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Raffinose Extract from Navy Beans Decreases Fermentation Time and Enhances Overall Quality of Yogurt

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Raffinose Extract from Navy Beans Decreases Fermentation Time and Enhances Overall Quality of Yogurt

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ABSTRACT

The consumption of pulses is gaining popularity in recent times due to the awareness of health benefits associated with this food group. Despite this awareness, pulses are still not being used. In order to increase utilization, and consumption of this product, different applications are considered. In this research, extracts prepared from navy beans were used to fortify yogurt. The aim of this study was to evaluate the effect of Navy bean extract on the quality of yogurt.

Navy bean extracts were prepared using water extraction procedure. Extract was added to 2% reduced fat milk (2.5% -10%). 2% milk and bean extract mixture was pasteurized at 90^{o}C for 10 mins, cooled to 42^{o}C, inoculated with yogurt culture (Danisco YO-MIX 883 LYO 500 DCU), and fermented for 4 – 8 hours at 42^{o}F. Physico-chemical analysis, microbiology, shelf-life study and quantification of raffinose in extracts were determined using standard procedures.

The results obtained showed raffinose concentration of up to 2.65g/l in the bean extract. Fortified samples reached the desired pH of 4.6 in 4 hours; there was a significant increase in acidity and decrease in pH 21-day period. A decrease in Total Soluble Solids (TSS) was observed with the addition of extract, however no effect was observed on the viscosity of yogurt. Microbiology results showed a slight increase in mean values for samples fortified with extract compared to control but this increase was not statistically significant over time (P>0.05).

Yogurt fortified with navy bean extract was shown to reduce fermentation time. Increase in acidification and decrease in pH levels shows increased probiotic activity thus, overall quality of yogurt was enhanced with respect to acidification and pH levels. Further studies should incorporate different probiotic strains and optimize extract concentrations.

Keywords: Pulses, Navy beans, Fortification, Raffinose Oligosaccharides.

Chapter One: Introduction

The consumption of pulses-legumes, and lentils are gaining popularity because of the awareness of health benefits associated with this food group. Following the declaration of 2016 as the Year of Pulses by the United Nations (Food and Agriculture Organization of the United Nations, n.d.), more emphasis is being placed on consumption, and usage of pulses in our daily diet; the increase in overall pulse consumption can help improve the nutrient quality and density of our diet and in the long run improve health and wellness of our society.

Pulses are plants, mostly dry seeds that include beans, lentils, peas, and chickpea. It is a staple food in most parts of the world especially developing countries and are very rich in nutrients and fiber. Pulses have been referred to in the past as “a poor man’s meat” (Leterme, 2002, p. 239), mainly because it was eaten in place of meat; in other countries, the chaff or by-product of beans is used to feed livestock. Pulse consumption has been low compared to other food groups because of its anti-nutritive effect; these effects arise as a result of oligosaccharides (raffinose) present in beans, which causesundesireable side effects. However, recent research has shown that oligosaccharides are considered prebiotics and is of great benefit to our health (McCrory, Hamakaer, Lovejoy, & Eichelsdoerfer, 2010). Some positive health benefits attributed to the consumption of pulses include weight management, reduction in the risk of heart disease, and cancer, as well as its important role in almost all vegetarian diets (Leterme, 2002).

The declaration of the year of pulses by the UN has opened up avenues, raised awareness and encouraged the consumption of beans, peas, chickpeas, and lentils; it has also encouraged the use of pulses in new product development, and research. As a result, ideas on how to use the different varieties of beans in creating new products, enhancing or substituting old products is ongoing. This has given birth to the idea of extracting some of the naturally occurring and abundant nutrients in bean, and using these extracts to enhance yogurt quality.

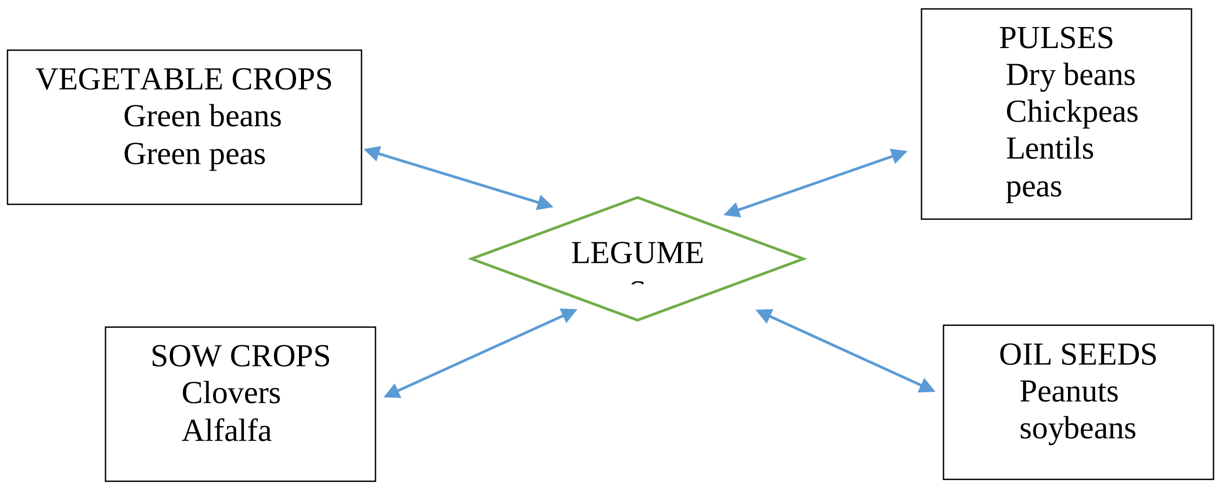

Figure 1a. Legume classification (McCrory et al. 2010).

Yogurt is a well sought after and popular snack because of its nutritional components, especially probiotics. Although it has been around for centuries, consumption has increased in recent times because of the awareness of its probiotic content, and the nutritional effect it has on gut microflora. Probiotics are organisms that feed on prebiotics, according to a research by Patil (2013), prebiotics travel through our digestive system untouched and are available for probiotic bacteria to use as a nutrient; bean has been seen to have an abundance of prebiotics. According to McCrory et al. (2010), Oligosaccharide found in pulse are considered prebiotics and are good for gut health; henceforth the prebiotics found in beans should be a good source of nutrients for probiotics in yogurt.

The objective of this study is to analyze the physio-chemical and textural effect of fortifying yogurt with navy bean flour extracts. Observations from this study can be used to develop a symbiotic drink that will be rich in both prebiotics and probiotics. Also, results from this study can be used as a reference in further research, the prebiotic content of the extract can be isolated and used to create other prebiotic rich products, and in general increase production and usage of pulses. Focus is given to navy beans because of its color and taste.

1.1 Research Question

Will there be any significant difference between navy bean extract fortified yogurt and plain yogurt?

1.2 Hypothesis

Navy bean extract will aid in increasing the overall quality of yogurt, support probiotic growth and improve the shelf life of yogurt.

1.3 Objectives of this research includes

1. Extract and quantify raffinose content in navy bean flour extract.
2. Investigate the optimum concentration of navy bean extract that can be added in yogurt.
3. Evaluate the effect of fortifying yogurt with navy bean flour extract.
4. Analyze the effect of navy bean flour extract on physico-chemical and microbial count and viscosity during a 21 day shelf life study.

Chapter Two: Literature Review

2.1 Pulses

Pulses are gaining popularity because of the health benefits research has found to be associated with them and it is eminent to know what they are. Pulses are dry leguminous plant seeds. They are very rich in nutrient composition, and a great source of protein. These nutritious plant seeds have been around for thousands of years and are cultivated in almost every part of the world, especially in countries and vegetarian societies that do not consume animal based protein, and as such, pulses make up an essential part of their daily diet (Thavarajah, 2012). Pulses comprise of beans, peas, lentils and chickpea that are rich in proteins, low molecular weight carbohydrates, essential amino acids, micronutrients and polyunsaturated fatty acids. Several studies have shown that the components of these pulses are essential for good health. Boye, Zare, and Pletch. (2010) indicate that the consumption of pulses can help in reducing the risk of cardiovascular disease, osteoporosis, diabetes, gastrointestinal disorder and a host of order diseases. Several other studies e.g. (McCrory et al. 2010; Ooi & Liong, 2010) also validate these claims. The positive effect of pulse consumption can be attributed to several properties of pulses which include:

1. The presence of resistant starch acts as fiber because it cannot be digested in the small intestine. It binds to fat cells in the large intestine, and helps with bowel movement, weight management, and cardiovascular disease.
2. Low glycemic index- The glycemic index is an index that can help in predicting foods impact on blood sugar levels. Pulses have a low glycemic index as such it does not cause immediate spikes in blood sugar levels (Leterme, 2002) when consumed, making them great for people living with diabetes. Low glycemic index foods like

beans can also help to curb sugar cravings, making you feel full longer because of the fiber content and can help lower the risk of heart diseases.

Despite the highly dense nutritional property of these pulses, they are not being consumed as much as they should. In the United States, pulse consumption has been below dietary recommendation. McCrory et al. (2010) state that the reasons behind the low consumption of this nutrient dense food may include food preference and taste, cook time of pulses, insufficient education about the nutritional benefits of these seeds, and most importantly the gastrointestinal disturbance that may result from high consumption of pulses. A study by Lucier, Lin, Allshouse and Kantor (2000) on the factors affecting dry bean consumption indicates that although dry beans consumption was lower after World War II, but a rise in consumption has been seen because of the increase in immigration of Hispanics whose staple food contains a good percentage of beans. Other factors affecting dry bean consumption can be linked to race, ethnicity, income level. Lucier et al. (2000) confirms that 27 percent of all cooked beans are consumed by people from low income families, families of Hispanic and Mexican decent consume more beans that American families. With all these factors affecting bean consumption, it is important to educate our communities on the importance of bean consumption and especially reduce the gap between the low-income and high-income families.

It is in light of this that the Food and Agricultural Organization (FAO) of United Nations declared 2016 the year of pulses. According to the information from the FAO website article, the declaration was to help increase the awareness of the nutritional benefits of these pulses and create ample opportunity for the utilization of pulse-based proteins, encourage global production of pulses and trade (Food and Agricultural Organization of the United Nations, n.d.).

2.2 Navy Beans Origin, Composition, and Properties

Several varieties of beans are available around the world. They differ in color, size, shape, taste and includes black beans, black eye peas, great northern, kidney beans, navy beans, pinto beans, lima beans, soybean etc.; all comprising of varying amount of nutrient and mineral concentrations. Navy bean is our primary focus for this study; the reasons for my choice of beans is that they are white in color and will be easy to incorporate into yogurt without changing the original color. Also, navy beans have a lite, palatable, and likeable flavor profile that is not likely to interfere with the original flavor of yogurt.

Navy beans are small white legume seeds that have an oval shape, and belong to a class of leguminous family called *Phaseolus Vulgaris* (Bobe et al., 2007). The name "Navy Bean" was given to this bean because they were used as a major part of the diet by the U.S Navy sometime in the 19^{th} century (Garden-Robinson & McNeal, 2013). Like all other bean varieties, navy beans are composed of protein, carbohydrates, sugars, soluble and insoluble fiber, saponins and phenols which act as antioxidant. Bobe et al. (2007) suggested that the presence of these components can have a cancer-preventive effect which could be exerted directly through chemo-protective functions or indirectly through microflora and pH changes. The insoluble and soluble fiber complex comprises polysaccharides, galacto-oligosaccharides and resistant starch (Brummer, Kaviani, and Tosh, 2014), which also have preventive effects against cancer and other diseases. Some of the component especially galacto-oligosaccharides have been termed undesirable in the past because of its ability to cause flatulence when consumed in excess however, research has shown that these fibers are good sources of prebiotics that are good for gut microflora (Brummer et al., 2014), thus can be used to improve overall health and wellness of an individual. This again, brings up the need to create awareness of the positive effect of beans, increase consumption, encourage production and utilization of dry pulses, and its extracts.

In consideration of the different forms and applications that can be derived from the use of beans, the potential for developing a bean based fermented product arises, also the option of producing extracts from beans that contain ample amount of prebiotics is developed and used to fortify probiotic drinks such as yogurt. The scope being that the functional prebiotics or oligosaccharides present in these extracts will improve probiotic growth and thus improve gut health and overall quality of the fermented drink.

2.3 Application of Navy Beans

Recent health trends have led to a lot of changes with respect to our diet and making healthier choices. This has come as a result of the high rate of obesity, food allergies and other life threatening diseases associated with the consumption of certain foods. The Navy bean is a pulse and has no know food allergies. It is gluten free and thus can be utilized by people who are intolerant to gluten; as earlier stated, navy beans are rich in nutrient and have a low glycemic index, also making them safe for people living with diabetes.

For centuries, navy beans have been a staple food in the United States. They have been used in the production of most of the baked beans product, which is a major ingredient for making chilies. Navy beans can be applied in a lot of other different ways to create healthier meal or snack options; although these options may have been thought of over time, no form of implementation had been made. Recent studies have shown different applications in which bean flour can be used and its effect on the quality of products such as tortillas and cookies. In a study by Anton, Fulcher and Arntfield (2008), the effect of adding bean flour to extruded snacks was analyzed. Though results showed that the addition of bean flour reduced expansion of extruded products, there was significant increase in the protein and fiber content with crude protein increasing with the increase in bean fortification. Nutritional effect of adding bean flour to wheat tortillas also showed an increase in the protein content and an enhanced amino acid profile, providing a significant difference between the control and bean fortified wheat tortillas (Anton, Ross, Lukow, Fulcher & Arntfield ,2007).

Positive results derived from the use of navy bean flour in these studies means that there are unlimited possibilities to how pulse flour and its derivatives can be utilized. Pulse flour can be used to substitute regular all-purpose flour in making of cakes and pastries. They can also be used in gravies, and of course used in fortifying probiotic drink or creating a fermented symbiotic drink.

2.4 Prebiotic Content of Navy Bean and Application

Prebiotics are “selectively fermented ingredients that allows specific changes in the composition / activity in the gastrointestinal microbiota” (Charalampopoulos & Rastall, 2012, p. 187). The changes exerted on the microbiota confers positive health benefits to the host cells. Prebiotics are considered functional foods because they have the ability to provide health benefits that supports good health. Health benefits derived from prebiotics are enormous, they include increase in the availability of minerals, preventing and improving infections in the gastrointestinal tract and aiding immune response (Charalampopoulos & Rastall, 2012). For a food substance to be considered a prebiotic, it has to be able to withstand gastric acid; this means that it should not be digestible or absorbed in the gastrointestinal tract. It also must be able to change and make the composition of the gut microflora healthier. (Kolida, Tuohy & Gibson, 2002).

There are different types of prebiotics that are available to us, each coming from a variety of sources. Table 2.4.1 shows a list of prebiotics and their sources. These prebiotics are able to stimulate the activity and growth of good bacteria in our gut.

Table 2.4.1 Types and sources of prebiotics

TYPES OF PREBIOTICS	SOURCES OF PREBIOTICS
Arabinoxylooligosaccharides	Wheat bran
Cyclodextrins	Water-soluble glucans
Enzyme-resistant dextrin	Potato starch
Fructooligosaccharides	Asparagus, sugar beet, garlic, chicory, onion, artichoke, wheat, honey, banana etc.
Galactooligosaccharides	Human milk and cow milk
Isomaltooligosaccharides	Starch
Isomaltulose	Honey, sugarcane juice, sucrose
Lactulose, lactosucrose	Lactose
Maltooligosaccharides	Starch
Palatinose	Sucrose
Raffinose oligosaccharide	Seeds of legumes (pulses e.g. Lentils, beans, peas), mustard, mallow composite
Soybean oligosaccharides	soybean
Xylooligosaccharides	Bamboo shoots, fruits, vegetables, milk

Source: Al-Sheraji et al., 2013.

Pulses have been found to be rich in prebiotics. These prebiotics are available as Raffinose oligosaccharides (RFOs). Raffinose according to Svejstil, Musilova and Rada (2015) is a trisaccharide that contains α-(1-6) galactosidic bond that is linked to glucose unit of sucrose. In the past, this form of oligosaccharides has been considered to be negative as they are the major cause flatulence in humans. Flatulence occurs because the α-galactosidase bond cannot be digested in the intestine and so undergoes fermentation (Tajoddin, Manohar & Lalitha 2012). As earlier stated, a substance must be able to resist gastric acid and be selectively fermented by colon microbiota. For this reason, raffinose oligosaccharide found in pulses including navy beans is considered prebiotics, and is according to Wang et al. (2013), one of the oligosaccharides approved by the U.S Food and Drug Administration to be Generally Recognized as Safe (GRAS).

The negative biological effect associated with consumption of pulses in humans has made it difficult for us to consider different ways of utilizing it, this singular effect may be the reason why prebiotics from pulses have not been used in fortifying fermented probiotic drinks / products. However, this research is aimed at using extracts derived from navy beans to fortify yogurt, fermented milk product rich in probiotics.

2.5 Extraction methods used for Raffinose Oligosaccharides

There are different extraction methods used for the extraction of raffinose and other sugars from pulses**.** Over time, extraction carried out on pulses especially beans have been to remove its “anti-nutritive” component. A simple method used in almost every home that can be referred to is the soaking of beans before cooking; water used for soaking is usually discarded because it is said to reduce flatulence when the bean is finally cooked and consumed. It has been established that this “anti-nutritive” component of pulses is its prebiotic content and is of great importance to gut health. In this study, extracts derived from navy bean flour will be use to fortify yogurt, a probiotic rich product made from milk.

Several methods have been described by different research. Brain (2010) in his research extracted raffinose from navy bean using the simple method of soaking and boiling. He found that soaking and boiling beans at high temperatures removed about 96% of raffinose from beans. Brain (2013) also extracted raffinose by hydrolysis using α-galactosidase. This method gave a 40 – 100% reduction of raffinose content of beans.

Extractions using solvent have been used in different studies including (Ekvall, Stegmark & Nyman, 2007; Johansen, Glitso & Knudsen, 1996; Wang et al, 2012). In each research, different concentration of ethanol was used to extract raffinose. Ekvall et al. (2007), used 50% and 80% v/v of ethanol to extract raffinose from vine peas. In this research, it was observed that using a lower concentration of ethanol (50%) was more effective than using 80% ethanol. Concentration of extracts derived from solvents like ethanol and methanol are as effective as extracts derived from water and there is no significant difference between the two methods (Johansen et al, 1996). This means that using water for extraction of raffinose as seen in different studies and domestically is as good as extraction methods that use solvents.

For the purpose of this research, water will be used to extract raffinose from navy bean flour sample. The extraction method as described by Bakr (2013) will be adopted and optimized for this study and extracts derived will be analyzed to ensure that raffinose is present. The presence of raffinose will mean creating a prebiotic rich extract that will be used to fortify yogurt and will lead to the creation of a more health drink packed with prebiotics and probiotic.

2.6 Yogurt and Probiotics

Fermented foods such as yogurt have been around for centuries. This concept originated in the middle east and has become a worldwide staple consumed by both young and old. Yogurt is a nutrient-dense food, rich in minerals, vitamins, proteins and probiotics; produced by fermenting milk at a regulated temperature with the addition of live bacteria cultures such as *Lactobacillus bulgaris* and *Streptococcus thermophiles* (Gahruie, Eskandar, Mesbahi, & Hanifpour, 2015). According to El-Abbadi, Dao, and Meydani (2014), yogurt is considered a probiotic drink because of the presence of live microorganisms in the culture, which can help improve gut functions and overall health.

To understand what probiotic are, we will look at several definitions from different studies. Schrezenmeir and De Vrese (2001) defined probiotics as “A preparation of, or a product containing viable, defined microorganisms in sufficient number, which alter the microflora in a compartment of the host and by that exert beneficial health effect in the host” (p.362S). Patil (2013) has an easier definition of this term; in his paper, he defines probiotics as “a viable microbial food supplement which beneficially influence the health of the host” (p.321). In other words, probiotics are good bacteria that are beneficial to our health especially the gut. Probiotics have been shown to ease constipation and allergic symptoms, aid in preventing cancer, aid in reducing cholesterol and concentration of triglycerides in our plasma (Schrezenmeir & De Vrese 2001), and aid in overall gut health all of which are beneficial for good health.

Nutrient intake or consumption is a general requirement for survival for any living creature; cells die off or reduce its functions when there is insufficient nutrient and the same holds true for microorganisms. Prebiotics as we have learned are substrates that microorganisms (probiotics) feed on. This means that with ample amount of prebiotics for probiotics to feed on, probiotics can survive better and therefore have improved functions in our gut. Navy bean, is a great source of prebiotics and has the potential of improving probiotic content in yogurt or any probiotic drink. Navy beans are cultivated worldwide and in several states in America; they are readily available for us and are cost efficient.

Pulse can be applied in so many different ways; as flour substitute in making pastries, bean extract for use in the production of symbiotic products and possible fermented drink. By incorporating more pulses into our diet, health and wellness will be greatly improved over time. With so much possibilities associated with pulses, this research looks at ways of utilizing the prebiotic content of navy beans extract to enhance yogurt quality. This will not only create a healthier symbiotic product that can be consumed by all age groups and families from all walks of life but, will indirectly increase bean production, utilization and consumption, therefore helping the FAO and UN to achieve part of its goal for the international year of pulses.

Chapter Three: Materials and Methods

3.0 Navy Bean and Extract Preparation

Navy beans used for this research was purchased on amazon from Mrs. Glee's gluten free foods (produced in Michigan). Navy bean extracts were prepared according to the method described by Bakr (2013). Briefly, 10g of bean flour was measured using an analytical weighing balance and dissolved in 100ml of distilled water. Mixture was homogenized using a magnetic mixer and incubated overnight (12 hours) in a water bath at 70^{o}C. After 12 hours, the mixture was spun in a centrifuge at 1500rpm for 15mins. Supernatant was drained out and used to fortify yogurt.

3.1 Quantification of Raffinose Oligosaccharides in Bean Extract

Commercially available Raffinose assay kit was ordered from Megazyme and used to determine the concentration in the extract. Content of the kit included a buffer solution containing sodium azide (0.02%w/v), NAD^{+}, D-Galactose dehydrogenase plus galactose mutarotase suspension, α-Galactosidase (pH 4.6) lyophilized powder, Galactose standard solution and raffinose control powder.

The reagents were prepared and used according to procedure provided in the manual. Using properly labeled test tubes, samples and reagents were pipetted according to the table below. Absorbance was read at 340nm using a spectrophotometer. Values for A_1 and A_2 were printed out and used for calculating the concentration of raffinose in the bean extract.

Calculations:

Absorbance $\Delta A = (A_2 - A_1)_{sample} - (A_2 - A_1)_{blank}$.

Determination of raffinose from sample:

$\Delta A_{raffinose} = \Delta A_{raffinose + free\ D\text{-}galactose} - \Delta A_{free\ D\text{-}gal}$.

Concentration of Raffinose and D-galactose was determined as follows.

$$\text{Concentration } C = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A$$

where:
V = final volume(mL)
MW = Molecular weight of the substance assayed (g/mol)
ε = extinction coefficient of NADH at 340nm
d = light path (cm)
v = sample volume(mL)

Table 3.1a. Raffinose assay procedure

Pipette into cuvette	Blank raffinose + free D-Gal	Raffinose + free D-Gal sample	Blank free D-Gal	Free D-Gal sample.
Sample solution	-	200µl	-	200µl
distilled water	200µl	-	300µl	100µl
Solution 4 (α-Gal)	100µl	100µl	-	-
Mix and incubate for 20mins.				
Solution 1 (buffer)	200µl	200µl	200µl	200µl
Distilled water	2000µl	2000µl	2000µl	2000µl
Solution 2 (NAD+)	100µl	100µl	100µl	100µl
Mix, read absorbance of solution (A_1) after 3 mins and start reaction by adding:				
Suspen. 3 (β-GalDH/GalM)	20µl	20µl	20µl	20µl
Mix, incubate at 40°C for 20mins. Read absorbance of the solution (A_2) at the end of reaction.				

Source: (Megazyme Raffinose/D-Galactose assay procedure p.5)

3.2 Yogurt Preparation and Fortification

Stater Culture Preparation

2% Great Value milk was purchased from Walmart and used for the preparation of culture and sample. Milk was pasteurized at 90^{o}C for 10mins. Freeze dried *Lactobacillus bulgaricus* and *Streptococcus thermophilius* culture mix purchased from Danisco (YO-MIX 883 LYO 500 DCU) was added to pasteurized milk and incubated for 8 hours. Prepared culture was used within three days of preparation.

Sample Preparation

2.5%. 5%, 7.5% and 10% of bean flour extracts were respectively used to fortify 2% milk; fortified milk was pasteurized. After pasteurization, milk was allowed to cool to less than 42^{o}C, cultured using starter culture and incubated for 8 hours. Physicochemical results of yogurt samples were observed and recorded at different time intervals.

3.3 Physicochemical Analysis

3.3.1 Percentage acidity.

Titratable acidity of the yogurt sample was measured for control and experimental samples. Measurement was determined according to standard AOAC procedure. The titratable acidity of a mixture of yogurt and distilled water (1:9 v/v) was titrated with 0.1N NaoH and 0.1% phenolphthalein indicator. When the value was reached, the volume of NaoH was recorded. Titration was repeated 3 times to achieve statistical average, acid percentage was calculated using the formula below:

Titratable acidity (TTA) = $\frac{\text{Titre value} \times M \times 90 \times 100}{\text{Volume of sample} \times 1000}$ (M = Molar concentration of NAOH)

3.3.2 Total soluble solids (TSS).

The total soluble solids of yogurt samples were analyzed using a portable Hanna Instrument refractometer expressed in degree brix. A portion of the sample was placed on the refractometer and readings were taken in triplicate. Average reading was calculated and recorded.

3.3.3 pH.

The pH of samples was measured at room temperature (27^{0}C) with a pH/ORP Meter (HI 9125, HANNA instruments Co., Ltd). The pH meter was calibrated using buffer standards of pH 4 and pH 7 prior to use. A portion of the sample was put in a beaker, pH probes were inserted into the sample and reading was taken when pH was steady. Triplicate readings were recorded and average taken to achieve statistical significance.

3.3.4 Viscosity

Viscosity measurement measured using a NDJ-9S Digital rotary viscometer. Viscosity was measured on the first day of sample preparation to ensure that no form of agitation had occurred. A no. 3 rotator was inserted into the sample and spun for 40 seconds at 30 RPM. Samples were controlled for temperature and triplicate readings were recorded.

3.4 Microbiological Tests.

Microbiological analysis was conducted to estimate the probiotic content (Lactic acid bacteria) in both control and bean fortified yogurt sample. Samples were enumerated for the concentration of lactic acid bacteria utilizing MRS agar and *Streptococcus Thermophilus* using M17 agar. Different dilutions (10^4 -10^7) of the extracts were prepared and spread on culture plates. MRS samples were incubated at 37oC using a vacuum sealed anaerobic chamber. Plates were read after 72 hours and value recorded. M17 samples where incubated for 72 hours at 42oC in an aerobic chamber. Results were recorded in triplicates.

3.5 21 Day Shelf-Life study

A 21-day shelf life study was conducted during the course of this research. All parameters mentioned above (pH, Viscosity, percentage acidity, microbiology) were repeated over the cause of 21 days, results were measured and reported. The results from the 21-day shelf life study was used to evaluate the quality of yogurt over time.

3.6 Statistical Analysis

All experiments were carried out in triplicate (N=3). Data is expressed as Mean±SD. Statistical analysis was conducted using repeated measures ANOVA on SPSS (IBM SPSS Statistics 21). Measure of significance using a P value of 0.05 and was derived using Tukey test.

Table 3.1b shows a flow chat of method used in this experiment.

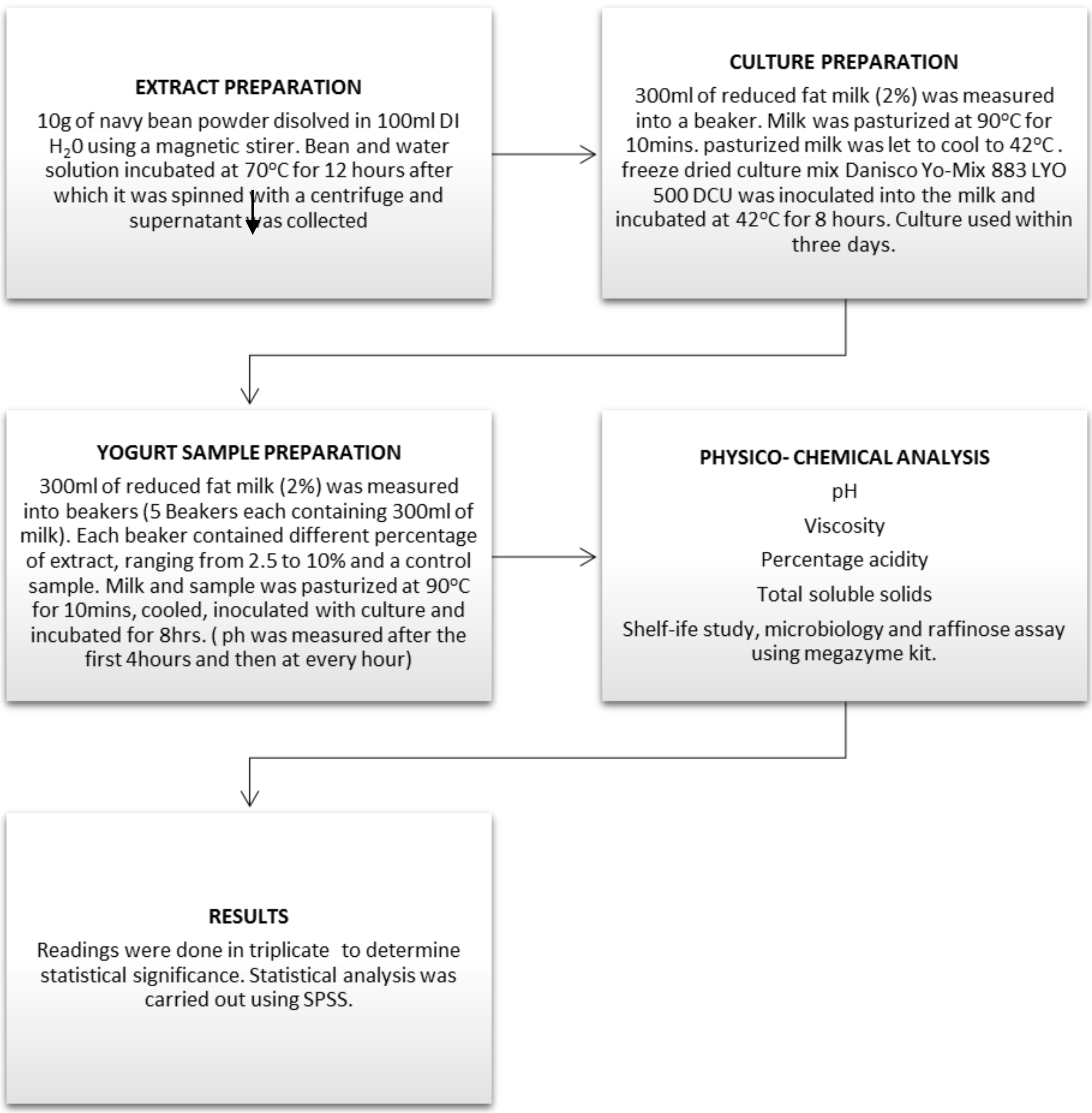

Fig.2a. Flow chart for yogurt preparation.

Chapter Four: Results and Discussion

4.1 Effect of extract on yogurt preparation.

The use of navy bean extract was chosen after attempts to use navy bean flour for fortification did not yield suitable results. Yogurt prepared using navy bean flour resulted in yogurt that had a distinct separation of bean and milk solids after homogenizing with an electronic mixer. Yogurt samples prepared with 2.5%- 10% bean extract showed good results as pH values of less than 4.0 in 8 hours compared to control sample. When controlled for time, samples with extracts reach the desired pH of 4.6 in four (4) hours compared to control (8 hours). Results obtained were similar to research by Zare, Champange, Simpson, Orsat and Boye (2011) where milk supplemented with pea protein, soy protein and soy flour reach a significant low pH after 4 hours of fermentation. Reduction in fermentation time is significant and important because it can save production time which is essential for economic purposes.

4.2 Determination of Raffinose in Navy Bean Extract

Results obtained from this study shows positive outcomes and possibilities that can be achieved with the utilization of navy bean extract in yogurt preparation and other food products. Raffinose assay was aimed at determining the concentration of raffinose in navy bean extract. Concentration was derived using the megazyme assay kit and values shown below were obtained after three repetitions of procedure.

Table 4.1. Concentrations of raffinose from navy bean extract

Control 1	Blank raffinose + free D-Gal	Raffinose + free D-gal +sample	Blank free D-Gal	Free D-Gal + sample
A1	0.0328	0.0402	0.0305	0.029
A2	0.028	0.7982	0.0217	0.0248
A2-A1	-0.0048	0.758	-0.0088	-0.0042

ΔA raffinose = Raffinose + Free D-Gal +sample -Free D-Gal + sample = 0.758- (-0.0042) = 0.7622
Concentration of raffinose in control = 0.7622 x 1.049 = 0.7995g/l

Sample 1	Blank raffinose + free D-gal	Raffinose + free D-gal + sample	Blank free D-gal	Free D-gal + sample
A1	0.0359	1.4501	0.0265	1.4902
A2	0.0251	3.9493	0.0269	1.5392
A2-A1	-0.0108	2.4992	0.0004	0.049

ΔA raffinose = Raffinose + Free D-Gal +sample -Free D-Gal + sample = 2.4992-0.0490 = 2.4502
Concentration of raffinose in the sample = 2.4502 x 1.049 = 2.5703g/l

Sample 2	Blank raffinose + free D-gal	Raffinose + free D-gal + sample	Blank free D-gal	Free D-gal + sample
A1	0.0279	1.2677	0.0283	0.9968
A2	0.0268	3.6061	0.0406	1.0074
A2-A1	-0.0011	2.3384	0.0123	0.0106

ΔA raffinose = Raffinose + Free D-Gal +sample -Free D-Gal + sample = 2.3384-0.0106 = 2.3278
Concentration of raffinose in sample = 2.3278 x 1.049 = 2.4419g/l

Control 3	Blank raffinose + free D-gal	Raffinose + free D-gal + sample	Blank free D-gal	Free D-gal + sample
A1	0.0285	0.0276	0.0264	0.0242
A2	0.0591	0.8056	0.0306	0.0384
A2-A1	0.0306	0.778	0.0042	0.0142

ΔA raffinose control = Raffinose + Free D-Gal +sample -Free D-Gal + sample = 0.7780 - 0.0142 = 0.7638
Concentration of raffinose in control = 0.7638 x 1.049 = 0.8012

Sample 3	Blank raffinose + free D-gal	Raffinose + free D-gal + sample	Blank free D-gal	Free D-gal + sample
A1	0.0241	0.9853	0.026	0.9222
A2	0.0364	3.8734	0.0296	0.9832
A2-A1	0.0123	2.8881	0.0036	0.061

ΔA raffinose = Raffinose + Free D-Gal +sample -Free D-Gal + sample = 28881- 0.0610 = 2.8271
Concentration of raffinose in sample = 2.8271 x 1.049 = 2.9656

Average concentration of raffinose in navy bean extract

	concentration of raffinose in g/l				
Control	C1	C2	C3	Average	SDev
	0.7995	0.7788	0.8012	0.7931	0.0124
Samples	S1	S2	S3		
	2.5703	2.4419	2.9656	2.6592	0.2729

As seen from the tables above, extracts made contained ample concentration of raffinose, suggesting that there is a high amount of prebiotics in the extract. From the average concentration obtained, amount of raffinose used for fortification was 13.29mg, 26.59mg, 39.88mg and 53.18mg (2.5%, 5%. 7.5% and 10%) respectively.

In the recent past, people who consume beans would soak and drain the bean water to reduce flatulence and other discomfort caused by bean consumption. The process of extraction used in this study is somewhat similar to the process used in most homes and shows that a good amount of raffinose is lost in the process of draining water from soaked bean. Although reasons behind this waste has been “for the good of consumers”, research has shown that raffinose oligosaccharide is a good component of beans as it acts as a prebiotic and thus great for gut health. This means that the discomfort experienced when bean in consumed is not really a bad thing. According to an article from the nutrition fact website by Greger (2011), our body is able to adapt to the discomfort caused by beans with time; He also noted in his article the health-promoting effects of bean consumption (prebiotic and colon health) and encourages consumption.

Knowing that it is very challenging to persuade consumers into embracing all the discomfort from whole bean consumption, this research was aimed at utilizing bean extract rich in raffinose to fortify yogurt. The concentrations used for samples preparation contained about

13.29mg – 53.18mg (2.5% - 10% extract) which should be enough to encourage probiotic growth and conferring its positive effect without causing excess flatulence or discomfort.

4.3 Effect of Extract on Fermentation time and pH.

Results obtained after fermentation shows that pH of yogurt fortified with navy bean extract varies with different concentrations. Samples which contained extracts reached a pH of 4.6 in four (4) hours compared to the control and pH values were significantly different across experimental groups P = 0.05.

After 21 days of shelf life study, mean values for samples were statistically different across the different days except for the days 14 and 21 where no significant difference was observed. Results shown are somewhat similar to results obtained by Zare et al (2011) who found the pH of yogurt to reduce with the addition of pulse and soy; also in this study, pH of 4-4.5 was reached in four (4) hours of incubation compared to control.

Table 4.2. Effect of navy bean extract on pH of yogurt after 21days shelf life study (mean values and ± standard deviation

Treatment	Day 0	Day 7	Day 14	Day 21
1	4.63±0.03Aa	4.58±0.02Aab	4.52±0.02Ac	4.55±0.01Acb
2	4.55±0.02Ba	4.37±0.00Bb	4.26±0.01Bc	4.32±0.01Bd
3	4.44±0.00CDa	4.29±0.00Cb	4.24±0.01Bc	4.25±0.00Cc
4	4.48±0.01Ca	4.36±0.00Bb	4.26±0.01Bc	4.23±0.01CDc
5	4.39±0.01Da	4.24±0.00Db	4.18±0.01Cc	4.20±0.01Dc

Mean Values followed by the same letter (ABC) in a column are not statistically significant.
Mean Values followed by the same letter (abc) in a row are not statistically significant.
P = 0.05.
Treatments 1,2,3,4,5 represents Control, 2.5%, 5%, 7.5% and 10% respectively.

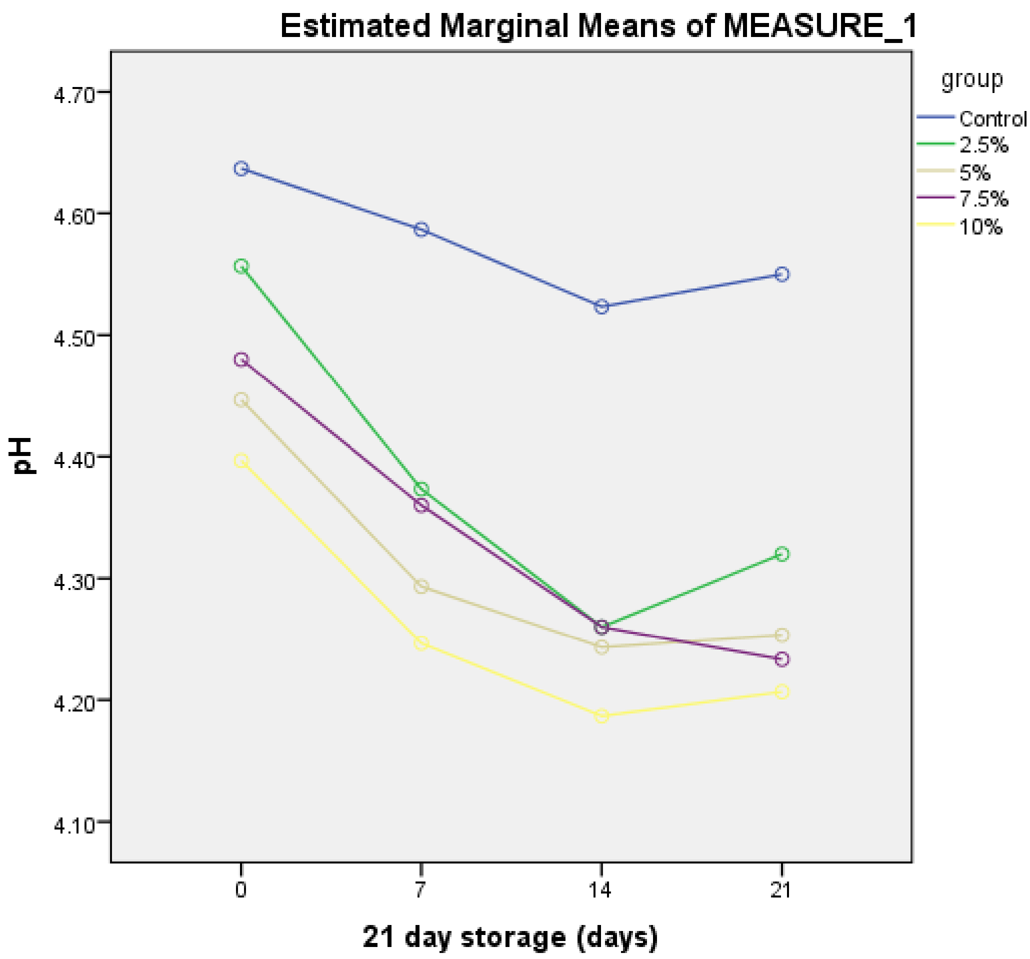

Fig. 3a Change in mean values of pH with respect to time (days)

Table 4.1 and chart of estimated marginal means above shows mean values for pH with respect to time. Results are similar to Aryana and McGrew (2007) who observed a decline in pH of yogurt sample with the addition inulin and oligofructose (prebiotics), indicating that low pH could be as a result of probiotics consumption of short chain prebiotics which results in the production of lactate, therefore showing that addition of prebiotics such as inulin and in this case raffinose has a positive effect on probiotic microorganism growth.

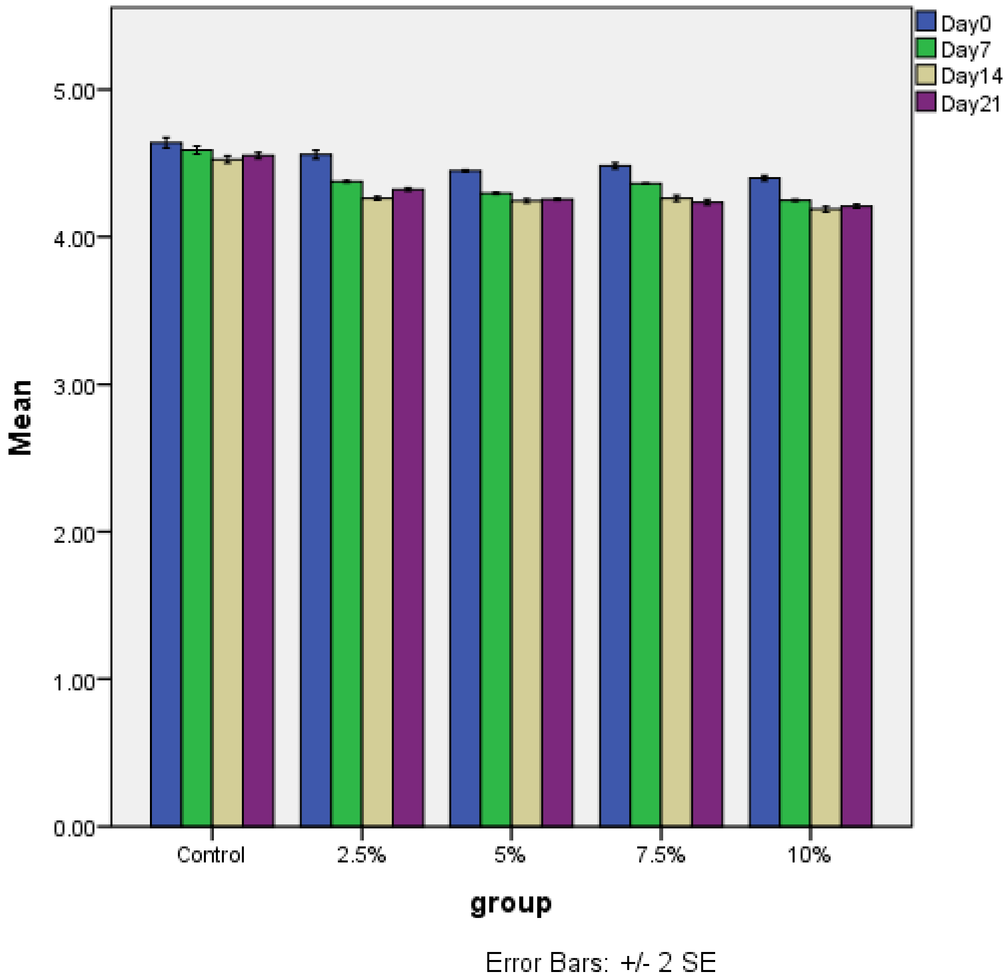

Fig. 3b Histogram of Mean pH values of samples over a 21 days storage period.

4.4 Effect of Fortification on Titrable acidity (%)

An increase in titrable acidity was observed between groups and time with the addition of navy bean extract. Results in table 4.2 shows titratable acidity increased with increase in storage time. Increase in acidity was significant between days 0, 7 and 14 but days 14 and 21 had no significant increase ($P \geq 0.05$), however there was slight increase in acidity on those days.

Table 4.3 Effect of navy bean extract on titrable acidity of yogurt after 21days shelf life study (mean values and ± standard deviation)

Treatments	Day 0	Day 7	Day 14	Day 21
1	0.462±0.03Aa	0.831±0.04Ab	0.567±0.01Ac	0.630±0.00Ad
2	0.543±0.027Ba	0.888±0.01Ab	0.702±0.00Bc	0.759±0.02Bc
3	0.570±0.10Ba	0.906±0.27Ab	0.0711±0.02Bc	0.711±0.00BCc
4	0.501±0.03ABa	0.615±0.05Bb	0.069±0.01Bc	0.747±0.01Bc
5	0.564±0.03Ba	0.0600±0.00Ba	0.741±0.02Bb	0.675±0.03ACc

Mean Values followed by the same letter (ABC) in a column are not statistically significant.
Mean Values followed by the same letter (abc) in a row are not statistically significant.
P = 0.05.
Treatments 1,2,3,4,5 represents Control, 2.5%, 5%, 7.5% and 10% respectively.

As pH decreased, acidity increased over the same period of time with respect to the experimental groups. Samples that were fortified with navy bean extracts showed greater decrease than the control. According to Agil et al (2013), lactic acid is produced when there are viable probiotics bacteria in a sample therefore, increase in titrable acidity among groups can be said to be influenced by the prebiotic content of bean extract. Fig.2.1 shows a long spike in acidity for Control. 2.5% and 5% on day 7, this high increase was probably due to experimental errors however, increase in acidity was observed across the experimental groups.

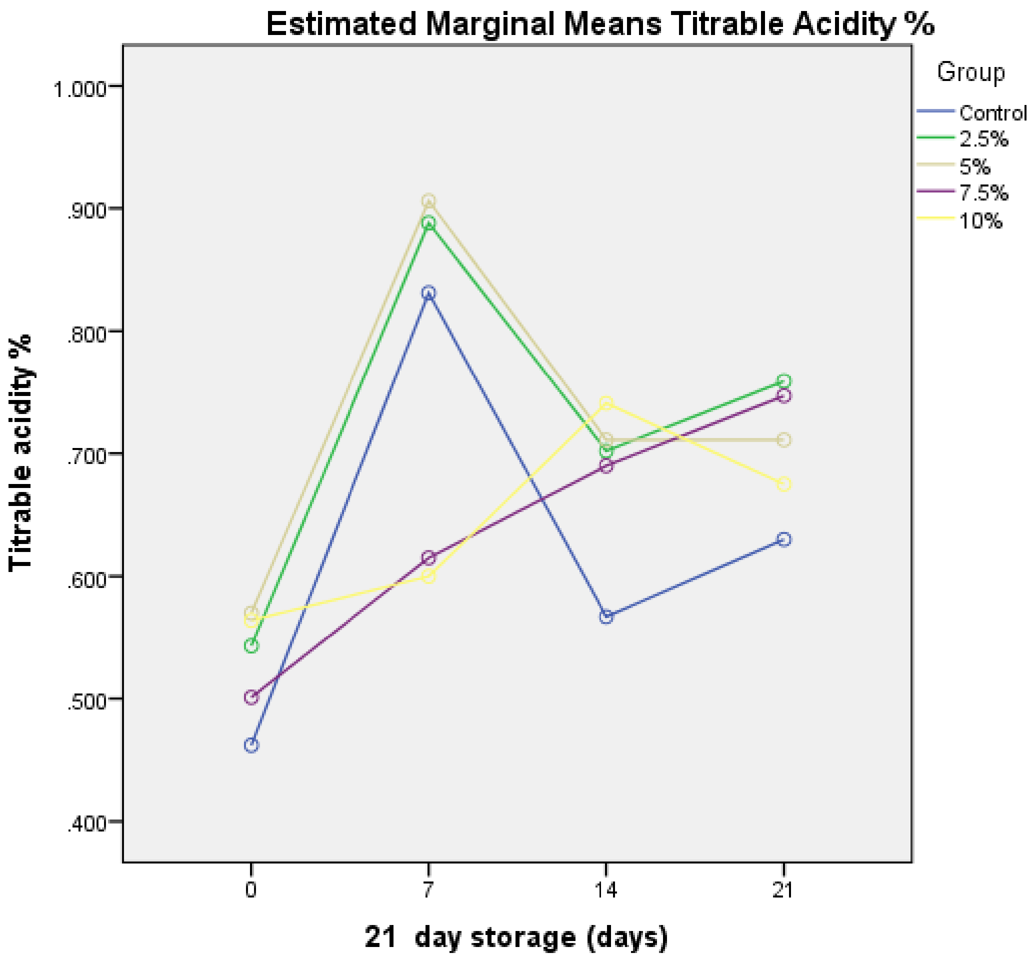

fig 4a Change in mean values of titrable acidity with respect to time (days)

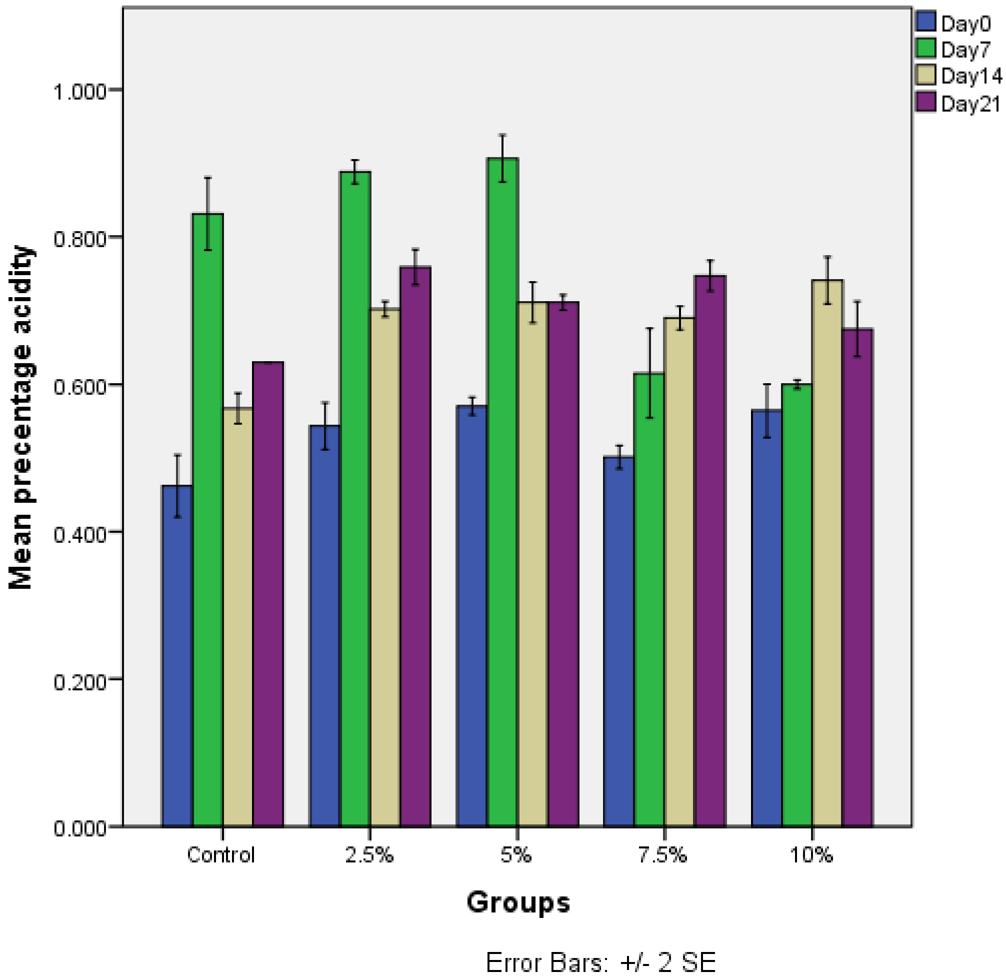

fig 4b Histogram of mean values of titrable acidity over 21day storage period.

4.5. Effect of Fortification on Viscosity.

Viscosity is the measure of thickness of a substance. Results from this study showed viscosity to increase with the addition of navy bean extract although there was no statistical difference across groups ($P > 0.5$). Lack of statistical significance might be as a result of liquid extracts being used for fortification. Research from Aryana and Mcgrew (2007) had similar results in their study. They found that the addition of inulin and oligofructose to yogurt did not have any effect on viscosity despite increasing the fiber content of the yogurt.

Table 4.4 Effect of navy bean extract on Viscosity (pa) of yogurt after 21days shelf life study (mean values and ± standard deviation)

Treatments	Day 0	Day 7	Day 14	Day 21
1	2.541±0.88Aa	3.900±0.00Ab	3.904±0.00Ab	3.708±0.20Ab
2	3.616±0.45Aa	3.906±0.00Aa	3.904±0.00Aa	3.8693±0.06Aa
3	2.725±0.55Aa	3.872±0.05Aab	3.905±0.00Aab	3.901±0.00Ab
4	3.426±0.44Aa	3.904±0.00Aa	3.906±0.00Aa	3.902±0.00Aa
5	2.709±0.78Aa	3.905±0.00Aa	3.906±0.00Aa	3.652±0.43Aa

Mean Values followed by the same letter (ABC) in a column are not statistically significant.
Mean Values followed by the same letter (abc) in a row are not statistically significant.
P = 0.05.
Treatments 1,2,3,4,5 represents Control, 2.5%, 5%, 7.5% and 10% respectively.

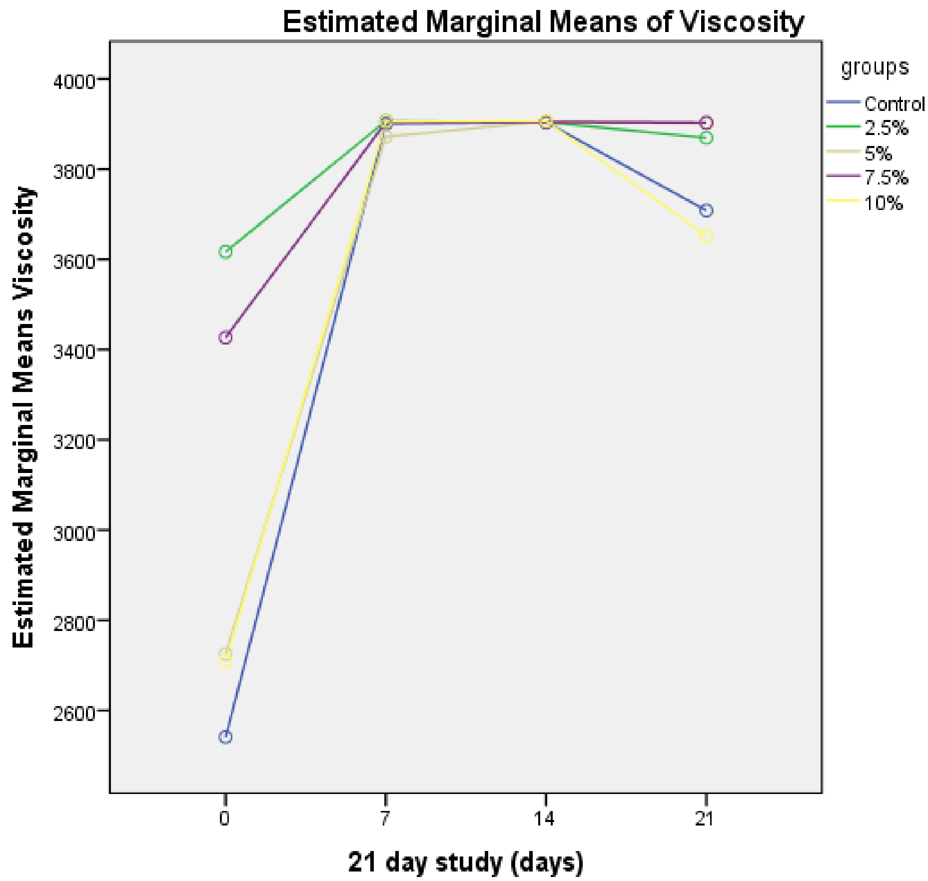

Fig. 5a Change in mean values of viscosity with respect to time.

Fig. 5a about shows the difference in viscosity over a 21day storage period. A highly significant increase was observed between day 0 and day 7 of storage. This increase stayed constant over day 14 and 21 except for the control and 10% samples which decreased on day 21, although reasons for the decrease is not known. Overall, there was no significant difference between control and samples that were fortified with navy bean extract across the 21day storage period.

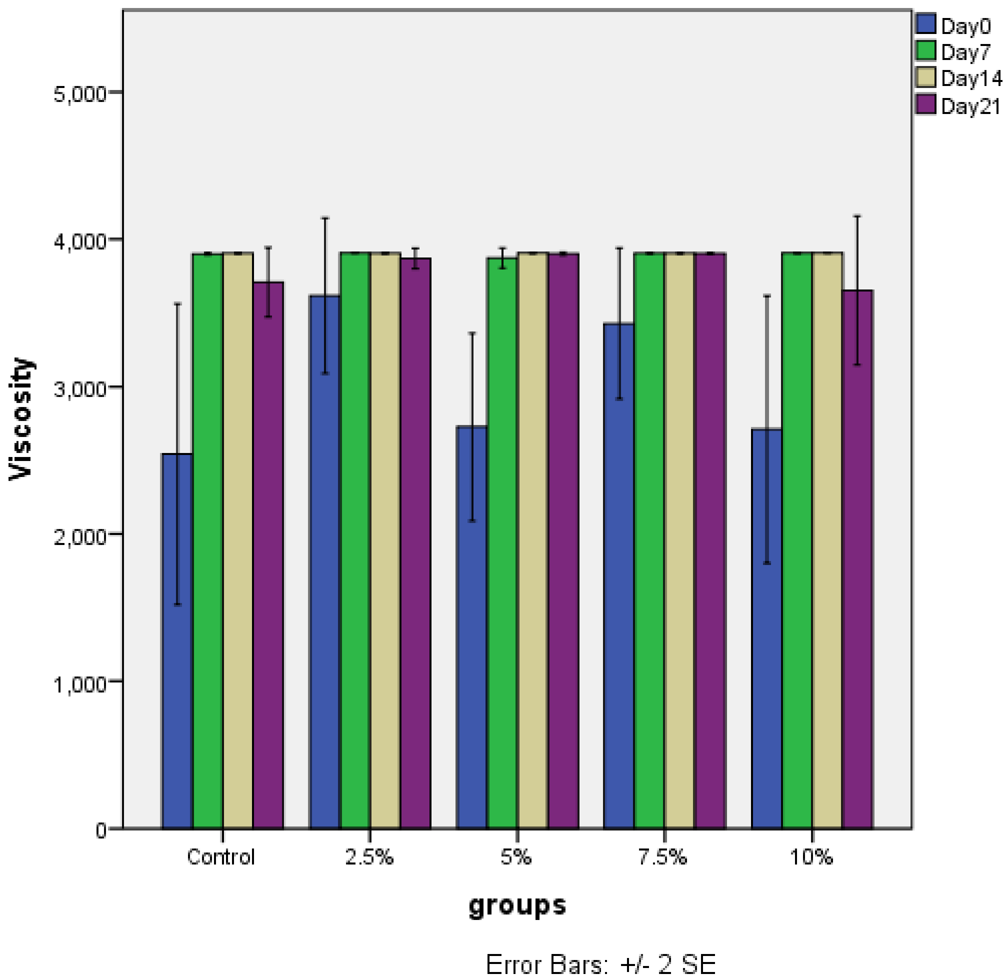

Fig.5b Histogram of mean values of viscosity over 21days storage period.

4.6 Effect of Extract on Total Soluble Solids (TSS)

Total soluble solids (TSS) is the amount of solids present in a liquid sample and may include some amount of acid, salt and sugars, but mostly sugars. TSS is measured using a refractometer and expressed in degree brix. Results for TSS in this study shows no significant difference between days ($P>0.05$) but between groups, there was a decrease in the number of soluble solids across treatment groups. Although not significant, the decrease observed may have been as a result of adding liquid extract to the milk before incubation. Extract were made using water and so it inferred that the water may have reduced the amount of solids present in the yogurt.

Table 4.5 Effect of navy bean extract on Total Soluble Solids of yogurt after 21 days shelf life study (mean values and ± standard deviation).

Treatments	Day 0	Day 7	Day14	Day21
1	6.13±0.11Aa	6.60±0.00Ab	6.83±0.58Ab	6.30±0.10Aab
2	6.33±0.15Aa	6.36±0.05ABa	6.43±0.05Aa	6.43±0.10Aa
3	6.20±0.10Aa	6.43±0.05ABa	6.20±0.20Aa	6.33±0.15Aa
4	6.03±0.11Aa	6.36±0.28Aba	6.13±0.30Aa	6.26±0.23Aa
5	6.13±0.15Aa	6.10±0.17Ba	5.93±0.11Aa	6.30±0.17Aa

Mean Values followed by the same letter (ABC) in a column are not statistically significant.
Mean Values followed by the same letter (abc) in a row are not statistically significant.
P = 0.05.
Treatments 1,2,3,4,5 represents Control, 2.5%, 5%, 7.5% and 10% respectively.

Results for Total soluble solids according to fig 6a. shows a fluctuation in the amount of solids in the samples. As observed, the control sample has the highest increase in soluble solids compared to the other fortified samples. Although not significant, the increase in control and decrease on experimental group shows that the addition of navy bean extract had no effect on the total soluble solids of yogurt. As a result of the decrease in total soluble solids, further study should aim at evaporating water and using powder derived from this process to fortify yogurt.

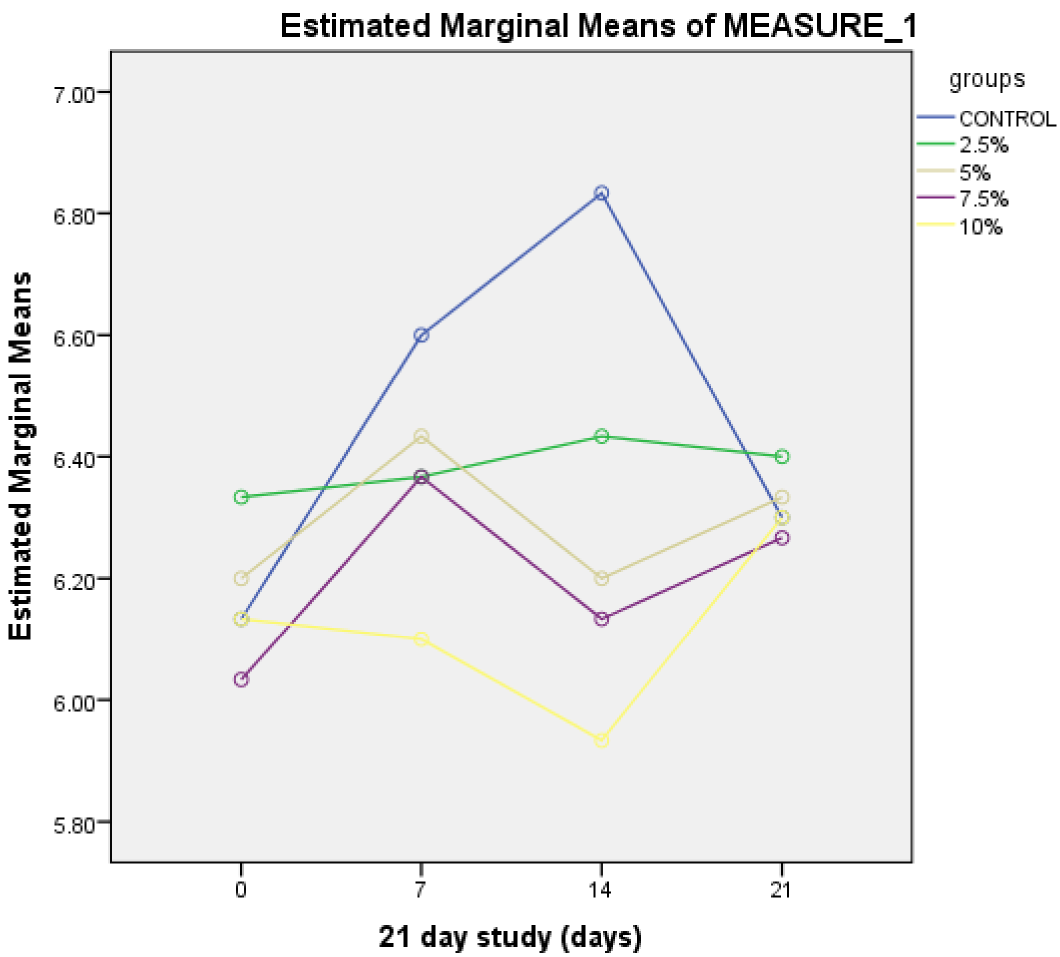

Fig.6a Mean values of total soluble solids across days

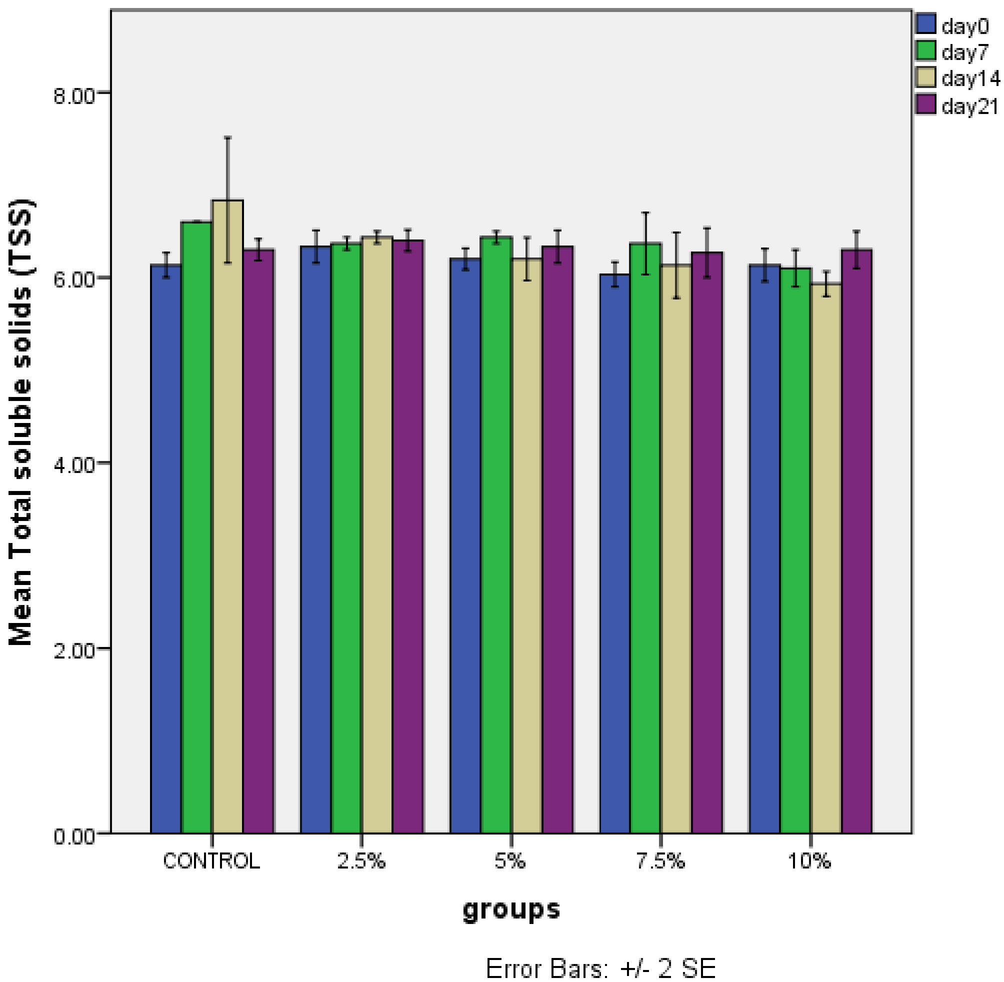

Fig. 6b Histogram showing mean difference in total soluble solids over 21day storage time.

4.7 Effect of navy bean extracts on *L. Bulgaricus* count using MRS.

Lactobacillus Bulgaricus along with Streptococcus thermophilus were the two main components of the starter culture used, and studies have shown that they encourage probiotic growth in yogurt. *L.Bulgaricus* grows in anaerobic conditions. Results from *L. Bulgaricus* count showed an increase in the amount of viable counts between control and fortified samples on day 0. An increase was also observed across other days except for day 14 where there was a drop in microbial count. Although these changes were not significant within treatment groups and across time (P>0.05), most of the samples had a CFU/ml of 10^8. According to Zare et al (2011), it is recommended that fermented milk products have at least 10^8 CFU/serving. Similar results for cell counts were also observed in this study, Zare et al (2011) also observed that the addition of lentil to yogurt increased acidification but did not significantly affect CFU counts and shows same for this study.

Table 4.6 Effect of navy bean extract on *L.Bulgaricus* Microbial count of yogurt after 21 days shelf life study (mean values and ± standard deviation)

Treatments	Day 0	Day 7	Day14	Day21
1	4.74±4.13Aa	5.12±4.43Aa	8.06±0.43Aa	8.54±0.20ABa
2	7.64±1.42Aa	4.93±4.31Aa	8.47±0.68Aa	8.26±0.08ACa
3	5.61±4.86Aa	8.14±0.72Aa	2.96±5.13Aa	8.73±0.10BDa
4	8.72±0.23Aa	7.84±0.15Aa	2.96±5.12Aa	8.04±0.20Ca
5	5.86±5.08Aa	7.93±0.38Aa	4.86±4.21Aa	9.14±0.96Da

Mean Values followed by the same letter (ABC) in a column are not statistically significant.
Mean Values followed by the same letter (abc) in a row are not statistically significant.
P = 0.05.
Treatments 1,2,3,4,5 represents Control, 2.5%, 5%, 7.5% and 10% respectively.

Table 4.5 and fig. 7a shows results for the mean values of log CFU/ml over 21-day storage period. Fluctuations in cell count might be as a result of pH and acidity levels of samples. This inference can be supported by Bakr (2013) who stated that composition of cell can be affected by the type of milk, and pH levels of yogurt. Although results are not statistically significant, an increase in viable cells was observed across group and time, suggesting that the addition of navy bean flour has the potential of increasing viable cell counts, and therefore improving the probiotic quality of yogurt.

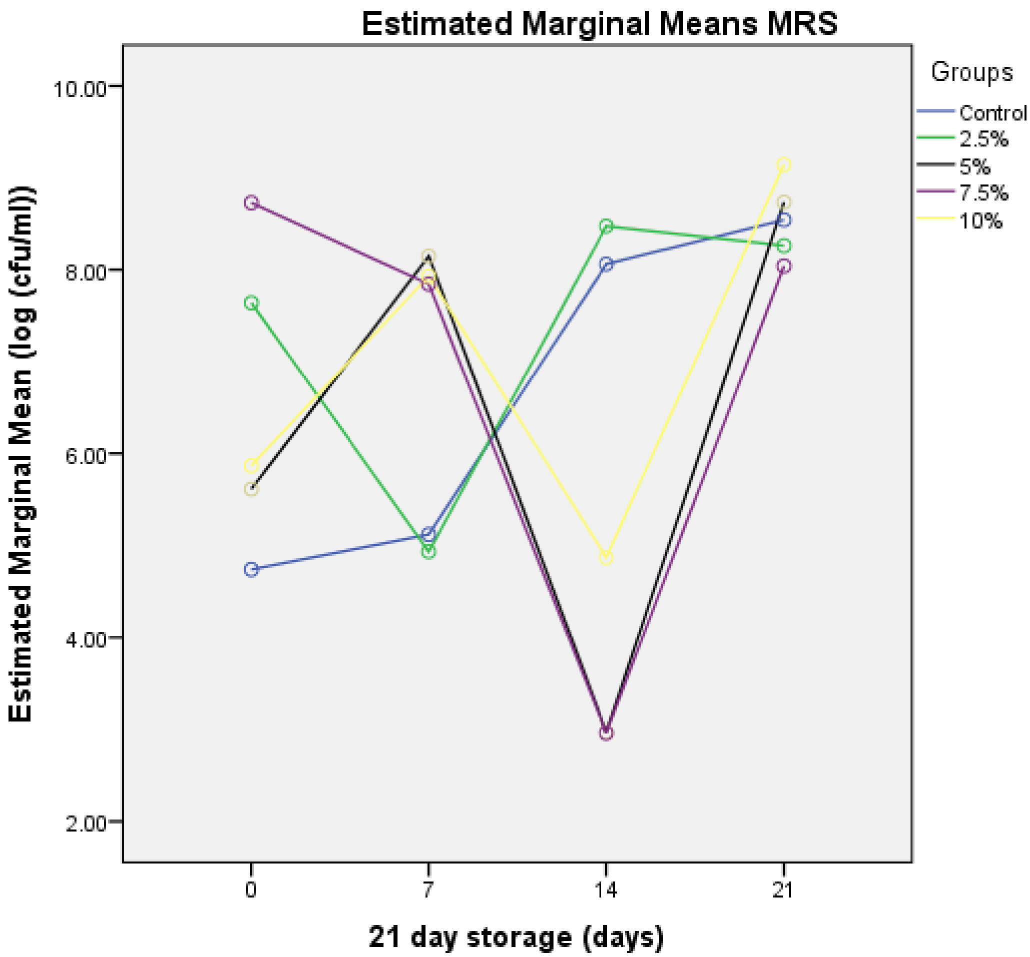

Fig. 7a Mean values of Log CFU/ml of L. Bulgaricus using MRS

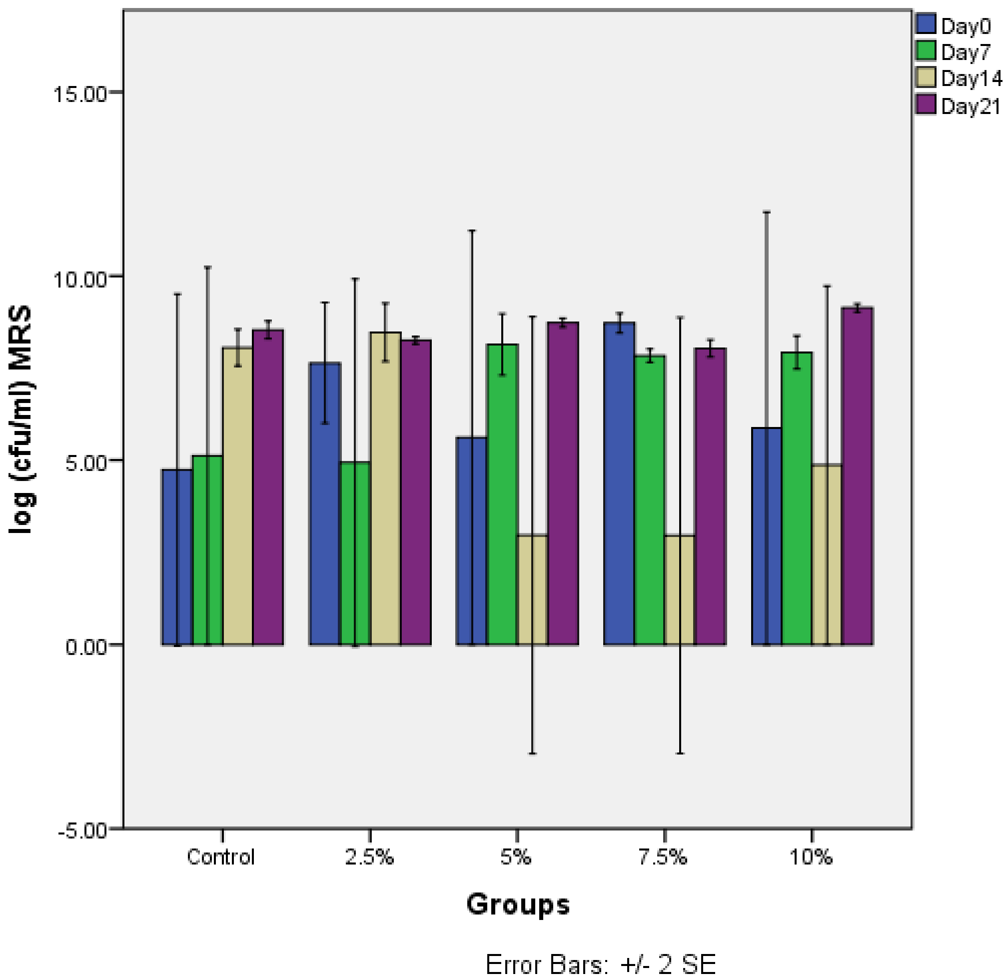

Fig.7b. Histogram showing logCFU/ml of L. Bulgaricus spp across 21day storage period.

4.8 Effect of Extract on *Streptococcus Thermophilus* Count Using M17agar

Using aerobic conditions, *S. thermophilus,* cell counts were taken for control and fortified samples. Results showed no significant difference between groups and across 21day storage period. There was no steady increase or decrease in the samples, reasons for this fluctuations are not properly understood although proper control measures were taken during sample preparations. According to Agil et al. (2013), some oligosaccharides (fructose oligosaccharides) increases growth of specific started cultures but does not support that of *S. thermophiles*. This might be the case for raffinose but further research needs to be conducted to understand this process. However, recommendations by Agil et al (2013) states that levels of viable bacteria in yogurt after approximately 4 weeks of refrigeration should be between 6 to 8 log CFU/ml and samples fortified with navy bean extract exhibited bacterial counts within this range.

Table 4.7 Effect of navy bean extract on *S. Thermophilus* microbial count M17 of yogurt after 21days shelf life study (mean values and ± standard deviation)

Treatments	Day 0	Day 7	Day14	Day21
1	6.25±0.75Aa	5.03±4.38Aa	6.97±0.60Aa	6.74±0.08Aa
2	8.36±0.21Ba	8.29±0.11Aa	8.28±0.22Aa	7.18±0.12Aa
3	6.94±0.10Aba	7.48±0.59Aa	8.00±0.50Aa	6.99±0.20Aa
4	8.28±0.37Ba	5.12±4.45Aa	8.02±0.41Aa	4.86±4.21Aa
5	7.91±0.98Ba	7.51±0.35Aa	7.89±0.61Aa	4.78±4.23Aa

Mean Values followed by the same letter (ABC) in a column are not statistically significant.
Mean Values followed by the same letter (abc) in a row are not statistically significant.
P = 0.05.
Treatments 1,2,3,4,5 represents Control, 2.5%, 5%, 7.5% and 10% respectively.

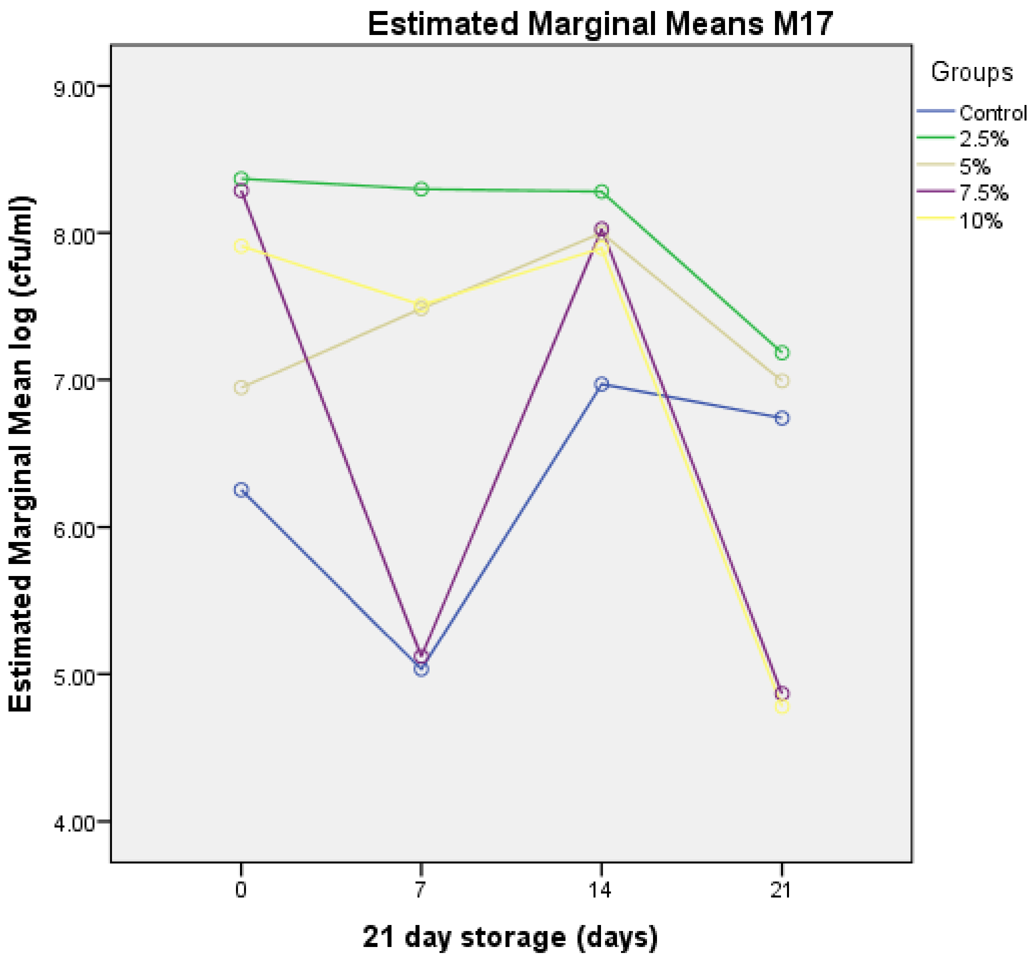

Fig.8a Mean values of log CFU/ml of S.thermaphilus using M17

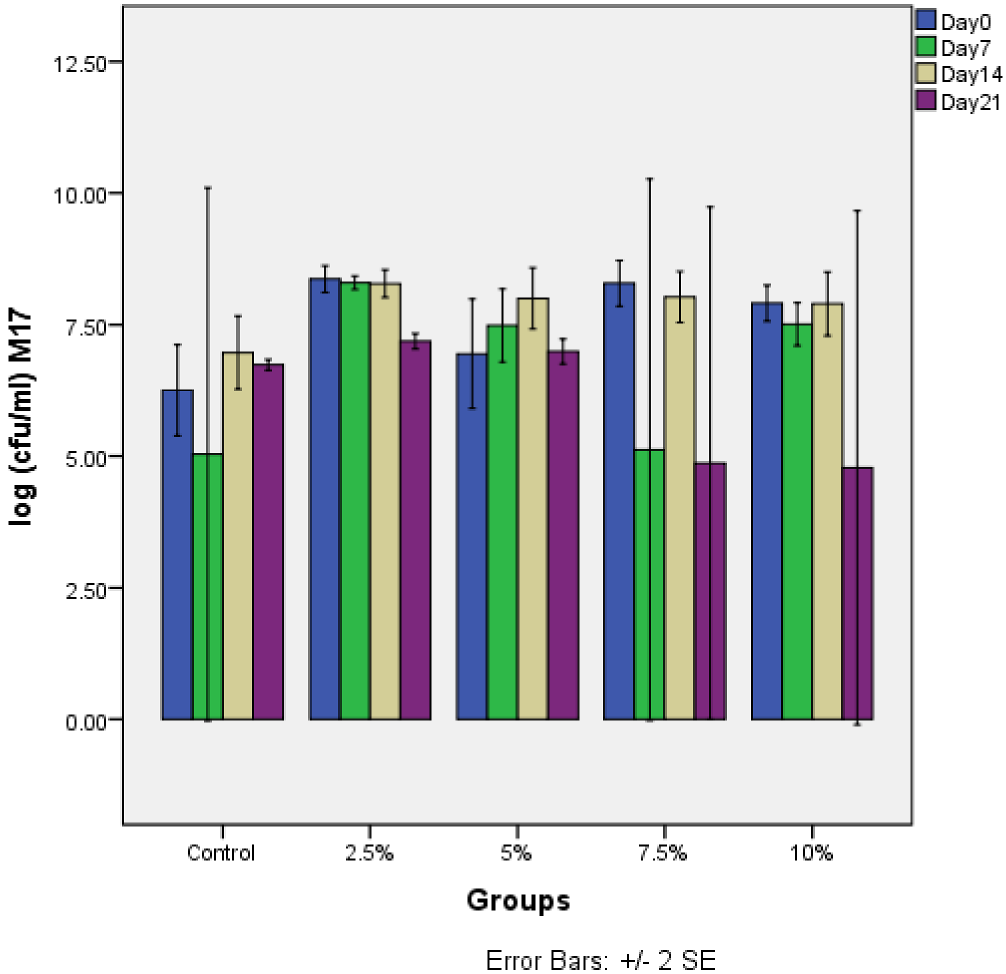

Fig.8b Histogram showing mean values of S. thermophiles counts in log CFU/ml after 21 days of storage.

5.0 Conclusion

The wide varieties of pulses identified have been shown by different studies to be beneficial to human health. Despite the research and enormous benefits associated with pulses, consumption is still low compared to other grains. The Food and Agricultural Organization in conjunction with United Health Organization declared 2016 to be the year of pulses and thus encourages research concerning the use of pulses and its derivatives.

The aim of this research was to study the effect of fortifying yogurt with navy bean flour extract. Extracts were found to contain a good amount of raffinose oligosaccharide which is the prebiotic component of beans; results from this study also showed that there is a beneficial interaction between the prebiotics in extracts and probiotics in yogurt.

The benefit of fortification was seen in the increase in acidification and reduction in fermentation time of yogurt fortified with bean extract. This is of great importance to industries as its application can help reduce time for yogurt production and increase economic value of the product. Also viable cell count logs were maintained between groups despite fluctuations in data obtained. The viable cell count over time suggests that probiotics did feed on the available prebiotics from the extracts which made them to strive more than control samples. Although the increase in cell count was not statistically significance, the addition of navy bean flour extract did show to increase the viability of probiotics in the yogurt thereby increasing the quality of yogurt and overall, has great potential for the production of a healthy prebiotic/probiotic yogurt that is great for gut health.

Further studies should aim at isolating raffinose powder from the water extract. The powder can be used to fortify yogurt and results compared to other prebiotic powders available in the market. This powder can be used as an alternative to inulin which is presently used as a source of prebiotic for fortifying yogurt. The use of prebiotics from beans would encourage by-product utilization from industries, increase production and utilization as well as consumption of bean and other pulses. Further studies can also evaluate the effect of extract and raffinose powder on different probiotic strains, as some microorganisms may have selective effect; extracts can be prepared from different variety of beans and lentils and used for both yogurt fortification or preparations of symbiotic products. Sensory analysis can be carried out in the future to determine consumer acceptability. The utilization of pulses in our daily diet will not only create healthy food options for the society but will help increase utilization, production and economic value of pulses and its derivatives.

Despite the short comings experienced during the course of this research, water extract from beans has shown to have positive effect when used to fortify yogurt. This goes a long way to show that bean can be used in many different ways and still have great benefits to both on probiotic growth and gut health. With respect to this, extracts have been shown to improve quality of yogurt and shows promising results for future studies.

References

Agil, R., Gaget A., Gilwa, J., Avis T.J., Willmore, W.G., & Hosseinian F. (2013). Lentils enhance probiotic growth in yogurt and provide added benefit of antioxidant protection. *LWT- Food Science and Technology*, *50,* 45-49.

Al-Sheraji, S.H., Ismail, A., Manap, M.Y., Mustafa, S., Yusof, R.M., & Hassan, F.A. (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods, 5,* 1542-1553. doi:10.1016/j.jff.2013.08.009.

Anton, A.A., Fulcher, R.G., & Arntfield, S.D. (2008). Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus Vulgaris L.*) flour: Effect of bean addition and extrusion cooking. *Journal of Food Chemistry, 113,* 989-996. doi:10.1016/j.foodchem.2008.08.050.

Anton, A.A., Ross, K.A., Lukow, O.M., Fulcher, R.G., & Arntfield, S.D. (2007). Influence of added bean flour (*Phaseolus vulgaris* L.) on some physical and nutritional properties of wheat flour tortillas. *Journal of Food Chemistry, 109,* 33-41. doi:10.1016/j.foodchem.2007.12.005.

Aryana, K.J., & McGrew, P. (2007). Quality attributes of yogurt with Lactobacillus Casei and various prebiotics. *LWT- Food Science and Technology, 45,* 1804-1814. doi:10:1016/j.lwt.2007.01.008.

Bark, S.A. (2013). Nutritional and therapeutical values of chickpea water extract enriched yogurt made from cow and camel milk. *American Journal of Drug Discovery and Development*, 1-13. doi: 10:3923/ajdd.2013.

Bobe, G., Barrett, K.G., Mentor-Marcel, R.A., Saffiotti, U., Colburn, N. H., Young, M.R.,…Lanza, E. (2007). Dietary cooked navy beans and their fractions attenuate colon carcinogenesis in azoxymethane-induced ob/ob mice. *Nutrition and Cancer, 60(3),* 373-381. doi: 10.1080/01635580701775142.

Boye, J., Zare. F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*, 414-431. doi:10.1016/j.foodres.2009.09.003.

Brummer, Y., Kaviani. M., & Tosh, S.M. (2014). Structural and functional characteristics of dietary fiber in beans, lentils, peas and chickpea. *Food Research International, 67*, 117-125. doi:10.1016/j.foodres.2014.11.009.

Charalampopoulos, D., & Rastall, R.A. (2012). Prebiotics in foods. *Journal of Current Opinion in Biotechnology, 23*, 187-191. doi:10.1016j.copbio.2011.12.028.

Ekvall, J., Stegmark. R., & Nyman. M. (2006). Optimization of extraction methods for determination of the raffinose family oligosaccharides in leguminous vine peas (*Pisim Sativum L.*) and effects of blanching. *Journal of Food Composition and Analysis, 20*, 13-18. doi: 10.1016/j.jfca.2006.06.010.

El-Abbadi, N. H., Dao, M.C., & Meydani, S.N. (2014). Yogurt: Role in healthy and active aging. *The American Journal of Clinical Nutrition, 99,* 1263S-1279S. doi: 10.3945/ajcn.113.073957.

Food and Agriculture Organization of the United Nations. (n.d). 2016 International year of pulses. Retrieved from http://www.fao.org/pulses-2016/en/

Gahruie, H. H., Eskandari, M. H., Mesbahi, G., & Hanifpour, M., A. (2015). Sceintific and technical aspects of yogurt fortification: A review. *Food Science and Human Wellness,4,* 1-8.

Garden-Robinson, J., & McNeal, K. (2013). All about beans: Nutrition, health benefits, preparation and use in menus. *The Bean Instirute.* 1-16. Retrieved from http://www.ag.ndsu.edu/pubs/yf/foods/f

Greger, M. (2011). Bean and gas: Clearing the air. Retrieved from http://nutritionfacts.org/2011/12/05/beans-and-gas-clearing-the-air/

Johansen, H.N., Glitso, V., & Knudsen, K.E. B. (1996). Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by high-perfoemance liquid chromatography. *Journal of Agric. Food Chemistry, 44,* 1470-1474.

Kolida, S., Tuohy, K., & Gibson, G.R. (2002). Prebiotic effects of inulin and oligofructose. *British Journalof Nutrition, 87(2),* S193-S197. doi:10.1079/BJN/2002537.

Leterme, P., (2002). Recommendations by the health organizations for pulse consumption. *British Journal of Nutrition, 88(3),* S239-S242. doi:10.1079/BJN2002712.

Lucier, G., Lin, B-H.,Allshouse, J., & Kantor, L S. (2000). Factors affecting dry bean consumption in the United States. *Economic Research Service/USDA*. 26-34. Retrieved from http://www.agmrc.org/media/cms/DryBeanConsumption_06EF5442C497B.pdf

McCrory, M.A., Hamakaer, B.R., Lovejoy, J.C., & Eichelsdoerfer, P.E. (2010). Pulse consumption, satiety, and weight management. *Advances in Nutrition, 1,* 17-30. doi:10.3945/an.110.1006.

Ooi, L-G., & Liong, M-T. (2010). Cholesterol-lowering effect of probiotics and prebiotics: A review of in vivo and in vitro findings. *International Journal of Molecular Sciences, 11,* 2499-2522. doi:10.3390/ijms11062499.

Patil, S. (2013). Probiotics and prebiotics: Fabulous nutritional supplements. *International Journal of Science & Engineering Research, 4(4)*, 318-333. Retrieved from http://www.ijser.org/

Schrezenmeir, J., & De Vrese, M. (2001). Probiotics, prebiotics and symbiotics- approaching a definition. *American Journal of Clinical Nutrition, 73*, 361S-364S.

Tajoddin, M., Manohar, S., & Lalitha, J. (2012). Laboratory experimets illustrating evaluation of raffinose family oligosaccharides of mug beans (*Phaseolus aureus L.*) cultivars. *Indian Journal of Innovations and Development, 1(5)*, 390-394.

Thavarajah, D., & Thavarajah, P. (2012). *2012 U.S. pulse quality survey*, Retrieved from http://www.pea-lentil.com/core/files/pealentil/uploads/files/2012%20U_S_%20Pulse%20Quality%20Survey.pdf

Wang, Q-Q., Wang, P-F., Qin, M-J., Qi, J-J., Peng., Sun.,…Li X-E. (2012). Optimization of extraction method with alkali and determination of stachyose, sucrose and raffinose in fresh rehmannia (*Rehmannia glutinosa Libosch*) using high-performance liquid chromatography with evaporative light scattering detection. *Journal of Medicinal Plants Research, 7(29)*, 2170-2176. doi:10.5897/JMPR12.165.

Zare, F., Champagne, C.P., Simpson, B.K., Orsat, V., & Boye, J.I. (2011). Effect of the addition of pulse ingredients to milk on acid production by probiotics and yogurt starter cultures. *LWT- Food Science and Technology, 45*, 155-160. doi:10:1016/j.lwt.2011.08.012.