EVALUATION OF THE QUALITY CHANGES OF COMMON BEANS (*Phaseolus vulgaris* L.) VARIETIES DURING STORAGE IN HERMETIC POLYETHYLENE BAGS

MOMANYI R. MICAH

A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Food Science of Egerton University

EGERTON UNIVERSITY

JULY, 2022

DECLARATION AND RECOMMENDATION

Declaration

I declare that this thesis is my original work and has not been presented in this or any other university for any degree.

Sign  Date 06/07/2022

Micah Rambeka Momanyi

KM16/3350/12

Recommendation

This thesis has been submitted for examination with our approval as the official university candidate's supervisors.

Signature…**……**Date…17/06/2022……..…

Dr. John Masani Nduko, **PhD**

Department of Dairy and Food Science and Technology,

Egerton University.

Signature……. Date…04/07/2022……

Prof. Mary Omwamba**, PhD**

Department of Dairy and Food Science and Technology,

Egerton University.

Signature……. Date……

Prof. Abdul Faraj, **PhD (Posthumous)**

Department of Dairy and Food Science and Technology,

Egerton University.

DEDICATION

This thesis is dedicated to my family: Dad and Mum, My wife Josephine, my children Amos, Ruth, Felix, and Enock.

ACKNOWLEDGEMENTS

Honour and glory go to the almighty God for His endless love, mercy, care, strength, and guidance during my study. I wish to acknowledge Egerton University through the Department of Dairy and Food Science and Technology for their enormous support during my research. I sincerely thank *icipe*for offering me a chance to conduct my experiment. My sincere gratitude also is registered in the animal science department and specifically Mr. Mutumba, for the unimaginable assistance he gave me during my chemical and biochemical analyses. Most obliged to my supervisors Dr. John Masani Nduko, Prof. Mary Omwamba, and the late Prof. Abdul Faraj, without whom my work would have been a mirage. Their professional and technical guidance throughout my career was immense. I wish to thank my wife, Josephine, for guaranteed moral support, love, and prayers during my studies. Special thanks also go to my course mates and colleagues Dennis Mwan, Nobert Wafula, and Johnstone Mwove for the support they offered during the term of my research. Finally, I can't fail to recognize my entire family for the overwhelming support during the period.

ABSTRACT

Common beans (*Phaseolus vulgaris* L.) are extensively cultivated in sub-Saharan Africa as a cheap source of proteins, starch, dietary fiber, and excellent mineral salts and vitamins. However, beans are lost due to poor post-harvest handling and storage practices that provide insufficient protection against storage insect pests. Therefore, farmers are forced to apply insecticides more than once in a storage period to achieve protection. Excessive use of chemicals is not safe for health and is uneconomical. Hermetic techniques for storage have attracted increasing attention in recent years as chemical-free methods to preserve food grains and protect them against storage insect pests. Whereas substantial research that evaluates how hermetic technologies can lower quantitative losses arising from insect damage exists, there are barely any investigations that assess the effect on the quality of the stored produce, especially in hot and humid regions. This study aimed to evaluate the impact of hermetic storage (PICS®) conditions on the quality of beans in chemical and biochemical composition, grain texture, hard-to cook-defect, *in-vitro* nutrient digestibility, and aflatoxin contamination. Three varieties of common beans: *Rosecoco*, *Nyayo,* and *small red*, in three moisture levels (12%, 15%, and 18%) were stored hermetically for seven and a half months. Sampling and analyses were done every 45 days and PPB were used as control bags. The beans were analyzed for physicochemical properties, biochemical constituents, texture and cooking quality, moulds, and aflatoxin contamination using standard methods. Data analysis was done using Stata software to perform variance analysis while means were separated using the Least Square Difference at $\alpha=0.05$. The *Nyayo*beans data were subjected to principal component analysis to validate the interrelationships between the variables and treatments of each experimental condition.PICS® bag was significantly different from PPB bags at $p<0.001$ in terms of preserving the quality of beans during storage: The PICS® had 22%, 23%, and 18% higher total soluble sugars, in-vitro starch, and protein digestibility respectively than the PPB bags during storage. The study found out that a hermetic bag was significant in reducing the bean hardness during cooking compared to the control bag. Similarly, the activation energy required during cooking was significantly higher in PPBs than PICS®. Furthermore, it was observed in the study that incidences of molds were higher in PPB bags than PICS bags for *Aspergillus* spp*., Fusarium* spp*., Penicillium* spp., and *Rhizopus spp*. Hermetic bag was better than the ordinary PPB bag in terms of nutrient and texture preservation as well as for mould and aflatoxin control, hence recommended for beans storage.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION AND ACRONYMS

AACC	American Association for Cereal Chemists
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
DH	Degree of Hydrolysis
DMSO	Dimethyl sulfoxide
DRBC	Dichloran Rose Bengal Chloramphenicol agar
FAN	Free Amino Nitrogen
FFA	Free Fatty Acids
HDPE	High-Density Polythene
HS	Hermetic Storage
HTCD	Hard- to- Cook Defects
IF	Indigestible Fraction
IPM	Integrated Pest Management
IVISP	In vitro insoluble protein
EDTA	Ethylene Diamine Tetra Acetic Acid
MINAGRI	Ministry of Agriculture
NaClO	Sodium hypochlorite
OTA	Ochratoxin A
DTPA	Diethylenetriamine Penta Acetic acid
PDA	Potato Dextrose Agar
PICS	Purdue Improved Crop Storage
PVC	Polyvinylchloride
T	Tonnes
TPC	Total Phenolic Compouns
UK	United Kingdom
USA	United States of America
USAID	United States Agency for International Development.
MAP	Modified Atmosphere Package

CHAPTR ONE
INTRODUCTION

1.1 Background Information

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes worldwide, with a market value exceeding all other legumes (Rodríguez et al., 2021). Common bean is a major source of nutrients to more than 300 million people in parts of Latin America and Eastern Africa, where it represents 65% of proteins consumed, 32% energy, and is a major source of micronutrients such as iron, zinc, thiamin and folic acid (Amongi *et al*., 1970). It is also important for the household economy of smallholders in Eastern and Southern Africa. Common bean has potential of alleviating poverty and enhancing food security of smallholder farmers. Although beans can be consumed fresh after maturity, much of the grain is dried and stored for future consumption. However, grain storage in sub-Saharan Africa is one of the key points of loss in the harvested grain's supply chain with an average of 13.5% of harvested grains lost post-harvest valued at USD 4 billion annually, aggravating hunger (Agarwal *et al*., 2021). Most losses occur due to insect attacks and mold infections at post-harvest (Olorunfemi & Kayode, 2021).

For many years, farmers and other actors have used chemical methods for protecting food grains during storage. According to Hasan *et al*. (2020), Methyl Bromide and Phosphine are the most widely used chemical fumigants for insect control in stored grains. Many common storage insect pests however, are now known to develop resistance to common pesticides. Moreover, the toxicity of some pesticides and the need for qualified applicators for others, such as methyl bromide used as a chemical fumigant, present limitations for use and the safety of the preserved food. Because of these reasons, hermetic technologies are increasingly being seen as providing a unique advantage. Hermetic storage (HS) technologies are environmentally safe and sustainable, and simple to use. Some HS units are characterized by their ease of installation, cheap costs, ease of relocation, and very modest infrastructure requirements (Ndemera *et al*., 2020).

Hermetic storage has been used for a long time but has only re-emerged in recent years as an important alternative method for grain storage (Mutungi & Affognon, 2022). There are three different hermetic storage forms: Organic hermetic storage, hermetic vacuum fumigation, and gas-hermetic fumigation. Organic-hermetic storage consists of a sealed storage system containing a modified atmosphere that develops due to metabolic and respiration effects. Living forms, including insects, microflora, and the commodity itself use up oxygen while emitting

carbon dioxide, eventually resulting in low oxygen and high carbon dioxide environment. The soft permeability envelope also maintains a constant moisture environment within the bags. Vacuum-hermetic fumigation, on the other hand, uses a vacuum pump to rapidly create a deficient pressure atmosphere for accelerated disinfestation of non-crushable commodities through asphyxiation. In contrast, gas-hermetic fumigation uses an external gas source, usually carbon dioxide, to create an oxygen-free environment (Mutungi & Affognon, 2022).

The technology for storage in silos well serves the world's highly developed economies generally located in temperate climates. However, in hot, humid climates in tropical and semi-tropical regions, silo technology has produced negative results by causing condensation and humidity damage to the stored commodities. Hasan *et al*. (2020b) studied farmers' adoption and willingness to pay for post-harvest technologies in Tanzania and discussed the effects of extreme weather on stored produce.

The Purdue Improved Crop Storage (PICS®) bags technology is a scaled-down form of hermetic storage that uses two layers of relatively thick (80 μm) polyethylene sheets placed in a polypropylene bag. The technology was initially developed to solve losses due to storage insect infestations in cowpeas in Central and West Africa. It is highly effective, has also been shown to effectively protect other grains, including maize, pigeon beans, common beans, and green grams in East Africa (Cherotich, 2021). Thus, the technology is increasingly being regarded as an alternative chemical-free solution for long-term storage of cereal and legume grains. Some studies have also indicated that hermetic storage could arrest mold growth, suggesting a possibility to lower contamination by storing in hermetic containers *(*Alemayehu *et al.*, 2020).

Generally, storage conditions are known to influence the nutritive value and quality of many legumes (Gu *et al.,* 2022). There is, however, limited knowledge on the effect of hermetic storage systems on quality of common beans. Main focus has been on effectiveness and economics of hermetic storage on grains and cereals than the nutritive value and quality (Baributsa & Njoroge, 2020). When looking at the nutritional and ant nutritional factors during the storage process of common bean observed that the storage time was a major factor that had influence on the content of protein, phytates, tannins and calcium by either reducing or increasing their values as a function of time. When common beans are stored under high temperature and high relative humidity for a long time, they develop a hardening phenomenon

that reduces cookability (hard-to-cook defect). Beans with this defect are characterized by poor soaking characteristics, longer cooking times, poor cooked texture, are of lower nutritive value, and less acceptable to the consumer *(*Bassett *et al*., 2020).

Storage conditions could thus affect the nutritional quality, food value, and the economy of processing of common beans. A key characteristic of hermetic storage is the use of impervious material to retain airtight conditions, which also causes retention of moisture level constancy within the system. According to *Chigoverah et al*. (2016), pesticide-free hermetic grain storage is an environmentally benign alternative to synthetic pesticides, currently being used in many countries. The principle behind hermetic storage is to shut the produce in airtight bags together with all microorganisms where they compete for air. Kalsa *et al.* (2019) observed that limited information exists on postharvest preservation strategies of stored wheat and nutrient retention.

Depending on agro-ecological conditions and the extent to which farmers adhere to best pre-storage drying practices, adverse micro-environments within the hermetic systems may be created, causing undesirable effects. There are, however, no substantial studies that demonstrate how hermetic storage might influence the quality of these grains. This study focused on the effects of hermetic storage on the chemical, microbial, and bio-chemical quality of beans to fill the knowledge gap beside pest control.

1.2 Statement of the Problem

The main storage problem in food grains is attack by storage insect pests. In sub-Saharan Africa Countries, huge post-harvest grain losses valued at USD 4 billion are registered annually. Most failures occur due to insect attacks and mold infections. To avoid insect attacks where long-term storage is desired, insecticides are commonly added as protectants. The use of insecticides, however, has safety and environmental sustainability concerns. Other methods such as refrigeration and freezing are expensive in terms of initial cost and energy needs and challenging to maintain, and therefore not cost-effective at the farmer level. Similarly, indigenous preservation methods using local products such as wood ash, animal dung, and botanicals, among others, have limited efficacy. Hermetic storage is emerging as a chemical- free alternative method of grain storage.

However, there are limited studies that demonstrate how hermetic storage influences the quality of these grains since; storage conditions affect the nutritional value and quality of many legumes.

Common beans stored under high temperature and relative humidity for a long time, develop (hard-to-cook defect). A key characteristic of hermetic storage is waterproof material to retain tight air conditions, which also causes retention of moisture level constancy within the system. Depending on agro-ecological conditions and the extent to which farmers adhere to best pre-storage drying practices, adverse micro environments within the hermetic systems may be created, causing undesirable effects. The aim of this study was to evaluate the effects of hermetic storage on the chemical, microbial, and bio-chemical quality of beans to inform its applicability on grain and legume storage.

1.3 Objectives

1.3.1 General objective

To evaluate the quality changes of common beans varieties stored in hermetic polyethylene bags in order to enrich the aspirations of Sustainable development goal number 2 (Zero Hunger) and contribute to the big 4 agenda (Food and Nutritional Security).

1.3.2 Specific Objectives

i) Determine the effect of hermetic polyethylene storage on physicochemical properties and biochemical constituents of common beans.
ii) Determine the effect of hermetic polyethylene bag storage on the texture and cooking quality of common beans.
iii) Determine the effect of hermetic polyethylene bag storage on mold infection and aflatoxin contamination.

1.4 Hypotheses

i. There is no effect of hermetic polyethylene storage on the physicochemical properties and biochemical constituents of common beans.
ii. There is no effect of hermetic polyethylene storage on the texture and cooking quality of common beans stored.
iii. There is no effect of hermetic polyethylene storage on mold infection and aflatoxin contamination of common beans.

1.5 Justification and significance of the study

Common beans are an essential diet for many low-income households in sub-Saharan Africa. They are a cheap source of proteins, starch, dietary, and fiber an excellent source of

mineral salts and vitamins. Production of beans is seasonal; hence, storage is a critical post-harvest undertaking. Therefore, reducing post-harvest losses of food grains at the storage level has been demonstrated in several recently published works (Mogale *et al*., 2020). The nutritional value and functional quality of many legumes, including common beans, are often influenced by storage conditions, thus defeating the very logic for storing them. High relative humidity and temperatures that favour the growth and proliferation of storage pests also tend to lead to quality deterioration, especially if best storage practices are not followed. Preference for stored beans depends on quality aspects, including cooking time, cooked texture, grain damage, and discoloration, among other characteristics that correlate with the specific physicochemical and biochemical change that relate directly to nutritional value. Thus, there is a need to validate the performance of hermetic storage, especially from the point of view of quality preservation of the stored produce. This research is aligned to the big four agenda aspirations and will contribute to Sustainable Developments Goal number 2 of zero hunger and ensuring food and nutritional security. The findings of this study may be of help to the farmers in rural areas participating in beans production and storage.

CHAPTER TWO
LITERATURE REVIEW

2.1 Botanical diversity of common beans

Common bean (*Phaseolus vulgaris* L.) is an herbaceous annual plant grown worldwide for its edible fruit, either the dry seed or the unripe fruit, both of which are referred to as beans. The leaves are also used as a vegetable, and the straw can be used for fodder. Along with other bean genus species, it is classified botanically into the legume family *(Fabaceae*), most of whose members acquire nitrogen through an association with rhizobia, a species of nitrogen-fixing bacteria (Zhao *et al*., 2021).

The common bean includes several species, both wild and cultivated. All the wild species members have a climbing habit, but there are many cultivated varieties, classified as bush bean or pole bean, depending on their particular style of growth (Parker & Gepts, 2021). Kidney bean, small red bean, pinto bean/red mottled, cranberry bean, navy bean, and wax bean are some types of common beans named based on their fruit and seed characteristics. Common bean includes highly variable species with a long history. Wild varieties form perpendicular bushes 20-60cm tall. Altogether, the types that belong to this group bear alternate green or purple leaves: tri-oval smooth-edged leaflets, each 6-15cm long and 3-11cm long. They also bear white, pink, or purple flowers about 1cm long, which give way to pods 8-20cm long and 1-1.5cm wide. The fruits are plump, kidney-shaped up to 1.5cm long with a wide range of colors, and others mottled in two or more colors (Figure1).

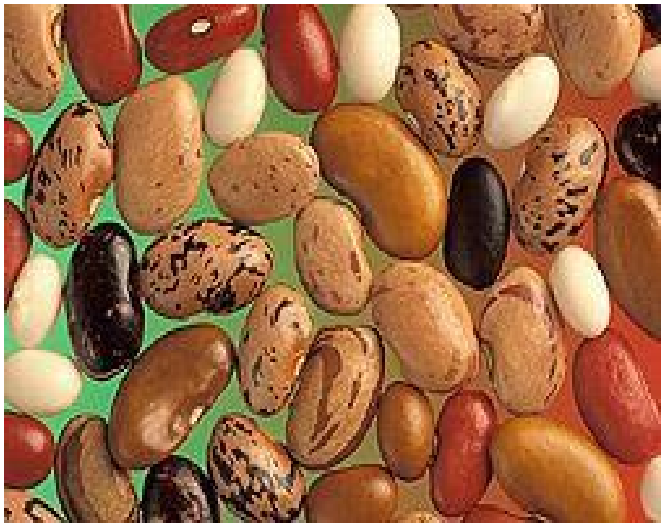


Figure 1*: Appearance of diverse varieties of common dry beans*

Source: Gentry (1969)

2.2 Production of common bean in Kenya

Common bean is an essential component of the production systems and a significant protein source in world and more so the poor population in Eastern and Southern Africa. India produces more dry beans than any other country on Earth (6,390,000T), followed by Myanmar (5,466,166T), Brazil (3,033,017T), and the United States (1,625,900T), Venter (2019). By 2009, Kenya was the leading producer of common beans in East Africa, followed by Uganda and Tanzania (Katungi*et al.*, 2009). However, as per 2017 the republic of Tanzania was the leading producer (1,140,444T) followed by Uganda (1,024,742T) and Kenya (846,000T), Venter (2019). There are many different varieties of common beans grown in Kenya. About 80 types were identified as of the 1970s (Osdaghi *et al*., 2020).However, six varieties are the most popular. They include the red/red-purple mottled (known in different local names as *Roseccoco*, Red mottled (*Nyayo*), or Small red (*Wairimu*) based on the local dialect. The other common variety is the grey/purple speckled (locally referred to as *Mwezi Moja*) and Pinto'sugar' bean (locally known as *Mwitemania*). Rosecoco and the Canadian wonder are the most commonly grown varieties. This is because of their early maturity, sensory appeal, high palatability, and attractive bean pigmentation and size (Wafula *et al*., 2020). They require heavy rain and high levels of soil fertility.

2.3 Nutritional importance of common beans

Common beans are rich in starch, protein, and dietary fiber and an excellent source of iron, potassium, selenium, molybdenum, thiamine, vitamin B6, and folate. Genetic diversity among bean varieties is responsible for compositional variations, which could also be exploited to ensure beans provides the critical nutritional requirement (Abay, 2021). Consumption of common beans is high, mostly because it is relatively cheap compared to animal protein sources. For resource-poor households, common beans play a strategic role in alleviating malnutrition by complementing other foods (e.g., maize) that are primary sources of carbohydrates. In Eastern and Central Africa, the annual per-capita consumption of common beans is estimated at 40 - 60 kg. Daily consumption is high, and common beans provide many proteins, calories, and micro nutrients (Siddiq *et al.,* 2022). This fact also was supported by Ntatsi *et al*. (2018) while looking at the cultivation of legumes and the value chain to produce either dry seeds for human consumption, also known as pulses or animal fodder.

Health organizations now promote regular consumption of common beans and other pulses because it reduces the risk of cancer, diabetes, or coronary heart diseases (Mullins & Arjmandi, 2021). Recent research has shown that black beans provide exceptional support for digestive tract health, particularly the colon. The indigestible fraction (IF) in black beans was larger than the IF in either lentils or chickpeas. The indigestible fraction is fermented by colon microbiota into short-chain fatty acids such as butyric acid, which is essential for the colon's health. Common beans also act as an appetite suppressant because of the high content of slowly digestible starch, which causes a low sustained increase in blood sugar. Despite these advantages, the nutritional value of common beans is affected by several factors, including low levels of sulfur amino acids and tryptophan, low protein digestibility, and the presence of anti-nutritional factors such as proteinase inhibitors (Mayer *et al.*, 2021).

2.4 Storage of common beans

The common bean (*Phaseolus vulgaris)* has significant nutritional and economic importance in many regions of Eastern and Southern Africa (Catarino *et al*., 2021). On-farm storage is short term, though, mostly accredited to severe losses (up to 40% in less than 6 months) due to pests in inadequately protected grain stores. To minimize storage losses, extension staffs have promoted different methods to combat these storage pests. These pest control methods include insecticide use and/or solar disinfection and the application of certain botanicals. However, the low efficacy of the botanicals, safety concerns with the use of insecticides and the labor intensity of solar disinfection practices limits broad application of these methods for common bean storage(Thakur *et al*., 2021). A new technology, Purdue Improved Crop Storage (PICS) triple-layer hermetic storage bags, has been proposed as an improved alternative for insecticide-free, long-term storage of common beans with minimal grain damage (Chakraborty *et al*., 2021).

2.5 Different methods for hermetic storage of food grains

Several different forms of HS have been developed over time, including concrete and metal silos. Organic hermetic storage method has been used to preserve products such as rice bran, basmati, coffee, cocoa, peanuts, and spices, as well as rice, maize, pulses, and seeds (Villers *et al*., 2018). The technology for storage in silos has been observed to work well in developed economies generally located in temperate climates. However, hot, humid climates in tropical and semi-tropical regions have produced negative results by causing condensation and humidity damage to the stored commodities. The cocoons, usually made from specially formulated flexible PVC and

sealed with a unique zipper, are the most widely used HS for storing bagged grain, although the large size limits these (Villers *et al.*, 2006). Cocoons are produced in capacities ranging from 5 to 1000 tones, although in the Philippines, Ghana, Sri-Lanka, and Rwanda, cocoon™ of up to 300 metric tons has been used. In the Philippines, milled rice could be preserved for up to 12 months, whereas in Rwanda, up to 30 months of safe storage was reported (Villers *et al*., 2006) without loss of germination potential. The use of HS has been enhanced in recent years through scaled-down forms such as Super Grain®and PICS® bags, which have smaller capacities of 5000 kg. The development of Super Grain® and PICS® bags allowed better storage on transportation and distribution (Rickman & Aquino, 2004). Hermetic Bunkers is another form of hermetic storage with capacities ranging from 10,000 to 20,000 tons. It has been used to store wheat at or below its critical moisture content of 12.5% without significant quality degradation, including baking qualities, for up to two years (Ling *et al*., 2020). In barley storage, bunkers preserved quality for three years, with total losses of 0.66 to 0.98% and germination remaining above 88% (Roy, 2021).

Purdue Improved Crop Storage (PICS) grain storage technology (Figure 2) is one of the hermetic storage technologies developed in the 1980s to store cowpeas in West Africa. The technology relies on an organic creation of a modified atmosphere by the organisms in a sealed environment. The modified atmosphere created within a PICS system hinders the survival of life-form. The technology applies a double-layered envelope made up of two 80 microns thick high-density polythene (HDPE) liners fitted inside a woven polypropylene sack to create an airtight seal (Sahu *et al*., 2015).By shutting the produce within the PICS bags, the bags' oxygen level drops due to utilization by insects and other micro-flora within the bags. In contrast, the level of carbon dioxide increases dramatically. Once the oxygen levels have dropped sufficiently, the insects stop feeding and eventually die. Lane *et al*. (2017), while studying small hermetic bags (50 and 100 kg capacities) used by smallholder farmers in several African countries, have proven the bags to be a low-cost solution for preventing storage losses due to insects.

***Figure 2**: Hermetic storage technology.*

Source: ICIPE

Several mechanisms are responsible for preventing insect survival under hermetic storage. According to Affognon *et al*. (2016), decreasing oxygen and increasing carbon dioxide concentrations slowed down the rate of feeding of *Callosobruchus maculatus.* The oxygen concentrations of feeding became extremely low or even ceased. Under oxygen deprivation, oxidative metabolism is suppressed, and *Callosobruchus maculatus* cannot generate metabolic water for support of vital life processes and cellular or *Callosobruchus maculatus* tissue integrity Mutungi *et al.* (2014). Consequently, death eventually occurs due to desiccation. Simultaneous exposure to low oxygen and high carbon dioxide concentrations was thought to have a synergistic effect on insect mortality in this study.

The deadly action of carbon dioxide is explained by the fact that increased carbon dioxide solubility in insects' body fluids subsequently lowering the pH. A drop in pH is believed to coincide with the development of lesions in the cell membranes of larvae and adult insects, which causes loss of cellular integrity (Bayley *et al*., 2018). It has also been shown that a dramatic increase in the uptake of CO_2under some pressure causes expansion and evaporation from the liquid when the pressure is reduced, resulting in lesions in the cell membranes of adults and larva (Stejskal *et al.*, 2021). According to Barbieri *et al*. (2020), a high carbon dioxide level is also shown to slow the ventilation rate and reduce the absorption of oxygen.

On the other hand, a fall in oxygen levels and the simultaneous rise in carbon dioxide concentration could bring about oxidative stress for *C. maculatus*, causing the larvae to enter a hypometabolic state. In that condition, survival continues, but development and metamorphosis

are retarded, and insect fertility is severely reduced (Baoua *et al*., 2012). Elgersma *et al*. (2018), while researching hermetic storage technologies, concluded that information on his findings was relevant for harvest planning and storage. In the hypo metabolic state, the low demand for energy may be supplied by anaerobic metabolism, which, as argued by some, eventually causes accumulation of toxic metabolites leading to physiological dysfunction and eventual death (Luisetto *et al*., 2019).

2.6 Changes in whole bean composition during storage

A few studies have been conducted to investigate the qualitative changes of hermetically stored produce. According to Borém *et al.* (2019),investigated the sensory properties of coffee beans stored under hermetic conditions for six months. Sensory evaluation of coffee stored in the hermetic storage showed that the coffee-maintained quality, aroma, and taste over the storage period. In another study, peanuts stored in hermetic conditions for up to eight months showed quality integrity with constant moisture content and germination rates similar to those stored under refrigeration (Sultana *et al.*, 2021). Cocoa beans stored in PICS bags achieve low oxygen percentages of about 2% in less than two weeks, preventing the deterioration of free fatty acids (FFAs) due to oxidation (Ashong, 2020).

Beans are considered a living seed until they have been processed so that the integrity of the bean has been destroyed. It has been demonstrated that minimal changes occur in beans during storage of intact whole beans. The storage of the entire whole beans has little impact on the protein or fat components. According to Hall *et al*. (2020), there is a non-significant decrease in black beans' protein stored for two years at room temperature. Hou *et al.* (2020) observed that only a slight reduction in lipid content was noted during bean storage. The other essential components of beans with a significant impact on nutrition are the anti-nutritional components. According to Liang *et al*. (2020), a phytic acid content reduction of approximately 21% after six months of storage.

These studies suggest that beans' storage has a significant impact on the nutritional, chemical, and biochemical constituents of beans. The other components of beans, such as pectin, a part of the soluble dietary fiber, sometimes referred to as the glue that holds plant cell walls together, remains soluble and interacts with water to form a gelatinous material during cooking, causing a softening. However, if pectin becomes insoluble, the bean does not absorb as much water

resulting in a bean that does not soften during cooking resulting in a hard- to-cook defect (Wu *et al.*, 2018). On the other hand, pectin solubility may decrease significantly during storage. The increase in seed hardness during storage was also correlated to the pectins' reduced solubility (Chu *et al.*, 2020).The action mechanism dictates that the soluble fiber composition converts into the insoluble form of dietary fiber during bean storage .

The development of hard-to-cook defects (HTCD) is a significant quality challenge for the storage of beans in tropical regions. To avoid the deficiency, it is recommendable that beans be stored at the lowest possible moisture content and in a dry and cool environment. Several hypotheses have been put forth to explain the cause of bean hardening: First is lipid oxidation or polymerization, the formation of insoluble pectates, lignification of the middle lamella, and several mechanisms (Chen *et al*., 2021).The cell wall's cellular ultrastructure and cotyledon tissue reflect a significant difference between the hard and the soft beans. The development of HTCD is characterized by cotyledon cells' failure to separate during cooking (Affrifah *et al*., 2021).The defect has also been associated with phenolic compounds. A study by Chigwedere *et al*. (2019), on different degrees of HTCD in differently stored navy beans indicated that storage induced HTCD was higher in beans with higher hydroxycinnamic acids (especially ferulic acid) than the control beans.

In addition, in other studies, HTCD was hypothesized to result from the interaction between storage proteins and starch granules (Jombo *et al*., 2021). A study by Duijsens *et al*. (2021), reported that the hard-to-cook defect could be reversed by storing beans exhibiting this defect at refrigeration temperatures. The HTC beans (common beans and cowpeas) stored at 6.5°C and 71% RH showed progressive shorter cooking times during storage between six to twelve months. He also concluded that soaking HTC common beans in a solution of either PA or EDTA reduced the cooking time to that of control beans. DTPA (Diethylenetriamine Penta Acetic acid) and EDTA are chelating agents. Any chelating agent capable of removing Calcium ions from Calcium pectate during the cooking process should reduce cooking time because Calcium ions and pectin makes calcium pectate, which is part of what causes bean hardening. Soaking in aqueous salt solution before cooking was also reported to significantly decrease beans' textural hardness, demonstrating the HTC defect by these scientists. These researchers explained the beneficial results obtained by combining ion exchange and chelation mechanisms between monovalent cations (Na+, K+) in solution and divalent cations (Ca+2, Mg+2) in pectates of the

middle lamella. The altered pectates were considered to be more water-soluble and heat-labile, thus facilitating cell separation during cooking. These mechanisms, however, were not the only ones responsible for the reported textural improvements.

Koriyama *et al*. (2017) studied the influences of soaking treatment and storage conditions on the softening of cooked beans, namely, soy beans and red kidney beans. It was revealed that the easing of fresh soy beans and fresh red kidney beans was suppressed during subsequent boiling after soaking treatment at 50 and 60° C. A study conducted by Cichy *et al*. (2019), indicated that the lower storage temperature can control the Hard to Cook effect for both bean varieties. Overall, phytate content can be an indicative factor for the cookability of beans when the relationship between variety and storage conditions has been determined.

Beans possess some critical antioxidants. The Antioxidant phenolics in beans can exist in free or bound forms. Studies have equally demonstrated that 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. Second, an increase in esterified phenolic acids in the seed coat was shown to be related to bean hardening and the hard to cook phenomenon. Pectin fraction's phenolic acid content was two times higher in hard-to-cook beans than in regular beans (Mubaiwa *et al*., 2019).

2.7 Mold infection and contamination of common beans.

Seeds are commonly infected with various molds and contaminated with mycotoxins, their secondary metabolites. Williams *et al*. (2014), while studying storing maize in regions of the world without sufficient drying and storage capacity, found it challenging due to the potential risk of aflatoxin contamination produced by *Aspergillus flavus*. Most studies on mold and mycotoxin contamination have been focused on cereals, while research on beans (*Phaseolus vulgaris L*.) is limited. However, Rangjaroen *et al.* (2019), in their study, concluded that many types of fungi are seed parasites, including stored seeds. Their invasion can lead to various damages, including quantitative, qualitative discolorations, mycotoxins production, and total decay. Sudini *et al.* (2015) found out that seeds are prone to quality deterioration and damage due to improper storage. He was working on peanut seed storage when he concluded that hermetic storage could preserve the seed's quality. In a research that was designed to identify seed-borne fungi on bean (*Phaseolus vulgaris L.*) crops grown in 13 counties of the Republic of

Croatia and their association with Ochratoxin A (OTA) production. A study by Alves *et al.* (2020),found fungi and mycotoxins in most of the bean samples that were collected. No such studies have been conducted in Kenya to identify incidences of fungi and their associated mycotoxins. It is therefore important that hermetic storage technology's effect on bean quality be assessed for recommendation to endusers.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was a laboratory-scale storage trial based at ICIPE Duduville center, Nairobi (Appendix II). *Icipe* is a recognized research center based at Kasarani Nairobi. It has a modern laboratory and all the equipment required for the study. The experiment took 7.5 months, and sampling was done after every 45 days

3.2 Experimental design and statistical analysis

The experiment employed a four factor factorial experiment in a random Complete Block Design (RCBD) (*Appendix I*). The experimental design had Factor A (α) which was the bag type (2 types; PPB and PICS), Factor B (β) was the bean variety (Rose *coco, Small red* and *Nyayo*), factor C (γ) was the storage moisture (three levels: 12, 15 and 18%), and factor D (σ) was the storage period (7 levels; 0, 45, 90, 135, 180, 225, and 270 days of storage). The control samples were bean varieties stored in normal polypropyrene bags (PPB). The experiment was done in three replicates.

$$\mathbf{Y_{ijklm}=\mu+\alpha_i+\beta_j+\alpha\beta_{ij}+\gamma_k+\alpha\gamma_{ik}+\beta\gamma_{jk}+\alpha\beta\gamma_{ijk}+\delta_l+\gamma_{ijklm}}$$

Where:

$Y_{ijklm=}$ Observation on the response,

μ = Overall mean,

α_i= Effect of the i^{th} bag,

βj= Effect of the j^{th} bean variety,

γ_k=Effect of the k^{th} moisture level,

$\alpha\beta_{ij}$=Interaction effect between the i^{th} bag on the $_j{}^{th}$ bean variety,

$\alpha\gamma_{ik}$ = Interaction effect between the i^{th} bag on the k^{th} moisture level,

$\beta\gamma_{jk}$ = Interaction effect between the j^{th} bean variety on the k^{th} moisture level,

$\alpha\beta\gamma_{ijk}$ = Interaction effect between the i^{th} bag and j^{th} bean variety on the k^{th} moisture level,

δ_l=Effect of the l^{th} storage time (blocks) and

γ_{ijklm}= Random term component.

3.3 Sample treatment

Three varieties of common beans namely *Rosecoco*, small red (*Wairimu*), and red mottled (*Nyayo*) were selected based on their wide local popularity with farmers and consumers and used for this study. Freshly harvested beans were sourced from *Icipe* contract farmers; after screening to remove impurities and broken grains, three batches formed for each variety. Each set was equilibrated to moisture contents of 12.0%, 15%, or 18% by spraying with the predetermined amounts of tap water over the grains spread in a thin layer in a plastic bowl. The grains were thoroughly mixed by hand after wetting, taking care not to leave any water in the bowl. The moistened samples were then tightly wrapped in plastic bags (10 kg per bag) and stored at 4 °C for two weeks. During this time, each bag was shaken for a few minutes every day. About 5 kg of the beans at the different moisture contents was packed into 10 kg mini-bags made of polypropylene or double-layer polyethylene/PICS (hermetic storage) and stored under ambient laboratory conditions for seven and a half months (in the abstract you said 7 and half months). Samples were drawn at 45 days intervals and analyzed for various physical, nutritional, and biochemical parameters.

3.4 Determination of storage conditions

In this section temperature, humidity and due point were measured and recorded throughout the experiment. Readings were done both inside the bags and outside (storage room).

3.4.1 Temperature and relative humidity during storage

An EL-USB-2 data logger (Lascar Electronics Inc., Pennsylvania, USA) programmed to take data every 60 minutes was placed in each of the bags before closing to record the temperature and relative humidity during the entire storage period.

3.4.2 Oxygen and carbon dioxide concentration in hermetic bags

Oxygen and carbon dioxide concentrations in the hermetic polyethylene bags were measured at five days intervals using a Mocon Pac Check® Model 325 portable oxygen/carbon dioxide analyser (MOCON Inc., Minneapolis, USA). The inner HDPE liner of the triple hermetic (PICS bag) was punctured with the analyser needle at the top, middle, and bottom to take measurements.

The needle holes were sealed with 10 mm diameter adhesive pads after the measurements. Subsequent measurements were performed from the same spot by lifting and replacing the place.

3.5 Proximate composition of beans

In this section, proximate composition was done to all samples initially to confirm how the samples compared to the standards. They were found to be within the standards in literature.

3.5.1 Sampling and Preparation of Samples

Grain samples (50 g) were taken by pushing a hollow tube to the bottom of the bag so that a representative column of seeds from the four corners and center of the bags was taken. The samples were mixed (250 g) and quartered on a flat surface. One quarter of (60 g) was randomly selected and finely ground into flour using a laboratory mill (Knife Mill Cup KM 400, city, UK). The milled samples were stored at –20 °C in zip-lock polyethylene bags awaiting analysis.

3.5.2 Moisture Content

The oven method (AOAC, 1995 method No. 950.46, AOAC, 1990 was used. About 2.0 g of samples were accurately weighed and transferred into aluminum dishes. The samples were dried in a dry air oven at 105°C to constant weight and cooled in a desiccator for 10 minutes. Moisture content was calculated as the percent ratio of weight loss to the original weight of the sample. The amount of moisture in percentage was calculated as follows:

$$\%\ \text{Moisture content} = \frac{W1 - (W3 - W2)}{W1} \times 100$$

3.5.3 Crude Protein

Crude protein content (N × 6.25) was determined according to the improved Kjeldahl method (Method 46-12A; AACC, 2000) with slight modifications. About 0.5 g ground sample of known dry matter content was accurately weighed in a nitrogen free-filter paper, folded carefully, and placed in a Kjeldahl flask. One tablet of Kjeldahl catalyst and 20 mL of concentrated H_2SO_4 was added to the flask. The mixture was digested in a fume cupboard for about 2 hours until a clear solution is obtained. A blank sample of only a filter paper and sulphuric acid was also digested. After cooling, enough distilled water was added to increase the mixture's volume to three-quarters of the Kjeldahl flask. Two to three drops of phenolphthalein indicator (5%) were added. The Kjeldahl flask connected to the distillation unit and added enough 40% sodium hydroxide

(NaOH) solution to change the solution's colour. Distillation was carried out until a distillate drop did not react with Nessler's reagent placed in a test tube. The distillate was collected in a 400 ml conical flask containing 50 mL of 0.1 mol/L hydrochloric acids (HCL) solution and 2-3 drops of methyl orange indicator. The excess hydrochloric acid (HCL) solution in the distillate was back titrated with 0.1 mol/L sodium hydroxide (NaOH). The percent nitrogen obtained was multiplied by 6.25 to convert to percent protein.

% nitrogen = normality HCL x (mL acid for sample – mL acid for blank) x 14.007 g of sample % protein = % nitrogen × 6.25

3.5.4 Crude Fiber

Approximately 2g ground sample of known dry matter content was accurately weighed into a graduated 600 mL beaker and about 100 mL boiling distilled water and 2.04 mol/L H_2SO_4 solution added. The mixture's volume was made up to 200 mL with boiling distilled water and maintained at this volume while boiling for 30 minutes on a hot plate. The mixture was then filtered using a funnel lightly packed with glass wool. The residue was washed three times with boiling distilled water. The residue and the glass wool were transferred quantitatively back to the beaker, and about 100 mL of boiling distilled water and 25 mL of 1.73 mol/L potassium hydroxide (KOH) solution were added. The volume was made up to 200 mL with boiling distilled water, and this volume was maintained while boiling on a hot plate for 30 minutes. The mixture was filtered again using glass wool and was washed three times with boiling distilled water. The residue was further washed three times with small amounts of ethanol. The residue and glass wool were transferred quantitatively to a porcelain dish and dried in an air oven at 105°C for 2 hours. The sample was cooled and weighed in the porcelain dish before igniting at 550°C in a muffle furnace to constant weight. The sample was cooled in the dish and weighed. The crude fiber content was calculated and expressed as a percentage of the sample dry matter content.

3.5.5 Crude Fat

Crude fat was determined by the Soxhlet method (AOAC Method No. 24.005; AOAC, 1984) with slight modifications. Approximately 5 g ground sample of known dry matter content was weighed accurately into an extraction thimble and covered with cotton wool. The thimble was placed into the Soxhlet extractor, and the fat was extracted into a tared flask for eight hours using petroleum ether (BP 40-60°C). The solvent was then evaporated in a rotary evaporator and the

residue dried in an air oven at 105°C for one h before weighing. The crude fat content was calculated and expressed as a percentage of the sample dry matter content.

3.5.6 Total Carbohydrates

The difference between 100 and the sum of values for moisture content, fat, protein, crude fiber, and ash was estimated as the total carbohydrate content.

3.5.7 Total Starch

Total starch content was determined using the Megazyme Total Starch Assay Procedure: AACC Method 76.13 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). The sample was ground to less than 0.5 mm and 0.1-1 g transferred into a 100 mL flask. Dimethylsulphoxide (DMSO) (20 mL) and 8 mL 8 mol/L hydrochloric acid (HCL) were added, and the covered flask was incubated for 30 min at 60 °C. Distilled water (50 mL) was added to the flask, and the pH adjusted to 4-5 with five mol/L sodium hydroxide. The solution was cooled to room temperature and diluted with 100 mL with distilled water. Absorbencies of starch assay reagent and glucose assay reagent were then read at 340 nm. This was used to calculate starch concentration according to the following equation:

$$SC \text{ (mg/mL)} = (\Delta A) \text{ X } (TVGA/SVGA) \text{ X } (F) \text{ X } (0.052)$$

Where: *SC* is starch concentration, ΔA is test absorbance minus total blank absorbance, *TVGA* is total assay volume from glucose assay in mL, *SVGA* is sample volume from glucose assay in mL and F is dilution factor from sample preparation, and 0.052 is constant.

3.5.8 Ash Content

Ash content was determined using AOAC Method No. 942.05 (AOAC, 1984) was used where 2.0 g of sample was accurately weighed and placed into silica crucibles. The samples were ashed in a muffle furnace at 550^0C for 3 hours. The ash was cooled in a desiccator to room temperature and weighed. Ash content was calculated as a percentage of the dry sample.

3.6 Determination of effects of storage on selected quality parameters

3.6.1 The moisture content of stored beans

A Dickey-John mini GAC® plus moisture tester (DICKEY-john Corporation, Illinois, USA) was used for quick determination of the beans' moisture content during storage. Grains were sampled and about 400 g of grain-filled into the tester cup, and moisture content recorded.

3.6.2 Total soluble sugars

Total soluble sugars were estimated by the phenol-sulphuric acid method (Mutungi*et al.*, 2009). A sample aliquot of 20 µL was diluted in 10mL deionized water, vigorously homogenized, and 100 µL aliquot was drawn and diluted in 400 µL deionized water in a separate test tube. The diluted sample was then be mixed with 500 µL of 5%(w/v) phenol prepared in 0.1 mol/L hydrochloric acids (HCL), after which 2.5mL 97% sulphuric acid (H_2SO_4) (v/v) was added, stirred on a vortex mixer. It was then allowed to cool to 25^{o}C before reading absorbance at 490nm against similarly treated glucose standards containing 0, 10, 20, 30, 40, and 50 µg glucose monohydrate in 500µL deionized water. The concentration of total soluble sugars was determined from the glucose standard curve.

3.6.3 *In Vitro* Starch Digestibility

In vitro starch digestibility (IVSD) was determined by dissolving a 5 mg sample in 1 mL of 0.2 mol/L phosphate buffer (pH 6.9). Porcine pancreatic α- amylase (20 mg) was suspended in 50 ml of the same buffer, and 0.5 mL added to the sample suspension and incubated at 37°C for 2 hours. The sample suspension was analyzed for reducing sugar content against glucose monohydrate standards using Nelson-Somogyi alkaline copper reduction method (Nelson, 1944). Aliquots (50µL) of the homogenized sample were added to 450µL of deionized water,mixed with 500µL copper solution (4 g copper sulfate, 0.185 g sodium sulfate, 23.96 g sodium carbonate, 15.96 g sodium bicarbonate, and 15.96 g 12.14 g sodium potassium tartrate dissolved in 1000mL of distilled water) and heated in a boiling water bath for 60 minutes. The mixture was cooled to 25°C and reacted with 500-µL asernomolybdate solution (49.43 g of ammonium molybdate tetrahydrate, 5.93 g sodium arsenate diabasic heptahydrate, and 756 mmol/L H_2SO_4 in 1000 mL distilled water). The content of reducing sugars was determined by reading the absorbance at 546 nm against standards containing 0, 40, 80, 120, 160, and 200 µg glucose monohydrate in 500 µL deionized water. The degree of hydrolysis (DH) in percentage was calculated by dividing the difference between the reducing value of the enzyme blank by the

difference between the total carbohydrate content of an equivalent sample and total carbohydrates content of the enzyme blank multiplied by100.

3.6.4 In Vitro Soluble Protein Digestibility

In vitro soluble protein digestibility (IVPD) was determined by adding a 200 mg sample to a 100 ml Erlenmeyer flask containing 35 ml 0.1 mol/l sodium citrate tribasic (pH 2.0) with pepsin (1.5 g pepsin/1, Sigma P-7012). The mixture was incubated for 2 hours in a shaking water bath at 37^0C, and then centrifuged at 10,000 rpm for 15 minutes. The residue was washed in 10 ml 0.1 mol/l phosphate buffer (pH 7.0) and re centrifuged at 10,000 rpm for 15 min, then resuspended in 35 ml 0.1 mol/l phosphate buffer (pH 8.0) with pancreatin solution (1.5 g pancreatin/1, Sigma P-1750). The mixture was incubated in a shaking water bath at 37^0C for 1 hour. This step was followed by centrifugation at 10,000 rpm for 15 minutes, washing the residue in 10 ml phosphate buffer (pH 7.0), and re centrifugation at 10,000 rpm for 15 minutes. The residue was collected on nitrogen-free filter paper and washed with 10 ml phosphate buffer (pH 7.0). The dried residue wasanalyzedfornitrogenbytheKjeldahlmethodasdescribedin3.3.3.Residualproteinwas subtracted from total protein, and the difference was expressed as a percent of the complete protein and reported as IVSP digestibility.

Soluble nitrogen was determined by weighing 1 g sample into 50 ml centrifuge tube and dispersed in 20 ml distilled water. The dispersion was mechanically shaken for 1 hour and centrifuged at 10,000 rpm for 15 minutes before collecting the supernatant. The residue was resuspended and centrifuged twice in 10 ml distilled water. The combined supernatants were analyzed for soluble nitrogen by the Kjeldahl method.

In vitro insoluble protein (IVISP) digestibility was calculated as: ((Insoluble protein – Residual protein) / Insoluble protein x 100)), where insoluble protein = total protein – soluble protein; residual protein = protein remaining after pepsin hydrolysis.

3.6.5 Free Amino Nitrogen

Milled samples (1 g) were added to 40.0 ml 5% trichloroacetic acid at 30°C, and extraction was carried out for one h at 30°C. At 15 min intervals, the extraction tubes were swirled to suspend the contents. Ten (10) ml of extract was centrifuged at 4,500 g for 10 min, and 1 ml of clear supernatant was diluted to 25 ml with distilled water. These samples were subjected to ninhydrin assay according to AOAC Method No 10.180 (AOAC, 1980). The sample (1 ml) was diluted to

100ml with water, and 2 ml of the diluted sample was transferred to each of three 10 × 150 mm test tubes to obtain 1-3 mg FAN/l in a diluted solution. Ninhydrin color reagent was prepared by dissolving 10 g sodium hydrogen phosphate, 6 g potassium dihydrogen phosphate, 0.5 g 1, 2, 3-indantrione. H_2O, and 0.3 g fructose in water and diluted to 100 ml. The ninhydrin color reagent (1 ml) was added to the sample and heated exactly for 16 min in a boiling water bath. This was then cooled for 20 min in a 20±1°C bath, and 5 ml dilution solution (2 g potassium iodate dissolved in 600 ml water and 400 ml alcohol added) was added. After mixing thoroughly, the absorbance was read at 570 nm against water within 30 min. A standard curve was prepared by dissolving 107.2 mg glycine in water and diluted to 100 ml for the stock solution, and 1 ml of this solution was diluted to 100 ml with water at various dilutions from 1:10 to 1:50. FAN in the samples was calculated by:

$$FAN(mg) = \frac{Net\ absorbance\ of\ the\ sample\ solution \times 2\ \times Dilution}{Net\ absorbance\ of\ the\ standard}$$

3.6.6 Tannins

Tannins were extracted by shaking a 1 g sample in 10 ml acidified methanol (1 ml concentrated hydrochloric acid (HCL)/100 ml methanol) in centrifuge tubes at 25^0C for 20 minutes. After centrifuging the sample for 15 minutes at 10,000 rpm 1ml was pipetted into a test-tube and mixed with 5ml of vanillin-hydrochloric acid reagent. Vanillin- hydrochloric acid reagent was prepared by mixing equal portions of vanillin solution (4g vanillin/100 ml methanol) and acidified methanol (8 ml concentrated hydrochloric acid/100 ml methanol). Absorbance of the vanillin-hydrochloric acid reagent and sample mixture was read in 1-cm cuvettes using a spectrophotometer at 500nm after 20 minutes against vanillin – hydrochloric acid reagent as blank. To correct for the interference of natural pigments, sample blanks were prepared by subjecting the original extract to the reaction conditions but without the vanillin – hydrochloric acid reagent. A standard curve was prepared by adding 1 g tannic acid (FlukaChemieGmbH, Buchs, Switzerland) to 100 ml acidified methanol, and this stock solution was used at various dilutions from 1:10 to1:50.

3.6.7 Phytic acid content

Phytic acid content was determined as phytic phosphorus using the indirect spectrophotometric method, according to Mirjana *et al.* (2012). A calibration curve was then generated using a sequence of regular phytic acid sodium salt solutions. 0.5 g of powdered sample was extracted for 3 hours with continuous stirring in 100 ml of 2.4 percent HCl. The extract was filtered using Whatman filter paper No. 41. The ammonium iron (III) – sulfate solution (0.2 g of NH4Fe (SO4)2.12H2O dissolved in 100 ml of 2 mol/L HCl and filled to label with purified water) was then applied to 0.5 ml of extract in a glass tube with stopper. The closed glass tube was put in a boiling water bath for 30 minutes and then cooled in an ice bath for 15 minutes before further cooling to room temperature, followed by centrifugation at 3000 r/min. One ml of supernatant was mixed with 1.5 ml 2, 2'-bipyridine solution (10 g 2, 2'-bipyridine dissolved in 10 ml thioglycolic acid and filled to mark with purified water) and absorbance estimated at 519 nm at after a predetermined amount of time.

3.6.8 Grain Hardness and Cooking Time

A CT3 texture analyzer (10000 g maximum load, Brookfield Engineering Laboratories, MA,USA) was used to measure the hardness and monitor the effect of cooking time on hardness by compressing the grains using a cylindrical probe (TA25/1000 cylinder 50.8mm D, 20 mm L) over a target distance and measuring the force to crush and withdraw from the grains. A hundred grams of beans were soaked for 15 h in 200 ml of distilled water. The soaking water was drained, and 50 ml of boiling distilled water added to the beans in a beaker covered with a watch glass and heated in a boiling water bath. At 15, 30, 45, 60, 90, and 120 min, a sample of 20 g beans were removed using a spoon. The beans were be placed in a single layer on a small plastic tray, covered with a paper towel, and cooled for 10 min at room temperature. They were then punched using the above-described probe. The force used to crush and withdraw from the grains was measured and recorded. The recorded data was used to calculate the activation energy according to the formula used by Jing *et al.* (2011).

3.6.9 Mold Contamination

In this section changes in oxygen and carbon dioxide levels in PICS bags and PP bags was monitored throughout the experiment. Mold incidences and prevalence was analyzedinall samples. Mold contamination and aflatoxin infection was alsoanalyzed.Potato dextrose agar with chloramphenicol (PDA-C)/Dichloran rose bengal chloramphenicol agar (DRBC) were used to evaluate mold growth. One hundred grains were taken from each sample, and the surface was disinfected for 1 minute in 1% NaClO solution and rinsed twice with sterile water. Ten (10) grains of beans were plated onto each petri dish with a double blotter.

The bean samples were milled and serially diluted before plating. One gram of powder was then suspended in 9 mL sterile distilled water and serially diluted up to a dilution of 10-4. One (1) mL of the 10-3 and 10-4 dilutions was plated in PDA Agar. The plates were then incubated at 25°C for up to 14 days. The number of kernels showing the growth of fungal species in each petri dish was counted, and the number of colonies expressed per plate. The colonies from the dilution plates were also be measured and expressed as colony-forming units per gram (CFU/g). Typical *Aspergillus flavus* counts have been enumerated on *A. flavus* and *Aspergillus parasiticus* agar. Fungal colonies were sub-cultured on PDA Agar for 7 to 14 days. They were identified to species level based on cultural and morphological characteristics like a colony, color, conidiophores, and phialides presence, and vesicles' size (Watanabe, 1994).

3.6.10 Aflatoxin Analysis

Samples were milled into a fine powder using a laboratory-scale Knife Mill Cup KM 400 MRC Lab (MRC International, city, UK), and then stored at -15°C awaiting analysis. The Ridascreen® ELISA kit for total Aflatoxin (R-Biopharm AG, Germany) was used to test for aflatoxin contamination. The milled samples (2 g) were put into a 50 mL screw cap centrifuge tube and mixed with 10 mL of methanol/ distilled water (70/30 v/v). The mixture was agitated gently on a vortex mixer at room temperature for 10 min, centrifuged at 3000 × g, and the supernatant recovered. Fifty (50) µLof the supernatant and equal volumes of the calibrated aflatoxin standards (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, and 4.05 ppb) was added in separate duplicate wells of the anti-aflatoxin antibody-coated microtitre plate. Enzyme conjugate (50 µL) was added, followed by another 50 µL antibody solution to each well and mixed gently by shaking the container manually. The plates were covered with aluminum foil and incubated for 30 min at room temperature (20-25 °C) in a dark cabinet. The plate wells' liquid was poured off and the wells filled with 250 µL washing buffer (10 mM phosphate buffer, pH 7.4 containing 0.05% Tween 20). The washing procedure was repeated two times and semi-dried by tapping the plate gently against the adsorbent paper. A hundred (100) µL of substrate/ chromogen solution was added to the wells, mixed gently by shaking the container manually. The plate was then incubated for 15 min at room temperature in a dark cabinet, after which 100 µL of stop solution (1 mol/L sulfuric acid) was added. After mixing gently by shaking the plate manually and resting it for 20 minutes, the absorbance at 450 nm was measured using a UT-6100 auto microplate reader (MRC International, UK). A standard curve prepared using the known standard was used to determine the aflatoxin concentration of the samples.

3.7 Data Analysis

The SAS software version 9.1 was used to evaluate the results. The data was subjected to the Kolmogorov–Smirnoff test for normality and the Levene test for homogeneity of variances (Goberna *et al*., 2005). The General Linear Model (PROC GLM) protocol was used for the analysis of variance (ANOVA), the PROC NPAR1WAY method for Komolgorov–test, Smirnoff's and PROC GLM with LEVENE's choice for Levene's test. Tukeys' Honestly Significant Difference (HSD) at $P \leq 0.05$ was used to separate the treatments. To verify the interrelationship between the variables and treatments of each experiment, the *Nyayo* bean's data was used for principal component analysis. Biplots were produced with the first two main

components. Principal component analysis was performed with the aid of the Past 4.03 software (Hammer *et al*., 2001). When the interaction term's coefficient were significant, it was concluded that there was a significant difference between treatments over the storage period, and ANOVA was then conducted for each period to compare treatments. Means were separated using Least Square Difference at a 95% confidence interval.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 The effect of hermetic polyethylene storage on physico-chemical properties and biochemical constituents of common beans.

Storage conditions are known to influence the nutritive value and quality of many legumes in this section, the chemical and biochemical properties of beans stored under hermetic conditions was investigated.

4.1.1 Moisture content, time, temperature, humidity and dew point monitoring in PICS and PPB storage environment

A EL-USB-2 data logger (Lascar electronics Inc., Pennsylvania, USA) designed to take data every 60 min was inserted into each of the bags before closure to monitor the temperature and relative humidity throughout the storage period. Oxygen and carbon dioxide concentrations in the PP bags and PICS®bags was taken at five days intervals using a Mocon Pac Check® Model 325 portable oxygen/carbon dioxide analyzer (MOCON Inc., Minneapolis, USA). To take measurements, the inner HDPE liner of the triple hermetic (PICS bag) was punctured with the analyzer needle at the top, center and bottom. In addition, the needle holes were then patched with 10 mm diameter adhesive pads after the measurements. Subsequent measurements were performed from the same spot by lifting and replacing the pad.

The conditions in the experimental room and inside the bags including moisture content (Table1), were measured and recorded. Temperature, humidity and dew point on, selected PICS bags and PP bags were monitored throughout the storage period as shown in figure 4, 5(a) and 5(b) respectively. The parameters i.e. Temperature, dew point and humidity were fairly constant in the PICS bags from day one to day 270. However, this was not the case with measurements recorded in the laboratory room and inside the PP bags, where the parameters kept changing with time.

Table 1: Actual recorded internal moisture in PICS bags

Variety	Moisture category	Actual recorded moisture level (%)
Nyayo	Low	11.52±0.04
	Medium	14.92±0.02
	High	18.15±0.04
Rosecoco	Low	12.16±0.04
	Medium	14.88±0.04
	High	17.56±0.04
Small Red	Low	11.62±0.04
	Medium	14.67±0.05
	High	17.48±0.00

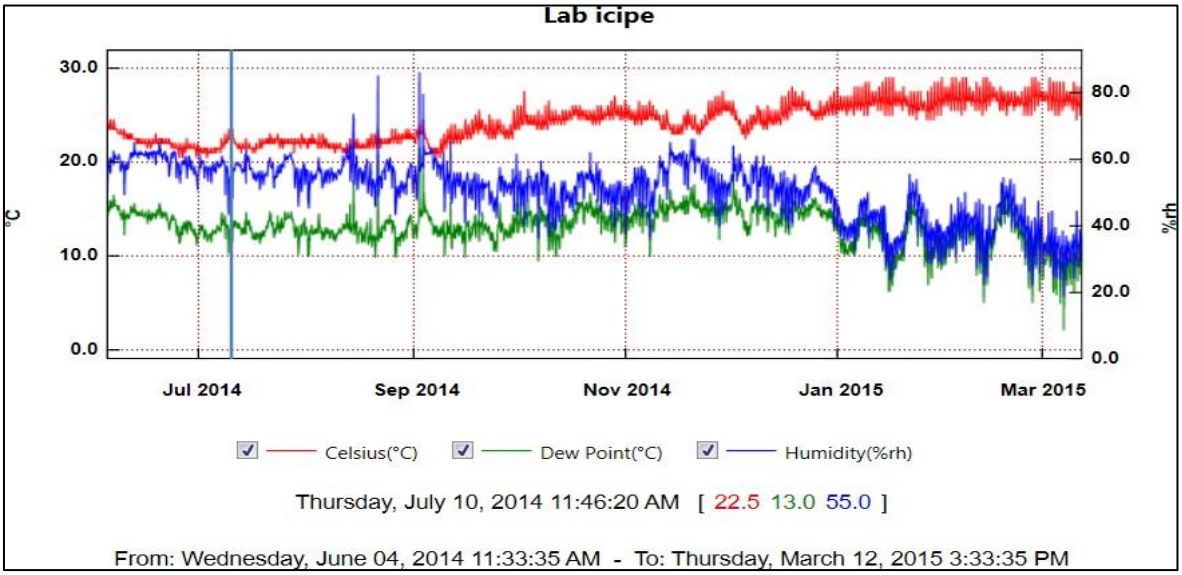
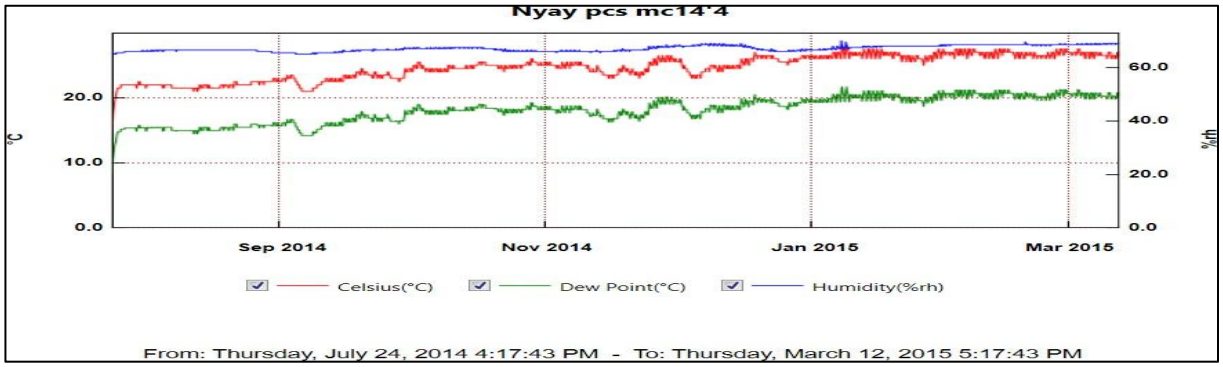


Figure 3: *Room storage conditions*

(a)



(b)

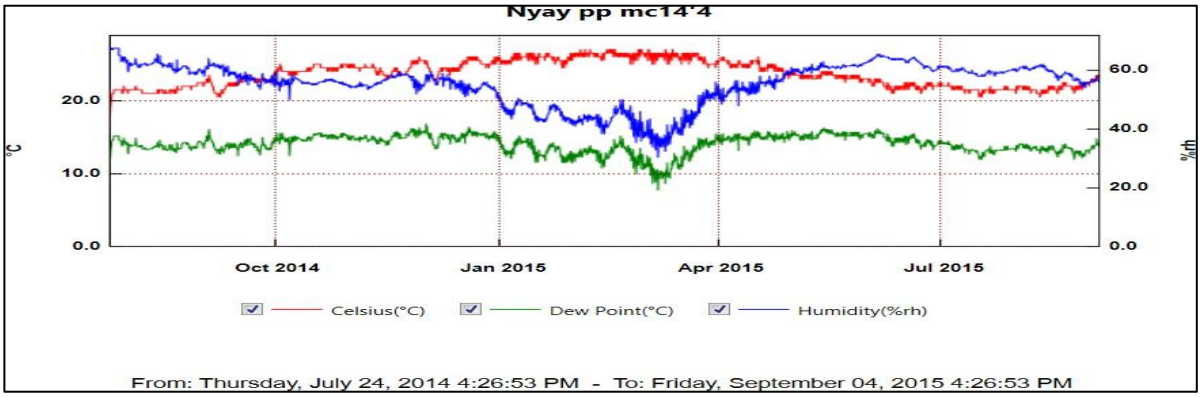


Figure 4: *Temperature, humidity and dew point monitoring for PICS (a) and PP (b) for beans stored at 14% moisture content.*

Table 2 shows the initial biochemical properties of various bean varieties. The chemical properties of the various varieties studied did not vary significantly at P≤0.05. *Rosecoco*, on the other hand, had the highest IVPD while *Nyayo* had the highest IVSD and FAN but lowest TSS even though none of the parameters were statistically different at P≤0.05.

In vitro starch digestibility is a very good nutritional indicator of the metabolic glycemic response. The values got from the three varieties studied were within the range of the most literature especially the work that was done by (Giuberti *et al.,* 2019); from the 13 bean varieties he worked on, IVSD range was (41.83-52.77).The results of this study was (48.95-50.07). However, IVPD, TSS and FAN were slightly lower in this study compared to what he achieved. Differences could be attributed to genetic diversity, farming practices and soil type where the beans were planted. These findings also agrees with (Wang *et al*., 2012). While looking at genetic diversity of common beans germplasm among different ecological zones.

Table 2: Initial biochemical properties of different bean varieties before storage

Variety	TSS (°Brix)	IVSD (%)	IVPD (%)	FAN (mM)
Nyayo	4.16±0.10[a]	50.07±1.50[a]	51.94±1.66[a]	4.09±0.14[a]
Rosecoco	4.18±0.11[a]	49.41±1.47[a]	54.74±1.50[a]	4.02±0.13[a]
Small red	4.18±0.10[a]	48.95±1.63[a]	52.24±1.42[a]	4.05±0.13[a]

Values are means ± stderr of triplicate measurements. Means with the same letter along the columns are not significantly different. TSS= Total Soluble Sugars; FAN= Free Amino Nitrogen, IVSD= *In-Vitro* Starch Digestibility, IVPD= *In-Vitro* Protein Digestibility, and Stderr= standard error.

The overall effect of storage bag type on the biochemical properties of different bean varieties is shown in Table 3. The bean variety had no significant (p> 0.05) effect on the biochemical properties of the beans but, beans stored in PICS irrespective of the variety had significantly higher ($p \leq 0.05$) TSS, IVSD, and IVPD than their counterparts stored in PPB bags. However, the storage bag type had no significant (p> 0.05) effect on FAN, phytic acid, and tannin contents. *Rosecoco* variety stored in PICS bags had the highest levels of TSS, IVSD, IVPD, and FAN compared with the other types, while the small red variety had the highest phytic acid and tannin contents. PICS do not allow moisture loss/gain and air (O_2) interaction during storage unlike PPB. Hence grains stored in pics had minimal changes in chemical properties. This is similar to the result obtained by Nkunda (2018) where storage of beans in PICS resulted in better quality beans in terms of water absorption capacity, total polyphenols and proteins, the beans were preferred by most assessors than those stored in ordinary bags. The storage bag however did not have significant difference. The possible reason for this would be that phytic acids and tannin had migrated from the seed coat to the cotyledons where they cross-linked with macro molecules or components of the cell wall and middle lamella during storage as also reported by Reyes-Moreno *et al*. (1993).

Table 3: Comparison of the mean values of biochemical properties of common bean varieties stored in two types of storage bags

Variety	Bag	TSS (°Brix)	VSD (%)	VPD (%)	FAN (mM)	Phytic acid (µg/kg)	Tannin (µg/kg)
Nyayo	PICS	4.45±0.14[a]	55.23±1.28[a]	57.89±2.17[a]	4.18±0.17[a]	1.10±0.06[a]	1.59±0.04[a]
	PPB	3.89±0.09[b]	47.68±2.24[b]	49.14±2.48[b]	4.01±0.17[a]	1.09±0.07[a]	1.60±0.03[a]
Rosecoco	PICS	4.67±0.15[a]	56.07±1.10[a]	58.69±1.88[a]	4.32±0.15[a]	1.02±0.08[a]	1.59±0.03[a]
	PPB	3.91±0.12[b]	46.07±2.16[b]	52.29±2.43[b]	3.90±0.17[a]	1.18±0.12[a]	1.66±0.04[a]
Small red	PICS	4.56±0.13[a]	55.29±2.00[a]	57.44±1.88[a]	4.12±0.15[a]	1.20±0.10[a]	1.63±0.03[a]
	PPB	3.77±0.09[b]	46.79±2.25[b]	50.17±1.71[b]	3.93±0.16[a]	1.05±0.09[a]	1.41±0.07[a]

Values are means ± stderr of triplicate measurements. Means with the same letter along the columns within each beans variety are not significantly different at $p \leq 0.05$. TSS= Total Soluble Sugars; FAN= Free Amino Nitrogen, IVSD= *In-Vitro* Starch Digestibility, IVPD= *In-Vitro* Protein Digestibility.

Overall effect of the bag, variety and moisture content of beans during storage on chemical properties is shown in Table 4. The type of storage bag had a significant effect on total soluble sugars, in-vitro starch digestibility, *in-vitro* protein digestibility and free amino nitrogen at (P=0.001). It also significantly affected Free Amino Nitrogen. Bean variety had a significant effect on only the tannin content while the level of moisture content had a significant effect on all properties except the tannin content. Beans storage time in days significantly affected the total soluble sugars and phytic acid content. In addition, total soluble sugars were significantly affected by the interaction between bean variety, moisture content of storage and type of the bag used in the storage. Lastly the interaction between bag, variety and MOC also significantly affected total soluble sugars at (p= 0.05).This results are in tandem with those realized by Mutungi *et al*. (2020) where they concluded that the interaction between bean variety, environment(moisture) and storage bag had significant effect on the parameters of beans stored.

Table 4: Mean squares of Analysis of Variance (ANOVA) of storage bag type, variety, moisture content and storage time on biochemical properties of beans

S.O.V	DF	TSS	In-vitro Starch	In-vitro Protein	Tannin	Phytic acid	Free Amino Nitrogen
Bag	1	36.097^{***}	5703.756^{***}	4125.630^{***}	0.079^{ns}	0.051^{ns}	5.694^{*}
Variety	2	0.004^{ns}	23.130^{ns}	169.542^{ns}	0.329^{**}	0.195^{ns}	0.079^{ns}
Moc	2	7.725^{***}	6102.095^{***}	4053.375^{***}	0.158^{ns}	3.764^{***}	15.288^{***}
Day	2	1.487^{*}	174.253^{ns}	159.100^{ns}	0.119^{ns}	0.994^{**}	0.416^{ns}
Bag*Variety	2	0.362^{ns}	50.987^{ns}	154.588^{ns}	0.720^{***}	0.414^{ns}	0.724^{ns}
Bag*Moc	2	0.830^{ns}	253.038^{*}	339.366^{*}	0.071^{ns}	0.167^{ns}	5.256^{**}
Variety*Moc	4	1.929^{**}	33.514^{ns}	7.792^{ns}	0.185^{*}	0.152^{ns}	1.243^{ns}
Bag*Variety*Moc	4	1.802^{**}	31.231^{ns}	136.407^{ns}	0.167^{ns}	0.291^{ns}	0.846^{ns}
Error	193	0.520	84.827	110.218	0.075	0.308	1.070

Key: TSS= Total Soluble Sugars; Moc= Moisture content; SOV= Source of Variations; FAN= Free Amino Nitrogen; ns= Not Significant; *=Significant at P≤0.05; **=Significant at P<0.01and ***=Significant at P<0.001).

Effect of type of storage bag on the TSS, *in-vitro* starch digestibility, *in-vitro* protein digestibility and free amino nitrogen is shown in Table 5. Total soluble sugars, *in-vitro* starch digestibility, in-vitro protein digestibility and free amino acids levels were significantly higher in beans stored in hermetic PICS bags than in beans stored in ordinary PPB. This means that storage environments, such as the form of storage bag used, directly influence the nutritional components of beans. PICS® bag had 22%, 23%, and 18% higher total soluble sugars, in-vitro starch digestibility, and *in-vitro* protein digestibility, respectively, than the PP bags (Table 5).

The findings in this study agreed with the results found by Bento *et al*. (2021) while looking at the factors affecting the cooking quality of carioca beans (*Phaseolus vulgaris*) during hermetic storage. He found out that browning of the grain integument and the cooking time mainly depended on the environmental conditions including storage environment. Such kind of

browning is an indicator of prior chemical reactions that may affect the chemical and nutritional composition of the stored product. Nkunda (2018) when comparing the performance the two storage bags found out that beans stored in PICS bags had significantly ($p<0.01$) a higher water absorption capacity than those stored in PP bags. This was true also to this to the findings of this research which found out that all parameters analyzed were better preserved in PICS than PPs. It therefore implied that the tightness of the PICS bags did not allow the moisture loss during storage unlike the woven PP bags. In his study also, he found out that the depletion of Total Phenolic Compounds was significantly higher for PP bags ($p<0.05$) than PICS bags. The TPC is often associated with the antioxidant activity of foods which is a nutritional advantage of PICS bags. Williams et al. (2017) also found out that maize stored in PICS had no signs of deterioration compared to the woven PPB bags in terms of specific metrics of grain quality. The storage bag type however had no significant ($p > 0.05$) effect on FAN, phytic acid, and tannin contents. This could be because the FAN, phytic acid, and tannin contents could have migrated from the seed coat to the cotyledons, where they cross-linked with macromolecules or components of the cell wall and middle lamella during storage as reported by Paredez-Lopez (1993) and Reyes-Moreno(1993)

Table 5: Mean ± Stderr of TSS, in-vitro starch digestibility, in-vitro protein digestibility and free amino nitrogen of beans between the two storage bags

Bag	TSS	In-vitro Starch	In-vitro Protein	FAN
PICS	4.58±0.09[a]	54.62±0.94[a]	57.34±1.12[a]	4.22±0.10[a]
PPB	3.76±0.05[b]	44.34±1.32[b]	48.60±1.22[b]	3.89±0.11[b]

TSS= Total Soluble Sugars; FAN= Free Amino Nitrogen, Stderr= standard error of the mean and means with same letter are not significantly different

Effect of type of beans storage bag on the TSS, in-vitro starch digestibility and in-vitro protein digestibility over a storage period of 270 days is shown in Table 6. During the whole period of storage, total soluble sugars, in-vitro starch digestibility and in-vitro protein digestibility levels were significantly higher in beans stored in hermetic PICS bags than in beans stored in ordinary PPB. Prasantha (2014) evaluated the suitability of hermetic storage for mung bean over a period of six months. The results he got are similar to those arrived at in this research. At 6-months, cooking time and grain hardness of the hermetic samples were similar to the initial samples

while those in the ordinary bag were much higher. These data also indicated that, insect infestation and HTC characteristics can be effectively controlled by hermetic storage of mung bean while maintaining its desirable market quality. The hard to cook effect experienced in ordinary bags is due to complex chemical changes which are also detrimental to bean quality and nutritional stability

.

Table 6: The Mean ± Std err of TSS, in-vitro digestible starch and in-vitro digestible protein contents in beans from PIC® bags and PP bags at different days during storage.

Component	Bag	Time (days)					
		45	90	135	180	225	270
TSS	PICS	4.28±0.91[a]	4.52±1.08[a]	5.02±0.90[a]	4.61±0.98[a]	4.51±0.98[a]	4.56±0.90[a]
	PPB	3.55±0.79[b]	3.83±0.71[b]	4.06±0.56[b]	3.73±0.62[b]	3.67±0.44[b]	3.74±0.52[b]
In-vitro starch	PICS	54.90±9.86[a]	52.35±12.3[a]	55.83±15.3[a]	54.12±4.54[a]	57.28±8.30[a]	53.02±8.96[a]
	PP	42.83±12.9[b]	42.81±15.4[b]	43.73±12.9[b]	40.17±14.4[b]	48.97±8.39[b]	47.53±14.5[b]
In-vitro protein	PICS	58.83±10.8[a]	56.83±10.5[a]	56.44±14.3[a]	52.91±12.6[a]	62.83±9.84[a]	56.17±11.1[a]
	PP	48.89±11.6[b]	49.67±10.0[b]	48.39±14.0[b]	47.89±14.6[b]	50.06±14.7[b]	46.72±12.3[b]
Free amino Nitrogen	PICS	4.31±0.24[a]	4.30±0.26[a]	4.51±0.25[a]	3.84±0.26[a]	4.32±0.27[a]	4.02±0.18[a]
	PP	3.53±0.28[a]	3.91±0.24[b]	3.86±0.27[b]	4.08±0.29[a]	3.94±0.26[a]	4.01±0.30[a]

Key: TSS= total soluble sugars, Stderr= standard error of the mean and means with same letter are not significantly different.

Interaction effect of type of storage bag and moisture content at which beans were stored on the in-vitro starch digestibility, in-vitro protein digestibility and free amino nitrogen is shown in Table 7. Beans stored in PICS bags had significantly higher levels of in-vitro starch digestibility, in-vitro protein digestibility and free amino Nitrogen than beans stored in PP bags in all storage moisture contents. However, all the parameters studied tremendously decreased with increase in moisture level regardless of the bag type. The least IVSD, IVPD and Free Amino Nitrogen compositions were recorded at 18% moisture level and the highest levels were recorded at 12% moisture level. This scenario was true also in the research conducted by Likhayo *et al.* (2018) while looking at the effect of moisture in maize stored in hermetic bags. In their conclusion, they agreed that storage in moisture level of (14–18%) in hermetic bags may pose health risk due to grain discoloration and contamination by microflora which thrive at elevated moisture levels. The case was not the same to maize stored at 12% moisture level.

Table 7: The Mean±Stderr of in-vitro starch digestibility, in-vitro protein digestibility and free amino nitrogen due to interaction between moisture content and type of bag for storage

MOC	Bag	In-vitro starch	In-vitro protein	Free amino Nitrogen
12%	PICS	59.01±0.82[a]	62.94±1.82[a]	4.42±0.15[a]
	PPB	49.52±1.61[b]	57.75±1.89[b]	4.55±0.15[b]
15%	PICS	58.80±1.02[a]	56.94±1.69[a]	4.57±0.15[a]
	PPB	51.81±1.81[b]	49.50±1.86[b]	3.64±0.17[b]
18%	PICS	46.04±1.81[a]	52.14±1.96[a]	3.66±0.19[a]
	PPB	31.68±2.24[b]	38.56±1.19[b]	3.48±0.19[b]

Key: TSS=Total Soluble sugars, MOC=Moisture Content and means with same letter are not significantly different.

Table 8 shows the interaction effect due to moisture content, storage time, and storage bags used during the study for *Nyayo* beans. At moisture content of 12%, 90 days of storage in both PICS and PBB the total soluble sugars and in vitro starch digestibility were high. In vitro protein digestibility and free amino nitrogen for the two different storage bags was higher at the interaction of day 0 and 15% moisture.

Elevated levels of phytic acid and the tannins were recorded in both bags at 12% moisture, and 45 days for *Nyayo* variety. Free amino acids nitrogen was relatively high at 18% moisture content, 270 days of storage in the PPB bags. Generally, there was significant difference in the means at $p \leq 0.05$. However, in some instances the parameters had no particular trends. From the study, it was apparent that the storage moisture, time and bag had an effect on the chemical and anti-nutrient composition of the beans, which is in line with the results obtained by Nkunda (2018), where there was an increase in cooking time in beans after storage, due to the hard-to-cook effect. The hard-to-cook effect occurs when there is impermeability and difficulty of softening of the grains as a result of the formation of metabolites/interaction of nutrients and anti-nutrients in the grains which could have occurred in this study (Uebersax & Siddiq, 2013). The inconsistent trends in the results observed could be attributed to the interaction effect of the treatments, although this needs to be investigated further.

Table 8: Biochemical properties of *Nyayo* stored in different types of bags at different moisture levels

Mc	Storage	TSS		IVSD		IVPD	
		PICS	PPB	PICS	PPB	PICS	PPB
12%	0	$4.30^{ef}\pm0.01$	$4.30^{b}\pm0.01$	$64.10^{a}\pm0.01$	$64.10^{a}\pm0.01$	$76.96^{a}\pm0.07$	$76.89^{a}\pm0.07$
	45	$3.19^{g}\pm0.00$	$4.14^{bc}\pm0.26$	$62.19^{bc}\pm0.23$	$35.47^{e}\pm1.75$	$74.78^{a}\pm3.57$	$51.61^{e}\pm5.60$
	90	$5.69^{ab}\pm0.01$	$4.61^{a}\pm0.56$	$58.29^{c}\pm3.04$	$60.12^{ab}\pm7.55$	$56.51^{de}\pm3.51$	$46.06^{f}\pm4.06$
	135	$5.43^{b}\pm0.00$	$4.31^{b}\pm0.60$	$60.31^{bc}\pm0.81$	$49.67^{c}\pm8.96$	$54.61^{e}\pm4.50$	$53.36^{e}\pm1.05$
	180	$5.69^{ab}\pm0.86$	$3.62^{d}\pm1.03$	$56.82^{cd}\pm3.73$	$52.44^{c}\pm2.43$	$61.69^{bc}\pm3.44$	$71.20^{b}\pm2.21$
	225	$2.97^{g}\pm0.13$	$3.92^{cd}\pm0.39$	$67.58^{a}\pm1.57$	$50.13^{c}\pm0.01$	$58.63^{de}\pm2.63$	$66.00^{c}\pm6.00$
	270	$3.84^{f}\pm0.65$	$4.05^{bc}\pm0.26$	$56.88^{cd}\pm4.31$	$56.36^{bc}\pm3.06$	$60.44^{e}\pm4.56$	$51.56^{e}\pm4.45$
15%	0	$4.50^{de}\pm0.00$	$4.50^{ab}\pm0.00$	$53.20^{bc}\pm0.00$	$53.20^{bc}\pm0.00$	$74.78^{a}\pm0.12$	$74.96^{ab}\pm0.12$
	45	$4.05^{f}\pm0.69$	$3.62^{d}\pm0.00$	$51.00^{de}\pm6.73$	$50.42^{c}\pm1.73$	$53.39^{e}\pm8.39$	$66.26^{c}\pm9.52$
	90	$3.84^{f}\pm0.56$	$4.18^{bc}\pm0.47$	$54.76^{cd}\pm1.05$	$50.39^{c}\pm9.03$	$45.51^{f}\pm7.50$	$60.38^{d}\pm8.38$
	135	$4.70^{d}\pm0.30$	$4.14^{bc}\pm0.52$	$60.98^{cd}\pm0.21$	$60.12^{ab}\pm2.30$	$55.66^{de}\pm2.24$	$44.50^{f}\pm4.50$
	180	$5.13^{c}\pm0.65$	$3.71^{cd}\pm0.34$	$54.33^{cd}\pm4.79$	$51.27^{c}\pm0.11$	$58.51^{de}\pm5.51$	$46.70^{f}\pm7.95$
	225	$3.92^{f}\pm0.30$	$3.66^{d}\pm0.13$	$62.76^{b}\pm2.14$	$56.16^{bc}\pm6.26$	$59.48^{d}\pm0.56$	$32.84^{h}\pm4.96$
	270	$4.14^{ef}\pm1.03$	$3.79^{cd}\pm0.00$	$58.94^{bc}\pm2.68$	$57.04^{b}\pm7.26$	$65.56^{b}\pm6.30$	$35.22^{gh}\pm1.78$
18%	0	$3.80^{f}\pm0.00$	$3.80^{cd}\pm0.00$	$61.90^{a}\pm0.00$	$61.90^{a}\pm0.00$	$50.78^{e}\pm0.22$	$50.78^{ef}\pm0.22$
	45	$4.48^{de}\pm0.43$	$2.76^{f}\pm0.09$	$46.29^{e}\pm5.09$	$26.47^{f}\pm1.84$	$27.39^{g}\pm2.61$	$35.06^{gh}\pm4.94$
	90	$4.66^{d}\pm0.09$	$3.28^{e}\pm0.17$	$49.42^{de}\pm2.51$	$41.50^{d}\pm6.18$	$54.30^{e}\pm8.94$	$38.14^{g}\pm3.64$
	135	$5.78^{a}\pm0.95$	$3.84^{cd}\pm0.22$	$35.38^{f}\pm2.93$	$20.66^{g}\pm4.27$	$51.57^{e}\pm5.35$	$32.18^{h}\pm4.84$
	180	$4.35^{e}\pm0.39$	$3.84^{cd}\pm0.99$	$52.02^{d}\pm7.17$	$28.82^{f}\pm3.18$	$58.48^{de}\pm7.52$	$30.10^{h}\pm2.90$

	225	4.96^{cd}±0.04	3.58^{d}±0.47	45.91^{e}±4.22	43.88^{d}±1.11	58.05^{de}±0.18	32.71^{h}±2.50
	270	4.01^{f}±0.65	3.97^{c}±0.34	46.80^{e}±3.56	31.14^{ef}±7.55	58.67^{de}±4.34	35.22^{gh}±0.53
		FAN		Phytic		Tannin	
		PICS	PPB	PICS	PPB	PICS	PPB
12%	0	4.47^{e}±0.01	4.47^{d}±0.01	1.27^{bc}±0.01	1.27^{c}±0.01	1.47^{b}±0.00	1.47^{ab}±0.00
	45	4.54^{e}±0.17	4.21^{fg}±1.17	1.43^{ab}±0.12	1.64^{ab}±0.08	1.38^{bc}±0.15	1.44^{ab}±0.03
	90	5.30^{b}±0.24	4.08^{g}±0.16	1.19^{bc}±0.16	1.21^{cd}±0.10	1.49^{b}±0.14	1.44^{ab}±0.03
	135	5.58^{a}±0.04	5.00^{c}±0.70	1.08^{c}±0.73	1.05^{cd}±0.22	1.59^{ab}±0.07	1.57^{ab}±0.09
	180	3.28^{l}±0.72	4.39^{de}±0.35	1.07^{c}±0.01	1.11^{cd}±0.04	1.49^{b}±0.10	1.53^{ab}±0.01
	225	3.57^{k}±0.32	5.25^{b}±0.29	1.37^{ab}±0.27	1.33^{bc}±0.26	1.78^{ab}±0.08	1.58^{ab}±0.05
	270	4.63^{de}±0.24	4.12^{g}±1.08	1.50^{ab}±0.09	1.13^{cd}±0.08	1.65^{ab}±0.05	1.82^{a}±0.05
15%	0	4.10^{h}±0.01	4.10^{g}±0.01	1.30^{bc}±0.01	1.30^{c}±0.01	1.60^{ab}±0.00	1.60^{ab}±0.00
	45	4.46^{f}±0.07	2.93^{k}±0.60	1.57^{a}±0.50	1.47^{bc}±0.10	1.81^{ab}±0.12	1.73^{a}±0.03
	90	4.97^{c}±0.08	4.23^{f}±1.31	1.25^{bc}±0.04	1.08^{cd}±0.14	1.57^{ab}±0.12	1.60^{ab}±0.015
	135	4.96^{c}±0.90	2.06^{l}±0.33	1.04^{c}±0.20	1.53^{ab}±0.35	1.67^{ab}±0.09	1.50^{ab}±0.01
	180	3.86^{i}±1.02	3.96^{g}±0.93	0.64^{d}±0.26	1.91^{a}±0.38	1.88^{a}±0.25	1.65^{ab}±0.17
	225	4.26^{g}±0.63	4.43^{d}±0.26	1.34^{b}±0.27	0.85^{de}±0.36	1.53^{ab}±0.17	1.46^{ab}±0.05
	270	4.69^{d}±0.29	4.33^{e}±0.14	0.86^{c}±0.20	0.70^{e}±0.14	1.73^{ab}±0.07	1.49^{ab}±0.11
18%	0	3.80^{ij}±0.01	3.80^{h}±0.01	1.27^{bc}±0.01	1.27^{c}±0.01	1.32^{bc}±0.00	1.32^{b}±0.00
	45	3.73^{j}±1.16	3.36^{i}±0.99	1.39^{ab}±0.25	0.37^{fg}±0.12	1.50^{ab}±0.02	1.75^{a}±0.09
	90	2.80^{m}±0.78	3.21^{j}±0.70	0.77^{d}±0.11	0.99^{d}±0.75	1.83^{ab}±0.07	1.68^{ab}±0.47
	135	3.95^{hi}±1.59	4.12^{g}±0.93	0.75^{d}±0.41	0.56^{f}±0.29	1.75^{ab}±0.24	1.53^{ab}±0.05

180	2.56^{m}±0.20	3.29^{i}±1.11	0.74^{d}±0.32	0.98^{d}±0.17	1.65^{ab}±0.03	1.64^{ab}±0.15
225	5.01^{c}±0.06	3.31^{i}±1.58	0.79^{d}±0.29	0.88^{de}±0.07	1.59^{ab}±0.09	1.77^{a}±0.19
270	3.20±1.20	5.62^{a}±0.90	0.43^{e}±0.12	0.33^{g}±0.04	1.01^{c}±0.51	1.65^{a}±0.04

The values are means ± stderr of triplicate measurements. Means with the same letter along the columns are not significantly different at $p \leq 0.05$. TSS= Total Soluble Sugars; FAN= Free Amino Nitrogen, IVSD= *In-Vitro* Starch Digestibility, IVPD= *In- Vitro* Protein Digestibility

Table 9 shows the interaction effect of moisture content, storage time in days and storage bags used for *Rosecoco* variety. Generally, throughout the storage period and all moisture levels of this variety, phytic acid and tannin content were almost the same and their difference was insignificant. At the interaction of 12% moisture content, and 135 days for the PICS bags, total soluble sugars were higher which was also noted at 12% moisture and day 0 for the PPB bags. In vitro starch digestibility was higher in the beans stored at 12% moisture in day 0 for both storage bags. The trend was similar for the in vitro protein digestibility at 12% moisture content at day 0. Higher levels of Free amino nitrogen was recorded at 12% moisture for PPB bags at day 0 and 45 days of storage while for PICS, it was higher at 15% moisture for the same period. Generally, there were significant differences in the means at $p \leq 0.05$. Similar to results for the *Nyayo* bean variety, for the *Rosecoco* variety, storage moisture, time and bag type also affected the chemical and anti-nutrient composition of the beans in no particular manner, which is in line with other studies (Nkunda, 2018).

Table 9: Biochemical properties of *Rosecoco* stored in different types of bags at different moisture levels

MC	Storage	TSS		IVSD		IVPD	
		PICS	PPB	PICS	PPB	PICS	PPB
12%	0	$5.20^{c}\pm0.00$	$5.20^{a}\pm0.00$	$63.40^{a}\pm0.01$	$63.40^{a}\pm0.00$	$79.01^{a}\pm0.01$	$79.01^{a}\pm0.01$
	45	$5.52^{bc}\pm0.95$	$4.70^{b}\pm0.99$	$58.15^{bc}\pm2.90$	$53.46^{c}\pm3.33$	$65.71^{c}\pm6.39$	$50.73^{d}\pm4.51$
	90	$5.17^{c}\pm0.34$	$4.18^{cd}\pm0.65$	$58.26^{bc}\pm3.48$	$33.49^{f}\pm1.04$	$70.7^{b}\pm7.94$	$48.00^{de}\pm5.11$
	135	$6.03^{a}\pm0.86$	$3.92^{d}\pm0.30$	$61.64^{b}\pm0.66$	$46.82^{d}\pm0.99$	$54.02^{d}\pm0.30$	$75.78^{a}\pm2.77$
	180	$5.22^{c}\pm0.30$	$4.01^{d}\pm0.04$	$51.94^{cd}\pm1.35$	$46.80^{d}\pm3.56$	$53.40^{d}\pm1.14$	$69.26^{b}\pm8.85$
	225	$5.69^{b}\pm0.60$	$3.41^{ef}\pm0.22$	$58.26^{bc}\pm1.55$	$58.25^{b}\pm3.09$	$74.44^{ab}\pm0.56$	$68.00^{b}\pm8.00$
	270	$5.30^{c}\pm0.73$	$3.23^{f}\pm0.13$	$57.81^{bc}\pm5.14$	$51.93^{cd}\pm3.98$	$62.83^{c}\pm8.83$	$58.60^{c}\pm4.83$
15%	0	$4.50^{e}\pm0.00$	$4.50^{bc}\pm0.00$	$60.00^{bc}\pm0.00$	$60.00^{ab}\pm0.00$	$61.50^{c}\pm0.00$	$61.50^{bc}\pm0.00$
	45	$3.36^{gh}\pm0.09$	$3.10^{g}\pm0.43$	$59.39^{bc}\pm2.77$	$55.38^{bc}\pm5.02$	$61.28^{c}\pm2.95$	$49.29^{de}\pm0.71$
	90	$4.83^{d}\pm1.12$	$4.05^{d}\pm0.60$	$61.00^{b}\pm6.71$	$53.05^{cd}\pm2.11$	$55.31^{d}\pm3.91$	$55.87^{c}\pm4.99$
	135	$4.83^{d}\pm0.26$	$4.14^{d}\pm0.26$	$66.38^{a}\pm2.87$	$50.07^{cd}\pm0.06$	$65.94^{bc}\pm2.17$	$55.52^{cd}\pm4.74$
	180	$4.18^{f}\pm1.25$	$3.71^{de}\pm0.17$	$58.30^{bc}\pm3.72$	$49.93^{cd}\pm4.94$	$43.51^{e}\pm6.50$	$45.26^{ef}\pm5.18$
	225	$4.66^{d}\pm1.03$	$3.92^{d}\pm0.65$	$60.62^{bc}\pm3.57$	$48.93^{d}\pm0.73$	$68.72^{bc}\pm1.72$	$45.61^{e}\pm0.39$
	270	$4.53^{e}\pm0.73$	$4.44^{c}\pm0.39$	$56.23^{c}\pm6.33$	$39.95^{e}\pm9.71$	$56.27^{d}\pm9.95$	$46.50^{de}\pm0.50$
18%	0	$5.30^{c}\pm0.00$	$5.30^{a}\pm0.00$	$55.70^{c}\pm0.00$	$55.70^{bc}\pm0.00$	$54.40^{d}\pm0.00$	$54.70^{cd}\pm0.00$
	45	$3.71^{g}\pm0.52$	$3.49^{ef}\pm0.04$	$52.34^{cd}\pm2.44$	$34.03^{f}\pm3.56$	$56.32^{d}\pm5.99$	$41.69^{f}\pm5.84$
	90	$3.97^{f}\pm0.00$	$3.58^{e}\pm0.22$	$48.48^{d}\pm2.79$	$22.04^{g}\pm2.48$	$54.93^{d}\pm7.92$	$46.22^{de}\pm4.44$
	135	$4.18^{f}\pm0.65$	$3.36^{ef}\pm0.17$	$53.54^{c}\pm1.72$	$33.54^{f}\pm9.23$	$55.27^{d}\pm4.84$	$40.46^{fg}\pm0.90$
	180	$3.32^{h}\pm0.04$	$3.41^{ef}\pm0.65$	$52.15^{cd}\pm2.25$	$16.81^{h}\pm2.52$	$47.11^{e}\pm5.16$	$36.26^{g}\pm0.41$

	225	4.18^{f}±0.22	3.53^{e}±0.34	46.20^{d}±1.49	36.35^{ef}±1.42	48.11^{e}±1.31	46.21^{de}±1.20
	270	4.35^{ef}±0.30	3.06^{g}±0.04	37.75^{e}±1.27	49.54^{cd}±0.34	43.74^{e}±7.84	43.55^{ef}±2.11
		FAN		Phytic		Tannin	
		PICS	PPB	PICS	PPB	PICS	PPB
12%	0	4.10^{g}±0.00	4.10^{f}±0.00	1.45^{a}±0.00	1.45^{d}±0.00	1.33^{b}±0.00	1.33^{b}±0.00
	45	4.85^{c}±0.27	4.76^{e}±0.29	1.44^{a}±0.11	1.71^{c}±0.14	1.62^{ab}±0.01	1.54^{ab}±0.12
	90	4.57^{e}±0.40	3.71^{i}±0.68	1.14^{bc}±0.11	1.45^{d}±0.09	1.33^{b}±0.09	1.60^{ab}0.03
	135	4.69^{de}±1.17	3.61^{j}±0.58	1.33^{ab}±0.49	0.63^{fg}±0.31	1.85^{a}±0.23	1.88^{ab}±0.01
	180	4.84^{c}±0.38	4.82^{d}±0.10	1.23^{ab}±0.15	0.82^{fg}±0.49	1.75^{ab}±0.12	1.69^{ab}±0.08
	225	5.18^{a}±0.11	5.19^{c}±0.35	1.35^{ab}±0.28	1.16^{e}±0.20	1.73^{ab}±0.24	1.94^{ab}±0.24
	270	4.60^{e}±0.10	5.62^{a}±0.33	0.96^{c}±0.63	1.11^{e}±0.01	1.67^{ab}±0.08	1.70^{ab}±0.10
15%	0	5.30^{a}±0.00	5.30^{b}±0.00	0.78^{c}±0.00	0.78^{fg}±0.00	1.54^{ab}±0.00	1.54^{b}±0.00
	45	5.13^{ab}±0.42	3.81^{h}±1.07	1.08^{bc}±0.75	1.74^{c}±0.29	1.64^{ab}±0.15	1.56^{b}±0.10
	90	4.35^{f}±0.33	3.71^{i}±1.35	0.65^{c}±0.31	1.17^{e}±0.00	1.50^{ab}±0.02	1.57^{ab}±0.06
	135	4.85^{c}±0.96	3.94^{g}±0.09	1.23^{ab}±0.03	2.22^{b}±1.88	1.82^{a}±0.01	1.83^{ab}±0.17
	180	3.63^{j}±0.38	3.55^{jk}±1.18	1.13^{bc}±0.50	0.81^{fg}±0.24	1.51^{a}±0.04	1.60^{b}±0.15
	225	4.07^{g}±1.64	2.86^{o}±0.02	1.36^{ab}±0.21	0.79^{fg}±0.30	1.55^{a}±0.09	2.04^{a}±0.02
	270	3.70^{ij}±0.28	3.15^{m}±0.60	1.21^{b}±0.13	2.52^{a}±1.50	1.49^{ab}±0.03	1.64^{b}±0.06
18%	0	3.90^{h}±0.00	3.90^{gh}±0.00	0.40^{d}±0.00	0.40^{g}±0.00	1.42^{ab}±0.00	1.42^{b}±0.06
	45	2.82^{m}±0.06	3.02^{n}±0.90	0.98^{c}±0.69	1.56^{cd}±0.08	1.51^{ab}±0.17	1.71^{ab}±0.07
	90	3.18^{l}±0.96	3.26^{i}±0.65	0.80^{c}±0.39	0.85^{f}±0.66	1.73^{ab}±0.07	1.79^{ab}±0.15
	135	4.74^{d}±0.81	2.53^{p}±0.08	1.05^{bc}±0.63	0.62^{g}±0.20	1.71^{ab}±0.02	1.83^{ab}±0.22

180	5.04^{b}±0.83	3.02^{n}±0.86	0.30^{d}±0.04	0.84^{fg}±0.10	1.63^{ab}±0.06	1.73^{ab}±0.05
225	3.79^{i}±0.45	3.48^{k}±0.92	0.63^{c}±0.05	0.65^{fg}±0.12	1.50^{ab}±0.18	1.89^{ab}±0.05
270	3.41^{k}±0.80	3.42^{k}±0.33	0.96^{c}±0.08	1.74^{c}±1.25	1.48^{ab}±0.20	1.73^{ab}±0.21

The values are mean ± stderr of triplicate measurements. Means with the same letter along the columns are not significantly different at $p \leq 0.05$. TSS= Total Soluble Sugars; FAN= Free Amino Nitrogen, IVSD= *In-Vitro* Starch Digestibility, IVPD= *In-Vitro* Protein Digestibility.

As shown in Table 10, largely, tannins were higher throughout the storage period and all moisture levels in PICS bags. On day 0, the PICS bags depicted relatively higher *in vitro* starch digestibility at 12% and 15% moisture levels. Equally, on days 0 and 90, TSS was higher at 12% and 15% moisture levels, respectively. FAN was higher at 12% moisture level on 135 for the PPB bag and day 45 at 15% moisture. Phytic was seen to be higher at 12% moisture on day 0 for both PICS and PBB. There was a significant difference in the means at $p \leq 0.05$.For the other varieties, the results for the small red bean variety indicates that the storage moisture, time and bag type had no particular pattern on their effect on the chemical and anti-nutrient composition. This could indicate an interaction effect as observed for the other bean varieties in this study.

Table 10: Biochemical properties of small red beans stored in different types of bags at different moisture levels stored for up to 270 days

MC	Storage	TSS		IVSD		IVPD	
		PICS	PPB	PICS	PPB	PICS	PPB
12%	0	$4.10^{e}\pm0.00$	$4.10^{b}\pm0.00$	$68.40^{ab}\pm0.00$	$68.40^{b}\pm0.00$	$73.89^{a}\pm0.22$	$73.89^{a}\pm0.22$
	45	$4.74^{cd}\pm0.78$	$3.28^{de}\pm0.43$	$63.47^{bc}\pm8.31$	$55.51^{d}\pm2.63$	$63.55^{b}\pm3.45$	$54.84^{c}\pm6.17$
	90	$4.05^{e}\pm0.26$	$3.84^{bc}\pm0.30$	$55.18^{c}\pm1.08$	$42.55^{f}\pm7.18$	$61.34^{bc}\pm7.77$	$60.12^{bc}\pm6.12$
	135	$5.43^{b}\pm0.86$	$4.35^{ab}\pm0.47$	$57.46^{c}\pm2.11$	$51.19^{de}\pm1.06$	$76.18^{a}\pm3.82$	$45.49^{de}\pm0.08$
	180	$4.61^{cd}\pm0.13$	$3.71^{c}\pm0.34$	$55.77^{c}\pm3.42$	$41.90^{f}\pm1.19$	$52.59^{cd}\pm0.75$	$52.37^{cd}\pm3.37$
	225	$5.47^{b}\pm0.47$	$3.58^{cd}\pm0.04$	$62.74^{bc}\pm3.16$	$56.61^{cd}\pm2.02$	$72.90^{a}\pm1.88$	$53.51^{c}\pm5.51$
	270	$5.86^{a}\pm0.34$	$3.92^{bc}\pm0.04$	$59.34^{c}\pm3.99$	$48.67^{e}\pm5.13$	$58.78^{bc}\pm7.00$	$64.35^{b}\pm1.10$
15%	0	$4.30^{de}\pm0.00$	$4.30^{ab}\pm0.00$	$73.20^{a}\pm0.00$	$73.20^{a}\pm0.00$	$62.26^{bc}\pm0.15$	$62.26^{b}\pm0.15$
	45	$4.87^{c}\pm0.13$	$3.97^{bc}\pm0.69$	$59.16^{c}\pm2.72$	$48.02^{e}\pm1.64$	$53.23^{cd}\pm4.88$	$48.77^{d}\pm2.77$
	90	$5.78^{a}\pm0.60$	$3.92^{bc}\pm0.56$	$54.58^{d}\pm2.12$	$54.13^{d}\pm0.84$	$59.86^{bc}\pm9.86$	$54.32^{c}\pm5.91$
	135	$4.44^{d}\pm0.47$	$4.57^{a}\pm0.34$	$66.05^{b}\pm8.66$	$48.76^{e}\pm5.83$	$42.89^{e}\pm5.89$	$43.00^{e}\pm5.79$
	180	$5.22^{b}\pm0.39$	$3.84^{bc}\pm0.39$	$52.92^{de}\pm2.79$	$47.04^{e}\pm2.62$	$60.12^{bc}\pm4.88$	$41.63^{ef}\pm3.63$
	225	$4.83^{c}\pm0.34$	$3.66^{c}\pm0.30$	$63.39^{bc}\pm2.62$	$52.67^{de}\pm4.47$	$63.17^{b}\pm3.05$	$55.50^{c}\pm5.50$
	270	$4.83^{c}\pm0.43$	$3.79^{c}\pm0.17$	$57.61^{c}\pm2.27$	$59.29^{c}\pm9.28$	$57.02^{c}\pm3.02$	$55.04^{c}\pm7.03$
18%	0	$4.00^{e}\pm0.00$	$4.00^{bc}\pm0.00$	$49.20^{l}\pm0.00$	$49.20^{e}\pm0.00$	$51.11^{d}\pm0.23$	$51.11^{cd}\pm0.23$
	45	$4.53^{d}\pm0.13$	$2.97^{e}\pm0.47$	$42.13^{f}\pm4.92$	$26.67^{i}\pm5.35$	$47.10^{d}\pm3.10$	$41.40^{ef}\pm4.60$
	90	$2.72^{g}\pm0.13$	$2.80^{e}\pm0.13$	$31.14^{g}\pm3.54$	$28.04^{i}\pm7.09$	$53.18^{cd}\pm9.82$	$38.15^{f}\pm5.35$
	135	$4.35^{de}\pm0.47$	$3.88^{bc}\pm0.69$	$40.70^{f}\pm9.18$	$32.69^{h}\pm9.45$	$52.50^{cd}\pm2.51$	$46.83^{de}\pm4.37$

	180	3.71^{f}±0.34	3.62^{cd}±0.26	52.82^{de}±1.67	26.53^{i}±8.00	41.00^{e}±0.22	38.63^{ef}±2.41
	225	3.92^{ef}±0.47	3.75^{c}±0.56	49.91^{e}±2.23	37.77^{g}±0.54	62.29^{bc}±7.35	41.00^{ef}±9.00
	270	4.01^{e}±0.04	3.36^{d}±0.60	45.82^{ef}±9.34	33.84^{gh}±8.47	41.38^{e}±7.37	31.52^{g}±2.73
		FAN		Phytic		Tannin	
		PICS	PPB	PICS	PPB	PICS	PPB
12%	0	3.80^{i}±0.00	3.80^{gh}±0.00	2.67^{a}±1.34	2.67^{a}±1.34	1.26^{bc}±0.00	1.26^{bc}±0.00
	45	4.87^{d}±0.19	4.54^{d}±0.35	1.38^{cd}±0.09	1.67^{c}±0.27	1.68^{ab}±0.07	1.70^{ab}±0.05
	90	4.22^{g}±1.03	5.01^{c}±0.76	0.90^{ef}±0.12	1.54^{cd}±0.17	1.62^{ab}±0.13	1.58^{ab}±0.08
	135	3.75^{i}±0.77	5.43^{a}±0.60	1.88^{b}±0.24	0.75^{f}±0.02	1.56^{b}±0.08	1.89^{a}±0.12
	180	3.05^{h}±0.19	4.10^{f}±1.51	1.55^{c}±0.40	1.01^{e}±0.13	1.70^{ab}±0.15	1.67^{ab}±0.07
	225	4.29^{fg}±0.44	3.89^{g}±0.43	0.87^{ef}±0.47	0.44^{gh}±0.17	1.79^{ab}±0.21	1.76^{ab}±0.10
	270	3.86^{hi}±0.83	4.20^{e}±0.17	1.19^{d}±0.51	1.65^{c}±0.10	1.97^{a}±0.07	1.51^{b}±0.06
15%	0	4.20^{g}±0.00	4.20^{e}±0.00	1.48^{c}±0.00	1.48^{cd}±0.00	1.38^{b}±0.00	1.38^{bc}±0.00
	45	5.43^{a}±0.44	2.06^{l}±0.07	1.73^{bc}±0.20	1.49^{cd}±0.14	1.69^{ab}±0.08	1.26^{bc}±0.59
	90	5.02^{c}±1.50	4.46^{d}±0.43	1.41^{cd}±0.00	1.31^{d}±0.00	1.53^{ab}±0.25	1.06^{c}±0.53
	135	4.55^{e}±0.03	3.96^{fg}±0.05	1.18^{d}±0.64	0.64^{fg}±0.26	1.86^{ab}±0.03	0.87^{c}±0.80
	180	4.88^{d}±0.66	4.21^{e}±1.33	0.77^{ef}±0.13	0.61^{fg}±0.18	1.49^{b}±0.04	1.23b^{c}±0.42
	225	5.30^{b}±0.41	4.19^{ef}±0.08	1.01^{de}±0.28	0.91^{ef}±0.07	1.61^{ab}±0.05	1.93^{a}±0.16
	270	4.16^{g}±0.15	3.77^{h}±0.16	1.37^{cd}±0.38	0.82^{ef}±0.25	1.56^{b}±0.05	1.73^{ab}±0.01
18%	0	3.60^{i}±0.00	3.60^{i}±0.00	1.40^{cd}±0.00	1.40^{d}±0.00	1.55^{b}±0.00	1.55^{ab}±0.00
	45	2.93^{m}±0.49	3.12^{j}±1.07	1.20^{d}±0.26	0.64^{fg}±0.35	1.79^{ab}±0.05	1.07^{c}±0.32
	90	4.33^{f}±0.15	3.55^{i}±0.30	0.79^{ef}±0.50	0.90^{ef}±0.02	1.34^{b}±0.22	1.09^{c}±0.46

135	3.59^{j}±0.01	4.24^{e}±0.16	0.68^{ef}±0.30	0.51^{g}±0.07	1.65^{ab}±0.03	0.86^{c}±0.43
180	3.42^{k}±0.91	5.38^{b}±0.16	0.59^{f}±0.32	0.62^{fg}±0.16	1.63^{ab}±0.00	0.99^{c}±0.46
225	3.42^{k}±1.47	2.88^{k}±0.11	0.62^{f}±0.19	0.24^{h}±0.02	1.91^{ab}±0.16	1.40^{bc}±0.05
270	3.91^{h}±0.08	1.89^{m}±0.51	0.66^{f}±0.34	2.18^{b}±1.10	1.70^{ab}±0.34	1.76^{ab}±0.16

Values are mean± stderr of triplicate measurements. Means with the same letter along the columns are not significantly different at $p \leq 0.05$. TSS= Total Soluble Sugars; FAN= Free Amino Nitrogen, IVSD= *In-Vitro* Starch Digestibility, IVPD= *In-Vitro* Protein Digestibility

Principal component analysis (PCA) was done for the *Nyayo* bean variety to determine the variables which were strongly correlated with each component, where a correlation above 0.5 was deemed important (Fig. 1) (Coradi *et al*., 2020). Biplot for TSS, IVPD, and phytic acid indicated that PICS loaded positively for both component 1 and component 2 while for PPB, they loaded positively for component 1 but negatively for component 2 (Fig. 1A, C and E). This was an indication that for PICS samples, values for TSS, IVPD, and phytic acid could vary with storage period and moisture content while for PPB stored samples, values could not be affected by storage period but reduced with storage moisture content of the grains. The first PC had large positive associations with 12% storage moisture content and shorter storage period (A-E) but a negative association with 18% moisture content (for TSS, IVPD, and phytic acid), so this component measured TSS, IVPD, and phytic acid retention in the beans depending on storage moisture and duration. The second component had large negative associations with 12% moisture content for TSS, 15 and 18% moisture contents for IVPD, and 15% for phytic acid, so this component primarily measured TSS and IVPD as dependent on the storage moisture content of the beans.

The biplots for IVSD and tannins indicated that PPB loaded positively for both PC1 and PC 2 while for PICS, it loaded positively for PC 1 but negatively for PC 2 (Fig. 1B and F). This was an indication that for PPB samples, values for IVSD and tannins varied with both storage period and moisture content while for PICS stored samples, values could not be affected by storage period but reduced with storage moisture content of the grains. The first PC had large positive associations with the lower storage moisture content (12 and15%) and shorter storage period (18A) for IVSD but with high storage moisture contents (15 and 18%) and longer storage period (12 F and 12G) for tannins. PC 1 had a negative association with 18% storage moisture content for IVSD but with low storage moisture content (12%) and shorter storage period (A-C) for tannins. The first PC therefore measured IVSD and tannins of the beans dependent on the storage moisture and duration. The second PC had negative associations with 12F, 12B and 18E for IVSD and 15E, 15G and 18D, so this PC measured IVSD and tannins as dependent on the storage moisture content of the beans.

Biplots for FAN indicated that PICS loaded positively for both PC1 and PC2 while for PPB, it loaded negatively for PC1 but positively for PC2 (Fig. 1D). This was an indication that for PICS samples, values for FAN varied with both storage period and moisture content while for PPB

stored samples, values could not be affected by storage period but reduced with storage moisture content of the grains. The first PC had large positive associations with the higher storage moisture content (15–18%) and medium storage period (B, D, and F) but a negative association longer storage period (12F and 18G). This component therefore measured FAN in the beans depending on storage moisture and duration. The second PC had negative associations with 18B, 18C and 18E, so this PC measured FAN as dependent on the storage moisture content of the beans. This indicated that for samples stored in PICS, FAN was protected with little variation while those in PPB could be affected most by storage moisture but little affected with storage period with FAN retention being most at 12% moisture content.

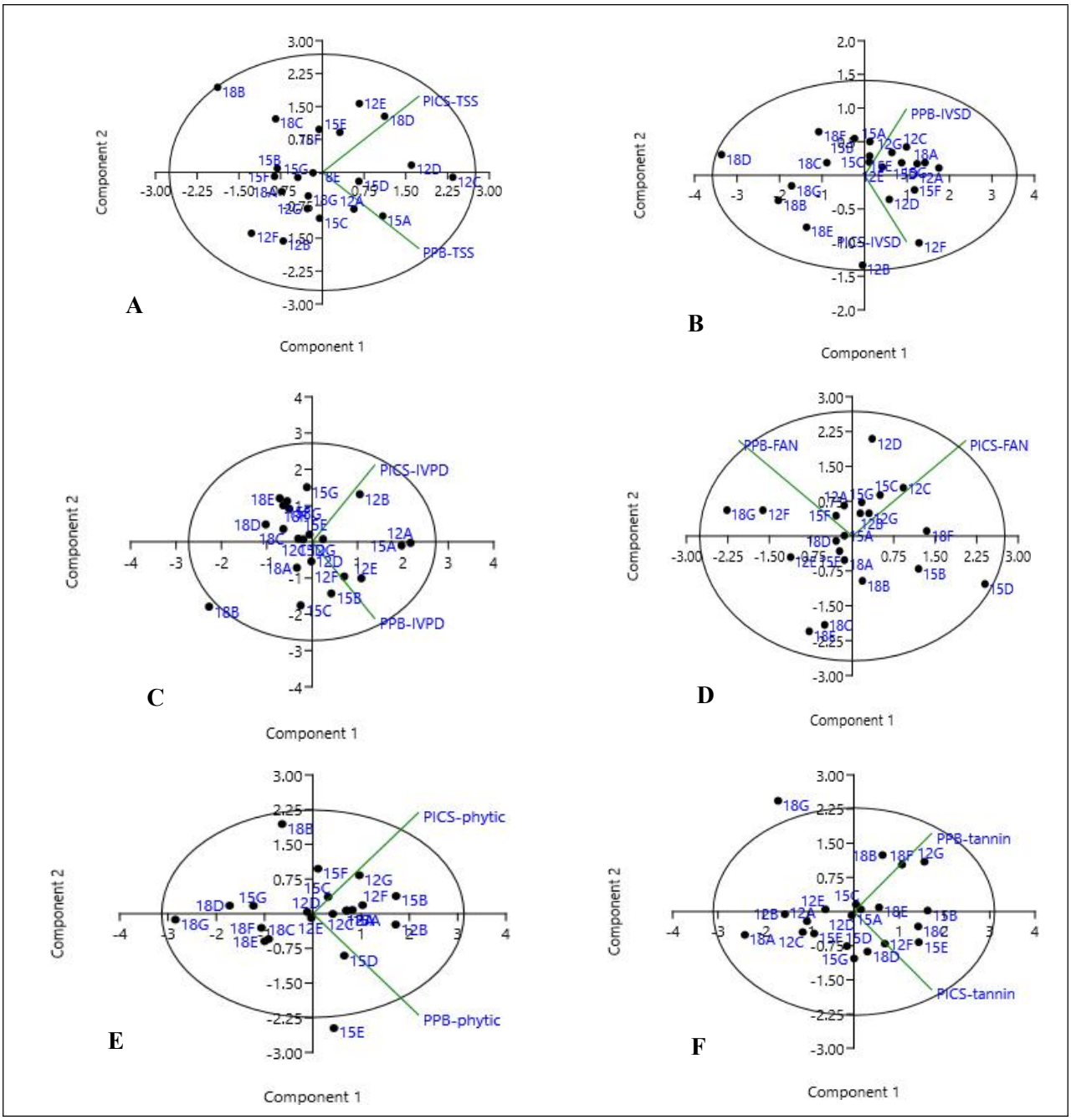


***Figure 5*:(A-F):** *Biplots for Principal component Analysis*

Figure Legends: Principal component analysis biplots of stored *nyayo* beans quality under PICS and PPB bags. Ellipses are the 95% confidence boundaries. 12, 15 and 18 are percentage storage moisture contents. The alphabets in the figures are bean storage periods (A, B, C, D, E, F, and G are for 0, 45, 90, 135, 180, 225, and 270 days, respectively). Figures; **A**, Total soluble solids (TSS); **B**, *in- vitro* starch digestibility (IVSD); **C**, *in-vitro* protein digestibility (IVPD); **D**, Free amino Nitrogen (FAN); **E**, phytic acid; **F**, tannins.

Effect of beans variety on the tannin content is shown in Figure 7. *Rosecoco* variety had significantly higher levels of tannin than small red variety when checking the beans stored in PPB bags. However, no difference was noted in the PICS bags in terms of tannin content. Small red has a thick skin and most beans with thick skin also have higher content of tannin content according to Giuberti *et al.* (2019) and Lamichaney *et al.* (2019). Lectins, phytic acid and condensed tannins exert major anti-nutritional effects in common bean when grains are consumed as a staple food.

They are endowed with biological activities for health that can be beneficial or exert serious anti-nutritional effects. It is voted to contribute to inhibitory effect of tannins on Fe bio-availability at the intestinal level.

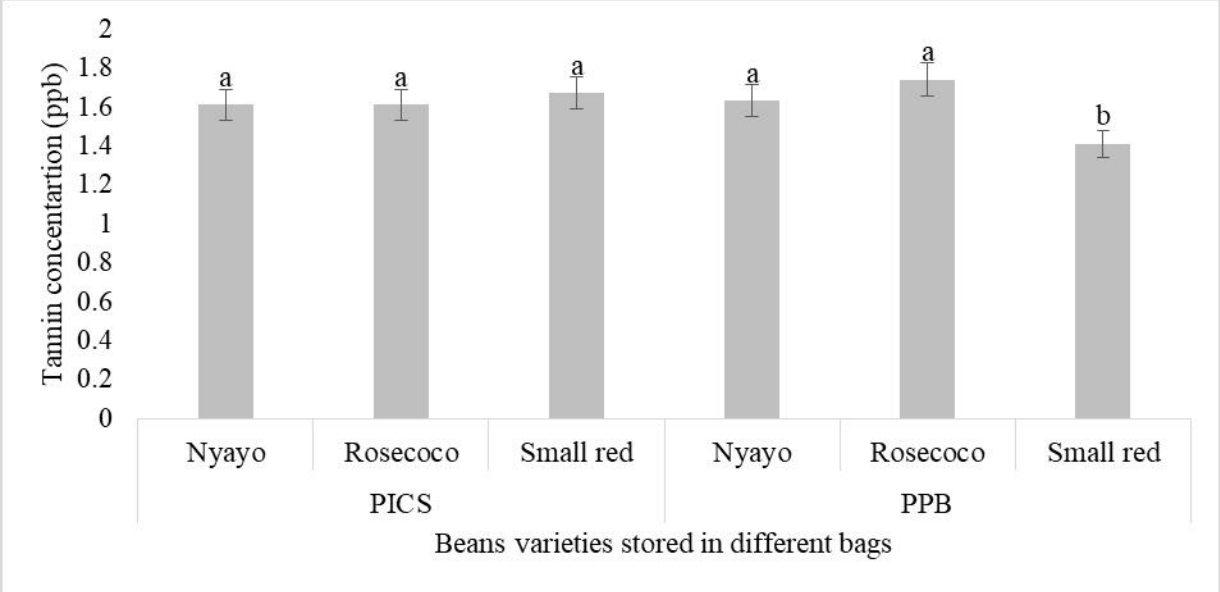


Figure 6: *Effect of bean variety on the tannin content*

The total soluble sugars (TSS), high *in-vitro* starch digestibility and *in-vitro* protein digestibility, and free amino nitrogen are the beans' desired nutritional parameters. Simultaneously, tannin and phytic acid are the anti-nutritional components that adversely affect the bio availability of the dietary ingredients.

The PICS® bag was significantly different from PP bags at $p<0.001$ on the effect of total soluble sugars, in-vitro starch digestibility, and in-vitro protein digestibility shown in Table 3. This indicates that the storage condition, i.e., the type of storage bag, has a significant effect on the beans' nutritional components. The PICS® bag had 22%, 23%, and 18% higher total soluble sugars, in-vitro starch digestibility, and in-vitro protein digestibility, respectively, than PP bags

during storage (Table 5). Therefore, the hermetic storage technology was superior to the ordinary bags in the preservation of the nutritional components. For both types of bags, there is a gradual increase of TSS from day 45 up to day 135 and then followed by a gradual decrease to day 270. The in-vitro starch digestibility remained relatively the same during the storage period for both bags; however, day 225 had the highest content. Similarly, the in-vitro digestible protein behaved the same way as in-vitro starch digestibility (Table 5). This indicates that there are continual biochemical changes during the storage of beans, which also continually alter the composition of the components.

Beans stored at 18% moisture content were found to have significantly lower total soluble solids from those held at 12% and 15% moisture content by 14% and 10%, respectively (Table .8). In addition, the beans stored at 18% moisture content had significantly lower *in-vitro* starch digestibility content by 40% and 42% compared to beans stored at 12% and 15% moisture content. However, the protein content varied significantly at $p \leq 0.05$ among beans at all moisture contents during the storage. The in-vitro protein digestibility was reduced considerably, with an increase in beans' moisture content during storage (Table 8). This shows that storage conditions affect the components in beans and, by extension, the nutritional content.

In addition, the duration of beans storage affects the beans' components, whereby the beans stored in PICS® bags had optimal starch and protein content at day 225 and only proteins for PP bags. The beans stored at 12% moisture content had the highest in-vitro starch digestibility and in-vitro protein digestibility, while the ones stored at 18% had the least for PICS® bags (Table 3). On the contrary, for the PP bags, beans stored at 15% moisture content had the highest *in-vitro* starch digestibility and *in-vitro* protein digestibility than beans stored at 12% and 18%. However, the storage in PICS® at 12% had significantly higher *in-vitro* starch digestibility and *in-vitro* protein digestibility than beans stored at 15% in PP bags. Therefore, it can be concluded that the hermetic storage bags are superior in retaining nutrients to PP bags.

On the other hand, the type of bag did not significantly affect the beans' tannin and phytic acid content during the storage period. The tannin content was marginally higher in the PICS® bag by 2.5%, but the phytic content was marginally lower by 2.8% compared to beans stored in PP beans over the storage period. Tannin content was found to vary significantly at $p<0.01$ among

the beans' varieties, whereby the mean tannin content was 1.62±0.24, 1.68±0.20, and 1.54±0.41% for *Nyayo*, Rose coco, and Small red bean varieties, respectively.

4.2 The effect of hermetic polyethylene bag storage on the texture and cooking quality of common beans.

The study found out that the hermetic bag was significant in reducing the punching pressure of cooked beans compared to ordinary storage bags showing that the PICS® technology had a lesser effect on hardness (hard-to-cook phenomena) beans stored in it were quickly cooked. At higher moisture levels and more extended storage periods, PPB had higher texture values than PICS, indicating that PICS bags may perform much better in reducing the bean hardness even at higher moisture storage environments.

Different studies have indicated that storage conditions, time and moisture content of stored beans affected their texture and cooking quality (Sánchez-Arteaga *et al*., 2015). In this section, texture, cooking quality and activation energy needed during cooking of common beans stored hermetically were investigated.

4.2.1 The effect of storage bag and storage time on the texture and cooking quality of bean

The mean texture for bean varieties at different moisture contents, stored for different number of days and cooked at different times are shown in Table 11. The storage bag significantly ($p < 0.05$) affected the texture of stored beans. The study found out that the hermetic bag was responsible for significantly low punching pressure in cooked beans as compared to ordinary storage bags. The beans stored in PICS were much easy to cook. The overall punching force values for PICS was 186.88 ± 4.31 which was significantly lower than that for PPB bags which was 206.06 ± 5.47. PICS bags showed the lowest force values for all bags except for small red variety where the texture values were not significantly different. Freitas *et al.* (2011) while assessing the Quality of kidney beans stored under hermetic conditions at constant moisture and high and low temperature for 6 months concluded that hardness, fracturability, gumminess, chewiness, springiness and cohesiveness were higher in samples stored at elevated temperatures than those stored at hermetic conditions. Puncture forces followed approximately a normal distribution curve, and there was always some overlap between hermetically stored and control beans.

Storage time significantly affected the texture and cooking quality of beans as shown in figure 8. Longer storage resulted in an increase in punching pressure/force. These results are consistent

with literature which states that cooking quality of beans deteriorates rapidly with storage at ambient conditions (23–25 °C and 30–50% relative humidity and worsens with time (Santos *et al*., 2013). Punching force at day zero was 162.10 ± 7.85 and 162.99 ± 7.84 for PICS and PPB bags. Statistically, these values were no significantly different. The punching force increased to 204.18 ± 8.77 and 229.74 ± 12.52 after 135 days of storage in the PICS and PPB bags respectively. Despite the increase in both storage bags with time, the increase was significantly higher in the beans stored in PPB than in PICS.

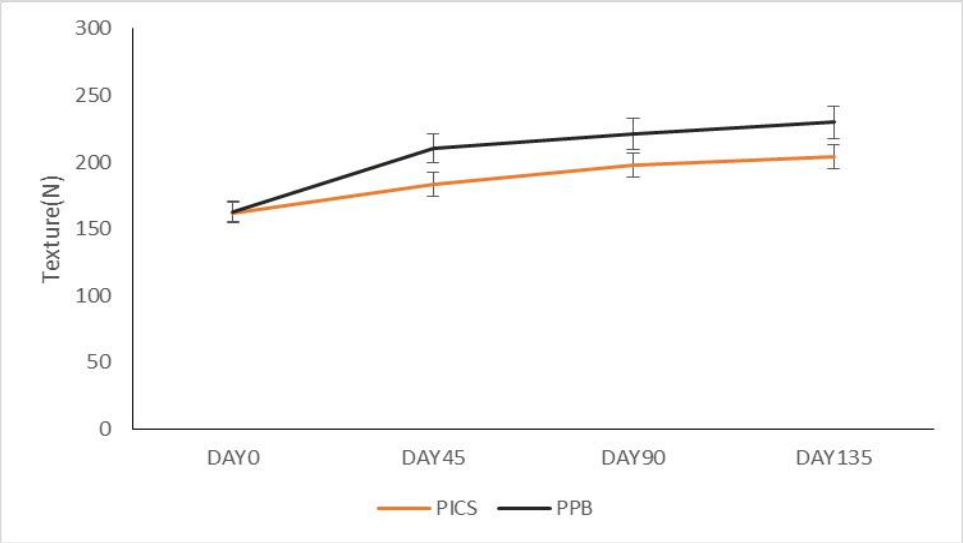


Figure 7: *The effect of storage time in different bags on cooked bean texture*

Table 11: Comparison of texture (punching pressure) in terms of variety, moisture content, cooking time, and storage time in both PICS and PPB storage bags.

		PICS	PPB
Variety	*Nyayo*	188.34 ± 8.37^{ab}	205.89 ± 9.79^{b}
	Rosecoco	196.04 ± 8.67^{a}	227.07 ± 11.01^{a}
	Smallred	176.26 ± 4.67^{b}	185.24 ± 7.14^{c}
Moisture content	12%	211.00 ± 10.25^{a}	224.67 ± 11.24^{a}
	15%	179.09 ± 6.05^{b}	204.06 ± 9.00^{b}
	18%	170.55 ± 4.88^{b}	189.46 ± 7.83^{c}
Cooking Time (Min)	30	434.27 ± 15.96^{a}	544.03 ± 17.41^{a}
	60	184.14 ± 3.15^{b}	193.70 ± 3.18^{b}
	90	144.65 ± 3.02^{c}	142.29 ± 2.19^{c}
	120	126.56 ± 2.94^{d}	131.37 ± 8.41^{cd}

	150	120.11 ± 4.12^{d}	114.22 ± 3.29^{de}
	180	111.53 ± 3.93^{d}	110.77 ± 4.97^{e}
Temperature	75 ^{o}C	185.80 ± 5.99a	213.10 ± 9.22a
	85 ^{o}C	192.56 ± 5.05a	206.21 ± 6.96a
	95 ^{o}C	182.27 ± 10.30a	198.89 ± 11.68a
Storage time (Days)	0	162.10 ± 7.85^{c}	162.99 ± 7.84^{c}
	135	204.18 ± 8.77^{a}	229.74 ± 12.52^{a}
	45	183.60 ± 8.86^{b}	210.41 ± 11.20^{b}
	90	197.63 ± 8.82^{ab}	221.11 ± 11.34^{ab}

Means in the same column for each variable with the same superscript are not significantly different (P>0.05)

The variety of beans stored significantly affected the resulting texture. This agrees with Wainaina *et al.* (2021), who found that bean cultivars required different cooking times to achieve the same softness. Comparing PICS and PPB storage for each bag revealed that the PPB bags' texture was significantly more demanding than in PICS bags in both *Rosecoco* and *Nyayo* varieties. These results confirm earlier findings by Turner *et al*. (2020) who in their study to evaluate the hard-shell percentage in seven common beans (*Phaseolus vulgaris* L) cultivars, by using the Burr cooking method before and after soaking, they found significant differences ($p<0.05$) among the cultivars concerning hard-shell and cooking time. However, the storage bag did not significantly affect the texture in Small red varieties.

Nevertheless, the texture was higher in the PPB bag. This shows that varieties respond differently to different storage conditions. In this research, Small red was least affected by the storage time and conditions. In contrast, *Rosecoco* and *Nyayo* were highly involved, resulting in a massive variation in stored beans' texture. Generally, storage conditions influence the quality of many legumes (Gu *et al*., 2022). According to Diaz *et al.* (2021), the lower storage temperature can control the Hard to Cook effect for both bean varieties. Overall, phytate content can be an indicative factor for the cookability of beans when the relationship between variety and storage conditions has been determined.

In this research, storage conditions significantly affected the texture of stored beans in both storage bags. PICS technology achieved significantly lower punching force values at all moisture

levels as compared to the PPB except at the 12% moisture level in which there was no significant difference in the mean punching force between the two bags. This indicates a significant interaction between beans' hardness and texture during storage for both PICS and PPB bags.

4.2.2 The effect of bean variety and storage conditions on the texture and cooking quality

The results of the effect of bean variety and storage conditions on texture of beans is shown in Figure 9 below. The variety of beans used significantly affected the resulting texture. In both bags, *Rosecoco* had the highest significant punching pressure reading at 196.04±8.67 and 227.07±11.01 followed by *Nyayo* at 188.34±8.37 and 205.89±9.79 and Smallred 176.26± 4.67 and 185.24 ± 7.14 in PICS and PPB bags respectively. However, the punching pressure in PICS for *Rosecoco* and *Nyayo* were not significantly different.

Small red had least punching pressure in both bags as shown in figure 9. These results confirm earlier findings by Corrêa *et al*. (2010). In their study to evaluate the hard-shell percentage in seven common beans (*Phaseolus vulgaris L*) cultivars, by using the Burr cooking method before and after soaking, they found significant differences ($p<0.05$) among the cultivars in relation to hard-shell and cooking time. However, storage bag did not significantly affect the texture in Small red varieties. Nevertheless, texture was higher in the PPB bag. This shows that varieties respond differently to different storage conditions. In this research, Small red was least affected by the storage time and conditions, while *Rosecoco* and *Nyayo* were highly affected resulting in a huge variation in texture of stored beans. Generally, storage conditions are known to influence the quality of many legumes (Paredes-López, 1993; Reyes-Moreno 1993). In this research, storage conditions significantly affected the texture of stored beans in both storage bags. These results also are in tandem with those obtained by Kinyanjui *et al.* (2015) while studying hard to cook varieties and easy to cook common bean varieties.

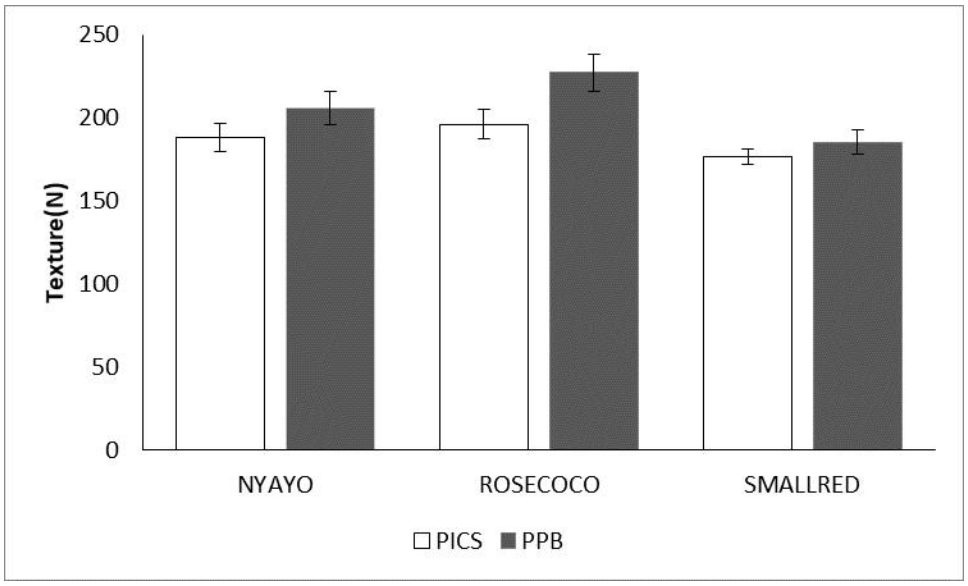


Figure 8: *The effect of bean variety on the texture of cooked beans.*

The moisture content of stored beans significantly affected the texture and cooking quality of beans as shown in figure 10. Lower moisture content resulted in punching force values in both PICS and PPB storage bags.

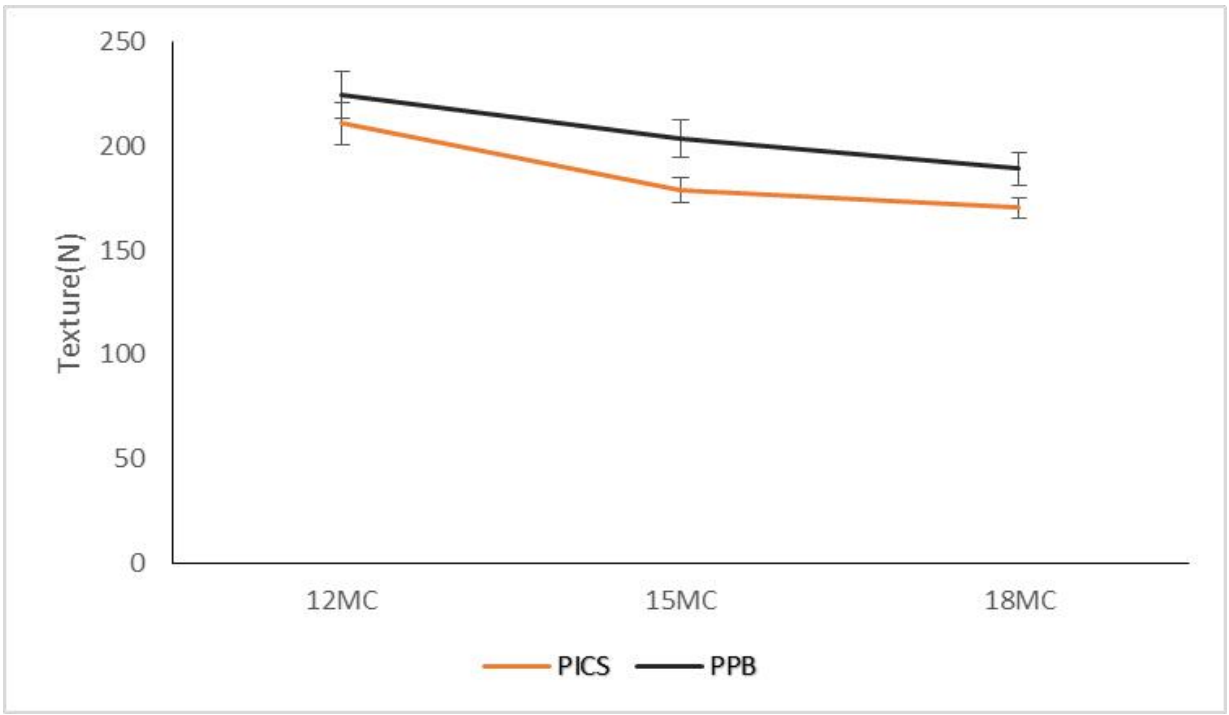


Figure 9*: The effect of storage moisture content on texture of cooked beans*

Increasing the moisture content seemed to reduce the texture value. Punching force values were highest at 12% moisture content at 211.00 ± 10.25 and 224.67 ± 11.24 and lowest in 18% moisture at 170.55 ± 4.88 and 189.46 ± 7.83 in PICS and PPB bags respectively.

PICS technology achieved significantly lower punching pressure values at all moisture levels as compared to the PPB except at the 12% moisture level in which there was no significant difference in the mean texture between the two bags. This indicates there is a relationship between hardness and texture of beans during storage for both PICS and PPB bags.

4.2.3 The effect of cooking time and temperature on the texture and cooking quality

The cooking time significantly affected the texture and cooking quality of beans. Increasing the cooking time significantly resulted in reduced texture values for both PICS and PPB bags. At 30 minutes, texture values were 434.27 ± 15.96 and 544.03 ± 17.41 which reduced to 111.53 ± 3.93 and 110.77 ± 4.97 at 180 Minutes in PICS and PPB bags respectively. Nevertheless, at cooking times above 90 minutes, there was no significant difference in texture values recorded between the two bags despite the higher texture values in the beans stored in PPB bags as shown in Figure 11.This indicates that cooking times as high as 90 minutes are sufficient to achieve similar hardness in cooked beans irrespective of storage bag used. This agrees with Kouemene (2013), in their research on evaluation of temperature and mechanical properties of beans during the cooking process, they found that hardness reduced with increase in cooking time. However, their results differ in time taken to achieve similar hardness between the various varieties used. In their research, cooking time from 20 minutes or 40 minutes was sufficient depending on varieties used to produce hardness that did not vary significantly. The difference can be explained by the different varieties used as well as the different storage conditions. There was no significant difference in texture at different cooking temperatures. Moreover, there was also no significant differences in texture at different cooking temperatures between the two storage bags. Nevertheless, beans stored in PPB bags were harder than those stored using the PICS technology.

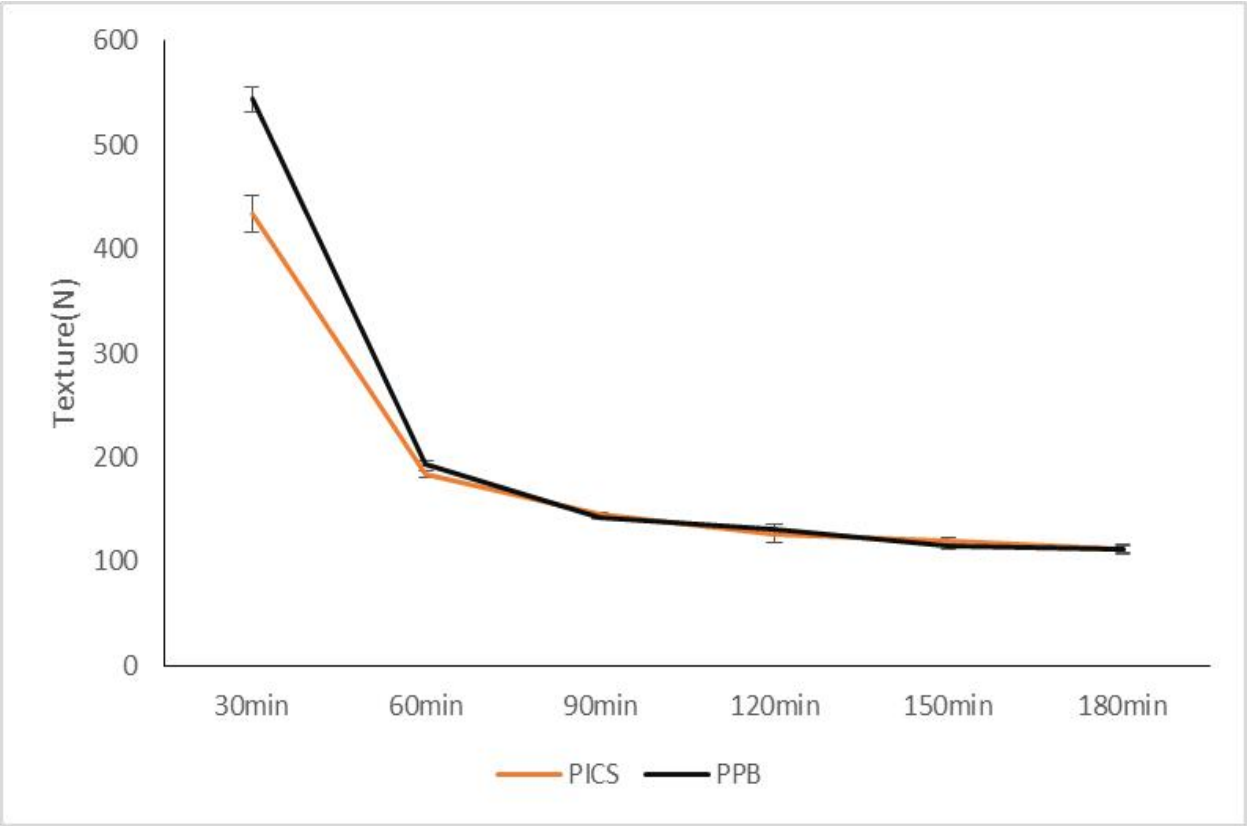


Figure 10: *The effect of the PICS and PPB storage bags on cooking time of beans*

4.2.4. The effect of bag on activation energy of three-bean varieties storage

The type of storage bag significantly affected the activation energy (Table 12). The activation energy was significantly higher in PP as compared to PICS. Moreover, similar trends were observed in both NY and RC, except SR in which the activation energy was higher in the PICS bags. The activation energy represents the least amount of energy needed for a chemical reaction to take place (Peng *et al*., 2014). Since it was significantly higher in PPB bags as compared to PICS bags, it therefore means that the beans stored in PPB bags would require more energy to achieve similar softness. The activation energies were high and were not comparable to those reported by Dolan *et al.* (2004) for dark kidney beans. While studying the softening kinetics of cooked dry beans at temperatures below 100C, these researchers reported dark kidney beans to have activation energies at 28,933 and 94,223 KJ/mol.K using the 1st order isothermal and non-isothermal models, respectively.

Table 12: The effect of storage bag on activation energy of three bean varieties

	Overall activation energy (J/mol)	NY	RC	SR
PICS	68218±57128[b]	37330±18304[b]	40159±25848[b]	127165±6003[a]
PPB	75807±37513[a]	69753±33551[a]	57644±22824[a]	100024±40834[b]

The cooking time significantly affected the texture and cooking quality of beans. Increasing the cooking time significantly resulted in reduced hardness for both PICS and PPB bags. At cooking times above 90 minutes, there was no significant difference in texture values recorded between the two pockets. This indicates that cooking times as high as 90 minutes are sufficient to achieve similar hardness in cooked beans irrespective of the storage bag used. This agrees with Pasqualone *et al.* (2020) who in their research on the evaluation of beans' temperature and mechanical properties during the cooking process, they found that hardness reduced with an increase in cooking time. However, their results differ in the time taken to achieve similar hardness between the various varieties used. In their research, cooking time from 20 minutes or 40 minutes was sufficient depending on the types used to produce hardness that did not vary significantly. The difference can be explained by the different varieties used as well as the other storage conditions

4.2.5 The effect of storage time on activation energy for three varieties of beans stored in both PPB and PICS

There was a gradual decrease in activation energy from day 0 to day 270 regardless of the bag used (Table 13). Storage time significantly affected the activation energy in both PP and PICS bags. In both cases, there was a gradual decrease in activation energy from day 0 to day 270.

The activation energy, which represents the least amount of energy needed for a chemical reaction to occur, was significantly higher in PPB bags than in PICS bags showing that the beans stored in these bags would require more energy to achieve similar softness. The activation energies were high and comparable to those reported by Dolan *et al.* (2004) for dark kidney beans. While studying the softening kinetics of cooked dry beans at temperatures below 100C, these researchers reported dark kidney beans to have activation energies at 28,933 and 94,223 KJ/mol.K using the 1st order isothermal and non-isothermal models, respectively.

Table 13: The effect of storage time on activation energy for three varieties of beans stored in both PP and PICS

Storage time (Days)	Overall activation energy(J/mol)	PICS	PPB
0	100581±61261[a]	100806±76539[a]	100357±43222[a]
45	85487±52506[b]	78907±65389[b]	92067±36174[ab]
90	68886±44808[cd]	65933±64126[bc]	65960±29336[cd]
135	77027±48154[bc]	74984±51885[b]	79070±45532[bc]
180	64421±35968[d]	64049±40728[bc]	64794±31694[d]
225	58730±37367[de]	57765±46075[c]	59695±27385[d]
270	48955±37540[e]	35373±25692[d]	68706±31520[cd]

4.2.6 The effect of bean variety on activation energy in both PP and PICS

Regardless of the bag used for storage, SR significantly required higher activation energy while there was no significant difference in activation energy between NY and RC (Table 14). The highest significant activation energy was observed in SR for both bags. In PPB bags, SR was followed by NY while RC had significantly lowest activation energy. Moreover, there was no significant difference between NY and RC stored in the PICS bags.

Table 14: The effect of bean variety on activation energy in both PP and PICS

Bean variety	Overall activation energy(J/mol)	PICS	PPB
NY	53541±31425[b]	34810±22830[b]	69753±33551[b]
RC	48902±25782[b]	42803±21059[b]	57644±22824[c]
SR	113595±52822[a]	127165±60031[a]	100024±40834[a]

There was a significant interaction between the moisture level and the storage time (Table 15). At day 0, activation was significantly different at all moisture levels tested. Highest storage moisture recorded the highest activation energy followed by the medium storage and finally the lowest storage moisture. However, as storage was increased to 45 days through to 135 days, there was no significant difference in activation energy observed at the 3 moisture levels.

Increasing the storage to 270 days, there was a significant difference in activation energy between lowest storage moisture and both the medium and high storage moisture levels.

Table 15: Activation energy at different moisture storage levels during storage

Moisture	0	45	90	135	180	225	270
H	144394±75098[a]	89973±57694[a]	68988±32253[a]	81320±51001[a]	56299±26768[b]	61516±29223[a]	52156±52611[a]
M	93106±38628[b]	73251±38183[a]	77743±60134[a]	79948±41742[a]	77143±50698[a]	62362±46591[a]	56609±30057[a]
L	64245±34841[c]	93236±61111[a]	59926±39481[a]	69812±54299[a]	59822±23621[ab]	52313±36746[a]	38101±24680[b]

4.2.7 The effect of storage moisture on activation energy in both PP and PICS

Storage of beans at high moisture resulted in significantly higher activation energy (79235.568 ± 55856.596J/mol) although that was not significantly different from the medium storage moisture level (74309.206 ± 44428.293) as shown in Table 16. Lowest activation energy was found in beans stored at 12% moisture level at 62494.102 ± 42832.657J/mol.

Storage moisture significantly affected the activation energy. In the PP bags, increasing storage moisture significantly increased the activation energy. However, in the PICS bags, there was no significant difference between activation energy at the highest and medium storage moisture and between the highest and the lowest storage moisture. The effect of storage moisture on the activation energy may have been lower in the PICS as compared to the PP.

Table 16*:* The effect of storage moisture on activation energy in both PP and PICS

Moisture level	Overall activation energy(J/mol)	PICS	PPB
H	79235±55856^{a}	69968±62008^{ab}	91146±42526^{a}
M	74309±44428^{a}	73386±59669^{a}	72712±28556^{b}
L	62494±42832^{b}	61425±49494^{b}	63563±35524^{c}

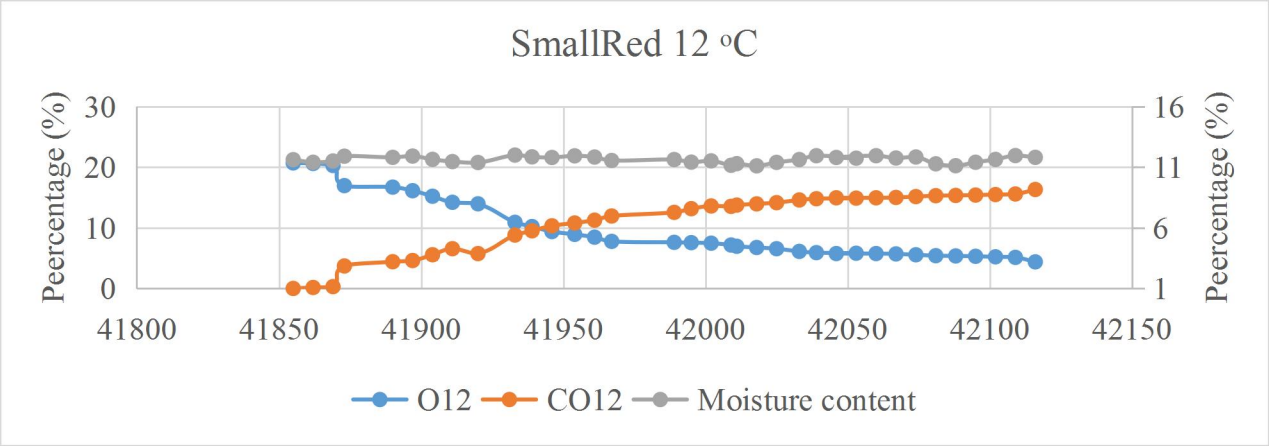
4.3 The effect of hermetic polyethylene bag storage on mold infection and aflatoxin contamination

Molds and aflatoxin producing fungi have been found in hermetically stored common grains in the previous experiments.in this section changes in oxygen and carbon dioxide levels in the storage bag was measured. Incidences of moulds was studied and presence of aflatoxins in stored beans was confirmed.

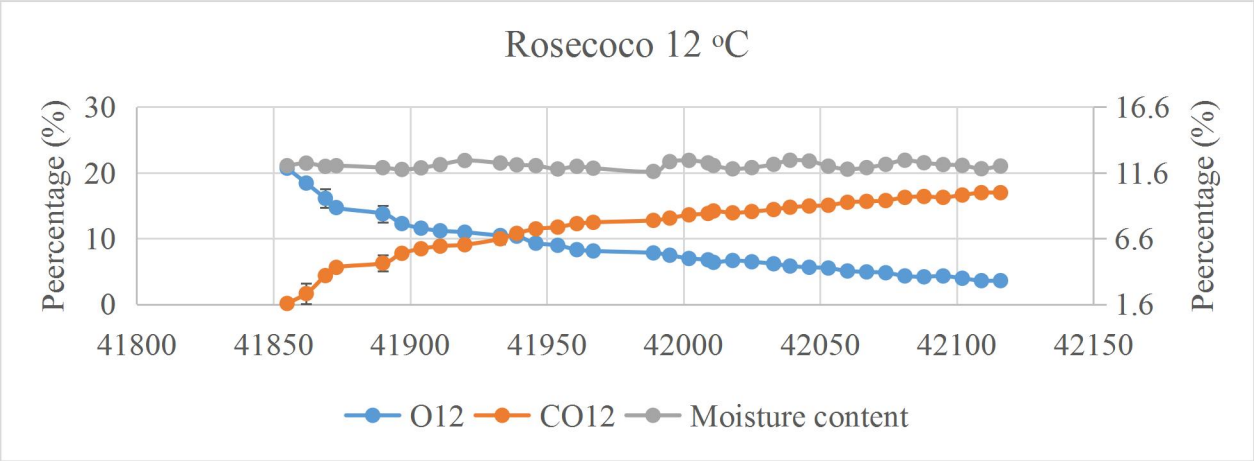
4.3.1 Changes in oxygen and carbon dioxide levels in PICS bags during storage for three varieties of beans stored at three temperature environments

Figure 12 (a-i) shows the changes in oxygen and carbon dioxide levels in PICS bags during storage for three varieties of beans stored at three temperature environments. Oxygen concentration at the beginning of storage ranged between 20.63±0.05 to 20.795±0.015%. Carbon dioxide levels at the beginning ranged between 0 to 0.07%. Oxygen levels decreased gradually to reach levels between 0.1 and 4.7% while the carbon dioxide increased gradually reaching 15.495 - 19.55%. Carbon dioxide tended to build more in bags with higher moisture content

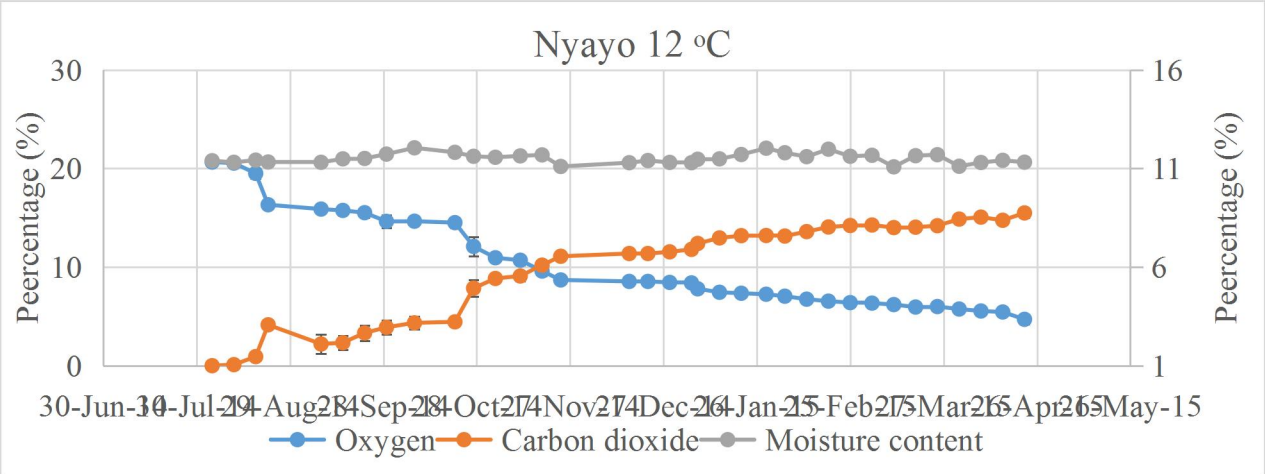
(a)


SmallRed 12 °C
Peercentage (%)
30
20
10
0
16
11
6
1
Peercentage (%)
41800
41850
41900
41950
42000
42050
42100
42150
O12
CO12
Moisture content

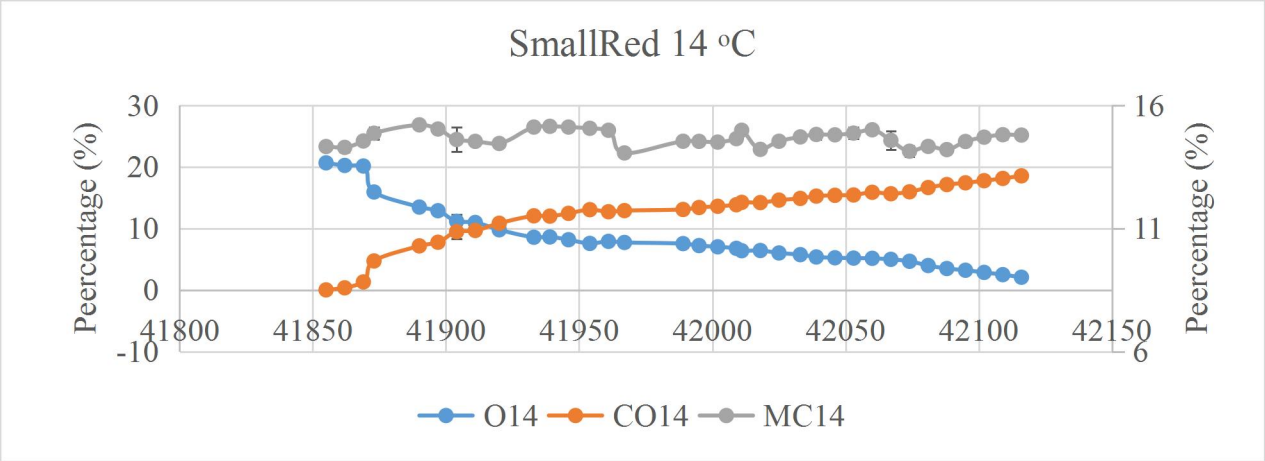
(b)


Rosecoco 12 °C
Peercentage (%)
30
20
10
0
16.6
11.6
6.6
1.6
Peercentage (%)
41800
41850
41900
41950
42000
42050
42100
42150
O12
CO12
Moisture content

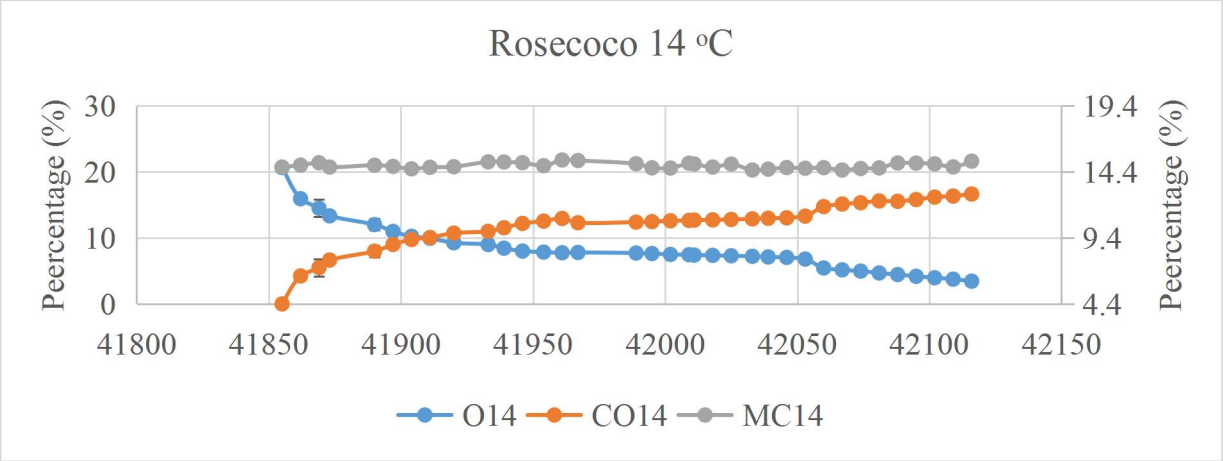
(c)


Nyayo 12 °C
Peercentage (%)
30
20
10
0
16
11
6
1
Peercentage (%)
30-Jun-14
10-Jul-14
29-Aug-14
28-Sep-14
28-Oct-14
27-Nov-14
27-Dec-14
26-Jan-15
25-Feb-15
27-Mar-15
26-Apr-15
26-May-15
Oxygen
Carbon dioxide
Moisture content

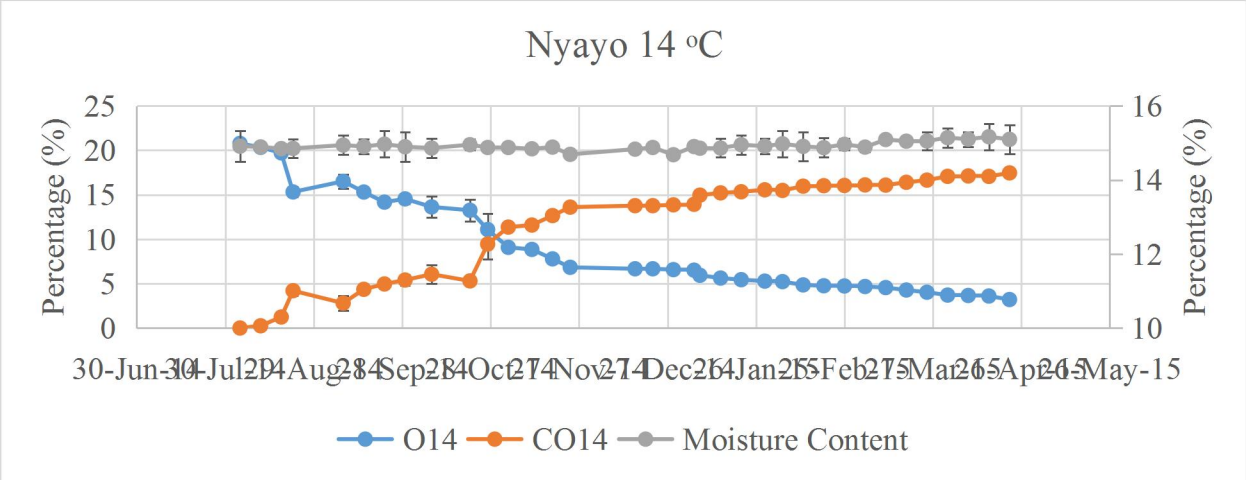
(d)


SmallRed 14 °C
Peercentage (%)
30
20
10
0
-10
16
11
6
41800
41850
41900
41950
42000
42050
42100
42150
Peercentage (%)
O14
CO14
MC14

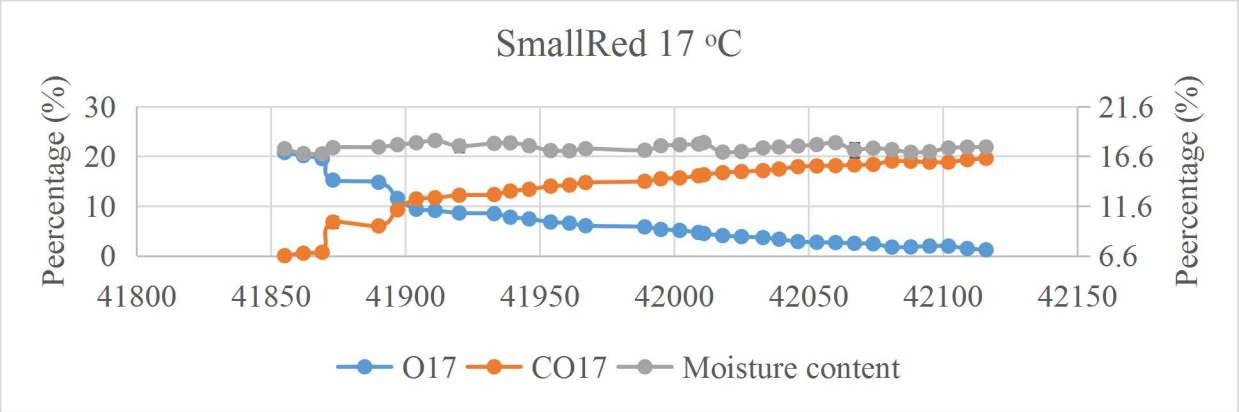
(e)


Rosecoco 14 °C
Peercentage (%)
30
20
10
0
19.4
14.4
9.4
4.4
41800
41850
41900
41950
42000
42050
42100
42150
Peercentage (%)
O14
CO14
MC14

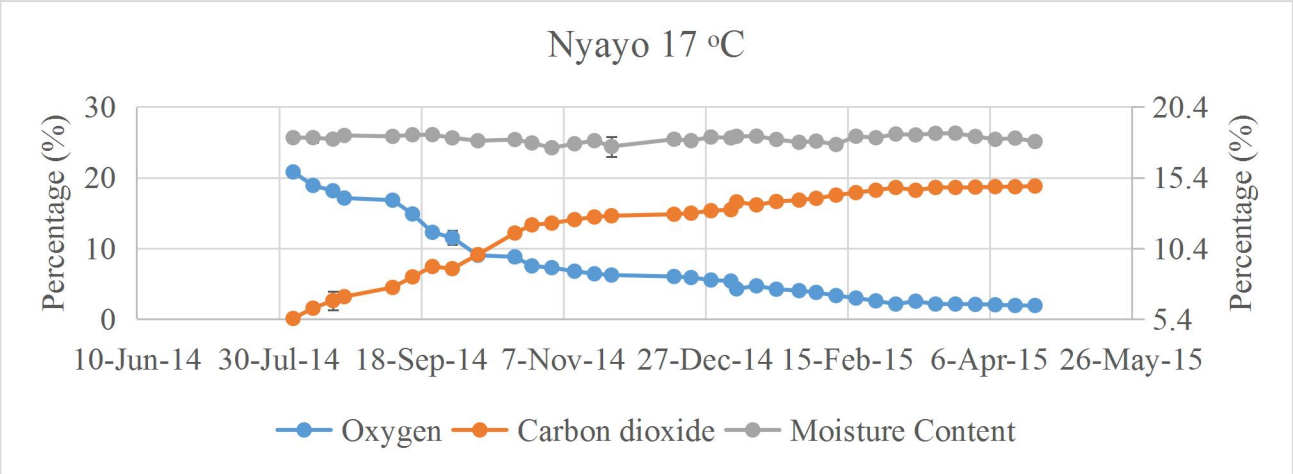
(f)


Nyayo 14 °C
Percentage (%)
25
20
15
10
5
0
16
14
12
10
30-Jun-14
Aug-28-14
Sep-28-14
Oct-27-14
Nov-27-14
Dec-26-14
Jan-25-15
Feb-27-15
Mar-26-15
Apr-26-15
May-15
Percentage (%)
O14
CO14
Moisture Content

(g)



(h)



(i)

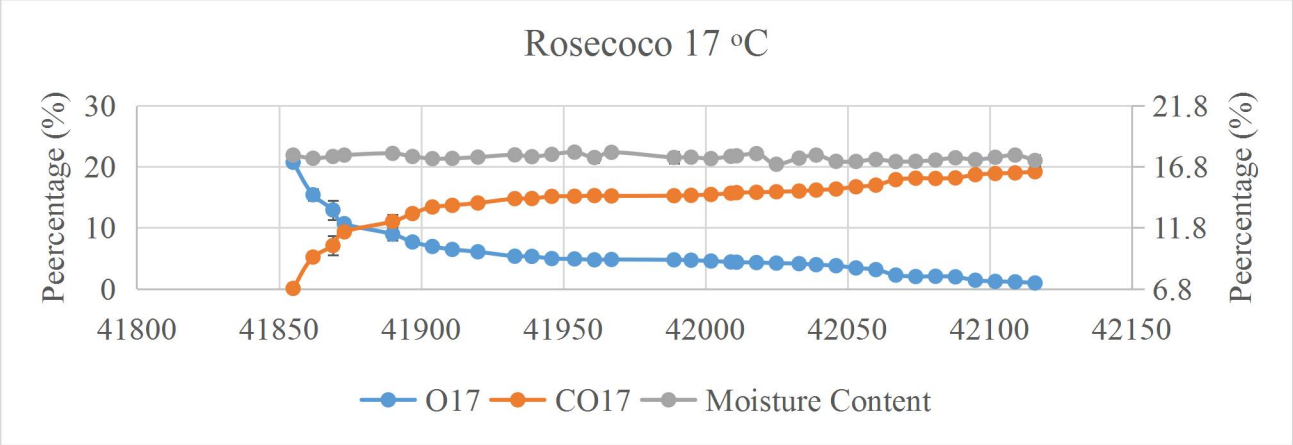


Figure 11: *(a-i): Changes in oxygen and carbon dioxide levels in PICS bags during storage for three varieties of beans stored at three temperature environments. (O=Oxygen, CO=Carbon dioxide*

4.3.2 Overall Incidences of Molds in Two Types of Storage Bags.

The overall incidence of molds in both types of storage bags for the beans is shown in Figure 13. During the storage period, the incidence of *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. was decreasing while other types of molds which were predominantly *Cladosporium* spp. and *Alternaria* spp were increasing. *Penicillium* spp. and *Aspergillus* spp. are the main storage fungi in common bean and usually invade the seeds during and after maturation, causing damage as soon as they find appropriate conditions. The primary colonizer is *Aspergillus* spp., which subsequently allows the development of *Penicillium* spp. (Faiad *et al*., 1996). *Cladosporium* and *Alternaria* spp. are airborne spores and in this study they were considered among other types of mould spp as indicated in Figure 13. As observed in Grinn-Gofroń and Rapiejko (2009) they formed the majority of moulds that were recorded in this experiment.

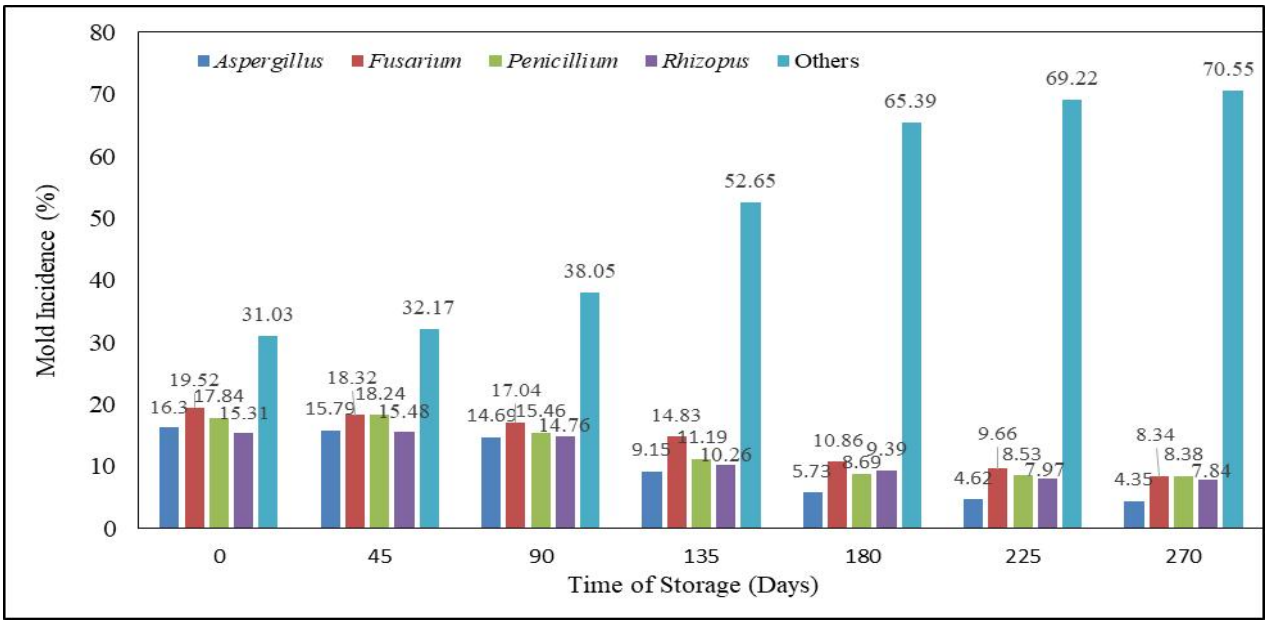


Figure 12: *The effect of storage time on mold incidences (%) in beans from day 0 (baseline) up to day 270 in both types of bags.*

The effect of the type of storage bag for the beans on incidence of molds is shown in Figure 14. Beans stored in PICS bags had lower incidences for *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. compared to those stored in PPB bags. Other types of mold included *Cladosporium* spp. and *Alternaria* spp. The aflatoxin producing *Aspergillus* spp was the least prevalent mold in both bags, however, it was higher in the PPB than in PICS.

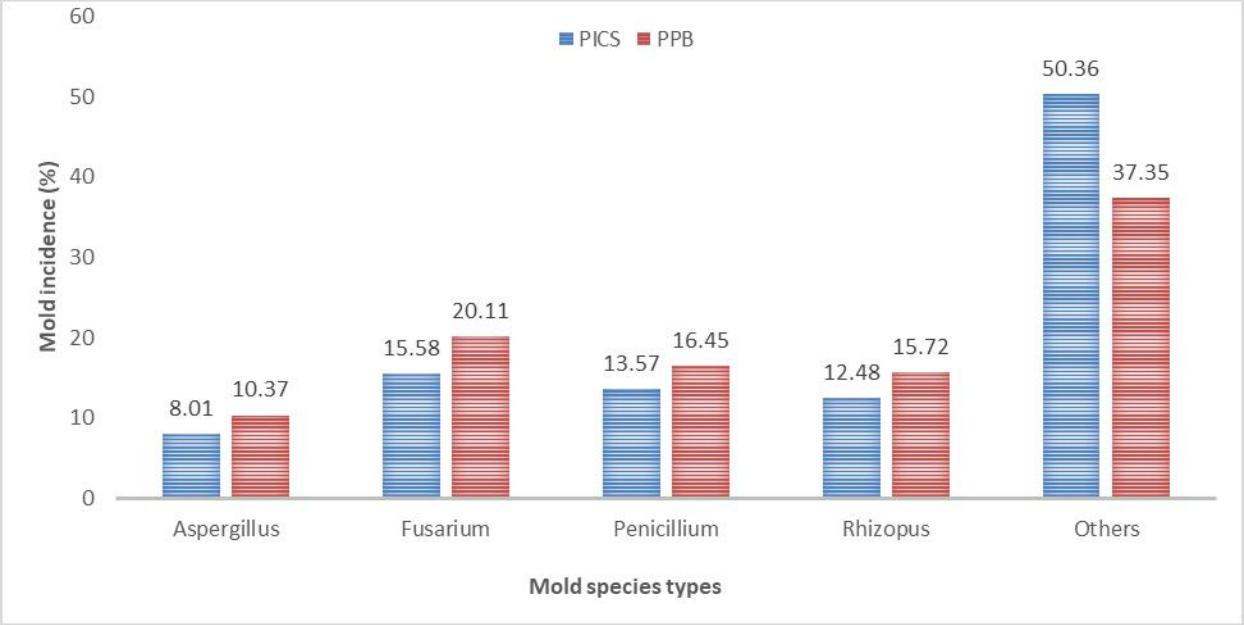


Figure 13: *The effect of type of storage bag on the overall Incidence (%) of molds in beans stored in PICS and PPB bags*

The effect of bean varieties on the overall mold incidence (%) during storage is shown in Figure 15. The bean varieties had no effect on the incidence of for *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp.

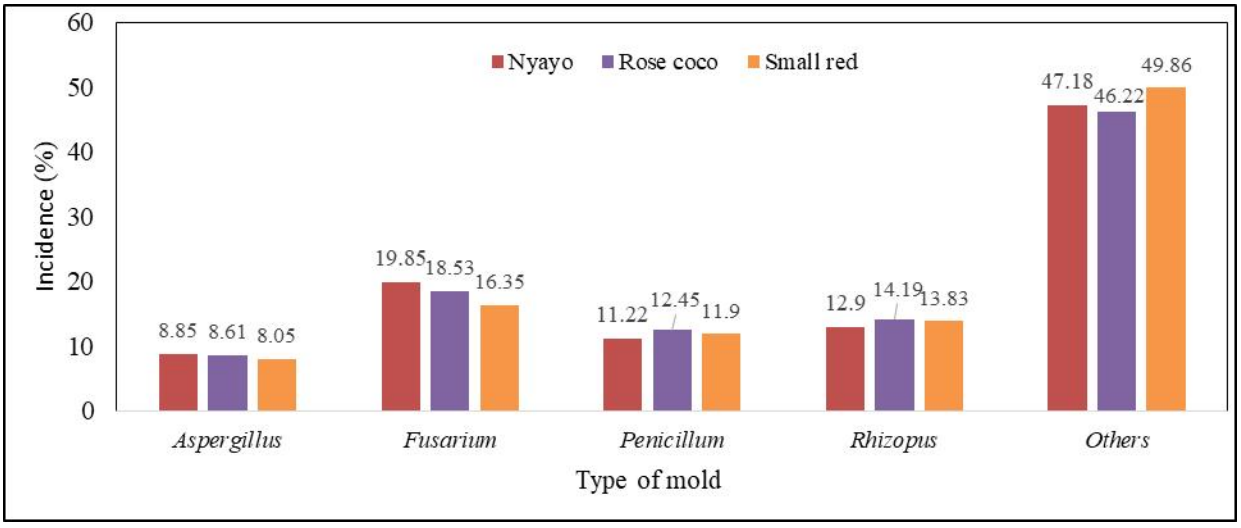


Figure 14: *The effect of bean varieties on the overall mold incidence (%) during storage*

Incidences of mold growth in the two types of bags at different moisture content of beans during storage is shown in Figure 16. For both bags, incidences of *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. were highest for the beans stored at 18% moisture content

level and least for those stored at 12% moisture content level. However, at all moisture levels of storage, the incidences of *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. were higher in beans stored in the PPB bags than in beans stored in PICS bags.

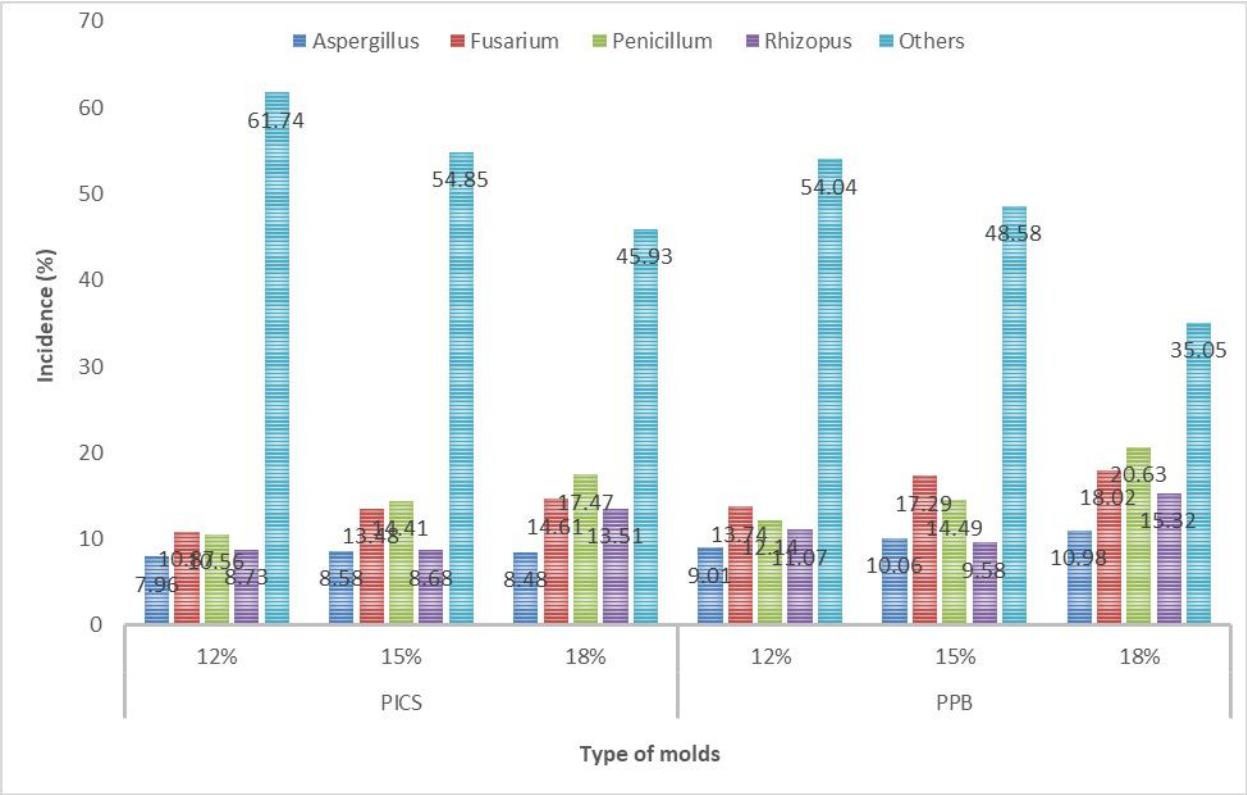


Figure 15: *Incidence (%) of molds in beans stored in PICS and PPB bags at 12%, 15% and 18% moisture content levels*

The mean, minimum, maximum and median mold counts for the beans stored in PPB and PICS bags at 12%, 15%, 18% and overall are shown in Table 17. In both PPB and PICS bags, the mold counts increased significantly with increase of moisture content at which beans were stored. However, mold counts for beans stored in the PICS bags at all levels of moisture content were significantly lower than beans stored in PPB bag as shown in Table 18. The overall mold counts of beans stored in PPB bags of 15.94±1.12 cfu/6 beans was significantly higher compared to 8.64±0.97 cfu/6beans.

Table 17: Mean±Std Error, Minimum, Maximum and Median mold counts for the beans stored in two different bags at 12%, 15%, 18% and overall.

Bag	MOC	Mean±Std Error	Min.(Mold count)	Max.(Mold Count)	Median(mold count)
PICS	12%	5.14±0.87^{e}	0	18	2.00
	15%	8.02±1.34^{d}	0	25	3.50
	18%	12.76±2.32^{bc}	0	42	4.00
	Overall	8.64±0.97^{cd}	0	42	3.50
PPB	12%	10.76±1.38^{c}	2	26	7.50
	15%	15.88±1.96^{b}	1	40	12.00
	18%	21.19±2.12^{a}	4	44	18.50
	Overall	15.94±1.12^{b}	1	44	12.00

MOC; Moisture content, Std Error; Standard error of the mean, Min; Minimum mold count, Max; Maximum mold count and means along the column with same letter are not significantly different at $P<0.05$.

The effect of the type of bag for beans storage on the mold counts during storage is shown in Figure 17. The mold counts in beans decreased with time for both types of bags though the counts were significantly lower in PICS bags.

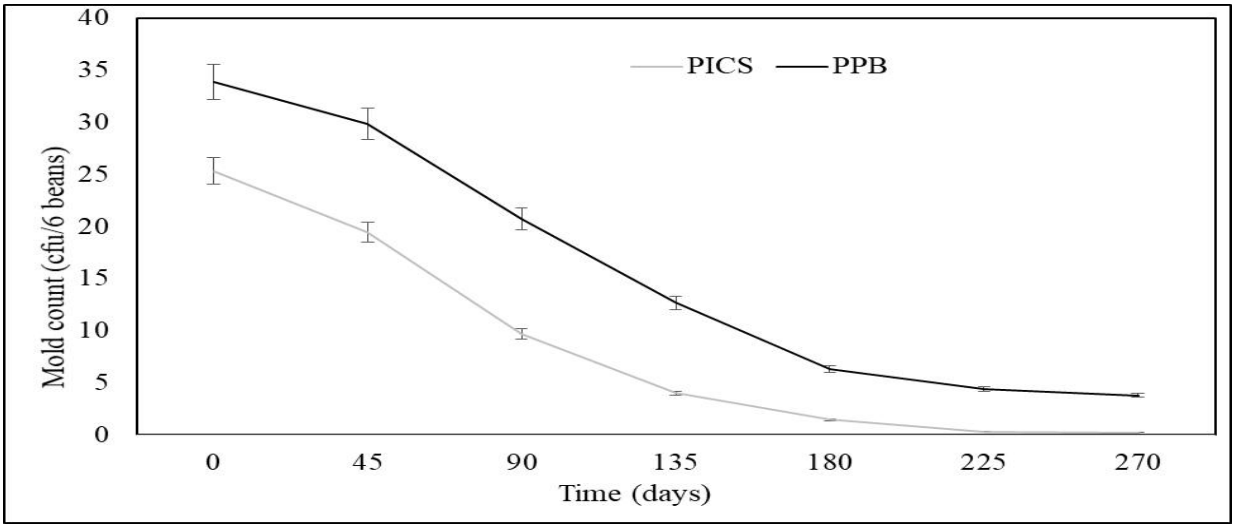


Figure 16: *Mold counts for beans in PPB and PICS bags during storage*

This study found out that irrespective of the storage bag, the main molds that grew were *Aspergillus* spp*., Fusarium* spp*., Penicillium* spp., and *Rhizopus* spp. However, their incidences were decreasing throughout storage. The probable reason for molds in beans could be contamination during handling from harvesting time to storage. Soils are known to harbor microorganisms, including molds, which usually find their way into the food through contamination. Besides grounds, there are airborne mold spores in the air, which could be another source of contamination. But it was observed that the incidences of these main mold species present, their incidences decreased with storage time. This could be due to insurgences of unfavorable conditions such as change beans moisture content and depletion of oxygen and increased carbon dioxide in the storage bags. A study by Bradford *et al.* (2018) showed that storage in PICS bags prevented survival due to decreasing oxygen and increasing carbon dioxide concentrations slowed down organisms' metabolic activities. Oxidative metabolism is suppressed under oxygen deprivation, unable to generate metabolic water for support of vital life processes and cellular or tissue integrity. Consequently, death eventually occurs due to desiccation, Simultaneous exposure of insects to low oxygen, and high carbon.

It was also observed in the study that molds' incidences were higher in PPB bags than PICS bags for *Aspergillus* spp*., Fusarium* spp*., Penicillium* spp., and *Rhizopus* spp. Therefore, the type of bag storage affected the incidence of mold. This could be due to the ability of PICS bags ability to control abiotic factors that could favor the growth of molds that PPB bag. In both packs, the moisture levels at which were stored affected the level of mold incidences. The higher the moisture content at which beans were stored, the higher the incidences of molds. Though moisture content of storage increased mold incidences, it was higher for beans stored in PPB bags. The moisture content of food plays a significant role in microbial growth. These findings agree with the conclusions from Suleiman *et al*. (2018) while studying the impact of moisture content and maize weevils on maize quality during hermetic and non-hermetic storage. He concluded that the multiplication of *acidophilus zeamais* in stored maize corresponded with the amount of moisture. More growth was observed in higher moisture samples.

Bean variety did not affect the incidences of molds. The type of storage bag and moisture content at which the beans were stored affected the mold incidences and the counts. Molds numbers were higher in beans stored in PPB bags than in PICS. Also, in each type of bag, mold counts

increased with an increase in moisture content at which beans were stored. Molds decreased in numbers as the storage time progressed in both bags, but more pronounced in PICS bags.

Consequently, aflatoxin contamination in beans during storage was affected by the type of bag and the storage moisture content. Aflatoxin contaminations were higher in beans stored in PPB bags because of higher counts and higher incidence of *Aspergillus* spp. Molds. In both packs, the aflatoxin contamination levels increased with the moisture level at which beans were stored. Concentrations of aflatoxin increased with time of storage, probably due accumulation effect. This increase was much higher in beans stored in PPB bags, attributed to the higher mold counts and incidence of *Aspergillus* spp. observed than in PICS bags.

4.3.3 Effect of storage bag on aflatoxin contamination of stored common beans

The mean, minimum, maximum and median aflatoxin levels for the beans stored in PPB and PICS bags at 12%, 15%, 18% and overall are shown in Table 18. In both PPB and PICS bags, the mean aflatoxin levels increased significantly with increase of moisture content at which beans were stored. However, the mean aflatoxin levels for beans stored in the PICS bags at all levels of moisture content were significantly lower than beans stored in PPB bag as. The overall aflatoxin levels of beans stored in PPB bags of 6.30±1.92 µg/Kg were significantly higher compared to 1.97±0.41µg/Kg.

Table 18: Mean±Std Error, Minimum, Maximum and Median aflatoxin content for the beans stored in two different bags at 12%, 15%, 18% and overall

Bag	MOC	Mean±Std Error	Min.	Max.	Median
PICS	12%	1.27±0.44^{e}	0.00	11.88	0.00
	15%	1.61±0.58^{e}	0.00	15.59	0.00
	18%	3.03±0.99^{d}	0.00	34.07	0.00
	Overall	1.97±0.41^{e}	0.00	34.07	0.00
PPB	12%	3.87±0.84^{d}	0.00	16.56	1.82
	15%	4.71±1.30^{c}	0.00	30.25	1.71
	18%	5.55±5.56^{a}	0.00	34.35	1.08
	Overall	4.71±0.64^{b}	0.00	34.35	1.60

MOC; Moisture content, Std Error; Standard error of the mean, Min; Minimum aflatoxin level, Max; Maximum aflatoxin level and means along the column with same letter are not significantly different at P<0.05.

Effect of the two types of bags, bean varieties, storage moisture content of the beans and their interactions on the mold counts and aflatoxin levels in beans during storage period is shown in Table 19. Effect of PICS and PPB bags on beans during storage differed significantly at P<0.05 on the moisture content levels, mold counts and the aflatoxin levels. Also, beans variety and moisture content at which beans were stored had a significant effect at P<0.05 on moisture content of beans during storage, aflatoxin levels and mold count except for the *Aspergillus* spp. Number of days during storage of beans also significantly at P<0.001 affected the moisture content of beans during storage, mold counts and aflatoxin levels. Moisture at which beans were stored had a significant effect at P<0.05 for the moisture content of beans during storage and mold counts except for the *Aspergillus.*

Effect of the PICS and PPB bags on the moisture content of beans, mold counts and levels of aflatoxin during storage is shown in Table 19. Beans stored in PPB had significantly higher moisture content during storage, all mold counts and aflatoxin levels.

Table 19: Effect of the two types of the bags on the Mean±SE of the moisture content of beans, mold counts and levels of aflatoxin during storage

Bag	MOC	*Aspergillu s* spp.	*Fusarium* spp.	*Penicilliu m* spp.	*Rhizopus* spp.	Other spp.	Aflatoxin
PPB	15.94±1.12[a]	10.07±0.74[a]	17.02±0.96[a]	13.45±0.82[a]	12.06±0.83[a]	45.89±2.18[a]	4.71±0.64[a]
PICS	8.64±0.97[b]	7.34±0.77[b]	10.27±1.05[b]	8.26±0.86[b]	7.17±0.76[b]	40.39±3.11[b]	1.97±0.41[b]

Key: Means with the same letter along the column are not significantly different at P<0.05. MOC; Moisture content

The effect of the type of bag for beans storage on the aflatoxin levels during storage is shown in Figure 16. The aflatoxin levels in beans increased with time for both types of bags though the counts were significantly lower in PICS bags.

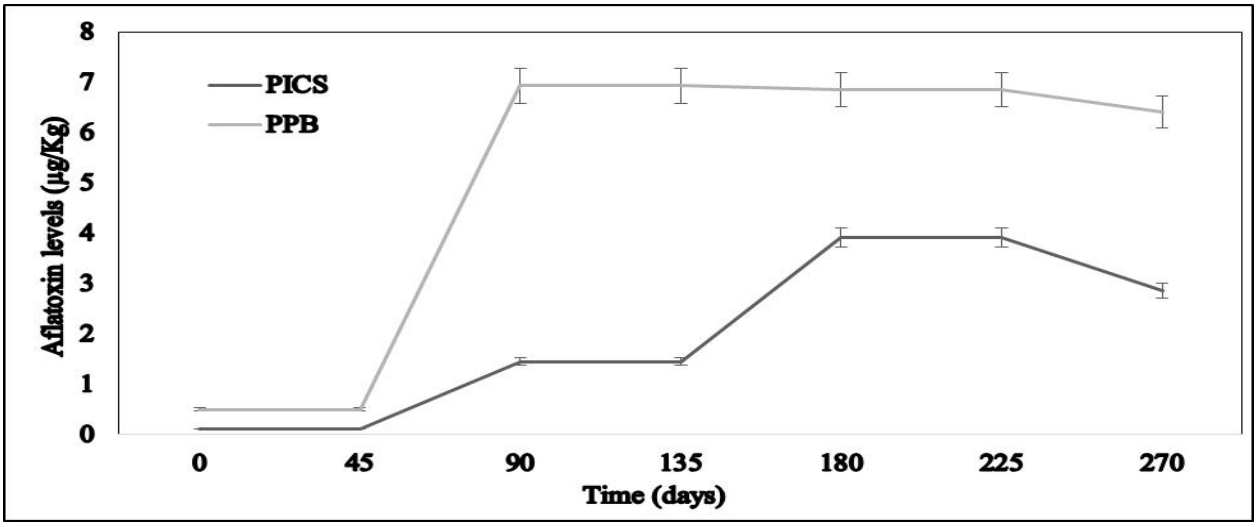


***Figure 17**: Aflatoxin levels for beans in PPB and PICS bags during storage*

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

This chapter presents the conclusions and recommendations from the research work. This chapter also gives suggestions for further research in this area.

5.1 Conclusions

Based on the findings of this study, the following are the conclusions made:

i. Hermetic bags were better in preserving the total soluble sugars, *in-vitro* starch digestibility, in- vitro protein digestibility, and free amino acids in beans than PPB bags during storage. However, their effect on tannin content did not differ.

ii. Beans stored in hermetic bags had used low punching pressure and had better cookability than beans stored in ordinary bags.

iii. Hermetic bags-controlled mold growth better and consequently had low aflatoxin contamination levels than ordinary PPB bags.

5.2 Recommendations

Based on the findings of this study, the following are the recommendations made:

Since Hermetic bags are better in preserving beans' chemical and biochemical properties, control the hard to cook defect and growth molds. The study recommended that they be presented to small-scale farmers to check on post-harvest and nutritional losses of common beans.

5.3 Further Research

The study suggested that tannin content in common beans and its possible relationship with flatulence should be further investigated

REFERENCES

Abay, M. M. (2021). *Effects of storage temperature and relative humidity on physico chemical qualities of selected common bean (phaseolus vulgaris l.) varieties.* Masters' Thesis Haramaya University, Addis Ababa, Ethiopia.

Affognon, H. D., Njoroge, A. W., Mutungi, C. M., Manono, J., Baributsa, D., & Murdock, L. L. (2014, August). Storage of pigeonpea grain (Cajanus cajan (L.) Millsp.) In hermetic triple-layer bags prevents losses caused by Callosobruchus maculatus (F.)(Coleoptera: Bruchidae). In XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1120 (pp. 245-252).

Affrifah, N. S., Chinnan, M. S., Saalia, F. K., & Phillips, R. D. (2021). Hydrothermal treatments affect the development of the hard-to-cook defect in cowpeas. *Legume Science, 1, 1-15* https://doi.org/10.1002/leg3.126

Agarwal, M., Agarwal, S., Ahmad, S., Singh, R., & Jayahari, K. M. (2021). Food Loss and Waste in India: The Knowns and the Unknowns. *World Resources Institute, 1, 1-36.* https://doi.org/10.46830/wriwp.20.00106, http://www.wri.org/publication/food-loss-and-waste-in-india.

Alemayehu, S., Abay, F., Ayimut, K. M., Assefa, D., Chala, A., Mahroof, R., Harvey, J., & Subramanyam, B. (2020). Evaluating different hermetic storage technologies to arrest mold growth, prevent mycotoxin accumulation and preserve germination quality of stored chickpea in Ethiopia. *Journal of Stored Products Research,* 85, 101526. https://doi.org/10.1016/j.jspr.2019.101526

Alves, S., Fonseca Alvarenga Pereira, R. G., de Azevedo Lira, N., Micotti da Glória, E., Chalfoun, S. M., & Batista, L. R. (2020). Fungi associated to beans infested with coffee berry borer and the risk of ochratoxin A. *Food Control*, 113, 107-204. https://doi.org/10.1016/j.foodcont.2020.107204

Amongi, W., Kato, F., Male, A., Musoke, S., Acam, C., Kabwama, A., Nakyanzi, B., Sebuliba, S., Williams, M., Mbiu, J., Baguma, G., & Mukankusi, C. (1970). Development of white common beans for the processing industry in East Africa: Adaptability, resistance to selected diseases, cooking time and canning quality. *African Crop Science Journal*, *29*(3), 401–431. https://doi.org/10.4314/acsj.v29i3.6

Ashong, A. G. (2020). P*revalence of mycotoxigenic fungi and mycotoxins (ochratoxin a) in dried cocoa beans*. Masters' Thesis, University of Ghana, Accra, Ghana.

Barbieri, J. F., Gáspari, A. F., Teodoro, C. L., Motta, L., Castaño, L. A. A., Bertuzzi, R., Bernades, C. F., Chacon-Mikahil, M. P. T., & de Moraes, A. C. (2020). The effect of an airflow restriction mask (ARM) on metabolic, ventilatory, and electromyographic responses to continuous cycling exercise. *PLOS ONE*, *15*(8), e0237010. https://doi.org/10.1371/journal.pone.0237010

Baributsa, D., & Njoroge, A. W. (2020). The use and profitability of hermetic technologies for grain storage among smallholder farmers in eastern Kenya. *Journal of Stored Products Research*, 87, 1-10. https://doi.org/10.1016/j.jspr.2020.101618

Bassett, A., Dolan, K. D., & Cichy, K. (2020). Reduced retort processing time improves canning quality of fast-cooking dry beans (*Phaseolus vulgaris* L.). *Journal of the Science of Food and Agriculture, 100*(10), 3995–4004. https://doi.org/10.1002/jsfa.10444

Bayley, J. S., Winther, C. B., Andersen, M. K., Grønkjær, C., Nielsen, O. B., Pedersen, T. H., & Overgaard, J. (2018). Cold exposure causes cell death by depolarization-mediated Ca $^{2+}$ overload in a chill-susceptible insect. *Proceedings of the National Academy of Sciences*, *115*(41), 1-8. https://doi.org/10.1073/pnas.1813532115

Borém, F. M., Ribeiro, F. C., Figueiredo, L. P., Giomo, G. S., Siqueira, V. C., & Dias, C. A. (2019). Sensory analysis and fatty acid profile of specialty coffees stored in different packages. *Journal of Food Science and Technology, 56*(9), 4101–4109. https://doi.org/10.1007/s13197-019-03879-3

Bradford, K. J., Dahal, P., Van Asbrouck, J., Kunusoth, K., Bello, P., Thompson, J., & Wu, F. (2018). The dry chain: Reducing postharvest losses and improving food safety in humid climates. *Trends in Food Science & Technology*, 71, 84–93. https://doi.org/10.1016/j.tifs.2017.11.002

Catarino, S., Brilhante, M., Essoh, A. P., Charrua, A. B., Rangel, J., Roxo, G., Varela, E., Moldão, M., Ribeiro-Barros, A., Bandeira, S., Moura, M., Talhinhas, P., & Romeiras, M. M. (2021). Exploring physicochemical and cytogenomic diversity of African cowpea and common bean. *Scientific Reports*, *11*(1), 1-14. https://doi.org/10.1038/s41598-021-91929-2

Chakraborty, A., Chander, S., Sehgal, M., Malik, M., & Sachan, M. S. (2021). Management of Stored Grain Pests—*Novel Strategies. 4*(2), 25-32.

Chen, D., Pham, U. T. T., Van Loey, A., Grauwet, T., Hendrickx, M., & Kyomugasho, C. (2021). Microscopic evidence for pectin changes in hard-to-cook development of common beans during storage. *Food Research International*, 141, 110115. https://doi.org/10.1016/j.foodres.2021.110115

Chigwedere, C. M., Njoroge, D. M., Loey, A. M., & Hendrickx, M. E. (2019). Understanding the Relations Among the Storage, Soaking, and Cooking Behavior of Pulses: A Scientific Basis for Innovations in Sustainable Foods for the Future. Comprehensive *Reviews in Food Science and Food Safety,18*(4), 1135–1165. https://doi.org/10.1111/1541-4337.12461

Chu, J., Ho, P., & Orfila, C. (2020). Growth Region Impacts Cell Wall Properties and Hard-to-Cook Phenotype of Canned Navy Beans (*Phaseolus vulgaris*). *Food and Bioprocess Technology, 13(5),* 818–826. https://doi.org/10.1007/s11947-020-02436-7

Cichy, K. A., Wiesinger, J. A., Berry, M., Nchimbi-Msolla, S., Fourie, D., Porch, T. G., Ambechew, D., & Miklas, P. N. (2019). The role of genotype and production environment in determining the cooking time of dry beans (*Phaseolus VULGARIS* L.). *Legume Science, 1*(1). 1-15. https://doi.org/10.1002/leg3.13

Diaz, S., Ariza-Suarez, D., Ramdeen, R., Aparicio, J., Arunachalam, N., Hernandez, C., Diaz, H., Ruiz, H., Piepho, H.-P., & Raatz, B. (2021). Genetic Architecture and Genomic Prediction of Cooking Time in Common Bean (*Phaseolus vulgaris* L.). *Frontiers in Plant Science,* 11, 622213. https://doi.org/10.3389/fpls.2020.622213

Duijsens, D., Gwala, S., Pallares, A. P., Pälchen, K., Hendrickx, M., & Grauwet, T. (2021). How postharvest variables in the pulse value chain affect nutrient digestibility and bioaccessibility. *Comprehensive Reviews in Food Science and Food Safety, 20*(5), 5067–5096.https://doi.org/10.1111/1541-4337.12826

Francisco, F. G., & Usberti, R. (2008). Seed health of common bean stored at constant moisture and temperature. *Scientia Agricola,* 65, 613-619.

Gu, J., Bk, A., Wu, H., Lu, P., Nawaz, M. A., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2022). Impact of processing and storage on protein digestibility and bioavailability of

legumes. *Food Reviews International,* 1–28. https://doi.org/10.1080/87559129.2022.2039690

Hall, C., Vatansever, S., & Biswas, A. (2020). *Storage of pulses and the effect on structure and functionality*. Doctor of Philosophy Thesis, Dalhousie University, Toronto, Canada.

Hasan, M. M., Aikins, M. J., Schilling, M. W., & Phillips, T. W. (2020a). Comparison of Methyl Bromide and Phosphine for Fumigation of Necrobia rufipes (*Coleoptera: Cleridae*) and Tyrophagus putrescentiae (S*arcoptiformes: Acaridae*), Pests of High-Value Stored Products. *Journal of Economic Entomology, 113*(2), 1008–1014. https://doi.org/10.1093/jee/toz319

Hasan, M. M., Aikins, M. J., Schilling, M. W., & Phillips, T. W. (2020b). Comparison of Methyl Bromide and Phosphine for Fumigation of Necrobia rufipes (Coleoptera: Cleridae) and Tyrophagus putrescentiae (Sarcoptiformes: Acaridae), Pests of High-Value Stored Products. *Journal of Economic Entomology,113*(2), 1008–1014. https://doi.org/10.1093/jee/toz319

Hou, D., Zhao, Q., Yousaf, L., Khan, J., Xue, Y., & Shen, Q. (2020). Consumption of mung bean (*Vigna radiata* L.) attenuates obesity, ameliorates lipid metabolic disorders and modifies the gut microbiota composition in mice fed a high-fat diet. *Journal of Functional Foods,* 64, 103687. https://doi.org/10.1016/j.jff.2019.103687

Jombo, T. Z., Emmambux, M. N., & Taylor, J. R. N. (2021). Modification of the functional properties of hard-to-cook cowpea seed flours and cooked prepared pastes by γ-irradiation. *Journal of Food Science and Technology, 58*(1), 22–33. https://doi.org/10.1007/s13197-020-04509-z

Liang, Q., Wang, K., Shariful, I., Ye, X., & Zhang, C. (2020). Folate content and retention in wheat grains and wheat-based foods: Effects of storage, processing, and cooking methods. *Food Chemistry,* 333, 127459. https://doi.org/10.1016/j.foodchem.2020.127459

Ling, B., Cheng, T., & Wang, S. (2020). Recent developments in applications of radio frequency heating for improving safety and quality of food grains and their products: *A review. Critical Reviews in Food Science and Nutrition, 60*(15), 2622–2642. https://doi.org/10.1080/10408398.2019.1651690

Luisetto, M., Almukhtar, N., Rafa, A., Ahmadabadi, B., & Mashori, G. (2019). Role of plants, environmental toxins and physical neurotoxicological factors in Amyotrophic lateral

sclerosis, Alzheimer Disease and other Neurodegenerative Diseases. *Journal of Neuroscience and Neurological Disorders, 3*(1), 001–086. https://doi.org/10.29328/journal.jnnd.1001019

Mayer, I.-C., Frøkiær, H., & Sandberg, A.-S. (2021). Nutritional and antinutritional composition of fava bean (Vicia faba L., var. Minor) cultivars. *Food Research International,* 140, 110038. https://doi.org/10.1016/j.foodres.2020.110038

Mogale, D. G., Kumar, S. K., & Tiwari, M. K. (2020). Green food supply chain design considering risk and post-harvest losses: A case study. *Annals of Operations Research, 295*(1), 257–284. https://doi.org/10.1007/s10479-020-03664-y

Mubaiwa, J., Fogliano, V., Chidewe, C., & Linnemann, A. R. (2019). Influence of alkaline salt cooking on solubilisation of phenolic compounds of bambara groundnut (*Vigna subterranea* (L.) Verdc.) in relation to cooking time reduction. *LWT, 107,* 49–55. https://doi.org/10.1016/j.lwt.2019.02.067

Mullins, A. P., & Arjmandi, B. H. (2021). Health Benefits of Plant-Based Nutrition: Focus on Beans in Cardiometabolic Diseases. *Nutrients, 13*(2), 5-19. https://doi.org/10.3390/nu13020519

Mutungi, C., & Affognon, H. (2013). Mitigating food losses in Benin: status and way forward for postharvest research and innovations. *ICIPE policy brief*; no. 2/13.

Ndemera, M., De Boevre, M., & De Saeger, S. (2020). Mycotoxin management in a developing country context: A critical review of strategies aimed at decreasing dietary exposure to mycotoxins in Zimbabwe. *Critical Reviews in Food Science and Nutrition, 60*(4), 529–540. https://doi.org/10.1080/10408398.2018.1543252

Olorunfemi, B. J., & Kayode, S. E. (2021). Post-Harvest Loss and Grain Storage Technology- A Review. *Turkish Journal of Agriculture - Food Science and Technology, 9*(1), 75–83. https://doi.org/10.24925/turjaf.v9i1.75-83.3714

Osdaghi, E., Young, A. J., & Harveson, R. M. (2020). Bacterial wilt of dry beans caused by Curtobacterium flaccumfaciens pv. flaccumfaciens: A new threat from an old enemy. *Molecular Plant Pathology, 21*(5), 605–621. https://doi.org/10.1111/mpp.12926

Parker, T. A., & Gepts, P. (2021). Population Genomics of *Phaseolus* spp.: A Domestication Hotspot. *Springer International Publishing,* 1, 1-83. https://doi.org/10.1007/13836_2021_89

Pasqualone, A., Costantini, M., Coldea, T. E., & Summo, C. (2020). Use of Legumes in Extrusion Cooking: *A Review. Foods, 9*(7), 958. https://doi.org/10.3390/foods9070958

Rangjaroen, C., Lumyong, S., Sloan, W. T., & Sungthong, R. (2019). Herbicide-tolerant endophytic bacteria of rice plants as the biopriming agents for fertility recovery and disease suppression of unhealthy rice seeds. *BMC Plant Biology, 19*(1), 580. https://doi.org/10.1186/s12870-019-2206-z

Rodríguez Madrera, R., Campa Negrillo, A., Suárez Valles, B., & Ferreira Fernández, J. J. (2021). Phenolic Content and Antioxidant Activity in Seeds of Common Bean (*Phaseolus vulgaris* L.). Foods, *10*(4), 864. https://doi.org/10.3390/foods10040864

Roy, K. (2021). *The effect of germination conditions on growth of Fusarium graminearum and secretion of deoxynivalenol during floor malting of barley*. Masters' Thesis, Dalhousie University, Halifax, Nova Scotia, Toronto, Canada.

Sahu, S., Ghosh, S., Fujita, D., & Bandyopadhyay, A. (2015). Live visualizations of single isolated tubulin protein self-assembly via tunneling current: Effect of electromagnetic pumping during spontaneous growth of microtubule. *Scientific Reports, 4*(1), 7303. https://doi.org/10.1038/srep07303

Siddiq, M., Uebersax, M. A., & Siddiq, F. (2022). Global Production, Trade, Processing and Nutritional Profile of Dry Beans and Other Pulses. In M. Siddiq & M. A. Uebersax (Eds.), Dry Beans and Pulses, 1st ed., pp. 1–28. Wiley. https://doi.org/10.1002/9781119776802.ch1

Stejskal, V., Vendl, T., Aulicky, R., & Athanassiou, C. (2021). Synthetic and Natural Insecticides: Gas, Liquid, Gel and Solid Formulations for Stored-Product and Food-Industry Pest Control. *Insects, 12*(7), 590. https://doi.org/10.3390/insects12070590

Sultana, R., Kunusoth, K., Amineni, L., Dahal, P., & Bradford, K. J. (2021). Desiccant drying prior to hermetic storage extends viability and reduces bruchid (*Callosobruchus chinensis* L.) infestation of mung bean (*Vigna radiata* (L.) R. Wilczek) seeds. *Journal of Stored Products Research, 94*, 101888. https://doi.org/10.1016/j.jspr.2021.101888

Thakur, K., Sharma, A., & Sharma, K. (2021). Management of agricultural insect pests with physical control methods. *The Pharma Innovation Journal*; SP-10(6): 306-314

Turner, E. R., Luo, Y., & Buchanan, R. L. (2020). Microgreen nutrition, food safety, and shelf life: A review. *Journal of Food Science, 85*(4), 870–882. https://doi.org/10.1111/1750-3841.15049

Villers, P., De Bruin, T. O. M., & Navarro, S. (2004). *Advances in hermetic storage as a methyl bromide replacement. In Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast Australia.* FTIC Ltd. Publishing, Israel, pp. 207-223. 8e13th August.

Wafula, E. N., Wainaina, I. N., Buvé, C., Nguyen, N.-D.-T., Kinyanjui, P. K., Saeys, W., Sila, D. N., & Hendrickx, M. (2020). Application of near-infrared spectroscopy to predict the cooking times of aged common beans (Phaseolus vulgaris L.). *Journal of Food Engineering*, 284, 110056. https://doi.org/10.1016/j.jfoodeng.2020.110056

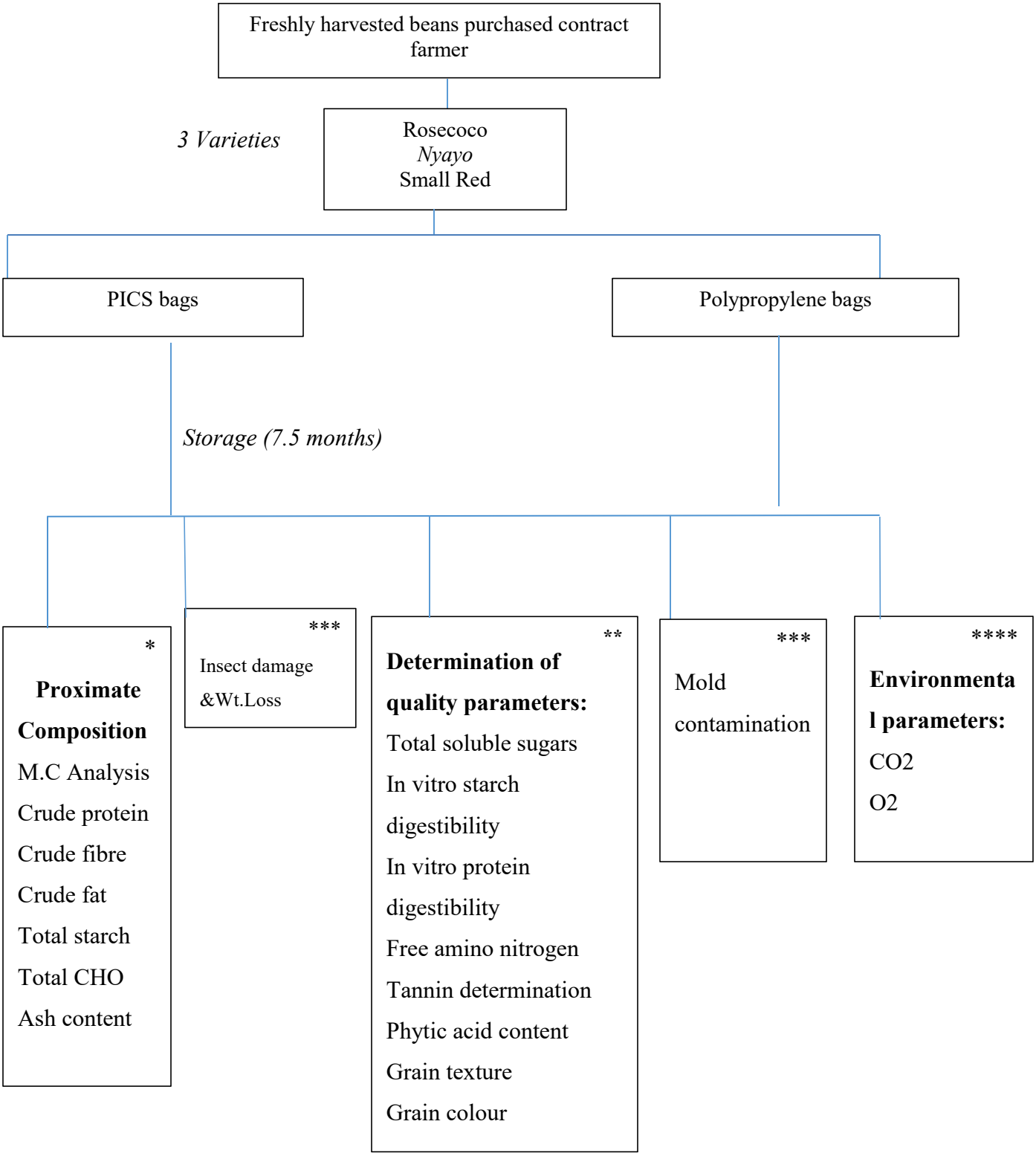
Wainaina, I., Wafula, E., Sila, D., Kyomugasho, C., Grauwet, T., Van Loey, A., & Hendrickx, M. (2021). Thermal treatment of common beans (Phaseolus vulgaris L.): Factors determining cooking time and its consequences for sensory and nutritional quality. *Comprehensive Reviews in Food Science and Food Safety, 20*(4), 3690–3718. https://doi.org/10.1111/1541-4337.12770

Wu, X., Song, M., Qiu, P., Li, F., Wang, M., Zheng, J., Wang, Q., Xu, F., & Xiao, H. (2018). A metabolite of nobiletin, 4′-demethylnobiletin and atorvastatin synergistically inhibits human colon cancer cell growth by inducing G0/G1 cell cycle arrest and apoptosis. *Food & Function, 9*(1), 87–95. https://doi.org/10.1039/C7FO01155E

Zhao, Y., Zhang, R., Jiang, K.-W., Qi, J., Hu, Y., Guo, J., Zhu, R., Zhang, T., Egan, A. N., Yi, T.-S., Huang, C.-H., & Ma, H. (2021). Nuclear phylotranscriptomics and phylogenomics support numerous polyploidization events and hypotheses for the evolution of rhizobial nitrogen-fixing symbiosis in Fabaceae. *Molecular Plant, 14*(5), 748–773. https://doi.org/10.1016/j.molp.2021.02.006.

APPENDICES

Appendix 1: Lab set up



Appendix 2: ANOVA tables

Source	DF	Squares	Mean Square	F Value	Pr> F
Model	22	76.2932407	3.4678746	6.66	<.0001
Error	193	100.4356019	0.5203917		
BAG	1	36.09671296	36.09671296	69.36	<.0001
BREED	2	0.00842593	0.00421296	0.01	0.9919
MOC	2	15.45009259	7.72504630	14.84	<.0001
DAY	5	7.43356481	1.48671296	2.86	0.0163
BAG*VARIETY	2	0.72453704	0.36226852	0.70	0.4998
BAG*MOC	2	1.65953704	0.82976852	1.59	0.2057
BREED*MOC	4	7.71407407	1.92851852	3.71	0.0062
BAG*BREED*MOC	4	7.20629630	1.80157407	3.46	0.0093

The GLM

Procedure Dependent Variable:

STARCH

Source	DF	Sum of Squares	MeanSquare	F Value	Pr>F
Model	22	19692.49903	895.11359	10.55	<.0001
Error	193	16371.70512	84.82749		
Corrected Total	215	36064.20415			

R-Square	Coeff Var	Root MSE	STARCH Mean
0.546040	18.61486	9.210184	49.47759

Source	DF	TypeISS	MeanSquare	FValue	Pr>F
BAG	1	5703.75556	5703.75556	67.24	<.0001
BREED	2	46.25984	23.12992	0.27	0.7616
MOC	2	12204.19027	6102.09514	71.94	<.0001
DAY	5	871.26283	174.25257	2.05	0.0729
BAG*BREED	2	101.97398	50.98699	0.60	0.5492
BAG*MOC	2	506.07674	253.03837	2.98	0.0530
BREED*MOC	4	134.05634	33.51408	0.40	0.8120
BAG*BREED*MOC	4	124.92347	31.23087	0.37	0.8311

The GLM Procedure

Dependent Variable: PROT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	14931.66667	678.71212	6.16	<.0001
Error	193	21272.16667	110.21848		
Corrected Total	215	36203.83333			

R-Square	Coeff Var	Root MSE	PROT Mean
0.412433	19.81888	10.49850	52.97222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BAG	1	4125.629630	4125.629630	37.43	<.0001
BREED	2	339.083333	169.541667	1.54	0.2174
MOC	2	8106.750000	4053.375000	36.78	<.0001
DAY	5	795.500000	159.100000	1.44	0.2104

BAG*BREED	2	309.175926	154.587963	1.40	0.2485
BAG*MOC	2	678.731481	339.365741	3.08	0.0483
BREED*MOC	4	31.166667	7.791667	0.07	0.9908
BAG*BREED*MOC	4	545.629630	136.407407	1.24	0.2963

The GLM Procedure

Dependent Variable: TANNIN

Source	DF	Sum of Squares	MeanSquare	F Value	Pr>F
Model	22	4.64007222	0.21091237	2.81	<.0001
Error	193	14.46991111	0.07497363		
Corrected Total	215	19.10998333			

R-Square	CoeffVar	RootMSE	TANNINMean
0.242809	17.00409	0.273813	1.610278

Source	DF	TypeISS	MeanSquare	FValue	Pr>F
BAG	1	0.07935000	0.07935000	1.06	0.3049
BREED	2	0.65880000	0.32940000	4.39	0.0136
MOC	2	0.31628611	0.15814306	2.11	0.1241
DAY	5	0.59678889	0.11935778	1.59	0.1641
BAG*BREED	2	1.43923333	0.71961667	9.60	0.0001
BAG*MOC	2	0.14132500	0.07066250	0.94	0.3914
BREED*MOC	4	0.73990556	0.18497639	2.47	0.0463
BAG*BREED*MOC	4	0.66838333	0.16709583	2.23	0.0674

Dependent Variable: PHYTIC

Source	DF	Sum of Squares	MeanSquare	F Value	Pr> F
Model	22	15.87764630	0.72171120	2.34	0.0011
Error	193	59.48175185	0.30819561		
Corrected Total	215	75.35939815			

R-Square	Coeff Var	Root MSE	PHYTIC Mean
0.210692	51.17060	0.555154	1.084907

Source	DF	Type I SS	Mean Square	F Value	Pr> F
BAG	1	0.05102963	0.05102963	0.17	0.6845
BREED	2	0.39063704	0.19531852	0.63	0.5317
MOC	2	7.52842870	3.76421435	12.21	<.0001
DAY	5	4.97169815	0.99433963	3.23	0.0080
BAG*BREED	2	0.82744815	0.41372407	1.34	0.2636
BAG*MOC	2	0.33401204	0.16700602	0.54	0.5825
BREED*MOC	4	0.60841574	0.15210394	0.49	0.7405
BAG*BREED*MOC	4	1.16597685	0.29149421	0.95	0.4386

The GLM Procedure

Dependent Variable: AMINO

Source	DF	Sum of Squares	MeanSquare	F Value	Pr>F
Model	22	58.8225380	2.6737517	2.50	0.0005
Error	193	206.5200505	1.0700521		
Corrected Total	215	265.3425884			

R-Square	CoeffVar	RootMSE	AMINO Mean
	25.51152	1.034433	4.054769

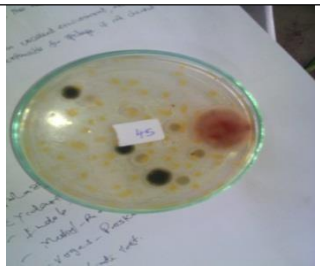
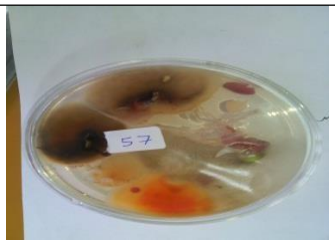
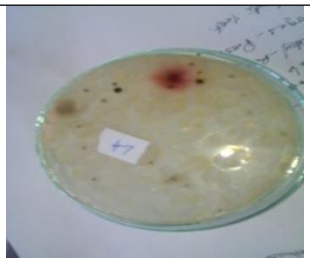
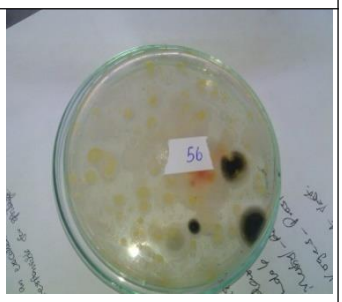
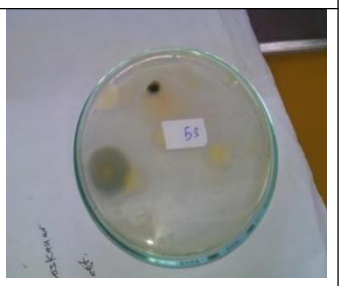
Source	DF	TypeISS	MeanSquare	FValue	Pr>F
BAG	1	5.69400417	5.69400417		
BREED	2	0.15728426	0.07864213	0.07	0.9292
MOC	2	30.57601204	15.28800602	14.29	<.0001
DAY	5	2.07894120	0.41578824	0.39	0.8563
BAG*BREED	2	1.44730833	0.72365417	0.68	0.5097
BAG*MOC	2	10.51200833	5.25600417	4.91	0.0083
BREED*MOC	4	4.97242130	1.24310532	1.16	0.3291
BAG*BREED*MOC	4	3.38455833	0.84613958	0.79	0.5325

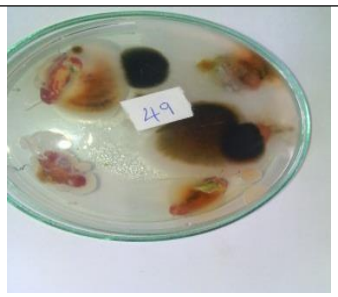
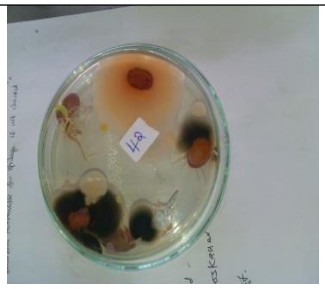
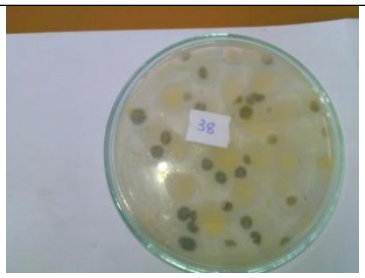
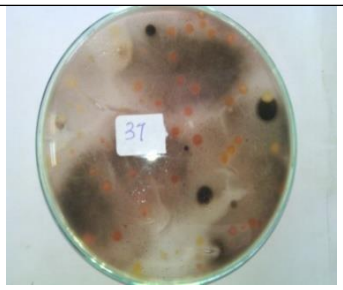
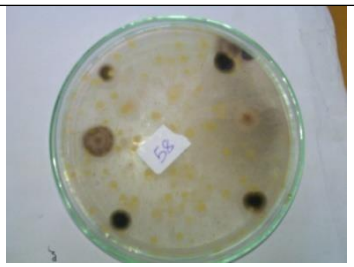
Appendix 3. Mean squares for the analysis of variance (ANOVA)

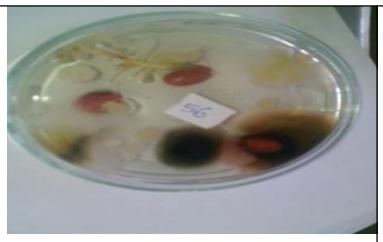
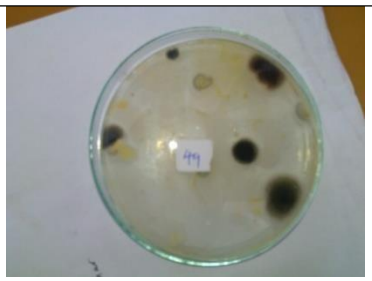
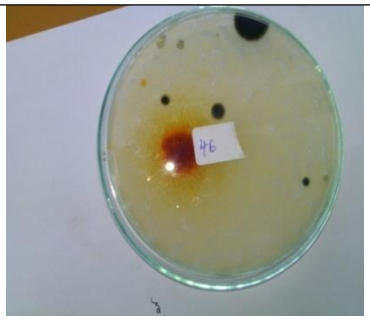
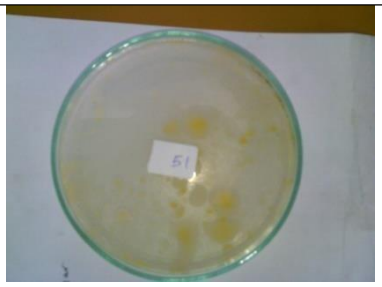
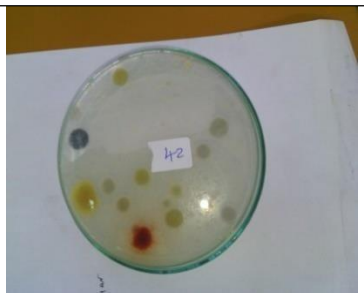
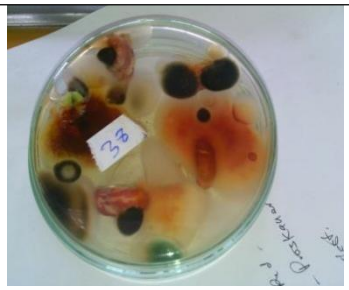
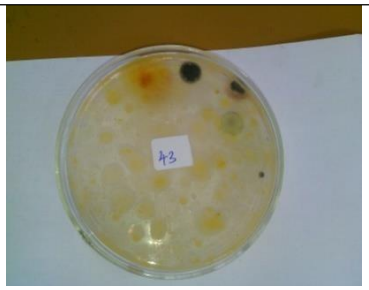
S.O.V	DF	MOC	ASPG	FUS	PENI	RHIZ	Others	Aflatoxin
Bag	1	3358.73***	468.31***	2866.73**	1698.01**	1506.17**	1910.26*	471.09**
Var	2	29.19***	1.36ns	536.12***	70.12**	242.32***	1952.44*	165.79**
SMC	2	1717.35***	1.06ns	180.68*	1202.99**	231.90***	5274.81***	39.29ns
Day	6	4510.89***	1787.50***	919.67***	1495.20**	1266.73**	5334.94***	169.69**
Rep	1	24.14	56.56	19.86	6.35	26.50	1251.03	17.64
Bag*Var	2	43.98***	323.55***	91.12ns	94.56***	676.96***	1690.89*	47.46ns
Bag*SMC	2	46.30***	91.37***	570.07***	724.56***	179.35***	704.23ns	27.11ns
Bag*Day	6	85.55***	45.30***	301.24***	141.30***	89.27***	10140.93***	41.25ns
Var*SMC	4	14.99**	191.99***	501.81***	170.83***	199.69***	652.53ns	69.12ns
Var*Day	12	12.21***	28.44***	139.93***	59.65***	76.01***	842.40ns	30.81ns
SMC*Day	12	162.37***	31.11***	99.50*	71.95***	52.46***	717.08ns	42.62ns
Bag*Var*SMC	4	9.84ns	110.44***	737.21***	77.54***	115.12***	172.40ns	79.16*
Bag*Var*Day	12	5.36ns	56.31***	127.70**	45.15***	43.11**	494.80ns	13.26ns
Var*SMC*Day	24	9.29***	55.10***	94.86*	32.67***	67.87***	425.96ns	51.92ns
Bag*Var*SMC*Day	36	14.35***	21.06***	108.69***	68.62***	49.48***	442.39ns	19.37ns
Error	125	4.36	9.64	50.88	14.80	18.32	476.41	29.65
C.V		16.98	35.70	52.28	35.44	44.52	50.60	162.99
R^2		0.9857	0.9339	0.8177	0.9227	0.8927	0.7399	0.6138

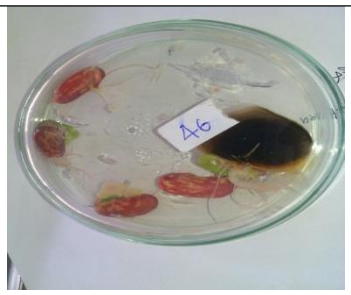
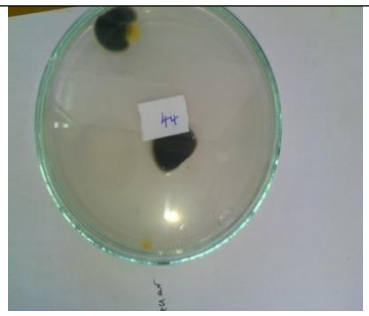
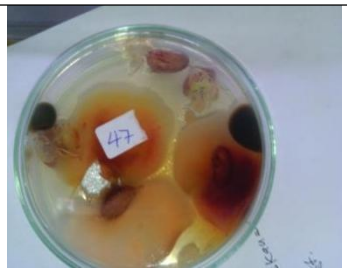
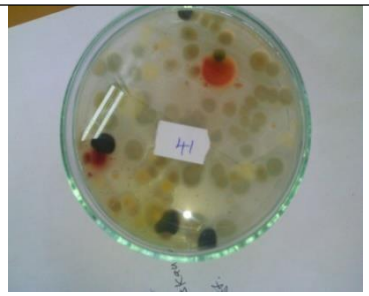
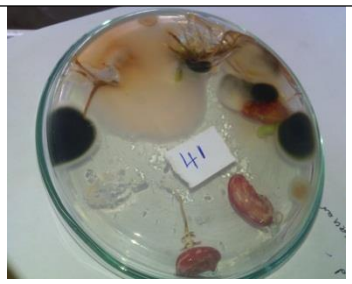
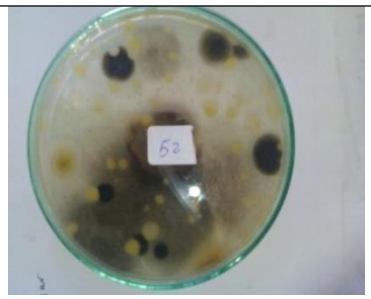
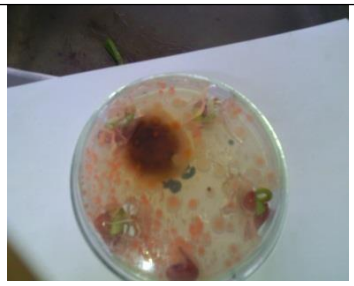
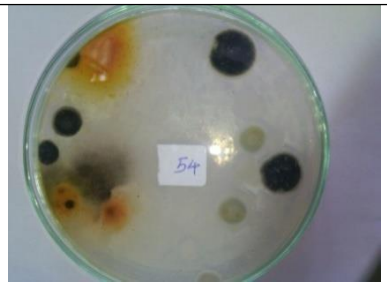
Key: S.O.V; Source of Variations, DF; Degree of Freedom, MOC; Moisture content, ASPG; *Aspergillus* spp., FUS; *Fusarium* spp., PENI; *Penicillium* spp., RHIZ; *Rhizopus* spp., Var; Beans variety, SMC; Moisture Content at which beans were Stored.

Appendix 4.Mold Growth

Sample	Colony counts For with beans		Sample	Colo ny counts For with beans	
37	6		45	10	
57	6		47	23	
	4		56	5	
44	5		53	4	

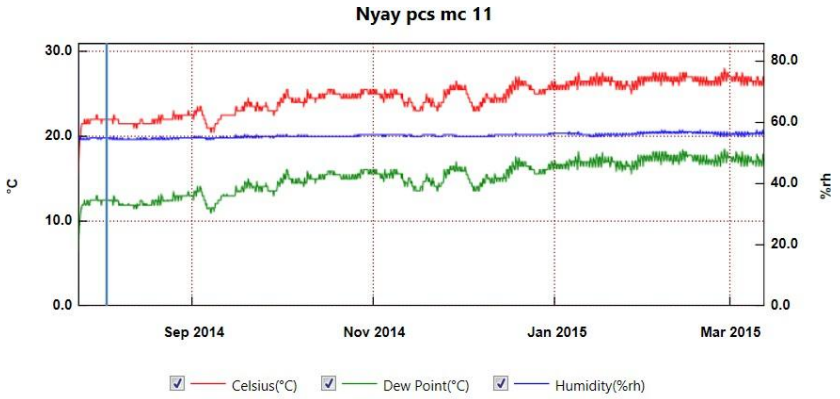
49	9		40	13	
42	9		39	10	
55	8		38	35	
52	5		37	8	
43	7		58	9	

56	5		49	8	
51	5		46	8	
39	5		51	0	
40	5		42	14	
38	13		43	5	

46	1		44	5	
47	7		41	62	
54	3		50	9	
41	8		52	11	
58	3		54	12	

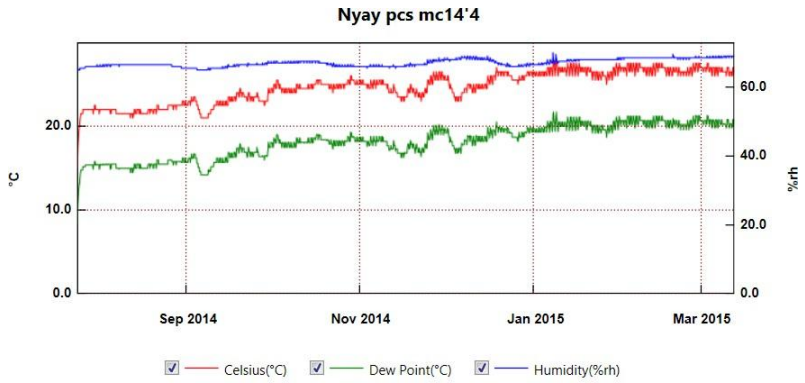
Appendix 5: Temperature Celsius, Humidity, and Dew Point monitoring

A. PICS

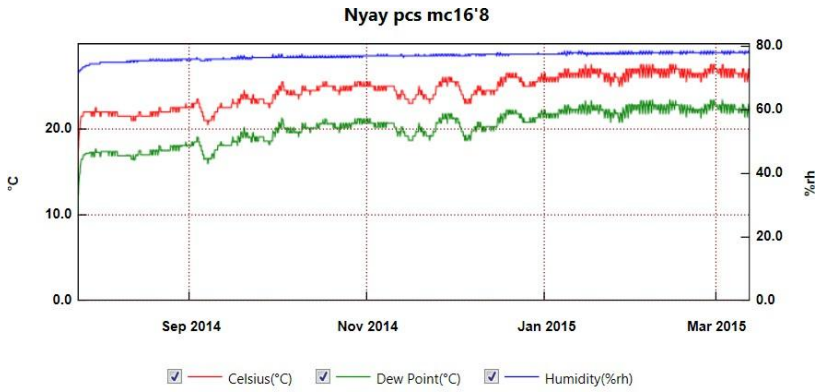


Sunday, August 03, 2014 7:39:18 AM [22.0 12.5 55.0]

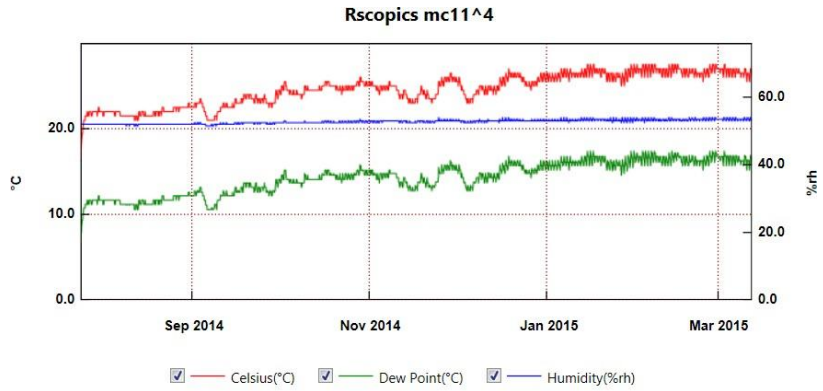
From: Thursday, July 24, 2014 4:04:03 PM - To: Thursday, March 12, 2015 5:04:03 PM



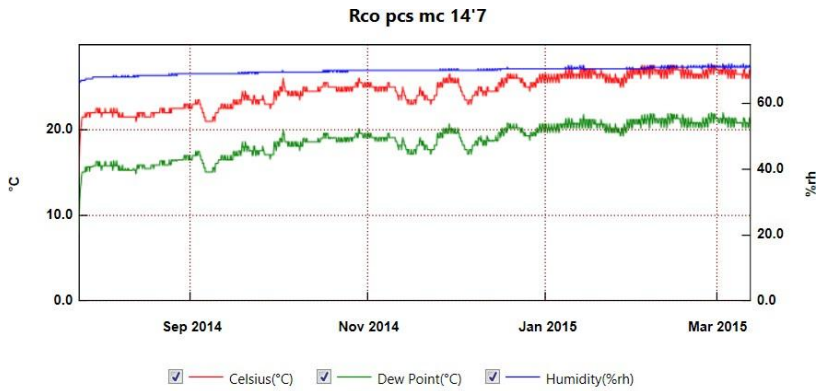
From: Thursday, July 24, 2014 4:17:43 PM - To: Thursday, March 12, 2015 5:17:43 PM



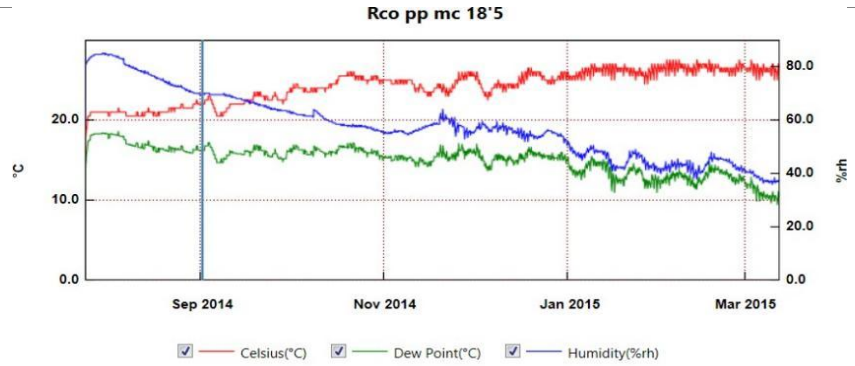
From: Thursday, July 24, 2014 4:21:28 PM - To: Thursday, March 12, 2015 5:21:28 PM



From: Thursday, July 24, 2014 4:09:17 PM - To: Thursday, March 12, 2015 5:09:17 PM

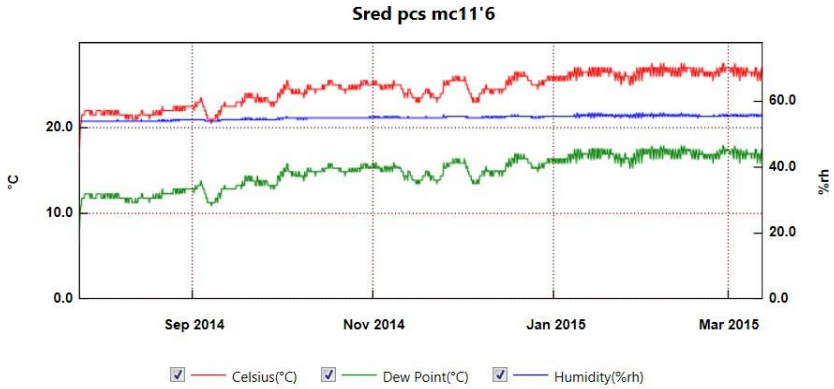


From: Thursday, July 24, 2014 4:18:56 PM - To: Thursday, March 12, 2015 5:18:56 PM

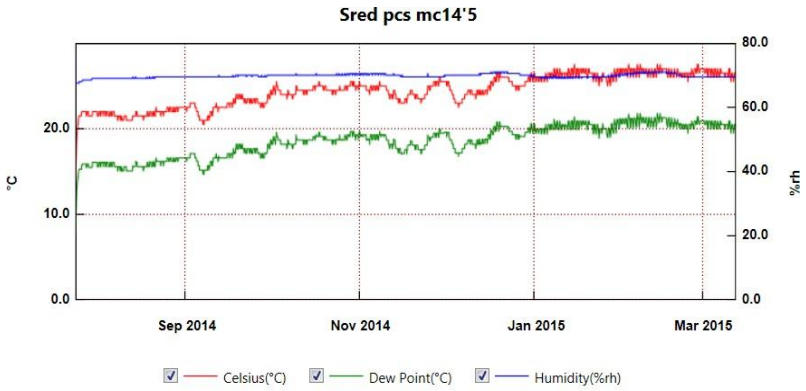


Monday, September 01, 2014 6:13:46 PM [22.0 16.3 70.0]

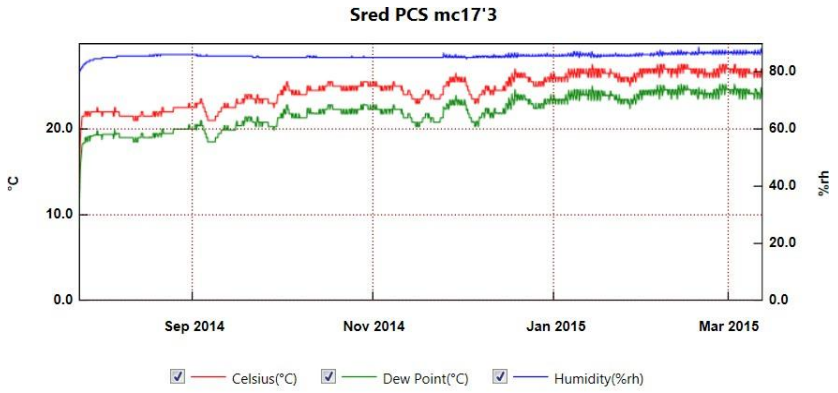
From: Thursday, July 24, 2014 4:32:18 PM - To: Thursday, March 12, 2015 3:32:18 PM



From: Thursday, July 24, 2014 4:12:59 PM - To: Thursday, March 12, 2015 5:12:59 PM

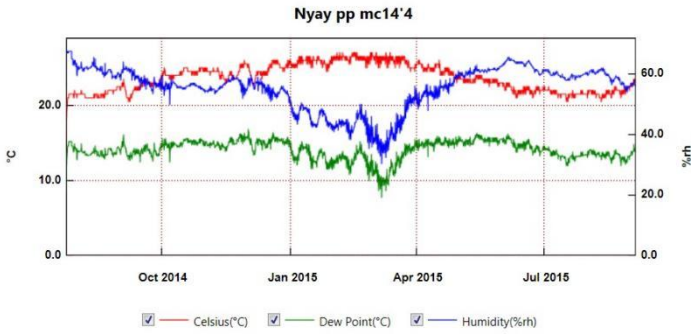


From: Thursday, July 24, 2014 4:20:12 PM - To: Thursday, March 12, 2015 5:20:12 PM



From: Thursday, July 24, 2014 4:23:36 PM - To: Thursday, March 12, 2015 5:23:36 PM

PP



From: Thursday, July 24, 2014 4:26:53 PM - To: Friday, September 04, 2015 4:26:53 PM

*A*ppendix 6: Abstract of the Published Paper

ELSEVIER

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Effect of hermetic Purdue Improved Crop Storage (PICS) bag on chemical and anti-nutritional properties of common Bean (*Phaseolus vulgaris* L.) varieties during storage

Micah Rambeka Momanyi *, John Masani Nduko, Mary Omwamba

Department of Food Science and Technology, Egerton University, P.O Box 536-20115, Egerton, Kenya

ARTICLE INFO

Keywords:
Common beans
Digestibility
Hermetic polyethylene bags
Nutritional quality
PICS
Storage moisture

ABSTRACT

Storage conditions influence the nutritive value and quality of many legumes. The aim of this study was to evaluate the quality of beans stored under hermetic conditions as a strategy for preserving the quality of beans post-harvest. Three bean varieties [*Rosecoco*, small red (*Wairimu*)], and red mottled (*Nyayo*)] were adjusted to three moisture levels (12%, 15% and 18%) and stored in hermetic bags and ordinary polypropylene bags and sampled after 0, 45, 90, 135,180, 225 and 270 days for chemical and anti-nutritional analysis. Total soluble sugars, *in-vitro* starch and protein digestibility, free amino nitrogen, tannin content and phytic acid content of the beans were determined using standard methods. Results showed that the beans in hermetic bags had 22%, 23% and 18% higher total soluble sugars, *in-vitro* starch and protein digestibility, respectively, than those in polypropylene bag during storage. On day 225 of storage, beans in hermetic bags had the optimal *in-vitro* starch and protein digestibility, and tannin content. Principal component analysis indicated that nutrient and anti-nutrient retention of the beans was achieved with lower storage moisture and duration in hermetic bags. The results of this study can be used to explain the superiority of the hermetic storage technology over ordinary methods of beans storage, and by extension other legumes, in nutrient retention during storage.