

**CHEMICAL CHANGES DURING THERMAL PROCESSING OF
UNFERMENTED AND FERMENTED RED KIDNEY BEANS
(*PHASEOLUS VULGARIS*) AND EFFECTS ON *IN VITRO* PROTEIN
DIGESTIBILITY.**

BY

**PanmunFilibus WULAM, B.Sc BIOCHEMISTRY (JOS)
2009.M.Sc/SCIE/46287/2012-2013**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF
POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA.**

**IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF
SCIENCE IN NUTRITION**

**DEPARTMENT OF BIOCHEMISTRY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA.**

NOVEMBER, 2015

ABSTRACT

Many developing countries in the world are living in abject poverty, a constraint to abundant and commercial food production. Insufficient food production is a major cause of malnutrition. The need to source for affordable, high quality nutritious food becomes imperative. Red kidney bean is not widely consumed in Nigeria due to its geographical distribution and longer period of processing. This study was aimed at evaluating the effect of thermal processing on the chemical contents of unfermented and fermented red kidney beans (*Phaseolus vulgaris*) and the effects of the resulting changes on the *in vitro* protein digestibility. Unfermented and fermented *P. vulgaris* were boiled using ordinary cooking pot and a pressure pot and the chemical contents were evaluated by standard methods. *In vitro* protein digestibility was carried out by pepsin digestion. Fermentation significantly ($p < 0.05$) decrease the traditional cooking time in the ordinary cooking pot. The protein content of the fermented sample was significantly ($p < 0.05$) increased. The *in vitro* protein digestibility value was significantly ($p < 0.05$) increased by more than 30% with greater increase evident in fermented samples. Sulphur containing amino acids, methionine and cysteine were the limiting amino acids but their contents appreciated by 6.64% and 10.92% respectively after fermentation. Total ash, crude fibre, fat and carbohydrate contents of *P. vulgaris* were all significantly ($p < 0.05$) affected during the open fermentation and cooking of unfermented beans. During the different processing methods, , potassium, calcium, iron and zinc all significantly ($p < 0.05$) decreased. Phytate, alkaloids, oxalates, cyanides and tannins contents of *P. vulgaris* significantly ($p < 0.05$) decreased most in boiled fermented samples compared with the other processing methods. There was overall improvement in the *In vitro* protein digestibility, reduction of cooking time and antinutritional factors when *P. vulgaris* was fermented and cooked. This justifies the fact that combining both fermentation and cooking

results in the overall improvement in the nutritional value of *P. vulgaris* as against cooking without fermentation.

CHAPTER ONE

1.0 INTRODUCTION

Red kidney beans are so named because of their shape which is very similar to that of the human kidney. They have an earthy flavour and solid texture. Kidney beans and other beans such as pinto beans, navy beans and black beans are known scientifically as *Phaseolus vulgaris* (Debouck *et al.*, 1993). They are referred to as "common beans" owing to the fact that they are all derived from a common bean ancestor that originated in Peru. They spread throughout South and Central America as a result of migrating Indian traders who brought kidney beans with them from Peru. Red kidney bean was introduced into Europe in the 15th century by Spanish explorers returning from their voyages to the new World. Subsequently, Spanish and Portuguese traders introduced kidney beans into Africa and Asia (Wortmann, 2006). As beans are a very inexpensive form of good protein, they have become popular in many cultures throughout the world. Today, the largest commercial producers of dried common beans are India, China, Indonesia, Brazil and the United States (Debouck *et al.*, 1993).

In Nigeria, red kidney bean is widely cultivated in Plateau State. It is called 'kwakil longtong' in 'Mwaghavul' language. Just like other bean, it is also called 'wake' in 'Hausa' language, and in 'Igbo', it is called 'fiofio'.

Since this dark red bean holds its shape really well during cooking, they are a favourite bean to use in simmered dishes (Queiroz *et al.*, 2002). Kidney bean is an important

component of the daily diet, providing carbohydrates, protein, dietary fibre (DF) and many vitamins. Whole grains of kidney bean are also rich in vitamins, especially B vitamins, and good sources of minerals, particularly trace minerals (Rehman and Shah 2004; Yin *et al.*, 2008). Whole grains are also sources of many phytochemicals including phenolic compounds, antioxidants and gamma amino butyric acid (GABA) (Miller *et al.*, 2000).

Kidney bean is a very good source of cholesterol-lowering fibre as are most other beans (Bazzano *et al.*, 2003). In addition to lowering cholesterol, kidney beans' high fibre content prevents blood sugar levels from rising too rapidly after a meal making these beans an especially good choice for individuals with diabetes, insulin resistance or hyperglycaemia (McIntosh and Miller, 2001). When combined with whole grains such as rice, kidney beans provide virtually fat-free high quality protein. Kidney bean is an excellent source of the trace mineral, molybdenum, an integral component of the enzyme sulphite oxidase which is responsible for detoxifying sulphites. Sulphites are a type of preservative commonly added to prepared foods like salad bars. Persons who are sensitive to sulphites in these foods may experience rapid heartbeat, headache or disorientation if sulphites are unwittingly consumed. For people who have ever reacted to sulphites, it may be because their molybdenum stores are insufficient to detoxify them (Ensminger *et al.*, 1986).

Generally, legumes have been reported to have low nutritive value due to low amounts of sulphur-containing amino acids, low protein digestibility and presence of anti-nutritional factors. Cooking is usually done before the use of legumes in a human diet. This improves the protein quality by destruction or inactivation of the heat-labile anti-nutritional factors

(Chau *et al.*, 1997; Wang *et al.*, 1997; Vijayakumari *et al.*, 1998, Loggerenberg, 2004). However, cooking causes considerable losses in soluble solids especially vitamins and minerals (Barampama and Simard, 1994). Increasing the time and temperature of processing has been reported to reduce the nutritive value and available lysine of legumes (Kon and Sanshuck, 1981).

Soaking, cooking, germination, fermentation or irradiation treatments may be used to improve protein nutritional value (Jood *et al.*, 1985; Sathe and Salunkhe, 1989; Guzman-Maldonado and Paredes-Lopez, 1998; Soetan and Oyewole, 2009). Barampama and Simard (1994) studied the effect of processing on oligosaccharides in legumes and found that soaking, cooking, germination or fermentation can be used to reduce the levels of anti-nutritional factors.

The aim of this research therefore, was to evaluate the effect of thermal processing on the chemical compositions of fermented and unfermented red kidney beans (*Phaseolus vulgaris*) as well as the effects of these changes on *in vitro* protein digestibility.

1.1 Statement of Research Problems

Many developing countries in the world are living in abject poverty, a constraint to abundant and commercial food production. Insufficient food production is a major cause of malnutrition. The need to source for affordable, high quality nutritious food becomes imperative.

In Mangu local government area and other local governments of Plateau state, Nigeria where red kidney bean is a staple food, women and their children labouriously carry wood as domestic cooking fuel for long distances, frequently in mountainous terrain, to their villages or rural homes. This is due to lack of sufficient modern cooking techniques such as pressure pot compared to developed countries that have access to them. Traditional cooking of dry red kidney bean in these areas (villages) involves excessive expenditure of time and fuel. The development of appropriate preparation technologies for use at the household and village-level would facilitate processing and dietary availability of red kidney beans and other legumes.

Beans are important sources of macronutrient, micronutrient and antioxidant compound with a great potential for human and animal nutrition (Gloria *et al.*, 2003). Consumption of red kidney beans is however, limited by the lengthy cooking period (about three times greater than for cowpea) and presence of several antinutritional factors, adversely affecting its consumption and bioavailability of nutrients (Bressani, 2003; Pusztai *et al.*, 2004). This necessitates the need to explore different processing methods with a view to increasing its nutritional potentials through the reduction of its cooking time and antinutritional factors.

1.2 Justification

Fermentation is one of the oldest and most economical methods of producing and preserving food. In addition, fermentation provides a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive

value and appearance of the food, to reduce the energy required for cooking and to make a safer product (Hiran *et al.*, 2011).

Fermentation of food has been found to reduce the risk of having food intoxication arising from toxicants found in food. In addition to this, several experiments have demonstrated that fermentation of legumes enhances their nutritive value (Zamora and Fields, 1979; Akpanpunamand Achinewha, 1985), reduces some anti nutritional endogenous compound such as phytic acids (Mahajan and Chauhan, 1988; Kozłowska *et al.*, 1996; Soetan and Oyewole, 2009) and exert beneficial effect on protein digestibility and biological value of legumes (Lopez *et al.*, 1983).

The process of heating a food induces physical changes and chemical reactions such as starch gelatinisation, protein denaturation and browning, which in turn affect the sensory characteristics such as colour, flavour and texture, either advantageously or adversely (Brennan, 2006).

The development of appropriate preparation technologies for use at the household and village-level would facilitate processing and dietary availability of beans and other legumes. Valuable time could thus be devoted to more effective childcare or additional income-generating activities. The integrative need for research that transcends traditional bean production limitations (germplasm, adaptation, yield and disease) and more fully addresses the social and cultural implications of improved food utilization has been imbedded within the USAID (Graham *et al.*, 2003).

1.3 General Objective

The general aim of this study, is to evaluate the effect of thermal processing on the chemical compositions of fermented and unfermented red kidney beans (*Phaseolus vulgaris*) and the effects of the changes on *in vitro* protein digestibility.

1.4 Specific objectives

The objectives of this study are to determine the

- Chemical contents of unfermented and fermented *P. vulgaris*.
- Amino acid contents of fermented and unfermented *P. vulgaris*.
- Effect of cooking time on the chemical contents of fermented and unfermented *P. vulgaris*.
- Effects of cooking time and the associated changes on the *in vitro* protein digestibility of *P. vulgaris*.

1.5 Hypothesis

H₀: Thermal processing and fermentation do not have effects on the chemical contents and *in vitro* the protein digestibility of *Phaseolus vulgaris*

H_A: Thermal processing and fermentation have effects on the chemical contents and the *in vitro* protein digestibility of *Phaseolus vulgaris*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Red Kidney Beans (*Phaseolus vulgaris*)

2.1.1 History of kidney beans

Dry beans (*Phaseolus spp. L.*) are the most important grain legumes for human consumption. Dry beans have been cultivated for thousands of years and have been reported to play an important role in the traditional diets of many regions throughout the world (Zamindar *et al.*, 2011). Beans are less significant in western diets compared to most of the developing countries. The daily per capita consumption of all bean products is 9g in the United States compared to about 110 g in Asia (Boateng *et al.*, 2008).

Phaseolus vulgaris originated from Central and South America, where it was cultivated as early as 6000 BC in Peru and 5000 BC in Mexico (Wortmann, 2006). It was introduced to the old World by the Spaniards and Portuguese. It is now widespread and cultivated as a major food crop in many tropical, subtropical and temperate areas of the Americas, Europe, Africa and Asia (Wortmann, 2006).

Kidney beans gained wide acceptance during the history of Americas before the appearance of significant European influences on the American continents (Pre-Colonianian period). Early chroniclers indicated that great importance was given to this species in the Aztec and

Incan empires. The people of Axocopan used dry beans to pay tributes at the early colonial period in North America (Wu, 2002).

2.1.2 Geographical distribution of *Phaseolus vulgaris*

The common bean is a warm season growing legume that does better under subtropical and temperate conditions. It can be found in tropical areas but does not do well under very wet conditions that cause fungal attacks and flower drop, and it could be advantageously replaced by cowpea (*Vigna unguiculata*) in such conditions (Ecoport, 2013). Common bean sows from sea level up to 2200m to 3000m altitude in places where annual rainfall is between 300mm and 4300 mm with optimum between 500 and 1500 mm, and where average temperatures range between 15°C and 23°C. It can grow under higher temperatures (35°C) but this may hamper seed production. It has slight frost tolerance but growth stops below 10°C and frost hinders yield at various stages of growth (Smoliak *et al.*, 1990; Wortmann, 2006). Dry weather during the maturing stage is suitable for seed preservation. The common bean grows well on a large variety of soils with pH ranging from 4 to 9. It does better on well-drained, sandy loam, silt loam or clay loam soils, rich in organic content (Ecoport, 2013). Generally, it cannot withstand waterlogging though some cultivars do well in standing water (Ecoport, 2013). It is sensitive to aluminium (Al), boron (B), manganese (Mn) and high levels of sodium (Na). Deficiencies in zinc (Zn), magnesium (Mg)

and molybdenum(Mo) may arise in calcareous soils and sandy soils respectively (Wortmann, 2006; Ecoport, 2013).

Worldwide statistics on common beans are difficult to collect, as various *Phaseolus* and *Vigna* species are often lumped together. According to FAO, dry beans production (theoretically only *Phaseolus* species) was about 23 million tones in 2012, cultivated on 29 million ha. Myanmar, India, Brazil, China, USA, Mexico and Tanzania represented 2/3 of the world production of dry beans while China was the main producer of fresh beans (*Phaseolus* and *Vigna* species: 17 million tones in 2011, 77% of the world production) (FAO, 2013). According to other sources, 30% of common bean production comes from South America. Common bean is less known in Asia where other grain legumes are preferred (Ecoport, 2013). However, production in China is important: estimated acreage in the 2010s was about 0.6 million ha (Cheng and Tian, 2011).

2.1.3 Botanical study of *Phaseolus vulgaris*

The bean plant belong to the genus *Vigna savi*, (Willis, 1988) and the family *Leguminosae-papilionoidae* and the tribe *Phaseoleae* which is made up of about 80-100 species that are tropical especially in Africa and Asia (Mbagwu and Edeoga, 2006).

Common beans usually refer to the food legumes of the genus *Phaseolae*, family *leguminosae*, subfamily *papiliomoideae*, tribe *Phaseolae* and subtribe *Phaseolinae*. The

genus *Phaseolae* contains some 50 wild growing species distributed only in the Americas (Asian *Phaseolus* has been reclassified as *Vigna*) (Gepts *et al.*, 2008).

The genus also contains five domesticated species in decreasing order of preference, common beans (*Phaseolus vulgaris* L) Lima bean (*Phaseolus lunatus* L), runner bean (*Phaseolus coccineus* L.), tepary bean (*P. acutifolius* A. Gray), and year bean (*P. polyanthus* Greenman), with distinct adaptations and reproductive systems: moist and temperate, predominantly self pollinated; warm and humid, predominantly self pollinated; hot and dry, cleistogamous and cool and humid, out crossing, respectively. Lima bean is phylogenetically more distant from the other domesticated species which are sibling species and constitute a syngameon. The principal species economically and scientifically is common bean and originated in Latin America where its wild progenitor (*P. vulgaris* var. *inexicanus* and var. *aborigineus*) has a wide distribution ranging from northern Mexico to north-western Argentina (Gepts *et al.*, 2008). All species of the genus are diploid and most have 22 chromosomes ($2n = 2 \times 22$) and a few species show an aneuploid reduction to 20 chromosomes. The genome of common bean is one of the smallest in the legume family at 625 Mbp per haploid genome (Gepts *et al.*, 2008).

2.1.4 Chemical composition of *Phaseolus vulgaris*

Beans are excellent sources of proteins (20-30%) and carbohydrates (50-60%) and fairly good sources of minerals and vitamins (Rehman and Shah 2004; Yin *et al.*, 2008). Beans contain two or three times more proteins than cereals and offer a more practical way of eradicating protein malnutrition than cereal based diets (Carmona-Garcia *et al.*, 2007).

Legumes also contain higher amount of resistant starch in comparison to cereals and tubers (Yadav *et al.*, 2010). Resistant starch is important due to its various beneficial health properties mostly mediated by short chain fatty acids produced during its fermentation in the large intestine as they result in decrease in intestinal pH (Fernandes *et al.*, 2010). The merit of dry bean is thus attributed to its high caloric value and protein content. Low concentrations of phytates and phenolic compounds (which are present in beans) can be protective against cancer and cardiovascular diseases. (Ramirez-Cardenas *et al.* 2010).

Beans are the rich source of B vitamins, folate, riboflavin and valuable mineral substances like potassium, calcium, magnesium, phosphorus and iron salts (Souci *et al.*, 2000). Thus, they are important components of a healthy diet. However, their nutritional quality is indirectly impacted by the presence of heat labile and heat-stable antinutritional factors (ANF) that exhibit undesirable physiological effects (Pusztai *et al.*, 2004).

2.1.5 Utilization of *Phaseolus vulgaris*

Red kidney beans (*Phaseolus vulgaris* L.), play an important role in human diet in Africa, Latin-America and Asian countries, improving the nutritional status of many low income populations (Milan-Carrillo *et al.*, 2007). Kidney beans are consumed as cooked dried beans or canned beans (cooked, baked or refried). Also they are used in the fruit and vegetable processing industry in the production of frozen or canned food. Beans are highly nutritious food able to compete with meat. In the western hemisphere, kidney beans are used in salads, soups and other food products (Kahlon *et al.*, 2005).

Dry common bean (*Phaseolus vulgaris L*) is a legume widely consumed throughout the world and it is recognized as the major source of dietary protein in many Latin-American and African countries (Shellie-Dessert and Bliss, 1991). A large variability exists in common bean seeds, colour and size are, two important quality characteristics for the consumers. Seed size and weight depend on genetic variations, cultivar and environmental conditions (Gonzalez de Mejia *et al.*, 2005). The seed colour of beans is determined by the presence and concentration of flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) (Beninger and Hosfield, 2003; Apancio-Fernandez *et al.*, 2005). Recently, common bean is gaining increasing attention as a functional or nutraceutical food due to its rich variety of phytochemicals with potential health benefits such as fibre, polyphenolic compounds, lectins, unsaturated fatty acids, trypsin inhibitors, phytic acid, among others (Guzmán-Maldonado and Paredes-Lopez, 1998).

Numerous special and unique methods of preparation are established within a given region or culture; however, beans are commonly soaked and cooked in an open pot of water (steel or ceramic) over a low heat fire. This provides for a long-term slow (up to eight hours) cooking process and yields a palatable and nutritious product. The requirements for high quality water and sufficient wood fuel for cooking are major constraints. Several traditional methods of bean preparation such as germination and fermentation in African and Asian countries have found wide acceptability. These methods produce highly

specialized and culturally distinctive products and are recognized for improving beans' digestibility and reducing antinutritional factors. Reddy *et al.* (1982a) indicated that legume-based fermented foods are very popular in Southeast Asia, the Near East, and parts of Africa and examined the production of various legume-based fermented foods and critically assesses their nutritional quality. These products form an appreciable part of daily diets of people as a main source of protein, calories and certain vitamins.

Preparation of legume-based fermented foods has remained, to some extent an art, and their nutritional quality has been of interest to both professionals and laymen. The fermentation process aids in improvement of the organoleptic quality of legumes and enhances nutritional quality. Davila *et al.* (2003) reviewed the use of germination and fermentation to improve the nutritional value of legumes with particular reference to the generation of functional foods and functional ingredients. Germination and fermentation are presented as alternatives that are able to reduce or inactivate anti-nutritional factors in legumes, preserve and possibly enhance the content of isoflavones in legumes and improve the potential of legumes as functional foods and as ingredients for use in functional foods. The regions of highest bean consumption include all of Latin America, where legume consumption ranges from 1 kg/capita per year (Argentina) to 25 kg/capita per year (Nicaragua). Common beans dominate and account for 87% of the total legume product consumption (Leterme and Muñoz, 2002). Sub-Saharan Africa utilizes a wide range of dry beans and other legume crops (cowpea). These are typically water cooked and eaten as porridge. The subcontinent of India uses the greatest quantity and most diversity of legume-based foods. These are characteristically prepared and processed in very specialized recipes and formats (Khader

and Uebersax, 1989). Throughout Southeast Asia, consumption of legumes is moderate and a great variety of species are produced and used as mature seeds and immature vegetative pods. Sprouted grains are consumed fresh or dehulled and roasted or ground for use in soups or side dishes(Leterme and Muñoz, 2002).

Dry beans are important source of protein, dietary fiber, iron, complex carbohydrates, minerals, and vitamins for millions of people in the world. They are one of the basic food categories in the diet of the indigenous populations in South America, Asia and Eastern/Southern Africa. The per capita dry bean consumption has been increasing in the United States in the past 20 years. Factors contributing to this continuous trend in the dry bean market include the increasing awareness and changes in the traditional American diet, an increase in immigration of Hispanic population, and the increasing interests in ethnic foods featuring dry beans. According to the Continuing Survey of Food Intakes of Individuals, compiled by USDA's Agriculture Research Service, about 4% of the population consumes kidney bean on any given day, which is among the highest of any dry bean consumption (Lucier *et al.*, 2000; Belshe *et al.*, 2001).

About 80% of the total proteins found in dry beans are storage proteins and plays a functional role to the plant. These proteins supply the young seedling with nitrogenous compounds and amino acids. Dry beans are deficient in sulphur-containing amino acids such as methionine and cysteine and have small deficiencies in valine, leucine, isoleucine and threonine. All dry beans are good sources of lysine, indicating that dry beans could be added to lysine-deficient cereal products (Loggerenberg, 2004).

2.1.5.1 Dry edible beans as weaning foods

The long-standing nutritional challenge that positively affect an infant's transition from nursing to solid foods is complex and entails an array of socio-economic factors. The dynamic of the mother child relationship and the availability of appropriate foods are well documented in numerous cultures. The use of dry beans to enhance the protein and energy needs required for weaning periods has been extensively researched. Generally, digestibility and flatulence-producing components are important factors to consider when feeding legumes to children. Preliminary processing techniques include: (1) dehulling, (2) finegrinding, (3) roasting (4) germination and fermentation, and (5) prolonged cooking. These techniques have been found to increase digestibility, improve protein to energy ratio and reduce flatulence from beans. Further, high viscosity associated with bean pastes and gruels has been a deterrent to use in weaning foods. Pre-processing protocols (extractions, enzymatic digestions fermentation, and germination) have been proposed to reduce starch and oligosaccharide content. These procedures have generally reduced viscosity, enhanced overall acceptability and improved digestibility (Uebersax and Occena, 1997). Thus, pre-processing (in-home or centrally within the local marketplace) may enhance potential for bean-based weaning foods.

A number of traditional weaning food mixtures can be prepared using pre-heated legumes and cereals. These products must incorporate calculated ratios of selected ingredients to optimize the nutritive value (characteristically designed for improved amino acid profiles), enhance preparation time and possess stable shelf-life and are designed for economical

accessibility. Numerous studies have demonstrated the diversity of approaches taken to achieve suitable weaning food products. Drum-dried bean meals prepared from split beans, a low grade by-product of whole bean markets, demonstrated potential for pre-cooked, prolonged shelf-life weaning food formulations which can provide both protein and energy to the infants as well as offer preparation convenience (Occena *et al.*, 1997). Mbithi-Mwikya *et al.*,(2000) evaluated sprouted kidney beans (up to 96 hours at 30°C) for inclusion into a weaning food formulation. During the sprouting period, starch content decreased; reducing and non-reducing sugars increased; tannins, trypsin inhibitor and phytates decreased; and in-vitro digestibility increased. Mensa-Wilmont *et al.*,(2001) developed six cereal/legume mixtures with the aid of computer-assisted optimization software. Three processing schemes (roasting, amylase digestion and extrusion cooking) were employed. Nutrient composition indicated that these blends were nutritionally adequate as weaning foods. Ghanaian mothers of weaning children evaluated sensory attributes of the formulations and found the convenience of a weaning food made from local staples processed on village/market scale to be very attractive.

Mbithi-Mwikya *et al.*, (2000) studied the effects of sprouting, autoclaving and fermenting of kidney beans and finger millet during the processing of a weaning food. Sprouting resulted in a significant decrease in lysine in kidney beans. Autoclaving caused significant decreases in histidine, while fermentation significantly decreased phenylalanine and increased tryptophan in finger millet. Leucine to lysine ratio was significantly improved in finger millet by both sprouting and fermentation. Rodriguez-Burger *et al.*, (1998) developed a nutritious weaning food with the use of fermented black beans combined with

rice. Raw beans were coarsely ground, soaked, cooked, fermented (up to 25 hours) and then homogenized to obtain a supernatant and a precipitate. Cooking improved protein digestibility and decreased the levels of lectin and trypsin inhibitor. The oligosaccharide content was reduced by fermentation. The weaning food product prepared using dry weight ingredients (27% fermented dry beans / 73% cooked rice) had an in vitro protein digestibility of 86% and a very low content of oligosaccharides.

2.1.5.2 Utilization of Dry Beans as Processed Foods in Developed Regions and Diets

A comprehensive assessment of strategies and procedures used for processing dry beans is prerequisite to improved utilization of dry beans. Implementation of a given protocol can be maximized through an understanding of the physical and chemical components, the inherent constraints and diversified processing techniques available to develop economically viable alternative and innovative products (Uebersax *et al.*, 1991).

The typical means of presentation of bean-based products are sensitive to differences among various regions and countries. The appreciable differences in bean costs and availability may be attributed to significant differences in global supply chains. These differences may be particularly acute for specific commercial classes of beans. There is considerable opportunity to expand overall bean utilization by improving quality of products derived from non-traditional production areas and world trading partners such as China and Thailand. Canned products consistently dominate bean usage (based on individual frequencies of use data and total sales volume) compared with dry beans distributed in pre-packaged lots or bulk dispensing. Dry beans account for the

greatest volume of legumes used in Europe; however, much variance among beans' commercial classes is apparent by individual country. These differences are illustrated by predominant use of 'Navy beans' in the UK, preference for large white beans (Great Northern, White Kidney) in France and special use of coloured beans (Cranberry and Dark Red Kidney) in Italy. The per capita consumption of legume-based food products in the United States, Europe (encompassing the EU) and other industrialized economies has generally and consistently been substantially lower than that observed in other regions of the world. However, as an aggregate (with much internal variability) there has been an overall slight increase observed in recent years. Specific regional responses are noteworthy and illustrate this variability. Western European countries (e.g. Spain, France and the UK), account for about 60 % of the total bean consumption within the European Union (Schneider 2002).

Cooked bean consumption is recognized to be greatest in the southern and western areas of the United States. About 55% of black beans, one of the fastest growing classes in terms of per capita use, are consumed in the southern region of the country. Although people of Hispanic origin represented approximately 11% of the population, they account for 33% of all cooked dry edible bean product consumption. Relative to their share of the population, low-income consumers consume substantially more navy, lima, and pinto beans than those consumed by mid or high income groups (Lucier *et al.*, 2000).

2.1.6 Constraints limiting the utilization of red kidney beans

2.1.6.1 Protein quality

One of the primary constraints militating against beans is the protein quality. Bean protein as in the case of other legume proteins is deficient in sulphur containing amino acids with a level of 1.2g/100g and 1.8g/100g protein for methionine and cysteine respectively. The primary deficiency of methionine and cysteine in many instances is further complicated by a secondary deficiency of tryptophan (Evans and Bandier, 1967).

This makes beans protein inferior when compared to protein from animal source. However the protein quality can be improved by supplementation with methionine (Onayemi and Potter, 1976). Beans are very rich in lysine and the amino acid profiles compliment those of the cereal proteins to give a high protein quality (Bressani *et al.*, 1974).

2.1.6.2 *Physical constraints*

The processing of beans for preparation of bean products like ‘moi-moi’, bean cake (akara), bean soup (gbegiri) poses some problems. The seed coat has to be removed before it can be grounded into paste. This means steeping the beans seed in water to loosen the seed coat or devising other means for involving the coat. observation have shown that removing cowpea seed coat can be tedious and time consuming especially for those who use cowpea to prepare dishes on a large scale. For instance, it takes as long as 90 minutes soaking time to remove the seed coat of Sampea 6 (IAR Report, 1994). Therefore the ease with which the coat can be removed is an important factor.

Beans require some cooking before they are eaten. This thermal process provides tenderization of the cotyledon which increase product palatability, digestibility and

inactivates endogenous toxic factors that would markedly limit the final nutritional value (Sefa-Dede *et al.*, 1978; Uebersax and Ruengsakulrach, 1991). Investigation showed that cooking time which is long varies for different varieties. While Sampea 7 and sampea 4 cook under one hour, sampea 6 takes over one hour to cook. The cooking time is particularly important in these days of shortage and high cost of cooking fuel (IAR report, 1994).

Another physical constraint associate with beans consumption is the storage induced hard-to-cook defect. This term indicates a resistance of seeds to softening during cooking. This phenomenon is normally associated with certain legumes stored under high temperature and high humidity. The hard-to-cook seeds require longer cooking time than soft seeds and decrease the nutritional quality (Tuan and Phillips, 1991).

2.1.6.3 Antinutritional factors (ANFs)

The ANFs are structurally different compounds broadly divided into two categories: proteins (such as lectins and protease inhibitors) and others such as phytate, tannins or proanthocyanidins, oligosaccharides, saponins and alkaloids. In general raw beans contain far higher levels of ANFs than their processed forms hence processing is necessary before the incorporation of these grains into food or animal diets (Hajos and Osagie, 2004). Few studies about the industrial process of dehydration after soaking and cooking treatments have been carried out in order to investigate the nutritional improvement of beans (Martin-Cabrejas *et al.*, 2006).

Trypsin Inhibitors

It has been well understood that thermal inactivation of trypsin inhibitors is essential for use in animal and human food (Gomes *et al.*, 1979; Genovese and Lajolo, 1996). Birk (1996) discussed protein protease inhibitors as widely distributed in legumes. The Kunitz soybean trypsin inhibitor (STI) and the Bowman-Birk trypsin-chymotrypsin inhibitor (BBI) have been characterized. The STI has been responsible for induction of the pancreatic enlargement. The BBI trypsin-chymotrypsin inhibitors from soybeans and from chickpeas inhibit insect midgut proteases, supporting the hypothesis that proteinase inhibitors comprise the defense mechanism of the seed against insects. The findings on the involvement of proteinase inhibitors such as BBI in prevention of tumorigenesis suggest a possible positive contribution of the active inhibitors to the nutritional or health value of legume seeds.

Phytohaemagglutinins

An important anti-nutrient is phytohemagglutinin (PHA), a heat-labile lectin known to depress the nutritional quality of dry beans (Thompson *et al.*, 1986). Lajolo and Genovese (2002) studied the nutritional significance of lectins and enzyme inhibitors from legumes with reference to contents of enzyme inhibitors and lectins in legumes, nutritional and physiological effects, inactivation through processing, resistance to proteolysis, effects of chronic intake of low dietary levels, nutritional utilization of enzyme inhibitors and lectins and possible useful biological activities of these compounds. Coffey *et al.* (1985, 1992 and 1993) used two processes (1) extrusion and (2) cooking in a high pH medium to achieve the inactivation of PHA. Extrusion was relatively ineffective in reducing the activity

of PHA in whole red kidney or black beans. Extrusion was more effective but highly variable in reducing PHA activity of bean flours (25-80%). However, soaking and cooking beans at high pH was very effective, significantly reducing the activity of PHA and also reducing the time required to reach a palatable texture. Soaking and cooking dry beans at high pH also caused significant changes in the saline soluble protein extract as determined by gel electrophoresis. It was concluded that high pH cooking treatment could be useful in improving nutritional quality of dry beans.

Tannins

Tannins are ubiquitous in nature and although a lot of attention has been given to their study, the term “tannin” continues to be difficult to define precisely. Indeed, whereas related phenolic compounds such as simple phenolics, neolignans and flavonoids are characterized and classified according to their chemical structure, tannins are a diverse group of compounds that are related primarily in their ability to complex with proteins (Fahey & Jung, 1989). Thus, tannins are usually defined as water-soluble polyphenolic substances that have high molecular weight and that possess the ability to precipitate proteins. Tannins have diverse effects on biological systems because they are potential metal ion chelators, protein precipitating agents, and biological antioxidants. Because tannins can play such varied biological roles and because of the enormous structural variation among tannins, it has been difficult to develop models which allow accurate prediction of the effects of tannins in any system (Loggerenberg, 2004).

The high affinity of tannins for proteins lies in the formers' great number of phenolic groups. These provide many points at which bonding may occur with the carbonyl groups of peptides. The formation of such complexes is specific, both in terms of the tannin and protein involved, the degree of affinity between the participating molecules residing in the chemical characteristics of each (Rolando, 1999). With respect to tannins, the factors promoting the formation of complexes include their relatively high molecular weight and their great structural flexibility (Mueller-Harvey & McAllan, 1992). The proteins that show the most affinity for tannins are relatively large and hydrophobic, have an open, flexible structure and are rich in proline.

The complexes formed between tannins and proteins or other compounds are generally unstable. The bonds uniting them continually break and re-form (Mueller-Harvey & McAllan, 1992). The complexes that could come through four types of bond: (1) hydrogen bonds (reversible and dependent on pH) between the hydroxyl radicals of the phenolic groups and the oxygen of the amide groups in the peptide bonds of proteins, (2) by hydrophobic interactions (reversible and dependent of pH) between the aromatic ring of the phenolic compounds and the hydrophobic regions of the protein, (3) by ionic bonds (reversible) between the phenolate ion and the cationic site of the protein, and(4) by covalent bonding (irreversible) through the oxidation of polyphenols to quinones and theirsubsequent condensation with nucleophilic groups of the protein (Rolando, 1999). For a long time it was believed that the formation of tannin-protein complexes was owed mainly to hydrogen bonds. However, it is now known that hydrophobic interactions are important.

Phytic Acids

Phytic acid (myoinositol, 1, 2, 3, 4, 5, 6 hexa-dihydrogen phosphate) and phytate (salts of phytic acid) are widespread in plant seed grains (also including cereals), roots, tubers (Graf, 1989; Lasztity and Lasztity, 1990) and legumes (Reddy *et al.*, 1982b). Phytate accumulates in the seeds during the ripening period and is the main storage form of both phosphate and inositol in plant seeds and grains (Loewus, 2002).

The phytate molecule is negatively charged at physiological pH and is reported to bind with essential, nutritionally important divalent cations such as Fe^{2+} , Zn^{2+} , Mg^{2+} and Ca^{2+} etc., and forms insoluble complexes, thereby making minerals unavailable for absorption (Rimbach *et al.*, 1994). It also forms complexes with proteins and starch and inhibits their digestion (Oatway *et al.*, 2001).

The dephosphorylation of phytate is a prerequisite for improving nutritional value because removal of phosphate groups from the inositol ring decreases the mineral binding strength of phytate. These results increased bioavailability of essential dietary minerals (Sandberg *et al.*, 1999).

Various food processing and preparation techniques such as decortications, soaking, cooking, germination and fermentation are the major efforts made to reduce the amounts of phytate in foods (Elmaki *et al.*, 2007; Sangronis and Machado, 2007; Liang *et al.*, 2008;

Khatab and Arntfield, 2009; Kumar *et al.*, 2010 and Wanget *al.*, 2010). The most effective treatments are fermentation (Marfo *et al.*, 1990) and germination (Honke *et al.*, 1998)but their application remains limited because of the additional workload they imply or the particular organoleptic properties they induce. Sangronis and Machado (2007)evaluate the effect of germination on some nutrients as well as on some antinutritional factors of white beans (*Phaseolus vulgaris L.*), black beans (*Phaseolus vulgaris L.*) and pigeon beans (*Cajanus cajan L. Mill sp.*) and found that the reduction of phytic acid was more than 40% for the three grains germinated and these variations in the content of nutrients and antinutrients of the germinated grains are attributed to the joint effect of the germination and previous soaking. Shimelis and Rakshit (2007) also obtained a notable reduction (over 75%)in phytic acid in three kidney bean varieties after 4 days of germination. The purpose of this work was to investigate the effect of individual or combined processing methods on the reduction/elimination of phytic acid content in soybean, mung bean and kidney bean. This would help in determining simple and cost-effective processing options for developing countries in order to improve the nutritional value of such beans.

2.2 Thermal Processing

2.2.1 Meaning of thermal processing

Thermal processing involves heating food, either in a sealed container or by passing it through a heat exchanger followed by packaging. It is important to ensure that the food is

adequately heat treated and to reduce postprocessingcontamination (PPC). The food should then be cooled quickly and it may require refrigerated storage or be stable at ambient temperature. The heating process can be either batch or continuous. In all thermal processes, the aim is to heat and cool the product as quickly as possible. This has economic implications and may also lead to an improvement in quality. Heat or energy (J) is transferred from a high to a low temperature, the rate of heat transfer being proportional to the temperature difference. Therefore, high temperature driving forces will promote heat transfer. SI units for rate of heat transfer (J s^{-1} or W) are mainly used but Imperial units (BTU h^{-1}) may also be encountered. The heating medium is usually saturated steam or hot water. For temperatures above 100°C , steam and hot water are above atmospheric pressure. Cooling is achieved using either pipe borne water, chilled water, brine or glycol solution. Regeneration is used in continuous processes to further reduce energy utilisation (Brennan, 2006).

2.2.2 General principles of thermal processing

Thermal destruction of microorganisms is traditionally established to take place following a first order semi-logarithmic rate. Therefore, theoretically, a sterile product cannot be produced with certainty no matter how long is the process time. Targeting a product that is completely void of microorganisms would render the product unwholesome or inferior in quality. Industrially, thermal processes are designed by processing authorities to provide commercially sterile or shelf-stable products. Commercial sterility (as defined by the United States Food and Drug Administration (FDA) or shelf-stability (U.S. Department of Agriculture (USDA)) refers to conditions achieved in a product by the application of heat

to render the product free of microorganisms that are capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution. Designing a sound thermal process requires extensive understanding of process methods, the heating behavior of the product and its impact on a target microorganism. Thus, the severity of any thermal process must be known and depend on factors such as: (i) the physical characteristics of the food product including thermo-physical properties, shape and size of the container holding the product, (ii) the type and thermal resistance of the target microorganisms that are likely to be present in the food, and (iii) the pH, water activity (a_w) and salt content of the food (Awuah *et al.*, 2007).

Changes in the intrinsic properties of food, mainly salt, water activity and pH are known to affect the ability of microorganisms to survive thermal processes in addition to their genotype. Due to health-related concerns on the use of salt, there is increased demand to reduce salt levels in foods. The United States Food and Drug Administration (FDA) has classified foods in the federal register (21 CFR Part 114) as follows: (i) acid foods, (ii) acidified foods and (iii) low acid foods. Acid foods are those that have a natural pH of 4.6 or below (Awuah *et al.*, 2007). Acidified foods (e.g., beans, cucumbers, cabbage, artichokes, cauliflower, puddings, peppers, tropical fruits and fish) are low acid foods to which acid(s) or acid foods are added with a water activity greater than 0.85 and a finished equilibrium pH of 4.6 or below. Low-acid foods have been defined as foods, other than alcoholic beverages, with a finished equilibrium pH greater than 4.6 and a water activity greater than 0.85. Scientific investigations have revealed that spores of *Clostridium botulinum* will not germinate and grow in food below pH 4.8 (Awuah *et al.*, 2007). To provide sufficient buffer,

a pH of 4.6 has generally been accepted as the point below which *C. Botulinum* will not grow to produce toxin. Thus, a pH of 4.6 represents a demarcating line between low and high acid foods. During thermal processing of low acid foods ($\text{pH} \geq 4.6$), attention is given to *C. botulinum*: the highly heat resistant, rod-shaped, spore former that thrives comfortably under anaerobic conditions to produce the *botulism* toxin. Commercial sterility is achieved when *C. botulinum* spores are inactivated to satisfy regulatory requirements. However, other heat resistant spores (generally referred to as *thermophiles*) such as *Clostridium thermosaccolyticum*, *Bacillus stearothermophilus*, and *Bacillus thermoacidurans* have the potential to cause spoilage and economic losses when processed cans are stored under “abuse” storage conditions of temperature. However, *thermophiles* would be of no consequence provided one can guarantee that processed cans would be stored at temperatures below 30°C (Awuah *et al.*, 2007).

2.2.3 Reasons for heating of foods

Foods are heated for a number of reasons, the main one being to inactivate pathogenic or spoilage microorganisms. It may also be important to inactivate enzymes, to avoid the browning of fruit by polyphenol oxidases and minimise flavour changes resulting from lipase and proteolytic activity. The process of heating a food also induces physical changes and chemical reactions such as starch gelatinisation, protein denaturation or browning, which in turn affect the sensory characteristics such as colour, flavour and texture, either advantageously or adversely. For example, heating pretreatments are used in the production of evaporated milk to prevent gelation and age-thickening and for yoghurt manufacture to achieve the required final texture in the product. Heating processes may

also change the nutritional value of the food. Thermal processes vary considerably in their intensity, ranging from mild processes such as thermisation and pasteurisation through to more severe processes such as in-container sterilisation. The severity of the process affects both the shelf life and other quality characteristics. Foods which are heat-treated can be either solid or liquid, so the mechanisms of conduction and convection may be involved. Solid foods are poor conductors of heat, having a low thermal conductivity and convection is inherently a much quicker process than conduction (Brennan, 2006).

Fluids range from those having a low viscosity (1–10 mPa s), through to highly viscous fluids; and the presence of particles (up to 25 mm in diameter) further complicates the process as it becomes necessary to ensure that both the liquid and solid phases are at least adequately and if possible equally heated. The presence of dissolved air in either of the phases is a problem as it becomes less soluble as temperature increases and can come out of solution. Air is a poor heat transfer fluid and hot air is rarely used as a heating medium. Attention should be paid to removing air from steam e.g. venting of steam retorts and removing air from sealed containers (exhausting) (Brennan, 2006).

2.2.4 Traditional methods of thermal processing of beans

Conventional thermal processing applied on whole seeds or milling of pulse seeds to flours has been used for decades, for processing many food products (Bar-Yosef, 1998). Some of the drawbacks of large-scale processing include slow heat conduction, long processing times and poor commercial stability of the product. This has led food processors to look for alternative technologies. Therefore, a comparative study of traditional processes such as

roasting and boiling employed with legumes, is reviewed below. Each method can provide different physiochemical and structural changes, as well as improve the nutritional quality of the final product.

Roasting

Roasting is a rapid processing method that uses dry heat for short period of time. Studies have shown that after roasting, grains may exhibit improved texture, enhanced crispiness and increases in volume by puffing (Hoke et al., 2007). Roasting is a common heating processes applied to legumes which generally leads to a significant reduction in insoluble dietary fiber and total dietary fiber but an increase in soluble dietary fiber (Azizah and Zainon, 1997 and Mahadevamma and Tharanathan, 2004). Gahlawat & Sehgal, (1992) reported that roasting may also improve the digestibility, colour, flavor, shelf life and reduces the anti-nutrient factors of cereals and legumes. Commercial food processing by applying heat to seed products helps to improve their protein quality by destroying certain anti-nutritional compounds. Thermal treatment such as roasting of flour by applying dry heat for 6-8 min at 104-105°C has been shown to reduce its enzyme activity and lower its trypsin inhibitor and haemagglutinin activities (Aguilera et al., 1982; Smith and Circle, 1972). Therefore, roasting being a simple and cost effective processing method can be used in developing countries in order to achieve maximum nutrient utilization from legumes like lentils. Under conventional heating methods, thermal energy is transferred from the product surface towards its center 10-20 times more slowly than in a microwave-heated product. Roasting has both positive and negative impacts on legume seeds and flours according to its duration.

Boiling (Hydrothermal processing)

Boiling is one of the commonest methods to cook any form of comestible legumes. It can be done by two methods: (i) open pan boiling, or (ii) pressure boiling.

In the traditional process, comestible legumes are cooked by open pan boiling where violent heating may result in loss of water soluble nutrients. Consequently, turning down the heat once the water starts boiling, in order to slow down the cooking process, is recommended. Appropriate cooking times for legumes can be affected by genetic factors, physical structure, chemical composition and processing (Iyer *et al.*, 1989). Pariharet *al.*, (1999) reported that proportionately more proteins were retained under both forms of boiling than fats or carbohydrates. Preliminary studies by Xu and Chang, (2008) reported that the boiling process significantly reduced total phenolic contents, free radical scavenging capacity in cool season comestible legumes.

Porreset *al.*, (2003) found that by pressure cooking lentils at 120°C for 30 min reduces concentrations of trypsin inhibitor activity, phytate, and tannin content by 76%, 8%, and 12% respectively. In-vitro protein digestibility was improved by 81% after pressure cooking compared to open pan boiling (Naveeda and Jamuna, 2006). Ur-Rehman and Salariya, (2005) studied that by ordinary boiling of different comestible legumes

improved protein digestibility by 86.0-93.3% when compared to uncooked legumes. Ma *et al.*, (2011) reported that processing by boiling of different legume flours resulted in varying compositions. Protein content was significantly lowered for unhulled green and red lentils compared to hulled ones. A significant reduction in total trypsin inhibitor after boiling has been reported for all pulse flours.

2.2.5 Effects of heat on quality and nutritional attributes

Quality issues revolve around minimising chemical reactions and loss of nutrients and ensuring that sensory characteristics (appearance, colour, flavour and texture) are acceptable to the consumer. Quality changes which may result from enzyme activity must also be considered. There may also be conflicts between safety and quality issues. For example, microbial inactivation and food safety is increased by more severe heating conditions, but product quality in general deteriorates. To summarise, it is important to understand reaction kinetics and how they relate to microbial inactivation, chemical damage, enzyme inactivation and physical changes (Brennan, 2006).

Although some changes may be desirable, the rather harsh temperature for an extended period of time would trigger chemical reactions and loss of nutrients and sensory characteristics such as appearance, color, flavor and texture (Awuah *et al.*, 2007).

Effects of Heat on Proteins

The effect of thermal processing on proteins can be divided into two: those responsible for altering the secondary, tertiary and quaternary structure of proteins and those that alter the primary structure. Breaking the secondary, tertiary and quaternary structures unfolds the proteins and improves their bioavailability since peptide bonds become readily accessible to digestive enzymes. Modifications of primary protein structures on the other hand may lower digestibility and produce proteins that are not biologically available (Awuah *et al.*, 2007).

Effects of Heat on Vitamins

Vitamins are among the most sensitive food component to be affected by heat sterilization. Vitamin degradation during heat treatment is not simple and dependent on other agents such as oxygen, light and water solubility. In addition, vitamin degradation depends on pH and may be catalyzed by chemicals present, metals, other vitamins and enzymes. Heat sensitive vitamins are the fat-soluble Vitamins A (in the presence of oxygen), D, E and β -carotene, and water-soluble Vitamin C (ascorbic acid), Vitamins B1 (thiamine), B2 (riboflavin) in acid environment, nicotinic acid, pantothenic acid and biotin C. In general, the largest loss of Vitamin C in non-citrus foods occurs during heating. In canned juices, the loss of Vitamin C tends to follow consecutive first-order reactions that is, a rapid oxygen-dependent reaction that proceeds until oxygen is depleted, followed by anaerobic

degradation. Of the heat-sensitive vitamins, thiamine appears to have the most stable denaturation kinetics. Negligible losses are associated with vitamin losses in aseptically processed milk while lipids, carbohydrates and mineral are virtually unaffected (Awuah *etal.*, 2007).

Maillard Reaction

Even mild heat treatment can trigger Maillard reactions which are a complex series of reactions between proteins and reducing sugars via Amadori re-arrangements. The initial Maillard reaction is characterized by colorless solution but after several reactions, a brown or black insoluble compound called melanoidins are formed. Although such reactions may be desirable in generating characteristic flavors identified with some cooked products, the nutritional value of the product will be compromised by protein damage and loss of amino acids, including lysine, L-arginine, and L-histidine. The loss of lysine is important due to its essentiality in diet. Maillard browning can be inhibited by decreasing moisture to very low levels or by increasing dilution, lowering pH and temperature if the product is in the form of a liquid. Browning can also be reduced by removing one of the substrates responsible for it, which is usually, the sugar component. Studies in retortable pouches to investigate the relationship between temperatures and browning for different pouch thickness shows that minimum browning was achieved at 130°C for 20 mm, 135°C for 15mm and 140°C for 8mm thick pouch (Awuah *etal.*, 2007).

Effects of Heat on Colour

The colour of processed foods plays a role by influencing consumer acceptability. Natural occurring pigments in foods are susceptible to changes or degradation from heat. Chlorophylls (in photosynthetic tissues), anthocyanins (the red and blue hues associated with many fruits and vegetables), carotenoids (found in fruits, dairy products, egg, fish and vegetables) and betanins (present in red beet roots and meat) form the major classes of pigments. Chlorophylls are converted to pyropheophytin via pheophytin in fruits and vegetables, while carotenoids are isomerized from 5,6-epoxides to 5,8-epoxides which have less color intensity. Anthocyanins are changed by heat to brown pigments while traditional retorting can change some of these pigments due to prolonged heat exposure. High-temperature short-time operations can be expected to minimize these changes considerably. One major pigment that has been researched enormously is the chlorophyll content of green vegetables. These products would benefit from aseptic processing for better retention of green color. All indications point to vitamins as the most sensitive food component that would probably continue to be used as yardstick for quality evaluation of processed foods. Notwithstanding the above observation, product-specific quality attributes will play a vital role in dictating consumer acceptance of sterilized foods (Awuah *et al.*, 2007).

2.2.6 Health effects of thermally processed foods

The two most important issues connected with thermal processing are *food safety* and *food quality*. The major safety issue involves inactivating pathogenic microorganisms which are of public health concern. The World Health Organisation estimated that there are over 100 million cases of food poisoning each year and that one million of these result in death. These pathogens show considerable variation in their heat resistance: some are heat-labile,

such as *Campylobacter*, *Salmonella*, *Listeria* and of more recent concern *Escherichia coli* 0157, which are inactivated by pasteurisation, while of greater heat resistance is *Bacillus cereus* which may survive pasteurisation and also grow at low temperatures. The most heat-resistant pathogenic bacterial spore is *Clostridium botulinum*. In addition to these major foodborne pathogens, it is important to inactivate those microorganisms which cause food spoilage such as yeasts, moulds and gas-producing and souring bacteria. Again there is considerable variation in their heat resistance, the most heat-resistant being the spores of *Bacillus stearothermophilus*. The heat resistance of any microorganism changes as the environment changes, for example pH, water activity or chemical composition changes; and foods themselves provide such a complex and variable environment. New microorganisms may also be encountered such as *Bacillus sporothermodurans*. Therefore it is important to be aware of the type of microbial flora associated with all raw materials which are to be heat-treated. After processing it is very important to avoid reinfection of the product, generally known as ppc, which can cause problems in both pasteurisation and sterilisation (Brennan, 2006).

Therefore, raw materials and finished products should not be allowed in close proximity to each other. Other safety issues are concerned with natural toxins, pesticides, herbicides, antibiotics, growth hormones and environmental contaminants. Again, it is important that steps are taken to ensure that these do not finish up in the final product. Recently, there have been some serious cases of strong allergic reactions, with some deaths, shown by some individuals to foods such as peanuts and shellfish. These are all issues which also need to be considered for heat-treated foods (Brennan, 2006).

2.3 Fermentation

2.3.1 Meaning of fermentation

From a biochemical point of view, fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, and using microbes as enzyme source and an endogenous electron acceptor, which is usually an organic compound. However, to the microbiologist, the term “fermentation” describes a form of energy yielding microbial metabolism in which organic substrate, usually a carbohydrate, is incompletely oxidized, and an organic hydrate acts as the electron acceptor (Adams, 1990).

2.3.2 Classification of fermentation

Fermentation processes may be classified in a number of different ways. The first systematic approach was proposed by Gale, (1947) who grouped microbiological processes in a series of type groups, oxidation, reduction, hydrolysis, etc. Such an arrangement though fundamentally attractive is only suitable for specific reactions operating on specific substrates to yield specific products. Unfortunately, many commercially important fermentation processes cannot be so neatly described. Therefore, Gale’s classification scheme has been later on extended to a more detailed breakdown of ‘type reactions’ (Stodola, 1958). In this scheme, microorganisms or more specifically their enzyme complements are looked at as added means for controlled organic synthesis. Again, this concept is not applicable to most of the fermentation processes now practiced commercially, at least at the present level of knowledge regarding mechanisms.

A different approach was proposed by Gaden, (1956). Here fermentation processes rather than specific reactions are grouped together and the overall free energy change involved is the basis for classification. The primary advantage of this scheme is technological; it coincides with the general classification of fermentation rate patterns suggested earlier. Experience has shown that fermentation processes fall more or less into three kinetic groups which may be designated 'types I to III' for convenience.

i. Type I: processes in which the main product appears as a result of primary energy metabolism. Examples of this type of system are most common in the older branches of fermentation technology, for instance: Aerobic yeast propagation (mass propagation of cells in general), Alcoholic fermentation, Oxidation of glucose to gluconic acid, and Dissimilation of sugar to lactic acid.

ii. Type II: processes in which the main product arises indirectly from reactions of energy metabolism. In systems of this type the product is not a direct residue of oxidation of the carbon source but the result of some side-reaction or subsequent interaction between these direct metabolic products. Examples are: Formation of citric acid, and Formation of certain amino acids

iii. Type III: processes in which the main product does not arise from energy metabolism at all but is independently or accumulated by the cells. It is perfectly true that carbon, nitrogen, etc., provided in essential metabolites appear in product molecules but the major products of energy metabolism are CO_2 and water. Antibiotic synthesis is a prime example of this type.

Another important classification of fermentation could be done based on the source of microorganisms used in the process. Based on that classification, there is open fermentation, natural fermentation and controlled fermentation.

a. **Open fermentation:** the fermentation process which is performed in an open air and uses the microorganisms present on the substrate (e.g food legumes) and those present in the atmosphere surrounding the fermentation vessel as fermenting microorganisms.

b. **Natural fermentation:** this is the fermentation process mostly operated in a fermenter and is spontaneously and aseptically initiated with the microbiota naturally present on the substrates (Granito *et al.*, 2002).

c. **Controlled fermentation:** this is the fermentation process that is controlled by the use of specific cultures or starters from a batch of previously fermented products (Baramparna and Simard, 1994; Ibrahim *et al.*, 2002)

Microbial fermentation can also be classified based on the mode of operation of the bioprocess as batch fermentation, fed-batch fermentation and continuous fermentation.

2.3.3 Fermentation vessels

Laboratory scale fermentations are carried out in shaker flasks and flat bed bottles. Large scale fermentations are carried out in glass or stainless steel tank fermenter. A fermentation vessel should be cheap not allow contamination of the contents, be non-toxic to the microorganism used for the process, be easy to sterilize, be easy to operate, be robust and

reliable, allow visual monitoring of the fermentation process, allow sampling, and be leak proof.

i. Shaker Flasks: these are conical vessels made of glass and are available in different sizes. The typical volume of these flasks is 250ml. There are different types of shaker flasks such as baffled, unbaffled or Erlenmeyer flask and flying saucer. Shaker flasks are used for the screening of microorganisms and cultivation of microorganisms for inoculation. Shaker beds or shaker tables are used to allow oxygen transfer by their continuous rotary motion. Baffled flasks are used to increase the oxygen transfer. Shaker flasks need to be plugged to prevent contamination with other microorganisms. Cotton- wool, polyurethane foam, glass and synthetic plugs are commonly used. Femwald shaker flasks and flat bed Thompson bottles are expensive and are not commonly used (Brian and Linda, 2008).

ii. Stirred Tank Fermenters: These are the most commonly used fermenters. They are cylindrical vessels with a motor driven agitator to stir the contents in the tank. The Top-entry stirrer (agitator) model is most commonly used because it has many advantages like ease of operation, reliability and robustness. The Bottom-entry stirrer (agitator) model is rarely used (Brian and Linda, 2008).

iii. Air-Lift Fermenters: These fermenters do not have mechanical agitation systems (motors shaft, impeller blades) but contents are agitated by injecting air from the bottom. Sterile atmospheric air is used if microorganisms are aerobic and “inert gas” is used if microorganisms are anaerobic. This is a gentle method of mixing the contents and is most suitable for fermentation of animal and plant cell cultures since the mechanical agitation

produces high shearing stress that may damage the cells. Air-lift fermenters are most widely used for large-scale production of monoclonal antibodies. Draft tubes are used in some cases to provide better mixing, mass transfer, and to reduce bubble coalescence by inducing circulatory motion (Brian and Linda, 2008).

iv. **Fixed Bed Fermenters:** These are also called immobilized cell fermenters. The cells are absorbed onto or entrapped in the solid surfaces like plastic beads, glass or plastic wool and solidified gels to render them immobile. Fixed bed fermenters are most commonly used for waste water treatment and as biological filters in small aquarium water recycling systems and production of amino acids and enzymes (Brian and Linda, 2008).

v. **Tower Fermenters:** Tower fermenters are simple in design and easy to construct. They consist of a long cylindrical vessel with an inlet at the bottom, an exhaust at the top and a jacket to control temperature. They do not require agitation hence there are no shafts, impellers or blades. Tower fermenters are used for continuous fermentation of beer and yeast (Brian and Linda, 2008).

2.3.4 Nutritional value of fermented foods

Generally, a significant increase in the soluble fraction of a food is observed during fermentation. The quantity as well as quality of the food proteins as expressed by biological value, and often the content of water soluble vitamins is generally increased, while the anti-nutritional- factors show a decline during fermentation (Paredes-López and Hany, 1988). Fermentation results in a lower proportion of dry matter in the food and the concentrations

of vitamins, minerals and protein appear to increase when measured on a dry weight basis (Adams, 1990). Single as well as mixed culture fermentation of pearl millet flour with yeast and lactobacilli significantly increased the total amount of soluble sugars, reducing and non-reducing sugar content, with a simultaneous decrease in its starch content (Khetarpaul and Chauhan, 1990). So fermentation causes significant changes in food composition as follow.

Proteins

The protein efficiency ratio (PER) of wheat was found to increase on fermentation partly due to the increase in availability of lysine. A mixture of wheat and soybeans in equal amounts would provide improved pattern of amino acids. The fermentation process rose the PER value of the mixture to a level which was comparable to that of casein (Hesseltine and Wang, 1980). Fermentation may not increase the content of protein and amino acids unless ammonia or urea is added as a nitrogen source to the fermentation media (Reed, 1981). The relative nutritional value (RNV) of maize increased from 65% to 81% when it was germinated and fermentation of the flour made of the germinated maize gave a further increase in RNV to 87% (Lay and Fields, 1981). Fermentation of legumes for making 'dhokia' and fermentation of millet for making 'ambali' did not show any improvement in values reported for PER, TD, BV and NPU in relation to the unfermented products (Aliya and Geervani, 1981). So fermentation has an important effect on the protein content of foods.

Vitamins

During fermentation certain micro-organisms produce vitamins at a higher rate than others do. The content of thiamine and riboflavin in 'dhokia' and 'ambali' was about 50% higher after fermentation. Fermented milk products in general showed an increase in folic acid content and a slight decrease in vitamin B12 while other Bvitamins were affected only slightly (Alm, 1982) in comparison to raw milk. The levels of vitamin B12, riboflavin and folacin were increased by lactic acid fermentation of maize flour while the level of pyridoxine was decreased (Murdock and Fields, 1984). Fermented whole onion plant retained 97% of vitamin A activity while fermented egg plant only retained 34% of the vitamin A activity (Speek *et al.*, 1988). Kefir made from ten different kefir grain cultures showed significant (>20%) increase for pyridoxine, cobalamin, folic acid and biotin and reduction exceeding 20% for thiamin, riboflavin, nicotinic acid, and pantothenic acid depending on the culture used.

Minerals

The mineral content is not affected by fermentation unless some salts are added to the product during fermentation or by leaching when the liquid portion is separated from the fermented food. Sometimes, when fermentation is carried out in metal containers, some minerals are solubilised by the fermented product which may cause an increase in mineral content. Vaishali *et al.*, (1997) who studied effect of natural fermentation on *in vitro* zinc bioavailability in cereal-legume mixtures found that fermentation increased the zinc solubility (2-28%) and the zinc uptake by intestinal segment (1-16%) to a significant level.

Antinutrients

Phytate content in bread was lowered when the amount of yeast or the fermentation time was raised (Harland and Harland, 1980). Phytate content in locust bean seeds was lowered from 0.51 mg/g to 0.31 mg/g by fermentation (Eka, 1980). Natural lactic fermentation of maize meal decreased phytate phosphorus by 78% (Chompeeda and Fields, 1984).

In bambaranut milk (Obizoba and Egbuna, 1992), tannin content could be reduced by fermentation. There was a marked increase in protein availability and concentration during fermentation of 'siljo', a traditional Ethiopian fermented food. A study on the effect of fermentation of cowpea (*Vigna unguiculata*) on the nutritional quality of the cowpea meal showed that 72h fermentation increased content of protein, ash and lipid levels while decreasing the levels of tannin and phytate (Nnam, 1995). Trypsin inhibitors, thiamine and riboflavin were reduced significantly during fermentation. A decrease in protein content was observed during the first 2 days of fermentation and thereafter the decrease was not significant (Gupta *et al.*, 1998).

2.3.5 Health effects of fermented foods

2.3.5.1 Probiotic effect

In the late 19th century, microbiologists identified microflora in the gastrointestinal tracts (GIT) of healthy individuals that differed from those found in diseased individuals. These beneficial microflora found in the GIT were termed probiotics. Probiotics, literally meaning 'for life', are micro-organisms proven to exert health-promoting influences in humans and animals (Parvez *et al.*, 2006).

Most probiotics fall into the group of organisms' known as lactic acid-producing bacteria and are normally consumed in the form of yoghurt, fermented milks or other fermented foods. Some of the beneficial effect of lactic acid bacteria consumption include: (i) improving intestinal tract health; (ii) enhancing the immune system, synthesizing and enhancing the bioavailability of nutrients; (iii) reducing symptoms of lactose intolerance, decreasing the prevalence of allergy in susceptible individuals and (iv) reducing risk of certain cancers (Parvez *et al.*, 2006). The mechanisms by which probiotics exert their effects are largely unknown but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, competing for pathogen binding and receptor sites as well as for available nutrients and growth factors, stimulating immunomodulatory cells and producing lactase (Parvez *et al.*, 2006).

2.3.5.2 Anticholesterolemic effect

Hepner *et al.*, (1979) reported hypercholesteremic effect of yoghurt in human subjects receiving a one-week dietary supplement. Studies on supplementation of infant formula with *Lactobacillus acidophilus* showed that the serum cholesterol in infants was reduced from 147 mg/ml to 119mg/100 ml (Harrison and Peat, 1975). In an *in vitro* study, the ability of 23 strains of lactic acid bacteria isolated from various fermented milk products the bacterial cells to bind cholesterol was investigated. No cholesterol was found inside the cells (Taranto *et al.*, 1997). Poppel and Schaafsma (1996) have also reported the ability of yoghurt to lower the cholesterol in serum by controlled human trials. Possible role of lactic

acid bacteria in lowering cholesterol concentration and various mechanisms by which it may be possible has been discussed by Haberer *et al.*, (1997). Brigidi *et al.*, (1995) have cloned a gene encoding cholesterol oxidase from *Streptomyces lividans* into *Bacillus*, *Lactobacillus* and *E. coil*.

2.3.5.3 Effect on transit time, bowel function and glycemic index

The transit time for 50% (t_{50}) of the gastric content was significantly reduced for regular unfermented milk (42 ± 10 min) in comparison with a fermented milk product indigenous to Sweden called “langfil” or ropy milk (62 ± 14 min). Another study (Wilhelm, 1993) reports increase in transport time and improved bowel function in patients with habitual constipation. The number of defecations per week increased from three during control period to seven using conventional fermented milk and fifteen when acidophilus milk was served. Regular unfermented milk also gave significantly higher increase in glycemic index curve than fermented milk product called langful (Strandhagen *et al.*, 1994). Liljeberg *et al.*, (1995) have shown that presence of acid, especially acetic or lactic acid would lower the glycemic index in breads to a significant level. ‘Koji’ which is prepared from *Aspergillus oryzae* and ‘beni-koji’ made from *Monascus pilosus* were found to express rises in blood pressure (Tsuji *et al.*, 1992).

2.3.5.4 Anticarcinogenic effect

There are interesting data on anticarcinogenic effect of fermented foods showing potential role of *Lactobacilli* in reducing and eliminating procarcinogens and carcinogens in the

alimentary canal (Reddy *et al.*, 1983; Shahani, 1983; Mitajl and Garg, 1995). The enzymes β -glucuronidase, azoreductase and nitroreductase, which are present in the intestinal canal, are known to convert procarcinogens to carcinogens (Goldin and Gorbach, 1984). Oral administration of *Lb rhamnosus* GG was shown to lower the faecal concentration of β -glucuronidase in humans (Salminen *et al.*, 1993) implying a decrease in the conversion of procarcinogens to carcinogens. Fermented milk containing *Lactobacillus acidophilus* given together with fried meat patties significantly lowered the excretion of mutagenic substances compared to ordinary fermented milk with *Lactococcus* fed together with fried meat patties (Lidbeck *et al.*, 1992). The process of fermentation of foods is also reported to reduce the mutagenicity of foods by degrading the mutagenic substances during the process. Lactic acid bacteria isolated from 'dadih', traditional Indonesian fermented milk, were found to be able to bind mutagens and inhibit mutagenic nitrosamines.

2.3.5.5 Food safety aspects of fermented foods

It has been estimated that more than 13 million infants and children under five years of age die annually in the tropical regions of the world. After respiratory infections, diarrhoea diseases are the commonest illnesses and have the greatest negative impact upon the growth of infants and young children. The causes of diarrhoea have traditionally been ascribed to water supply and sanitation (Motarjemi *et al.*, 1993). Foods prepared under unhygienic conditions and frequently heavily contaminated with pathogenic organisms play a major role in child mortality through a combination of diarrhoea diseases, nutrient malabsorption and malnutrition. All food items contain microorganisms of different types and in different amounts. The microorganisms that will dominate depend on several factors, and

sometimes microorganisms initially present in very low numbers in the food, for example lactic acid bacteria (LAB), will outnumber the other organisms inhibiting their growth. In contrast to fermented meat, fish, dairy and cereal products, fermented vegetables have not been recorded as a significant source of microbial food poisoning.

2.3.5.6 Effect of fermentation on pathogenic organisms

Over a study period of nine months, a group of children fed with lactic acid fermented gruel had a mean number of 2.1 diarrhoea episodes compared to 3.5 for the group fed with unfermented gruel (Lorri and Swanberg, 1994). Although Salmonella, Vibrio, Yersinia and Escherichia are the most common organisms associated with bacterial diarrhoea disease. Other enterotoxigenic genera include *Pseudomonas*, *Enterobacter*, *Klepsiella*, *Serratia* and *Proteus*. *Providencia*, *Acromonas*, *Achromobacter* and *flavobacterium* have also been reported (Nout *et al.*, 1989). In addition, it was found that there was no significant difference between the behaviour of the pathogens in fermented porridge or acid-supplemented non fermented porridge which implies that the antimicrobial effect is due to presence of lactic and acetic acids at reduced pH and that other anti-microbial substance do not play a detectable role (Nout *et al.* 1989). Similarly, Adams (1990) suggested that lactic acid bacteria are inhibitory to many other microorganisms when they are cultured together and this is the basis of the extended shelf life and improved microbiological safety of lactic-fermented foods. Lactobacillus species can produce a variety of metabolites, including lactic and acetic acids which lower pH, that are inhibitory to competing bacteria, including psychrotrophic pathogen (Lu *et al.*, 2010) This effect could be due to a combination of many factors as shown in Table 2.1.

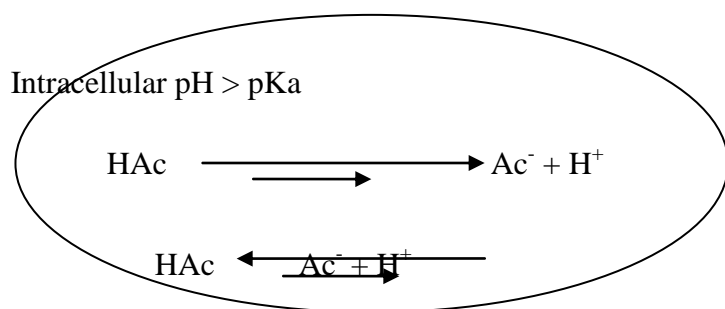
The inhibition by organic acids has been attributed to the protonated form of these acids which are uncharged and may therefore cross biological membranes (Figure 2.1). The resulting inhibition of growth may be due to acidification of the cytoplasm and/or accumulation of anions inside the cell (Adams, 1990; Russei, 1992; Lu *et al.*,2010). The ability of an acid to inhibit bacteria depends principally on the pKa of the acid; the higher the pKa of the acid, the greater the proportion of undissociated acid, and the more inhibitory the acid is likely to be. On this basis, one would expect acetic acid (pKa = 4.75) to be a more effective antimicrobial agent than lactic acid (pKa 3.86) (Adams, 1990).

Table 2.1: Pathogenic and Food Spoilage Organisms

Products	Main target organism
<u>Organic Acid</u>	
Lactic acid	Putrefactive and Gram-negative bacteria, some fungi
Acetic Acid	Putrefactive bacteria, clostridia, some yeasts and some fungi
Hydrogen peroxide	Pathogens and spoilage organisms, especially in protein-rich foods
<u>Enzymes</u>	
Lactoperoxidase system with hydrogen peroxide	Pathogens and spoilage bacteria (milk and dairy products)
Lysozyme (by recombinant DNA)	Undesired Gram positive bacteria
<u>Low-molecular-weight metabolites</u>	
Reuterin	Wide spectrum of bacteria, yeasts and molds
Diacetyl	Gram-negative bacteria
Fatty acids	Different bacteria
<u>Bacteriocins</u>	
Nisin	Some LAB and gram-positive bacteria, notably endospore-formers

Other	Gram-positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type
-------	---

Culled from Lu *et al.*, (2010)



Extracellular pH ≤ pKa

Figure 2.1: The diffusion of a weak organic acid into a microbial cell and its dissociation yielding protons (H⁺) and potentially toxic anions (A⁻), Culled from Adams, (1990).

2.3.6 Toxins and Toxic-Producing Organisms in Fermented Foods

Lactic starter cultures were found to be effective in preventing the formation of botulin toxin even in the absence of nitrate (Shahani, 1983). No aflatoxin production was reported

in ‘Tempe’ and ‘miso’ prepared using *Rhizopus oligosporus* and *Aspergillus oryzae* on soya bean, chickpea and horsebean (Paredes-Lopez and Hany, 1988). *Aspergillus flavus* grown in broth had a lower aflatoxin production when 10% cell free supernatant culture fluid from lactobacilli was added. This effect could not be explained on the basis of pH or competition (Karunarama *et al.*, 1990). Studies, mainly with *Aspergillus oryzae* have shown no traces of aflatoxin production in traditional mould-fermented products (Wang and Hesseltine, 1981). However when an aflatoxin producing strain was inoculated at the same time, large amounts of aflatoxin was found. The aflatoxin production of *Aspergillus parasiticus* was studied and found to increase in the presence of *Lactococcus lactis genome*” (Hofmann *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection and preparation of plant sample

Matured *Phaseolus vulgaris* (red kidney bean seeds) were purchased from local farmers in Mangu Local Government Area of Plateau state, Nigeria. The identity of the bean was

confirmed at the herbarium of Biological Science Department, Ahmadu Bello University, Zaria, Nigeria (Voucher Number 2403). The beans were picked, cleaned of all debris and broken seeds and then stored in a plastic container at room temperature (27-30°C) for subsequent analysis.

3.1.2 Chemicals

The chemicals used for this research are:

Pepsin

Hydrochloric acid

Trioxonitrate (iv) acid

Tetraoxosulphate (vi) acid

Tetraoxochlorate (vii) acid

Petroleum ether (60-80°C)

Sodium hydroxide

Catalyst

Boric acid

Ammonium hydroxide

Calcium chloride.

All other chemicals and reagents used were of analytical grades and were purchased from sigma.

3.1.3 Equipments

The major equipments used for this research include:

Oven (Gallenhap England)

Murffle Furnace (Model SXL, SearchTech)

Soxhlet Extraction Unit

Kjedahl Flask

Markham Semi Nitrogen Steel Tube

Technicom Sequential Multi-Sample Amino Acid Analyzer

Atomic Absorption Spectrophotometer

Flame Photometer

pH metre

Weighing Balance (AR2130, Ohaus Cooperation, Japan)

3.2 Methods

3.2.1 Open fermentation

Red kidneybeans sample was rinsed with distilled water and dried in an oven at 55°C for 24hours. The rinsed beans were placed into a transparent plastic container and three cups of cold water for every one cup of dried red kidney beans was added. The beans were then allowed to soak for three (3) days uncovered and was fermented by atmospheric microorganisms (Wittenberg, 2007), during which the seed coat remains intact. After fermentation, the microbial growth was terminated by drying at 55°C in an oven for 24 hours (Fadahunsi, 2009).

3.2.2 Thermal processing

The unfermented and fermented bean samples were boiled using ordinary cooking pot and pressure cooker. Boiling of unfermented bean was for 143.42 and 40.32 minutes using ordinary cooking and pressure pots respectively while 84.91 and 39.27 minutes were for fermented bean samples using ordinary pot and pressure cooking pot respectively after which it was filtered and rinsed with water in each case. The boiled beans were then dried in an oven at 55°C for 24h after which the bean sample was ground in a laboratory bench mill and kept in a cool dry rubber container for subsequent analysis.

3.2.3 Determination of proximate composition

3.2.3.1 Determination of moisture

The method employed for the determination of moisture content of the sample was that which is based on the measurement of the loss in weight due to drying at a temperature of 105°C as described by AOAC, (1990).

Six (6) watch glasses were washed and dried in an oven at 105°C after which they were cooled and weighed empty. Two grammes (2.0g) of each sample were weighed into their respective watch glasses. The watch glasses and their contents were dried in an air circulated oven at about 105°C to a constant weight. The watch glasses and their contents were cooled in desiccators and reweighed.

The percentage moisture content of each sample was calculated using the expression

$$\% \text{ moisture} = \frac{\text{Loss of weight on drying(g)} \times 100}{\text{Initial sample weight}}$$

3.2.3.2 Ash content determination

The term ash refers to the residue left after the combustion of the oven dried sample and is a measure of the total mineral content. Determination of ash content was done according to the method described by AOAC, (1990).

Six crucibles were preheated in a muffle furnace at 550°C. Each crucible was cooled in a desiccator and weighed. One gramme (1.0g) of each sample was weighed into different crucibles. The crucibles and their contents were transferred into the muffle furnace and the temperature set at 550°C and allowed to stay for 5 hours. The weights of the crucible and their contents were taken and recorded before ashing and then the weight of the ash was calculated in each case.

Percentage ash was calculated using the expression below

$$\% \text{ ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of dry sample}}$$

3.2.3.3 Determination of lipid content

The lipid content of each sample was determined by the procedure described by AOAC, (1990). A clean dry round bottom flask containing anti bumping granules was used. Exactly 210cm³ of petroleum ether (60 – 80°C) was dispensed into a flask fitted with soxhlet extraction unit. A weighed sample (2.0g) was transferred into a thimble and fixed into the Soxhlet extraction unit fitted with a condenser. Cold water was put into circulation. The heating mantle was switched on and the heating rate adjusted until the solvent began to reflux at a steady rate. Extraction was carried out for 8hours.

The sample was removed and dried to a constant weight in an oven, cooled in a dessicator and reweighed and the percentage crude lipid content was determined using the formula:

$$\% \text{ lipid} = \frac{\text{Weight of lipid extracted} \times 100}{\text{Weight of dry sample}}$$

Where the weight of lipid extracted was the loss in weight of the sample after extraction, drying in an oven and cooling in a dessicator.

3.2.3.4 Determination of crude fibre

Crude fibre was determined by the method of AOAC, (1990). Two grammes (2.0g) of ground sample was placed in a round bottom flask, 100ml of 0.25M H₂SO₄ was added and

the mixture was boiled under reflux for 30 minutes. The insoluble matter was washed several times with hot water until it was acid free. It was then transferred into a flask containing 100 ml of 0.25 M NaOH solution. The mixture was boiled again under reflux for 30 minutes and filtered under suction. The insoluble residue was washed with hot water until it was base free and oven dried to a constant weight (C_1). It was then ashed in a furnace at 550°C for 2 hours. The furnace was then put off and allowed to cool down. The sample was then removed and cooled in a desiccator and weighed (C_2). The crude fibre content was then calculated as loss of weight in ashing. Weight of original sample was used as W .

$$\% \text{ crude fiber} = \frac{C_1 - C_2}{W} \times 100$$

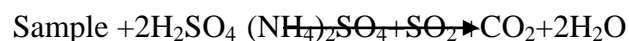
3.2.3.5 Determination of nitrogen content and crude protein

Principle

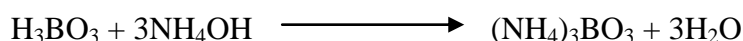
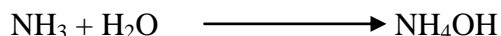
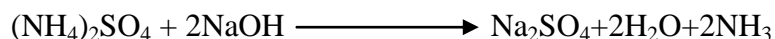
Proteins are major compounds containing nitrogen primarily in the form of amino acids which are their building blocks. Nitrogen is used as an index termed crude protein as distinct from true protein. The Kjeldahl method of AOAC (1990) was used for the crude protein determination.

Steps for determination

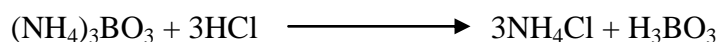
A. mineralization steps of organic substance in boiling sulphuric acid.



B. Distillation Steps of Ammonium Sulphate after alkalisation of the boric acid solution



C. Titration of ammonium borate with hydrochloric acid of standardize concentration



Method.

Exactly 2.0g of each sample was weighed into 100ml Kjeldahl flask and a few anti bumping granules were added. One gramme of the mixed catalyst (CuSO_4 and K_2SO_4 in the ratio 8:1 respectively) and 15ml of concentrated sulphuric acid were added. The flask was placed on a Kjeldahl digestion rack and heated on a heating black until a clear solution was obtained. At the end of the digestion the flask was cooled and the sample was quantitatively transferred to a 100ml volumetric flask and made up to the mark with distilled water. Ten millilitres of the digest was pipetted into Markham semi micro nitrogen steel tube, 10ml of 40% NaOH solution was then added cautiously. The sample was then steam distilled liberating ammonia into a 100ml conical flask containing 10ml of 4% boric acid and a drop of methyl blue indicator until the colour changed from pink to green. Exactly 30ml of sample volume was then collected. The content of the conical flask was then titrated with 0.1M HCl. The end point was indicated by a colour change from green to pink and the volume (v) of the acid for each distillate was noted. Percentage nitrogen per sample was calculated using the expression below

$$\% \text{nitrogen} = \frac{M \times v \times 14 \times 100 \times 100}{\text{Weight of sample} \times 1000 \times 10}$$

Where, M = Molarity of HCl

V = Titre volume

14 = Atomic weight of nitrogen.

100 = Total volume of digest taken for distillation.

100 = % conversion.

10 = Volume of the digest taken.

1000 = Conversion to litre.

The crude protein was calculated as

$$\% \text{ Protein} = 6.25 \times \% \text{ nitrogen.}$$

3.2.3.6 Determination of carbohydrate content

The percentage carbohydrate was obtained by difference.

$$\text{NFE} = 100 - (\% \text{moisture} + \% \text{CP} + \% \text{CF} + \% \text{Ash} + \% \text{Fat})$$

Where, NFE = Nitrogen Free Extracts

%CP = Percentage Crude Protein.

%CF = Percentage Crude Fibre

3.2.4 Determination of amino acid profile

The amino acid profile in the sample was determined using the method described by Adeyeye and Afolabi, (2004). The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM).

Defatting of sample:

The sample was defatted using chloroform/methanol mixture of ratio 2:1. Four grammes (4.0g) of the sample was put in extraction thimble and extracted for 15 hours in Soxhlet extraction apparatus (AOAC, 2006).

Nitrogen Determination

This was done according to the method of AOAC 1990 as described in 3.2.3.5.

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. Exactly 7ml of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to

avoid possible oxidation of some amino acids during hydrolysis (e.g methionine and cystine). The glass ampoule was then sealed using Bunsen burner flame and placed in an oven preset at $105^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for 22 hours. The ampoule was allowed to cool, broken open at the tip and the content was filtered. Tryptophan was destroyed by 6N HCl during hydrolysis.

The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved in 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles which were kept in the freezer.

Loading of the hydrolysate into TSM analyzer

Exactly 7.5 microlitres of the hydrolysate was dispensed into the cartridge of the analyzer which separated and analyzed free amino acids (i.e neutral, acidic and basic) of the hydrolysate. The period of an analysis was 76 minutes.

Method of Calculating Amino Acid Values from the Chromatogram Peaks.

An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

$$\text{NE} = \frac{\text{Area of Norleucine Peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture:

Where $S_{std} = NE_{std} \times \text{Molecular weight} \times \mu\text{MAA}_{std}$

μMAA_{std} = Micromole Atomic Mass Standard.

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

Concentration (g/100g protein) = $NH \times W \text{ at } NH/2_{ss} \times S_{std} \times C$

$$\text{Where } C = \left(\frac{\text{Dilution} \times 16}{\text{Sample weight (g)} \times 10 \times \text{Volume loaded}} \right) \div (NH \times W(n\text{leu}))$$

Where: NH = Net height

W = Width at half height
nleu = Norleucine.

3.2.5 Determination of mineral contents

Magnesium, calcium, zinc and iron were determined using the atomic absorption spectrophotometry as described by AOAC(1990). Two grammes of the sample was digested with 23ml of a mixture of HNO₃ (SG 1.57), H₂SO₄ (SG1.84) and HClO₄ (7:8:8 by volume). The cooled digest was then diluted to 50ml with distilled water, filtered through a

Whatman 45 filter paper and made up to 100ml with distilled water in a glass volumetric flask. This final solution was then used for atomic absorption spectrophotometry.

Sodium and potassium were analysed using flame photometry.

3.2.6 Determination of anti nutritional factors

3.2.6.1 Determination of Cyanide

The cyanide content was determined according to the method of AOAC (1984)

Exactly 2.0g of sample was weighed into a flask and 100ml of distilled water was then added to it and allowed to hydrolyse for 1hour. Exactly 10ml of 2.5% NaOH was measured carefully and poured to the sample holder. The soxhlet apparatus was set up and distilled into the sample holder containing the 2.5% NaOH until about 70ml was collected. It was then carefully transferred to a 100ml volumetric flask and the sample holder rinsed with distilled water into the volumetric flask and made up to the 100ml mark. Exactly 25ml of the distillate was pipetted into a conical flask; 2ml of 6molar NH_4OH was added as well as 0.5ml of 10% KI solution. It was then titrated with 0.02M AgNO_3 to first turbid colour.

1ml of 0.02M AgNO_3 is equal to 1.08g of cyanide.

3.2.6.2 Determination of phytic acid

The phytic acid was determined using the procedure described by Lucas and Markakas (1975).

Exactly 2.0g of each sample powder was weighed into 250ml conical flask, 100ml of 2% HCl acid was used to soak each sample in the conical flask for 3hrs and then filtered through a double layer of harden filter paper. Then 50ml of each filtrate in 250ml beaker and 107ml of distilled water was added in each case to give proper acidity.

Ten millilitres of 0.3% ammonium thiocyanide solution was added into each solution as indicator and titrated with standard iron chloride solution which contains 1.95mg/ml of iron. The end point was a brownish colour persisting for 5minutes. The percentage of phytic acid was calculated using the formula.

$$\% \text{ phytic acid } \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{Y \times 1.19 \times 100}{\text{Sample weight}}$$

Where Y = titre value x 1.95mg.

3.2.6.3 *Determination of alkaloids*

The gravimetric method as described by AOAC (1984) was adopted.

Exactly 5.0g of each sample was dispensed into 50ml of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for 4hrs before filtering. The filtrate was evaporated to one quarter of its original volume. Concentrated NH₄OH solution was added drop wise to precipitate the alkaloid. The precipitate was filtered off with a

weighed filter and then dried on the filter paper in an oven at 69°C for 30mins and reweighed. By weight difference, the weight of alkaloid was determined and expressed by;

$$\% \text{ alkaloids} = \frac{W_2 - W_1}{W} \times 100$$

Where, W_1 =weight of precipitate before drying

W_2 =weight of precipitate after drying

W =weight of sample.

3.2.6.4Determination of oxalates

Oxalate was determined using the method of Oke (1969). The total oxalic acid of the powdered sample was determined by weighing 2g of sample into 250ml of conical flask, then 190ml of distilled water and 10ml of 6MHCl was then added. The mixture was warmed for 1hour on a boiling water bath, cooled, transferred into a 250ml volumetric flask, diluted to volume and filtered. Four drops of methyl red indicator was added, followed by concentrated ammonia till the solution turned faint yellow. It was then heated to 100°C, allowed to cool and filtered to remove precipitate containing ferrous iron. The filtrate was then boiled and 10ml of 5% CaCl₂ was added with a constant stirring and was allowed to stand overnight.

The mixture was filtered through Whatman No.4 filter paper. The precipitate was then washed several times with distilled water and transferred to a beaker and 5ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80°C and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. A blank was also run for the test sample. From the amount of potassium permanganate used, the oxalate content of the unknown sample was calculated using the equation below:

1ml potassium permanganate = 2.24mg oxalate.

3.2.6.5 Determination of tannins

The tannin content of each sample was determined using the method described by Krishnaiah *et al.*, (2009).

Exactly 50µL of sample solution for each sample was taken in a test tube and the volume was made up to 1ml with distilled water. Then 0.5ml of Folin Clocalteu reagent was added and mixed properly. Then 2.5ml of 20% sodium carbonate solution was added, mixed and kept for 40 minutes at room temperature. Optical density was then taken at 725nm on the spectrophotometer and the concentrations was obtained from the standard curve. Percentage concentration of tannin was determined using the expression:

$$\% \text{ Tannin} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{A_n \times C \times D_f}{A_s \times W} \times 100$$

Where,

A_n = Absorbance of test sample

A_s = Absorbance of standard tannic acid

C = Concentration of standard tannic acid (mg/ml)

D_f = Dilution factor V_{ex}/V_a

W = Weight of test sample (mg)

V_{ex} = Volume of extract

V_a = Volume of extract analysed

3.2.7 Determination of *in vitro* protein digestibility

The *in vitro* protein digestibility was carried out according to the method of Mertz *et al.*, (1984). Two hundred (200) milligramme of the powdered bean sample was dispersed in 20cm³ of pepsin reagent (1.5mg/ml in 0.1M phosphate buffer of pH 2.0) and shaken vigorously. All the tubes were kept in a water bath at 37°C for three hours with constant shaking at 15 minutes interval. After three hours, the digestion was stopped by removing the tubes from the water bath and placing them in ice bath for 30 minutes. The samples were then filtered through whatman No.1 filter paper and the residue washed with buffer and dried at 80°C for 2 hours. The dried residue was placed in a 50cm³ micro-kjedahl flask and analysed for nitrogen by micro-kjedahl digestion. The indigestible nitrogen was

subtracted from total nitrogen of the sample to obtain digestible nitrogen using the following:

Digestible N (mg) = Total N in sample (mg) – N in residue (mg).

Digestible protein = Digestible N (mg) × Conversion factor.

$$\% \text{ in vitro digestibility} = \frac{\text{Digestible protein}}{\text{Total protein in sample}} \times 100$$

3.3 Statistical Analysis

Data obtained is expressed as mean ± standard deviation (SD). Statistical analysis was done by the One Way Analysis of Variance (ANOVA) and Paired Sample T-test using SPSS (version 20). Duncan Multiple Range Test was used to determine the source of variance at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of the Different Processing Methods on Cooking Time of Red Kidney Beans (*Phaseolus Vulgaris*)

Figure 4.1 shows the various cooking time for the different processing methods viz: Unfermented beans boiled with ordinary cooking pot and pressure pot and fermented beans boiled with ordinary cooking pot and pressure pot. Based on the result, there was a significant ($p < 0.05$) decrease in the cooking time of fermented beans cooked with the ordinary cooking pot when compared with the unfermented beans cooked with the ordinary cooking pot. There was also a significant ($p < 0.05$) decrease in the cooking time of both unfermented and fermented beans cooked with the pressure pot compared with the unfermented and fermented beans cooked with the ordinary cooking pot respectively. There was on the other hand no significant ($p > 0.05$) change in the cooking time of unfermented and fermented beans cooked with pressure pot.

4.2 Proximate Contents of Raw and Differently Processed Whole Grains of *Phaseolus vulgaris*.

Table 4.1, 4.2 and 4.3 show the proximate compositions of raw and differently processed whole grains of *P. Vulgaris*. The ash, crude fibre and fat contents were observed to be significantly ($p < 0.05$) lower in the fermented bean samples compared to the raw bean

samples. On the other hand, the crude protein and carbohydrate contents were observed to be significantly $\{p < 0.05\}$ higher in the fermented bean samples when compared to the raw bean samples. There was no significant $(p > 0.05)$ change in the moisture contents of fermented bean samples when compared to the raw bean samples. (Table 4.1).

The ash, crude fibre and fat contents were observed to be significantly $(p < 0.05)$ Lower in unfermented bean samples boiled with the ordinary cooking pot (BUC) and pressure pot (BUP) compared to raw. However, there was no significant $(p > 0.05)$ difference in the ash, fat and crude fibre contents of BUC when compared to BUP. The crude protein contents of BUC was also observed to be significantly $(P < 0.05)$ lower when compared to the raw bean samples and the BUP. The carbohydrate contents the bean samples was observed to be significantly $(p < 0.05)$ higher in BUC and BUP when compared to the raw. The carbohydrate contents of BUC was significantly $(p < 0.05)$ higher when compared to BUP. There was no significant $(P > 0.05)$ change in the moisture contents of the raw when compared to BUC and (BUP) (Table 4.2).

In the fermented beans boiled with ordinary cooking pot (BFC) and the fermented beans boiled with pressure pot (BFP), the ash, fat and crude fibre contents were observed to be significantly $(p < 0.05)$ lower when compared to the raw bean samples. Protein content of the fermented bean samples and samples fermented and boiled with the ordinary cooking pot (BFC) was observed to be significantly $(p < 0.05)$ higher when compared to the raw bean samples and the fermented bean samples boiled with the pressure pot (BFP). The carbohydrate contents of BFC and BFP were observed to be significantly $(p < 0.05)$ higher compared to the raw bean samples. The ash, fat, crude protein, crude fibre and carbohydrate contents show no significant $(p > 0.05)$ change in BFC when compared to fermented bean

samples. However, the moisture content was observed to be significantly ($p < 0.05$) higher in BFC when compared to the fermented bean samples. The fat content was observed to be significantly ($p < 0.05$) lower in BFP when compared to BFC. The carbohydrate content was observed to be significantly ($p < 0.05$) lower in BFC compared to BFP. The ash and crude fibre contents were not significantly ($p > 0.05$) different in BFC compared to BFP. (Table 4.3).

4.3 Effects of Open Fermentation of Whole Grains on the Amino Acid Profile of Red Kidney Beans (*Phaseolus vulgaris*).

The seventeen (17) amino acids determined were observed to be not significantly ($p > 0.05$) different in fermented bean samples when compared to the raw bean samples. In both fermented and unfermented bean samples, glutamic acid was the highest (13.71 and 13.18 respectively) followed by aspartic acid (12.31 and 11.20 respectively), the least amino acid was cystein (1.32 and 1.19 respectiely) (Table 4.4).

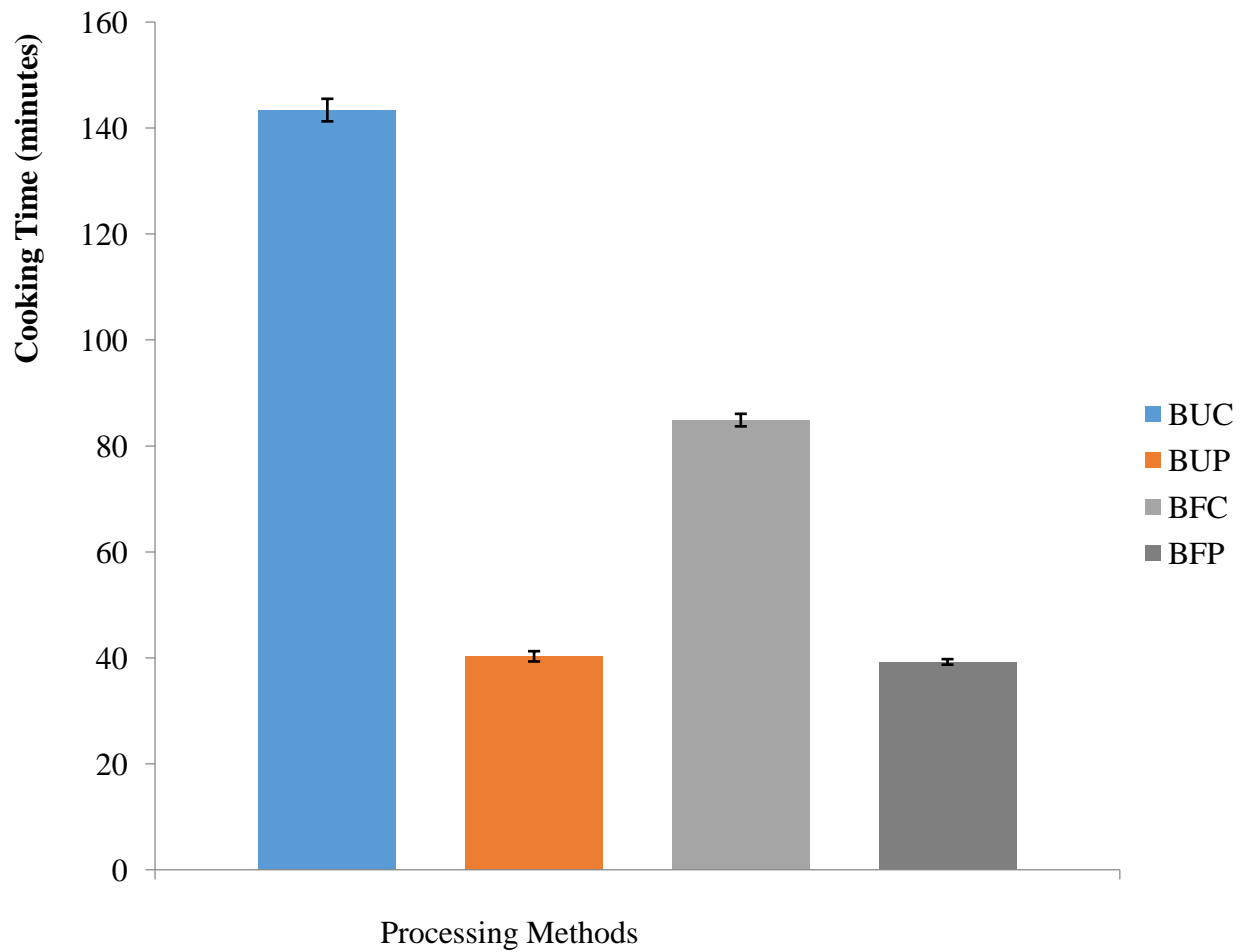


Figure 4.1: Cooking Time of the Different Processing Methods

BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot. BFC - Fermented beans boiled with ordinary cooking pot. BFP - Fermented beans boiled with pressure pot.

Proximate Composition

Processing Method	Ash (%)	Crude Protein (%)	Fat (%)	Moisture (%)	Crude Fibre (%)	Carbohydrate (%)
Raw	5.29± 0.05 ^a	24.62± 0.21 ^b	10.97 ±0.13 ^a	2.24 ±0.05 ^a	7.02± 0.47 ^a	50.86 ±0.29 ^b
Fermented	3.45± 0.28 ^b	26.12 ±0.19 ^a	8.92± 0.27 ^b	2.53± 0.25 ^a	3.80 ±0.25 ^b	55.18± 0.65 ^a

Table 4.1: Effect of Fermentation on the Proximate Contents of *Phaseolus vulgaris*

Values are Mean ± Standard Deviation. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05)

Table 4.2: Effects of Boiling On the Proximate Contents of Unfermented *P. Vulgaris*

Processing Method	Proximate Composition					
	Ash (%)	Crude Protein (%)	Fat (%)	Moisture (%)	Crude Fibre (%)	Carbohydrate (%)
Raw	5.29 ±0.05 ^a	24.62± 0.21 ^a	8.92 ±0.27 ^a	2.24± 0.05 ^a	7.02 ±0.47 ^a	50.86 ±0.29 ^c
BUC	4.01± 0.12 ^b	22.32±0.44 ^b	7.32± 0.61 ^b	2,54± 0.18 ^a	4.45± 0.39 ^b	62.83± 1.32 ^a
BUP	4.17 ±0.05 ^b	24.45± 0.26 ^a	6.63± 0.42 ^b	2.15± 0.08 ^a	4.85± 0.32 ^b	57.76 ±0.25 ^b

Values are mean ± standard deviation. Values having the same alphabet in the same column are statistically the same (P>0.05). While values having different alphabets in the same column are statistically different (P<0.05). BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot.

Proximate Composition

Processing Method	Ash (%)	Crude Protein (%)	Fat (%)	Moisture (%)	Crude Fibre (%)	Carbohydrate (%)
Raw	5.29± 0.05 ^a	24.62 ±0.21 ^b	10.97± 0.13 ^a	2.24± 0.05 ^b	7.02± 0.47 ^a	50.86 ±0.29 ^c
Fermented	3.45± 0.28 ^b	26.12 ±0.19 ^a	8.92± 0.27 ^b	2.53± 0.25 ^b	3.80± 0.25 ^b	55.18± 0.65 ^b
BFC	3.16± 0.03 ^b	26.0±1 0.10 ^a	8.69± 0.41 ^b	3.45± 0.04 ^a	3.32± 0.33 ^{bc}	55.29± 0.16 ^b
BFP	3.21± 0.12 ^b	24.80 ±0.22 ^b	6.93± 0.10 ^c	2.38± 0.06 ^b	2.87± 0.19 ^c	59.81± 0.68 ^a

Table 4.3: Effects of Cooking on the Proximate Contents of Fermented *P. Vulgaris*

Values are mean ± standard deviation. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05). BFC - Fermented beans boiled with ordinary cooking pot. BFP - Fermented beans boiled with pressu pot.

Table 4.4: Effect of Fermentation on the Amino Acid Profile of *P. vulgaris*

Amino Acid	Concentration (g/100g of Protein)	
	Unfermented	Fermented
Lysine	6.31± 0.11 ^a	6.64 ±0.14 ^a
Histidine	3.41± 0.09 ^a	3.59 ±0.13 ^a
Arginine	7.60± 0.13 ^a	8.28 ±0.08 ^a
Aspartic Acid	11.20± 0.15 ^a	12.31 ±0.02 ^a
Threonine	3.70 ±0.14 ^a	4.30 ±0.00 ^a
Serine	4.01± 0.02 ^a	4.20± 0.10 ^a
Glutamic Acid	13.18± 0.09 ^a	13.71± 0.09 ^a
Proline	3.31± 0.01 ^a	3.31± 0.01 ^a
Glycine	4.08± 0.01 ^a	4.54± 0.06 ^a
Alanine	3.91± 0.03 ^a	3.99± 0.05 ^a
Cystein	1.19 ±0.03 ^a	1.32± 0.00 ^a
Valine	5.44± 0.21 ^a	5.56± 0.10 ^a
Methionine	1.25± 0.02 ^a	1.33± 0.06 ^a
Isoleucin	3.49 ±0.01 ^a	3.58± 0.05 ^a
Leucine	7.70 ±0.00 ^a	8.00± 0.17 ^a
Tyrosine	3.18 ±0.06 ^a	3.49 ±0.09 ^a
Phenyl Alanine	5.74 ±0.03 ^a	5.99± 0.06 ^a

Values are mean ± standard deviation. Values having the same alphabet in the same row are statistically the same (P>0.05). While values having different alphabets in the same row are statistically different (P<0.05).

4.4 Mineral Contents of the Different Processing Methods.

The potassium, calcium, iron and zinc contents were observed to be significantly ($p < 0.05$) lower in fermented bean samples when compared to the raw bean samples. Sodium and magnesium were observed to be not significantly ($p > 0.05$) lower in the fermented bean samples when compared to the raw bean samples (Table 4.5).

The potassium, calcium, magnesium and zinc were observed to be significantly ($p < 0.05$) lower in BUC and BUP when compared to the raw bean samples. The iron content of BUC was also observed to be significantly ($p < 0.05$) lower compared to the raw bean samples. While in the case of BUP, there was no significant ($p > 0.05$) difference when compared to the raw bean samples. Potassium, iron and zinc were observed to be significantly ($p < 0.05$) lower in BUC when compared to the BUP. Calcium and magnesium contents were observed not to be significantly ($p > 0.05$) different in BUC when compared to BUP. The iron content of BUP was observed not to be significantly ($p > 0.05$) lower compared to the raw bean samples. There was no significant difference in the sodium contents of the raw, BUC and BUP. (Table 4.6).

The potassium and zinc contents of BFC and BFP were observed to be significantly ($p < 0.05$) lower when compared to the fermented bean samples and raw. Calcium and iron were observed to be significantly ($p < 0.05$) change in BFC and BFP when compared to the raw, but show no significant ($p > 0.05$) lower compared to the fermented bean samples. There was no significant ($p < 0.05$) difference in the sodium and magnesium content of the raw bean samples, fermented bean samples, BFC and fermented BFP. Potassium, sodium,

calcium, magnesium, iron and zinc contents of fermented bean samples, BFC were insignificantly ($p>0.05$) different when compared to BFP. (Table 4.7)

Table 4.5: Effect of Open Fermentation on the Mineral Composition of *P.vulgaris*

Mineral Contents						
Processing Method	Potassium (%)	Sodium (%)	Calcium (ppm)	Magnesium (%)	Iron (ppm)	Zinc (ppm)
Raw	1.20 ± 0.02 ^a	1.45 ± 0.01 ^a	926.69 ± 0.21 ^a	0.19 ± 0.20 ^a	744.11 ± 0.14 ^a	82.46 ± 0.01 ^a
Fermented	0.93 ± 0.02 ^b	1.38 ± 0.02 ^a	729.32 ± 0.23 ^b	0.18 ± 0.44 ^a	597.06 ± 0.06 ^b	71.47 ± 0.18 ^b

Values are mean ± standard deviation.. Values having the same alphabet in the same column are not statistically different (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05).

Table 4.6: Effect of boiling on the Mineral Contents of Unfermented *P. vulgaris*

Processing Method	Mineral Content					
	Potassium (%)	Sodium (%)	Calcium (ppm)	Magnesium (%)	Iron (ppm)	Zinc (ppm)
Raw	1.2 ± 0.02 ^a	0.15 ± 0.0 ^a	926.69 ± 0.21 ^a	0.19 ± 0.20 ^a	744.11 ± 0.14 ^a	82.46 ± 0.01 ^a
BUC	0.88 ± 0.01 ^c	0.16 ± 0.01 ^a	522.56 ± 0.02 ^b	0.15 ± 0.27 ^b	597.06 ± 0.11 ^b	58.28 ± 0.06 ^b
BUP	0.95 ± 0.05 ^b	0.15 ± 0.11 ^a	597.24 ± 0.04 ^b	0.16 ± 0.30 ^b	714.71 ± 0.07 ^a	38.28 ± 0.10 ^c

Values are mean ± standard deviation. BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot. Values having the same alphabet in the same column are not statistically different (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05).

Table 4.7: Effects of Cooking on the Proximate Contents of Unfermented *P. Vulgaris*

Processing Method	Mineral Content					
	Potassium (%)	Sodium (%)	Calcium (ppm)	Magnesium (%)	Iron (ppm)	Zinc (ppm)
Raw	1.20 ± 0.02 ^a	0.15 ± 0.01 ^a	926.69 ± 0.21 ^a	0.19 ± 0.20 ^a	744.11 ± 0.14 ^a	82.46 ± 0.01 ^a
Fermented	0.93 ± 0.02 ^b	0.14 ± 0.02 ^a	729.32 ± 0.23 ^b	0.18 ± 0.44 ^a	597.06 ± 0.06 ^b	71.47 ± 0.18 ^b
BFC	0.63 ± 0.08 ^c	0.14 ± 0.05 ^a	714.66 ± 0.19 ^b	0.18 ± 0.02 ^a	567.65 ± 0.04 ^b	45.10 ± 0.26 ^c
BFP	0.60 ± 0.07 ^c	0.14 ± 0.02 ^a	723.31 ± 0.0022 ^b	0.17 ± 0.09 ^a	538.24 ± 0.04 ^b	45.10 ± 0.07 ^c

Values are mean ± standard deviation. Mean percentage increase. Mean percentage decrease BFC - Fermented beans boiled with ordinary cooking pot. BFP – Fermented beans boiled with pressure pot. Values having the same alphabet in the same column are not statistically different (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05).

4.5 Antinutrient Contents of the Different Processing Methods

The phytates, alkaloids, oxalates, cyanides and tannins contents of the fermented bean samples were observed to be significantly ($p < 0.05$) lower compared to the raw bean samples (Table 4.8).

The phytates, alkaloids, oxalates, cyanides and tannins contents were observed to be significantly ($p < 0.05$) lower in unfermented bean samples boiled with the ordinary cooking pot (BUC) and unfermented bean samples boiled with the pressure pot (BUP) compared to the raw bean samples. Phytates, alkaloids, oxalates and cyanides were observed to be significantly ($p < 0.05$) lower in BUC compared to BUP, the tannin content however, show no significant ($p > 0.05$) change (Table 4.9).

Phytates, alkaloids, oxalates and cyanides were observed to be significantly ($p < 0.05$) lower in fermented bean samples boiled with the ordinary cooking pot (BFC) and fermented bean samples boiled with the pressure pot (BFP) compared to the raw and fermented bean samples. The tannin content of the fermented bean sample show no significant ($p > 0.05$) change lower BFP when compared to the fermented bean samples. Phytates alkaloids and tannins were observed to be significantly ($p < 0.05$) lower in BFC when compared to BFP. On the other hand, oxalates and cyanides were observed to be significantly ($P < 0.05$) lower in BFP compared to the BFC (Table 4.10).

Table 4.8: Effects of Open Fermentation on the Antinutrients Contents of *P. vulgaris*

Processing Method	Antinutrient Content				
	Phytic Acids (%)	Alkaloids (%)	Oxalates (mg/100g)	Cyanides (mg/100g)	Tannins (%)
Raw	0.31 ± 0.01 ^a	5.13 ± 0.11 ^a	19.58 ± 0.06 ^a	14.52 ± 0.09 ^a	1.72 ± 0.05 ^a
Fermented	0.26 ± 0.01 ^b	2.10 ± 0.03 ^b	18.23 ± 0.05 ^b	10.93 ± 0.11 ^b	1.20 ± 0.03 ^b

Values are mean ± standard deviation. Values having the same alphabet in the same column are not statistically different ($P > 0.05$) while values having different alphabets in the same column are statistically different ($P < 0.05$).

Table 4.9: Effects of Cooking on the Antinutrient Contents of Unfermented *P. Vulgaris*

Processing Method	Antinutrient Content				
	Phytic Acids (%)	Alkaloids (%)	Oxalates (mg/100g)	Cyanides (mg/100g)	Tannins (%)
Raw	0.31 ± 0.01 ^a	5.13 ± 0.11 ^a	19.58 ± 0.06 ^a	14.52 ± 0.09 ^a	1.72 ± 0.05 ^a
BUC	0.23 ± 0.00 ^b	3.33 ± 0.03 ^c	16.88 ± 0.03 ^c	11.42 ± 0.08 ^c	1.50 ± 0.14 ^c
BUP	0.25 ± 0.00 ^c	4.34 ± 0.06 ^b	18.90 ± 0.02 ^b	12.43 ± 0.14 ^b	1.60 ± 0.02 ^b

Values are mean ± standard deviation. BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot. Values having the same alphabet in the same column are not statistically different (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05)

Table 4.10: Effects of Cooking on the Antinutrient Contents of Fermented *P. vulgaris*

Processing Method	Antinutrient Content				
	Phytic Acids(%)	Alkaloids (%)	Oxalates (mg/100g)	Cyanides (mg/100g)	Tannins (%)
Raw	0.31 ± 0.01 ^a	5.13 ± 0.11 ^a	19.58 ± 0.06 ^a	14.52 ± 0.09 ^a	1.72 ± 0.05 ^a
Fermented	0.26 ± 0.01 ^b	2.10 ± 0.03 ^b	18.23 ± 0.05 ^b	10.93 ± 0.11 ^b	1.20 ± 0.03 ^b
BFC	0.12 ± 0.01 ^d	1.51 ± 0.03 ^d	13.88 ± 0.06 ^c	8.75 ± 0.01 ^c	0.94 ± 0.05 ^d

BFP	0.19 ± 0.00^c	1.80 ± 0.02^c	9.80 ± 0.10^d	6.74 ± 0.04^d	1.09 ± 0.04^c
-----	-------------------	-------------------	-------------------	-------------------	-------------------

Values are mean \pm standard deviation.. BFC - Fermented beans boiled with ordinary cooking pot. BFP – Fermented beans boiled with pressure pot. Values having the same alphabet in the same column are not statistically different ($P>0.05$) while values having different alphabets in the same column are statistically different ($P<0.05$)

4.6 Effects of the Different Processing Methods on the Protein Digestibility Profile of Red Kidney Beans (*Phaseolus vulgaris*).

The digestibility value was observed to be significantly ($p < 0.05$) higher in unfermented bean samples boiled with the ordinary cooking pot (BUC) and unfermented bean samples boiled with the pressure pot (BUP) when compared to the raw bean samples. However, digestibility value of unfermented bean samples boiled with the pressure pot (BUP) was observed to be not significantly ($p > 0.05$) higher compared to the digestibility value of BUC. The digestibility value was observed to be significantly ($p < 0.05$) higher in the fermented bean samples compared to the digestibility value of raw bean samples, unfermented BUC and BUP. The digestibility value of fermented bean samples boiled with the ordinary cooking pot (BFC) and fermented bean samples boiled with the pressure pot (BFP) were observed to be significantly ($p < 0.05$) higher compared to the raw BUC and unfermented BUP. The digestibility value of BFC was observed to be significantly ($p < 0.05$) higher compared to the fermented bean samples, while the digestibility value of BFP was observed to be not significantly ($p > 0.05$) higher compared to the fermented bean samples (Table 4.11)

Table 4.11: Digestibility Profile of the Differently Processed Red Kidney Beans (*P. vulgaris*)

Processing Method	%Protein in Undigested Sample	%Protein in Digested Sample	Digestibility Value (%)
Raw	24.67±0.21	8.01±0.25	32.51±0.69 ^d
BUC	22.32±0.44	9.78±0.42	43.86±0.99 ^c
BUP	26.45±0.26	11.94±0.18	45.39±0.99 ^c
Fermented	26.12±0.19	14.36±0.22	54.87±1.29 ^b
BFC	26.01±0.19	15.37±0.13	59.07±0.02 ^a
BFP	24.80±0.22	13.53±0.07	57.60±0.79 ^b

Values are mean ± standard deviation. BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot. BFC - Fermented beans boiled with ordinary cooking pot. BFP – Fermented beans boiled with pressure pot. Values having the same alphabet in the same column are not statistically different ($P>0.05$) while values having different alphabets in the same column are statistically different ($P<0.05$)

CHAPTER FIVE

5.0 DISCUSSION

There was significant ($p < 0.05$) decrease in the cooking time of fermented beans with pressure pot and ordinary cooking pot as well as that of unfermented beans cooked with pressure pot (Figure 4.1). The decrease in cooking time of fermented beans cooked with both pressure pot and an ordinary cooking pot. This finding is consistent with the findings of Uрга *et al.*, (2006) who worked on the effects of blanching and soaking on some physical characteristics of grass pea. Uрга *et al.* (2006) attributed the reduction in cooking time to the increased permeability of the seed coats to hot water which could be as a result of softening and disruption of the seed coats.

The results showed that ash, fat and crude fibre contents significantly ($p < 0.05$) decreased during the combined processing methods of fermentation and cooking. Crude protein also decreased significantly ($p < 0.05$) during cooking with the ordinary cooking pot but increased significantly during fermentation. Carbohydrate was observed to significantly ($p < 0.05$) increase during all the processing methods. The decrease in fat during fermentation agrees with the findings of Elmaki *et al.*, (2007) and Afify *et al.*, (2012d) who worked on the effects of different processing methods on the chemical compositions of different sorghum cultivars. This decrease in fat during fermentation also agrees with the findings of Babalola and Giwa (2012) who worked on the effects of fermentation on the nutritional and antinutritional properties of fermenting soybeans. The decrease in fat during cooking agrees with the findings of Mubarak (2005), Okrah (2008) and Afify *et al.* (2012d) who worked on effects of processing on chemical composition of different legume cultivars. These decrease

in fat contents during fermentation might be as a result of decrease in most fatty acids during cooking and fermentation process. Decrease in crude fibre contents during the processing methods agrees with the findings of Alemu (2009) and Afify *et al.*, (2012d) who observed decrease in crude fibre contents during fermentation and cooking of different sorghum cultivars. This decrease in the crude fibre contents during fermentation could be due to the activities of methabolising organisms using fibre as energy source hence decreasing the fibre contents (Ganiyu, 2006; Babalola and Giwa, 2012; Difo *et al*(2014). The decrease in fibre contents during cooking could be as a result of increase in the soluble components due to high temperature during thermal processing. The decrease in ash content during the processing methods is consistent with the findings of Mubarak; (2005), Okrah, (2008); Alemu (2009); Afify *et al* (2012d) and Difo *et al* (2014) but disagrees with the findings of Babalola and Giwa, (2012), who attributed it to theleaching of some of the minerals in the processing water. Increase in the protein contents during fermentation is consistent with the findings of Afify *et al* (2012d);Babalola and Giwa, (2012) and Difo *et al* (2014). This could be due to production of enzymes by methabolizing microbes, hence increasing the protein contents (Babalola and Giwa, 2012). Protein was observed to decrease during cooking and this agrees with the findings of Afify *et al* (2012b) who attributed it to the possible leaching of some soluble nitrogen into the cooking water. Increase in the carbohydrate contents during the processing methods could be attributed to the increase in the soluble contents due to actions of fermenting microbes (Babalola and Giwa, 2012; Difo *et al* 2014) and high thermal processing temperature during cooking.

Open fermentation of whole grains of red kidney beans has no significant ($p < 0.05$) effect on the amino acid compositions of red kidney beans (Table 4.4). Like other legumes sulphur containing amino acids, cysteine and methionine were the limiting amino acids (Chau et al., 1997). When combined with other protein sources, *P. vulgaris* could serve as a good source of amino acids. Red kidney bean was also found to contain high amount of Aspartic acid, glutamic acids, leucine and lysine in comparison to the other amino acids contained. This slight increase in the amino acid contents could be due to the increase in protein solubility (Afify et al 2012d).

The minerals determined in this research include: sodium, potassium, calcium, magnesium, iron and zinc. Based on the results obtained, there was a significant ($p < 0.05$) decrease in the calcium, zinc, iron and potassium contents of the beans during the open fermentation of whole grains while there was no significant ($p > 0.05$) decrease for sodium and magnesium. This decrease in minerals agrees with the findings of Alemu, 2009; Afify et al., (2012d); Difo et al., (2014) who attributed it to the leaching of these minerals in the fermenting water. The decrease in these minerals could also be as a result of utilization of these minerals by fermenting micro organisms (Zamora and Fields, 1979). Sodium, potassium, calcium, magnesium, iron and zinc all significantly ($p < 0.05$) decreased during the different cooking methods. This is consistent with the findings of Saharan et al., (2001); Alemu, (2009) and Afify et al., (2012d). This decrease in minerals could be as a result of the increase in solubility of these minerals during the cooking process (Saharan et al., 2001) (Table 4.5, 4.6 and 4.7).

Phytic acids, alkaloids, oxalic acids, cyanides and tannins were evaluated during this research. During the open fermentation of the whole grains, phytic acids, alkaloids, cyanogenic glycosides and tannins all significantly ($p < 0.05$) decreased. This is in agreement with the findings of Afify *et al* (2012d); Babalola and Giwa, (2012) and Difo *et al.*, (2014) who attributed this decrease to degradative actions of fermenting micro organisms (Table 4.8). The reduction in these antinutritional factors could also be as a result of leaching of these antinutrients into the fermentation water (Osman, 2007; Soetan and Oyewole, 2009). There was significant ($p < 0.05$) decrease in the phytic acids, alkaloids and cyanides of red kidney beans boiled with ordinary cooking pot (Table 4.9). The significant decrease in the the above mentioned antinutrients could be as a result of leaching of these antinutrients in the cooking water since it was drained of the water after cooking (Osman, 2007). Boiling of the fermented beans with ordinary cooking pot shows significant ($p < 0.05$) decrease in all the antinutrients determined while three of the antinutrients (Phytic acids, oxallic acids and cyanogenic glycosides) for red kidney bean smple boiled with pressure cooking pot significantly decreased (Table 4.10). This could be attributed to the leaching of these antinutrients in the boiling water (Osman, 2007) which could be as a result of hydrolysis of the antinutrients in the boiling water (Soetan and Oyewole, 2009).

The result of this research shows that cooking significantly ($p < 0,05$) increased the digestibility values of both unfermented and fermented red kidney beans (Table 4.11). This finding agrees with that of Nakitto *et al.* (2015) but disagrees with that of Carbonaro *et al.*, (1997) and Sulieman *et al.*, (2008). Nakitto *et al.* (2015) attributed the low digestibility values to the high tannins and phytate contents which bind to the proteins and thus limiting

their solubility and reducing the binding of the proteases to the proteins. The digestibility of boiled bean samples (either cooked with the pressure pot or the ordinary cooking pot) was found to significantly ($p < 0.05$) increase. Sulieman *et al.*, (2008) however, reported a decrease in the digestibility values of protein in lentils and faba beans during cooking of the beans in contrast to Nakitto *et al.*, (2015) who reported an improvement in the *in vitro* protein digestibility of a 'k131 bean variety' during cooking especially when it was dehulled. Nikitto *et al.*, (2015) attributed this increase in protein digestibility of the beans to reduction of phytate and tannin levels beyond detection as a result of dehulling.

The digestibility values of fermented bean samples (either boiled or unboiled) was found to significantly ($p < 0.05$) increase more than those of the unfermented boiled bean samples. Fermentation thus, improved the *in vitro* protein digestibility. This finding agrees with Lopez *et al.*, (1983) report. This improvement in the digestibility of the fermented sample could be as a result of decrease in the phytate and tannin contents which could improve the solubility of the protein hence enhancing its digestibility. This improvement in protein digestibility could also be attributed to the degradation of complex molecules like fibre during fermentation hence increasing access of substrates to the active sites of digestive enzymes. The improvement in the *in vitro* protein digestibility could also be as a result of the synergistic reduction in tannin contents during combined processing methods of cooking and fermentation (Soetan and Oyewole, 2009). Cooking and fermentation have been reported to result in the break down tannin-enzyme and protein-tannin complexes and released free tannins which subsequently leached out the products (Ikemefuna *et al.*, 1991), hence, increasing the access to substrates to the active sites of digestive enzymes.

This research result showed significant ($p < 0.05$) reduction in cooking time of *P. vulgaris*. This could be of economic importance as it will help reduce the cost of processing through reduction in the amount of cooking fuel used hence encouraging increase in consumption of *P. vulgaris*. Although, the fermentation period of three (3) days might be discouraging, *P. vulgaris* can be fermented in large quantity after harvest and kept for future use as this helps to disrupt the seed coats hence, softening the seed coats. Although there was significant ($p < 0.05$) reduction in the ash, fat and crude fibre content during these processing methods, the combined method of fermentation and cooking can be employed and alternate sources of these nutrients be used to replenish these nutrients when *P. vulgaris* is processed for consumption. Despite the significant reduction in protein contents when unfermented *P. vulgaris* is cooked with the ordinary cooking pot (BUC), there was overall improvement in protein content and protein quality when the combined processing method of fermentation and cooking was employed. The amino acid contents and the protein digestibility value increased during these processes. The improvement in the *in vitro* protein digestibility could be as a result of decrease in tannins which precipitate proteins making it difficult for their digestion to take place, hence, increasing its solubility and absorption in the body system when consumed. Decrease in the contents of minerals determined during the combined processing methods could be countered by the reduction in the level of antinutrients (Such as phytates and tannins) which limit the bioavailability of these nutrients (Minerals). The decrease in cyanide content during the combined processing methods could help in the transport of oxygen in the body system, hence, reduction in risk of suffocation. This is because cyanides as an antinutrient inhibits the complex of electron

transport chain hence, preventing the transport of oxygen, subsequently resulting in suffocation.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

- This research findings showed that fermentation significantly ($p < 0,05$) reduced cooking time of red kidney beans cooked with ordinary cooking pot compared to the unfermented cooked with the same ordinary cooking pot.
- There was no significant ($p > 0.05$) difference in the cooking time of fermented and unfermented beansamplescooked with the pressure pot pot’
- Open fermentation of *P. Vulgaris* whole grain resulted in a significant ($P < 0.05$) increase in its protein content, but boiling of unfermented beans with the ordinary cooking pot significantly($p < 0.05$) decrease the crude protein content

- Total ash, crude fat and crude fibre contents all significantly ($p < 0.05$) decreased during the different processing methods while carbohydrate contents were insignificantly affected.
- The amino acid contents were not significantly ($p > 0.05$) increased during fermentation. Sulphur containing amino acids, methionine and cysteine were the limiting amino acids but appreciated by 6.64% and 10.92% during fermentation respectively.
- During the different processing methods, potassium, calcium, iron and zinc all significantly ($p < 0.05$) decreased.
- The antinutrients of *P. vulgaris* all significantly ($p < 0.05$) decreased during the open fermentation and the cooking of the fermented and unfermented beans by the two boiling methods.
- *P. vulgaris* was found to have low digestibility value which significantly ($p < 0.05$) increased during cooking and fermentation with more increase evident in the cooked fermented bean samples.

6.2 CONCLUSION

There was overall improvement in the *In vitro* protein digestibility, reduction of cooking time and antinutritional factors when *P. vulgaris* was fermented and cooked. This justifies

the fact that combining both fermentation and cooking results in the overall improvement in the nutritional value of *P. vulgaris* as against cooking without fermentation.

6.3 RECOMMENDATIONS

- Since the presoaking of the red kidney beans reduced the cooking time significantly when the traditional cooking method was employed, this processing method is recommended to be used in the preparation of *P. vulgaris*.
- The processed beans can be used in the preparation of ‘moi moi’ with improved nutritional quality since the seed coats are soaked during the fermentation process.
- Further work should be done to determine the *in vivo* protein digestibility of *P. vulgaris*.

REFERENCES

- Adams, M.R (1990): topical aspects of fermented foods. Trends in Food Science and Technology 1, 141-144.
- Adeyeye, E.I. and E.O. Afolabi, (2004). Amino acid composition of three different types of land snails consumed in Nigeria. *Journal of Food Chemistry* 85, 535-539.
- Afify AMR, El-Beltagi HS, Abd El-Salam SM, Omran AA (2012b). Protein solubility, digestibility and fractionation after germination of sorghum varieties. *PLoS ONE* 7(2), 31154, 1-6.
- Afify AMR, El-Beltagi HS, Abd El-Salam SM, Omran AA (2012d). Effect of Soaking, Cooking, Germination and Fermentation Processing on Proximate Analysis and

Mineral Content of Three White Sorghum Varieties (*Sorghum bicolor* L. Moench).
Not Bot Horti Agrobo, 2012, 40(2), 92-98

Aguilera, J. M., E. W. Lusas., M. A. Uebersax., M. E. Zabik(1982). "Roasting of Navy Beans (*Phaseolus vulgaris*) by Particle-to-Particle Heat Transfer." *Journal of Food Science*47(3), 996-1000.

Akpanpunam, M. N. and Achinewha, S. C. (1985) Physical and Chemical Characteristics of refined vegetable oils from rubber seeds (*Hevea brasiliensis*) and bread-fruit (*Arthocapus altilis*). *Qualitas plantarum plant foods human nutrition* 35, 353-358.

Alemu MK (2009). The Effect of Natural Fermentation on Some Antinutritional Factors, Minerals, Proximate Composition and Sensory *Characteristics in Sorghum Based Weaning Food. M.Sc. Thesis, Addis Ababa, University., Ethiopia*, p 83.

Aliya, S. and Geervani, P. (1981): An assessment of the protein quality and vitamin B content of commonlu used fermented products of legumes and millets. *Journal of the science of food and agriculture* 32, 837-842.

Alm, L. (1982). Effect of fermentation of B-vitamin content of milk in Swenden. *Journal of dairy science* 65,353-359.

Anuraga, M., Duarsa, P., Hill, M.J., & Lovett, J.V.(1993). Soil moisture and temperature affect condensed tannin concentrations and growth in *Lotus corniculatus* and *Lotus pedunculatus*. *Australian Journal of Agricultural Research*, 44, 1667-1681.

AOAC (1984) Official Methods of Analysis. Association of Official Analytical Chemists.

AOAC (1990) Official Methods of Analysis. Association of Official Analytical Chemists.

AOAC (2006) Official Methods of Analysis. 15th edition, Association of Official Analytical Chemists, Washington DC.

Aparicio-fermandez X., Yousef G.G., Loarca-Pina G., Gonzalez de Mejia E., Lila M.A., (2005). Characterization of polyphenolics in the seed coats of Black jamapa bean (*Phaseolus vulgaris* L.). *Journal of agricultural food chemistry*, 53, 465-4622.

Awuah, G. B., Ramaswamy, H. S. And Economides, A. (2007) Thermal Processing and Quality: Principles and Overview. *Journal of Chemical Engineering and Processing*. 46, 584-682

Azizah, A.H and Zainon, H. (1997).—Effect of processing on dietary fiber contents of selected legumes and cereals|| . *MalaysianJournal of Nutrition*, 3 (1), 131-136.

- Babalola, R. O. and Giwa, O. E. (2012). Effect of fermentation on nutritional and anti-nutritional properties of fermenting Soy beans and the antagonistic effect of the fermenting organism on selected pathogens. *International Research Journal of Microbiology (IRJM)* (ISSN: 2141-5463) Vol. 3(10) pp. 333-3
- Barampama, Z., and Simard, R.E (1994). Oligosaccharide, anti-nutritional factors and protein digestibility of dry beans as affected by processing. *Journal of food science*. 59, 833-838
- Bar-Yosef, O.(1998). "The Natufian culture in the Levant, threshold to the origins of agriculture." *Evolutionary Anthropology: Issues, News, and Reviews*65, 159-177.
- Bazzano LA, He J, Ogden LG, Loria CM, Whelton PK. (2003) Dietary fiber intake and reduced risk of coronary heart disease in US men and women: the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Arch Intern Med*. 2003 Sep 8;163(16):1897-904.
- Belshe, D. M., Boland, S. D., & O'Brien, D. (2001). Economic Issues with Dry Edible Beans. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Kansas State University.
- Beninger, C.W. hosfield, G.L.(2003). Antioxidant activity of extract, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *Journal of Agricultural Food Chemistry*. 51, 7879-7883.
- Birk Y. (1996). Protein proteinase inhibitors in legume seeds – overview. *Arch Latinoam Nutr*. 44 (4),26S-30S.
- Boateng, J., Verghese, M., Walker, L.T., & Ogutu, S. (2008). Effect of processing on antioxidant contents in selected dry beans (*Phaseolus spp.* L.). *LWT Food Science and Technology*, 41, 1541–1547.
- Brennan, J. G. (2006) *Food Processing Handbook*.. Copyright © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 3-527-30719-2. Pp 33-35.
- Bressani, R. (2003). Grain quality of common beans. *Food Reviews International*, 9(2), 237-297.
- Bressani, R., Murillo, B., & Elias, L. G. (1974). Whole Soybeans As A Means Of Increasing Protein And Calories In Maize-Based Diets. *Journal of Food Science*, 39(3), 577-580.

- Brigidi, P., Bolognani, F. Rossi, M., Cerre, C. and Matteuzzi, D. (1995) Cloning of gene for Cholesterol oxidase in *Bacillus spp.*, *Lactobacillus reuteri* and its expression in *Escherichia coli*. *Letters in applied microbiology* 17, 61-64.
- Brian, M. & Linda, H. M. (2008). *Practical fermentation technology*. Wiley.
- Carmona-Garcia, R., Osorio-Dyaz, P., Agama-Acevedo, E., Tovar, J., & Bello-Perez, L.A. (2007). Composition and effect of soaking on starch digestibility of *Phaseolus vulgaris* L. cv. 'Mayocoba'. *International Journal of Food Science and Technology*, 42, 296–302.
- Carbonaro, M., Cappelloni, M., Nicoli, S., Lucarini, M., & Carnovale, E. (1997). Solubility-digestibility relationship of legume proteins. *Journal of Agricultural and Food Chemistry*, 45(9), 3387-3394.
- Chau, C. F., Cheung, P. C., & Wong, Y. S. (1997). Effect of cooking on content of amino acids and antinutrients in three Chinese indigenous legume seeds. *Journal of the Science of Food Agriculture*, 75, 447–452.
- Cheng X. and Tian J., (2011). Status and future perspectives of *Vigna* (mungbean and azuki bean) production and research in China. In: Tomooka N, Vaughan DA, eds. 14th NIAS int. workshop on genetic resources – Genetic resources and comparative genomics of legumes (*Glycine* and *Vigna*). Tsukuba: National Institute of Agrobiological Science. Pp83–86.
- Chompeeda P., T. and Fields, M. L. (1984) Effects of heat and fermentation on the extractability of minerals from soyabean meal and corn meal blends. *Journal of Food Sciences* 49, 566-568.
- Coffey DG, Uebersax MA, Hosfield GL and Brunner JR. (1985). Evaluation of the hemagglutinating activity of low-temperature cooked kidney beans. *Journal-of-Food-Science*. 50(1), 78-81, 87.
- Coffey DG, Uebersax MA, Hosfield GL and Bennink MR. (1992). Stability of Red Kidney Bean Lectin. *Journal of Food Biochemistry*. 16(1), 43-57.
- Coffey DG, Uebersax MA, Hosfield GL and Bennink MR. (1993). Thermal Extrusion and Alkali Processing of Dry Beans (*Phaseolus-Vulgaris* L). *Journal of Food Processing and Preservation*. 16, 421-431.
- Davila MA, Sangronis E, Granito M. (2003). Germinated or fermented legumes: food or ingredients of functional food. *Arch Latinoam Nutrition* 53(4), 348-54.

- Debouck, D. G, Toro, O. Paredes O.M, Johnson WC, Gepts P (1993). Genetic diversity and ecological distribution of *Phaseolus vulgaris* (Fabaceae) in northwestern South America. *Econ. Bot.* 47, 408–423.
- Difo, V. H., Onyike, E., Ameh, D. A., Njoku, G. C., & Ndidi, U. S. (2014). Changes in nutrient and antinutrient composition of *Vigna racemosa* flour in open and controlled fermentation. *Journal of Food Science and Technology*, 1-6.
- Ecoport, (2013). Ecoport database. Ecoport .
- Edmundson, A. B., Ehy, Kathryn, Sly., Dayle, A., Wesiholin, A.F. Powers, AD., and Liener, I.E. (1971). Isolation and characterization of coneanavalin A polypeptide chains. *Biochemistry* 10, 3554 - 59.
- Eka O. U. (1980): Effect of fermentation on the nutrient status of locust beans. *Food Chemistry* 5, 303- 308.
- Elkowicz, K. and Sosulski, F. W. (1982). Antinutritive factors in eleven legumes and their air-classified protein and starch fraction. *Journal of Food Science.* 47, 524 - 528.
- Elmaki, H. B.; S. M. Abdel-Rahman; W. H. Idris; A. B. Hassan ; E. E. Babiker and A. H. El- Tinay (2007). Content of antinutritional factors and HCl-extractability of mineral from white bean (*Phaseolus vulgaris*). Cultivars: Influence of soaking and/or cooking. *Food Chemistry*, 100, 362–368.
- Ensminger AH, Esminger M. K. J, Kondale JE and Robson JRK..(1986) *Food for Health: A Nutrition Encyclopedia*. Clovis, California: Pegus Press. PMID.1, 5210.
- Evans, R. J. and Bandier, S. L. (1967). Nutritive value of legume seed protein. *J. Agric. Food* (hem. 15, 439-443.
- Fadahunsi, I. F. (2009). The effect of soaking, boiling and fermentation with *Rhizopus oligosporus* on the water-soluble vitamin content of Bambara groundnut. *Pakistan Journal of Nutrition*, 8(6), 835-840.
- Fahey, G.C., & Jung, H.G. (1989). Phenolic compounds in forages and fibrous feedstuffs. *Toxicants of Plant Origin*. (Vol IV. pp.123). CRC Press, Inc. Boca Raton, Fla.
- FAO, (2013). FAOSTAT. Food and Agriculture Organization of the United Nations’
- Fernandes, A.C., Nishida, W., & Costa Proenca, 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, 2209–2218.
- Gaden, E. L. (1956). *Chemical technology of fermentation*. McGraw-Hill.
- Gahlawat, P., and S. Sehgal(1992). —Phytic acid, saponins and polyphenols in weaning foods prepared from oven heated green gram and cereals.*Cereal Chemistry*69(4): 463–464.
- Gale, B. F. *Chemical Activities of the Bacteria* (1947). New York: Academic Press.
- Ganiyu Oboh (2006). Nutritional and antinutrient composition of produced from some fermented underutilized legumes. *J. Food Biochem.* 4514 - 4517.
- Gbadamosi, S. O., Abiose, S. H., & Aluko, R. E. (2012). Amino acid profile, protein digestibility, thermal and functional properties of Conophor nut (*Tetracarpidium conophorum*) defatted flour, protein concentrate and isolates. *International Journal of Food Science & Technology*, 47(4), 731-739.
- Genovese MI and Lajolo, FM. (1996). Effect of bean (*Phaseolus vulgaris*) albumins on phaseolin in vitro digestibility, role of trypsin inhibitors. *Journal of food biochem.* 20, 275-294.
- Gepts, P., Aragão, F. J., De Barros, E., Blair, M. W., Brondani, R., Broughton, W., & Yu, K. (2008). Genomics of *Phaseolus* beans, a major source of dietary protein and micronutrients in the tropics. In *Genomics of tropical crop plants* (pp. 113-143). Springer New York.
- Gloria, U., Jesse, M. P., Aranda, P., Lopez – Jurado, M., (2003) Effect of natural and controlled fermentation on chemical composition and nutrient dialyzability from beans (*Phaseolus vulgaris*). *Journal of Agricultural Food Chemistry* 51, 5144-5149.
- Goldin B. K and Gorbach S. L (1984): The effect of oral administration on *Lactobacillus* and antibiotics on intestinal bacterial activity and chemical induction of large bowel tumors. *Developments in Industrial Microbiology* 25,139-150.
- Gomes JC, Koch U and Brunner-JR. (1979). Isolation of a trypsin inhibitor from navy beans by affinity chromatography. *Cereal-Chemistry*; 56 (6) 525-529, 45 ref.
- González de Mejia S U., Vaiu, M.C., Reynoso-Camacho I.T and Loarca-Pifia G. (2005). Tannins, trypsin inhibitors and lectin cytotoxicity in tepaay (*Phaseolus acnE jfolius*) and common (*Phaseolus vulgaris*) beans. *Plant Foods Human Nutrition*,60, 137-145.

- Graf, E. (1989). Phytic Acid—Chemistry and Applications, Pilatus Press, Minneapolis, USA.
Cited from: Hidvegi M. and R. Lasztity (2002). Phytic acid content of cereals and legumes and interaction with proteins. *Periodica Polytechnica Chemical Engineering*, 46, 59-64.
- Graham PH, Hall AE and Coyne DP. (2003). Research Highlights of the Bean/Cowpea Collaborative Research Support Program, 1981-2002. *Field Crops Research* 82, special issues 2-3, 79-242.
- Granito, M., Frias J., Champ, M., Guerra M. Doblado r. And Vidal-Valverde, C. (2002) Nutritional Improvement of beans (*Phaseolus vulgaris*) by natural fermentation. *European Food Resource Technology* 214, 226-231.
- Gupta, U., Rudramma, Rat E. R. And Joseph, R (1998): Nutritional quality of lactic acid, fermented bitter gourd and fengrek leaves. *International Journal of food Sciences and Nutrition* 49, 101-108.
- Gaden, B. L., Jr. *Chem. Engng.* (April, 1956), 159
- Guzmán-Maldonado S.H. and Parcides-López O. (1998). Functional products of plants indigenous of Latin America: amaranth, quinoa, common beans and botanicals. In: *Functional Foods. Bicheinical and Processing Aspects*; Mazza G. (Eds). Technomic. Lancaster, PA. Pp: 39-328.
- Haberer P., Hoizapfei W. H. and Wagner H. (1997): Moegliche Rolle von Milchsacurebakterien bei der Cholesterinsenkung im Blutserum. *Mittcilungsblatt der B undesanstali fiier Fleisch/hrschung, Kulnihach* 36, 202-207.
- Hajos, G., and Osagie, A. U. (2004). Technical and biotechnological modifications of antinutritional factors in legumes and oilseeds. *Proceedings from AFLO '04: The fourth international workshop on antinutritional factors in legume seeds and oilseeds.* Wageningen: EAAP.
- Harland B. F. and Harland J. (1980): Ferinentative reduction of phytate in rye, white, and whole wheat breads. *Cereal ('heniisliy* 57, 226-229.
- Harrison V. C. and Peat G. (1975): Serum cholesterol and bowel flora in the newborn. *American Journal of Chnical nutrition* 28, 135 1 - 1 355.
- Hepncr G., Fried R. R., Jeor S. S., Fuseli L. and Morin R. (1979): Hypercholesteremic effect of yoghurt and milk. *American Journal of Clinical Nutrition* 32, 19.

- Hesseltine, C. W., & Wang, H. L. (1980). The importance of traditional fermented foods. *BioScience*, 30(6), 402-404.
- Hiran, P., Kerdchoechuen, O. and Laohakunjit, N. (2011) Change of Nutritional Value and Antioxidant Activity of Fermented Germinated Red Kidney Bean. *Agricultural Science Journal*.42(2)(Suppl.), 321-324.
- Hofmann, G., McIntyre, M., & Nielsen, J. (2003). Fungal genomics beyond *Saccharomyces cerevisiae*. *Current Opinion in Biotechnology*, 14(2), 226-231.
- Hoke, K., Houška, M., Průchová, J., GabrovskáD.,Vaculová, K, and Paulíčková, I.(2007). "Optimisation of puffing naked barley.*Journal of Food Engineering*. 80(4): 1016-1022 .
- Honke, J.; H. Kozłowska; J. F. Vidal-Valverde and R. Gorecki (1998).Changes in quantities of inositol phosphates during maturation and germination of legume seeds. *Zeitschrift fur lebensmittelUntersuchung und-Forschung A*, 206, 279–283
- IAR Cropping scheme meeting (1994). Report on food science and technology Research programme, IAR, ABU, Zaria.
- Ibrahim S. S., Habiba R. A., Shata A. A. and Embaby H. F. (2002). Effect of Soaking, germination, cooking and fermentation on anti-nutritional factors in cowpeas. *Nahrung* 46, 92- 95.
- Ikemefuna C, Obizoba J, Atii JV (1991). Effects of soaking, sprouting, fermentation and cooking on nutrient composition and some antinutritional factors of sorghum (*Guinea*) seeds. *Plant Foods for Human Nutrition*, 41, 203-212.
- Iyer, V. S.S. Kadam and D.K. Salunkhe, Cooking, D.K. Salunkhe, S.S. Kadam, (1989) Editors , CRC “Handbook Of World Food Legumes: Nutritional Chemistry, Processing Technology And Utilization.|| Boca Raton: CRC Press Inc, Florida, 141–163.
- Jood, S., U. Mehta, R. Singh, and C.M. Bhat. (1985). Effect of processing on flatus-producing factors in legumes. *Journal of Agriculture and Food. Chemistry*. 33, 268-271.
- Kahlon, T.S., Smith, G.E., & Shao, Q. (2005). In vitro binding of bile acids by kidney bean (*Phaseolus vulgaris*), black gram (*Vigna mungo*), Bengal gram (*Cicer arietinum*) and mothbean (*Phaseolus aconitifolius*). *Food Chemistry*, 90, 241-246.

- Karunarama A., Wczenberg F. and Bullerman L. B. (1990): Inhibition of mold growth and aflatoxin production by *Lactobacillus* spp. *Journal of Food Protection* 53, 230-236.
- Kazanas N., and Fields M. L (1981). Nutritional Improvement of Sorghum by Fermentation. *Journal of Food Science*. 46, 819- 821.
- Khader V. and Uebersax M.A. (1989). Legumes in Indian Diets. *Michigan Bean Digest*. 13(4), 10-13.
- Khattab, R. Y. and S. D. Arntfield (2009). Nutritional quality of legume seeds as affected by some physical treatments. 2. Antinutritional factors. *LWT – Journal of Food Science and Technology*, 42, 1113–1118.
- Khetarpaul, N., & Chauhan, B. M. (1990). Improvement in HCl-extractability of minerals from pearl millet by natural fermentation. *Food chemistry*, 37(1), 69-75.
- Kon, S., & Sanshuck, D. W. (1981). Phytate content and its effect on cooking quality of beans. *Journal of Food Processing and Preservation*, 5, 169–178.
- Kozłowska, H., Honke, J., Sadowska, J., Finas, J., Vidal – Valverde, c. (1996) *Journal of Science, Food, Agriculture*, 71, 367-375.
- Krishnaiah, T. Devi, A. Bono and R. Sarbatly, (2009) *J. Medicin. Plant Res*, 3(2), 067-07.
- Kumar, V.; A. K. Sinha; H. P.S. Makkar and K. Becker (2010). Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, 120, 945–959.
- Lajolo FM and Genovese MI. (2002). Nutritional significance of lectins and enzyme inhibitors from legumes. *Journal of Agriculture and Food Chemistry*. 50(22), 6592-8.
- Lasztity, R and Lasztity, L. J.(1990). Phytic acid in Cereal Technology, In: *Advances in Cereal Science and Technology*, Pomeranz, Y., Ed., AACC Publ., and St. Paul, MN. USA, Chapter 5. pp. 10-23.
- Lay. M. M. G. and Fields ML (1981): Nutritive value of germinated corn and corn fermented after germination. *Journal of Food Science* 46, 1069-1073.
- Leterme P and Muñoz LC. (2002). Factors influencing pulse consumption in Latin America. *British Journal of Clinical Nutrition*.;88,(Suppl 3):S251-S254.
- Liang, J.; B. Z. Han; M. J. R. Nout and R. J. Hamer (2008). Effects of soaking, germination and fermentation on phytic acid, total and in vitro soluble zinc in brown rice. *Food Chemistry*, 110, 821–828.

- Lidbeck A., Overvik B., Rafter B., Nord C. B. and Gustafson S. A. (1992): Effect of *Lactobacillus acidophilus* supplements on nutrient excretion in faeces and urine in humans. *Microbial Ecology in health and Disease* 5,59-67.
- Liener, L.B. (1974). Phytohemagglutinins: their nutritional significance. *Journal of Agriculture and Food Chemistry*. 22,17-22.
- Liljeberg H. G. M., Loenner C. H. and Björck I. M. F. (1995): Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans. *Journal of Nutrition* 125, 1503-1511.
- Loggerenberg, M. V. (2004). Development and application of a small-scale canning procedure for the evaluation of small white beans (*Phaseolus vulgaris*). (Doctoral Dissertation, University of the Free State, Bloemfontein).
- Loewus, F. (2002). Biosynthesis of Phytate in Food Grains and Seeds. In: Food Phytates (Eds.) Reddy N. R. and S. K. Sathe, CRC Press, Florida, USA: pp. 53–61.
- Lopez, I., Gordon, D. T., Fields, M. (1983) Release of phosphorus from phytate by natural lactic acid fermentation. *Journal of Food Science* 48, 953-954
- Lorri, W. And Swanberg, U. (1994) Lower prevalence of diarrhoea in young children fed with lactic acid fermented cereal gruels. *Food and Nutrition Bulletin*. 15, 57-63.
- Lu, Z., Altermann, E., Breidt, F., & Kozyavkin, S. (2010). Sequence analysis of *Leuconostoc mesenteroides* bacteriophage Φ1-A4 isolated from an industrial vegetable fermentation. *Applied and environmental microbiology*, 76(6), 1955-1966.
- Lucas GM, Markakas P. (1975). Phytic acid and other phosphorus compounds of bean (*Phaseolus vulgaris*). *Journal of Agricultural Education and Chemistry*. 23, 13 – 15.
- Luchene R.H. and Harrigan, W. F. (1990) Growth of and aflatoxin production by *Aspergillus parasiticus* when in the presence of either *Lactococcus lactis* or lactic acid at different initial pH values *Journal of applied bacteriology* 69,512-519.
- Lucier, G., Lin, B-H., Allshouse, J., & Kantor, L.S. (2000). Factors Affecting Dry Bean Consumption in the United States. In *Vegetables and Specialties Situation and Outlook*, U.S. Department of Agriculture, Economic Research Service, NGS-280, April 2000, pp.26-34.

- Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Monpetit, D., & Malcolmson, L. (2011). Thermal processing effects on the functional properties and microstructure of lentil, chickpea, and pea flours. *Food Research International*, 44(8), 2534-2544.
- Mahadevamma, S. and R. N. Tharanathan (2004). "Processing Of Legumes:Resistant Starch And Dietary Fiber Contents. *Journal of Food Quality* 27(4), 289-303.
- Mahajan, S., & Chauhan, B. M. (1988). A research note effect of natural fermentation on the extractability of minerals from pearl millet flour. *Journal of Food Science*, 53(5), 1576-1577.
- Marfo, E. K.; B. K. Simpson; J. S. Idowu and O. L. Oke (1990).Effect of local food processing on phytate levels in cassava, cocoyam, yam, maize, sorghum, rice, cowpea and soybean. *Journal of Agricultural and Food Chemistry*, 38,1580–1585.
- Martin-Cabrejas, M. A., Aguilera, Y., Benitez, V., Molla, E., Lopez-Andreu, F. J., & Esteban, R.M. (2006). Effect of industrial dehydration on the soluble carbohydrates and dietary fibre fractions in legumes. *Journal of Agricultural and Food Chemistry*, 54, 7652–7657.
- Mbagwu, F. N., & Edeoga, H. O. (2006). Anatomical studies on the root of some Vigna savi species (Leguminosae-Papilionoideae). *Agri cultural Journal*, 1(1), 8-10.
- Mbithi-Mwikya S, Ooghe W, Camp J-van, Ngundi D and Huyghebaert A. (2000). Amino acid profiles after sprouting, autoclaving, and lactic acid fermentation of finger millet (*Eleusine coracana*) and kidney beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*. 48(8), 3081-3085.
- McIntosh M and Miller C. A (2001) diet containing food rich in soluble and insoluble fiber improves glycemic control and reduces hyperlipidemia among patients with type 2 diabetes mellitus. *Nutritional Review* 2001 Feb;59(2), 52-5.
- Mensa-Wilmot Y, Phillips RD and Hargrove JL. (2001). Protein quality evaluation of cowpea-based extrusion cooked cereal/legume weaning mixtures. *Nutrition Research*. 21(6), 849-857.
- Mertz, E. T., Hassen, M. M., Cairns-Whittern, C., Kirleis, A. W., Tu, L., & Axtell, J. D. (1984). Pepsin digestibility of proteins in sorghum and other major cereals. *Proceedings of the National Academy of Sciences*, 81(1), 1-2.
- Milan-Carrillo, J., Valdez-Alarcon, C., Gutierrez-Dorado, R., Cardenas-Valenzuela, O.G., Mora-Escobedo, R., Garzon-Tiznado, J.A., Reyes-Moreno, C. (2007). Nutritional

- properties of quality protein maize and chickpea extruded based weaning food. *Plant Foods for Human Nutrition*, 62, 31–37.
- Miller, H.E., Rigelhof, F., Marquart, L., Prakash, A. and Kanter, M., (2000) Whole-grain Products and Antioxidants, *Cereal Food World*, 45, 59x63.
- Mitall, B. K., & Garg, S. K. (1995). Anticarcinogenic, hypocholesterolemic, and antagonistic activities of *Lactobacillus acidophilus*. *Critical reviews in microbiology*, 21(3), 175-214.
- Motarjemi Y., Käferstein F., Moy G. and Quevedo F. (1993): Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. *Bulletin of the World Health Organization* 71, 79-82.
- Mubarak AE (2005). Nutritional composition and antinutritional factor of mung beans (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chemistry*. 89, 489-495
- Mueller-Harvey, I., & McAllan, A. B. (1992). Tannins their biochemistry and nutritional properties. *Advances in Plant Cell Biochemistry and Biotechnology*, 1, 151-217.
- Murdock F. A. and Fields M. L. (1984): B-vitamin content of natural lactic acid fermented torn meal. *Journal of food Science* 49, 373-375.
- Nakitto, A. M., Muyonga, J. H., & Nakimbugwe, D. (2015). Effects of combined traditional processing methods on the nutritional quality of beans. *Food science and nutrition*, 3(3), 233-241.
- Naveeda , K. and P. Jamuna., (2006). Nutritive value and sensory profile of microwave and pressure cooked decorticated legumes (dals). *Journal of Food Processing and preservation* 30 (1), 299-313.
- Nnam, N. M. (1995). Evaluation of nutritional quality of fermented cowpea (*Vigna unguiculata*) flours *Ecology of food and Nutrition*. 33, 273-279.
- Nout M. J. K., Rombouts F. M. and Ilavelaar A. (1989): Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. *International Journal of Food Microbiology* 8, 351-361.
- Oatway, L.; T. Vasanthan and J. H. Helm (2001). Phytic acid: A Review. *Food Reviews International*, 17, 419–431.

- Obizoba I. C. and Egbuna I. (1992): Effect of germination and fermentation on the nutritional quality of bambara nut (*Voandzeia subterranea* L. Thouars) and its product (milk). *Plant Foods for Human Nutrition* 42, 13-23.
- Occena LG, Bennink MR, Uebersax MA, Chung YS. (1997). Evaluation of drum-dried meals prepared from split beans (*Phaseolus vulgaris* L.): Protein quality and selected antinutritional factors. *Journal of Food Processing and Preservation*. 21(4): 335-344.
- Oke, O. L. (1969) The Role of Hydrocyanic Acid In Nutrition. *World Review of Nutrition and Dietetics* 11, 170-198
- Okrah SG (2008). Screening of Six Local Sorghum Varieties for their Malting and Brewing Qualities. M.Sc. Thesis, Kwame Nkrumah Univ. Sci. Tech., Ghana, p87.
- Onayemi, O and Potter, N. N. (1976) Cowpea powders dried with methionine preparation, storage stability, organoleptic properties, nutritional quality. *Journal Food science*. 41, 48-53.
- Osman, M. A. (2007). Effect of different processing methods, on nutrient composition, antinutritional factors, and in vitro protein digestibility of *Dolichos lablab* bean [Lablab purpureus (L) Sweet]. *Pakistan Journal of Nutrition*, 6(4), 299-303.
- Paredes-Lopez O. and Hany G. L (1988): Food biotechnology review traditional solid-state fermentations of plant raw materials - application, nutritional significance and Future prospects. *CRC! Critical Reviews in Food Science and Nutrition* 27, 159-187.130.
- Parihar, P., A. Mishra., O. P. Gupta., L. P. S., Rajput., A. Singh., P. Parihar., and A. Singh., (1999). —Effect of cooking processes on nutritional quality of some common pulses|| . *Advances in Plant Sciences* 12 (2), 15-20.
- Parvez S., Malik K. A., Ali Kang S. and Kim H. Y. (2006): Probiotics and their fermented Food products beneficial for health. *Journal of Applied Microbiology* 100, 1171-1185.
- Poppel G. v. and Schaafsma G. (1996): Cholesterol lowering by a functional yoghurt Food Ingredients Europe, Conference proceedings, 31-32.131.
- Porres, J. M., M. López-Jurado., P. Aranda., G. Urbano (2003). "Effect of Heat Treatment and Mineral and Vitamin Supplementation on the Nutritive Use of Protein and Calcium From Lentils (*Lens culinaris* M.) in Growing Rats. *Nutrition*. 19(5), 451-456.

- Pusztai, A., Bardocz, S., & Martín-Cabrejas, M. A. (2004). The mode of action of ANFs on the gastrointestinal tract and its microflora. Proceedings from AFLO '04: The fourth international workshop on antinutritional factors in legume seeds and oilseeds. Wageningen: EAAP.
- Queiroz Kda S, de Oliveira AC, Helbig E, Reis SMPM, and Carraro F. (2002) Soaking the common bean in a domestic preparation reduced the contents of raffinose-type oligosaccharides but did not interfere with nutritive value. *Journal of Nutrition Science Vitaminol.* Aug;48(4):283-9.
- Ramírez-Cárdenas, L., Leonel, A. J., Costa, N. M., & Reis, F. P. (2010). Zinc bioavailability in different beans as affected by cultivar type and cooking conditions. *Food Research International*, 43(2), 573-581.
- Reddy NR, Pierson MD, Sathe SK, Salunkhe DK. (1982a). Legume-based fermented foods: their preparation and nutritional quality. *Crit Rev Food Science Nutrition* 17(4), 335-70.
- Reddy, N. R.; S. K. Sathe and D. K. Salunkhe (1982b). Phytate in legumes and cereals. *Advances in Food Research*, 28, 1-9.
- Reddy B. S., Ekelund G., Bohe M., Engle A. and Domellof L. (1983): Metabolic epidemiology of colon cancer: Dietary pattern and fecal sterol concentrations of three populations. *Nutrition cancer* 5, 34-40.
- Reed G. (1981): Use of microbial cultures: yeast products. *Food Technology* 35, 89-94.
- Rehman, Z.U., & Shah, W.H. (2004). Domestic processing effects on some insoluble dietary fibre components of various food legumes. *Food Chemistry*, 87, 613-617.
- Rimbach, G.; H. J. Ingelmann and J. Pallauf (1994). The role of phytase in dietary bioavailability of minerals and trace elements. *Ernährungsforschung* 39, 1-10.
- Rodríguez-Burger AP, Mason A and Nielsen S. (1998). Use of fermented black beans combined with rice to develop a nutritious weaning food. *Journal of Agricultural and Food Chemistry* 46(12), 4806-4813.
- Rolando, B. R. (1999). Condensed tannins in tropical forage legumes: Their characterisation and study of their nutritional impact from the standpoint of structure-activity relationships. Thesis (Ph. D.).
- Russei J. B (1992): Another explanation of the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. *Journal of Applied Bacteriology* 73.

- Saharan K, Khetarpaul N, Bishnoi S (2001). HCl-extractability of minerals from rice bean and faba bean: Influence of domestic processing methods. *Innovative Food Science Emerging Technology* 2(4):323-325
- Salminen S., Deighton M. and Gorbach S. (1993): Lactic acid bacteria in health and disease. Lactic acid bacteria. S. Salminen and A. von Wright. 237-294. New York, USA: Marcel Dekker Inc.
- Sandberg, A. S. ; M. Brune; N. G. Carlsson ; L. Hallberg ; E. Skoglund and L. Rossander-Hulthen (1999). Inositol phosphates with different number of phosphate groups influence iron absorption in humans. *American Journal of Clinical Nutrition*, 70, 240–246.
- Sangronis, E. and C. J. Machado (2007). Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. *LWT Journal Science Technology*, 40:116-120.
- Sathe, S.K. and D.K. Salunkhe. (1989). Technology of removal of unwanted components of dry legumes, pp. 249, 251. In D.K. Salunkhe and S.S. Kadam (eds.). CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume III. CRC Press, Florida.
- Schneider AVC.(2002). Overview of the market and consumption of pulses in Europe. *British Journal of Clinical Nutrition*. 88(Suppl 3):S243-S250.
- Sefa, Dede, S., Stanley, D. W., and P. W. (1978). Effect of storage time and conditional on the hard —to-cook defect in cowpea (*vigna unguiculate*). *Journal of Food Science* 44, 790-793.
- Shahani K. M. (1983): Nutritional impact of lactobacillic fermented foods. Nutrition and the Intestinal Flora. ed. B. Hallgren ISBN 91 (22) 593-5.
- Shellie-Dessert K.C., Bliss F.A. (1991). Genetic improvement in food quality factors.
- Shimelis, E. A. and S. K. Rakshit (2007). Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chemistry*, 103, 161–172.
- Smith, A.K. and S. J. Circle (1972). —Soyabeans. Chemistry and technology. || AVI Publishing Company, Westport.

- Smoliak, S.; Ditterline, R. L.; Scheetz, J. D.; Holzworth, L. K.; Sims, J. R. Wiesner, J. R.; Baldrige, D. E.; Tibke, G. L., (1990). Common bean. Montana State University, Animal & Range Sciences Extension Service, Forage extension program, Bozeman, USA.
- Soetan, K. O. and Oyewole, O. E. (2009) The need for adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science*. 3 (9),. 223-232,
- Souci, S. W., Fachmann, W., & Kraut, H. (2000). Food composition and nutrition tables. Stuttgart: Medpharm Scientific Publishers.
- Speek A. J., Spee Saichua S and Shreurs W. H. P (1988): Total carotenoid and beta-carotene contents of Thai vegetables and the effect of processing. *Food Chemistry* 27, 245-257.
- Stodola, F. H. Chemical Transformations by Microorganisms. (1958). New York: Wiley
- Stolz, D. R. (1982). The health significance of mutagens in foods. in: Stich, H. F. ed. "carcinogens and mutagens in the environment. 3,75- 82.
- Strandhagen E., Lia A., Liridstrand S., Bergstrom P., Lundstroem A., Fonden R. and Andersson H. (1994): Fermented milk (ropy milk) replacing regular milk reduces glycemic response and gastric emptying in healthy subjects. *Scandinavian Journal of Nutrition* 38, 117—121.
- Sulieman, M. A., Hassan, A. B., Osman, G. A., El Tyeb, M. M., El Khalil, E. A. I., El Tinay, A. H., et al. (2008). Changes in total protein digestibility, fractions content and structure during cooking of Lentil cultivars. *Pakistan Journal of Nutrition*, 7 801–805
- Tang, C. H. (2008). Thermal denaturation and gelation of vicilin-rich protein isolates from three Phaseolus legumes: a comparative study. *LWT Food Science and Technology*, 41, 1380–1388.
- Taranto M. P., Sesma F., Pesce de RUIZ Holgado A. and Valdez G. F. D. (1997): Bile salts hydrolase plays a key role on cholesterol removal by Lactobacillus reuteri. *Biotechnology* 19, 845-847.
- Thompson LU, Tenebaum AV and Hui-H. (1986). Effect of lectins and the mixing of proteins on rate of protein digestibility. *Journal-of-Food-Science*; 51 (1), 150-152, 160.

- Tsuji K., Ichikawa T., Tanabe N., S., Tarui S. and Nakagawa Y. (1992): Effects of two kinds of koji on blood pressure in spontaneously hypertensive rats. *Journal of the Agricultural Chemistry of Japan* 66, 123 1-1246.
- Tuan, Y. H. and Phillips, R. D. (1991). Effect of the hard-to-cook defect and processing on protein and starch digestibility of cowpeas. *Cereal Chemistry*. 68, 4 13-417.
- Uebersax MA, Ruengsakulrach S and Occena LG. (1991). Strategies and procedures for processing dry beans. *Food Technology*. 45(9), 104-108, 110-111.
- Uebersax MA and Occena LG. (1997). Composition and Nutritive Value of Dry Edible Beans: *Commercial and World Food Relief Applications. Michigan Bean Digest*. 15(5): 2-12, 28.
- Uebersax, M. A., Ruengsakulrach, S., and Occena, L.G. (1991). Strategies and procedure for processing Dry beans. *Food Technology* 45. 104-111.
- Urga K1, Fufa H, Biratu E and Gebretsadik M. (2006). Effects of Blanching and Soaking on Some Physical Characteristics of Grass Pea (*Lathyrus sativus*). *African Journal of Food Agriculture, Nutrition And Development*. 6(1), 1-17
- Ur-Rehman, Z. and A. M. Salariya (2005). "The effects of hydrothermal processing on antinutrients, protein and starch digestibility of food legumes. *International Journal of Food Science and Technology* 40(7): 695-700.
- Vaishali V. A, Medha K. G. and Shashi A C (1997) Effect of natural fermentation on in vitro bioavailability in cereal-legume mixtures. *International Journal of Food Science and Technology* 32, 29-32.
- Vijayakumari, P., Siddhuraju, P., Pugalenti, M., & Janardhanan, K. (1998). Effect of soaking and heat processing on the levels of antinutrients and digestible proteins in seeds of *Vigna sinensis*. *Food Chemistry*, 63, 259–264.
- Wang H. L. and Hcsseltinc C. W. (1981): Use of microbial cultures. *Legume and Cereal Product & Food Technology* 35, 79-83.
- Wang, N., Lewis, M. J., Brennan, J. G., & Westby, A. (1997). Effect of processing methods on nutrients and anti-nutritional factors in cowpea. *Food Chemistry*, 58, 59–68.
- Wang N.; D. W. Hatcher; R. T. Tyler; R. Toews and E. J. Gawalko (2010). Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). *Food Research International*, 43, 589-594.

- Wilhelm S. G. (1993). Studies on the therapeutic properties of acidophilus milk. Symposium of the Swedish Nutrition Foundation.
- Willis, W. H. (1988). Early agriculture and sedentism in the American Southwest: Evidence and interpretations. *Journal of World Prehistory*, 2(4), 445-488.
- Wittenberg, M. M. (2007). *New Good Food: Essential Ingredients for Cooking and Eating Well.*; Iowa State University Extension -- Prepare Dried, Beans, Peas, and Lentils.
- Wortmann, C. S., (2006). *Phaseolus vulgaris* L. (common bean). Record from PROTA4U. Brink, M. & Belay, G. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands .
- Wu, X. (2002). *Correlation of physio-chemical characteristics in the seed coat and canning quality in different dark red kidney bean (Phaseolus vulgaris L.) cultivars* (Doctoral dissertation, University of Wisconsin-Stout).
- Xu, B. J. and S. K. C. Chang (2008). "Total Phenolic Content and Antioxidant Properties of Eclipse Black Beans (*Phaseolus vulgaris* L.) as Affected by Processing Methods." *Journal of Food Science* 73(2), H19-H27.
- Yadav, B.S., Sharma, A., & Yadav, R.B. (2010). Resistant starch content of conventionally boiled and pressure-cooked cereals, legumes and tubers. *Journal of Food Science and Technology*, 47, 84–88.
- Yin, S.W., Tang, C.H., Wen, Q.B., Yang, X.Q., & Li, L. (2008). Functional properties and in vitro trypsin digestibility of red kidney bean (*Phaseolus vulgaris* L.) protein isolate: effect of high-pressure treatment. *Food Chemistry*, 110, 938–945.
- Zamindar, N., Shahedi, M., Nasirpour, A., & Sheikhzeinoddin, M. (2011). Effect of line, soaking and cooking time on water absorption, texture and splitting of red kidney beans. *Journal of Food Science and Technology*, 10, 1-7.
- Zamora, A. F., & Fields, M. L. (1979). Nutritive quality of fermented cowpeas (*Vigna sinensis*) and chickpeas (*Cicer arietinum*). *Journal of Food Science*, 44(1), 234-236.

APENDICES

Appendix I: Plates Showing Raw and Differently Processed *P. vulgaris*



Plate 1: Raw *P. vulgaris*



Plate 2: Fermented *P. vulgaris*

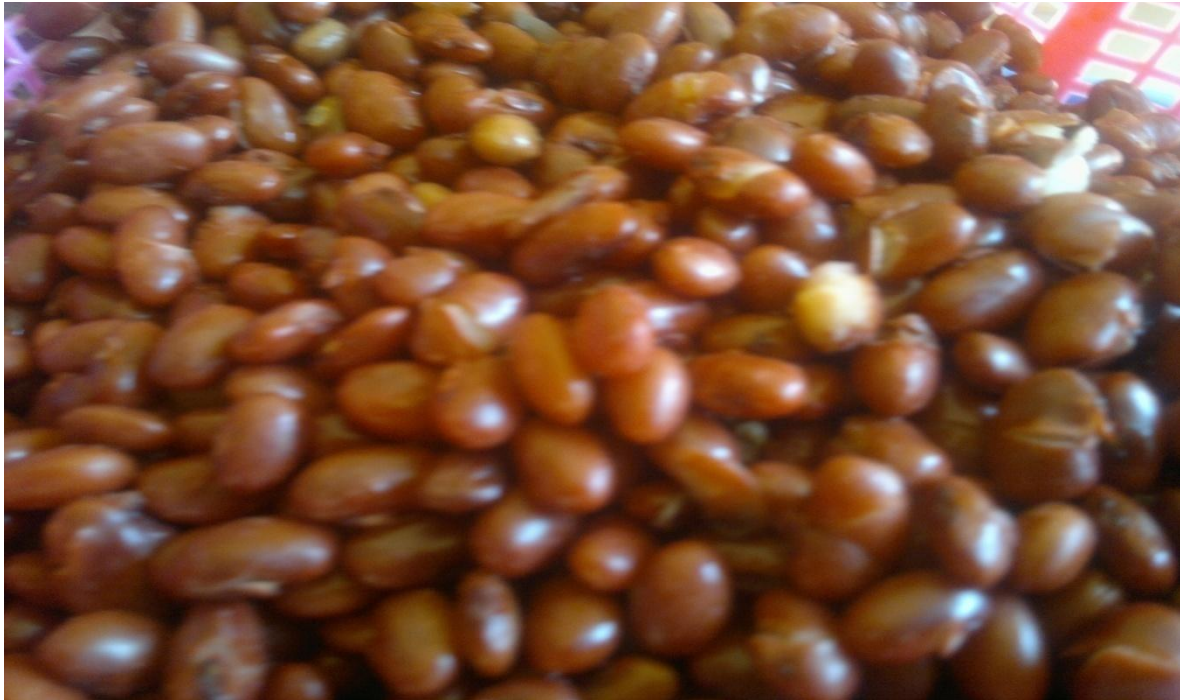
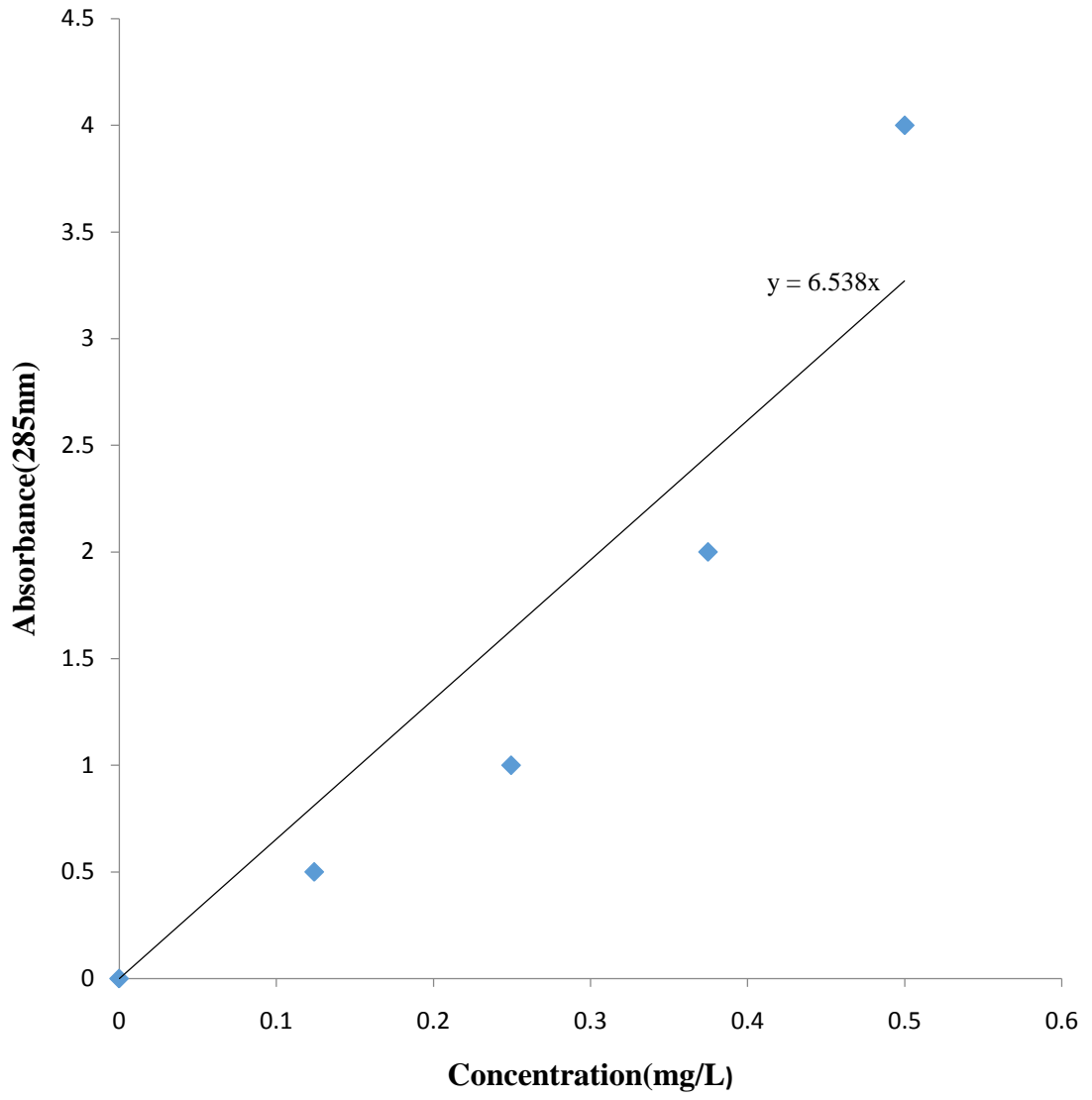
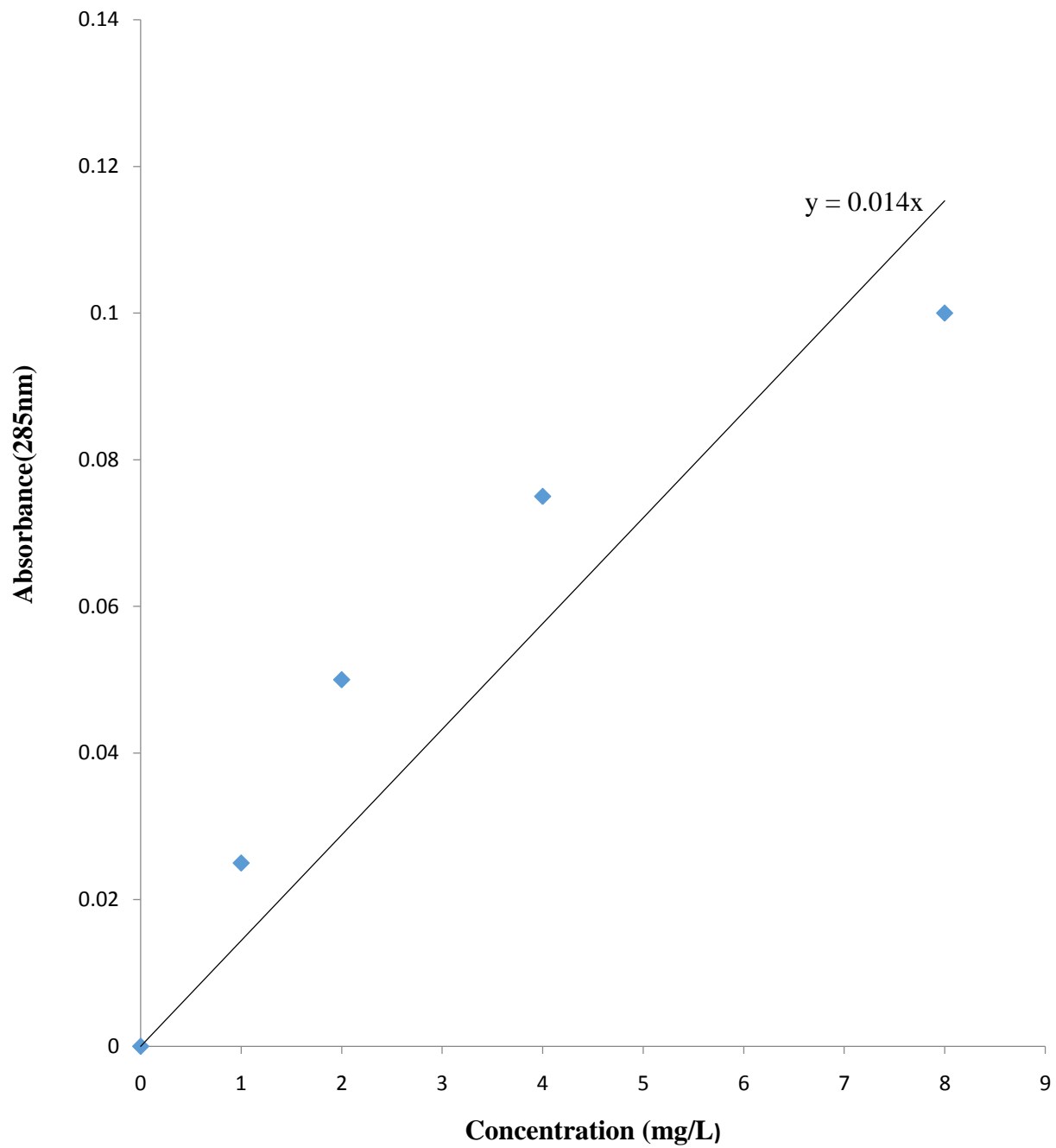


Plate 3: Boiled *P. Vulgaris*.

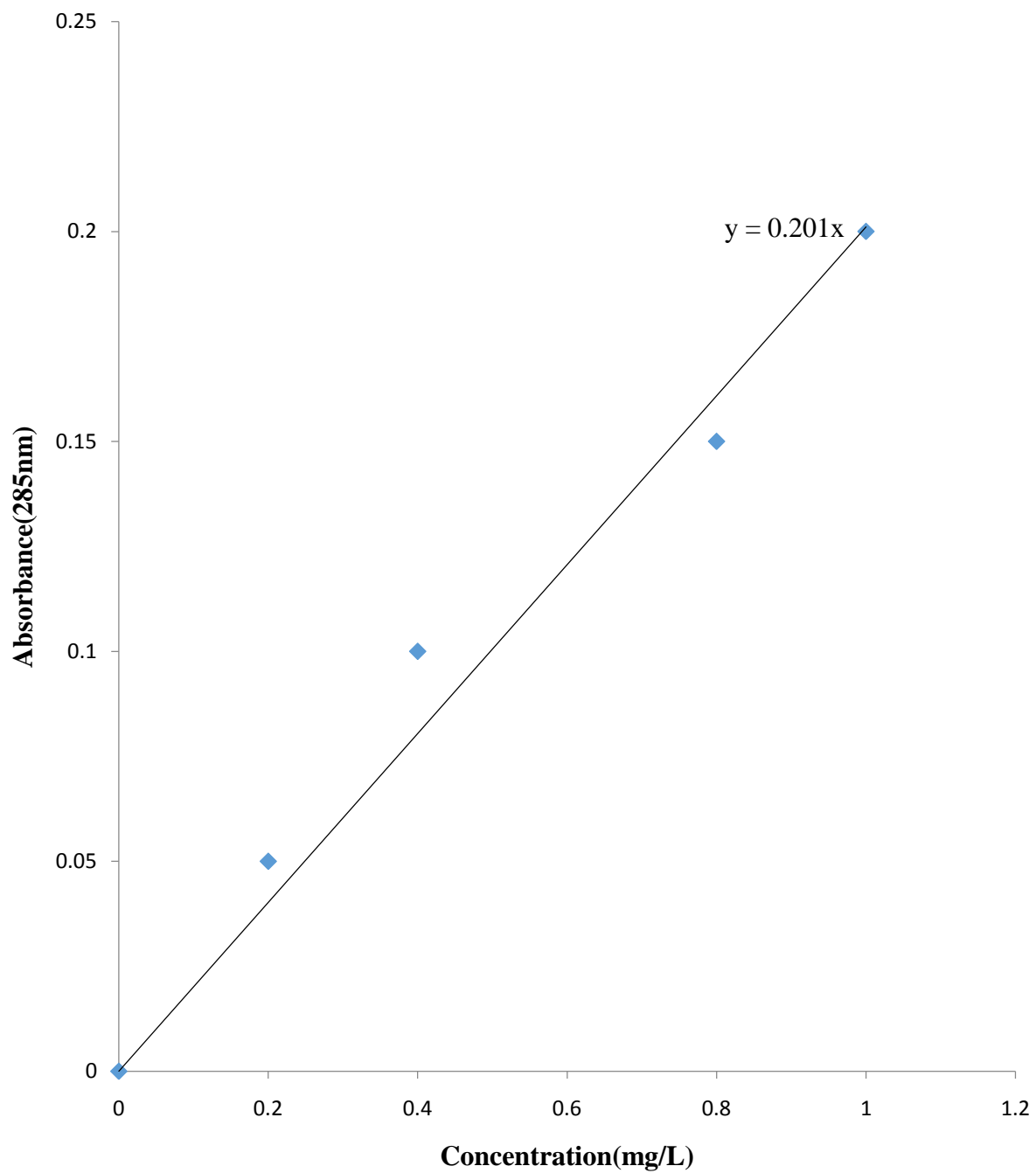
Appendix II: Calibration Curve for Determination of Magnesium



Appendix III: Calibration Curve for Determination of Iron(Fe)



Appendix IV: Calibration Curve for Determination of Zinc (Zn)



Appendix V: Calibration Curve for Determination of Calcium

