PHYSICOCHEMICAL AND FUNCTIONAL CHARACTERISATION OF THREE LABLAB BEAN (*Lablab purpureus* L. (Sweet) VARIETIES GROWN IN KENYA

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Physicochemical and functional characterisation of three lablab bean(*Lablab purpureus* L. (Sweet) varieties grown in Kenya

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DECLARATION

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

Signature ……. Date……

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

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DEDICATION

I dedicate this work to my dear daughter, Sharon who has continually encouraged me that it is indeed possible to achieve one's goals.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
ASAL	Arid and Semi -Arid areas
AOAC	Association of Official Analytical Chemists
FAO	Food and Agriculture Organization
GoK	Government of Kenya
HPLC	High Performance Liquid Chromatography
IFAD	International Fund for Agricultural Development
IVPD	*In-vitro* protein digestibility
IVSD	*In-vitro* starch digestibility
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KALRO	Kenya Agricultural and Livestock Research Organisation
KDHS	Kenya Demographic Health Survey
UN-ECA	United Nations Economic Commission for Africa
USAID	United States Agency for International Development
WFP	world Food Programme

ABSTRACT

The Lablab bean can significantly enhance the Food and nutrition security situation, particularly among the communities in the ASAL areas. However, the different varieties of Lablab beans grown in Kenya have received little research attention in comparison to other food crops. The objective of this study was to assess the physical properties, nutritional composition, cooking characteristics and the protein functional properties of three Lablab varieties (KAT/DL-1, KAT/DL-2 & KAT/DL-3) and compare them with that of KAT/X69 *Phaseolus vulgaris* variety (*Rosecoco).* Samples were obtained from KALRO-Katumani Dryland Research Station. The beans were evaluated for their physical characteristics, proximate composition, minerals, anti-nutrients and bioactive components, cooking characteristics, and functional properties of proteins. A fermented product, tempeh was developed and evaluated for its nutrition quality. The data was subjected to analysis of variance and Duncan Test used for mean separation at 5% significant level. The results showed that KAT/DL-1 had significantly higher seed weight and seed porosity (29.0g; 42.8) compared to KAT/DL-2 and KAT/DL-3. KAT/DL-3 had significantly higher hydration and swelling capacities (192.9 and 143.9 respectively) compared to the other two varieties. The three lablab beans had protein content of (25%,24%,22% while KAT/X69 had 21%... Linoleic acid was the most abundant fatty acid. KAT/DL-2 had significantly higher content of phytic acid (723mg/100g), tannins (0.33g/100g) and trypsin inhibitor (13 TIU/mg) compared to the other two lablab beans. Cooking and germination reduced the anti-nutrients significantly. KAT/DL-3 was adequately cooked at 140 minutes while KAT/X69 was cooked at 120 minutes. KAT/DL-1 had better taste compared to KAT/DL-2 KAT/DL-3 and KAT/X69. In conclusion, KAT /DL-1 had significantly higher seed weight and porosity, while KAT/DL 3 had significantly higher sphericity and hydration capacity. The lablab beans had significantly higher protein content compared to KAT/X69. KAT/DL-1 had significantly higher protein and starch digestibility compared to the other two varieties. KAT/ DL-2 had significantly higher levels of phytic acid and tannins, while KAT/DL-1 had significantly higher flavonoids than the other two varieties. All the lablab bean varieties had significantly lower trypsin inhibitory activity and tannins than the common Rosecoco bean. Cooking and germination were found to significantly reduce anti-nutrient levels in the bean varieties. KAT/DL-3 had a significantly shorter cooking than the other varieties. time to cook. It is therefore recommended that Lablab bean utilization should be up-scaled particularly in the arid and semi-arid regions due to its nutritional value. Breeding of easy to cook and low ant-nutrient beans need to be encouraged while more research is required on other Lablab varieties found in Kenya.

CHAPTER ONE

INTRODUCTION

1.1. Background information

The global population suffering from undernourishment is about 795 million, which has been contributed to by low productivity and low incomes of the smallholder family farmers. This has led to low economic growth, which is a key success factor for reducing undernourishment (FAO, IFAD & WFP, 2015).

Kenya experiences recurrent famine and food insecurity in the arid and semi-arid areas that constitute about 80% of the country's land mass (GOK, 2004; USAID, 2014). The country relies mainly on rain fed agriculture for its food production. It is within this context that the country has policies (UN-ECA, 2013) that promote the production of drought tolerant crops for improved food and nutrition security of the population especially in Arid and Semi -Arid areas (ASAL). Among the drought tolerant crops are the pulses and legumes. These are also referred to as the "poor man's meat" since they are a cheap source of proteins (Mortuza & Tzen, 2009). Research has led to successful utilization of legumes in child feeding programmes and food and feed formulations (Kamatchi *et al*., 2010).

Lablab is among the drought tolerant crops in Kenya. The legume crop is believed to have originated in Asia and introduced to Africa from South East Asia in the eighth century (Deka & Sarkar, 1990). It is a herbaceous, climbing and warm-season annual or short-lived plant with a vigorous taproot. It has a thick, herbaceous stem that can grow up to 3 feet, and the climbing vines may stretch up to 25 ft. (Valenzuela & Smith, 2002). Lablab (*Lablab purpureus*) has been reported to have the potential of reducing protein deficiency in developing nations (D'souza, 2013; Kimani, Wachira, & Kinyua, 2012). It can be utilized in enhancing food security and reducing the high prevalence of malnutrition.

The nutritional composition of the lablab bean varies depending on the varieties (Mortuza & Tzen 2009). As is the case with other legume grains, the protein content may vary markedly among cultivars of a single species (Alghamdi, 2009). It has been reported to range between 18–25% with a good amino acid balance (Subagio, 2006). This makes the bean a good source of dietary proteins and as well a suitable source of functional proteins. The bean has also been reported to be a good source of minerals such as iron, zinc, potassium, manganese and calcium as well as essential and conditional amino acids (Alabi & Alausa, 2006) .

The nutritive value of grain legumes depends primarily on their nutrient contents, and presence or absence of anti-nutritional and or toxic factors (Ramakrishna, Rani, & Rao, 2006). Different processing methods may be employed to remove the anti-nutrient factors (ANF) for effective utilization of the legume nutrients as human food (D'souza, 2013). The ANF in the untreated lablab bean may include trypsin inhibitors, hydrogen cyanide, tannins, and phytic acid, as these are usually found in legume grains (Subagio, 2006a). These antinutrients adversely affect the utilization of the nutrients in the beans. For instance, D'souza, (2013) reported that the trypsin inhibitors at high levels can prevent metabolism of proteins, while phytates compromise mineral absorption and tannins chelate the minerals.

Among the processing methods that have been used in the reduction of anti-nutritional factors in legume grains include soaking, germination, roasting, cooking and autoclaving (Osman, 2007). Heat processing of legumes generally improves the nutritive value of proteins by inactivating the anti-nutrients such as trypsin inhibitors and hemagglutinins (Tharanathan & Mahadevamma, 2003) . Cooking has been reported to be most effective in the reduction of trypsin inhibitors in Lablab beans while roasting gives better results for phytic acid reduction (D'souza, 2013).

The cooking characteristics of the beans have been attributed to the physical characteristics such as the seed size, colour, volume, density, hydration capacity and

swelling capacity (Mortuza & Tzen, 2009). These physical characteristics are also important in the processing and functional properties of the bean. Mortuza & Tzena, (2009) reported that the hydration and swelling capacities of the bean reflect the ability to imbibe water in a reasonable period of soaking and thus affect the cooking time.

A survey on Lablab beans carried out by Kamotho *et a*l. (2006) in Kinyua & Kiplangat (2012) established the various challenges, utilization and the most utilized varieties in various regions of Kenya. The results showed low production where some farmers would grow the crop on terraces as a cover crop even though its yields were found to be relatively higher than those of the common bean. Its utilization was adversely affected by the bitter taste among some of the varieties and the long cooking time. However, the crop was reported to command a higher price in the Kenyan local markets than other legumes. The bean varies in colour and size. The colours include black, cream, brown and dirty brown (Kinyua & Kiplangat, 2012). According to the Kenya Agricultural Research Institute plant genetic resource, there were three varieties of Lablab bean varieties released between the year 1978 and 1995. They are maintained at the KARI-Katumani station namely KAT/DL-1, KAT/DL-2 and KAT/DL-3. All the varieties can do well in an altitude of 1000 to 2000 metres above sea level and have maturation period of between 3-4 months.

1.2. Problem statement

Kenya experiences a relatively high prevalence of food insecurity and undernutrition, particularly in the arid and semi-arid regions of the country. The latest Kenya Demographic and Health Survey (KDHS) revealed a prevalence of stunting of 26% among children under the age of five (KDHS, 2014) . Stunting is a commonly used indicator of chronic undernutrition or malnutrition. The arid and semi-arid parts of the country have been classified under ‘stressed’ and ‘criitical’ by the Integrated Food Security Phase Classification (IPC, 2012). In 2014, approximately 1.5 million Kenyans required emergency food assistance while about 350,000 children had acute malnutrition contributed by inadequate rainfall (USAID, 2014). The Lablab bean is one

of the relatively drought tolerant crops that grow in such regions, with a potential to contribute towards the alleviation of the malnutrition and food insecurity. However, currently Lablab is grown mainly at subsistence level. It has not received as much research attention as the common bean. The Kenya Plant Health Inspectorate released three varieties of lablab beans for as fodder crops in the year 1978. However, there is no information on the nutritional composition, anti-nutrients and the functional properties of these varieties. Further, there is also lack of information on the cooking and physical characteristics of these beans as well as how certain treatments like soaking; germination and storage duration affect their nutritional quality and the cooking time. In addition, there is insufficient data on the various methods of eliminating the anti-nutrients, effects of these methods on nutritional composition and the ways of improving the cooking characteristics of these beans. Among the factors that limit the utilization of beans generally, include presence of anti-nutrient factors, long cooking periods and flatulence (Díaz *et al*., 2010; Osman, 2007). Therefore, addressing these issues is likely to result in increased utilization, demand and production of these beans.

1.3. Justification

Grain legumes are characterized by presence of soluble and insoluble dietary fibre, slow digestive starch, prebiotic oligosaccharides and phenolic compounds that have been associated with chronic disease prevention and management. They have capacity to regulate glycaemia and gastrointestinal function while other components act as antioxidants. This gives them the potential to act as novel ingredients in nutritional quality improvement of foods necessitating the need for research on the presence and quantity of such components in Lablab.

Lablab is classified among the orphaned crops of Africa that have received minimal research attention due to their perceived limited economic importance. The heat and drought tolerant legume is extensively used by subsistence farmers and its utilization

could be improved by informed decisions on its nutrient composition, cooking and functional properties.

Lablab is a drought tolerant crop that can be grown in a wide variety of climate and soil conditions. It is well adapted to the semi-arid climates hence it is suitable for most parts of Kenya. They also act as cover crops and nitrogen fixing agents. This is because unlike other plants, legumes have a better symbiotic nitrogen fixation. This has an economic significance due to low crop failure in areas that receive minimal rainfall.

In order to stimulate wider adoption of lablab, there is need to enhance research to make it attractive to the producers and users. It will also increase the legume's potential to feed the population that is projected to double by the year 2050.

Food processing and preservation requires effective and efficient equipment and methodologies that are affordable to both the processor and consumer without compromising the quality of the product. Research on nutritional, cooking and functional characteristics of Lablab will create a better understanding on various processing techniques and novel foods that can be obtained from the legume and hence enhance its production and utilization. The information obtained will also be useful to policy makers in integrating lablab production into the food and agricultural system.

1.4. Objectives

1.4.1. Overall objective

The main objective of the study was to assess the nutritional quality, cookability and protein functional properties of three Lablab *(Lablab purpureus L.* Sweet*)* bean varieties and the effects of processing on these characteristics, and the applicability of the beans in product development.

1.4.2. Specific objectives

1. To determine and compare the physical characteristics of the lablab bean varieties in Kenya.
2. To determine and compare the nutrient composition of Lablab bean varieties grown in Kenya.
3. To determine and compare the protein and starch digestibility of the bean varieties grown in Kenya.
4. To determine and compare the antinutrient and bioactive components in the lablab bean varieties in Kenya.
5. To determine and compare the protein functional properties a of the Lablab bean varieties grown in Kenya.
6. To determine and compare the cookability and sensory characteristics of Lablab bean varieties grown in Kenya.
7. To determine the effect of processing (soaking, germination and boiling) on anti-nutrients and bioactive components in the Lablab bean varieties.
8. To assess the applicability of Lablab beans in formulation of lablab tempeh.

1.5. Null Hypothesis

1. There is no significant difference in the nutritional composition, protein functional properties, anti-nutrients and cookability among Lablab bean varieties grown in Kenya and also between the lablab beans and the common bean, Rosecoco.
2. There is no significant difference on the effect of soaking and germination and cooking on the nutrition composition, anti-nutrients and cookability among the Lablab bean varieties and also between the lablab beans, and the common bean, Rosecoco.

CHAPTER TWO

LITERATURE REVIEW

2.1. Drought resistant Legumes as a mitigation to hunger and food insecurity

2.1.1. Lablab as a neglected crop

Lablab is classified among the neglected crops of Africa that can solve the basic problems of hunger, malnutrition, rural poverty, environmental destruction (National research council, 2006). This is because besides provision of nutrients, they act as a cover crops (Maass *et al*., 2010). They also act as weed suppressors and soil erosion retardants (D'souza & Devaraj, 2011). This innovative approach can help to eradicate food shortages and improve the lives of subsistence farmers in tropical regions (Small & Raizada, 2017). If these practices are put into consideration they will contribute to the goal of ending hunger, achieving food security together with improved nutrition, and promoting sustainable agriculture. (Small & Raizada, 2017). Legumes are known to form a symbiotic relationship which can hide the atmospheric nitrogen gas like ammonia, which is known as biological nitrogen fixation (BNF) (Morton, 2007). According to Mubiru and Coyne, (2009), biological nitrogen fixation enables the drought resistant legumes to be rich in nitrogen, a building block organic nitrogen fertilizer and edible protein to restore soils fertility. These drought resistant legumes can be used as food and at the same time as a way of reducing the need of artificial fertilizers., They can also prevent soil erosion and in most cases suppress growth of weeds that grow on bare and dry soil. The practice of cover cropping has not been practiced traditionally in most of subtropical areas which causes the biological nitrogen fixation to be inhibited by dry seasons. Most of the subsistence farmers all over the world benefit from drought resistant legumes which are able to maintain nitrogen fixation in dry periods (Mubiru & Coyne, 2009).

Lablab can do well in diverse climatic conditions (arid, semi-arid, subtropical and humid temperatures). This drought resistance legume can grow in lands which are dry

and limited to rainfall. The crop acts as a good source of carbohydrates and proteins (National research council, 2006).

2.1.2. Role of Legumes

Most of the subtropical regions experience extended dry reason which limits growth of most crops in these areas hence causing seasonal scarcity of food. Worldwide about 600 million peasant and landless labourers are faced with hunger during dry seasons (Morton, 2007). Approximately 45% of global agricultural land is vulnerable to intense surface run off especially during transition from one season to the other (dry season to rainy season). Erosion causes soil infertility and nutrient mining. Drought-resistant legumes are able to mitigate these problems.

Legumes are considered as a meat alternative in many parts of the world (Robotham & Chapman, 2015) They are nutritionally rich containing proteins with amino acids. They form at least 60% of complex carbohydrates. Legumes contain minimal fats providing less than 5% energy except peanuts chickpeas and soybeans (Maphosa & Jideani, 2016). Additionally, legumes such as lablab provide important minerals and vitamins. Apart from their rich nutritional content, they are associated with cultural, physiological, economical and medicinal benefits because of their strong possession of important bioactive compounds (Kaosar *et al*., 2007). Research shows that legumes contain antioxidant characteristics which are vital in cancer prevention, osteoporosis, heart diseases and many degenerative diseases (Gebrelibanos *et al*., 2013). These antioxidant components make legumes attractive people in need of weight management and diabetic. The incorporation of drought resistant legumes in diets in Kenya and other parts of the world can play a major role in fighting protein-energy malnutrition.

2.2. Characteristics, domestication and varieties of Lablab beans

2.2.1. Characteristics of Lablab beans

Lablab purpureus L. (Sweet) is one of the most ancient crops among cultivated plants that belong to the Fabaceae family. It is a twining plant with alternate and trifoliate leaves whose pods and seeds vary considerably in colour and size (Guretzki & Papenbrock, 2014). The herbaceous, perennial plant normally has a bushy, semi-erect and prostate growth pattern with white, pink, red or purple flowers. The bean can grow up to one metre tall with long stem climbing types extending to six metres long. They are predominantly self-fertilising crops with strong taproot and many lateral and adventitious roots. Lablab is adaptable to diverse climatic conditions ranging from arid, semi-arid, sub-tropical and humid regions where temperatures vary between 22°C–35°C and soils of varying pH (Abdel-Wahab, Shabeb, & Younis, 2002).

Figure 2.1: Flowering lablab bean plants
Source: (www.Lablab.org-University of Agricultural Science, Bangalore, 2012).

Lablab bean grows in areas with rainfall as low as 400mm where deep soils are available. They also do well in excess rainfall 750 mm but not above 2500 mm.

2.2.2. Domestication, synonyms and common names for Lablab

The ancient crop Lablab can be traced from around 1500 BC. It is a native of Africa and Asia (Pengelly & Maass, 2001). Many tropical botany publications have described

Lablab's origin to have been Asia. This is because the Asian continent especially south and southeast Asia have made great developments and utilisation of the plant (National Research Council, 2006). However research by Robotham and Chapman (2015) has shown that this legume crop originated in East Africa and spread to other parts of the world and might have been introduced to Asia more than once.

The description of different varieties of Lablab as summarised in the University of Agricultural Science, Bangalore, (2012) web page www.Lablab.org started in 1754 by Linnaeus who described the species under *Dolichos* L. while Adanson phrased the name Lablab for Dolichos L. in 1763. In the year 1832, Roxburgh described seven varieties of Dolichos Lablab in India where five of them were cultivated. These were further divided into two categories of *Dolichos Lablab* var. *typicus* and *Dolichos Lablab* var. *lignosus* by Barker (1911) as cited by (Savitha, Ravikumar, & Shinde, 2012). Further work by Verdcourt (1970) recognized three sub-species namely: *unicinatus*, *purpureus* and *bengalensis* which were differentiated in terms of chromosome numbers and size of pods. The *spp purpureus* was the commercial variety (Verdcourt, 1971). In the year 1980, Verdcourt assigned Dolichos *L.* the name Lablab widely known as *Lablab purpureus* L. (Sweet) though sometimes referred to it as *Dolichos Lablab* hence the interchangeable use of the names (Fuller, Korisettar, Venkatasubbaiah, & Jones, 2004). The name Lablab is derived from Egyptian word that describes the dull clattering of the seeds inside the dry-pods.

Synonyms: *Lablab purpureus* L. *Sweet; Dolichos Lablab* L*; Dolichos purpureus* L*; Dolichos Lablab* ssp *ensiformis* Thunb; *Dolichos cultratus* Thunb; *Dolichos bengalensis* Jacq; *Dolichos Lablab* var*; hortensis* Schweinf and Muschler; *Dolichos albus* Lour; *Dolichos uniflorus; Dolichos Lablab* ssp *bengalensis* Jacq; *Lablab niger* Medik; *Lablab vulgaris* Savi; *Lablab leucocarpos* Davi*; Lablab purpureus* ssp *purpureus* Verdc; *Lablab vulgaris* var; *niger* DC; *Lablab purpureus* ssp *uncinatus* Verdc; *Lablab perennans* DC;*Lablab nankinicus* Savi; *Lablab purpureus ssp*

bengalensis (Jacq.) Verdc. (Islam, Prodhan, & Sarwar, 2007; University of Agricultural Science,Bangalore, 2012).

Common names: Almost every country uses a different common name for Lablab bean (Murphy & Colucci, 1999). The multiplicity of the names is indicative of its global distribution, ancient existence and, cultivation for human food and animal forage.

Table 2.1: Different names used for Lablab bean in the world

Dolichos bean	*Hyacinth bean*	*Bonavist bean*	*Seim bean*	*Chimbolo Verde (Costa Rica*
Field bean	*Mochakotta*	*Seim bean*	*Ataque, D. du Soudan*	*Amora guaya*
Lablab bean	*Macululu (Angola)*	*Poor man bean (Australia)*	*Poroto bombero (Chile)*	*Njahe (Kenya)*
Common bean	*Pendal bean*	*Chikkudu (Telugu, India)*	*P.indien (Mauritius)*	*Poroto bombero (Chile)*
Pole bean,	*Waby bean (English)*	*Avare*	*Chapparadavare*	*Chikkadikai (Kannada, India)*
Avara,	*Louria (Cyprus*	*Bounavista pea*	*Dolic (d' Egypte)*	*Fiwi bean*
Ballar (Hindi, India)	*Shim (Bengali, India)*	*Val (Gujarathi, India)*	*Mochai (Tamil, India)*	*Sin bean (Assam,India)*
Batau, Japanese	*Australian pea*	*Bannabees (Guyana)*	*Itab (Philippines)*	*Agni quangoahura (Ivory coast*
Papaya bean	*Wal (Marathi, India)*	*Kerara (Indonesia)*	*Gallinita (Mexico)*	*Dauvan*
Sem (Trinidad)	*Bunabis (Grenada.)*	*Seam*	*Butter bean*	*Caraota Chivata*
Indian bean	*Cabellero (Salvador)*	*Frijol bocon*	*F chileno (Peru)*	*F.de la tierra (Cuba)*
Egyptian kidney bean	*Kekara (Malaysia)*	*Tonga bean*	*Gallinazo blanco (Venezuela)*	*Fuji-mame (Japan)*
Gerenga (Ethiopia)	*Gueshrangaig (Egypt)*	*Haricot cutelinho (Portugal)*	*Helmbohne (Germany)*	*Kashrengeig (Sudan)*
Cumandiata	*Labe-labe (Brazil)*	*Lubia bean (Ethopia)*	*Macape (Malag)*	*Macululu (Angola)*

Source: (Murphy & Colucci, 1999).

2.2.3. Major *Lablab purpureus* commercial cultivars of the world

There are three main cultivars of *Lablab purpureus* spread across the world with over 500 germplasm accessions (University of Agricultural Sciences, 2013).

2.2.3.1. Rongai variety

The Rongai cultivar was derived from material from the Rongai District of Kenya according to Cameron (1988) and was released in New South Wales, Australia in 1962 (Karachi, 1997). The variety is a summer growing, widespread and vigorously twining herbaceous annual or short-lived perennial. The stems of the plant can trail, reaching 3 to 6 m in length. The leaves are almost even smooth on the upper surface and have short hairs on the lower surface. Petioles are long and slender and inflorescence lax, fascicled, of many flowered racemes on elongated peduncles. Its smooth pods are 4-5 cm long containing two to four seeds (FAO, 1997; Venkatesha *et al*., 2007). The seeds are buff or pale brown in colour with a conspicuous white hilum 1.0 cm long and 0.7 cm broad (Smith, Rouquette, & Pemberton, 2008). The brown, ovoid and laterally compressed seeds number 3600-4300 per kg (Barnard, 1972). Rongai is a late maturing white flowering cultivar that will continue to grow until cut or damaged by frosts. In the absence of frost, flowering may continue for several months (Cameron, 1988). Crosses have been made to develop a more endurance variety to winter seasons.

Figure 2.2: Picture of Rongai Lablab variety
Source: Graham *et al*., 1986)

2.2.3.2. Highworth Lablab

This is a high seed yield variety that was introduced in Australia from South India for grain production. It is characterised by high foliage dry matter, purple flowers and blackseeds (Graham *et al*., 1986). The Highworth cultivar originated from Coimbatore, South India and is morphologically similar to Rongai. Contrasting with the green foliage, white flowers and light brown seeds of Rongai, foliage of Highworth has a purple band near the leaf axil, purple flowers and black seeds. Highworth is an early flowering line with high seed-yielding ability; it is suitable for pulse production and forage uses. It was originally intended for grain production in districts where early frosts prevented the seeding of Rongai (Cameron, 1988). This variety has also been traced to have originated from Kenya (National Research Council, 2006).

Figure 2.3: Photo of high worth Lablab bean
Source: Graham *et al*., 1986).

2.2.3.3. Koala Lablab

This was introduced into Australia from France by the Queensland Department of Primary Industries in 1962 as a forage crop (Mullen, Holland, & Heuke, 2003). Koala is an early maturing plant with pale mauve of flowers, which are borne in terminal racemes 20–30 cm long and cream coloured seeds. The pods of Koala are 3– 6 cm long, usually comprising 2– 5 seeds with a seed weight of 20– 24 g/100 seeds (National Research Council, 2006) . Koala Lablab is adapted to a wide range of soils, from sandy loams to heavier textured, well drained clay loam soils and it is suitable for food and fodder.

Figure 2.4: Photo of Koala Lablab bean
Source: Graham *et al*., 1986).

Many tropical botany publications have pointed out that Lablab beans probably originated from Asia (National research council, 2006). This is because it has found

greatest development in South Asia and Southeast Asia (Graham *et al*., 1986; University of Agricultural Sciences, 2012). However, the centre of diversity of genus Lablab is Africa (National Research Council , 2006) where the native seeds were found across the Indian Ocean. It is suitable for almost all the regions of Sub-Saharan Africa.

2.3. Drought tolerance of lablab beans

Drought is major limiting factor to agricultural productivity (D'souza & Devaraj, 2011). Lablab thrives under high heat and humidity (National Research Council, 2006). The drought tolerance of crops is related to quantitative and qualitative changes in the antioxidant systems and other metabolic adjustments (D'souza & Devaraj, 2011). The drought mitigation strategies employed by lablab include biochemical, physiological, morphological and developmental processes (Myrene & Devaraj, 2013). During drought the Lablab bean produces various enzymes that reduce peroxide which may be detrimental to plant life during the drought period. Additionally, D'souza and Devaraj (2011) found out that several defence mechanisms function in the leaf and in the shoots to moderate oxidative stress in dry season rendering the plant drought resistant (Akpapunam, 2011). These advantages form part of major reasons for the use of this bean in tropical zones (National Research Council , 2006). It is grown under a warm climate, humid conditions and at a temperature of 18-30 degrees Celsius. Temperature below 20˚C will reduce the growth of the plant. These drought adaptive characteristics among others make lablab bean promising for land restoration and sustainable agricultural systems (National Research Council, 2006). This is a major factor in addressing food insecurity and malnutrition.

2.4. Production, and use of the Lablab bean

Lablab production is widely distributed globally since it has a wide range of soil adaptability and pH range of between 4.5 to7.5 (National Research Council , 2006). The plant is drought tolerant when established and will grow where rainfall is <500 mm and temperatures of 18-30ºC. Lablab bean is a multipurpose legume which can be utilised as a seed, fodder and as well cover crop (Maass , et al., 2010).

Despite its ancient existence and cultivation, Lablab is not one of the crops featured in the production statistics (www.faostatistics.org) because it has been considered as a minor legume for a long time (Hill, Hiu, & Hill, 1989). Within India, the crop is largely

cultivated in districts in Tamil Nadu. It has an expected production of about 18,000 tonnes from an area of 85,000 hectares (University of Agricultural Sciences, 2013). In tropical Africa, Lablab is widespread but less popular than some other leguminous vegetables and pulses. Though the seeds and pods are available in the African local markets, there are no statistical data available on production or trade (Adebisi & Bosch, 2004).This is because the bean is still considered a minor crop in legume classification (Kimani *et al*., 2012; Maass, *et al*., 2010; Maass, Robotham, & Chapman, 2016).

In some places Lablab has been produced for forage use rather than human food (Agyemang *et al*., 2000; Konduri, Godwin, & Liu, 2000). Its increased production as forage has resulted from the high demand for meat and meat products that require more cultivated pastures (Aganga & Tshwenyane, 2003). Lablab presents an opportunity of improving the livestock feed in quantity and quality at the time when natural grasses have become less (Ewansiha *et al*., 2007). The crop is fast growing and can give up to 10 tonnes of fodder per hectare(National research council, 2006). This high-protein livestock feed has been incorporated in the fodder bank technologies for beef-fattening and dairy-production (Chigariro, 2004). The crop can be fed to other small animals like the goats, sheep and pigs (Ajayi, Babayemi, & Taiwo, 2008).

In most cases, Lablab is intercropped with maize, millet or sorghum (Hassan *et al*., 2014). It is a good cover crop and nitrogen fixer. In an experiment carried out (Ojiem *et al*., 2007), Lablab showed the greatest resilience in N_2-fixation and net nitrogen input across different agro-ecological and soil fertility gradients. The crop provides a dense green cover which can protect the soil against desiccation and a source of manure according to (Kimani *et al*., 2012). Studies on intercropping of vegetables and cereals with *Lablab purpureus* have indicated high yields realisation (Amole *et al.*, 2013; Haque, Roy, & Sikdar, 2004).

Lablab is one of the commonly consumed leguminous vegetables in India (Khanum, Swamy, Krishna, Santhanam, & Viswanathan, 2000; Maass , et al., 2010). In the

tropical Africa the young green pods and immature seeds are eaten boiled while the young leaves are used as a leafy vegetable (Adebisi & Bosch, 2004). In other places like in northern Nigeria and Kenya, the dry seeds are consumed as pulses while in Madagascar and Mauritius, Lablab is grown on a small scale for both the green and dry seeds.

2.5. Nutritional composition of Lablab beans

Legume seeds are rich in many nutrient components including protein, starch, dietary fibre, certain fatty acids and micronutrients (Baginsky *et al*., 2013; Troszyńska & Ciska, 2002). As is the case with other legume grains, Dolichos (*Lablab purpureus*) is a good source of these nutrients and has the potential of reducing protein deficiency in developing nations (D'souza, 2013) and malnutrition in general (National research council , 2006). It can therefore be utilized in food security and malnutrition interventions due to its supply of a variety of nutrients. The seeds generally contain a moderate protein content of 18–25% with a good amino acid balance (Subagio, 2006a; Arora, 2014) hence a good source of dietary proteins. The bean is also be considered a suitable source of functional protein (Kaosar *et al.*, 2007). The bean has about 6-7% of lysine hence can complement cereal diets (National Research Council, 2006).

The nutritional composition of the Lablab bean may vary depending on the varieties. Data obtained by Mortuza and Tzen (2009) on proximate composition exhibited variations on crude protein, crude fat, carbohydrates and the moisture content of the different cultivars. It has been reported previously that the protein content in edible legumes may vary markedly among cultivars of a single species (Alghamdi, 2009).

Legumes have total lipids ranging from 1.0% to 46.7% (Sridhar & Seena, 2006). Lablab has a low-fat content compared to groundnuts and soybeans. Mortuza & Tzen, (2009b) reported lipid levels of between 3.14 – 3.84 g /100 g in different lablab varieties. Chau, Cheung, and Wong (1998) on the other hand found lipid content of 2.6 g/100g in samples of lablab.

Lablab beans also act as good sources of minerals such as potassium, sodium, calcium, zinc, magnesium, manganese and copper. Varieties of lablab obtained from Tamil Nadu Agricultural University had potassium content of 1725 mg/100g, 575 mg/10g calcium while zinc and iron were at 2.6 mg/100g and 10.2 mg/100g. Lablab bean samples analysed by Alabi and Alausa (2006) were found to contain 4.2 mg/100g of zinc and 1048 mg/100g of potassium.

Dolichos lablab are also a good source of energy with carbohydrate content of about 60% (Arora, 2014). The bean has been commended for diabetic patients due to its content of dietary fibre (Bhattacharya & Malleshi, 2012). The beans are believed to have appetite suppressive peptides that can induce satiety by stimulating cholecystokinin (CCK) secretion (Kaosar *et al*., 2007).

2.6. Anti-nutritional factors (ANF) and bioactive components in Lablab beans

2.6.1. Anti-nutrients in lablabs

The nutritive value of grain legumes depends primarily on their nutrient contents, and presence or absence of anti-nutritional and / or toxic factors (Ramakrishna, Rani, and Rao, (2006). The term anti-nutrient or natural toxicants describes the defence metabolites that protects the seeds exposed to oxidative damage by many environmental factors such as light, oxygen, free radicals and metal ions (Troszyńska & Ciska, 2002).The ANF are substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms which exert effects contrary to optimum nutrition (Soetan & Oyewole, 2009).The observed biological effects normally depends on the structures of the individual compounds that range from high molecular weight proteins to simple amino acids and the oligosaccharides which are a form of carbohydrates. The ANF may be divided into two major categories i.e. proteins (such as lectins and protease inhibitors) and other substances which are not sensitive to normal processing temperatures like polyphenolic compounds non-protein amino acids and galactomannan gums (Aremu, Ibrahim, & Ekanem, 2016).

Phytic acid, also known as myo-inositol hexakisphosphoric acid is a naturally occurring compound in plants (Gilani, 2005) . Phytates is the phytic acid bound in seed minerals and is the storage form of phosphorus in nuts, seeds and legumes (Uebersax, 2006). Phytic acid content varies in terms of the seed type and environmental conditions such as soil quality, and climate (Konietzny, 2003). In bean seeds, the phytates are concentrated in the cotyledon layer hence mechanical processes may not effectively remove the phytates (Konietzny, 2003; Gilani, 2005). This compound is considered as an antinutrient because of its inhibitory effects on dietary mineral bioavailability (Konietzny, 2003). Phytates bind minerals in the gut making them unavailable for absorption. Mostly phytates affect zinc, iron, calcium and manganese bioavailability (Gebrelibanos *et al*., 2013; Konietzny, 2003).

Tannins are polymeric flavonoids comprising of a small part of the broad and diverse group of plants phenolic compounds (Díaz *et al*., 2010).They can be grouped into hydrolysable (gallic acid derived) and condensed tannins which are flavan-3,4 diol derived (Díaz *et al*., 2010; Sieniawska & Baj, 2017). They can precipitate proteins and complex with iron in the gastrointestinal lumen, reducing the absorption, digestibility and availability of these nutrients (Díaz *et al*., 2010). On the other hand, anthocyanin which are also generated by the flavonoid biochemical pathway are considered as anti-inflammatory, vasotonic, and anti-oxidant compounds that play an important role in the prevention of degenerative illnesses (Uebersax, 2006). Condensed tannins are oligomers of catechin or epicatechin mostly found in plant legumes. They are low molecular weight compounds best assayed using the methanol treatment (Sieniawska & Baj, 2017). Coloured bean seeds have been found to contain higher levels of tannins (Sandberg, 2002).

Saponins are complex compounds composed sugar and steroid moieties occurring in legumes and cause bitter taste in the beans when in high concentration (Aremu, Ibrahim & Ekanem, 2016). Saponins also cause hypocholesterolaemia through binding of cholesterol thus making it unavailable for absorption (Soetan & Oyewole, 2009). These

anti-nutrients have effects on the bioavailability of proteins in that they bind digestive enzymes such as trypsin and chymotrypsin (Aremu, *et al.,* 2016). Saponins have also been attributed to spermal plasma damage through inhibition of acrosine activity of human sperms (Khalil & El-Adawy, 1994).

Trypsin inhibitors are widely distributed across the genera and species of the Leguminosae family (Konietzny, 2003) . They inhibit the activities of trypsin affecting the digestibility of the proteins. The analysis of the trypsin inhibitor activity should serve as additional criterions for the selection of appropriate *L. purpureus* genotypes (Guretzki & Papenbrock, 2014). Two types of trypsin inhibitors have been identified in beans: the Kunitz trypsin inhibitor and Bowman-birk inhibitor (BBI) (Savage & Morrison, 2003). Kunitz is a peptide with about 181 amino acids and is easily inactivated by heat and gastric juices (Savage & Morrison, 2003). Bowman birk, widely distributed in legume seeds, is double headed hence inhibits both trypsin and chymotrypsin and has been found to be resistant to heat and gastric juices (Savage & Morrison, 2003). The bean structure has been suggested to affect the removal of the trypsin inhibitors. Other inhibitors, including haemagglutinins cause depression of growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract (Soetan & Oyewole, 2009).

The bitter taste in Lablab bean has been attributed to cyanogenic glucosides (Wanjekeche, Wakasa, & Mureithi, 2003). These toxic compounds are carbohydrate derivates of cyanohydrins produced by plants (Gebrelibanos *et al*., 2013). Upon hydrolysis they yield toxic hydrocyanic acid (HCN). The cyanide ions inhibit several enzyme systems; depress growth through interference with certain essential amino acids and utilization of associated nutrients (Soetan & Oyewole, 2009). They can also cause acute toxicity, neuropathy and death. According to results obtained by Kimani *et al*, (2012), uncooked lablab beans analysed for cyanides were found to contain at least 10 ppm of the toxin. Different processing methods are normally employed to remove the antinutrient for safe utilization of the legume nutrients as human food (D'souza, 2013).

2.6.2. The role of bioactive components of Lablab beans in human health

Legumes in general play a vital role in providing phytochemicals important for human health (McCrory *et al*., 2010). They are associated with reduced risk of mortality related to chronic diseases including cardiovascular diseases, diabetes, cancer, obesity and gut health. Observation studies attribute this to improvements in blood pressure, lipid profile, blood sugar metabolism and body weight (Foyer *et al*., 2016b).

Lablab as a legume can be recommended in dietary therapy for Diabetes Mellitus management (Gebrelibanos *et al*., 2013). Animal studies have suggested that Lablab has hypoglycaemic and hypolipidemic activity (Singhal, Kaushik, & Mathur, 2014). Most of the studies have consistently shown lower glucose and insulin responses to consumption of controlled amounts of legumes compared with other foods (Uebersax, 2006).

A meta-analysis of eleven (11) studies showed that daily consumption of legumes for more than 4 weeks resulted in a significant reduction in fasting blood glucose and insulin (Reddy, Andrapradesh, Kante & Reddy, 2013). Better results could be obtained when the legumes are consumed as part of low glycaemic index diets (Singhal *et al*., 2014). Diabetes is a major risk factor for several cancers and neurodegeneration conditions (Viguiliouk *et al*., 2017). The future health of ageing populations may therefore be dependent on a food system that provides legumes in an affordable, palatable and sustainable way (Singhal *et al.*, 2014). Legume consumption has been linked to lower risk of bowel cancer. Increasing evidence suggests that legumes can act as prebiotics that potentially alter bowel flora, affecting production of gut hormones and consequently appetite (Reddy et al., 2013).

The dietary fibre in legumes which encompasses soluble and insoluble fibre is of health benefit. Insoluble fibre normally is associated with the faecal bulking through its water holding capacity (McCrory *et al*., 2010). On the other hand the soluble part of dietary fibre in beans undergoes fermentation in the colon resulting in short chain fatty acids

(SCFA), lowered pH and potential microbiota changes (McCrory *et al*., 2010). The SCFA increase the blood flow, muscle activity and water absorption in the colon, and also act partly as food for the gut microbiota. SCFA nourish the colon lining preventing degradation hence improves the colon health (McNabney & Henagan, 2017).

Dolichos lablab has shown pharmacological effects such as antimicrobial, antioxidant, anticancer, hypolipidemic, immunological, anti-inflammatory properties (Verma *et al*., 2017). The phenolic compounds, saponins and other metabolites possess antidiabetic, anti-inflammatory, analgesic, antioxidant, cytotoxic, hypolipidemic, antimicrobial, hepatoprotective, antilithiatic, and antispasmodic effects (Al-snafi, 2016; Vadde, Pochana, & Pillutla, 2007).

In their review, Singhal *et al*. (2014) explored some legumes and their pharmacological effects. It was found that some animal studies had suggested hypoglycaemic and hypolipidemic activity of Lablab bean also known as field bean. Reports by Murugananthan *et al.* (2013) indicate that there is a growing importance of immunomodulators. The immune-modulating properties of plants are being studied extensively with ever-increasing interest due to the benefits of immune system modulation for disease prevention and cure.

Masayuki *et al.* (1998) isolated glycoside fraction of the saponins, the oleanane-type triterpene bisdesmosides called Lablabosides A, B and C (Figure 2.5) from *Dolichos Lablab*. These components were found to exhibit inhibitory effects on alcohol and sugar absorption as well as anti-inflammatory, anti-allergic and hepatoprotective activity. Further studies found out that the glycoside mixture of white dolichos Lablab showed potent adjuvant activity, the ability to accelerate, prolong, or enhance antigen-specific immune responses when used in combination with specific vaccine antigens.

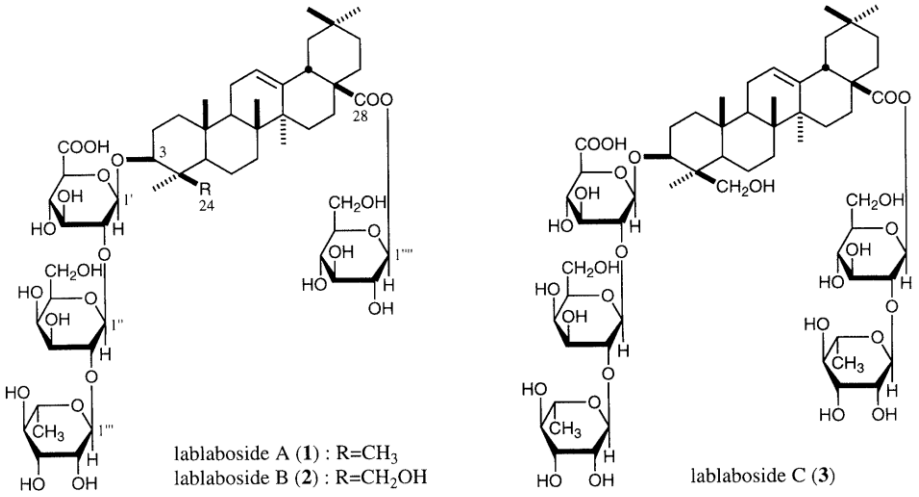

Figure 2.5: Oleanane-type triterpene bisdesmosides -Lablabosides A, B and C
Source: masayuki et al. (1998)

2.7. Functional properties of proteins

Fernández *et al*. (1997) defines functional proprieties of proteins as '*those physical and chemical properties, which affect the behaviour of proteins in food systems during storage, processing, preparation and consumption'.* They form those characteristics which influence the quality and organoleptic attributes in food (El-Adawy, 2000). The functional properties of a protein are affected by both intrinsic and extrinsic factors. In order for legume proteins to be a successful ingredient in foods, they must possess suitable functional properties, as well as having a good protein quality and sensory characteristics (Akhtar *et al*., 2014). The functionality of proteins is affected by the protein source, environmental factors and protein concentration (Zayas, 1997).

Functional properties of legume proteins include solubility, water hydration capacity, foaming capacity, emulsion capacity and gelation properties. The solubility of a protein is an important functional property that enhances the applicability of the protein in food systems (Adebowale & Lawal, 2004). Functional properties such as emulsification, foaming, and gelation are dependent on the solubility of proteins (Lawal, 2005).

The water holding capacity (WHC) is that ability of the foods to hold its own or added water during processing (Zayas, 1997). It is an important factor to consider in functionality of proteins since the formulated food should be able to prevent water from being released (Zayas, 1997). The water retention also plays a key role in sensory attributes. Fat absorption capacity influences the textural properties. In plants, fat absorption can be affected by the protein concentration and the number of the non-polar sites (Zayas, 1997). High oil absorption capacity in plant-based proteins signifies large proportions of hydrophobic as compared to hydrophilic groups on the protein molecules surfaces (Subagio, 2006).

The ability of protein to form gels and to provide a structural matrix for holding water, flavours and sugars is useful in new foodstuff development, thereby providing an added dimension to protein functionality. Gelation is an aggregation of denatured molecules, a three dimensional cross-linked network with partial unfolding of proteins (Zayas, 1997).

2.8. Physical and cooking characteristics

Heat processing of legumes generally improves the nutritive value of proteins by inactivating the antinutrients such as trypsin inhibitors and haemagglutinins (Tharanathan & Mahadevamma, 2003). The cookability and organoleptic qualities of beans are important attributes affecting preference, selection and acceptance of bean varieties. Güzel and Sayar (2012) define cookability as the condition where beans achieve a degree of tenderness during cooking acceptable to consumers. When the beans are hard to cook, they may undermine their consumption. Prolonged cooking has effects on the taste and the nutritive value of the beans especially vitamins and certain amino acids according to Shivachi *et al.* (2012) who reported that various genotypes vary in their cooking time with a range of 70 minutes to about 200 minutes. Some of the genotypes showed a relationship between the cooking time and acceptability.

The cooking characteristics of the beans have been attributed to the physical characteristics such as the seed size, colour, volume, density, hydration capacity and swelling capacity (Mortuza & Tzen, 2009). These physical characteristics are also important in other processing methods and functional properties of the bean. Mortuza and Tzen (2009), argue that the hydration and swelling capacities of the bean reflect the ability to imbibe water in a reasonable period of soaking and thus affect the cooking time.

2.9. Effect of processing treatments on nutrients and antinutrients in lablab beans

2.9.1. Effect of processing on antinutrients

The nutritional potential of lablab beans can be limited by the presence of antinutrients such as phytic acid, tannins and trypsin inhibitors (Urbano *et al*., 2005). Certain processing methods including soaking, germination, roasting, cooking and autoclaving have been employed to remove such antinutrients. Soaking and germination are older methods of processing cereals and legumes. They offer the advantage of saving energy and reducing the flatulence producing oligosaccharides. Applied prior to cooking, they are cost effective treatment methods that can be used to reduce antinutrients in legumes. Germination, soaking and pre-soaked cooking of beans has been associated with a higher potential of improving the nutritional value by reducing anti-nutritional factors such as trypsin inhibitors and phytic acid and thereby enhancing its utilization (Jain *et al.,* 2009). Liu and Markakis (1987) documented that soaking of soybeans in water at 22˚C for 24 hours could reduce the level of other anti-nutrients but had no effect on the level of trypsin inhibitor.

According to Rehman and Shah (2005) phytates form insoluble complexes with zinc, iron, magnesium and calcium at physiological pH. When the beans are soaked, germinated and cooked, there is significant reduction in the level of phytic acid thus the availability of these minerals increase (D'souza, 2013). According to D'souza (2013),

cooking has the highest effect on the reduction of trypsin inhibitors on Lablab while roasting gives better results for phytic acid reduction. A study carried out by Khokhar and Apenten (1997) showed that soaking the seeds of moth bean in plain water and mineral salt solution for 12 hours reduced phytic acid by 46–50%. Bean germination for 60 hours was found to have pronounced effects on saponin levels of up to 46% reduction (Soetan & Oyewole, 2009). D'souza (2013) reported improved availability of iron in malted cereals after germination as a result of reduced phytic acid. According to El-Adawy (2000), germination had a less reducing effect on trypsin inhibitor, hemagglutinin activity, tannins and saponins but more effective in reducing phytic acid, stachyose and raffinose. Alonso, Aguirre, and Marzo (2000) cited extrusion as one of the best methods to eliminate trypsin, chymotrypsin, α-amylase inhibitors and hemagglutinating activity without modifying protein content.

2.9.2. Effect of processing methods on nutrients of Lablab bean.

Lablab (*Lablab purpureus*) like other beans can be utilized as a good source of protein and also processed to flour and starch for several food applications (Borijindakul & Phimolsiripol, 2013). However, the nutritional benefits of this bean could be maximised by employing different processing methods. Traditional processing methods like sprouting, popping or puffing of legumes are still used in the preparation of food mix and have gained special attention among consumers as they are economical and simple (Vasantha & Sangeetha, 2017). Soaking then cooking the beans in an open pot of water either made of steel or ceramic over a low heat fire yields palatable and nutritious beans (Kinyanjui, 2016).

Protein content normally increases during the germination process as a result of increased water activity and the hydrolytic enzymes. Germination has also shown to improve invitro protein digestibility (Kanensi *et al.,* 2011). Fermentation on the other hand has been cited to increases the content of protein due to break down of complex protein through hydrolysis by protease enzyme (Premarani & Chhetry, 2011). Thermal

processing such as boiling and roasting have an effect of increasing crude protein content and protein digestibility (Adu *et a*l.,). The minor decrease in protein content during soaking and cooking might be attributed to the leaching of soluble proteins (Wang *et al*., 2010). Other studies have shown varying changes in the amino acid composition (Adu et al., 2014; Amaechi & Ngozi, 2016).

Different cooking methods result in varied nutrient retention (Nasar-Abbas *et al.*,2010: Wang *et al.,* 2010). Cooking has been reported to cause significant decreases in fat, total ash, carbohydrate fractions, sulphur-containing amino acids as well as B-vitamins (Alajaji & El-Adawy, 2006). Alonso *et al.* (2000) cited extrusion method as an effective treatment for improving protein and starch digestibility when compared with dehulling, soaking and germination.

Fermentation process has been shown to reduce the carbohydrate content according to Hachmeister and Fung (1993). This was attributed to the utilization of the carbohydrates by microorganisms during respiration. A similar observation was also made for fat content as described by Liu (1996), who suggested that as the mould strain grows, it continues to use fat as a source of energy and as a result fat content in the fermented grain decreases.

Dehulling process has the effect of reducing the amount of fibre in the dehulled bean. Elleuch *et al.* (2010) reported that the seed coat of the bean is rich in fibre, and removing the coat results into significant reduction in the fibre content in the processed bean product.

2.10. Legumes in *tempeh* production

2.10.1 Origin of tempeh

The word *tempeh* appears to have originated in Central Java, in today's Indonesia where it is also called '*tempeh kedele'* and its origin can be traced back to the beginning of the 18th century as documented by Shurtleff and Aoyagi (2011). In Indonesia, *tempeh* processing could be the oldest food technology in the history of Javanese people and is now their most popular soy-protein food (Astuti *et al*., 2000). *Tempeh*-like products have also been produced in China, such as soybean *koji* (Shurtleff & Aoyagi, 2001) or *Douchi* made from black or yellow beans fermented by *Mucor spp*., *Aspergillus spp*. or *Rhizopus oligosporus* (Feng, 2006).The Japanese have processed '*natto*' a soybean product fermented with *Bacillus subtilis* (Quílez & Diana, 2017). Lablab bean (*Lablab purpureus*) have been processed into uncommon Lablab *tempeh*, also known as '*tempeh koro wedus'*.

Tempeh was introduced to Europe through the Dutch who had once colonized Indonesia in 1895 (Shurtleff & Aoyagi 2011). A Dutch microbiologist and chemist, Prinsen Geerlings made an attempt to identify the *tempeh* mould. In the USA, *tempeh* came to be known in the 1950s during the search for possible protein sources to feed the children in the underdeveloped Countries (Autret, Pilarm, & Van-Veen,1955).This was meant to introduce *tempeh* as cheap source of protein in developing countries (Africa and South-America). The efforts however failed since the local population had no experience with mould-fermented foods (Autret *et al*., 1955). Nevertheless, current trends indicate that the interest for *tempeh* is increasing due to health, nutrition and the need for vegetarian diets (Quílez & Diana, 2017).

The term *'tempeh'* is currently used for various plant materials that have been subjected to fermentation using a fungus belonging to the genus *Rhizopus* (Shurtleff & Aoyagi, 2011; Dinesh *et al*, 2009)). The most important characteristics of *tempeh* fermentation are that the key microorganism belongs to the genus *Rhizopus* and that the final products are mycelial-knitted compact cakes. This occurs due to the substrate overgrowth by a culture of *Rhizopus* species, which produces thick white mycelium,

binding the seeds together into a compact and sliceable cake as described by Starzyńska *et al*. (2014). Most desirable characteristics for a good *tempeh* would be an attractive flavour and texture, certain nutritional properties, and the reduced cooking time compared with the raw materials (Shurtleff & Aoyagi, 2001). This traditional Indonesian food is well known in western countries as a meat replacement product in vegetarian diets, served either as a main dish or as a salad ingredient. Its popularity in Eastern Europe continues to increase owing to the growing interest in multicultural cuisine. According to Shurtleff and Aoyagi, (2001), *tempeh* owes its good flavour, sliceable meat like texture and excellent nutritional properties to the fermentation process.

Fermentation involves food modification by use of safe microorganisms. These organisms grow and reproduce through consumption of part of the substrate, the food by which they enrich it with the products of their metabolism (Yadav, Sharma & Singh 2012). It is an ancient technology desirable for processing and preserving foods that has remained most applicable because of its low cost, low energy requirements, and high yield, with acceptable and diversified attributes for human consumption (Starzyńska *et al*., 2014).

Fermentation process in legumes can lead to microbial synthesis of enzymes which hydrolyse the grain constituents leading to development of desirable texture, flavour and aroma. Marshall *et al.* (2007) explains that the process may cause reduced anti-nutrients or complete elimination in the legumes consequently improving the overall nutritional quality of the legume grains. Microbial fermentation which is considered as one of the oldest methods for food processing and preservation has been majorly applied in legume fermentation. Fermentation can effectively reduce phytic acid levels in grains and legumes though the extent of reduction may be dependent on the type and variety (Makokha *et al,* 2002). Fermentation also has the potential to increase the availability of certain minerals in grains and legumes as well as improving the invitro digestibility (Makokha *et al*., 2002).

2.10.1. The process of tempeh production

Tempeh production can use different substrates such as cereals and legumes. However, the basic fermentation process is similar for all substrates. The process includes soaking, dehulling (where necessary), boiling and fermenting. The main differences between different substrates used in tempeh fermentation are the selection of ideal pre-treatments. These would include modification of grain surface area by cutting, cracking or pearling to enable optimum growth of mould. In most cases inoculation involves use of *Rhizopus oligosporus* which is the dominant tempeh fungus. However, *R. oryzae* and *Mucor spp*, may as well contribute to the flavour, texture or nutritive value of the product. *R. oryzae* has the ability to break down the α-galactosides and utilize them as carbon source contrast to *Rhizopus oligosporus* (Wiesel, Rehm, & Bisping, 1997). *R. oryzae* is also associated with significant reduction of stachyose and raffinose. These two flatulence-causing sugars could therefore be reduced by the enzymatic activity of fungus improving the utilisation of the legume tempeh as examined by Rehms and Barz (1995). This makes a mixture of the inoculum more beneficial since there is nutrition competition among the different strains of the fungi.

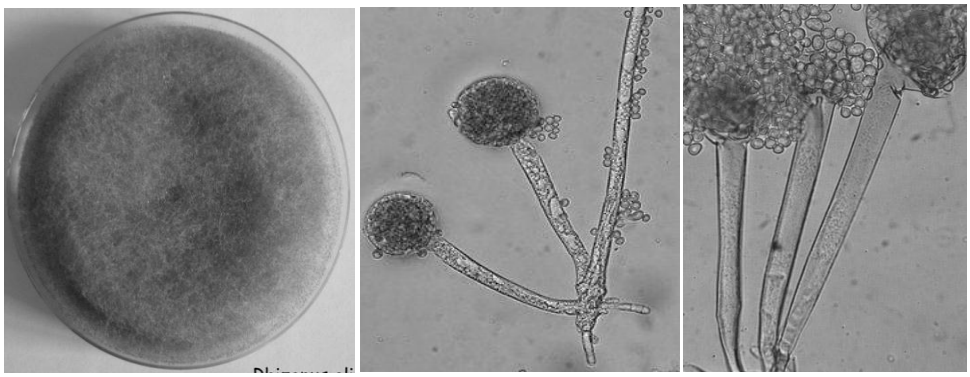

Figure 2.6: Morphology of R*hizopus* oligosporus
Source: Feng, 2006

2.10.2. Nutritional and health benefits of tempeh

Tempeh like other fermented products has various nutritional and health benefits to the consumer. The microorganisms involved in legume fermentation hydrolyse and metabolize seed constituents resulting in the production of beneficial products (Feng, 2006). These have the ability to produce antimicrobial compounds and desirable organic acids that can preserve the food by the suppression of growth and survival of undesirable microbial flora. This makes the fermentation process more advantageous over other conventionally feasible methods of legume processing, in addition to being less expensive (Astuti *et al*., 2000). Fermentation is likely to increase the bioavailability of legume proteins through reduction of trypsin inhibitory activity (TIA), amylase inhibitor activity (AIA) phytic acid, and tannins (Onuoha, Orukotan, & Ameh, 2017; Osman, 2011). Fermentation increases the hydrolysis of complex protein by protease (Somishon & Thahira, 2013).

Fermented foods exert beneficial health effects is through bioactive molecules that confer a biological action, resulting from chemical changes during the fermentation process (Fujita, Yamagami, & Ohshima, 2001). Phenolic compounds act as natural antioxidants and immune modulators (Martins *et al*., 2011). The antimicrobial compounds secreted by *Rhizophus oligosporus* during fermentation may inhibit the growth and toxin accumulation of some undesirable microorganisms (Shanna & Ye, 2014). Other studies have shown Gamma-aminobutyric acid (GABA) formation in *tempeh* fermentation (Quílez & Diana, 2017). GABA is an inhibitory neurotransmitter in the brain which regulates brain excitability acting like a "brake" during times of runaway stress.

CHAPTER THREE

METHODOLOGY

3.1. Research design

The study was laboratory based, where different varieties of Lablab beans were analysed in the laboratory for their nutritional value and effect of various processing methods on the nutrients and antinutrients.

3.2. Sample acquisition

Three varieties of Lablab *(Lablab purpureus)* namely KAT/DL-1, KAT/DL-2 and KAT/D-3 were obtained from the Kenya agricultural and livestock research organisation (KALRO), Katumani dryland research station in Machakos. A common bean variety *Phaseolus vulgaris* (*Rosecoco*) KAT/X69 was also obtained for comparison.

KAT/DL-1 **KAT/DL-2** **KAT/DL-3** **KAT/X69**

Figure 3.1: The lablab bean varieties and Rosecoco variety

3.3. Sampling

The three lablab sample varieties, KAT/DL-1, KAT/DL-2 and KAT/D-3 were purposively selected as they were the varieties that had been developed by KALRO, yet information on their relevant nutritional characteristics was not available. KAT/X69 was selected for comparison since its one of the generally used common bean. For each variety a sample of 2 kg was obtained from the 2014 harvest season.

3.4. Sample preparation

3.4.1. **Cleaning**: The raw samples were cleaned to remove the foreign materials and damaged seeds. This was done by rubbing the beans in a dry cloth and passing them through a sieve to remove dust.

3.4.2. **Grinding**: Part of the cleaned samples were ground using a blender mill to pass through a sieve of 0.5mm then packed into airtight containers for chemical analysis.

3.4.3. **Soaking**: Some of the cleaned seed samples were washed with tap water before soaking for 12 and 24 h at room temperature (28°C). The ratio of 1: 4 (seed: water) was used. The soaked seeds were removed from soaking water, dried in the oven (Memmert, Germany UF110) at 50˚C for 24 hours then ground and packed for analysis.

3.4.4. **Germination**: Some beans were germinated following the method of Martín-Cabrejas et al. (2008) with slight modification. From each variety, five hundred grams of Lablab seeds were washed with 0.7% sodium hypochlorite solution and then soaked in 1,500 mL tap water at room temperature for 24 h. After that, the excess water was removed. The drained Lablab seeds were placed on moist cotton cloth and allowed to germinate at 25±2ºC for 48 h. The germinated Lablab samples were oven dried (Memmert, Germany UF110) at 50ºC for 24 h, milled and packed in tightly in aluminium bags.

3.4.5. **Cooking:** Part the cleaned soaked (12 h and 24 h soaking) and un-soaked beans from each of the lablab varieties and common bean rosecoco were subjected to standard cooking at 96.5 °C. This was done in thermostatic water bath (Memmert WBU - 45, Germany. Pressing the beans between fingers was used to measure cookability. The cooked samples were then oven dried (Memmert, Germany UF110) at 50ºC for 24 h, ground and packed for analysis.

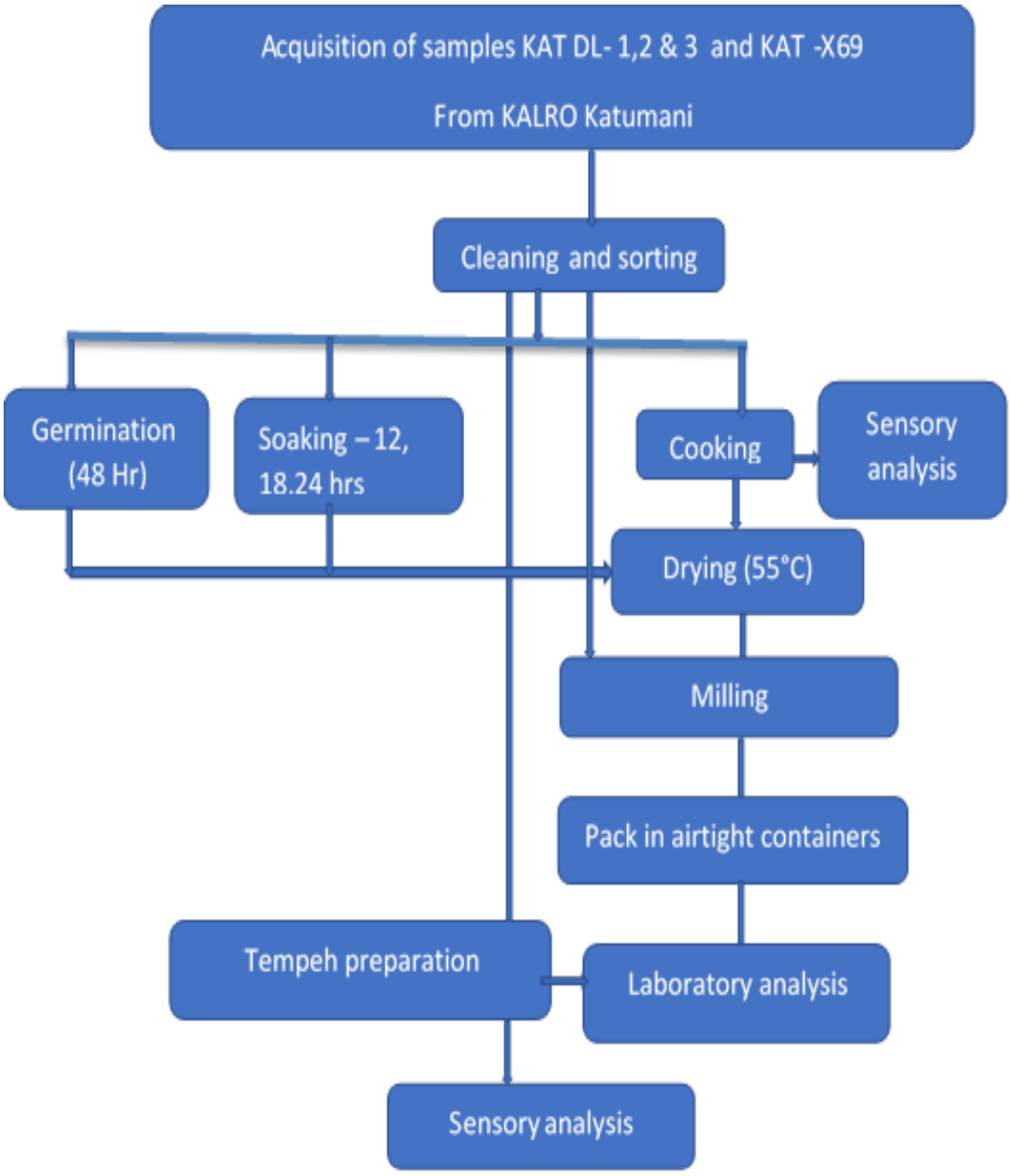

Figure 3. 2: Flow diagram of sample preparation

3.5. Data collection

3.5.1. Physical properties of Lablab bean varieties and Rosecoco (KAT/X69)

3.5.2. Seed dimensions of Lablab and beans

The seed dimensions of randomly selected seeds of from the sample beans were measured using a Vernier calliper reading to 0.01 mm. The three principal dimensions of length (L), width (W) and thickness (T) were determined using ten seeds of each variety, according to a modified method of Berrios, Swanson and Cheong (1999).

Geometric and arithmetic mean diameter

The geometric and arithmetic mean diameter (D_g) of the Lablab bean was calculated by using the following relationship (Kenghe, Nimkar, & Shirkole, 2013).

$$D_g = (LWT)^{1/3}$$

$$D_a = \frac{(L+W+T)}{3}$$

where,

Dg = Geometric mean diameter, mm

Da = Arithmetic mean diameter, mm

L = Length, mm

W = Width, mm

T= Thickness, mm

Sphericity

This shows the shape character of the object relative to the sphere having the same volume.

The geometric mean diameter (*Dg*) and sphericity (ϕ) of bean grains were calculated using the following relationships in centimetres (Mohsenin, 1970)

$$\text{Sphericity } (\phi) = \frac{Dg}{L} * 100$$

3.5.3. Seed weight

A hundred randomly selected seeds were weighed in triplicate for each variety of beans and the average recorded as the 100 seed weight (Martín-Cabrejas *et al.*, 1997).

3.5.4. Seed density

The principle of liquid displacement of Asoegwu *et al.*, (2006) was employed to obtain the true density .The true density (P_t) of grain is defined as the ratio of the mass of a sample of a grain to the solid volume occupied by the sample ((Deshpande, Bal, & Ojha, 1993; Wani *et al*., 2014). A 500 ml beaker was filled to the 350 ml mark with water. Approximately 100 g of beans were immersed in the water. The mass of the water displaced is the balance reading with the seed submerged minus the mass of the beaker with water. The seed volume was estimated by dividing the mass of displaced water (g) by the density of water (g/cm ^{3}). Seed density was determined by dividing the seed mass by the measured seed volume. This was repeated thrice for each variety of beans. The immersion was for a few seconds to avoid the seeds absorbing moisture.

$$\text{True/Seed density} = \frac{\text{weight of seeds (g)}}{\text{volume of displaced water (cm}^3\text{)}}$$

3.5.5. Bulk density

The bulk density (ρ_d) is the ratio of the mass of a sample of a grain to its total volume (Suthar & Das, 1996). The AOAC method reported by (Ogunjimi, Aviara, & Aregbesola, 2002) was adopted for bulk density determination. A measuring cylinder (500 mL) was filled with seeds to a height of 15 cm and then the content was weighed. This was repeated five times for each variety. Bulk density was calculated as the ratio of the bulk weight and the volume of the container (g/ml) (Asoegwu, *et al*., 2006).

$$\text{Bulk density} = \frac{\text{weight of seeds (g)}}{\text{volume of the bulk seeds (cm}^3\text{)}}$$

3.5.6. Seed Porosity

Porosity is the percentage of volume of voids in the test sample at a given moisture content. Seed porosity (Φ) is the property of the grain which depends on its bulk and kernel densities. The formula method of (Mpotokwane *et al*., 2008) was used to calculate the seed porosity: It was calculated as the ratio of the difference in true density and bulk density to the true density value and expressed in percentage for Lablab beans and rosecoco bean

$$Porosity\ \Phi = \left(1 - \left(\frac{bulk\ density}{true\ density}\right)\right) * 100$$

3.5.7. Hydration coefficient

The hydration coefficient was determined by soaking 100 bean seeds (weight taken) in deionized water (ratio of 1:10) at 25 °C in an incubator. After 18 h the beans were removed from the soaking water followed by free water removal by using a blotting paper and reweighing. Weight gain was taken as the amount of water absorbed and expressed as the hydration coefficient (Nasar-Abbas *et al.*, 2008)

$$\text{Hydration coefficient} = \frac{\text{weight of bean seeds after soaking}}{\text{weight of bean seeds before soaking}} * 100$$

3.5.8. Swelling coefficient

The volume of raw bean seeds before and after soaking in deionized water for 18 hours at 25 °C was determined by measuring the volume of water displaced before and after soaking using a graduated cylinder and expressed as the swelling coefficient (Nasar-Abbas *et al*., 2008).

$$Swelling\ coefficient = \frac{Volume\ of\ beans\ after\ soaking}{Volume\ of\ beans\ before\ soaking} * 100$$

3.5.9. Water absorption capacity

Water absorption in the Lablab and rosecoco bean samples was determined by placing a sample of approximately 10 g (weighed exactly) in a 50 mL beaker containing 40 mL of deionised water. The experiment was based on the moisture gradient on the centre of the beans. It is a time temperature function that was determined by calculating the difference in seed weight before and after soaking (6, 12, 18 and 24) at room temperature (25ºC). At the different soaking times, the beans were removed from the soaking solution, drained for 2 min, blotted with tissue paper and weighed. The weight gain was calculated and the beans were returned to the soaking solution at the defined temperature. All soaking tests were done in triplicate and average results were used to calculate the percentage moisture gain (Sayar, Turhan, & Gunasekaran, 2001).

$$Water\ absorption\ capacity = \frac{Seed\ weight\ after\ soaking - Seed\ weight\ before\ soaking}{Seed\ weight\ before\ soaking}$$

3.5.10. Proximate composition of Lablab and Rosecoco beans

3.5.11. Moisture content

Whole bean flour samples (n=4) were dried in an oven at 105 °C for 5 h, and then weighed after cooling in a desiccator. Thereafter weight measurements were carried out after every 30 min until constant weight was achieved. The moisture content was calculated as:

$$\%\ Moisture\ content = \frac{(weight\ before\ drying - \ weight\ after\ drying)}{weight\ before\ drying} * 100$$

3.5.12. Crude Protein

Protein content was determined using the semi-micro Kjeldahl AOAC method 976.05 (AOAC, 2005). 2 g of sample was accurately weighed into a digestion flask together with a combined catalyst of 5 g K_2SO_4 and 0.5 g of $CuSO_4$ and 15 ml of concentrated H_2SO_4. The mixture was heated in a fume chamber till the digest colour became blue. The blue colour signified end of the digestion process. The digest was then cooled, transferred to 100 ml volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalysts was also made. Then 10 ml of diluted digest was transferred into the distilling flask and washed with about 2 ml of distilled water. Thereafter, 15 ml of 40% NaOH was then added and this was also washed with 2 ml of distilled water. Distillation was done to a volume of about 60 ml distillate. The distillate was titrated using 0.02N HCl to an orange colour of the mixed indicator, which signifies the end point.

$$\%\ Nitrogen = (v1 - v2) * N * f * 0.014 * \frac{100}{v} * \frac{100}{s}$$

Where: $\boldsymbol{v1}$ is titer for sample in ml, $\boldsymbol{v2}$ is titer for blank in ml, $\boldsymbol{N}$ is normality of standard HCl solution (0.02), $\boldsymbol{f}$ is the factor of the standard HCl solution, $\boldsymbol{v}$ is the

volume of diluted digest taken for distillation (10 ml), ***s*** is weight of sample taken (1 g). Protein was calculated as follows:

$$\%\ protein = \%\ Nitrogen * 6.25$$

3.5.13. Ash content

Ash content was determined by incinerating the sample in a muffle furnace (AOAC, 2000) Method 923.03. Sample weights of about 5 g were weighed accurately in pre-conditioned crucibles. First the samples were charred by a flame to eliminate smoking before being incinerated at 550°C in a muffle furnace, to the point of white ash. The residue was cooled in a desiccator and the weights taken.

$$\%\ crude\ ash = \frac{weight\ of\ ash}{weight\ of\ sample} * 100$$

3.5.14. Crude fat

Crude fat was obtained by Soxhlet's extraction method. About 5 g of dry sample was put in cellulose thimbles and oil extracted for 16 hours using petroleum spirit at 40-60 ºC, (AOAC 1995), Method 920.85-32.1.13. The solvent was removed by rotary vacuum evaporator, and the crude fat dried at 70 ºC for 30 minutes and weighed. It was expressed as a percentage of the sample weight.

$$\%\ Crude\ fat = \frac{weight\ of\ fat}{weight\ of\ sample} * 100$$

3.5.15. Estimation of carbohydrates and energy content

The carbohydrates in the samples were estimated by subtracting the sum of dry matter percentage of crude protein, crude fat, crude ash and moisture from 100. To obtain energy values [in kcal/g], the Atwater specific factors (WHO/FAO, 2002) were used. The protein percent in the Lablab beans was multiplied by 3.47, fats multiplied by 8.37 and carbohydrates by 4.07.

3.5.16. Oligosaccharide determination

Extraction of oligosaccharides was performed according to Kuo, Van Middlesworth, and Wolf (1988). About 1 g of whole bean flour was extracted with 20 mL of 80% ethanol at 70 °C for 30 minutes with continuous shaking. The suspension was then centrifuged (H–2000C, Japan) at 3500 *g* at room temperature for 15 minutes. The supernatant was decanted, and an aliquot centrifuged at 9300 *g* for 10 minutes. A sample of the centrifuged supernatant was diluted with deionized water and passed through a 0.45-µm filter (Econ filter PTFE, Agilent Technologies). The samples (20 µL), were auto injected into the HPLC system (Shimadzu CBM 20A, Japan) fitted with an APS-2 HYPERSIL carbohydrate (C-18) column (5 µm, 150 × 4.6 mm, Thermo Fisher) and a pulsed array detector (PAD) (Shimadzu PAD-20A). Acetonitrile/water (65:35) was used as mobile phase at 1 mL/min. Column and detector temperatures were maintained at 35 °C. Standard curves were determined using raffinose and stachyose standards (Sigma Aldrich) and used to quantify the oligosaccharides content in the beans.

3.5.17. Minerals determination

The mineral content of the bean samples was determined by dry ash method (AOAC, 2000). About five grams of samples were ashed at 550ºC for 8 hours then drops of 6N HCl were added and evaporated. The samples were incinerated further for 1 hour and diluted using 1N nitric acid. The samples were placed in 100 mL volumetric flasks and made to 100 mL using 1N nitric acid. Standards were prepared using the 1N nitric acid and the absorbance read in the atomic absorption spectrophotometer (Shimadzu, AA-6200, Tokyo, Japan).

3.5.18. Fatty acid profile

The fatty acid profile of the bean samples was determined by gas chromatography (GC) after fat extraction using a modified method by Bligh and Dyer, (1959). Five grams of the bean sample was put in a 250 mL glass stoppered centrifuge tube and methanol and chloroform were then added in a ratio of 2:1. The contents were then placed in an

electronic shaker for 8 hours then centrifuged at 30,000 rpm for 10 minutes. The supernatant was transferred to a conical flask and 15mL of chloroform was added to the remnant. The contents were centrifuged at 30,000 rpm for 10 minutes and the first and second supernatants were combined and then passed through a defatted cotton wool. The contents were then put in a rotary flask and evaporated to dryness. Five millilitres of 95% methanol and 5% hydrochloric acid were added followed by refluxing at 100ºC for 1 hour. The contents were then cooled in running water before transferring to a separating funnel where 10 mL of hexane was added followed by shaking vigorously. The contents were then left to settle where the upper layer of hexane was collected in a conical flask and the lower layer was further re-extracted using hexane and the two hexane layers were mixed. The hexane layers were put in a separating flask and washed with water. The contents were then passed through a plugged funnel with cotton wool and anhydrous sodium sulphate. The contents were evaporated in a rotary evaporator to 0.5-1 microlitre and put in vials. 1 µl of the sample was drawn and injected into a gas chromatography (Shimadzu GC-9A, No.41991A, Tokyo, Japan). Known concentrations of fatty acids standards were fed into the GC (column initial temperature of 170°c, flame ionisation detector at 240°c and injection temperatures of 220°c) and identification and quantification done by peak area integration or rather comparing the retention time and the reference spectra.

3.5.19. Determination of anti- nutrients and bioactive components

3.5.20. Phytate determination

Phytate determination was done by HPLC according to the method of Camire and Clydesdale (1982). Approximately 50 mg of whole bean flour sample was weighed into a 125 mL Erlenmeyer flask and 10 mL of 3% H2SO4 was added. The flasks were placed on a shaker (Model KS 250 basic, Germany) at a moderate speed of 1500 revolutions per minute (rpm) for 30 minutes at 25 °C and filtered using a 0.45-µm filter (Econofilter PTFE, Agilent Technologies). The filtrate was transferred to a boiling water bath for 5 minutes and 3 mL of a $FeCl_3$ solution (6 mg ferric iron per mL in 3%

H_2SO_4) was added. A second boiling water bath heating was done for 45 minutes to complete precipitation of the ferric phytate complex. Centrifugation followed at 2500 rpm (H–2000C, Japan) for 10 minutes and the supernatant was discarded. The precipitate was washed with 30 mL distilled water, centrifuged and the supernatant discarded. Three mL of 1.5 N NaOH were added to the residues and the volume brought to 30 mL with distilled water. Heating was done for 30 minutes in a boiling water bath to precipitate the ferric hydroxide. Cooled samples were centrifuged and the supernatant transferred into a 50 mL volumetric flask. The precipitate was rinsed with 10 mL distilled water, centrifuged and the supernatant was added to the contents of the volumetric flask. Samples of 20 µL of the supernatant were auto-injected into an HPLC (Shimadzu CBM 20A plus, Japan) fitted with an RP-18 (5µm) column at 30 °C and a refractive index detector (RID) (Shimadzu RID-20MA, Japan). 0.005N sodium acetate in distilled water mobile phase was used at a flow rate of 1 mL min-1. A stock solution of the phytate standard containing 10 mg /mL of sodium phytate (inositol hexaphosphoric acid C6H6(OPO3Na2)6+H2O) in distilled water was prepared. Serial dilutions were made for the preparation of a standard curve (50-1000 ppm) for quantification.

Mg/100 = ($/M\ SW$ × 1000 100)

$$\frac{mg}{100} = {}^{Y/M}\Big/_{SW} * \frac{1000}{100}$$

Where

Y- height of the peak

M - gradient of the standard curve

SW-sample weight

3.5.21. Tannin determination

Tannins determination was done by the Vanillin-HCl method (Price, Scoyoc, & Butler, 1978). Approximately 0.25 g of ground whole bean flour sample was accurately weighed into an Erlenmeyer flask. 10 mL of 4% HCl in methanol was pipetted into each of the flasks and the flask sealed. The flasks were gently shaken for 20 minutes on a shaker; the resulting extracts were centrifuged for 10 minutes at 4500 rpm (H–2000C, Japan). The supernatant aliquots were transferred to 25 mL volumetric flasks. Second extractions were done by adding 5 mL of 1% HCl in methanol to the residue from the first extraction and repeating the extraction process. The aliquots of the first and second extracts were combined and made up to 25 mL volume using methanol. A set of catechin (Sigma) standard solutions was prepared ranging from 100 to 1000 ppm using methanol as the solvent. One mL of each respective standard and sample extract was pipetted in to test tubes and 5mL of freshly prepared Vanillin-HCl reagent added. To correct for interference of natural pigments in dry bean seed coat, a blank sample was prepared by subjecting the original extract to the reaction conditions without the vanillin reagent. They were prepared by adding 5 mL of 4% HCl in methanol to 1 mL of the aliquots of the extracts pipetted into the test tubes. The absorbance of the standard solutions, sample extracts and blanks were read in a UV-VIS spectrophotometer (Shimadzu, UV mini 1240, Japan) at 500 nm exactly 20 minutes after adding Vanillin-HCl reagent to the samples and standards. A standard curve was prepared from the readings of the catechin standards solutions. Tannin content was expressed in mg of catechin equivalent (CE) per g of sample (mg CE/g).

$$mg\,\frac{CE}{100g} = \left(\left(\frac{YM}{SW}\right) * 1\,\frac{1000}{100}\right)$$

Y- absorbance.

M - gradient of the standard curve

SW- sample weight

3.5.22. Determination of trypsin inhibitor activity (TIA)

The Trypsin inhibitor activity was determined according to Kakade et al. (1974) method with modifications (Mbithi-Mwikya *et al*., 2000). Some 50 mL of 0.01 mol/L NaOH was used to extract one gram of the sample flour for 1.5 h. Portions (0, 0.6, 1.0, 1.4 and 1.8 mL) of the suspension were pipetted into duplicate sets of test tubes and adjusted to 2.0 mL with water. 2mL of trypsin solution (4mg trypsin in 200mL 0.001 mol/L HCl) was added to each tube before placing into a water bath at 37˚C. To each tube, 5mL of benzoyl-DL-arginine-p-nitroaniline (BAPA) solution (40 mg of benzoyl-DL-arginine-p-nitroaniline in 100 mL water with 1 mL dimethyl sulphoxide) previously warmed to 37 0C was and the reaction terminated after 10 minutes by adding 1 mL of acetic acid, filtered and the absorbance of the solution measured at 410 nm wavelengths against a reagent blank.

$$TIA\ \left(\frac{mg}{100}\right) = \frac{(2.632 * D * A1)}{S}$$

Where

A1 = change in absorbance due to trypsin inhibition/mL diluted sample extract,

D = dilution factor and

S = weight of sample (g).

3.5.23. Determination of flavonoids

Sample extraction for analysis of flavonoids and antioxidant activity was done as described by Harborne (1973). About 5 g of freshly ground bean samples were weighed into a 100 mL conical flask and about 50 mL methanol added. The flask was closed securely using parafilm and covered all-round with aluminium foil. The samples were put in a shaker and shaken for about 3 hours. They were then kept in the dark and left to extract for 72 hours.

After 72 hours, the samples were filtered through Whatman No. 4 filter paper, and then the filtrate made to 50 mL. The extract was transferred into bottles and securely stoppered and covered.

Qualitative analysis: A preliminary qualitative analysis was first carried out to establish the presence of flavonoids. This was done according to the method of Harborne (1973). Five mL of dilute ammonia solution was added to a portion of aqueous filtrate of extract followed by addition of concentrated H_2SO_4. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Quantitative analysis: Aluminium chloride colorimetric method was used for determination of flavonoids (Jagadish *et al.*, 2009). In a 10 mL volumetric flask, 4 mL of distilled water and 1 mL of bean extract were added. After 3 minutes, 0.3 mL of 5 % sodium nitrite solution was added. After 3 minutes, 0.3 mL of 10 % aluminium chloride was added. After 5 minutes, 2 mL of 1 M sodium hydroxide was added and the volume made up to 10 mL with water. Absorbance was measured at 415 nm using UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The total flavonoids were calculated from a calibrated standard curve prepared from quercetin.

3.5.24. Determination of free radical scavenging activity

The radical scavenging activities of the plant extracts against 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical (Sigma-Aldrich) were determined by UV spectrophotometer at 517 nm (Molyneux, 2004). The extracts were obtained as in 3.5.7.4 above. The following concentrations of the extracts were prepared:0.1, 1.0, 2.0, 5 and 10 mg/mL in methanol (Analar grade). Vitamin C was used as the antioxidant standard at concentrations similar to the extract concentrations. One mL of the extract was placed in a test tube, and 3 mL of methanol was added followed by 0.5 mL of 0.5 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Methanol was used to zero the spectrophotometer and the absorbance was read at 517 nm after 15 minutes in UV-Vis spectrophotometer (Shimadzu model UV –

1601 PC, Kyoto, Japan). The radical scavenging activity was calculated using the following formula:

$$\%\ \textit{Inhibition}\ \text{of DPPH} = \frac{\left(A_B - A_A\right)}{A_B} \times 100$$

Where A_B is the absorption of blank sample and A_A is the absorption of tested extract solution.

The results were expressed as percentage inhibition of DPPH and mean inhibitory concentrations (IC_{50}) determined from a plot of % inhibition of DPPH versus concentration of extract.

3.5.25. In vitro protein and starch digestibility

3.5.26. In vitro protein digestibility (IVPD)

The In vitro protein digestibility of the Lablab samples was determined following the modified pepsin method described by Mertz et al., (1984) using pepsin enzyme. The method involved determination of protein content before and after digestion of the samples with pepsin enzyme. Pepsin (1:3000, from HOG Stomach, Loba Chemie) was used for digesting the samples.

Total protein content: The total protein content (before pepsin digestion) of the lablab and rosecoco beans was determined by the Micro-Kjeldahl method (Method #979.09) (AOAC, 2000).

Pepsin digestion: About 0.2g of the raw and cooked sample were weighed into a centrifuge tube and then suspended in 35 ml of a solution of pepsin (1.5 mg/ml) in 0.1M phosphate buffer (pH 2.0); the mixture was incubated in a water bath shaker (model SHA – C, temp range: Room temperature) with gentle shaking at 37ºC for 2h. The tubes were then placed in an ice bath for 30 min to attain a temperature of 4ºC followed by centrifugation (Type 20 000, Kokusan corporation, Tokyo, Japan) at 12,000 x g for 15 minutes at 4ºC. The supernatant was discarded and 10ml of the buffer solution added,

then shaking and centrifugation was done again using the same conditions. The supernatant was discarded and the residue filtered using a Whatman filter paper No. 3. The residue in the centrifuge tube was washed into the funnel with 5ml of the phosphate buffer. The filter paper with the residue was dried for 30 minutes in an oven and then rolled and inserted into a Kjeldahl flask. A blank was prepared in the same way but without a sample.

Digestible protein content: To determine the digestible protein content of the samples, digestion, distillation and titration of the residue were conducted according to the semi-micro Kjeldahl method. A mixture of potassium sulphate and copper sulphate (5.5 g) and concentrated sulphuric acid (15ml) were added to the Kjeldahl flask containing the sample and heated on a heater under fume hood until a green-blue colour was formed. The digest was then transferred into a 100-ml volumetric flask and topped up with distilled water. Ten mL of the diluted digest was pipetted into a distillation flask, 15mL of 40% NaOH added and then distilled into 4% boric acid. Finally, the distillate was titrated with 0.02N HCl.

The digested protein of sample was calculated by subtracting residual protein from total protein of the sample:

Digested protein (%) = (A – B)/A

Where

A = % Protein content in the sample before pepsin digestion

B = % Protein content in the sample after pepsin digestion

%Protein digestibility = Digested protein/Total protein*100.

3.5.27. Starch digestibility (IVSD)

This was carried out according to the method described by Siddhuraju and Becker (2001) with modifications. The sample flour was made into slurry using a homogenizer. The slurry was poured into Brabender viscograph bowl and heated from 30 to 95°C, maintained at 95°C for 20 min and then cooled to 30°C, at a rate of 1.5° C per min with constant stirring. The cooked flour paste was then cooled to room temperature and then processed as.

- Freeze dried (0 h),
- Kept at 4°C for 24 h and then freeze dried (24 h) and
- Kept at 4°C for 48 h and then freeze dried (48 h).

The samples were stored at 4°C until used for the experiments. The *in-vitro* starch digestibility (IVSD) was determined in Lablab and rosecoco bean flours by dispersing the prepared samples in 50 ml of water and treated with Termamyl (100 µl) and incubated in boiling water bath for 10 min, cooled and equilibrated at 60°C. Solubilized starch was then hydrolysed by adding glucosidase (6 mg in 0.6 ml acetate buffer pH 4.6) and incubated in shaking water bath at 60°C for 2 h. The samples were centrifuged to inactivate the enzyme and filtered. The supernatant was made up to a known volume. Triplicate aliquots of 0.5 mL were incubated with a glucose oxidase/peroxidase reagent (Catalog no. 510-A, Sigma Chemical Co.). The glucose was converted into starch by multiplying by 0.9. Percentage of starch digestibility was calculated as percent starch hydrolysed from the total starch content of the sample.

Determination of Resistant Starch (RS): Sample flour (100 mg,) was suspended in water (50 ml) and treated with Termamyl (100 µl) at 95°C for 45 min, cooled, centrifuged and supernatant was discarded. The residue was hydrolysed with protease (10 mg in phosphate buffer pH 7.5) and amyloglucosidase (10 mg 0.1 M acetate buffer pH 4.75) to remove proteins and hydrolyse starch, respectively. The residues were dissolved in 2 M KOH (50 mg of sample, 6 mL of KOH constant shaking), incubated

with amyloglucosidase for 35 min at 60°C to hydrolyse RS. Glucose content in the above samples was determined using glucose oxidase peroxidase kit. Digestible starch was calculated as the difference between total starch and resistant starch.

Total Dietary Fibre: For the total dietary fibre (TDF), the Lablab flour samples were treated in autoclave with heat stable amylase, amyloglucosidase, and protease to remove starch and protein according to the AOAC Official Method 2009.xx (Mccleary et al., 2011). Enzymatically undigested fibre was precipitated by ethanol and filtered. Residue was dried, weighed, ashed, and reweighed. A second portion of sample was refluxed with neutral detergent and treated with α-amylase from porcine pancreas to remove water soluble carbohydrates and protein. Residue was dried, weighed, ashed and reweighed. Total dietary fibre (TDF) was calculated as sum of the 2 residues. For insoluble and soluble dietary fibre (IDF and SDF), enzyme was filtered, and the residue (IDF) washed with warm water, dried and weighed. For SDF, combined filtrate and washes were precipitated with alcohol, filtered, dried, and weighed. TDF, IDF, and SDF residue values were corrected for protein, ash and blank.

3.5.28. Functional properties of Lablab beans

The functional properties investigated include protein solubility, emulsion capacity and its stability, foaming capacity and stability, gelation and the water and oil absorption of the protein isolate from the Lablab beans and the common bean Rosecoco.

3.5.29. Preparation of defatted Lablab flour for functional properties

The four samples were milled in a laboratory mill into flour to pass through a 0.5-mm screen. The defatting process involved cold extraction where the flour samples were soaked into hexane at the ratio 1:10 (flour: hexane). The samples were stirred in mechanical shaker for 48 hours with exchange of solvent in between. The mixture was filtered and the oil free flour was dried at 55˚C for 16 hours then reground to pass through 0.4-mm mesh screen and stored at 4 ºC for analysis of functionality of proteins.

Protein isolates for the Lablab bean varieties were prepared from defatted seed flours using the method modified by Zhong *et al*. (2012).

The defatted flours were dispersed in distilled water 1:5 (w/v) flour to water ratio and the suspension adjusted to pH 9.0 using 1 N NaOH. The mixture was stirred at room temperature for 20 minutes and the insoluble matrices separated by centrifugation at 4000 g/20 min and discarded. Extraction and centrifugation procedures were repeated on the residue. The supernatant was adjusted to pH 4.0 using 1.0 N HCl and stirred at room temperature for another 20 min. The mixture was centrifuged (4000 g/20 min). The precipitate was washed several times, using distilled water to free it from salts. It was then neutralized to pH 7.0, using 1.0 N NaOH. The neutralized precipitate was left over night in a refrigerator (4°C), freeze-dried and ground into powder using a ceramic mortar and pestle then stored at room temperature for analysis.

3.5.30. Water absorption capacity (WAC) of lablab and rosecoco bean isolates

The Water absorption capacity (WAC) of the samples was determined following a modified method of Zhong *et al*. (2012) where 1 g of each sample was placed into a pre-weighed centrifuge tube mixed by vortex with 10 mL distilled water. The solutions were allowed to stand for 30 min before centrifuging (2000 g, 20min). The supernatant was poured out and the sample reweighed. The water absorption capacities were expressed as the number of grams of water bound by gram of flour.

3.5.31. Oil absorption capacity (OAC) of lablab and rosecoco bean isolates

The method of Zhong *et a*l. (2012) was used in OAC determination. 0.5 g of protein isolates were mixed with 3.0 mL of corn oil and stirred for 1 min. After a holding period of 30 min, the mixture was centrifuged at 3000g for 20 min and the volume of free oil was read. The oil absorption capacity was expressed in percentage as the amount of corn oil bound by a 100-g sample (Lin & Humbert, 1974) .

3.5.32. Protein solubility of lablab and rosecoco bean isolates

Protein solubility refers to the protein content that is soluble in salt solution. It can be measured both in water and in 3% NaCl solution. The protein solubility for Lablab beans was carried out according to the method described by Lawal *et al*. (2005). 100mg of protein isolate samples were dispersed in 20 ml of distilled water and the pH of the suspension was adjusted to in a range of 2–10 with 0.5 M NaOH or 0.5 M HCl. The suspensions were agitated with a magnetic stirrer for 30 min at room temperature before centrifuging at 1200 g for 20 min at 48˚C. The amount of protein content in the supernatant were determined using the micro Kjeldahl method. Analysis were carried out in triplicate and the solubility profile obtained by plotting the means of protein solubility (%) against pH.

$$Protein\ Solubility = \frac{Protein\ content\ in\ supernatant}{Protein\ content\ in\ sample} \times 100$$

3.5.33. Emulsifying properties of lablab and rosecoco bean isolates

Emulsifying activity and stability were determined using the method described by Zhong et al. (2012) where 5 mL portions of protein solution (10 mg/ml) were homogenized with 5 mL oil (corn oil) for 1 min. The emulsions were centrifuged at 1100 rpm for 5 min. The height of emulsified layer and that of the total contents in the tube was measured. The emulsifying activity was calculated as:

$$Emulsion\ capacity\% = \frac{\text{Height of emulsified layer in the tube}}{\text{Height of the total contents in the tube}} * 100$$

The emulsion stability was determined by heating the emulsions at 80˚C for 30 min before centrifuging at 1100 rpm for 5 min.

$$Emulsion\ Stability\ \% = \frac{\text{Height of emulsified layer after heating}}{Height\ of\ emulsified\ layer\ after\ heating} * 100$$

3.5.34. Foaming properties of lablab and rosecoco bean isolates

The foaming capacity and stability was analysed using the method of Coffmann and Garcia, (1977). About 2 g of the sample was added to 50 mL distilled water in a 100 mL graduated cylinder. The suspension was then mixed and shaken for 5 min to foam. The volume of foam at 30 s after whipping was expressed as foaming capacity using the given formula below.

Volumes were recorded before and after whipping. The percentage volume increase, which serves as index of foam capacity, was calculated according to the following equation.

$$\%\ \text{Foaming capacity}\ \frac{\text{V2} - \text{V1}}{V1} * 100$$

V_1 =volume of solution before whipping

V2=volume of solution after whipping

The volume of foam was recorded one hour after whipping to determine foaming stability as a percentage of the initial foam volume.

$$\%\ \text{Foaming stability}\ \frac{\text{V3}}{V1} * 100$$

Where

V3= Volume after one hour of whipping.

3.5.35. Gelation Properties of lablab and rosecoco bean isolates

The method of Lawal *et al* (2005) was used to determine the gelation properties of the protein isolate. The isolate sample was suspended in distilled water at different concentrations (2-20%) and the pH of suspensions was adjusted to the desired value (2-10). Each sample suspension (10 mL) was taken into the test tube and heated for 1 hr. in boiling water bath and then cooled rapidly in cold water then cooled further to 4° C for

2 h. The test tubes were then inverted and analysed for gelling ability. The concentration at which the sample did not slip or fall from the inverted test tubes was expressed as least gelation concentration.

3.5.36. Cooking characteristics of Lablab beans

3.5.37. Cooking assessment by Finger pressing method

Two hundred seeds from each of the three Lablab bean varieties, un-soaked and soaked (6 h, 12h and 24 h) were subjected to boiling at 96.5 °C in a thermostat water bath (Memmert WBU-45, Germany). For each test, ten seeds were withdrawn at 30 min intervals and cooled in a cold-water bath for 1 min before determining the cooking status by finger pressing. The softness/hardness (cookability) of the cooked beans was determined subjectively by pressing them between the thumb and forefinger (Shomer, Paster, Lindner, & Vasiliver, 1990). The beans were classified as cooked when the cotyledons disintegrated on applying slight pressure with the two fingers. The percentage of cooked beans in the batch was determined as a function of time. The data obtained were used to generate cooking curves for the different Lablab bean varieties.

3.5.38. Cooking assessment by Cutting test (Tensile) method

The cookability of the beans were also tested using the Instron Universal Testing Machine that was set up to puncture one bean at a time, by using the distance cycling controls on the machine. The beans were cooked for 60 minutes after which about 10 beans were subjected to the puncture test after every 20 minutes up to 180 minutes. The punch is caused to cycle between two pre-set distance limits at a predetermined speed, permitting the operator to devote full attention to placing and removing beans in the puncture cup.

The system uses a cutting probe which could measure up to a maximum force of 100 N (10 kg) at a speed of 100 mm min^{-1}. The maximum force registered during the cutting of the cooked beans was recorded. The experimental value was obtained by averaging

10 measurements (seeds) for a given sample. The data obtained were used to generate cooking curves for the different varieties indicating the average cutting force as a function of time. Testing was carried after cooking the beans for 60 minutes and after every 20 minutes up to 180 minutes of cooking.

3.5.39. Sensory characteristics and acceptability of cooked Lablab and rosecoco beans

Evaluation of sensory characteristics and acceptability was conducted using appropriate descriptions with well-defined numbering scores for appearance, texture, taste, and overall acceptability. The beans were cooked in a hot plate until they were soft. The cookability of the beans was checked by finger pressing method of Shomer *et a*l. (1990). The cooked beans were subjected to a panellist for ranking. A commonly consumed variety of *Phaseolus vulgaris*, Rosecoco, used as a control was among the samples introduced to the panellists for ranking. A five-point hedonic measure as described by Singh-Ackbarali and Maharaj, (2014) was used as shown in Table 3. **1**.

1	2	3	4	5
dislike extremely	dislike moderately	neither like nor	like moderately	like extremely

Table 3. 1: Five-point hedonic scale

Thirty-five (35) untrained panellists consisting of men and women from Karatina sub county in Nyeri County, central Kenya where the Lablab beans are frequently consumed participated in the sensory evaluation. Before ranking the samples, the meaning of the descriptions was explained and panellists were instructed to rank the appearance first, then taste, texture and then give the overall acceptability of the four samples. Water was provided to rinse the palate between samples, and expectoration cups with the cover were provided for panellists who did not wish to swallow the between samples. The evaluation was carried out in cool and quiet room. The panellists had not smoked, eaten or drunk for a minimum of 30 minutes prior to the test.

3.5.40. Development and evaluation of Lablab tempeh

3.5.41. Acquisition of tempeh starter and preparation of the Lablab beans

The tempeh starter comprising *Rhizopus oligosporus, Rhizopus oryzae* and rice flour was shipped from Jakarta Indonesia (*Ragi IndoPal tempeh starter*).

The preparation of *tempeh* was done according to the method of Nout and Kiers (2005) with modifications on the dehulling and cooking procedures The Lablab beans were cleaned and soaked in deionised water dosed with 7ml of vinegar for 24 hours. The soaked Lablab beans were then dehulled and split. The dehulled split beans were then boiled in a saucepan for 40 minutes. The beans were drained of the boiling water and blotted dry with a clean tea towel. Cooling was done to inoculation temperatures of 35˚C. The cooked beans were placed in a dry bowl and inoculated with the tempeh starter in ratio of 500 g beans: 2.5 g inoculum. The inoculated beans were mixed thoroughly and placed in perforated polythene bags. These polythene bags were sealed properly. The incubator was set at 30˚C. Incubation was carried out for 48 h while monitoring the temperatures. Part of the fermented product (*tempeh*) was then dried at 50˚C for 24 h and milled for nutrient analysis. The other part of the product was subjected to microbial and sensory analysis.

3.5.42. Total microbial load of Lablab *tempeh*

The determination of the total plate count was done using Nutrient agar. The agar was weighed and dissolved in distilled water, covered with cotton wool then mixed thoroughly by shaking. The agar solution was autoclaved at 120˚C for five minutes. The agar was allowed to cool to 45-50˚ C before it was poured into sterile petri dishes under sterile surfaces. A volume of 10ml solution was made using 5 g of the *tempeh* product made by homogenising in a blender to obtain the crude sample mixture. Serial dilutions of 1ml were made in sterile diluents in 3 test tubes (10 mL) using a micropipette. 0.1ml inoculum using a micropipette was put into a sterile Petri plates containing the media at

45-50˚C aseptically and the plates carefully swirled to mix the inoculum and allowed to solidify. The plates were then incubated for 48 hours at 25 ˚C. The results then taken by counting and recording the colonies of each dilution factor using a colony counter.

3.5.43. Sensory evaluation of Lablab tempeh

Evaluation of sensory characteristics and acceptability was conducted using appropriate descriptions with well-defined numbering scores for appearance, texture, taste, and overall acceptability. This was done to evaluate whether there was significant appeal in sensory characteristics of a Lablab bean tempeh. A nine-point Hedonic scale as described by Singh-Ackbarali and Maharaj (2014) was used (Table 3.2).

Table 3.2: Nine-point hedonic scale

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Thirty (30) untrained panellists consisting of students and staff from Karatina University main campus were used in the sensory evaluation. Before the assessment, the meaning of the descriptions was explained and panellists instructed to assess the appearance first, then taste, texture and then give the overall acceptability of the sample. Water was provided to rinse the palate between samples, and expectoration cups with the cover was provided for the panellists who did not wish to swallow the between samples. The evaluation was carried out in cool and quiet room. The panellist had not smoked, eaten or drunk for a minimum of 30 minutes prior to the test. The data obtained was then analysed. Means and standard deviations calculated. Analysis of variance then carried out to detect significant differences ($p < 0.05$) in appearance, taste, texture and overall acceptability of the Lablab bean *tempeh*.

3.6. Data analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using GenStat (Version 12.0). The mean values were displayed with standard deviation (SD). The statistical comparison between means for the treatments were made using Duncan's Multiple Range Test (DMRT) and significant difference was reported at P=0.05. The sensory data was evaluated using analysis of variance (ANOVA) and the Tukey Kramer or Tukey's Honest Significant Difference (Tukey HSD) multiple comparison procedure was performed to determine which samples were significantly different (Appendix 2).

CHAPTER FOUR

RESULTS

4.1. Physical characteristics of the bean samples

4.1.1. Colour and seed dimensions of the bean samples

KAT/DL-2 variety was black in colour while the other two varieties KAT/DL-1 and KAT/DL-3 were brown and cream in appearance, respectively. The variety KAT/DL-1 had a significantly higher length (10.75 mm) and width (7.45 mm) than the other two varieties (P=0.05), but also a lower thickness (5.25 mm) as shown in table 4.1. In comparison to the Rosecoco bean, the Rosecoco bean (KAT/X69) had significantly higher length (10.75 mm) than the lablab bean varieties, but was not significantly different in width compared to KAT/DL-1 and KAT/DL-2. It was also not significantly different in thickness in comparison to KAT/DL-3 and KAT/DL-2.

Table 4.1: Colour and seed dimensions

Variety	Colour	Length (mm)	Thickness (mm)	Width (mm)
KAT/DL-1	*Brown*	$10.75^{b}\pm0.98$	$5.25^{a}\pm0.05$	$7.45^{b}\pm0.18$
KAT/DL-2	*Black*	$10.32^{ab}\pm0.69$	$5.62^{b}\pm0.08$	$7.2^{ab}\pm0.14$
KAT/DL-3	*Cream white*	$9.75^{a}\pm0.24$	$5.50^{b}\pm0.25$	$6.88^{a}\pm0.51$
KAT/X69	*Red with white flecks*	$15.17^{c}\pm0.04$	$5.75^{b}\pm0.1$	$7.28^{b}\pm0.38$

Values are means ±standard deviation of 10 measurements. Means with similar letters in the same column are not significantly different (p=0.05)

4.1.2. Seed weight, density, porosity and sphericity

Results in table 4.2 show a significant difference in seed weight among the bean samples where KAT/DL-1 had a significantly higher 100 seed weight of 29.0 g (P=0.05) compared to the other two Lablab varieties. The Rosecoco bean had significantly higher seed weight (45.3/100 seeds) than the lablab bean varieties. There

was no significant difference in the density of all the bean varieties, including that of the Rosecoco bean. KAT/DL-3 had a significantly higher sphericity (P=0.05) than the other two lablab varieties. The lablab beans had significantly higher sphericity than the rosecoco bean. For seed porosity, KAT/DL-1 had a significantly higher porosity (p=0.05) than all the other bean varieties.

Table 4.2: Seed weight, density, sphericity and porosity

Bean variety	**100 Seed weight (g)**	**True density (P_t)**	**Sphericity (ϕ)**	**Bulk density(ρ_d)**	**Seed porosity(Φ)**
KATDL-1	29.0^{b}±0.2	1.4^{a}±0.1	64.8^{b}±0.5	0.8^{a}±0.0	42.8^{d}±0.6
KATDL-2	27.5^{a}±0.2	1.1^{a}±0.0	66.6^{b}±0.4	0.8^{a}±0.0	27.7^{a}±2.3
KATDL-3	26.7^{a}±0.3	1.3^{a}±0.1	75.9^{c}±0.5	0.8^{a}±0.0	37.3^{c}±1.2
KAT-X69	45.3^{c}±0.2	1.1^{a}±0.0	57.4^{a}±0.6	0.7^{a}±0.0	32.3^{b}±1.0

Values are means ±standard deviation of 10 measurements. Means with similar letters in the same column are not significantly different (p=0.05)

4.1.3. Swelling and hydration coefficients of the beans

The results in table 4.3 indicate a significantly higher hydration (192.9%) and swelling (143.9%) coefficient (P=0.05) in KAT/DL-3 as compared to KAT/DL-1 and KAT/DL-2. However, the Rosecoco bean variety KAT/X69 had significantly (P=0.05) higher hydration (204.7%) and swelling (171.7%) coefficients than all the lablab bean varieties. It also had a significantly higher water absorption capacity than the lablab bean varieties.

Table 4.3: Swelling and Hydration of bean seeds (after 12h)

Bean variety	**Hydration coefficient**	**Swelling coefficient**
KATDL-1	122.6^{a}±12.6	116.8^{a}±1.4
KATDL-2	155.2^{b}±11.16	123.5^{b}±6.80
KATDL-3	192.9^{c}±10.23	143.9^{c}±3.57
KAT-X69	204.7^{d}±13.06	171.7^{d}±2.40

Values are means ±standard deviation of three measurements. Means with similar letters in the same column are not significantly different (p=0.05)

The Water absorption capacity of the bean samples increased with soaking time. The highest absorption capacity was recorded at 24 hours while the least was at 6 hours (figure 4.1). KAT/DL-3 exhibited a higher absorption capacity among the three Lablab beans while Rosecoco (KAT/X69) overall had significantly absorption capacity than the lablab bean varieties (P=0.05).

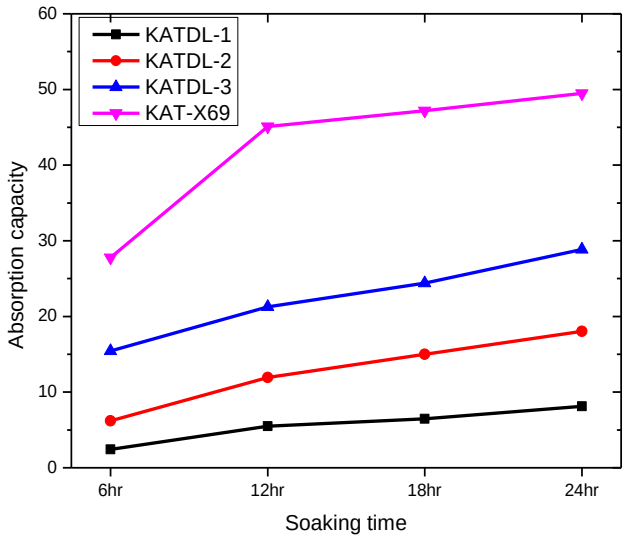

Figure 4. 1: Water absorption capacity during soaking

4.2. Nutrient composition and energy content of Lablab beans

4.2.1. Proximate composition

The proximate composition which includes protein, ash, moisture, fats and carbohydrates is presented in table 4.4. The protein content of raw lablab KAT/DL-1 (27.6 %) was significantly higher than the other two lablab bean varieties (P=0.05). There was significant difference in the amounts of proteins for lablab and rosecoco where protein in rosecoco was significantly lower (23.6%) compared to lablab beans. The rosecoco (KAT/X69) bean exhibited higher ash content at P=0.05. There was no significant difference in fat content among all the bean varieties, which was relatively low ranging from 2.5 to 2.7 %. The lablab beans were found to have significantly higher amounts of energy in kilocalories in comparison to the rosecoco bean.

Table 4.4: Proximate composition and energy content of the bean varieties.

Variety	Moisture g/100g	Proteins (g/100g)	Ash (g/100g)	Fats (g/100g)	CHOs (g/100g)	Energy Kcal/g
KAT/DL-1	$8.4^{a}\pm0.04$	$27.6^{d}\pm0.4$	$3.9^{a}\pm0.07$	$2.6^{a}\pm0.12$	$57.5^{a}\pm0.3$	$351.0^{c}\pm0.6$
KAT/DL-2	$8.1^{a}\pm0.01$	$26.6^{c}\pm0.2$	$4.1^{ab}\pm0.27$	$2.6^{a}\pm0.15$	$58.6^{ab}\pm0.2$	$352.7^{c}\pm0.6$
KAT/DL-3	$9.8^{b}\pm0.2$	$24.9^{b}\pm0.5$	$4.4^{ab}\pm0.13$	$2.7^{a}\pm0.04$	$582^{ab}\pm0.6$	$345.4^{b}\pm0.5$
KAT/X69	$10.5^{c}\pm0.3$	$23.6^{a}\pm0.4$	$4.5^{b}\pm0.34$	$2.5^{a}\pm047$	$58.9^{b}\pm1.07$	$342.6^{a}\pm1.4$

Values are means ±S. D of three determinations. Means with similar letters in the same column are not significantly different (p=0.05)
Carbohydrates calculated by difference (100- (crude proteins+ ash and fats)
Kcal/g calculated by Atwater specific factors (proteins *3.47, fats *8.37 CHOs* 4.07)

4.2.2. Mineral content in Lablab beans

The mineral quantities for the lablab and rosecoco beans in respect to potassium, calcium, magnesium, iron zinc copper and manganese as tabulated in table 4.5. Potassium (K) was found to be significantly higher at P=0.05 in KAT/DL-1 compared to the other two lablab beans and the rosecoco. There was no significant difference in

the levels of calcium, copper and iron among the lablab beans. Rosecoco had significantly higher iron content (6.19mg/100g) than the three varieties of lablab beans (P=0.05). However, the content of manganese and zinc were significantly lower in rosecoco (0.86mg/100g;1.42 mg/100g) at P=0.05 in comparison to the lablab beans. Rosecoco was found to significantly (P=0.05) have lower levels of zinc (2.19mg/100g) as compared to the lablab bean varieties. The four bean varieties did not differ significantly in the amounts of magnesium (mg/100).

Table 4.5: Mineral content in three Lablab and Rosecoco bean varieties (mg/100g)

	K	Ca	Mg	Fe	Zn	Cu	Mn
KAT/DL-1	$1293^{c}\pm7$	$115.5^{a}\pm7$	$161.8^{b}\pm7$	$5.75^{a}\pm0.3$	$2.95^{c}\pm0.1$	$1.14^{b}\pm0.1$	$1.83^{b}\pm0.1$
KAT/DL-2	$1104^{ab}\pm6$	$112.1^{a}\pm3$	$140.4^{a}\pm2$	$5.36^{a}\pm0.2$	$2.45^{b}\pm0.2$	$1.12^{b}\pm0.1$	$1.95^{b}\pm0.1$
KAT/DL-3	$986^{a}\pm7$	$110.4^{a}\pm9$	$152.3^{ab}\pm3$	$5.63^{a}\pm0.1$	$2.19^{a}\pm0.2$	$1.08^{b}\pm0.1$	$1.89^{b}\pm0.1$
KAT/X69	$1218^{bc}\pm3$	$137.7^{b}\pm_{1}$	$154.9^{ab}\pm15$	$6.19^{b}\pm0.4$	$2.78^{bc}\pm0.3$	$0.86^{a}\pm0.1$	$1.42^{a}\pm0.1$

Values are means ±S. D of three determinations. Means with similar letters in the same column are not significantly different (p=0.05).

4.2.3. Fatty acid profile of the lablab and rosecoco bean

The fatty acid profile of samples KAT/DL-1, KAT/DL-2, KAT/DL-2and KAT/X69 is shown in table 4.6. Palmitic, oleic, linoleic and α linolenic acids were found to be the most abundant in the samples. The unsaturated fatty acids (UFA) were the most abundant fatty acids in Lablab beans constituting more than half of the total fatty acids. KAT/DL-2 sample showed the highest level of linoleic acid (56%) when compared to the other two Lablab varieties. KAT/DL-3 had the highest oleic acid content (8%) while α linolenic acid was highest in KAT/DL-1 at 9%. The differences among the samples were significant (p= 0.05). The levels of short chain fatty acids (lauric, myristic and myristoleic acid) were below 1% each across all the four sample.

Table 4.6: Fatty acids in Lablab and Rosecoco bean varieties

Fatty acids	g 100 g^{-1} of total fatty acids in crude fat			
	KAT/DL-1	KAT/DL-2	KAT/DL-3	KAT/X69
Lauric (C12:0)	0.22^{a}	0.19^{a}	0.21^{a}	0.28^{b}
Myristic (C14:0)	0.94^{c}	0.39^{a}	0.33^{a}	0.68^{b}
Myristoleic acid (C14:1)	0.36^{c}	0.26^{b}	0.17^{a}	0.44^{d}
Palmitic (C16:0)	25.75^{b}	25.39^{b}	24.93^{a}	25.46^{b}
Palmitoleic (C16:1)	0.25^{a}	0.34^{b}	0.36^{b}	0.35^{b}
Stearic (C18:0)	2.95^{a}	3.66^{b}	3.24^{b}	2.97^{a}
Oleic (C18:1n9c)	6.55^{b}	5.94^{a}	8.16^{c}	6.82^{b}
Linoleic (C18:2n6c)	53.32^{a}	55.76^{c}	54.79^{b}	53.78^{a}
α Linolenic (18:3, n-3)	9.36^{c}	8.07^{b}	7.82^{a}	8.97^{b}

Values are means of three determinations. Means with similar superscripts in the same row are not significantly different (p=0.05)

4.2.4. Dietary fibre, soluble fibre and starch levels in lablab and rosecoco beans

The content of dietary fibre, soluble and insoluble fibre and total starch of bean samples is presented in table 4.7. For total dietary fibre, KAT/DL-1 and KAT/DL-2 had significantly higher levels than KAT/DL-3 (p=0.05). Similar results were also observed for total starch content. For soluble fibre, KAT/DL-1, had significantly higher levels than the other lablab varieties, while for insoluble fibre, it was KAT/DL-2 that had significantly higher levels than the other two lablab varieties. The same variety

(KAT/DL-2) had significantly lower soluble fibre levels than the other two other lablab varieties. The Rosecoco bean had significantly (P=0.05) lower total dietary fibre (TDF) (13.96g/100g) compared to KAT/DL-1 and KAT/DL-2, but significantly higher levels than KAT/DL-3. There was no significant difference in the insoluble fibre content between the Rosecoco bean and KAT/DL-1. Resistant starch did not differ significantly between KAT/DL-2 and KAT/DL-3.

Table 4.7: Dietary fibre and total starch in the bean varieties (g/100g)

Bean varieties	***TDF***	***SF***	***IF***	***TS***	***RS***
KAT/DL-1	15.55^{c}±1.0	2.77^{c}±0.4	12.78^{b}±1.1	39.10^{c} ±0.6	4.49^{b} ±0.1
KAT/DL-2	16.71^{d}±1.0	0.8^{a} ±0.1	15.91^{c}±0.5	35.87^{b} ±0.5	3.57^{a} ±0.2
KAT/DL-3	11.86^{a}±0.3	1.95^{b}±0.1	9.91^{a} ±0.5	32.76^{a} ±0.3	3.49^{a} ±0.3
KAT/X69	13.96^{b}±0.6	1.41^{b}±0.3	12.54^{b}±0.7	36.66^{b} ±1.4	4.21^{b} ±0.3

Means within column, followed by the same letter are not significantly different (p=0.05) from each other

TDF- Total dietary fibre
SF- Soluble fibre
IF- Insoluble fibre
TS- Total starch
RS- Resistant starch

4.2.5. In-vitro digestibility of protein and starch of the lablab and rosecoco beans

As shown in table 4.8, the protein digestibility for the bean samples was significantly (p=0.05) higher in KAT/DL-1, compared to the other two lablab bean varieties. KAT/DL-3 also had a significantly higher protein digestibility than KAT/DL-2. In comparison to the Rosecoco beans, KAT/DL-1 and KAT/DL-3 had significantly (P=0.05) higher protein digestibility than the Rosecoco bean. However, there was no significant difference (P=0.05) in protein digestibility between the Rosecoco bean and KAT/DL-2. Similarly, KAT/DL-1 had significantly (P=0.05) higher starch digestibility than the other lablab bean varieties (P=0.05). Further, KAT/DL-2 had significantly

higher starch digestibility than KAT/DL-3. There was no significant difference in starch digestibility between the Rosecoco bean variety and KAT/DL-2.

Table 4.8: In vitro digestibility *(*g/100g of Protein/starch*)* of Lablab and Rosecoco beans

Bean varieties	***KAT/DL-1***	***KAT/DL-2***	***KAT/DL-3***	***KAT/X69***
Protein digestibility (g/100g)	54.57^{c} ±0.8	48.18^{a}±1.7	51.39^{b}±0.5	48.85^{a}±2.5
Starch digestibility (g/100g)	35.60^{c}±1.5	32.30^{b}±0.2	29.27^{a}±1.6	32.45^{b}±1.9

Means within rows followed by the same letter are not significantly different (P=0.05) from each other

4.3. Antinutrients and bioactive components in the bean samples

The levels of antinutrients including tannins, phytic acid and trypsin inhibitor for the three lablab bean varieties and common bean rosecoco are tabulated in table 4.9. The Table gives results also for flavonoid content in the four bean varieties. The KAT/DL-2 variety had significantly (p=0.05) higher phytic acid (723.6mg/100g) and tannin levels (0.33g/100g) than varieties KAT/DL-1 and KAT/DL-3 (P=0.05). The results showed no significant (p=0.05) difference in trypsin inhibitor activity among the three Lablab varieties. KAT/DL-3 had significantly (p=0.05) lower levels of phytic acid compared to the other lablab bean varieties. Compared with the Rosecoco bean variety, the lablab bean varieties had significantly lower tannin levels and trypsin inhibiting activity than the rosecoco bean (P=0.05). The results also indicate that KAT/DL-1 had significantly (p=0.05) higher levels of flavonoids (1492mg/100g) compared to other two lablab beans and KAT/X69.

Table 4.9: Anti-nutrient and flavonoid content in Lablab beans and Rosecoco

Bean variety	**Phytic acid**	**TIA(TIU/mg)**	**Tannins**	***Flavonoids***

	mg_100g		**(g/100)**	***(mg/100g)***
KAT/DL-1	687.8 ^{c}±15.4	11.5 ^{a}±1.8	0.26^{b}±0.04	1492 ^{c} ±13
KAT/DL-2	723.6^{d} ±17.0	13.1^{a} ±1.9	0.33 ^{c} ±0.07	1137 ^{ab} ±30
KAT/DL-3	533.4^{a} ±16.0	12.5 ^{a}±1.0	0.23 ^{a} ±0.03	1092 ^{a} ±25
KAT/X69	617.7^{b}±19.1	22.3^{b}±2.2	0.39^{d}±0.03	1243 ^{b} ±16

Values are means ±S. D of three determinations. Means with similar letters in the same column are not significantly different (P=0.05)
TIA-trypsin inhibitor activity
TIU-trypsin units inhibited per mg sample.

The lablab beans and common bean rosecoco were also analysed for their free radical scavenging ability. High % inhibition of DPPH is an indication of high free radical scavenging activity (FRSA) of the samples. Though the methanol extracts of the Lablab bean flours showed potential of free radical scavenging activity against DPPH, it was, however, considerably lower compared to vitamin C, the standard used. The inhibition was found to be concentration dependent in that inhibition increased with increase in concentration. At the concentration of 0.1mg/ml, the varieties had a percent inhibition of about 5%. The bean samples recorded the highest inhibition activity (19%) at 10 mg/ml concentration. The difference in inhibition among the varieties was not significant (p=0.05) across the concentrations as shown in figure 4.2.

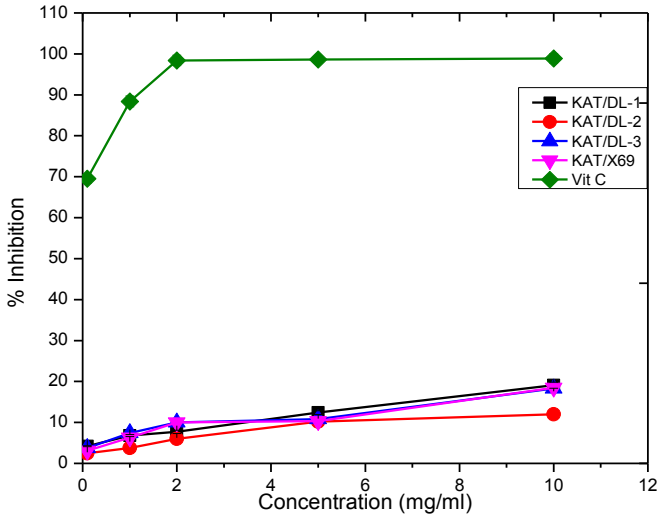

Figure 4.2: Anti-oxidant activity of the bean extracts

4.3.1. Functional properties of Lablab and rosecoco beans

4.3.2. Protein solubility

The results of protein solubility for the four bean samples is shown in figure 4.3 where across all the pH values KAT/DL-1 samples had the lowest solubility. The protein solubility increased at pH 6, pH 8 and pH 10, but was lowest at pH 4 for all the bean samples. The mean highest solubility was 76% obtained at pH 10. The bean protein has a good solubility that can be manipulated in product formulation. The Rosecoco bean had higher protein solubility than the lablab bean varieties.

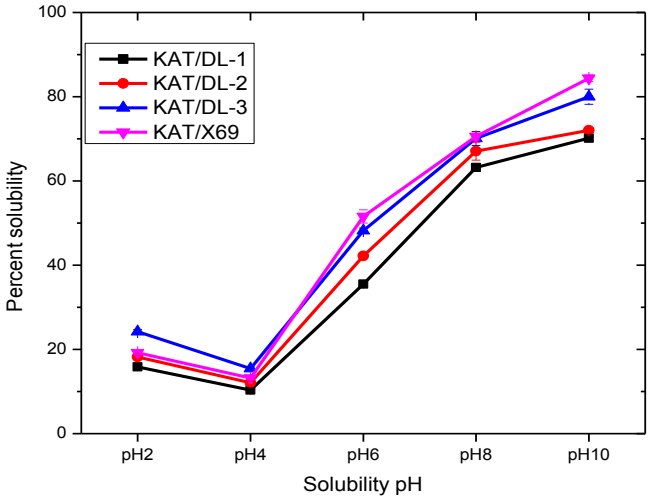

Figure 4.3: Protein solubility of Lablab protein isolate (%)

4.3.3. Emulsion capacity and stability of bean protein isolates

The emulsion capacity of the bean proteins varied with the pH. Increase in pH from pH 2 to pH 10 led to a significant increase in emulsion capacity except at pH 4 where a decrease was observed. KAT/DL-3 had significantly higher emulsion capacity compared to other Lablab beans as indicated in figure 4.4. Similarly, KAT/DL-1 had lower emulsion capacity than the other two varieties.

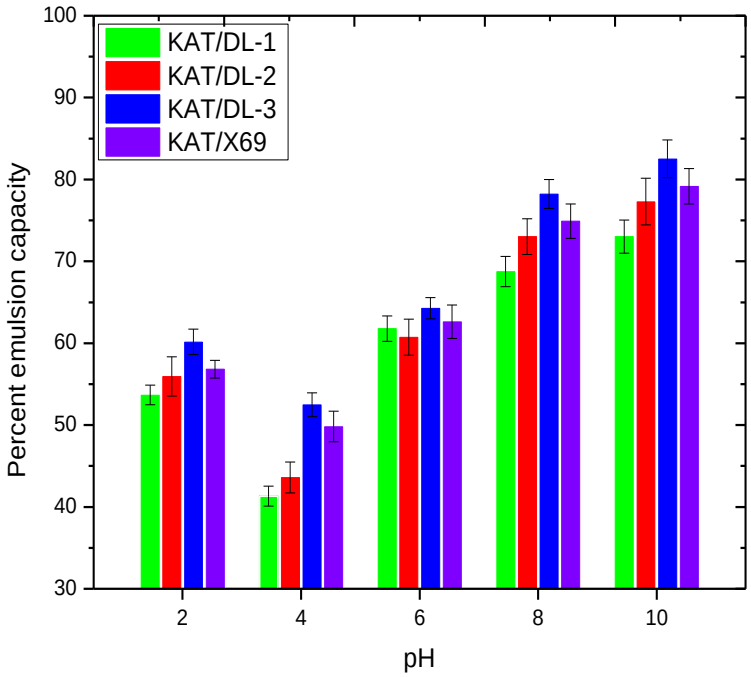

Figure 4. 4: Emulsion capacity (%) of the Lablab bean protein isolates

The emulsions were then evaluated for their stability after being left to stand for one hour. The results indicate a significant difference between emulsion stability of KAT/DL-3 and the other bean samples. Though KAT/DL-3 had a significantly higher emulsion capacity than the other two varieties, this emulsion had lower stability in comparison to the stability of the other two varieties (table 4.10).

Table 4.10: Emulsion stability of the bean protein isolate

Bean variety	**Emulsion stability (%)**
KAT/DL-1	86.09^{b}±0.5
KAT/DL-2	86.16^{b}±0.3
KAT/DL-3	84.74^{a}±0.5
KAT/X69	85.91^{b}±0.6

Means with similar letters in the same column are not significantly different (P=0.05)

4.3.4. Gelation capacity of the protein isolates

The gelation capacity of the four bean protein isolates differed significantly among the varieties. The protein isolates for KAT/DL-1 had significantly higher gelling capacity compared to KAT/DL-2 and KAT/DL-3. There was no significant difference (P=0.05) between the gelation capacities of KAT/X69 and KAT/DL-3 as indicated in table 4.11.

Table 4.11: Gelation properties of the bean protein isolates

Bean variety	Gelation capacity (%)
KAT/DL-1	18.20^{a} ±0.78
KAT/DL-2	19.24^{b}±1.09
KAT/DL-3	20.59^{c}±1.03
KAT/X69	20.13^{c}±1.78

Means with similar letters in the same column are not significantly different (P=0.05)

4.3.5. Foaming capacity and stability

The Lablab bean protein isolates exhibited foaming capacity of about 50% and there was no significant difference among the varieties. The mean foam stability was about 67 %, and there was no significant difference among the varieties as shown in table 4.12.

Table 4.12: Foaming capacity and stability of bean protein isolates

Bean variety	**Foaming capacity (%)**	**Foaming stability (%)**
KAT/DL-1	51.47^{a}±1.5	66.41^{b}±2.8
KAT/DL-2	55.54^{d}±1.0	65.36^{a}±1.1
KAT/DL-3	52.27^{b}±1.2	68.91^{d}±1.7
KAT/X69	53.29^{c}±1.8	67.00^{c}±1.3

Means with similar letters in the same column are not significantly different (P=0.05)

4.3.6. Water and oil absorption

The protein isolates of the four samples were analysed for their water and oil absorption. Water absorption was found to be significantly higher (6.2%) in KAT/DL-3 protein isolates compared to the other three bean protein isolates (p=0.05). The mean oil absorption was 4% and was significantly lower in KAT/DL-1. There was no significant difference in oil absorption for KAT/DL-2, KAT/DL-2 and KAT/X69 (Rosecoco) as indicated in figure 4.5.

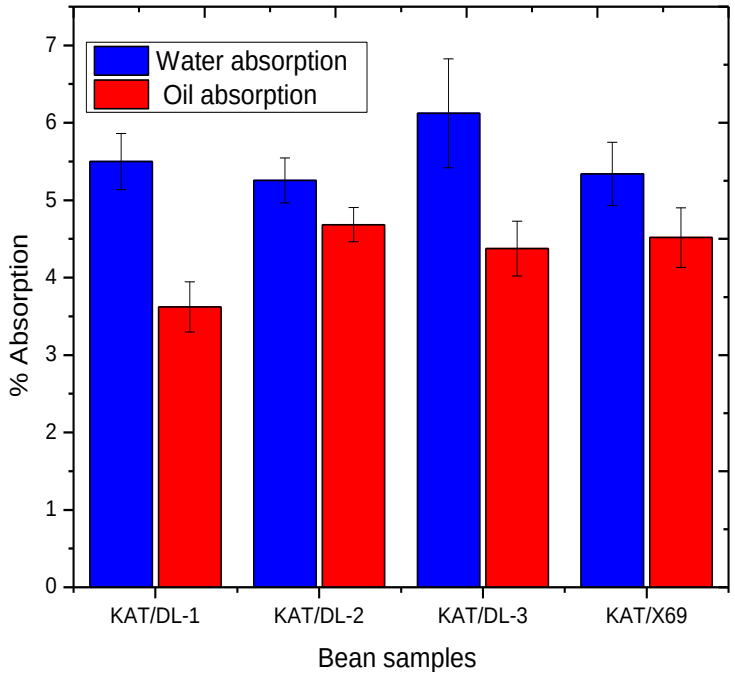

Figure 4.5: Water and oil absorption capacities of the bean protein isolates

4.4. Cooking and sensory characteristics of lablab and rosecoco bean varieties

4.4.1. Cooking characteristics of the Lablab and Rosecoco beans

4.4.1.1. Cooking time by finger pressing test

When the finger press method was used to determine the cooking time adequate cooking time for the beans ranged from 120 to 160 minutes. KAT/DL-3 cooked faster compared to the other two Lablab varieties. In comparison to the Rosecoco bean (KAT/X69) the Rosecoco common bean cooked at a faster rate than the lablab bean varieties, as it was adequately cooked after 120 minutes.

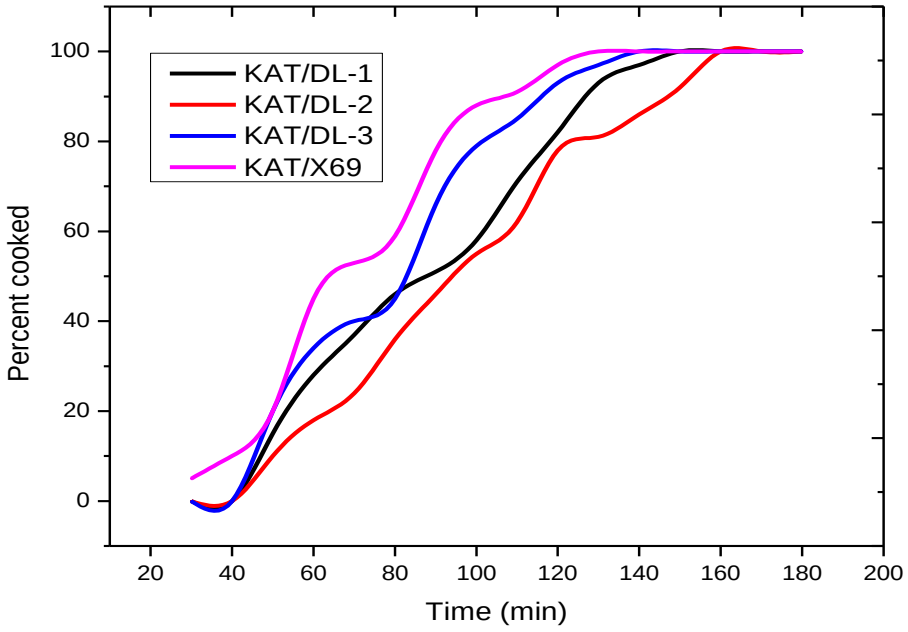

Figure 4.6: Bean cookability using finger press method

4.4.1.2. Cooking time: Cutting test (Tensile)

When cooking time was determined using the tensile or cutting test, similar results to those observed using the finger pressing test (figure 4.6) were found as presented in figure 4.7 where KAT/DL-2 was the slowest in attaining the requirement of a minimum force of 0.5 mm/min to penetrate the cooked bean.

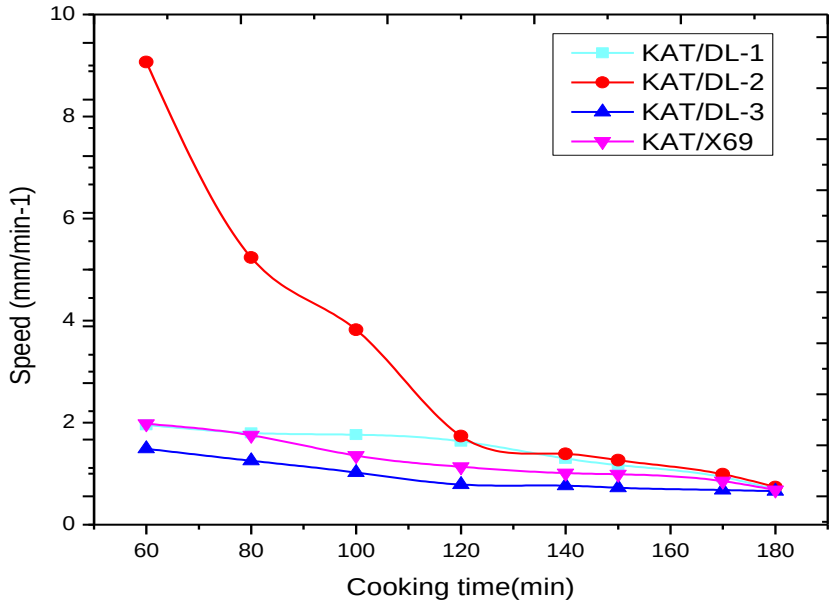

Figure 4.7: Bean cookability using the cutting (tensile) method

4.4.2. Cooking time for raw and soaked lablab and rosecoco beans

Soaking of the beans for at least 12 hours significantly reduced cooking time of the beans. However, there was no difference in cooking time when the beans were soaked for only six hours in comparison to the raw beans. After soaking for 12, 18 and 24 hours, KAT/X69 which is the common bean Rosecoco variety, cooked at a significantly shorter period of 110, 100 and 80 minutes, respectively, in comparison to the cooking time for the lablab bean varieties while KAT/DL-3 cooked at 90 minutes with a 36% decrease in cooking time at 24 hours as shown in table 4.13. The results also show that there was no significant difference in cooking time for Lablab beans soaked for 6 hours and the un-soaked beans (P=0.05). Among the lablab bean varieties, KAT/DL-3 had a significantly shorter cooking time than the other two varieties after the various soaking periods. On the other hand, KAT/DL-2 had a significantly longer cooking time.

Table 4.13: Cooking time for soaked and un-soaked beans

Bean variety	Un-soaked	Soaked 6Hr	Soaked 12 Hr	Soaked 18Hr	Soaked 24 Hr
	Cooking time in minutes				
KAT/DL-1	150^{c}±7	150^{c}±5	130^{b}±5	120^{b}±3	115^{c}±5
KAT/DL-2	160^{d}±10	160^{d}±7	145^{c}±7	130^{c}±5	115^{c}±6
KAT/DL-3	140^{b}±3	140^{b}±8	130^{b}±5	125^{bc}±3	90^{b}±8
KAT/X69	130^{a}±4	120^{a}±5	110^{a}±6	100^{a}±5	80^{a}±5

Values are means of three cooking times. Means with similar letters in the same column are not significantly different (P=0.05)

4.4.3. Sensory characteristics of the cooked bean seeds

There were four sensory attributes analysed for the cooked bean samples. Ranking of the cooked beans was done by untrained panellists for their appearance, taste, texture and the overall acceptability. The ranking results for appearance indicated no significant difference among all the beans samples (p=0.05). However, the results showed that there was significant difference in ranking when subjected to Tukey's Honest Significant Difference test (HSD) in taste, texture and general acceptability of the four beans. Both KAT/DL-1 and KAT/DL-3 had significantly better test than KAT/DL-2 and Rosecoco. (P=0.05). For the texture, that of KAT/DL-2 was significantly less preferred in comparison to that of the other three bean varieties (p=0.05). For general acceptability, KAT/DL-1 and KAT/DL-3, had a significantly higher acceptability than KAT/DL-2 and rosecoco ((KAT/X69)). However, there was no significant difference (p=0.05) between the taste and general acceptability of KAT/DL-1 and KAT/DL-3 as shown in figure 4.8.

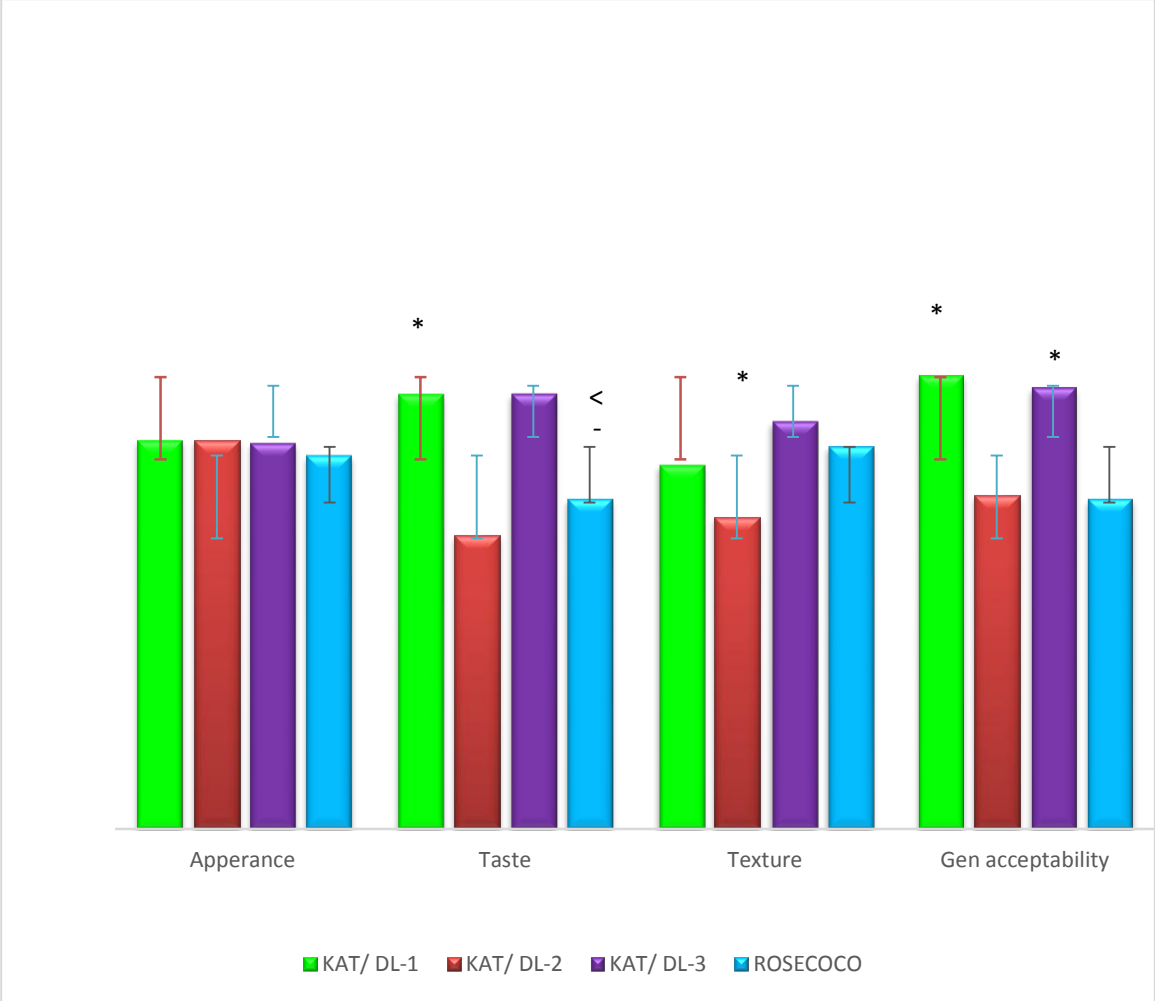

Figure 4.8: Sensory attributes of four cooked bean samples

4.5 Effect of processing methods on anti-nutritional factors, bioactive components and in vitro (protein and starch) digestibility of Lablab (*Lablab purpureus*) beans grown in Kenya

4.5.1 Effect of soaking, cooking and germination on anti-nutrient and flavonoid composition

Bean samples were cooked using the boiling method, some germinated, and others soaked for 6, 12 and 24 hours respectively then analysed for the phytic acid, tannins, trypsin inhibitor and flavonoids. The results showed a significant ($p = 0.05$) decrease in

phytate content upon soaking, cooking and germination (table 4.14). Significant reduction in phytic acid was achieved through cooking and germination.

Table 4.14: Effect of processing on phytic acid (mg/100g)

	Treatments					
Variety	Raw	Boiling	Soaked 6 h	soaked 12 h	Soaked 24 h	Germinated
KAT/DL-1	687.8^{e}±5.1	345.0^{c}±4	620^{d} ±10	541.4^{c}±4	461.6^{b}±7	429.2^{a} ±9
KAT/DL-2	723.6^{f}±7.0	388.6^{a} ±3	673^{e} ±7	582.4^{d} ±3	562.5^{c} ±2	473.5^{b} ±5
KAT/DL-3	533.4^{e} ±1.6	272.4^{a} ±9	473.6^{d} ±2	442.0^{c} ±5	443.1^{c} ±4	365.6^{b} ±3
KAT/X69 (Rosecoco)	617.7^{f}±5	317.3^{a}±6	559.1^{e}±6	504.2^{d}±4	477.8^{c}±5	355.9^{b}±9

Different superscripts within a row indicate significant differences p = 0.05

The various methods of processing (soaking, boiling and germination) were also found to reduce the tannin levels in the four bean samples. Cooking had a high significant reduction of the tannins as compared to soaking (p=0.05) across the four bean samples. There was no significant (p=0.05) difference in the levels of tannins between 6 h and 12 h soaking for beans KAT/DL-1 and KAT/DL-2 unlike for the KAT/DL-3. Similarly, there was no significant difference in the reduction levels in cooking and germination for the KAT/DL-3 and *Rosecoco* bean varieties as indicated in table 4.15.

Table 4.15: Effect of processing on tannin content (g/100g)

	Treatments					
	Raw	**Cooked**	**Soaked 6 h**	**soaked 12 h**	**Soaked 24 h**	**Germinated**
KAT/DL-1	0.27^{d}±0.04	0.15^{a}±0.07	0.24^{c}±0.08	0.25^{c}±0.08	0.24^{c}±0.07	0.20^{b}±0.07
KAT/DL-2	0.33^{d}±0.07	0.20^{a} ±0.02	0.32^{d}±0.08	0.3^{d} ±0.05	0.26^{c} ±0.08	0.22^{b} ±0.01
KAT/DL-3	0.23^{c} ±0.03	0.11^{a} ±0.03	0.21^{a}±0.09	0.15^{b} ±0.06	0.12^{a} ±0.00	0.11^{a} ±0.05
KAT/X69 (Rosecoco)	0.4^{e}±0.03	0.2^{a}±0.01	0.34^{d}±0.01	0.29^{c}±0.01	0.25^{b}±0.01	0.22^{a}±0.01

Different superscripts within a row indicate significant differences (p =0.05)

Likewise, for the trypsin inhibitor activity, all the three treatments caused significant reduction (p = 0.05). Cooking was the most effective in the reduction of the levels of trypsin inhibitor activity where a percent reduction of >85% was achieved across all the four beans samples as shown in table 4.16.

Table 4.16: Effect of processing on trypsin inhibitor activity (TIU/mg)

	Treatments					
	Raw	**Cooked**	**soaked 6 h**	**Soaked 12 h**	**Soaked 24 h**	**Germinated**
KAT/DL-1	11.5^{c}±0.1	1.7^{a}±0.02	8.5^{b}±0.1	8.2^{b}±0.03	8.0^{b}±0.13	8.4^{b}±0.1
KAT/DL-2	13.1^{c}±0.1	1.5^{a} ±0.1	8.5^{b} ±0.1	7.2^{b} ±0.1	8.8^{b} ±0.7	8.3b ±0.1
KAT/DL-3	12.5^{c}±0.1	6.7^{a} ±0.1	8.7^{b} ±0.1	7.5^{ab} ±0.8	7.3^{ab} ±0.9	8.3^{b} ±0.3
KAT/X69 Rosecoco	22.3^{f}±0.4	9.5^{a}±0.5	18.8^{e}±0.6	13.7^{d}±0.46	12.6^{c}±0.45	10.9^{b}±0.3

Different superscripts within a row indicate significant differences (p = 0.05)
TIU- trypsin units inhibited per mg. of sample.

Cooking and soaking resulted in significant reduction of flavonoids in all the bean varieties (p=0.05). However, germination had the opposite effect, resulting in a significant increase in the flavonoid levels in all the bean varieties. The flavonoid content in the four bean samples varied significantly during treatments. The results in table 4.17 shows expressively high contents in germinated beans across all the four bean samples. Flavonoids increased by 45% and 43% in KAT/DL-3 and KAT/X69 respectively when samples were germinated. Cooking and soaking reduced the flavonoid content significantly at p = 0.05.

4.5.2 Effect of treatment on anti-oxidant inhibition activity

The inhibition activity for the bean samples was also analysed for soaked, cooked and germinated samples. The inhibition activity was seen to vary with the concentration. 10mg/ml had the highest inhibition compared to 0.1mg/ml table 4.18). Cooking realised the highest inhibition activity among the four treatments while germination recorded lower levels of inhibition. Soaking the beans was found to have a positive effect on the

inhibition activity. The results however, expressed lower levels of inhibition when compared to the vitamin C inhibition which served as the radical scavenger for this particular experiment.

Table 4.17: Treatment effect on flavonoid content (mg/100g)

Treatment	**Raw**	**Cooked**	**Germinated**	**Soaked 6 h**	**Soaked 12 h**	**Soaked 24 h**
Variety	Mean Concentration (mg/100g)					
KAT/DL-1	1492^{d}±13	810^{b}±28	1747^{e}±24	686^{a}±8	925^{c}±12	919^{c}±16
KAT/DL-2	1137^{d}±30	930^{c}±16	1439^{e}±27	744^{a}±8	900^{c}±14	820^{b}±10
KAT/DL-3	1092^{c}±25	911^{b}±19	1588^{d}±23	900^{b}±7	942^{b}±9	737^{a}±11
KAT/X69	1243^{d}±16	679^{a}±11	1788^{e}±32	977^{c}±12	829^{b}±13	842^{b}±11

Different superscripts within a row indicate significant differences p = 0.05

Table 4.18: Anti-oxidant activity of the lablab and rosecoco beans (Inhibition (%) of DPPH)

		Inhibition (%) of DPPH against concentration of extracts of bean flour and vitamin C				
Treatment	**variety**	**10mg/ml**	**5mg/ml**	**2mg/ml**	**1mg/ml**	**0.1mg/ml**
Raw	KAT/DL-1	19.1	12.4	7.7	6.8	4.2
	KAT/DL-2	12.0	10.2	6.0	3.8	2.5
	KAT/DL-3	18.3	10.8	10.0	7.4	3.8
	KAT/X69	18.5	12.3	10.0	6.3	3.1
Cooked	KAT/DL-1	46.8	35.6	31.5	28.8	27.2
	KAT/DL-2	42.3	33.9	32.6	31.2	26.2
	KAT/DL-3	39.5	35.0	29.5	25.6	23.7
	KAT/X69	45.5	28.3	19.7	18.0	15.6
Germinated	KAT/DL-1	18.8	9.0	10.9	8.6	4.2
	KAT/DL-2	19.9	10.7	7.4	6.3	4.6
	KAT/DL-3	15.3	10.6	9.4	8.2	5.4
	KAT/X69	22.4	14.8	11.7	9.1	6.9
Soaked (6 h)	KAT/DL-1	28.8	24.8	21.5	20.0	14.0
	KAT/DL-2	20.6	15.5	8.8	5.8	2.9
	KAT/DL-3	22.2	20.1	15.9	11.0	6.7
	KAT/X69	41.5	26.3	17.4	10.9	6.4
Soaked (12 h)	KAT/DL-1	42.9	32.8	29.1	25.8	22.7
	KAT/DL-2	38.6	32.2	28.6	26.1	20.0
	KAT/DL-3	37.8	33.5	28.4	26.0	22.2
	KAT/X69	39.1	30.4	26.4	23.8	22.1
Soaked (24 h)	KAT/DL-1	32.8	28.3	24.3	22.8	18.4
	KAT/DL-2	35.0	27.9	21.9	18.4	16.1
	KAT/DL-3	34.8	29.4	21.0	18.4	15.3
	KAT/X69	34.0	21.3	18.2	16.9	14.2
	Vitamin C	98.9	98.9	98.6	98.5	79.5

4.5.3. Effect of processing on in-vitro digestibility of protein and starch

The effect of cooking and germination on the in-vitro digestibility of starch and proteins were assessed. Raw, cooked and germinated samples were subjected to digestibility analysis. The starch and protein results indicate a significant increase in protein digestibility when subjected to germination and cooking. Variety KAT/DL-1 achieved an increase of 51% and 38% protein digestibility through germination and cooking respectively. Rosecoco had significant 61% increase in protein digestibility after germination table 4.19. On the other hand, cooked samples significantly increased the starch digestibility more than germinated samples as shown in table 4.20. The results do not show a significant difference in starch digestibility of cooked samples and those soaked prior to cooking.

Table 4.19: Protein digestibility of raw, cooked and germinated beans

VARIETY	RAW	COOKED	GERMINATED
KAT/DL-1	54.6^{a} ±0.7	76.1^{b}±1.2	82.6^{c} ±0.8
KAT/DL-2	48.2^{a}±1.7	76.0^{b}±1.1	79.2^{c}±2.8
KAT/DL-3	51.4^{a}±0.5	76.2^{b}±2.8	81.7^{c}±1.6
KAT/X69	48.9^{a}±2.5	75.2^{b}±1.6	78.7^{c}±1.8

Means within same row per variety and treatment, followed by the same letter are not significantly different (p=0.05) from each other

Table 4.20: Starch digestibility of raw, cooked and germinated lablab and rosecoco beans

Variety	**Raw**	**Germinated**	**Cooked**	**Soaked& cooked**
KAT/DL-1	35.60^{a}±1.5	52.97^{b}±0.3	64.24^{c}±1.4	63.27^{c}±0.4
KAT/DL-2	32.30^{a}±0.2	45.75^{b}±0.3	65.09^{c}±1.5	67.03^{c}±0.2
KAT/DL-3	29.27^{a}±1.6	44.76^{b}±0.4	55.32^{c}±0.8	61.09^{c}±0.5
KAT/X69	32.45^{a}±1.9	51.03^{b}±0.2	60.15^{c}±1.2	60.14^{c}±0.6

Means within same row per variety and treatment, followed by the same letter are not significantly different (p=0.05) from each other.

4.6. Lablab bean-based tempeh products, and their quality characteristics

The lablab beans were subjected to mould fermentation where lablab-based tempeh was processed from the three Lablab bean varieties. Figure 4.9 shows the inoculated beans in an incubator while figure 4.10 is a photograph of the final Lablab *tempeh* product prepared using the mould *Rhizopus oligosporus.*

Figure 4.9: Inoculated Lablab beans in an incubator

Figure 4.10: Lablab based *tempeh*

4.6.1. Nutritional quality of Lablab tempeh

4.6.1.1. Proximate composition

The fermented lablab *tempeh* was analysed for its proximate composition and energy. The results as shown in (table 4.21) indicated a significant difference in all the parameters (p =0.05) analysed between the tempeh and the raw lablab bean variety samples. The fermented product had a significant (p =0.05) increase in protein content compared to the raw samples. Seemingly, the fat contents significantly (p=0.05) decreased during fermentation consequently affecting the overall kilocalories of the fermented product. The lablab *tempeh* from KAT/DL-1 had significantly higher protein content compared to KAT/DL-2 and KAT/DL-3. The energy calories in the lablab tempeh was not significantly different among the three bean varieties.

Table 4.21: Proximate composition of Lablab beans and Lablab *tempeh*

Varieties	***Moisture %***	***Proteins %***	***Ash %***	***Fats %***	***% CHOs***	***Energy (Kcal)***
Raw						
KATDL-1	$8.4^{a}\pm0.04$	$27.6^{d}\pm0.4$	$3.9^{a}\pm0.07$	$2.6^{a}\pm0.12$	$57.5^{a}\pm0.3$	$351.0^{c}\pm0.6$
KATDL-2	$8.1^{a}\pm0.01$	$26.6^{c}\pm0.2$	$4.1^{ab}\pm0.27$	$2.6^{a}\pm0.15$	$58.6^{ab}\pm0.2$	$352.7^{c}\pm0.6$
KATDL-3	$9.8^{b}\pm0.2$	$24.9^{b}\pm0.5$	$4.4^{ab}\pm0.13$	$2.7^{a}\pm0.04$	$582^{ab}\pm0.6$	$345.4^{b}\pm0.5$
Fermented – Lablab tempeh						
KATDL-1	$10.11^{b}\pm0.05$	$30.65^{e}\pm0.3$	$3.59^{a}\pm0.1$	$1.62^{a}\pm0.2$	$54.03^{a}\pm0.1$	$339.8^{a}\pm1.3$
KATDL-2	$9.89^{a}\pm0.13$	$29.30^{d}\pm0.5$	$3.73^{ab}\pm0.4$	$1.63^{a}\pm0.2$	$55.45^{b}\pm0.2$	$341.0^{a}\pm1.8$
KATDL-3	$10.24^{b}\pm0.3$	$27.03^{b}\pm0.6$	$3.89^{ab}\pm0.3$	$1.83^{ab}\pm0.1$	$57.01^{c}\pm0.7$	$341.1^{a}\pm1.2$

Superscripts Values are means ± SEM of proximate data
Different superscripts within a column indicate significant differences (p =0.05)
Carbohydrates calculated by difference (100- (crude proteins+ ash and fats)
Kcal/g calculated by Atwater specific factors (proteins *3.47, fats *8.37 CHOs* 4.07)

4.6.1.2. Protein digestibility of Lablab tempeh

The lablab *tempeh* was evaluated for its invitro digestibility of protein to understand how fermentation affected digestibility. The fermented tempeh product was shown to have better digestibility as compared to raw and cooked beans as indicated in table 4.22. The digestibility significantly (p= 0.05) increased by 60% and 63% for KAT/DL-1 and KAT/DL-3, respectively, when the beans were subjected to fermentation.

Table 4.22: In-vitro digestibility of protein of raw beans, cooked beans and lablab *tempeh*

Variety	Raw	Cooked	Fermented (tempeh)
KAT/DL-1	54.57^{a} ±0.7	76.14^{b}±1.22	87.2^{c} ±0.84
KAT/DL-2	48.18^{a}±1.7	76.00^{b}±1.14	82.7^{c}±2.85
KAT/DL-3	51.39^{a}±0.5	76.18^{b}±2.82	84.2^{c}±1.60

Different superscripts within a column indicate significant differences P = 0.05

4.6.1.3. Oligosaccharide content

The fermented lablab product was analysed for stachyose and raffinose oligosaccharides.

There was significant decrease in this flatulence causing oligosaccharide content in the fermented Lablab *tempeh* (p=0.05). The stachyose content reduced by a range of 3.5 g/100g in the raw beans to less than 0.5g/100g in all the Lablab tempeh samples (figure 4.11). in the raw beans. The raffinose level reduced from a range of 1.5 – 2.0 g/100g to less than 0.5 g/100 g in all the fermented samples (figure 4.12).

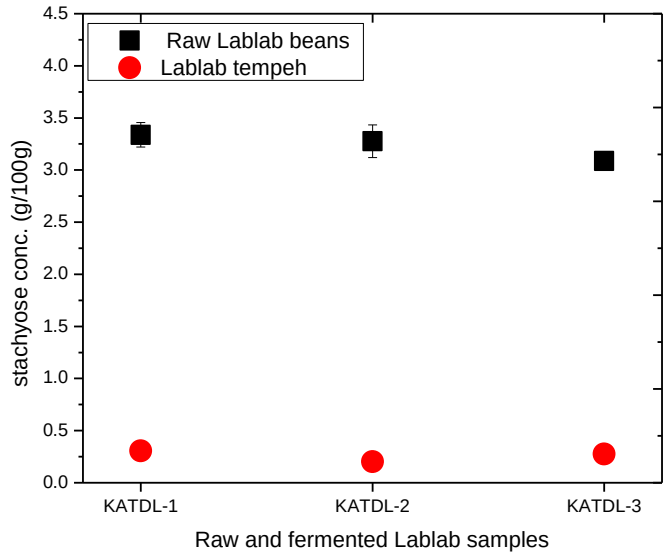

Figure 4.11: Stachyose content in raw and fermented lablab beans

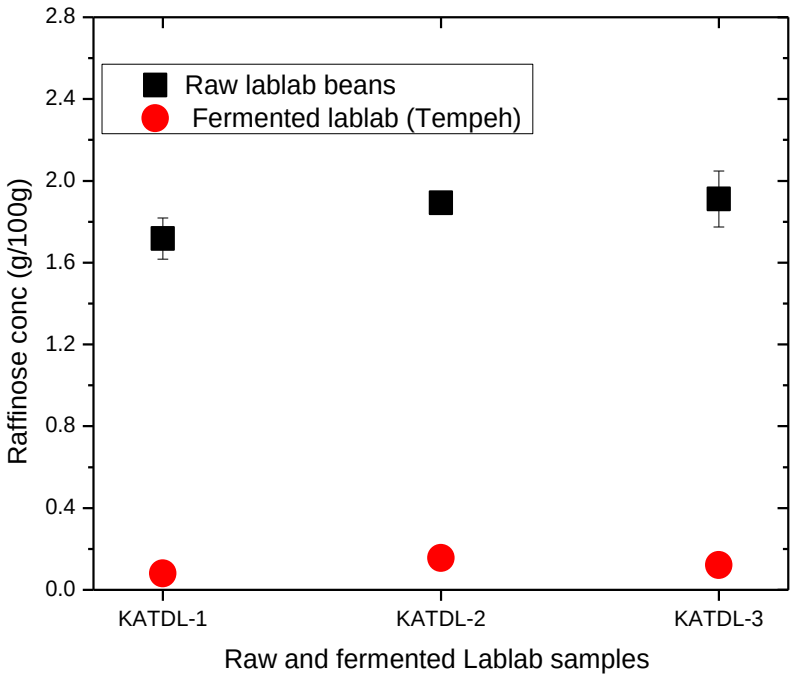

Figure 4.12: Raffinose content in raw and fermented lablab beans

4.6.2. Microbial quality of Lablab tempeh

Results on microbial analysis in table 4.23 indicate non detectable level of bacteria in the tempeh product while moulds were present since the product had been inoculated with Rhizopus mould. The number of moulds reduced with increased dilution factor.

Table 4.23: Microbial analysis of Lablab *tempeh*

Lablab Tempeh	Bacteria Total plate count (cfu/g) x 10^2	**Yeast and mould count (cfu/g) x 10^1**		
	Dilution factors	10^{-1}	10^{-2}	10^{-3}
KAT/ DL-1	N. D	305	110	75
KAT/DL-2	N. D	300	110	70
KAT/DL-3	N. D	340	125	85

N.D – Not detected

4.6.3. Sensory characteristics of Lablab tempeh

The Lablab tempeh from the three Lablab bean varieties was subjected to sensory evaluation. The samples were evaluated for flavour, texture, taste and overall acceptability using a hedonic scale. The samples did not have significant ($P \leq 0.05$) difference in texture. However, there was a significantly ($P \leq 0.05$) higher preference for the taste and overall acceptable of KAT/DL-1 Tempeh in comparison to the *tempeh* from the other two lablab varieties (figure 4.13).

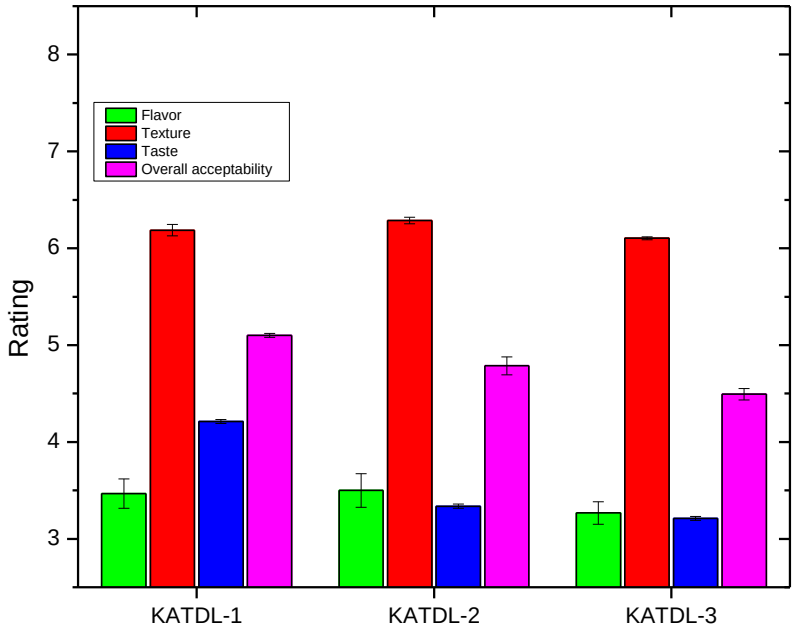

Figure 4.13: Sensory attributes of tempeh from three varieties of Lablab beans

CHAPTER FIVE

DISCUSSION

5.1. Physical Characteristics of Lablab and Rosecoco beans

Legume seeds are structurally similar but they differ in physical characteristics including seed size, shape colour, thickness and height, seed swelling and hydration coefficient (Mortuza & Tzena, 2009). These physical characteristics are important in various ways, including in mechanisation of seed processing (Giczewska & Borowska, 2003). The dimensions of legumes also vary widely with growing location, season and variety. The seeds in this study differed significantly (p=0.5) in their seed weight, length and width as indicated in Table 4.1. The *Phaseolus vulgaris* variety (Rosecoco) used for comparison had significantly higher seed weight, length, hydration and swelling coefficient in comparison to the lablab seed varieties. Other studies on Lablab physical properties have reported similar results (Deka & Sarkar 1990;Subagio 2006) for the seed width and length of lablab seeds, but Subagio found lower lablab seed thickness (4.0 mm) than what was observed in this study.

The hydration and swelling coefficients in beans are important in processing. Mortuza and Tzen (2009) observed higher hydration (199.9) and swelling coefficients (220.8) than those observed for the lablab beans in this study. The results were comparable to those for the Rosecoco variety in this study. The hydration processes occur before or during cooking aiding the seeds to soften and starch to gelatinize, the two characteristics that define a cooked seed. According to Mortuza and Tzen (2009), hydration and swelling capacities reflect the ability of the seeds to imbibe water in a reasonable soaking time. Swelling and hydration therefore act as indicators of the amount of water taken to cook the beans.

The seed weight and volume give indication of the space the beans occupy as well as their bulkiness. Since the dimensions and seed weight of the four bean cultivars were

significantly different, equal quantities of each variety will occupy varying space (Hamid *et al.*, 2014). The seed bulkiness also determines the number of seeds for a given weight (kilogram) hence KAT/DL-1 will fetch more in terms of cost compared to KAT/DL-3. The seed dimensions will also influence the cost of packaging and transportation if based on space occupied (Hamid et al., 2014). The seed dimensions also act as a guide for the design of relevant machines and facilities for handling and processing of the beans (Rahman, 2014). The size and shape are important in designing of separating, harvesting, sizing and grinding machines (Altuntaş, Özgöz, & Taşer, 2005). Dehulling of the seeds for reduction of antinutrients or for fermentation like in *tempeh* can be mechanised with the understanding of physical characteristics (Giczewska & Borowska, 2003).

Bulk density and porosity affect the structural loads of the legumes. The porosity of the legumes which is the percentage of airspace affects the resistance to airflow through bulk solids. This in turn affects the performance of systems designed for forced convection drying of bulk solids and aeration systems used to control the temperature of the solids (Hazbavi *et a*l., 2015). Thus the porosity of legumes affects artificial drying. (Kenghe *et al*., 2013). It is also important in grain storage as it can be used to determine the filling method in case of bulk storage (Sahin & Servet, 2007).

The sphericity of the Lablab beans is important in separation or bean cleaning (Sahin & Sumnu, 2006). Sphericity is an expression of a shape of a solid relative to that of a sphere of the same volume (Jideani, Wyk, & Cruywagen, 2009). It was found that sphericity was significantly different among the Lablab bean varieties, with KAT-DL-3 having the highest sphericity. This implies that this particular variety would roll more rapidly in a spiral separator since it has a better regular shape than the other two lablab varieties. The lablab bean varieties also had better sphericity than the *Rosecoco* variety. It is thus easier to clean or separate Lablab seeds in comparison to the *Rosecoco* beans as they have better sphericity (Sahin & Servet, 2007).

Most of the physiological and chemical changes in beans occur during soaking with moisture content increasing from around 15% to about 55% (Perlas & Gibson, 2002). The increase in weight and seed dimensions is due to the hydration of the beans. Increasing the temperatures of the soaking water can also enhance the seed weight and seed dimension (Shafaei, Masoumi, & Roshan, 2016; Turhan, Sayar, & Gunasekaran, 2002). Understanding the hydration capacity and its effects on the seed dimensions could be useful in canning the Lablab beans (Mannam, 2013). The hydration and absorption capacities of legume grains can be enhanced through thermal treatment and blending with cereal (Fasoyiro *et al*., 2010; Nawaz *et al*., 2015; Walle & Moges, 2017). The thermal treatment increases the water due to starch gelatinization and protein denaturation. Blending with cereal grain results in increased polar amino acids that have more affinity for water molecules leading to higher water absorption capacity (Gamel *et al.,* 2006).

Water absorption during soaking was observed to increase with soaking time. There was significant increase in absorption rate with time for all the lablab beans though for the rosecoco bean absorption remained constant after 12 h of soaking. This is similar to observations made by Marques Corrêa *et al.* (2010) for some common bean cultivars who reported that water absorption increased with soaking time though the varieties presented distinct behaviour patterns in this respect. The Lablab beans did not reach a constant absorption similar to other studies for hard to cook common beans and black bean varieties (Marques Corrêa *et al*., 2010). Absorption rates could be affected by the characteristics of the grain tegument or viral matrix of the bean which include thickness, weight, adherence to cotyledons, elasticity, porosity and colloidal properties in water absorption by beans (Wani *et al*., 2014). Other factors may include the genotype and environmental conditions that the beans are subjected to during development.

5.2. Proximate and mineral composition

The three varieties of Lablab beans had significantly higher amounts of protein and energy than the Rosecoco bean. However, there was no significant difference in the amount of ash and fat content among the four bean varieties. Similar studies from other parts of the world have shown more or less the same results (Chau *et al*., 1998; D'souza, 2013; Gouveia *et al*., 2014). Osman (2007) found 26% protein content in raw Lablab beans in Saudi Arabia. However, (Kotue *et al*., 2018) found protein content of 29% in Black turtle bean (Phaseolus vulgaris L.) of Cameroon. Kalpanadevi and Mohan (2013) reported higher levels of crude fat (5.6%) but lower protein content (20%) in brown Lablab beans obtained from Tamil Nadu- Asia. Mortuza and Tzen (2009) reported protein content of 29% and 3.8% fat content in Lablab cultivars grown in Bangladesh. Similarly Shaahu, Carew, and Ikurior, (2015) reported higher protein and fat levels in the Highworth variety of Lablab purpureus seeds. Hossain, Ahmed, Bhowmick, Mamun, and Hashimoto, (2016) found similar results for proteins (24%) for a black variety of Lablab beans though with lower fat content (1%) unlike for this study (2.6%). Similar results to those of this study were also reported by Hossain *et al*., (2016) for fats , carbohydrates and ash. The high level of protein in the lablab beans is often an important source of this vital nutrient among communities of low socio-economic status who may not afford animal sources of protein.

The Lablab seeds exhibited high carbohydrate and energy content comparable to those of the common bean *Phaseolus vulgaris* (*Rosecoco*) variety. Hence when used instead of the common bean, it will supply similar amounts of energy (Asif *et al.*, 2013; Nakitto, Muyonga, & Nakimbugwe, 2015; Nazni & Devi, 2016;Romero-Arenas *et al*. 2013;Sánchez-Chino *et al*. 2015).

The carbohydrates in legume grains have also been reported to have anti-diabetic properties (Reddy *et al*. 2013) and hence Lablab beans have the potential to be used in therapeutic diets for diabetes patients. This is attributed to their low post prandial glucose response and slow starch digestibility (Hedges & Lister, 2008).

The lablab and rosecoco beans were found to contain considerable amounts of minerals. These included zinc, iron, manganese, copper, and macro minerals like calcium, magnesium and potassium. Lablab had significantly higher amounts of manganese and copper compared to rosecoco (common bean). The levels of magnesium, calcium, potassium and iron for this particular studies generally low compared to results of Kamatchi *et al*. (2010). However the zinc and copper values compared to Kamatchi *et al*. (2010) values of 2.6mg/100g and 1.6mg/100g respectively. The iron and copper content in this study compared (5.6 and 1.2mg/100g respectively) with the results of Shaahu *et a*l.(2015) for Rongai white and Highworth lablab varieties. The zinc, iron and manganese levels also compared to results common bean varieties evaluated by Gouveia et al. (2014). The iron (Fe) content of the beans in this study were comparable to those observed by Deka and Sarkar (1990) and Abdul *et al*.(2017). The mineral content obtained for the beans would meet the dietary requirements of a population (NRC, 1989) especially if consumed with other foods that enhance mineral absorption (Felix *et al*., 2001; Kaur, 2016; Ademola & Abioye, 2017).The values are adequate for the recommended dietary allowance of 10- 15 mg for children, 12 mg for men and 18 mg for women per day (USDA, 2013; Jáuregui-Lobera, 2014).

5.3. Fatty acid profile of lablab and rosecoco beans

Though the overall fat content of the Lablab bean was low, as is the case with most of the other pulses, the concentration of polyunsaturated fatty acids (PUFA) was high accounting for approximately 70% of the total fatty acids. It implies therefore that consumption of lablab beans contributes to increased intake of PUFA. The results agree with documented literature that polyunsaturated and mono-unsaturated fatty acids are the dominant fatty acids in beans accounting for up to 87% of the total lipids (Deshpande, 2003). These act as precursors to eicosapentaenoic (EPA), docosahexaenoic acid (DHA, C22:6, ω-3) and arachidonic acid (AA, C20:4, ω-3) (Hossain et al., 2016). The derivatives of these highly unsaturated fatty acids referred to as lipid mediators play important physiological roles, including anti- inflammation in

the human body (Bennett and Gilroy, 2016; Hashimoto *et al*., 2009). These results are consistent with other studies (USDA, 2015) where it was reported that beans contain the essential PUFA, linoleic and linolenic acid (55%.; 12%).

5.4. Protein digestibility of Lablab and Rosecoco beans

The three Lablab beans showed relatively higher protein digestibility compared to the common bean variety, Rosecoco. These results are similar to other findings, including those of Murphy and Colucci (1999), who reported protein digestibility of 80% in lablab beans. The digestibility was also higher than that of other legume grain varieties - *Vigna subterranean, Phaseolus coccineus,* and *Phaseolus lunatus* reported by Aremu, Ibrahim, and Ekanem (2016). Similarly, Rehman and Shah (2005) observed lower protein digestibility (36-42%) values for chick peas, lentils and red and white kidney beans. In their study, Omer, Mohamed, and Abdalla (2007) reported 48.1% and 58% protein digestibility for brown and white seed cultivars of Lablab, respectively, in Khartoum, Sudan. Luo and Xie (2013) reported higher digestibility (73%), even though the tannin content in their Faba bean samples was also considerably high (0.56g/100g), suggesting other factors could be responsible for protein digestibility of legumes. In Pigeon pea (*Cajanus cajan*) varieties, protein digestibility has been found to range between 35-47% (Duhan, Khetarpaul ,& Bishnoi, 2000). Other than the anti-nutrients, factors such as cell wall rigidity and fibre content may influence the protein digestibility of legumes while high soluble dietary fibre and low insoluble dietary fibre are likely to improve the invitro protein digestibility (Duhan, et al., (2000).

5.5. Starch composition and oligosaccharides in Lablab beans and Rosecoco

Legume grains are generally considered to contain starch with lower digestibility compared to cereal starches (Tharanathan & Mahadevamma, 2003). Starch can be classified as total dietary fibre, resistant starch, soluble fibre, insoluble fibre and digestible starch. Resistant starch (RS) is a form of starch that cannot be digested in the small intestines. In the current study the Lablab bean samples had resistant starch and digestible starch of below 40% for all the four bean samples. The resistant starch in

the lablab beans makes them suitable in managing blood glucose due to their slow release.

High levels of oligosaccharides has been reported to account for reduced consumption of pulses, due to the flatulence associated with them (Kannan *et al* 2018) . Tsung *et al* (1988) reported that oligosaccharides are found in most of the plant seeds in varying concentrations except in sesame and castor beans. The results in the current study showed presence of stachyose and raffinose forms of oligosaccharides in Lablab and *Rosecoco* beans while verbascose levels were below detection limits. These oligosaccharides could limit utilisation of the beans thus reduction or elimination methods are required to improve utilisation.

Processing methods like soaking, germination and cooking may lead to significant reduction of oligosaccharides in legumes as reported by Shimelis and Rakshit, (2007) and Fernandes *et al* (2010). Other methods, including molecular approaches have also applied to reduce oligosaccharide levels (Kannan *et al*., 2018).

5.6. Anti-nutrient and bioactive components in Lablab beans

5.6.1. Anti-nutrient in lablab and rosecoco beans

Low nutritive value of the grain legumes has been associated with presence of some antinutritional substances which include tannins, phytates and trypsin inhibitors. Common beans and Lablab beans have been found to have relatively high quantities of anti-nutrients, especially tannins and phytic acid (Deka & Sarkar 1990;Wang *et al*. 2010). These were found to be present in varying concentration in the lablab beans in this study. In comparison, the *Rosecoco* beans had significantly higher tannin content and trypsin inhibitor activity than the Lablab beans. There was no significant difference in the trypsin inhibitory activity among the three Lablab bean varieties. Studies have shown that legumes contain varying amounts of tannins, phytates and trypsin inhibitors. The phytates or phytic acid in this study ranged from 533 mg/100g (KAT/DL-3) to 723 mg/100 g (KAT/DL-2). These amounts compare to the levels (0.6 to 2.1%) documented

in literature (Deshpande, 2003). The phytic acid levels in this study were lower compared to 1.2% and 2.1% obtained for Highworth and Rongai lablab varieties by Shaahu, et al. (2015). Phytic acid reduces the bioavailability of some essential minerals (Rehman & Shah, 2005) with most affected minerals being calcium, iron and zinc. Tannins on the other hand inhibit the digestibility of protein by forming protein cross-links (Rehman & Shah, 2005). The tannin levels in this study were lower compared to Shaahu, et al.(2015) findings (1.95;1.75g/100g) for Highworth and Rongai lablab varieties. Tannins in rosecoco beans were significantly ($p<0.05$) higher compared to lablab beans. This may contribute to colour differences, as argued by Deshpande (2003) who contends that tannins content vary with the colour of the seed coat. Some studies have found a strong correlation between easy to cook beans and high tannin content (Parmar, Singh, Kaur, & Thakur, 2017; Stanley, 1992).

Lablab and *Phaseolus* beans contain trypsin inhibitors. Many of these inhibitors also inhibit chymotrypsin. The trypsin inhibitors are grouped in to two; the kunitz and the bowman birk inhibitors where the latter is widely distributed in legume seeds (Savage & Morrison, 2003). Levels of trypsin inhibitor activity (TIA) were significantly higher in common bean rosecoco as compared to lablab beans (22 TIU/mg). The TIA content for lablab in the present study were lower than (38 TIU/mg) those found by Shaahu, et al.(2015) for the Highworth and Rongai lablab varieties.

Like tannins, flavonoids belong to the family of polyphenolics. They are bioactive components (phytochemicals) of the beans (Yao *et al*., 2004) . They are widely distributed in most of the legumes. The results in this study of the levels of flavonoids in the beans are similar to those reported for Faba beans (Baginsky *et a*l., 2013). They are also comparable to those of soy beans (1200mg/100g) as reported by Uwem, Babafemi, & Sunday (2017). However, the flavonoid content in this study were higher than those obtained for *Delonix regia* seeds (3mg/100g) (Oyegbile, Yola, & Abdullahi, 2017) . Flavonoids have been found useful in prevention of various diseases affecting the heart, brain, and other disorders, including those leading to cancer (Benouis, 2017).

Flavonoids and phenolic acids also have antioxidant properties. Genistein, a flavonoid found in lablab beans may lead to increased activity of antioxidant enzymes including superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase (Morris, 2009).

5.6.2. Anti-oxidant activity and flavonoids in Lablab beans

The results in this study showed that the Lablab beans had some anti-oxidant activity Reyes-Bastidas *et al.*(2010) reported similar (19% inhibition) results for common beans while Garretson, Tyl and Marti (2018) found DPPH of 70% in pinto beans. There is growing evidence of the role of antioxidants in prevention of coronary heart disease (Heart Protection Study Collaborative Group, 2002). A study on phenolic compounds in Lablab indicated a hypocholesteroleamic effect on rats (Vadde Ramakrishna & Rani, 2006). Lablab beans have also been reported to have beneficial cytotoxic effect (Habib *et al*, 2012) and (Al-Snafi, 2017b). Moreover, a review (Al-Snafi, 2017a) shows that the antioxidant components in lablab beans contribute to inhibition of chronic inflammation (cycooxygenase-2 suppression) which is thought to play a role in tumour development and promotes a healthier immune function.

5.7. Functional properties of lablab and rosecoco bean proteins

5.7.1. Overview functional properties of bean protein

Functional properties reflect the complex interaction between the composition, structure, molecular conformation and physico-chemical properties of food components and the nature of environment in which these properties are measured (Siddiq et al., 2009). Proteins are the basic functional components of various high protein processed food products and thus determine textural, sensory and nutritional properties (Zayas, 1997). There are three main groups of protein solubility. That is (i) properties related with hydration (water and oil absorption, solubility), (ii) properties related with the protein structure and rheological characteristics (viscosity, elasticity, gelation), and (iii) properties related with the protein surface characteristics (emulsifying, foaming). Ojukwu, Olawuni and Iwouno (2012).

5.7.2. Protein solubility of lablab and rosecoco protein isolate

The solubility of a protein is the most important functional property since the protein needs to be soluble in order to be applicable in food systems. It is considered as that proportion of nitrogen in a protein product which is in the soluble state under specific conditions (Zayas, 1997). The protein solubility of the lablab beans was (85%) at pH 10. The protein solubility of the Lablab protein isolates was found to be pH dependent (Shaw *et al*., 2001), and it was found to be at its lowest point at pH 4. This could be related to the isoelectric point of proteins which has been found to be between pH 4 and pH 5(Shaw *et al*., 2001). Normally at the isoelectric point, proteins have a net zero charge, attractive forces predominate, and molecules tend to associate, resulting in insolubility (Zayas, 1997). Similar observations have been presented by Adebowale, Adeyemi, and Oshodi, (2005) for Mucuna beans. The isoelectric point is the intermediate pH where the amino acid is evenly balanced between the two forms, as the dipolar zwitterion with a net charge of zero. This isoelectric point is dependent on the amino acid structure (Trevino, Scholtz & Pace, 2007). Therefore, the prevalent charge on the constituent amino acids of proteins at various pH values determines protein solubility. In order for the lablab bean protein to be incorporated into the food systems, alkali treatment would be necessary to improve the solubility.

5.7.3. Water and oil absorption capacity (WAC and OAC)

Water and oil absorption properties can be termed as hydration properties. It is the ability of the food protein to hold its own and added water during the application of forces, pressing, centrifugation, or heating (Zayas, 1997). Since proteins have both hydrophilic and hydrophobic sites, they have ability to interact with both water and oil in foods. Protein isolates of the lablab and rosecoco beans in this study showed water absorption capacity of 5-6%. This is an indication that the protein isolates of all the four bean varieties can retain sensory properties when the bean proteins are incorporated in functional foods. The results in this study are comparable to those obtained for Lablab samples in other studies of Mortuza and Tzen (2009) who reported WAC of 5% and

OAC of 4%. Borijindakul and Phimolsiripol (2013), on the other hand obtained WAC and OAC values of 6.4% and 6.2% for Lablab protein isolates. The values attained were higher in comparison to those obtained for Mucuna bean flours (Adebowale et al., 2005). Ojo and Ade-omowaye, (2015) reported higher values (7-9%) of oil absorption capacities for legume proteins *Canavalia ensiformis*, *Vigna racemosa* and *Sphenostylis sterocarpa*. Water and Oil absorption capacity plays an important role for they act as flavour retainer and improves the mouthfeel of foods Fasoyiro *et al*., 2010). These properties also facilitate reduction of moisture and fat losses of extended meat products (Mohammed, Mathew, & Samaila, 2016) . The results show that Lablab has similar flavour-retaining ability to conventional legumes due to the capacity of oil absorption

5.7.4. Gelling properties of Lablab and rosecoco bean protein isolates

The capacity of protein to form three dimensional network (gels) and provide a structural matrix that has ability to hold water, flavours and other ingredients is useful in new foodstuff development (Mortuza & Tzen, 2009). The results showed that the lablab protein had gelling capacities of between 18 and 20%. Proteins with good gelling properties have gelling capacities of between 0.5% to 25%. Similar results (14-18%) were observed by Habib *et al* (2017) for the Lablab Highworth variety. These values are also similar to those reported for Mucuna bean (14 to 20%) by Adebowale *et al* (2005), cereal-legume flour blends (Nawaz *et al*., 2015) and cashew nut (Ogunwolu *et al.*, 2009).

Protein gelation is a key functional property in modification of the structure and texture of foods. Gelation occurs when the functional groups within the protein are exposed causing group interaction and a three-dimensional network. Gels are characterized by a relatively high viscosity, plasticity and elasticity (Mohammed *et al*., 2016). Variation in gelling properties has been associated with the ratio of different constituents such as protein, lipids and carbohydrates in different legumes (Gamel *et al*., 2006). A direct correlation has been suggested to exist between gelation capacity and the level of globulins in legume seeds Fernandez-Quintela *et al*., 1997). Moreover, Raikos *et*

al.(2007) explained the formation of a gel as being dependent on the protein concentration, amount of water present, ionic strength, temperature and the pH. Modification of protein hydrogen bonding though not evaluated in this study has been proposed to increase the gelation strength. This can be achieved through addition of salts or optimising the temperature and protein particle size (Osen et al., 2014).

5.7.5. Foaming capacity and stability

Foam stability is important because the usefulness of whipping agents depends on their ability to maintain the whip as long as possible. The Lablab bean flours tested in this study exhibited relatively good foaming capacities and foam stability of 50% and 60 %, respectively. Mortuza and Tzen (2009) reported foaming capacity of 43-63 and stability of 37-64% in Lablab beans while Chau *et al* (1997) reported 60% foam stability for lablab beans . Similar values have been obtained for other legumes; 60.5% for cashew nuts by Ogunwolu et al.(2009) and 37-63% for chickpea (Maqbool, Naik, & Hussain, 2017). On the other hand (Dobhal & Raghuvanshi, 2018) reported higher values of foam stability but low foaming capacity (95% and 14%, respectively). Similarly Habib et al.(2017) observed low foaming capacity (27%) and high foam stability (96) in Highworth Lablab samples.

Foaming capacity and stability are also pH dependent (Lawal *et al*., 2005). This is because foaming is a surface-active function of protein and depends on the isoelectric points of the amino acids present in the protein. A good foaming capacity has been linked to protein flexibility with reduced surface tension and high protein concentration (Yuliana *et al*., 2014). Lawal *et al* (2005) further reported the occurrence of protein denaturation that results from the whipping process aiding in foam formation through unfolding of protein molecules. Foaming capacity and foam stability are important qualities in food products such as breads, cakes, crackers, meringues, ice creams and several other bakery items as they help maintain their texture and structure during or after processing.

5.7.6. Emulsion capacity and emulsion stability

Emulsifying properties are an expression of the interfacial area stabilized per unit weight of protein, which characterises the ability of a protein to absorb both water and oil or have an oil−water interface. Noticeable from the study, emulsion capacity was lowest (40-55%) at pH 4 and highest (70-83%) at pH 10 for all the four samples investigated. There was significant difference in emulsion capacity, as KAT/DL-3 had better emulsion capacity compared to other bean samples. Similar studies on Lablab beans have shown emulsion activity and stability 57% and 64% (Borijindakul & Phimolsiripol, 2013) and 49% and 85% (. Habib *et al*., 2017) respectively. Studies carried out on other legumes have reported similar emulsion activity values of 120 for *Caragana bean* (Zhong e*t al*., 2012), 75% in African yam bean (. Lawal et al., 2005) and 61% in Pigeon pea (Ohizua *et al*., 2017). The differences among the emulsion activities and emulsion stabilities are related to the protein content (soluble and insoluble) and other components, such as starch, fat, and sterol content, of the legume flour (Oyewole, Abu, & Enujiugha, 2017) . Protein solubility and emulsification properties are a function of protein–water interactions occurring in the polar amino acid regions of protein molecules (Chau *et al*., 1997). Most proteins contain several polar side chains with peptides on the parent chains, making them hydrophobic.

5.8. Cooking and sensory characteristics of the Lablab beans

5.8.1. Cooking characteristics

Cooking time is one of the main considerations used for evaluating pulse cooking quality. Longer cooking times result in a loss of nutrients and higher expenses on cooking fuel. Hence, consideration of cooking time is paramount in determining the energy cost for preparation of meals. The time taken to cook legumes is affected by the permeability of seed coat and cotyledons to hot water.

All the lablab bean varieties had been adequately cooked after three hours (180 minutes). The common bean (Rosecoco) variety took a shorter time to cook compared to the Lablab beans. Among the lablab bean varieties, KATDL-3 cooked in comparable

minutes to the common bean. The results are similar to those reported by Shivachi *et al*., (2012) who reported a range of cooking time of 70 to 197 minutes for lablab beans and Mortuza and Tzen (2009) where nine different varieties of Lablab beans cooked between 114-179 minutes. Shivachi *et al*., (2012) noted that the black/ dark coloured variety took the longest time to cook which agrees with the results in this study for the black variety KAT/DL-2. Similar observations were recorded for black seeded pigeon pea by Fasoyiro *et al*. (2005). The *Rosecoco* bean whose cooking time is comparable to KAT/DL-3 has been classified as easy to cook bean due to the shorter cooking time (Kinyanjui, 2016).

Different factors have been associated with variation in bean cooking time. Such factors include the growing conditions, length and temperature of storage, chemical composition, bean microstructure (seed coat and cotyledon cell walls) and thickness of the palisade layer (Emire, 2006). In this study the lengthy cooking time observed in some varieties could be attributed to their genetic makeup since the experiment was carried out under constant conditions (Bitjoka, Teguia & Mbofung, 2008). The long cooking time of most dark seeded varieties could also be attributed to high anti-nutritive levels in their seed coats. Pengelly and Maass (2001) related lablab seed colour to anti-nutritive levels, and found dark seeded types to contain higher amounts of these substances than white seeded genotypes. Fasoyiro *et al*, (2005) linked prolonged cooking time to the high amounts of energy required to eliminate the anti-nutrients in dark varieties.

The results indicated a significant reduction in cooking time upon soaking of the beans. Soaking has been associated with uniform expansion of seed coat and cotyledon, cooking and bean tenderness. The rate of hydration of beans determines their cookability and that the hydration and swelling capacities of the soaked beans would be related to required cooking time. Faster hydration and higher hydration capacity of pulse grains is associated with shorter cooking time (Urga *et al.,* 2006).

5.8.2. Sensory characteristics of Lablab beans

There were significant differences among the cooked bean varieties in the taste, texture and overall acceptability. However, there was no significant difference in the ranking of the appearance of the samples. The sensory quality traits of appearance, texture and taste greatly affect consumers' choice for Lablab genotypes. Previous findings have alluded to white and cream white colours being more preferred to black, citing anti-nutritional factors and bitter taste as reasons for low acceptance (Pengelly & Maass, 2001).

The results on ranking of the sensory attributes show a higher general acceptance for KAT/DL-1 and KAT/DL-3 as indicated by their texture and taste preference. The results therefore are in agreement with Pengelly and Maass, (2001) where genotypes of lablab were less preferred in respect to taste and texture. Similar findings were reported by Shivachi *et al*. (2012) for various accessions of Lablab beans and pigeon peas (Fasoyiro *et al*., 2010).

5.9. The effect of processing methods on nutritional quality and anti-nutritional factors of Lablab (*Lablab purpureus*) beans

5.9.1 Processing methods for beans

Different methods of processing have been used on legumes to enhance their palatability and nutritional quality. Common methods that have been employed for various legumes include thermal processing (Alagbaoso *et al*., 2016; Wang *et al*., 2010) fermentation (Ayemhenre & Ignatius, 2015; Olanipekun, Otunola, & Oyalade, 2015) soaking, and germination (Duhan *et al*., 2000; Smith, 2015; Soetan & Oyewole, 2009). In the current study, thermal treatment (boiling), soaking and germination were applied to investigate their effects on nutrients and anti-nutrients factors in the Lablab beans in comparison to common bean *(Rosecoco*).

5.9.2 Effect of processing on anti-nutrients

One of the limiting factors for legume grain utilization is the presence of anti-nutrients such as phytic acid, tannins and trypsin inhibitors. These anti-nutrients were present in

both lablab and rosecoco beans. Higher levels of tannins were observed in the dark coloured lablab (KAT/DL-2) and in the common bean (Rosecoco) varieties, while relatively lower amounts were in the cream- white KAT/DL-3 variety. Similar trends were also observed for phytic acid and trypsin inhibitor contents, where the darker variety had higher levels of these anti-nutrients.

Physical and chemical methods are normally employed to reduce or remove the anti-nutritional factors enhancing the nutritional value of the legume beans (Soetan & Oyewole, 2009). Soaking of the beans at varying hours significantly reduced the phytates variably. Reduction in phytates increasing with increasing number of soaking hours. This trend was also reported by Osman (2007) for lablab beans where a decrease of 22% was realised after overnight soaking. Upon germination, Osman (2007) realised 48% reduction in phytates while 38% was realised for the present study. Similar observations were for chickpea (Ghavidel & Prakash, 2007). The present study realised a 50% reduction in phytates upon cooking by boiling method which is comparable to Osman (2007). 24.0–35.1% phytic acid contents were reduced upon cooking by the ordinary boiling method of kidney beans and chickpea (Rehman & Shah, 2005). The three methods (boiling, soaking and germination) were therefore found to significantly reduce phytate content in the four bean varieties. Phytates in leguminous seeds are concentrated in the cotyledon (Konietzny, 2003). Germination leads to breakdown of stored phytates by the enzyme phytase hence the phytates reduction (Bello, Sunday, & Azubuine, 2017; Konietzny, 2003). Soaking on the other causes phytates reduction through loosening the water-soluble phytates (Shetty, 2010).

Inactivation of trypsin inhibitors increases the digestibility and nutrient availability of beans. Reduction in trypsin inhibitors was found to be highest among the cooked beans through boiling. A decrease of 81% -59% was achieved by boiling of the beans which is comparable to 66% TIA reduction by boiling reported for lablab beans by Osman (2007). Similar results on TIA reduction through boiling of lablab were reported by (Ramakrishna, Rani, & Rao, 2008). Other cooking methods such as autoclaving and

extrusion have been reported to fully inactivate TIA ((Savage & Morrison, 2003). According to Osman (2007), soaking had the least (6%) effect on TIA while germination achieved 19% reduction. However, soaking and germination the present study realised 27% and 24% decrease in TIA respectively.

The three methods of treatment, - soaking, cooking and germination - were found to significantly reduce tannin content in the lablab and rosecoco beans. Soaking was found to reduce the tannins by 11%. Cooking significantly (p=0.05) reduced the amounts of tannins from 0.23g/100g to 0.11g/100g (54% decrease). However unlike in this present study Ramakrishna *et al*. (2006) reported 75% reduction upon boiling of lablab beans varieties . Reduction in tannin through germination and cooking was also reported for common beans by Nakitto *et al.* (2015) for common beans and soybeans by Sharma *et al.* (2013). These results also agree with those obtained for Lablab beans by Osman (2007), jack beans (Doss *et a*l. 2011) and amaranth seeds (Kanensi *et al*., 2011) who obtained varied degree of reduction in tannins through germination and cooking. Germination, cooking and soaking can therefore produce beneficial effects on nutritional quality of lablab and common beans as observed in this study since the reduction of tannins reduces the formation of insoluble enzyme resistant complexes (Soetan & Oyewole, 2009). Therefore, soaking, cooking beans and germinating hold a good potential for improving the nutritional value of lablab bean by reduction in antinutritional factors such as trypsin inhibitors, tannins and phytates.

5.9.3 Effect of processing on bioactive components

Bioactive compounds have been reported to possess anti-inflammatory, antioxidant, antibacterial, antifungal, and antimalarial activities. Besides provision of nutrients, beans are sources of bioactive compounds essential for good health. Free radical-mediated oxidation could result in food quality deterioration and has been implicated in many human diseases and in ageing process (Egbuonu, 2017). Antioxidants in foods help to reduce the oxidative activity of free radicals. Flavonoids are among the bioactive components in foods with beneficial effects. In this study germination resulted in the

highest levels of flavonoids while soaking reduced the flavonoids. These findings are in agreement with those obtained for heirloom bean varieties (Garretson *et al*., 2018). Higher rates of water uptake during soaking have been linked to lower total phenolics content in the seed coat. Plant sourced flavonoids also act as potent antioxidants hence the beans were found to have some level of inhibition both lablab and common bean rosecoco which increased with increased concentration as observed in other studies for cowpeas (Hedges & Lister, 2008). The bioactive components and anti-oxidative activity in beans play role in enhancing the health benefits with positive effects on hepatoprotective and cytotoxicity as suggested in previous studies (Al-Snafi, 2017a; Foyer *et al*., 2016a; McCrory *et al*., 2010; Vadde *et al*., 2007; Verma *et al*., 2017).

5.10 Nutritional quality and sensory characteristics of lablab tempeh

5.10.1 Nutrition quality of Lablab tempeh

Tempeh is a general term used to describe collectively various legume/pulse grain based foods that have been subjected to fermentation using a fungus belonging to the genus *Rhizopus* (Shurtleff & Aoyagi, 2011). The primary objective of fermenting legumes may not be so much their preservation, but rather the modification of their organoleptic and nutritional properties. The Lablab beans when subjected to fermentation using *Rhizopus oligosporus*, *Rhizopus oryzae* resulted in a tempeh product of better nutrition value than the unfermented beans. From the proximate analysis, there was significant ($p = 0.05$) increase in protein content as compared to raw and cooked Lablab beans. However, the energy levels in kilocalories in the tempeh reduced. This could be attributed to the utilisation of carbohydrates and fats during fermentation. There was significant increase in protein content upon fermentation suggested to be as a result of utilisation of carbohydrates by the microorganisms causing production of carbon dioxide as a by- product which in turn would cause concentration of nitrogen (Onyango, Noetzold, Bley, & Henle, 2004). The protein and starch digestibility of the fermented lablab *tempeh* was found to be higher than the raw beans. These results are similar to those reported by Shanna and Ye (2014) who found out that the protein digestibility,

protein efficiency ratio and net protein utilization increased after tempeh fermentation. The improvement in the digestibility would be associated to the reduction of non-nutritive compounds that inhibit digestive enzymes (trypsin and chymotrypsin) and promote protein crosslinking (phenolics) (Çabuk *et al*., 2018). The production of microbial protease also degrade and release some of the proteins from the matrix (Çabuk *et al.*, 2018). The starch digestibility increases due to the loosening of the starch granules by the microbes making their active cites more accessible to the amylase enzymes (Onyango *et al*., 2004).

Further, there was also a significant decrease in oligosaccharide content of the fermented Lablab tempeh in comparison to the raw beans. This significant reduction in raffinose and stachyose flatulent causing sugars can be attributed to the enzymatic degradation action of *R. oryzae* (Wiesel *et al.*, 1997). The microorganisms *Rhizophus oligosporus*, the dominant fungus in tempeh together with *Rhizophus oryzae* and *Mucor* species are also responsible for the improved texture and flavour of the Tempeh. (Shanna & Ye, 2014). The results indicate that lablab *tempeh* is a better protein source compared to the raw and cooked lablab beans. Some literature have suggested that the tempeh is a better source of essential amino acids (Babu *et al* 2009) while others have likened it to have similar qualities of meat (Somishon & Thahira, 2013).

Consumption of *tempeh* in Indonesia has been linked to reduced risks of cancer, high blood pressure and digestive disorders and weight management (Liu, Han, & Zhou, 2011; Shanna & Ye, 2014).

5.10.2. Sensory characteristics of Lablab tempeh

One of the reasons of processing legumes is to improve the sensory characteristics besides the nutritional value. The *tempeh* products from the three Lablab bean varieties had no significant difference in the ranking of texture and flavour. However, KAT/DL-1 and KAT/DL-3 had significantly better taste and overall acceptability among the panellists than the other two bean varieties. The reasons for the preference was not clear though, as taste is due to various components in the food (Roland et al., 2017).

Normally as food undergoes fermentation process, there occurs a multitude of mainly catabolic microbial and enzymatic activities that results in the formation of various compounds that contribute to flavour and aroma of the fermented products. These attributes depend on the concentration of the compounds, their interactions and perceptions (Javier, 2012). Yeasts are well known for their contribution to sensory attributes. Their oxidative and fermentative metabolism of sugars results in carbon dioxide and water in the first case (oxidative) and to ethanol and CO_2 in the second fermentation. Yeast uses sugars and amino acids as substrates to produce a wide range of compounds contributing to the flavour of fermented foods (Javier, 2012).

The tempeh product can be fried or baked into cakes or patties enhancing their flavour and aroma. This product can be used as a main meal or as snack (Shanna & Ye, 2014) . There is great potential of increasing the utilization of lablab beans through development of novel lablab *tempeh* products in Kenya since the beans are available and the fermentation technology is accessible. Fermentation also reduces flatulence, improves digestibility hence improved utilisation.

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

There were significant differences in the seed dimensions of length, width and thickness among the three lablab bean varieties. KAT/DL-1 had a significantly higher seed weight compared to the other two Lablab varieties. The Rosecoco bean had significantly higher seed weight than all the lablab bean varieties. KAT/DL-3 had significantly higher hydration and swelling coefficients in comparison to the other two lablab varieties, though all the three varieties had a significantly lower hydration and swelling coefficients than the Rosecoco bean variety.

KAT/DL-1 had significantly higher protein levels than the other two Lablab varieties, all the lablab bean varieties had higher protein levels than the Rosecoco bean. The fat content was not significantly different in all the bean varieties. All the three lablab bean varieties had significantly higher energy levels than the Rosecoco bean.

The KAT/DL-1 lablab bean variety had significantly higher protein and starch digestibility than the other two lablab varieties. There was no significant difference in starch digestibiity between the lablab beans and the Rosecoco bean.

KAT/DL-2 lablab variety had significantly higher phytic acid and tannin levels than the other two lablab varieties. However, there was no significant difference in trypsin inhibitory activity among the three lablab varieties. Compared with the Rosecoco bean, the Rosecoco had significantly higher tannin levels and trypsin inhibitory activity than all the lablab bean.

KAT/DL-3 cooked faster compared to the other two Lablab varieties. However, the Rosecoco bean cooked faster than the three lablab bean varieties.

There was signficantly higher preference for the taste, texture and overall acceptability of KAT/DL-1 in comparison to the other lablab bean varieties.

Cooking, soaking and germination all caused significant reduction in the tannins, phytates and trypsin inhibitory activity in all the bean varieties.

The tempeh made from the lablab beans had higher protein digestibility and lower levels of oligosaccharides in comparison to the unfermented beans.

6.2 Recommendations

Communities in Kenya should be encouraged to increase the utilisation of Lablab beans since they are a good source of proteins with a good blend of amino acids and are rich in both macro and trace minerals.

Different methods of processing such as soaking, germination and cooking should be applied in lablab preparation since they reduce the antinutrients content and improve digestibility.

Breeding of Lablab beans should focus on easy to cook varieties in order to save on fuel and time. Beans that are low in phytic acid and tannins will also enhance in vitro-digestion. Breeding should also focus on Lablab beans that are low in oligosaccharides.

Fermentation of Lablab beans should be encouraged since it reduces flatulence and produces products that are of higher nutritional value as compared to the cooked lablab beans. These products can be baked or fried to enhance sensory attributes. The product is also ideal for people with gastritis issue who experience heart burns and bloating upon consumption of the cooked lablab beans.

Further research is required on other varieties of Lablab beans found in Kenya to determine the best varieties that could be utilised for maximum nutrition security. Further work should also be done in profiling the amino acid composition of lablab beans.

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APPENDICES

Appendix i: Questionnaire for sensory attributes and acceptability of the lablab and rosecoco beans

Name:_____ Date:_____

You are presented with four coded samples of cooked beans. Please give a score of between 1 to 5 for each parameter. The scores are explained in the table below.

1	2	3	4	5
Dislike extremely	Dislike moderately	Neither like nor	Like moderately	Like extremely

Please note:

1. An expectoration cup with a cover has been provided in case you do not wish to swallow the beans.
2. Rinse you mouth after each sample before tasting the next one.

Indicate Male/Female

PARAMETER	**SAMPLE CODE**			
	10001	**10002**	**10003**	**1005**
Appearance				
Texture				
Taste				
General acceptability				
Any other comment				

Thank you for your participation.

Appendix ii: ANOVA and Tukey test (HSD) procedure for sensory evaluation

Anova: Single Factor ***Sensory attribute – Appearance***

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
1001	*35*	*126*	*3.6*	*1.835294*
1002	*35*	*126*	*3.6*	*1.305882*
1005	*35*	*121*	*3.457143*	*1.078992*
1009	*35*	*125*	*3.571429*	*2.310924*

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	*0.485714*	*3*	*0.161905*	*0.099159*	*0.960359*	*2.671178*
Within Groups	*222.0571*	*136*	*1.632773*			
Total	*222.5429*	*139*				

ANOVA across the rows

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	*2927163*	*35*	*83633.21*	*42207.42*	*1.1E-142*	*1.58603*
Within Groups	*142.6667*	*72*	*1.981481*			
Total	*2927305*	*107*				

TUKEY TEST (HSD) PROCEDURE FOR SENSORY ANALYSIS

Tukey Test procedure for taste (P=0.05) and $\mathbf{Q}_{.05}$*=3.63*

	k = 4	*df for No. observations =136*	
comparison	*absolute difference*	*critical range*	*Results*
KAT/DL-1 to KAT/DL-2	*1.314285714*	*0.683916882*	*TRUE*
KAT/DL-1 to Rose coco	*0.971428571*	*0.683916882*	*TRUE*
KAT/DL-1 to KAT/DL-3	*0*	*0.683916882*	*FALSE*
KAT/DL-2 to Rose coco	*0.342857143*	*0.683916882*	*FALSE*
KAT/DL-2 to KAT/DL-3	*1.314285714*	*0.683916882*	*TRUE*
Rose coco to KAT/DL-3	*0.971428571*	*0.683916882*	*TRUE*

Tukey test procedure for texture (P=0.05) and $Q_{.05}$*=3.63*

	k = 4	*df for No. observations =136*	
Mean comparison	*Absolute difference*	*critical range*	*Results*
KAT/DL-1 to KAT/DL-2	*0.48571429*	*0.763484*	*FALSE*
KAT/DL-1 to Rose coco	*0.17142857*	*0.763484*	*FALSE*
KAT/DL-1 to KAT/DL-3	*0.4*	*0.763484*	*FALSE*
KAT/DL-2 to Rose coco	*0.65714286*	*0.763484*	*FALSE*
KAT/DL-2 to KAT/DL-3	*0.88571429*	*0.763484*	*TRUE*
Rose coco to KAT/DL-3	*0.22857143*	*0.763484*	*FALSE*

Tukey test procedure for general acceptability (P=0.05) and $\mathbf{Q}_{.05}$*=3.63*

	k = 4	*df for No. observations =136*	
Mean comparison	*Absolute difference*	*critical range*	*Results*
KAT/DL-1 to KAT/DL-2	*1.142857*	*0.642066*	*TRUE*
KAT/DL-1 to Rose coco	*1.142857*	*0.642066*	*TRUE*
KAT/DL-1 to KAT/DL-3	*0.114286*	*0.642066*	*FALSE*
KAT/DL-2 to Rose coco	*0.028571*	*0.642066*	*FALSE*
KAT/DL-2 to KAT/DL-3	*1*	*0.642066*	*TRUE*
Rose coco to KAT/DL-3	*1.028571*	*0.642066*	*TRUE*

k=set of means

df = Degrees of freedom

$\mathbf{Q}_{.05}$=3.63 = studentized range statistic Table (4:136)

Absolute difference = value difference of two means

Critical range =3.63 square root [mean variation within groups * number of panellists]

TRUE= There significant difference between means at P=0.05

FALSE= There is no significant difference between means at P=0.05.