010).

**3.9 STATISTICAL ANALYSIS OF DATA**

Statistical differences between the samples were determined by analysis of variances (ANOVA), mean separation by DUNCAN multiple range test, (SAS, 2003) and statistical level of significance difference of products were accepted at (p<0.05).

**CHAPTER 4**

**RESULTS AND DISCUSSION**

**4.1 PROXIMATE COMPOSITION OF YOGHURT PRODUCED FROM BLENDS OF RICE MILK AND SOY MILK**

The results of the proximate analysis of the composition of rice-soy milk yoghurt samples are presented in Table 4.1.

**Moisture**

The results showed that the moisture content of the yoghurt samples differed significantly (p<0.05) ranging from 73.35 % in the control to 91.27 % in sample 1350. With exception of the control, the moisture content of the rice-soy milk yoghurt samples were higher than the range (82 to 84 %) recommended for yoghurt (Ahmad, 1994). This high moisture contents could reduce their viscosity and possibly affect texture and mouth feel (Ehirim and Onyeneke, 2013). It could also affect the stability and safety of the yoghurt samples with respect to microbial growth and proliferation hence they require cold storage.

**Fat**

The fat content of the yoghurt samples ranged from 0.80 to 1.60 % with sample 1350 recording the highest value and sample 350 having the least value. However, the fat content of some samples were not significantly different (p>0.05) from the control (1.58 %). Generally, the fat content of the rice-soy milk yoghurt samples were quite lower than the values reported for goat milk (3.7 %) and cow milk (4.1 %) yoghurts by Ihekoronye and Ngoddy (1985). It was also evident that increased addition of soy milk caused an increase in fat content suggesting that soybean contains more fat than rice. Fat plays an important role in improving the consistency of yoghurt while providing twice as much energy as the same quantity of carbohydrates and protein (Gregher, 1985). Thus, the lower value of fat in the rice-soy milk yoghurts is an indication of reduced available total energy.

**Protein**

The protein content of the yoghurt samples ranged from 0.53 to 7.08 % with soy milk recording the highest value while rice milk had the least value and was insignificantly different (p>0.05) from the values recorded for sample 1150 (0.58 %) and 350 (0.53 %). The protein content of sample 1350 (7.08 %) recorded as the highest was higher than the value (4.80 %) reported for soy milk drink by Udeozor (2012). The results also revealed that increased substitution of rice milk with soy milk resulted to an increase in the level of protein in the imitation yoghurt samples. This result is expected since soybean is a richer source of protein than rice.

**Carbohydrate**

The carbohydrate content of the yoghurt samples showed significant differences (p<0.05) ranging from 0.03 % in sample 1350 to 22.96 % in the control. Furthermore, the values recorded for the rice-soy milk yoghurt samples were very low compared to the control. This could be attributed to the process of fermentation which converts carbohydrate to acid (Igbabul *et al*., 2014). This makes yoghurt an ideal food for lactose intolerant individuals (Ehirim and Ndimantang, 2004).

**Table 4.1: Proximate composition of rice-soy milk yoghurts**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Yogurt Samples** | **Moisture** | **Fat** | **Ash** | **Protein** | **Carbohydrate** |
| Y | 73.35f±0.09 | 1.58a±0.04 | 0.01a±0.00 | 2.10d±0.11 | 22.96a±0.02 |
| 350 | 87.34e±0.07 | 0.80d±0.02 | 0.01a±0.00 | 0.53e±0.04 | 11.32b±0.08 |
| 550 | 90.48c±0.07 | 1.20bc±0.04 | 0.01a±0.00 | 0.59e±0.01 | 7.72d±0.04 |
| 750 | 90.93b±0.01 | 1.50a±0.00 | 0.03a±0.02 | 3.75b±0.05 | 3.79f±0.01 |
| 950 | 91.06b±0.02 | 1.30b±0.14 | 0.03a±0.00 | 3.12c±0.04 | 4.49e±1.27 |
| 1150 | 87.94d±0.05 | 1.10c±0.00 | 0.04a±0.00 | 0.58e±0.00 | 10.34c±0.06 |
| 1350 | 91.27a±0.01 | 1.60a±0.00 | 0.01a±0.00 | 7.08a±0.005 | 0.03e±0.00 |

Values are means ± standard deviation of duplicate determinations expressed as percentage. Two means along the same column with different superscripts are significantly different (p<0.05)

**Key**

**Sample Y =** Control (Hollandia Yoghurt), **Sample 350** = 100 % rice milk

**Sample550 =** 80 % rice milk + 20 % soy milk yogurt

**Sample 750 =** 25 % rice milk + 75 % soy milk yogurt

**Sample 950 =** 50 % rice milk + 50 % soy milk yogurt

**Sample 1150 =** 75 % rice milk + 25 % soy milk yogurt

**Sample 1350 =** 100 % soy milk yogurt

**4.2 CHEMICAL PROPERTIES OF YOGHURT PRODUCED FROM BLENDS OF RICE MILK AND SOY MILK**

Table 4.2 shows the physico-chemical properties of rice-soy milk yoghurts.

**Milk-solid-non-fat (MSNF):** The range of milk-solid-non-fat of the yoghurt samples was found to be from 1.701 % in sample 350 to 18.144 % in sample 950. There were no regular pattern regarding the effect of substitution of rice milk with soy milk and vice versa. According to USDA (2001) and FDA (2009), yoghurt should contain not less than 8.25 % solid-non-fat before the addition of bulky flavours. With exception of sample 350, other yoghurt samples in this study conforms to these standards therefore suggesting that rice milk alone cannot be used to produce yoghurt of improved milk-solid-non-fat. Low milk-solid-non-fat in yoghurts might result to loss of overrun (Umelo *et al*., 2014)

**Total solids:** The total solid content of the samples ranged from 3.44 % in sample 1350 to 12.66 % in sample 350. Dublin-Green and Ibe (2005) reported that the total solid content for fruit and natural yoghurts should range from 15.0 to 22.8 % and 13.6 to 18.8 % respectively. However, in this study, only sample 350, 1150 and the control conformed to these specifications and it was reflected in their firmer bodies compared to other yoghurt samples with lower values of total solids content.

**pH:** The pH of the yoghurt samples ranged from 6.423 to 6.780 with sample 1350 and 350 recording the highest and least values respectively. These values were near neutral pH and below the specifications for pH of yoghurt (4.6 or lower) as recommended by FDA (2009). Lower pH (acidic) in foods helps to reduce the activity of spoilage microorganisms. It therefore implies that the yoghurt samples may have low shelf stability. Thus there is need for cold storage in order to extend their shelf stability.

**Total acidity:** The acidity of the yoghurt samples ranged from 0.315 % in the control to 0.980 % in sample 1350. The percentage acidity did not follow a reverse trend with the pH. According to FDA (2009), the recommended standard for total acidity of yoghurt should be 0.7 %. Hence, sample 1150 and the control did not conform to this standard. Yoghurts with low total acids could encourage the growth of lipolytic and proteolytic bacteria which are implicated for deterioration of yoghurt if not adequately refrigerated (Umelo *et al*., 2014).

**Specific gravity (SG):**  The specific gravity of the yoghurts ranged from 1.002 to 1.062 with the control recording the highest value while sample 1350 had the least value. There was no observable trend regarding the effect of substitution of rice milk with soy milk and vice versa. With exception of the control, the values obtained for specific gravity of the rice milk-soy milk yoghurts were in agreement with the range (1.01 to 1.03) stated for all beverages, non-alcoholic (including soft drinks and juices) and fruit drinks (low calories and undiluted) by FAO/WHO (2011). The highest value of specific gravity recorded in the control might be attributed to its lower moisture content and high total solids content.

**Refractive index (RI):** The refractive index of the samples ranged from 1.333 in sample 1350 to 1.357 in the control. There were no significant difference (p>0.05) sample 350, 550, 750, 950 and 1150. The refractive index is related to the ease with which light passes through fat (Abdel *et al*., 2013) in the yoghurt samples.The values obtained for refractive index of the yoghurt samples were slightly above the range (1.3440 to 1.3485)reported for milk at 20˚C suggesting that the yoghurt samplescontained more water, proteins, lactose and other minor constituents compared to milk at 20˚C (Singh *et al*., 1997).

**Viscosity:** The viscosity of the yoghurts was found to range from 2000 to 4200mp. The highest value was recorded in the control while sample 750 had the least value which was insignificantly different (p>0.05) from sample 1350. Viscosity is related to overrun and it is known to contribute to the texture and mouth-feel of yoghurt through the fat, casein and whey proteins. Hence, yoghurts with higher viscosities in this study would have good rheological properties and better texture than those with lower values.

**Table 4.2: chemical properties of rice milk-soy milk yoghurts**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Yogurt  Samples | MSNF  (%) |  | Total solid  (%) | pH | % Acidity | SG | RI | Viscosity  (Mp) |
| Y | 24.948a±0.008 |  | 26.25a±0.26 | 4.500f±0.028 | 0.315d±0.005 | 1.062a±0.004 | 1.357a±0.009 | 4200a±282.8 |
| 350 | 1.701g±0.002 |  | 12.66b±0.29 | 6.423e±0.007 | 0.800bc±0.000 | 1.012d±0.002 | 1.341ab±0.007 | 3900ab±141.4 |
| 550 | 11.340f±0.108 |  | 9.52d±0.18 | 6.517d±0.008 | 0.770bc±0.007 | 1.012d±0.000 | 1.339ab±0.002 | 3500bc±141.4 |
| 750 | 14.742d±0.002 |  | 9.06e±0.12 | 6.558c±0.012 | 0.630c±0.001 | 1.016bc±0.005 | 1.339ab±0.002 | 2000e±282.8 |
| 950 | 18.144b±0.011 |  | 5.94f±0.08 | 6.616b±0.005 | 0.890ab±0.070 | 1.023b±0.000 | 1.337ab±0.002 | 2800d±0.0 |
| 1150 | 16.433c±0.032 |  | 12.06c±0.16 | 6.483d±0.018 | 0.330d±0.169 | 1.024b±0.005 | 1.339ab±0.004 | 3200cd±141.4 |
| 1350 | 12.474e±0.015 |  | 3.44g±0.08 | 6.780a±0.007 | 0.980a±0.000 | 1.002e±0.002 | 1.333b±0.000 | 2150e±70.7 |

Values are means ± standard deviation of duplicate determinations. Means along the same column with different superscripts are significantly different (p<0.05)

**Key**

**Sample Y =** Control (Hollandia Yoghurt),

**Sample 350** = 100 % rice milk

**Sample550 =** 80 % rice milk + 20 % soy milk Yogurt

**Sample 750 =** 25 % rice milk + 75 % soy milk Yogurt

**Sample 950 =** 50 % rice milk + 50 % soy milk Yogurt

**Sample 1150 =** 75 % rice milk + 25 % soy milk Yogurt

**Sample 1350 =** 100 % soy milk Yogurt

**4.3 MINERAL CONTENT OF YOGHURT PRODUCED FROM BLENDS OF RICE MILK AND SOY MILK**

Table 4.3 shows the mineral content of yoghurt produced from varied proportions of rice milk and soy milk. The mineral content of the yoghurts varied among the blends. However, the values of mineral contents of most of the blends obtained were lower when compared with standards recommended for dairy whole milk by the International Dairy Federation (2008).

**Magnesium:** The magnesium content ranged from 9.83 mg/100g in the control to 47.83 mg/100g in sample 1350. These values were different from the range (80 to 131 mg/kg) reported for plain yoghurt by Buttriss (1997). Although the results did not reveal a trend regarding the effect of substitution of rice milk with soy milk, it indicated that addition of soy milk to the yoghurt improved their magnesium content.

**Calcium:** This was the most abundant mineral analyzed and it followed a similar trend as magnesium ranging from 19.60 mg/100g in the control to 95.67 mg/100g in sample 1350. These values were lower than 101 mg/100g reported for tigernut-soy milk extract by Awonorin and Udeozor (2014). However, the results indicated that addition of soy milk to rice milk improved the calcium content of the yoghurts. Calcium is important in the body because it is the main structural component of bones and teeth (Wardlaw and Kessel, 2002).

**Potassium:** This was not detected in the control but it was however, found to range from 5.00 mg/100g in sample 550 to 0.45 mg/100g in sample 750. These values were below 1297 mg/kg reported for plain yoghurts by Moreno Rojas *et al*., (1993). Thus combination of rice milk with soy milk resulted to yoghurts with low potassium content.

**Copper:** The highest value for copper was recorded in the control (1.30 mg/100g) while sample 750 recorded the least value (0.45 mg/100g) and was insignificantly different (p>0.05) from sample 550 (0.47 mg/100g) and 350 (0.46 mg/100g). There was no specific trend regarding the effect of substitution but the result clearly showed that addition of rice milk decreased the copper content of the yoghurts.

**Iron:** There was no trace of iron in the control but the highest value was recorded for sample 350 (0.28 mg/100g) while the least value was recorded for sample 950 (0.03 mg/100g). These values were different from the value (0.5 mg/kg) reported for plain yoghurt by Garcia Martinez *et al*., (1998). Hence the yoghurts could be classified as food of less nutritional value because of its low contribution of iron in the diet value (Schneider, 1994). The addition of soy milk was found to improve the iron content of the yoghurts. Iron functions as hemoglobin in the body; in cellular respiration, it functions as essential component of enzymes involved in biological oxidation such as cytochromes (Malhotra, 1998).

**Cadmium:** This was totally absent in the control. However, in sample 1350 it was higher (0.36 mg/100g) compared to other samples but sample 750 recorded the least value of 0.02 mg/100g.

**Zinc:** This was not detected in the control but it was detected in the rice-soy milk yoghurts and it was found to be highest in sample 1350 and 1150 as they both recorded the value of 0.12 mg/100g while the least was found in sample 350 (0.06 mg/100g) which was insignificantly different (p>0.05) from sample 750 (0.07 mg/100g). Zinc dependent enzymes are involved in macronutrient metabolism and cell replication (Arinola, 2008).

**Table 4.3: Mineral content of rice-soy milk yoghurts**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Yogurt  Samples | Mg | Ca | K | Cu | Fe | Cd | Zn |
| Y | 9.83g±0.04 | 19.60g±0.14 | - | 1.30a±0.14 | - | - | - |
| 350 | 14.33e±0.05 | 30.67e±0.04 | 4.95a±0.04 | 0.46c±0.02 | 0.28a±0.08 | 0.05bc±0.02 | 0.06b±0.02 |
| 550 | 20.50c±0.42 | 41.00c±0.00 | 5.00a±0.14 | 0.47c±0.01 | 0.03bc±0.01 | 0.07b±0.01 | 0.08ab±0.00 |
| 750 | 18.50d±0.07 | 37.00d±0.28 | 0.45d±0.07 | 0.45c±0.00 | 0.09bc±0.04 | 0.02cd±0.01 | 0.07ab±0.02 |
| 950 | 12.00f±0.02 | 24.00f±0.56 | 0.55d±0.00 | 1.17ab±0.04 | 0.03bc±0.00 | 0.03bcd±0.01 | 0.08ab±0.01 |
| 1150 | 30.67b±0.05 | 61.30b±0.28 | 1.15c±0.05 | 0.37c±0.04 | 0.07bc±0.02 | 0.03bcd±0.00 | 0.12a±0.14 |
| 1350 | 47.83a±0.00 | 95.67a±0.24 | 1.65b±0.07 | 1.03b±0.01 | 0.12b±0.00 | 0.36a±0.02 | 0.12a±0.02 |
| IDF Reference  for Whole Milk | 0.8-1.5 mg/100g | 120 mg/100g | 87 mg/100g | - | 80 mg/100g | - | 7 mg/100g |

Values are means ± standard deviation of duplicate determinations expressed as mg/100g. Means along the same column with different superscripts are significantly different (p<0.05)

**Key**

**Sample Y =** Control (Hollandia Yoghurt), **Sample 350** = 100 % rice milk, **Sample550 =** 80 % rice milk + 20 % soy milk Yogurt

**Sample 750 =** 25 % rice milk + 75 % soy milk Yogurt, **Sample 950 =** 50 % rice milk + 50 % soy milk Yogurt

**Sample 1150 =** 75 % rice milk + 25 % soy milk Yogurt+, **Sample 1350 =** 100 % soy milk Yogurt

**4.4 MICROBIOLOGICAL QUALITY OF YOGHURT PRODUCED FROM BLENDS OF RICE MILK AND SOY MILK**

The results of the microbiological quality of the rice-soy milk yoghurts are given in Table 4.4. The total viable count (TVC) ranged from 1.7 to 3.0 x 101 CFU/ml with sample 750 recording the least value of 1.7 x 101 CFU/ml while sample 350 and 1150 had the highest value of 3.0 x 101 CF0U/ml. These values were lower than the range (1.1 to 3.8 x 101 CFU/ml) reported for yoghurts sold in Gwagwalada by Akanbi and Oyediji (2015). Furthermore, the TVC of the rice-soy milk yoghurts were compared with International Commission on Microbiological Specifications for Foods (1996) standard and they were found to be of acceptable microbiological quality since their TVC were below 103 CFU/ml. The low bacterial count of the yoghurts could be attributed to adequate hygienic measures in processing. The results also showed that there were no coliform or fungi present in the samples suggesting that quality raw materials and adequate heating process was employed during yoghurt production.

**Table 4.4: Microbial load (CFU/ml) of rice milk-soy milk yoghurts**

|  |  |  |  |
| --- | --- | --- | --- |
| Yogurt  Samples | Total Viable Count | Coliform count | Total Fungal Count |
| Y | 2.4c x 101 CFU/ml | - | - |
| 350 | 3.0a x 101 CFU/ml | - | - |
| 550 | 2.7b x 101 CFU/ml | - | - |
| 750 | 1.7d x 101 CFU/ml | - | - |
| 950 | 2.5bc x 101 CFU/ml | - | - |
| 1150 | 3.0a x 101 CFU/ml | - | - |
| 1350 | 2.3c x 101 CFU/ml | - | - |

Values are means of duplicate determinations. Means along the same column with different superscripts are significantly different (p<0.05)

**Key**

**Sample Y =** Control (Hollandia Yoghurt),

**Sample 350** = 100 % rice milk

**Sample550 =** 80 % rice milk + 20 % soy milk Yogurt

**Sample 750 =** 25 % rice milk + 75 % soy milk Yogurt

**Sample 950 =** 50 % rice milk + 50 % soy milk Yogurt

**Sample 1150 =** 75 % rice milk + 25 % soy milk Yogurt

**Sample 1350 =** 100 % soy milk Yogurt

* 1. **SENSORY EVALUATION OF YOGHURT PRODUCED FROM BLENDS OF RICE MILK AND SOY MILK**

The sensory scores for rice-soy milk yoghurt obtained by varying proportions are given in Table 4.5. All the samples rated alike in almost all the sensory attributes evaluated indicating the minimal effect of varying proportion had on the organoleptic properties of the yoghurts.

**Appearance:** The result showed significant difference (p<0.05) between the colour of the samples with sample 350 recording the highest score of 7 which translates to like moderately on the 9-point hedonic scale while sample 750 had the least score (5.25) which translates to neither like nor dislike. The colour was based on how the appearance appealed to the panelist. The preference of sample 350 to other samples could be due to its very white colour which appealed most to the panelists.

**Aroma:** The sensory rating for aroma followed a different trend as sample 1350 recorded the highest score of 6.55 which translates to like moderately while sample 350 had the least score of 4.60 which translates to neither like nor dislike. The results showed that addition of soy milk improved the aroma of yoghurts.

**Taste:** The taste of sample 350 was most preferred by the panelists as it recorded the highest score of 6.70 which translates to like moderately while sample 1350 had the least score of 4.60 translating to neither like nor dislike. The lower scores recorded for the yoghurts containing soy milk might be attributed to their beany taste.

**Mouthfeel:** The sensory score for mouthfeel showed that sample 750 had the least score (5.25) which translates to neither like nor dislike while sample 1350 recorded the highest score (6.10) which translates to like slightly. However, all the samples were insignificantly different (p>0.05) suggesting that viscosity had no effect on mouthfeel.

**Beany taste:** A residual beany taste is always associated with soy foods. In this work, sample 1350 had the highest score (5.75) translating to like slightly while sample 550 had the least score of 5.10 translating to neither like nor dislike. The results also indicated that decreased addition of soy milk resulted to decrease in beany taste. However, the decrease was insignificant.

**General acceptability:** Sample 350 was most preferred as it had the highest score (6.85) translating to like moderately while sample 750 had the least score of 5.25 translating to neither like nor dislike. The preference of sample 350 to other samples might be attributed to its appearance and lack of beany taste.

**Table 4.5: Sensory attributes of rice milk-soy milk yoghurts**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Yogurt**  **Samples** | **Appearance** | **Aroma** | **Taste** | **Mouthfeel** | **Beany taste** | **General Acceptability** |
| Y  350 | 7.20a  7.00a | 5.60ab  6.05a | 7.05a  5.95ab | 5.5a  5.55a | 5.00a  5.60a | 7.30a  6.85a |
| 550 | 6.60ab | 5.35ab | 5.40ab | 5.85a | 5.10a | 6.00ab |
| 750 | 5.25c | 4.60b | 4.60b | 5.25a | 5.15a | 5.25b |
| 950 | 5.85abc | 5.60ab | 5.30ab | 5.90a | 5.75a | 5.30b |
| 1150 | 6.55ab | 5.65ab | 6.10ab | 5.40a | 5.55a | 6.30ab |
| 1350 | 5.70bc | 6.55a | 6.70ab | 6.10a | 5.35a | 6.30ab |

**Key**

**Sample Y =** Control (Hollandia Yoghurt)

**Sample 350** = 100 % rice milk

**Sample550 =** 80 % rice milk + 20 % soy milk Yoghurt

**Sample 750 =** 25 % rice milk + 75 % soy milk Yoghurt

**Sample 950 =** 50 % rice milk + 50 % soy milk Yoghurt

**Sample 1150 =** 75 % rice milk + 25 % soy milk Yoghurt

**Sample 1350 =** 100 % soy milk Yoghurt

**CHAPTER 5**

**CONCLUSION AND RECOMMENDATION**

**5.1 CONCLUSION**

The results of this research showed that yoghurts prepared from a combination of rice milk and soy milk at 25:75 and 50:50 have higher nutrient contents compared to other rice-soy milk yoghurts thereby making them a perfect dairy free alternative food. It also shows that the addition of soymilk to rice milk improve the nutrient deficiency in rice yoghurt. However, between all the yoghurts studied 100 % soy milk yoghurt was found to have highest nutrient content while 100 % rice milk yoghurt had the least nutrient content.

The results on microbiological quality revealed that the yoghurts were of high quality as they conformed to the standards of the International Commission on Microbiological Specifications for foods and they were also free from coliforms and fungi.

Based on the sensory evaluation, the yoghurts were acceptable although 100 % rice milk yoghurt was most acceptable. The lower preference for yoghurts containing soy milk was due to the residual beany flavor associated with soy foods.

**5.2 RECOMMENDATION**

Based on the findings of this study, it is recommended that:

Flavors should be added to reduce the beany taste of the yoghurts

The yoghurts should be fortified with nutrients especially minerals and protein in order to improve their nutritional quality.

More studies should be carried out on the possibility of using non-dairy milk from other cereals, legumes and nuts for production of yoghurt of high nutritional quality that may address the issue of nutrient deficiency prevalent in developing nations.

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