EFFECT OF LACTIC ACID BACTERIA FERMENTATION ON THE NUTRITIONAL AND ANTI-NUTRITIONAL CONTENT OF SOAKED AND BOILED WHOLE RED HARICOT BEAN (*Phaseolus Vulgaris* L.).

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DECLARATION

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

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DEDICATION

To my beloved parents Mr.Simon Chemweno and Mrs. Jane Chemweno whom I am forever indebted to. Dad, your love, belief in me and advice have been a propelling force throughout this journey. Mum, your constant prayers and support were the anchor I needed in the most turbulent stages of this academic endeavor.

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LIST OF ABBREVIATIONS and ACRONYMS

AOAC	Association of Official Analytical Chemists
CFU	Coliform Forming Unit
DWB	Dry weight basis
FAOSTAT	Food and Agriculture Organization Corporate Statistical Databases
GIT	Gastrointestinal tract
GRAS	Generally Regarded as Safe
IBSF	Inoculated Boiled Salt solution Fermentation
IBSSF	Inoculated Boiled Salt-Sugar solution Fermentation
ISSF	Inoculated Soaked Salt solution Fermentation
ISSSF	Inoculated Soaked Salt-Sugar solution Fermentation
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LAB	Lactic acid bacteria
Lb.plantarum	*Lactobacillus plantarum*
LCEFoNS	Legume Centre of Excellence for Food and Nutrition Security
LC-MS	Liquid chromatography mass spectrophotometry
MRS	Mann Ragosa Sharpe
NR-NCDs	Nutrition related Non-Communicable Diseases
PCA	Plate Count Agar

PDA	Potato Dextrose Agar
PEM	Protein Energy Malnutrition
RFOs	Raffinose Family Oligosaccharides
SEM	Standard Error of Mean
STDEV	Standard Deviation
TAC	Total Aerobic Count
TTA	Total Titratable Acidity
UBSF	Spontaneous Boiled Salt-only solution Fermentation
UBSSF	Spontaneous Boiled Salt-Sugar solution Fermentation
UN	United Nations
UNICEF	United Nations Children's Fund
USSF	Spontaneous Soaked Salt-only solution Fermentation
USSSF	Spontaneous Soaked Salt-Sugar solution Fermentation
VRBGA	Violet red bile agar

ABSTRACT

Common beans are a leguminous plant of the genus *Phaseolus*. They are grown worldwide making them easily available and affordable. In addition to providing high protein, energy and minerals, their consumption confers health benefits ranging from lowering blood cholesterol, maintaining blood glucose balance, alleviating constipation and contributing to the improvement of the gastrointestinal microbiome. Utilization of common bean has however been poor due to high anti-nutrient content that results in reduced carbohydrate and protein digestibility and mineral bioavailability. Flatulence after consumption is also a huge deterrent to bean consumption. Fermentation is a food processing technique that uses microorganisms and their enzymes to break down complex food structures resulting in desirable nutrient and compositional changes as well as detoxification of foods. Lactic acid fermentation is the most common form of food fermentation with the Lactobacilli *spp* dominating most spontaneous food fermentations. The objective of this study was to determine the effect of Lactic acid bacteria (LAB) on the nutritional, anti-nutritional and flatulence causing oligosaccharides composition of red haricot bean. A factorial research design was used in the study. Red haricot beans were sorted, soaked overnight and boiled for 60 minutes. The soaked and boiled beans were fermented in salt-only and salt-sugar solutions at 2% solute concentration for 120 h. Experimental batch was inoculated with *Lb. plantarum* BFE 5092 and the control batch spontaneously fermented. Microbial growth and pH were monitored during the fermentation. After fermentation, the beans were dried and milled and the flour subjected to proximate and anti-nutrient composition analysis. Analysis of variance was done using SPSS statistics 23. Salt-sugar solutions lowered pH significantly ($P<0.05$) after 72 h of fermentation whereas salt-only solutions recorded a slow decline with fermentation time. The total aerobic bacteria and LAB counts significantly increased ($P<0.05$) with fermentation time. Coliform counts decreased significantly ($P<0.05$). Yeast and mold were not detected in all the batches. Fermentation of the boiled beans increased ash and protein content and lowered the crude fiber, and carbohydrate content. Soaking and boiling lowered the tannin content to 306.82 mg/100 g and 274.78 mg/100 g respectively. Fermentation further lowered them to 109.50-54.04 mg/100 g and 97.07-68.47 mg/100 g respectively. Phytic acid content decreased significantly after soaking and boiling to387.25 mg/100 g and 350.11 mg/100 g respectively. Fermentation further lowered the phytate content to 242.52-163.43 mg/100 g and 160-109.07 mg/100 g in the soaked and boiled beans respectively. Spontaneous fermentation resulted in more phytate and tannin reduction. Soaking and boiling lowered stachyose and raffinose content. Fermentation of soaked beans resulted in up to 35.82% and 42.63% loss of stachyose and raffinose respectively. Fermentation of boiled beans resulted in up to 76.53% and 78.56% decrease in stachyose and raffinose respectively. Salt-sugar solution provided the best condition for growth of LAB which dominated the fermentation, lowered pH and suppressed growth of coliforms. Spontaneous fermentation resulted in more anti-nutrient and oligosaccharide loss compared to inoculation with *Lb. plantarum* BFE 5092. Fermented boiled beans recorded higher anti-nutrient losses.

KEY WORDS*: Lb. plantarum* BFE 5092, Salt-only solution, Salt-sugar solution, Fermentation, Soaking, Boiling

CHAPTER ONE

INTRODUCTION

1.1 Background information

The year 2016 was declared an international year of pulses by the United Nations (UN) with the aim of increasing public awareness on the benefits of consuming pulses. Pulses are edible seeds of the legume family in which common beans belong. Common beans (*Phaseolus vulgaris* L) are leguminous plants of the genus *Phaseolus*. They are widely grown around the world, making them available and affordable. Their seeds are consumed at maturity either as green or dry grains and are a good source of protein rich in lysine (Suárez-martínez *et al*., 2016) which is the limiting amino acid in cereal grains. Therefore, complimenting cereal based diets with common beans results in meals with a balanced amino acid ratio. Common beans are also rich in essential minerals (Díaz, Caldas, & Blair, 2010) hence a solution to hidden hunger which plagues many populations. In addition, they have also been associated with numerous health benefits (Chaudhary & Sharma, 2013) including, lowering blood cholesterol and stabilizing blood sugars. As a result of their highly nutritious nature, beans have the potential to be developed into a multiple use product (Nyombaire, Siddiq, & Dolan, 2002) with nutritional benefits.

Research has shown that even though beans are nutritionally rich, they are also high in anti-nutrients. Anti-nutrients are biologically active compounds that when consumed reduce protein and starch digestibility and reduce the bioavailability of minerals (Adeniran, Farinde & Obatolu, 2013) and may cause flatulence to the consumer. The anti-nutrients in common beans include phytates which lowers mineral bioavailability, tannins which affect nutrient digestibility and the raffinose family oligosaccharides (RFOs) sugars which cause flatulence (Nyombaire *et al*., 2002). In addition to the shunning away from common beans due to stomach discomforts and eructation which is

a social problem as a result of flatulence, the other anti-nutrients reduce the potential of common beans to meet the nutritional needs of the populations that heavily depend on them as an affordable source of protein and minerals (Nyombaire, Siddiq & Dolan, 2002).

Several pre-processing techniques like soaking, boiling, decortication and germination have been adapted to lower the anti-nutrients in foods (Tope, 2014). In addition, fermentation has also been reported to be effective in detoxification of food. Fermentation is a metabolic process in which energy and carbon is derived from organic compound (Bourdichon *et al*., 2012). It is mainly carried out by fermentation of microorganisms which could grow naturally in food or added intentionally under specified conditions. Fermented foods account for about 25% of the total foods consumed around the world (Adebayo, Aderiye & Akpor, 2014) with most of these foods being prepared at household levels using natural fermentation methods. Natural fermentation techniques have been used for ages in making different products but as the world population increases, there is an increased demand for commercialization of these products to meet consumer needs as well as improve food security.

Lactic acid bacteria (LAB) have been isolated as the major microorganisms in many of the naturally fermented food products (Chelule, Mokoena & Gqaleni, 2010). This is a group of gram-positive bacteria that are non-spore forming, anaerobic and acid tolerant. They are closely associated with the human gastro intestinal tract and are generally regarded as safe (GRAS). Many strains of LAB species have been identified as probiotic which means they confer additional health benefits to the host on consumption (Pundir *et al*., 2013). LAB has a vast number of enzymes ranging from proteases, amylases and α-galactosidases which enable them to hydrolyze a number of complex carbohydrate substrates (Chelule *et al.*, 2010). The hydrolysis results in the production of lactic acid, acetic acid and other volatile compounds depending on the microorganism present in the fermentation. The acid produced lowers the pH which inhibits the growth of the other microorganisms hence preserving the ferment from spoilage.

Salt serves the purpose of selecting for salt tolerant LAB such as *Lb.plantarum* (McFeeters & Pérez-Díaz, 2010). It helps to draw water and sugar from the plant tissue into the fermentation liquor. The released water and sugars consequently promotes fermentation reactions hence increasing the rate of fermentation (Henney *et al*., 2013). As the sugars diffuse into the brine, LAB growth increases rapidly, this results in the production of organic acids and other antimicrobial compounds which consequently inhibits growth of acid intolerant bacteria (Mcmurtrie & Johanningsmeier, 2018).

This research endeavored to solve the issue of common bean underutilization by lowering anti-nutrient and RFOs composition through LAB fermentation and therefore improving the nutritional benefits of consuming common beans.

1.2 Problem statement

Many developing countries still grapple with the burden of Protein Energy Malnutrition (PEM) and micronutrient malnutrition as a result of poor and inadequate diets (Aworh, 2008; Ugen *et al.*, 2009). In addition to PEM, the additional burden of nutritionally related non communicable diseases (NR-NCDs) is increasingly weighing in on to the malnutrition crisis. The NR-NCDs are majorly attributable to increased consumption of refined, high sugar and fatty foods in the diets. Majority of the population in developing countries live below the poverty line earning less than a dollar per day. These people heavily depend on low cost food products for their nutrients (Audu *et al*., 2013). Consumption of common beans has been limited chiefly because of its high anti-nutritional content (Mohamed *et al*., 2011). Flatulence is a major problem associated with consumption of beans. This has been attributed to the high oligosaccharide content in common beans. Fermentation of oligosaccharides by the colonic microflora results in gas production in the gastro-intestinal tract (GIT). In addition to abdominal discomfort, the increased flatus results in social problems such as boborygmi and eructation (Granito & Alvarez, 2006; Granito *et al.*, 2003). Beans are also high in phytates, tannins, lecithin, trypsin inhibitors, and amylase inhibitors (Mohamed *et al*., 2011). These compounds

result in reduced carbohydrate and protein digestibility as well as lowers mineral bioavailability hence minimizing the nutritional benefit of bean diet to its consumers (Granito & Alvarez, 2006). Either one or a combination of these problems associated with bean consumption has resulted in populations shunning bean consumption. This has led to the underutilization of this important nutrient dense food product.

In a bid to reduce the problems associated with consumption of beans, pre-processing techniques like soaking, decortication, boiling, cooking, autoclaving and fermentation have been applied. Studies show that these processes are able to lower the content of anti-nutritional compounds in beans. However, a majority of these studies have been applied of bean flours and bean milk. Information on the effect these processes on whole common beans (*Phaseolus vulgaris* L) is scanty. Since common beans are mostly consumed whole it means that the populations continue to grapple with reduced nutrient digestibility and bioavailability as well as flatulence on consuming common bean while good proportion of the population avoid its consumption all together. It is therefore important to scale up research and establish ways to ferment common beans whole. This will increase the utilization of the common beans and hence unlock the potential of common beans in alleviating PEM, NR-NCDs and other nutritional concerns in the population.

1.3 Justification

Beans are widely grown throughout the world and can be consumed either as green or dry grains. The dry grain is the most commonly consumed form and it has a longer shelf life. This makes beans available to the populations at a lower cost (Agbenorhevi *et al*.,2010; Udensi *et al*., 2010) in comparison to other protein sources like those from animals. Beans are a rich source of proteins (Reddy *et al*., 1985) having a high content of lysine the limiting amino acid in cereals. This property makes it an important food for complementation with cereal grains to achieve a balanced amino acid ratio (Njoroge *et al.*, 2015). They also are a good source of calories and minerals (Adeniran *et al.*, 2013;

Reddy *et al*., 1985). The excellent nutritional value of common beans makes it an important complimentary food to the majorly root and tuber based African diets (Udensi *et al*., 2010). World Cancer Research Fund International., (2014) reported that consumption of predominantly plant based diets reduces the risk of developing obesity, diabetes, cardiovascular diseases and some forms of cancer. Due to its nutrient composition, common beans hold the promise of unlocking the double burden of malnutrition. However a good portion of the population do not benefit from this powerhouse as they shy away from consuming common beans (Audu & Aremu, 2011). Though beans are widely recognized as a low-cost nutrient dense food product, they are underutilized. The underutilization has been attributed to their high energy and time consumption during preparation, presence of flatulence causing oligosaccharides and high anti-nutrient levels that result in reduced nutrient digestibility and mineral bioavailability. Fermentation is a processing technique that has been used from time immemorial to preserve foods as well as develop new products with desired flavor, texture and nutritive properties. Increased scientific and clinical research continue to unmask the potential health benefits of fermented food products and will result in increased consumption of these foods (Hutkins, 2006). Lactic acid bacteria (LAB) fermentations improves digestibility and nutritional value of the ferment. LAB was used because of its good fermentation attributes and ability to break down complex carbohydrates. The results of this study provide a way out of the hurdle of flatulence effect in common beans and improve the beans' nutritional value consequently contributing to the UN's goal of increasing consumption of common beans. It is also a window towards alleviating PEM and curbing the ever-rising burden of NR-NCDs and hence will be of great significance to the nutrition and public health policy makers. It also provides yet another avenue for exploiting the benefits of LAB fermentation for the benefit of the human population.

1.4 Objectives

1.4.1 Main objective

To determine the effect of Lactic acid bacteria (LAB) fermentation on the nutritional, anti-nutritional and oligosaccharide composition of soaked and boiled whole red haricot bean (*Phaseolus vulgaris* L).

1.4.2 Specific objectives

1. To determine the growth of behavior of Lactic acid bacteria and its effect on quality parameters such as acidity during the fermentation of soaked and boiled whole red haricot bean (*Phaseolus vulgaris* L)
2. To determine the effect of pretreatment (soaking and boiling) of whole red haricot bean (*Phaseolus vulgaris* L) before fermentation on the nutrient, anti-nutrient and oligosaccharide composition of the beans
3. To determine the effect of fermentation of pretreated whole red haricot bean (*Phaseolus vulgaris* L) on the nutrient, anti-nutrient and oligosaccharide composition of the beans

1.5 Hypotheses

H_0: Lactic acid bacteria (LAB) fermentation has no effect on the nutritional, anti-nutritional and oligosaccharide composition of soaked and boiled whole red haricot bean (*Phaseolus vulgaris* L).

H_a: Lactic acid bacteria (LAB) fermentation has an effect on the nutritional, anti-nutritional and oligosaccharide composition of soaked and boiled whole red haricot bean (*Phaseolus vulgaris* L).

CHAPTER TWO

LITERATURE REVIEW

2.1 Cultivation, consumption and nutritional value of common beans

Legumes are widely cultivated in the world and especially the tropics where most developing countries are situated (Apata, 2008). As a source of food, legumes rank second after cereal grains (Onwurafor *et al*., 2014) hence important in the diets of people in many parts of the world (Shimelis & Rakshit, 2005; Adewumi & Odunfa, 2009; Tope, 2013). Bean is a common name for the genera *Fabaceae* (Agbenorhevi *et al*., 2010*;* Ganesan & Xu, 2017). The common bean (*Phaseolus vulgaris* L.), is one of the five species of the family *Phaseolus* cultivated worldwide (Berrios *et al.*, 2010, Audu & Aremu, 2011) and represent the most important economic variety of the family (Chaudhary& Sharma, 2013) . East Africa has the highest production of common bean (*Phaseolus vulgaris* L) in sub-saharan Africa (Nakitto *et al*., 2015) which constitute an important part of the these countries' diet (Granito & Alvarez, 2006; Díaz *et al*., 2010). Kenya is the third highest producer of common bean in Africa after Tanzania and Uganda (FAOSTAT, 2016). The widespread production of common beans makes them available and affordable (Udensi *et al*., 2010; Audu *et al*., 2013) to the large populations living below the poverty line who are forced to depend on starchy root tubers and cereal grains for their energy and protein needs (Audu *et al*., 2013; Ganesan & Xu, 2017). East Africans are among the highest bean consumers in the world at 40-60kg per capita. In Kenya the annual common bean consumption per capita is 50-60kg (Ugen *et al*., 2009; Legesse *et al.* 2015). It is commonly consumed as *githeri*-a mixture of boiled beans and maize or stewed as an accompaniment for various starches.

Common beans contain practically almost all nutrients and have the potential to be developed as multi-use product beyond their traditional consumption (Nyombaire *et al*., 2002). They are composed of 20-40% proteins with high amounts of lysine, the limiting

amino acid in cereal grains (Ugen *et al*., 2009; Suárez-Martínez *et al*., 2016), 50-60% complex carbohydrates, vitamin B, folic acid (Mcphee *et al.*, 2002; Suárez-Martínez *et al*., 2016) , dietary fiber and minerals including iron, copper, zinc, phosphorus, potassium, magnesium and calcium (Legesse *et al*., 2015; Ganesan & Xu, 2017).Therefore, common bean holds the promise of unlocking the burden of PEM by providing an affordable; readily available alternative source of protein (Udensi *et al*., 2010) to compliment the starchy staple diets to the 36.1% of the Kenyan population living below the poverty line(world bank, 2018). Common beans are also known to confer a wide range of health benefits to their consumers due to the abundant phytochemicals in the seeds (Reyes-Bastidas *et al*., 2010; Agarwal, 2016; Ganesan & Xu, 2017). These phytochemicals act as antioxidants with antidiabetic, anti-carcinogenic, anti-obesity and cardio-protective activities (Ganesan & Xu, 2017). Their carbohydrate fraction is composed of a high proportion of resistant starch, soluble fiber and insoluble fiber (Messina, 2014; Niba & Rose, 2003). This results in stabilizing of blood sugar due to low glycemic index (Martin-Cabrejas *et al*., 2004; Ganesan and Xu, 2017) making them important in protecting and managing diabetes and obesity (Niba & Rose, 2003; Ugen *et al*., 2009; Lattimer & Haub, 2010). The soluble and insoluble fiber also may result in weight loss (Lattimer & Haub, 2010). They also have been associated with potential to lower blood cholesterol (Martin-Cabrejas *et al*., 2004), reduced heart diseases, lowered brain and immune dysfunction (Reyes-Bastidas *et al*., 2010; Chaudhary & Sharma, 2013) Improved gastrointestinal integrity and improved bowel health (Berrios *et al*., 2010). Common beans are also low in fat content (Martin-Cabrejas *et al*., 2004, Chaudhary & Sharma, 2013; Ganesan & Xu, 2017) hence making it nutritionally beneficial. Cruz-Bravo *et al*. (2011) also reported the epidemiological evidence of dietary fiber from beans to protect against colorectal cancer. This means that common bean can be exploited for prevention and management of nutrition related non communicable diseases (NR-NCDs).

Despite the affordability, nutritional and health benefits conferred by common beans, they are not only underutilized but research shows that their consumption is declining among the populations (Granito, Champ, Guerra, & Frias, 2003; Messina, 2014). This trend has been associated with the flatulence effect and abdominal discomfort resulting from their consumption (Nyombaire *et al*., 2002; Granito *et al*., 2003; Granito & Alvarez, 2006; Apata, 2008). The low-income earners who are the highest consumers of common beans (Messina, 2014) also do not enjoy the full nutritional potential of the pulse. This is due to nutrient indigestibility and unavailability (Tope, 2014) as a result of their high anti-nutrient level (Agarwal, 2016).

2.2 Anti-nutrients in common beans

Anti-nutrients are highly bioactive chemical compounds synthesized naturally in foods through different mechanisms during normal metabolism of the plant species (Akande *et al*., 2010; Fekadu, 2014). Anti-nutrients in food are of great concern since their metabolism interferes with the process of nutrient intake and utilization (Granito & Alvarez, 2006 ; Adeniran *et al*., 2013) thereby preventing optimal exploitation of nutrients present in food. Decreased nutrient value of foods as a result of anti-nutritional factors interferes with the health status of the consumers. The major anti-nutrients inherent in common beans include tannins, phytic acid, trypsin inhibitors and amylase inhibitors indigestible starch and α-oligosaccharides (Mcphee *et al.*, 2002). These compounds are endogenously produced by the beans in response to environmental stress (Admassu, 2008). The presence of these anti-nutrients negatively impact on the nutritional value of common beans (Adewumi & Odunfa, 2009). The presence of these anti-nutrients in legumes necessitates application of pre-processing techniques that can result in the alteration of the physical and chemical properties of the legumes (Niba & Rose, 2003)

2.2.1 Tannins

Tannins refer to any large polyphenolic compound containing hydroxyls and other suitable groups that enable them to form strong complexes with proteins and other macromolecules (Fekadu, 2014). They are heat stable and water soluble phenolic compounds with high molecular weight majorly located in the seed coats and account for a considerable portion of dry bean (Reddy *et al*., 1985; Díaz *et al*., 2010; Akande *et al*., 2010; Fekadu, 2014). Tannins have received considerable attention as a result to their possible influence on the nutritional quality of foods (Reddy *et al*., 1985). They have the ability to precipitate proteins through hydrogen bonding and hydrophobic interactions and hence decrease their digestibility (Akande *et al*., 2010; Fernandes *et al*., 2010). They not only form complexes with dietary proteins lowering their digestibility but also inhibit digestion by binding to digestive enzymes (Admassu, 2008; Akande *et al*., 2010; Díaz *et al*., 2010; Starzyńska-Janiszewska *et al*., 2014) ultimately resulting in growth depression (Reddy *et al*., 1985). Research has shown that tannins are responsible for decreased feed intake, growth rate and feed efficiency among rats (Fekadu, 2014). Tannins can also bind to cobalamin (vitamin B_{12}) and interfere with iron absorption resulting in negative biochemical and physiological influence on their consumers. Nakitto *et al*. (2015) reported a higher iron and zinc extractability with decreased tannin content.

2.2.2 Phytates

Phytic acid (Myo-inositol 1,2,3,4,5,6-hexa-kis dihydrogen phosphate), is widely distributed in the grains of mature pulses (Jany *et al*., 2006 ; Ramadan, 2012) and is an important source of phosphate during the germination of the seeds (Agarwal, 2016). It is primarily available as a salt of the mono and divalent cations K^+ , Mg^{2+} and Ca^{2+} and accumulates in the seeds during ripening (Jany *et al*., 2006; Fekadu, 2014). Most of the legumes' phosphorus (46-73%) occurs as organically bound phytin phosphorus commonly known as phytic acid (Akande *et al*., 2010). Accumulation of phytic acid in

the seed cause chelating of other minerals and this result in a complex salt known as phytate (Akande *et al*., 2010). Phytate has been found to decrease calcium bioavailability by forming complexes hence inhibiting calcium absorption in particular (Adeniran *et al*., 2013). The human digestive system lacks enzyme phytase to break down the phytates and hence Phosphorus from beans remains largely unavailable to the consumer. In addition, phytic acid is a strong chelator and hence forms protein-mineral-phytic acid complexes in the diet reducing the protein and mineral availability in the diet (Jany *et al*., 2006; Akande *et al*., 2010; Ramadan, 2012). Its presence in food also impairs the utilization of other micronutrients including Iron, Calcium, Magnesium, Manganese, Copper and Zinc (Fernandes *et al*., 2010; Fekadu, 2014; Agarwal, 2016). Jany *et al*. (2006) and Akande *et al.* (2010) pointed out that major concerns over phytic acid in human nutrition is its effect is on zinc bioavailability. Nakitto *et al.* (2015) reported increased zinc extractability in beans with reduced phytate content. Research has also indicated that phytic acid inhibit the activity of tyrosinase, trypsin, pepsin, lipase and amylase enzymes (Jany *et al*., 2006). In legumes, phytate has been associated with the hard to cook defect in beans (Agarwal, 2016). Hydrolysis of phytates in the gastrointestinal tract may be done by phytases from either dietary sources or bacteria in the colon (Jany *et al*., 2006). Removal of the phosphate group from the inositol ring decreases the mineral binding capacity of the phytate. Dephosphorylation of phytate is therefore a prerequisite for improving the nutritional value of legumes. Alternatively, fermentation and germination treatments are effective in the removal of phytate from foods (Mohamed *et al*., 2011). Despite all the anti-nutritional effects, phytate has also been associated with prevention of kidney stones, protection against dental carries, atherosclerosis, coronary heart disease, diabetes mellitus and some cancers (Jany *et al*., 2006)

2.2.3 Flatulence causing oligosaccharides in common beans

The carbohydrate content in common beans is majorly made up of starch, soluble sugars and dietary fiber (Berrios *et al*., 2010; Winham & Hutchins, 2011). Dietary fiber

component is composed of oligosaccharides that cannot be broken down by the enzymes in the digestive tract and hence provide bulk in the diet and helps promote healthy bowel movement. Among these are the raffinose family oligosaccharides (RFOs) which include raffinose, stachyose and verbascose. They belong to the family of α-galactosides (Granito & Alvarez, 2006; Apata, 2008) and consist of one, two or three α-1,6-D-galactose units respectively linked to sucrose (Sumarna, 2008; Gänzle & Follador, 2012). The RFOs in proper ratio with sucrose provide cell wall stability during seed maturation and desiccation (Mcphee *et al*., 2002). During germination stage they serve as a carbon source for the new seedlings (Agarwal, 2016). Raffinose family oligosaccharides are considered anti-nutritional due to their flatulence causing properties. Generally, RFOs account for 2-10% of legume grain weight (Gänzle & Follador, 2012). Mcphee *et al*., (2002) reported raffinose and stachyose content range between 2-5 mg/g and 18-38 mg/g respectively in common beans and undetectable or presence in trace amounts for verbascose. This makes stachyose the major RFO in common beans (Mcphee *et al*., 2002; Saraswathy & Sadasivam, 2010; Cruz-Bravo *et al*., 2011). Sucrose is important in the biosynthetic pathway for RFOs and was detected in the range of 15-27 mg/g by Mcphee *et al*., (2002). Raffinose and stachyose are regarded as derivatives of sucrose because of similarity in the glysosidic linkages between D-glucose and D-fructose units in each of the three sugars (Sumarna, 2008). Fructose on the other hand was detected in little amounts and was not directly involved in the biosynthetic pathway. Mcphee *et al*. (2002) reported that raffinose was a precursor for stachyose hence attributing their accumulation in common beans to be closely related and possibly controlled by a common mechanism. Raffinose family oligosaccharides have α-D-1,6-galactosidic linkages which are broken down by enzyme α-galactosidase (Berrios *et al*., 2010; Kumar *et al*., 2012). The enzyme α-galactosidase cleaves α-1,6 linked galactose from α-D-galactosidases which include the RFOs, melibiose and branched polysaccharides like galactomannans and galactoglucomannans (Kumar *et al*., 2012). The human digestive system lacks α-galactosidase enzyme and hence cannot hydrolyze the oligosugars (Sumarna, 2008; Devindra *et al*., 2012;

Saraswathy & Sadasivam, 2010). They are therefore passed into the colon where they are anaerobically fermented by bacteria such as *Clostridium* spp. (Granito *et al*., 2003; Granito & Alvarez, 2006 ; Adewumi & Odunfa, 2009; Berrios *et al*., 2010; Kumar *et al*., 2012) to produce H_2, CO_2 and CH_4 which results in the discomfort and embarrassment caused by flatulence (Apata, 2008; Agbenorhevi *et al.*, 2010; Ramadan, 2012; Djaafar *et al*., 2013; Agarwal, 2016). In some cases the flatulence inducing property of legumes may be accompanied by diarrhea, headache and dyspepsia (Adewumi & Odunfa, 2009). A survey conducted in Ghana by Agbenorhevi *et al*., (2010) established that 64.4% of the participants experienced flatulence after consuming a bean meal. He also reported that as a result of the anticipated negative effects after the consumption of beans, some people opt to avoid them completely (Agbenorhevi *et al*., 2010). Winham and Hutchins (2011) reported that though flatulence effects increase with increased fiber intake, the body will adjust to the added fiber with persisted regular consumption of legumes. The major flatulence causing factor in common beans is thought to be the RFO (Ramadan, 2012; Agbenorhevi *et al*., 2010). It is due to this flatulence causing property that makes RFOs to be considered as an anti-nutrient in legumes. Decomposition of these sugars is therefore desirable (Starzyńska-Janiszewska *et al*., 2014) in a bid to increase the consumption of legumes. Use of bacteria with α-galactosidase activity in legume fermentation enables the hydrolysis of indigestible RFOs into disaccharides and monosaccharides. These simple sugars are absorbable and hence improves the nutritional value of the legumes (Granito *et al*., 2003; Sumarna, 2008; Adewumi & Odunfa, 2009). The simple sugars are also utilized by the fermenting microorganisms for their metabolism (Sumarna, 2008). Gänzle and Follador (2012) reported that RFOs metabolism by lactobacilli results in their conversion to α-galacto-oligosaccharides which may prevent their flatulence causing property without eliminating their prebiotic property. Agarwal (2016) reported that RFOs play an important role in reducing the risk of intestinal cancer in addition to increasing stool weight and frequency.

Alpha-galactosidase is an inducible enzyme (Kumar *et al*., 2012) whose production is generally induced in the presence of various sugars such as dextrose, maltose, lactose, sucrose, raffinose and starch. However various works seem to suggest that for different microorganisms, different sugars induce the biosynthesis of α-galactosidase in different micro-organisms. For Bacillus SPE10 and SPE15, Kumar *et al*. (2012) found raffinose to be the best inducer compared to the other sugars. Whereas Shivam, Tripathi, and Mishra (2009) reported that a combination of galactose and raffinose induced more enzyme production in *Aspergillus parasiticus* MTCC 2796, and that melibiose also did induce the enzyme production. Saraswathy and Sadasivam (2010) on the other hand reported that galactose and melibiose moderately inhibited activity of α-galactosidase from *Penicillium purpurogenum*, whereas fructose enhanced its activity. Sucrose and raffinose was reported not to inhibit but as to whether it enhanced the enzyme activity were not reported. Optimum conditions for enzyme production also differs from one microbe to the other. Shivam *et al*. (2009) reported that the presence of zinc, cobalt and copper ions inhibited enzyme production in *Aspergillus parasiticus* MTCC 2796, whereas zinc and copper had no inhibiting effect in *Penicillium purpurogenum* as reported by Saraswathy and Sadasivam, (2010). Saraswathy and Sadasivam, (2010), reported that most fungal α-galactosidases showed optimal activity between pH 4.5 and 5.5 whereas those from bacteria were found to be active at near neutral pH. Decline of enzyme activity with incubation time is associated with inhibition of microbial cellular activity due to depletion of nutrients, enzyme deactivation with lowered pH and aging of the microbes (Sumarna, 2008; Shivam *et al*., 2009). α-galactosidase has various industrial applications including raffinose degradation in beet sugar industry, and removal of flatulence causing oligosaccharides in legumes (Saraswathy and Sadasivam, 2010).

2.3 Fermentation

Advances in food biotechnology has significantly increased the production of quality and nutritious foods with long shelf life that are safe and less reliant on artificial

additives (Soomro, Masud, & Anwaar, 2002). Fermentation is one of the food biotechnology technique through which a desirable product is obtained by modifying raw materials by use of microorganisms (Soomro *et al*., 2002; Hil & Nout, 2005). It is an old food processing technique that dates back to thousands of years and has been used to solve issues of food spoilage in the world (Chelule *et al*., 2010; Rhee, Lee, and Lee, 2011; Bourdichon *et al*., 2012; Adeniran *et al.*, 2013; Gan *et al*., 2017) and for household and commercial level to produce a variety of foods (Onwurafor *et al* 2014). In addition, fermentation is a simple and inexpensive processing technique (Martin-Cabrejas *et al.*, 2004; Ejigui *et al*., 2005). During fermentation, the microbes utilize the food as a substrate for their growth (Chelule *et al*., 2010) resulting in biochemical modification of the food (Issofou, 2010, Onwurafor *et al*., 2014). This contributes to increased shelf-life (Chelule *et al.*, 2010), improved flavor, texture (Martin-Cabrejas *et al*., 2004; Ongol, 2012; Adeniran *et al.*, 2013) detoxification and lowered anti-nutrient level (Porres *et al*., 2003; Hil & Nout, 2005, Chelule *et al*., 2010) of the fermented product. This consequently improves the nutritional quality (Granito & Alvarez, 2006; Bourdichon *et al*., 2012; Akpor & Aderiye, 2014) of the fermented product. Fermentation also increases the economic value of the product due to the resulting value addition (Hutkins, 2006; Ongol, 2012). Fermented foods are also known to have health promoting bacteria known as probiotics (Soomro *et al*., 2002). These are beneficial microorganisms that helps maintain favorable microflora balance in the gastrointestinal tract by replenishing suppressed bacteria and inhibiting growth of pathogenic microorganisms (Soomro *et al*., 2002; Anuradha & Rajeshwari, 2005; Onwurafor *et al*., 2014) consequently boosting the immune system and improving resistance to infection. The action of probiotics is through a variety of mechanisms including production of organic acids, bacteriocins and reuterin which poses inhibitory properties against growth of pathogenic microorganisms (Soomro *et al*., 2002; Pundir *et al*., 2013). Ongol, (2012) reported that regular consumption of probiotics improves health by restoring and maintaining microbial balance in the respiratory, gastrointestinal and urogenital tracts thus preventing colonization by pathogenic bacteria. Fermented food products are

continually increasing in popularity across the world. This especially is aided by the increase in cultural and cuisine interactions (Hutkins, 2006).

Lactic acid bacteria (LAB) are the most common fermentative microorganism in plant based material fermentation (Granito & Alvarez, 2006). They are a diverse group of non-motile, catalase negative, gram positive rods or cocci. These bacteria utilize a wide variety of carbohydrates as carbon source and produce lactic acid as a major or sole metabolic product (El-din, 2010). The fermenting microorganism produce enzymes that hydrolyze the complex structures in food resulting in the simple, non-toxic products (Chelule *et al.*, 2010). These products have improved digestibility and bioavailability (Assohoun *et al*., 2013). Textural and compositional changes result in the softening of the fermented product (Chelule *et al.*, 2010, Onwurafor *et al*., 2014) hence lowering the energy required to cook.

Various fermentation techniques can be applied on food including solid state fermentation and submerged culture fermentation (Onwurafor *et al*., 2014; Gan *et al*., 2017).There are also different forms of fermentations classified on the basis of their end products. They include alcohol fermentation, lactic acid fermentation, alkaline fermentation, acetic acid fermentation and peptide sauce fermentation (Chelule *et al.*, 2010). Fermentation can be initiated spontaneously by use of indigenous microbiota in the food or by use of specific starter cultures from a previously fermented product (Onwurafor *et al*., 2014, Porres *et al*., 2003). Inoculated fermentation can also be done by addition of GRAS microbes into the food (Gan *et al*., 2017). Pre-processing techniques such as cleaning, soaking, or thermal treatment is necessary before inoculated fermentation (Gan *et al*., 2017). This is necessary to lower the load of preexisting microbes. However for natural fermentation it is important to maintain these microbiota for successful fermentation to occur (Rhee *et al*., 2011; Gan *et al*., 2017). Fermentation may be combined with other culinary and processing techniques to improve the nutritive value of foods (Porres *et al.*, 2003). For efficient fermentation to occur, it is paramount that fermentation conditions like inoculum size, temperature, oxygen availability and

substrate content be carefully controlled (Gan *et al*., 2017). This will vary depending on the nature of fermentation and the specific demands of the fermenting microbes.

2.3.1 Use of salt during fermentation

The use of salt during fermentation of plant material is key (Henney *et al.*, 2013). Salt can be applied to fermentation either as dry salt or dissolved in fermentation liquor to make brine (Vatansever *et al*., 2017). Salt helps to draw water and sugar from the plant tissue into the fermentation liquor. The released water and sugars consequently promotes fermentation reactions hence increasing the rate of fermentation (Henney *et al*., 2013). In addition, salt helps to select for LAB which are salt tolerant unlike other spoilage microorganisms (Henney *et al*., 2013.; Mcmurtrie & Johanningsmeier, 2018). As the sugars diffuse into the brine, LAB growth increases rapidly, this results in the production of organic acids and other antimicrobial compounds which consequently inhibits growth of acid intolerant bacteria (Mcmurtrie & Johanningsmeier, 2018). Higher brine concentrations ensures better anaerobic conditions which is favorable for LAB growth (Vatansever *et al*., 2017). However, the amount of salt used during fermentation will determine the type and rate of fermentation. Higher salt concentration above 10% will result in product preservation by the salt itself and not through fermentation, this is because it will cause osmotic shock to the bacteria (FAO, n.d). Use of salt in fermentation liquor also results in decreased water activity. When the water activity goes below optimum values for growth, then a linear decrease in microbial growth rate will be observed (Johanningsmeier, Franco, Perez-diaz, & Mcfeeters, 2012). Johanningsmeier *et al*. (2012) reported than use of more than 5 g/L of Sodium Chloride (NaCl) concentration during the fermentation of cucumber resulted in slowed growth of microbial cells. Chin & Koehler, (1986) also reported that low salt of 5% concentration supported higher LAB growth compared to 10 % NaCl concentration. FAO (n.d) also reported 2-5% NaCl concentration to best support LAB fermentation. Homo-fermentative LAB have a lesser tolerance to salt compared to the hetero-fermentative LAB. Therefore NaCl can be used to select for homo-fermentative LAB during

fermentation (Johanningsmeier *et al*., 2012). Cultivation of LAB in environments with lower NaCl concentration exhibits a positive growth effect though in higher levels, the salt will hamper growth of the bacteria (Johanningsmeier *et al*., 2012)

2.3.2 Lactic acid fermentation

Lactic acid fermentation is the most dominant fermentation in food (Chelule *et al*., 2010) and consequently in legume fermentation. *Bacillus subtilis,* molds and yeasts have also been used in fermentation of some legumes (Gan *et al*., 2017). Lactic acid fermentation results in the production of lactic acid which lowers pH and increases titratable acidity of the system. This results in the inhibition of acid-intolerant pathogenic microorganism (Porres *et al*., 2003; Onwurafor *et al*., 2014) such as *E.coli, Salmonella and Shigella* spp. This makes the food resistant to microbial spoilage and toxin development (Chelule *et al*., 2010; Rhee *et al*., 2011). Acid production is also contributes to the flavor development and stability of the product (Rhee *et al*., 2011; Onwurafor *et al*., 2014) hence increasing the shelf life of fermented product by preserving it from harvest to consumption. Lactic acid bacteria influence the nutritive and health attributes of the fermented products (Ongol, 2012). Chelule *et al*. (2010) reported that consumption of Lactic acid fermented products prevents diarrheal diseases by modifying the composition of intestinal microorganisms as a result deterring pathogenic microbes. Additionally, LAB is credited with alleviating lactose intolerance as a result of lactase activity on lactose in dairy products; reduction of serum cholesterol through various mechanisms including reduction of intestinal absorption of dietary cholesterol and inhibiting cholesterol synthesis in the liver; prevention of antibiotic associated complications; improved quality of life for persons with inflammatory bowel disease, lowered colon and bladder cancer development (Ongol, 2012). Lactic acid bacteria is reportedly a major source of enzyme phytase which is important in degradation of phytate during fermentation (Ongol, 2012)

Lactic acid fermentation is carried out by Lactic acid bacteria (LAB) which include species of *Lactobacilli, Pediococcus, Aerococcus, Streptococcus, Leuconostoc* and *Enterococcus* (Chelule *et al.,* 2010). These are a group of Gram positive, non-sporulating rods or cocci that produce lactic acid on fermenting carbohydrates (Hutkins, 2006; Kocková *et al*., 2011; Ongol, 2012; Pundir *et al*., 2013). Lactic Acid Bacteria are mesophilic with optimum growth between 30°C and 40°C though some strains may grow at temperatures below 5°C while others may do well in temperatures above 45°C (Kocková *et al.,* 2011). They could be aerotolerant, microaerophilic or facultatively anaerobic (Kocková *et al.,* 2011) though LAB generally do well in the latter two environments (Gan *et al*., 2017). Lactic acid bacteria have been isolated from most food fermentations and it is believed that they are responsible for most of the beneficial characteristics of fermented foods (Chelule *et al*., 2010; Kocková *et al*., 2011; Djaafar *et al*., 2013). These bacteria as generally regarded as safe (GRAS) under the US Food and Drug Administration (FDA) guidelines (Ongol, 2012). Lactic acid bacteria have the ability to ameliorate diseases like allergy, Crohn's disease, ulcerative colitis and some cancers (Ongol, 2012) and account for the majority of microorganisms used as probiotics (Pundir *et al*., 2013).

Lactic acid bacteria are non-spore forming rods that occupy a wide range of habitats ranging from vegetables, dairy, meats, juices, grains, cereals as well as the human and animal gut (Hutkins, 2006). They are the most prominent microorganisms in the intestinal flora and are the most commonly used group of microorganisms for their potential benefits as probiotics. They are known to reduce lactose intolerance, alleviate some diarrheas, lower blood cholesterol and improve immune response (Shori & Baba, 2012; Pundir *et al*., 2013). These advantages have resulted in their exploitation as probiotics and ranks them as the largest probiotic bacteria (Amutha & Kokila, 2015). Lactic acid bacteria are known to produce α-galactosidase enzyme that enables them to hydrolyze α-galactoside linkages found in oligosaccharides (Djaafar *et al.,* 2013). The proteolytic and lipolytic activity of the LAB results in the production of aromatic

compounds which contributes to improved taste and flavor of fermented foods (Kocková *et al.*, 2011). They are also known to produce antibacterial substances which have a positive influence on the shelf life of fermented products (Shori & Baba, 2012; Kocková *et al*., 2011). Amutha and Kokila, (2015) reported *Lb. plantarum* isolated from cow milk had cholesterol lowering ability. All these beneficial characteristics have made LAB a focus of intensive food research (Shori & Baba, 2012). The LAB are divided into three groups depending on their metabolism. Homo-fermentative LAB anaerobically metabolize hexose sugars through the glycolytic embden-myerhoff pathway. This way they convert more than 90% of the substrate into lactic acid. Hetero-fermentative LAB metabolize the hexose sugars through the pentose-phosphate pathway. On the other hand, they convert up to 50 % of substrate to lactic acid and the rest is converted to acetic acid, formic acid and ethanol. The third group is the facultatively hetero-fermentative LAB. These bacteria metabolize hexose sugars via both the glycolytic embden-myerhoff and the pentose-phosphate pathways. During scarcity of fermentable sugars, the pentose-phosphate pathways dominates (Kocková *et al*., 2011). In legume fermentation using LAB, the starter culture is inoculated mostly between 1% and 10 % ($Log_{10}6$ and $Log_{10}7$ cfu/ml) of original sample. Temperature is also maintained at 37°C for optimal growth of the bacteria. Fermentation time of between 48 h and 96 h is the most commonly used duration for legume fermentation. Addition of an extra source of energy through the addition of sucrose or glucose is also common to accelerate bacterial growth (Gan *et al*., 2017). Sucrose is widespread and most abundant sugar in grain cereals. A majority of LAB have been reported to harbor at least one of the three functional sucrose metabolic pathway. The presence of these two or more alternative metabolic pathways for sucrose metabolism and higher fructo-oligosaccharides indicates that they are highly preferred substrates. Gänzle *et al.* (2007) indicated that oligosaccharide metabolism is essential for ecological fitness of lactobacilli. However, all these require optimization based on the specific LAB strain used and legume variety.

Lactic acid bacteria have the ability to hydrolyze RFOs to sucrose due to the presence of α-galactosidase enzyme. This enzyme is encoded by MelA, a glycosyl-hydrolase. (Gänzle & Follador, 2012). The presence of two or more alternative sucrose metabolic pathways in lactobacilli strains, results in complete degradation of the RFOs to constituent di- and monosaccharides (Granito *et al*., 2003: Adewumi & Odunfa, 2009; Gänzle & Follador, 2012). Enzymes levansucrase and fructofiranosidase responsible for sucrose metabolism have also shown activity with RFO substrates. Lactobacillus strains expressing *melA*, levansucrase and either of the alternative sucrose metabolic pathways-sucrosephosphorylase or fructofiranosidase exist for RFO degradation (Gänzle & Follador, 2012). Raffinose family oligosaccharides can be degraded through (i) Extracellular conversion of RFO to corresponding α-galacto-oligosaccharide (α-GOS) and fructose through the action of Levansucrase. This is followed by the uptake of the α-GOS by the bacteria and consequently their hydrolysis. (ii) The bacteria take up the RFOs and hydrolyses them to sucrose and galactose through *melA* activity. This is then followed by sucrose conversion through sucrose phosphorylase or fructo furanosidase metabolic pathways (Teixeira *et al* 2012; Gänzle and Follador, 2012). The metabolic diversity of individual lactobacilli strains and species determines their capacity for oligosaccharide metabolism. They are majorly mesophilic with optimum growth temperature of 30°C-45°C, though some psychrotropic and thermoduric lactobacilli strains exist (Hutkins, 2006). They are also known to be acid tolerant and generally fastidious with complex nutritional requirements (Hutkins, 2006)

2.3.3 *Lactobacillus plantarum*

Lactobacillus plantarum is an important LAB in the fermentation of many plant products (Maina *et al*., 2008). It is a homofermenter and the highest acid producing LAB species (Rhee *et al*., 2011). At low temperature, *Lb. plantarum*'s acid production is lower and hence prevents over ripening of fermented products as well as extends the period of optimal flavor (Rhee *et al*., 2011). *Lb. plantarum* has been isolated from diverse ecosystems ranging from vegetable, dairy, meat, fish as well as the

gastrointestinal tract (Siezen & Vlieg, 2011). They have a wide range of industrial application owing to their phenotypic, genomic and metabolic diversity (Oguntoyinbo *et al*., 2016). *Lb. plantarum* strains are known to produce several bacteriocins which can act as antimicrobial agents against some Gram-positive bacteria (Anderssen *et al*., 1998; Cho *et al*, 2010), *Lb. plantarum* strains have the best fermentation characteristics as they are tolerant to gastric and bile acid in the human gut with probiotic effects (Ammor & Mayo, 2007; Xiong *et al*., 2012). Adewumi and Odunfa, (2009) reported that *Lb. plantarum* was able to produce α-galactosidase enzyme which is able to cleave the α-1, 6-glycosidic linkages in RFOs and branched polysaccharides. Alpha-galactosidase enzyme is active as a homotetramer and recognizes unbranched oligosaccharides including raffinose and stachyose as subtrates. It is encoded by *MelA*, a glycosyl-hydrolase in GH36 family in *Lb.plantarum* (Gänzle & Follador, 2012). Alpha-galactosidase hydrolyses RFOs to release sucrose and hence complete degradation of these sugars is dependent on the presence of sucrose metabolic enzymes (levansucrase and fructofuranoside) in the bacteria (Gänzle and Follador, 2012)

Lb.plantarum BFE 5092 strain was isolated from *kule naoto* a traditionally fermented milk product from the Maasai community of Kenya (Maina *et al*., 2004) It has a genome size of 3,285094bp with 433 of its genes involved in sugar metabolism (Oguntoyinbo *et al*., 2016). Maina *et al.* (2008) showed the ability of *Lb. plantarum* BFE 5092 to survive through the gastrointestinal tract in an in-vitro model hence its probiotic potential. It also has been shown to possess genes for production of plantaricins EF, JK and N (Oguntoyinbo *et al*., 2016; Cho *et al*., 2010) hence potential to inhibit gram positive pathogens. *Lb. plantarum* BFE 5092 possesses 433 genes involved in sugar metabolism, especially those for phosphoenolpyruvate or phosphotransferase system for utilization of various sugars such as N-acetylglucosamine, mannose, sucrose, maltose, and glucose, beta-glucoside sugars, cellobiose, trehalose, mannitol, galactitol, fructose, as well as glucitol and sorbitol sugars (Oguntoyinbo *et al*., 2016). Currently studies on its application as a starter culture for indigenous African leafy vegetables are underway.

2.4 Processing techniques for lowering anti-nutrients in common bean

Various processing techniques have been exploited in the endeavor to lower the anti-nutrient composition in common bean including soaking, dehulling, germination, boiling as well as fermentation (Ramadan, 2012). Fermentation has been shown to lower the RFOs and other anti-nutritional components in legumes and their products (Assohoun, *et al* 2013; Osman & Gassem, 2013). Edible seed require pre-treatment or a combination of the pre-processing techniques before fermentation. These pre-processing techniques range from soaking, splitting, cooking, grinding, sieving or thermal treatment (Gan *et al*., 2017). Water soluble and thermo-labile anti-nutrients can be eliminated by the traditional method of soaking overnight and boiling (Mcphee *et al*., 2002; Fernandes *et al*., 2010; Ramadan, 2012). The RFOs are however not completely eliminated (Niba and Rose, 2003; Ramadan, 2012). Due to the discomfort and social embarrassment associated with the RFOs in beans, some people opt to completely avoid consumption of beans (Agbenorhevi *et al*., 2010). Reduction of these oligo sugars promises improved utilization of common bean. This resonate with the desire of 80% of the participants of the Ghana survey by Agbenorhevi *et al*. (2010). Consequently, higher consumption of common bean will result in the improvement of the nutritional and health status of the world. Fermented legume products are popular due to their desirable properties. These include improved organoleptic properties, enhanced digestibility (Tope, 2013), removal of anti-nutritional compounds and consequently increased nutritional value (Granito & Alvarez, 2006). Additionally, fermented legumes have been reported to contain increased beneficial bioactive components (Gan *et al*., 2017). Though common bean is consumed whole, fermentations on beans has been mostly done on flours and extracted milk. To increase the utilization of common bean in its conventional way, this research was devoted to exploit the possibility of lactic acid fermentation on whole common beans. Submerged fermentation using different brine solutions was applied and their effect on the growth of LAB and influence on microbial safety. The food biotechnology field has recently developed a number of commercial probiotic products (Shori & Baba,

2012). Fermented whole common bean will definitely be an important addition to the industry.

2.5 Gaps in knowledge

Underutilization of beans has been an issue of concern with various methods being proposed to alleviate the causal problems. Though fermentation has been used over time its major focus has always been on new product development to diversify diets. The use of fermentation for solving anti-nutrient and cooking quality of common beans has not been fully exploited. Lactobacilli spp. has been shown to possess attributes that can breakdown anti-nutritional components and importantly the flatulence causing polysaccharides. This research seeks to fill this gap in knowledge by exploiting the LAB's ability to ferment whole common bean grains and providing a solution to the problems causing the poor utilization of common beans.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research design

A four factorial research design was used in this study. The research design outlook is presented in Figure 3.1 and the fermentation set up is presented in Figure 3.5 respectively. The factors in this study were pre-treatment, fermentation solution, and fermentation type and time. The levels in pre-treatment were soaking and boiling after soaking.

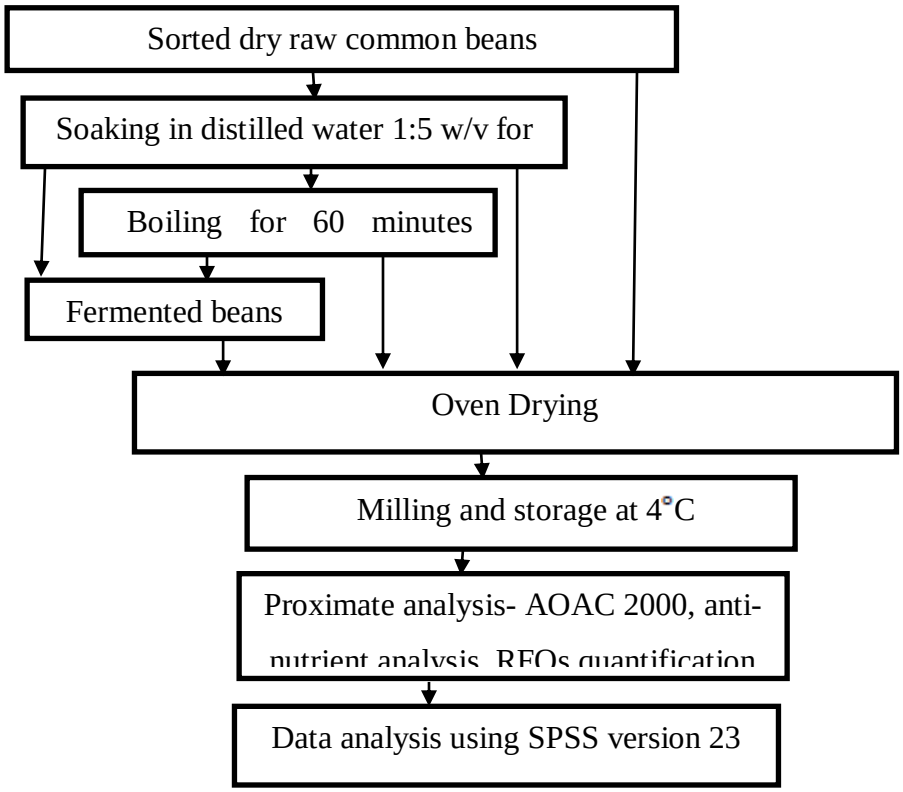


Figure 3.1: Research design outlook

The levels in Fermentation solution were 2% salt-only solution and 2% salt-sugar solution at a ratio of 1:1. The levels in fermentation type were inoculation with *Lb. plantarum* BFE 5092 and spontaneous fermentation. The levels in fermentation time were 0 h, 24 h, 72 h and 120 h. Assessment of the main effect of factors and their interaction effect on the nutrient, anti-nutrient (tannins and phytates) and RFOs (raffinose and stachyose) was done.

3.2 Materials

Ten kilograms of red haricot beans (locally in Kenya referred to as *Wairimu*) was acquired from the National Cereals and Produce Board (NCPB). The beans were transported in a gunny bag to Jomo Kenyatta University of Agriculture and Technology where they were transferred to airtight plastic containers and stored in a cold room at 10°C awaiting analysis. *Lb.plantarum* BFE 5092 strain was isolated from *kule naoto* a traditionally fermented milk product from the Maasai community of Kenya (Maina *et al*., 2004) by Dr. Julius Maina Mathara of the department of Food Science, Jomo Kenyatta University of Agriculture and Technology. The table salt (Kensalt) and local sugar (Kabras) used in this study were purchased locally.

3.3 Pretreatment of red haricot beans before fermentation

The red haricot beans (Figure 3.2) were hand sorted to remove dirt and defective grains. About 500 g of the beans was drawn as control sample, milled raw and stored in freezer awaiting biochemical analysis.

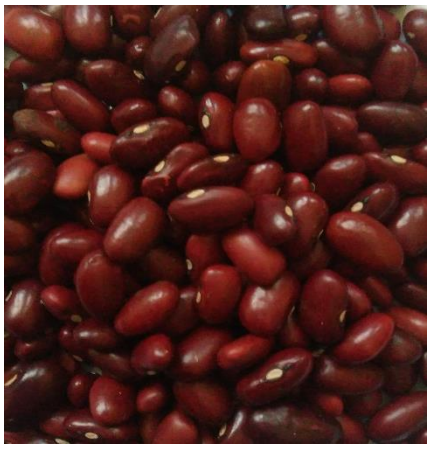


Figure 3.2: Raw red haricot bean (*Phaseolus vulgaris* L)

3.3.1 Soaking of the beans

About 2 kg of the sorted red haricot beans was washed in distilled water and all floats removed. The beans were then soaked in distilled water (Figure 3.3) at a ratio of 1:5 weight per volume for 15 h at room temperature. The soaking water was drained and rinsed in distilled water. The soaked beans were then divided into six portions, 5 portions of 200 g each and 1 portion of 1 kg. From the 5 portions of 200 g, 1 portion of soaked beans was dried at 60°C for 10 h in an oven (Memmert UF 110, Germany), milled then stored in a freezer awaiting biochemical analysis. The other 4 portions of soaked beans were put in zip lock bags awaiting fermentation. The sixth portion of 1 kg of soaked beans was subjected to boiling.

Figure 3.3: Red haricot bean (*Phaseolus vulgaris* L) in soaking water

3.3.2 Boiling of the beans

The 1 kg portion of soaked beans from 3.3.1 was further divided into 5 portions of about 200 g. These portions were then transferred into 5 sterile fermentation bottles with 400 ml of distilled water (1:2 w/v) (Figure 3.4.). They were then boiled on a hot plate for 60 minutes as presented in Figure 3.4. After boiling, the boiling water was drained and the beans allowed to cool in the fermentation bottles before fermentation. Boiled beans from one bottle were removed from the bottle, rinsed in distilled water, and then dried for 60°C for 10 h in an oven. The dried boiled beans were then milled with a steel mill then stored in a freezer awaiting biochemical analysis

Figure 3.4: Red haricot bean (*Phaseolus vulgaris* L) during boiling

3.4 Fermentation protocol for red haricot bean

Preliminary study was carried out on the fermentation of whole red haricot bean (*Phaseolus vulgaris* L) and the results informed the fermentation protocol adopted. The fermentation protocol used in this study is presented diagrammatically in Figure 3.5.

3.4.1 Preparation of fermentation solutions

A 2% salt-only solution was prepared by dissolving 12 g of salt into four clean fermentation bottles containing 600 ml of distilled water each. A salt-sugar solution was prepared by dissolving 12 g of salt and sugar 1:1 w/w in four clean fermentation bottles containing 600 ml of distilled water each. This resulted in 2% salt-only and salt-sugar fermentation solutions. The bottles with the fermentation solutions were then sterilized by autoclaving at 121 °C for 15 min. They were allowed to cool to room temperature in readiness for fermentation experiments.

3.4.2 Starter culture preparation

About 100 µl of cryopreserved *Lb. plantarum* BFE 5092 starter culture were transferred into 9 ml MRS broth and incubated at 30 °C for 24 h. The broth was then vortexed and a loop-full streaked on MRS agar plate and incubated for 24 h at 30 °C to check for purity. A pure colony was then transferred into MRS broth and incubated for 24 h at 30 °C. Approximately 0.6 ml of broth was then transferred into eight 1.5 ml Eppendorf tubes and vortexed at 13,000 rpm for 5 min. The supernatant was discarded and the pellet dissolved in 600 µl sterile ringer solution. This was used as starter culture for the batches to be inoculated with *Lb. plantarum* BFE 5092.

3.4.3 Fermentation

3.4.3.1 Preparation of soaked beans for fermentation

From the 4 portions of 200 g of soaked beans prepared in 3.3.1, two portions were each transferred into two fermentation bottles each containing 600 ml of 2 % salt-only fermentation solution prepared in 3.4.1. Exactly 600 µl of *Lb. plantarum* BFE 5092 starter culture prepared in 3.4.2 was then added to one of these fermentation bottles. This was labeled inoculated soaked salt-only fermentation (ISSF). The remaining fermentation bottle with 200 g of soaked beans in salt-only solution was labeled un-inoculated soaked salt-only fermentation (USSF). The other two portions of 200 g of soaked beans described in 3.3.1 were each transferred into two fermentation bottles each containing 600 ml of 2 % salt-sugar fermentation solution prepared in 3.4.1. Exactly 600 µl of *Lb. plantarum* BFE 5092 starter culture prepared in 3.4.2 was then added to one of these fermentation bottles. This was labeled inoculated soaked salt-sugar fermentation (ISSSF). The remaining fermentation bottle with 200 g of boiled beans in salt-sugar solution was labeled un-inoculated soaked salt-sugar fermentation (USSSF). The ISSF, ISSSF, USSF and USSSF bottles are presented in Figure 3.6

Figure 3.5: Soaked red haricot bean (*Phaseolus vulgaris* L) in fermentation solutions

3.4.3.2 Preparation of boiled beans for fermentation

Exactly 600 ml of 2 % salt-only fermentation solution prepared in 3.4.1 was transferred into 2 fermentation bottles with 200 g of boiled beans each described in 3.3.2. Exactly 600 µl of *Lb. plantarum* BFE 5092 starter culture prepared in 3.4.2 was then added to one of these fermentation bottles. This was labeled inoculated boiled salt-only fermentation (IBSF). The remaining fermentation bottle with 200 g of boiled beans in salt-only solution was labeled un-inoculated boiled salt-only fermentation (UBSF). Exactly 600 ml of 2 % salt-sugar fermentation solution prepared in 3.4.1 was transferred into the remaining 2 fermentation bottles with 200 g of boiled beans each described in 3.3.2. Exactly 600 µl of *Lb. plantarum* BFE 5092 starter culture prepared in 3.4.2 was then added to one of these fermentation bottles. This was labeled inoculated boiled salt-sugar fermentation (IBSSF). The remaining fermentation bottle with 200 g of boiled beans in salt-sugar solution was labeled un-inoculated boiled salt-sugar fermentation (UBSSF). The IBSF, IBSSF, UBSF and UBSSF bottles before fermentation is presented in Figure 3.7.

Figure 3.6: Boiled red haricot bean (*Phaseolus vulgaris* L) in fermentation solutions

The fermentation bottles were then transferred into a sterile bench and the beans were left to ferment at 25±2 °C for 120 h. Brine samples were drawn aseptically using a pipette and sterile pipette tips at 0 h, 24 h, 72 h and 120 h for pH determination and microbial enumeration.

Fermentation set-up is diagrammatically presented in Figure 3.5 below.

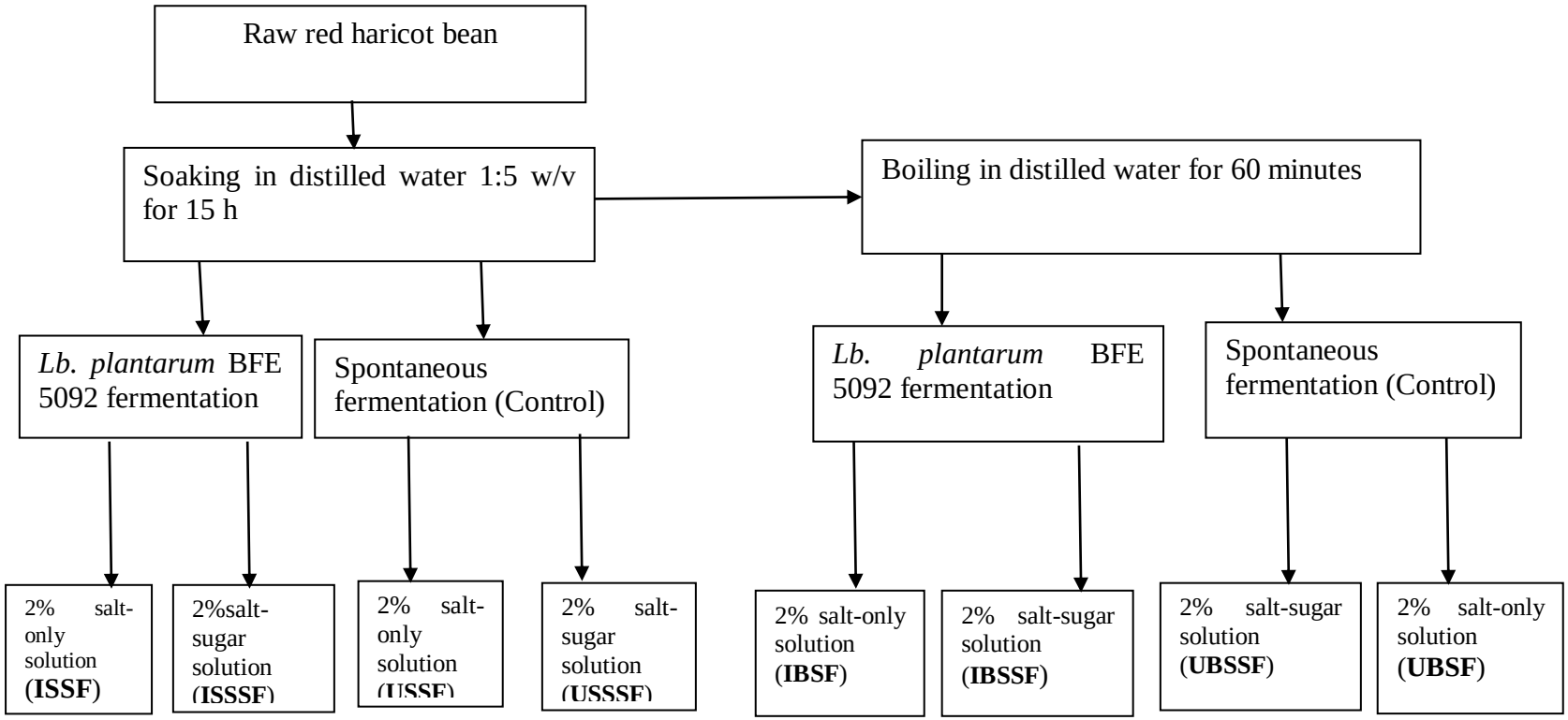


Figure 3.7: Flow chart presentation of fermentation setup

3.5 pH determination

Approximately 5 ml of the fermentation solution drawn at 0 h, 24 h, 72 h and 120 h of fermentation was used to monitor the pH. The pH of the fermentation solution was determined using a pH meter (HI 2211, Hanna Instruments, Japan).

3.6 Microbial enumeration during fermentation

The 5 ml of the fermentation solution were transferred into a sterile 9 ml test tube and vortexed properly to mix. About 1 ml 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}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}. These preparations were thoroughly mixed by vortexing. Exactly 10 µl of the aliquots from different dilutions were transferred to petri dishes containing, Mann Ragosa Sharpe (MRS) agar for LAB enumeration, Violet red bile agar (VRBGA) for enterobacteria enumeration, Plate count agar (PCA) for Total aerobic bacteria enumeration and Potato dextrose agar (PDA) for yeast and molds enumeration. They were then spread plated followed by incubation of the agar plates at respective temperatures (PCA and VRBG at 37ºC, MRS at 30ºC and PDA at 25ºC). The counting was performed in triplicate with the detection limit of 1 x 10^2 CFU/ml (log 2). The colonies formed in the incubated plates were counted after incubation and used to determine the bacterial colony forming units (CFU) present in the sample. The CFU was calculated using the following Equation 1 below

$$\textbf{Colony forming unit (cfu/ml)} = \frac{\textbf{Number of colonies}}{\textbf{Volume of inoculum}} \textbf{ X Dilution factor} \quad .$$

Equation 1

3.7 Sample preparation for biochemical analysis after fermentation

After 24 h, 72 h and 120 h of fermentation, the fermentation solution was discarded and the fermented red haricot bean (*Phaseolus vulgaris* L) spread on clean trays. They were then dried in an oven at 60 °C for 10 h. The dried beans were then milled using a stainless-steel blender. The resulting flour was stored at 4°C in polyethylene bags.

3.8 Proximate Analysis

The moisture content, crude ash, crude protein, crude fat, crude fiber and carbohydrate content of the raw, soaked, boiled and fermented bean flour were determined using the AOAC (2000) methods. Moisture content was determined by oven drying method at 105°C, where 3 g of sample was dried until constant weight was obtained. Crude ash content was determined by incineration, where 3 g of sample were dry ashed in an electric muffle furnace (Shimadzu KL-420, Japan) at 600°C for five hours until constant weight. Protein content was determined according to Kjeldahl method and conversion factor of 6.25 used to calculate the crude protein content from Nitrogen content in the samples. Crude fat was extracted from 5 g of the samples using Soxhlet apparatus and petroleum ether as the solvent. The crude fat content was then quantified gravimetrically. Crude fiber content was determined by sequential digestion of samples using 1.25% H_2SO_4 followed by 1.24% NaOH. The samples were then filtered through glass wool and the residue dried in an oven for one hour then fiber content was determined gravimetrically. The carbohydrate content was determined using the difference method in Equation 2 below.

$$\%\ carbohydrates = 100 - (\%\ Moisture\ content + \%\ ash + \%\ protein + \%\ fat + \%\ fiber$$

Equation 2

3.9 Anti-nutrient determination

3.9.1 Determination of tannin content on dry weight basis

Tannin content was determined using the Vanilin-HCL method of Price *et al*. (1978). About 0.2 g of bean flours were weighed into clean and dry centrifuge tubes. About 10 ml of 4% methanolic HCL was then added into each of the tubes and then shaken for 20 minutes on a shaker. The sample mixtures were then centrifuged at 2500 rotations per minute (rpm) for 10 minutes in a centrifuge (Hettich, D-78532 Tuttingen, Gemany). The supernatants were transferred into 25ml volumetric flasks. About 5 ml of 1% methanolic HCL was then added to the precipitate in the centrifuge tubes and centrifuged as described above. The supernatants were then

transferred into the 25 ml volumetric flasks above and topped up to the mark using 1% methanolic HCL. About 1 ml of the supernatants was then transferred into two test tubes each. To one test-tube, 5 ml of freshly prepared mixed reagent (8% methanolic HCL + 4% vanillin in methanol) was added. To the other test-tube, 5 ml of 4% methanolic HCL was added. A series of catechin standards 0.1 mg/ml, 0.08 mg/ml, 0.06 mg/ml, 0.04 mg/ml 0.02 mg/ml and 0.01 mg/ml was prepared. Exactly 1 ml of each of the standard concentration was transferred into a test tube and 5 ml of the mixed reagent added. The samples extracts and standards were allowed to sit for 20 minutes for color development. The absorbance of the sample extracts and standard solutions were read at 500 nanometers using UV-vis photospectrophotometer (UV mini 1240 model, Shimadzu, Japan). The standard curve is shown in (Appendix 1). The mg/100 g of tannins were calculated using equation 3 below

$$\%\ catechin = \left(\frac{Absorbance * dilution\ factor}{1000 * sample\ weight}\right) * 100 \quad \textbf{.....Equation 3}$$

3.9.2 Determination of phytic acid content on dry weight basis

Phytic acid was determined using the method of Adeniran *et al*. (2013) with modification. About 0.5 g of milled bean flours were weighed into clean and dry centrifuge tubes. About 10 ml of 3% sulfuric acid was added into the sample and shaken for 30 minutes. The contents were filtered and the filtrate transferred into a boiling bath for 5 minutes. Exactly 3 ml of Ferric chloride solution was then added into the sample which was then heated for 45 minutes to precipitate the ferric phytate complex to completion. The sample mixtures were then centrifuged at 2500 rpm for 10 minutes and the supernatant discarded. The precipitate was washed with 30 ml of distilled water and centrifuged as described above. The supernatant was then discarded and 3 ml of 1.5N NaOH solution added to the precipitate. This was then topped up to 30 ml using distilled water. The contents were then heated for 30 minutes in a boiling water bath to precipitate Ferric chloride. The sample mixture was then cooled and centrifuged (Hettich, D-78532 Tuttingen, Germany) at 2500

rpm for 10 minutes. The resulting supernatant was transferred into a 50 ml volumetric flask and the precipitate rinsed with 10 ml distilled water. This was then centrifuged as above and the resulting supernatant transferred into the 50 ml volumetric flask above. The sample extracts were then filtered through a 0.45 micro-filter. Liquid Chromatography- Mass Spectrophotometry (LC-MS) (Genevac, DNA-23050-A00, England) analysis was done using Shimadzu Refractive Index Detector (RID 6A). The mobile phase was 0.005 N sodium acetate in distilled water at a flowrate of 0.5µl/minute. The standard curve for phytic acid is presented in (Appendix 2). Quantification of phytic acid was calculated as in Equation 4 below.

$$\frac{Mg}{100g} = \left(\frac{\frac{Y}{M}}{SW} * 1000/100\right) \text{.....Equation 4}$$

Where:

Y= Height of peak

M= Gradient of standard curve

SW= Weight of sample

3.10 Raffinose family oligosaccharide extraction and quantification

Extraction of raffinose and stachyose was done using the method of Antonio, Pinheiro, Manuela, Ortu, and Qu, (2008) with adjustments. About 0.01 g of the raw and processed bean flours were weighed into micro-centrifuge tubes containing 250µL ice-cold chloroform:methanol (3:7 v/v) . The contents were then vortexed in a vortex (Iwaki mixer, TM-151, Japan) for one minute and the mixture incubated at -4°C for two hours to stop metabolism and extract water soluble metabolites. After incubation 200 µL ice-cold water was added and the tubes shaken for thirty minutes in a shaker (Ika Labortechnik, KS-250 B, Germany). The samples were then topped up to 1000 µL with cold distilled water and centrifuged in a micro-centrifuge (Genevac, DNA-23050-A00, England) at 17,900×g, at 4°C for 10 minutes. The upper phase was transferred into sample vials (2.0 mL) followed by liquid chromatography ion trap mass spectrometric analysis in an LC-MS machine

(Genevac, DNA-23050-A00, England). The liquid chromatography-ion trap mass spectrophotometry conditions are presented in (Appendix 5). The standard curves are shown in (Appendix 3 and Appendix 4). Quantification of the sugars were calculated as in Equation 5 below:

$$Calculated\ concentration = \left(\frac{(Peak\ area - Intercept\ on\ y\ axis)}{gradient}\right)$$

Mg/100 g = (((calculated concentration*volume used)/ sample weight)*100/1000)
……Equation 5

3.11 Statistical analysis

Data was presented in means and standard error of means or standard deviation. Separation of means was done using LSD method at P≤ 0.05. Contrast ANOVA was conducted to compare the main effect of pretreatment, fermentation solution, fermentation type and fermentation time and their interaction effect on microbial growth, nutrients (proximate composition) , anti-nutrients (tannins and phytic acid content) and RFOs (raffinose and stachyose concentration). Post estimation tests were done using pairwise comparison of estimated marginal means at P≤0.05. Statistical analysis was carried out using SPSS statistics version 23.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 To determine the growth of Lactic acid bacteria and microbial safety during the fermentation of soaked and boiled whole red haricot bean (*Phaseolus vulgaris* L.)

4.1.1 Effect of fermentation solution and fermentation time on the growth of Lactic acid bacteria during the fermentation of whole red haricot bean (*Phaseolus vulgaris* L.)

The growth of LAB during fermentation of soaked red haricot bean in salt-only and salt-sugar solutions are presented in Figure 4.1. The growth of LAB increased significantly ($P<0.0001$) with increase in fermentation time in inoculated soaked salt-only fermentation (ISSF) and inoculated soaked salt-sugar fermentation (ISSSF) batches from $\log_{10}$ 6.5 to $\log_{10}$ 8.2 and $\log_{10}$ 8.6 respectively after 24 h of fermentation. The LAB counts continued to increase significantly ($P<0.0001$) with increased fermentation time in the ISSF batch to $\log_{10}$ 8.6 after 120 h of fermentation.

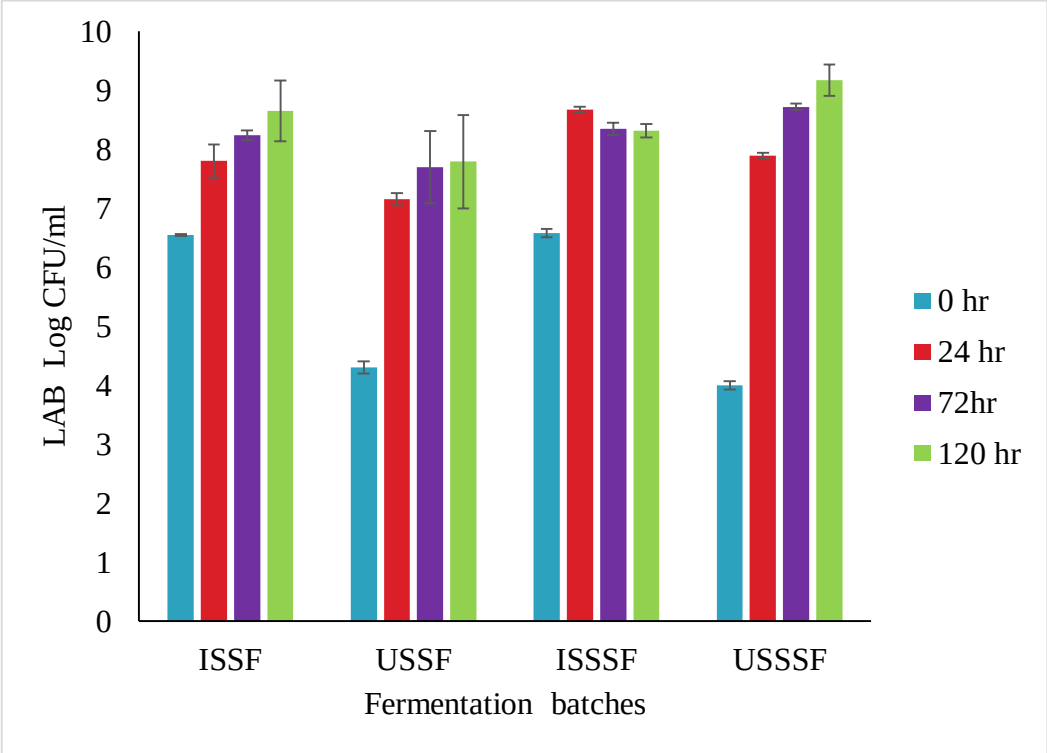


Figure 4.1: Effect of fermentation time and solutions on the growth of LAB during the fermentation of pre-soaked whole red haricot bean in Inoculated soaked salt-only fermentation (ISSF), spontaneous soaked salt-only fermentation (USSF), Inoculated soaked salt-sugar fermentation (ISSSF) and (Spontaneous soaked salt-sugar fermentation (USSSF) batches presented as Log CFU/ml ± SEM (*n*=3)

In the ISSSF batch, the LAB counts began to even out after 72 h of fermentation and remained unchanged by the end of 120 h of fermentation. In the spontaneously fermented batches; Spontaneous soaked salt-only fermentation (USSF) and spontaneous soaked salt-only fermentation (USSSF), there was a significant increase ($P<0.0001$) in the LAB counts after 24 h of fermentation from $\log_{10}$ 4.3 and $\log_{10}$ 4.0 to $\log_{10}$ 7.15 and $\log_{10}$ 7.9 respectively. With increased fermentation time, the LAB counts continued to increase in the USSF batch. This change was however not significantly different between 72 h and 120 hr where the counts were $\log_{10}$ 7.7 and $\log_{10}$ 7.8 respectively. In the USSSF batch, increased fermentation time resulted in

further significant increase in LAB counts resulting in $\log_{10}$ 9.2 at the end of 120 h of fermentation. This accounted for the highest counts in comparison to the other batches during fermentation. Higher LAB growth in salt-sugar brine could be attributed to the addition of sugar to the brine which provides an extra energy source for the bacteria's metabolism hence accelerated growth (Gan *et al*., 2017).

Growth of LAB during fermentation of boiled whole red haricot bean (*Phaseolus vulgaris* L) is presented in figure 4.2.

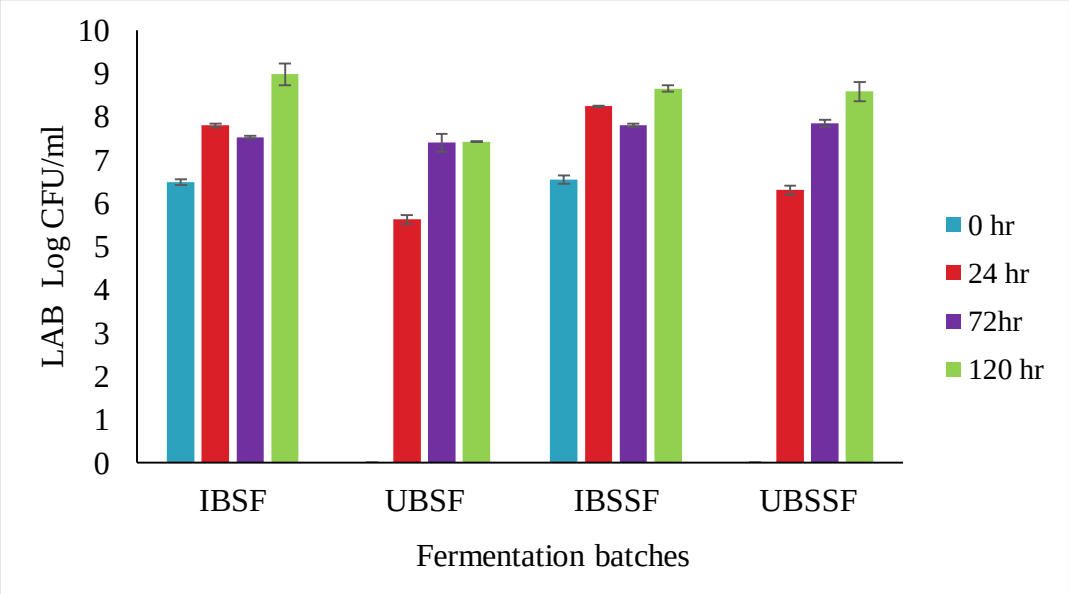


Figure 4.2: Effect of fermentation time and solutions on the growth of LAB during the fermentation of pre-boiled whole red haricot bean in Inoculated boiled salt-only fermentation(IBSF), Spontaneous boiled salt-only fermentation (UBSF), Inoculated boiled salt-sugar fermentation (IBSSF) and Spontaneous boiled salt-sugar fermentation (UBSSF) batches presented as Log CFU/ml ± SEM (*n*=3)

The growth of LAB increased significantly ($P<0.0001$) with increase in fermentation time in inoculated boiled salt-only fermentation (IBSF) and inoculated boiled salt-sugar fermentation (IBSSF)batches from $\log_{10}$ 6.5 to $\log_{10}$ 7.8 and $\log_{10}$ 8.2 respectively after 24 h of fermentation.

The LAB counts then leveled out after 72 hr of fermentation in both the batches with no significant changes to $\log_{10}$ 7.5 and $\log_{10}$ 7.8 in IBSF and IBSSF batches respectively. With increased fermentation time in IBSF and IBSSF batches a significant ($P<0.0001$) increase in the LAB counts was observed. At the end of 120 h of fementation, the LAB counts were at $\log_{10}$ 9.0 and $\log_{10}$ 8.6 in the IBSF and IBSSF batches respectively. At the start of spontaneous fermentation of boiled red haricot bean; spontaneous boiled salt-only fermentation (UBSF) and spontaneous boiled salt-sugar fermentation (UBSSF), no LAB was detected. This is because of boiling which killed any pre-existing LAB. After 24h of fermentation, significant increase ($P<0.0001$) in the LAB counts occured reaching $\log_{10}$ 5.6 and $\log_{10}$ 6.3 in the UBSF and UBSSF batches respectively. With increased fermentation time, the LAB counts continued to increase in the UBSF batch with no significant change observed between 72 h and 120 h plateuing at $\log_{10}$ 7.4. In the UBSSF batch, increased fermentation time resulted in further significant increase ($P<0.0001$) in LAB counts resulting in $\log_{10}$ 8.5 at the end of 120 h of fermentation..

4.1.2 Effect of fermentation solution and fermentation time on the growth of Total Aerobic count during the fermentation of whole red haricot bean (*Phaseolus vulgaris* L)

The growth of total aerobic count during fermentation of soaked red haricot bean in salt-sugar and salt-sugar solutions are presented in Figure 4.3. The total aerobic count increased significantly ($P<0.0001$) within the first 24 h of fermentation in ISSF and ISSSF batches from $\log_{10}$ 6.6 to $\log_{10}$ 8.6 and $\log_{10}$ 6.8 to $\log_{10}$ 8.8 respectively. Total aerobic count started to decline marginally with increased fermentation time in the ISSSF batches to $\log_{10}$ 8.7 after 120 h of fermentation. In the ISSF batch, the LAB counts decreased to $\log_{10}$ 8.3 after 72 h of fermentation and then increased to $\log_{10}$ 8.6 by the end of 120 h of fermentation. The changes in total aerobic counts after 72 h and 120 h of fermentation were not significantly different in the ISSF and ISSSF batches. In the spontaneously fermented batches; USSF and USSSF, there was a significant increase ($P<0.0001$) in the total aerobic counts after 24 h of fermentation from $\log_{10}$ 4.5 and $\log_{10}$ 5.0 to $\log_{10}$ 7.9 and $\log_{10}$ 8.0 respectively. With increased fermentation time, a slow increase of total aerobic count in the USSF batch

was observed. This change was however not significantly different between 72 h and 120 h where the counts were $\log_{10}$ 8.3 and $\log_{10}$ 8.5 respectively. In the USSSF batch, increased fermentation time resulted in further significant increase of total aerobic count resulting in $\log_{10}$ 9.4 at the end of 120 h of fermentation. This accounted for the highest counts in comparison to the other batches during fermentation and was comparable with the LAB counts in the USSSF batch.

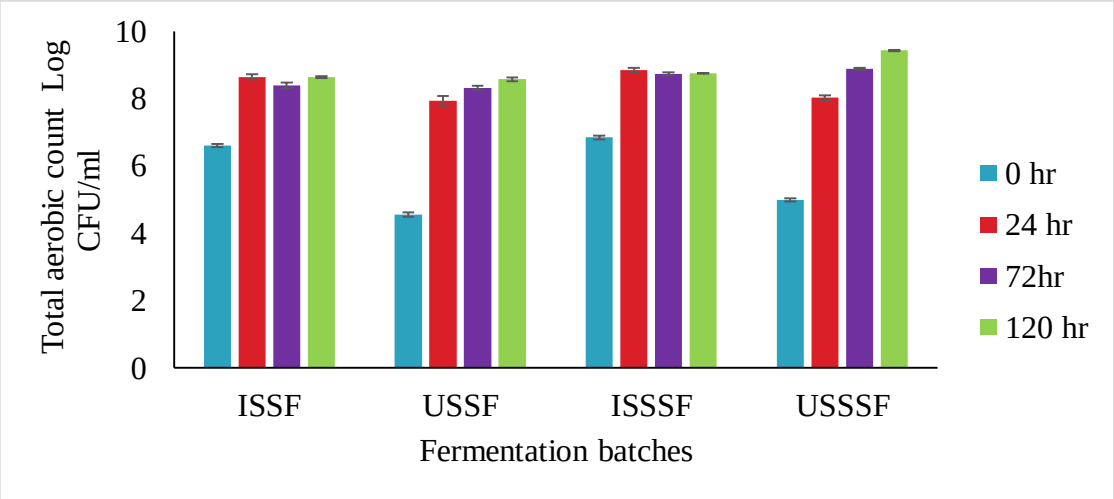


Figure 4.3: Effect of fermentation time and solutions on the growth of total aerobic count during the fermentation of pre-soaked whole red haricot bean in Inoculated soaked salt-only fermentation (ISSF), spontaneous soaked salt-only fermentation (USSF), Inoculated soaked salt-sugar fermentation (ISSSF) and (Spontaneous soaked salt-sugar fermentation (USSSF) batches presented as Log CFU/ml ± SEM (*n*=3)

The total aerobic count during fermentation of boiled whole red haricot bean (*Phaseolus vulgaris* L) is presented in Figure 4.4. Total aerobic counts increased significantly ($P<0.0001$) with increase in fermentation time in IBSF and IBSSF batches from $\log_{10}$ 6.6 to $\log_{10}$ 8.5 and $\log_{10}$ 6.7 to $\log_{10}$ 9.2 respectively after 24 h of fermentation. The total aerobic count in the IBSF batch then increased to $\log_{10}$ 8.7 after 72 h of fermentation followed by a slight decline after 120 h of fermentation. Total aerobic count in the IBSSF batch started to decline after 72 h and 120 h of

fermentation to log_{10} 7.9 and log_{10} 7.6 respectively. At the start of spontaneous fermentation of boiled red haricot bean; UBSF and UBSSF, total aerobic count was not detected. This is because of boiling which killed any pre-existing bacteria. After 24 h of fermentation, significant increase ($P<0.0001$) in total aerobic count occured reaching log_{10} 3.5 and log_{10} 4.0 in the UBSF and UBSSF batches respectively. With increased fermentation time, total aerobic count continued to increase significantly ($P<0.0001$) in both the UBSF and UBSSF batches. At the end of 120 h of fermentation of boiled whole red haricot bean, total aerobic counts in the UBSF and UBSSF were log_{10} 8.6 and log_{10} 9.4 respectively.

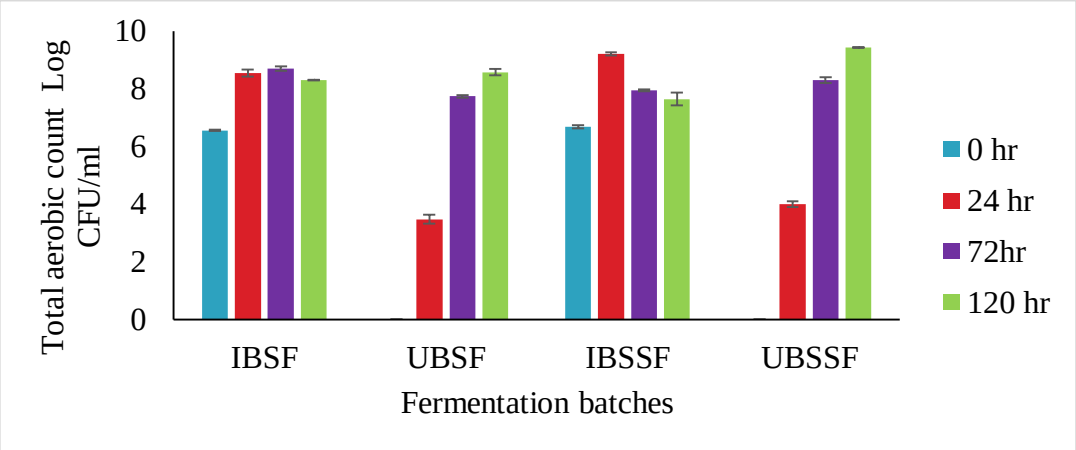


Figure 4.4: Effect of fermentation time and solutions on the growth of total aerobic count during the fermentation of pre-boiled whole red haricot bean in Inoculated boiled salt-only fermentation(IBSF), Spontaneous boiled salt-only fermentation (UBSF), Inoculated boiled salt-sugar fermentation (IBSSF) and Spontaneous boiled salt-sugar fermentation (UBSSF) batches presented as Log CFU/ml ± SEM (*n*=3)

4.1.3 Effect of fermentation of whole red haricot bean (*Phaseolus vulgaris* L) on the pH of the fermentation solution

The changes in pH of the fermentation solutions during the fermentation of soaked whole red haricot bean is presented in Figure 4.5. The pH of fermentation solution at the beginning of fermentation was 6.06. A significant decrease ($P<0.0001$) in the pH

was observed with increase in fermentation time in all the batches. After 24 h of fermentation pH of 5.7 was recorded in the ISSF and USSF batches whereas the pH in the ISSSF and USSSF was 5.1 and 4.7 respectively. The pH continued to decrease significantly with increase in fermentation time in all the batches. pH at the end of 120 h of fermentation was 4.7 and 4.8 in the ISSF and USSF batches respectively. Lowest pH of 3.9 was recorded in the ISSSF and USSSF fermentation batches. This reduction in pH is comparable to the changes reported by Onwurafor *et al*. (2014) during the fermentation of mung bean. The decline in pH continued with increased fermentation time though it was not significantly different (P>0.05) after 120 h of fermentation. When salt brines were used, a slow decline in the pH was reported. This decline was significant after 24 h and 120 h of fermentation in both the SSF and the inoculated BSF systems.

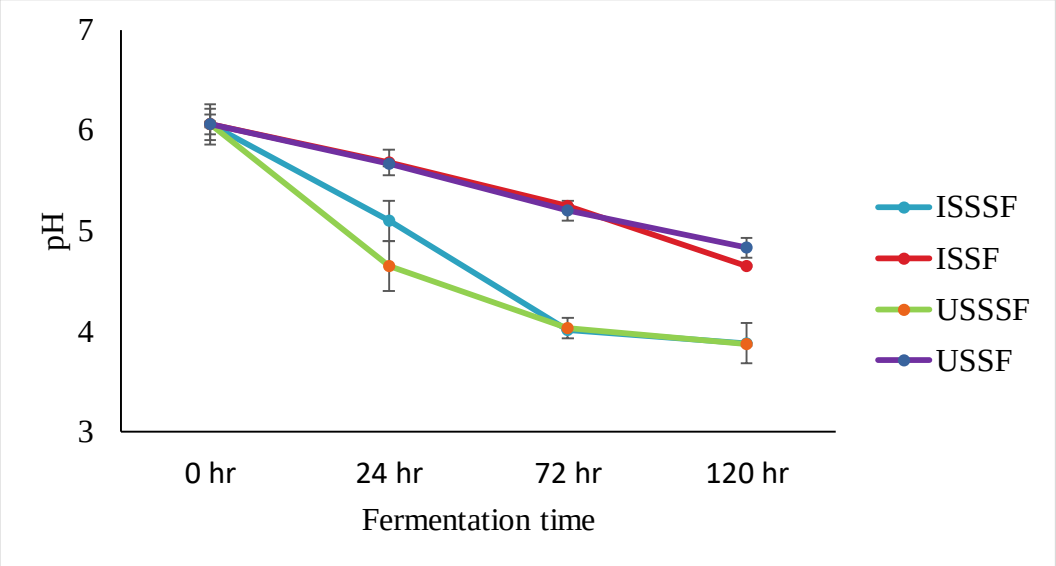


Figure 4.5: Effect of fermentation time and solutions on pH during the fermentation of pre-soaked whole red haricot bean in Inoculated soaked salt-only fermentation (ISSF), spontaneous soaked salt-only fermentation (USSF), Inoculated soaked salt-sugar fermentation (ISSSF) and (Spontaneous soaked salt-sugar fermentation (USSSF) batches presented as Log CFU/ml ± SEM (*n*=3)

The changes in pH of the fermentation solutions during the fermentation of boiled whole red haricot bean is presented in Figure 4.6. The pH of fermentation solution at the beginning of fermentation was 6.1. A significant decrease ($P<0.0001$) in the pH was observed with increase in fermentation time in all the batches. After 24 h of fermentation pH of 5.8 and 6.0 was recorded in the IBSF and UBSF batches respectively whereas pH in the IBSSF and UBSSF was 5.2 and 4.8 respectively. The pH continued to decrease significantly with increase in fermentation time in all the batches. The pH at after 120 h of fermentation was 4.8 and 5.3 in the ISSF and USSF batches respectively. Lowest pH of 3.8 and 3.9 was recorded in the ISSSF and USSSF batches respectively after 120 h of fermentation. The decline of pH with fermentation is an indicator of lactic acid production by the fermenting microorganisms (Ejigui *et al*., 2005).

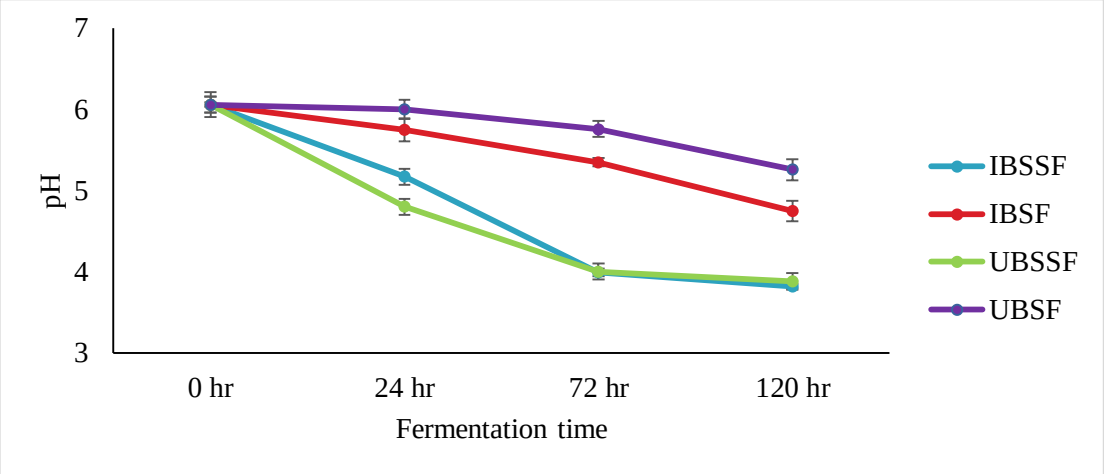


Figure 4.6: Effect of fermentation time and solutions on pH during the fermentation of pre-boiled whole red haricot bean in Inoculated boiled salt-only fermentation(IBSF), Spontaneous boiled salt-only fermentation (UBSF), Inoculated boiled salt-sugar fermentation (IBSSF) and Spontaneous boiled salt-sugar fermentation (UBSSF) batches presented as Log CFU/ml ± SEM (*n*=3)

4.1.4 Effect of the growth of Lactic acid bacteria during the fermentation of whole red haricot bean (*Phaseolus vulgaris* L) on growth of coliforms and microbial safety of the fermented beans

Mold and yeast were not detected in all the fermentation systems used. This is observation is similar to the findings of Granito and Alvarez (2006) after the fermentation of black beans for 48 h. When the boiled beans were fermented, no coliform was detected (P=1.000) throughout the fermentation period. This is attributable to the effect of heat during boiling on coliforms which are heat labile. The growth of coliforms during the fermentation of soaked beans is presented in Figure 4.7. At the start of fermentation of soaked whole red haricot bean (*Phaseolus vulgaris* L), the coliform count was at $\log_{10}$ 4. This is comparable to the findings of Granito and Alvarez (2006) who reported 62% of total aerobic count to be coliforms at the start of black bean fermentation. The coliform counts then increased significantly (P<0.0001) in number to $\log_{10}$ 8.1 in the ISSF and USSF batches after 24 h of fermentation. The coliform count in ISSF remained unchanged after 72 h of fermentation, then declined to $\log_{10}$ 7.3 at the end of 120 h of fermentation.

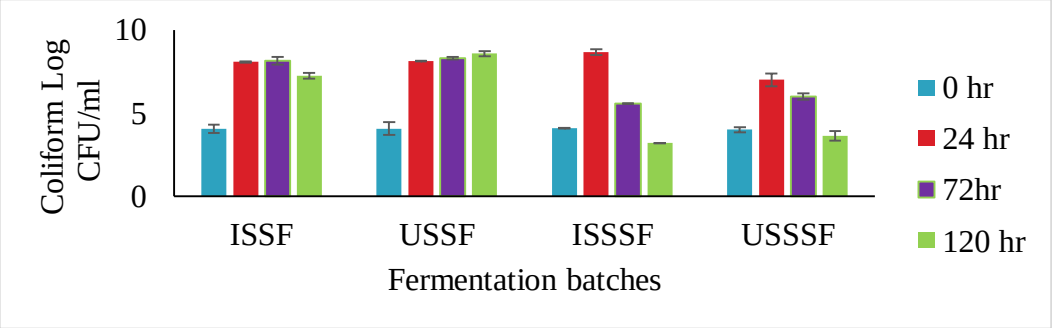


Figure 4.7: Effect of fermentation time and solutions on the growth of coliform during the fermentation of pre-soaked whole red haricot bean in Inoculated soaked salt-only fermentation (ISSF), spontaneous soaked salt-only fermentation (USSF), Inoculated soaked salt-sugar fermentation (ISSSF) and (Spontaneous soaked salt-sugar fermentation (USSSF) batches presented as Log CFU/ml ± SEM (*n*=3)

The coliform count in the USSF batch continued to increase with increase in fermentation time with significant change (log_{10} 8.5) occuring after 120 h of fermentation. In the ISSSF batch, a significant increase ($P<0.0001$) in the coliform count was recorded after 24 h of fermentation from log_{10} 4 to log_{10} 8.7. This was the followed by a significant reduction ($P<0.0001$) in the number of coliforms to log_{10} 5.6 CFU/ml and log_{10} 3.2 CFU/ml after 72 h and 120 h of fermentation. A similar trend was observed in the USSSF batch where a significant increase ($P<0.0001$) in coliform count from log_{10} 4.0 to log_{10} 7.0 was made after 24 h of fermentation. This was then followed by a significant decrease in the coliform count to log_{10} 6.0 and log_{10} 3.6 after 72 h and 120 h of fermentation. The significant decrease in the coliform counts in the ISSSF and USSSF batches during fermentation is attributable to the acid production by lactic acid bacteria (Granito and Alvarez, 2006) this was evidenced by low pH (Figure 4.5) in these batches. Coliforms are sensitive to low pH and are inhibited from pH below 4.4 (Onwurafor *et al*., 2014). The pH of 3.8 and 3.9 in the ISSSF and USSSF batches (Figure 4.5) is lower than the pH 4.4 from where the coliform growth is inhibited. This explains the decreased coliform count after 72 h of fermentation. The high pH of 4.8 and 5.3 in the ISSF and USSF batches (Figure 4.5) is the reason behind the high coliform count as it favors their growth.

4.2 Effect of soaking and boiling of whole red haricot bean (*Phaseolus vulgaris* L) on the nutrient, anti-nutrient and Raffinose Family Oligosaccharides composition

4.2.1 Effect of soaking and boiling on the nutrient composition of whole red haricot beans (*Phaseolus vulgaris* L) on dry weight basis

The effect of soaking and boiling on nutrient composition of whole red haricot bean (*Phaseolus vulgaris* L.) is presented in Table 4.1. The crude fat content of the raw bean was 1.70%. This was between the range of 15.8% reported by Audu *et al*. (2013) and 1.5% reported by Chaudhary and Sharma (2013) for raw whole red kidney bean. The difference could be attributed to varietal differences of the beans. The low fat content in red haricot beans shows that this bean variety has positive

nutritional value (Chaudhary & Sharma, 2013). Soaking of common beans for 12 h did not result in a significant effect (P=0.653) on the crude fat content of the beans compared to the raw bean. When the red haricot beans were boiled, the fat content decreased to 1.62 % (Table 4.1). This decrease was however not significant (P=0.653) compared to that of raw red haricot bean. The decrease in fat content as a result of boiling compares favorably with the findings of Audu and Aremu (2011), Ramadan (2012) and Udensi *et al*. (2010) who attributed the decrease to and increased activity of lipases which break down the fats to simpler triglycerides and their leaching out into the boiling water. The crude fiber content of the raw bean was 3.64% (Table 4.1), this compared favorably with the 3.6 % reported by Audu and Aremu (2011) for red kidney beans. Soaking of the beans lowered the crude fiber content of common beans significantly (P<0.0001) to 3.22 %. This compares with the findings of Fernandes *et al*. (2010) who reported a decrease of fiber content with soaking of beans and of Udensi *et al*. (2010) during the soaking of *Mucuna flagellipe* flour. Boiling increased the crude fiber content significantly (P<0.05) to 4.42%. this was in agreement with (Olanipekun *et al.*, 2015) who reported an increase from 4.51% to 6.25% after boiling of red kidney beans. Ramadan (2012) also reported similar effect on crude fiber after boiling soaked soy beans from 7.12% to 9.83%. The ash content of raw red haricot bean (*Phaseolus vulgaris* L) was 3.08% (Table 4.1) an amount slightly lower than 3.69% reported by Olanipekun *et al*. (2015) and 3.6% reported by Chaudhary and Sharma (2013) in whole kidney bean seeds. The difference could be attributed to varietal as well as the agronomical differences of the beans used in these studies.

Table 4.1: Change in nutrient composition of whole red haricot bean after soaking and boiling (DWB)

Treatment	Crude fat %	Crude fiber %	Ash %	Protein %	Carbohydrates %
Raw red haricot bean	1.70±0.06[a]	3.64±0.50[a]	3.08±0.22[a]	19.10±0.56[a]	61.55±0.50[a]
Soaked	1.67±0.13[a]	3.22±0.22[b]	2.77±0.02[a]	18.27±0.80[b]	64.70±0.56[b]
Boiled	1.62±0.07[a]	4.42±0.30[c]	2.67±0.04[a]	17.76 ±0.16[c]	63.51±0.75[c]
P. value	0.653	<0.0001	0.293	0.003	0.001

Values are means of triplicate determinations ± SD. Means in the same column followed by the same superscript are not significantly different P<0.05. Mean comparison for the treatments was done using the LSD test.

After soaking, the ash content decreased insignificantly (P=0.293) to 2.77% (Table 4.1). This decrease could be attributed to the leaching out of water-soluble minerals into the soaking water. A further reduction of the ash content to 2.67% (Table 4.1) was recorded when the beans were boiled. This reduction was however not significant (P=0.293) in comparison to the raw beans. The reduction of ash content as a result of soaking and boiling was similar to the findings reported by Udensi *et al*. (2010), Audu and Aremu (2011) and Ramadan (2012). This could be attributed to the leaching out of water-soluble minerals into the soaking and boiling water.

The protein content of the raw red haricot bean was 19.10 % (Table 4.1) this was in the range of 20.9% reported by Olanipekun *et al.* (2015) and 15.3% reported by Audu and Aremu (2011) for raw whole red kidney beans. The difference could be attributed to the varietal differences of the beans. The high protein content confirms that red haricot beans are a good potential source of protein (Martin-Cabrejas *et al*., 2004). Soaking lowered the protein content significantly (P=0.003) to 18.27% as presented in Table 4.1. This is in agreement with Udensi *et al*. (2010) who reported that soaking resulted in reduction in protein content in *Mucuna flagellipe* seeds. When the beans were boiled, further significant (P=0.003) decrease to 17.76% (Table

4.1) was observed. This was in agreement with the findings of Chaudhary and Sharma (2013) who reported a decrease in protein content from 23.63% to 21.7%. The decrease in protein content as a result of soaking and boiling could be attributed to hydrolysis of proteins to simpler compounds, progressive solubilization and leaching out of nitrogenous compounds into the soaking and boiling water (Udensi *et al*., 2010, Chaudhary & Sharma, 2013)

The carbohydrate content of raw red haricot bean (*Phaseolus vulgaris* L) was 61.55% (Table 4.1). This compared favorably with the 60.09 % carbohydrate content reported by Olanipekun *et al*. (2015). This high carbohydrate content indicates that red haricot bean is an important source of energy. Soaking of the beans resulted in a significant increase (P=0.001) to 64.70%. Boiling of the beans resulted in a significant increase (P=0.001) in carbohydrate content to 63.51% compared to the raw bean. Similar results were reported by Udensi *et al*., (2010); Chaudhary and Sharma (2013) after soaking and boiling of *Mucuna flagellipe* seeds and red kidney beans for 60 minutes. This increase could be attributed to the reduction on fat content, ash content and the protein content of the beans as a result of boiling of the beans.

4.2.2 Effect of soaking and boiling on the anti-nutrient composition of whole red haricot bean (*Phaseolus vulgaris* L) on dry weight basis

The effect of soaking and boiling on the tannin concentration is presented in Table 4.2. The Tannin content in the raw red haricot bean (*Phaseolus vulgaris* L) was 333.68 mg/100 g. This was lower compared the 4533 mg/100 g reported by Chaudhary and Sharma (2013) and higher than 210 mg/100 g reported by Olanipekun *et al*. (2015) tannin content in whole red kidney beans. The difference could be associated with varietal differences of the beans. When the whole red haricot beans were soaked in distilled water, tannin content reduced significantly (P<0.0001) to 306.82 mg/100 g. The reduction is in agreement with the findings of Reddy *et al*. (1985), Ferreira *et al*. (2014) and Fernandes *et al*. (2010) who established that soaking of beans in water and discarding the water eliminates a percentage of tannins. This is as a result of leaching out of the tannins into the soak

water (Mohamed *et al*., 2011; Chaudhary & Sharma, 2013). Boiling further significantly (P<0.0001) reduced the tannin content to 274.8 mg/100 g. This is in agreement with the findings of Omenna *et al*. (2016). Reddy *et al*. (1985) reported that the decrease in tannin content after cooking could be as a result of a change in their chemical reactivity or solubility and not necessarily due to actual loss from the beans.

The content of phytic acid in raw red haricot bean was 482.99 mg/100 g as presented in Table 4.2. This was lower compared to the 543 mg/100 g reported by Chaudhary and Sharma (2013) for red kidney beans. When the whole red haricot beans were soaked, the phytic acid content reduced significantly (P<0.0001) to 387.25 mg/100 g.

Table 4.2: Effect of boiling and soaking on the anti-nutrient composition of common bean (*Phaseolus vulgaris* L) on DWB

	Tannins (mg/100 g)	**Phytic acid (mg/100 g)**
Raw red haricot bean	333.68±6.3^{a}	492.99±23.51^{a}
Soaked	306.82±0.4^{b}	387.25±3.68^{b}
Boiled	274.78±9.5^{c}	350.11±45.18^{c}
P.value	<0.0001	<0.0001

Values are means of triplicate determinations ± SD. Means in the same column followed by the same superscript are not significantly different P<0.05. Mean comparison for the treatments was done using the LSD test.

The reduction of phytic acid content in the beans after soaking is in agreement with Reddy *et al*. (1985), Ferreira *et al*. (2014) and Fernandes *et al*. (2010) who established that soaking of beans in water and discarding the water eliminates a percentage of phytic acid. This was also observed in soybean (Ramadan, 2012) and in red kidney beans (Chaudhary & Sharma, 2013) due to leaching of phytate ions into soak water due to the water soluble nature of the phytate ion (Mohamed *et al*., 2011; Chaudhary & Sharma, 2013; Nakitto *et al*., 2015). Nakitto *et al*. (2015) also

reported that imbibition of water also activates phytase enzymes in the beans which results in the breaking down of phytates. A combination of these two effects contributes to the decrease in phytate content during soaking. Boiling of the whole red haricot beans further decreased the phytic acid content significantly ($P<0.0001$) to 350.11 mg/100 g. Chaudhary and Sharma (2013) reported that blanching enhances the activity of naturally occurring phytase enzyme in the beans which results in the breaking down of phytates. Discarding of soak water from the beans before boiling is more effective in the reduction of phytates (Fernandes *et al.*, 2010). This is because of the water soluble property of phytic acid (Mohamed *et al.*, 2011; Chaudhary & Sharma, 2013). Mohamed *et al*. (2011) reported a 15.6% loss of phytate after 12 h of soaking, and 17.1% loss after boiling of soaked seeds for 60 minutes. The losses during boiling was accredited to leaching into the boiling water, and the activity of phytase enzymes in the beans which degrades the phytate complex.

4.2.3 Effect of Soaking and boiling of whole red haricot bean (*Phaseolus vulgaris* L) on the raffinose family oligosaccharides on dry weight basis

The effect of soaking and boiling on raffinose and stachyose concentration in red haricot bean (*Phaseolus vulgaris* L) is presented in Table 4.3. The concentration of raffinose in the raw red haricot bean (*Phaseolus vulgaris* L) was 72.27 mg/100 g. This was between the range of 69 - 429 mg/100 g reported by Agbenorhevi *et al*. (2010) for cow pea. Soaking of the red haricot bean in distilled water resulted in a significant decrease ($P=0.001$) in raffinose concentration from 72.27 mg/100 g to 55.91 mg/100 g (22.64% decrease). This agrees with the findings of Agbenorhevi *et al*. (2010) who reported 52-210 mg/100 g raffinose concentration in soaked cowpea. Which accounted for 18.2%-53.6% loss in raffinose concentration. Agbenorhevi *et al*., 2010) soaked the cow pea in distilled water at 25°C conditions similar to those in this present study. Nyombaire *et al*. (2002) reported up to 80% decrease in raffinose content of soaked red kidney beans after 12 h of soaking. This high decrease and difference with the present study could be attributed to the different soaking conditions used by the researchers. Nyombaire *et al*. (2002) soaked the beans in distilled water with sodium bicarbonate and sodium polyphosphate at 77° C. Agbenorhevi *et al*. (2010) suggested that extent of oligosaccharide loss is varied due

to varietal differences which affects the degree of absorption of water. Which also explains the difference in the % decrease in raffinose content in the current study in comparison to the findings of other researchers. The reduction of oligosaccharides during soaking is attributable to their solubility in water. When the soak water is absorbed by the seeds during soaking, the oligosaccharides dissolve and are leached into the soak water (Devindra *et al*., 2012; Agbenorhevi *et al*., 2010; Nyombaire *et al*., 2002).

When the soaked beans were boiled, a further decrease of raffinose concentration from 55.91 mg/100 g to 50.30 mg/100 g (10.03% decrease) occurred (Table 4.3). The decrease was however not significantly different (P>0.05) in comparison to the soaked bean. Agbenorhevi *et al*. (2010) and Nyombaire *et al*. (2002) also reported further 23-68% and 16% loss in raffinose concentration as a result of boiling of the soaked beans.

Table 4.3: Change in the raffinose and stachyose concentration as a result of soaking and boiling of whole red haricot bean (*Phaseolus vulgaris* L) on DWB

	Raffinose (mg/100 g)	**% Decrease**	**Stachyose (mg/100 g)**	**% Decrease**
Raw red haricot bean	72.27±4.90[a]	ND	1264.45±59.28[a]	ND
Soaked	55.91±1.98[b]	22.64	1247.18±58.90[a]	1.37
Boiled	50.30±2.01[b]	10.03	995.76±84.23[b]	20.16
P.value	0.001		0.001	

Values are means of triplicate determinations ± SD. Means in the same column followed by the same superscript are not significantly different P<0.05. Mean comparison for the treatments was done using the LSD test

ND- Not determined

Decrease in raffinose concentration as a result of boiling could be attributed to non-enzymatic hydrolysis of the raffinose sugar to its constituent monomer sugar units-glucose, galactose, fructose and sucrose (Cruz-Bravo *et al*., 2011; Agbenorhevi *et al*., 2010) as a result of heat treatment. It could also be as a result of continued leaching out of the raffinose sugar into the boiling water.

The stachyose content of raw red haricot bean was 1264.45 mg/100 g (Table 4.5). This was lower compared to 2400 mg/100 g reported by Rupe (1998) for red bean and higher compared to 109-570 mg/100 g reported by Agbenorhevi *et al*. (2010) for cowpea. The high concentration of stachyose in red haricot bean agrees with the report of Granito *et al*. (2003) and Rupe (1998) that stachyose is the main oligosaccharide in most legumes. Soaking of the red haricot bean in distilled water resulted in an insignificant decrease ($P>0.05$) of 1.37% in the stachyose concentration of the red haricot beans. The stachyose concentration decreased from 1264.45 mg/100 g to 1247.18 mg/100 g. This was similar to the findings of Rupe (1998) who reported a 1.25% decrease in stachyose concentration from 2400 mg/100 g to 2370 mg/100 g after 16 h of soaking in tap water. It was also agreement with the findings of Niba and Rose, (2003) reported that there was minimal changes in oligosaccharide content as a result of soaking of adzuki and mung beans. The minimal decrease is attributable to leaching out of stachyose into soak water. This is because stachyose is water soluble (Agbenorhevi *et al*., 2010). When the soaked bean was boiled, a significant decrease ($P=0.001$) of 20.16% occurred reducing the stachyose content to 995.76 mg/100 g. This loss is comparable to the findings of Agbenorhevi *et al*. (2010) who reported a 20-58.1% decrease in stachyose concentration after boiling of cowpea. This decrease in stachyose concentration is attributable to non-enzymatic hydrolysis of the stachyose sugar to its constituent monomer sugars (Agbenorhevi *et al*., 2010; Rupe, 1998). It could also be due to further leaching of the stachyose to the boiling water due to its water-soluble nature.

4.3 Effect of LAB fermentation of soaked and boiled whole red haricot bean (*Phaseolus vulgaris* L) on the nutrient, anti-nutrient and Raffinose Family Oligosaccharide composition

4.3.1 Effect of LAB fermentation of soaked whole red haricot beans (*Phaseolus vulgaris* L) on nutrient composition on dry weight basis.

The effect of fermentation on soaked whole red haricot beans (*Phaseolus vulgaris* L) is presented in Table 4.4. In the ISSSF batch, an insignificant decrease (P=0.636) in crude fat content was observed with increase in fermentation time. At the end of 120 h of fermentation the crude fat content was 1.48% (Table 4.4). In the USSSF batch an insignificant decrease (P=0.506) in crude fat content was recorded with increase in fermentation time. At the end of 120 h of fermentation the fat content was 1.46%. Similar observation was made in the ISSF and USSF batches. There was an insignificant decrease (P=0.059) and (P=0.428) in the crude fat content from 1.67 to 1.50% and 1.56 % in the ISSF and USSF batches respectively. The decrease in fat content of soaked beans during fermentation was similar to the findings of those of Oladunmoye (2007), Reyes-Bastidas *et al*. (2010), Adebowale and Maliki (2011), Audu and Aremu (2011) and Onwurafor *et al*. (2014) during the fermentation of locust bean, *Phaseolus vulgaris* cv. *Azufrado higuera* pigeon pea, kidney beans and mung bean respectively. The decrease though could be attributed to action of lipolytic enzymes from the fermenting microorganisms (Adebowale and Maliki, 2011). It could also be as a result of hydrolysis by fermenting microorganisms for synthesis of cell biomass (Oladunmoye, 2007). From this current study, fermentation of soaked whole red haricot beans did not significantly affect the crude fiber in the beans

Fermentation of soaked whole red haricot bean in the ISSSF, USSSF, ISSF and USSF batches resulted in a significant increase (P<0.0001) in the ash content. The ash content increased from 2.77% to 4.23%, 4.17%, 4.37% and 4.33% after 24 h of fermentation in the ISSSF, USSSF, ISSF and USSF batches respectively (Table 4.4). This was comparable with the findings of Onwurafor *et al*. (2014) and Adebowale and Maliki (2011) during mung bean and pigeon pea fermentation respectively. The

higher ash content could also be as a result of the breaking down of phytates (Kocková *et al.*, 2011) (Figure 4.10) as a result of fermentation which is highest between 24 h and 72 h of fermentation. Presence of phytates in beans chelates other minerals (Akande *et al.*, 2010) and therefore its degradation results in the release of the minerals. The high ash content could also be attributed to the Sodium, Chloride and Iodine ions found in the table salt and other trace elements in the sugar that was added in the fermentation solution at 2% w/v that could have been absorbed or accumulated on the beans after draining of fermentation solution. With increase in fermentation time, a decrease in the ash content was observed. The change was however not statistically different ($P>0.05$) from that at 24 h in ISSSF, USSSF, ISSF and USSF fermentation batches. This decrease could be attributed to the leaching out of the minerals that were released after the breaking down of phytates.

There was an increase in the protein content during fermentation of soaked whole red haricot bean (*Phaseolus vulgaris* L) in the ISSSF and ISSF. A significant increase ($P<0.0001$) was recorded after 72 h of fermentation. The protein content increased from 17.94% to 18.57% in the ISSSF batch and 17.94 % to 18.65% in the ISSF batch. No significant change in the protein content was made after 120 h of fermentation in these batches. This increase in protein content could be as a result of increased microbial activity and growth during fermentation in the form of single cell proteins (Oladunmoye, 2007; Ojokoh *et al.*, 2013). Proteolytic activity of LAB as well as action by extracellular enzymes produced by the fermenting microbes could also be the reason behind the improved availability of proteins in this study (Kocková *et al.*, 2011; Tope, 2013). When the soaked whole red haricot beans (*Phaseolus vulgaris* L) were subjected to spontaneous fermentation (USSSF and USSF batches) a decrease in the protein content was observed. A significant decrease ($P<0.0001$) occurred after 24 h of fermentation in the USSSF and USSF batches from 17.94% to 17.12% and 17.04% respectively (Table 4.4). This decrease could attributed to breaking down of proteins during the fermentation as evidenced by putrefaction in the batches (Figure. 3.8).

Table 4.4: Change in nutrient composition with fermentation on soaked whole red haricot (*Phaseolus vulgaris* L) on DWB

Treatment	Fat %	Ash %	Protein %	Fiber %	Carbohydrates%
ISSSF (0 h)	1.67+0.13^{a}	2.77±0.02^{a}	17.94+0.32^{a}	3.22±0.22^{a}	65.04+0.29^{a}
ISSSF (24 h)	1.56+0.16^{a}	4.23±0.03^{b}	18.10+0.37^{a}	3.97±0.14^{b}	65.02+0.52^{a}
ISSSF (72 h)	1.43+0.05^{a}	3.96±0.11^{b}	18.57+0.10^{b}	3.98±0.35^{b}	64.16+0.21^{b}
ISSSF (120 h)	1.48+0.01^{a}	3.85±0.17^{b}	18.70+0.32^{b}	4.82±0.16^{c}	62.84+0.36^{c}
P value	0.636	<0.0001	<0.0001	<0.0001	<0.0001
USSSF (0 h)	1.67+0.13^{a}	2.77±0.02^{a}	17.94+0.36^{a}	3.22±0.22^{a}	65.04+0.29^{a}
USSSF (24 h)	1.49+0.13^{a}	4.17±0.04^{b}	17.12+0.30^{b}	3.55±0.10^{ab}	66.19+0.94^{b}
USSSF (72 h)	1.43+0.08^{a}	4.07±0.01^{b}	17.30+0.27^{b}	3.57±0.07^{ab}	66.07+0.55^{b}
USSSF (120h)	1.46+0.02^{a}	4.19±0.02^{b}	17.27+0.31^{b}	3.62±0.06^{b}	66.03+0.38^{b}
P value	0.506	<0.0001	<0.0001	0.0130	<0.0001
ISSF (0 h)	1.67+0.13^{a}	2.77±0.02^{a}	17.94+0.33^{a}	3.22±0.22^{a}	65.04+0.29^{a}
ISSF(24 h)	1.47+0.03^{a}	4.37±0.03^{b}	18.21+0.13^{ac}	3.85±0.15^{b}	63.44+0.19^{b}
ISSF(72 h)	1.48+0.03^{a}	4.22±0.33^{b}	18.65+0.05^{b}	4.55±0.06^{c}	63.11+0.38^{b}
ISSF(120 h)	1.50+0.07^{a}	3.84±0.44^{b}	18.43+0.15^{bc}	5.21±0.17^{d}	63.67+0.28^{b}
P value	0.059	<0.0001	0.002	<0.0001	<0.0001
USSF (0 h)	1.67+0.13^{a}	2.77±0.02^{a}	17.94+0.37^{a}	3.22±0.22^{a}	65.04+0.29^{a}
USSF(24 h)	1.46+0.12^{a}	4.33±0.02^{b}	17.04+0.39^{b}	3.57±0.10^{ab}	66.29+0.21^{b}
USSF(72 h)	1.50+0.06^{a}	4.25±0.04^{b}	17.23+0.07^{b}	3.75±0.18^{b}	66.83+0.36^{b}
USSF (120h)	1.56+0.07^{a}	4.19±0.04^{b}	17.24+0.41^{b}	3.84±0.60^{b}	66.61+0.39^{b}
P value	0.428	<0.0001	<0.0001	0.007	<0.0001

Values are means of triplicate determinations ± SD. Means in the same column in a treatment followed by the same superscript are not significantly different P<0.05. Mean comparison for the treatments was done using the LSD test.

ISSF-Inoculated soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

USSF- Spontaneous soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

ISSSF-Inoculated soaked salt-sugar fermentation for 0 h, 24 h, 72 h and 120 h

USSSF- Spontaneous soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

Since the micro flora in the spontaneous fermentation is vast, it is possible that the microorganisms present in these batches were proteolytic and hence broke down proteins in the beans during fermentation evidenced by putrefaction in these batches. Increased fermentation time did not result in any significant change in the protein content of the USSSF and USSF batches. Porres *et al*. (2003) also reported a significant reduction of soluble protein in naturally fermented beans compared to those fermented with *Lb. plantarum* (PL48 and PL48A). A phenomena they attributed to breaking down of legume protein by microbial or legume proteinases to smaller polypeptides that are not precipitated by the acidic conditions used to separate soluble protein from non-protein nitrogen during analysis (Porres *et al*., 2003).

The crude fiber content increased as a result of the fermentation of soaked whole red haricot beans (*Phaseolus vulgaris* L).in the ISSSF batch, crude fiber content increased significantly ($P<0.0001$) from 3.22% to 3.98% and 4.82% after 24 h and 72 h of fermentation respectively (Table 4.4). In the USSSF batch, significant increase ($P=0.013$) occurred after 120 h of fermentation increasing from 3.22% to 3.62% (Table 4.2). In the ISSF batch significant increase ($P<0.0001$) occurred after 24 h, 72 h and 120 h of fermentation. At the end of 120 h of fermentation the crude fiber content was 5.21%. In the USSF batch, crude fiber content increased significantly ($P=0.007$) after 72 h of fermentation from 3.22% to 3.75% (Table 4.4). Crude fiber increase was higher in the samples inoculated with *Lb. plantarum* BFE 5092 (ISSSF and ISSF) compared to those spontaneously fermented (USSSF and USSF). Increase in crude fiber content compares favorably with the findings of Onwurafor *et al*. (2014) who reported an increase from 3.01% to 4.05% and 4.50% in back-slopped and spontaneously fermented mung bean respectively. However in this present study, the crude fiber increase was higher in the inoculated samples compared to the spontaneously fermented samples contrary to the findings of Onwurafor *et al*. (2014). Audu and Aremu (2011) also reported an increase in crude fiber content of dehulled red kidney bean after 4 days of spontaneous fermentation from 3.6% to 4.8%. Fermentation resulted in a decrease in the carbohydrate content of the soaked whole red haricot bean (*Phaseolus vulgaris* L) in the

ISSSF and ISSF batches. This was similar with the findings of Martin-Cabrejas *et al*. (2004). Significant decrease ($P<0.0001$) in the ISSSF batch occurred after 72 h of fermentation from 65.04% to 64.16% (Table 4.4). Further significant decrease to 62.84 % occurred after 120 h of fermentation. In the ISSF batch, significant decrease ($P<0.0001$) occurred after 24 h of fermentation from 65.04% to 63.44%. In the ISSF batch, no significant change in carbohydrate content was observed after 24 h of fermentation. The decrease in carbohydrate content could be attributed to utilization by the fermenting microorganisms for metabolism (Tope, 2013; Ojokoh *et al*., 2013; Onwurafor *et al*., 2014). Lower loses could be attributed to the presence of testa on the whole red haricot bean which could have hampered the availability of the carbohydrates to the microorganisms for utilization. Higher LAB counts (Figure 4.1) could also mean that the LAB were able to utilize the solutes in the fermenting solutions to support their growth during fermentation of the soaked whole red haricot beans. In the USSSF and USSF fermentation batches, a significant increase ($P<0.0001$) in the carbohydrate content from 65.04% to 66.19% and 66.29% occurred after 24 h respectively. With increased fermentation time, no significant change was observed. This could be attributed to the preferential breaking down of proteins by the proteolytic microorganisms in these fermentation batches as presented in Table 4.4. It could also be as a result of the breaking down of raffinose (Figure 4.12) and stachyose (Figure 4.14) to glucose, galactose and sucrose (Granito & Alvarez, 2006). Similar to the innoculated batches higher LAB counts (Figure 4.1) in the USSSF and USSF also indicates that the LAB were able to utilize the solutes in the fermenting solutions to support their growth during fermentation of the soaked whole red haricot beans.

4.3.2 Effect of LAB fermentation of boiled whole red haricot beans (*Phaseolus vulgaris* L) on nutrient composition on dry weight basis.

The effect of fermentation on boiled whole red haricot (*Phaseolus vulgaris* L) is presented on Table 4.5. Fat content of boiled whole red haricot beans decreased with increase in fermentation time from 1.62% in the boiled bean to 1.47%, 1.49%, 1.42% and 1.43% in the IBSSF, UBSSF, IBSF and UBSF batches after 120 h of fermentation (Table 4.5). However, the decrease in fat content as a result of fermentation was not significantly ($P>0.05$) different from the boiled bean in the IBSSF, UBSSF, IBSF and UBSF batches. The decrease in fat content of soaked beans during fermentation was similar to the findings of those of Oladunmoye (2007), Reyes-Bastidas *et al*. (2010), Adebowale and Maliki (2011), Audu and Aremu (2011) and Onwurafor *et al*. (2014) during the fermentation of locust bean, *Phaseolus vulgaris* cv. *Azufrado higuera* pigeon pea, kidney beans and mung bean respectively. The decrease though could be attributed to action of lipolytic enzymes from the fermenting microorganisms (Adebowale & Maliki, 2011). It could also be as a result of hydrolysis by fermenting microorganisms for synthesis of cell biomass (Oladunmoye, 2007).

Table 4.5: **Change in nutrient composition with fermentation of boiled whole red haricot bean (*Phaseolus vulgaris* L) on DWB**

Treatment	Fat %	Ash %	Protein %	Fiber %	Carbohydrates%
IBSSF (0 h)	1.62±0.07^{a}	2.67±0.38^{a}	17.63+0.18^{a}	4.55±0.12^{ac}	63.31+0.40^{a}
IBSSF (24 h)	1.45+0.09^{a}	4.21±0.12^{b}	17.75+0.17^{ab}	3.92±0.62^{b}	64.98+0.13^{b}
IBSSF (72 h)	1.52+0.12^{a}	3.65±0.47^{c}	18.06+0.13^{bc}	4.42±0.24^{a}	64.11+0.28^{c}
IBSSF (120 h)	1.47+0.09^{a}	3.49±0.40^{c}	18.37+0.07^{c}	4.88±0.42^{ac}	62.43+0.94^{d}
P value	0.915	<0.0001	0.001	<0.0001	<0.0001
UBSSF (0 h)	1.62±0.07^{a}	2.67±0.38^{a}	17.63+0.22^{a}	4.55±0.12^{a}	63.31+0.40^{a}
UBSSF (24 h)	1.52+0.14^{a}	4.15±0.02^{b}	17.78+0.11^{ab}	3.64±0.01^{b}	66.30+0.75^{b}
UBSSF (72 h)	1.48+0.05^{a}	3.87±0.00^{b}	18.05+0.27^{bc}	3.78±0.14^{b}	64.37+0.50^{c}
UBSSF (120h)	1.49+0.10^{a}	3.74±0.06^{b}	18.25+0.40^{c}	3.83±0.18^{b}	64.30+0.50^{c}
P value	0.666	<0.0001	<0.0001	<0.0001	<0.0001
IBSF (0 h)	1.62±0.07^{a}	2.67±0.38^{a}	17.63+0.19^{a}	4.55±0.12^{a}	63.31+0.40^{a}
IBSF(24 h)	1.58+0.05^{a}	4.26±0.12^{b}	17.86+0.10^{ab}	4.67±0.09^{ab}	64.96+0.29^{b}
IBSF(72 h)	1.50+0.08^{a}	4.18±0.04^{bc}	18.13+0.07^{bc}	5.07±0.01^{b}	64.42+0.34^{b}
IBSF(120 h)	1.42+0.01^{a}	3.69±0.21^{c}	18.54+0.07^{c}	5.20±0.28^{b}	63.57+0.26^{b}
P value	0.932	<0.0001	<0.0001	0.001	<0.0001
UBSF (0 h)	1.62±0.07^{a}	2.67±0.38^{a}	17.63+0.23^{a}	4.55±0.12^{ab}	63.31+0.40^{a}
UBSF(24 h)	1.44+0.11^{a}	4.17±0.03^{b}	17.99+0.10^{ab}	4.30±0.03^{b}	66.96+0.29^{b}
UBSF(72 h)	1.48+0.09^{a}	4.17±0.01^{b}	18.09+0.07^{b}	4.78±0.06^{a}	65.90+0.24^{c}
UBSF (120h)	1.43+0.09^{a}	4.01±0.31^{b}	18.17+0.12^{b}	4.88±0.36^{a}	64.75+0.47^{d}
P value	0.988	<0.0001	0.023	0.012	<0.0001

Values are means of triplicate determinations ± SD. Means in the same column in a treatment followed by the same superscript are not significantly different P<0.05. Mean comparison for the treatments was done using the LSD test.

IBSF-Inoculated boiled salt-only fermentation for 0 h, 24 h, 72 h and 120 h

UBSF- Spontaneous boiled salt-only fermentation for 0 h, 24 h, 72 h and 120 h

IBSSF-Inoculated boiled salt-sugar fermentation for 0 h, 24 h, 72 h and 120 h

UBSSF- Spontaneous boiled salt-only fermentation for 0 h, 24 h, 72 h and 120

Ash content increased significantly (P<0.0001) after 24 h of fermentation of boiled whole red haricot bean (*Phaseolus vulgaris* L) from 2.67% to 4.21%, 4.15%, 4.26% and 4.17% in the IBSSF, UBSSF, IBSF and UBSF batches respectively (Table 4.5). The high ash content could also be as a result of the breaking down of phytates (Kocková *et al*., 2011) (Figure 4.11) as a result of fermentation which is highest after 24 h of

fermentation. Breaking down of phytates results in the release of minerals that were chelated in the phytate complex (Akande *et al*., 2010). The high ash content could also be attributed to the Sodium, Chloride and Iodine ions found in the Table salt and other trace elements in the sugar that was added in the fermentation solution at 2% w/v which could have been absorbed or accumulated on the beans after draining of fermentation solution. With increased fermentation time, a significant decrease ($P<0.0001$) in ash content to 3.65% was observed in the IBSSF batch after 72 h of fermentation and no significant change after 120 h of fermentation (Table 4.5) Significant decrease ($P<0.0001$) in ash content to 3.69% occurred in the IBSF batch after 120 h of fermentation. In the UBSSF and UBSF, the decrease in ash content was not significantly different from that at 24 h. These losses of ash after 72 h and 120 h of fermentation could be attributed to the leaching out of soluble minerals into the fermentation solution.

The protein content of the boiled whole red haricot beans (*Phaseolus vulgaris* L) increased during fermentation. Significant increase ($P<0.05$) in protein content occurred after 72 h of fermentation of the beans in all the fermentation batches. The increase was from 17.63% to 18.06 % in the IBSSF batch, 18.05% in the UBSSF batch, 18.13% in the IBSF batch and 18.09% in the UBSF batch (Table 4.5). Further increase in fermentation time resulted in increased protein content though the change was not significantly different from the protein content at 72 h of fermentation in all the batches. This increase in protein content could be as a result of increased microbial activity and growth during fermentation in the form of single cell proteins (Oladunmoye, 2007; Ojokoh *et al*., 2013). Proteolytic activity of LAB as well as action by extracellular enzymes produced by the fermenting microbes could also be the reason behind the improved availability of proteins in this study (Kocková *et al*., 2011; Tope, 2013).

Crude fiber content in the IBSSF batch decreased significantly from 4.55% to 3.92% after 24 h of fermentation. It then increased to 4.42% and 4.88% after 72 h and 120 h of fermentation. After 72 h of fermentation the crude fiber content was comparable to the content in boiled bean. A similar trend was observed in the UBSSF batch where the

crude fiber content decreased significantly to 3.64% after 24 h then started to increase to 3.78% and 3.84% after 72 h and 120 h of fermentation. By the end of fermentation however, the crude fat content was still significantly lower than that of the boiled bean. In the UBSF system, the crude fiber content decreased significantly to 4.30% after 24 h of fermentation then increased to levels comparable to that of the boiled bean. In the IBSF system, the crude fat content increased from the onset of fermentation to 5.20% at the end of fermentation. This phenomenon was similar to that of soaked whole bean in Table 4.4. The decrease in crude fiber content after 24 h of fermentation in the IBSSF, UBSSF and UBSF could be as a result of hydrolysis of pectic compounds and utilization of cellulose and hemicellulose as substrate by the fermenting microorganisms (Granito and Alvarez, 2006). The decrease also correlates with the breakdown of oligosaccharides after 24 h of fermentation as presented in (Figure 4.13 and 4.15)

The carbohydrate content increased significantly ($P<0.0001$) from 6.31% to 64.98%, 66.30%, 64.96% and 66.96% after 24 h of fermentation in IBSSF, UBSSF, IBSF and UBSF batches respectively (Table 4.5). This increase is attributable to the breaking down of raffinose and stachyose (Figure 4.13 and 4.15) to their constituent galactose, sucrose and glucose (Granito and Alvarez, 2006) which increases the carbohydrate content. Increased fermentation time resulted in a decrease in the carbohydrate content to 62.43%, 64.30%, 63.57% and 64.75% after 120 h of fermentation in the IBSSF, UBSSF, IBSF and UBSF batches respectively. This decrease could be as a result of the utilization of the carbohydrates in the beans by the fermenting microorganisms (Tope, 2013; Ojokoh *et al*., 2013; Onwurafor *et al*., 2014). Boiling of the beans could have softened and broken the testa hence making the carbohydrates available for utilization by the microorganisms.

4.3.3 Effect of LAB fermentation on anti-nutrients in soaked and boiled whole red haricot bean (*Phaseolus vulgaris* L)

4.3.3.1 Effect of fermentation of whole red haricot bean (*Phaseolus vulgaris* L) on tannin concentration on dry weight basis

The effect of fermentation of soaked whole red haricot bean is presented in Figure 4.8. Fermentation resulted in a decrease in tannin content in all the fermentation batches. In the batches inoculated with *Lb. plantarum* BFE 5092, the tannin content reduced from 306.82 mg/100 g in the soaked whole bean to 283.41 mg/100 g and 274.29 mg/100 g in the ISSSF and ISSF batches respectively after 24 h of fermentation.

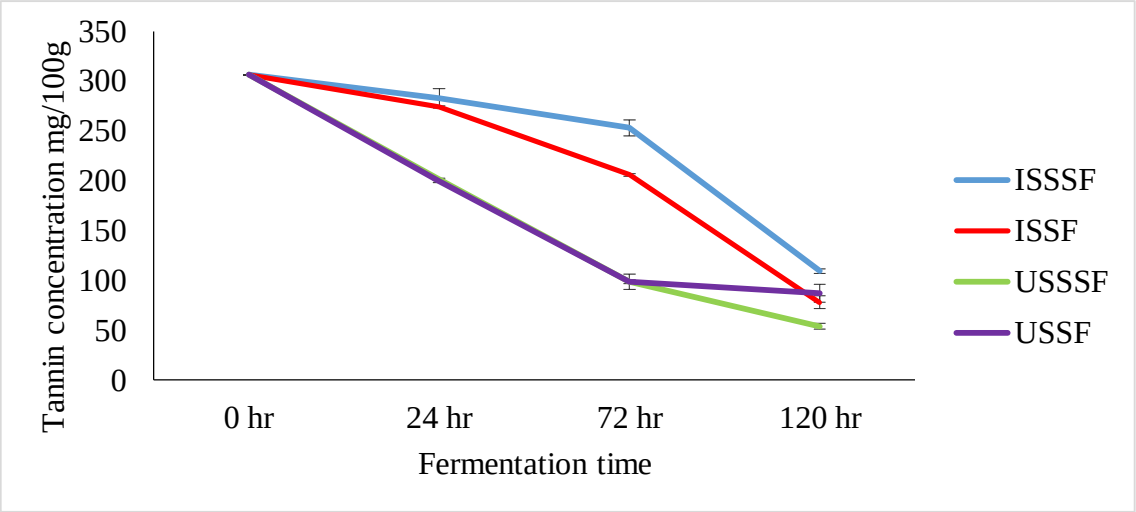


Figure 4.8: Effect of fermentation time and solutions on tannin concentration during the fermentation of pre-soaked whole red haricot bean in Inoculated soaked salt-only fermentation (ISSF), spontaneous soaked salt-only fermentation (USSF), Inoculated soaked salt-sugar fermentation (ISSSF) and (Spontaneous soaked salt-sugar fermentation (USSSF) batches presented as mg/100 g ± SEM (*n*=3)

Higher tannin reduction in the inoculated batches occurred between 72 h and 120 h of fermentation. At the end of fermentation the tannin content was 109.50 mg/100 g in the ISSSF batch and 78.15 mg/100 g in the ISSF batch. In the spontaneously fermented whole soaked beans batches; USSSF and USSF higher tannin losses occurred compared to the batches inoculated with *Lb. plantarum* BFE 5092. The tannin content decreased from 306.82 mg/100 g to 201.59 mg/100g in the USSSF batch and 199.76 mg/100 g in the USSF batch after 24 h of fermentation (Figure 4.8). The decrease was highest between 24 h and 72 h of fermentation. At the end of spontaneous fermentation, the tannin content in the USSSF and USSF batches was 54.04 mg/100g and 87.17 mg/100 g respectively. The effect of fermentation of boiled whole red haricot bean (*Phaseolus vulgaris* L) on tannin concentration is presented in Figure 4.9. Tannin concentration decreased with increased fermentation time in all the fermentation batches.

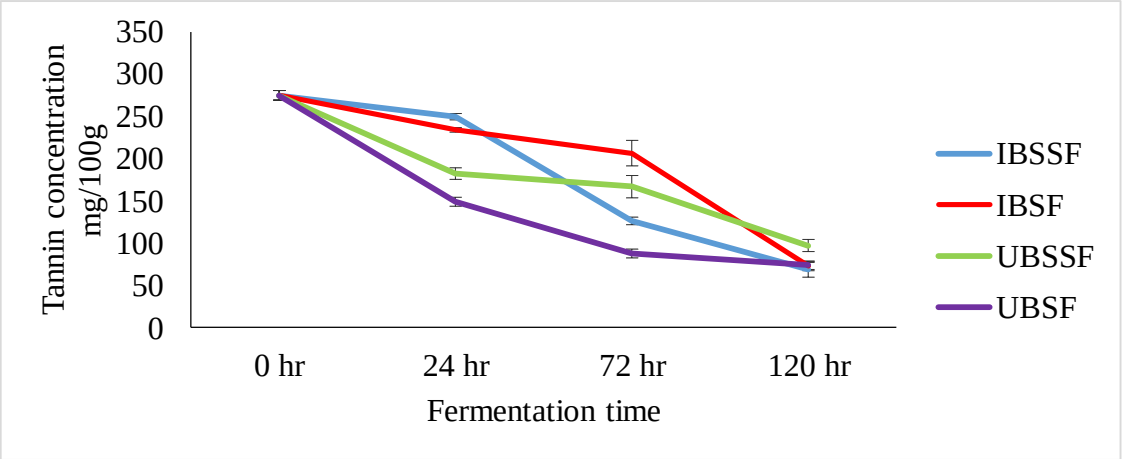


Figure 4.9: Effect of fermentation time and solutions on tannin concentration during the fermentation of pre-boiled whole red haricot bean in Inoculated boiled salt-only fermentation(IBSF), Spontaneous boiled salt-only fermentation (UBSF), Inoculated boiled salt-sugar fermentation (IBSSF) and Spontaneous boiled salt-sugar fermentation (UBSSF) batches presented as mg/100 g ± SEM (*n*=3)

The tannin concentration decreased from 274.76 mg/100 g to 249.52 mg/100 g in the IBSSF batch and 234.20 mg/100 g in the IBSF batch (Figure 4.9) after 24 h of fermentation. Highest loss in tannin concentration in the IBSSF batch occurred after 72 h of fermentation to 126.30 mg/100 g and at the end of 120 h of fermentation. In the IBSF batch, the highest loss in tannins occurred between 72 h and 120 h of fermentation resulting in 73.09 mg/100 g tannin concentration after 120 h of fermentation (Figure 4.9). When the boiled beans were spontaneously fermented, the tannin concentration losses were highest within the first 24 h of fermentation in comparison to the batches inoculated with *Lb. plantarum* BFE 5092 (Figure 4.9). The tannin concentration decreased from 274.76 mg/100 g in the boiled bean to 181.90 mg/100 g and 148.89 mg/100 g in the UBSSF and UBSF batches respectively. With increased fermentation time, the tannin concentration in the UBSSF and UBSF batches continued to decrease. At the end of 120 h of fermentation, the tannin concentration in these batches was 97.07 mg/100 g and 73.67 mg/100 g respectively. It was observed that though spontaneously fermented batches had highest tannin concentration losses within the first 24 h of fermentation, at the end of 120 h of fermentation, the loss in the inoculated batches were comparable to that of UBSF.

Fermentation significantly reduced the tannin content in all the fermentation systems of both the soaked and the boiled red haricot bean. This is in agreement with Granito and Alvarez (2006) and Adeniran *et al*. (2013) who reported an 83%, 89.5% and 68.42% decrease in tannin content of cooked fermented black beans, lima bean and locust bean respectively. The reduction of tannin as a result of fermentation can be attributed to the hydrolysis of polyphenolic compounds of tannin complexes during fermentation (Adeniran *et al*., 2013). In this current study however, it was observed that at the beginning of the fermentation the spontaneously fermented batches decreased tannin content more compared to the batches inoculated *with Lb. plantarum* BFE 5092. This could indicate that the bacteria present in the spontaneous fermentation batches were more adapted to hydrolysis of tannin complex in comparison to *Lb. plantarum* BFE 5092. Between 24h and 120 h of fermentation, *Lb. plantarum* BFE 5092 however, is

able to break down the tannin and to concentrations comparable with the spontaneously fermented batches in both the soaked and boiled beans. The antioxidant benefit of tannins in beans can be enjoyed by the consumer even after fermentation of the beans (Fernandes *et al*., 2010) as there is a little amount that is left after fermentation.

4.3.3.2 Effect of fermentation of whole red haricot bean (*Phaseolus vulgaris* L) on phytic acid concentration on dry weight basis

The phytic acid concentration of soaked whole red haricot bean during fermentation is presented in Figure 4.10. The phytic acid concentration decreased with increase in fermentation time in all the batches. In the ISSSF batch the phytic acid concentration decreased gradually from 387.25 mg/100 g to 242.52 mg/100 g at the end of fermentation. In the ISSF batch, the phytic acid decreased at a higher rate within the first 24 h of fermentation from 387.25 mg/100 g to 245.85 mg/100 g. Further fermentation of the beans in this batch resulted in gradual decrease of phytic acid to 203.71 mg/100 g at the end of 120 h of fermentation. In the USSSF batch, a steady decline in the phytic acid content was observed from the onset of fermentation. The phytic acid content in this batch decreased from 387.25 mg/100 g to 279.34 mg/100 g and 190.70 mg/100 g after 24 h and 72 h of fermentation. This was then followed with a slowed down reduction ending at 186.29 mg/100 g at the end of 120 h of fermentation. In the USSF batch, the phytic acid concentration decreased at a higher rate within the first 24 h of fermentation to 201.78 mg/100 g which was the lowest in comparison to all the soaked bean batches. Steady decline was then observed in this batch with increased fermentation time.

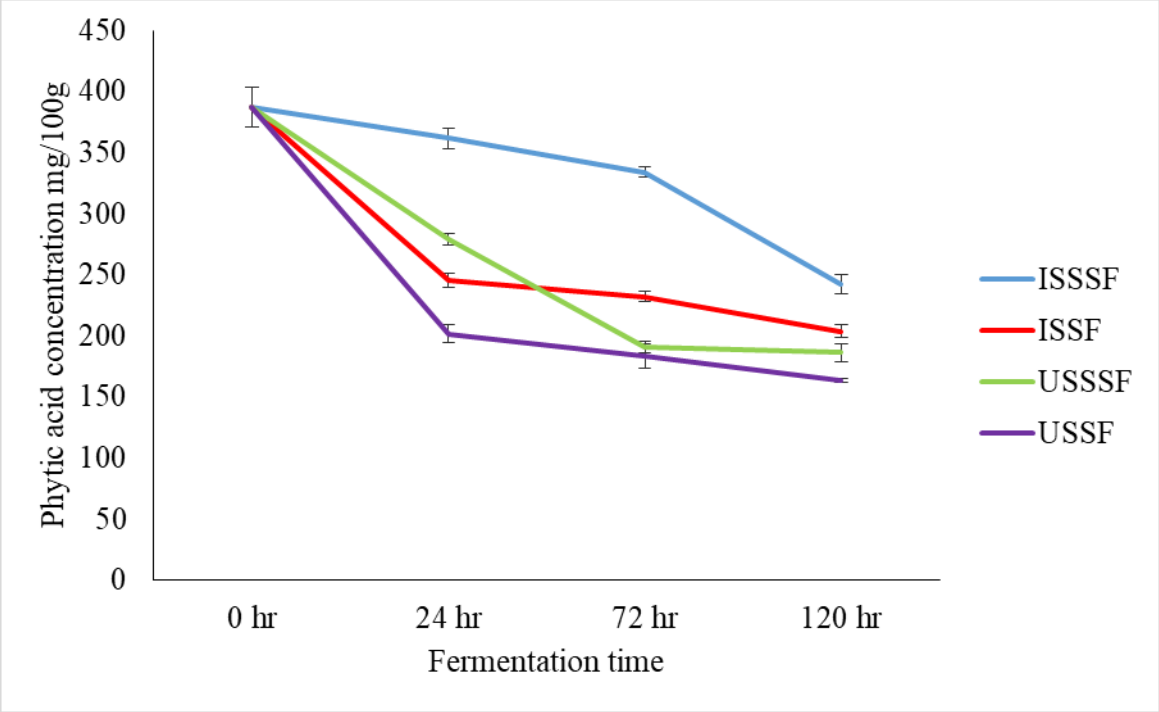


Figure 4.10: Effect of fermentation time and solutions on phytic acid concentration during the fermentation of pre-soaked whole red haricot bean in Inoculated soaked salt-only fermentation (ISSF), spontaneous soaked salt-only fermentation (USSF), Inoculated soaked salt-sugar fermentation (ISSSF) and (Spontaneous soaked salt-sugar fermentation (USSSF) batches presented as mg/100 g ± SEM (*n*=3)

At the end of fermentation, the phytic acid concentration in the USSF batch was 163.40 mg/100 g. This also was the lowest phytic acid content in comparison to all soaked bean fermentation batches (Figure 4.10). The effect of fermentation on the phytic acid concentration of boiled whole red haricot bean is presented on Figure 4.11. The phytic acid concentration decreased in all the boiled bean fermentation batches. In the IBSSF batch, the phytic acid decreased at a higher rate after 24 h and 120 h of fermentation from 350.11 mg/100 g to 257.74 mg/100 g and 159.96 mg/100 g respectively. However, in comparison to other fermentation batches, the phytic acid concentration after

fermentation were highest in this batch throughout the fermentation period. In the IBSF batch, the phytic acid decreased from 350.11 mg/100 g to 186.72 mg/100 g within the first 24 h of fermentation. Increase in fermentation time resulted in further reduction in the phytic acid concentration to 137.54 mg/100 g at the end of 120 h of fermentation.

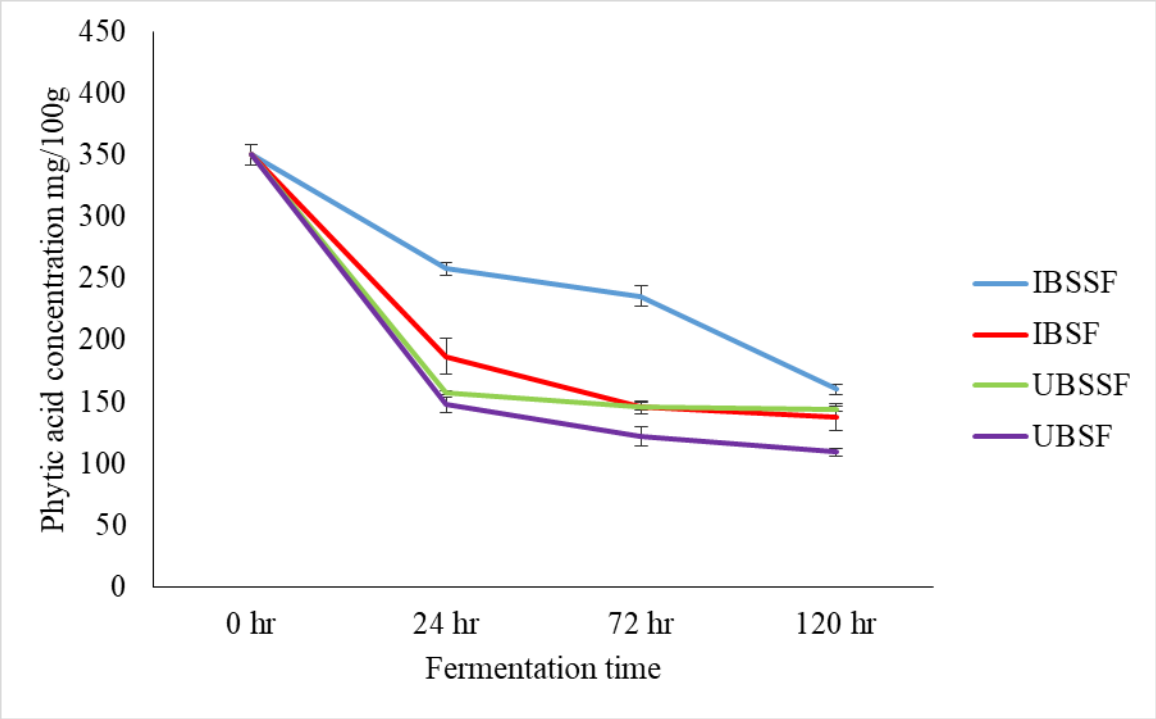


Figure 4.11: Effect of fermentation time and solutions on phytic acid concentration during the fermentation of pre-boiled whole red haricot bean in Inoculated boiled salt-only fermentation(IBSF), Spontaneous boiled salt-only fermentation (UBSF), Inoculated boiled salt-sugar fermentation (IBSSF) and Spontaneous boiled salt-sugar fermentation (UBSSF) batches presented as mg/100 g ± SEM (*n*=3)

In the UBSSF batch, higher reduction of phytic acid concentration occurred after 24 h of fermentation. This reduced the phytic acid concentration from 350.11 mg/100 g to 157.09 mg/100 g. This was followed with a slow reduction to 145.65 mg/100 g after 72

h of fermentation and 143.98 mg/100 g after 120 h of fermentation. In the UBSF batch, highest reduction of phytic acid concentration from 350.11 mg/100 g to 147.40 mg/100 g occurred after 24 h of fermentation. This was the highest reduction in comparison to the other boiled bean fermentation batches after 24 h of fermentation. Increased fermentation time resulted in further decrease in phytic acid concentration to 122.04 mg/100 g and 109.07 mg/100 g at the end of 72 h and 120 h of fermentation. At the end of fermentation, the UBSF batch had the lowest phytic acid concentration in comparison to the other boiled bean fermentation batches (Figure 4.11).

The decrease in phytic acid concentration as a result of fermentation of whole red haricot beans established by this current study is similar to the findings of Adeniran *et al*. (2013) who reported a 77.82% and 73.53% reduction of phytic acid in lima and locust bean respectively. The reduction of phytic acid during fermentation can be attributed to the activity of phytase enzyme from the beans, fermenting microorganisms or a combination of the two (Porres *et al*., 2003; Kocková *et al*., 2011; Ojokoh *et al*., 2013; Adeniran *et al*., 2013). Phytase enzyme hydrolyzes phytate into inositol and either orthophosphate or phosphoric acid. The latter process results in the release of phosphorus hence improving its bioavailability (Adeniran *et al*., 2013). Optimum activity for cereal phytase is between 5.1-5.3 drop in pH during fermentation may denature the enzyme phytase. Hence capacity to lower phytate level is optimum up to 48 h of fermentation (Ejigui *et al*., 2005). Mohamed *et al*. (2011) reported that lowered pH favored phytase activity resulting in 58.1% and 70.6% loss of phytate after 24 h and 72 h of *Lb. plantarum* fermentation of kidney beans respectively. The findings in this current study agrees with these researchers. It was observed that higher phytic acid concentration losses occurred between 24 h and 72 h of fermentation. In the soaked bean fermentation batches (Figure 4.10), ISSSF had lowest losses of phytic acid in comparison to the other batches. This could be attributed to the fact that the pH in this batch was below 5.1 after 24 h of fermentation (Figure 4.5) which continued to decrease with increase in fermentation time. It was also observed that higher phytic acid losses in the ISSF and USSF was highest after 24 h when pH in both the batches was at 5.6

(Figure 4.5). This indicates that phytase enzyme activity could be favored at higher pH than 5.3 as reported by Ejigui *et al*. (2005) . In the boiled bean fermentation batches, a similar trend was observed. The highest phytic acid loss occurred after 24 h of fermentation (Figure 4.11). At this time, the pH in all batches was above 5.2 except in the UBSSF batch (Figure 4.6). This indicates that activity of phytase enzyme was optimal and hence higher rates of breaking down of the phytic acid in the beans. The UBSSF batch had lower phytic acid throughout the fermentation, this corresponds to the low pH in this batch (Figure 4.5) throughout the fermentation. This low pH could have lowered the phytase activity through denaturation (Ejigui *et al*., 2005). In the USSSF and UBSSF batch, the phytic acid losses were high between 24 h and 72 h of fermentation respectively (Figure 4.10 and 4.11). This occurred even though their pH was at 4.5 and 4.8 in the USSSF and UBSSF batches respectively after 24 h of fermentation (Figure 4.5 and 4.6). This indicates that in addition to phytase enzyme, there was another action that resulted in phytic acid losses. Ejigui *et al*. (2005) reported that in addition to phytase enzyme activity, phytic acid of some cereals can also be eliminated through passive diffusion of water soluble phytate. This could therefore be the reason why losses in phytic acid was observed in the ISSSF, USSSF, IBSSF and UBSSF batches even after pH was lowered below 5.1. It was also observed in the current study the spontaneously fermented batches had higher phytic acid loss compared to the batches inoculated with *Lb. plantarum* BFE 5092. This is contrary to the findings of Onwurafor *et al*. (2014), who established that back-sloping caused in higher reduction of phytic acid compared to spontaneously fermented mung bean. Zamudio and Gonza (2001) reported that *Lb. plantarum* had highest extracellular phytase activity at pH 5.5 and 65°C. Since this study was done at room temperature, it is possible that the low temperatures could have resulted in low phytase activity in the inoculated batches. This observation could also be attributed to the adaptation of the bacteria in the spontaneous fermentation to cereal fermentation compared to the *Lb. plantarum* BFE 5092 which was isolated from fermented milk. Higher phytic acid losses was also observed in the boiled bean batches (Figure 4.11) compared to the soaked bean batches (Figure 4.10). This could be attributed to the softening and breaking of the testa as a

result of boiling which could have exposed the phytic acid to microbial phytases. Loss of phytic improves availability of minerals as they become available for utilization by the digestive system of the consumer.

4.3.4 Effect of LAB fermentation of whole red haricot bean (*Phaseolus vulgaris* L) on the raffinose family oligosaccharide

4.3.4.1 Effect of LAB fermentation of whole red haricot bean (*Phaseolus vulgaris* L) on the raffinose sugar concentration on dry weight basis

The effect of fermentation on the raffinose content of soaked whole red haricot bean is presented in Figure 4.12 whereas the percentage decrease of raffinose concentration is presented in Table 4.6. Fermentation resulted in a decrease in raffinose concentration in all the fermented batches. The raffinose concentration decreased from 55.91 mg/100 g to 50.58 mg/100 g (9.52% decrease) after 24 h of fermentation in the ISSSF batch. Increased fermentation time resulted in further decrease in the raffinose concentration of the beans in the ISSSF batch. The highest decrease of 26.85% (Table 4.6) occurred after 120 h of fermentation decrease the raffinose content to 32.85 mg/100 g. A similar trend was observed in the USSSF batch, raffinose content decreased from 55.91 mg/100 g to 50.22 mg/100 g (10.16% decrease) after 24 h of fermentation. Increased fermentation time resulted in further decrease in the raffinose concentration. At the end of 120 h of fermentation, the raffinose sugar concentration decreased by 24.78% to 32.58 mg/100 g.

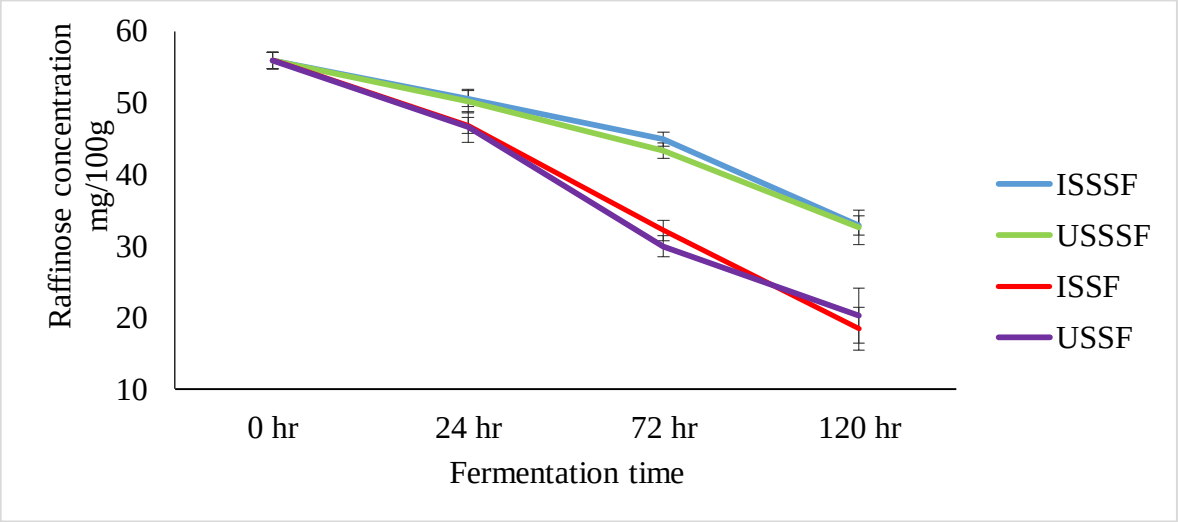


Figure 4.12: Effect of fermentation time and solutions on raffinose concentration during the fermentation of pre-soaked whole red haricot bean in Inoculated soaked salt-only fermentation (ISSF), spontaneous soaked salt-only fermentation (USSF), Inoculated soaked salt-sugar fermentation (ISSSF) and (Spontaneous soaked salt-sugar fermentation (USSSF) batches presented as mg/100 g ± SEM (*n*=3)

When the soaked whole beans were fermented in the ISSF and USSF batches, a 16.25% and 16.65% decrease in raffinose concentration respectively occurred. This lowered the raffinose concentration from 55.91 mg/100 g to 46.82 mg/100 g and 46.60 mg/100 g respectively. Increased fermentation time resulted in an increase in percentage decrease of raffinose by 31.34% and 35.77% respectively after 72 h of fermentation. At the end of 120 h of fermentation, highest decrease of 42.65% occurred in the ISSF batch. This decreased the raffinose content to 18.44 mg/100 g in the ISSF batch which was the lowest in comparison to the other soaked bean batches (Figure 4.12). In the USSF batch, the raffinose concentration decreased by 32.25% to 20.28 mg/100g after 120 h of fermentation (Figure 4.12). The effect of fermentation of boiled whole red haricot bean on raffinose concentration is presented in Figure 4.13. The percentage decrease in the

raffinose concentration during fermentation of boiled red haricot bean is presented in Table 4.6. When boiled beans were subjected to fermentation, a decrease in the raffinose concentration occurred (Figure 4.13). In the IBSSF batch, raffinose concentration decreased from 50.30 mg/100 g to 22.66 mg/100 g (54.94% decrease) after 24 h of fermentation. With increased fermentation time, the raffinose concentration continued to decrease at a decreasing rate (Table 4.6).

Table 4.6: Percentage decrease in raffinose concentration during the fermentation of soaked whole red haricot bean (*Phaseolus vulgaris* L)

	ISSSF	USSSF	ISSF	USSF
0 h	ND	ND	ND	ND
24 h	9.52	10.16	16.25	16.65
72 h	11.21	13.75	31.34	35.77
120 h	26.85	24.78	42.63	32.25

ISSF-Inoculated soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

USSF- Spontaneous soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

ISSSF-Inoculated soaked salt-sugar fermentation for 0 h, 24 h, 72 h and 120 h

USSSF- Spontaneous soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

ND- Not determined

At the end of 120 h of fermentation, the raffinose concentration was 14.04 mg/100 g. In the UBSSF batch the raffinose concentration decreased from 50.30 mg/100 g to 11.86 mg/100 g (76.42% decrease). Increased fermentation time resulted in further decrease in the raffinose concentration. At the end of 120 h of fermentation, the raffinose concentration had decreased to 1.81 mg/100 g (Figure 4.13). In the IBSF batch raffinose concentration decreased from 50.30 mg/100 g to 40.62 mg/100 g (19.24% decrease) after 24 h of fermentation. Increased fermentation time resulted in further decrease in the

raffinose concentration (Figure 4.13) by 52.77% and 62.68% decrease after 72 h and 120 h of fermentation. At the end of 120 h of fermentation the raffinose concentration was 7.16 mg/100 g. In the UBSF batch the raffinose concentration decreased by 81% after 72 h of fermentation. This lowered the raffinose concentration to 4.67 mg/100 g from 25.91 mg/100 g at 24 h of fermentation. After 120 h of fermentation, the raffinose concentration in this batch was 2.51 mg/100 g. The decrease in raffinose concentration as a result of fermentation in this current study is in agreement with that of Adewumi and Odunfa (2009). The decrease in raffinose concentration is attributed to the ability of the microbes to produce α-galactosidase enzyme that breaks down the α-1,6-glycosidic linkages (Adewumi and Odunfa, 2009).

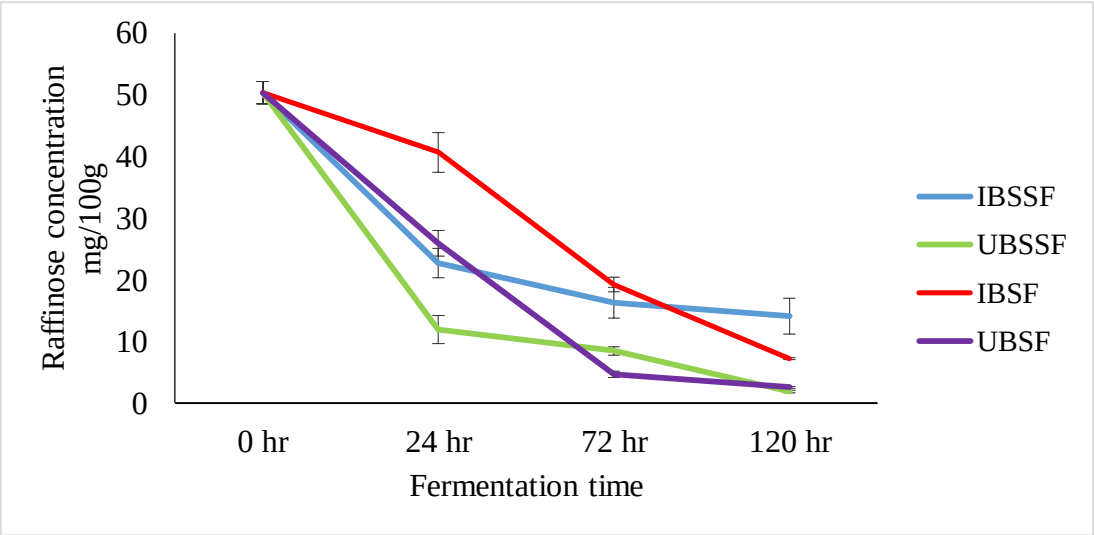


Figure 4.13: Effect of fermentation time and solutions on raffinose concentration during the fermentation of pre-boiled whole red haricot bean in Inoculated boiled salt-only fermentation(IBSF), Spontaneous boiled salt-only fermentation (UBSF), Inoculated boiled salt-sugar fermentation (IBSSF) and Spontaneous boiled salt-sugar fermentation (UBSSF) batches presented as mg/100 g ± SEM (*n*=3)

A-galactosidase is an inducible enzyme and rafinose sugar being one of its best inducer (Kumar *et al*., 2012). Therefore the presence of raffinose in the beans resulted in the production of α-galactosidase by the fermenting microorganisms. In the soaked bean batches (ISSSF, USSSF, ISSF and USSF), it was observed that the rate of raffinose reduction increased with fermentation time (Table 4.5) with highest decrease occurring after 120 h of fermentation. This was contrary to the findings of Adewumi and Odunfa (2009) who reported that raffinose content decreased significantly between 24 h and 72 h of fermentation of *Vigna unguiculata* beans with *Lb. plantarum.* Kumar *et al*. (2012) also reported that α-galactosidase activity was detected after 12 h incubation with maximum activity at 72 h beyond which production and activity of the enzyme declined. The higher rate of raffinose loss after 120 h could be attributed to difference in the fermenting microbes (Djaafar *et al*., 2013). It could also be attributed to the growth of LAB in these batches which were still increasing in number even after 72 h of fermentation (Table 4.1).

Table 4.7: Percentage decrease in raffinose concentration during the fermentation of boiled whole red haricot bean (*Phaseolus vulgaris* L)

	IBSSF	UBSSF	IBSF	UBSF
0 h	ND	ND	ND	ND
24 h	54.94	76.42	19.24	48.50
72 h	28.30	29.01	52.77	81.96
120 h	13.57	78.56	62.68	45.29

IBSF-Inoculated boiled salt-only fermentation for 0 h, 24 h, 72 h and 120 h

UBSF- Spontaneous boiled salt-only fermentation for 0 h, 24 h, 72 h and 120 h

IBSSF-Inoculated boiled salt-sugar fermentation for 0 h, 24 h, 72 h and 120 h

UBSSF- Spontaneous boiled salt-only fermentation for 0 h, 24 h, 72 h and 120 h

ND- Not determined

Higher decrease in raffinose concentration occurred in the ISSF and USSF batches in comparison to the ISSSF and USSSF batches. This could be attributed to the low pH of 3.9 in the ISSSF and USSSF batches (Figure 4.5) which could have denatured the α-galactosidase enzyme and lowered their activity. Carević *et al*. (2016) and Carrera-silva *et al*. (2006) reported that α-galactosidase enzyme had an optimum pH of 4.3-5.0.

In the boiled bean batches, it was observed that raffinose content decreased at a higher rate between 24 h and 72 h of fermentation. This was in agreement with the findings of Adewumi and Odunfa, (2009) who reported higher raffinose loss between 24 h and 72 h of fermentation. This correlated with the optimal activity time (12 h -72 h) for α-galactosidase that was reported by Kumar *et al*. (2012). The IBSSF and UBSSF batches had highest decrease in raffinose concentration after 24 h of fermentation. At this time the pH was at 5.1 and 4.8 respectively (Figure 4.6) which was favorable for α-galactosidase activity (Carević et al., 2016). In the IBSF and UBSF higher decrease in raffinose concentration was after 72 h when the pH was at 5.3 and 5.6 respectively (Figure 4.6). This decrease in raffinose concentration between 24 and 72 h could also be attributed to the exposure of the raffinose sugar to microbial α-galactosidases. This is due to the softening and breaking down of the testa during boiling.

4.3.4.2 Effect of fermentation on the stachyose sugar concentration of whole red haricot bean (*Phaseolus vulgaris* L) on dry weight basis

The effect of fermentation on soaked whole red haricot bean on stachyose sugar concentration is presented in Figure 4.14. The percentage decrease in stachyose sugar with fermentation time is presented in Table 4.7. Stachyose concentration decreased with increased fermentation time in all the soaked bean fermentation batches. In the ISSSF batch, the stachyose concentration decreased by 29.97% after 24 h of fermentation from 1247.17 mg/100g to 873.35 mg/100g (Figure 4.14). Increased fermentation time resulted with further decrease of 11.68% and 23.08% after 72 h and 120 h of fermentation. At the end of fermentation (120 h), the stachyose concentration in the ISSSF batch was at 593.33 mg/100 g.

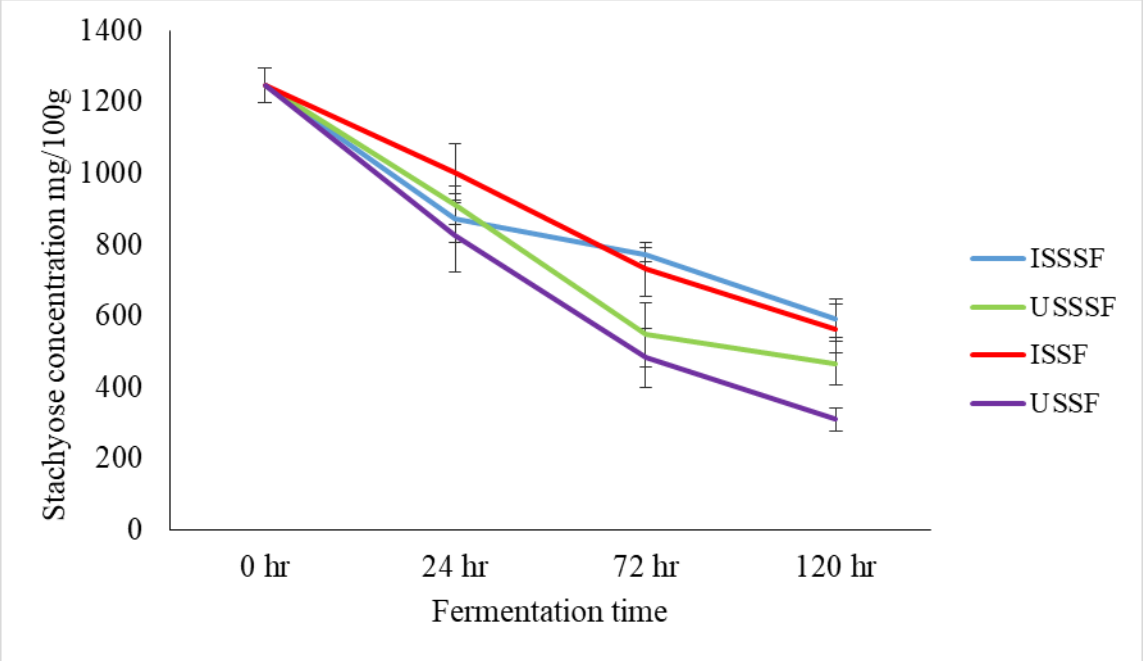


Figure 4.14: Effect of fermentation time and solutions on stachyose concentration during the fermentation of pre-soaked whole red haricot bean in Inoculated soaked salt-only fermentation (ISSF), spontaneous soaked salt-only fermentation (USSF), Inoculated soaked salt-sugar fermentation (ISSSF) and (Spontaneous soaked salt-sugar fermentation (USSSF) batches presented as mg/100 g ± SEM (*n*=3)

In the USSSF fermentation batch, the stachyose concentration decreased by 26.91% from 1247.17 mg100 g to 911.61 mg/100 g after 24 h of fermentation. Further decrease of 39.95% and 14.59% in stachyose concentration occurred after 72 h and 120 h of fermentation (Table 4.7). At the end of 120 h of fermentation, the stachyose concentration was at 467.49 mg/100 g in the USSSF batch. In the ISSF batch, the stachyose concentration decreased by 19.78% after 24 h of fermentation from 1247.17 mg/100 g to 1000.47 mg/100 g. Further losses in stachyose concentration occurred with increased fermentation time with highest loss of 26.94% occurring after 72h of fermentation (Table 4.7). At the end of fermentation (120 h), the stachyose concentration

in the ISSF batch was 564.16). In the USSF batch, stachyose concentration decreased by 33.87%, 41.37% and 35.82% after 24h, 72 h, and 120 h of fermentation respectively (Table 4.7). This was the highest decrease in comparison to the other batches. At the end of 120 h of fermentation, the stachyose concentration in the USSF batch was 310.36 mg/100 g (Figure 4.14)

Table 4.8: Percentage decrease in stachyose concentration during the fermentation of soaked whole red haricot bean (*Phaseolus vulgaris* L)

	ISSSF	USSSF	ISSF	USSF
0 h	ND	ND	ND	ND
24 h	29.97	26.91	19.78	33.87
72 h	11.68	39.95	26.94	41.37
120 h	23.08	14.59	22.82	35.82

ISSF-Inoculated soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

USSF- Spontaneous soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

ISSSF-Inoculated soaked salt-sugar fermentation for 0 h, 24 h, 72 h and 120 h

USSSF- Spontaneous soaked salt-only fermentation for 0 h, 24 h, 72 h and 120 h

ND- Not determined

The effect of fermentation of boiled whole red haricot bean (*Phaseolus vulgaris* L) is presented in Figure 4.15). The percentage decrease in stachyose concentration as a result of fermentation is presented in Table 4.8. Stachyose concentration decreased with increased fermentation time in all the boiled bean fermentation batches (Figure 4.15). In the IBSSF batch, the stachyose concentration decreased by 72.84% from 995.76 mg/100 g to 270.47 mg/100 g. Further decrease of 0.1% and 22.51% in stachyose concentration occurred after 72 h and 120 h of fermentation. At the end of 120 h of fermentation, the stachyose concentration was at 209.37 mg/100 g. Stachyose concentration in the

UBSSF batch decreased by 79.48% to 204.34 mg/100 g after 24 h of fermentation. The stachyose concentration further decreased by 15.30% and 76.53% after 72 h and 120 h of fermentation. At the end of fermentation (120 h), the stachyose concentration in the UBSSF batch was 40.61 mg/100 g (Figure 4.15). In the IBSF batch, the stachyose concentration decreased by 70.87% after 24 h of fermentation to 290.06 mg/100 g. Further decrease of 6.86% and 40.93% in stachyose concentration occurred after 72 h and 120 h of fermentation. At the end of 120 h of fermentation, the stachyose concentration had decreased to 159.56 mg/100 g in the IBSF batch. In the UBSF batch, the stachyose concentration decreased by 63.69% to 361.57 mg/100 g after 24 h of fermentation. A further decrease by 72.02% and 41.92% occurred after 72 h and 120 h of fermentation. The stachyose concentration in the UBSF batch decreased to 58.76 mg/100 g after 120 h of fermentation (Figure 4.15).

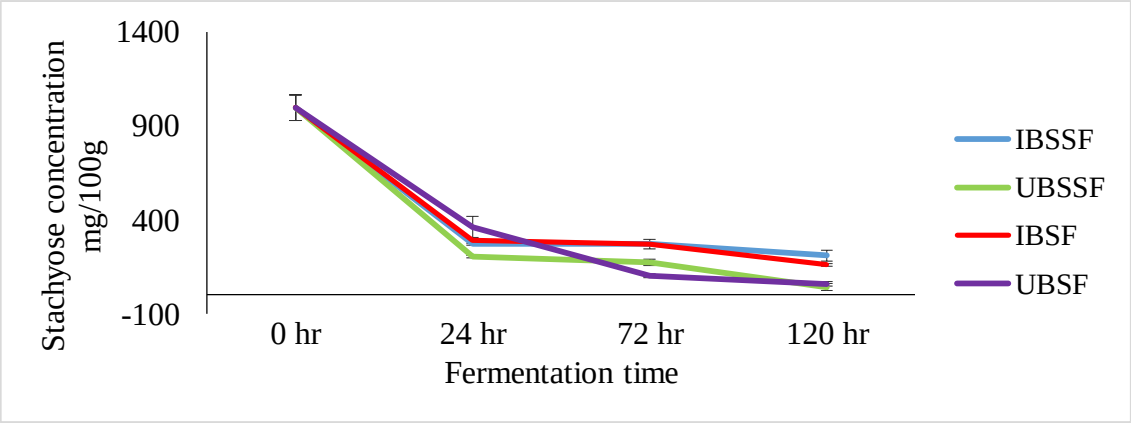


Figure 4.15: Effect of fermentation time and solutions on stachyose concentration during the fermentation of pre-boiled whole red haricot bean in Inoculated boiled salt-only fermentation(IBSF), Spontaneous boiled salt-only fermentation (UBSF), Inoculated boiled salt-sugar fermentation (IBSSF) and Spontaneous boiled salt-sugar fermentation (UBSSF) batches presented as mg/100 g ± SEM (*n*=3)

Fermentation resulted in decrease in stachyose concentration in both soaked and boiled beans. This is in agreement with the findings of Adewumi and Odunfa (2009) and Granito *et al*. (2003) who reported a decrease in stachyose concentration as a result of fermentation of *vigna ungiulata* beans and common beans (*Phaseolus vulgaris*) respectively. Highest decrease in stachyose concentration of the beans occurred between 24 h and 72 h in the soaked bean. However even after 120 h of fermentation appreciable amount of stachyose reduction continued to occur. In the boiled beans, highest reduction in stachyose concentration occurred after 24 h of fermentation. With further loses being recorded even after 120 h of fermentation. This correlates with the 12 h - 72 h optimal production and activity time span for α-galactosidase enzyme as reported by (Kumar *et al.*, 2012). After 72 h of fermentation the rate of stachyose reduction was slowed down in the USSSF, ISSF, USSF and UBSF batches. This could be as a result of a decline in α-galactosidase production and activity attributed to catabolic repression by nutrients present and also decline in pH (Kumar *et al*., 2012).

Table 4.9: Percentage decrease in stachyose concentration during the fermentation of boiled whole red haricot bean (*Phaseolus vulgaris* L)

	IBSSF	UBSSF	IBSF	UBSF
0 h	ND	ND	ND	ND
24 h	72.84	79.48	70.87	63.69
72 h	0.10	15.30	6.86	72.02
120 h	22.51	76.53	40.93	41.92

IBSF-Inoculated boiled salt-only fermentation for 0 h, 24 h, 72 h and 120 h

UBSF- Spontaneous boiled salt-only fermentation for 0 h, 24 h, 72 h and 120 h

IBSSF-Inoculated boiled salt-sugar fermentation for 0 h, 24 h, 72 h and 120 h

UBSSF- Spontaneous boiled salt-only fermentation for 0 h, 24 h, 72 h and 120 h

ND- Not determined

In the ISSSF, IBSSF, UBSSF, IBSF batches however, it was observed that the rate decreased after 72 h of fermentation and then increased after 120 h of fermentation. This could be as a result of the reduced LAB counts after 72 h of fermentation (Figures 4.1 and 4.2) followed by an increase in the LAB count after 120 h of fermentation. The reduced LAB count could have resulted in the decrease of α-galactosidase enzymes, hence a reduction in the rate of stachyose degradation by the enzymes. The spontaneously fermented beans recorded a relatively higher reduction of stachyose content compared to the inoculated batches. This is in agreement with the findings of Granito *et al*. (2003) who reported that spontaneous fermentation removed more stachyose compared to controlled fermentation.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Lactic acid bacteria counts were initially higher in the batches inoculated with *Lb. plantarum* BFE 5092, compared to the spontaneously fermented batches, however, with increase in fermentation time the LAB counts in the spontaneously fermented batches were comparable to those in the inoculated batches thus showing that inoculation only increases the pace of fermentation but eventually both spontaneous and inoculated samples would of similar quality. Salt-sugar solution batches performed better because they had the ability to lower the pH to below 4 after 72 h of fermentation. Lactic acid fermentation inhibited yeast and mold growth as no yeast nor mold were detected in all the batches throughout fermentation.

Pre-treatments of whole red haricot bean through soaking and boiling had no significant effect on the crude fat and ash content. They however lowered the crude fiber, protein, tannins, phytic acid, raffinose and stachyose contents significantly. Carbohydrate content increased as a result of soaking.

Fermentation resulted in significant reduction of the tannin, phytic acid, raffinose and stachyose concentrations. However boiling pretreatment of beans prior to fermentation resulted in higher anti-nutrient loses compared to fermentation after soaking pretreatment. Higher anti-nutrient loss was also observed in the spontaneously fermented batches compared to the inoculated batches. Highest loses occurred between 24-72 h of fermentation. This relates to the optimum activity and pH of α-galactosidase enzyme produced by LAB.

5.2 Reccomendations

I recommend that further studies be carried out to determine;

1. The sensory acceptability of the fermented whole red haricot bean
2. The benefits of reduced anti-nutrients and RFOs content on the potential consumers of the fermented bean product

REFFERENCES

Adebowale, O. J., & Maliki, K. (2011). Effect of fermentation period on the chemical composition and functional properties of Pigeon pea (Cajanus cajan) seed flour. *International Food Research Journal*, *18*(4), 1329–1333.

Adeniran, H. A., Farinde, E. O., & Obatolu, V. A. (2013). Effect of Heat Treatment and Fermentation on Anti-Nutrients Content of Lima Bean (Phaseolus lunatus) During Production of Daddawa Analogue, *3*(March 2010), 256–266.

Adewumi, G. A., & Odunfa, S. A. (2009). Effect of controlled fermentation on the oligosaccharides content of two common Nigerian Vigna unguiculata beans (drum and oloyin), *8*(11), 2626–2630.

Admassu, S. (2008). Variability in Phytochemicals , A -Galactosides , Sucrose Composition and in Vitro Protein Digestibility of Common Bean (Phaseolus vulgaris L .) Varieties. *East African Journal of Sciences*, *2*, 45–54.

Agarwal, A. (2016). Journal of Nutritional Disorders and Duality of Anti-nutritional Factors in Pulses. *Nutritionist and Aromatherapist, SNDT Women's University, Mumbai, India *Corresponding*, *6*(1), 1–2.

Agbenorhevi, J. K., Oduro, I., Ellis, W. ., Abodakpi, V. ., & Eleblu, S. . (2010). Effect of soaking, autoclaving and repeated boiling on oligosaccharides in cowpea. *Nigerian Food Journal*, *25*(2), 88–94.

Akande, K. E., Doma, U. D., Agu, H. O., & Adamu, H. M. (2010). Major antinutrients found in plant protein sources: Their effect on nutrition. *Pakistan Journal of Nutrition*, *9*(8), 827–832.

Akpor, O., & Aderiye, B. (2014). Assessment of bacterial and fungal spoilage of some Nigerian fermented and unfermented foods. *African Journal of Food*

Science, *8*(3), 140–147.

Amutha, K., & Kokila, V. (2015). Cholesterol lowering property of lactobacillus plantarum isolated from cow milk. *International Journal of Pharmaceutical Sciences and Research*, *6*(3), 1184-89

Anderssen, E. L., Diep, D. B., Nes, I. F., Eijsink, V. G. H., & Nissen-Meyer, J. (1998). Antagonistic activity of Lactobacillus plantarum C11: Two new two- peptide bacteriocins, plantaricins EF and JK, and the induction factor plantaricin A. *Applied and Environmental Microbiology*, *64*(6), 2269–2272.

Antonio, C., Pinheiro, C., Manuela, M., Ortu, M. F., & Qu, I. D. T. (2008). Analysis of carbohydrates in Lupinus albus stems on imposition of water deficit , using porous graphitic carbon liquid chromatography-electrospray ionization mass spectrometry, *1187*, 111–118.

Anuradha, S., & Rajeshwari, K. (2005). Probiotics in health and disease. *Journal, Indian Academy of Clinical Medicine*, *6*(1), 67–72.

APata, D. F. (2008). Effect of cooking methods on available and unavailable carbohydrates of some tropical grain legumes, *7*(16), 2940–2945.

Assohoun, M. C. N., Djeni, T. N., Marina., K., & Brou, K. (2013). Effect of Fermentation Process on Nutritional Composition and Aflatoxins Concentration of Doklu , a Fermented Maize Based Food. *Food and Nutrition Sciences*, *2013*(November), 1120–1127.

Audu, S. S., & Aremu, M. O. (2011). Effect of Processing on Chemical Composition of Red Kidney Bean (Phaseolus vulgaris L.) Flour. *Pakistan Journal of Nutrition*, *10*(11), 1069–1075.

Audu, S. S., Aremu, M. O., & Lajide, L. (2013). Influence of traditional processing

methods on the nutritional composition of lack turtle bean (Phaseolus vulgaris L.) grown in Nigeria. *International Food Research Journal*, *20*(6), 3211–3220.

Aworh, O. C. (2008). The Role of Traditional Food Processing Technologies in National Development: The West African Experience. *International Union of Food Science & Technology*, 1–18.

Berrios, J. D. J., P, M., Cámara.M, & Sánchez-mata.M C. (2010). Carbohydrate composition of raw and extruded pulse flours. *Food Research International*, *43*(2), 531–536.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., … Hansen, E. B. (2012a). Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology*, *154*(3), 87–97.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., … Hansen, E. B. (2012b). Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology*, *154*(3), 87–97.

Carević, M., Banjanac, K. A. T. A., Jakovetić, S. O. N. J. A., Milivojević, A. N. A., Sekulić, M. V., & Bezbradica, D. (2016). Selection of lactic acid bacteria strain for simultaneous production of α - and β -galactosidases, *Zaštita materijala*, *57*(2), 265-273.

Carrera-Silva, E. A., Silvestroni, A., LeBlanc, J. G., Piard, J. C., de Giori, G. S., & Sesma, F. (2006). A thermostable α-galactosidase from Lactobacillus fermentum CRL722: genetic characterization and main properties. *Current microbiology*, *53*(5), 374-378.

Chaudhary, R., & Sharma, S. (2013). Conventional nutrients and antioxidants in red kidney beans (phaseolus vulgaris L.): An explorative and product development endeavour. *Annals. Food Science and Technology*, *14*(2), 275-285.

Chelule, P., Mokoena, M., & Gqaleni, N. (2010). Advantages of traditional lactic acid bacteria fermentation of food in Africa. *Current Research, Technology*, *2*, 1160–1167.

Chin, K. H., & Koehler, P. E. (1986). Effect of Salt Concentration and Incubation Temperature on Formation of Histamine , Phenethylamine , Tryptamine and Tyramine During Miso Fermentation, *Journal of food protection*, *49*(6), 423-427.

Cho, G. S., Huch, M., Hanak, A., Holzapfel, W. H., & Franz, C. M. A. P. (2010). Genetic analysis of the plantaricin EFI locus of Lactobacillus plantarum PCS20 reveals an unusual plantaricin E gene sequence as a result of mutation. *International Journal of Food Microbiology*, *141*(SUPPL.), S117–S124.

Cruz-Bravo, R. K., Guevara-Gonzalez, R., Ramos-Gomez, M., Garcia-Gasca, T., Campos-Vega, R., Oomah, B. D., & Loarca-Piña, G. (2011). Fermented Nondigestible Fraction from Common Bean (Phaseolus vulgaris L.) Cultivar Negro 8025 Modulates HT-29 Cell Behavior. *Journal of Food Science*, *49*(6), 423-427

Devindra, S., Sreenivasa, R. J., Bhaskar, V., & Mulimani, V. H. (2012). Effect of heat treatment on α-galactoside content of red gram seeds (Cajanus cajan, L). *Journal of Food Processing and Preservation*, *36*(2), 97–103.

Díaz, A. M., Caldas, G. V., & Blair, M. W. (2010). Concentrations of condensed tannins and anthocyanins in common bean seed coats. *Food Research International*,

43(2), 595–601.

Djaafar, T. F., Cahyanto, M. N., Santoso, U., & Edang, S. R. (2013). Growth of indigenous lactic acid bacteria Lactobacillis plantarum-pentosus T14 and lactobacillus-pentosus T35 in keradang(Canavalia virosa) milk and changes of raffinose. *Malaysian Journal of Microbiology*, *9*(3), 213–218.

Ejigui, J., Savoie, L., Marin, J., & Desrosiers, T. (2005). Beneficial changes and drawbacks of a traditional fermentation process on chemical composition and anti- nutritional factors of yellow maize (Zea mays). *Journal of biological sciences*, *5*(5), 590-596.

El-din, I. M. K. (2010). *Characterization of Lactic Acid Bacteria from Egyptian Environment and their Bioactive Products Islam Mohamed Kamal El-Din*, Unpublished MSc thesis, Cairo: Cairo University.

Gemede, H. F., & Ratta, N. (2014). Antinutritional factors in plant foods: Potential health benefits and adverse effects. *International Journal of Nutrition and Food Sciences*, *3*(4), 284-289.

Fernandes, A. C., Nishida, W., & Da Costa Proença, R. P. (2010). Influence of soaking on the nutritional quality of common beans (Phaseolus vulgaris L.) cooked with or without the soaking water: A review. *International Journal of Food Science and Technology*, *45*(11), 2209–2218.

Ferreira, A. S. T., Naozuka, J., Kelmer, G. A. R., & Oliveira, P. V. (2014). Effects of the Domestic Cooking on Elemental Chemical Composition of Beans Species (Phaseolus vulgaris L.). *Journal of Food Processing*, *2014*, 1–6.

Gan, R. Y., Li, H. Bin, Gunaratne, A., Sui, Z. Q., & Corke, H. (2017). Effects of Fermented Edible Seeds and Their Products on Human Health: Bioactive Components and Bioactivities. *Comprehensive Reviews in Food Science and*

Food Safety, *16*(3), 489–531.

Ganesan, K., & Xu, B. (2017). Polyphenol-rich dry common beans (Phaseolus vulgaris L.) and their health benefits. *International Journal of Molecular Sciences*, *18*(11), 2331

Gänzle, M. G., & Follador, R. (2012). Metabolism of oligosaccharides and starch in lactobacilli: A review. *Frontiers in Microbiology*, *3*(SEP), 1–15.

Granito, M., & Alvarez, G. (2006). Lactic acid fermentation of black beans (Phaseolus vulgaris) : microbiological and chemical characterization, *1171*(November 2005), 1164–1171.

Granito, M., Champ, M., Guerra, M., & Frias, J. (2003). Effect of natural and controlled fermentation on flatus-producing compounds of beans (Phaseolus vulgaris), *1009*(October 2002), 1004–1009.

Greiner, R, & Konietzny, U, J. K.-D. (2006). 11. Phytate - an undesirable constituent of plant - based foods?. : *J Ernahrungsmedizin*, *8*(3), 18-28.

Henney, J. E., Taylor, C. L., & Boon, C. S. (n.d.). *Committee on Strategies to Reduce Sodium Intake Food and Nutrition Board*. United States: National Academies Press.

Hil, P. J. R. Den, & Nout, M. J. R. (2005). Anti-Diarrhoeal Aspects of Fermented Soya Beans. *Soybean and Health*, 383-406.

Hutkins, R. W. (2006). *Microbiology and Technology of Fermented Foods* (First edit). Carlton: Blackwell Publishing Ltd.

Jany, K., Greiner, R., & Konietzny, U. (2006). 11. Phytate - an undesirable constituent of plant - based foods?. : *J Ernahrungsmedizin*, *8*(3), 18-28.

Johanningsmeier, S. D., Franco, W., Perez-diaz, I., & Mcfeeters, R. F. (2012). Influence of Sodium Chloride , pH , and Lactic Acid Bacteria on Anaerobic Lactic Acid Utilization during Fermented Cucumber Spoilage, *77*(7), M397-M404.

Kinyanjui, P. K., Njoroge, D. M., Makokha, A. O., Christiaens, S., Ndaka, D. S., & Hendrickx, M. (2015). Hydration properties and texture fingerprints of easy- and hard-to-cook bean varieties. *Food Science & Nutrition*, *3*(1), 39–47.

Kocková, M., Gereková, P., Petruláková, Z., Hybenová, E., Šturdík, E., & Valík, Ľ. (2011). Evaluation of fermentation properties of lactic acid bacteria isolated from sourdough. *Acta Chimica Slovaca*, *4*(2), 78–87.

Kumar, D. J. M., Suresh, K., Saranya, G. M., Priyadharshini, D. A., Damodaran, R., & Kalaichelvan, P. T. (2012). Production, optimization and characterization of α-Galactosidase by Bacillus sp . SPE10 and Bacillus sp . SPE15 Isolated from soy effluent. *European Journal of Experimental Biology.*, *2*(3), 774–780.

Lattimer, J. M., & Haub, M. D. (2010). Effects of dietary fiber and its components on metabolic health. *Nutrients*, *2*(12), 1266–1289.

Legesse, H., Nigussie-Dechassa, R., Setegn, G., & G, B. (2015). Chemical Quality of Common Beans as Influenced by Genotype and Aluminium Rates Under Two Soil Liming Regimes. *African Journal of Food, Agriculture, Nutrition And Development*, *15*(2), 9872–9891.

Maina, J., Ulrich, M. Æ., Phillip, S. Æ., Mbugua, S. K., Guigas, Æ. C., Franz, Æ. C., & Holzapfel, Æ. W. H. (2008). Functional Properties of Lactobacillus plantarum Strains Isolated from Maasai Traditional Fermented Milk Products in Kenya, *Current Microbiology*, *56*(4), 315-321.

Martin-Cabrejas, M. A., Sanfiz, B., Vidal, A., Molla, E., Esteban, R., & Lopez-Andreu,

F. J. (2004). Effect of Fermentation and Autoclaving on Dietary Fiber Fractions and Antinutritional Factors of Beans (Phaseolus vulgaris L.). *Journal of Agricultural and Food Chemistry*, *52*(2), 261–266.

McFeeters, R. F., & Pérez-Díaz, I. (2010). Fermentation of cucumbers brined with calcium chloride instead of sodium chloride. *Journal of Food Science*, *75*(3), 291–296.

Mcmurtrie, E. K., & Johanningsmeier, S. D. (2018). Quality of Cucumbers Commercially Fermented in Calcium Chloride Brine without Sodium Salts, *2018*, 13.

Mcphee, K. E., Zemetra, R. S., & Brown, J. (2002). Genetic Analysis of the Raffinose Family Oligosaccharides in Common Bean, *127*(3), 376–382.

Messina, V. (2014). Nutritional and health benefits of dried beans 1 – 3. *The American Journal of Clinical Nutrition*, *100*(suppl_1), 437S-442S.

Mohamed, R. K., Arab, E. A. A.-, Gibriel, A. Y., Rasmy, N. M. H., & Ferial, M. (2011). Effect of legume processing treatments individually or in combination on their phytic acid content, *2*(2), 36–46.

Nakitto, A. M., Muyonga, J. H., & Nakimbugwe, D. (2015). Effects of combined traditional processing methods on the nutritional quality of beans, *131*, 233–241.

Nations, U. (n.d.). *Food and Agriculture Organization of the United Nations*. Rome: FAO.

Niba, L. L., & Rose, N. (2003). Effect of Soaking Solution Concentration on Resistant Starch and Oligosaccharide Content of Adzuki (V. angularis), Fava (V. Faba), Lima (P. lunatus) and Mung Bean (V. radiata L.). *Journal of Food*

Sciences. *1*, 4-8.

Njoroge, D. M., Kinyanjui, P. K., Christiaens, S., Sh-, A., Makokha, A. O., Sila, D. N., & Hendrickx, M. E. (2015). Effect of storge conditions on pectic polysaccharides in common beans(phaseolus vulgaris) in relation to the hard-to-cook defect. *Food Research International*, *76*, 105-113.

Nyombaire, G., Siddiq, M., & Dolan, K. (2002). Effect of Soaking and Cooking on the Oligosaccharides and Lectins in Red Kidney Beans (Phaseolus vulgaris L.).

Oguntoyinbo, F. A., Cho, G., Brinks, E., Fiedler, G., Kabisch, J., Koberg, S., & Bockelmann, W. (2016). Draft Genome Sequence of Lactobacillus plantarum BFE 5092 Isolated from Maasai Fermented Milk, *4*(3), 2007–2008.

Ojokoh, A. O., Daramola, M. K., & Oluoti, O. J. (2013). Effect of fermentation on nutrient and anti-nutrient composition of breadfruit (Treculia africana) and cowpea (Vigna unguiculata) blend flours, *8*(27), 3566–3570.

Oladunmoye, M. K. (2007). Effects of fermentation on nutrient enrichment of locust beans (Parkia biglobosa, Robert bam). *Research Journal of Microbiology*, *2*(2), 185-189.

Olanipekun, O. T., Omenna, E. C., Suleiman, O. O. A., & Omodara, O. G. (2015). Effect of boiling and roasting on composition of kidney beans seed flour the nutrient, *4*(2), 24–29.

Omenna, E. C., Olanipekun, O. T., & Kolade, R. O. (2016). Effect of boiling , pressure cooking and germination on the nutritional and antinutrients content of cowpea (Vigna unguiculata), *ISABB J Food Agric Sci*, *6*(1), 1-8.

Ongol, M. P. (2012). Lactic acid bacteria in health and disease. *Rwanda Journal of*

Health Sciences, *1*(1), 39–50.

Onwurafor, E. U., Onweluzo, J. C., & Ezeoke, A. M. (2014). Effect of Fermentation Methods on Chemical and Microbial Properties of Mung Bean (Vigna radiata) Flour. *Nigerian Food Journal*, *32*(1), 89–96.

Osman, M. A., & Gassem, M. (2013). Effects of domestic processing on trypsin inhibitor , phytic , acid , tannins and in-vitro Protein Digestibility of three sorghum varieties, *9*(5), 1189–1198.

Porres, J. M., Aranda, P., López-jurado, M., & Urbano, G. (2003). Effect of natural and controlled fermentation on chemical composition and nutrient dialyzability from beans (Phaseolus vulgaris L.). *Journal of Agricultural and Food Chemistry*, *51*(17), 5144–5149.

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, *26*(5), 1214–1218.

Pundir, R. K., Rana, S., Kashyap, N., & Kaur, A. (2013). Probiotic potential of lactic acid bacteria isolated from food samples: An in vitro study. *Journal of Applied Pharmaceutical Science*, *3*(3), 85–93.

Ramadan, E. A. (2012). Effect of Processing and Cooking Methods on the Chemical Composition, Sugars and Phytic Acid of Soybeans. *Food and Public Health*, *2*(1), 11–15.

Reddy, N. R., Pierson, M. D., Sathe, S. K., & Salunkhe, D. K. (1985). Dry bean tannins: A review of nutritional implications. *Journal of the American Oil Chemists Society*, *62*(3), 541–549.

Reyes-Bastidas, M., Reyes-Fernández, E. Z., López-Cervantes, J., Milán-Carrillo, J.,

Loarca-Piña, G. F., & Reyes-Moreno, C. (2010). Physicochemical, nutritional and antioxidant properties of tempeh flour from common bean (Phaseolus vulgaris L.). *Food Science and Technology International*, *16*(5), 427–434.

Rhee, S. J., Lee, J. E., & Lee, C. H. (2011). Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories*, *10*(SUPPL. 1), S1-S5.

Rupe, P. (1998). Oligosaccharides in raw and processed legumes, *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, *206*(2), 130-133.

Saraswathy, N., & Sadasivam, S. (2010). Degradation of flatulence-causing oligosaccharides in soymilk by α -galactosidase — A novel thermotolerant from Penicillium purpurogenum. *Indian Journal of Biotechnology*, *9*(April), 160–165.

Shimelis, E. A., & Rakshit, S. K. (2005). Proximate composition and physico-chemical properties of improved dry bean (Phaseolus vulgaris L.) varieties grown in Ethiopia. *LWT - Food Science and Technology*, *38*(4), 331–338.

Shivam, K., Tripathi, C. P. M., & Mishra, S. K. (2009). Culture conditions for the production of α-galactosidase by Aspergillus parasiticus MTCC-2796: A novel source. *Electronic Journal of Biotechnology*, *12*(4), 4-5.

Shori, A. B., & Baba, A. S. (2012). Viability of lactic acid bacteria and sensory evaluation in Cinnamomum verum and Allium sativum-bio-yogurts made from camel and cow milk. *Journal of the Association of Arab Universities for Basic and Applied Sciences*, *11*(1), 50–55.

Soomro, A., Masud, T., & Anwaar, K. (2002). Role of Lactic Acid Bacteria (LAB) in Food Preservation and Human Health – A Review. *Pakistan Journal of Nutrition*, *1*(1), 20–24.

Starzyńska-Janiszewska, A., Stodolak, B., & Mickowska, B. (2014). Effect of controlled lactic acid fermentation on selected bioactive and nutritional parameters of tempeh obtained from unhulled common bean (Phaseolus vulgaris) seeds. *Journal of the Science of Food and Agriculture*. *94*(2), 359-366.

Suárez-martínez, S. E., Ferriz-martínez, R. A., Elton-puente, J. E., De, K., Carbot, T., García-gasca, T., … García-gasca, T. (2016). Bean seeds : leading nutraceutical source for human health, *CyTA-Journal of Food*, *14*(1), 131-137.

Sumarna. (2008). Changes of raffinose and stachyose in soy milk fermentation by lactic acid bacteria from local fermented foods of Indonesian. *Malaysian Journal of Microbiology*, *4*(2), 26–34.

Tope, A. K. (2013). Microbiological and nutrient studies of fermented cooked lima bean (Phaseolus lunatus) seeds, *Intern J of Advanced Research*, *2*(2), 94–101.

Tope, A. K. (2014). Effect of fermentation on nutrient composition and anti-nutrient contents of ground Lima bean seeds fermented with Aspergillus fumigatus , Rhizopus stolonifer and Saccharomyces cerevisiae Materials and Methods ., *Intern J of Advanced Research*, *2*(7), 1208–1214.

Udensi, E. A., Arisa, N. U., & Ikpa, E. (2010). Effects of soaking and boiling and autoclaving on the nutritional quality of Mucuna flagellipes (“ukpo”). *African Journal of Biochemistry Research*, *4*(2), 47–50.

Ugen, M., Musoni, A., Cheminingwa, G., Kimani, P., & Mucharo, M. (2009). Utilization of Common Bean for Improved Health and Nutrition in Eastern and Central Africa. *Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA)*, 1–11.

Vatansever, S., Vegi, A., Garden-robinson, J., & Iii, C. A. H. (2017). The Effect of

Fermentation on the Physicochemical Characteristics of Dry-Salted Vegetables, *6*(5), 32–40.

Winham, D. M., & Hutchins, A. M. (2011). Perceptions of flatulence from bean consumption among adults in 3 feeding studies, *Nutrition journal*, *10*(1), 128.

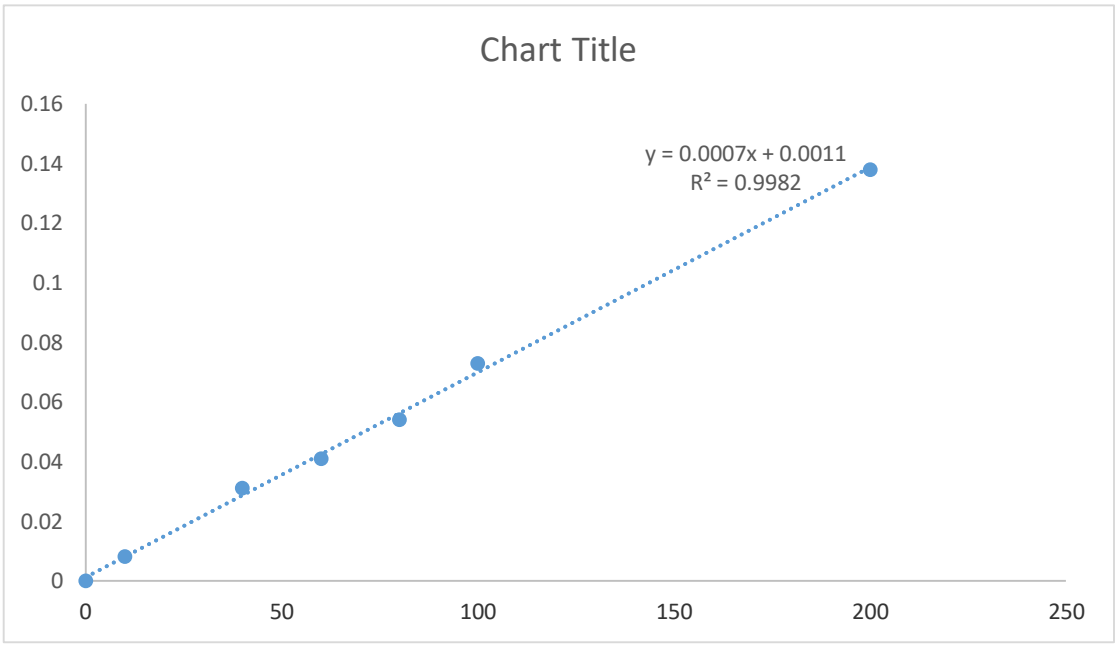
world bank. (2018). Poverty Incidence in Kenya Declined Significantly, but Unlikely to be Eradicated by 2030. Retrieved from, https://www.worldbank.org/en/country/ kenya/publication/kenya-economic-update-poverty-incidence-in-kenya-declined-significantly-but-unlikely-to-be-eradicated-by-2030

World Cancer Research Fund International. (2014). *the Science on the Connection between nutrition and ncds dietary patterns*. Retrieved from www.wcrf.org/nouRishing

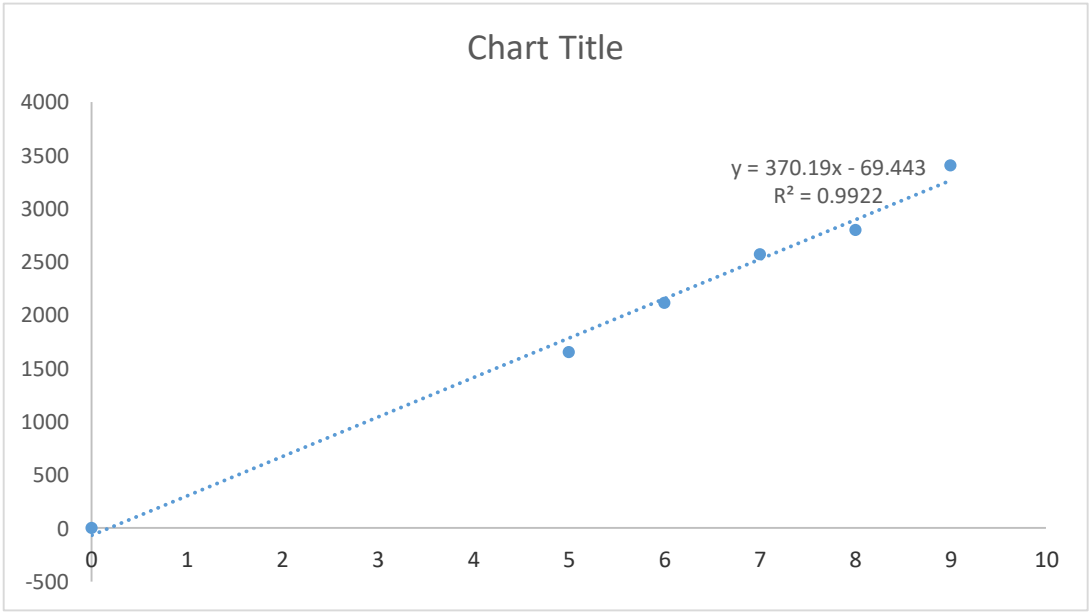
Zamudio, M., & Gonza, A. (2001). Lactobacillus plantarum phytase activity is due to non-speci ® c acid phosphatase, *International Food Research Journal*, *18*(4), 181–184.

APPENDICES

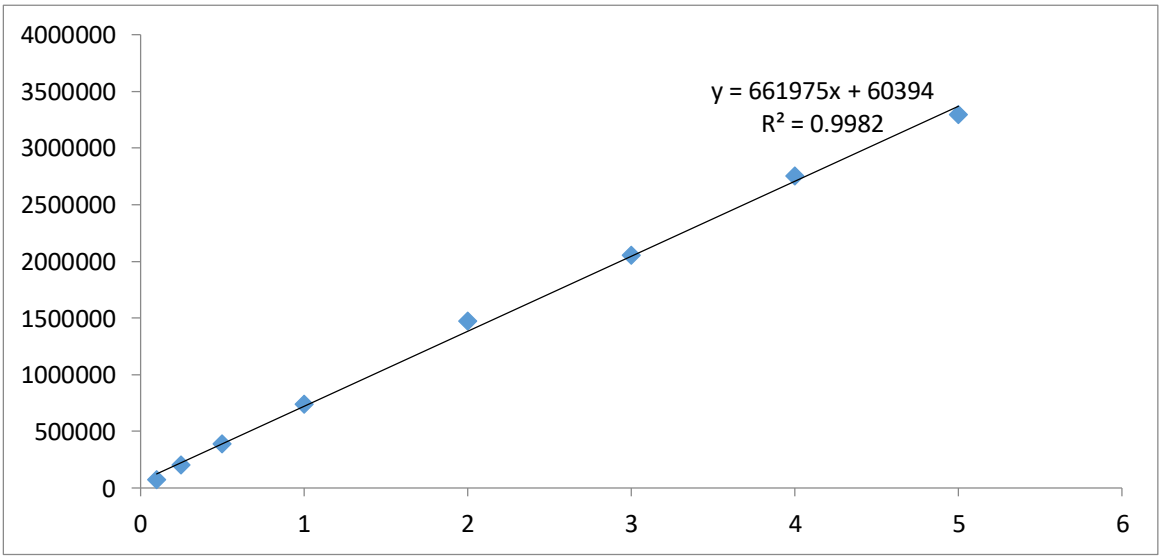
Appendix I: Tannins standard curve



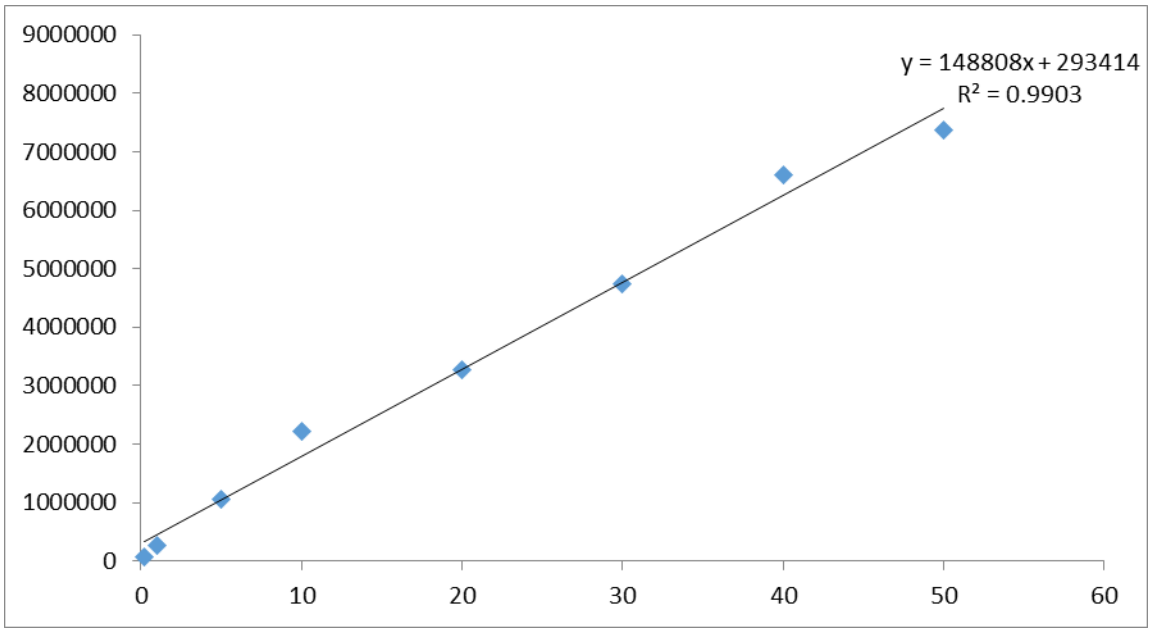
Appendix II: Phytic acid standard curve



Appendix III: Raffinose sugar standard curve



Appendix IV: Stachyose sugar standard curve



Appendix V: ANOVA table for raw, soaked and boiled red haricot bean

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Crude fat	Between Groups	.008	2	.004	.458	.653
	Within Groups	.053	6	.009		
	Total	.061	8			
Crude fiber	Between Groups	2.654	2	1.327	66.269	.000
	Within Groups	.120	6	.020		
	Total	2.774	8			
Ash	Between Groups	.345	2	.172	1.515	.293
	Within Groups	.683	6	.114		
	Total	1.028	8			
Proteins	Between Groups	2.078	2	1.039	18.267	.003
	Within Groups	.341	6	.057		
	Total	2.420	8			
Carbohydrates	Between Groups	14.931	2	7.466	29.380	.001
	Within Groups	1.525	6	.254		
	Total	16.456	8			
Phytates	Between Groups	30869.292	2	15434.646	42.756	.000
	Within Groups	2165.946	6	360.991		
	Total	33035.239	8			
Tannins	Between Groups	52.025	2	26.013	60.270	.000
	Within Groups	2.590	6	.432		
	Total	54.615	8			
Raffinose	Between Groups	782.205	2	391.103	30.688	.001
	Within Groups	76.467	6	12.745		
	Total	858.672	8			
Stachyose	Between Groups	293004.737	2	146502.368	31.219	.001
	Within Groups	28156.242	6	4692.707		
	Total	321160.978	8			

Appendix VI: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on crude fat content.

Tests of Between-Subjects Effects

Dependent Variable: Crude fat

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.026[a]	31	.033	1.061	.410
Intercept	215.353	1	215.353	6906.169	.000
Pre-treatment	.098	1	.098	3.144	.081
Fermentation solution	.010	1	.010	.337	.564
Fermentation type	1.430E-5	1	1.430E-5	.000	.983
Fermentation time	.120	3	.040	1.287	.286
Pre-treatment * Fermentation solution	.001	1	.001	.039	.844
Pre-treatment * fermentation type	.003	1	.003	.096	.758
Pre-treatment * fermentation time	.332	3	.111	3.551	.019
Fermentation solution* fermentation type	.004	1	.004	.129	.720
Fermentation solution* fermentation time	.037	3	.012	.395	.757
Fermentation type * fermentation time	.112	3	.037	1.194	.319
Pre-treatment * Fermentation solution* fermentation type	.003	1	.003	.080	.778
Pre-treatment * fermentation solution* fermentation time	.022	3	.007	.236	.871
Pre-treatment * fermentation type * fermentation time	.174	3	.058	1.862	.145
Fermentation solution* fermentation type* fermentation time	.021	3	.007	.228	.877
Pre-treatment * fermentation solution* fermentation type* fermentation time	.088	3	.029	.938	.428
Error	1.996	64	.031		
Total	218.374	96			
Corrected Total	3.022	95			

a. R Squared = .340 (Adjusted R Squared = .020)

Appendix VII: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on crude fiber content

Tests of Between-Subjects Effects

Dependent Variable: Crude fiber

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	35.574[a]	31	1.148	22.341	.000
Intercept	1651.993	1	1651.993	32161.389	.000
Pre-treatment	10.157	1	10.157	197.747	.000
Fermentation solution	3.029	1	3.029	58.962	.000
Fermentation type	5.131	1	5.131	99.900	.000
Fermentation time	6.496	3	2.165	42.155	.000
Pre-treatment * Fermentation solution	.949	1	.949	18.473	.000
Pre-treatment * fermentation type	.221	1	.221	4.303	.042
pre-treatment * fermentation time	3.803	3	1.268	24.679	.000
Fermentation solution* fermentation type	.028	1	.028	.555	.459
Fermentation solution* fermentation time	1.223	3	.408	7.938	.000
Fermentation type * fermentation time	3.088	3	1.029	20.042	.000
Pre-treatment * Fermentation solution* fermentation type	.181	1	.181	3.527	.065
Pre-treatment * fermentation solution* fermentation time	.439	3	.146	2.850	.044
Pre-treatment * fermentation type * fermentation time	.389	3	.130	2.527	.065
Fermentation solution* fermentation type* fermentation time	.111	3	.037	.720	.544
Pre-treatment * fermentation solution* fermentation type* fermentation time	.327	3	.109	2.122	.106
Error	3.287	64	.051		
Total	1690.855	96			
Corrected Total	38.862	95			

a. R Squared = .915 (Adjusted R Squared = .874)

Appendix VIII: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on ash content

Tests of Between-Subjects Effects

Dependent Variable: Ash

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	38.484[a]	31	1.241	19.277	.000
Intercept	1305.280	1	1305.280	20268.412	.000
Pre-treatment	.197	1	.197	3.052	.085
Fermentation solution	.389	1	.389	6.038	.017
Fermentation type	.364	1	.364	5.660	.020
Fermentation time	35.040	3	11.680	181.369	.000
Pre-treatment * Fermentation solution	.046	1	.046	.720	.399
Pre-treatment * fermentation type	.047	1	.047	.725	.398
pre-treatment * fermentation time	.146	3	.049	.757	.522
Fermentation solution* fermentation type	.003	1	.003	.042	.838
Fermentation solution* fermentation time	.287	3	.096	1.486	.227
Fermentation type * fermentation time	1.666	3	.555	8.622	.000
Pre-treatment * Fermentation solution* fermentation type	.005	1	.005	.072	.789
Pre-treatment * fermentation solution* fermentation time	.099	3	.033	.510	.677
Pre-treatment * fermentation type * fermentation time	.089	3	.030	.461	.711
Fermentation solution* fermentation type* fermentation time	.101	3	.034	.523	.668
Pre-treatment * fermentation solution* fermentation type* fermentation time	.006	3	.002	.029	.993
Error	4.122	64	.064		
Total	1347.885	96			
Corrected Total	42.606	95			

a. R Squared = .903 (Adjusted R Squared = .856)

Appendix IX: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on protein content

Tests of Between-Subjects Effects

Dependent Variable: Proteins

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20.990[a]	31	.677	13.546	.000
Intercept	30812.582	1	30812.582	616461.846	.000
Pre-treatment	.567	1	.567	11.337	.001
Fermentation solution	.007	1	.007	.147	.702
Fermentation type	5.551	1	5.551	111.049	.000
Fermentation time	2.628	3	.876	17.525	.000
Pre-treatment * Fermentation solution	.041	1	.041	.816	.370
Pre-treatment * fermentation type	5.423	1	5.423	108.500	.000
pre-treatment * fermentation time	2.297	3	.766	15.318	.000
Fermentation solution* fermentation type	.062	1	.062	1.231	.271
Fermentation solution* fermentation time	.254	3	.085	1.692	.178
Fermentation type * fermentation time	2.054	3	.685	13.699	.000
Pre-treatment * Fermentation solution* fermentation type	.005	1	.005	.105	.747
Pre-treatment * fermentation solution* fermentation time	.022	3	.007	.147	.931
Pre-treatment * fermentation type * fermentation time	1.834	3	.611	12.229	.000
Fermentation solution* fermentation type* fermentation time	.067	3	.022	.450	.718
Pre-treatment * fermentation solution* fermentation type* fermentation time	.178	3	.059	1.190	.321
Error	3.199	64	.050		
Total	30836.770	96			
Corrected Total	24.188	95			

a. R Squared = .868 (Adjusted R Squared = .804)

Appendix X: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on carbohydrate content

Tests of Between-Subjects Effects

Dependent Variable: Carbohydrates

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	192.864[a]	31	6.221	33.123	.000
Intercept	403292.469	1	403292.469	2147154.553	.000
Pre-treatment	17.126	1	17.126	91.182	.000
Fermentation solution	1.740	1	1.740	9.264	.003
Fermentation type	65.824	1	65.824	350.449	.000
Fermentation time	35.984	3	11.995	63.860	.000
Pre-treatment * Fermentation solution	1.361	1	1.361	7.248	.009
Pre-treatment * fermentation type	9.882	1	9.882	52.614	.000
pre-treatment * fermentation time	9.969	3	3.323	17.692	.000
Fermentation solution* fermentation type	3.202	1	3.202	17.050	.000
Fermentation solution* fermentation time	1.269	3	.423	2.251	.091
Fermentation type * fermentation time	24.311	3	8.104	43.145	.000
Pre-treatment * Fermentation solution* fermentation type	1.093	1	1.093	5.819	.019
Pre-treatment * fermentation solution* fermentation time	1.125	3	.375	1.996	.123
Pre-treatment * fermentation type * fermentation time	5.410	3	1.803	9.601	.000
Fermentation solution* fermentation type* fermentation time	6.289	3	2.096	11.161	.000
Pre-treatment * fermentation solution* fermentation type* fermentation time	8.279	3	2.760	14.692	.000
Error	12.021	64	.188		
Total	403497.354	96			
Corrected Total	204.885	95			

a. R Squared = .941 (Adjusted R Squared = .913)

Appendix XI: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on Tannin content

Tests of Between-Subjects Effects

Dependent Variable: Tannins

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7227.359[a]	31	233.141	177.900	.000
Intercept	31886.402	1	31886.402	24331.138	.000
Pretreatment	39.656	1	39.656	30.260	.000
Fermentation solution	140.471	1	140.471	107.187	.000
Fermentation type	16.210	1	16.210	12.369	.001
Fermentation time	6192.112	3	2064.037	1574.978	.000
Pretreatment * fermentation solution	.239	1	.239	.182	.671
Pretreatment * fermentation type	.001	1	.001	.001	.979
Pretreatment * Fermentation time	76.081	3	25.360	19.351	.000
Fermentation solution * fermentation type	118.182	1	118.182	90.180	.000
Fermentation solution * fermentation time	299.664	3	99.888	76.220	.000
Fermentation type * fermentation time	31.918	3	10.639	8.118	.000
Pretreatment * fermentation solution * fermentation type	2.770	1	2.770	2.114	.151
Pretreatment * fermentation solution * fermentation time	46.438	3	15.479	11.812	.000
Pretreatment * fermentation type * fermentation time	13.918	3	4.639	3.540	.019
Fermentation solution * fermentation type * fermentation time	235.478	3	78.493	59.894	.000
Pretreatment * fermentation solution * fermentation type * fermentation time	14.222	3	4.741	3.617	.018
Error	83.873	64	1.311		
Total	39197.635	96			
Corrected Total	7311.233	95			

a. R Squared = .989 (Adjusted R Squared = .983)

Appendix XII: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on phytic acid content

Tests of Between-Subjects Effects

Dependent Variable: Phytic acid

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	898623.791[a]	31	28987.864	129.199	.000
Intercept	6036854.639	1	6036854.639	26906.215	.000
Pre-treatment	37182.859	1	37182.859	165.724	.000
Fermentation solution	45174.437	1	45174.437	201.342	.000
Fermentation type	15935.952	1	15935.952	71.026	.000
Fermentation time	677228.315	3	225742.772	1006.134	.000
Pre-treatment * Fermentation solution	1.435	1	1.435	.006	.937
Pre-treatment * fermentation type	3160.323	1	3160.323	14.086	.000
Pre-treatment * fermentation time	73360.436	3	24453.479	108.989	.000
Fermentation solution* fermentation type	3939.948	1	3939.948	17.560	.000
Fermentation solution* fermentation time	18788.263	3	6262.754	27.913	.000
Fermentation type * fermentation time	7282.125	3	2427.375	10.819	.000
Pre-treatment * Fermentation solution* fermentation type	74.489	1	74.489	.332	.567
Pre-treatment * fermentation solution* fermentation time	1398.527	3	466.176	2.078	.112
Pre-treatment * fermentation type * fermentation time	8206.476	3	2735.492	12.192	.000
Fermentation solution* fermentation type* fermentation time	6520.420	3	2173.473	9.687	.000
Pre-treatment * fermentation solution* fermentation type* fermentation time	369.787	3	123.262	.549	.650
Error	14359.459	64	224.367		
Total	6949837.889	96			
Corrected Total	912983.251	95			

a. R Squared = .984 (Adjusted R Squared = .977)

Appendix XIII: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on raffinose concentration

Tests of Between-Subjects Effects

Dependent Variable: Raffinose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37001.102[a]	31	1193.584	70.278	.000
Intercept	127816.948	1	127816.948	7525.785	.000
Pre-treatment	16613.652	1	16613.652	978.202	.000
Fermentation solution	638.718	1	638.718	37.607	.000
Fermentation type	109.597	1	109.597	6.453	.014
Fermentation time	12574.440	3	4191.480	246.792	.000
Pre-treatment * Fermentation solution	156.657	1	156.657	9.224	.003
Pre-treatment * fermentation type	764.691	1	764.691	45.025	.000
pre-treatment * fermentation time	3444.777	3	1148.259	67.609	.000
Fermentation solution* fermentation type	65.111	1	65.111	3.834	.055
Fermentation solution* fermentation time	598.644	3	199.548	11.749	.000
Fermentation type * fermentation time	764.646	3	254.882	15.007	.000
Pre-treatment * Fermentation solution* fermentation type	19.505	1	19.505	1.148	.288
Pre-treatment * fermentation solution* fermentation time	206.945	3	68.982	4.062	.011
Pre-treatment * fermentation type * fermentation time	839.199	3	279.733	16.471	.000
Fermentation solution* fermentation type* fermentation time	117.988	3	39.329	2.316	.084
Pre-treatment * fermentation solution* fermentation type* fermentation time	86.532	3	28.844	1.698	.176
Error	1086.968	64	16.984		
Total	165905.018	96			
Corrected Total	38088.070	95			

a. R Squared = .971 (Adjusted R Squared = .958)

Appendix XIV: ANOVA results for the main effect of pre-treatment, fermentation solution, fermentation type and fermentation time and their Interaction effect on stachyose concentration

Tests of Between-Subjects Effects

Dependent Variable: Stachyose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15468583.790[a]	31	498986.574	40.609	.000
Intercept	36023060.519	1	36023060.519	2931.632	.000
Pre-treatment	5291288.647	1	5291288.647	430.616	.000
Fermentation solution	622090.067	1	622090.067	50.627	.000
Fermentation type	42469.780	1	42469.780	3.456	.068
Fermentation time	8020042.881	3	2673347.627	217.563	.000
Pre-treatment * Fermentation solution	142136.772	1	142136.772	11.567	.001
Pre-treatment * fermentation type	89629.804	1	89629.804	7.294	.009
Pre-treatment * fermentation time	549955.450	3	183318.483	14.919	.000
Fermentation solution* fermentation type	4953.343	1	4953.343	.403	.528
Fermentation solution* fermentation time	227383.868	3	75794.623	6.168	.001
Fermentation type * fermentation time	25500.819	3	8500.273	.692	.560
Pre-treatment * Fermentation solution* fermentation type	10780.032	1	10780.032	.877	.352
Pre-treatment * fermentation solution* fermentation time	290176.674	3	96725.558	7.872	.000
Pre-treatment * fermentation type * fermentation time	125047.757	3	41682.586	3.392	.023
Fermentation solution* fermentation type* fermentation time	6844.069	3	2281.356	.186	.906
Pre-treatment * fermentation solution* fermentation type* fermentation time	20283.826	3	6761.275	.550	.650
Error	786413.788	64	12287.715		
Total	52278058.098	96			
Corrected Total	16254997.579	95			

a. R Squared = .952 (Adjusted R Squared = .928)