SUITABILITY OF COMMON BEAN 'MILK' AS A NUTRITIOUS VEHICLE FOR PROBIOTIC BACTERIA

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Suitability of Common Bean 'Milk' as a Nutritious Vehicle for Probiotic Bacteria

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

To my amiable wife Emma, lovely daughter Chloe and others to come.

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LIST OF ABBREVIATIONS/ACRONYMNS

AOAC	Association of Official Analytical Chemists
CFU	Colony Forming Units
FAO	Food and Agricultural Organization
HPLC	High Pressure Liquid Chromatography
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LAB	Lactic acid bacteria
MRS	De Man, Rogosa Sharpe
NCD	Non-Communicable Diseases
PEM	Protein Energy Malnutrition
UNICEF	United Nations Children's Fund
UV-VIS	Ultra-Violet Visible Spectrophotometer
WHO	World Health Organization

DEFINITION OF TERMS

Bean milk Plant-based drink produced by soaking beans, removing hulls, grinding and boiling the mixture and filtering out the remaining particulates.

Common bean Herbaceous annual plant grown worldwide for its edible dry seeds.

Probiotic Live microorganism which when administered in adequate amounts confer a health benefit on the host.

ABSTRACT

In a bid to address the challenges of hard-to-cook phenomenon, oligosaccharides and anti-nutrients in common beans (*Phaseolus vulgaris* L.), the goal of the study was to evaluate the ability of selected pro-biotic bacteria in fermenting oligosaccharides and reducing anti-nutrients in fermented bean milk. The study aimed at determining nutritional and anti-nutritional composition of pinto, yellow kidney and red haricot dry beans and their corresponding fermented and non-fermented bean milk. The specific objectives of the study were (i) to determine nutritional properties of the selected dry beans and their corresponding milk extracts, (ii) to evaluate growth of probiotic bacteria during fermentation and their survival during storage of fermented common bean milk (iii) to determine the effect of fermentation on the nutritional properties of common bean milk and (iv) to evaluate sensory acceptability of fermented milk extracted from pinto beans, yellow kidney beans and red haricot beans Common bean milk was extracted from local dry grains of three varieties of common beans at the Food Biochemistry Laboratory of Jomo Kenyatta University of Agriculture and Technology. The samples were analyzed for their proximate composition, minerals (iron, zinc, calcium, phosphorus and magnesium), phenolic compounds (tannins, total phenols and flavonoids) and phytates, vitamin B complex (thiamine, riboflavin, niacin, pyridoxine and folic acid) and oligosaccharides. Each of the bean milk samples was inoculated with three starter cultures at 1g/l of sample and incubated at 45°C until a pH $\leq$4.3 was attained. The enumeration of probiotic bacteria contained in Yoba Fiti® culture was performed on MRS agar, MRS with 0.5% L-cysteine was used to enumerate bacteria contained in ABT® culture while M17 was used to enumerate probiotic bacteria present in YF L-903® culture. The effect of fermentation on chemical composition was determined. Carbohydrates concentration significantly increased in milk extracted from red haricot beans (66.3±0.5 – 77.0±0.2) and pinto beans (62.7±1.3 – 71.2±2.8), but was retained in milk extracted from yellow kidney beans (67.3±1.4 - 68.2±1.4), while crude ash decreased (4.6±0.1 – 1.9±0.1) and crude fiber was not detected. Protein significantly ($p<0.05$) increased in milk extracted from yellow kidney beans (20.8±1.3 – 26.0±1.7) but was retained in milk extracted from pinto beans while crude fat significantly ($p<0.05$) increased in all bean milk samples. However, protein reduced in milk extracted from red haricot beans (22.0±0.3 – 17.7±0.2). At the end of fermentation, bean milk fermented with ABT and YF L-903 cultures had colony forming units (CFU) greater than Log_{10} 6, but only milk extracted from pinto beans fermented with Yoba Fiti culture reached the recommended CFUs of Log_{10} 6. Stable survival of probiotic bacteria was found in bean milk samples fermented with Yoba Fiti culture while loss in survival of probiotic bacteria during storage was found in bean milk fermented with ABT and YF L-903 cultures ($p<0.05$). Bacterial survival in bean milk fermented with YF L-903 was greater than Log_{10} 6 during the 28 days storage in milk extracted from yellow kidney and red haricot beans. Fermentation with any of the three cultures caused significant reduction in raffinose, verbascose and stachyose sugars ($p<0.05$) in all bean milk samples. It was observed that regardless of the culture used, fermentation caused significant increase in niacin, pyridoxine and folic acid which is an

indication that probiotic bacteria could be used for vitamin biosynthesis in bean milk. Overall acceptability was statistically similar ($p>0.05$) for bean milk fermented with YF L-903 and ABT cultures (3.5±0.3 and 3.7±0.2 in milk extracted from yellow kidney beans, 4.1±0.1 and 4.0±0.1 in milk extracted from red haricot beans and 3.5±0.3 and 3.3±0.3 in milk extracted from pinto beans), while significantly lower sensory scores for overall acceptability, aftertaste and aroma were found in pinto beans fermented with Yoba Fiti culture. The findings from this study provide evidence that the three cultures used for fermentation improved the nutritional characteristics of common bean milk. However, in terms of probiotics, fermentation of bean milk with YF L-903 culture has great potential for commercial utilization since it led to better growth of CFUs and survival of the bacteria during storage to levels that could confer health benefit. Therefore, from the findings of the study it is recommended that (i) fermentation with probiotic LAB should be exploited in development of a nutritious probiotic common bean milk, (ii) specific health benefits of the fermented bean milk should be exploited and (iii) strategies to improve sensory acceptability should be exploited to enable consumer acceptability.

Key words

Common beans, bean milk, fermentation, probiotics

CHAPTER ONE

INTRODUCTION

1.1. Background

In developing countries, there are recurrent problems of protein energy malnutrition and inadequate mineral and vitamin intake (Müller & Krawinkel, 2005). These challenges are compounded by inadequate prenatal nutrition which contributes to deficiencies in both mother and fetus (Marangoni et al., 2016). There is also an increase in the prevalence of non-communicable diseases such as diabetes, cardiovascular diseases and cancer, and dietary habits and nutrition are expected to play a decisive role in the prevention of these disease conditions (Monemi, 2008; Peltzer et al., 2014; Rizkalla et al., 2002).

Food staples such as legumes which are produced and consumed in many developing countries can be optimized to enhance their quality in an effort to prevent NCDs and malnutrition (Bennink et al., 2009; Katungi et al., 2009). Legumes, especially common bean (*Phaseolus vulgaris* L.) in general is a major provider of the dietary energy and 20-25% protein in human diet (Rehman et al., 2001). The protein from common bean and other legumes provides sulfur-containing amino acids which are lacking in grain cereals (Landbouwcatalogus, 1990). Legumes contain type B starch which is less digestible and lower in glycemic index (Campos et al., 2009). Thus, common bean can lower post-prandial blood glucose levels leading to lower incidences of NCDs and reduced prevalence of protein energy malnutrition (Niba, 2003).

However, consumption and utilization of legumes including common beans is a distance second to cereals (Akibode & Maredia, 2011; Wortmann et al., 1998). This is attributed to phenomena such as long cooking time, tedious cooking procedure, anti-nutritional factors, and lack of interest in their exploitation as international cash crops (Niba, 2003; Reyes & Paredes, 1993). Socio-economic factors such as development of urban centers and migration from rural areas to such developed centers have contributed to changes in

lifestyle leading to consumption of refined foods and alteration in food habits and eating patterns (Niba, 2003). These changes have contributed to decline in consumption of the most cultivated legume including common bean and calls for expansion in bean utilization besides boiling which is the most practiced method of bean preparation (Wortmann et al., 1998).

Making vegetable milk from the common bean followed by fermentation can introduce variety and eliminate the undesirable attributes which hinder bean utilization (Nakitto et al., 2015). Fermented common bean preparations have shown increased protein content, essential amino acids and increased protein digestibility (Bastidas et al., 2010; Nakitto et al., 2015). They also showed high levels of mineral, shorter cooking time and reduced anti-nutrient attributes (Obadina et al., 2013; Ranilla et al., 2009). However, the fermentation outcome varies with the starter microorganism used for fermentation and the bean variety (Eneobong & Obizoba, 1996; Qin et al., 2010). The present study sought to evaluate suitability of milk extracted from yellow kidney beans, red haricot beans and pinto beans as a nutritious vehicle for probiotic bacteria. These bean varieties are popular in various parts of the country because they are high yielding and are well adapted to harsh environmental condition (Wortmann et al., 1998).

1.2. Problem statement

Though common bean is popular in small-holder communities, there are few products associated with it (Katungi et al., 2009). The presence of numerous anti-nutritional factors and hard to cook phenomena limit processing of common beans to heat processing or a combination of heat and other methods such as soaking and dehulling. The preferred method is boiling common bean for longer hours which has been observed to eliminate most of the anti-nutritional factors (Reyes & Paredes, 1993). However, boiling requires a lot of energy which cannot be accessed frequently and is expensive for the small-holder communities. In addition, boiled common bean is easily spoilt by microorganisms and cannot be stored for a long time like the dry beans, hence the need for alternative

processing techniques with potential to shorten the cooking time, prolong the shelf life of the cooked bean and eliminate or reduce the anti-nutrient contents (Urua et al., 2013).

Fermentation could offer a suitable alternative with unique potential to enhance the nutritional properties of the fermented common bean, reducing spoilage, and increasing shelf life and microbial food safety. However, fermentation has sparingly been utilized in improving the quality of common bean since only limited acceptable products have resulted from common bean seed or flour (Katungi et al., 2009). Therefore, changing the form of the bean from seed into vegetable milk can facilitate fermentation and introduce variety in utilization of common bean (Hasan & Sultan, 2014). Naidu et al. (2012), Mani et al. (2014), Bridgman et al. (2014) and Pieniz et al. (2014) have shown that products fermented with probiotic bacteria have health promoting properties such as preventing allergies, boosting immune system, treating gastrointestinal disorders and eczema.

1.3. Justification

Fermentation plays a significant role in enhancing nutrient quality of food products. Fermented products are widely accepted for their improved sensory qualities, especially, flavor, texture and color (Heller, 2001; Molin, 2001). Fermenting common bean milk with probiotic bacteria could potentially optimize both nutrient and sensory qualities as well as other health benefits. Most consumers prefer boiling common bean for longer hours to eliminate a number of undesirable attributes such as oligosaccharides, phenolic compounds, phytates, lectins, allergens, enzyme inhibitors, flatus factors and beany flavor (Reyes & Paredes, 1993). The boiling process consumes a lot of fuel which is not within the reach of most consumers thus limits bean utilization. Fermentation provides a cheaper alternative which when utilized effectively will remove the anti-nutrient factors in common bean and shorten the cooking time for the fermented bean milk. This may possibly provide diversity to common bean utilization since the fermented bean milk product can be consumed at home, and possibly opens up business opportunity for innovative and early adopter entrepreneurs.

Acid milk beverages and yoghurt are widely consumed worldwide by different races. However, in many parts of the world, especially developing countries, cow's milk is prohibitively expensive and is not readily available (Gerosa & Skoet, 2012; Niyibituronsa et al., 2018). Fermented common bean milk might well serve as a substitute in these areas to meet the food and nutrition needs of the population. Therefore, the current study offers much promise since it could lead to acceptable fermented common bean milk. Fermented products have longer, preservative free shelf-life which is a desired quality for consumables including common bean.

Fermentation with commercial starter cultures Yoflex (YF L-903) (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), ABT (*Streptococcus thermophilus, Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* BB-12) and Yoba Fiti (*Streptococcus thermophilus* and *Lactobacillus rhamnosus* GR1) could impart antioxidant and antimicrobial properties and thereby improve digestibility (Nakitto et al., 2015). They could also be of physiological importance in the gastrointestinal tract through fermentation of complex, non-digestive carbohydrates in common bean to produce beneficial short chain fatty acids (Niba, 2003). Indeed, with improved nutrient content, reduced undesirable quality attributes and longer shelf life there will be renewed interest in common bean utilization and thereby help in ending hunger and achieving food security and improved nutrition, which is one of the Sustainable Development Goals (SDGs) (Sachs et al., 2019). As a leading legume whose production is intensive where human population density is high, increased common bean utilization could play a significant role in meeting food security demands of the ever growing Kenyan population which is projected to increase from 47.s4 to 60.1 and 81.4 million in 2030 and 2050 respectively (United Nations Department of Economic and Social Affairs/Population Division, 2015). The results of the present study will therefore inform the consumer and the producer on the unique potential for common bean milk utilization and value addition opportunities offered by fermentation with probiotic bacteria.

1.4. Main objective

To determine nutritional and anti-nutritional composition of pinto, yellow kidney and red haricot dry common beans (*Phaseolus vulgaris* L.) and their corresponding fermented and non-fermented bean milk

1.4.1. Specific objectives

1. To determine the nutritional and anti-nutritional properties of pinto beans, yellow kidney beans and red haricot dry beans and their corresponding milk extracts
2. To evaluate growth of probiotic bacteria during fermentation and their survival during storage of fermented common bean milk
3. To determine the effect of fermentation on the nutritional and anti-nutritional properties of common bean milk
4. To evaluate sensory acceptability of fermented milk extracted from pinto beans, yellow kidney beans and red haricot beans

1.5. Hypotheses

1. H_o: Nutritional properties of pinto beans, yellow kidney beans and red haricot dry beans and their corresponding milk extracts does not differ with variation in beans type
2. H_o: There is no significant difference in the number of colonies forming units (CFU) after fermentation of bean milk and during storage
3. H_o: Nutritional properties of fermented common bean milk does not vary with the fermenting starter culture
4. H_o: Fermentation does not change the sensory acceptability of common bean milk

CHAPTER TWO

LITERATURE REVIEW

2.1. Common beans origin and description

Common bean (*Phaseolus vulgaris* L.) is a leguminous vine plant grown worldwide for its unripe or edible dry seed known as beans (Kiptoo et al., 2016). Common beans vary in growth habits from determinate beans to climbing indeterminate type. The most predominant bean type grown in East Africa and Kenya is the determinate bushy type (Karanja, 2016). Common bean is an annual plant that belongs to the genus, *Phaseolus*, with pinnately compound trifoliate large leaves (Katungi et al., 2009). Common bean is both self-pollinated and cross-pollinated plant through stigma contact with pollen coated bees. Common bean seeds are di-cotyledons that vary greatly in colour and size from small black wild type to large black, red, brown, white or mottled seeds (Wortmann et al., 1998).

2.2. Common bean varieties grown in Kenya

Common bean is highly variable with diverse seed types in Kenya. Approximately 80 different bean seeds have been identified in various parts of the country but only a few are popular (Katungi et al., 2009). They include pinto bean which is locally known as *Mwitemania*; red and yellow kidney beans which occur in different local names such as *Kitui, Wairimu, Nyayo* and *Rosecoco*; Canadian wonder, and red haricot beans. These varieties are high yielding and are well adapted to harsh environmental condition (Wortmann et al., 1998).

2.3. Common bean production in Kenya

Common bean production is widespread throughout the country, with counties within Nyanza and Rift Valley provinces equally contributing 25% of the total production, while counties within Central province account for 12%, Eastern 18% and Western 23% of the bean production (Ministry of Agriculture Livestock and Fisheries, 2015). This spread

indicates the staple nature of common bean for smallholder farmers with no limited concentration in any one region. In the last decade the common bean yields in Kenya have been relatively flat at around 0.5 MT/Ha as shown in Figure 2.1 (Kenya Country Stat, 2017). This is partly because majority of the common bean is intercropped with maize (Katungi et al., 2009). When grown as a single crop common bean yields are significantly high which implies that Kenya has a great potential to increase production beyond 0.5MT/Ha. Due to differences in agro economic practices and agri-climatic conditions, there is wide variation in average yields across counties, with some Western counties and Rift Valley counties averaging more than 1.0 MT/Ha which is more than twice the national average (Ministry of Agriculture Livestock and Fisheries, 2015). Most regions in Kenya grow common bean during both long rains (March to June) and short rains (September to December) seasons, but this is changing due to shifting climatic patterns

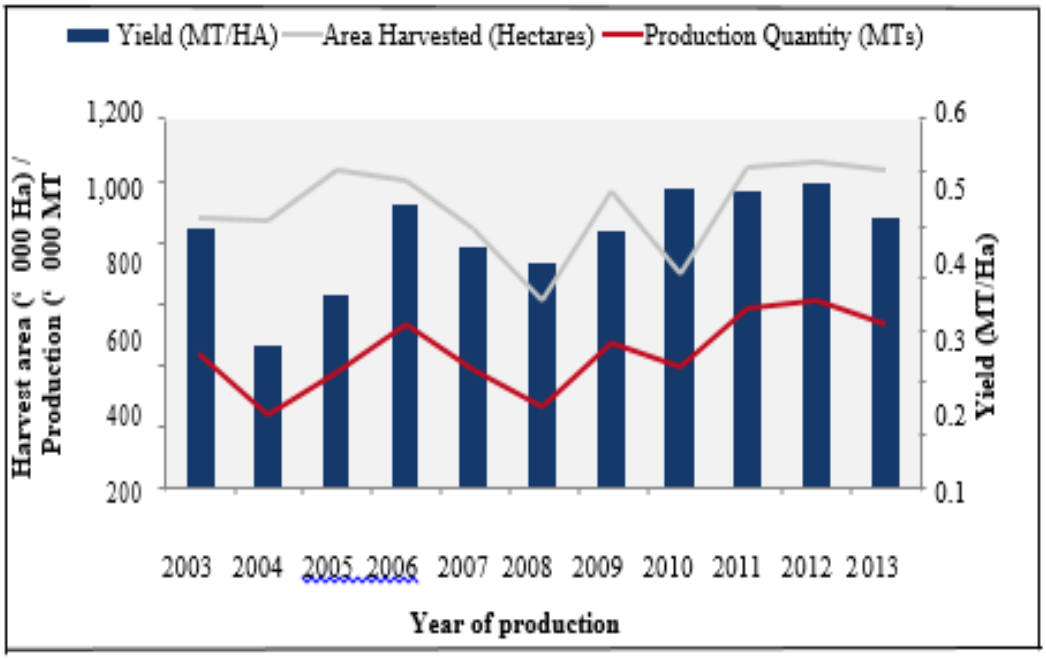

Figure 2.1: Common bean production in Kenya from 2003 to 2013

Source: Kenya Country Stat (viewed in February 2017).

2.4. Nutrient and anti-nutrient composition of common bean

Common bean contains 20 to 25% protein and 55 to 65% carbohydrate (Rehman et al., 2001). The major constituents of the carbohydrates are 22 to 45% starch and non-starch polysaccharides or dietary fiber (Hoover & Zhou, 2003). The non-starch polysaccharides in common bean comprise of soluble and insoluble fiber, inulin, gums, pectin and hemicellulose (Campos et al., 2009). Dietary fiber has health benefits which include increased fecal loss of bile acid, laxation and attenuation of blood cholesterol. It also contain minerals (Ca, Fe, Cu, Zn, P, K, and Mg) and vitamins (thiamine, riboflavin, niacin and vitamin B_6) (Kotue et al., 2018). However, common bean has a number of undesirable attributes such as oligosaccharides (0.12 mg/100 g to 3.2 mg/100 g) (Queiroz et al., 2002), phytates (17 mg/100 g to 915 mg/100 g) (Gibson et al., 2006), tannins (215 to 290 mg/100 g) (Mamiro et al., 2017), flavonoids (127 to 259 mg/100 g) (Akillioglu & Karakaya, 2010; Ren et al., 2012), lectins, enzyme inhibitors, hard-to-cook characteristic and beany flavor, which should be removed for effective utilization (Campos et al., 2009).

Oligosaccharide sugars in common beans comprise about 31% to 76% of the total sugars and are known to cause flatulence and discomfort in the stomach (Campos et al., 2013). Lectins, tannins, phytates and enzyme inhibitors such as α-amylase and trypsin inhibitors are anti-nutrients and the latter are known to reduce protein digestibility (Lajolo & Genovese, 2002). Previous studies revealed that lectins concentration in dry beans ranged from 3.4% to 5% while tannins ranged from 0.0 to 2.0% depending with beans shape and color (Salunkhe et al., 1990). Tannins form complexes with nutrients in foods such as carbohydrates, protein, polymers and metal ions under suitable conditions of concentration and pH as reported by Reddy & Pierson (1985) while lectins can depress nutrient absorption (Lajolo & Genovese, 2002). Phytates and polyphenols have metal chelating ability which is well-known to inhibit iron absorption (Laparra et al., 2009).

2.5. Common bean consumption

Common bean is the second most important staple crop after maize in central, southern and eastern Africa (Guerena, 2015). In East Africa the rural smallholder communities utilize common beans as the leading source of dietary protein and important source of essential minerals and vitamins (Karanja, 2016). Similarly, common bean is an important grain legume and source of protein for rural smallholder communities in Kenya (Kiptoo et al., 2016). On farm consumption of common bean accounts for about 60%, while 35% is sold in the local markets with only 5% utilized for commercial processing or exported (Context Network, 2016). In Kenya, common beans are mostly consumed in two forms, as *githeri*, a traditional Kenyan delicacy of legumes and maize boiled together in a cooking pot and as boiled beans. Consumption of common beans in the form of *githeri* or boiled beans is influenced by the cooking method that can potentially break the hard to cook phenomena of dry beans (Nakitto et al., 2015). Therefore, option for beans value addition to improve consumption could potentially be on modification of the beans processing methods.

2.6. Sensory characteristics and consumer preferences of common bean

In Kenya consumers prefer red, brown and purple colored common bean seeds because of the color imparted to food after cooking (Katungi et al., 2009). Commercial common bean producers grow beans of the preferred color which they can sell at high price (Jones, 2001). The method used to prepare common bean also determines the variety consumed (Katungi et al., 2009). For instance, dry common beans are often boiled to make stew or *githeri* because of their soft gravy. However, consumers can swap seed colour for superior traits such as palatability and good flavor since the preference are often not strongly exclusive and are mostly associated with the known cultivars (Wortmann et al., 1998). Cultivars with such superior traits are yellow kidney beans, pinto beans and red haricot beans which have a special niche in bean marketing in Kenya (Karanja, 2016). Grain quality of common bean is also decided by the consumer acceptability characteristics such as nutritive value, appearance, flavor and stability under storage conditions (Reyes &

Paredes, 1993). The quality of the developed common bean product such as bean milk should have most of these qualities if not all. The processing method used in the preparation of the common bean milk determines the final acceptability characteristics. For instance, presence of hulls contributes to discoloration of bean flours and thereby reduces the final color acceptability (Aminigo & Metzger, 2005).

2.7. Common bean processing method

Traditional food preparation methods such as soaking, de-hulling and boiling of common beans can eliminate most of the undesirable attributes, especially anti-nutrients but require high cooking fuel (Nakitto et al., 2015). Little research has been done to find out the importance of combined food processing methods such as soaking, de-hulling, fermentation and steaming on nutritional quality and production of nutritious fast cooking common bean (Nakitto et al., 2015). Fermentation of common bean with probiotic cultures can play a role in improving wealth and health by; (1) enhancing the nutritional properties of the fermented food and sequestrating anti-nutrient factors, (2) providing income to local producers who sell the product, (3) reducing spoilage, and (4) increasing shelf life and microbial food safety (Kort et al., 2015).

2.8. Probiotics

The internationally accepted definition of probiotics is live microorganism that when administered in adequate amount, confers health benefit to the host (FAO/WHO, 2002). Other definitions have been advanced over the years. These definitions are restrictive by specifications of site of action, delivery, mechanism of action, delivery method and the host (FAO/WHO, 2002; WHO & FAO, 2006). Probiotic is also a term derived from Latin preposition ‘pro’ which means ‘for’ and the Greek word ‘biotic’ meaning ‘bios’ or ‘life’ (Reid et al., 2003). The term probiotic therefore means ‘for life’. They are non-pathogenic beneficial live microorganisms which include yeast and bacteria (Hoveyda et al., 2009). The most commonly used probiotics in food are *Saccharomyces boulardii*, *bifidobacterum* and *Lactobacillus* (Islam, 2016). *Saccharomyces boulardii* is a type of yeast while

Bifidobacterium and *Lactobacillus* are Gram positive facultative anaerobic bacteria. *Lactobacillus* comprises a number of species which include *L. casei, L. reuteri, L. bulgaricus, L. acidophilus* and *L. rhamnosus*. *Lactobacillus rhamnosus* has strains such as *L. rhamnosus* GG and *L. rhamnosus yoba* (Sybesma et al., 2013). However, not all species of probiotics are part of intestinal microbiota and the beneficial effect associated with one probiotic strain cannot be generalized to others (Doron & Snydman, 2015). Foods that contain probiotics include fermented milk products such as *kefir* and pickled vegetables and fruits such as *sauerkraut*.

2.9. History of probiotics in food

The use of probiotics in foods goes back to over a hundred years ago when people drank fermented milk for their health (Doron & Snydman, 2015). In 1899, Henry Tessler, a scientist from the Pauster Institute in Paris discovered that the intestines of breastfed infants contained *bifidobacterium* (Islam, 2016). Tessler observed that these infants had fewer episodes of diarrhea when compared with non-breastfed counterparts. However, it was Eli Metchnikoff, a Russian scientist who first proposed that probiotics have health benefits at the beginning of 20^{th} century (Molin, 2001). In 1907 he prepared yoghurt with lactic acid bacteria culture consisting of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. A decade later a strain of *Escherichia coli* (*E. coli* nissle 1917) was isolated and used to treat patients with shigellosis. Since then, the use of probiotics in foods, especially dairy products and its health benefits have been documented and are available in literature (Islam, 2016).

Consumers are receptive of probiotics in foods since they are sold in fermented foods which already have positive health image (Heller, 2001). Consumers are also familiar with the fact that fermented foods contain nonpathogenic live microorganisms (Sodini et al., 2002). For these reasons dairy products have often been used as carriers for probiotics. In addition, dairy products such as yoghurts (Doron & Snydman, 2015) have been optimized for survival of fermentation organisms. Dairy products have buffering capacity that ensures the survival of probiotics during fermentation and storage (Mani et al., 2014).

However, fermented dairy foods are associated with some health risks such as allergy to milk, high fat and high cholesterol content and lactose intolerance. (Kumar, Vijayendra & Reddy, 2015). Lactose intolerance (LI) which is also known as lactose malabsorption is the inability to digest lactose into its constituents, galactose and glucose as a result of low levels of lactase enzyme (Hauck et al., 2011). Symptoms of lactose intolerance include loose stool, flatulence, cramping and bloating, and some reports also suggest that LI may lead to irritable bowel syndrome (Kumar et al., 2015). These symptoms appear 30 minutes to 2 hours after consumption of dairy product (Joachim, 1999). Allergy to milk is associated with atopic dermatitis (AD), a common food allergy in children (Ricci et al., 2006). Some studies have noted a reduction in the severity of signs and symptoms of patients with AD after consuming dairy products fermented with selective strains of probiotics (Kumar et al., 2015; Moro et al., 2006).

Indeed, since the successful introduction of probiotic bacteria in food at the beginning of 20^{th} century, the incorporation of the probiotics in foods has focused on milk-based products. Example of these products include yoghurt, ice creams, milk based desserts, cheese, mayonnaise, powdered milk for infants and butter (Naidu et al., 2012). In 1980s *L. acidophilus* was incorporated into milk because the traditional yoghurt containing *L. delbruekii* subs. *bulgaris* and *S. thermophilus* did not survive well in the human gut (Heller, 2001). However, *L. acidophilus* did not multiply well in milk and prompted exploration of fermentation of non-milk products, this was also considered as an alternative to alleviate the disadvantages of dairy based fermented foods (Rivera-Espineza & Gallardo-Navaro, 2010). Since then a number of non-milk-based foods which contain high concentration of lactic acid bacteria have been discovered. They include cereals based, fruit and vegetable based and soy based fermented foods. Some of the fermented food of vegetable origin include salted gherkins, sauerkraut and brined olives (Molin, 2001). However, only few non-milk based products are in the market (Heller, 2001). Besides, most non-milk products are processed with heat before consumption thus, are not considered as suitable carriers of probiotics.

Attempts have been made to produce beverages which do not require further heat processing before consumption. One such product is *tongwa*, a lactic acid fermented beverage which is made from sorghum or maize. It is regularly consumed in Tanzania by young children and it has been observed to decrease the enteropathogens in rectal swabs and improved barrier function for the intestinal mucosa in children with acute diarrhea (Molin, 2001). Similarly, Wang et al. (1974) successfully fermented soybean milk with eight strains of two probiotic species; *L. acidophilus* and *L. delbruekii* subs. *bulgaricus* to produce fermented bean milk. However, there is scanty documentation on fermentation of common bean milk with probiotic microorganisms.

2.10. Probiotics growth and survival in food

The survival of probiotics depends on time, storage conditions and species of the probiotic bacteria. Mani et al. (2014) observed that *L. acidophilus* in fermented milk decreased by 1 log during storage but remained at the level recommended by WHO/FAO ($> 10^6$cfu/ml) to have beneficial effect as probiotics. The authors further observed that *L. reuteri* had good stability and viability in fermented milk and remained at the recommended level for 21 days of storage. It was observed that beyond 28 days the microbial survival in fermented cow milk reduced as pH decreased and acidity increased. The major constituent of the vegetable milk are carbohydrates, fats and protein as noted by Olujobi et al. (2012), thus the probiotic bacteria used to ferment vegetable milk must have characteristics which enable them to utilize these three nutrients (Antai & Ibrahim, 1986). Even though WHO and FAO recommend probiotics $> 10^6$cfu/ml to have beneficial effects, there are no standard daily dosage recommendations (WHO & FAO, 2006). Therefore, higher dosages based on CFU; 10 billion CFU for adults and 5 billion CFU for children per day have been associated with significant health outcome (Naidu et al., 2012).

2.11. Carbohydrates fermented by probiotic microbes

The preferred energy source for probiotic bacteria is non-digestible carbohydrates, though in absence of these carbohydrates' microbes ferment protein (Navarro, Abelilla & Stein,

2019). Non digestible carbohydrates include polysaccharides, resistant starch, oligosaccharides and non-starch and are collectively known as fiber (Robeefroid & Slavin, 2000). Intestinal Oligosaccharides consists of mannan oligosaccharides, fructo-oligosaccharides and galacto-oligosaccharides that cannot be digested by intestinal and pancreatic enzymes (Englyst, Liu & Englyst, 2007). Legumes contain large proportion of galacto-oligosaccharides which comprise of verbascose, stachyose and raffinose. In the structure of these sugars, a unit of sucrose is linked to one, two or three units of raffinose, stachyose and verbascose respectively (Slavin, 2013). Humans lack α-galactosidase enzyme that can breakdown the glycosidic bonds linking the monosaccharides that makes up these oligosaccharide sugars (Vaclavik & Christian, 2014). Bacteria contained in the large intestine including strains of *Bifidobacteria, Lactobacillus* and *Streptococcus* have α-galactosidase enzyme and have been reported to utilize galacto-oligosaccharides (Liener, 2000; Vaclavik & Christian, 2014). Since probiotics strains used in commercial production of fermented products have similar characteristics to corresponding strain of gut microbiota (Liener, 2000), they have potential to ferment galacto-oligosaccharides sugars in legumes, including common beans and extracted bean milk.

2.12. Common probiotics and cultures

The genera *Lactococcus, Pediococcus* and *Lactobacillus* belong to the LAB and are part of the commensal intestinal flora of animals and humans (Klare et al., 2007). Strains of these genera are used on large scale as probiotics or as commercial starter cultures. Selected LAB strains commonly used in probiotic applications belong to the genera *Bifidobacterium* and *Lactobacillus*. The most notable individual species of *Bifidobacterium* used as probiotics include *B animalis, B longum, B lactis and B infanti* (Reid et al., 2003). Similarly, the most common species of *Lactobacillus* used as probiotics include *L acidophilus, L casei, L reuteri, L bulgaricus* and *L rhamnosus* (Servin, 2004). They are used to ferment food products, especially dairy foods and food supplements that may improve the gut flora by anticarcinogenic activities, antimutagenic activities, stimulation of the immune response and competitive exclusion of gastrointestinal pathogens (Klare et al., 2007; Mercenier, Pavan & Pot, 2003).

Mechanisms and mode of actions of probiotic bacteria are strain specific and so is their indication and dosage (Molin, 2001). For instance, strains of *L rhamnosus, S boulardii, L acidophilus* and *L bulgaricus* are recommended for treatment of antibiotic associated diarrhea (Islam, 2016). Similarly, Islam (2016) also noted that *L reuteri* and *B infantis* have been used for treatment of acute infectious diarrhea in infants and children and irritable bowel syndrome respectively. *Lactobacillus rhamnosus* is also recommended for treatment of the latter diseases (Klare et al., 2007). Additionally, *L rhamnosus* has history of use in treating traveler's diarrhea and atopic dermatitis (Kort et al., 2015). Though consumers use probiotics to treat several health disorders, many of the claims made by probiotic manufacturers are not supported by scientific evidence and require further clinical studies. Besides, the available recommendations by WHO and Food Drug Administration (FDA) are sketchy and should be studied further (Islam, 2016).

2.13. Benefits and safety of probiotics

Probiotics adherence to cells permits them to have health benefit in the human host (Azizpour et al., 2009). This allows probiotic bacteria to enhance colonization resistance and to direct inhibitory effects against pathogens (Islam, 2016; Nagpal et al., 2012). They antagonize pathogens such as *Clostridium perfringens, Yersinia enterocolitica, Salmonella typhimurium* and *Staphylococcus aureus* directly through antibacterial and antimicrobial compounds like butyric acid and cytokins (Kailasapathy & Chin, 2000). Probiotic bacteria lowers the gut pH and competes with the pathogens for receptor and binding sites (Langhendries et al., 1995). This improves immune function and stimulates immunodulatory cells (Azizpour et al., 2009). Like in *tongwa* product, most consumers use probiotic products to treat gastrointestinal disorders. Hoveyda et al. (2009) demonstrated the ability of probiotics to treat irritable bowel syndrome, a chronic gastrointestinal disorder with weak responsiveness to drug therapies. Probiotics also play important role in the treatment of gastrointestinal gas, bloating, flatulence and abdominal pain (Hoveyda et al., 2009). Additionally, they can be used to treat several other conditions because they contain health promoting properties such as preventing allergies, boosting immune system, treating gastrointestinal disorders and eczema (Naidu et al., 2012).

Probiotics have potential to reduce spoilage and increase shelf-life and microbial food safety (Kort et al., 2015; Sullivan et al., 2002). They produce acids, hydrogen peroxide and bacteriocins which are antagonistic to growth of spoilage microbes and pathogens (Azizpour et al., 2009; Sullivan et al., 2002). Additionally, Azizpour et al. (2009) observed that since probiotic bacteria adheres to cells, they have the potential to exclude or reduce pathogenic adherence in gut. Probiotic bacteria also multiply and persevere harsh conditions, attributes which enhance their viability in food during storage (Langhendries et al., 1995).

As reported by Kailasapathy and Chin, (2000), the probiotic bacteria strains that are selected for probiotic milk products should fulfill certain requirements. They should be able to survive a pH of 1-4 of the gastric acidic conditions; be resistant to the action of the bile salts; resist degradation of lysozymes and other digestive enzymes present in the intestine; survive action of primary phenols and toxic metabolites produced during the digestion process, anaerobic growth condition, antibiotics and phage and storage conditions of the food carrier. It is also essential that the probiotic species be of human origin and carrier food contain not less than 1 million viable cells (Hasan & Sultan, 2014). Species of *L. acidophilus* and *L. rhamnosus* have shown ability to meet all the requirements for viable probiotics. Strains of *L. acidophilus* survive well at pH 3.0 or greater and the viable counts remain greater than 10^7 CFU/g after 3 h incubation (Kailasapathy & Chin, 2000). Different strains of *L. acidophilus* and strain of *L. rhamnosus* GG survive well in various concentration of bile up to 1.5% (Succi et al., 2005).

Historical data shows that lactobacilli probiotics consumed in foods are safe for human use (Gregor Reid et al., 2003) and that is why they were chosen for current study. Their occurrence as normal microorganisms in the human flora and their known safe use in diverse foods worldwide support this observation. However, probiotics are live organisms, thus it is possible that they could infect the host. Consequently, there are concerns with consumption of probiotics which include basic issues such as dosing and their mechanism of actions (Naidu et al., 2012). There is also concern on the lack of safety data for

probiotics, especially absence of data from clinical trials on safety of probiotics when consumed in large dosages (Doron & Snydman, 2015).

2.14. Effect of probiotics on nutrients and sensory characteristics of common bean

Today people are knowledgeable about functional foods and are interested in foods with health benefits beyond adequate nutrition. Probiotics are used in foods to provide health benefits in addition to nutrients. Dairy products are the major vehicles for probiotic bacteria to human because they provide a suitable environment for growth and viability (Stanton et al., 2001). In probiotic soy products, probiotics increase the concentration of free isoflavones and reduce the levels of some of the carbohydrates responsible for production in the intestinal system (Song, Ibrahim & Saeed, 2012).

Fermentation of common bean with probiotics has not been extensively studied but it has been observed that fermentation with probiotic *L. acidophilus* (ATCC 11974) reduced total soluble phenols and increased iron uptake in common bean seeds (Laparra et al., 2009). Tannin content and trypsin inhibitor activities decreased by 83 and 57% when fermented with LAB and *in vitro* protein digestibility increased by 12.55% (Granito & Alvarez, 2006). This is an indication that reduction in anti-nutrient compounds could lead to increase *in vitro* protein digestibility. Interaction of anti-nutrients such as phytates and tannins increases the extent of protein cross linking, reduces protein solubility and make protein components that are hardly susceptible to proteolytic attack (Reddy & Pierson, 1985). Lactic acid fermentation of common bean with probiotic bacteria increase insoluble fiber due to formation of retrograded starch and other complex molecules, lower soluble fiber due to hydrolysis and solubilization of some pectin compounds (Granito & Alvarez, 2006). Similar occurrence is noted with oligosaccharides, especially raffinose concentration which reduces considerably. Therefore, lactic acid fermentation with probiotic bacteria could be used as a functional starter culture for production of common bean milk.

There is scanty literature on the effect of probiotics on the sensory qualities of legumes including common bean. Soy bean milk fermented with *L. acidophilus* strains B-1910, B-1911, B-1912 and B-1858 showed significant variation in acidity, flavor and color. Fermentation of soybean milk with *L. acidophilus* strain B-1910 gave higher score in overall product acceptability, color, texture and flavor (Wang et al., 1974). The improved flavor suppressed the previously beany flavor and the product was generally accepted for sensory qualities by the evaluation panel.

CHAPTER THREE

CHEMICAL COMPOSITION OF THE SEED AND 'MILK' OF THREE COMMON BEAN (PHASEOLUS VULGARIS L.) VARIETIES

3.1. Introduction

Common bean is a leguminous vine plant grown worldwide for its edible dry seed (Campos et al., 2013). It is an important contributor of protein, carbohydrate, minerals and vitamins in the human diet (Karanja, 2016; Kiptoo et al., 2016; Rehman, Salariya & Zafar, 2001), especially among the less economically privileged (Katungi et al., 2009). Most smallholder farmers in Kenya grow common beans for subsistence and dry them in the sun to increase their shelf-life (Katungi et al., 2009; Mureithi, Gachene & Ojiem, 2003). Unfortunately, drying beans in the sun followed by storage in tropical climates with mean temperatures of ≥18^{o}C makes them resistant to cooking, a condition known as hard-to-cook phenomenon (Reyes & Paredes, 1993). Common beans also contain oligosaccharides and phytochemicals such as tannins, phytates and flavonoids (Campos et al., 2013; Laparra et al., 2009). These phytochemicals have recently elicited interest due to their ability to reduce the risk of aging-related non-communicable diseases (Campos et al., 2013). On the other hand, among the nutritionally deprived, they pose the disadvantage of forming complexes with metal ions and potentially inhibiting mineral absorption (Laparra et al., 2009). Tannins and phenolic substances also form complexes with carbohydrates and protein under certain conditions of concentration and pH (Akillioglu & Karakaya, 2010; Aminigo & Metzger, 2005; Salunkhe, Chavan & Kadam, 1990), and thus hinder protein and starch digestibility (Cruz et al., 2003). As a result, they are often regarded as anti-nutrients.

The most common cooking method for beans involves boiling for one to three hours depending on variety, age and size of the beans as well as the pre-cooking methods used (Nakitto et al., 2015). However, due to the hard-to-cook phenomenon, the long cooking hours involves high consumption of energy which is expensive. Traditional processing

methods such as soaking and de-hulling followed by thermal treatment can be used to reduce the cooking time and potentially eliminate most of the undesirable attributes (Nakitto et al., 2015). Moreover, legume-based milks are gaining popularity as alternatives to bovine milk, mainly because consumers consider them to be cholesterol free, low in fat, and to have bioactive peptides with antioxidant and health promoting properties (Sethi, Tyagi & Anurag, 2016). However, legume-based milks have limitations on their use due to beany flavor and flatulence factors. Common bean milk can be prepared by soaking and dehulling, followed by milk extraction and heat treatment, and thus affords the benefits of reducing anti-nutrients, reducing cooking times, and offering variety in the diet (Nakitto et al., 2015). The aim of this study was to determine the nutritional characteristics of three dry common bean varieties and bean milk produced from them by a process involving soaking, dehulling and heat treatment.

3.2. Materials and methods

3.2.1. Beans collection

Local dry grains of the three varieties of common beans (pinto beans, yellow kidney beans and red haricot beans) were purchased from retail outlet in Nairobi County, Kenya. Five kilograms (5 Kg) was purchased for each of the bean variety and transported to the laboratory wrapped in a Kraft paper. The dry beans were stored at room temperature (20°C to 25°C) in Kraft papers until use.

3.2.2. Preparation of bean milk

Common bean milk was prepared for each of the three common bean varieties at the Food Biochemistry Laboratory of Jomo Kenyatta University of Agriculture and Technology (JKUAT). The common bean milk preparation process was modified from the method described for preparation of soy milk by Ma et al. (2015) and Min et al. (2005) as shown in Figure 3.1. Common bean (100 g) was rinsed in a plastic bowl and soaked in a plastic pot containing 1000 ml of deionized water for 16 hours at room temperature (20 to 25°C).

The soaked common bean seeds were drained, rinsed in 1000 ml of deionized water, dehulled by hands and grounded in a Mika blender (model MBLR4314/WH, Dubai) with a blade speed of 550 rpm. Grinding was done for 3 minutes with 1L boiling water. The boiling water was added to make common bean slurry in a ratio of 1:10 on a weight basis. The resulting slurry was passed through 2 layers of muslin cloth to separate the water-soluble common bean milk material from the hulls and other insoluble matter. The strained milk was heated in a heavy bottom pan to 100°C and this temperature held for 20 minutes, stirring frequently to prevent sticking. The heated bean milk was placed at room temperature (20 to 25°C) and left to cool for 30 minutes and thereafter stored at -20°C until use.

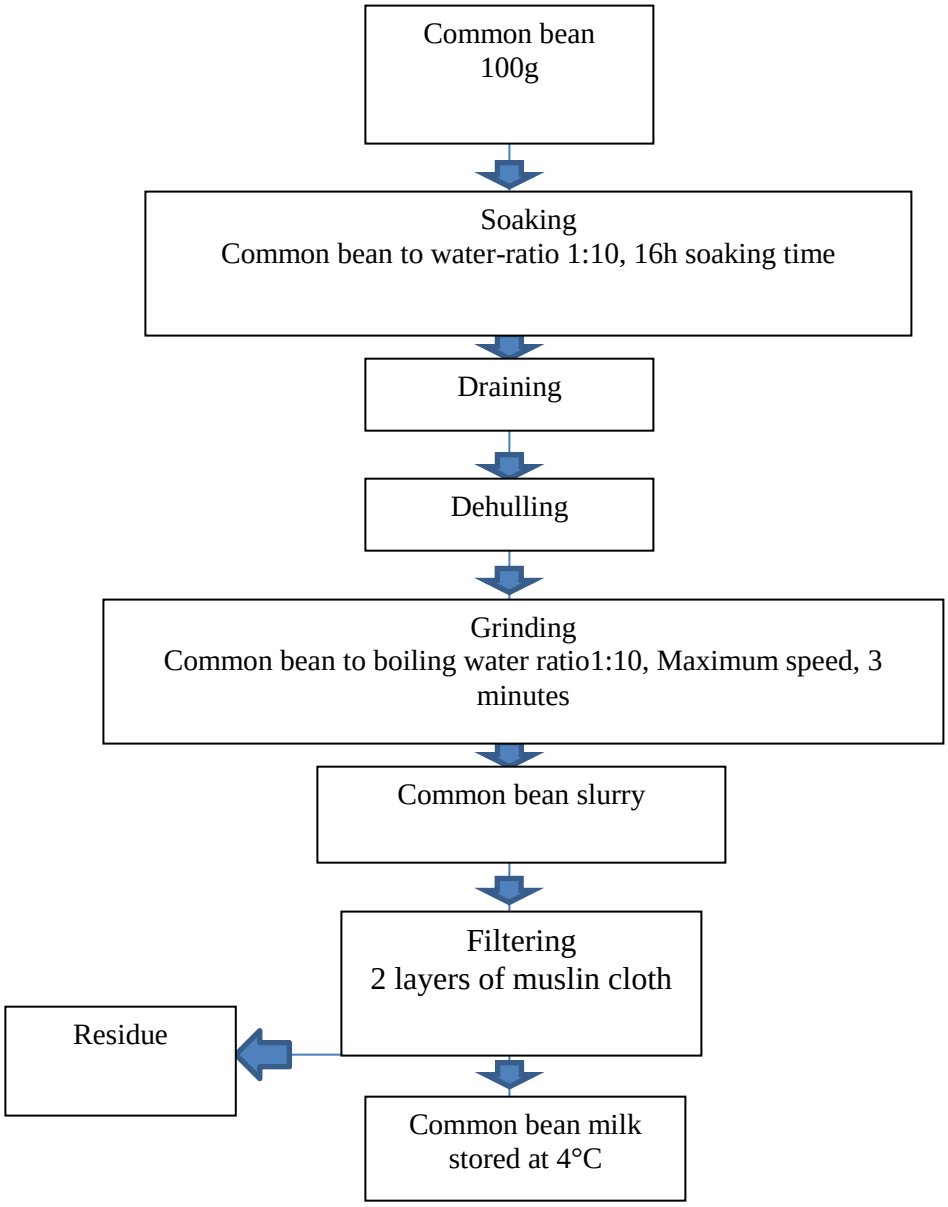

Figure 3.1: Flow diagram for common bean milk processing; modified from Min et al. (2005).

3.2.3. Experimental design

The study was designed as a factorial study design since two sets of independent variables were studied simultaneously. These variables, also referred to as factors or treatments were bean varieties and milk extraction. The specific beans varieties studied were red haricot beans, yellow kidney beans and pinto beans.

3.2.4. Determination of proximate composition

Proximate chemical composition analysis of samples including moisture, total ash, protein, crude fiber and fat content were performed according to Association of Official Analytical Chemist (AOAC) official methods 925.09, 923.03, 979,09, 978.10 and 920.29 respectively (AOAC, 2000). Micro-Kjeldahl method was used to determine the percentage nitrogen which was thereafter converted to crude protein by multiplying with a standard factor of 6.25 (Mosisa & Tura, 2017). The carbohydrate concentration was obtained by difference between 100 and total sum of the percentage of moisture, ash, fat, fiber and protein (AOAC, 2000). These analyses were performed in triplicates.

3.2.5. Determination of mineral concentration

Atomic absorption spectroscopy (UV-1800, Shimadzu Co-operation, Kyoto, Japan) was used to determine the minerals; iron, zinc, calcium, magnesium and phosphorous (Perkin Elmer Coorporation, 1996). To a 5 ml aliquot of bean milk sample in a 100 ml volumetric flask, 50 ml of 24% (w/v) tricarboxylic acid (TCA) was added. The samples were shaken at 5 min intervals for 30 min and filtered using filter paper No. 1. To a 5 ml aliquot of the filtrate transferred to a volumetric flask, 1 ml of 5% (w/v) lanthanum solution were added and made to volume with distilled water. A mixed standard containing 5.0 mg/L Fe, 5.0 mg/L Ca, 0.6 mg/L Mg, 1.6 mg/L Zn, 5.0 mg/L P, 500 mg/L La and 1.2% (w/v) TCA were prepared. All determinations were made versus a reagent blank containing 500 mg/L La and 1.2% TCA.

3.2.6. Determination of phytates, tannins, flavonoids and total phenols

The method of Laparra et al. (2009) was used to determine phytates in triplicates. A 1 ml of bean milk sample was centrifuged at 15000 rpm for 10 minutes to separate the soluble fraction. The supernatant was transferred to 15 ml tubes and 10 ml of H_2SO_4 (1.25%, v/v) was added to each sample. The mixture was agitated for 2 hours in a mechanical shaker and then centrifuged at 2000 rpm for 10 min. The supernatant was filtered through a 0.45 µm membrane filter before injection of 0.1 ml of the supernatant diluted with 0.9 ml of deionized water into the HPLC column (20A Series, Shimadzu Co-operation, Kyoto, Japan).

Phytates content was analyzed by injecting 25 µl into a HPLC system connected to a conductivity detector. The elution rate was set at 1.0 ml/min using gradient of mobile phases (A: deionized water; B: 200 mmol/L NaOH; gradient program 0 to 3 min: 87% A, 3 to 11 min: linear gradient up to 50% A; 11.1 to 15 min: 87% A). Phytates was obtained by matching 25 µl of phytates standards (Inositol, Sigma-P5681 MSDS) with the retention times of the peaks in the common bean milk chromatogram using calibration curves of aqueous standards containing 0.125% H_2SO_4.

The spectrophotometry method of Campos et al. (2009) was used to determine tannins in triplicates. A 1 g of lyophilized sample was placed in a 50 ml flask and mixed with 10 ml methanol. Tinted flask was used to protect the resulting mixture from light and shaken for 24 hours at 25°C. The samples were then centrifuged at 2500 rpm for 10 min. The condensed tannins expressed as mg/g of (+)-catechin equivalents (CE) were obtained by adding 5 ml of vanillin reagent (0.5% vanillin, 4% HCl in methanol) and put in a water bath heated at 30°C for 20 minutes. Condensed tannins were quantified by spectrophotometry using (+)-catechin (up to 0.2 mg/ml) as reference standard. Blank sample was prepared by subjecting the original extract to the same conditions without adding the vanillin reagent to correct for potential interferences from natural pigmentation in beans.

Flavonoids were determined in triplicates using the methods of Xu & Chang (2007). A mixture of 0.25 ml of the sample, 75 µl of 5% $NaNO_2$ solution and 1.25 ml of distilled water was allowed to stand for 6 min before adding 150 µL of 10% $AICl_3.6H_2O$ and allowing to stand for another 5 min. Thereafter, 0.5 mL of 1 M NaOH was added to the resulting solution and the mixture topped up with distilled water to 2.5 mL. The absorbance readings were taken at 510 nm using a UV-visible spectrophotometer. The results were expressed as (+)-catechin equivalents (CE).

Total phenols were determined in triplicates using the method described by Akillioglu & Karakaya (2010). A mixture of 3 ml distilled water, 750 µL of 7% $NaCO_3$, 250 µL of Folin-Ciocalteu's reagents and 50 µL of the sample was incubated at 25°C for 8 min. Thereafter, 950 µL of distilled water was added to the mixture and allowed to stand for 2 hours at 25°C. The absorbance was measured at 765 nm using a UV-visible spectrophotometer and expressed as Gallic acid equivalents (GAE).

3.2.7. Determination of molar ratio of phytates to minerals

The relative bioavailability of calcium, iron and zinc was estimated by the molar ratios of phytates to mineral, that is Phy:Ca, Phy:Fe and Phy:Zn. The molar ratio between phytates to minerals was determined by dividing the mole of phytates with the mole of minerals (Gemede et al., 2016). The mole of minerals and phytates was obtained after dividing the weight of minerals and phytates with their atomic weight (Ca: 40.08 g/mol; Zn: 65.38 g/mol, Fe: 55.85 g/mol and phytates: 660.3 g/mol).

3.2.8. Protein digestibility

The protein digestibility was determined in triplicates using pepsin method by Mertz et al. (1984). A 1 g of the sample was prepared and suspended in 35 ml of a solution of pepsin reagent (1.5mg/ml) in 0.1 M phosphate buffer of pH 2.0. With gentle shaking the mixture was incubated for 3 hours at 37°C. After 3 hours the digestion was stopped by removing the tubes from the water bath (D-91126, Memmert, Aubere Rittebacher, Germany) and

placing them in ice bath for 30 min. The samples were filtered with a filter paper and the residue washed with buffer and dried at 80^{o}C for 2 hours. To a 50 cm^3 micro-kjedahl flask, the dried sample was placed and analyzed for nitrogen by micro-kjedahl digestion. The indigestible nitrogen was subtracted from total nitrogen of the common bean milk sample to obtain nitrogen using the equation;

Digestible nitrogen (N) = Total N in sample – N in residue

Digestible protein = Digestible N (mg) x conversion factor

$$\% \text{ digestibility} = \frac{\% \text{ digestible protein}}{\% \text{ total protein in sample}} \; x \; 100$$

3.2.9. Determination of vitamin B complex (folic acid, pyridoxine, niacin, riboflavin and thiamine)

The methods used by Chase et al. (1993); Ekinci & Kadakal (2005) and Kamman, Labuza & Warthesen (1980) were modified and used to determine concentration of folic acid, pyridoxine, niacin, riboflavin and thiamine. These concentrations were determined in triplicates. A 20 ml of deionized water was added to each of the 50 ml volumetric flask, one containing 5 ml of bean milk sample and the other containing 5g of beans powder. The resulting solution in each case was evenly mixed using a homogenizer (PCU11/98000-18, Kinematica AG Littau, Switzerland) at medium speed for 1 min. The mixture was centrifuged for 15 min at 1500 rpm and water soluble vitamins extracted using Sep-Pak C_{18} (500 mg) cartridges method as described by Cho, Ko & Cheong (2000). Vitamin extracts were passed through FP 30/45 CA-S filters (Schleicher and Schuell, Darmstadt, Germany) with 0.45 μm micropore membrane. A syringe was used to inject 0.45 μm of the filtrate into HPLC column (20A Series, Shimadzu Co-operation, Kyoto, Japan) with a set flow rate of 0.1 mol L^{-1}. The column measurements for this chromatograph were C_{18} 150 mm x 4.6 mm. The mobile phase comprised of KH_2PO_4 at PH 7.0 and methanol in a ratio of 90:10 and was set in isocratic mode at 0.7 mL min^{-1}. The vitamins were identified by comparing their retention times and UV-visible spectra

with those of standards stored in a data bank at 282 nm for folic acid, 324 nm for pyridoxine, 261 nm for niacin, 266 nm for riboflavin, and 234 nm for thiamine.

3.2.10. Determination of oligosaccharides

Oligosaccharides sugars including stachyose, verbascose and raffinose were determined in triplicates using modified methods of Brenes et al. (2003) and Campos et al. (2009). A 100 ml of aqueous ethanol (100 ml, 80%, v/v) was used to homogenized 10 ml of common bean milk sample. A similar concentration was used to homogenize 10 ml of suspension of beans powder. The homogenized mixture was placed in a Soxhlet set at 80°C and monitored for 60 minutes. The extracts were recovered, concentrated under vacuum and thereafter water phase frozen. The frozen concentration was lyophilized and 7 mg of the lyophilized extract re-dissolved in 1 ml of deionized water. The solution was passed through FP 30/45 CA-S filters (Schleicher and Schuell, Darmstadt, Germany) with 0.45 µm micropore membranes and subjected to HPLC (20A Series, Shimadzu Cooperation, Kyoto, Japan) analysis. The mobile phase comprised of water and acetonitrile in a ratio of 65:35 while column flow rate was maintained at 0.1 mol L^{-1} and temperature set at 25°C. Detector temperature was also set at 25°C. A syringe was used to inject 20 µl of the extracted oligosaccharides into HPLC column to obtain peak areas. Verbascose, stachyose and raffinose standards were used to obtain standard curves. The HPLC column was connected to a refractive index detector fitted with Zorbax NH_2 pre-column (4.6 x 12.6 mm, 5 µm) and Zorbax column (250 x 4.6 mm).

3.2.11. Statistical analysis

STATA/SE 12.0 software was used to carryout two-way full factorial ANOVA. Bonferroni least significant difference was used for comparison of means for different treatment groups. The treatment groups comprised of dry bean varieties and milk extracts. Statistical significance was considered at $p<0.05$.

3.3. Results and discussion

3.3.1. Proximate composition of dry beans and bean milk

Proximate composition of beans and corresponding milk extracts was determined and presented in Table 3.1. The specific proximate compositions analyzed were protein, crude fat, crude fiber, crude ash and carbohydrates.

Table 3.1: Proximate composition (protein, crude fat, crude fiber, crude ash and carbohydrates) on dry matter basis of dry beans and corresponding bean milk derived from; red haricot (RH), yellow kidney (YK) and pinto (P) beans.

	Protein (%)		**Crude fat (%)**		**Crude fiber (%)**		**Crude ash (%)**		**Carbohydrates (%)**	
	Beans	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**
RH	22.0 ± 0.3^{b}	17.7 ± 0.2^{a}	1.8 ± 0.1^{a}	3.4 ± 0.1^{c}	5.4 ± 0.1^{b}	ND	4.6 ± 0.1^{b}	1.9 ± 0.1^{a}	66.3 ± 0.5^{b}	77.0 ± 0.2^{c}
YK	20.8 ± 1.3^{b}	26.0 ± 1.7^{c}	2.7 ± 0.1^{b}	3.9 ± 0.2^{cd}	4.6 ± 0.0^{a}	ND	4.6 ± 0.1^{b}	2.0 ± 0.1^{a}	67.3 ± 1.4^{b}	68.2 ± 1.4^{b}
P	23.5 ± 1.0^{bc}	22.4 ± 2.2^{bc}	2.1 ± 0.1^{a}	4.4 ± 0.4^{d}	7.4 ± 0.3^{c}	ND	4.4 ± 0.1^{b}	2.0 ± 0.3^{a}	62.7 ± 1.3^{a}	71.2 ± 2.8^{b}
P-value	<0.02		<0.03		<0.01		<0.01		<0.02	

Results are means ± standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p < 0.05$; ND, not detected).

Crude protein in beans and milk from different varieties ranged from 20.8±1.3% to 23.5±1.0% and 17.7±0.2% to 26.0±1.7%, respectively (Table 3.1). The dry bean crude protein values were within the range of 18.9% to 24.2% previously reported for different common bean varieties (Rehman, Salariya & Zafar, 2001; Vargas et al., 2004). Interestingly, there were varietal differences in protein extraction into bean milk: the crude protein content of milk from red haricot variety was significantly ($p<0.05$) lower than the dry beans; the opposite was true for yellow kidney beans; while milk and bean from pinto did not differ significantly ($p>0.05$). A previous study similarly found that cooked beans from red haricot variety had significantly lower crude protein than the dry beans (Teklehaimanot, 2004). Varietal differences in protein extraction during soymilk preparation have also been reported (Niyibituronsa et al., 2018). Heating during or prior to legume milk extraction causes denaturation of proteins, which decreases solubility and is expected to contribute to reduction of protein extraction into the milk (Mession et al, 2013; Tolano et al., 2016). However, the cause(s) of the varietal differences in protein extraction are not clear and a more detailed study to establish the reason(s) for increase in protein in milk extracted from yellow kidney beans is necessary. Nevertheless, the results imply that yellow kidney bean is a superior variety for extracting milk of higher protein concentration. This could be of nutritional importance since it has been suggested that protein obtained from seeds and vegetable meals is important in meeting a substantial proportion of a person's dietary protein of 0.8 grams of protein per kilogram of body weight and energy requirement (Urua et al., 2013) and, therefore, could be beneficial in combating protein energy malnutrition (PEM).

The crude fat content of the three varieties ranged between 1.8 and 2.7%, which was within the range of 0.6 to 2.9% previously reported for some 52 common bean landraces (Gouveia et al, 2014). Yellow kidney beans had 2.7% fat, which was significantly higher ($p<0.05$) than the other varieties (Table 3.1). In all the three varieties, the fat content of milk was significantly higher than that of dry beans. Heating helps to release fats from plant tissues (Kasmin, Lazim & Awang, 2016) which can explain the higher content of lipids in the milk than the dry seeds. The fat content of the dry beans of the three bean

varieties increased in the order red haricot < pinto < yellow kidney beans, which is the same order observed for the crude protein content in the milk from these varieties. Thus, fat content might influence the ease of extraction of the proteins. Consistent with this possibility, Saso et al. (1999) and Madukwe & Eme (2012) reported that hydrophobic substances including fatty acids or fish oil have inhibitory activity against protein denaturation, which, as mentioned above, contributes to reducing the solubility and extraction of proteins into bean milk.

The mean crude fiber concentration showed significant differences ($p<0.05$) among the three bean varieties. Except for the yellow kidney beans, the fiber concentrations were higher than the range of 4.0% to 5.0% previously reported for common beans (Mosisa & Tura, 2017). Crude fiber was not detected in the extracted bean milk which is consistent with a previous report on legumes which demonstrated that soymilk contained only a trace of crude fiber (Niyibituronsa et al., 2018). The elimination of crude fiber during bean milk preparation may lead to loss of some of the health benefits associated with bean consumption since dietary fiber has been associated with reduced constipation and reduced cancer incidence, particularly ovary, mouth, and pharynx cancer (Shankar & Lanza, 1991). Hence, when this milk is recommended for consumption it should be taken in combination with high fiber food. Nevertheless, crude fiber greatly underestimates dietary fiber (Tosh & Yada, 2010), and a more detailed study of the extent of retention of dietary fiber in bean milk would be necessary.

Dry beans were found to contain crude ash in the range of 4.4±0.1% to 4.6±0.1% (Table 3.1). These values were comparable to those reported for red haricot beans (4.7%), pinto beans (4.9%) and kidney beans (3.3% to 3.6%) (Maria et al., 2012). Extraction of milk from dry beans reduced crude ash concentration by more than 50% (Table 1). The reduction in ash concentration is expected to have occurred by leaching of soluble inorganic minerals during soaking. Moreover, dehulling also contributes to loss of minerals with the seed coats (Mosisa & Tura, 2017).

Carbohydrate concentration in milk from the three varieties was higher than the corresponding dry beans, and such differences were significant ($p<0.05$) for red haricot beans and pinto beans, but not yellow kidney beans. In all cases, the increased carbohydrate content in the milk is partly due to the elimination of crude fiber and reduction in ash (Table 3.1). Since pinto beans had significantly higher fiber than red haricot beans and yellow kidney beans ($p<0.05$), the loss in fiber reflected in a higher increase in carbohydrate concentration in the milk extracted from pinto beans variety. Likewise, the higher increase in carbohydrate in red haricot milk is explained by lower protein extraction from this variety. Therefore, bean milk could be a good source of energy.

3.3.2. Mineral concentration of dry beans and bean milk

The literature reviewed showed that legumes contain considerable proportions of iron, zinc, magnesium, calcium and phosphorus. Table 3.2 shows proportions of these minerals in dry beans and bean milk. They are essential for the body and they are needed for physiological functioning of the brain, heart and skeletal muscles (de Baaij, Hoenderop & Bindels, 2015). They are also important in the formation of new bones and repair of existing bones (Beto, 2015) as well as growth and maintenance of immune function (Walker et al., 2005).

Table 3.2: Mineral content (mg/100 g) of dry beans and corresponding bean milk derived from; red haricot (RH), yellow kidney (YK) and pinto (P) beans.

	Iron (mg/100 g)		Zinc (mg/100 g)		Calcium (mg/100 g)		Magnesium (mg/100 g)		Phosphorus (mg/100 g)	
	Beans	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**
RH	6.8 ± 0.6^{a}	1.5 ± 0.1^{b}	2.8 ± 0.5^{c}	0.7 ± 0.1^{a}	106.8 ± 5.3^{c}	41.5 ± 0.9^{a}	126.9 ± 5.7^{e}	23.4 ± 1.2^{a}	182.6 ± 6.6^{b}	17.9 ± 0.1^{a}
YK	6.4 ± 0.3^{a}	1.3 ± 0.1^{b}	1.5 ± 0.1^{b}	1.3 ± 0.1^{b}	99.0 ± 0.6^{c}	45.2 ± 0.2^{a}	72.2 ± 1.6^{d}	33.0 ± 1.6^{b}	183.1 ± 3.8^{b}	12.7 ± 0.2^{a}
P	6.0 ± 0.3^{a}	1.2 ± 0.1^{b}	3.7 ± 0.3^{c}	0.5 ± 0.1^{a}	109.7 ± 7.2^{c}	62.3 ± 4.1^{b}	65.1 ± 5.0^{d}	46.5 ± 1.8^{c}	343.0 ± 18.5^{c}	13.3 ± 0.2^{a}
P-value	<0.01		<0.02		<0.01		<0.01		<0.01	

Results are means ± standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p < 0.05$).

There were no inter-varietal significant differences ($p>0.05$) in the iron and calcium contents in dry beans of the three varieties. On the other hand, there were inter-varietal significant differences ($p<0.05$) in the magnesium concentrations of the bean milk. Iron concentrations in dry beans ranged from 6.0±0.3 mg/100 g to 6.8±0.6 mg/100 g and were within the range of 6.0 mg/100 g to 23.8 mg/100 g previously reported for different common beans (Lucia et al., 2012).

Dry red haricot and pinto beans contained the highest concentration of zinc (2.8±0.5 mg/100 g and 3.7±0.3 mg/100 g respectively) while yellow kidney beans contained the least (1.5±0.1 mg/100 g). A slightly higher range of 3.4±0.1 mg/100 g to 4.3±0.1 mg/100 g of zinc in beans was previously reported (Lucia et al., 2012). Calcium concentration in dry beans ranged from 99.0±0.6 mg/100 g to 109.7±7.2 mg/100 g. The results were within the range of 100 mg/100 g to 260 mg/100 g reported for different common beans (Moraghan, Etchevers & Padilha, 2006). Significantly higher ($p<0.05$) phosphorous concentration of 343.0±18.5 mg/100 g was contained in pinto beans. This value was lower than those reported by previous works for different common beans which ranged from 400 to 560 mg/100 g (Paredes, Becerra & Tay, 2009). The bean milks contained significantly lower ($p<0.05$) minerals than the dry beans. Minerals are components of the crude ash (Eneobong & Obizoba, 1996) and were therefore expected to be lost by leaching into the soaking water and as a result of dehulling. Therefore, beans and bean milk could be a good source of dietary minerals. The daily dietary reference intakes (DRI) for calcium in children below the age of 59 months range from 210 to 500 mg, 0.27 mg to 11 mg for iron, 30 mg to 80 mg for magnesium, 100 mg to 460 mg for phosphorous and 2 mg to 3 mg for zinc (USDA, 2011). Consumption of 200 mg of bean milk could meet a substantial portion of the recommended dietary allowance (RDA) of minerals in children.

3.3.3. Phenolic compounds and phytates of dry beans and bean milk

There are various types of phenolic substances in beans, including tannins and flavonoids. Table 3.3 shows the contents of phenolic substances and phytates found in the three bean varieties. The contents of tannins, flavonoids, total polyphenols and phytates in the bean milks were much lower than in the dry beans, consistent with leaching of these products into the soaking water and their removal with the seed coats during dehulling (Elmoneim et al, 2012).

Table 3.3: Phenolic compounds and phytates content (mg/100 g) of dry beans and corresponding bean milk derived from; red haricot (RH), yellow kidney (YK) and pinto (P) beans.

	Tannins (mg/100 g)		**Flavonoids (mg/100 g)**		**Total Phenols (mg/100 g)**		**Phytates (mg/100 g)**	
	Beans	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**
RH	288.6 ± 8.7^{f}	31.9 ± 1.8^{c}	217.4 ± 6.3^{e}	21.3 ± 1.9^{c}	530.60 ± 17.3^{d}	47 ± 1.4^{b}	256.6 ± 16.4^{d}	57.6 ± 2.4^{ab}
YK	238.3 ± 5.0^{d}	14.3 ± 0.4^{a}	179.2 ± 2.6^{d}	10.6 ± 1.2^{a}	540.4 ± 5.0^{d}	30 ± 2.1^{a}	223.3 ± 6.5^{c}	65.9 ± 1.5^{b}
P	251.9 ± 1.1^{e}	24.3 ± 0.9^{b}	256.7 ± 1.7^{f}	16.7 ± 1.1^{b}	440.7 ± 16.2^{c}	45 ± 2.3^{b}	274.8 ± 6.7^{d}	52.8 ± 1.3^{a}
P-value	<0.01		<0.01		<0.01		<0.01	

Results are means ± standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p < 0.05$).

The range of tannin contents in Table 3.3 (251.9 mg/100 g to 288.6 mg/100 g) was within the range of 215 mg/100 g to 290 mg/100 g which was previously reported for common beans (Mamiro et al., 2017; Silva et al., 2018). Phenolic substances especially tannins form complexes with nutrients such as carbohydrates and proteins, and thus hinder protein and starch digestibility (Cruz et al., 2003; Salunkhe et al., 1990). Flavonoids concentrations in dry beans ranged from 179.2±2.6 mg/100 g to 256.7±1.7 mg/100 g (Table 3.3) and were higher than those previously reported for selected common beans in the range of 127±0.1 mg/100 g to 140±0.1 mg/100 g (Akillioglu & Karakaya, 2010) but lower than 259 mg/100 g reported for kidney bean (Ren et al., 2012). The differences in flavonoids concentration observed in these studies could be due to genetic variation in the common bean type studied (Krishnaiah et al., 2009). Additionally, a wide range of values have been reported for the total phenols in common beans. For example Silva et al. (2018) found 188mg/100 g and 344 mg/100 g in two bean cultivars, while Yao et al. (2011) reported that common beans contained 859 mg/100 g total phenols. Thus, the range of 440-540 mg/100 g found in this study lies within the range of previously reported values.

On average, dry pinto beans (274.8±6.7 mg/100 g) and red haricot beans (256.6±16.4 mg/100 g) had significantly higher ($p<0.05$) phytates than yellow kidney beans (223.3±6.5 mg/100 g). These values were within the range of 17 mg/100 g to 915 mg/100 g previously reported by Gibson et al. (2010) for different common beans varieties. Flavonoids and phytates bind to metal ions and thus reduce bioavailability of minerals such as iron, zinc and calcium (Akillioglu & Karakaya, 2010; Gibson et al., 2010). Thus, it is important to compare the losses of minerals and the losses of these phytochemicals during bean milk preparation in order to determine whether losses in the phytochemicals led to improved mineral bioavailability.

3.3.4. Determination of molar ratio of phytates to minerals

Table 3.4 shows the molar ratios of phytates: iron, phytates: zinc and phytates: calcium. Phytates: minerals molar ratio is an important parameter used to estimate potential bioavailability of selected minerals (Kiewlicz & Rybicka, 2020). The molar ratio for

phytate: calcium should be <0.17, for phytates: zinc <15 and for phytates: iron <1 (preferably <0.4) (Peltzer et al., 2014).

Table 3.4: Comparison of molar ratio of phytates to minerals (iron, zinc and calcium) in dry beans and milk from three bean varieties.

	Phytates: Iron		**Phytates: Zinc**		**Phytates: Calcium**	
	Beans	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**
RH	3.25	3	9.75	9	0.15	0.09
YK	3.09	5	17	5	0.14	0.09
P	3.82	4	7	8	0.16	0.05

The literature data shows that the higher the molar ratio is, the lower mineral bioavailability occurs (Gemede et al., 2016; Hailu & Addis, 2016; Kiewlicz & Rybicka, 2020). The highest value of phytates: zinc was observed in yellow kidney beans. However, after extraction of milk it had a molar ratio lower than 15, indicative of higher mineral bioavailability in milk than seed of this bean variety. Phytates: iron molar ratios for both beans and milk of all the three bean varieties were >1 signifying poor iron bioavailability. This could be due to higher phytates content in beans and insufficient phytic acid degradation by boiling and soaking (Hailu & Addis, 2016; Norhaizan & Norfaizadatul, 2009). The phytates: calcium molar ratio in all the three bean varieties and their corresponding milk extract was >0.17, which is regarded as favorable for calcium absorption (Gemede et al., 2016), predicting that a good calcium bioavailability could be obtained from all the bean varieties and their corresponding milk extract.

3.3.5. Protein digestibility of dry beans and bean milk

Protein digestibility generally refers to how well protein is digested and its amino acids absorbed in the body (Nakitto et al., 2015). Protein digestibility is a potential indicator of

the quality of dietary protein (Bastidas et al., 2010). Table 3.5 shows the protein digestibility of dry beans and their corresponding milk extracts.

Table 3.5: Percentage protein digestibility of dry beans and corresponding bean milk derived from; red haricot, yellow kidney and pinto beans.

	% protein digestibility in dry beans	**% protein digestibility in milk (%)**
Red haricot	74.7 ± 1.4^{a}	86.7 ± 3.3^{c}
Yellow kidney	81.9 ± 1.6^{bc}	82.9 ± 0.1^{c}
Pinto	78.2 ± 1.8^{ab}	81.0 ± 3.3^{bc}
P-value	0.03	

Results are means ± standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p < 0.05$).

Milk from all the three bean varieties had higher protein digestibility than the corresponding dry beans, and the difference was significant ($p<0.05$) for red haricot but not the other varieties. The increase in protein digestibility could be due to structural modifications in the globulins during heating process leading to increased solubility and improved heat induced gelation (Clemente et al., 1998) as well as less interaction with tannins (Table 3.3). Therefore, milk extracted from red haricot beans has potential to provide quality dietary protein than the corresponding beans.

3.3.6. Vitamin concentration of dry beans and bean milk

Table 3.6 shows vitamin concentration in dry beans and bean milk. These vitamins play important roles in cell metabolism. Additionally, niacin plays a role in lipid and nucleic acid synthesis, pyridoxine aid in biosynthesis of neurotransmitters while folate is involved in erythropoiesis (Gu & Li, 2016). As expected, vitamin B values in dry beans was statistically higher ($p<0.05$) than concentration observed in bean milk.

Table 3.6: Vitamin content of dry beans and corresponding bean milk derived from; red haricot (RH), yellow kidney (YK) and pinto (P) beans.

	Thiamine (mg/100 g)			Riboflavin (µg/100g)			Niacin (mg/100 g)			Pyridoxine (mg/100 g)			Folic acid (mg/100 g)		
	RH	**YK**	**P**	**RH**	**YK**	**P**	**RH**	**YK**	**P**	**RH**	**YK**	**P**	**RH**	**YK**	**P**
Beans	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	215.3 ± 3.2^{e}	133.4 ± 1.1^{d}	119.8 ± 7.5^{d}	2.4 ± 0.1^{d}	1.5 ± 0.4^{b}	2.1 ± 0.2^{c}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.0^{b}	0.4 ± 0.1^{abc}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}
Milk	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	88.1 ± 1.6^{c}	52.2 ± 3.4^{b}	40.0 ± 4.7^{ab}	0.5 ± 0.0^{a}	0.4 ± 0.0^{a}	0.5 ± 0.1^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}
P-Value	<0.01			<0.01			0.02			<0.01			0.71		

Results are means ± standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p<0.05$).

As indicated in Table 3.6, dry common beans had 0.6 mg/100 g of thiamine and bean milk contained 0.2 mg/100 g concentration of thiamine. These values are within the range of 6±0.1 mg/100 g and 0.2 mg/100 g reported by Khalil & Mansour (1995) for dry and cooked faba beans respectively. However, compared to thiamine concentration contained in soymilk, bean milk contains low levels (Sarkar, Morrison & Somerset, 1998). The significant decrease ($p<0.05$) in thiamine content could be due to increased thiaminase activity in milk extracts and formation of complex due to heating of bean milk (Khetarpaul & Chauhan, 1989).

Red haricot beans contained significantly higher ($p<0.05$) riboflavin than yellow kidney beans and pinto beans in that order. Milk extracted from yellow kidney beans and red haricot beans had riboflavin concentration of 52.2±3.4 μg/100g and 88.1±1.6 μg/100g. These values were higher than 40 to 60 μg/100g reported for cooked kidney beans and red haricot beans (Kotue et al., 2018). The statistically high riboflavin content in milk extracted from red haricot beans and yellow kidney beans may be as a result of a more complete extraction of coenzyme forms, e.g. FAD and FMN (Arena et al., 2014).

Niacin concentration was significantly higher ($p<0.05$) in red haricot beans followed by pinto beans and then yellow kidney beans. Niacin concentration for cooked soybeans (0.4 mg/100 g) reported by Sarkar et al. (1998) was in agreement with the niacin values observed in bean milk. Niacin is heat stable (Barampama & Simard, 1995) hence the decrease in niacin concentration in bean milk could be due to effect of soaking and dehulling.

Pyridoxine concentration was statistically similar in dry beans (0.4 mg/100 g) as well as bean milk (0.1 mg/100 g). On the other hand, the present study found no significant change ($p>0.05$) in folic acid concentration during preparation of bean milk. However, on average, folic acid was higher in bean milk (0.3 to 0.4 mg/100 g) than those reported for cooked red haricot and pinto beans (0.1 to 0.2 mg/100 g) (Kotue et al., 2018). The study reveals that retention of Vitamin B-group in milk is not influenced by the bean variety

from which the milk sample was extracted. Instead, retention of the B-group vitamin is dependent with the effect of the processing method on the individual vitamins. B-group vitamin retention was observed in the order folic acid>riboflavin>thiamine>pyridoxine >niacin.

3.3.7. Oligosaccharides concentration of dry beans and bean milk

The major oligosaccharide sugars contained in bean and milk are raffinose, verbascose and stachyose sugars (Campos et al., 2009). They are non-digestible short-chain carbohydrates which are passed undigested to the colon of humans (Nnam, 1997). Here they are broken down by the gut flora to hydrogen, carbon dioxide and methane gases which cause abdominal bloating (Wang et al., 1974). The concentrations of these sugars in common beans and corresponding bean milk is presented in Table 3.7.

Table 3.7: Oligosaccharides content (mg/100 g) of dry beans and corresponding bean milk derived from; red haricot (RH), yellow kidney (YK) and pinto (P) beans.

	Raffinose (mg/100 g)		**Verbascose (mg/100 g)**		**Stachyose (mg/100 g)**	
	Beans	**Milk**	**Beans**	**Milk**	**Beans**	**Milk**
Red haricot	0.5 ± 0.0^{b}	0.4 ± 0.0^{a}	0.3 ± 0.1^{ab}	0.2 ± 0.0^{a}	4.0 ± 0.1^{b}	3.4 ± 0.1^{a}
Yellow kidney	0.6 ± 0.1^{b}	0.4 ± 0.0^{a}	0.3 ± 0.1^{bc}	0.4 ± 0.0^{c}	4.9 ± 0.1^{c}	4.2 ± 0.2^{b}
Pinto	0.5 ± 0.0^{b}	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	4.7 ± 0.0^{c}	3.6 ± 0.4^{ab}
P value	0.08		0.03		<0.01	

Results are means ± standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p < 0.05$).

Soaking beans prior to dehulling and cooking the extracted bean milk significantly ($p<0.05$) reduced the α-galactosides raffinose and stachyose (Table. 3.7). Raffinose levels was reduced by 20 to 33.3% during preparation of the bean milk. Queiroz et al. (2002) observed 25% reduction in raffinose of cooked common beans which had been soaked for 9 hours. Raffinose can cause flatulence in susceptible people leading to abdominal

discomfort but other studies have shown that positive effects can be attributed to oligosaccharides. Raffinose can be used as source of carbon and energy by *Bifidobacteria* in the micro-biota (Zartl et al., 2018) and due to their antagonistic effect they could inhibit activity of the colonic putrefactive bacteria, reducing the formation of toxic fermentation products. Thus, additional studies on reduction of raffinose as a flatulence factor to susceptible people considering its bifidogenic benefits could be explored.

Verbascose was the oligosaccharide found in the smallest amount in beans and bean milk (Table 3.7). This concurs with previous findings which had demonstrated low levels of verbascose sugars (Oboh et al., 2000). Soaking, dehulling and cooking did not cause significant change in verbascose concentration of dry common beans and bean milk. This is contrary to findings reported by Queiroz et al. (2002) who demonstrated reduction in verbascose concentration of soaked and cooked beans. The reduction could be attributed to cooking procedure since in the later study cooking water was discarded which was not the case in bean milk preparation procedure used in the current study.

Stachyose was quantified in the highest amount in both dry common beans and bean milk (Table 3.7). This agrees with the previous study by Queiroz et al. (2002) which reported significantly higher stachyose concentration (3.2 mg/100 g) in common beans compared to other oligosaccharide sugars (raffinose, 0.4 mg/100 g and verbascose, 0.12 mg/100 g). Significant reduction ($p<0.05$) in stachyose concentration was observed in bean milk. This may be attributable to soaking which has been reported to activate α-galactosidases activities (Oboh et al., 2000) leading to cleaving of the galactose unit of low molecular weight oligosaccharides into soluble fraction. The significantly lower stachyose concentration in milk extracted from red haricot beans could be due to the difference in the degree of α galactosidases activity of the bean cultivars, red haricot beans and yellow kidney beans.

3.4. Conclusion

All the three common beans varieties were found to contain high dietary protein, carbohydrates, fiber, B-group vitamins and minerals (calcium, phosphorous, iron, zinc and calcium). The three bean varieties also had high protein digestibility and low amount of crude fat making beans to be healthy food in the diet. However, they contained high amount of anti-nutrients which included tannins, phytates, flavonoids and total phenols and oligosaccharides (raffinose and stachyose). Common bean milk from the three varieties had high amount of protein and carbohydrates but reduced B-group vitamins, minerals, oligosaccharides, phenolic substances and phytates. The reduction in minerals and vitamins during milk preparation was less than the reduction of phytates, and thus the bean milk may have better vitamin and mineral bioavailability than the dry beans. Additionally, the protein digestibility of the milk was higher than the dry beans, an indication of higher bioavailability of protein in bean milk than the dry beans.

CHAPTER FOUR

GROWTH AND SURVIVAL OF PROBIOTIC LACTIC ACID BACTERIA IN COMMON BEAN 'MILK'

4.1. Introduction

Consumption of foods containing probiotic microorganisms is increasingly becoming popular worldwide due to their perceived health benefits (LeBlanc et al., 2011). Probiotic microorganisms do not only positively alter the quality attributes of the food products on which they are used, but also contribute to the gastrointestinal tract health and the overall wellbeing of the consumers (Hasan & Sultan, 2014). Studies involving probiotic lactic acid bacteria (LAB) fermentation of vegetable milk extracted from soybeans showed significant reduction in the anti-nutrients and flatulence factors caused by oligosaccharide sugars (Farnworth et al., 2007; Onwurafor, Onweluzo & Ezeoke, 2014). Zartl et al. (2018) reported that fermentation of legumes with probiotics reduced non-digestible raffinose family oligosaccharides and galactomannans by more than 50%. Indeed, with the increasing consumer knowledge on the health benefits of fermented product (LeBlanc et al., 2010), fermentation with probiotic bacteria provides a unique opportunity for enhancing diversity of common bean consumption.

A probiotic product must contain live microorganisms which when administered in adequate amount confers health benefits (WHO & FAO, 2006) such as enhancement of immune response, improvement of intestinal health, reduction of serum cholesterol and prevention of non-communicable diseases (Kechagia et al., 2013). This implies that probiotics are viable by definition, hence as noted by Lahtinen (2012), survival of the probiotics is prerequisite for health benefits. This study was designed to evaluate growth and survival of probiotic bacteria in common bean milk during and after fermentation.

4.2. Materials and methods

4.2.1. Raw materials

Dry beans for each of the bean varieties were purchased from a local retail store in Nairobi, Kenya and transported to JKUAT as described in Section 3.2.1.

4.2.2. Probiotic culture, microbial media and reagents collection

Yoba Fiti culture which contained *Streptococcus thermophilus* and *Lactobacillus rhamnosus* GR1 was obtained from Yoghurt Fiti production unit of JKUAT. Yoflex (YF L-903) which contained *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and mixed probiotic bacteria starter cultures ABT which contained *Streptococcus thermophilus, Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* BB-12 were obtained from Sigma-Aldrich. Similarly, De Man, Rogosa, Sharpe (MRS) and M17 microbial cultures and all other chemicals used were obtained from Sigma-Aldrich.

4.2.3. Preparation of bean milk

Bean milk was prepared using the modified method described for preparation of soymilk by Ma et al. (2015) and Min et al. (2005). Detailed description of this method is presented in Section 3.2.2.

4.2.4. Experimental design

The study was designed as a factorial study design since three independent variables were studied simultaneously. These variables, also known as factors or treatments were bean varieties, extraction of milk and fermentation.

4.2.5. Fermentation of bean milk

Each of the bean milk samples was put in four 250 ml plastic bottles as described by Mpofu et al. (2014) and inoculated with three starter cultures at 1g/l of sample. The three starter cultures used were; mixed starter cultures YF L-903, ABT and Yoba Fiti. The controls were not inoculated with the starter culture strains. The fermentations were carried out in triplicates using three independent bean milk samples.

All samples were incubated at 45°C in a Heartherm microbiological incubator (IS62, Yamato Scientific Co. LTD., Tokyo, Japan) for 17 hours or until a pH ≤4.3 was attained as described in the instruction for use in the starter culture sachets and then refrigerated for 28 days at 4°C consistent with previous studies (Heller, 2001; Mani et al., 2014; Mpofu et al., 2014). The samples for analysis were collected at 2 hours interval for the first 16 hours, 7 day, 14 day, 21 day and 28 day for pH monitoring. The progress of the fermentation was determined by microbial enumeration on different types of media and titrable acidity determination at 0 day of storage, 7 days of storage, 14 days of storage, 21 days of storage and 28 days of storage under a sterile clean bench (PAU-1300BG, Dalton Corporation, Indiana).

4.2.6. Microbial enumeration during storage of the fermented bean milk samples

The enumeration of probiotic bacteria present in Yoba Fiti culture was performed on MRS agar (Mani et al., 2014). MRS with 0.5% L-cysteine was used to enumerate bacteria contained in ABT culture while M17 was used to enumerate probiotic bacteria present in YF L-903 culture (Mani et al., 2014). The cultures were prepared as described in Appendix 1. The microbes in the naturally fermented samples were enumerated on MRS, MRS-L-Cysteine and M17. The experiment was performed in triplicate. Five milliliters of the fermentation solution were transferred into a sterile 9 ml test tube and vortexed to mix (PCU11, Kinematica AG Littau, Switzerland). One milliliter of the mixture was transferred to 9 ml test tube containing quarter-strength Ringer's solution to make serial 10-fold dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Ten microliters (10 µl) of the

aliquots from different dilutions were transferred to autoclaved (AL-300, Sibata Scientific Technology LTD., Tokyo, Japan) petri dishes containing MRS, MRS with 0.5% L-Cysteine, and M17 agar and spread plated. A 50 μg/ml of fusidic acid (CEM-102) was added to each of the petri dishes containing agar broth for its inhibitory activities against MRS strains (Jones et al., 2011). This was followed by incubating the agar plates in a Heartherm microbiological incubator (IS62, Yamato Scientific Co. LTD., Tokyo, Japan) at their respective growth temperature and conditions (Appendix 1). The incubated plates were counted daily for up to 4 days to determine the bacterial colony forming (CFU), using the following formula:

$$\text{CFU/ml} = {}^{Number\ of\ colonies}/_{Volume\ of\ inoculum} X\ Dilution\ factor$$

4.2.7. Determination of pH of fermented bean milk

The pH of the common bean milk samples were determined by electrode immersion with a pH meter (Henshall, 2012). Fifty milliliters of each bean milk samples were used to determine pH using pH meter. The pH meter was calibrated with potassium hydrogen phthalate and phosphate standard buffer solutions at pH 4.0 and pH 7.0 respectively before pH measurement was taken.

4.2.8. Determination of titrable acidity of fermented bean milk

The method of Falade, Ogundele & Ogunshe (2015) was used to determine titrable acidity. Twenty-five milliliters (25 ml) of each of the bean milk samples was pipetted into conical flasks and two drops of 0.1 N phenolphthalein indicator added. The resulting mixture was titrated against 0.1 N NaOH until the first permanent pink colour appeared. The titrable acidity was then calculated and expressed as percent lactic acid (AOAC, 2000).

4.2.9. Statistical analysis

The results are averages of three independent fermentations. Statistical analysis for enumeration of probiotic LAB was conducted on log transformation of bacterial counts. Analysis of variance on STATA/SE 12.0 was used to analyze the data to show significant treatment effects. Mean separation was done using Benferroni's method and comparisons were considered significantly different if $p<0.05$.

4.3. Results and discussion

4.3.1. Growth of probiotic bacteria present in starter cultures

Figure 4.1 presents CFU counts for bean milk inoculated with probiotic bacteria while Figure 4.2 presents CFU counts at the end of fermentation (pH=4.3). Initial inocula ranged from Log_{10} 4.1±0.2 to Log_{10} 4.9±0.3 cfu/ml and was statistically similar ($p>0.05$) for corresponding culture of each milk extract.

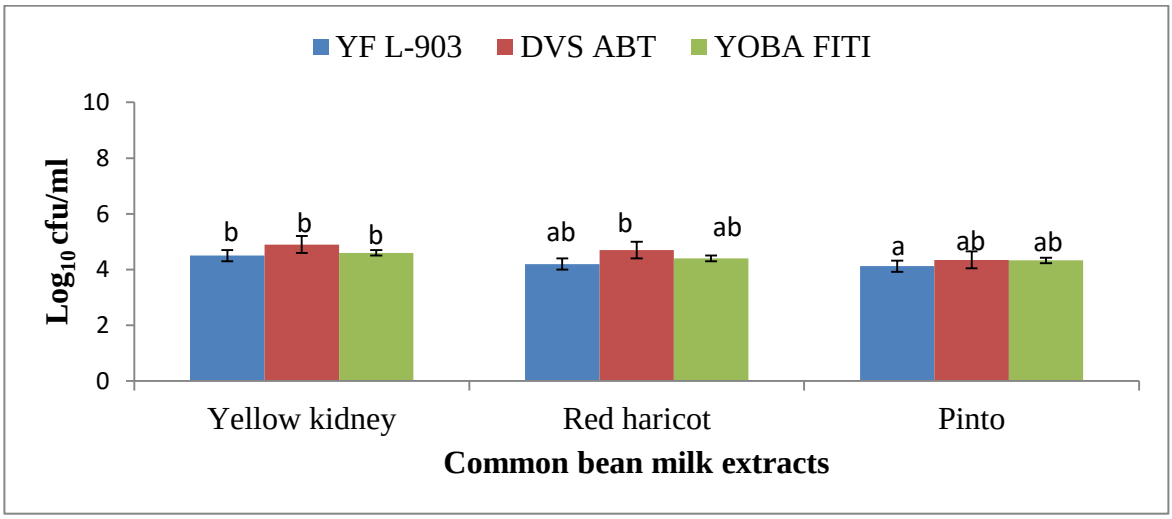

Figure 4.1: Initial CFU counts for probiotic bacteria starter cultures: YF L-903, ABT and Yoba Fiti at inoculation.

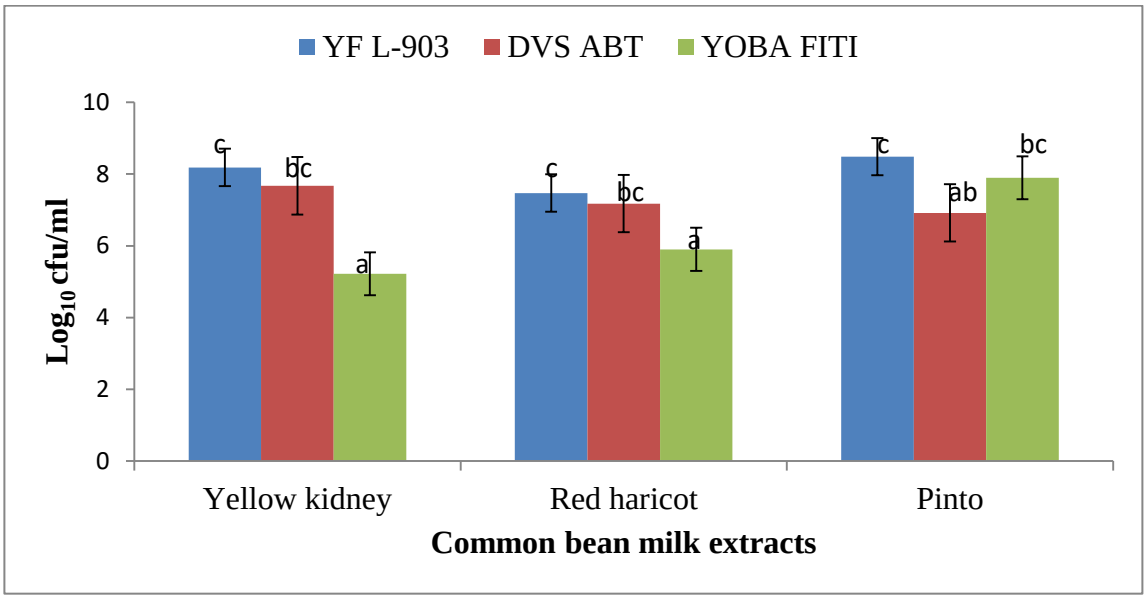

Figure 4.2: CFU counts for probiotic bacteria starter cultures: Yoflex (YF L-903), ABT and Yoba Fiti in milk extracts at the end of fermentation (at pH=4.3) at the end of fermentation. Values are averages of three independent fermentations. Bars are standard deviation of averages.

At the end of the fermentation, counts varied from Log_{10} 5.2±0.2 to Log_{10} 8.5±0.6 cfu/ml. Colony counts greater than Log_{10} 6 which is the recommended dose by Shin, Lee & Pestka (2000) to receive the health benefits of a probiotic product was observed in bean milk fermented with YF L-903 culture which had log_{10} 7.5±0.3 to log_{10} 8.5±0.6 cfu/ml and ABT culture which had log_{10} 6.9±0.7 to log_{10} 7.7±0.6 cfu/ml (Figure 4.2). However, probiotic bacteria counts greater than log_{10} 6 in milk fermented with Yoba Fiti culture was only observed in milk extracted from pinto beans which had log_{10} 7.9±0.4 cfu/ml. LAB probiotic counts in milk fermented with YF L-903 and ABT cultures were within the range of 2 x 10^7 to 2 x 10^9 reported for cow's milk fermented with different probiotic starter cultures (Mani et al., 2014). Counts of probiotic bacteria in milk extracted from pinto beans after fermentation with Yoba Fiti culture was also within the range reported by Mani et al. (2014) for cow milk fermented with different strains of probiotic bacteria. HPLC analysis of oligosaccharide sugars by Adeyemo & Onilude (2014) showed that soy bean

milk is a good substrate for growth of probiotic bacteria since they used these sugars to support their growth. Such studies have hardly been reported for bean milk, nevertheless, bean milk could be a suitable substrate for the growth of probiotic bacteria present in YF L-903 and ABT cultures because *L. delbrueckii* subsp. *bulgaricus, L. acidophilus* La-5 and *B. animalis* BB-12 use sucrose and oligosaccharides sugars as their main source of energy due to the presence of β-galactosidase (Da et al., 2006). Besides, bacteria contained in the large intestine including strains of *Bifidobacteria, Lactobacillus* and *Streptococcus* have α-galactosidase enzyme and have been reported to utilize galacto-oligosaccharides (Liener, 2000; Vaclavik & Christian, 2014). Probiotic bacteria present in YF L-903 and ABT cultures grew well in milk extracted from red haricot and yellow kidney beans (Figure 4.2). Interestingly, milk extracted from these beans had significantly higher stachyose concentration of 4.2±0.2 and verbascose concentration of 0.4±0.0 (Table 3.7), but after fermentation they had similar concentrations ($p>0.5$) to other milk extracts. This could be an indication that these probiotics assimilated well stachyose and verbascose sugars leading to better growth in the two milk samples. However, this led to reduction in prebiotics which are necessary for gut flora and growth of probiotics contained in fermented bean milk (Moro et al., 2006). Consumption of bean milk along with prebiotic fiber food could potentially compensate for the loss of prebiotics in fermented bean milk.

4.3.2. Survival of probiotic bacteria present in starter cultures

Survival of probiotic bacteria was monitored over a 28-day refrigerated (4°C) storage period after fermentation. Significant decline in number of colonies was observed as storage period increased except in milk extracted from red haricot beans fermented with YF L-903 (Figure 4.3). In contrast, there were no colonies observed in naturally fermented milk extracted from common beans during the 28 days storage period.

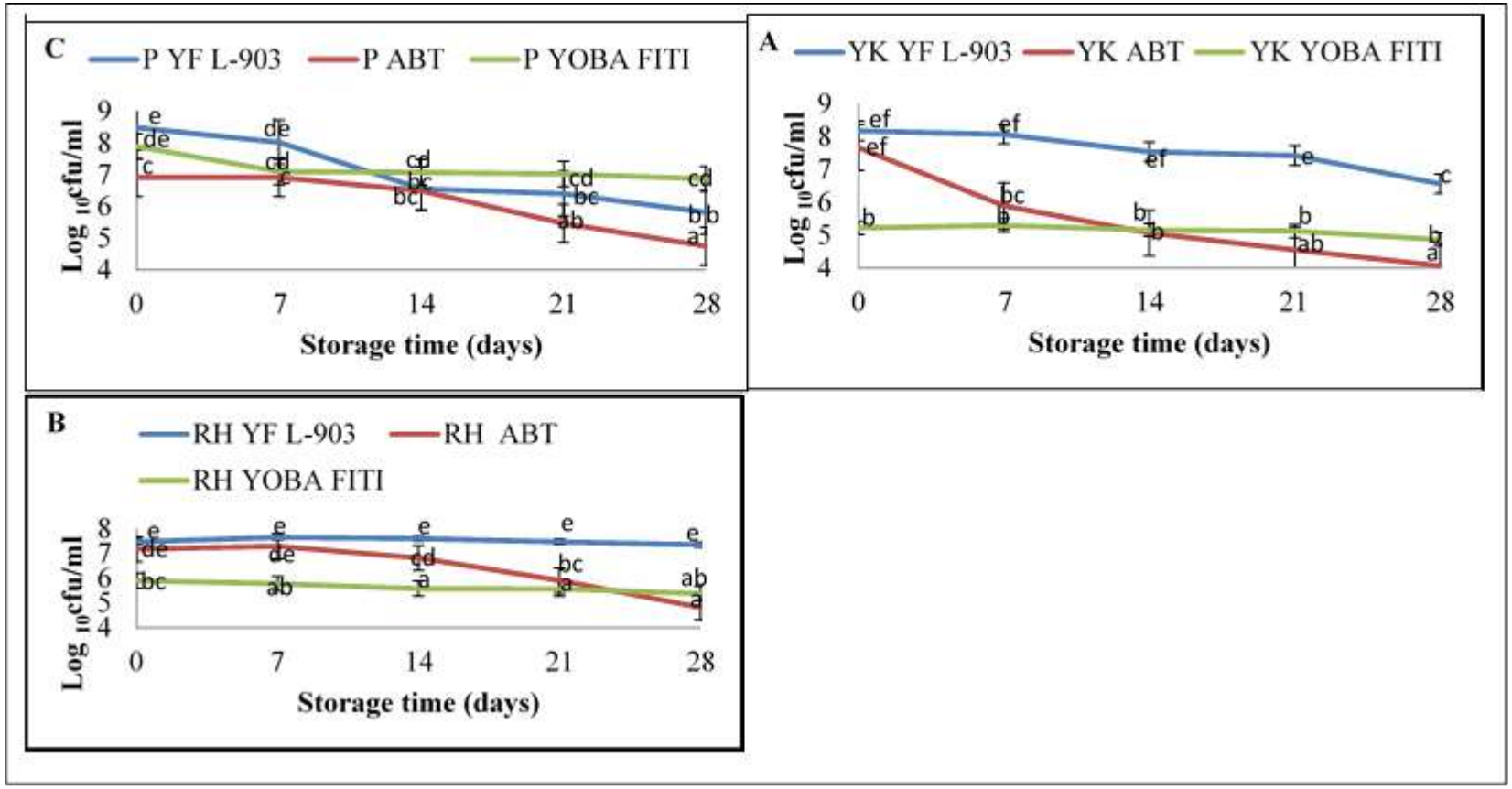

Figure 4.3: Characterization of survival of probiotic starter cultures: Yoflex (YF L-903), ABT and Yoba Fiti in milk extracts from yellow kidney beans (YF) (Figure A), red haricot beans (RH) (Figure B) and pinto beans (P) (Figure C). Values are averages of three independent fermentations. Bars are standard deviation of averages.

To maintain confidence in a probiotic product, it is important to demonstrate a good survival of the probiotic bacteria throughout the shelf-life of the product (Kort et al., 2015). Probiotic bacteria present in bean milk fermented with YF L-903 was greater than $\log_{10}$ 6.6 cfu/ml during the 28 days refrigeration storage in milk samples extracted from yellow kidney beans and red haricot beans (Figure 4.3a and b) and greater than $\log_{10}$ 6.4 cfu/ml within 21 days of storage in milk samples extracted from pinto beans (Figure 4.3c). These values indicate loss in viability of $\log_{10}$ 1.6 cfu/ml in milk samples extracted from red haricot beans and $\log_{10}$ 2.6 cfu/ml in milk samples extracted from pinto beans during the 28 days refrigeration storage at 4°C. The viable cells though remained at levels greater than $\log_{10}$ 6 cfu/ml, which is the recommended level for a probiotic product to confer beneficial health effects. Viable cells of probiotics contained in bean milk fermented with Yoba Fiti culture was greater than $\log_{10}$ 6.6 cfu/ml during the 28 days storage in milk samples extracted from pinto beans (Figure 4.3c) and had a stable viability with loss of < $\log_{10}$ 1 cfu/ml. On the other hand, viable cells of probiotic bacteria in milk fermented with ABT culture was greater than $\log_{10}$ 6 cfu/ml within the first week of storage in milk samples extracted from yellow kidney beans (Figure 4.3a) and after the second week of storage in milk samples extracted from red haricot beans and pinto beans (Figure 4.3b and c). Refrigerated storage for 28 days at 4°C reduced probiotic population in bean milk fermented with ABT culture by $\log_{10}$ 2.2 cfu/ml in samples extracted from pinto beans and $\log_{10}$ 3.6 cfu/ml in samples extracted from yellow kidney beans. Damin et al. (2008) observed loss of $\log_{10}$ 2 cfu/ml in yoghurt fermented with *L. acidophilus* and Mani et al. (2014) reported loss of $\log_{10}$ 1.5 cfu/ml in cow milk fermented with *L. acidophilus* after 28 days of storage and $\log_{10}$ 2 cfu/ml to $\log_{10}$ 3 cfu/ml loss in plain yoghurt fermented with *L. reuteri*.

The decline in probiotic population could be due to the cessation of metabolic activity of probiotic bacteria due to long cold temperature storage. Sengupta et al. (2013) observed that low temperature of refrigeration inhibited growth of LAB, especially those bacteria with an optimum temperature range of 30°C to 32°C. Decline in probiotic population may also be attributed to post acidification. The extent of loss in survival due to post

acidification is influenced by individual probiotic bacteria present in the fermenting culture (Shah, 2000). Dave and Shah (1997) reported antagonistic effect against probiotics of *L. delbrueckii* subs. *bulgaricus* and *L. acidophilus* due to secretion of inhibition metabolites such as bacteriocins and hydrogen peroxide which can partially damage the probiotic cells. As noted by Kailasapathy and Chin (2000) the tolerance of probiotics to the product is strain specific hence suitable probiotic strains are those that can maintain their survival and stability during production and storage of a product. In general loss of viability in the study was influenced by the bean variety, strain type and the culture mixture. These are the same factors that influence the shelf life of a food product (Odu, Egbo & Okonko, 2012). Therefore, the fermenting culture with the longest shelf-life of 21 days refrigerated storage at 4°C was YF L-903. Based on these findings it is recommended that YF L-903 culture should be used in fermenting bean milk in order to confer health benefits by the time of consumption.

4.3.3. pH of fermented bean milk

pH concentration of the bean milk reduced significantly during fermentation (Figure 4.4). Initially bean milk fermented with ABT and YF L-903 cultures had faster decrease in pH, regardless of the bean variety (Figure 4.4), but later comparable milk extracts fermented with Yoba Fiti culture had a faster decrease rate so that the pH became comparable at 16 hours (Figure 4.4c). These bean milk samples attained the desired pH $\leq$4.3 within 16 hours of fermentation. Reduction in pH is expected during LAB fermentation due to acidification by the fermenting bacteria. Reports on changes in pH of soymilk during fermentation with starter cultures containing single probiotic LAB indicated that pH reduced from 6.5 to 4.5 after 12 hours of fermentation (Farnworth et al., 2007). In the current study, the desired pH of 4.3 was attained within 16 hours of fermentation with probiotic LAB starter cultures.

However, as shown in Figure 4.5, during 28 days of cold storage (4°C), pH concentration of the fermented bean milk was very stable for both LAB fermented milk and naturally fermented milk samples. Similar reports showed reduction in pH (4.16 and 4.40) of two commercial probiotic yoghurts during 35 days refrigeration storage at 5°C, when the initial pH values were 4.3 and 4.6 respectively (Dave & Shah, 1997). Mani et al. (2014) obtained pH of 4.0 to 4.4 for 16 fermented milk samples stored at 5°C, when the initial pH was pH 4.4 to 4.6.

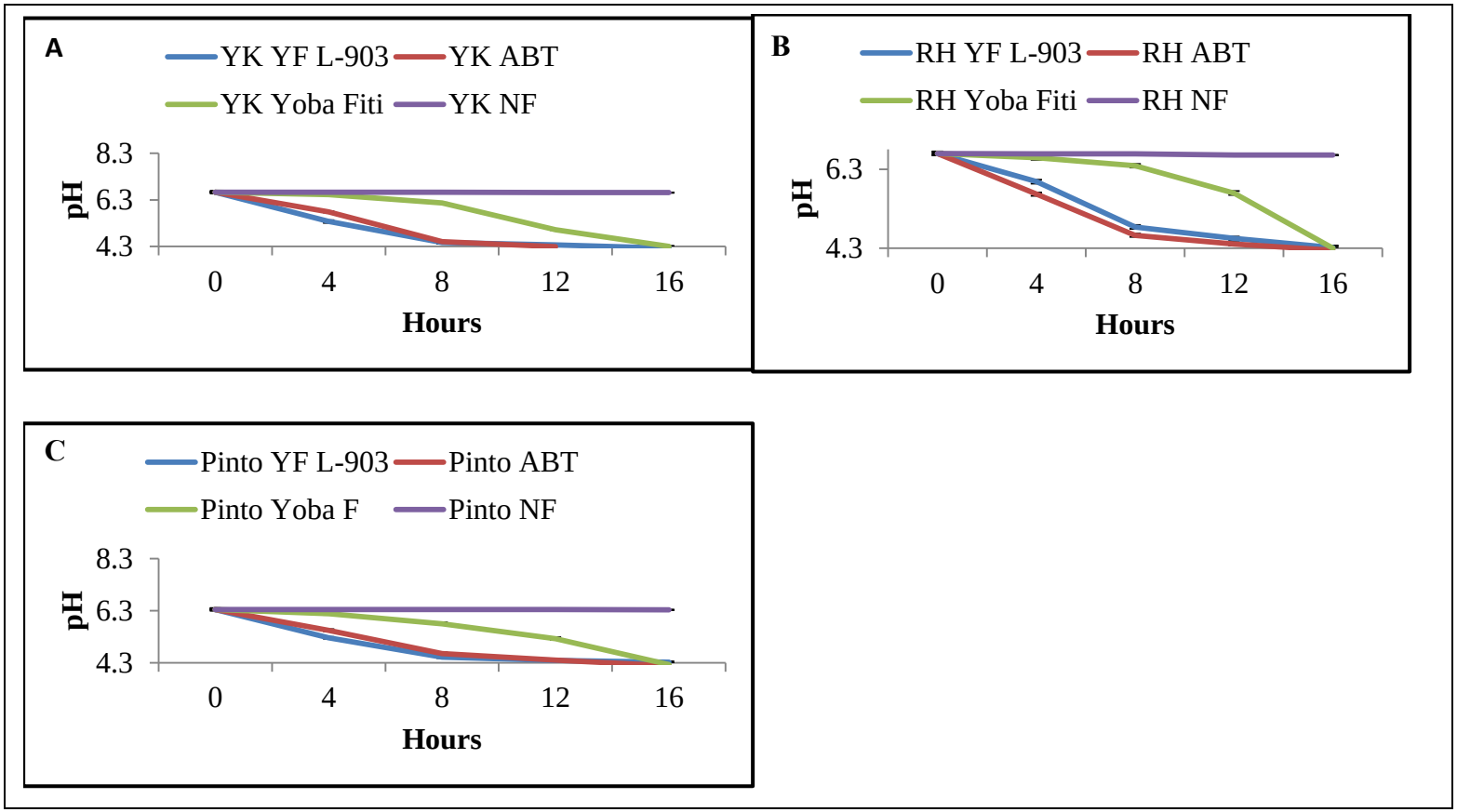

Figure 4.4: Fermentation time to reach pH 4.3 in milk extracted from yellow kidney beans (YF) (Figure A), red haricot beans (RH) (Figure B) and pinto beans (P) (Figure C) during natural fermentation (NF) and fermentation with probiotic bacteria starter cultures: Yoflex (YF L-903), ABT and Yoba Fiti. Values are averages of three independent fermentations. Bars are standard deviation of averages.

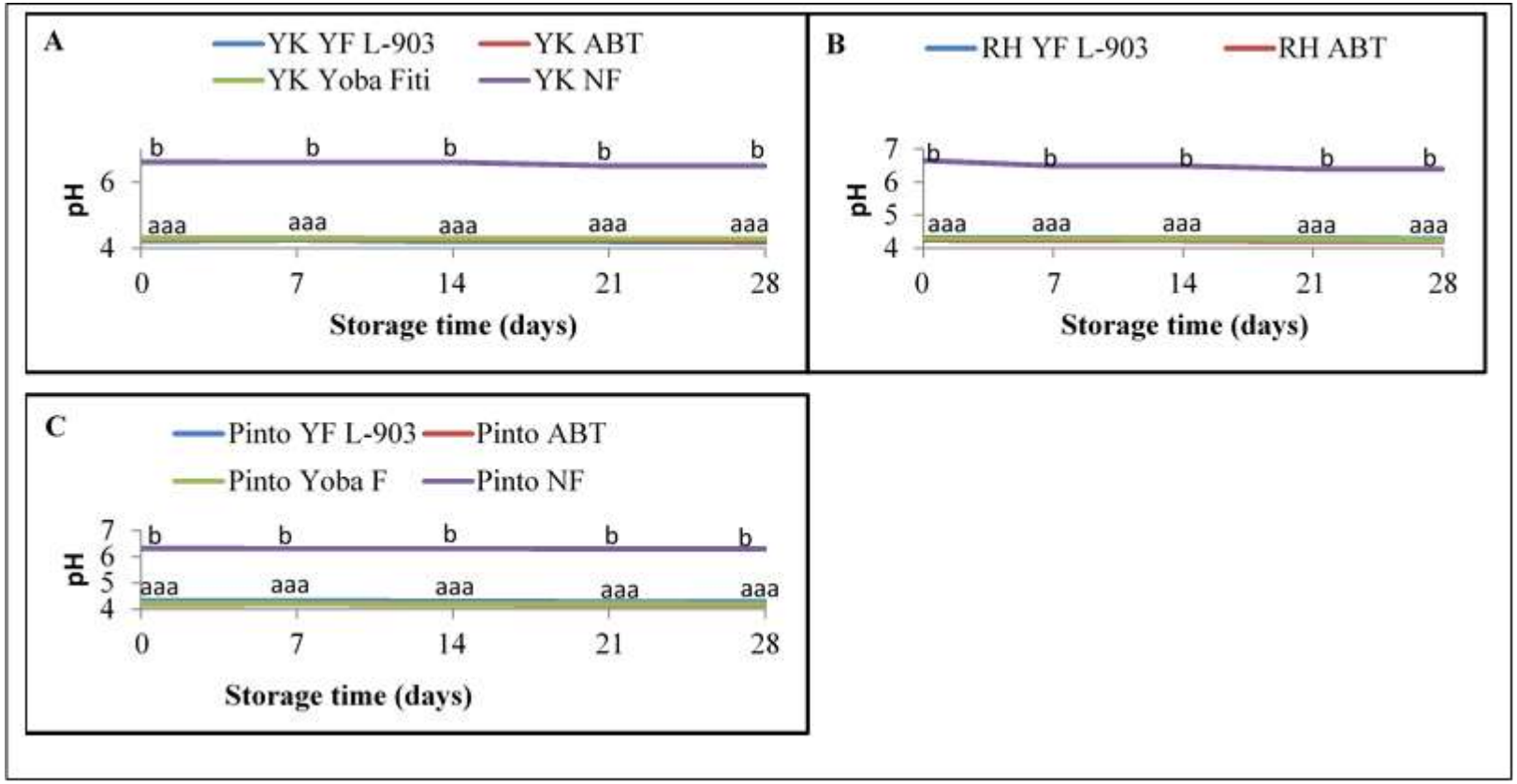

Figure 4.5: Changes in pH of milk extracted from yellow kidney beans (YF) (Figure A), red haricot beans (RH) (Figure B) and pinto beans (P) (Figure C) fermented naturally (NF) and with probiotic bacteria starter cultures: Yoflex (YF L-903); ABT and Yoba Fiti during 28 days refrigerator storage. Values are averages of three independent fermentations. Bars are standard deviation of averages.

The reduction in pH values was reported to be due to residual acidification during formation of lactic acid (Mani et al., 2014). The reduction in pH reported in these studies contrast stable pH values during refrigeration storage at 4°C obtained in the current study. Stijepić et al. (2013) who reported stable pH value for probiotic plain soy yoghurt during 20 days refrigeration storage at 4°C demonstrated similar results to those observed in the current study. Therefore, the current study confirms existence in variation in residual acidification which could probably be due to differences in the LAB used for yoghurt fermentation (Damin et al., 2008). Differences in the storage temperatures and duration of storage of the fermented milk samples could also be possible reasons for variation in the pH values. pH concentration of a product was reported to be important quality for viability of fermenting microorganism (Shin et al., 2000). However, in the current study viability of the probiotic bacteria present in the fermenting culture was independent of the pH concentration.

4.3.4 Titrable acidity of fermented bean milk with storage

Titrable acidity was measured in fermented bean milk samples. Overall, titrable acidity concentration for the three bean milk samples increased with storage time as shown in Figure 4.6.

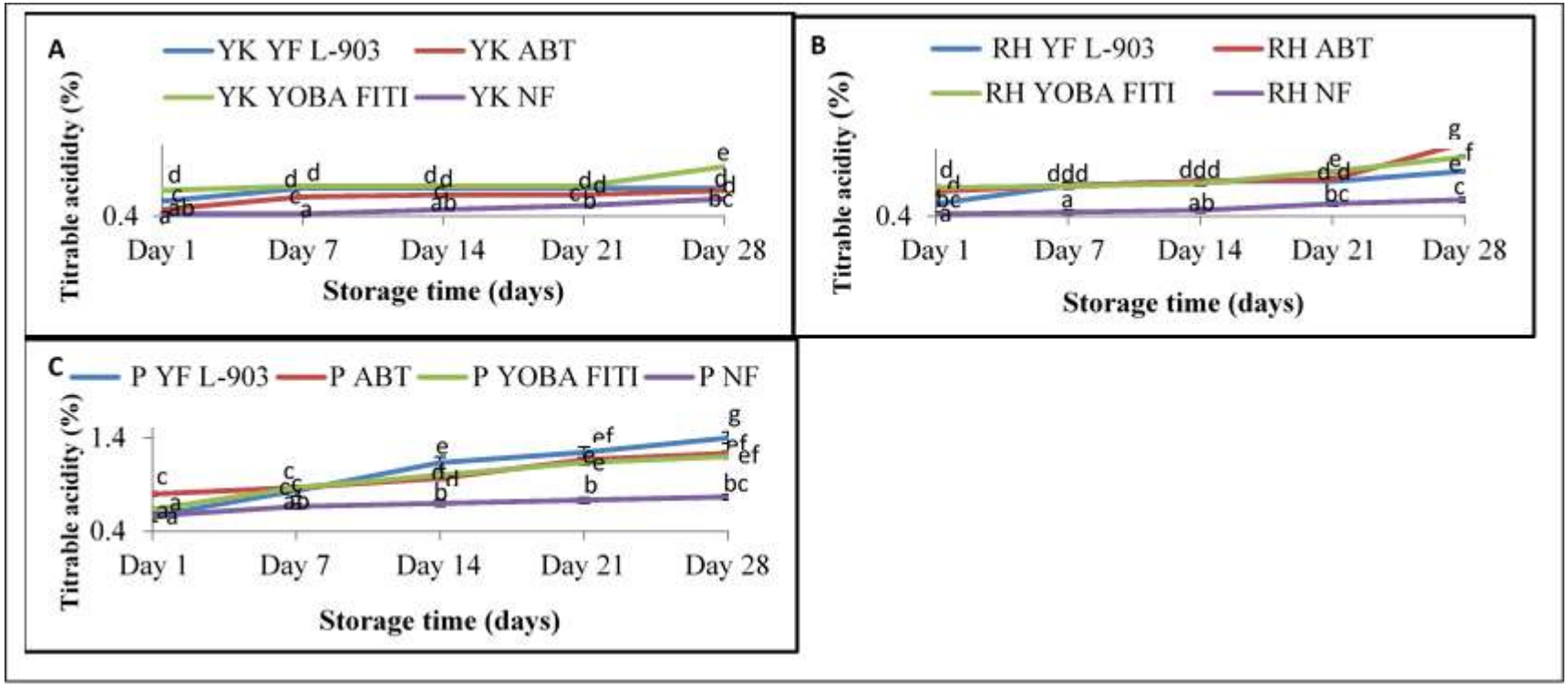

Figure 4.6: Changes in titrable acidity (TTA) of milk extracted from yellow kidney beans (YF) (Figure A), red haricot beans (RH) (Figure B) and pinto beans (P) (Figure C) fermented naturally (NF) and with probiotic bacteria starter cultures: Yoflex (YF L-903), ABT and Yoba Fiti. Values are averages of three independent fermentations. Bars are standard deviation of averages.

Titrable acidity concentration ranged from 0.4 to 1.2% in fermented milk samples extracted from yellow kidney beans (Figure 4.6a), 0.4 to 1.4% in fermented milk samples extracted from red haricot beans (Figure 4.6b) and 0.6 to 1.4 in fermented milk samples extracted from pinto beans (Figure 4.6c). Titrable acidity concentration in naturally fermented milk was significantly different from those fermented with starter cultures. They ranged from 0.4% to 0.8% in naturally fermented bean milk and 0.5% to 1.4% in bean milk samples fermented with starter cultures. These values are within the range of 0.8% to 1.47% for day 1 and day 8 respectively reported by (Osunddahunsi et al., 2007) for plain soy yoghurt. Falade, Ogundele and Ogunshe (2015) reported a gradual increase of 1.5% to 1.7% from day 1 to day 9 in titrable acidity of soy yoghurt stored at 7°C and a faster increase of 1.5% to 2.5% in soy yoghurt samples stored at 27°C. Additionally, Murevanhema (2012), reported a faster increase in titrable acidity of bambara groundnut milk stored at 15°C to 25°C. They attributed increased titrable activity during storage to growth of the bacteria in fermented milk. However, as there was no microbial growth observed during the storage period (Figure 4.3), increase in titrable acidity in the present study could have been due to other factors. Schmidt et al. (1996) indicated that in addition to bacterial growth and pH changes, factors such as age and protein content can impact the titrable acidity of milk and its associated products. Therefore, as protein of fermented milk increased (Table 5.1), so did the titrable acidity value potentially due to buffering effect and accumulation of organic acids from breakdown of proteins by metabolic activities of the bacteria present in the fermenting culture (Navarro et al., 2019). The organic acids present harsh environment for viability of probiotic bacteria but may favor development of yeasts and moulds leading to spoilage (Kaddumukasa et al., 2017).

4.4. Conclusion

Common bean milk is a suitable substrate for probiotic lactic acid bacteria since CFUs enumerated were more than the recommended counts for a probiotic product to have health benefit. Fermented bean milk could confer health benefits such as such as preventing allergies, boosting immune system, treating gastrointestinal disorders and prevention of atopic dementia in children due to the presence of probiotics. However,

survival of the probiotic LAB during 28 days refrigerated storage at 4°C was only attained in milk extracted from red haricot and yellow kidney beans fermented with common dairy starter culture YF L-903 and milk extracted from pinto beans fermented with Yoba Fiti culture. pH did not change during storage but post acidification was observed in all the fermented bean milk regardless of the fermenting starter culture used. Therefore, the study recommends milk extracted from pinto beans as the most suitable vehicle for probiotics studied and YF L-903 starter culture as the most appropriate in fermenting bean milk due to its ability to sustain survival to levels that could confer health benefit for longer storage duration. These potential health benefits include improved immune system, reduced allergic reactions, reduced gastrointestinal disorders and reduced eczema.

CHAPTER FIVE

CHEMICAL COMPOSITION OF FERMENTED 'MILK' EXTRACTED FROM YELLOW KIDNEY, RED HARICOT AND PINTO COMMON BEAN (PHASEOLUS VULGARIS L.) VARIETIES

5.1. Introduction

Milk extracted from common beans (*Phaseolus vulgaris* L.) is a nutrient-rich food that contains 15 to 28% protein and 62 to 81% carbohydrates (Gouveia et al., 2014; Kotue et al., 2018). Bean milk also contains vitamins (especially B-complex) and minerals (Gouveia et al., 2014). In low resource households, common bean consumption supplies on average 5 to 11% of the daily calorie demand and 28% of the daily protein (Silva et al., 2012). The protein from common bean milk provides sulfur-containing amino acids which are lacking in grain cereals (Landbouwcatalogus, 1990). Bean milk contain type B starch which is less digestible and lower in glycemic index (Campos et al., 2009). Thus, bean milk can lower post-prandial blood glucose levels to lower incidences of non-communicable diseases (NCDs) and reduce prevalence of protein energy malnutrition (Niba, 2003).

However, the plant based forms of nutrients such as protein, minerals and vitamins are reported to be less bioavailable than the equivalent forms in animal products, potentially due to phytates and phytochemicals (Offiah, Abuh & Yusufu, 2017). Household preparation methods such as fermentation could be used to improve the quality of bean milk (Gibson, Perlas & Hotz, 2006). Fermentation has been advocated for as a suitable readily adaptable food processing method at house level with potential to enhance nutrient and energy densities (Offiah et al., 2017). Both natural and controlled fermentation of soymilk has been reported to increase protein, ash and total solids content (Obadina et al., 2013). Gabriel, Akinyosoye & Adetuyi (2011) observed increased mineral content in jack beans fermented with lactic acid bacteria (LAB). The effect of fermentation as an additional processing method to bean milk has not been extensively studied. The aim of

this research was to determine the proximate composition, phytates and phenolic compounds, minerals, B-complex vitamins and oligosaccharide composition of fermented bean milk.

5.2. Materials and methods

5.2.1. Raw materials

Dry beans for each of the bean varieties were purchased from a local retail store in Nairobi, Kenya and transported to JKUAT as described in Section 3.2.1.

5.2.2. Probiotic culture, microbial media and reagents collection

Probiotic culture, microbial media and reagents were collected as described in Section 4.2.2.

5.2.3. Preparation of bean milk

Bean milk was prepared using the modified method described for preparation of soymilk by Ma et al. (2015) and Min et al. (2005). Detailed description of this method is presented in Section 3.2.2.

5.2.4. Experimental study design

The experimental study design adopted is as described in Section 4.2.4.

5.2.5. Fermentation of bean milk

Each of the bean milk samples (200 ml) was put in four 250 ml plastic bottles. Three of these bottles containing bean milk were inoculated with three starter cultures at 1g/l of sample. The fermentations were carried out in triplicate for each of the bean milk samples. The remaining samples were not fermented and were used as control. All the inoculated

samples were incubated at 45°C in a Heratherm microbiological incubator (IS62, Yamato Scientific Co. LTD., Tokyo, Japan) until a pH ≤4.3 was attained.

5.2.6. Determination of proximate composition for fermented and non-fermented bean milk

Proximate chemical composition analysis of fermented and non-fermented bean milk samples was performed according to AOAC as presented in Section 3.2.4.

5.2.7. Determination of mineral concentration for fermented and non-fermented bean milk

Atomic absorption spectroscopy (UV-1800, Shimadzu Co-operation, Kyoto, Japan) was used to determine the minerals; iron, zinc, calcium, magnesium and phosphorous as described in Section 3.2.5 using the methods of Perkin Elmer Coorporation (1996).

5.2.8. Determination of phenolic compounds and phytates for fermented and non-fermented bean milk

Phenolic compounds and phytates were determined as described in Section 3.2.6.

5.2.9. Determination of vitamin B complex (thiamine, riboflavin, pyridoxine and folic acid) for fermented and non-fermented milk

Thiamine, riboflavin, pyridoxine, niacin and folic acid were extracted using methods described by Chase et al. (1993); Ekinci and Kadakal (2005); Kamman et al. (1980). Detailed description of these methods is presented in Section 3.2.9.

5.2.10. Determination of oligosaccharides for fermented and non-fermented milk

The modified methods of Brenes et al. (2003) and Campos et al. (2009) presented in Section 3.2.10 were used to determine verbascose, stachyose and raffinose concentration.

5.2.11. Statistical analysis

All data were subjected to two-way full factorial ANOVA using STATA/SE 12.0 software for windows to identify significant treatment effects. Percent change in proportions of bean milk parameters (minerals, vitamins, phytates, phytochemicals and oligosaccharides) was determined by dividing the difference in weight of beans and milk with the corresponding weight of beans. Comparison among means for different groups was made using Bonferroni least significant difference (LSD) test at $p \leq 0.05$.

5.3. Results and discussion

5.3.1. Proximate composition of fermented bean milk

Table 5.1 shows the effect of fermentation on proximate composition of bean milk. The results show that crude protein increased for each variety when compared to its control, but the increase was not significant ($p > 0.05$) except in milk extracted from pinto beans fermented with YF L-903 which had significantly ($p < 0.05$) higher protein.

Table 5.1: Proximate composition of fermented milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties fermented with probiotic starter cultures; Yoflex (YF L-903), ABT and Yoba Fiti (YF).

	Crude protein (%)			Crude ash (%)			Crude fat (%)			Carbohydrate (%)		
	RH	**YK**	**P**	**RH**	**YK**	**P**	**RH**	**YK**	**P**	**RH**	**YK**	**P**
NF	17.7 ± 0.2^{a}	26.0 ± 1.7^{bc}	22.4 ± 2.2^{b}	1.9 ± 0.1^{b}	2.0 ± 0.1^{b}	2.0 ± 0.3^{b}	3.4 ± 0.1^{a}	3.9 ± 0.2^{abc}	4.4 ± 0.4^{bcd}	77.0 ± 0.2^{c}	68.2 ± 1.4^{ab}	71.2 ± 2.8^{b}
YF L-903	18.3 ± 0.2^{a}	28.0 ± 1.0^{c}	27.1 ± 0.5^{c}	1.9 ± 0.2^{b}	1.9 ± 0.2^{b}	2.3 ± 0.2^{b}	3.8 ± 0.1^{ab}	4.0 ± 0.1^{bc}	4.5 ± 0.1^{d}	76.0 ± 0.1^{c}	66.1 ± 1.1^{a}	66.1 ± 0.4^{a}
ABT	18.2 ± 0.6^{a}	27.7 ± 0.8^{c}	25.1 ± 0.6^{b}	1.8 ± 0.1^{b}	1.7 ± 0.0^{a}	1.8 ± 0.0^{b}	3.9 ± 0.2^{ab}	3.9 ± 0.1^{b}	4.7 ± 0.0^{d}	75.9 ± 0.9^{c}	66.6 ± 0.7^{a}	68.4 ± 0.6^{b}
YF	18.0 ± 0.7^{a}	26.9 ± 0.2^{c}	23.9 ± 0.8^{b}	1.6 ± 0.0^{a}	2.2 ± 0.2^{b}	2.0 ± 0.2^{b}	3.5 ± 0.1^{a}	3.9 ± 0.1^{b}	4.4 ± 0.1^{cd}	76.8 ± 0.8^{c}	67.0 ± 0.3^{a}	69.7 ± 0.6^{b}
P	0.02			0.04			0.03			0.04		

Results are means ± standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p<0.05$; NF, non-fermented).

Previous studies have reported different outcome of fermentation on protein concentration. On one hand, decrease in protein in controlled fermentation of *Vigna racemosa* flour was reported by Difo et al. (2015). On the other hand, a study on lactic acid fermentation of South African maize based porridge found significant increase in protein concentration (Chelule et al., 2010). Additionally, Obadina et al. (2013) reported increase in protein concentration of soy milk after natural fermentation due to anabolic processes leading to polymer build up and microbial cell proliferation. As earlier presented in Figure 4.4c milk extracted from pinto beans fermented with YF L-903 had significantly higher microbial cell proliferation which could be the reason for the significantly higher protein compared to those fermented with the rest of the fermenting culture.

Crude ash contained in non-fermented milk extracted from the three bean varieties were statistically similar at $p>0.05$ (Table 5.1). No definite trend was observed on the ash content of fermented bean milk. Fermentation significantly reduced crude ash in milk extracted from red haricot beans and yellow kidney bean varieties fermented with Yoba Fiti and ABT cultures respectively. However, fermentation did not cause significant changes in other ash values of bean milks irrespective of the fermenting culture. The decrease in crude ash was probably due to increase in crude protein. Difo et al. (2015) found similar results in crude ash of fermented pearl millet.

There was no significant change in crude fat upon fermentation for all bean varieties regardless of the fermenting culture. This result concurs with that reported by Difo et al. (2015) who found no significant changes in crude fat concentration of fermented pearl millet. Contradictory report on crude fat content of fermented milk extracted from soy beans and tamarind seeds are available. Obadina et al. (2013) reported that natural fermentation significantly reduced fat content of fermented soy milk while Omotola et al. (2017) observed increase in crude fat content of tamarind seed. Unlike other proximate compositions of legumes, little has been reported in literature on change in crude fat after fermentation. This may be due to the fact that legumes are generally low in crude fat

(Gouveia et al., 2014). However, such studies are essential for common beans due to their high crude fat content compared to most legumes.

Carbohydrate in non-fermented milk varied from 68.2±1.4 to 77.0±0.2%. There was insignificant change in carbohydrates of fermented bean milk except milk extracted from pinto beans fermented with YF L-903 culture, which had significantly less carbohydrates than corresponding fermented milk, possibly due to significantly higher microbial growth (Figure 4.3). Osman (2011) studied changes in carbohydrates of pearl millet and observed decrease in carbohydrates concentration after 8 to 12 h fermentation. The reduction in carbohydrate was attributed to the action of microbial α and β amylases. Additionally, Obadina et al. (2013) reported reduction in carbohydrate concentration of soy milk by 1.5% at 0 h to 0.6% at 72 h of fermentation due to increased activities of the fermenting microorganisms. Since fermentation took place as indicated by increased microbial growth (Figure 4.1 and 4.2), reduced pH (Figure 4.4) and oligosaccharide sugars (Table 5.5), the insignificant change in carbohydrates could be attributed to the difference method used to estimate carbohydrates concentration. This method involved obtaining carbohydrate by difference between 100 and total sum of the percentage of crude ash, crude fat, crude fiber and crude protein (AOAC, 2000). Therefore, the changes in carbohydrates concentration is highly influenced by the outcome of other proximate compositions.

5.3.2. Minerals composition of fermented bean milk

The mineral compositions determined were iron, zinc, magnesium, calcium and phosphorus. These minerals play important roles in humans. For instance, magnesium is needed for physiological functions of the brain, heart and skeletal muscles (de Baaij et al., 2015) while both calcium and phosphorus are needed for formation of new bones and repair of existing bones (Beto, 2015). Zinc and iron are essential micronutrients for growth and for maintenance of immune function (Walker et al., 2005). The effect of fermentation on these mineral compositions are presented in Table 5.2.

Table 5.2: Mineral composition (mg/100 g) in milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties fermented with probiotic starter cultures; Yoflex (YF L-903), ABT and Yoba Fiti (YF).

	Iron (mg/100 g)			**Zinc (mg/100 g)**			**Calcium (mg/100 g)**			**Magnesium (mg/100 g)**			**Phosphorous (mg/100 g)**		
	RH	YK	P	RH	YK	P	RH	YK	P	RH	YK	P	RH	YK	P
NF	1.5 ± 0.1^{b}	1.3 ± 0.1^{ab}	1.2 ± 0.1^{a}	0.7 ± 0.1^{ab}	1.3 ± 0.1^{c}	0.5 ± 0.1^{a}	41.5 ± 0.9^{a}	45.2 ± 0.2^{ab}	62.3 ± 4.1^{c}	23.4 ± 1.2^{a}	33.0 ± 1.6^{b}	46.5 ± 1.8^{d}	17.9 ± 0.1^{a}	12.7 ± 0.2^{a}	13.3 ± 0.2^{a}
YFL-903	1.7 ± 0.1^{de}	1.3 ± 0.0^{a}	1.5 ± 0.0^{c}	0.8 ± 0.0^{b}	1.5 ± 0.0^{d}	1.2 ± 0.1^{c}	44.8 ± 1.2^{ab}	64.6 ± 1.7^{c}	65.6 ± 3.6^{c}	31.9 ± 1.6^{b}	49.5 ± 1.1^{de}	53.6 ± 1.1^{e}	53.4 ± 4.6^{cd}	37.5 ± 1.9^{b}	42.8 ± 6.2^{bcd}
ABT	1.7 ± 0.1^{de}	1.3 ± 0.1^{ab}	1.5 ± 0.0^{c}	0.8 ± 0.1^{b}	1.5 ± 0.0^{d}	1.2 ± 0.1^{c}	49.1 ± 1.1^{b}	64.1 ± 2.5^{c}	74.2 ± 5.7^{c}	33.3 ± 1.0^{b}	44.9 ± 2.9^{d}	56.7±0.9f	43.6 ± 1.8^{bc}	34.1 ± 1.1^{b}	40.5 ± 1.5^{b}
YF	1.9 ± 0.0^{f}	1.5 ± 0.0^{c}	1.6 ± 0.0^{d}	1.1 ± 0.1^{c}	1.6 ± 0.0^{e}	1.5 ± 0.0^{d}	57.5 ± 2.1^{c}	62.1 ± 3.9^{c}	85.3 ± 2.1^{e}	37.0 ± 0.7^{c}	47.8 ± 1.0^{d}	62.2 ± 1.9^{f}	53.2 ± 2.2^{d}	40.6 ± 1.0^{b}	54.1 ± 1.0^{d}
P	0.01			0.01			0.03			0.02			0.03		

Results are means ± standard (SD). Different superscript letters within the same column and row for corresponding parameters indicate statistical significance (Bonferroni, $p<0.05$; NF, non-fermented).

Iron concentration significantly increased ($p=0.01$) by 13.3 to 26.7% in fermented milk extracted from red haricot beans and 25 to 33.3% in pinto bean variety. Gabriel, Akinyosoye and Adetuyi (2011) and Obadina et al. (2013) reported increase in iron concentration of fermented jack beans and soymilk respectively due to their release from chelated complex compound through the activities of microorganisms. Iron values in corresponding fermented milk were statistically similar irrespective of the fermenting culture except in milks fermented with Yoba Fiti culture which had statistically higher iron ($p<0.05$) levels. This implies that appropriate selection of the fermenting culture may be exploited in bio-fortification of iron in beans and bean products and Yoba Fiti culture could be the most suitable for common beans.

Fermentation significantly ($p<0.05$) increased zinc concentration in milk extracted from yellow kidney beans (15.4 to 23.1%) and pinto bean varieties (140 to 200%). There was no change ($p>0.05$) in zinc concentration of fermented milk extracted from red haricot beans except in milk fermented with Yoba Fiti culture which had 57.1% increase. An earlier study reported more than 50% increase in zinc concentration of naturally fermented soy milk (Obadina et al., 2013). Zinc ions are well-known to exist as chelated complex compounds with the polyphenols and phytates. Fermentation may have increased the activity of the enzyme phytase which may have hydrolyzed the complexes and increased the solubility and extractability of zinc from the milk sample (Reddy, Sathe & Salunkhe, 1982). Hence increase in zinc concentration is an indication that zinc ions were released from chelated complex compounds. Fermentation with Yoba Fiti culture raised zinc concentration to significantly higher levels ($p<0.05$) than the corresponding milk fermented with other cultures. Thus, Yoba Fiti culture may be a suitable means for zinc bio-fortification in beans and beans extracts or products.

Fermentation increased calcium concentration by a range of 5.3% to 42.9%. These values are lower than the range of 8.8% to 129.3% reported by Onwurafor, Onweluzo & Ezeoke (2014) for spontaneous and back slopping fermentation of mung beans, and 56.2% for

natural fermentation of African yam beans milk (Nnam, 1997). Nnam (1997) and Onwurafor et al. (2014) suggested that the increase in calcium could be due to increased activity of microbial enzymes which led to dry matter reductions. This is possible because lactic acid bacteria can metabolize large molecular sugars in legumes to release calcium and increase its extraction rate (Obadina et al., 2013). Calcium values in corresponding fermented milk were comparable irrespective of the fermenting culture except in milk obtained from red haricot beans and pinto bean variety fermented with Yoba Fiti cultures which had significantly higher ($p<0.05$) calcium concentration than the corresponding fermented milk extracts. This is an indication that Yoba Fiti culture could be a superior bio-fortifier than the common dairy starter culture YF L-903, Yoba and ABT cultures.

Magnesium in fermented milk increased ($p<0.05$) from 23.4±1.2 to 37.0±0.7 mg/100 g; 33.0±1.6 to 49.5±1.1 mg/100 g and 46.5±1.8 to 62.2±1.9 mg/100 g in milk extracted from red haricot beans, yellow kidney beans and pinto beans respectively. The biggest increase of 58.1% was found in milk extracted from red haricot beans fermented with Yoba Fiti culture. There are limited studies on the effect of fermentation on magnesium concentration of milk extracted from plants. However, fermentation of tamarind seed showed remarkable increase in magnesium by 10.3% (Omotola et al., 2017). The trend observed in this study was probably due to the breakdown of the polyphenols and phytates compounds (Table 5.3). Fermentation is known to provide optimum pH conditions for the enzymatic degradation of phenolic compounds which are present in the bean milk in the form of complexes with the polyvalent cations such as magnesium rendering them unavailable (Afoakwa et al., 2013). Reduction in these polyphenols may have increased the amount of soluble magnesium.

The phosphorus levels in fermented milk ranged from 43.6±1.8 to 53.4±4.6 mg/100 g in milk extracted from red haricot beans; 34.1±1.1 to 40.6±1.0 mg/100 g in milk extracted from yellow kidney beans and 40.5±1.5 to 54.1±1.0 mg/100 g in milk extracted from pinto beans. These values indicate significant increase ($p<0.05$) in phosphorous by more than

two fold in all the bean milk samples. Chompreeda & Fields (1984) and Nnam (1997) reported increased extractability of phosphorous due to fermentation in soybean meal and African Yam Bean milk. The higher phosphorous in fermented bean milks maybe explained by the favorable attributes of fermentation which probably released phosphorous from their originally bound complexes. During fermentation, the enzyme phytase is produced (Reddy et al., 1982) which hydrolyzes phytates to inositol and phosphoric acid. This may have led to an increase in the extractability of phosphorous.

5.3.3. Phenolic compounds and phytates of fermented bean milk

The phenolic compounds determined in the study were tannins, flavonoids and total phenols. Effect of fermentation on these phenolic compounds and phytates are presented in Table 5.3. As shown, fermentation significantly reduced these compounds.

Table 5.3: Phenolic compounds and phytates (mg/100 g) in milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) beans fermented with probiotic starter cultures; Yoflex (YF L-903), ABT and Yoba Fiti (YF).

	Tannins (mg/100 g)			**Flavonoids (mg/100 gm)**			**Total Phenols (mg/100 g)**			**Phytates (mg/100 g)**		
	RH	YK	P	RH	YK	P	RH	YK	P	RH	YK	P
NF	31.9 ± 1.8^{l}	14.3 ± 0.4^{j}	24.3 ± 0.9^{k}	21.3 ± 1.9^{k}	10.6 ± 1.2^{i}	16.7 ± 1.1^{j}	47.0 ± 1.4^{i}	30.0 ± 2.1^{h}	45.0 ± 2.3^{i}	57.6 ± 2.4^{h}	65.9 ± 1.5^{i}	52.8 ± 1.3^{h}
YF L-903	3.0 ± 0.1^{e}	5.0 ± 0.1^{h}	3.6 ± 0.0^{g}	6.8 ± 0.1^{g}	4.4 ± 0.2^{d}	5.0 ± 0.1^{e}	13.1 ± 0.1^{d}	14.5 ± 0.1^{e}	13.4 ± 0.1^{d}	4.3 ± 0.2^{f}	3.3 ± 0.0^{e}	2.8 ± 0.0^{d}
ABT	2.0 ± 0.1^{c}	3.3 ± 0.0^{f}	7.7 ± 0.2^{j}	6.5 ± 0.2^{fg}	3.3 ± 0.1^{a}	4.9 ± 0.2^{de}	15.7 ± 0.1^{f}	14.2 ± 0.1^{e}	11.7 ± 0.0^{b}	4.9 ± 0.0^{g}	2.1 ± 0.4^{ab}	1.8 ± 0.0^{a}
YF	1.5 ± 0.1^{b}	1.0 ± 0.0^{a}	2.6 ± 0.1^{d}	6.0 ± 0.2^{fg}	6.0 ± 0.1^{f}	4.7 ± 0.1^{de}	12.6 ± 0.0^{c}	10.3 ± 0.0^{a}	10.6 ± 0.0^{a}	2.4 ± 0.1^{bc}	1.8 ± 0.1^{a}	1.7 ± 0.1^{a}
P	<0.01			0.01			0.02			0.02		

Results are means ± SD. Different superscript letters within the same column and row for corresponding parameters indicate statistical significance (Bonferroni, $p<0.05$; NF, non-fermented).

Fermentation reduced ($p<0.05$) tannins by more than threefold irrespective of the fermenting culture and the largest reduction was found in bean milk fermented with Yoba Fiti culture. Onwurafor et al. (2014) reported similar results upon fermentation of mung beans. These authors attributed reduction in tannins content of the fermented product to the activity of enzymes associated with seeds. Tannins form complexes with nutrients such as carbohydrates and protein. These complexes are known to hinder protein and starch digestibility. The formed complexes also play significant roles in depression of protein and carbohydrate foods (Reddy & Pierson, 1985) and inhibit digestive enzymes which affects protein utilization (Singh & Raghuvanshi, 2012). Therefore, bean milk fermented with LAB maybe a suitable source of high-quality protein in the diet due to their low tannin content.

The fermented bean milk contained lower levels of flavonoids. Fermentation reduced ($p<0.05$) flavonoids by a range of 43.4% to 71.9%. The least and largest reductions were observed in milk extracted from yellow kidney beans and pinto beans fermented with Yoba Fiti culture. Flavonoids compounds have been found to limit the nutritional benefit of biofortified iron in black beans due to their ability to chelate metal ions (Tako et al., 2014). Hence the low flavonoids concentration in fermented milk maybe an indication that bean milk is a promising vehicle for increasing bioavailable iron.

Total phenols were lower ($p<0.05$) in fermented bean milk than in the non-fermented milk. The highest reduction of more than 76% was observed in milk extracted from pinto beans fermented with Yoba Fiti culture. Suazo and Davidov-pardo (2014) observed 62.6% reduction in total phenols of fermented cocoa. They suggested that fermentation reduced phenols due to oxidation reactions of single polyphenols to insoluble complex tannins, a process catalyzed by oxidase enzymes. However, contrary to this suggestion, there was reduction in tannins as well (Table 5.3). Total phenols inhibit the activities of digestive enzymes and so their presence even in low levels is not desirable (Vadivel & Pugalenthi, 2008). On the other hand, phenolic compounds have antioxidant activities which are considered to be salutary (Chen et al., 2019). There are contradictory reports on effect of fermentation on total phenols. Adetuyi and Ibrahim (2014) observed increase in total

phenolics of okra seeds upon LAB fermentation. This could be an indication that some LAB does not oxidize phenolic but hydrolyses them into biologically more active phenols.

Fermentation reduced ($p<0.05$) phytates by more than 91% in bean milk irrespective of the fermenting culture. This could be due to enzymatic hydrolysis of phytase which is activated during fermentation. Phytase hydrolyzes phytates to inositol and phosphoric acid and releases polyvalent minerals such as magnesium, calcium, zinc and phosphorous from their organically bound form for utilization (Table 5.2). Nnam (1997) reported similar phenomenon during fermentation of milk extracted from African Yam Beans.

5.3.4. Vitamin concentration of fermented bean milk

Composition of vitamin B-complex contained in non-fermented and fermented bean milk was determined. The specific vitamins studied were thiamine, riboflavin, niacin, pyridoxine and folic acid. Effects of fermentation on vitamin concentration of the bean milk are presented in Table 5.4. There were significant differences ($p<0.05$) in folic acid and riboflavin concentrations of raw bean milk. However, there were no significant inter-varietal differences in pyridoxine, niacin and thiamine concentration of raw milk. Thiamine was also not quantifiable in fermented bean milk regardless of the culture used. This is consistent with the results of Granito et al. (2002) who observed significant losses in thiamine after natural fermentation of lentils and red beans.

Table 5.5: Vitamin concentration in milk extracted from pinto (P), yellow kidney (YK) and red haricot (RH) beans varieties fermented with probiotic starter cultures; Yoflex (YF L-903), ABT and Yoba Fiti (YF).

	Thiamine (mg/100 g)			**Riboflavin (µg/100g)**			**Niacin (mg/100 g)**			**Pyridoxine (mg/100 g)**			**Folic acid (mg/100 g)**		
	RH	YK	P	RH	YK	P	RH	YK	P	RH	YK	P	RH	YK	P
NF	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	88.1±1.6^{c}	52.2±3.4^{b}	40.0±4.7^{ab}	0.5±0.0^{a}	0.4±0.0^{a}	0.5±0.1^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.3±0.0^{a}	0.4±0.0^{b}	0.4±0.0^{bc}
YF L-903	ND	ND	ND	50.1±0.2^{b}	60.1±10.6^{b}	33.9±4.7^{ab}	3.7±0.4^{d}	2.8±0.2^{cd}	3.0±0.4^{cd}	0.2±0.0^{b}	0.2±0.0^{b}	0.2±0.0^{b}	0.5±0.0^{c}	0.9±0.1^{e}	0.6±0.1^{cd}
ABT	ND	ND	ND	433.0±63.7^{d}	1121.9±15.9^{f}	670.9±26.1^{e}	2.8±0.2^{cd}	2.2±0.1^{c}	2.8±0.1^{cd}	1.0±0.1^{f}	0.5±0.1^{de}	1.0±0.0^{f}	0.6±0.0^{d}	0.9±0.1^{e}	0.7±0.1^{de}
YF	ND	ND	ND	23.8±0.6^{a}	27.8±1.1^{a}	21.2±0.1^{a}	3.6±0.3^{d}	2.4±0.4^{c}	3.0±0.8^{cd}	0.4±0.0^{d}	0.3±0.0^{c}	0.4±0.1^{cd}	0.5±0.0^{c}	0.8±0.0^{e}	0.5±0.0^{c}
P	0.1			0.01			0.03			0.01			0.01		

Results are means ± standard deviation (SD). Different superscript letters within the same column and row for corresponding parameters indicate statistical significance (Bonferroni, $p<0.05$; NF, non-fermented; ND, not detectable).

Fermentation with mixed probiotic bacteria cultures led to either increase or decrease on the riboflavin content of beans (Table 5.4) which is consistent with previous report by Burgess, Smid and Sinderen (2009) who reported that probiotic strains can either utilize or produce individual vitamin B molecules. These authors also reported that the ability of probiotic bacteria to synthesize vitamin B molecule depends on the genome of the fermenting bacteria. Fermentation with Yoba Fiti culture significantly reduced ($p<0.05$) riboflavin in milk extracted from the three bean varieties by 46.7% to 73%. An earlier study by Elmadfa et al. (2001) showed that most probiotic strains of lactobacilli consume riboflavin thereby decreasing its bioavailability. Additionally, riboflavin biosynthesis has been shown to occur when the four genes; ribG, ribB, ribA and ribH are present in the microbes genome (Bacher et al., 2015). However, absence of the ribG is previously reported for *L. rhamnosus* GR1, *L. rhamnosus yoba, L. bulgaricus* and *S. thermophilus* (Thakur et al., 2015; Valle et al., 2014). The bean variety from which milk was extracted had great influence on the riboflavin concentration of the fermented milk. For example, fermentation with YF L-903 significantly reduced riboflavin content in milk extracted from red haricot and pinto beans but not in milk extracted from yellow kidney beans, while fermentation with ABT caused great increases of this vitamin in milk extracted from all the varieties, especially in milk extracted from yellow kidney beans (>2000% increase). *Bifidobacterium animalis Bb-12* and *L. acidophilus La-5* which are the fermenting bacteria in the mixed probiotic ABT culture contain the four gene operons needed to catalyze biosynthesis of riboflavin (Thakur et al., 2015). Thus, appropriate selection of species and/or strains is essential in increasing riboflavin of fermented bean milk.

Fermentation caused significant increase ($p<0.01$) in niacin concentration of the milk extracted from the three bean varieties. The highest increase in niacin concentration, an increase of 640% was found in milk extracted from red haricot beans fermented with YF L-903 culture. Increase in niacin values of cheese and yoghurt fermented with lactic acid producing bacteria was earlier reported (Gu & Li, 2016). These strains may be useful in enriching niacin composition of bean milk and could be exploited for other legumes.

With regards to pyridoxine the highest concentration was quantified in milk fermented with ABT culture (Table 5.4). Similarly, the highest increase of 900% was found in milk extracted from red haricot and pinto beans fermented with ABT cultures. A previous study by Vajaranant & Fields (1989) reported increase ($p<0.05$) in pyridoxine values of corn meal (0.52±0.0 to 0.72±0.1 mg/100 g) fermented with different strains of *Bacillus licheniformis*. Similarly, fermentation of soy with different species and strains of *Streptococcus thermophilus, Lactobacillus helveticus and Bifidobacterium longum* was previously reported to cause significant increases in pyridoxine concentration (Champagne et al., 2010). The biosynthesis of pyridoxine was previously reported to depend on the microbial ecological niche (Qaidi et al., 2013). This could imply that *Streptococcus thermophilus, Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* BB-12 have got specific metabolic properties that make them more efficient in the biosynthesis of folic acid than other LAB (Table 5.4).

Milk extracted from yellow kidney beans and pinto beans had statistically higher ($p<0.05$) folic acid of 0.4±0.0 mg/100 g than those extracted from red haricot beans which contained 0.3±0.0 mg/100 g. Folic acid was significantly higher ($p<0.05$) in corresponding fermented bean milk than the non-fermented milk (Table 5.4). This agrees with earlier studies which reported increase in folic acid in milk fermented with *L. rhamnosus* (Hugenschmidt et al., 2010), *S. thermophilus* (Lai~no, LeBlanc & Savoy, 2012), *L. acidophilus* (Lai~no et al., 2014), and *B. animalis* (Pompei et al., 2007). Milk extracted from yellow kidney beans exhibited relatively higher increase in folate than the other two bean varieties. Additionally, the highest increase in folic acid in each milk category was found in those fermented with ABT culture, an increase of 75 to 125%. *L. acidophilus* strains is reported to contain folate biosynthesis cluster which converts 6-hydroxymetheyl-7,8-dihydropterin (DHPP) to folic acid biosynthesis precursor parabenzoic amino acid (pABA) (Gu & Li, 2016). However, *L. rhamnosus* and *S. thermophilus* use an alternative biosynthesis pathway (KEGG, 2014) which could be less efficient in the biosynthesis of folic acid (Table 5.4).

5.3.5. Oligosaccharides concentration of fermented bean milk

There are various types of sugars in bean milk including oligosaccharide sugars such as raffinose, verbascose and stachyose. The oligosaccharide sugars are known to cause flatulence and are partly the reason for low consumption of common beans and its associated products (Paredes & Harry, 1989). Table 5.5 shows the effects of fermentation on oligosaccharides concentration of common bean milk extracted from three bean varieties.

Table 5.5: Oligosaccharides concentration (mg/100 g) in milk extracted from pinto (P), red haricot and yellow kidney beans fermented with probiotic starter cultures; Yoflex (YF L-903), ABT and Yoba Fiti.

	Raffinose (mg/100 g)			**Verbascose (mg/100 g)**			**Stachyose (mg/100 g)**		
	RH	YK	P	RH	YK	P	RH	YK	P
NF	0.4 ± 0.0^{d}	0.4 ± 0.0^{d}	0.4 ± 0.0^{d}	0.2 ± 0.0^{b}	0.4 ± 0.0^{d}	0.3 ± 0.0^{c}	3.4 ± 0.1^{g}	4.2 ± 0.2^{h}	3.6 ± 0.4^{gh}
YF L-903	0.2 ± 0.0^{ab}	0.2 ± 0.1^{abc}	0.2 ± 0.0^{b}	0.2 ± 0.1^{abc}	0.1 ± 0.1^{ab}	0.1 ± 0.0^{a}	2.6 ± 0.4^{f}	1.6 ± 0.1^{de}	1.1 ± 0.2^{acd}
ABT	0.1 ± 0.1^{ab}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.9 ± 0.2^{ab}	1.0 ± 0.1^{ab}	0.8 ± 0.0^{a}
Yoba Fiti	0.2 ± 0.0^{ab}	0.2 ± 0.1^{abc}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	1.6 ± 0.2^{bcde}	1.1 ± 0.1^{abc}	1.0 ± 0.1^{ab}
P	0.03			0.02			0.03		

Results are means ± SD. Different superscript letters within the same column and row for corresponding parameters indicate statistical significance (Bonferroni, $p<0.05$; NF, non-fermented).

A narrow range of values have been reported for raffinose in legumes. For instance, Difo et al. (2015) found that *racemosa* seeds contained 1.9±0.0 mg/100 g of raffinose sugars while Akinyele and Akinlosotu (1991) reported concentration of 2.0±0.0 mg/100 g in cowpeas (*Vigna unguiculata)*. Thus, the concentration of 0.4±0.0 mg/100 g contained in non-fermented bean milk was far much lower than the previously reported values for most legumes. Similar to the results of Da et al. (2006) stachyose (3.4±0.1 to 4.2±0.2 mg/100 g) was the most abundant oligosaccharide sugar in non-fermented bean milk (Table 5.5). The highest reduction in raffinose concentration (75%) was recorded in pinto bean milk fermented with ABT culture. A previous study by Granito and Alvarez (2006) reported similar results when black beans varieties were fermented with lactic acid bacteria. The reduction in raffinose could be attributed to the utilization of the oligosaccharides for energy by the microorganisms through the activities of α galactosidase enzyme. The current finding is of great interest as it suggests that fermentation could be used to reduce flatulence causing raffinose.

Fermentation with ABT and Yoba Fiti cultures caused significant decreases ($p<0.05$) in verbascose concentration of the milk extracted from red haricot beans ($p>0.05$) (Table 5.5). Fermented milk extracted from yellow kidney and pinto beans were also found to contain statistically lower ($p<0.05$) verbascose values on average (0.1±0.0 mg/100 g) than non-fermented milk (0.4±0.0 mg/100 g) and (0.3±0.0 mg/100 g) respectively. This represents a 75% reduction in verbascose concentration in milk extracted from yellow kidney beans. This agrees with earlier reports which had shown reduction in verbascose values of common beans upon fermentation (Starzynska, Bozena & Mickowska, 2014). LAB contains α galactosidase enzyme which potentially enables them to utilize verbascose sugars. However, there was variation in the utilization rate of verbascose (Table 5.5) among the fermenting LAB probiotic starter cultures which could be due to differences in the expression of the α-galactosidase enzyme.

Fermentation triggered significant reduction ($p<0.05$) in stachyose values for the three-bean milk, with the highest reduction of 77.8% observed in milk extracted from pinto bean variety fermented with ABT culture (Table 5.5). Stachyose could have been hydrolyzed by α-galactosidase into sucrose and galactose, and the latter metabolized through the galactose-utilization system (Da et al., 2006). Additionally, significantly higher ($p<0.05$) stachyose value was found in milk extracted

from red haricot beans fermented with YF L-903. This could be an indication that the ability of fermenting microorganisms to hydrolyze bonds in oligosaccharide moieties is dependent on enzymatic properties of the bacterial strain and the efficiencies of the α-galactosidase activity of that particular strain. Thus, appropriate selection of the fermenting culture is important in reducing stachyose in fermented bean milk.

5.4. Conclusion

Apart from milk extracted from pinto beans fermented with YF L-903 which demonstrated significant increase in protein and significant reduction in carbohydrates, fermentation did not cause significant change in crude protein, crude fat and carbohydrate. Fermentation improved the micronutrient composition of bean milk as there was significant increase in mineral concentration (iron, calcium, magnesium, phosphorous and zinc) and vitamin B complex concentration (pyridoxine, niacin and folic acid). Therefore, this study recommends use of probiotic cultures for biofortification of minerals and synthesis of specific B complex vitamins. However, thiamine was non-quantifiable in fermented milks while riboflavin values were lowered for all the fermenting cultures, except ABT culture. This implies that combination of probiotic strains of *Lactobacillus acidophilus La-5, Bifidobacterium animalis Bb-12* and *Streptococcus thermophilus* could be exploited for natural fortification of riboflavin in bean milk. It was also observed that fermentation significantly lowered phytates and phenolic compounds as well as the oligosaccharide compositions of stachyose, raffinose and verbascose. Thus, fermentation of bean milk with any of the three cultures could be utilized for removal of the flatulence causing oligosaccharides and thereby enhance bean consumption.

CHAPTER SIX

EVALUATION OF SENSORY ATTRIBUTES OF FERMENTED MILK EXTRACTED FROM THREE COMMON BEAN (PHASEOLUS VULGARIS L.) VARIETIES

6.1. Introduction

Acid milk beverages and yoghurt are widely consumed worldwide by different races (Azizpour et al., 2009). However, in many parts of the world, especially developing countries, cow's milk is expensive and is not readily available (Gerosa & Skoet, 2012). Additionally, cow milk cause allergic reactions and lactose intolerance which prevent individuals from consuming the recommended servings of nutrient rich dairy foods (Palacios et al., 2009). Fermented common bean milk might well serve as a substitute in these areas to meet the food and nutrition needs of the population.

Chen et al. (2019) reported low phytic and phenolic compounds in common bean milk. Phenolic compounds are reported to sequestrate minerals lowering their bioavailability (Brigide, Canniatti & Silva, 2014) and protein digestibility (Granito et al., 2002). Additionally, Obadina et al. (2013) reported insignificant concentration of verbascose, raffinose and stachyose sugars in fermented bean milk. These oligosaccharide sugars are contained in common beans seed and are known to cause flatulence and social discomforts as a result of stomach upsets (Da et al., 2006) and thereby limiting utilization of common beans seed (Petry et al, 2015). Hence their low concentration in fermented bean milk presents an opportunity for improved utilization of common beans.

However, as noted by Palacios et al. (2009), acceptance and usage constitute an additional consideration for milk substitute beverages. Leng et al. (2004) observed that sensory attributes such as taste influences food choices and may lead to altered consumption of a food and the nutrients it provides. Wrick (2003) reported low annual growth rate in the consumption of raw soymilk. Some of the factors limiting acceptance of soymilk are rancid flavor, beany flavor and bitter taste (Min et al., 2005). Thus, in addition to nutritional content, the milk products must taste good and generally be acceptable to consumers or the product and the nutrients it has to offer will

not be consumed. Reports on acceptance of bean milk are scarce hence the current study was designed to evaluate sensory qualities of common bean milk.

6.2. Materials and methods

6.2.1. Raw materials

Dry beans for each of the bean varieties were purchased from a local retail store in Nairobi, Kenya and transported to JKUAT as described in Section 3.2.1.

6.2.2. Probiotic culture, microbial media and reagents collection

Probiotic culture, microbial media and reagents were collected as described in Section 4.2.2.

6.2.3. Preparation of bean milk

Bean milk was prepared using the modified method described for preparation of soymilk by Ma et al. (2015) and Min et al. (2005). Detailed description of this method is presented in Section 3.2.2.

6.2.4. Experimental design

The study adopted experimental design described in Section 4.2.4

6.2.5. Fermentation of bean milk

Bean milk was fermented as described in Section 4.2.5.

6.2.6. Sensory evaluation

Sensory evaluation was carried out 6 hours after fermentation using method of Ma et al. (2015) for milk extracted from three common bean varieties fermented with probiotic bacteria. Naturally fermented bean milk sample was used as a control. A group of 50 panelist was randomly drawn from research students in Department of Food Science and Technology at JKUAT. The panelists received 6 hours of training sessions and practice in milk evaluation by a trained assessor in milk

evaluation. The training comprised of sessions where panelists evaluated and discussed sensory characteristics of commercial soymilk. The parameters discussed and evaluated during training were adapted from Lawrence, Lopetcharat and Drake (2016). Panelists discussed and assigned definitions and reference for sensory attributes including color, taste, aroma, texture, flavor, aftertaste and overall acceptability. Analysis of variance was carried out during the practice sessions to tell whether the judges could differentiate sensory properties of soymilk.

Evaluations by the panelist were carried out and repeated three times on different sessions for each of the fermented bean milk samples. At the start of each evaluation session, judges were attuned with a soymilk sample which they had earlier evaluated during the training sessions. To avoid biasness, evaluations were done in sensory evaluation bench, by one panelist at a time. A sensory evaluation bench had a tray containing 100 ml of each of the fermented bean milk samples placed in lidded 200 ml plastic jars coded with three-digit random numbers. The fermented milk samples were presented in a random order in each tray. The milk samples were kept at 4°C for 6 hours before sensory evaluation in order to get a feel for traditional yoghurt taste style. The judges tested one sample at a time and rinsed their mouths with distilled water between samples. Two minutes wait time was ensured after characterizing each of the fermented bean milk samples. Judges were asked to record scores 60 seconds after swallowing for each of the seven attributes. The scores were recorded for each of the characterized attributes using a 9-point hedonic scale of Poste et al. (1991) as shown in Appendix 2. This scale was used to measure how much the participant liked or disliked the product. The minimum attribute dislike extremely denoted not intense or not much liked and the maximum attribute of like extremely denoted very intense or very much liked (Poste et al., 1991). The mean score for each of the seven parameters evaluated was used to describe acceptance of fermented bean milk.

6.2.7. Statistical analysis

Statistical analyses were conducted using STATA/SE version 12.0. A two-way analysis of variance (ANOVA) was used to carryout analysis of descriptive data followed by principal component analysis (PCA) using the correlation matrix. A PCA is an extensively used analytical statistical method, designed to reduce a set of dependent variables to a smaller number based on the original

variable correlation pattern (Lawrence et al., 2016). The two-way ANOVA was performed on consumer liking data with bean varieties and fermentation treatments as variables to determine interactions. Mean separation was carried out using Bonferroni's method. All statistical analyses were performed at a 95% confidence interval.

6.3. Results and discussion

6.3.1. Evaluation of bean milk sensory qualities

The mean scores for sensory attributes are presented in Table 6.1. There was no significant difference ($p>0.05$) in colour of the corresponding bean milk irrespective of the kind of culture used for fermentation. Odu, Egbo & Okonko (2012) reported that color acceptability of soymilk was influenced by heating duration during soymilk processing. These authors observed that heat induced color changes were brought about by Maillard browning reaction. Therefore, statistical similarities in mean scores reported for colour could be due to similar heat treatment of bean milk during milk preparation.

Table 6.1: Sensory evaluation means scores for bean milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties fermented with probiotic starter cultures; YF L-903, ABT and Yoba Fiti. Naturally fermented bean milk (NF) was used as control.

Milk	Color	Taste	Aroma	Texture	Flavor	Aftertaste	Overall acceptability
YK Yoba Fiti	5.6 ± 0.1^{h}	3.0 ± 0.2^{a}	3.4 ± 0.1^{ab}	4.6 ± 0.2^{ef}	3.4 ± 0.2^{ab}	3.3 ± 0.3^{ab}	3.7 ± 0.1^{b}
YK ABT	5.4 ± 0.3^{gh}	3.0 ± 0.2^{a}	3.8 ± 0.2^{bc}	4.6 ± 0.1^{f}	3.6 ± 0.2^{bc}	3.3 ± 0.3^{ab}	3.5 ± 0.3^{ab}
YK YF L-903	5.6 ± 0.1^{h}	3.2 ± 0.2^{a}	3.7 ± 0.3^{bc}	4.6 ± 0.1^{f}	3.6 ± 0.2^{bc}	3.4 ± 0.3^{ab}	3.7 ± 0.2^{bc}
YK NF	5.8 ± 0.1^{hi}	3.7 ± 0.1^{bc}	4.4 ± 0.1^{e}	4.9 ± 0.2^{fg}	4.0 ± 0.2^{cd}	4.1 ± 0.0^{e}	4.2 ± 0.0^{d}
RH Yoba Fiti	6.0 ± 0.1^{i}	3.6 ± 0.2^{bc}	3.7 ± 0.1^{b}	4.7 ± 0.2^{fg}	3.5 ± 0.2^{ab}	3.2 ± 0.2^{a}	3.2 ± 0.1^{a}
RH ABT	5.9 ± 0.1^{i}	4.0 ± 0.1^{cd}	4.2 ± 0.3^{ce}	5.0 ± 0.1^{g}	3.7 ± 0.3^{bcd}	3.6 ± 0.2^{bc}	4.1 ± 0.1^{d}
RH YF L-903	5.6 ± 0.2^{hi}	4.1 ± 0.3^{cde}	4.2 ± 0.3^{ce}	5.1 ± 0.2^{fg}	4.0 ± 0.1^{bcd}	3.7 ± 0.2^{bc}	4.0 ± 0.1^{cd}
RH NF	5.5 ± 0.3^{ghi}	4.0 ± 0.0^{d}	4.1 ± 0.2^{ce}	4.7 ± 0.1^{f}	4.1 ± 0.3^{bcde}	3.7 ± 0.1^{b}	3.7 ± 0.3^{bcd}
P Yoba Fiti	5.2 ± 0.3^{fgh}	3.7 ± 0.3^{bc}	3.7 ± 0.1^{bc}	4.5 ± 0.2^{ef}	3.7 ± 0.2^{bc}	3.4 ± 0.3^{a}	3.7 ± 0.3^{bcd}
P ABT	5.3 ± 0.3^{gh}	3.3 ± 0.2^{ab}	3.9 ± 0.0^{c}	4.1 ± 0.2^{cde}	3.7 ± 0.2^{bc}	3.7 ± 0.3^{bcd}	3.5 ± 0.3^{abc}
P YF L-903	5.0 ± 0.2^{fg}	3.4 ± 0.3^{ab}	3.7 ± 0.3^{bc}	4.1 ± 0.2^{cde}	3.6 ± 0.1^{b}	3.2 ± 0.3^{ab}	3.3 ± 0.3^{ab}
P NF	5.2 ± 0.3^{fg}	4.4 ± 0.0^{e}	3.9 ± 0.2^{c}	4.5 ± 0.2^{ef}	3.7 ± 0.1^{b}	3.7 ± 0.3^{bcd}	3.7 ± 0.3^{bcd}
SE	0.08	0.08	0.08	0.08	0.07	0.07	0.08

Results are means ± SD. Different superscript letters within the same column indicate statistical significance (Bonferroni, $p<0.05$).

Taste score was statistically higher ($p<0.05$) for bean milk which had been left to ferment naturally compared to all corresponding milk extracted from yellow kidney beans and pinto beans fermented with probiotic culture. Ma et al. (2015) found negative correlations between total glycitein and sweetness of soymilk ($r=0.3$). Milk fermented with LAB culture were reported to contain high amount of isoflavones and glycitein (Murphy et al., 1999). These compounds adversely affected flavor and sweetness of fermented soymilk due to their least taste threshold value (Kudou et al., 1991). Aftertaste scores for milk extracted from yellow kidney beans was comparable for all corresponding milk fermented with probiotic culture irrespective of the kind of culture used for fermentation. However, these scores were statistically lower ($p>0.05$) than that in corresponding milk fermented naturally. The difference could be due to alterations in the isoflavones and glycitein compositions of the fermented milk (Murphy et al., 1999). Aftertaste scores for milk extracted from red haricot beans and pinto beans were statistically similar irrespective of the kind of culture used for fermentation or fermentation type used, except in corresponding milk fermented with Yoba Fiti culture which had statistically lower ($p>0.05$) scores. Therefore, as observed by the panelists, addition of sweeteners can greatly improve acceptability of these products by improving both taste and aftertaste.

Aroma in naturally fermented milk extracted from yellow kidney beans was statistically higher ($p<0.05$) compared to its equivalent matches fermented with probiotic culture. Additionally, aroma score was statistically lower for milk extracted from red haricot beans fermented with Yoba Fiti culture compared to its corresponding naturally fermented milk. Poysa and Woodrow (2002) observed that superior soymilk lines had higher total soluble solids content than inferior lines which could imply that naturally fermented milk extracted from yellow kidney beans had higher soluble solid content. Moreover, Lim et al. (1990) found that high soluble total solids are desired characteristics for consumers since it is an important parameter for beverage evaluation. Addition of flavor enhancers was pointed out by the judges as one of the possible ways to improve aroma of the fermented bean milk. This can be exploited in future studies to establish the most suitable flavor enhancer and proportions that can enhance acceptance of fermented bean milk.

Texture was statistically similar ($p>0.05$) for all the bean milk irrespective of the fermenting culture or fermentation type. Similarly, flavor score was comparable for all bean milk fermented with probiotic culture irrespective of the kind of culture used for fermentation. However, for milk extracted from yellow kidney beans, fermentation with Yoba Fiti culture yielded statistically lower ($p<0.05$) score than that which was naturally fermented. According to a report by Ma et al. (2015), this difference in flavor could be due to changes in protein and fat composition after fermentation.

The average mean score for overall acceptability ranged from 3.2±0.1 to 4.2±0.0. The maximum mean score corresponds to naturally fermented milk extracted from yellow kidney beans, whereas, the lowest mean score for overall acceptability corresponds to milk extracted from red haricot beans fermented with Yoba Fiti culture. Naturally fermented milk extracted from yellow kidney beans was the most accepted by the sensory panel with regard to taste, aroma and aftertaste quality of the bean milk (Table 6.1). Interestingly, milk extracted from red haricot beans fermented with Yoba Fiti culture had the least scores for aroma and aftertaste among the corresponding milk (Table 6.1). Higher overall acceptability was previously reported in soymilk with consistently superior sensory attributes due to the reduced beany flavor (Abagoshu et al., 2017) and improved taste and aroma (Odu et al., 2012). Fermented bean milk scored poorly on all these parameters regardless of the starter culture used. The panelist picked out the beany flavor in all the fermented milk samples and attributed the low score on flavor to this factor. Since bean milk was cooked prior to fermentation a detailed study on strategies to eliminate beany flavor and improve aroma and taste will go a long way in enhancing acceptance of fermented bean milk. As suggested by the panelist, addition of flavor enhancers and sweeteners could be a good starting point but also understanding the association among the sensory attributes could play a critical role in enhancing acceptance of plant based milk product (Ma et al., 2015). Association among the bean milk sensory attributes are as indicated in Table 6.2.

Table 6.2: Correlation coefficients (r) among fermented bean milk sensory attributes for bean milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties.

Sensory attribute	Color	Taste	Aroma	Texture	Flavor	After-taste
Taste	0.2					
Aroma	0.3	0.7				
Texture	0.8	0.5	0.6			
Flavor	0.1	0.7	0.8*	0.4		
Aftertaste	0.2	0.6	0.9*	0.4	0.8	
Overall acceptability	0.5	0.6	0.7	0.9*	0.5	0.7

*represent the significance level at $p<0.05$ (Bonferroni)

Correlation coefficients from the averaged data of bean milk sensory evaluation parameters were positively associated with each other. The results in Table 6.2 indicated that bean milk aroma was significantly associated with flavor and aftertaste. It was also observed that overall acceptability was significantly ($p<0.05$) associated with texture. This could be an indication that aroma is ideal indicator of bean milk flavor and aftertaste sensory qualities. Also, texture could be an ideal indicator of overall acceptability of bean milk. These findings differed with the previous report of Ma et al. (2015), who demonstrated that overall acceptability of soymilk was significantly positively associated with other soymilk parameters such as smoothness, thickness, sweetness and color. The observed difference could be due to variation in consumption habits (Villegas, Caronell & Costell, 2009).

6.3.2. Principal components for sensory attributes of bean milk

Table 6.3: Principal components for sensory attributes of bean milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties naturally fermented and controlled fermented with probiotic starter culture

Variable	Comp1 (55.7%)	Comp2 (12.8%)	Comp3 (8.7%)
Color	0.1	0.9	-0.2
Taste	0.6	0.0	-0.3
Aroma	0.6	0.0	-0.1
Texture	0.1	-0.1	0.9
Flavor	0.5	-0.1	-0.1
Aftertaste	0.5	-0.2	-0.2
Overall acceptability	0.2	-0.2	-0.2

Table 6.3 shows results of Principal Component Analysis (PCA). In this study, seven principle components were identified and the first three could explain 77.2% of the total variance. As shown in Table 6.3, the first component (Comp1) explaining 55.7% of the total variance was described as the bean milk overall sweetness factor as it was mainly associated with taste, aroma, flavor and aftertaste (r=0.5 to 0.6). Study by Ma et al. (2015) associated taste, aroma and flavor to sweetness. The second component (Comp2) explaining 12.8% of the total variance was described as the bean milk appearance factor, as it was primarily associated with bean milk color (r=0.9). The third component (Comp3) explaining 8.7% of the total variance was described as the bean milk mouth feel factor for its strong association with bean milk texture (r=0.9). The above results were mainly based on the preference of bean milk by the Kenyan consumers. However, for Chinese consumers, owing to the different consumption habits, the first principal component was primarily associated with mouth feeling of soy milk (Villegas et al., 2009). Additionally,

for Western consumers, the first component was strongly associated with colour and appearance of the soy milk (Ma et al., 2015). These implied that the most important sensory attributes are mouth feeling of soy milk for Chinese consumers and color and appearance for Western consumers. In contrast, for Kenyan consumers the sweetness of the bean milk was the most important attribute. Therefore, improving the sensory characteristics of the bean milk according to different consumer habits could be possible through useful bean milk value addition programs.

6.4. Conclusion

The product scored poorly in sensory evaluation with the highest mean score of 5.5 indicating neither like nor dislike recorded for color. The reasons for poor scores on sensory attributes pointed out by the panelist include the beany flavor and lack of taste. Indeed, after principal component analysis (PCA), sweetness factor which comprised of taste, aftertaste and aroma parameters explained more than half of the total variance in bean sensory qualities. Therefore, the sweetness factor was the most important attribute for consumers suggesting that value addition to bean milk should target sweetness factors to improve acceptance of fermented bean milk. This could be done by addition of sweeteners and flavor enhancers.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

The study sought to evaluate suitability of common bean milk extracted from yellow kidney beans, red haricot beans and pinto beans as a nutritious vehicle for probiotic bacteria. All the three common beans varieties were found to contain high dietary protein, carbohydrates, fiber, B-group vitamins and minerals (calcium, phosphorous, iron, zinc and calcium). The three bean varieties also had high protein digestibility and low amount of crude fat making beans to be healthy food in the diet. However, they contained high amount of anti-nutrients which included tannins, phytates, flavonoids and total phenols and oligosaccharides (raffinose and stachyose). Common bean milk from the three varieties had high amount of protein and carbohydrates but less B-group vitamins, minerals, oligosaccharides, phenolic substances and phytates. The reduction in minerals and vitamins during milk preparation was less than the reduction of phytates, and thus the bean milk may have better vitamin and mineral bioavailability than the dry beans. Additionally, the protein digestibility of the milk was higher than the dry beans, an indication of higher bioavailability of protein in bean milk than the dry beans. However, since fiber was not detected, bean milk should be consumed accompanied with fiber rich food. This is important because reduction in fiber could lead to increased incidence of constipation.

Common bean milk is a suitable substrate for probiotic lactic acid bacteria since CFUs enumerated were more than the recommended counts for a probiotic product to have health benefit. However, survival of the probiotic bacteria during 28 days refrigerated storage at 4°C was only attained in milk fermented with common dairy starter culture YF L-903. pH changes during storage were not significant for all the milk samples though post acidification was observed in all the fermented bean milk samples regardless of the fermenting starter culture used. Hence survival of probiotics is reduced during storage of

bean milk due to increased acidity but spoilage could still occur through infestations with yeasts and fungi. Therefore, the amounts of probiotics in fermented bean milk will depend on when the consumer drinks it.

Fermentation did not cause significant change in proximate composition of bean milk but significantly increased mineral concentrations (iron, zinc, magnesium, calcium and phosphorous). Vitamins concentrations (pyridoxine, niacin and folic acid) of the three bean milks were also increased by fermentation. However, thiamine was non-quantifiable in fermented milks while riboflavin values were lowered for all the fermenting cultures, except ABT culture. This implies that combination of probiotic strains of *Lactobacillus acidophilus La-5, Bifidobacterium animalis Bb-12* and *Streptococcus thermophilus* could be exploited for natural fortification of riboflavin in bean milk. It was also observed that fermentation significantly lowered phytates and phenolic compounds as well as the oligosaccharide compositions of stachyose, raffinose and verbascose which are well-known to cause flatulence. Although removal of oligosaccharides can reduce flatulence, the negative effect will be that they are important in growth of probiotics and gut health.

Taste, aftertaste and aroma parameters were the qualities which showed largest coefficient of variation. Indeed, after principal component analysis (PCA), sweetness factor which comprised of taste, aftertaste and aroma parameters explained more than half of the total variance in been sensory qualities. Therefore, the sweetness factor was the most important attribute for consumers suggesting that value addition to bean milk should target sweetness factors to improve acceptance of fermented bean milk.

7.2. Recommendations

From the study, it is recommended that;

i. Fermentation with probiotic bacteria should be exploited in development of a nutritious probiotic common bean milk, especially for people with various deficiencies.

ii. Specific health benefits of the fermented bean milk should be exploited.

iii. Strategies to improve sensory acceptability should be exploited to enable consumer acceptability.

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APPENDICES

Appendix I: Ingredients and preparation for culture media (MRS, MRS with 0.5% L-cysteine and M17) and growth temperature and conditions for the starter cultures, Yoba Fiti, ABT and Yoflex (YF L-903).

Starter culture	Culture media	Growth temperature and conditions
Yoba Fiti	**MRS** **Ingredients:** Peptone 10.0; Lab lemco powder 8.0; yeast extract 4.0; glucose 20.0; sorbitan mono-oleate 1 ml; di-potassium hydrogen phosphate 2.0; sodium acetate $3H_20$ 5.0; In-ammonium citrate 2.0; magnesium sulphate $7H_20$ 0.2; manganese sulphate $4H_2O$ 0.05; agar 10.0. **Preparation:** 62g of MRS media was suspended in 1 litre of distilled water and then boiled until the media dissolved. The resulting solution was dispensed into 250 ml flask and sterilized by autoclaving at 121°C for 15 minutes.	30°C
ABT	**MRS with 0.5% L-cysteine** **MRS** **Ingredients:** Peptone 10.0; Lab lemco powder 8.0; yeast extract 4.0; glucose 20.0; sorbitan mono-oleate 1 ml; di-potassium hydrogen phosphate 2.0; sodium acetate $3H_20$ 5.0; In-ammonium citrate 2.0; magnesium sulphate $7H_20$ 0.2; manganese sulphate $4H_2O$ 0.05; agar 10.0. **Preparation:** 62g of MRS media was suspended in 1 litre of distilled water and then boiled until the media dissolved. A 0.5% of L-cysteine (Cysteine Hydrochloride Monohydrate; m.w. 175.64) was added	30°C, Anaerobic conditions

	and the resulting solution was dispensed into 250 ml beaker and sterilized by autoclaving at 15Ibs pressure, 121°C for 15 minutes.	
YF L-903	**M17** **Ingredients:** Papaic digest of soybean meal 5 g/l; peptic digest of animal tissue 5 g/l; yeast extract 2.5 g/l; beef extract 5 g/l; lactose 5 g/l; ascorbic acid 0.5 g/l; magnesium sulphate 0.25 g/l and agar 10 g/l. **Preparation:** 33.25g was suspended in 1000 ml distilled water. Thereafter, 19g of Disodium-β-glycerophosphate was added. The resulting mixture was boiled until the medium completely dissolved. The solution was then sterilized by autoclaving at 15Ibs pressure, 121°C for 15 minutes.	30°C
ALL	**Ringers solution powder** **Preparation:** 8.9g was suspended in 100 ml distilled water. The mixture was heated until the medium completely dissolved. The resulting solution was dispensed into 250 ml beakers and sterilized by autoclaving at 15Ibs pressure, 121°C for 15 minutes.	

Source; instructions for use.

Appendix II: Sensory evaluation of fermented bean milk

You are invited to participate in a research study of perception of bean milk yoghurt. Kindly read this form and ask any questions that you may have before agreeing to be in the study.

This is a voluntary exercise to determine acceptability of fermented bean milk.

The results of your performance as a panelist will be kept strictly confidential.

Kindly fill in your details in the section below:

1. Gender

   Male ☐ Female ☐

2. Age

   <20 ☐ 21-25: ☐ 26-30: ☐ 31-35: ☐

   36-40 ☐ >41 ☐

3. How often do you consume beans?

   Daily ☐ Weekly ☐ Fortnightly ☐ Monthly ☐

   Never ☐

Statement of consent

I have read the information about the conditions of this sensory evaluation and my concerns about the study have been addressed. I hereby give my voluntary consent for participation in this study.

Name: …… Date: ……

Signature……

Sensory evaluation questionnaire

You have been provided with 12 samples of fermented bean milk.

Please take a sip of water to clean your palate before and after tasting the sample.

Sip the fermented bean milk and hold in the mouth for 5 seconds.

Record your perception by using the scale below. Please look and taste each of the (12) coded fermented bean milk samples. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicating your reference number (1-9) in the column against each attribute.

9 – Like extremely

8 – Like very much

7 – Like moderately

6 – Like slightly

5 – Neither like nor dislike

4 – Dislike slightly

3 – Dislike moderately

2 – Dislike very much

1 – Dislike extremely

Attributes	**Sample codes**											
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12
Color/appearance												
Taste												
Aroma												
Texture												
Flavor												
Aftertaste												
Overall acceptability												
Would you prefer to buy this product? (Yes or No)												

Additional comments:-

Thank you for participating in the study.

Appendix III: Images of common bean varieties and corresponding milk extracts

Red haricot beans

Yellow kidney beans

Pinto beans

Corresponding milk extracts

Appendix IV: List of Publications

1. **Anino, C**., Onyango, A., Imathiu, S. & Maina, J. (2019). Effect of Lactic Acid Bacteria Starter Cultures on Vitamin and Oligosaccharide Composition of Milk Extracted from Three Common Bean (*Phaseolus vulgaris* L.) Varieties. *Journal of Food Research*, *8*(3), 103–110. https://doi.org/10.5539/jfr.v8n3p103
2. **Anino, C**., Onyango, A. N., Imathiu, S., Maina, J. & Onyangore, F. (2019). Chemical composition of the seed and 'milk' of three common bean (*Phaseolus vulgaris* L.) varieties. *Journal of Food Measurement and Characterization*, *7*(3), 1–10. https://doi.org/10.1007/s11694-019-00039