EFFECT OF STORAGE CONDITIONS AND PRE PROCESSING TREATMENTS ON PHYSICAL, COOKING AND NUTRITIONAL PROPERTIES OF TWO COMMON BEAN VARIETIES IN KENYA

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Effect of storage conditions and pre-processing treatments on physical, cooking and nutritional properties of two common bean varieties in Kenya

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DECLARATION

I hereby declare that this is my original work and has not been submitted for a degree in any other University.

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

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DEDICATION

This thesis has been dedicated to my family with lots of love for the great support and encouragement.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION & ACRONYMS

AAS	Atomic absorption Spectroscopy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BBI	Bowman-Birk trypsinchymotrypsin Inhibitor
ETC	Easy to cook
DW	De ionized water
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agricultural Organization statistics
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
HTC	Hard to cook defect
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LDL	Low density lipoprotein
NC	Sodium carbonate
PEM	Protein energy malnutrition
PHA	Phytohemagglutinin
PPM	Parts per million
RH	Relative humidity
SD	Standard deviation
STI	Soybean trypsin inhibitor
UV-Vis	Ultra violet visible spectrophotometer
USDA	United States Developments of Agriculture
WHO	World Health Organization

ABSTRACT

Common beans (*Phaseolus vulgaris*) are highly nutritious and widely consumed in Kenya. They are good sources of nutrients such as proteins, vitamins, minerals, complex carbohydrates and polyunsaturated free fatty acids. The objective of this study was to determine effects of storage conditions (25 ˚C and 75% RH, 45 ˚C and 75% RH and 35 ˚C and 83% RH) and pre- processing treatments on the physical, cooking and nutritional quality in the common beans. The tropical climate ambient conditions of 25 ˚C and 75% RH were the control and the conditions of 45 ˚C and 75% RH and 35 ˚C and 83% RH) were the accelerated conditions of storage. Two common bean varieties harvested in the same season, Rose coco (GLP 2) and Red Kidney (GLP 24) were obtained from Kenya Agricultural and Livestock Research Organization (KALRO), Thika. The beans were subjected to a combination of varying temperature (25 ˚C, 35 ˚C and 45 ˚C) and relative humidity (75% RH and 83% RH) storage conditions in incubation chambers for a maximum storage period of six months. The desired levels of relative humidity were maintained by use of saturated salt solutions of sodium chloride and potassium chloride for 75% and 83% RH respectively. Beans from each treatment condition were sampled at different time intervals after which analysis of physical properties, cooking properties and nutritional properties were performed. Soaking pretreatment in deionized water, sodium carbonate, and calcium chloride and subsequent cooking were carried out to determine their effects on nutritional and cooking properties. For physical properties; dimension, weight and density characteristics were not significantly different for both bean varieties during the storage period. However, colour characteristics differed significantly. Soaking characteristics: hydration and swelling coefficient for both varieties decreased significantly while leached solutes and electrical conductivity increased. Cooking profiles for Rose coco and Red kidney revealed that under the accelerated conditions of storage, cooking time increases with increased storage time. The pre-processing treatment of Na_2CO_3 shortened the cooking time significantly while soaking in 0.1M $CaCl_2$ resulted in beans that did not cook within 5hrs. The pre-processing treatment of Na_2CO_3 and deionized water also reduced the levels of antinutrients in the beans such as phytates and tannins. In both varieties; crude protein and mineral content did not have significant change while significant

decrease was observed for moisture uptake, protein digestibility and phytates. On the other hand, tannins had a significant increase. From this study, the accelerated storage conditions of (35 ˚C and 45 ˚C) and relative humidity (75% RH and 83% RH) caused development of the HTC defect as was evident from some of the physical, cooking and nutritional properties. In conclusion, the results of this study revealed that storage at elevated temperature and RH led to the development of the HTC defect. Soaking pre-treatments for beans not only reduces the cooking time but also reduces the antinutrients content. Breeding for bean varieties without the HTC trait should be done and these varieties passed onto other stakeholders in the bean value chain for utilization.

Key words: Common bean, storage conditions, hard to cook defect

CHAPTER ONE

INTRODUCTION

1.1 Background information

The common bean *Phaseolus vulgaris* was domesticated more than 7000 years ago in two centers of origin; Mesoamerica (Mexico and America) and the Andean region. Scientists believe dry beans, along with maize, squash and amaranth began as weeds in fields planted with cassava and sweet potatoes in Central America. Beans are found growing from sea level up to 3000m above sea level and are cultivated either in monoculture, associations or in rotations (Broughton *et al.,* 2003).

The common bean refers to food legumes of the genus *Phaseolus,* family *Leguminosae,* subfamily *Papilio-noidae,* tribe *Phaseoleae,* subtribe *Phaseolinae* (Gepts, 2004). The genus *Phaseolus* contains 50 wild growing species and also contains five domesticated species: in decreasing order of importance, common bean *(Phaseolus vulgaris L*.), lima bean (*P. lunatus L*.), runner bean (*P. coccineus L*.), tepary bean (*P.acutifolius* A. Gray), and year bean *(P. polyanthus* Greenman (Broughton *et al.,* 2003)..

Food legumes play a vital part in the farming systems and diets of poor people globally and hence are ideal crops for simultaneously achieving three developmental goals in the population: reducing poverty, improving human health and nutrition, and enhancing ecosystem resilience (Akibode and Maredia, 2011). In dietary terms, dry beans are nutritious (Hangen and Bennink, 2002) and complement cereal crops as a source of proteins and minerals (Baudoin and Maquet, 1999). Agronomically, they serve as rotation crop with cereals, reducing soil pathogens and supply the essential nitrogen to the cereal crop. They also play a vital role as a source of animal feed in smallholder livestock systems thus play the dual role of being human food and animal feed (Gepts, 2004).

Temperature and relative humidity management are referred to as maintaining the cold chain during the postharvest handling and marketing (Adaskaveg, 2002). The storage of grains, such as beans, in the natural environment of tropical countries presents larger problems due to the temperature conditions and relative humidity when compared to areas having cold or temperate climate (Abba and Lovato, 1999). In tropical countries such as Kenya, it is estimated that each year between 25 and 40% of stored agricultural products is lost due to inadequate farm- and village- level storage (Hayma, 2003). Therefore, in order to minimize the losses during storage it is important to know the optimum environmental conditions for storage (Hayma, 2003). Much of the theory of the storage of agricultural products depends on the relative humidity of the air, temperature and the moisture content of the product (Hayma, 2003). Relative humidity is a percentage measurement of the amount of moisture in the air as compared to the maximum amount of moisture which air could hold at that temperature (Hayma, 2003). The safe moisture content for beans is in the range of 13 to 15% though this may vary slightly depending on the variety. In tropical countries, the ambient storage temperatures are observed above 20 ˚C and below 30 ˚C while relative humidity is at 65 to 75 % (Kilmer *et al.,* 1994; Dhingra *et al.*, 2001).Higher temperatures require lower moisture content (Hayma, 2003).

Legumes are generally stored for varying periods of time before use for domestic consumption. In contrast to cereals, legumes are not easy to preserve and consequently storage at warm temperatures (30 - 40 ˚C) and high RH (> 75%) results in development the Hard to cook (HTC) defect (Kilmer *et al.,* 1994). Under these conditions of high temperature and RH, the physicochemical properties of the seed components are modified (Desphande and Damodaran, 1990). For example, a study by Ndung'u *et al.* (2012*)* showed that storage of cowpeas under conditions of high temperature (30 – 40 ˚C) and RH (60 - 80%) leads to development of the HTC defect. The storage varies from several months up to even one year or more and this has adverse consequences on cooking time (Kaur and Singh, 2007). Warm and humid conditions in many regions of the world with subtropical and tropical climates accelerate development of the HTC

defect in stored beans (Berrios *et al.,* 1998). The main observed alterations due to the HTC defect are deterioration of texture and flavor and an increase of the cooking time due to the hardening of the bean (Batista *et al.,* 2010). The HTC phenomenon inhibits cell separation during the cooking process and this in turn affects the texture of the cooked seeds and limits the protein availability due to the physical restriction for the access of digestive enzymes and hence lowering nutritional attribute of the beans (Alonso *et al.,* 2001).

1.2 Problem Statement

In Kenya, the common beans can play a strategic role in alleviating food and nutrition insecurity among the poor who cannot afford animal proteins (Katungi *et al.,* 2008). This is because the plant proteins such as those from common beans are relatively cheap. Therefore, if factors that limit bean utilization are addressed, food and nutrition security can be increased. The HTC defect is one of the major factors that reduce utilization as its development makes beans have long cooking time. Consequently, for groups of people who are low on the economic scale, increased fuel consumption leads to decrease in consumption of the common beans.

This study investigates the effect of accelerated storage conditions of temperature (35 ˚C and 45 ˚C) and relative humidity (75% RH and 83% RH) on the development of the HTC defect.

Soaking of beans in water prior to cooking is a common practice in Kenya which is done so as to shorten the cooking time. However there are limited documented studies on the effect of using different soaking solutions on the cooking and nutritional properties of the beans. Thus the effect of different soaking solutions on cooking time and antinutrients content was addressed by this study

1.3 Justification

The vision 2030 of the government of Kenya and the sustainable development goals (SDGs) clearly identify food and nutrition security as part of the economic pillar. Beans

have been identified as one of the priority crops under the Agricultural development strategy of Kenya. Many initiatives that exist target increased production and utilization of beans. However, very few cover the HTC problem which becomes more severe with increased storage under the tropical weather conditions in Kenya. The HTC problem reduces utilization of the beans and hence contributes to food and nutrition insecurity.

Common beans have a high content of protein, carbohydrates, fibers, minerals and vitamins (Batista *et al.,* 2010) hence have a beneficial effect on consumer's health. They have been associated with weight management, lowered risk to heart diseases, diabetes, cancer and hypertension (Katungi *et al.,* 2008). For low income groups, the reduction or increase in energy costs is a vital defining factor for purchase of common beans (Corrêa *et al.,* 2010). Moreover, cutting fuel consumption for bean cooking will greatly aid in environmental conservation efforts as less fuel would be needed for cooking. Therefore, the resulting environmental conservation will benefit present and future populations. Finally, control of HTC defect could also aid in reducing postharvest losses of beans as more beans will meet the consumer acceptability criteria.

1.4 Objectives

1.4.1 Main objective

To determine effects of: storage temperature (35 and 45 ˚C), relative humidity (75 and 83% RH) and pre-processing treatments on the physical, cooking, nutritional and anti-nutritional properties of beans.

1.4.2 Specific objectives

1. To determine the effect of storage conditions on the physical, nutritional (protein content, mineral content and protein digestibility) and anti- nutritional (phytates and tannins) properties of Rose coco and Red kidney beans.
2. To determine the effect of storage conditions on the development of the HTC defect (cooking behavior) of common beans

3. To determine effect of pre- processing treatments on the cooking, nutritional and anti- nutritional properties of Rose coco and Red kidney.

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1.5 Hypothesis

1. Storage conditions have no effect on physical and nutritional properties of Rose coco and Red kidney beans.
2. Storage conditions have no effect on the development of the HTC defect (cooking behavior) of common beans
3. Pre-processing treatments have no effect on cooking and nutritional properties of Rose coco and Red kidney

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Common beans are legumes that supply a significant amount of protein for a great part of the world's population (Batista *et al.,* 2010) and more so in Kenya. Common bean is the most important food legume and a major source of dietary protein (De-Mejia *et al.,* 2005). The global total bean production exceeds 23 metric tons of which 7 million metric tons are produced in Latin America and Africa (Broughton *et al.,* 2003). In addition, beans are an excellent source of complex carbohydrates and polyunsaturated free fatty acids; linoleic and linolenic (Reyes-Moreno *et al.,* 1994). Furthermore beans have recently been attributed with conferring numerous positive health benefits; hypocholesteromic response, mitigation of diabetes and colonic cancer and weight control when properly positioned in diet (Uebersax, 2006).

On the other hand, beans have several undesirable attributes, such as presence of anti-nutritional factors such as; enzyme inhibitors (trypsin inhibitor), phytates, polyphenols (condensed tannins and anthocyanins), flatulence causing oligosaccharides, lectins and allergens (Towo *et al.,* 2003). The low protein digestibility of beans is attributed to the presence of some of these factors (Carvahlho, 1998). However , there is increasing evidence that these antinutritional components are implicated in the prevention of some chronic diseases such as cancer, heart disease and diabetes (Regina, 2003; Azevedo *et al.,* 2003). Furthermore, trypsin inhibitors may inhibit carcinogenesis (Malkowicz *et al.,* 2001) and can be used as chemo preventive and antiproliferative agents in transformed cells (Garcia-Gasca, 2002). Moreover, condensed and hydrolysable tannins are considered effective antioxidants, antimutagenic and anticarcinogenic agents (Galoti, 2004; Cardador- Martinez, 2002).

Despite the positive health benefits associated with beans, leguminous seeds once subjected to long periods of storage at high temperature and humidity undergo gradual loss of nutritional and textural quality (Shiga *et al.*,, 2009). This phenomenon is known as the HTC defect and is characterized by extended cooking times for cotyledon softening (Liu, 1995). The HTC defect is one of the most vital acceptability characteristics because the cooking time required for beans to reach acceptable texture also greatly influences consumer perception of bean quality (Affrifah and Manjeet, 2006). The HTC defect is attributed to multiple mechanisms such as lipid oxidation, insoluble pectates formation and middle lamella lignifications (Liu, 1995).

Preparation of bean for consumption involves two vital steps: soaking and thermal treatment (Bernal-Lugo, 1997). The soaking of beans in water prior to cooking is a common practice that softens the seeds of most cultivars and therefore shortens the cooking time (Martinez-Manrique *et al.*, 2011). The cooking time involved in the thermal treatment stage involves the bean reaching a degree of acceptable texture to the consumers and this is referred to as the cooking quality (Desphande and Damodaran, 1990). Therefore, beans with extended cooking times will be rejected and this is the case for beans with the HTC defect. Three events can be distinguished during the thermal treatment: solubilization and /or degradation of pectin substances, starch gelatinization and cytoplasm protein denaturation (Bernal-Lugo, 1997). These fluctuations in order disorder transitions are said to contribute to the softening of the seed and to the acquisition of texture and flavor (Desphande and Damodaran, 1990). Reduced cooking quality has been observed in stored legumes and is said to result from: slow rate of pectin β-degradation and faster protein denaturation than starch gelatinization during cooking (Desphande & Damodaran, 1990).

2.2 Distribution of the common bean

Common beans are a food and nutrition security crop and a source of cash income in East, Central and Southern Africa. The top 10 producers according to FAO (2008) are as indicated in Table 2.1. In terms of production, Kenya comes second after Uganda and this is as a result of a relatively favourable biophysical environment in Uganda (FAO, 2008).

Table 2.1: Top 10 producers of common bean in terms of area in Africa in 2000-2007

Country Average area (Ha)	Average area (Ha)	Average production (Tonnes)
Kenya	910,478	412,381
Uganda	794,375	478,625
Tanzania	373,125	285,414
Rwanda	340,055	231,882
Angola	290,391	92,786
Burundi	249,375	229,607
Democratic Republic of Congo	205,958	110,404
Malawi	197,605	87,593
Ethiopia	188,000	143,414
Madagascar	82,096	77,273

Source: FAOstat at www.fao.org

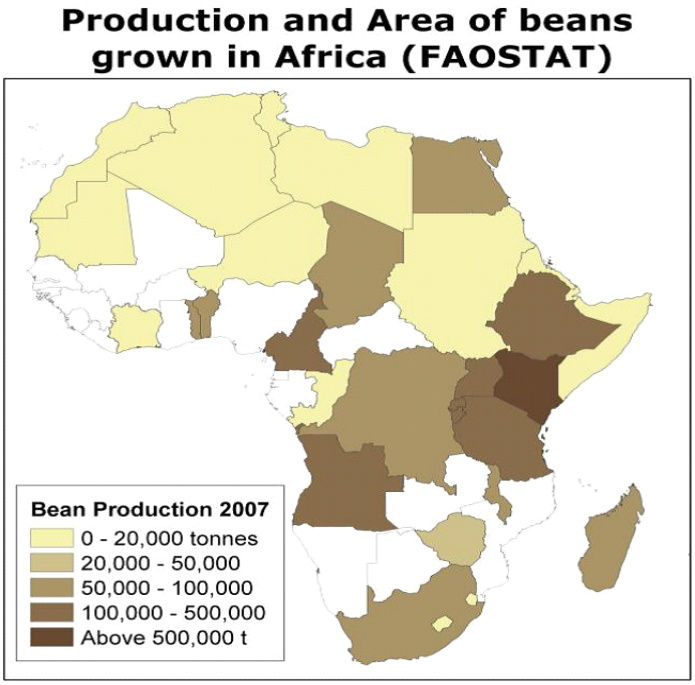

Figure 2.1: Production and Area of beans grown in Africa (FAOSTAT)

Source: FAOstat at www.fao.org

Figure 2.1 shows common bean production in Africa. In Eastern Africa, common beans are grown twice a year, with sowing seasons running from March to April and from September to October (Katungi *et al.*, 2009). Production of common beans in Kenya is mainly in the highlands and midlands with approximately 75% of the annual cultivation occurring in three regions: Rift valley, Nyanza and Eastern Province (Katungi *et al.*, 2009). The Rift valley contributes the biggest share, accounting for 33% of national output, followed by Nyanza and Western province with 22% each, while the output from Eastern parts of the country and coast is constrained by adverse climatic conditions (Katungi *et al.*, 2009).

In the Eastern Africa region, beans are mainly grown by small scale farmers with a very minimum input except the seed (Rubyogo *et al.*, 2008). According to FAO (2005), despite a slight increase in yield trend, beans productivity and yield levels at farm level

have remained relatively low and even decreasing in some areas. In recent years, the crop production trend has not kept pace with the annual growth rate (estimated above 2%) in population in some countries due to a number of biotic, abiotic and socio-economic constraints (Kambewa, 1997; Xavery *et al.,* 2006). Drought is the major and common abiotic cause and constraint across Eastern and Southern Africa (Katungi *et. al.,* 2009). With global climatic change threatening to exacerbate the drought problem in some parts, rapid population growth and increasing cost of livestock products, food and nutritional insecurity in Sub-Saharan Africa has the potential to increase (Katungi *et. al.,* 2009)

2.3 Utilization of the common bean

Common beans are a short duration crop (2.5 to 4 months) and thus are a key for helping to shorten the hunger periods and providing quick cash and hence improving livelihoods. Rubyogo *et al.*,(2008) stated that their early maturity and capacity to provide a range of food products (leaves as well as, fresh pods and dry grain) also helps provide a more balanced diet to vulnerable community members (under five, pregnant women and the chronically ill). Rubyogo *et al.,* (2008) further stated that, in recent years, bean consumption is on the rise as a result of increasing scarcity of animal proteins and increasing poverty among inhabitants of rural and urban areas. Per capita consumption is estimated at 14 kg/ year in Kenya, but can be as high as 66 kg/ year in Western Kenya (Katungi *et al.,* 2009). It is commonly believed that demand for beans is income-inelastic, that is, consumption drops as economic levels rise (Broughton *et al.,* 2003).

There exists an impressive diversity of common bean seed types in Kenya with about 80 different seed types distinguished in different parts of the country (Njuguna *et al.,* 1980). However only six are popular to date, namely: red and red/ purple mottled (occurring in different local names such as Rosecoco, Nyayo, Wairimu, Kitui), Purple/grey speckled (Mwezi moja) and Pinto (Mwitemania). Rosecoco was the most widely grown followed by Canadian wonder/Red kidney type because they are high yielding but require heavy rains and high soil fertility (Katungi *et al.,* 2007). As expected, the varieties have been

losing area because of increased problem of soil fertility and associated diseases and are thus being replaced by varieties like Pinto and Red haricots that are adapted to poor soil conditions (Odendo *et al.*, 2001).

Cooking is fundamental for bean consumption (Corrêa *et al.*, 2010). It increases digestibility, inactivates antinutritional factors, increases nutrient biological value and imparts sensorial quality that consumers approve (Tharanathan and Mahadevamma, 2003).

"*Githeri*" is a popular Kenyan food incorporating a mixture of maize and beans (Katungi *et al.,* 2009). It is a mixture of maize and beans in equal ratios or more of either one, depending on personal preferences. The mixture is boiled for around two hours with the beans having been subjected to overnight soaking in most cases. Cooking time usually shortens as soaking time is increased (Corrêa *et al.,* 2010). The cooked mixture can then be eaten on addition of salt. Alternatively it can be stir fried with cooking oil, spices and vegetables depending on personal tastes.

2.4 Nutrient content of beans

The common bean is rich in a host of nutrients. Consequently, both rural and urban populations benefit. This is in agreement with the fact that it is generally recognized that for food products to have significant nutritional impact there must be broad social acceptance (Uebersax, 2006). Key elements that are associated with the nutritional value of beans are focused on the importance of digestibility of proteins, carbohydrates (starch and fiber) and micro nutrients which include iron, zinc and folic acid (Uebersax, 2006). Beans are especially rich in iron and protein and this is a clear indication that beans can make a vital contribution to health especially in Sub –Saharan Africa where 40% of women suffer from iron deficiency (Broughton *et al* ., 2003)

2.4.1 Carbohydrates

Carbohydrates content ranges from 50 to 60% of common beans dry weight and starch is the major constituent (Reyes-Moreno *et al.,* 1993). The starch content ranges from 35 to

60%, dietary fiber ranges from 14 to 19% and oligosaccharides ranges from 2 to 6% of the dry weight (Reyes-Moreno *et al.,* 1993). Pectic substances, arabinogalactans and xyloglucans are other carbohydrates present in common beans (Reddy *et al.,* 1984 and Sathe *et al.,* 1985). The influence of carbohydrates on cookability is centered around three fractions: starch granule, middle lamellae/cell wall and dietary fiber components.

2.4.1.1 Starch

Legume starch is a glucose polymer stored as microscopically small granules in seeds and roots (Sathe *et al.,* 1985). It is comprised of high levels of linear amylose and less of the branched amylopectin (Uebersax 2006). Fructose, together with the oligosaccharides raffinose, stachyose and verbascose represent the major reserves of soluble carbohydrates in seeds (Kigel, 1999). Starch is a vital determinant of end product quality due to the extent of hydration and swelling during soaking and cooking (Uebersax 2006).

2.4.1.2 Fiber

Dietary fiber is comprised of indigestible components of food fermented by microorganisms in the colon. Beans are high in both soluble and insoluble dietary fiber. Dietary fiber interaction with protein and minerals has been shown to reduce apparent bioavailability. Gomes *et al.,* (1997) assessed the in vitro digestibility of bean proteins in relation to the interaction of amino acids with dietary fiber components. It was found that digestibility of proteins associated with fiber fractions was found to be low and this was related to the protein/cellulose ratio. Further, Hughes *et al.,* (1996) investigated the ability of dietary fiber and tannins to lower protein utilization in dry beans. It was found that tannins significantly reduced protein utilization and that soluble dietary fiber was responsible for the reduction in protein digestibility that is associated with dietary fiber in foods. Thus dietary fiber and tannins contribute to the low nutritional value of bean protein as compared to animal protein. Garcia – Lopez (1985) assessed influence of dietary fiber on iron absorption. The results indicated that ability of fiber to decrease

iron absorption appeared to depend on; fiber source, particle size and concentration (presence of competing minerals and iron status of animal).

2.4.2 Protein

Protein content values ranging from 16 to 33% have been reported after analyzing many diverse bean lines (Reyes-Moreno *et al.,* 1993). Other researchers have documented a protein content of 18-22% which is among the highest in vegetable sources (Uebersax, 2006).) Most of this protein content is made up of the storage protein phaseolin which is a major determinant of both quantity and nutritional quality of proteins in bean seeds (Broughton *et al.,* 2003). Similar to other seed proteins of the legume family, phaseolin is deficient in sulphur containing amino acids such as methionine (Broughton *et al.,* 2003). Thus, methionine is the limiting amino acid in dry beans. Cereal grains like corn have the amino acid lysine as the limiting amino acid (Broughton *et al.,* 2003). The combined consumption of cereals and legumes generally alleviates these mutual deficiencies and ensures a balanced diet when cereals and legumes are consumed (Bressani, 1983). Consequently increasing legume yield and in particular beans has vital repercussions on improving nutrition and health of hundreds of millions of bean consumers in the world and especially in developing countries (Broughton *et al* ., 2003). Legumes in general have a lower protein digestibility than proteins of animal origin and this is attributed to presence of anti-nutritional factors such as trypsin inhibitors, polyphenols and phytic acid (Jood *et al.,* 1998).

2.4.3 Minerals

Beans are much more superior to cereals as sources of micronutrients (Welch *et al.,* 1975). Beans are vital source of iron (5.0 to 8.2 mg per 100 g), magnesium (138 to 176 mg per 100g, zinc (2.2 to 3.6 mg per 100 g), copper (0.69 to 1.20 mg per 100 g) and calcium (83 to 147 mg per 100g) (Augustin *et al.,* 1981; Augustin and Klein, 1989 , Broughton *et al.,* 2003 and USDA,2012). This is because legumes have higher initial content of minerals and many cereals are polished before eating to remove the seed coat that contains a significant amount of minerals (Broughton *et al.,* 2003). Additionally

common beans are mostly consumed whole and hence mineral content is conserved (Broughton *et al.,* 2003).

2.4.4 Lipids

Depending on the common bean species, lipid content ranges from 1 to 3% (Patte *et al.,* 1982 and Sathe *et al.,* 1985). Neutral lipids which are made up primarily of triglycerides are the predominant class of lipids in common beans, ranging from 32 to 45% of total lipids while phospholipids vary from 28 to 34% of total lipids (Takayama *et al.,* 1965; Mahadevappa and Raina, 1978). Common bean lipids show variation in their fatty acid composition and contain substantial amounts of unsaturated fatty acids (Patte *et al.,* 1982 and Sathe *et al.,* 1985). The major unsaturated fatty acids range from 65 to 87% of total lipids and are oleic (7 to 10%), linoleic (21 to 28%) and linolenic (37 to 54%) (Reyes-Moreno *et al.,* 1993). Linoleic and linolenic cannot be synthesized by animals and human but are required for normal growth, cell structure, tissue function and prostaglandin synthesis (Lehninger, 1975 and Sathe *et al.,* 1985). High content of linoleic and linolenic have been attributed to lower serum cholesterol levels in rats (Mahadevappa and Raina, 1978).

2.4.5 Vitamins

Reyes-Moreno *et al.,*1993 postulate that raw common beans are a good source of water soluble vitamins which include, thiamine (0.86 to 1.14 mg per 100 g), riboflavin (0.136 to 0.266 mg per 100 g), niacin (1.16 to 2.68 mg per 100g), vitamin B_6 (0.336 to 0.636 mg/100 g) and folic acid (0.171 to 0.579 mg per 100 g). During cooking, the nutrient retention values vary from 70.9% (vitamin B_6) to 75.9% (riboflavin) (Augustin *et al.,* 1981; Augustin and Klein, 1989).

2.5 Health benefits of bean consumption

The popular perception is that beans are a “poor man’s meat”, however this notion should appropriately and deservedly change to “healthy peoples meat” (Maryange *et al.,* 2010).

Epidemiological and demographic studies indicate that populations with largest consumption of beans have a reduced risk of mortality from breast prostate and colon cancers (Machado, 2008). This evidence is further supported by several authors who have mentioned that beans contain potentially bioactive micro-constituents such as phenolic compounds that have shown anti-carcinogenic and antioxidant properties in animal models and also *in-vitro* (Hangen and Bennink, 2002; Benninger and Hosfield, 2003; Azevedo *et al.,* 2003; Diaz-Batalla *et al.,* 2006). Beans have been cited for imparting diverse positive health responses (hypocholesteromic response, mitigation of diabetes mellitus and colonic cancer and weight control) when properly positioned in diet (Geil and Anderson, 1994; Uebersax, 2006). Furthermore, beans are a good source of protein and energy that if deficient causes the public health concern of protein energy malnutrition (PEM) that manifests itself as either kwashiokor or marasmus. Evidently, the nutrient composition of dry beans makes them ideally suited to meet two major dietary recommendations for good health: increased intake of starches and complex carbohydrates and decreased consumption of fat (Uebersax, 2006).

2.5.1 Hypoglycemic response

Low glycemic index have been observed for legumes compared with other starchy foods such as bread, potato and certain breakfast cereals (Uebersax, 2006). Research designed to assess low glycemic responses attributed to consumption of bean products clearly demonstrates positive impact associated with slow glucose release (Jenkins *et al.,* 1983).

In a study conducted by Jenkins *et al.,* (1983), volunteers took carbohydrate portions of eight varieties of dried legumes and 24 common foods. Both the mean peak rise in blood glucose concentration and mean area under the glucose curve of the subjects provided

with beans were at least 45% lower than those of subjects receiving the other foods. These results thus suggest a potentially valuable role for dried leguminous seeds in carbohydrate exchanges for people with impaired carbohydrate tolerance.

Leathwood and Pollet (1988) studied the effects of the slow release carbohydrates from ingested bean flakes on plasma glucose and satiety. After a meal containing potato, plasma glucose levels rose sharply, but fell below initial levels 2 to 3 hours later. In contrast, there was a slow, sustained increase in blood glucose after consumption of bean puree. It was thus concluded that consumption of bean puree delayed the return of hunger and decreased ratings for desire to eat a snack.

2.5.2 Hypocholesteromic response

Dry beans are an excellent source of dietary fiber. There is considerable evidence that foods high in soluble dietary fiber can reduce blood cholesterol (Anderson and Gustafson, 1988; Anderson *et al.,* 1999; Kritchevsky, 2001). According to Uerbersax (2006), the precise mechanism of action of the hypocholestremic effect of fiber has not been fully elucidated but some proposed mechanisms include; reduction of fat and energy intake, reduction of cholesterol and fat absorption, changes in endocrine response, effects of colonic metabolites and increased bile acid excretion.

Clinical research associated with bean consumption has been conducted on hypercholesterolemic men (Anderson *et al.,* 1984). Bean supplemented diets (115 g dried beans/day; 50 g total and 20 g soluble fiber) were found to selectively lower atherogenic LDL(low density lipoprotein) cholesterol fractions while preserving anti-atherogenic HDL(high density lipoprotein) cholesterol levels (Anderson *et al.*,, 1984). Uebersax (2006) stated that this probably relates to the high soluble storage polysaccharide content of legumes. Additionally, short chain fatty acid fermentation products of soluble fibers may attenuate hepatic cholesterol synthesis.

2.5.3 Weight management

Weight control and weight management strategies involve highly complex physiological and psychological interactions (Uebersax, 2006). A total lifestyle change in terms of diet and increased activity level contribute to healthy weight management rather than one particular food. However, beans have been found to contribute to weight management due to the relative low glycemic index, high resistant starch and high satiety level (Leathwood *et al.*, 1988; Ludwig *et al.*, 1999).

2.5.4 Colon cancer mitigation

There is increasing evidence that suggests an inverse relationship between bean consumption and colon cancer in humans (Uebersax, 2006). Furthermore, Hughes *et al.*, (1997) stated that epidemiological studies show a low incidence of colon cancer in many Latin American countries where dry bean consumption is high. Further, research using rats was conducted to test effects of dry beans on the inhibition of colon carcinogenesis. The carcinogen, azoxymethane (AOM) was used. This study by Hughes *et al.*, (1997) demonstrated that dry beans contain anticarcinogenic compounds capable of inhibiting AOM- induced colon cancer in rats. Additional studies by Hangen and Bennink (2002) have shown that consumption of black beans reduce colon carcinogenesis in rats. Rats were fed a modified AIN-93G diet (control) or diets containing 75% black beans for 4weeks, and then colon cancer was initiated by azoxymethane administration. Thus it was concluded that eating black beans significantly reduced colon cancer and multiplicity (Hangen and Bennick, 2002).

2.6 Changes in whole bean composition during storage

2.6.1 Pectin

Pectin is a family of complex polysaccharides present in all plant primary cell walls and they have multiple functions in plant growth and development (Ridley *et al.*, 2001). Pectin is part of the soluble dietary fiber and is the glue that holds plant cell walls together. As long as the pectin remains soluble it will interact with water to form a gelatinous material during cooking causing a softening. If pectin becomes insoluble, the

bean does not absorb as much water resulting in a bean that does not soften easily during cooking. This is referred to as the HTC phenomenon. A loss of solubility of pectins was associated with the hard to cook phenomenon (Moscoso *et al.,* 1984; Hentges *et al.,* 1991). The increase in seed hardness during storage correlated to reduced solubility of the pectins (Shiga *et al.,* 2004). This suggests that some of the soluble fiber components convert into the insoluble form of dietary fiber during bean storage.

2.6.2 Phenolic compounds

Phenolic acids and their derivatives are widely distributed in legumes and can be present in either free (extractable) and/or bound (both extractable and non- extractable) form (Srisuma *et al.,* 1989). These different forms can be affected by storage. Varriano-Marston and Jackson (1981) reported that as free phenolic acid content increases in the beans, seed viability decreases. Secondly, an increase in esterified phenolic acids in the seed coat related to bean hardening and the HTC phenomenon. Garcia *et al.,* (1998) observed that the phenolic acid content in the pectin fraction was two times higher in HTC beans than in normal beans. Maurer *et al.,* (2004) also observed an increased binding between phenolic acids and pectin in the HTC beans. Therefore, the interaction between phenolic acids and pectin during storage contributes to bean hardening. The benefit of phenolic acids present in beans against oxidative stress conditions in the human body could therefore be affected by the HTC defect (Machado *et al.,* 2008).

2.7 Factors limiting common beans utilization

2.7.1 Hard- to - cook phenomenon

Prolonged storage of beans especially under high temperature (>25 °C) and high relative humidity (>65% RH) promotes the HTC defect which is an irreversible change that increases the time and fuel needed for cooking beans (Kigel, 1999). Hardening results in a corresponding high energy cost to soften the legumes prior to consumption (Kaur and Singh, 2007). On the other hand, the overcooking in turn causes essential nutrient loss (Kigel, 1999).

According to Hohlberg and Stanley (1987), the need for prolonged cooking time is attributed to two distinct processes. The first one is hard shell which occurs when the seeds do not absorb enough water during cooking and thus do not soften when cooked. This can be due to low permeability of the seed coat to water. The second is HTC defect which occurs when the seeds absorb enough water but fail to soften upon soaking and cooking.

Documentation of the HTC phenomenon was began by Mattson *et al.* (1946) where they proposed that divalent cations released from phytate hydrolysis increase pectin stability by formation of cross-links. Further, Hohlberg and Stanley (1987) reported the events that occur during soaking and cooking of soft and hard beans and related these events to the physical and chemical changes resulting from the mechanism responsible for development of the HTC defect.

The mechanism of the HTC phenomenon is very complex and involves changes in the intracellular, cell wall and middle lamella polysaccharides and other components (Galiotou-Panayotou, 2008). According to the same author, the mechanisms include ; insolubilization of pectic substances depending on the phytate concentration in the seed cells, lignifications and other changes in the middle lamella and cell wall, multiple mechanisms due to the activity of pectinesterases, polyphenolases and lipoxygenases and changes in storage protein phaseolin.

Majority of research presented in literature indicates that the main mechanism involves the interaction of phytate and divalent cations with pectin and is therefore in agreement with the first theory (Galiotou-Panayotou, 2008). This theory has been reported to be based on the phytase-phytate-pectin hypothesis whose explanation was first provided by Mattson (1946). According to this hypothesis, pectin cements the plant cells together and exists mainly in a water soluble form, permittimg water uptake by the legume seeds. Further, calcium and magnesium ions can crosslink the carboxyl groups of soluble pectin, transforming them into insoluble calcium and magnesium pectates. Phytate has six strong phosphate groups and these prevail over the weak carboxyl groups of pectin

and chelates preferentially with the divalent cations. Therefore under these conditions, pectin remains soluble and hence the legume seeds are easy to cook. However when phytate is hydrolysed by phytase, the chelation disappears and allows cross-linking of pectic substances via calcium and magnesium bridges. Calcium and magnesium pectates formed do not dissolve readily on heating, thus restricting cell separation, inhibiting water uptake and thus resulting in hard to cook defect in beans and legumes in general. Therefore, conditions that accelerate the activity of phytase, such as increased temperature and relative humidity, lead to development of the HTC defect (Ockenden *et al.,* 1997). The HTC phenomenon is of great importance nutritionally and subsequently has commercial consequences (Jones & Boulter, 1983)

2.7.2 Digestibility and flatulence

Digestion rate is affected by factors which slow or prevent digestive enzymes from gaining access to legume starch glycosidic bonds (Uebersax, 2006). Consequently, substantial legume starch may reach the stomach and ileum while still in intact cell walls. Consequently, as stated by Uebersax (2006) there are two factors that hinder starch digestion. The fiber matrix of cell walls is a primary hindrance to starch digestion since amylase must penetrate the cell wall prior to amylolysis. Secondly, hydration state of starch affects digestion rate as starch granules must be hydrated to allow digestion. Starch will imbibe water if sufficient energy is present to break the internal hydrogen bonds and heating water serves this purpose. Breakage of hydrogen bonds chains allows the starch molecules to relax and the granule to swell and this will allow gelatinization which may determine the rate of starch digestion (Uebersax, 2006).

Flatulence is often the result of ingesting foods high in raffinose, stachyose and verbascose (Broughton *et al.,* 2003). Although these sugars are indigestible, micro-flora of the lower intestine ferments the sugars producing gas (Kigel, 1999)

2.7.3 Antinutritional factors

Several antinutritional factors are present in common beans. Antinutritional factors have long been recognized as concerns and require appropriate processing conditions to

alleviate adverse effects. The compounds are intrinsic to the bean and serve several vital physiological processes during growth and development. The compounds may contribute to acute and chronic health effects and must therefore be rendered inactive so as to ensure nutrient absorption and positive health benefits derived from bean consumption (Uebersax, 2006). Beans need to be soaked and cooked to make them palatable. Cooking inactivates heat labile antinutritional compounds as well as permits the digestion and assimilation of proteins and starch (Kigel, 1999).

2.7.3.1 Phytates

Phytate is considered as an antinutrient because it can significantly decrease bioavailability of essential elements such as calcium, iron, magnesium, copper and zinc (Kigel, 1999). The complexes formed between phytic acid and the metal ions are poorly absorbed and this therefore leads to reduced availability of the minerals (Uebersax, 2006). Phytic acid is a chelating agent and a main reservoir of 60-90% phosphorus (Lolas *et al.,* 1976) and other minerals in the seed that are mobilized during germination.

Coelho *et al.*, 2007 stated that during the storage period, the phytate content decreases owing to the gradual action of the phytase enzyme, which hydrolyses a portion of the phosphate ester groups. Consequently, these phosphate esters will no longer chelate magnesium and calcium ions. Once freed and diffused into the middle lamella, these ions can cause insolubilization of pectinic acid due to the formation of more thermally stable calcium pectates. Consequently, the softening of the beans during the cooking process is restricted because the middle lamella of the cotyledon cell wall does not separate easily (Coelho *et al.*, 2007).

Phytic acid also acts as an antioxidant during dormancy, stores cations and cell wall precursors (Uebersax, 2006). Possible therapeutic properties of phytate in the prevention of breast and colon cancer probably due to its anti-oxidant properties exists (Broughton *et al.,* 2003). Further, it has been implicated in the reduction of cholesterol and other lipids due to its presence in high fiber diets (Broughton *et al.,* 2003).

2.7.3.2 Tannins

Tannins are a group of polyphenols in plants and are composed of high molecular weight compounds with hydroxyl clusters (Coelho *et al.*, 2007). Phenolic compounds and tannins increase during seed development but decrease thereafter in parallel to polymerization of tannins (Coelho and Lajolo, 1993). Tannin content and polyphenoloxidase activity change during storage at rates dependent on storage temperatures (Iaderoza *et al.*, 1989). Condensed tannins have been reported to impair iron availability as has phytate compounds (Towo, 2003). Tannin content changes with seed colour (Broughton *et al.*, 2003). It also changes during storage at rates that depend on storage temperatures (Kigel, 1999). Tannins have a possible role in development of the HTC defect as some researchers have shown that tannins may migrate from the seed coat to the cotyledon and react with proteins and /or carbohydrates and hence participate in polymerization reactions that lead to HTC defect (Moreno *et al.*, 2000). Moreover, Coelho *et al.*, (2007) states that this cross linking of tannins and macromolecules in the cotyledon may occur during storage at high temperatures (40 ˚C). The tannin content of common beans is mainly found in the seed coat and range from 0 to 2% depending on the species and colour of the seed coat (Coelho, 2007).

2.7.3.3 Phytohemagglutinin (PHA) or "Lectins"

Phytohemagglutinin (PHA) is a heat labile lectin known to depress the nutritional quality of dry beans (Thompson *et al.*, 1986). In mammalian systems, the detrimental effects of lectins are attributed to the binding of these molecules to the surface of the small intestine epithelial cells, and this leads to interference with the digestive, absorptive and secretory function of the ileum and also anatomical abnormalities (Oliveira *et al.*, 2004). The nutritional significance of lectins and enzyme inhibitors from legumes was studied by Lajolo and Genovese (2002). This was done with reference to contents of enzyme inhibitors and lectins in legumes, nutritional and physiological effects, inactivation through processing and resistance to proteolysis, nutritional utilization of enzyme inhibitors and lectins and possible useful biological activities of

these compounds. This was an extension of earlier research carried out by Coffey *et al.,* (1985, 1992 and 1993) who had used extrusion and cooking in high pH medium to achieve inactivation of PHA. Extrusion was ineffective in reducing the activity of PHA in whole red kidney or black beans. However, soaking and cooking beans at a high pH was very effective, significantly reducing the activity of PHA and also reducing the time required to reach a palatable texture. It was therefore concluded that high pH and cooking treatment could be useful in improving nutritional quality of dry beans.

2.7.3.4 Protease inhibitors

Trypsin and chymotrypsin inhibitors are present in beans and they inhibit the activity of protease enzyme;trypsin and therefore negatively affect protein digestion. Thermal inactivation of trypsin inhibitors is essential in animal and human food (Gomes *et al.,* 1979; Genovese and Lajolo 1996). Birk (1996) discussed protein proteinase inhibitors as widely distributed in legumes. Consequently the Kunitz soybean trypsin inhibitor (STI) and Bowman-Birk trypsinchymotrypsin inhibitor (BBI) have been characterized. The STI has been responsible for induction of the pancreatic enlargement. The BBI trypsin-chymotrypsin inhibitors from soybeans and from chickpeas inhibit insect mid gut proteinases and thus support the hypothesis that proteinase inhibitors comprise the defense mechanism of the seed against insects. Of interest is the finding that proteinase inhibitors, such as BBI, have a role in prevention of tumor genesis, thus suggesting a possible positive contribution of the active inhibitors to the nutritional or health value of legume seeds (Birk, 1996).

Gupta (1987) stated that additional factors affecting legume digestibility include, saponins, lathyrogens, favism and cyanogenic glucosides. Arcelins and α -amylase inhibitors are also antinutrients. Although arcelins and α- amylase render the bean seeds less palatable, they serve protective functions as they have insecticidal properties (Broughton *et al.,* 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 Research site

Five kilograms each of dry beans of a recent harvest (Red kidney (GLP 24) and Rose Coco (GLP 2)) were purchased from Kenya Agricultural and Livestock Research Organization (KALRO) Thika, Kenya. The processing and analysis were carried out in Jomo Kenyatta University of Agriculture and Technology (JKUAT) laboratories.

3.2 Bean storage conditions

A 1kg reference sample of the dry beans was stored at -20 ˚C for each variety after equilibration at 25 ˚C and 75% RH for two weeks. The control conditions were thus 25 ˚C and 75%. The remaining beans were stored at accelerated storage conditions of 35 ˚C and 83% RH, and 45 ˚C and 75% RH, for a maximum of 6 months. These conditions were selected to ensure complete hardening of the bean in a relatively short amount of time. Analysis of the physical, cooking and nutritional properties was carried out at 0, 2, 4 and 6 months. The respective relative humidity was achieved using saturated salt solutions of potassium chloride (83% relative humidity) and sodium chloride (75% relative humidity).

3.3 Sample preparation

Dry beans were soaked for 16 h at a ratio of 1:5 (w/v) (bean weight: soaking solution) in de-ionized water, 0.1 M calcium chloride and 0.025 M sodium carbonate. The calcium chloride concentration was chosen according to the modified method of Clemente *et al.* (1998) while the 0.025 M sodium carbonate was chosen due to the retention of natural color of the beans unlike higher concentrations which gave a darker color (Kinyanjui *et al*., 2015).

Subsequently, the beans were subjected to a thermal treatment at a temperature of 96.5 ˚C in a shaking water bath (SHA-C, IKA Labortechnik, Japan). Beans were considered

to be fully cooked when the cotyledon could disintegrate on pressing between the thumb and the fore-fingers (Kinyanjui *et al*., 2015). The number of cooked seeds was recorded continuously for 5 hours. This was after every 15 min for sodium carbonate soaked beans and 30 min for deionized water soaked beans. Calcium chloride soaked beans did not cook for the experimental time of 5 hours. Consequently, the cooking profiles of the beans were generated based on the percentage cooked seeds as a function of time in all the differently pretreated beans.

For nutritional analysis, the samples were milled into flour to facilitate the different nutritional analysis procedures.

3.4.0 Physical properties

The physical tests were carried out after 0, 2, 4 and 6 months as explained below:

3.4.1 Characteristic dimensions of seeds

This was carried out using a vernier caliper (Mitutoyo, Tokyo, Japan) where length and width in millimeters (mm) of each variety was assessed using a representative sample of ten seeds from each variety.

3.4.2 One hundred seed weight

The weight of a hundred seeds in grams (g) was determined using a weighing balance (Libror AEG220 Shimadzu, Tokyo, Japan) in triplicate and averaged for each of the two bean varieties documented.

3.4.3 True Seed density

True seed density was calculated by recording the weight of twenty seeds in triplicate for each bean variety (Giami and Okwechime, 1993). The weight of a 100 ml volumetric flask was recorded and so was its weight with water filled up to the mark. Each of the twenty seeds was then placed into the volumetric flask and water added up to the mark and the weight recorded. The seed volume (V) was estimated by dividing the mass of the displaced water (g) by the density of water (g/cm^3). Seed density was calculated from the values obtained for weight (g) of twenty seeds and volume (cm^3).

$$\text{True seed density} = \frac{\text{weight of 20 seeds}}{\text{volume of 20 seeds}} \quad \text{.....(1)}$$

3.4.4 Bulk Seed density

Bulk density (BD) was determined using the method of Wang and K insella (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}) calculated as:

$$\text{Bulk Density} = \frac{(\text{cylinder and sample weights}) - (\text{cylinder weight})}{\text{volume of cylinder}} \quad \text{.....(2)}$$

3.4.5 Seed Porosity

Seed porosity is the property of the grain which depends on its bulk and true densities. Mohsenin (1980) presents the formula for its calculation as shown below:

$$P = 100\left(1 - \frac{BD}{TSD}\right) \text{..... (3)}$$

Where BD is bulk density (g/cm^3) and TSD is true seed density (g/cm^3)

3.4.6 Hydration coefficient

The twenty seeds of each variety were weighed in triplicate and soaked in deionized water at 25 ˚C for 16 h at a ratio of 1:5 (w/v) (bean weight to water). After soaking, the beans were cut into half along the fissure. The testa and cotyledon were separated and free water was removed using a blotting paper. The seeds were reweighed. Gain in weight was taken as the amount of water absorbed and expressed as the hydration coefficient (El-Refai *et al.,* 1988)

$$\text{Hydration coefficient} = \frac{\text{Weight of beans after soaking}}{\text{Weight of beans before soaking}} \times 100 \text{.....(4)}$$

3.4.7 Swelling coefficient

Twenty seeds of each variety were weighed in triplicate and soaked in deionized water at 25 ˚C for 16 h at a ratio of 1:5 (w/v) (bean weight to water) The volume of raw bean seeds before and after soaking in deionized water was determined by water volume

displaced in a graduated cylinder and expressed as the swelling coefficient (El-Refai *et al.*, 1988).

$$\text{Swelling coefficient} = \frac{\text{Volume of beans after soaking}}{\text{Volume of beans before soaking}} \times 100 \ldots\ldots\ldots\ldots\ldots (5)$$

3.4.8 Electrolyte and solute leaching

Twenty seeds of each variety were soaked in deionized water for 16 h at 25 ˚C. The soaking water was then collected and leached electrolytes were quantified by assessing conductivity (mohm/cm) with a digital conductivity meter (Sisabata model SC – 179, Tokyo, Japan). The solutes leached from beans were quantified by evaporating the soaking water by drying in a hot air oven at 105 ˚C, followed by cooling in a desiccator and weighing. Results were expressed as mg/g dry weight of beans (Hentges *et al.*, 1991)

3.4.9 Grain colour

A colorimeter (Minolta chromameter-CR-200b) was used to take colour measurements.

The instrument assesses color in Hunter (L^* a^* b^*) form; Hunter L-values, (whiteness/lightness), Hunter-a value (redness) and Hunter-b value (yellowness). The instrument was first calibrated using standard white plate with transparent paper placed over the standard plate. After calibration, colour measurements were taken at random in triplicates.

3.4.10 Moisture uptake

Twenty seeds of each variety were weighed, placed in perforated bags and cooked in triplicate. After 30 min interval, they were removed and re-weighed to determine the weight gain which reflects the moisture uptake during cooking.

3.5 Proximate Composition

3.5.1 Ash content

Ash content was determined by incinerating in an electric muffle furnace (KL-420 ADVANTEC). This was according to (AOAC, 2000) method 923.03-32.1.05. About 5 g

of sample were weighed in pre-conditioned pre-weighed crucibles. First, the sample was charred by a flame to eliminate carbons before being incinerated at 550 ˚C in a muffle furnace, to the point of white ash. The residues were cooled in a dessicator and the weights taken.

$$\% \text{ Crude ash} = \left(\frac{W1}{W2}\right) \times 100 \quad \ldots (5)$$

W1~weight of ash

W2~weight of the sample

3.5.2 Moisture content

Moisture was determined according to AOAC methods specification 950 46, method 925.10-32.10.03 (AOAC, 1995). About 5 g of sample, accurately weighed into a moisture dish was transferred in a hot-air oven (DSO-500D MRC Ltd) previously heated to around 105 ˚C and then drying done for one hour. Final weight of sample was taken after drying and cooling in a desiccator. The residue was taken as the total solids and loses in weight as the moisture content of the sample. This formula was adopted:

$$\% \text{ Moisture} = \frac{W1 - W2}{W1} \times 100 \quad (6)$$

W1~weight of sample before drying

W2~weight of sample after drying

W1~weight of sample before drying

3.5.3 Protein content

Protein was determined using the semi-micro Kjeldahl method, specification 950.46 method 20.87-37.1.22 (AOAC, 2000). Approximately 2 g of sample was weighed into a digestion flask together with a combined catalyst of 5 g K_2SO_4 and 0.5 g of $CuSO_4$ and 15 ml of concentrated H_2SO_4. The mixture was heated in a fume hood until the digest color turned blue. This signified end of the digestion process.

The digest was cooled, transferred to 100 ml volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalysts was also made. 10 ml of diluted digest was transferred into the distilling flask and washed with about 2 ml of distilled water. 15 ml of 40% NaOH was then added and this washed with 2 ml of

distilled water. Distillation was done to a volume of about 60 ml distillate. The distillate was titrated using 0.02 N HCl to an orange color of the mixed indicator, which signified the end point.

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

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 (2g)

3.6 Mineral Determination

The ash was cooled after determination. Then 15 ml of 6 N HCl was added to samples in crucibles before transferring to 100 ml volumetric flasks. Distilled water was used to top up to the mark, before mineral analysis (AOAC, 1995). Atomic Absorption Spectroscopy (AAS) (Model AA-6200, Shimadzu, Corp., Kyoto, Japan) was used for all the minerals.

3.7 Antinutrients

3.7.1 Estimation of phytates

This was done by HPLC combining the column/mobile phase conditions established by Tanjendjaja *et al.,* (1980), with modification as detailed by Camire and Clydesdale (1982). A known amount of the milled sample (0.05 g) was weighed into a 125 ml Erlenmeyer flask, extracted with 25 ml of 3% sulphuric acid for 30 min on a shaker bath at medium speed and at room temperature. The slurry was filtered through fast filter paper (Whatman No. 41) and rinsed using a fine jet stream from a squeeze bottle, with a

small volume of extracting solvent. The filtrate was transferred to a 50 ml screw cap centrifuge tube and placed in a boiling water bath (BWB) for 25 minutes (to aid in the precipitation of ferric phytate), before addition of 3 ml of a ferrous chloride solution containing 6 mg ferric iron per ml in 3% sulphuric acid. The tubes were heated in a BWB for 45 minutes to allow for complete precipitation of the ferric phytate complex. This was centrifuged at 2,500 revolutions per minute (rpm) for 10 minutes and supernatant discarded, while the precipitate was washed once with 30 ml of distilled water, centrifuged again and supernatant discarded. Afterwards, 3 ml of 1.5 N NaOH and 1 ml of distilled water were added to the precipitate. A glass rod was used to break the precipitate and then sonication was done to completely disperse the precipitate, which was then topped to 30 ml. The samples were cooled, centrifuged (Centrifuge model: KOKU SAN-H-200C) and the supernatant quantitatively transferred to 50 ml volumetric flask. The precipitate was rinsed once with approximately 10 ml of distilled water, centrifuged and the supernatant added to the volumetric flask.

Preparation of standard curve: A stock solution containing 10 mg/ml of sodium phytate in distilled water was prepared. Serial dilutions were made to contain from 1 g/100 ml to 100 mg/100 ml. The sample and standard dilutions were injected to the HPLC using a 20-µl sample loop.

Instrumentation: Analysis was performed using a HPLC Model LC-10AS, Shimadzu Corp., Kyoto, Japan equipped with a UV detector at 205-340 nm filter, 250 mm X 4.6 mm ID column containing spherisorb ODS C18 10 µ packing and oven temperature at 35 ˚C. 0.005 M sodium acetate in distilled water mobile phase was used at a flow rate of 0.5 ml/minute and injection volume 20 µl. Shimadzu software was used to calculate the peak areas.

$$Phytate\ content\ (mg\ per\ g) = \left(\frac{y}{b}\right) \times \left(\frac{dilution\ factor}{weight\ of\ sample}\right) \quad (8)$$

y = peak area b = concentration

3.7.2 Tannin Determination

This was done using the Vanillin Hydrochloric acid method (Price *et al.,* 1978) with modifications according to Ochanda *et al.,* (2010). Approximately 0.25 g of ground sample was weighed into an Erlenmeyer flask. 10 ml of 4% HCl in methanol was pippeted into each of the flasks and the flasks sealed with parafilm. The flasks were then gently shaken for 20 min on a shaker (KS 250 basic, Germany) and the resulting extracts centrifuged (Centrifuge model: KOKU SAN-H-200C) for 10 min at 4500 rpm. The supernatant aliquots were transferred to 25 ml volumetric flasks. Consequently, the second extraction was done by adding 5 ml of 1% HCl in methanol to form the first extraction and repeating the extraction process. The aliquots of the first and second extracts were combined and made up to 25 ml volume. Approximately 1 ml of each respective standard and sample extract was pipetted into test tubes 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 standard solutions, sample extracts and blanks was read in a UV-VIS spectrophotometer (Shimadzu, UV mini 1240, Japan) at 500 nm 20 min after adding vanillin HCL reagent to the samples and standards. A standard curve was then prepared from the readings of the catechin standard solutions. The blank absorbance was subtracted from the samples absorbance and the corrected absorbance substituted into the regression equation (y = a + bx) in order to calculate the concentration of the sample extracts. The concentration in µg per ml was converted into mg catechin per ml. the per cent catechin equivalents (% CE) was calculated as follows:

$$\% CE = 100\left(\frac{CC \times VM}{VE \times Wt}\right) \quad (9)$$

Where

CC= catechin concentration (mg/ml)

VM= Volume made up (25ml)

VE- Volume of extract (1ml)

Wt= weight of sample (0.025g)

3.8 In Vitro Protein Digestibility

In vitro protein digestibility was determined as described by Hsu *et al.,* (1977) using a multi enzymatic solution containing 1.6 mg trypsin (Type IX Sigma T-303 with 13,000-20,000 BAEE units/mg protein), 3.1 mg chymotrypsin (Type II Sigma C 4129 with≥ units/mg powder) and 1.3 mg peptidase (III grade Sigma P-7,500 with 50-100 units/g powder) per millitre. Changes in pH were measured with a potentiometer after 10 min. Apparent in vitro digestibility (Y) was measured with

$$Y = 210.464 – 18.103X$$

Where, X= protein suspension pH immediately after digestion with multi- enzymatic solution for 10 min.

3.9 Experimental flow chart

The figure below summarizes the steps followed in the project.

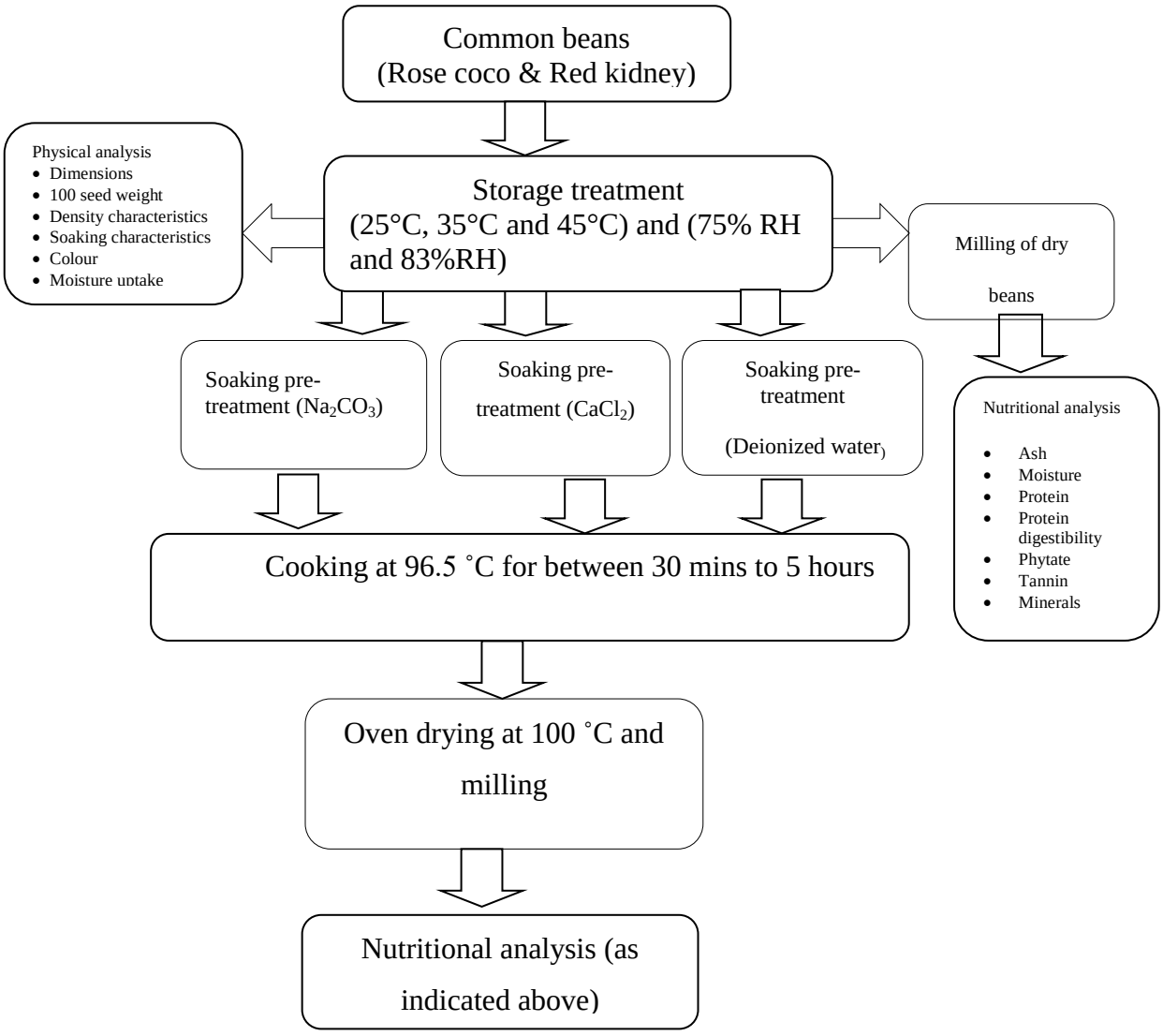

Figure 3.1 Experimental Flow Chart

3.10 Data management and analysis

Each analytical process was carried out in three independent replications. The data obtained was subjected to Analysis of Variance (ANOVA) and mean comparisons for

treatments were made using Duncan's Multiple Range Tests using Genstat (14^{th} edition 2012). Significant difference was accepted at $P<0.05$. (Steel and Torrie, 1980)

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of storage conditions on physical properties

The physical properties of the beans samples are presented in Table 4.1 to 4.4.

Table 4.1 illustrates the dimension characteristics of Rose coco. It had a length that ranged from 14.8 mm to 15.4 mm and a width of 7.3 mm to 7.7 mm. There were no significant ($p < 0.05$) differences noted on the length and width with storage for all conditions. The 100 seed weight ranged between 45.4 g to 50.4 g and there were no significant ($p < 0.05$) differences across the storage conditions over the six month period.

Table 4.2 outlines the physical properties of Red kidney. This bean had 100 seed weights of 30.4 – 31.7 g which was not significantly ($p < 0.05$) different across the storage conditions over the six month period. The length range was of 14.0 mm to 13.3 mm and a width of 6.8 mm to 7.5 mm. There were no significant ($p < 0.05$) differences noted on the length and width with storage for all conditions.

These results are in agreement with findings of Martin-Cabrejas (1997) who found a range of 20 g to 50 g for similar bean types.

Table 4.1 Effect of storage conditions on dimension and weight characteristics of Rose coco

Bean Type	Storage condition (˚C/%RH)	Storage time (months)	Length (millimeters)	Width (millimeters)	100 Seed weight (grams)
Rose coco	25/75	0	$15.4^{a} \pm 0.10$	$7.7^{a} \pm 0.50$	$48.9^{ab} \pm 0.60$
	35/83	2	$15.1^{a} \pm 0.30$	$7.6^{a} \pm 0.50$	$48.9^{ab} \pm 0.50$
	35/83	4	$15.2^{a} \pm 0.10$	$7.5^{a} \pm 0.20$	$47.6^{b} \pm 0.60$
	35/83	6	$15.1^{a} \pm 0.10$	$7.5^{a} \pm 0.20$	$45.4^{a} \pm 1.20$
	25/75	0	$15.4^{a} \pm 0.10$	$7.7^{a} \pm 0.50$	$48.9^{ab} \pm 0.60$
	45/75	2	$15.3^{a} \pm 0.50$	$7.6^{a} \pm 0.20$	$49.0^{ab} \pm 1.60$
	45/75	4	$15.5^{a} \pm 0.50$	$7.4^{a} \pm 0.30$	$47.9^{b} \pm 0.40$
	45/75	6	$14.8^{a} \pm 0.40$	$7.3^{a} \pm 0.30$	$47.1^{b} \pm 1.30$

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Table 4.2 Effect of storage conditions on dimension and weight characteristics of Red kidney

Bean Type	Storage condition (˚ C/%RH)	Storage time(months)	Length (millimeters)	Width (millimeters)	100 Seed weight (grams)
Red kidney	25/75	0	$13.7^{a} \pm 1.50$	$6.8^{a} \pm 0.70$	$31.3^{a} \pm 0.40$
	35/83	2	$13.7^{a} \pm 0.90$	$6.9^{a} \pm 0.50$	$31.2^{a} \pm 0.90$
	35/83	4	$14.1^{a} \pm 0.50$	$7.5^{a} \pm 0.60$	$31.2^{a} \pm 0.70$
	35/83	6	$13.9^{a} \pm 0.40$	$7.0^{a} \pm 0.40$	$30.4^{a} \pm 0.90$
	25/75	0	$13.7^{a} \pm 1.50$	$6.8^{a} \pm 0.70$	$31.3^{a} \pm 0.40$
	45/75	2	$14.0^{a} \pm 1.10$	$6.9^{a} \pm 0.50$	$31.7^{a} \pm 1.40$
	45/75	4	$14.0^{a} \pm 1.20$	$7.5^{a} \pm 0.60$	$30.5^{a} \pm 0.70$
	45/75	6	$13.3^{ab} \pm 0.20$	$7.0^{a} \pm 0.40$	$30.8^{a} \pm 0.80$

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Density characteristics which included true seed density, bulk seed density and porosity parameters are presented in Table 4.3 and 4.4.

Table 4.3 illustrates the density characteristics of Rose coco. There was no significant ($p > 0.05$) difference in the bulk density of Rose coco with a range of 0.77 g/cm^3 to 0.8 g/cm^3 after storage at 45˚ C/75% RH. No significant ($p > 0.05$) differences were noted for Rose coco true seed density with a range of 1.22 g/cm^3 to 1.25 g/cm^3. These values were lower in comparison to values of 1.28 g/cm^3 to 1.3 g/cm^3 for Rose coco reported by Martin-Cabrejas (1997). Rose coco porosity parameters had no significant ($p < 0.05$) difference with a range of 36.4% to 37.4%

Table 4.4 illustrates the density characteristics of Red kidney. The Red kidney bulk seed density had no significant ($p > 0.05$) difference with a range of 0.76 g/cm^3 to 0.82 g/cm^3. Similarly, true seed density had no significant ($p > 0.05$) difference with a range of 1.25 g/cm^3 to 1.28 g/cm^3. Red kidney porosity had no significant ($p > 0.05$) difference with a range of 33.1% to 36.9 %.These values were comparable to those of Martin-Cabrejas (1997) for Red kidney.

Table 4.3 Effect of storage conditions on density characteristics of Rose coco

Bean Type	Storage condition (˚ C/%RH)	Storage time(months)	True seed density (g/cm^3)	Bulk seed density (g/cm^3)	Porosity (%)
Rose coco	25/75	0	1.23^a±0.1	0.77^a±0.1	37.4^a±0.8
	35/83	2	1.22^a±0.1	0.77^a±0.1	35.6^a±1.3
	35/83	4	1.23^a±0.2	0.78^a±0.2	37.2^a±0.8
	35/83	6	1.22^a±0.1	0.78^a± 0.1	36.3^a±1.8
	25/75	0	1.23^a±0.1	0.77^a±0.1	37.4^a±0.7
	45/75	2	1.25^a±0.1	0.77^a±0.1	35.5^a±1.4
	45/75	4	1.24^a±0.1	0.81^b±0.1	37.4^a±0.7
	45/75	6	1.23^a±0.2	0.81^b±0.1	36.4^a±1.7

Table 4.4 Effect of storage conditions on density characteristics of Red kidney

Bean Type	Storage condition (˚ C/%RH)	Storage time(months)	True seed density (g/cm^3)	Bulk seed density (g/cm^3)	Porosity (%)
Red kidney	25/75	0	1.25^a± 0.2	0.82^a± 0.1	34.4^a± 0.7
	35/83	2	1.25^a±0.1	0.80^a± 0.3	36.1^a± 1.4
	35/83	4	1.28^a± 0.3	0.76^a± 0.1	35.5^a±1.8
	35/83	6	1.23^a± 0.1	0.79^a± 0.2	33.1^a±0.9
	25/75	0	1.25^a± 0.2	0.82^a± 0.1	34.4^a± 0.7
	45/75	2	1.28^a± 0.3	0.82^a± 0.3	36.9^a± 1.2
	45/75	4	1.26^a±0.2	0.82^a± 0.4	36.9^a± 1.2
	45/75	6	1.27^a± 0.2	0.82^a± 0.1	36.9^a± 1.2

Means within the same column with different superscripts were significantly ($P<0.05$) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Table 4.5 and 4.6 illustrate the soaking characteristics of Rose coco and Red kidney respectively.

Table 4.5 Effect of storage conditions on soaking characteristics of Rose coco

Bean Type	Storage condition (˚C/%RH)	Storage time(months)	Hydration coefficient (%)	Swelling coefficient (%)	Conductivity (mmh/cm)	Leached solutes (%)
Rose coco	25/75	0	211.3^{f}±0.9	267.8^{g}±13.5	1.8^{a}±0.1	0.03^{a}±0.01
	35/83	2	211.8^{g}±0.8	256.7^{e} ±0.6	2.9^{c}±0.3	0.09^{d}±0.01
	35/83	4	197.0^{d}±1.7	234.4^{c}±4.4	4.2^{e}±0.3	0.1^{f}±0.09
	35/83	6	189.4^{a}± 0.2	229.9^{b}±10.8	4.1^{d}±0.5	0.1^{e}±0.01
	25/75	0	211.3^{f}±0.9	267.8^{g}±13.5	1.8^{a}±0.1	0.03^{a}±0.01
	45/75	2	203.7^{e}± 2.7	259.4^{f} ±4.4	2.6^{b}±0.4	0.06^{c}±0.01
	45/75	4	196.6^{c}± 1.1	252.2^{d}±12.9	3.4^{c}± 0.2	0.09^{d}±0.01
	45/75	6	191.7^{b}± 1.4	203.7^{a}±12.8	5.5^{f}± 0.8	0.05^{b}±0.02

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Table 4.6 Effect of storage conditions on soaking characteristics of Red kidney

Bean Type	Storage condition (˚C/%RH)	Storage time(months)	Hydration coefficient (%)	Swelling coefficient (%)	Conductivity (mmh/cm)	Leached solutes (%)
Red kidney	25/75	0	205.6^{a}± 0.7	266.7^{g}±14.4	2.7^{a}±0.4	0.05^{a}±0.01
	35/83	2	195.8^{c}± 0.9	246.7^{f}± 5.8	5.3^{d}±0.2	0.1^{d}±0.04
	35/83	4	191.7^{d}±7.2	222.5^{b}± 3.5	5.6^{e}±0.5	0.05^{a}±0.04
	35/83	6	188.3^{f}±1.03	219.0^{a}±1.4	6.8^{f}±0.8	0.1^{d}±0.02
	25/75	0	205.6^{a}± 0.7	266.7^{g}±14.4	2.7^{a}±0.4	0.05^{a}±0.01
	45/75	2	201.9^{b}± 6.1	242.4^{e}±16.5	4.1^{b}±0.5	0.09^{c}±0.04
	45/75	4	190.2^{e}± 0.4	225.0^{d} ±7.1	5.1^{c}±0.4	0.1^{d}±0.01
	45/75	6	184.9^{g}± 3.2	224.4^{c}±13.5	7.5^{g}±0.7	0.07^{b}±0.05

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

In hydration, swelling coefficients, electrical conductivity and leached solutes considerable differences ($p < 0.05$) were observed. Both Rose coco and Red kidney showed a decreasing trend for hydration and swelling coefficients and an increasing

trend for conductivity and leached solutes parameters as storage time increased. Rose coco hydration coefficient ranged between189.4% and 211.2% while Red kidney range was 184.9% to 205.6%. The swelling coefficient was from 203.7% to 267.8% for Rose coco and 266.7% to 219% for Red kidney.

Both Rose coco and Red kidney showed a decreasing trend for hydration and swelling coefficients and an increasing trend for conductivity and leached solutes parameters. Significant differences ($p < 0.05$) were observed in these parameters. Rose coco hydration coefficient had a range of 189.4% to 211.2% while Red kidney had a range of 184.9% to 205.6%. Swelling coefficient for Rose coco ranged from 203.7% to 267.8% while Red kidney ranged from 219% to 266.7%

Hydration and swelling coefficients reflect the capacity to imbibe water in a reasonable amount of time (Abbas, 2008). For both bean varieties, hydration and swelling coefficients decreased with increased storage time. After 16 h soaking the hydration coefficients were lower in samples stored under accelerated storage conditions of temperature and humidity compared to fresh beans at 0 month (control). Similarly, the swelling coefficient reduced as it mainly depends on the amount of water absorbed (Abbas, 2008).

Conductivity values for Rose coco ranged from 1.8 mohm/ cm to 6.8 mohm/ cm while those of Red kidney ranged from 2.7 mohm/ cm to7.5 mohm/ cm. Leached solutes values for Rose coco ranged from 0.03% to 0.1% while Red kidney ranged from 0.05% to 0.1%. Therefore, electrical conductivity and amount of leached solutes increased with increased storage temperature and humidity for both bean varieties. After 16 h soaking the loss of solids and electrolytes from beans at accelerated storage conditions of temperature (35 ˚C and 45 ˚C) and relative humidity (75% and 83% RH) was high as compared to that of the control samples. This was an indication of develpoment of the HTC defect with storage. Evidently, a link between hydration and swelling coefficients can be drawn from this observation. The reduced hydration coefficient was partially due to solutes leached from the cotyledons during water

imbibition (Abbas, 2008) and this was greater in beans stored for 6 months which were harder. Jones and Boulter (1983) stated that leached solids may affect hydration rate of beans in two ways. On the one hand, the leached solids in the soaking water may increase the concentration of the solution which in turn affects water absorption rate. On the other hand, solute leakage may reduce water affinity and water holding capacity as is stipulated by osmotic principles.

Table 4.7 and 4.8 illustrate the colour characteristics for Rose coco and Red kidney respectively. The colour readings were determined in terms of Hunter -L values (whiteness), Hunter-avalues (redness) and Hunter-b values (yellowness). Rose coco Hunter-L values ranged from 46.9 to 27.9 while the range for Red kidney was 43.8 to 23.4. Hunter-a values for Rose coco ranged from 11.8 to 17 while Red kidney range was 4.9 to 13.3. Hunter-b values for Rose coco ranged from 4.6 to 13.4 while the Red kidney range was 1.8 to 4.3. Hunter- L values for both varieties decreased significantly ($p < 0.05$) indicating loss of colour lightness and darkening which is associated with hardening of the bean (Reyes- Moreno *et al.,* 2000). Hunter- a and b values increased significantly ($p < 0.05$) indicating an increase in redness and yellowness respectively. This was in agreement with the findings of Uebersax and Bedford (1980) who observed that Hunter L values decreased with increased relative humidity, storage time and temperature. Similarly, Hellevang and Henson (2000) found that Hunter-a and b values increased with increased relative humidity, temperature and storage time. There is a close relationship between cooking quality and grain darkening, thus the colour changes of stored beans could be used as an indicator of the HTC defect development (Reyes-Moreno *et al.,* 2000). Consumers tend to associate colour with storage time due to the fact that it correlates well with physical, chemical and sensory evaluations (Pedreschi *et al.,* 2006). In further agreement with this is the fact that shade, uniformity and degree of lightness of colour are important indicators of bean quality (Yousif, 2007). Generally, the colour changes reported correspond to non-enzymatic darkening and may be related to polymerization reactions of phenolic compounds of low molecular weight to tannins of high molecular weight (Reyes-Moreno *et al.,* 2001).

Table 4.7 Effect of storage conditions on colour characteristics of Rose coco

Bean Type	Storage condition (˚ C/%RH)	Storage time(months)	L	a	b
Rose coco	25/75	0	$46.9^{d} \pm 2.4$	$11.8^{a} \pm 0.5$	$4.6^{a} \pm 1.1$
	35/83	2	$48.2^{d} \pm 4.6$	$10.4^{a} \pm 2.4$	$4.2^{a} \pm 1.7$
	35/83	4	$43.1^{bcd} \pm 2.7$	$10.7^{a} \pm 0.8$	$3.6^{a} \pm 0.6$
	35/83	6	$37.4^{b} \pm 1.9$	$17.0^{b} \pm 2.4$	$13.4^{b} \pm 2.9$
	25/75	0	$46.9^{d} \pm 2.4$	$11.8^{a} \pm 0.5$	$4.6^{a} \pm 1.1$
	45/75	2	$45.9^{cd} \pm 2.4$	$9.1^{a} \pm 0.3$	$3.9^{a} \pm 0.4$
	45/75	4	$40.4^{bc} \pm 2.7$	$11.2^{a} \pm 1.3$	$3.8^{a} \pm 2.5$
	45/75	6	$27.9^{a} \pm 5.4$	$15.7^{b} \pm 1.0$	$12.3^{b} \pm 0.5$

Table 4.8 Effect of storage conditions on colour characteristics of Red kidney

Bean Type	Storage condition (˚ C/%RH)	Storage time(months)	L	a	B
Red kidney	25/75	0	$43.8^{b} \pm 0.6$	$4.9^{a} \pm 0.6$	$1.8^{b} \pm 0.3$
	35/83	2	$44.1^{b} \pm 0.4$	$4.7^{a} \pm 0.7$	$1.2^{ab} \pm 0.1$
	35/83	4	$40.2^{b} \pm 2.6$	$5.3^{a} \pm 1.4$	$1.1^{ab} \pm 0.5$
	35/83	6	$23.4^{a} \pm 3.8$	$8.3^{b} \pm 0.7$	$4.3^{c} \pm 1.5$
	25/75	0	$43.8^{b} \pm 0.6$	$4.9^{a} \pm 0.6$	$1.8^{b} \pm 0.3$
	45/75	2	$41.4^{b} \pm 1.7$	$3.9^{a} \pm 0.3$	$0.3^{a} \pm 0.2$
	45/75	4	$41.8^{b} \pm 3.7$	$5.8^{a} \pm 0.5$	$0.8^{ab} \pm 0.7$
	45/75	6	$23.9^{a} \pm 1.3$	$13.3^{c} \pm 2.2$	$4.2^{c} \pm 1.6$

Means within the same column with different superscripts were significantly ($P<0.05$) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

4.2 Effect of storage conditions on cooking properties

4.2.1 Effect of storage time on cooking properties

The control beans (0 month storage time) had shorter cooking times than beans stored for

2, 4 and 6 months at accelerated storage conditions. The cooking profiles for beans 45 ˚C/75% RH (Figure 4.1) and 35 ˚C/83% RH (Figure 4.2) shows an increase in cooking time along the six month storage period in both bean varieties in comparison to cooking time at the start of the experiment. The cooking profiles for beans stored at 45 ˚C/75% RH (Figure 4.1) and 35 ˚C/83% RH (Figure 4.2) shows an increase in cooking time with storage period for both varieties. Specifically, for Rose coco beans stored at 45 ˚C/75% RH the cooking times were 120 min at 0 month, 150 min at 2 months, 180 min at 4 months and 240 min at 6 months. This translated into 100% increase in cooking time after 6 months of storage. Similarly, there was a 100% increase in cooking time for Red kidney beans stored at 45 ˚C/75%. For Rose coco bean stored at 35 ˚C/83% RH, the cooking times were 120 min at 0 month, 150 min at 2 months, 180 min at 4 months and 210 min at 6 months. This was a 75% increase in cooking time. For Red kidney beans stored at 35 ˚C/83% RH the cooking times were 150, 180, 210 and 240 after storage for 0, 2, 4 and 6 months respectively. This was a 60% increase in cooking time. This is an indication that increased storage time under the accelerated storage conditions (45˚C/75% RH and 35˚C/83% RH) plays a vital role in the hardening process of beans as is depicted by increase in cooking time. This is in line with Berrios *et al.,* (1999), Hentges *et al,* (1991), Takayama *et al.*, (1964) and Burr and Morris (1968) and findings.

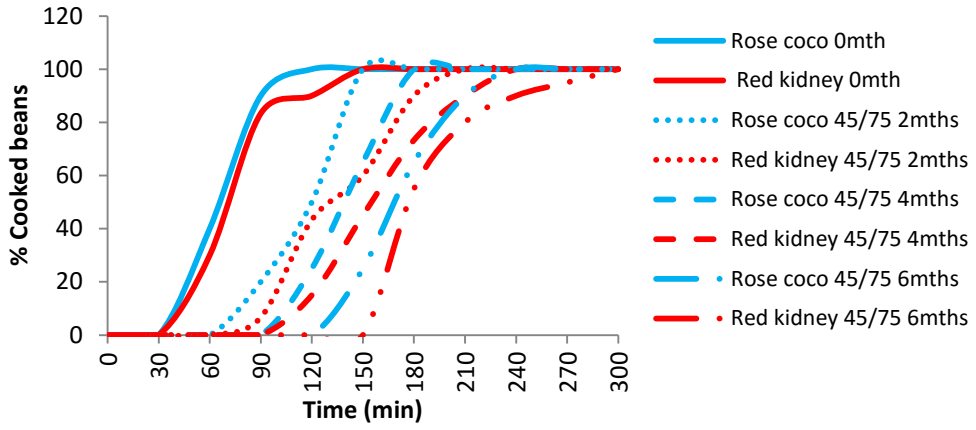

Figure 4.1 Cooking profiles for Rose coco and Red kidney beans stored at 45 ˚C / 75% RH for 0, 2, 4 and 6 months

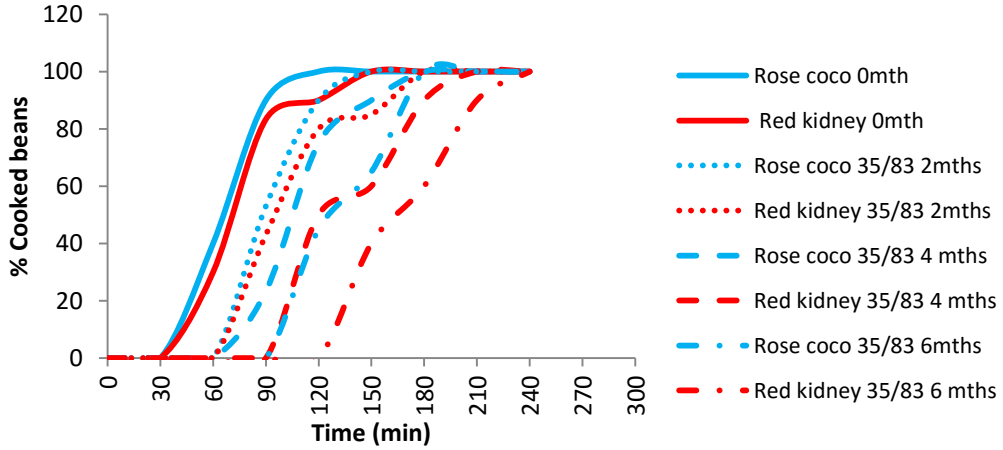

Figure 4.2 Cooking profiles for Rose coco and Red kidney beans stored at 35˚C / 83% RH for 0, 2, 4 and 6 months.

4.2.2 Effect of Relative Humidity on cooking quality

Storage of common beans in high relative humidity conditions (75% and 83%) led to development of the HTC defect (Figure 4.3). The cooking time for Rose coco beans stored at 75% RH was 150 min while for beans stored at 83% was 180 min. This translates to a 20% increase in cooking time at high RH. For the Red kidney beans, the cooking times were 210 and 240 min for seeds stored at 75%RH and 83% RH,

respectively This corresponded to a 14% increase in cooking at high RH (83%) .. This is in agreement with Kilmer *et al.*, (1994) who found that, high humidity (> 75%) results in the HTC condition. In addition, research by Ndung'u *et al.*, (2012) found that even a relative humidity of (60% - 80%) can lead to development of the HTC defect.

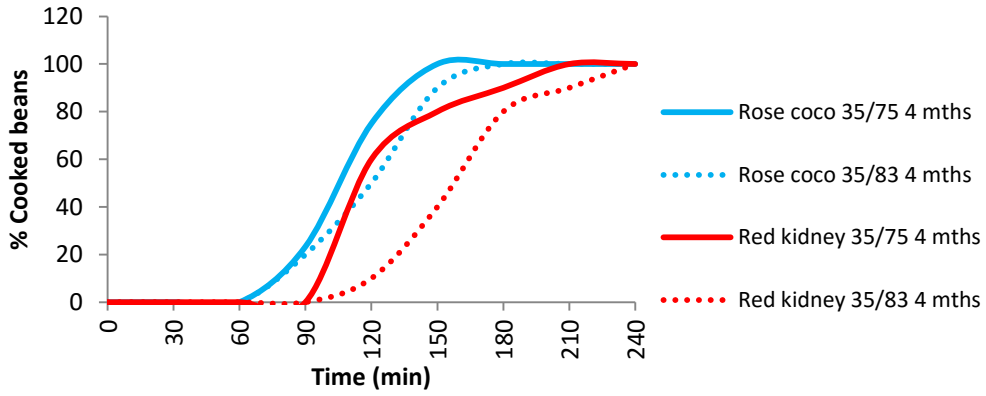

Figure 4.3 Cooking profile for Rose coco and Red kidney beans stored at 35˚C/83% RH and 35˚C/75% RH for 4 months.

4.2.3 Effect of temperature on cooking quality

Increase in storage temperature resulted to increased cooking time (Figure 4.4). The increase in cooking time indicates that the beans developed the HTC defect. Storage of beans for 6 months at 45 °C increased the cooking times by 40 % for Rose coco and 33 % for Red kidney, compared to the cooking times for beans stored at 25 °C. High temperature during storage result to deteriorative effects on common bean seed quality. Nasar-Abbas *et al.*, 2008 states that the main form of deterioration is increased hardness of cotyledons or loss of ability to soften with cooking followed by deterioration of colour, texture and loss of nutritive value. In addition, changes associated with HTC phenomenon are accelerated under high storage temperature and mainly lead to reduced hydration and swelling coefficients which in turn reduce cookability of seeds (Nasar-Abbas *et al.*, 2008).

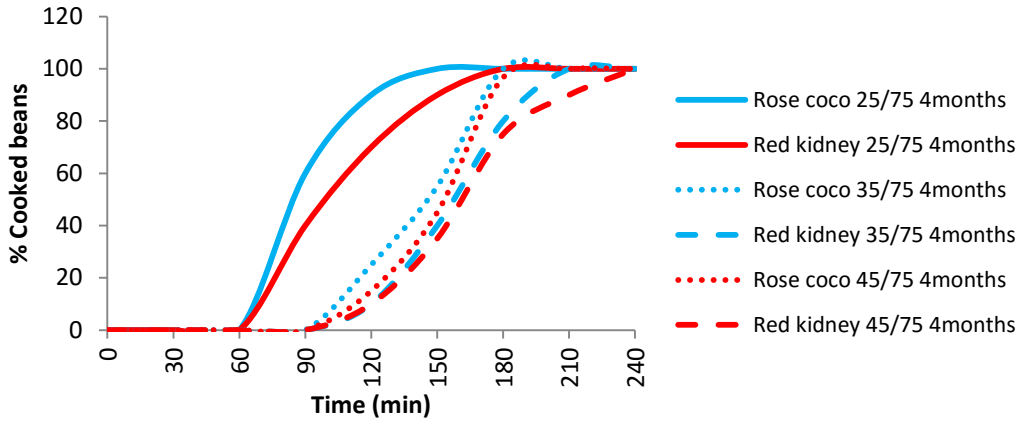

Figure 4.4 Cooking profile for Rose coco and Red kidney beans stored at 35 ˚C and 45 ˚C and constant relative humidity of 75% stored for 4 months

4.2.4 Effect of soaking pretreatments on cooking time

In the experiment, the soaking pre-treatment solutions used were de-ionized water, Na_2CO_3 and $CaCl_2$.

Figure 4.5 represents the cooking profiles for Rose coco and Red kidney dry beans not subjected to accelerated storage conditions under the three soaking pre-treatments and also beans not subjected to any soaking pre-treatment. Soaking in a $CaCl_2$ solution resulted in beans that did not cook within the experimental set time of five hours. Beans that had not been soaked prior to cooking took a longer time to cook than beans soaked in either de-ionized water or Na_2CO_3. Specifically, Rose coco cooked after 180 min while Red kidney took 210 min to cook with no soaking pre-treatment. However, soaking in de-ionized water resulted in Rose coco cooking after 120 min and Red kidney after 150 min. This was a 33.3% decrease in cooking time for Rose coco and 28.6 % reduction for Red kidney. Furthermore, beans soaked in a Na_2CO_3 solution cooked after 60 and 90 min for Rose coco and Red kidney, respectively. This was a 66.7% decrease in cooking time for Rose coco and 55.1 % reduction for Red kidney.

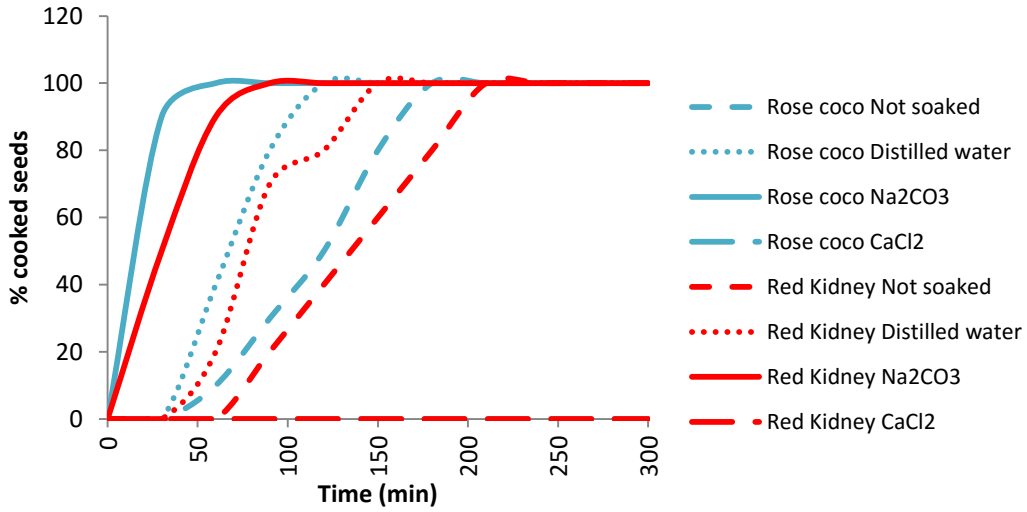

Figure 4.5 Effect of soaking pre-treatments on the cooking time of Rose coco and Red kidney at the control conditions of 25˚C/75% RH.

Figure 4.6 and 4.7 represents the cooking profiles of Rose coco and Red kidney stored at 35 ˚C /83% RH and 45˚C/75% RH, respectively, for six months.

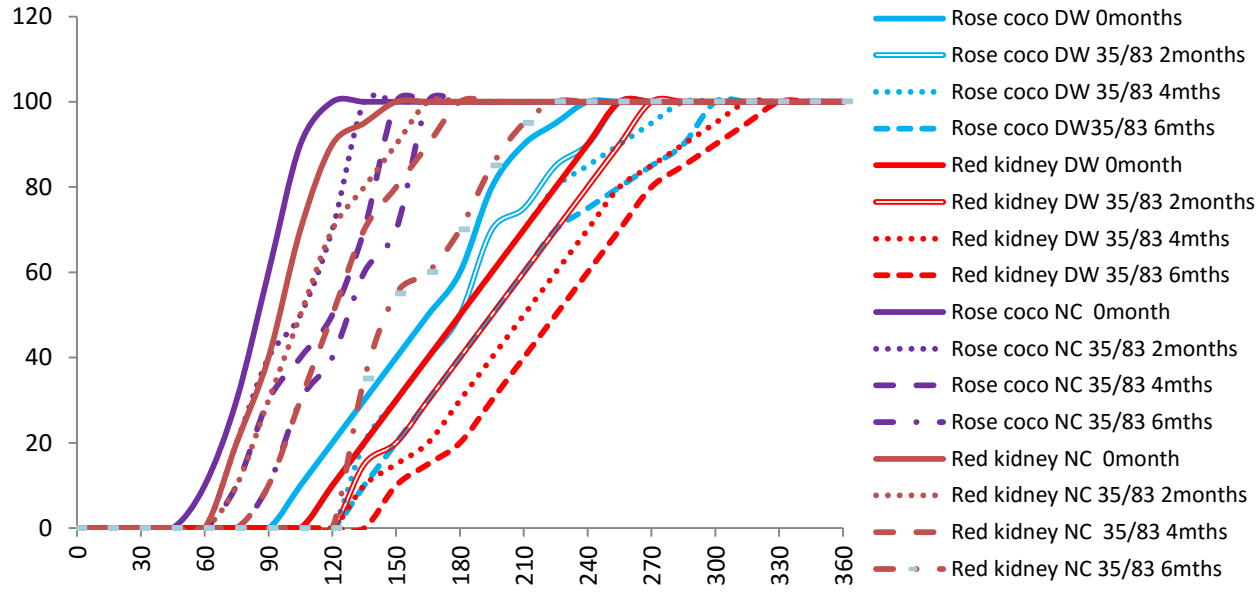

Figure 4.6 Effect of soaking pretreatments on Rose coco and Red kidney beans stored at 35 ˚C/83% RH

The most significant improvement in cooking time was that obtained when the beans had been soaked in Na_2CO_3 as compared to de-ionized water. Sodium carbonate had the most effect and de ionized water the least effect on softening the texture of the beans. This confirms the beneficial effect of Na_2CO_3 in decreasing the cooking time of both fresh and hardened beans.

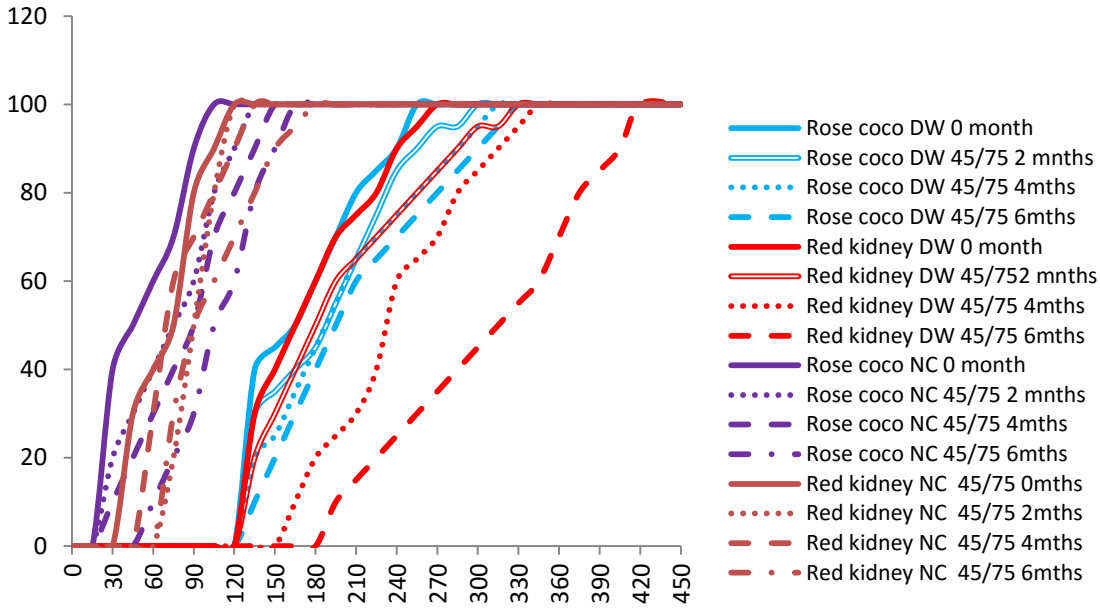

Figure 4.7 Effect of soaking pretreatments on Rose coco and Red kidney beans stored at 45 ˚C /75% RH

According to De Leon *et al.*, (1992), several mechanisms could be involved during the soaking and cooking process of the beans in the salt solution. Among this is ionic interaction whereby Na^+ tends to migrate into the bean and the Mg^{2+} and K^+ tend to leave the bean. With regard to improved cooking time, Coyoy Gonzalez (1987) states that the saline solutions improve heat transfer properties from the beans to its surroundings (diffusivity and thermal conductivity). De Leon *et al.*, (1992) gives evidence that sodium carbonate increase water absorption capacity and hence reduced cooking time. Additionally, the saline solutions

increase the water holding capacity of the beans (Garcia- Vela and Stanley, 1989).

Soaking in water is a common practice to soften texture and hasten the cooking process. The effect of the commonly used processing technique of soaking followed by cooking was able to determine the time it would take for the beans to cook. Cooking time is the most vital commercial quality characteristic of beans as consumers prefer bean varieties with shorter cooking times (Martinez- Manrique *et al.,* 2011). Prior grain soaking is directly related to cooking time which tends to decrease as beans remain immersed (Corrêa *et al.*, 2010).

4.2.5 Effect of storage conditions on moisture uptake

The moisture uptake curves for Rose coco and Red kidney beans stored at 45˚C/75% RH and that of 35 ˚C/83% RH are shown in Figure 4.8 and 4.9 respectively. Both bean varieties at 0 month showed faster moisture uptake as compared to subsequent moisture uptake after the 2, 4 and 6 months storage times. For Rose coco bean stored at 45°C/75% RH, the moisture uptake reduced from 118% at 0 month to 89% at 6 months while Red kidney bean in the same conditions had a reduction from 114% at 0 month to 75% at 6 months. For Rose coco bean stored at 35 ˚C / 83% RH, the moisture uptake reduced from 118% at 0 month to 99% at 6 months while Red kidney bean in the same conditions had a reduction from 114% at 0 month to 91% at 6 months. In both storage conditions, Rose coco beans had higher moisture uptake rates as compared to the Red kidney bean. At 0 month, the uptake differs only slightly but the differences become significant with increase in storage time. Burr *et al.* (1968), Jackson and Varriano-Marston (1981) also reported faster initial water absorption rate for fresh beans when compared to the initial water absorption rate for HTC beans. Generally, the faster initial water/moisture uptake and shorter cooking time exhibited by the beans at 0 month compared to the beans in the subsequent months may be due to micro structural differences of the stored bean (Berrios *et.al.,* 1999).

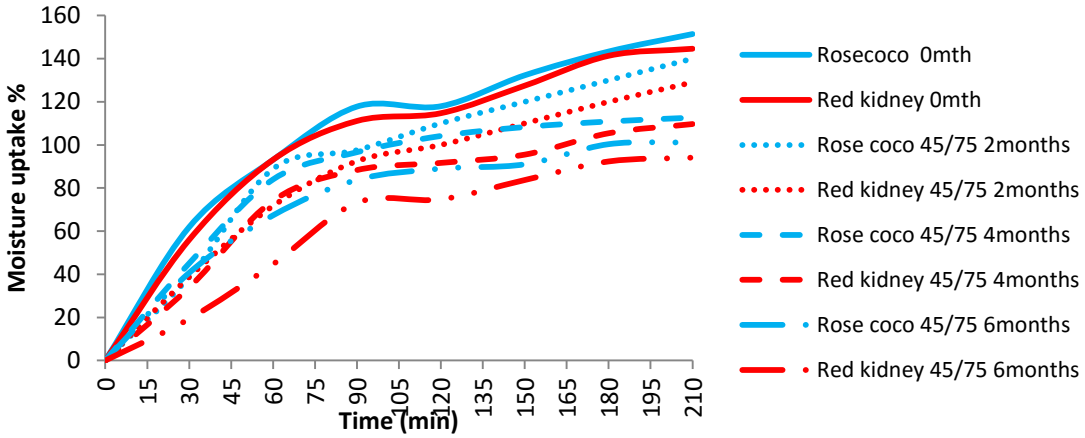

Figure 4.8 Moisture uptake curves for Rose coco and Red kidney beans stored at 45 ˚C/ 75 % RH for 0, 2, 4 and 6 months

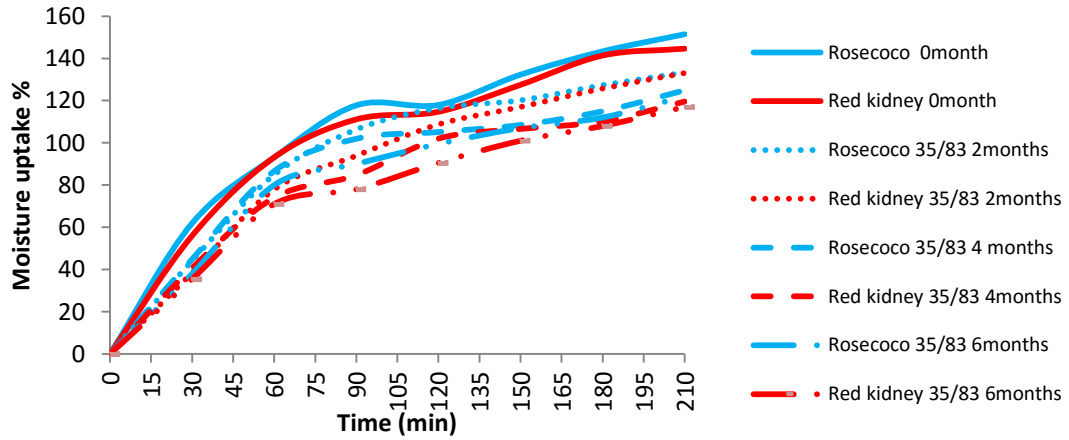

Figure 4.9 Moisture uptake curves for Rose coco and Red kidney beans stored at 35 ˚C/ 83 % RH for 0, 2, 4 and 6 months

4.3 Effect of storage conditions on proximate composition

All the results are on a dry weight basis.

4.3.1 Ash content

There was no significant ($p < 0.05$) variations in ash content for both Rose coco and Red kidney with storage for 6 months (Table 4.9 and 4.10). However, the general trend was a

gradual reduction in ash content with increased storage time. Rose coco ash content ranged from 4.0% to 2.6% while Red kidney had a range of 3.6% to 3.2%. Nevertheless, Rose coco beans had higher ash content in comparison to Red kidney.

4.3.2 Moisture content

There was a significant ($p < 0.05$) variation in moisture content for both Rose coco and Red kidney with storage (Table 4.9 and 4.10) for 6 months. The general trend was a reduction in moisture content with increase in storage time. Specifically, Rose coco had a moisture content range of 13.9% to 8.8% while Red kidney had a range of 12.8% to 8.4%.

4.3.3 Protein content

There was no significant ($p < 0.05$) change in protein content for both bean varieties with storage (Table 4.9 and 4.10). However, Rose coco had higher protein content with a range of to 19.2% to 19.3% while Red kidney had a range of to 14.1% to 14.3%. The protein content for both varieties is in disagreement with findings from some researchers who have found higher protein contents in common beans. Martin-Cabrejas (1997) who investigated protein content in Rose coco and Red kidney beans found a range of 20.2% to 23.3% for Rose coco and a range of 19.8% to 21.4% for Red kidney. However, Rose coco protein content is within the range reported by other researchers. Meiners *et al.*, (1976) investigated protein content in 3 lots of 10 kinds of mature common beans and reported a range of 17.8% to 26.4%. Deshpande *et al.,* (1984) studied 10 types of common beans and found a range of 18.1% to 23.4%. Koelher *et al.,* (1987) investigated 36 cultivars which represented 8 types of common beans and reported a range of 19.6% to 32.2%.

Table 4.9 Effect of storage conditions on proximate composition of Rose coco

Bean Type	Storage condition(˚ C/%RH)	Storage time (months)	Ash (%)	Moisture (%)	Protein content (%)
Rose coco	25/75	0	4.01^{b}±0.03	13.9^{e}± 0.3	19.3^{a} ± 0.01
	35/83	2	3.81^{b}±0.01	9.8^{cd} ± 0.20	19.2^{a} ± 0.03
	35/83	4	3.71^{b}±0.38	9.3^{b} ± 0.08	19.2^{a} ± 0.03
	35/83	6	2.96^{b}±0.54	8.8^{a} ± 0.30	19.2^{a} ± 0.01
	25/75	0	4.01^{b}±0.03	13.9^{e}± 0.30	19.3^{a} ± 0.01
	45/75	2	3.65^{b}±0.01	10.1^{d} ± 0.10	19.1^{a} ± 0.10
	45/75	4	3.1^{ab} ±0.10	9.4^{b}± 0.10	19.2^{a} ± 0.02
	45/75	6	2.6^{a} ± 1.84	9.6^{bc} ± 0.30	19.2^{a} ± 0.06

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Table 4.10 Effect of storage conditions on proximate composition of Red kidney

Bean Type	Storage condition (˚ C/%RH)	Storage time (months)	Ash (%)	Moisture (%)	Protein content (%)
Red kidney	25/75	0	3.56^{b} ±0.01	12.8^{c}± 0.98	14.1^{a} ± 0.22
	35/83	2	3.32^{ab}±0.16	9.56^{b}± 0.16	14.3^{a} ± 0.01
	35/83	4	3.47^{b} ±0.12	9.58^{b}± 0.41	14.2^{a} ± 0.02
	35/83	6	2.55^{a}±0.89	9.08^{ab}±0.42	14.3^{a} ± 0.01
	25/75	0	3.56^{b} ±0.01	12.8^{c}±0.98	14.1^{a}±0.22
	45/75	2	3.33^{ab}±0.17	8.74^{a} ±0.03	14.3^{a} ± 0.30
	45/75	4	3.21^{ab}±0.98	8.86^{ab}±0.11	14.4^{a} ± 0.04
	45/75	6	2.74^{a} ± 0.10	8.4^{a} ± 0.22	14.1^{a} ± 0.01

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

4.4 Effect of storage conditions on minerals

Mineral content results for Rose coco and Red kidney bean varieties are indicated in Table 4.11and 4.12 respectively. All the results are on a dry weight basis.

Mineral composition of the bean varies mainly due to genotypes, agricultural practices and environmental conditions (Mesquita *et al.*, 2007). This could therefore explain differences between earlier research results and current ones.

Table 4.11 shows the mineral composition of Rose coco. The iron content range was 5.8 to 6.7 mg/100g. In Table 4.12, the range for red kidney was 5.0 to 5.7 mg/100g. According to USDA (2012) the iron content for beans ranges between 5.0 and 8.2 mg/100g. Siddiq and Uebersax (2013) recently had similar results. However, previous studies have reported 10mg of iron per 100 g of beans (Amir *et al*,. 2007; Siddiq *et al.*,2010; Wang *et al.*, 2010).

The Mg content for Rose coco (Table 4.11) ranged from 172.5 to 178.5 mg/100g. While that for Red kidney (Table 4.12) ranged from 170.1 to 187.5 mg/100g According to USDA (2012), the Mg content in beans is in the range of 138 to 176 mg/ 100g. Thus the results of the current study are in agreement with this. However, El Maki *et al.,* (2007) reported levels of 289 to 312 mg/100g which was higher than the current study results.

Table 4.11 shows the Calcium content range for Rose coco as 123.4 to 143.6 mg/100g. Calcium content range for Red kidney in Table 4.12 is 105.5 to 118.7 mg/100g. According to USDA (2012), the Calcium content ranges from 83 to 147 mg/100g. The beans in this study were within this range. However, El Maki *et al.*, (2007) reported Calcium levels of 321 to 377 mg/100g in common beans which were much higher than results in the current study.

The Zinc content range for Rose coco (Table 4.11) is 5.1 to 6.8 mg/100g while that for Red kidney (Table 4.12) is 6.0 to 6.2 mg/100g.According to USDA (2012) the Zinc contents ranges from Zn 2.28 to 3.65 mg 100g. All the beans analyzed in this study were above this range. However, El maki *et al.*, (2007) reported Zn levels of 2.46 to 2.75 mg/100g in common beans. These values are lower than those in the current study.

Table 4.11 Effect of storage conditions on mineral content of Rose coco

Bean Type	Storage condition (˚C/%RH)	Storage time (months)	Iron(mg/100g)	Magnesium (mg/100g)	Calcium (mg/100g)	Zinc (mg/100g)
Rose coco	25/75	0	6.69^{a}±0.20	172.5^{a}±5.30	142.4^{a}±3.20	5.1^{a}±0.20
	35/83	2	6.29^{a}±0.30	178.5^{a}±6.50	123.4^{a}±7.30	6.6^{a}±0.60
	35/83	4	5.81^{a}±0.20	176.2^{a}±2.20	141.5^{a}±3.80	6.7^{a}±0.70
	35/83	6	6.28^{a}±0.20	174.1^{a}±4.00	143.2^{a}±4.20	5.9^{a}±0.40
	25/75	0	6.69^{a}±0.20	172.5^{a}±5.30	142.4^{a}±3.20	5.1^{a}±0.20
	45/75	2	6.22^{a}±1.40	174.3^{a}±3.10	141.3^{a}±2.10	6.5^{a}±0.50
	45/75	4	5.79^{a}±1.20	177.8^{a}±0.30	143.6^{a}±1.40	6.8^{a}±0.60
	45/75	6	6.19^{a}±0.10	167.2^{a}±4.10	140.2^{a}±1.00	5.8^{a}±0.40

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Table 4.12 Effect of storage conditions on mineral content of Red kidney

Bean Type	Storage condition (˚ C/%RH)	Storage time (months)	Iron (mg/100g)	Magnesium (mg/100g)	Calcium (mg/100g)	Zinc (mg/100g)
Red kidney	25/75	0	5.7^{ab}±0.90	187.5^{a}±2.20	106.5^{b}±1.00	6.2^{a}±0.10
	35/83	2	5.0^{b}±0.20	186.1^{a}±2.30	118.3^{ab}±8.20	6.3^{a}±0.20
	35/83	4	5.7^{a}±0.10	189.7^{a}±0.30	118.7^{a}±2.60	6.0^{ab}±1.90
	35/83	6	5.1^{a}±0.20	185.3^{a}±0.30	116.5^{ab}±8.60	5.7^{b}±0.10
	25/75	0	5.7^{ab}±0.90	187.5^{a}±2.20	106.5^{b}±1.00	6.2^{a}±0.10
	45/75	2	5.4^{a}±0.20	170.1^{a}±14.60	107.5^{ab}±0.60	6.0^{a}±0.30
	45/75	4	5.3^{a}±0.40	179.1^{a}±2.20	104.2^{a}±0.30	6.1^{a}±0.10
	45/75	6	5.2^{a}±0.10	181.0^{a}±7.50	105.5^{a}±0.20	6.2^{a}±0.10

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

4.5 Effect of storage conditions on antinutrients

4.5.1 Phytates

The phytate content decreased significantly ($p < 0.05$) as the storage time and temperature increased for both Rose coco and Red kidney. However for both varieties the decrease is not consistent and continuous during storage. This is indicated in table 4.13 and 4.14. This is in agreement with the work of Martin – Cabrejas (1997) who state that this may be due to the fact that phytic acid may only be important in the first stages

of the HTC defect. The works of Hentges *et al.,* 1991 and Mafuleka *et al.,* 1993 are also in agreement with these findings.

Rose coco flour from uncooked beans had the highest phytate content in comparison with the flour from beans subjected to soaking pre-treatments of de ionized water and sodium carbonate. Sodium carbonate (Na_2C0_3) soaking pre-treatment had the highest reduction in phytate content.

Rose coco raw bean under the storage conditions of 35˚C/83% RH had a phytate content range of 258.2 to 236.0 mg/ 100g while Rose coco bean cooked without any soaking pre-treatment had a range of 188.2 to 117.7 mg/ 100g. Rose coco bean soaked in deionized water had a range of 243.5 to 112.7 mg/ 100g while Rose coco soaked in sodium carbonate had a range of 316.9 to 98.o mg/ 100g. There was 60% reduction in phytate content when soaking pre-processing treatment of sodium carbonate and cooking was used in comparison to the raw beans.

Rose coco raw bean under the storage conditions of 45˚C/75% RH had a phytate content range of 258.2 to 236.0 mg/ 100g while Rose coco bean cooked without any soaking pre-treatment had a range of 188.2 to 92.9 mg/ 100g. Rose coco bean soaked in deionized water had a range of 243.5 to 122.2 mg/ 100g while Rose coco soaked in sodium carbonate had a range of 316.9 to 165.6 mg/ 100g. There was 30% reduction in phytate content when soaking pre-processing treatment of sodium carbonate and cooking was used in comparison to the raw beans.

Red kidney raw bean under the storage conditions of 35˚C/83% RH had a phytate content range of 263.2 to 102.0 mg/ 100g while Red kidney bean cooked without any soaking pre-treatment had a range of 166.9 to 167.9 mg/ 100g. Rose coco bean soaked in deionized water had a range of 213.4 to 133.4 mg/ 100g while Rose coco soaked in sodium carbonate had a range of 233.8 to 109.3 mg/ 100g. There was 61% reduction in phytate content when soaking pre-processing treatment of sodium carbonate and cooking was used in comparison to the raw beans.

Red kidney raw bean under the storage conditions of 45˚C/75% RH had a phytate content range of 263.2 to 242.7 mg/ 100g while Red kidney bean cooked without any soaking pre-treatment had a range of 166.9 to 130.7 mg/ 100g. Rose coco bean soaked in deionized water had a range of 213.4 to 98.9 mg/ 100g while Rose coco soaked in sodium carbonate had a range of 233.8 to 70.6 mg/ 100g. There was 73% reduction in phytate content when soaking pre-processing treatment of sodium carbonate and cooking was used in comparison to the raw beans.

The results indicate that cooking significantly reduces the phytate content and additional soaking pre-treatment further reduce the phytate content. This is in agreement with the works of Sattar *et al.,* (1989) and Vijayakumari *et al.,* (1998).

Table 4.13 Effect of storage conditions on phytate content of Rose coco

Bean Type	Storage condition (˚C/%RH)	Storage time (months)	Raw (mg/100g)	Cooked without soaking pretreatment (mg/100g)	Soaked in deionized water & cooked(mg/100g)	Soaked in sodium carbonate & cooked(mg/100g)
Rose coco	25/75	0	258.2^{b}±0.80	188.2^{e}±0.30	243.5^{g}± 0.70	316.9^{g}±0.50
	35/83	2	278.1^{f}±0.80	117.9^{c}±0.50	112.7^{a}±0.60	73.3^{a}± 0.70
	35/83	4	267.7^{d}±0.40	119.2^{d}±0.90	125.4^{c}±0.10	124.9^{d}±0.80
	35/83	6	236.0^{a}±0.60	117.7^{c}±0.70	138.5^{d} ±0.70	98.0^{c}± 0.90
	25/75	0	258.2^{b}±0.80	188.2^{e}±0.30	243.5^{g} ±0.70	316.9^{g}±0.50
	45/75	2	291.6^{g}±0.60	92.9^{a}± 0.40	195.8^{e}±0.60	145.3^{e}±0.80
	45/75	4	272.8^{e}±0.60	107.9^{b}±0.50	122.2^{b} ±0.90	95.8^{b} ± 0.90
	45/75	6	265.2^{c}±0.60	201.8^{f}±0.40	233.0^{f}± 0.60	165.6^{f}±1.30

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Table 4.14 Effect of storage conditions on phytate content of Red kidney

Bean Type	Storage condition (˚C/%RH)	Storage time (months)	Raw (mg/100g)	Cooked without soaking pretreatment (mg/100g)	Soaked in deionized water & cooked(mg/100g)	Soaked in sodium carbonate & cooked(mg/100g)
Red kidney	25/75	0	263.2^{e}±0.40	166.9^{c}±0.80	213.4^{e}±0.30	233.8^{g} ±0.80
	35/83	2	213.6^{b}±0.70	182.2^{d}±0.90	146.4^{c}±0.30	144.2^{d} ±0.30
	35/83	4	102.0^{a}±0.20	167.9^{c}±0.40	133.4^{b} ±0.10	130.3^{c}±0.20
	35/83	6	222.4^{c}±0.60	195.0^{f}±0.60	175.1^{d} ±0.40	109.3^{b}±0.10
	25/75	0	263.2^{e}±0.40	166.9^{c}±0.80	213.4^{e}±0.30	233.8^{g} ±0.80
	45/75	2	242.7^{d}±0.60	161.0^{b}±0.60	146.4^{c}±0.50	173.4^{f} ±0.10
	45/75	4	262.7^{e}±0.60	130.7^{a}±0.50	98.9^{a} ± 0.60	70.6^{a} ±0.30
	45/75	6	273.0^{e}±0.50	184.1^{e}±0.70	244.1^{f} ±0.80	168.9^{e} ±0.90

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Research done by Nyakuni (2008) found an inverse relationship between changes in cooking time and the phytic acid content of common beans. Phytic acid reduction has thus been related to the reduction in cooking quality and the development of the HTC defect in legumes (Bhatty, 1990). From this experiment, it was concluded that that the phytic acid losses could have been due to destruction by heat or losses in soaking and cooking water.

4.5.2 Tannins

In general, storage of beans under adverse storage conditions tended to significantly ($p < 0.05$) increase tannins over the six month storage period (Table 4.15 and 4.16).

Rose coco raw bean after six months of storage at 35˚ C/83% RH had tannin content range of 98 mg/100g to 120 mg/ 100g. While Rose coco bean cooked without any

soaking pre-treatment had a range of 40.5 to 63.5 mg/ 100g. Rose coco bean soaked in deionized water had a range of 87.5 to 89.9 mg/ 100g while Rose coco soaked in sodium carbonate had a range of 35.1 to 32.3 mg/ 100g. There was a 75% reduction in tannin content when soaking pre-processing treatment of sodium carbonate and cooking was used in comparison to the raw beans.

Rose coco raw bean after six months of storage at 45˚C/75% RH had a tannin content range of 98 to 120 mg/ 100g while Rose coco bean cooked without any soaking pre-treatment had a range of 40.5 to 50.4 mg/ 100g. Rose coco bean soaked in deionized water had a range of 87.5 to 69.3 mg/ 100g while Rose coco soaked in sodium carbonate had a range of 35.1 to 29.5 mg/ 100g. Similarly, there was a 75% reduction in tannin content when soaking pre-processing treatment of sodium carbonate and cooking was used in comparison to the raw beans.

Red kidney raw bean after six months of storage at 35˚C/83% RH had a tannin content range of 120-170 mg/ 100g while Red kidney bean cooked without any soaking pre-treatment had a range of 41.4 to 46.2 mg/ 100g. Rose coco bean soaked in deionized water had a range of 35.7 to 43.1 mg/ 100g while Rose coco soaked in sodium carbonate had a range of 23.4 to 22.2 mg/ 100g. There was 88% reduction in tannin content when soaking pre-processing treatment of sodium carbonate and cooking was used in comparison to the raw beans.

Red kidney raw bean under the storage conditions of 45˚C/75% RH had a tannin content range of 120 to 150 mg/ 100g while Red kidney bean cooked without any soaking pre-treatment had a range of 41.4 to 51.3 mg/ 100g. Rose coco bean soaked in deionized water had a range of 35.7 to 40.6 mg/ 100g while Rose coco soaked in sodium carbonate had a range of 23.4 to 28.2 mg/ 100g. There was 80% reduction in tannin content when soaking pre-processing treatment of sodium carbonate and cooking was used in comparison to the raw beans.

Reyes-Moreno *et al.,* (2000) had a study on chick peas that found that seed coat tannin content decreased significantly ($p < 0.05$) while cotyledon tannin content increased significantly ($p < 0.05$). Rodriguez and Mendoza (1990) reported similar behavior in common beans.

Table 4.15 Effect of storage conditions on tannin content of Rose coco

Bean Type	Storage condition (˚ C/%RH)	Storage time (months)	Raw (mg/ CE/100g)	Cooked without soaking pretreatment (mg/ CE/100g)	Soaked in deionized water & cooked(mg/ CE/100g)	Soaked in sodium carbonate & cooked(mg/ CE/100g)
Rose coco	25/75	0	98^{ab}±0.05	40.5^{b}±0.02	87.5^{f}±0.02	35.1^{e}±0.30
	35/83	2	97^{a}± 0.01	63.5^{g}±0.05	47.2^{a}±0.03	32.3^{d}±0.10
	35/83	4	99^{ab}±0.01	45.3^{c}±0.30	89.9^{g}±0.04	23.5^{a}±0.01
	35/83	6	120^{c}±0.03	61.4^{f}±0.02	65.4^{d}±0.10	29.8^{c}±0.06
	25/75	0	98^{ab}±0.05	40.5^{b}±0.02	87.5^{f}±0.02	35.1^{e}±0.30
	45/75	2	99^{ab}±0.05	47.3^{d}±0.02	69.3^{e}±0.04	29.5^{c}±1.40
	45/75	4	120^{b}±0.01	38.6^{a}±0.03	50.1^{b}±0.02	26.3^{b}±0.02
	45/75	6	120^{c}±0.08	50.4^{e}±0.07	53.1^{c}±0.01	25.9^{b}±0.03

Table 4.16 Effect of storage conditions on tannin content of Red kidney

Bean Type	Storage condition(˚ C/%RH)	Storage time (months)	Raw (mg/ CE/100g)	Cooked without soaking pretreatment (mg/ CE/100g)	Soaked in deionized water & cooked(mg/ CE/100g)	Soaked in sodium carbonate & cooked(mg/ CE/100g)
Red kidney	25/75	0	120^{a}±0.02	41.4^{e}±0.40	35.7^{b}±0.04	23.4^{d}±0.20
	35/83	2	140^{b}±0.03	34.6^{c}±0.20	40.9^{c}±0.02	22.2^{c}±0.20
	35/83	4	160^{d}±0.01	39.3^{d}±0.70	43.1^{d}±0.03	21.4^{b}±0.40
	35/83	6	170^{e}±0.02	46.2^{f}±0.80	42.7^{d}±0.04	19.9^{a}±0.01
	25/75	0	120^{a}±0.02	41.4^{e}±0.40	35.7^{b}±0.04	23.4^{d}±0.20
	45/75	2	140^{b}±0.01	33.4^{b}±0.40	24.1^{a}±0.02	28.2^{g}±0.20
	45/75	4	140^{b}±0.04	25.3^{a}±0.10	35.1^{b}±0.90	27.1^{f}±0.10
	45/75	6	150^{c}±0.01	51.3^{g}±0.10	40.6^{c}±0.10	25.9^{e}±0.01

Means within the same column with different superscripts were significantly ($P<0.05$) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

It was observed that soaking and cooking of beans significantly reduced the tannin content. Soaking in sodium carbonate had the most significant ($p < 0.05$) decrease in tannin content. Martin- Cabrejas (1997) states that the tannin compounds are derived from small molecular weight non tannin material which thus indicates post-harvest biochemical activity during storage and hence causing the observed increase in tannins. Such activity might include that of peroxidases enzymes which may be active in the moist tissues which result from storage in high humidity and high temperatures (Fry, 1986).Thus the results of this study indicate a possible role of tannins in the tendency for development of HTC defect in common beans.

4.6 Protein Digestibility

In general, the accelerated storage conditions of 35˚ C/83% RH and 45˚ C/75% RH reduced the protein digestibility (Table 4.17 and 4.18). Rose coco beans stored at 35˚ C /83% RH had a range from 88 to 23.1% and this was a 64.9% reduction in protein digestibility. At 45˚ C /75% RH, the range for Rose coco was 88 to 57.8% and this was a 30.2% reduction in protein digestibility. For the Red kidney beans, at 35˚ C /83% RH the range was 79.6 to 13.9% this was a 65.8% reduction. At 45˚ C /75% RH the range was 79.6 to 41.6% and this was a 55.2% reduction. Overall Red kidney had a higher reduction in protein digestibility.

Legumes have lower protein digestibility than proteins of animal origin (Nyakuni *et al.*, 2008). This is due to the presence of antinutritional factors such as trypsin inhibitors, polyphenols and phytic acid (Jood *et al,* .1998).

Table 4.17 Effect of storage conditions on protein digestibility of Rose coco

Bean Type	Storage condition (˚ C/%RH)	Storage time (months)	Protein digestibility (%)
Rose coco	25/75	0	88.0 ^{g} ± 0.05
	35/83	2	85.4 ^{f} ± 0.01
	35/83	4	52.3 ^{b}± 0.10
	35/83	6	23.1^{a} ± 0.02
	25/75	0	88.0 ^{g}± 0.05
	45/75	2	73.4^{e} ± 0.02
	45/75	4	61.8 ^{d}± 0.02
	45/75	6	57.8 ^{c}± 0.02

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Table 4.18 Effect of storage conditions on protein digestibility of Red kidney

Bean Type	Storage condition (˚ C/%RH)	Storage time (months)	Protein digestibility (%)
Red kidney	25/75	0	79.6 ^{g} ± 0.02
	35/83	2	44.3 ^{e}± 0.10
	35/83	4	31.85 ^{c}± 0.01
	35/83	6	13.85^{a} ± 0.03
	25/75	0	79.6^{g} ± 0.02
	45/75	2	46.3 ^{f}± 0.02
	45/75	4	41.6^{d} ± 0.03
	45/75	6	24.4 ^{b}± 0.02

Means within the same column with different superscripts were significantly (P<0.05) different. Values are presented as Mean ± SD, n=3. Each value is a mean of 3 replicates. S.D (standard deviation)

Low digestibility of the bean seed protein is one of the main drawbacks limiting the nutritional quality of beans. In the current study, *in vitro* protein digestibility decreased with increased storage time for both bean varieties. The highest reduction was in Red kidney, the variety that also exhibited the greatest increase in cooking time. The lowest decrease in protein digestibility was in Rose coco, the variety that showed lowest increase in total cooking time after six month storage under accelerated storage conditions. Nielson (1991) states that protein digestibility in common beans is influenced by changes in the structure of proteins and complexing of the protein with the

starch, hemicelluloses, minerals and other proteins, all which occur during prolonged storage. It has been suggested that the factors that control digestibility are similar to those that cause an increase in cooking time (Nyakuni *et al.,* 2008). Khatoon and Prakash (2004) observed that a reduction in protein digestibility could also be related to the possible buildup of disulphide bonds between sulphur containing amino acids, which are resistant to action of digestive enzymes and also cause protein-protein folding which in turn hinders action of enzymes

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From the study carried out it was established that high temperature (35 ˚C and 45 ˚C) and relative humidity (75% and 83%) storage conditions lead to minimal changes in physical and nutritional properties of Rose coco and Red kidney common bean varieties. The storage conditions did not have an effect on the length and width, bulk and true densities, proteins and minerals. However, storage conditions caused reduced hydration and swelling coefficient which in turn means that water uptake during cooking reduces. Colour changes were also depicted by reduction of hunter L values and increase of hunter- a and b values which confirmed development of the HTC defect. There was a decrease of protein digestibility and phytates while there was increase of leached solutes, electrical conductivity and tannins. Overall, the digestibility of proteins in legumes decreases with increased storage.

Storage conditions of high temperature and relative humidity led to increased hardening as exemplified by the increased cooking time of the two common bean varieties with Red kidney having the most increase in cooking time. This is synonymous with development of the HTC defect.

Soaking pre-treatment reduced cooking time with the most profound reduction observed in Na_2Co_3 soaking. Additionally, the soaking pre-treatments of de-ionized water and sodium carbonate followed by cooking reduced the levels of antinutrients (phytates and tannins) in the common beans. This helped improve the nutritional value of the common bean.

5.2 Recommendations

Further studies should look into the exact ideal storage conditions of beans. Soaking of common beans before cooking should be encouraged as it not only reduces overall cooking time but also reduces the antinutrients content.

More experiments need to be conducted to develop easy guidelines for identifying the HTC defect in beans. This study can be extended to include other legumes.

Further research should be done in the area of bean breeding to come up with varieties free from the HTC trait and this should be passed on to the farmers for small and large scale implementation and subsequent utilization.

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