INFLUENCE OF STORAGE CONDITIONS ON THE DEVELOPMENT OF HARD-TO-COOK DEFECT AND THE NUTRITIONAL QUALITY OF SELECTED COMMON BEANS (*Phaseolus vulgaris* L.) GROWN IN KENYA

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Influence of storage conditions on the development of hard-to-cook defect and the nutritional quality of selected common beans (*Phaseolus vulgaris* L.) grown in Kenya

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DECLARATION

I hereby declare that this that is my original work and has never been submitted for an award of degree in any other University.

Signature: …… Date: …….

Elizabeth Namaemba Wafula

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

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DEDICATION

I dedicate this work to my husband, Moses Simiyu Kerre and to our children Leo Kerre and Lucia Khaoma. To Mr. and Mrs. Wafula thanks for the love and support you have shown.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Chemists
ADF	Acid Detergent Fibre
ETC	Easy to cook
FAO	Food and Agricultural Organization
HPLC	High Performance Liquid Chromatography
HTC	Hard to cook
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KALRO	Kenya Agricultural Livestock and Research Organization
MASL	Metres above Sea Level
MG	Miligram
ML	Mililitres
NARO	National Agricultural Research Organization
PHA	Phytohemagglutinins
PME	Pectin methyl esterase
PUFA	Polyunsaturated fatty acids
RH	Relative humidity

ABSTRACT

Common beans form one of the main sources of proteins in poor sub-Saharan Africa. However, common beans tend to develop the hard-to-cook (HTC) defect, which has been attributed to storage at temperatures higher than 25˚C and relative humidity (RH) of more than 70%. This results in increased cooking time, fuel consumption and water use thus reducing the rate of utilization of beans at domestic level. The main objective of this study was to understand the development of the hard to cook defect in Pinto and Red Haricot bean varieties during storage at varying temperatures and relative humidity (RH), and its effect on physical and nutritional quality. Samples were collected from Kenya Agricultural and Livestock Research Organization, (KALRO) Thika Station. The hard- (Pinto) and easy- (Red haricot) to-cook beans were stored at varying temperatures and RH (25˚C, 45˚C at 75% RH and 35˚C at 83% RH) levels using suitable incubators and salt solutions. The conditions were selected based on the temperature ranges that can be found in some regions across Kenya i.e. normal (25˚C), moderate (35˚C) and extremely high temperature (45˚C). the relative humidity were also selected on the same basis with 75% being the almost normal RH conditions while 85% being the extreme condition found in some regions in Kenya. Three (3) incubators were used for each set of temperature and RH, and sampling was carried out at 0, 2, 4 and 6 months. Pre-treatment with sodium carbonate, distilled water and calcium chloride was done followed by thermal treatment at 96.5˚C. The samples were assessed for physical (seed density, seed porosity, characteristic dimension of beans, hydration coefficient, and swelling coefficient), chemical (ash and proteins), nutritional (minerals and protein digestibility) and anti-nutritional changes (tannins and phytate). Beans stored at 45 ˚C/75% RH were harder than the fresh beans (25˚C /75%) and the ones stored at 35 ˚C/83%. The hydration coefficient and swelling coefficient significantly decreased ($P<0.05$) with increasing storage time. Soaking in distilled water and sodium carbonate for 16 hours reduced the cooking time from 3 hours to 1 hour for Pinto and 3 hours to 30 minutes for Red haricot while soaking in calcium chloride prolonged

the cooking time for both to more than 5 hours. The increased relative humidity from 75% RH to 83% RH caused an increase in cooking time from 180 minutes to 270 minutes in Red haricot; and 210 minutes to 270 minutes in Pinto. Bean hardness, solutes and electrolytes leaching after 16 hours soaking significantly increased with increased storage temperature. The phytic acid content decreased significantly ($P<0.05$) with soaking and cooking. For Pinto beans stored at 35/83/6, the phytic acid content reduced by 33.2% after cooking without soaking, 39.3% after soaking in distilled water followed by cooking, and 65% after soaking in sodium carbonate followed by cooking. For Red haricot beans stored at 35/83/6, the phytic acid content reduced by 13.9% after cooking without soaking, 27.6% after soaking in distilled water followed by cooking, and 51.2% after soaking in sodium carbonate followed by cooking. The tannin content also decreased by 23.2% after cooking without soaking, 60.2% after soaking in distilled water followed by cooking, and 71.1% after soaking in sodium carbonate followed by cooking. For fresh Red haricot beans stored at 25/75/0, the tannin content reduced by 65.6% after cooking without soaking, 77.9% after soaking in distilled water followed by cooking, and 81.9% after soaking in sodium carbonate followed by cooking. There was no significant difference ($P>0.05$) in mineral content with increased storage. There was significant decrease ($P<0.05$) in protein digestibility with increased storage time. After storage for 6 months at 45/75, the digestible protein content reduced by 24.2% for Pinto and 28.2% for Red haricot, while at 35/83, the digestible protein content reduced by 28.2% for Pinto and 23.8% for Red haricot. It was concluded that the longer the beans are stored at higher temperatures and relative humidity, the more they develop the HTC defect. The high temperature and relative humidity caused a relative decrease in protein digestibility however, the protein and mineral content were not altered.

CHAPTER ONE

INTRODUCTION

1.1 Background information

The common bean (*Phaseolus vulgaris* L.) is a grain legume consumed in great quantities throughout the world especially in Latin America and Africa (FAO, 2009). It belongs to Plantae kingdom, order *Fabales*, family *Leguminoseae*, subfamily *Papilionoideae*, tribe *Phaseoleae*, subtribe *Phaseolina*e, genus Phaseolus and species *P. vulgaris.* Beans are smooth, plump, and kidney-shaped, up to 1.5 cm long, range widely in color. The common bean is the best known and most important in the world (Broughton *et al*., 2003; Graham & Vance, 2003). Common beans are considered to be nearly perfect food due to their high levels of proteins, dietary fibre and excellent sources of complex carbohydrates among other nutrients. Their protein content varies between 17 and 34% (dry weight basis) which includes the metabolic, structural and storage proteins (Broughton *et al*., 2003; Graham & Vance, 2003; Huma *et al.*, 2008). The large amounts of water soluble fibre are particularly effective in lowering cholesterol in the blood, whereas the water insoluble fibre provides bulk, pushing food through the digestive system at a faster rate (Morrow, 1991). Regular consumption of common bean and other pulses is currently promoted by health organizations because it reduces the risk of diseases such as cancer, diabetes or coronary heart diseases (Leterme & Munoz, 2002). This is because common bean is low in fat and is cholesterol free, an appetite suppressant because it digests slowly and causes a low sustained increase in blood sugar and it can delay the reappearance of hunger for several hours, enhancing weight-loss programs (Katungi, 2009). In Kenya, Malawi and Tanzania, beans are commonly consumed as boiled dry beans (either as stew or *Githeri*), making the varieties with soft grain when cooked, and thin skins more preferred. Varieties with thin soft seed coats are associated with less cooking time and give soft gravy (Katungi, 2009). However, common beans contain compounds that can negatively affect their nutritional value such as trypsin inhibitors, lectors, phytates, polyphenols (especially tannins in beans) and

oligosaccharides (raffinose and stachyose). Some of these are thermolabile disappearing after proper cooking, such as trypsin inhibitors and lectins. Others are thermostable but their concentrations are reduced by dissolution in water (Silva & Silva, 1999, 2000). These should be addressed through concerted research activities. HTC development is associated with storage of beans under conditions of high temperature (≥ 25°C) and high relative humidity (≥ 65%) (Shiga *et al.,* 2004). Beans susceptibility to hardening during storage directly affects their marketing, trade and consumption. Although beans are rich in nutrients that make their consumption advantageous (Cardador-Martínez *et al*., 2002; Oomah *et al*., 2010) they are often overlooked by less nutritious, fast-cooking or precooked foods. This change in dietary habits of the population calls for serious strategy to reduce the cooking time and/or introduce fast cooking bean types (Leterme & Muñoz, 2002). Cooking beans which have developed the defect takes several hours to prepare and requires large amounts of water and fuel (Shiga *et al.,* 2004). Several changes occur during development of the hard to cook defect, some of these changes include: increase in water activity and moisture content, a decrease in water absorption capacity and loss of cooking ability of cotyledons and seed coat and alterations in texture, color, and flavour (Pirhayati *et al*., 2011).

1.2 Problem Statement

Food insecurity and malnutrition have continued to ravage the Kenyan population for a long time. This is partly due to lack of access to adequate and diversified diet which results in various forms of nutritional problems. Despite common bean varieties being considered as one of the staples that can help in mitigating the food and nutrition insecurity problem, they are susceptible to hardening phenomenon when stored under adverse conditions of temperature (> 25°C) and relative humidity (>85%), this leads to extended cooking times which translates to high processing/cooking costs due to high fuel consumption. In addition it also reduces the nutritional quality and changes the textural quality of legumes. Therefore their palatability and acceptability to consumers is reduced (Pirhayati *et al*., 2011). The hard to cook defect has been associated with complexion of different nutrients such as proteins and minerals with anti-nutrients. Climate change, high-energy prices and

globalization are rapidly redefining food affordability. These factors are transforming food consumption, production and markets. Due to this prolonged cooking times, poor people further limit their consumption and shift to even less balanced diets and less frequent meals thereby affecting health and nutrition both in the short and long term. The poor cooking quality can be attributed to either the hard shell or the hard to cook phenomenon. Hard shell may be due to impermeability of the tests to water while the hard to cook defect may be a result of the inability of the cotyledon to be hydrated during the cooking process (Katungi *et al*., 2009; Nasar-Abbas *et al*., 2008; Pirhayati, Soltanizadeh, & Kadivar, 2011). Hardening of beans has also been associated with increased post-harvest bean losses (20%-30%) (Jones, 1999).

1.3 Justification

Malnutrition is one of the manifestations of food insecurity which can be exacerbated by the unavailability of sufficient nutrients from foods consumed. The vision 2030 of the Kenya's government identifies food and nutrition security as part of its economic pillar. Beans have been identified as one of the main crops under the Agricultural development Strategy. Some of the beneficial effects of beans on human health include; weight management due to its relative low glycemic index and risk reduction of a host of chronic diseases (Leterme & Munoz, 2002). Increased consumption of beans could help impart these benefits to more individuals in the population. This is only possible if variety is added to the bean products. However, the nutritional value of legumes is compromised because of the presence of antinutritional factors, such as trypsin inhibitors, chymotrypsin, hemagglutinin, phytic acid, tannin, flatulence oligosaccharides, saponins, phenolic compounds, lectins, and allergens (Morales-De Leon *et al*., 2007). Presence of these factors reduces the bioavailability of the nutrients in legumes, especially the proteins and minerals, particularly iron. Efforts to develop technological processes for transforming the hard-to-cook beans into edible and useful products including decreasing cooking time, increasing nutritive value, and improving sensory properties would have great nutritional and economic impact.

Since traditional cooking of dry edible beans in developing countries involves excessive expenditure of time and fuel, there is need to increase the acceptability and consumption of beans by tailoring existing and new processes to produce bean-based products that address this problem. This requires innovative scientific and technological approaches for changing the physicochemical properties of beans to meet certain functionalities (Kinyanjui *et al.*, 2014).

1.4 Objectives:

1.4.1 Main objective

To determine factors that influence the development of the HTC defect in Pinto and Red Haricot bean varieties during storage, and its effect on physical and nutritional quality.

1.4.2 Specific objectives

- To determine effect of storage temperature and humidity on the physical properties of Red Haricot and Pinto beans
- To determine the effect of storage temperature and humidity on the development of hard to cook defect in Red Haricot and Pinto beans
- To assess the impact of pre-treatment techniques on cooking time and the hard to cook defect.
- To determine the chemical, nutritional and anti-nutritional changes during the development of hard to cook defect

1.5 Hypothesis

- Storage temperature and humidity has no effect on the physical properties of Red Haricot and Pinto
- Storage temperature and humidity has no effect on the hard-to-cook defect
- Pre-treatment techniques have no effect on cooking time and the hard-to-cook defect

- Development of the hard-to-cook defect has no effect on the nutritional quality of beans.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Pulse is used to describe legumes that bear edible dry seed that is directly used by man. Some of the most commonly consumed bean varieties include: black bean (*Phaseolus vulgaris*), black-eyed pea (*Vigna unguiculata)*, fava beans, kidney beans (*Phaseolus vulgaris*), lentils (*Lens culinaris*), lima beans (*Phaseolus lunatus)*, mung beans, peanuts (*Arachis hypogaea*), green and yellow peas (*Pisum sativum*) , Pigeon peas (*Cajanus cajan*), pinto beans (*Phaseolus vulgaris*), soy beans (*Glycine max*), Broad beans (*Vicia faba*), and scarlet runner bean (*Phaseolus coccineus*) (Laura & Jan, 2006). FAO recognizes 11 primary pulses namely dry beans (*Phaseolus spp.* including several species now in Vigna) (kidney bean, haricot bean, pinto bean, navy bean (*Phaseolus vulgaris*), lima bean, butter bean (*Phaseolus lunatus*), azuki bean, adzuki bean (*Vigna angularis*), mung bean, golden gram, green gram (*Vigna radiata*), black gram, urad (*Vigna mungo*), scarlet runner bean (*Phaseolus coccineus*), rice bean (*Vigna umbellata*), moth bean (*Vigna acontifolia*), tepary bean (*Phaseolus acutifolius*)); dry broad beans (*Vicia faba*) (horse bean (*Vicia faba equina*), broad bean (*Vicia faba*), field bean (*Vicia faba*); dry peas (*Pisum spp*.) (garden pea (*Pisum sativum var. sativum*), Protein pea (*Pisum sativum var. arvense*)); chickpea, garbanzo, bengal gram (*Cicerarietinum*); dry cowpea, black-eyed pea, black eye bean (*Vigna unguiculata*); pigeon pea, arhar /toor, cajan pea, congo bean, gandules (*Cajanus cajan*); lentil (*Lens culinaris*); bambara groundnut, earth pea (*Vigna subterranea*); vetch, common vetch (*Vicia sativa*); lupins (*Lupinus spp*.); minor pulses, including: lablab, hyacinth bean (*Lablab purpureus*), jack bean (*Canavalia ensiformis*), sword bean (*Canavalia gladiata*), winged bean (*Psophocarpus teragonolobus*), velvet bean, cowitch (*Mucuna pruriens var. utilis*) and yam bean (*Pachyrrizus erosus*) (FAO, 2007).

2.2 Common bean

Common bean is a warm-season crop that does not tolerate long periods of exposure to near-freezing temperatures at any stage of growth. It's not affected by high temperatures if adequate soil water is present, although high nocturnal temperatures will inhibit pollination (Buruchara, 2007). The crop requires moderate amounts of rainfall (300 – 600 mm) but adequate amounts are essential during and immediately after the flowering stage. Common bean is a short-season crop with most varieties maturing in a range of 65 to 110 days from emergence to physiological maturing (Buruchara, 2007). Maturity period can continue up to 200 days after planting amongst climbers that are used in cooler upland elevations (Gomez, 2004). The crop grows in fertile, well-drained soils and does not have conditions that interfere with germination and emergence (Wortmann *et al,* 2006). In Africa, crop cultivation is concentrated at altitude above 1000 masl, with adequate amounts of precipitation (> 400 mm of rain) during crop growing season and soil pH above 5.5. These are the cooler highlands and the warmer mid-elevation areas of East, Central and Southern Africa. However, crop area in low elevation area (<1000 masl) has also been increasing following population pressure.

2.2.1 Botany of common beans

Common bean (*Phaseolus vulgaris* L.), also known as dry bean, is a leguminous plant with pinnately trifoliate large leaves. The beans are smooth, plump, and kidney-shaped, up to 1.5 cm long. The leaves, borne on long green petioles, are green or purple in colour and trifoliate. Leaflets are 6-15 cm long and 3-11 cm broad. The inflorescences are axillary or terminal, 15-35 cm long racemes. The flowers are arranged in pairs or solitary along the rachis, white to purple and typically papillonaceous (Wortmann, 2006). Once pollinated, each flower gives rise to one pod. Pods are slender, green, yellow, black or purple in colour, sometimes striped. They can be cylindrical or flat, straight or curved, 1-1.5 cm wide and up to 20 cm in length (Wortmann, 2006). The pods may contain 4 to 12 seeds. The seeds are 0.5-2 cm long, kidney-shaped and highly variable in colour depending on the variety: white, red, green, tan, purple, gray or black. The flowers are flat (butterfly or keel

shaped) and develop seeds of various size, shape and colour, which are produced in a single cavity pod (Uebersax, 2006). Common bean shows variation in growth habits from determinate bush to indeterminate, extreme climbing types. The bushy type bean is the most predominant type grown in Africa (Buruchara, 2007).

2.2.2 Common bean production and consumption

The world leader in production of dry bean is Burma, followed by India and then Brazil. In Europe, the most important producer is Germany. The regions of highest bean consumption in the world include 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 & Muñoz, 2002).

Table 2.1: Top Ten Dry Bean Producers in the world — as at 2013

Country	Production (Tons)
Burma	3,800,000
India	3,630,000
Brazil	2,936,444
People's republic of china	1,400,000
Mexico	1,294,634
Tanzania	1,150,000
United States	1,110,668
Kenya	529,265
Uganda	461,000
Rwanda	438,236
World	**23,139,004**

Source: United Nations Food And Agricultural Organization: Economic And Social Department: The Statistical Division (FAO, 2007)

Cultivation of common bean in Africa is widespread, but production (approximately 80% of African bean production) is concentrated in 10 countries (Table 2). In terms of area, Kenya is the leading producer of common bean in Africa followed by Uganda and then Tanzania (Table 2). Malawi and Ethiopia rank eighth and ninth, respectively according to FAO statistics (FAO, 2007) However, in terms of production, Kenya comes second after Uganda, with Tanzania keeping its third position. Common bean yields are higher in Uganda than in Kenya because of a

relatively favourable biophysical environment (such as weather condition) in Uganda compared to Kenya. In the latest figures from FAO for 2007, however, the production in Kenya has moved above 500,000 tonnes.

Table 2.2: Top 10 producers of common bean in terms of area in Africa in 2000-2007

Country	Average area (Ha)	Average production (Tons)
Kenya	910 478	412 381
Uganda	7943 75	478 625
Tanzania	373 125	285 414
Rwanda	340055	231882
Burundi	249 375	229 607
Angola	290 391	92 786
DRC	205 958	110 404
Malawi	197 605	87 593
Ethiopia	188 000	143 414
Madagascar	82096	77 273

Source: FAOstat at www.fao.org (FAO, 2007)

In Eastern Africa, common bean is grown twice a year, with sowing seasons running from March to April and from September to October, except in parts of Ethiopia where the main growing season is June to August (Ferris & Kaganzi, 2008). June and August (Meher seasons) in Ethiopia are wetter months and therefore most reliable while the rain between March and April (Belg season) is considered too unreliable to invest in commercial common bean production.

Common bean is used almost entirely for human consumption but beans require processing before they are eaten to degrade the toxic compound, lectin phyto-

hemagglutinin, which would otherwise cause severe gastric upset (Ferris & Kaganzi, 2008). In Eastern and Southern Africa, common bean is important for staggering food supply: leaves, pods, green grains and dry beans. It is consumed as boiled green leaves, green immature pods and/or dry grains. The fresh form of grain is the most preferred because of its fresh flavour, good taste, and requires considerably little time to cook (approximately 40 min). However, fresh beans are difficult to keep, and as such they are consumed for a short time only in season before beans dry. Consequently, beans in Eastern and Southern Africa are consumed as cooked or boiled dry grains, prepared in a wide range of recipes (Katungi *et al.*, 2009).

2.2.3 Bean Based Products

Some of the recipes made of common bean across Eastern and Southern Africa according to Katungi *et al*., 2009 include: In a stew or broth and served with Ugali, bananas, cassava, sweet potatoes, sorghum; Mixed and cooked with a staple food (e.g. whole maize grains), banana, cassava, sweet potatoes etc) and boiled together. This form of consumption is cheaper and quicker to prepare since it uses less fuel energy to prepare as well as shorter time than the stew form. When mixed with maize, it is called *Githeri* in Kenya, Ngata in Malawi, *Kande* in Tanzania and is also present in Uganda. *Githeri* is now a growing form of urban food, especially among the low-income class; Dry common bean can also be soaked, coats removed, boiled and mashed alone like in Malawi to form *Chipere* or in mixture with other foods like milk in Kenya or sweet potatoes in Eastern in Uganda; Cooked as green immature pods and eaten alone or with other foods particularly in restaurants in Tanzania; Boiled grain and consumed as snacks or main dish called *Nifro* in Ethiopia. *Nifro* made of haricot beans alone or blended with other foods and finally leaves boiled and eaten as spinach.

The form of preparation influences the varieties preferred for domestic use. In Kenya Malawi and Tanzania, beans are commonly consumed as boiled dry beans (either as stew or *Githeri*), making the varieties with soft grain when cooked, and thin skins more preferred. Varieties with thin soft seed coats are associated with less cooking time and give soft gravy (Katungi, 2009). Bean pigmentation and size are also

important in consumers' acceptance of a particular bean in these countries. Many consumers in almost all the four countries prefer large brownish/purple or reddish colour seeded beans. Reddish colour is normally preferred because of the red colour it imparts to the food after cooking. Nevertheless, trade-off of seed colour with other superior traits is possible in specific locations and there is a variety of seed colour (Wortmann *et al*., 2006).

2.2.4 Pre and Post-harvest factors that influence bean quality

Green beans should be harvested before reaching maturity, usually 2-3 weeks after blooming, but the time required varies with weather conditions. When ready, the pods are fully grown but the seeds are small. Ideally, green beans should be harvested before the seeds become large enough to cause the pod to bulge around the seeds. Marketable pods are fleshy, tender, and green for only a short period. Most varieties become tough and stringy if left on the plants until the seed develops to a considerable size (Thompson & Kelly, 1980). Beans should be picked after any morning dew is off the plants and they are thoroughly dry. Picking beans when wet can spread bean bacterial blight, a disease that seriously damages the plants.

Harvesting can be done by hand or by machine, though picking by hand is preferable. Hand-harvesting allows for multiple harvests of a field. Beans should be removed from the plants cleanly without tearing them or causing undue damage to the pods or plants. Over-handling or rough handling of the pods will result in both visible and latent damage. In addition, harvested pods should never be tightly packed into harvesting containers or allowed to remain in the sun for extended periods. Basic rules for manual harvesting include: Keeping your hands clean. Remember that you are handling a food product. You should wash your hands after each visit to the rest station; Pick all mature pods on the bush before moving on to the next bush; Harvest only those pods that are ready. Leave immature pods for the next harvest; Avoid overfilling your hands; do not squeeze or roll the pods; Do not put trash or cull pods into the container; Never allow harvested pods to remain in the sun (North Carolina Cooperative Extension Service, 2011).

Machine-harvesting, a one-time operation because the plants are destroyed in the process, usually follows after the first hand-harvest. Mechanical harvesters must be carefully adjusted and operated to reduce the amount of trash and unacceptable pods. Most harvesters have no means of discriminating between quality levels and will harvest immature, over mature, diseased, or damaged pods that would ordinarily be discarded during hand-picking. Eliminating large quantities of unacceptable pods and foreign matter in the packing house is difficult, expensive, and requires the harvested crop to be handled excessively. Under the best circumstances, a mechanical harvester and the required sorting machines subject bean pods to some damage. Any additional handling can so adversely affect the shelf life and appearance of the product that it will be discounted or refused by buyers (North Carolina Cooperative Extension Service, 2011).

1. Grading and packing

Regardless of the harvest method used, minimal handling of bean pods is desirable. Careful supervision of labour is the key to ensuring uniform cleaning, sizing, and packing of hand-harvested green beans. Smaller-acreage growers may use a grading table or belted conveyor located at a packing shed to remove trash and culls. Spreading in-shell beans on a belt or flat surface helps to dissipate field heat before packing and shipping. Pod diameter, not length, is the best indicator of quality. Buyers prefer pods with no bulge or only a slight bulge, indicating tender, young seeds. Over mature green beans with bulging pods are tough and fibrous, while immature pods (pin beans, sieve sizes one and two) are more susceptible to wilting. Uniform sizing of beans in the crate or carton is critical for acceptance by fresh market buyers. The shape must be fairly straight, the color bright, and the appearance fresh and without blemishes. Freshness is evidenced by a distinct, audible snap when the bean is broken (North Carolina Cooperative Extension Service, 2011). Different growers take different approaches to packing. Some growers conduct field packing so that the beans can be quickly moved from field to cooler with minimal handling. Some growers use 13.5 kilo/30 pound (net weight) waxed or unwaxed cartons, depending on the grower or shipper requirement. Other growers use smaller wire-

bound wooden or cardboard cartons (Adsule, Deshpande, & Sathe, 1998; Vegetable Research & Information Centre, 2011).

2. Storage

Green beans are highly perishable and should be cooled quickly after harvest, preferably to 4-5º C/39-41º F. Cooling greatly maintains quality and substantially lengthens shelf life. In addition, prompt and thorough cooling can reduce the effects of dehydration and lessen damage caused by decay-producing organisms. Still, while post-harvest cooling is essential for maintaining quality, it will not improve the quality of a poor product (North Carolina Cooperative Extension Service, 2011). Beans may be room cooled or forced-air-cooled, but hydro-cooling is preferable not only because cold water cools beans rapidly but also because the moisture helps to prevent wilting or shriveling (Gorini, Borinelli, & Maggiore, 1974). A delay in post-harvest cooling and exposure to the sun leads to quality deterioration, resulting in shriveling and weight loss. Therefore harvested beans should be shaded while they are held in the field. Limiting the time between harvesting and cooling to no more than 1 or 2 hours will help maximize shelf life.

If refrigeration is not immediately available, alternatives such as shade, harvesting during the coolest part of the day, and drenching the produce with cold well water after harvesting should be employed. Field-packed containers, if properly cleaned and destined for immediate sale, may be cooled with well water. Wetting may also initiate evaporative cooling if sufficient air circulation is present. Once the beans have been packed in cartons and wetted, air circulation must continue until the products are properly refrigerated. The placement of field-warm beans in a refrigerated space, known as room cooling, may be used but it is only recommended as a last resort. Room cooling may be of some benefit but is slow because it relies only on natural conduction and convection to transfer heat. Palletized and bulk containers of snap beans may require more than 16 hours to cool sufficiently in cooling rooms. To promote cooling and prevent the build-up of respiration heat, the green bean containers should be loosely stacked, leaving space between the pallets for air circulation. In forced-air cooling systems, circulating fans are used in a

cooling room to pull refrigerated air through produce containers, greatly improving the cooling rate. Experiments have shown that forced-air cooling is five to eight times faster than standard still-air room cooling (North Carolina Cooperative Extension Service, 2011).

Hydro-cooling, the process of bringing large quantities of chilled water into contact with the produce, is the preferred method for cooling green beans. It is particularly useful where large volumes of beans must be cooled quickly for shipment to distant markets. Because water is a much better heat transfer medium than air, hydro-cooling is very rapid. In this process, the produce is wetted either with a commercial hydro-cooler that rains the water onto the produce containers as they pass on a conveyor or by immersion into a tank of chilled water. The disadvantage of hydro-cooling is that the beans are wetted. Significant post-harvest disease problems will inevitably occur if the produce is allowed to re-warm after hydro-cooling or if the water is not properly chlorinated. Warm, wet beans are particularly liable to develop any of a host of post-harvest diseases. These include nesting (caused by *Pythium* species or *Rhizopus* species), gray mold (caused by *Botrytis cinerea*), and watery soft rot (caused by *Sclerotinia* species). Although hydro-cooling is the preferred cooling method, it should not be used unless adequate refrigeration facilities are available for continuous cooling and storage (North Carolina Cooperative Extension Service, 2011).

Although the skin of beans offers considerable protection against infection, pathogens (disease-causing organisms) can enter the produce through a variety of openings. Wounds such as punctures, cuts, and abrasions as well as stems and stem scars provide potential points of entry. The probability of pathogens entering the produce increases with the size of the opening, the depth of submergence, the length of time in the water, and the water temperature (North Carolina Cooperative Extension Service, 2011). Always use chlorinated water when washing and hydro-cooling beans and peas. Chlorine is a germicidal agent that can control decay-causing organisms found on produce. A free chlorine concentration of about 55 to 70 ppm at pH 7.0 (neutral) is recommended for sanitizing most fruits and vegetables. It may be necessary to add chlorine to the solution more often if the pH is higher and if the

temperature of the solution is more than 27º C/80º F. In practice, free chlorine concentrations of 150 ppm and more have been used.

Green beans should be stored at 3-7º C/37-45º F. and 95% relative humidity. Under these conditions, green beans will maintain quality for 4-10 days. However, temperatures of 3º C/37º F. and lower may cause significant chilling injury to beans. Chilling injury may appear later during distribution as obvious surface pitting and make beans unmarketable. Equally, chilling injury can occur when beans are stored for longer than 5- 6 days, resulting in a general opaque discoloration of the entire bean. The most common symptom of chilling injury is the appearance of discrete rusty brown spots which occur in the temperature range of 5-7.5º C/41-45º F. These lesions are very susceptible to attack by common fungal pathogens. Beans can be held about 2 days at 1ºC/34º F., 4 days at 2.5º C/36º F or 8-10 days at 5º C/41º F before chilling symptoms appear (Postharvest Technology Research Centre, 2011). When beans are to be stored or transported in mixed loads with other commodities, it is important to consider the compatibility of the produce in regard to temperature, humidity, and the presence of ethylene gas. Ethylene is given off by some fruits (apples, cantaloupes, bananas, and tomatoes) and will hasten the maturity and decline in quality of green beans. Storing beans with ethylene-producing items is discouraged. Beans also readily adsorb the odour of peppers, onions, and cantaloupes. Common storage and shipment with these items should also be avoided (Postharvest Technology Research Centre, 2011).

2.2.5 Effect of temperature and relative humidity on bean quality

Legume seeds are mostly preserved in dry storage and ambient temperatures to maintain all year round supply of this important protein food source. High temperatures during storage in countries such as Australia where air temperatures in summer may rise up to 40˚, can cause deteriorative effects on legume seed quality. The main form of deterioration in increased hardness of cotyledons or loss of cookability (ability to soften with cooking), followed by deterioration of colour, texture, and loss of nutritive value (Martin-Cabrejas *et al.*, 1997; Yousif *et al.*, 2003a). Hardness of cotyledons is commonly described as the ‘Hard-to-cook’ (HTC)

phenomenon and is characterized by a requirement for extended cooking time. HTC beans need additional energy during preparation and may have inferior nutritional qualities in terms of proteins, fats and mineral contents. Long cooking time is also one of the factors responsible for wider underutilization of legume seeds (Deshpande *et al*., 1984). Water absorption, soluble solids and electrolytes leaching are important quality factors associated with the bean hardness defect (Berrios *et al*., 1999) and are good indicators of the loss in bean quality during storage. Improper storage can also alter the nutritional composition of most grains. Storage conditions at high temperature and high humidity favour the appearance of the hard-to-cook (HTC) phenomenon, the main characteristic of which is the extension of cooking time (Plaami, 1997, Machado *et al*. 2008). This is further supported by the findings of Berrios *et al.* (1999) that reported that dry legume seeds stored in such conditions lose the ability to soften during cooking. For example, beans stored for six months at 37°C and 76% RH, did not soften after 300 min of cooking. Beans with this defect require more time to cook, and hence more fuel, so consumers reject them, and most importantly they may also be lower in nutritional value (Machado *et al.* 2008). Improper storage can alter the content of many compounds in seeds and can increase the effect of inhibitors on bioavailability and in the case of tannin it can lead to a decrease because of oxidative degradation but can also alter the seed coat structure leading to bean hardening.

The shade, uniformity and degree of lightness of colour are important indicators of adzuki bean quality (Yoshida *et al.,* 1991). Due to the fact that the colour of adzuki ann paste is influenced by the colour of the adzuki bean seed coat pre-processing,(Taira *et al*., 1989) adzuki seed coat colour plays an important role in determining adzuki bean quality for making Japanese traditional confectioneries. The colour of adzuki beans is related to the variety, growing conditions, and cultivation methods. Variety has a large effect on seed coat colour. Kato & Meguro, 1994; Yoshida *et al*., 1991 and Kato *et al*., 1992 compared the seed coat colour of adzuki beans produced in Hokkaido, the main adzuki growing area in Japan, and found that varieties, cultivation years, and districts of production affected the seed coat colour. Yoshida *et al*., 1991 studied the seed coat colour of seven varieties of adzuki and

found that the values of b* (chromaticness in the direction of blue (−) to yellow (+), L* and a* differed by 34%, 14%, and 8%, respectively, among the seven varieties.

Adzuki seed size is an important factor for both the commercial value, and the functional processing quality of the adzuki. It has been observed to be negatively correlated with the 'a' colour value of the seed coat and small beans show a slower hydration than larger beans. Kato *et al*., 1992; Yoshida & Satoh (1990) collected samples of adzuki seeds from different growers and districts of the Japanese island of Hokkaido (main growing area for adzuki in Japan); they found that the mean seed size varied with district and growers in the same district by as much as 62% and 38%, respectively. These differences may be attributed to cropping cycle, fertilization and harvest period. Seed size has an effect on the rate of water imbibition. Small-sized adzuki (< 4 mm) seeds imbibe water faster than the larger Dainagon type (>4.8 mm). However, smaller seeds have a higher percentage of "hard shell" (HS) defect, which is defined as the failure of seeds to absorb water (at room temperature) within a reasonable soaking time. Contrary to the above, it has been observed that in some cases, adzuki stored for 3 or 4 years at room temperature (∼20C) absorbed more water (at room temperature) than fresh beans of the same variety in the first 11–15 hours. This may be explained by the collection of free water between the seed coat and the cotyledon and in the fissure between the two cotyledons (Fig. 2).(Plhak *et al,* 1989) However, after 24 hours, the amount of water absorbed by aged beans was smaller than that absorbed by fresh beans for all the varieties tested.(Shiota *et al.,* 1983).

The recommended moisture level for adzuki beans during storage is 13% (Duan, 1989). At lower moisture levels, adzuki beans exhibit hardening and slower water uptake during cooking. The relative humidity during storage has a significant effect on the moisture content of adzuki beans. High humidity (RH>80%) storage conditions maintain high moisture content, which can cause mould growth, while relatively dry conditions such as 12.4–33.6% RH decrease the moisture content of adzuki to below 8%. Cooking trials indicated that adzuki beans stored under 12.4 to 33.6% RH, remained partially uncooked even after 90 minutes of cooking (Hayakawa & Breene, 1989.

2.3 Nutritional importance of beans

Common bean (*Phaseolus vulgaris L.*) is an excellent food from a nutritional stand point since it provides essential nutrients such as proteins, iron, calcium, vitamins (especially B complex), carbohydrates, fibre and lysine, which is an essential amino acid (Mesquita *et al.,* 2007). Beans in general are widely grown and consumed as a source of plant protein throughout the world. These are considered as one of the cheapest and richest source of dietary protein, which can be substituted for dietary animal protein (Anderson, 2004). However, proteins in legumes are higher in non-essential amino acids compared with proteins from animal foods (Newby, 2009); and their nutritive value has been known to be low because of their deficiency of sulfur amino acids, poor digestibility and the presence of antinutritional factors (Khattab *et al*., 2009; Abd El-Hady & Habiba, 2003). Yet, it is suggested that sulfur amino acids deficiency can be solved by complementing legume with cereals (Michaels, 2004); and food processing can improve the nutritional quality and increases the bioavailability of nutrients, by inactivating antinutritional factors (Machaiah & Pednekar, 2002; Xu & Chang, 2008). Thus, legumes, with the exception of soybeans, are not a complete protein like meat; however, when paired with grains or another complementary food, they provide a complete source of amino acids (James, 2010). In developing countries, legumes complement the lack of proteins from cereals, roots and tubers (Kalogeropoulos *et al*., 2010). Their most vital role is that of supplying most of the protein in regions of high population density and in balancing the deficiencies of cereal protein (Fabales, 2012). Legumes and cereals are considered ideal dietary partners because the amino acids making up their proteins are very good complements (Michaels, 2004).

Cereal proteins are deficient in certain essential amino acids, particularly lysine. On the other hand, legumes have been reported to contain adequate amounts of lysine, but are deficient in sulfur-containing amino acids (methionine, cystine and cysteine). Therefore, intentional combination of cereals and legumes at meals offers cheap, compared to meat, plant-based diets with balanced amino acid composition (Michaels, 2004; Machaiah & Pednekar, 2002; Iqbal *et al.,* 2006). Accordingly, supplementing cereal-based diets with legumes improves overall

nutritional status and is one of the best solutions to protein calorie malnutrition in the developing countries (Machaiah & Pednekar, 2002).

Legumes constitute a part of the diet of nearly all humans and their nutrient content (protein, carbohydrate and micronutrients) contribute to address under-nutrition (Venter & Eyssen, 2001). They contain complex carbohydrates (oligo-saccharides, dietary fibers and resistant starch- i.e. starch that escapes digestion in the small intestine and may ferment in the large intestine), protein with a good amino acid profile (high lysine), important vitamins (B vitamins, folates, ascorbic acid and tocopherols), minerals as well as antioxidants, polyphenols and numerous other phytochemicals endowed with useful biological activities (Tiwari *et al.,* 2011; Chen *et al*., 2011; Boschni & Arnoldi, 2011; Carvalho *et al.,* 2011). They are good sources of water-soluble vitamins, especially thiamine (*Vitamin* B_1), riboflavin (*Vitamin* B_2), niacin (*Vitamin* B_3), pyridoxine (*Vitamin* B_6) and folate, but poor sources of fat-soluble vitamins and vitamin C. They are low in sodium but are excellent sources of other minerals, including calcium, copper, iron, magnesium, phosphorus, potassium and zinc (Venter & Eyssen, 2001; Mirabelli & Shehab, 2008). Phosphorus in beans is present in the form of phytic acid; however its level can be reduced by processing. Even after cooking, beans provide substantial amounts of the recommended daily allowance for several minerals. According to Wang *et al.,* 2010, cooking of beans in boiling water increased the amount of Mn and P (determined by dry weight basis) but loses in K and Mg were also observed. In general, the ash content of red kidney bean was found to decrease after cooking due to diffusion of certain minerals into the cooking water (Wang *et al*., 2010) They have desirable characteristic such as low fat (except oilseeds), high concentration of polyunsaturated fatty acids and a long shelf life (Sridhar & Seena, 2006; Kalogeropoulos *et al*., 2010). The predominant fatty acid is linoleic acid, although they also contain α-linolenic acid (Venter & Eyssen, 2001). Thus, legumes contain many nutritionally important and health-promoting components responsible for addressing under-nutrition and promotion of health. Zinc is the most abundant intracellular trace element with an important role in many fundamental cellular processes (Gibney *et al*., 2002). It is involved in the synthesis of different growth hormones, collagen as well as alkaline phosphatase and its

deficiency is considered a public health problem (Ramírez-Cárdena *et al.*, 2010). A deficiency of this element can account for impaired immune function and growth retardation (House 1999). After iron, zinc is the micronutrient that is most often deficient in developing countries (Umeta *et al.*, 2005).

Iron presents itself in two forms: haem iron and non-haem iron, the first of animal origin and the second of plant origin. According to Shimelis & Rakshit (2005), beans are one of the best sources of non-haem iron. The "Recommended Daily Allowance" (RDA) for Fe is 10-15 mg, which is hardly reached in diets solely based on non-haem iron. The efficiency of absorption of non-haem iron is lower than for haem iron, merely 2% to 20% (House 1999) because of the different ways in which the two forms enter the body. One of the interesting aspects of iron metabolism in humans is the inefficiency of the body to excrete iron, for which its uptake is tightly regulated to prevent excesses. There are two mechanisms of absorption of such mineral in the duodenum (Zimmermann & Hurrell 2007). Haem iron is absorbed into cells of the mucosae as ferrous iron that is immediately transported by haem iron transporter (HCP1), the expression of which is regulated according to the iron status of the individual (House, 1999), whereas non-haem iron enters the body in the ferric form that must be reduced prior to absorption (House 1999).

2.3.1 Factors influencing nutritional quality of beans

Different chemical characteristics can be found in beans according to the genetic variety (Barampama & Simard, 1993; Kigel, 1999). Genetic research concerning bean culture is mainly aimed at improving productivity and resistance to field pests and environmental stress. Although, there is evidence that it may also change physicochemical characteristics of seeds and affect their nutritional value, studies concerning these changes in beans are still rare and limited to few cultivars (Augustin *et al.*, 1981; Barampama & Simard, 1993; Nunez-Gonzalez *et al.*, 2002).

Nutrient profile of beans may also be affected by environmental factors (Samman *et al.*, 1999), which could make it difficult to obtain standard nutritional values for seeds cultivated in different regions and from different harvests. However, some studies in cereal cultivars (oat, rice, and wheat) indicate that despite the changes in

absolute values due to environmental factors, cultivars may keep a standard compositional pattern, i.e. higher or lower starch or protein content (Storck *et al*., 2005).

Besides these changes in chemical characteristics due to genetic and environmental factors, certain processing techniques, such as cooking and storage conditions, can also produce important changes in starch, fiber, and other components of legume seeds (Kutos *et al*., 2003; Vargas-Torres *et al*., 2004). These changes may affect the digestibility and nutritional value of foods containing common bean seeds. All this variability has remarkable importance for strict diets that are based on food composition tables, which usually do not show specific values for different bean cultivars, nor their possible changes in the course of harvest years. In addition, bean seeds of better nutritional quality could be supplied for low-income populations around the world. Carbohydrates are the major component of beans. Current knowledge on nutritional features of starch indicates that a fraction of this carbohydrate is resistant to enzyme digestion in the small intestine. This resistant starch can be a substrate for the fermentation process in the colon with important benefits for human health, similar to the dietary fiber. However, relatively few studies evaluated resistant starch and dietary fiber content of common beans (Kutos *et al.*, 2003; Vargas-Torres *et al*., 2004; Landa-Habana *et al.*, 2004), while most studies evaluated total starch content and crude fiber content that do not give a real picture of the nutritional implications of these components (Antunes *et al*., 1995; Castellón *et al*., 2003; Lemos *et al*., 2004).

2.4 Health benefits of consuming a high beans diet

Beans are important for a variety of dietary health benefits. These benefits transcend the need for protein and energy and include concerns for risk reduction of a host of chronic diseases prevalent in affluent societies (Kushi *et al*., 1999). The inclusion of beans in daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer (Tharanathan & Mahadevamma, 2003). It has been reported that the protective effect of dry beans in disease prevention such as against cancer,

may not be entirely associated to dietary fibre, but to phenolics and other non-nutritive compounds (Oomah, Tiger, Olson & Balasubramanian, 2006), as polyphenols from dry beans may possibly act as antioxidants hindering the formation of free radicals (Boateng, Verghese, Walker, & Ogutu, 2008).

Studies have shown that intake of whole-grain legumes are associated with lowered risk of chronic diseases such as cardiovascular disease, type-2 diabetes, and cancers (Bazzano *et al*., 2011; Mckeown *et al.*, 2002; Mellen *et al*., 2008; Nagura *et al*., 2009; Schatzkin *et al.*, 2008). The minor constituents including phenolic compounds in whole grain foods are thought to play important role in disease prevention. Phenolic compounds are also known to contribute to appearance, sensory traits, functionality of food products particularly when interacting with proteins (O'Connel & Fox, 2001)

Legumes may offer benefits in the prevention of diabetes and in the clinical management of established diabetes. They reduce the risk of developing diabetes because of their high-fibre, low-fat content and low glycaemic indices. They are slowly digested and produce uniformly low blood glucose response that result in low glycaemic and insulin responses (Tiwari *et al*., 2011; Setchell & Radd, 2000; Venter & Eyssen, 2001; Kalogeropoulos *et al*., 2010) .Glycemic index is a measure of the potential for carbohydrates in different foods to raise blood glucose levels. Generally, foods with high glycemic index (such as white rice or potatoes) cause rapid rise in blood glucose levels, which result in greater insulin secretion by the pancreas.

Chronically elevated blood glucose levels and excessive insulin secretion are thought to play important roles in the development of type 2 diabetes. Several large prospective studies have shown the associated of low-glycemic index diets like legumes with reduced risk of developing type 2 diabetes (Mebrahtom *et al.*, 2012). Obesity is another important risk factor for type 2 diabetes; and it is claimed that low-glycemic diets result in significantly more weight or fat loss than high-glycemic diets. Numerous clinical trials have shown that the consumption of foods with low-glycemic index delays the return of hunger, decreases subsequent food intake, and

increases the sensation of fullness compared to high-glycemic index foods (Mebrahtom *et al.,* 2012).

Thus, diets rich in legumes may decrease the risk of diabetes by improving blood glucose control, decreasing insulin secretion, and delaying the return of hunger after a meal (Anderson, 2004). Legumes contain anthocyanins (Spanou *et al.,* 2010) which may lower blood glucose by improving insulin resistance, protecting β cells, increasing secretion of insulin and reducing digestion of sugars in the small intestine (Sancho & Pastore, 2012). It is reported that clinical use of inhibitors of intraluminal α-amylase inhibitors has importance because controlled reduction of starch digestion theoretically could influence carbohydrate uptake in diabetes mellitus and obesity (Kumanan *et al*., 2010).

Currently, great efforts are focused on reducing the risk of coronary heart disease (CHD) and cardiovascular disease (CVD) through dietary interventions (Kamal & Moazzami, 2009). Dietary interventions to reduce the risk of CVD include attention to the consumption of types of fatty acids, dietary fibre, isoflavones and antioxidants. Legume foods contribute to all these areas (Venter & Eyssen, 2001) and legumes phytochemicals such as carotenoids and tocopherols may prevent the risk of CVD (Boschni & Arnoldi, 2011). The promising link between the intake of leguminous foods and reduced risk of CVD and CHD has been reported in several prospective cohort studies. A number of dietary agents, including soluble fibres and plant sterols lower cholesterol levels in serum. Plant sterols inhibit cholesterol absorption and viscous fibers increase bile acid excretion (Kamal & Moazzami, 2009). Legumes are loaded with fiber and provide a good mix of water soluble and insoluble fibers. The soluble-fiber is known to have a cholesterol-lowering effect and thereby can decrease risk of cardiovascular diseases (James, 2010; Anderson, 2004).

Generally, among the mechanisms that have been proposed to explain the cholesterol-lowering effect of legume seeds, the most commons are the physiological action of legume components such as phytic acid, dietary fiber, saponins, phytosterols, proteins, peptides, and their amino acid profiles (Frota *et al.,* 2008). Legumes contain high levels of antioxidants, vitamin B6, folic acid (James,

2010) and they are fairly good sources of thiamin and niacin (Graham & Vance, 2003). High levels of homo-cystine damage the walls of the arteries; and vitamin B6 and folic acid aid in lowering the levels of homocystine, which in turn can reduce the risk of stroke, heart attack or vascular disease. Folic acid is also important in preventing birth defects (James, 2010; Anderson, 2004) and in reducing the risk of neural tube defects. Moreover, legumes are generally, low in sodium and rich in minerals such as potassium, calcium, copper, and magnesium. Low dietary intake of sodium and high dietary intake of potassium, calcium, and magnesium have been associated with a reduced risk of cardiovascular disease, by helping to lower blood pressure, in epidemiologic studies (James, 2010; Anderson, 2004; Bazzano *et al*., 2001). The low glycemic index values of legumes means that they are less likely to raise blood glucose and insulin levels, which may also decrease cardiovascular disease risk (Anderson, 2004).

Legume polyphenolic compounds act as cancer chemo preventive agents, especially by their antioxidant properties (Spanou *et al*., 2010) as legumes can serve as an excellent dietary source of natural antioxidants for health promotion and cancer (Xu & Chang, 2012). Legume phytochemicals, claimed to demonstrate anti carcinogenic activity, include natural antioxidants, γ-tocopherol, ascorbic acid, soy isoflavones, phytosterols, phytates (inositol hexaphosphate), saponins, fibers, protease inhibitors, lectins, and a variety of polyphenols and phenolic acids. These phytochemicals, if consumed in sufficient quantities, may help to reduce tumour risk and could potentially account for a protective effect. Ascorbic acid, phenolic acids and polyphenols have been associated with reduced cancer risk (Venter & Eyssen, 2001; Boschni & Arnoldi, 2011; Kalogeropoulos *et al*., 2010; Kolonel *et al*., 2000). Phytate has proven anti-cancer action (James, 2010); antioxidant effects, and may lower the risk of colon and breast cancer.

2.5 Challenges faced with the utilization of beans

2.5.1 Hard-to-cook phenomenon

When beans are stored under high temperature (≥ 25°C) and relative humidity (≥ 65%) they become susceptible to hardening which results to extended cooking times,

low acceptability by the consumers and inferior nutritive value (Shiga *et al*., 2004). Long cooking times are a major constraint to wider acceptance and use of pulses. Furthermore, overcooking of pulses has been reported to result in a reduction in the nutritive value of the protein (Pirhayati *et al*., 2011). Several changes occur during development of the hard to cook defect, some of these changes include: increase in water activity and moisture content, a decrease in water absorption capacity and loss of cooking ability of cotyledons and seed coat and alterations in texture, color, and flavour (Pirhayati *et al*., 2011). Shehata (1992) observed a condition where some beans even did not hydrate during soaking despite scarification or removal of seed coats. This phenomenon, in which the cotyledon does not absorb water while the seed coat is permeable, was called 'sclerema' and was attributed to enzymatic activity during storage at high temperature and high relative humidity. Reyes-Moreno *et al.* (2000) and Nasar-Abbas *et al.* (2008) described that extended storage at high temperature and humidity has been associated with the development of HTC defect in some legume varieties. Several mechanisms are proposed for HTC defect in legume seeds including: (i) lipid oxidation and ⁄ or polymerisation; (ii) autolysis of cytoplasmic organelles, weakling plasmalemma integrity and lignifications of middle lamella; (iii) phytin catabolism and pectin demethylation with subsequent formation of insoluble pectate; and (iv) interactions of proteins and polyphenols and polymerisation of polyphenolic compounds (Maurer *et al*., 2004). Another phenomenon that may be involved in HTC defect is the formation of new interactions between cell wall polymers that may reduce cell separation of the plant tissue during cooking (Shiga *et al.*, 2004). Shehata (1992) stated that mechanisms such as interactions between phytates and phenolic compounds with divalent cations (Ca^{2+}, Mg^{2+}) as well as with pectic substances and proteins, starch, lipids and lignin may have been also involved as a secondary effect. HTC defect in legume is basically reversible. Storage of legumes at desirable conditions (low temperature, low humidity) (Hentges *et al.*, 1991), radiation (Fasina *et al*., 2001), soaking in monovalent cations solutions (such as Na^{+}; De Leon *et al*., 1992) and extrusion, as it cause increase in water absorption (WA) and digestibility (Batista *et al*., 2010), are methods suggested to improve the texture HTC legumes.

Abass, 2007 carried out a study to determine the level of ADF and Lignin contents in beans during storage. There was substantial increase in ADF and lignin contents with increased storage temperatures suggesting that, HTC development influences the levels of ADF and lignin. From the study, the varietal differences seemed to be more related to the size of the bean grain, with the variety with small seeds exhibiting higher HTC development. Small seeded beans tend to lose moisture faster than bigger ones resulting in concentration of bean hardening enzymes (phytases and poly-methyl esterases) which accelerates liginification of the beans (Stanley *et al*., 1990).

However, the HTC defect can be overcome through reversibility of the HTC defect when legume seeds are stored at low temperature and high relative humidity. The increased moisture content of seeds stored at low temperature and high relative humidity seems to be a factor to the reversibility of HTC defect, just as high moisture content of stored seeds at high storage temperature relates to the development of HTC defect. If enzymatic activity is pronounced at high temperature and high moisture content to bring about the HTC phenomenon, then low temperature storage may deactivate or slow down any enzymatic activity. But, Maurer *et al*., 2004 and Hentges *et al.,* 1990a have proposed that the pathway by which the reversal of HTC defects occurs may not be the same as that by which HTC defect develops.

Other ways of controlling the long cooking time of hard-to-cook legumes seeds have been reported. Some inorganic chemicals are added to the cooking water during legume food processing. In Africa, for example in Ghana and Nigeria, calcium bicarbonate ($CaHCO_3$) and crude rock salt of carbonates (kaun) are used traditionally to alleviate the long cooking period; however, this method results in increased browning of legume seeds. Browning occurs as a result of these added carbonate salts dissolving in the water medium then altering the concentration of the cooking water by making it more basic.

Micronisation is a precooking technique that utilizes infrared (IR) heat treatment of moisture conditioned seeds. Micronisation has been reported as effective in reducing the long cooking time of cowpeas because it enhances starch gelatinization and

denatures proteins (Mwangwela *et al*., 2006, 2007). Even though micronisation has been effective in reducing cooking time of legumes, affordability of the microniser by all is questionable.

2.5.2 Flatulence

Legumes contain relatively high amounts of dietary fibre and resistant starches. Dry beans contain Raffinose and stachyose (oligosaccharides). Values for sucrose, Raffinose and stachyose content in bean seeds reported in the literature varied from 15-27 mg/100g, 2-5 mg/100g, and 18-38 mg/100g dry weight respectively (McPhee & Myers., 1996; Barampama & Simard, 1993). The soluble oligosaccharides found in legumes are not digestible by human intestinal enzymes; instead, they are broken down by bacterial fermentation in the intestines (Suarez & Levitt, 2000). Although some rectal gas is due to the ingestion of air, the majority of flatulence is produced from bacterial fermentation. The byproducts of this degradation are hydrogen, carbon dioxide, methane, and sometimes sulfur, depending upon the bacteria. Normal intestinal processes move these gases out of the body in the form of flatus (Kurbel *et al*., 2006). Some species of mold produce α-galactosidase, an oligosaccharide hydrolyzing enzyme, which humans can take to facilitate digestion of oligosaccharides in the small intestine. This enzyme can be added to food or consumed separately. Raffinose family oligosaccharides of legumes are synthesized by sequential addition of galactose units to sucrose (Peterbauer & Richter, 2001) and are α-galactosyl derivates of sucrose (Stoddard *et al*., 2010). These α-galactosides or oligosaccharides are a common carbohydrate present in the seed of grain legumes (Hill, 2003). Raffinose is the first member of the series. Verbascose, stachyose and ajugose are the next higher RFOs (Peterbauer & Richter, 2001). Raffinose family oligosaccharides of legumes produce flatulence**.** Humans do not have the enzyme α-galactosidase to cleave the α-galactosyl linkage and the intact oligosaccharide is not absorbed by the digestive tract. These oligosaccharides accumulate in the large intestine where the α-galactosidase containing intestinal bacteria degrade them and subsequent anaerobic fermentation results in production of H_2, CO_2 and traces of CH_4. These gases cause abdominal discomfort due to a flatus effect and sometimes result in diarrhoea (Stoddard *et al*., 2010). Traditional methods of processing grain

legumes prior to their consumption significantly reduced the level of oligosaccharides (Hill, 2003); and the flatulence side effect of legume oligosaccharides can be minimized in different ways such as processing, plant breeding, incorporation of microbial or plant α-galactosidase, or by changing the water in which beans are boiled one or more times (Venter & Eyssen, 2001; Stoddard *et al*., 2010).

2.5.3 Antinutrients

2.5.3.1 Lectins

Lectins are proteins that bind to carbohydrates or to molecules containing carbohydrates (Michaels, 2004). Lectins (or phytohemagglutinins), are present, paralleling the distribution of protease inhibitors, in the seed of a number of legume species that are consumed by humans (Hill, 2003; Liener, 2003). Protease inhibitors often inhibit the digestive enzyme trypsin, but may act more broadly by inhibiting chymotrypsin and other serine proteases (Michaels, 2004). They can retard growth by interfering with dietary protein digestion; and as a mechanism to adapt to this situation, the secretory activity of the pancreas can be stimulated and its size enlarged leading to an overall physiological effect of impaired growth and enlarged pancreas (Liener, 2003). Young humans and other animals are more susceptible to the effects of protease inhibitors than adults and are primary concern as these can impair nutrient utilization and reduce growth rates in the young groups. Lectins differ in the severity of their impact (toxic, only growth inhibitory or essentially nontoxic) (Michaels, 2004). Their antinutritional effects lies in the fact that dietary lectins strongly resist proteolytic degradation in the gut and bind to surface receptors (Michaels, 2004; Hill, 2003) on the cells lining the small intestines and interfere with the absorption of nutrients; the result is a failure in growth and eventual death (Liener, 2003). Fortunately, in many cases, protease inhibitors and lectins are heat labile and rendered innocuous by usual methods of cooking. Both are heat-labile and can be effectively eliminated or inactivated, essentially under the same conditions, by the heat treatment involved in domestic cooking and commercial processing (Michaels, 2004; Liener, 2003).

2.5.3.2 Trypsin/chymotrypsin inhibitors

A trypsin inhibitor is a type of serine protease inhibitor that reduces the biological activity of trypsin. Trypsin is an enzyme involved in the breakdown of many different proteins, including as part of digestion in humans and other animals. As a result, protease inhibitors that interfere with its activity can have an antinutritional effect. Protease inhibitors, in particular trypsin inhibitors, have generally been considered as anti-nutritional due to the long-standing observations that feeding animals raw beans causes growth depression and reduces nitrogen retention

2.5.3.3 Phytic acid

Phytic acid is one of the anti-nutrients in dry beans and serves as the main phosphorus reservoir. It accounts for up to 80% of the total phosphorus in common beans and occurs as a complex with mono- and divalent cations in discrete regions of the bean. Most of the phytic acid is located in the cotyledons and not on the seed coat Phytic acid, also known as inositol hexasphosphate or phytate when in the salt form, is the storage form of phosphate in many plant tissues. It is not digested by humans and is therefore not a dietary source of inositol or phosphate. They bind iron, zinc, calcium and magnesium. In presence Ca^{2+} and Mg^{2+}, it forms insoluble complexes which are not readily absorbed by the gastrointestinal tract (Akande *et al*., 2010; Agbaire & Emoyan, 2012). On germination of grains, the phytate reduces due to enzymatic breakdown of phytate that improves iron availability.

The ability of phytate to bind metal ions is lost when the phosphate groups are removed by hydrolysis through the action of phytase. Heat alone is relatively ineffective in reducing the phytate content of plant materials, but the phytate content can be reduced by taking advantage of the endogenous phytase that accompanies the phytate in separate compartments of the plant tissue (Liener, 2003). Traditional fermented dishes have reduced levels of phytate, presumably due to the action of phytase produced by various molds, bacteria or yeasts involved in the fermentation. The germination of mature seeds of various legumes results in a great increase in phytase activity with a concomitant reduction in phytate (Liener, 2003).

2.5.3.4 Tannins

Tannins are a group of water-soluble polyphenols of intermediate to high molecular weight. Tannins are highly hydroxylated molecules and can form insoluble complexes with carbohydrates, proteins and digestive enzymes, thereby reducing food digestibility. They can also bind cellulose and many mineral elements (Santos-Buelga & Scalbert, 2000). Tannins are also considered bioactive compounds because of their antioxidant capacity (Xu *et al.,* 2007; Xu & Chang, 2009) however they may have beneficial or adverse nutritional effects (Xu *et al*., 2007).

The anti-nutritional effects of tannins include: depression of food intake, formation of tannin complexes with dietary protein and other food components, inhibition of digestive enzymes, increased excretion of endogenous protein, effect of tannin on digestive tract and toxicity of absorbed tannins and other metabolites. Several processing methods and chemical treatments have been used to eliminate tannins from legumes. These include physical removal of tannins by milling and separation hulls, cooking, germination, soaking, plant breeding, addition of agents that aid in metabolic detoxification of tannins, and chemical treatment of samples (Reddy *et al*., 1985). Soaking beans in water and discarding the soak water may eliminate a percentage of these compounds. Some studies (Oliveira *et al*., 2001ab; Ramirez-Cardenas *et al.*, 2008) found greater reduction in the content of tannins, phytates and oligosaccharides in beans that were soaked and cooked without the soaking water.

Some of its uses include: In leather industry, the art of tannin i.e. converting animal hide or skin to leather is considered to be the first leather manufacturing process (Marion & Roy, 2006); tannins finds application in ink manufacture, dye industry, plastic resins, water purification, manufacture of adhesives, surface coatings, manufacture of gallic acid etc. (Bisanda *et al*. 2003), a number of tannin bearing raw materials are used extensively in the preparation of folk and Indian medicine.

CHAPTER THREE

METHODOLOGY

3.1 Experimental Set up

Pinto (GLPX92) and Red Haricot (GLP585) varieties of beans were obtained from Kenya Agricultural Livestock and Research Organization (KALRO) Thika in Kenya. The varieties were selected since they are among the most popular bean varieties in Kenya and had all been bred and grown at KALRO Thika station under similar and controlled agronomic conditions (Kinyanjui *et al*., 2015). They were also selected based on their cooking abilities, Pinto is a hard to cook and red haricot is an easy to cook bean. Manipulation of storage conditions was done by inducing hardness through storage (temperature, RH conditions). A control sample of freshly harvested beans was stored at -20 ˚C for each variety after the equilibration at 25 ˚C and 75% RH for two weeks. The different bean varieties were subjected to the different storage temperature of 35 ˚C and 45 ˚C and relative humidity of 75% and 83%. The conditions were selected based on the temperature ranges that can be found in some regions across Kenya i.e. normal (25˚C), moderate (35˚C) and extremely high temperature (45˚C). the relative humidity were also selected on the same basis with 75% being the almost normal RH conditions while 85% being the extreme condition found in some regions in Kenya. The accelerated storage conditions were 35 ˚C 83% RH and 45 ˚C 75% RH. These conditions were achieved by using storage incubators pre-conditioned to the desired temperature. The respective relative humidity was achieved using concentrated salt solutions of potassium chloride (83% relative humidity) and sodium chloride (75% relative humidity). 3 incubators were used for each set of temperature and RH, and sampling was carried out at 0, 2, 4 and 6 months.

Plate 1: Pinto Plate 2: Red haricot Source: Wafula, 2012

3.2 Impact of pre-treatment technique on cooking time

The seeds were soaked separately in distilled water, 0.025 M Sodium carbonate and 0.1 M calcium chloride for 16 hours at room temperature. A seed to water ratio of 1:5 (w/v) (bean weight: soaking solution) was used. The calcium chloride concentration was according to the modified method of Clemente *et al*., 1998, while the 0.025 M sodium carbonate was due to the retention of natural color of the beans unlike higher concentrations which gave them a darker color (Kinyanjui *et al*., 2015). The remaining water was discarded and the soaked seeds were subjected to subjective hardness tests.

3.3 Subjective hardness test: use of fingers (tactile method)

A sample was placed in perforated bags and cooked in boiling water (Water bath shaker SHA-C, No: 10706004) at a temperature of 96.5 ˚C. Triplicate sub samples (20 seeds each) were taken using a spoon at 30 minutes intervals and tested for softness by squeezing the seed between the forefinger and thumb as described by Kinyanjui *et al.*, 2015. A seed was deemed to be cooked when the cotyledon could disintegrate on pressing between the thumb and the fore-fingers (Kinyanjui *et al*., 2015). A panel consisting of two researchers carried out the cooking tests using the method. A graph of the number of cooked beans against time was drawn to determine the cooking profiles of the different bean varieties.

3.4 Determination of physical properties

3.4.1 Hydration coefficient (imbibition value)

Hydration coefficient was determined by soaking 20 seeds of each variety at 25 ˚C in deionized water (ratio of 1:5 w/v) (bean weight to water volume). The beans were removed after 16 hours from the soaked water, cut into two halves along the fissure

and the testa separated from the cotyledon followed by free water removal using a blotting paper and re-weighing. Gain in weight was taken as the amount of water absorbed and expressed as the hydration coefficient (El-Refai *et al*., 1988):

$$Hydration\ coefficient = \frac{Weight\ of\ beans\ after\ soaking}{Weight\ of\ beans\ before\ soaking} \times 100 \quad (1)$$

3.4.2 Swelling coefficient

The volume of raw bean seeds before and after soaking in deionized water at 25 ˚C for 16 hours at a ratio of 1:5 (w/v) (bean weight to water)was determined by water volume displaced in a graduated cylinder and expressed as the swelling coefficient (El-Refai *et al*., 1988):

$$Swelling\ coefficient = \frac{Volume\ of\ beans\ after\ soaking}{Volume\ of\ beans\ before\ soaking} \times 100 \quad (2)$$

3.4.3 Electrolytes and solutes leaching

Twenty seeds of each variety were soaked in deionized water for 16 hours at 25 ˚C. After 16 hours soaking in deionized water the soak water was collected and leached electrolytes were quantified by assessing conductivity with a digital conductivity meter (Sisabata model SC-179, Tokyo, Japan) in megohm/cm at 25 ˚C (Hentges *et al*., 1991). To measure solutes leached from beans during soaking, soak water was evaporate, dried in a hot air oven (105˚C), cooled in a dessicator, weighed and expressed as mg/g dry weight of beans.

3.4.4 Moisture content

This was done according to AOAC methods specification 950 46, method 925.10-32.10.03 (AOAC, 2000). 100 seeds of each variety were weighed and milled. Triplicates of 2 g were placed in crucibles and incubated for 16 hours at 105 ˚C in a hot air oven (DSO-500D Mrc Ltd, Tokyo, Japan). The samples were cooled in a dessicator for 1 hour and weighed to determine moisture loss. The moisture content of seeds in percentage dry weight was calculated as:

$$Moisture\ (\%) = 100\left(\frac{W1-W2}{W1}\right) \quad (3)$$

Where W_1 is weight of sample before drying, W_2 is weight of sample after drying

3.4.5 Colour

Seeds packed in transparent polythene bags for the purpose of analysis were selected for their colour determination using colour L.A.B equipment (Minolta Chroma Metre CR-200b). The instrument was first calibrated using standard black and white plates (with transparent papers placed on the side plates). After calibration, colour measurements were randomly taken in triplicates and the values interpreted as follows:

L represents lightness/darkness, **a** represents red/green and **b** represents yellow/blue. A positive **ΔL** value is lighter and a negative value is darker, a positive **Δa** value is redder and a negative value is greener and a positive **Δb** value is yellower and a negative value is bluer.

3.4.6 Characteristic dimension of beans

Seeds were selected randomly and their width and length determined using a Vernier caliper (Mitutoyo, Tokyo, Japan) to an accuracy of 0.001 mm, in triplicate.

3.4.7 100 Seed weight

100 seeds were selected in triplicate and their weights (g) recorded using a weighing balance (Libror AEG220 Shimadzu, Tokyo, Japan).

3.4.8 Bulk density

Bulk density (BD) was determined using the method of Wang & Kinsella (1976). An empty graduated measuring cylinder (250 ml) was weighed. Seed material of common bean was placed in the graduated measuring cylinder and packed by gentle tapping of the cylinder on a bench top. This weight was recorded (g). Bulk density ($g/cm^{3)}$ was calculated as:

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$$\text{Bulk Density} = \frac{(\text{cylinder and sample weights}) - (\text{cylinder weight})}{\text{volume of cylinder}} \quad (4)$$

3.4.9 True seed density

Seed/ true density was obtained by liquid displacement using a top loading balance. Seeds were immersed in distilled water in a beaker. The mass of the displaced water was the balance reading with the seed submerged minus the mass of the beaker plus water. The seed volume was estimated by dividing the mass of displaced water (g) by the density of water (g/cm^3). Seed density was determined by dividing the seed mass (g) by the measured seed volume (cm^3).

$$True\ seed\ density = \frac{\text{weight of 20 seeds in (g)}}{\text{volume of 20 seeds (cm3)}} \quad (5)$$

3.4.10 Seed porosity

This is the property of the grain which depends on its bulk and kernel densities. It was calculated as:

$$P = 100\left(1 - \frac{BD}{TSD}\right) \quad (6)$$

Where BD is bulk density (kg/m^3) and TSD is seed density (kg/m^3)

3.5 Determination of chemical, nutritional and anti-nutritional changes during storage

3.5.1 Chemical changes

3.5.1.1 Crude protein

This was done by semi-micro Kjeldahl method. 1g of the sample was weighed and placed in digestion flasks in triplicate. 5.5g Kjeldhal catalyst (5g K_2SO_4 and 0.5g $CuSO_4$) and 15ml concentrated H_2SO_4 were added and flasks placed in inclined position in the fume hood during heating until solution changed from black-brown-green-blue. It was cooled and transferred to 100ml volumetric flask and completely filled to the mark with distilled water. Distillation followed (Parnas-Wagner's method), the excess acid was distilled with NaOH solution. The distillate was titrated with 0.02N HCL solution until the colour changed from blue-dirty green-orange (AOAC, 2000).

$$\%\ Nitrogen = (V1 - V2) \times N \times f \times 0.014 \times \frac{100}{V} \times \frac{100}{s} \quad (7)$$

V_1= Titre for sample (ml)

V_2= Titre for blank (ml)

N=Normality of standard HCL solution (0.02)

f= Factor for standard HCL solution

v= volume of diluted digest taken for distillation (10ml)

s= weight of sample taken (g)

3.5.1.2 Crude ash and minerals

The analysis for minerals was done using Atomic Absorption Spectrometer (AAS) at the beginning and end of the storage period using AOAC method (AOAC, 2000). The minerals that were determined were calcium, iron, zinc and magnesium. 5 grams of the sample was weighed into clean dry crucibles in triplicates. The crucibles was placed on a hot plate under a fume hood and the temperature increased slowly until smoking cease and the samples were thoroughly charred. They were then put in an electric muffle furnace (KL-420 Advantec) and temperature increased gradually to 250° and heated for 1 hour. The temperature were increased to 550° and incinerated for about 5 hours. The temperature was then decreased to 300°, the crucibles removed and cooled to room temperature. The ash was transferred quantitatively to 100 mL beaker using 20 mL of 1N HCL, then heated at 80-90 °C on a hot plate for 5 minutes. This was then transferred to 100 mL volumetric flask and filled to the mark using 1N HCL. Insoluble matter was filtered and the filtrate kept in a labeled polyethylene bottle. The absorbance of the solutions was read by Atomic Absorption Spectrophotometer (AAS). The various mineral standards were also prepared to make the calibration curve.

$$\%\ \text{Crude ash} = \left(\frac{W1}{W2}\right) \times 100 \quad (8)$$

W1~weight of ash

W2~weight of the sample

3.5.2 Nutritional changes

3.5.2.1 In Vitro protein digestibility

This was done by Hori *et al.*, 1990 method. The nitrogen contents of samples were determined by the semi micro Kjeldahl method. A factor of 6.25 was used to convert nitrogen to protein. 100 mg of Samples were weighed and incubated with 0.5ml pepsin solution (10mg pepsin/ml) (pepsin from porcine stomach mucosa (Sigma Chemical Co.., USA), 1:10000), 10ml Walpole buffer (pH 1.5) and 2 drops of toluene at 37 ºC for 2 hours with constant shaking (Water bath shaker SHA-C). The samples were then neutralized with 0.5 N NaOH until approximately pH 8 was attained using a pH paper. Further digestion was done with 0.5ml trypsin solution (5mg trypsin/ml) (DIFCO Laboratories USA, 1:10000) and 10ml 0.1M sodium phosphate buffer (pH 8) for an additional 24 hours. At the end of digestion period the digests were transferred into conical flasks using 10% trichloroacetic acid to precipitate the protein. Samples were left standing overnight and filtered using Whatman No.5. The nitrogen in the filtrate was determined using the semi-micro Kjeldhal method.

3.5.3 Anti-nutritional changes

3.5.3.1 Tannin determination

Tannins were estimated by Vanillin-HCl method of Price *et al*. (1978) as modified by Ochanda *et al*., 2010. Approximately 0.25g of bean flour was accurately weighed into Erlenmeyer flask. 10 ml of 4% HCL in methanol was pipette into each flask, sealed with parafilm and shaken (KS 250 basic, Germany) for 20 minutes, centrifuged (HPLC-CTO-10AVP Shimadzu; detector used- Shimadzu RID6A Refractive Index Detector) for 10 minutes at 4500 rpm and supernatants transferred to 25 ml volumetric flasks. Second extraction using 5 ml of 1% HCL in methanol was added to the residue of the first extraction and process repeated. The aliquots of the first and the second extracts were combined and made up to 25 ml volume using methanol. A set of catechin (Sigma) standards solutions was prepared ranging from

100 to 1000 ppm using methanol as a solvent. 1 ml of suitably diluted extract was taken in a test tube and 5ml of freshly prepared vanillin-HCl reagent added. To correct for interference of natural pigments in dry bean seed coat, a blank sample was prepared by subjecting the original extract to the reaction conditions without the vanillin reagent. They were prepared by adding 5 ml of 4% HCL in methanol to 1 ml of the aliquots of the extracts pipetted into the test tubes. The absorbance of the standard solutions, sample extracts and blanks were read in a UV-VIS Spectrophotometer (Shimadzu, UV mini 1240, Japan) at 500 nm exactly 20 minutes after adding Vanillin-HCL reagent to the samples and standards. A standard curve was prepared from the readings of the catechin standard solutions. Tannin content was expressed in mg of catechin equivalent (CE) per g of sample (mg CE/g).

3.5.3.2 Phytic acid determination

This was done by HPLC analysis of phytic acid according to Camire and Clydesdale, 1982 and modified by Ochanda *et al*., 2010. Approximately 50 mg of sample was weighed into 125 ml Erlenmeyer flasks in triplicate, 10 ml 3% sulphuric acid added and placed on shaker (Model KS basic, Germany) for 30 minutes at 1500 rpm then filtered using 0.45-µm filter paper (Econofilter PTFE, Agilent Technologies). Filtrate was transferred to a boiling water bath for 5 minutes then 3ml of $FeCl_3$ solution (6 mg iron per ml in 3% H_2SO_4) added. A second boiling water bath heating was done for 45minutes to complete precipitation of the ferric phytate complex. Centrifugation followed at 2500 rpm (H-2000C, Japan) for 10 minutes and supernatant discarded. The precipitate was washed with 30 ml distilled water, centrifuged, and the supernatant discarded. 3 ml of 1.5N NaOH was added to the residues and volume brought up to 30ml with distilled water. Heating was done for 30 minutes to precipitate ferric hydroxide. Cooled samples were centrifuged and supernatant transferred into 50 ml volumetric flasks. The precipitate was rinsed with 10 ml distilled water, centrifuged and supernatant added to the contents of the volumetric flask. 20 µl of the supernatant was injected into HPLC (Shimadzu CBM 20A plus, Japan) fitted with a 50377 RP-18 (5µm) column cat at an oven temperature of 30˚C and RID-6A detector model. The mobile phase was 0.005N sodium acetate in distilled water, at a flow rate of 1 µl/min and injection volume 20 µl. A stock

solution of the standard containing 10 mg/ml of sodium phytate (inositol hexaphosphoric acid C_6H_6 $(OPO_3Na_2)_6+H_2O$) in distilled water was prepared. Serial dilutions were made to contain from 1 g/100 ml to 100 mg/100 ml for the preparation of a standard curve. Phytate values was calculated as per the calculations of Vohra *et al*., 1965.

$$Phytate\ content\ (mg\ per\ g) = \left(\frac{y-c}{b}\right) \times \left(\frac{dilution\ factor}{weight\ of\ sample}\right) \quad (9)$$

y = peak area b = concentration c=intercept y

4.0 Data analysis

Differences between varieties and various treatments was determined using one way analysis of variance (ANOVA) using Genstat (14^{th} edition – 2012). Mean comparisons for treatments were made using Duncan's Multiple Range Tests. The least significant difference (LSD) at 5% probability level was used for mean separation. Correlation between the cooking time and other parameters was also carried out to determine their relationship.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of storage temperature and relative humidity on physical properties

4.1.1 Soaking characteristics of Pinto and Red Haricot bean varieties.

Substantial differences occurred in physical characteristics of common beans stored at different conditions as shown in (Table 3 to 6) for 6 months. The storage conditions played a very important role in the hardening process of beans. Beans stored at 45°C/75% RH for 6 months were significantly harder than those stored at the same condition for 0, 2 and 4 months as well as those stored at 35ºC/83% after 0, 2, 4, and 6 months. After 16 hours soaking followed by 2 hours cooking, the finger pressing force required for seeds stored at 45 °C/75% was higher. According to Garcia *et al*., 1998, it develops in dry beans kept under adverse conditions. Liu & Borne, 1995 suggest that the main cause of the HTC phenomenon is the change in the cell wall polysaccharide structure and organization. As there may be a relationship between the insolubility of non-starch polysaccharides and bean hardening, knowledge in the composition and the structure of the polysaccharides in defective beans may help to explain the HTC phenomenon and its consequence for the nutritional quality of legume seeds (Shiga *et al*, 2003; 2004).

Solutes and electrolytes leaching after 16 hours soaking substantially increased with storage (Table 3). Storage of beans at 45°C/75% for 2, 4 and 6 months exhibited a higher solute leakage than the ones stored at 35 ºC/83% for the same period of time and fresh beans stored at 25°C/75% for 0 month. Berrios *et al.,* 1999 found after 12 hours soaking the loss of solids and electrolytes from black beans stored at refrigeration temperatures (4-5˚C) for two years was 10.5 mg/g compared with 18.6 mg/g for beans stored at 23-25˚C for the same period. Hentges *et al.,* 1991 also reported that cowpeas (*Vigna unguiculata*) and dry beans (*P. vulgaris*) stored at 29˚C for 24 months lost more solids and electrolytes than seeds stored at 5˚C.

There was significant difference (P>0.05) in conductivity as it increased with increased storage (Table 3). Pinto ranged from 4.7mohm/cm to 8.7mohm/cm with a mean of 5.75mohm/cm, while Red haricot ranged from 4.5mohm/cm to 9.63mohm/cm with a mean of 6.14mohm/cm. This is in line to what Berrios *et al*, 1999 found, that electrical conductivity of the soaked water after 12 hours soaking of HTC black beans (stored at 23˚C-25˚C) was nearly twice (818 ohms) that of beans (414 ohms) stored at 4-5˚C for 2 years. Similarly, after accelerated aging of cowpeas, at 40˚C and 100% relative humidity solute leakage and electrical conductivity showed progressive increases as aging period increased from 1 to 6 days. (Asiedu *et al*., 2000)

Hydration and swelling coefficients that reflect the capacity to imbibe water in a reasonable length of soaking time was substantially affected by storage temperature. After 12 hours soaking, the hydration coefficient for fresh beans varied significantly across the table and swelling coefficient was significantly (P<0.05) lower in samples stored at higher temperatures, especially those stored at 45°C/75%, compared to the fresh samples and those stored at 35ºC/83% (Table 3). These results are in line with what Yousif *et al*., 2002 found, Adzuki beans (*Vigna angularis*) stored at 30˚C for 6 months absorbed less water than those stored at 20˚C and 10˚C. According to Liu *et al.*, 1992, low hydration and swelling coefficients following storage at high temperature can be due to structural and chemical changes in the testa making it harder and less permeable to water so that it acts as a barrier preventing water reaching the cotyledons.

Table 4.3: Soaking characteristics of Pinto and Red Haricot bean varieties.

Storage conditions	Hydration coefficient (%)		Swelling coefficient (%)		Conductivity (mohm/cm)		Leached solutes (%)	
˚C/% RH/months	Pinto	Red haricot	Pinto	Red haricot	Pinto	Red haricot	Pinto	Red haricot
25/75/0	174.7 ± 5.8^{b}	180.6 ± 2.6^{ab}	207.1 ± 10.1^{a}	220.4 ± 10.3^{a}	4.7 ± 0.7^{a}	4.5 ± 0.3^{a}	0.7 ± 0.1^{a}	0.9 ± 0.1^{a}
45/75/2	195.6 ± 5.1^{c}	202.6 ± 9.4^{d}	204.2 ± 7.2^{a}	200.0 ± 0.0^{a}	5.1 ± 0.1^{a}	5.5 ± 0.5^{b}	1.7 ± 0.2^{a}	1.4 ± 0.3^{ab}
45/75/4	191.1 ± 2.0^{c}	198.9 ± 3.9^{cd}	200.0 ± 16.7^{a}	206.3 ± 10.1^{a}	6.4 ± 0.1^{b}	6.2 ± 0.6^{b}	2.1 ± 0.6^{a}	2.39 ± 0.7^{ab}
45/75/6	164.1 ± 1.4^{a}	169.7 ± 3.9^{a}	197.6 ± 4.1^{a}	188.4 ± 11.1^{a}	8.7 ± 0.2^{c}	9.63 ± 0.1^{c}	2.8 ± 2.6^{a}	2.64 ± 1.2^{b}
35/83/2	169.7 ± 6.8^{ab}	177.6 ± 5.3^{ab}	205.6 ± 9.6^{a}	229.2 ± 7.2^{a}	4.8 ± 0.6^{a}	5.3 ± 0.2^{ab}	1.8 ± 0.7^{a}	1.62 ± 0.7^{ab}
35/83/4	190.3 ± 2.5^{c}	185.2 ± 7.3^{bc}	204.2 ± 5.9^{a}	215.7 ± 13.7^{a}	4.9 ± 0.1^{a}	5.7 ± 0.2^{b}	2.1 ± 0.4^{a}	1.9 ± 0.7^{ab}
35/83/6	166.4 ± 3.5^{a}	168.4 ± 1.8^{a}	202.4 ± 8.3^{a}	193.3 ± 0.0^{a}	5.6 ± 0.2^{ab}	6.06 ± 0.9^{b}	2.4 ± 1.3^{a}	2.45 ± 0.9^{ab}
LSD (5%)	7.9	13.7	29.6	43.5	1.06	0.99	1.9	1.4
Mean	178.85	183.3	206.5	207.6	5.75	6.14	1.77	1.92

Means within the same column with different superscripts were significantly ($P<0.05$) different. Values are presented as Mean ± SD, n=3.

4.1.2 Colour characteristics and moisture content of Pinto and Red Haricot bean varieties.

Colour reflects the (L) darkness/lightness, (B) yellowness/blueness and (A) greenness/redness of a substance. From table 4, we can see a decrease in L value with increasing relative humidity, storage time, and temperature, and an increase in both A and B values. This is because the longer the beans were stored the darker they became, and for Pinto it became more yellow while Red Haricot became more red. This can be attributed to non-enzymatic browning of beans and polymerisation reactions (Reyes-Moreno *et al.,* 2000). Storage of faba bean at high temperatures (≥ 30˚C) accelerated colour darkening especially at ≥37˚C. This supports earlier evidence that high temperature storage is an important factor causing colour darkening in faba bean and other legume seeds (Amarowicz *et al.,* 2004; Sorour and Uchino, 2004). Davies, 1994 also found out that storage of faba beans at 40˚C caused a substantial increase in colour darkening. Adzuki beans (Yousif *et al.,* 2003b), also darkened when stored at 30˚C.

There was a general trend of decreasing moisture content with increased storage time. Fresh beans had the highest moisture content which decreased with increased storage time which can be attributed to the increase in bean hardness. There was significant difference ($P>0.05$) in the moisture content among the different storage conditions. The moisture content of beans ranged from 4.9% to 10.9% (Table 4). For Pinto, the moisture content ranged from a minimum of 4.9% to a maximum of 10.9%, while for red haricot the moisture content ranged from 5.14% to 10%. There was no significant difference in bulk and seed density during storage at different conditions (Table 6).

Table 4.4: Colour characteristics and moisture content of Pinto and Red Haricot bean varieties.

Storage conditions	Colour (L)		A		B		Moisture Content (%)	
˚C/% RH/months	Pinto	Red haricot	Pinto	Red haricot	Pinto	Red haricot	Pinto	Red haricot
25/75/0	53.9 ± 2.8^{b}	43.7 ± 1.9^{cd}	6.6 ± 0.3^{a}	7.7 ± 1.4^{ab}	9.7 ± 0.8^{a}	0.4 ± 0.7^{a}	10.9 ±0.26^{e}	8.22 ±0.2^{de}
45/75/2	52.0 ± 2.1^{b}	42.1 ± 2.1^{cd}	7.5 ± 1.0^{a}	7.8 ± 2.7^{ab}	9.9 ± 0.5^{a}	0.5 ± 0.1^{a}	8.54 ± 2.0^{c}	7.62 ± 3.7^{cd}
45/75/4	47.6 ± 2.5^{b}	38.6 ± 1.1^{c}	7.8 ± 0.7^{a}	10.2 ± 1.4^{bc}	10.1 ± 0.6^{a}	0.8 ± 0.6^{a}	6.45 ± 0.6^{b}	7.16 ± 0.1^{bc}
45/75/6	35.8 ± 3.7^{a}	21.8 ± 4.9^{a}	13.4 ± 0.3^{c}	12.8 ± 2.9^{c}	17.6 ± 1.7^{b}	2.2 ± 1.3^{ab}	4.9 ± 0.8^{a}	5.14 ± 0.1^{a}
35/83/2	51.7 ± 0.7^{b}	41.9 ± 4.0^{cd}	6.8 ± 0.5^{a}	9.8 ± 0.9^{abc}	10.4 ± 1.3^{a}	0.7 ± 0.4^{a}	9.6 ± 0.4d	10.0 ± 0.2^{f}
35/83/4	49.5 ± 4.1^{b}	44.8 ± 1.6^{d}	7.6 ± 0.4^{a}	5.7 ± 0.3^{a}	10.9 ± 0.6^{a}	0.9 ± 0.5^{a}	7.56 ± 1.2^{c}	8.78 ± 0.1^{e}
35/83/6	40.1 ± 5.2^{a}	28.6 ± 4.0^{b}	9.9 ± 1.1^{b}	10.6 ± 3.1^{bc}	15.9 ± 1.7^{b}	4.3 ± 4.0^{b}	5.89 ± 0.3^{ab}	6.36 ± 0.1^{b}
LSD (5%)	7.4	4.7	1.5	3.8	2.6	3	2.2	0.26
Mean	47.25	37.35	8.42	9.26	11.75	1.42	6.82	4.08

Means within the same column with different superscripts were significantly ($P<0.05$) different. Values are presented as Mean ± SD, n=3.

4.1.3 Dimensional characteristics of Pinto and Red Haricot bean varieties

There was no significant ($P<0.05$) difference in length and width during the 6 months storage (Table 5). However, the width varied slightly for Red haricot stored at 35ºC/83% at 4 and 6 months. There was no significant difference ($P<0.05$) in seed weight during storage however, slight variation were recorded. The weight of the 100 seeds ranged from 20.0 to 40.0 g, these result is similar to other African bean cultivars (Giami & Okwechime, 1993). The weight of Pinto is slightly above the weight of Red haricot because Pinto is classified as a large seeded bean variety while Red haricot is a small seeded bean variety

Table 4.5: Dimensional characteristics of Pinto and Red Haricot bean varieties

Storage conditions	Length		Width		100 seed weight	
˚C/% RH/months	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**
25/75/0	11.4 ± 1.0 [a]	11.4 ± 0.6 [a]	8.7 ± 0.9 [a]	6.9 ± 0.3 [ab]	39.35 ± 0.4 [ab]	22.2 ± 0.6 [a]
45/75/2	11.8 ± 0.8 [a]	10.9 ± 1.4 [a]	8.9 ± 0.8 [a]	6.6 ± 0.5 [ab]	39.68 ± 0.8 [b]	25.7 ± 0.8 [b]
45/75/4	11.8 ± 0.7[a]	10.6 ± 0.5 [a]	8.7 ± 0.4 [a]	6.5 ± 0.5 [ab]	37.66 ± 2.8 [ab]	24.3 ± 0.3 [b]
45/75/6	11.9 ± 0.8 [a]	10.8 ± 0.7 [a]	8.1 ± 0.6 [a]	6.7 ± 0.2 [ab]	37.55 ± 0.4 [ab]	24.6 ± 0.7 [b]
35/83/2	11.6 ± 0.1 [a]	10.0 ± 0.7 [a]	8.5 ± 0.5 [a]	6.3 ± 0.5 [a]	38.06 ± 1.0 [ab]	25.3 ± 1.9 [b]
35/83/4	10.8 ± 0.5 [a]	10.8 ± 0.8 [a]	8.1 ± 0.3 [a]	7.18 ± 0.7 [b]	37.35 ± 0.8 [a]	24.3 ± 0.8 [b]
35/83/6	11.4 ± 0.9 [a]	10.2 ± 0.3 [a]	8.7 ± 0.5 [a]	6.5 ± 0.3 [ab]	37.41 ± 0.6 [a]	24.5 ± 0.0 [b]
LSD (5%)	1.54	1.29	0.8	0.67	2	1.63
Mean	11.52	10.72	8.48	6.69	38.4	24.41

Means within the same column with different superscripts were significantly ($P<0.05$) different. Values are presented as Mean ± SD, n=3.

There was no significant ($P<0.05$) difference in bulk density among the two bean varieties during the 6 months storage. Factors which commonly affect bulk density are insect infestations, excessive foreign matter and high percentage moisture content which were not present in this study (WFP, 2006). According to Mpotokwane *et al*., 2008, bulk density could be used as an indication of quality during storage of beans. There was no significant difference in the true seed density which ranged between 1.16 g/cm^3 - 1.33 g/cm^3 (Table 6). This is in agreement with what Alabadan, 1996 reported a range between 1.22 – 1.45 g/cm^3. There was no significant change in porosity with storage time. Porosity is the fraction of the space in the bulk seeds which is not occupied by the seeds (Coskuner & Karababa, 2007), it depends on the geometry and surface properties of the material (Mpotokwane *et al*., 2008) and allows fluid to pass through the bulk.

Table 4.6: Density characteristics of Pinto and Red Haricot bean varieties.

Storage conditions	bulk seed density (g/cm^3)		True seed density (g/cm^3)		Porosity	
˚C/% RH/months	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**
25/75/0	0.77 ± 0.0 ^{a}	0.74 ± 0.0 ^{a}	1.23 ± 0.0 ^{abc}	1.24 ± 0.0 ^{bc}	37.03 ± 1.0 ^{bc}	38.6 ± 0.7 ^{c}
45/75/2	0.78 ± 0.0 ^{b}	0.75 ± 0.0 ^{b}	1.24 ± 0.0 ^{abc}	1.22 ± 0.0 ^{b}	37.48 ± 0.9^{bc}	38.4 ± 1.1 ^{c}
45/75/4	0.79 ± 0.0 ^{c}	0.75 ± 0.0 ^{b}	1.24 ± 0.0 ^{abc}	1.3 ± 0.0 ^{c}	36.46 ± 1.5 ^{bc}	42.2 ± 2.5 ^{d}
45/75/6	0.79 ± 0.0 ^{c}	0.82 ± 0.0 ^{f}	1.31 ± 0.1 ^{bc}	1.26 ± 0.1 ^{bc}	39.78 ± 5.8 ^{c}	34.9 ± 3.9 ^{b}
35/83/2	0.88 ± 0.0 ^{d}	0.79 ± 0.0 ^{c}	1.16 ± 0.0 ^{a}	1.12 ± 0.0 ^{a}	24.97 ± 2.2 ^{a}	26.67 ± 1.5 ^{a}
35/83/4	0.88 ± 0.0 ^{d}	0.81 ± 0.0 ^{e}	1.22 ± 0.0 ^{ab}	1.28 ± 0.1 ^{bc}	28.04 ± 7.1 ^{a}	37.97 ± 1.7 ^{bc}
35/83/6	0.88 ± 0.0 ^{d}	0.80 ± 0.0 ^{d}	1.33 ± 0.0 ^{c}	1.44 ± 0.0 ^{d}	33.89 ± 1.4 ^{b}	44.49 ± 2.2 ^{d}
LSD (5%)	0	0	0.09	0.06	5	2.9
Mean	0.82	0.78	1.25	1.26	33.83	37.91

Means within the same column with different superscripts were significantly ($P<0.05$) different. Values are presented as Mean ± SD, n=3.

4.2 Effect on chemical and nutritional changes during development of hard to cook defect

4.2.1 Crude protein

Beans provide significant amounts of protein as seen on Table 7. The crude protein content of beans ranged from 14% to 19% with Red haricot showing a slight variation in protein content during the 6 months storage, however, there was no significant difference in the content with increased storage ($P>0.05$). According to Esteves, 2000, the protein content of beans on a dry basis is between 22% and 26%. The results obtained in this study are quite lower. However, appreciable decreases in crude protein levels have been reported in different beans by some researchers. Crude protein content of Faba bean stored in tin cans at room temperature decreased from 29.2 % to 19.8% in 9 months (El-Reffai *et al.,* 1988). Protein content decreased from 9.3% (good cooking lentils) to 8.8% in seed coat of poor cooking lentils (*Lens culinaris*) (Bhatty, 1995). Comparable protein content range in 8 cultivars of Common bean from Ethiopia (17.96% to 22.07%) was reported by Shimelis & Rakshit (2005). De Alameida Costa *et al*. (2006) reported a protein content of 20.9% for common bean *Cv. IAC carioca Ete* from Brazil, comparable to the protein content of Biofort2 (20.89%).

4.2.2 Protein digestibility

The storage conditions, processing treatments all had significant effects on the in vitro protein digestibility. There was significant decrease in protein digestibility with increased storage time ($P<0.05$). At 45 ˚C/75% 0 months, the digestible protein was at 76.6% for Pinto and 80.92% for Red Haricot, after 6 months storage it was 52.4% and 52.7% respectively. At 35 ˚C/83% 0 months, the digestible protein was 75.8% for Pinto and 80.3% for Red Haricot, after 6 months storage it was 57.7% and 57.1% respectively, this reduced with increased storage time (Table 7). According to Khatoon & Prakash (2004), a reduction in protein digestibility could also be related to the possible build-up of disulphide bonds between sulphur-containing amino acids, which are highly resistant to hydrolytic action of the digestive enzymes used and could cause protein-protein folding, thus hindering action of enzymes.

Table 4.7: Nutritional contents of different bean varieties stored at 45ºC 75% and 35ºC 83% for 0, 2,4 and 6 months

Storage conditions	Ash (%)		Proteins (%)		Protein digestibility (%)	
˚C/% RH/months	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**
25/75/0	3.98 ± 0.03 ^{a}	4.2 ± 0.1 ^{bc}	15.7 ± 0.13 ^{a}	18.4 ± 0.1 ^{b}	76.6 ± 6.9 ^{b}	80.92 ± 1.4 ^{e}
45/75/2	3.78 ± 2.62 ^{a}	3.68 ± 0.01 ^{bc}	15.13 ± 0.7 ^{a}	17.1 ± 0.5 ^{ab}	74.2 ± 4.1 ^{b}	65.0 ±0.1 ^{c}
45/75/4	3.41 ± 0.8 ^{a}	3.1 ± 0.6 ^{ab}	14.6 ± 0.9 ^{a}	18.1 ± 0.1 ^{ab}	56.5 ± 2.7 ^{a}	63.3 ± 0.3 ^{c}
45/75/6	2.9 ± 0.7 ^{a}	3.09 ± 1.14 ^{ab}	15.3 ± 0.6 ^{a}	17.7 ± 1.7 ^{ab}	52.4 ±3.6 ^{a}	52.7 ± 7.1 ^{a}
35/83/2	3.8 ± 0.1 ^{a}	4.57 ± 0.02 ^{c}	14.2 ± 0.8 ^{a}	16.6 ± 1.5 ^{a}	75.8 ± 2.8 ^{b}	80.3 ± 0.8 ^{e}
35/83/4	3.38 ± 0.1 ^{a}	3.8 ± 0.5 ^{bc}	14.98 ± 1.6 ^{a}	17.9 ± 0.4 ^{ab}	71.2 ± 2.7 ^{b}	71.6 ± 1.4 ^{d}
35/83/6	2.9 ± 0.5 ^{a}	2.27 ± 0.5 ^{a}	15.3 ± 0.6 ^{a}	18.1 ± 0.9 ^{ab}	57.7 ± 0.6 ^{a}	57.1 ± 2.7 ^{b}
LSD (5%)	1.9	1	1.5	1.6	5.1	4
Mean	3.29	3.56	15.02	17.71	66.32	67.24

Means within the same column with different superscripts were significantly ($P<0.05$) different. Values are presented as Mean ± SD, n=3.

4.2.3 Ash and Mineral content

There was small but continuous reduction in ash content (sum of mineral contents) with increased storage. The reason remains unknown but our results are in agreement to those of El-Refai *et al.*, 1988 who demonstrated that storage of faba bean for 9 months led to a moderate reduction in ash content with no loss of phosphorus, calcium, iron or magnesium contents. At 25 ˚C/75% 0 months the ash content was 3.98% for Pinto and 4.2% for red haricot, after 6 months it came down to 2.9% and 3.09% respectively. At 35 ˚C/83% 2 months the ash content was 3.8% for Pinto and 4.57% for red haricot, after 6 months it was 2.9% and 2.27% respectively. The ash content values of 2.2 to 4.9% are comparable with those of Shimelis & Rakshit (2005) who reported 3.12% to 4.26% for 8 cultivars of common bean from Ethiopia, but marginally lower than those reported for common bean grown in Idaho, 4.65 % (Berrios *et al.* 2009).

4.2.3.1 Calcium

There was no significant difference in calcium content with increased storage time ($P>0.05$). Among the 2 varieties, Pinto had the highest calcium content with increased storage. For fresh beans (25˚C/75%) the calcium content for pinto and red haricot bean was 140.54 mg/100 g and 141.11 mg/100 g respectively and after 6 months at 45 ˚C/75% storage it was

160.44 mg/100 g and 161.41 mg/100 g for Pinto and red haricot respectively; and at 35 ˚C/83% it was 151.16 mg/100gand 116.64 mg/100g for pinto and red haricot respectively. Sali *et al*., 2011 reported higher calcium levels of between 296.60 and 186.20 mg/ kg.

4.2.3.2 Iron

There was no significant difference in iron content between the two varieties and during storage ($p>0.05$). For fresh beans (25˚C/75%), the iron content for Pinto and red haricot was 5.19 mg/100 g and 6.07 mg/100 g respectively. After 6 months storage at 45 ˚C/75% the iron content was 4.41 mg/100 g and 5.95 mg/100 g for Pinto and red haricot respectively; and at 35 ˚C/83% it was 4.82 mg/100 g and 5.33 mg/100 g for Pinto and red haricot respectively. An almost identical range of Fe contents but higher, 6.18 mg/100g to 8.4 mg/100g (Shimelis & Rakshit 2005), was reported in 8 cultivars of Ethiopian common bean. These values were higher than those reported for different legumes from Pakistan, 3 mg/100g, 26 mg/100g, 31 mg/100g and 23 mg/100g for chickpea, cowpea, lentil and green pea respectively (Iqbal *et al*. 2006). Hemalatha *et al*. (2007) reported 4.9 mg/100g for red gram and 6.4 mg/100g for black gram, suggesting a greater Fe content in black Vigna mungo cultivars. The only black bean present in the study, Sen46 was also the accession with the highest Fe content. Biofort and Biofort 2, which are the same biofortified cultivar but of different harvesting dates, showed some differences in mineral composition but this may be attributed to varying soil and climatic conditions during plant development. Seed mineral concentration is believed to be a stable heritable trait in which different soil conditions may be responsible for different mineral concentrations of the same cultivars (Blair *et al*. 2010). Biofort presented the lowest values for Fe content which was expected to present a higher Fe content than the other accessions, due to its fortification. The ability of the plant to absorb more Fe, is strongly correlated to the soil pH, the plant`s ability to absorb Fe and the soil profile as well, taking into account the availability of nutrients present. The source of nitrogen in the plant can also greatly affect the uptake of Fe, as a high incidence of nitrate can alkalize the rhizophere, limiting its capacity to take up iron (Barker & Pilbeam 2007).

4.2.3.3 Zinc

There was no significant difference ($P>0.05$) in zinc content between the two varieties. Zinc amounts ranged between 4.33 mg/100 g and 8.72 mg/100 g among the varieties at different storage times (Table 8). The Zn content reported in this study, are much higher than those reported for black and white cowpea, 4.6 mg/100g and 4.9 mg/100g, respectively (Mitchikpe

et al. 2008) and for two cultivars of common bean in Northern Nigeria, Baki wake (2.75 mg/100g) and Kwakiul (2.46 mg/100g) (Onwuliri & Obu 2002). A lower range of values was reported by Shimelis & Rakshit (2005) in their 8 Ethiopian cultivars of common bean, 1.54 mg/100g to 2.82 mg/100g. Zinc content of beans is one of the highest among vegetable sources, which is important structural, enzymatic and regulatory functions in living cells (Cozzolino, 2007).

4.2.3.4 Magnesium

There was no significant variation ($P<0.05$) in the magnesium content during storage. Magnesium amounts ranged between 162.5 mg/100 g -187.44 mg/100 g in Pinto and 167.49 mg/100 g–191.93 mg/100 g in Red haricot at different storage times (Table 8). According to USDA, 2012, the Mg content in beans is in the range of 138 to 176 mg/100g, this is in line to what was obtained in this study. El Maki *et al.*, 2007 reported Mg content in the range of 289 to 312 mg/100g which is higher than what was obtained in this study.

Table 4.8: Mineral contents of different bean varieties stored at 35ºC 83% and 45ºC 75% for 0, 2,4 and 6 months

Storage conditions	Ca content (mg/100g)		Fe content (mg/100g)		Mg content (mg/100g)		Zn content (mg/100g)	
˚C/% RH/months	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**
25/75/0	140.54±3.51^{b}	141.11±23.4^{e}	5.19±1.66 ^{c}	6.07±1.38^{ef}	187.44±3.24^{e}	191.93±1.41^{f}	5.52±0.28^{a}	5.09±0.89^{a}
45/75/2	147.33±15.19^{d}	133.62±13.06^{c}	5.40±1.19 ^{d}	5.60±5.56^{c}	182.00±1.69^{d}	187.39±0.56^{e}	4.33±1.91^{a}	6.10±2.00^{ab}
45/75/4	138.56±26.71^{a}	125.31±2.76^{b}	6.42±4.70 ^{e}	6.13±1.10^{f}	183.35±6.91^{d}	185.35±12.44^{d}	5.32±0.55^{a}	6.97±0.11^{b}
45/75/6	160.44±39.15^{e}	151.16±47.21^{f}	4.41±2.21 ^{a}	5.95±6.91^{e}	162.5±042^{a}	181.56±7.34^{c}	6.38±1.16^{a}	5.83±0.94^{ab}
35/83/2	163.48±20.56^{f}	134.49±54.22^{c}	5.43±6.78 ^{d}	4.51±12.98^{a}	169.05±9.26^{b}	167.49±0.14^{a}	5.69±0.51^{a}	4.97±3.21^{a}
35/83/4	145.46±37.76^{c}	138.94±14.28^{d}	5.50±3.31 ^{d}	5.76±10.22^{d}	163.2±22.01^{a}	179.86±6.91^{b}	5.63±0.11^{a}	8.72±0.39^{c}
35/83/6	161.41±28.09^{e}	116.64±14.05^{a}	4.82±0.28 ^{b}	5.33±3.04^{b}	173.98±1.69^{c}	184.05±7.19^{d}	4.58±0.17^{a}	7.08±0.17^{b}
LSD (5%)	1.34	1.34	1.9	1.34	1.77	1.34	1.9	1.34
Mean	151.03	134.47	5.32	5.62	174.36	182.51	5.35	6.39

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3.

4.3 Effect on anti-nutrients on development of hard to cook defect during storage

4.3.1 Tannin content

Tannin content of pinto and red haricot beans has been summarized on table 9. From the table, tannin content decreases with soaking and cooking. For fresh pinto beans stored at 25/75/0, the tannin content reduced by 23.2% after cooking without soaking, 60.2% after soaking in distilled water followed by cooking, and 71.1% after soaking in sodium carbonate followed by cooking. For fresh red haricot beans stored at 25/75/0, the tannin content reduced by 65.6% after cooking without soaking, 77.9% after soaking in distilled water followed by cooking, and 81.9% after soaking in sodium carbonate followed by cooking this is in line with Ramirez-Cardenas *et al*., 2008 who found a greater tannin reduction in beans that were soaked and cooked without soaking water. According to Ramakrishna *et al.*, 2006, the decrease in free phenolics during soaking may simply be due to leaching out into soaking solution and differences on distribution and contents of phenolic compounds in the seed coat and cotyledons between the tested seeds (Xu & Chang, 2008). While comparing soaked beans cooked with the soaking water vs. unsoaked beans, Nergiz & Gokgoz (2007) found lower tannin content in soaked beans. On the other hand, Toledo & Canniatti-Brazaca (2008) found the lower tannins in all unsoaked samples and all cooking methods. The authors justify that the greater loss is because of a longer cooking period, required when the beans are not previously soaked. However, when the soaked beans are compared, the same study shows lower values for soaked beans cooked without the soaking water. In the study done by Oliveira *et al*., (2001b), for whom tannin reduction was desirable, a greater reduction in tannin content was also obtained by discarding the soaking water. Soaking of beans in sodium bicarbonate or mixed salt solution gave a greater reduction in the antinutritional factors (tannins, phytic acid, and trypsin inhibitor activity) than did soaking in water, this is clearly shown from the results. Tannins may precipitate proteins from aqueous solution by inhibiting digestive enzymes (Soetan & Oyewole, 2009) and have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Tannins chelate iron and zinc irreversibly and interfere with their absorption. However, tannins did not affect the bioavailability of zinc and iron in the study done by Hemalatha *et al*., 2007.

The tannin content increased with increased storage time. According to Martinez, 2010, the lower values observed with increase in storage time may be attributed to oxidative degradation accelerated by improper storage conditions. The seed color difference present

between storage at 0 months and storage at 6 months, in which storage at 6 months presented a clear color darkening that may be attributed to air-and light-catalysed oxidations of phenolics, which further supports the hypothesis of an accelerated degradation.(Martinez, 2010). Mariotto-Cezar *et al*., 2013 also observed an increase in tannin content with increased storage.

Table 4.9: Tannin contents of different bean varieties stored at 35ºC 83% and 45ºC 75% for 0, 2,4 and 6 months

Storage conditions	Raw beans (Tannins in mg/g)		Soaked in distilled water (DW) (Tannins in mg/g)		Soaked in Na2CO3 (Tannins in mg/g)		Cooked not soaked (CNS) (Tannins in mg/g)	
˚C/% RH/months	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**	**Pinto**	**Red haricot**
25/75/0	75.13 ±6.73^{a}	135.4±8.42^{a}	29.89 ±11.79^{a}	29.89±6.73^{a}	21.69 ± 1.1^{ab}	24.47 ±1.1^{a}	57.67 ±5.05^{ab}	46.56±23.57^{ab}
45/75/2	58.46 ±7.27^{a}	131.5±21.89^{a}	32.27 ±13.47^{a}	54.5±5.51^{bc}	13.23±11.78^{a}	12.43±3.37^{a}	39.42 ±25.25^{a}	42.59±3.37^{ab}
45/75/4	80.69 ±21.89^{a}	137.8±17.17^{a}	45.77 ±13.47^{a}	29.1±4.37^{a}	25.13±10.1^{b}	22.75±6.73^{ab}	39.42±10.10^{a}	32.27±11.78^{a}
45/75/6	69.58±11.98^{a}	116.4±16.50^{a}	44.97 ±5.05^{a}	44.97±8.42^{ab}	26.72 ±3.36^{b}	33.07±6.73^{b}	36.24 ±8.42^{a}	48.15±10.1^{ab}
35/83/2	59.26 ±10.10^{a}	127.5±16.84^{a}	37.82 ±10.10^{a}	29.89±11.76^{a}	10.85 ±8.42^{a}	29.1±13.47^{ab}	45.77±10.1^{a}	54.5±16.49^{ab}
35/83/4	87.83±7.14^{a}	138.6±11.98^{a}	41.0 ±23.57^{a}	67.99±20.20^{c}	29.89 ±1.37^{b}	17.9±18.52^{ab}	57.67±3.37^{ab}	68.78±1.68^{b}
35/83/6	83.07±10.91^{a}	129.1±24.44^{a}	44.97 ±16.84^{a}	37.83±1.68^{ab}	24.33 ±3.37^{b}	31.48±5.05^{b}	72.75 ±23.57^{b}	49.74±13.47^{ab}
LSD (5%)	31.16	40.67	26.03	17.21	10.5	16.88	23.8	28.6
Mean	73.4	130.9	41.1	42	21.7	24.5	49.8	48.9

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3.

4.3.2 Phytic acid content

There were also significant differences ($P<0.05$) in the phytic acid contents among the 2 bean varieties during storage (Table 10). It was observed that phytic acid content decreases with soaking and cooking. For pinto beans stored at 35/83/6, the phytic acid content reduced by 33.2% after cooking without soaking, 39.3% after soaking in distilled water followed by cooking, and 65% after soaking in sodium carbonate followed by cooking. For red haricot beans stored at 35/83/6, the phytic acid content reduced by 13.9% after cooking without soaking, 27.6% after soaking in distilled water followed by cooking, and 51.2% after soaking in sodium carbonate followed by cooking. This is in line with Elmaki *et al*., 2007; Nergiz & Gokgoz, 2007; Ramirez-Cardenas *et al*., 2008; Toledo & Canniatti-Brazaca, (2008). Toledo & Canniatti-Brazaca, (2008) stated that phytate reduction was equal in samples with and without soaking, however, the phytate content varied according to cooking method. The greatest phytate content was found in beans that were cooked with the soaking water, followed by beans cooked without soaking and finally beans cooked without the soaking water. Among soaked beans and for all cooking methods, beans cooked without the soaking water always had statistically lower phytate content than those cooked with the soaking water. Similar results were found by Oliveira *et al*. (2001b) in an older study with common beans, and by Boateng *et al*. (2007) who studied the phytate content in another species of beans. According to Watzl & Leitzmann, (1999), phytate is also a beneficial phytochemical and has antioxidant activity in the body.

Phytic acid reduction may not be needed for utilization of some nutrients. Studies found that soaking and cooking had different effects on different legumes. Aranda *et al*., 2004 concluded that high consumption of phytates from beans had no significant negative effect on digestion of magnesium and calcium. However, another study showed cooking increased the metabolic utilization of calcium and magnesium. Chopra & Sankhala (2004) found a significant association between soaking and reduced phytate contents concurrent with increased iron bioavailability in horse gram (*Dolichos biflorus*) and moth bean (*Phaseolus aconitifolius*).

The phytate content decreased with increased storage time, this is in line to what Mariotto-Cezar *et al*., 2013 found that freshly-harvested beans presented higher phytate contents than the grains stored, thus indicating reduction of such antinutritional factor with the storage.

Granito *et al*., stored common bean during 150 days, and found reductions in phytate content of circa 20% lower than what was found herein, but confirming that the storage causes reduction in this antinutritional factor.

Table 4.10: Phytate contents of different bean varieties stored at 35 ºC 83% and 45 ºC 75% for 0, 2, 4 and 6 months

Storage conditions	Raw beans (Phytates in mg/g)		Soaked in distilled water (DW) (Phytates in mg/g)		Soaked in Na2CO3 (Phytates in mg/g)		Cooked not soaked(CNS) (Phytates in mg/g)	
˚C/% RH/months	**Pinto**	**Red Haricot**	**Pinto**	**Red Haricot**	**Pinto**	**Red Haricot**	**Pinto**	**Red Haricot**
25/75/0	226.4 ±5.5^{ab}	234.8±16.7^{b}	286.3 ± 21.3^{d}	256.9 ± 6.0^{d}	266.9 ± 35.7^{f}	231.3 ± 23.3^{d}	205.34 ± 15.8^{b}	180.3 ± 2.1^{b}
45/75/2	252.0 ± 5.4^{bc}	264.6 ± 34.1^{c}	158.71 ± 33.4^{b}	78.0 ± 9.3^{a}	196.4 ± 9.4^{e}	160.1 ± 15.9^{c}	226.5 ± 68.8^{b}	208.3 ± 40.3^{cd}
45/75/4	273.8 ± 12.0^{cd}	284.3 ± 0.42^{cd}	122.9 ± 12.5^{a}	130.1 ± 9.1^{bc}	44.6 ± 9.3^{a}	48.3 ± 3.7^{a}	123.2 ± 12.4^{a}	117.03 ± 0.1^{a}
45/75/6	273.5 ± 1.6^{cd}	297.0 ± 5.4^{d}	258.2 ± 1.5^{c}	244.3 ± 8.8^{d}	172.6 ± 2.0^{d}	157.8 ± 21.1^{c}	205.31 ± 10.5^{b}	227.3 ± 23.0^{d}
35/83/2	209.1 ± 6.2^{a}	231.4 ± 10.9^{b}	105.6 ± 2.9^{a}	153.6 ± 48.2^{c}	74.0 ± 7.1^{b}	210.0 ± 17.7^{d}	207.9 ± 6.7^{b}	182.5 ± 0.1^{b}
35/83/4	223.2 ± 43.0^{a}	108.7 ± 0.94^{a}	115.2 ± 9.3^{a}	117.3 ± 13.5^{b}	100.3 ± 8.7^{c}	143.1 ± 10.9^{c}	113.02 ± 3.9^{a}	111.1 ± 1.7^{a}
35/83/6	281.2 ± 10.9^{d}	219.0 ± 10.7^{b}	170.6 ± 7.3^{b}	158.6 ± 0.47^{c}	98.4 ± 0.4^{c}	106.8 ± 3.2^{b}	187.8 ± 6.5^{b}	188.5 ± 0.9^{bc}
LSD (5%)	25.64	23.88	20.4	31	21.86	22.09	42.2	23.26
Mean	248.8	234.3	173.9	162.7	136.2	151	181.8	173.6

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3.

4.4 Effects of storage conditions on moisture uptake

Storage of beans under unfavourable conditions affects their quality by reducing water uptake during cooking. The moisture uptake of beans stored at low temperature and low relative humidity was recorded and compared to beans stored at high temperature and high relative humidity for the same period of time. Figure 1 and 2 shows the differences in moisture uptake of ETC and HTC beans stored at different temperature and humidity for the same period of time. After cooking for 240 minutes, beans stored at low temperature and humidity had a much higher water uptake during cooking than for beans stored at high temperature and relative humidity. At 0 month, both bean varieties showed a much higher moisture uptake as compared to the subsequent 2, 4 and 6 months. For Red haricot stored at 45 ˚C/75% RH, the % moisture uptake reduced from 97.8% at 0 month to 58.7% at 6 months while Pinto in the same conditions had a reduction from 90.2% at 0 month to 54.2% at 6 months. For Red haricot stored at 35 ˚C/83% RH, the moisture uptake % reduced from 97.8% at 0 month to 67.1% at 6 months while Pinto in the same conditions had a reduction from 90.2% at 0 month to 61% at 6 months. In both storage conditions, Red haricot had higher moisture uptake rates as compared to the Pinto, this is in line with what Kinyanjui *et al*., 2004 found. This could partly explain the faster cooking rate of the ETC beans. This shows that beans stored at elevated temperature (45ºC) and low relative humidity (75%) exhibit the greatest loss in moisture during storage and increased bean hardness. Yousif *et al,* 2002 found that Adzuki bean stored at 30˚C for 6 months absorbed less water than those stored at 20˚C or 10˚C. Similarly, substantial reduction in water absorption capacities of chickpea varieties occurred when they were stored under favourable conditions of high temperature (33-35˚C) and relative humidity (75%) (Reyes-Moreno *et al*., 2000).

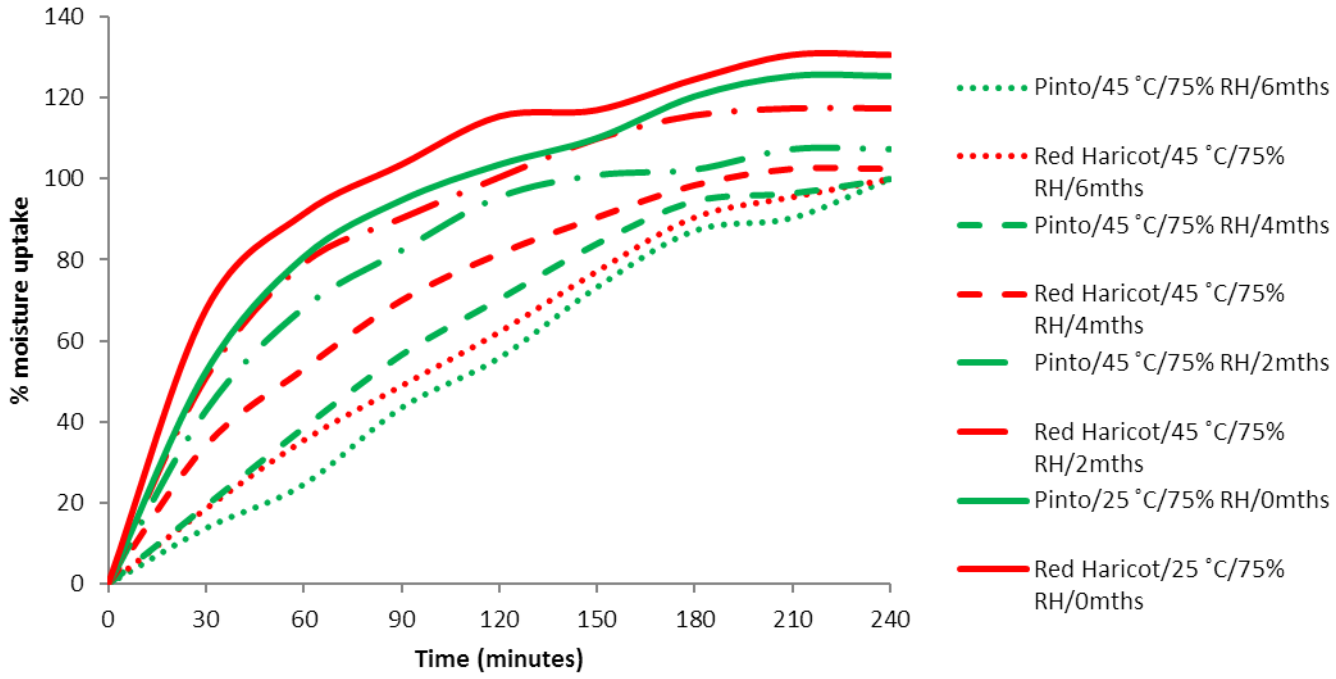

Figure 4.1: Percentage moisture uptake during cooking at 45 ˚/75%

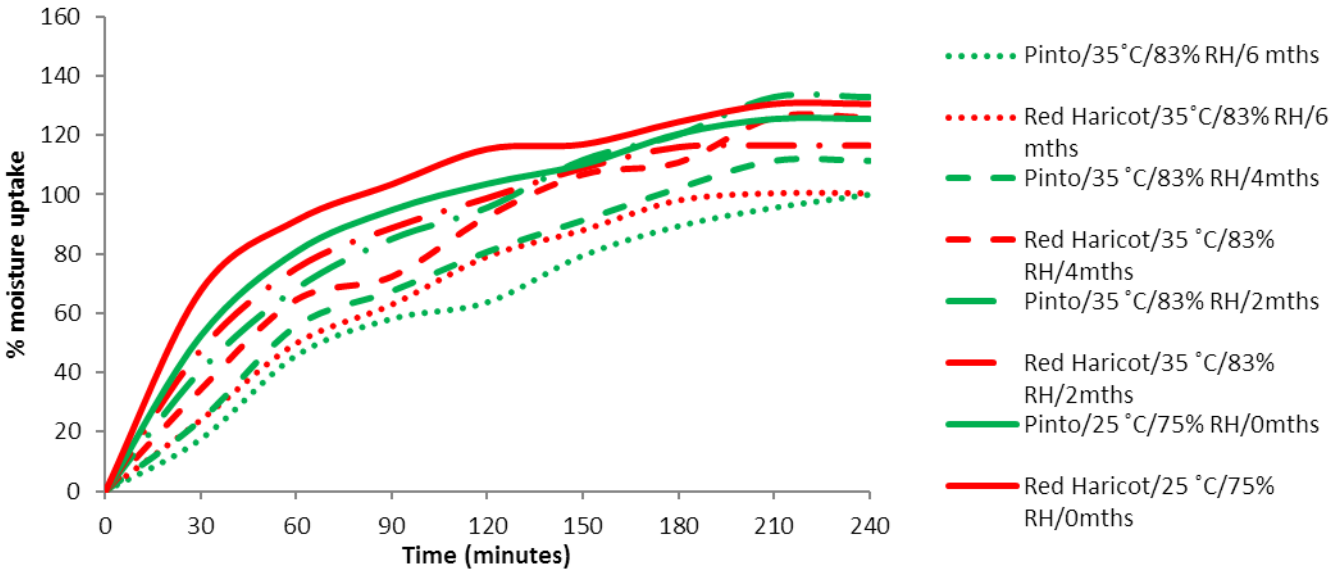

Figure 4.2: Percentage moisture uptake during cooking at 35 ˚/83%

4.5 Effects of storage time on cooking time

The cooking quality of beans is related to post harvest handling and storage conditions. Cooking time as well as cooked texture, appearance and flavour are considered to be important cooking quality characteristics. Figure 3 and 4 compares the effect of storage time

on cooking of an ETC and a HTC bean variety stored at 45 ˚C/75% and at 35 ˚C/83%. There is progressive increase in bean hardness as storage time increased with beans stored at 45 ˚C/75% for 6 months being harder than beans stored at the same temperature for 2 months (Figure 3). For Red haricot stored at 45 ˚C/75% RH the cooking time was 180 minutes for fresh beans, 210 minutes at 2 months, 300 minutes at 4 months and 300 minutes at 6 months. This was a 66.66% increase in cooking time. For Pinto stored at 45 ˚C/75% RH the cooking time was 180 minutes for fresh beans, 210 minutes at 2 months, 300 minutes at 4 months and 330 minutes at 6 months. This was 83.83% increase in cooking time, For Red haricot stored at 35 ˚C/83% RH, the cooking time was 180 minutes for fresh beans, 210 minutes at 2 months, 270 minutes at 4 months and 300 minutes at 6 months. This was also 66.66% increase in cooking time. For Pinto stored at 35 ˚C/83% RH the cooking time was 180 minutes for fresh beans, 240 minutes at 2 months, 270 minutes at 4 months and 330 minutes at 6 months. This was 83.83% increase in cooking time. These results are in line to what other researchers found. Maurer *et al*., 2004 found the cooking time of red and black beans (*P. vulgaris*) stored at 29˚C for 3.5 months was 2.45 and 2.41 times greater than those stored at 4˚C. black beans stored at 4-5˚C and 50-60% relative humidity exhibited quality characteristics of fresh beans, such as shorter cooking times and percentage of hard shell whereas beans stored at ambient conditions of 23-25˚C and 30-50% relative humidity lost these characteristics (Berrios *et al*., 1999). Similarly cooking time of three chick pea varieties substantially increased when stored for 160 days under unfavourable conditions of 33-35˚C and 75% relative humidity. It ranged from 112-142 minutes for fresh to 146-213 minutes for stored chick peas (Reyes-Moreno *et al*., 2000).

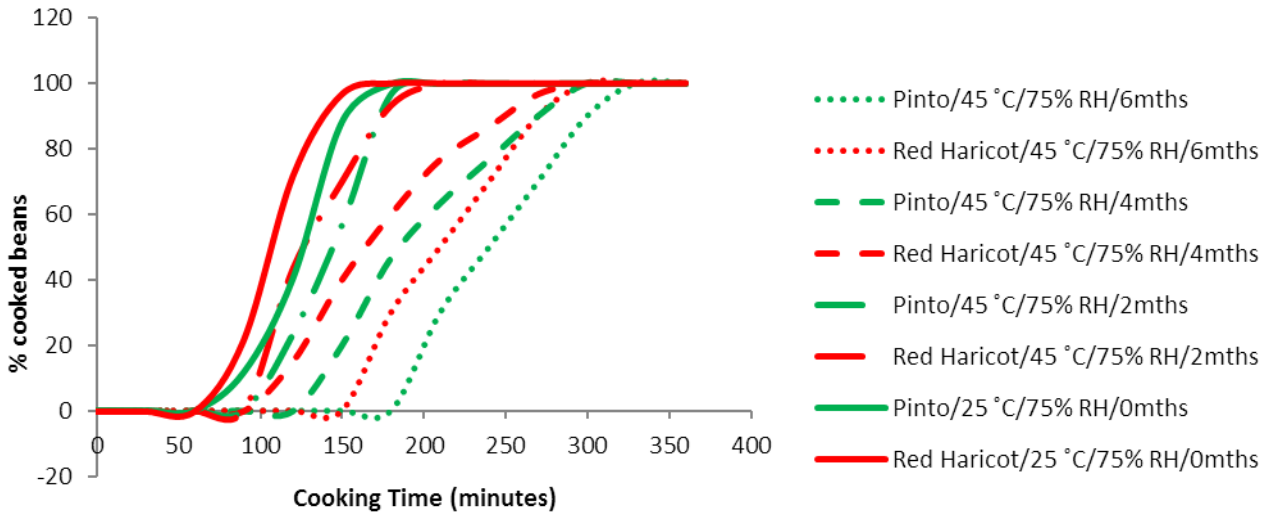

Figure 4.3: Effect of storage time on cooking of beans stored at 45 ˚/75% for 0, 2, 4, and 6 months

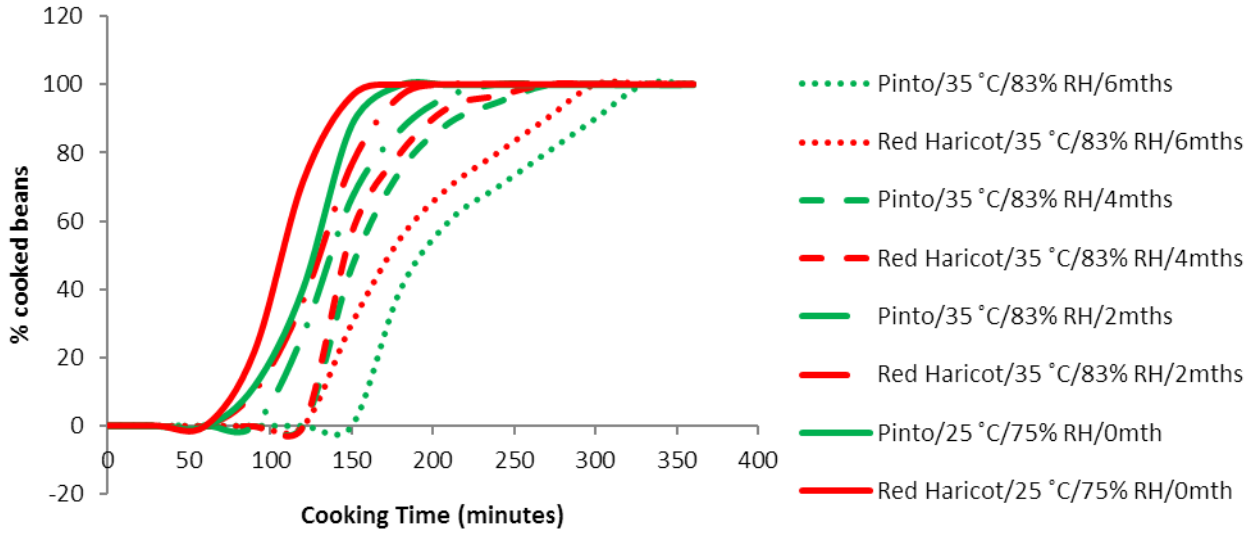

Figure 4.4: Effect of storage time on cooking of beans stored at 35 ˚/83% for 0, 2, 4, and 6 months

4.6 Effect of accelerated temperature on cooking time

Storage at an accelerated temperature of 45 ˚C for 4 months led to an increase in cooking time; this gradually decreases with reduced temperature (Figure 5). At 25 ˚C/75%, 74% of red haricot beans and 68% of pinto beans were cooked. At 35 ˚C/75%, 66% of red haricot and 60% of pinto beans had cooked while at 45 ˚C/75%, 51% of red haricot and 45% of pinto beans had cooked. Reyes-Moreno *et al*., 2000 also found an increase in cooking time for chickpeas stored at 33-35˚C as compared to lower temperatures. Beans stored at high temperature hydrate unevenly during cooking resulting in uneven bean softening and to the presence of HTC beans. HTC beans fail to soften enough to be eaten after cooking for a reasonable time.

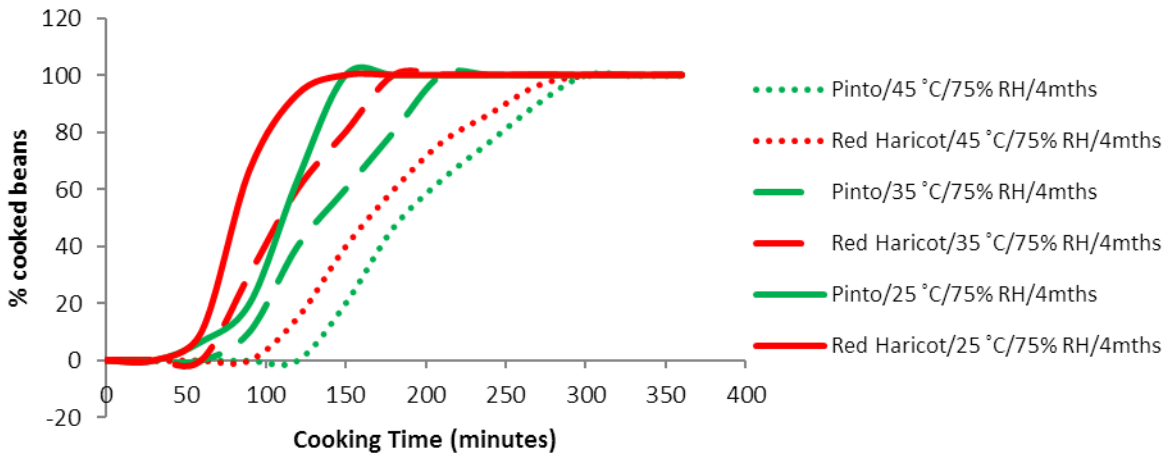

Figure 4.5: Effect of accelerated temperature (35˚C, 45˚C) on cooking of beans

4.7 Effect of relative humidity on cooking

High relative humidity results in increased bean hardness (Fig 6). The Relative humidity caused an increase in cooking time from 180 minutes at 75% RH to 270 minutes at 83% RH in Red haricot. Pinto had an increase from 210 minutes at 75% RH to 270 minutes at 83% RH. Storage of beans under high relative humidity (83%) renders them HTC; this in turn leads to increased cooking time. High relative humidity is known to favour bean hardening. Furthermore, proneness to HTC is a function of variety and storage conditions (Giselle *et al*., 2004). This results are in agreement with Kilmer *et al*., (1994) who found that, high humidity (> 75%) results in the hard to cook (HTC) condition. Ndung'u *et al*., (2012) also found that even a relative humidity of (60% - 80%) can lead to development of the HTC defect. According to Jones & Boulter, (1983), high moisture content and relative humidity stimulate phytase and pectin methyl esterase activity (PME). The phytase liberates Mg from phytic acid and PME hydrolyses pectin to pectinic acid and methanol. The pectinic acid combines with Mg forming magnesium pectate which cements cells together resulting in extended cooking time.

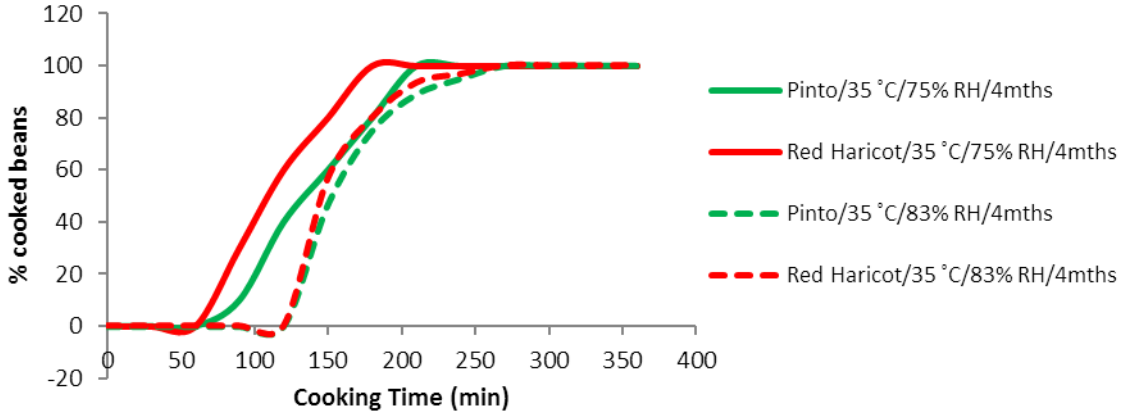

Figure 4.6: Effect of relative humidity (75% and 83%) on cooking of beans

4.8 Impact of pre-treatment techniques (soaking in $CaCl_2$, Na_2CO_3, and Distilled water) on cooking time

Figure 7 shows the effect of fresh beans (25 ˚C/75% at 0 month) not soaked, soaking beans in distilled water, sodium carbonate and calcium chloride on cooking time. For Pinto beans soaked in Na_2CO_3, distilled water, not soaked and soaked in calcium chloride the cooking times increased from 60 minutes to 210 minutes. For Red haricot beans soaked in Na_2CO_3, distilled water, not soaked and soaked in calcium chloride the cooking times increased from 30 minutes to 210 minutes. Sharma *et al.*, 2013 also found a decrease in cooking time for the overnight soaked soybean seeds were 65, 55 and 20 min for beans soaked in distilled water, 1% citric acid and 2% sodium bicarbonate soaking treatments respectively. Soaking in calcium chloride increased the cooking time to more than 5 hours for both bean varieties hence showing it has a significant hardening effect on the beans this can be attributed to cross-linking of pectin with calcium ions to form insoluble pectates which render the cells resistant to water absorption and the subsequent failure of adjacent cells to separate upon cooking. This hardening effect is in line with the HTC defect which manifests during cooking since it prevents the separation of cells in the cooked food materials (Nyakuni *et al.*, 2008). These results are in line with Onwuka & Okala (2003) who showed that adding calcium to the cooking water significantly increases the cooking time. Soaking in sodium carbonate and distilled water reduced the cooking time to less than 3 hours for both Pinto and red haricot hence proving it significant softening effect. However, soaking in sodium carbonate had the most effect and in distilled water the least effect on softening the texture of the beans. Hence, sodium carbonate had a significant effect ($P < 0.05$) on improving the hard texture of HTC beans while distilled water had the least effect on it. The softening effect was more

pronounced in Red Haricot than on Pinto. Studies indicated that soaking in acid/alkaline solution had increased the rate of water absorption possibly due to the enhanced permeability of the seed coat and softening of cotyledons. Soaking in $NaHCO_3$ has been reported to stimulate rapid water absorption in urdbean (Singh *et al.*, 2000). Aguilera & Rivera, 1990 indicated that soaking of hard beans in solutions such as sodium chloride, sodium bicarbonate, sodium carbonate, sodium phosphate and disodium ethylene diaminetetra acetic acid increased pectin water solubility via removal of divalent cations from the tissue and improved cooking ability of seeds.

De Leon *et al.*, (1992) also reported decreased cooking time of black bean with increasing ratio of monovalent to divalent cations in soaking solution and stated the ion exchange is the possible mechanism. Therefore, it seems the softening effect of sodium salt solutions is attributable to replacing of Ca and Mg with Na and subsequent formation of more soluble pectate in cell walls of bean and lentil.

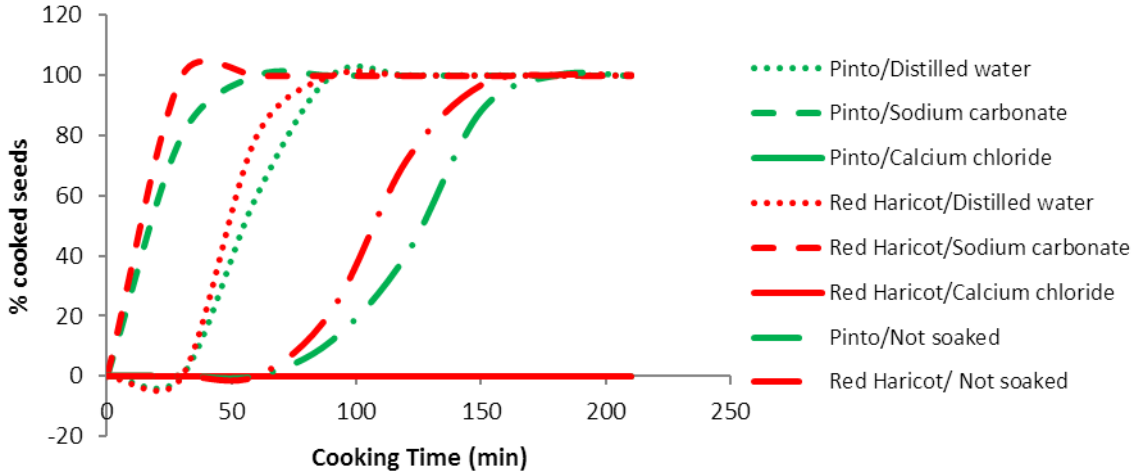

Figure 4.7: Comparison of soaking solutions of fresh beans (25 ˚C/75% at 0 month) in distilled water, Na_2CO_3, $CaCl_2$ and beans not soaked

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Storage of bean varieties under conditions of normal (25 ˚C) and high temperatures (35 ˚C and 45 ˚C) and relative humidity (75% and 83%) rendered them susceptible to hardening. Hardening resulted in decrease in the rate of water absorption, loss of colour lightness, development of browning and darkening, increase in solute and electrolyte leaching, reduction in hydration and swelling coefficients and an increase in conductivity. Hardening had no effect on the characteristic dimension of beans, seed weight and seed porosity. In line with that the bulk density and the true seed density were also not affected by hardening.

High temperatures and relative humidity resulted to development of HTC defect which caused a slow cooking rate of beans. The accelerated temperature resulted to an increase in cooking time this is because high temperatures result to uneven hydration of beans during cooking which results to uneven softening. High relative humidity increased the cooking time from 210 minutes (75%) to 270 minutes (83%) in Pinto. Hardening resulted to a decrease in water uptake during cooking by 36% in Pinto and 39% in red haricot.

Soaking in sodium carbonate and distilled water reduced the cooking time to less than 3 hours for both Pinto and red haricot hence proving its softening effect. However, soaking in sodium carbonate had the most effect and in distilled water the least effect on softening the texture of the beans. Hence, sodium carbonate had a great effect on improving the hard texture of HTC beans while distilled water had the least effect on it. The softening effect was more pronounced in Red Haricot than on Pinto. Soaking in calcium chloride increased the cooking time substantially hence had a hardening effect thus soaking of beans in sodium carbonate can be adopted by bean canners especially in bean varieties that easily mush during canning. Soaking of beans in distilled water and sodium carbonate helped in reducing the cooking time hence it may improve the food value of HTC common beans and reduce annual losses.

Storage of beans for six months did not significantly affect the crude protein, ash content and mineral content with increased storage ($P>0.05$). There was a relative decrease in protein

digestibility and this may be due to the interaction between proteins and phenolic acid. The latter are mobilized into the cell wall during development of the defect. The presence of certain anti-nutritional factors, in legumes, can decrease palatability, diminish protein digestibility and mineral bioavailability; and thereby limit the biological value and acceptance of legumes or pulses as a regular food item. Tannin content decreased with soaking and cooking, tannins of milled bean flour decreased during storage and that high RH accentuated this decrease. Levels of phytic acid content decreased with soaking and cooking. Soaking of beans and cooking the beans without the soak water is the most effective way of reducing levels of tannins and phytic acid in beans. Accelerated storage conditions resulted to hardening of beans after storage at 2, 4, and 6 months. The higher the temperature and relative humidity, the faster the deteriorative effect.

Storage under unfavourable conditions is a major cause of impaired bean hydration and cooking quality. It leads to the "hard shell" phenomenon, where beans fail to imbibe water when soaked and remain hard, and the "hard-to-cook" phenomenon, where the seeds that hydrate normally but the cotyledon fails to hydrate during cooking. The hard shell phenomenon is attributable to impermeability of the seed coat to water. Common bean consumers in Kenya can adopt to the ETC bean variety, red haricot in order to save fuel and cooking time.

5.2 Recommendations

1. A study of processing of mechanisms leading to reversibility of the HTC phenomenon would provide insight into the development of the defect and would aid in the search for appropriate methods to prevent it.
2. It is necessary to develop technological processes in order to transform the HTC beans into edible and useful products. Application of technological alternatives, such as dehulling, extrusion, and solid state fermentation, may improve the food value of HTC common beans and reduce annual losses.

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