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NUTRITIONAL, ANTI-NUTRITIONAL AND PHYSICO-CHEMICAL PROPERTIES OF DIFFERENT HARICOT BEAN VARIETIES ADAPTED UNDER SoRPARI's DIFFERENT RESEARCH CENTERS





Research team:

S.No	Name	Role
1	Abdulkarim Mohammed (Ass.Prof.)	Principal investigator
2	Mohammed Dol Ateye (MSc.)	Co-investigator
3	Hoodo Mohammed Jama (BSc.)	Co-investigator

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Signatures:

Abdulkarim Mohammed Ali (Ass.Prof.)

Principal Investigators

DG- SoRPARI

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EXECUTIVE SUMMARY

Haricot bean one the legume crop that widely consumed in Somali community. The study was conducted in Somali Regional state with objective to analyze macronutrient, micronutrient/minerals, antinutritional content, functional and physical of different haricot bean varieties adapted by Somali region pastoral and Agro-pastoral Research Institute. Six different types of samples such as *Awash mittin, Awash 2, Seri 119, Seri 125, Adi* and D Control as control with total of fifteen (15) samples were collected from three different research center namely Dolo-Ado, Fafan and Kelafo. The collected haricot samples were individually grounded to a fine powder (30 mesh size), packed in air tight polyethylene plastic bags and were stored at 4°C until further analysis. Proximate composition such as crude Protein, crude Fat, crude fiber, moisture content, carbohydrate, total energy, different minerals like Calcium, Iron, Zinc and Potassium were determined by AOAC. The Anti-Nutritional content such as tannins and phytate, functional and physical properties were also determined by different standard procedures. Based on the result obtained the moisture, ash, crude fiber, protein and fat are in the range of 7.915 (K-Seri 119) -11.533% (F-Adi), 4.218 (F-Awash-2) -5.606% (K-Seri 125), 2.230 (F-Seri 125) -4.480% (F-Awash - 2), 16.210 (K-Seri 125) -22.180% (D-Awash mittin) and 0.540 (D.Seri 125) – 2.250% (D-Awash -2) respectively and most of them are statistically different at $P<0.05$. The minerals content is also in the range of 65.96 (F-Adi) – 108.07mg/100g (D-control), 126.0 (D-Seri 119) – 152.0mg/100g (K-Seri 125), 3.092 (K-Awash 2) – 4.798mg/100g (D-Awash 2) and 4.306 (K-Seri 119)-6.571mg/100g (D-control) for calcium, potassium, zinc and iron respectively and more or less they were statistically different at $P<0.05$. Regarding the anti-nutritional content, the tannins and phytate content is in the range of 0.30 (F-Adi and F-Awash 2) - 34.95mg/g (D-Seri 119) and 2.193 (K-Seri 119) – 4.05mg/g (D-Seri 119) for tannins and phytate respectively and statistically different for tannins content at $P<0.05$. The functional and physical properties of different haricot bean varieties under study were more or less statistically different at $p<0.05$. Overall, from the finding we conclude that, adapted haricot bean varieties have better in nutritional content especially, ash and fiber and protein content especially varieties with D-Awash mittin. Varietal difference, agro-ecology, nature of soil, farming management and some other reason might be the factor for their different in their nutritional content. Haricot bean is reached in protein, minerals and other important nutrient and therefore, we recommend that, it can be used either alone or mix with those staple cereals as part of fighting against malnutrition and in the preparation of different recipe.

Key words: Haricot bean, macro-nutrient, minerals content, Anti-nutritional content, Functional Properties, Physical property

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Grain legumes occupy an important place in human nutrition, especially in the dietary pattern of low-income groups of people in developing countries. Legumes, considered as poor man's meat, are generally good sources of nutrients [1]. They are an important and inexpensive source of protein, dietary fiber and starch for a large part of the world's population, mainly in developing countries [2]. Among the commonly consumed food legumes, haricot beans (Phaseolus vulgaris L.) occupy an important place in human nutrition in East and Great Lakes regions of Africa [3]. Beans are also one of the best non-meat sources of iron, providing 23–30% of daily recommended levels from a single serving [4].

Ethiopia is known as the homeland of several crop plants. It is ranked 13th among pulse producing countries in the world [5]. The country produced about 1.3 million tons in 2014 [6]. Pulses play crucial economic, and food and nutrition security roles in Ethiopia. Recently, the production and supply of pulses, increased due to increased demand in both local and international markets, thus enhancing smallholders' income [5].

Haricot bean (Phaseolus vulgaris L.) has been an export pulse crop for Ethiopia for more than 50 years and probably been grown as food crop for a much longer period in the low and mid land altitude areas of the country [7]. The crop is widely grown in areas between 1400-2000 m.a.s.l. The main production areas include the East Hararghe, West Wellega, East shewa, West Arsi, Sidama, Wolayita, Wollo and East Gojam [8]. The crop is grown either as a sole crop and/or intercropped with either cereal or perennial crops [9]. There are a wide range of haricot bean types grown in Ethiopia, including the mottled, red, white and black varieties. The leading white bean varieties are the Awash 1, Awash melka and Mexican 142 varieties. The pure red and pure white colored beans are the most common commercial varieties [7]. Haricot bean is ranked as the second largest pulse crop in the country in terms of production with a share of 17 percent, next to Faba beans [10]. CSA 2014/15 report also shows that the production share of haricot bean has consistently been 19 percent of all the pulses for the last two years.

Common bean (*Phaseolus vulgaris L.*) is among one of the most important and well-known food legumes. It is the second most important the source of human dietary proteins and the third most important source of calories [11, 12].It is considered as “resource poor meat” and is important inexpensive sources of protein, dietary fiber, and starch. It contains almost two to three times more protein than cereals. Because of their high protein and lysine content, they also represent good sources of supplementary protein when added to cereal grains and root crops, which are low in essential amino acids. In addition to protein common beans are good source of dietary fiber, starch. Common Bean is rich in protein, carbohydrate, dietary fiber and is a good source of antioxidants, as well as vitamins and minerals [13, 14].

Animal proteins are better assimilated than vegetable proteins because plant proteins are deficient in sulphur amino acids (methionine and cysteine), and tryptophan. Therefore, a diet that combines protein-containing foods with complementary cereal amino acids is important to gain the complete amino acid pool [7, 8].Beans contain many bioactive substances that play important physiological roles *in vivo*, including, protein digestibility enzyme inhibitors, others types of enzyme inhibitors and fermentable non-digestible oligosaccharides [15].

In Ethiopia, Common bean (Phaseolus vulgaris L.) is the most important of major food legume which is used either as a source of protein for local consumption or as an export crop for generating foreign currency [16]. This crop was introduced to the northern parts of the country around the 16th century [17]. Common bean has a wide range of adaptation and its production is very heterogeneous in terms of ecology, cropping system and agronomic performance. It is one of the most important grain legumes grown in the low lands of Ethiopia particularly in the Central Rift Valley of Ethiopia. Ethiopian smallholder farmers grow white pea beans for export and food type-colored beans for house hold consumption. Common beans are important components of crop production in Ethiopia's smallholders’ agriculture, providing an economic advantage to smallholder farmers as an alternative source of protein, cash income, and food security. Common bean is often grown as cash crop by small scale farmers and used as a major food legume in many parts of the country where it is consumed in different types of traditional dishes [18].

1.2. Statement of the problem

Ethiopia is one of the poorest countries in the world with 85% of its population living in the rural parts of the country and employed in agriculture sector. The agriculture sector is very important in the Ethiopian economy that it accounts for roughly 46% of the country's Gross Domestic Product (GDP) and 90% of exports [19]. Agriculture is the core component and driver for Ethiopia's growth and long-term food security. Agriculture directly employs 80 percent of the total population, 43 percent of gross domestic product (GDP), and over 70 percent of export value [20]. Despite the considerable progress made in the sector, ensuring commercialized production remains one of the major challenges facing millions of people.

Haricot beans are a grain crop mainly produced in Ethiopia for home consumption and export purpose. It grows in most of the agro-ecology zones of low and mid altitude areas of the country. A market demand for the haricot beans both in the export market has become the main instrument for the growing trends in volume of production [21]. Haricot beans are among the most important grain legumes produced by small-scale farmers for both subsistence and cash, mainly in the lowlands and in the rift valley areas of Ethiopia. They are high in starch, protein, and dietary fiber, and are an excellent source of minerals and vitamins.

According to [22] world market prices for agricultural goods have been increasing. The reason for the increasing trend of agricultural world market prices is that global demand growth exceeds the growth in global supply, and this trend will continue in the foreseeable future. The global demand for food will continue to grow mainly for two reasons. One is the continued growth in world population; the other is the sustained growth in per capital incomes in developing and newly industrialized countries, with corresponding increase of per capital food consumption. This fact in turn has a positive impact on the home consumption and export of high value agricultural commodities and creates international market opportunity. Shortage in one end of the world becomes an opportunity in the other end of the world, this leads to increase the production of agricultural commodities in the potential production areas. Consequently, this might accelerate economic growth. Therefore, the competitiveness of agricultural commodities in global market are imperative for exportable products.

As indicated above, now adays SoRPARI adapted releasing the common beans specially haricot bean. So, we believe that based on literature and how haricot beans are important in terms of macro, micro-nutrient and anti-nutrient content, its mandatory to investigate its quality before home consumption and export purpose. Different research centers under Somali region pastoral and Agro-pastoral research institute have been working on the haricot bean varieties. Therefore, the aim of the present study will profile the nutritional value, antinutritional value, mineral contents, functional and physical properties of haricot bean varieties adapted and released by SoRPARI. Knowing the haricot bean nutritional diversity would have a direct impact on the improvement of haricot bean for quality breeding and for food product development to reduce the prevalence of food insecurity, malnutrition, and mineral deficiency, especially for those low-income communities.

1.3. Objective

1.3.1. General Objective

- The general objective of the study was to study the physicochemical properties, proximate and functional properties of haricot bean varieties produced/adapted under SoRPARI research centers.

1.3.2. Specific Objective

The specific objectives of the study were to;

- To analyze the proximate composition of different haricot bean varieties
- To Identify the Anti-nutritional content of different haricot bean varieties.
- To analyze the Physico-chemical properties of haricot bean varieties

1.4. Significance of the study

This study is important as it is the 1st in its nature to conduct such a study on different haricot bean varieties proximate composition, anti-nutritional, Physico-chemical properties. The outcome of the study will be important to get up-to-date data on the nutritional composition, anti-nutritional composition, physical, functional. The result obtained from this study will further be important for the future researchers as baseline information to conduct their research. The primary data obtained from this research will also helpful for industries, for product development

from haricot bean and other purposes. Based on the recommendation come out from this study, policymakers, government, non-Government organizations and other interested institutions working on the area will be expected to get up-to-date information from the study.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. The Haricot Bean Crop

Haricot bean (*Phaseolus vulgaris* L.) belongs to order Rosales, family Leguminosae subfamily Papilionideae, tribe Phaseolinae [23]. The common bean was originated in Tropical America (Mexico, Guatemala, and Peru), but there are also evidences for its multiple domestication within Central America [24]. The crop is now widely distributed throughout the world and consequently, it is grown in all continents except Antarctica [25]. In Ethiopia, it is most likely to be introduced by the Portuguese in the 16th century. It is well adapted to areas that receive an annual average rainfall ranging from 500–1500 mm with optimum temperature range of 16°C–24 °C, and a frost-free period of 105 to 120 days. Moreover, it performs best on deep, friable and well aerated soil types with optimum pH range of 6.0 to 6.8 [26]. Major haricot bean producing regions are Central, Eastern, and Southern parts of the country and in central Ethiopia; farmers grow early maturing white pea bean crop for export as their cash crop [27].

The common bean is cultivated primarily for its dry seeds, green pods (as snap beans), and green-shelled seed. Young tender leaves and flowers are also used as fresh vegetables in some Central and Eastern African, and in Latin America countries. The world demand for common bean is ever increasing because of its significance in human nutrition as a source of proteins, complex carbohydrates, vitamins, and minerals [28]. Their role in reducing blood cholesterol level and combating chronic heart diseases, cancers and diabetics is also gaining recognition from human health point of view. It is an important source of protein and energy in human diets in the tropical and sub-topical developing countries, particularly in the Americas and Eastern and Southern Africa [29].

Currently, haricot beans cover the dominant part of the Ethiopia's pulses export. However, the share of pulses in general in the export market has been limited by external demand for quality. In addition to other production constraints that limit the volume of production, lack of high yielding varieties with improved resistance to diseases and other biotic and abiotic constraints has been the major production constraint of common bean in Ethiopia in general [30].

2.2. Haricot Bean Production and its Economic Importance in Ethiopia

There is a wide range of haricot bean types grown in Ethiopia including mottled, red, white and black varieties [31]. The most commercial varieties are pure red and pure white coloured beans and these are becoming the most commonly grown types with increasing market demand [32]. To support both the growth in domestic and export bean markets, the Ethiopian Institute of Agricultural Research (EIAR) has developed a range of high yielding, multi-disease resistant bean varieties. The focus of this genetic improvement program has been on the pure red and white beans to support the commercial sector. Within the red bean types, the most favored and most commercially accepted varieties include Red Melka, a mottled medium sized red; Red Wolaita, a medium sized pure light red; and Nasser, a small pure dark red variety [33].

With regard to economic importance of haricot bean, it is used as source of foreign currency, food crop, means of employment, source of cash, and plays great role in the farming system. According to EPPA (2004) in the year 2000, 2001 and 2002 Ethiopia exported 23994.4, 32932.7 and 42127.0 tones and earning 8.2, 9.98 and 13.2 million USD respectively [6]. The main destination markets were Pakistan, Germany, Yemen, UK, South Africa, India and Mexico having 12.5, 7.8, 6.9, 5.79, 4, 4, 4 % share respectively [6]. The country's exports of haricot beans have increased over the last few years, from 58,126 MTs in 2005 to 78,271 MTs in 2007 and Ethiopia gets 63 million dollar from haricot bean market in 2005 [34]. White beans from the northern Rift Valley were sold into export markets to supply European canning factories and red beans were exported from the southern Rift Valley areas to supply drought affected areas in northern Kenya [35].

2.3. Health Benefits of Haricot Bean

Consumption of pulses on a regular basis has been associated with lower risks for the development of type -2 diabetes, heart attack risk by the soluble fiber provided from this legume, coronary heart disease and some forms of cancer, gives energy to burn while stabilizing blood sugar, iron for energy, copper and Manganese help with energy production plus antioxidant defenses, maintain your memory with thiamine (Vitamin B1) [36].

Navy beans, also called white beans, are small, oval, and white. Navy beans are good sources of folate, manganese, dietary fiber, protein, phosphorus, copper, magnesium, iron, and vitamin B1 (thiamin). Navy beans can lower your risk of heart attack, give you energy, stabilize blood sugar,

provide antioxidant benefits, and help maintain your memory. These small white beans are commonly used to make baked beans, but they are also good in soups, salads, and chili. They are relatively difficult to digest. Navy beans are so named because they were a food staple for the U.S. Navy in the 1800s [37].

Consumption of baked beans has been shown to lower total cholesterol levels and low-density lipoprotein cholesterol. This might be at least partly explained by high saponin content of navy bean. Saponins also exhibit antibacterial and anti-fungal activity, and have been found to inhibit cancer cell growth. Furthermore, navy bean is the richest source of ferulic acid and p-coumaric acid among the common bean varieties [38].

2.4. Proximate Composition of Haricot Beans

2.4.1. Moisture content

Seed moisture is an important parameter and is a result of harvest time and environmental conditions. The knowledge of morphology and size distribution and their dependence on the moisture content of haricot bean seeds is essential for accurate design of the equipment for cleaning, grading and separation. If haricot bean seed is harvested at moisture contents greater than 16%, it will have to be dried to prevent heating and molding in storage.

Wang et al. [39] reported that the moisture content of raw whole haricot bean is 14.2% and the surface area, one thousand seed mass, sphericity, seed porosity, terminal velocity, and angle of repose increased as the moisture content of haricot beans increased. However, the bulk density decreased as the moisture content increased. The moisture content of white haricot bean flours in this study was 7.58% for raw Awash-1 and 7.6 % for Chercher variety. The moisture content of the processed samples ranges from 6.91% to 8.9%.

2.4.2. Crude protein

Protein contents of legumes vary between 17 and 34 percent, which include metabolic, structural and storage protein. Legumes are rich sources of protein and the essential amino acid, lysine, but are usually deficient in sulphur containing amino acids, methionine and cystine. Protein concentration of haricot beans reportedly range from 21.1 to 24.5% [39]. In this study the crude protein content of Awash-1 variety was 29.4% and that of Chercher variety was 30.44%. The

crude protein content of these samples was increased when processing methods are used. The crude protein content of the processed samples ranged from 20.09% to 30.83%.

2.4.3. Total ash

Ash content of haricot beans ranges from 2.9 to 4.3% as reported by (Wang *et al*., 2009). The ash content of haricot beans is composed of relatively high levels of Mg, P, Ca, and S. In addition, haricot beans have a low Na and relatively high K contents, with a K: Na ratio of about 33:1 [40]. The total ash content of Awash-1 and Chercher varieties was 4.14% and 4.4% respectively. Whereas the total ash content of the processed samples was in the range of 4% to 4.58%.

2.4.4. Crude fat

Lipid content of legume is in the range of 1-6 percent and mainly depends upon variety, origin, location of growth, climate, season, environmental factors and soil type. Lipids found in legumes are mainly neutral lipids that mean triglycerides, di and mono glycerides, free fatty acids, sterols and sterol esters. According to Wang *et al*., [39] the lipid content of haricot beans is 1.5%. The crude fat content of Awash-1 and Chercher varieties were 2.57% and 2.46% in dry matter basis respectively. The crude fat content of the processed samples was in the range of 2.05% to 2.9%.

2.4.5. Crude fiber

Dietary fiber, defined as the sum of lignin and polysaccharides that are not digested by the endogenous secretions of the human digestive tracts is an important component of food. Haricot beans contain 6.6% crude fiber [39].Thermal processing or household cooking may alter the composition of these fibers and thus alter their physiological effects to human body. The crude fiber content of Awash-1 was 4.56% and that of Chercher variety was 3.35%. The crude fiber content of the processed samples ranges from 1.48% to 6.04%.

2.4.6. Carbohydrate

Major carbohydrates in legumes are starch and numerous other sugars. Sugars ranges between 6 and 12 percent, whereas starch vary from 24 to 41 percent. Wang *et al.,* [39] reported that the carbohydrate content of haricot beans ranges from 39.0 to 56.3%. In this study the utilizable carbohydrate content of the two varieties was 52.15% and 51.77% for Awash-1 and Chercher respectively.

2.5. Functional Properties

The term functional properties refer to the given component present in optimum concentration, subjected to processing at optimum parameters, contributes to the desirable sensory characteristics of the products usually by interacting with other food constituents. They indicate the ability of the

pertinacious material to hold oil or fat and water, to emulsify the same and to form products having a firm consistency upon heating and cooling. These include viscosity, dispersibility, emulsify, form gels, foam, produce films and absorb water and/or fat [41] (Maskus, 2010).

In order to increase the utilization and consumption of pulses, several researchers have recommended that pulses must be processed into flours and used as ingredients in food product applications. To consider pulses as ingredients, it is necessary to evaluate their functional properties (water absorption capacity, oil absorption capacity, foaming properties and emulsification properties) of the flours for use as ingredients in food product applications [41].

Legume flours, due to their amino acid composition and fiber content, are ideal ingredients for improving the nutritional value of bread and bakery products [42]. Haricot bean flour has potential for traditional and newer product developments with health benefits since it contains about 25% protein, 56% carbohydrate and 1.5% fat.

[43] Siddiq *et al.* (2009) reported that the water absorption capacity of haricot bean ranged from 2.25 to 2.65 g H_2O/g flour and the oil absorption capacity of haricot beans ranged from 1.23 to 1.52 g oil / g flour. Bulk density is a property of powders, granules, and other "divided" solids, especially used in reference to foodstuff, or any other masses of corpuscular or particulate matter. It is defined as the mass of many particles of the material divided by the total volume they occupy. The bulk density of soaked and dried, de-hulled haricot beans were 0.56 gml^{-1} and 0.53 gml^{-1} respectively.

2.6. Minerals

Mineral contents of legumes also showed significant reduction (18.99-39.50%) during the process of cooking due to leaching of minerals into cooking water [45] (Nuzhat *et al.*, 2008).Haricot bean is an excellent source of macronutrients (P, K, Ca, Mg), micronutrients (Fe, Zn, Cu, and Mn), and trace elements (Al, Cr, Ni, Pb , Co, Se, Mo). [40] Amany *et al*. reported that raw white bean seeds contain K, Mg, Ca, Fe and Zn with values 1293, 155.6, 154, 12.55 and 4.86 mg/100 g dry weight, respectively but soaked white beans contain K, Mg, Ca, Fe and Zn (1142, 145, 145, 7.98 and 3.38 mg/100 g dry weight respectively).

2.7. Anti-nutritional Factors of White Haricot Beans

The anti-nutrients like trypsin inhibitors, phytic acid, saponins, heamagglutinins and tannins are some of the undesirable components in legumes that could hinder utilization of important minerals including calcium, magnesium, iron and zinc by interfering with their absorption and utilization and thereby contributes to mineral deficiency [44]. Phytic acid and tannic acid are the two main anti-nutrients present in legumes.

2.6.1. Phytic Acid

Phytic acid (myoinositol, 1, 2, 3, 4, 5, 6- hexakisdihydrogen phosphate) and phytate (salts of phytic acid) are widespread in plant seed grains (also including cereals), roots, tubers (and legumes. Phytate accumulates in the seeds during the ripening period and is the main storage form of both phosphate and inositol in plant seeds and grains [46].

Phytic acid and its salts represent the majority of the phosphorus in plant legume seeds and monogastric species have a limited ability to hydrolyse phytates and release phosphate for absorption. In dicotyledonous seeds such as legumes, phytic acid is found closely associated with proteins and is often isolated or concentrated with protein fraction of this food. Phytic acid or phytate when in salt form is the principal storage form of phosphorus in plant tissues [47].

Phytic acid has been considered as an anti-nutrient which binds with other nutrients and makes them indigestible. Excessive phytic acid in the diet can have a negative effect on mineral balance because of the insoluble complexes it forms with essential minerals (Cu^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+} and Ca^{2+}), which causes poor mineral bioavailability (Urbano *et al*., 2000). Therefore, phytates have negative impact on enzyme activity and there is evidence of its negative impact on key digestive enzymes like lipase, α-amylase, pepsine, trypsin and chymotrypsin (Urbano *et al*., 2000). However, there are some beneficial effects of phytic acid, such as reduced bioavailability, and therefore toxicity of heavy metals (example, cadimium and lead) present in the diet [48].

2.7.2. Tannin

Tannins are defined as water soluble polymeric phenolics that precipitate proteins .Tannins in plant are involved in defense mechanism to environmental attack [49]. It exists in mixtures with many other classes of plant phenolic compounds. Tannins are usually subdivided into two groups: hydrolyzable tannins (HT**)** and proanthocyanidins (PA**).** Hydrolyzable tannins are more susceptible to enzymatic and non-enzymatic hydrolysis than proanthocyanidins, and usually are more soluble in water. Tannins inhibit the digestibility of protein, whereas phytic acid reduces the bioavailability of some essential minerals (Rehman and Shah, 2006) [50].

CHAPTER THREE

3. MATERIALS AND METHOD

3.1. Structure of the experiment work

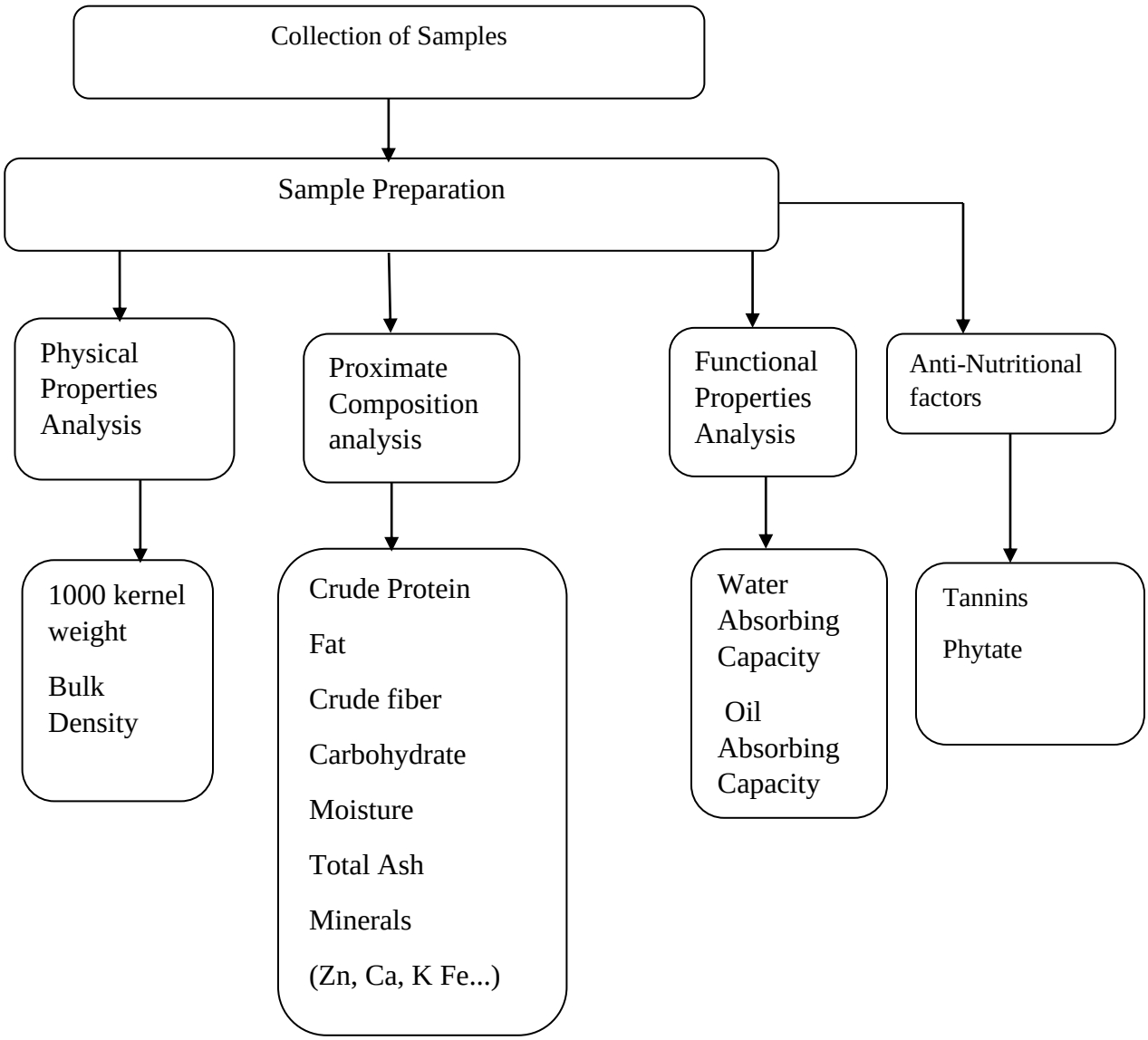


Fig 3.1: Experimental structure

3.1. Materials and Equipment

15 different varieties of haricot bean produced/adapted under SoRPARI were collected from all research centers. Five haricot bean varieties produced or adapted under SoRPARI were collected from Fafan, Dolo-Ado and Godey Centers/Kelafo Sub-center. Only haricot beans without any physical damage or insect infestation were selected for analysis. All equipment used in sample preparation will be expected to be obtained from SoRPARI laboratories and missed equipment can be used from local institutes. Further, after bringing to the laboratory, haricot bean samples were individually ground to a fine powder (30 mesh size), packed in airtight polyethylene plastic bags and were stored at 4°C until further analysis.

3.2. Physical properties

Hundred (100) kernel weight and bulk density

One thousand haricot bean kernels were randomly selected and weighed separately. For bulk density, 50 g of rice grains were weighed and was dropped into a graduated glass measuring cylinder from a constant height (approximately 30 cm). The volume occupied by rice samples was recorded and bulk density was calculated as g/ml ratio [51, 52]

3.3. Proximate Composition

Moisture content, total ash, crude fat, crude fiber and crude protein content were determined by [53][15] AOAC (2000) official methods of 925.10, 923.03, 920.39, 920.87 and 945.38; respectively. Whereas the difference was taken to determine total carbohydrate content. Percentage of total Carbohydrate content =100 – (Sum of Percentage Moisture, Protein, Fat + and total ash content). Energy value was quantified based on the three groups of nutrients (carbohydrates, fats and proteins). The gross food energy**: -**FE = {(%TC-%CF) x 4} + (%TFx9) +(%TP x4); -Where, FE=Food Energy in Kcal/g, TC = Total Carbohydrate, CF = Crude Fiber, TF = Total Fat and TP = Total Protein.

3.3.1. Moisture content

The moisture content of the samples was determined by drying 10g of sample in an air oven at 105^{o}C to constant weight (16 -18 h).

3.3.2. Protein content of the flours

Protein content was determined according to AOAC (2000) using the official method 979.09, which comprised three major steps: digestions, distillation and titration, and quantification.

Digestion

Fresh samples (in duplicate) of each 0.5g was added into Tecator tubes and 6ml of acid mixture (5parts of concentrated ortho-phosphoric acid and 95 parts of concentrated sulfuric acid) was added into each tubes and mixed, and 3.5ml of 30% hydrogen peroxide was added step by step, resulting in violent reaction. Following the cessation of violet reaction, the tubes should be shaken and placed back to the rack. Catalyst mixture (ground 0.5g of selenium metal with 100g of potassium sulfate) of 3g was added into each tube and was allowed to stand for about 10 minutes before digestion. Following the attainment of digester temperature at 370^{o}C, the tubes was low ill bed into the digester. The digestion was continued until a clear solution was obtained (~ after 4 hours). The tubes in the rack should be cooled in a fumehood. 25 ml of de-ionized water was added and the mixture should be shaken to avoid precipitation of sulfate in the solution.

Distillation and titration

The digested and diluted sample solution was distilled using boric acid and the distillate was subjected to titration using 0.1N hydrochloric acid until reddish color appeared.

Quantification of crude protein concentration

$$\text{Nitrogen } \% = \frac{\text{VHCl} * [\text{HCl}]}{\text{Wo}} * 14 * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$\text{Protein } \% = 6.25 * \% \text{ Nitrogen} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where; V is the volume of HCl consumed in liter, N is the normality of HCl (0.1N), W_O is the sample weight on dry mass basis and 14 is the molecular weight of nitrogen.

3.3.3. Experimental analysis of fat

The fat content of the complementary food samples (flours) was determined using a semi-continuous solvent extraction method (Soxhlet method) following the procedures of AOAC 960.39 (2000). In this method, the solvent builds up in the extraction chamber for 5-10 minutes and completely surrounds the sample and then siphons back to the boiling flask.

The crude fat content (percent weight by weight) was calculated as follows:

$$F = \left(\frac{W2 - W1}{W}\right) * 100\% \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where;

F= Crude fat content in percent weight by weight

W_1 = Weight of the extraction flask in gram (g)
W_2 = Weight of the extraction flask and the dried crude fat in gram (g)
W = Weight of sample in gram (g)

3.3.4. Experimental analysis of ash content

The ash content was determined based on AOAC (2000) using the official method 923.03. Briefly, porcelain crucible that is will behed using distilled water was placed in a muffle furnace at 550^{o}C for 30 minutes. Then, the crucibles were cooled by placing it in desiccators for 30 minutes at room temperature. Following this step, they were weighed to the nearest milligram (M_1) using the dried, cooled and weighed crucible, 2.5g of fresh sample together (M_2). Then the sample was thoroughly char in fume-hood by placing it on a hot plate, and increasing the temperature slowly until smoking ceases. After the completion of charring, the sample was placed into the muffle furnace at 550^{o}C for 5 hours. The ignition was continued by cooling for 1hour and weighing until a clean and white ash will be obtained, which was then measured to the nearest milligram (M_3).

$$\text{Total ash(in \% weight by weight)} = \left(\frac{M3-M1}{M2-M1}\right) * 100 \ldots\ldots\ldots\ldots\ldots (5)$$

Where:
(M_2-M_1) is the weight of sample in gram (g) on dry basis
(M_3-M_1) is the weight of ash in gram (g)

3.3.5. Experimental analysis of carbohydrates

The total available carbohydrate concentration was determined by difference according to AOAC official method, (922.02, 925. 05).

% Carbohydrate = 100% - (%moisture + %protein + %fat + %ash + % crude fiber). (6)

3.3.6. Experimental analysis of the crude fiber content

The crude fiber content of the complementary foods (flour) was determined by using the official method of AOAC (2000). The method involved the digestion of a fresh sample, filtration, will behing, drying and combustion. Following these steps, the crude fiber was calculated using the following formula:

$$\text{Crude fiber in } \left(\frac{g}{100g}\right) = \frac{[(W2-W3)*100]}{W1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Where;

W_1 = weight of the fresh sample

W_2 = weight of crucible with the sample after oven draying
W3 = weight of the crucible with the sample after ashing

3.3.7. Gross energy (total calorie)

Gross energy was determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors: 16.7 KJ/g (4 kcal/g) for protein, 37.4 KJ/g (9 kcal/g) for fat and 16.7 KJ/g (4 kcal/g) for carbohydrates and expressed in calories.

3.4. Mineral analysis

Mineral contents of powder sample were determined by atomic absorption spectrometry/flame photometry according to the methods [54]. For wet digestion of sample, 1g of the powdered sample was taken in digesting glass tube. 12ml of HNO_3 was added to the food samples and mixture was kept for overnight at room temperature. Then 4ml perchloric acid (HClO) was added to this mixture and was kept in for the fumes block for digestion. The temperature was increased gradually, starting from 50°C and increasing up to 250-300°C. The digestion completed in about 70-85min as indicated by the appearance of white fumes. The mixture was left to cool down and the contents of the tubes were transferred to 100ml volumetric flasks and the volumes of the contents were made to 100ml with distilled water. The wet digested solution was transferred to plastic bottles labeled accurately. Put the sample in many tube to centrifuge it at 3000rpm to 10min. Use supernatants for mineral determination.

The digested sample was analyzed for mineral contents by Atomic Absorption Spectrophotometer. Different electrode lamps were used for each mineral. The instrument was run for standard solutions of each mineral before and during determination to check that it is working properly. All minerals were performed with dilution factor of 100. For determination of Mg, further dilution of the original solution was done by using 0.5ml original solution and enough distilled water is added to it to make the volume up to 100ml. Also for the determination of Calcium, 1.0ml lithium oxide solution is added to the original solution to unmask Calcium from Magnesium. The concentrations of minerals recorded in terms of "ppm" are converted to milligrams (mg) of the minerals by multiplying the ppm with dilution factor and dividing by 1000, as follows:

$$MC = \frac{\text{Absorbency*Dry weight*D}}{\text{Weight of the sample*1000}}$$

Where, MC=mineral composition, D = dilution factor

Potassium (K) analysis of the sample were done by the method of flame photometry. The same wet digested food sample solutions as used in Atomic Absorption Spectrometry are used for the determination of Na and K. Standard solutions of 20, 40, 60, 80 and 100 milliequivalent/L are used for K. The calculations for the total mineral intake involve the same procedure as given in Atomic Absorption Spectrometry

3.5. Anti-nutritional factors determination

3.5.1. Determination of tannin content

Tannin content was ascertained by the Burns (1971) method modified by Gemed[55], using catechin as the tannin standard. Approximately 1.0 g of each treatment samples were weighed in triplicates in screw cap test tube and extracted with 10 mL of 1% HCl in methanol for 24 h at room temperature with mechanical shaking. After 24 h of shaking, the solution was centrifuged at 1000 rpm for 5 min. Mix 1 mL of supernatant with 5 mL of vanillin-HCl reagent (prepared by combining an equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol). D-catechin was used as a standard for tannin determination. A 0.01 g of D- catechin was measured and dissolved in 50 mL of 1% HCl in methanol, which was served as a stock solution. A 0, 0.2, 0.4, 3, 0.6, 0.8, and 1 mL of stock solution were taken in a test tube and the volume of each test tube was regulated to 1 mL with 1% HCl in methanol. To each test tube added 5 mL of vanillin-HCl reagent. After 20 min, the absorbance of sample solutions and the standard solution were evaluated at 500 nm (Perkin Elmer Lambda 950 UV/ Vis/NIR, UK) zero the spectrophotometer by using distilled water, and the calibration curve was formed from the series of standard solution as absorbance versus concentration and the slope and intercept were used for calculation.

3.5.2. Determination of Phytic acid

The phytate content in the samples was determined according to the method described in Deme *et al*.[56] Extract 100 mg of sample with 10 mL of 0.2 N HCl in a mechanical shaker for 1 h at room temperature. Centrifuged the extract at 3000 rpm for 30 min. The clarified supernatant was used for phytate estimation. Add 1 mL of Wade reagent (containing 0.03% solution of $FeCl_3 \cdot 6H_2O$ and 0.3% of sulfosalicylic acid in water) to 3 mL of the sample solution (supernatant) and

vortexed for 5 s. Absorption readings at 500 nm (Perkin Elmer Lambda 950 UV/Vis/NIR, UK) were taken against a blank solution (3 mL extract solution mixed with 2 mL of 2.4% HCl). The sodium salt of phytic acid (5– 36 mg/mL) was used as a standard for the construct calibration curve. Phytate concentration was determined from a standard curve and results were expressed as phytic acid in mg per 100 g dry matter.

3.6. Determination of functional properties

3.6.1. Oil and Water Absorption Capacity Determination

The centrifugal method of [57]Beuchat was used to determine the oil and water absorption capacities of the isolated bean starches and the bean flour. About two gram of sample was mixed with 10 mL distilled water/oil (Thermolyne, type 37600 mixer, Maxi mix II, Iowa, U.S.A) for 30 sec. The samples were then allowed to stand at 21 ^{o}C for 30 min, centrifuged at 5000 x g for 30 min, and the volume of the supernatant noted in a 10 mL graduated cylinder. Density of distilled water was assumed to be 1g/mL and that of oil (Rio, vegetable soya cooking oil, Thailand) was found to be 0.89 g/mL. Results were expressed on a dry weight basis.

3.7. Statistical analysis

All the analysis was performed in duplicate (unless stated otherwise) and presented as mean ± standard deviation. Statistical significance of the data obtained was analyzed by One-way analysis of variance (ANOVA) followed by Duncan test by using Genstat version 18.0. The level of significance was considered at $P < 0.05$.

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1. RESULT

4.1.1. Macronutrient content of different haricot bean varieties

Table 4.1: Proximate composition of different haricot bean varieties

Haricot bean Varieties	Results in (%) (100g)						
	Moisture	Ash	C.Fiber	C.Protein	C.Fat	CHO	Energy
DAwash2	8.095±0.5^{a}	5.500±0.2^{gh}	3.160±0.2^{f}	18.96±0.6^{i}	2.250±0.09^{k}	62.03±1.81^{b}	344.2±2.51^{fg}
D Awash - Mittin	8.141±0.3^{a}	5.473±0.2^{fgh}	3.230±0.2^{g}	22.18±0.8^{m}	1.620±0.07^{g}	59.36±1.22^{a}	340.7±3.01^{def}
D Control	8.395±0.6^{ab}	5.060±0.3^{def}	3.410±0.2^{hi}	17.25±0.5^{d}	1.740±0.07^{h}	64.15±2.11^{de}	341.2±3.12^{def}
D-Seri 119	8.782±0.7^{abc}	4.835±0.1^{bcd}	3.280±0.2^{g}	20.14±0.7^{k}	1.440±0.06^{f}	61.52±1.75^{b}	339.6±2.98^{de}
D-Seri125	8.584±0.5^{ab}	4.966±0.2^{cde}	3.350±0.3^{h}	18.77±0.6^{h}	0.540±0.02^{a}	63.79±2.08^{d}	335.1±2.87^{bc}
F-Adi	11.553±0.8^{e}	4.614±0.2^{abc}	2.760±0.1^{c}	19.63±0.7^{j}	1.400±0.06^{f}	60.04±1.32^{a}	331.3±2.68^{ab}
F-Awash 2	9.203±0.9^{bc}	4.218±0.1^{a}	4.480±0.3^{l}	18.02±0.6^{g}	0.790±0.03^{b}	63.29±1.90^{cd}	331.3±2.69^{ab}
F-Awash Mittin	10.954±0.8^{de}	4.516±0.2^{ab}	3.430±0.2^{i}	17.59±0.5^{e}	1.160±0.05^{d}	62.35±1.95^{bc}	330.2±2.56^{a}
F-Seri 119	10.613±0.7^{d}	4.296±0.1^{a}	3.620±0.2^{k}	16.75±0.4^{b}	1.070±0.04^{c}	63.65±2.06^{d}	331.2±2.68^{ab}
F-Seri 125	9.530±0.6^{c}	4.535±0.2^{ab}	2.230±0.1^{a}	20.29±0.8^{l}	1.310±0.05^{e}	62.10±1.98^{b}	341.4±3.03^{def}
K-Adi	8.689±0.5^{abc}	5.112±0.3^{defg}	2.400±0.1^{b}	17.88±0.5^{f}	1.740±0.07^{h}	64.18±2.32^{de}	343.9±3.56^{efg}
K-awash 2	8.678±0.6^{abc}	5.304±0.3^{efgh}	3.040±0.2^{e}	18.99±0.6^{i}	1.570±0.06^{g}	62.42±1.98^{bc}	339.8±3.04^{de}
K-awash Mittin	8.319±0.6^{ab}	5.284±0.3^{efgh}	3.230±0.2^{g}	17.59±0.5^{e}	1.910±0.08^{i}	63.67±2.21^{d}	342.2±3.23^{def}
K-Seri 119	7.915±0.3^{a}	5.160±0.2^{defg}	2.930±0.2^{d}	16.93±0.4^{c}	2.130±0.09^{j}	64.93±2.34^{e}	346.6±3.95^{g}
K-Seri 125	8.137±0.4^{a}	5.606±0.3^{h}	3.530±0.3^{j}	16.21±0.4^{a}	1.460±0.06^{f}	65.06±2.51^{e}	338.2±3.86^{cd}

Key: Same letter in the same column is not significantly different from each other at $p<0.05$
D: Dolo-Ado, F: Fafan, K: Kelafo Research center and Sub-centers. CHO: Carbohydrate, Energy: Energy in Calory unit obtained from consuming 100g of these haricot bean varieties

Table 4.1 shows proximate composition of different haricot bean varieties. The moisture content is the range of 7.915% (K-Ser 119) to 11.533% (F-Ada) variety. The moisture content of haricot bean collected from Fafan research center is highest as compared to those collected from Dolo

Ado and Kelafo research center and significantly different at P<0.05. Overall, there is no significance difference in moisture content of different haricot bean varieties collected both from Dolo-Ado and Kelafo research center at P<0.05. When it comes those varieties collected from Fafan research center, the moisture content F-Ada & F-Awash mittin are significantly different as compared to F-Awash 2, F-Ser 119 and F-Ser 125 at P<0.05

The ash content also depicted in Table 4.1 above, F-Awash 2 has the lowest (4.218%) and K-Seri 125 with highest (5.606%) ash content. Significant difference was observed between 15 different samples of haricot bean at P<0.05 while in between some samples there is no significant difference at P<0.05. When it comes to between research centers, those sample collected from Kelafo have highest ash content followed by Dolo-Ado and Fafan research center respectively as indicated in table 4.1. Significant different at P<0.05, were also observed in some of the same sample but collected from different research center as indicated in table 4.1.

With respect to crude fiber content, sample with F-Seri 125 has lowest in crude fiber content while sample with F-Awash 2 has highest in crude fiber content as indicated in table above. Both highest and lowest record is registered from same research center which Fafan. Except few haricot bean samples under study (D-Seri 125 and D-Control samples), the crude fiber content of most samples is significantly different at P<0.05. When it comes to comparison among different samples collected from different research centers, those samples collected from Fafan research center have highest crude fiber followed by Dolo-Ado and Kelafo research center respectively. The crude fiber content is significantly different at P<0.05, in some of the same sample but collected from different research center as indicated in table 4.1.

K-Seri 125 has lowest in crude protein content (16.21%) while D-Awash mittin has the highest in crude protein content (22.18%). There is no significant difference at P<0.05 between samples with code K-Awash mittin and F-Awash mittin. Except these two samples, the crude protein content is significantly different in between al samples at P<0.05. In comparing against area from where samples were collected, those haricot bean samples collected from Dolo-Ado research center have highest crude protein content followed by Fafan and Kelafo research centers. Even there is significant different in between same sample but collected from different research centers at P<0.05 as indicated in table 4.1 above. In general, the data shows, haricot bean is good source of crude protein.

The crude fat content is also indicated in table 4.1 above. D-Seri 125 has lowest in crude fat content (0.54%) while D-Awash-2 has the highest in crude fat content (2.25%). Both data is from same center which Dolo-Ado research center. There is no significant difference in between (K-Seri 125 & F-Adi), (D-Awash mittin & K-Awash 2) and (D-Control & K-Adi) at $P<0.05$. Except these mentioned samples, the crude fat content is significantly different in between others haricot bean samples as indicated in table 4.1. above at $P<0.05$. The crude fat content of those haricot bean samples collected from Kelafo research center is relatively highest followed by those samples collected from Dolo-Ado and Fafan Research centers respectively. Even there is significant different in crude fat content in between some of same samples but collected from different research centers at $P<0.05$. The result indicates that, haricot bean has lowest fat content in nature.

When it comes to carbohydrate content, K-Seri 125 has the highest in carbohydrate content (65.06%) while D-Awash mittin has the lowest in carbohydrate content which is 59.36% as indicated in table 4.1. Except few samples, the carbohydrate content is not significantly different at $P<0.05$. The carbohydrate content is almost comparable in between those samples collected from all three-research centers with fewer deviation when it comes to those samples collected from Fafan Research center as shown on above table.

Lastly, the energy in calory unit obtained from consuming 100 g haricot bean flour are indicated in table 4.1. above. Sample code with F-Awash mittin has the lowest in energy obtained (330.2Kcal/100g) while K-Seri 119 has the highest in energy content which is 346.6Kcal/100g. Except few samples, the energy obtained is not significantly different at $P<0.05$. The energy obtained is almost comparable in between those samples collected from all three-research centers with fewer deviation when it comes to those samples collected from Fafan Research center as shown on table 4.1 above.

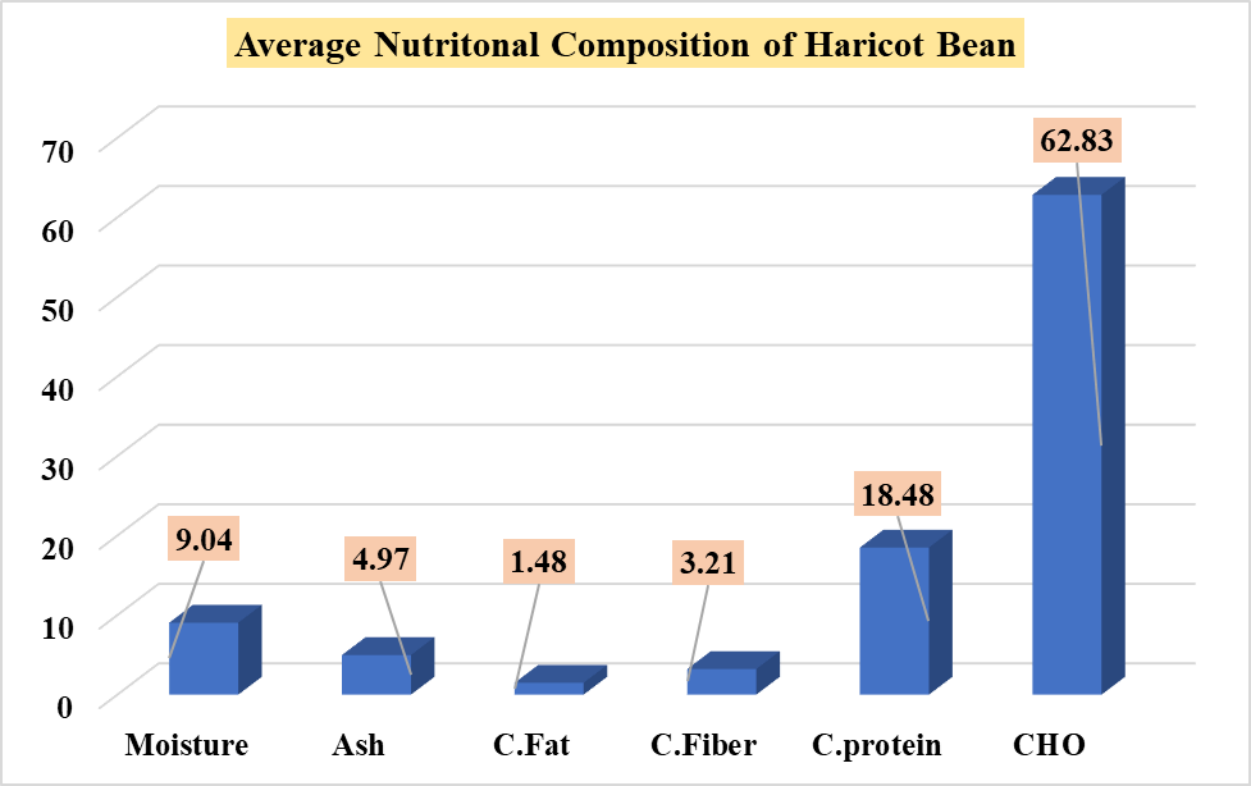


Figure 4.1: Average nutritional composition of haricot bean

Key: C-Crude, CHO-Carbohydrate

The general nutritional content indicated in figure 4.1 above. The figure indicates that, the average nutritional composition of 1.48%, 3.21%, 4.97%, 9.04%, 18.48% and 62.83% for crude fat, crude fiber, ash (Inorganic component), moisture, crude protein and carbohydrate in their order of increasing respectively.

4.1.2. The minerals content of different haricot bean varieties

The mineral content of different haricot bean varieties has indicated in table 4.2 below. Four different types of minerals were included in the experiment process namely calcium, zinc, iron and potassium. The calcium content is in the range of 65.96mg/100g for sample code F-Adi while the highest amount of calcium is registered for sample with code D-Control which is 108.07g/100g. Both lowest and highest calcium content is registered from haricot bean sample collected from same center which Dolo-Ado research center. Except few samples, significant difference was observed for calcium in most of the sample in a one-to-one comparison at $P<0.05$. But in general comparison, the calcium content is not significantly different except few samples at $p<0.05$ as shown in table above. In comparison between the source of the sample, sample

collected from Dolo-Ado research center have relatively highest value of calcium followed by those collected from Fafan and Kelafo center respectively. Relatively minor difference was observed in calcium content for same sample but collected from different sources or area.

Sample with code K-Awash-2 has the lowest in zinc content (3.092mg/100g) while D-Awash 2 has the highest in zinc content which is (4.798mg/100g) as indicated in table 4.2 below. Except few samples, there is no significant difference in zinc content if one-to-one and general comparison is considered at $P<0.05$. When it comes to zinc content comparison based on the sources where the haricot bean is collected, those sample collected from Dolo-Ado research center has relatively highest amount of zinc followed by those collected from Kelafo and Fafan research center respectively. Relatively minor difference was observed in zinc content for same sample but collected from different sources or area.

When it comes to iron content, sample with K-Seri 119 has lowest in iron content (4.306mg/100g) while sample with code D-control has the highest in iron content which is 6.571mg/100g as indicated in table below. Almost all no significant difference was observed in between most of the haricot sample when it comes to iron content at $P<0.05$. There is also no significant difference was observed in iron content for same sample but collected from different sources or area as shown below.

Table 4.2. Minerals content of different haricot bean varieties

Haricot bean Varieties	Results in (mg/100g)			
	Calcium	Zinc	Iron (Fe)	Potassium (K)
DAwash2	83.04±1.50^{fg}	4.798±0.98^{f}	5.227±0.99^{ab}	150.3±1.89^{e}
D Awash -Mittin	79.96±1.35^{defg}	4.152±0.82^{cdef}	4.543±0.79^{a}	147.8±1.79^{de}
D Control	108.07±1.85^{i}	3.739±0.79^{abcd}	6.571±1.04^{b}	145.1±1.71^{cde}
D-Seri 119	68.19±1.25^{ab}	4.422±0.92^{def}	5.666±0.99^{ab}	126.0±1.21^{a}
D-Seri125	65.96±1.21^{a}	4.106±0.80^{cdef}	5.219±0.99^{ab}	138.1±1.34^{bc}
F-Adi	85.32±1.62^{g}	3.780±0.78^{abcd}	4.534±0.75^{a}	135.6±1.32^{b}
F-Awash 2	80.93±1.42^{efg}	4.025±0.78^{cde}	4.538±0.81^{a}	133.3±1.31^{ab}
F-Awash Mittin	92.49±1.48^{h}	3.541±0.65^{abc}	5.671±0.97^{ab}	133.3±1.31^{ab}
F-Seri 119	72.79±1.29^{bc}	3.706±0.69^{abcd}	4.995±0.91^{ab}	131.0±1.30^{ab}
F-Seri 125	78.32±1.35^{def}	3.457±0.59^{abc}	6.343±1.02^{b}	140.3±1.61^{bcd}
K-Adi	83.01±1.50^{fg}	3.860±0.72^{bcde}	4.988±0.94^{ab}	145.0±1.71^{cde}
K-awash 2	83.42±1.52^{fg}	3.092±0.52^{a}	5.433±0.93^{ab}	147.5±1.76^{de}
K-awash Mittin	77.09±1.29^{cde}	3.256±0.58^{ab}	5.665±1.01^{ab}	145.1±1.72^{cde}
K-Seri 119	75.14±1.19^{cd}	3.941±0.76^{bcde}	4.306±0.71^{a}	147.5±1.78^{de}
K-Seri 125	84.55±1.42^{g}	4.582±0.91^{ef}	5.663±1.02^{ab}	152.1±1.95^{e}

Same letter in the same column is not significantly different from each other at $p<0.05$
D: Dolo-Ado, F: Fafan, K: Kelafo Research center and Sub-centers.

Lastly, the potassium content of different haricot bean samples in the range of 126.0mg/100g for sample with code D-Seri 119 and 152mg/100g for sample with code K-Seri 125 as indicated in table 4.2 above. Except few samples, there is no significant difference in potassium content if one-to-one and general comparison is considered at $P<0.05$. When it comes to potassium content comparison based on the sources where the haricot bean is collected, those sample collected from Kelafo research center has relatively highest amount of potassium followed by those collected from Dolo-Ado and Fafan research center respectively. Relatively minor difference was observed in potassium content for same sample but collected from different sources or area.

4.1.3. Anti-Nutritional factors of different haricot bean varieties

The anti-nutritional content (Tannins and phytate) of different haricot bean varieties has been indicated in table 4.3 below. Regarding tannins content, Sample with code F-Adi and F-Awash-2 has the lowest in tannins content (0.30mg/g) while D-Seri 119 has the highest in tannins content

which is 34.95mg/g. in addition to this, high level of tannin were obtained on sample code with K-Seri 125, K-Seri 119, D-Seri 125, F-Seri 125, F-Seri 119 and D-Control sample with values of 30.61mg/g, 28.85mg/g, 28.60mg/g, 26.31mg/g, 22.22mg/g and 13.65mg/g respectively. Except few samples, there is no significant difference in tannins content if one-to-one and general comparison is considered at $P<0.05$. When it comes to tannins content comparison based on the sources where the haricot bean is collected, those sample collected from Dolo-Ado research center has relatively highest amount of tannins followed by those collected from Kelafo and Fafan research center respectively. Relatively minor difference was observed in tannins content for same sample but collected from different sources or area except some samples. The tannins content of those Seri 119 and Seri 125 are significantly different as compared other haricot bean varieties at $P<0.05$.

When it comes to the phytate content, D-Seri 119 has the highest in phytate content (4.050mg/g) while k-Seri 119 has the lowest in phytate content which is 2.193mg/g as shown in table 4.3 below. As indicated in table, there is no significant difference between all haricot sample collected form all centers at $p<0.05$. When it comes to phytate content comparison based on the sources where the haricot bean is collected, those sample collected from Dolo-Ado research center has relatively highest amount of phytate followed by those collected from Kelafo and Fafan research center respectively.

Table 4.3 Anti-Nutritional factors of different haricot bean varieties

Haricot bean Varieties	Results (mg/g)	
	Tannins	Phytate
DAwash2	0.66±0.08[a]	2.663±0.28[a]
D Awash -Mittin	0.86±0.94[a]	3.594±0.36[a]
D Control	13.65±1.26[b]	2.539±0.26[a]
D-Seri 119	34.95±1.93[e]	4.050±0.43[a]
D-Seri125	28.60±1.87[d]	2.319±0.23[a]
F-Adi	0.30±0.05[a]	2.657±0.28[a]
F-Awash 2	0.30±0.05[a]	2.317±0.24[a]
F-Awash Mittin	0.52±0.07[a]	2.658±0.28[a]
F-Seri 119	22.22±1.57[c]	2.315±0.22[a]
F-Seri 125	26.31±1.51[cd]	2.310±0.21[a]
K-Adi	0.44±0.06[a]	2.309±0.20[a]
K-awash 2	1.91±0.09[a]	3.009±0.31[a]
K-awash Mittin	0.77±0.09[a]	2.662±0.29[a]
K-Seri 119	28.85±1.68[d]	2.193±0.21[a]
K-Seri 125	30.61±1.86[d]	2.784±0.30[a]

Key: Same letter in the same column is not significantly different from each other at $p<0.05$
D: Dolo-Ado, F: Fafan, K: Kelafo Research center and Sub-centers.

4.1.4. Functional properties of different haricot bean varieties

The functional properties of different haricot bean varieties have been indicated in table 4.4 below. With respect to water absorbing capacity, Sample with code K-Seri 125 has the lowest in water absorbing capacity (1.270) while D-control has the highest in water absorbing capacity which is 2.150. Except between few of the samples, there is no significant difference in water absorbing capacity if one-to-one and general comparison is considered at $P<0.05$. When it comes to water absorbing capacity comparison based on the sources where the haricot bean is collected, those sample collected from Dolo-Ado research center has relatively highest in water absorbing capacity and comparable between those collected from Kelafo and Fafan research center respectively. Almost no difference was observed in water absorbing capacity for same sample but collected from different sources.

Table 4.4 Functional properties of different haricot bean varieties

Haricot bean Varieties	Results	
	Water Absorbing capacity (g/g)	Oil Absorbing capacity (g/g)
D -Awash2	1.360±0.24^{a}	1.150±0.17^{ab}
D Awash -Mittin	1.865±0.43^{ab}	1.190±0.20^{ab}
D Control	2.150±0.56^{b}	1.875±0.41^{b}
D-Ser 119	1.735±0.41^{ab}	1.295±0.18^{ab}
D-Ser125	1.655±a0.38^{b}	1.460±0.22^{ab}
F-Ada	1.655±0.36^{ab}	1.325±0.21^{ab}
F-Awash 2	1.800±0.42^{ab}	0.910±0.12^{a}
F-Awash Mittin	1.340±0.23^{a}	1.285±0.19^{ab}
F-Ser 119	1.535±0.30^{ab}	1.060±0.14^{ab}
F-ser 125	1.380±0.21^{a}	0.860±0.11^{a}
K-Ada	1.580±0.31^{ab}	1.400±0.21^{ab}
K-awash 2	1.520±0.30^{ab}	1.205±0.18^{ab}
K-awash Mittin	1.735±0.42^{ab}	1.270±0.16^{ab}
K-Ser 119	1.820±0.44^{ab}	0.900±0.12^{a}
K-ser 125	1.270±0.22^{a}	0.865±0.11^{a}

Key: Same letter in the same column is not significantly different from each other at $p<0.05$

When it comes to the oil absorbing capacity, Sample with code F-Seri 125 has the lowest in oil absorbing capacity (0.860) while D-control has the highest in oil absorbing capacity which is 1.875 as indicated in table 4.3. above. Except between few of the samples, there is no significant difference in oil absorbing capacity if one-to-one and general comparison is considered at $P<0.05$. When it comes to oil absorbing capacity comparison based on the sources where the haricot bean is collected, those sample collected from Dolo-Ado research center has relatively highest in water absorbing capacity and comparable between those collected from Kelafo and Fafan research center respectively. Almost no difference was observed in oil absorbing capacity for same sample but collected from different sources.

4.1.5. Physical properties of different haricot bean varieties

Table 4.5 Physical properties of different haricot bean varieties

Haricot bean Varieties	Results	
	100 Kernel weight	Bulk density
D -Awash2	25.50±1.32^{e}	0.7550±0.03^{cdefgh}
D Awash -Mittin	17.00±1.08^{ab}	0.7245±0.02^{bcd}
D Control	20.50±1.12^{c}	0.7465±0.03^{bcdef}
D-Ser 119	24.70±1.31^{e}	0.6785±0.02^{a}
D-Ser125	25.50±1.32^{e}	0.7140±0.02^{abc}
F-Ada	24.50±1.30^{e}	0.7695±0.03^{defgh}
F-Awash 2	32.00±1.88^{g}	0.8265±0.04^{i}
F-Awash Mittin	22.75±1.20^{d}	0.7935±0.04^{fhi}
F-Ser 119	28.75±1.62^{f}	0.7020±0.2^{ab}
F-ser 125	29.50±1.73^{f}	0.7420±0.02^{bcde}
K-Ada	22.75±1.21^{d}	0.7750±0.03^{efgh}
K-awash 2	18.00±1.09^{b}	0.7580±0.03^{cdefgh}
K-awash Mittin	15.75±1.02^{a}	0.7465±0.02^{bcdef}
K-Ser 119	22.00±1.18^{d}	0.7465±0.02^{bcdefg}
K-ser 125	22.75±1.21^{d}	0.7295±0.02^{bcde}

Key: Same letter in the same column is not significantly different from each other at $p<0.05$

The physical properties of different haricot bean varieties have been indicated in table 4.5 below. When it comes to 100 kernel weight, Sample with code K-Awash mittin has the lowest in 100 kernel weight (15.75g) while F-Awash 2 has the highest in 100 kernel weight which is 32.00g. Significant difference was observed in most of the samples with 100 kernel eight parameter if one-to-one and general comparison is considered at $P<0.05$ except some few samples. When it comes to 100 kernel weight comparison based on the sources where the haricot bean is collected, those sample collected from Fafan research center has relatively highest in 100 kernel weight and followed by those collected from Dolo-Ado and Kelafo research center respectively. Significant difference was observed in 100 kernel weight for same sample but collected from different sources at $P<0.05$.

Regarding the bulk density, Sample with code D-Seri 119 has the lowest in bulk density 0.6785g/ml) while F-Awash 2 has the highest in bulk density which is 0.8265g/ml. Significant

difference was observed in most of the samples with bulk density parameter if one-to-one and general comparison is considered at $P<0.05$ except some few samples. With respect to bulk density comparison based on the sources where the haricot bean is collected, those sample collected from Fafan research center has relatively highest in bulk density and followed by those collected from Kelafo and Dolo-Ado research center respectively. Relatively minor difference was observed in bulk density for same sample but collected from different sources or research center.

4.2. DISCUSSION

4.2.1. Proximate Composition of Different Haricot Bean Varieties

Table 4.1 shows proximate composition of different haricot bean varieties. The moisture content is the range of 7.915% (K-Ser 119) to 11.533% (F-Adi) variety. The moisture content of haricot bean collected from Fafan research center is highest as compared to those collected from Dolo Ado and Kelafo research center and significantly different at $P<0.05$. When it comes those verities collected from Fafan research center, the moisture content F-Ada & F-Awash mittin are significantly different as compared to F-Awash 2, F-Ser 119 and F-Ser 125 at $P<0.05$. This might be due to difference in maturity during harvest, storage condition. According to the report of research done by Demirew Abera et.al., [53] and Shimelis and Rakshit [17], Moisture content concentration varied from 9.33 to 10.62 g/100g (DM) and 9.08 to 11.01g/100g (DM) respectively.

Ash is a part of the proximate composition and is related to mineral residue. The level of ash content represents total inorganic matters that are located in food ingredients. F-Awash 2 has the lowest (4.218%) and K-Seri 125 with highest (5.606%) ash content. This value is much higher with one reported by Demirew Abera et.al.,[53]. The difference might be due the fact that, agro-ecological difference, farming system or management difference, nature of soil and some others. Significant difference was observed between 15 different samples of haricot bean at $P<0.05$ while in between some samples there is no significant difference at $P<0.05$. Significant different at $P<0.05$, were also observed in some of the same sample but collected from different research center.

With respect to crude fiber Crude fiber is also one of the components of proximate composition that indicate the quality parameter of food. The percentage of the crude fiber suggested that diet prepared with common beans could help to ensure good gut movement of food through the gut to

provide energy and ensure break down of the food. Moreover crude fiber is known to influence production of high butyrate levels and butyrate has been linked to lower risks for cancer [58]. With respect to crude fiber content, sample with F-Seri 125 has lowest in crude fiber content (2.230%) while sample with F-Awash 2 has highest in crude fiber content which is 4.480%. Except few haricot bean samples under study (D-Seri 125 and D-Control samples), the crude fiber content of most samples is significantly different at $P<0.05$. The crude fiber content is significantly different at $P<0.05$, in some of the same sample but collected from different research centers. The crude fiber content of those fifteen haricot bean varieties were relatively lower than the one reported by Derese [59], Shimelis [17] and Demirew Abera et.al [53] were ranged from 4.86g/100g to 7.01g/100(DM), 4.66 to 5.95 g/100g (DM) and 4.40% to 8.89% respectively. This might be due difference in other content of the bean, agro-ecological difference, farm management and this result indicates that proximate composition varied from variety to variety.

Protein is another part of proximate composition which is the second dominant in percentage after carbohydrate that help in physical and mental growth and development as earlier reported by [60, 61]. From the fifteen haricot bean samples, K-Seri 125 has lowest in crude protein content (16.21%) while D-Awash mittin has the highest in crude protein content (22.18%). There is no significant difference at $P>0.05$ between samples with code K-Awash mittin and F-Awash mittin. Except these two samples, the crude protein content is significantly different in between all samples at $P<0.05$. In comparing against area from where samples were collected, those haricot bean samples collected from Dolo-Ado research center have highest crude protein content followed by Fafan and Kelafo research centers. Even there is significant different in between same sample but collected from different research centers at $P<0.05$ as indicated in table 4.1 above. In general, the data shows, haricot bean is good source of crude protein. The total protein mean content of Fifteen samples were lower than the research done by Demirew Abera et.al [53] on improved and released common bean varieties that were in the range of 18.62% - 25.98% But comparable with [17] which was in the range of 17.96g/100g to 22.07g/100g for the improved Ethiopian beans.

The ether extract fractions (crude fat or lipid) provides a very good source of energy and has a great role in transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cells processes [62, 63]. The crude fat content of fifteen haricot bean varieties is in the range of 0.54% (D-Seri 125)- 2.25% (D-Awash 2). There is no significant difference in between (K-Seri 125 & F-Adi), (D-Awash mittin & K-Awash 2) and (D-Control & K-Adi) at $P>0.05$. Except these mentioned samples, the crude fat content is significantly different in between others haricot bean samples at $P<0.05$. The crude fat content of those haricot bean samples collected from Kelafo research center is relatively highest followed by those samples collected from Dolo-Ado and Fafan Research centers respectively. Even there is significant different in crude fat content in between some of same samples but collected from different research centers at $P<0.05$. The result indicates that, haricot bean has lowest fat content in nature. The result obtained is almost similar with the research done on twenty three common bean varieties ranged between 0.84% and 2.86% by Demirew Abera et.al [53] for the Sari-I and Hundane, respectively. But the Lipid content of those common bean varieties were lower than 2.45 to 3.62 g range reported by Bhatty [64] and Siddiq [43]. Also, those Ethiopian common bean varieties showed relatively high fat content when compared with 0.60 to 2.38 g reported by several other laboratories [17, 65-67].

Carbohydrate is the first dominant part of proximate composition that has agreat role in providing good source of energyas reported in the literature by [60, 61]. K-Seri 125 has the highest in carbohydrate content (65.06%) while D-Awash mittin has the lowest in carbohydrate content which is 59.36%. (Table 4.1). Except few samples, the carbohydrate content is not significantly different at $P>0.05$. The carbohydrate content is almost comparable in between those samples collected from all three-research centers with fewer deviation when it comes to those samples collected from Fafan Research center. The result is comparable with research done by Demirew Abera et.al [53] on Twenty three Ethiopian improved and released common bean varieties which were in the range of 58.21%-66.36% and had also comparable carbohydrate content cited by [17] and [68] with the value of 62.05 to 65.08g/100g and 62.85 to 66.20g/100g respectively.

The energy in calory unit obtained from consuming 100 g haricot bean flour are indicated in table 4.1. above. Sample code with F-Awash mittin has the lowest in energy obtained

(330.2Kcal/100g) while K-Seri 119 has the highest in energy content which is 346.6Kcal/100g. Except few samples, the energy obtained is not significantly different at P<0.05. The energy obtained is almost comparable in between those samples collected from all three-research centers with fewer deviation when it comes to those samples collected from Fafan Research center as shown on table 4.1 above. Energy values were ranged from 336.42- 314.75Kcal/g, 1320.01–1375.74 kJ/100 g (DM) and 1348-1404 KJ/100g for twenty-three common beans, for eight and five common bean varieties according to research reported by Demirew Abera et.al [53], Shimelis [17] and Derese [59] respectively.

4.2.2. Mineral content of different haricot bean varieties

The mineral content of different haricot bean varieties has indicated in table 4.2above. The calcium content is in the range of 65.96mg/100g for sample code F-Adi while the highest amount of calcium is registered for sample with code D-Control which is 108.07g/100g. Except few samples, significant difference was observed for calcium in most of the sample in a one-to-one comparison at P<0.05. When it comes to the potassium content of different haricot bean samples in the range of 126.0mg/100g for sample with code D-Seri 119 and 152mg/100g for sample with code K-Seri 125 as indicated in table 4.2 above. Except few samples, there is no significant difference in potassium content if one-to-one and general comparison is considered at P>0.05. Relatively minor difference was observed in calcium and potassium content for same sample but collected from different sources or area. Range of calcium content was from 4.84-44.51mg/100g, 731.93 to 1629.77mg/kg (73.19-162.97mg/100g) according research report by Demirew Abera et.al [53], Shimelis and Rakshit [17] respectively and potassium content from 186.81-423.84mg/100g, according research report by Demirew Abera et.al [53].

Sample with code K-Awash-2 has the lowest in zinc content (3.092mg/100g) while D-Awash 2 has the highest in zinc content which is (4.798mg/100g) (table 4.2). Except few samples, there is no significant difference in zinc content if one-to-one and general comparison is considered at P>0.05. When it comes to iron content, sample with K-Seri 119 has lowest in iron content (4.306mg/100g) while sample with code D-control has the highest in iron content which is 6.571mg/100g (table 4.2). Almost all no significant difference was observed in between most of the haricot sample when it comes to iron content at P>0.05. Range of Iron content was from ND-8.58mg/100g, 61.81 to 83.99mg/kg (6.181-8.399mg/100g) and zinc content from 0.05-

1.4mg/100g, 15.36 to 28.22mg/kg (1.536-2.822mg/100g) according research report by Demirew Abera et.al [53], Shimelis and Rakshit [17]. Similarly the ranges of these results were also reported by Derese and shimelis [59] from 46.80 to 72.40mg/kg and from 14.30 (4.680mg/100g) to 22.90mg/kg (2.290mg/100g) for iron and Zinc content respectively.

4.2.3. Anti-Nutritional content of different haricot bean varieties

Regarding tannins content, Sample with code F-Adi and F-Awash-2 has the lowest in tannins content (0.30mg/g) while D-Seri 119 has the highest in tannins content which is 34.95mg/g. in addition to this, high level of tannin were obtained on sample code with K-Seri 125, K-Seri 119, D-Seri 125, F-Seri 125, F-Seri 119 and D-Control sample with values of 30.61mg/g, 28.85mg/g, 28.60mg/g, 26.31mg/g, 22.22mg/g and 13.65mg/g respectively Table 4.3. Except few samples, there is no significant difference in tannins content if one-to-one and general comparison is considered at $P<0.05$. Relatively minor difference was observed in tannins content for same sample but collected from different sources or area except some samples. The tannins content of those Seri 119 and Seri 125 are significantly different as compared other haricot bean varieties at $P<0.05$. The research done on different varieties of haricot bean indicate that, the tannins content is the range of 0.82 - 3.49mg/100g [69].

When it comes to the phytate content, D-Seri 119 has the highest in phytate content (4.050mg/g) while k-Seri 119 has the lowest in phytate content which is 2.193mg/g as shown in table 4.3 below. As indicated in table, there is no significant difference between all haricot sample collected form all centers at $p<0.05$. When it comes to phytate content comparison based on the sources where the haricot bean is collected, those sample collected from Dolo-Ado research center has relatively highest amount of phytate followed by those collected from Kelafo and Fafan research center respectively. The phytate content was in contradict with that of Phytate from 124.63- 217.44mg/100g, specifically that of Seri 119 and Seri 125 were 124.63 and 146.26mg/100g respectively, according to the research report by Demirew Abera et.al [53]. On the other hand, the research done on different varieites of haricor bean indicate that, the phytate content is the range of 122.26- 143.21mg/100g [69].

4.2.4. Functional properties of different haricot bean varieties

Water absorbing capacity, in the range of 1.270-2.150g/g K-Seri 125 and while D-control respectively. Except between few of the samples, there is no significant difference in water

absorbing capacity if one-to-one and general comparison is considered at P<0.05. Almost no difference was observed in water absorbing capacity for same sample but collected from different sources. It is known that water binding by starches is a function of several parameters including size, shape, conformational characteristics, steric factors, hydrophilic-hydrophobic balance in the starch molecule, lipids and carbohydrates associated with the proteins, thermodynamic properties of the system (energy of bonding, interfacial tension, etc.), physicochemical environment (pH, ionic strength, vapor pressure, temperature, presence/absence of surfactant etc.), solubility of starch molecules and others [70].

When it comes to the oil absorbing capacity, Sample with code F-Seri 125 has the lowest in oil absorbing capacity (0.860) while D-control has the highest in oil absorbing capacity which is 1.875 as indicated in table 4.3. above. Except between few of the samples, there is no significant difference in oil absorbing capacity if one-to-one and general comparison is considered at P<0.05. According to the research done on haricot bean flour by Shimelis and Rakshit [17], the water and oil absorbing capacity haricot bean flour in the range of 2.14- 2.45g/g and 2.49-3.52g/g respectively. Sosulski and Fleming [71] reported that water absorption by soybean flour, soybean concentrate, sunflower flour and sunflower concentrate to be 2.4, 3.6, 1.8, and 3.9 g/g; respectively. The result looks contradicting with this particular research.

4.2.5. Physical properties of different haricot bean varieties

The physical properties of different haricot bean varieties have been indicated in table 4.5 above. When it comes to 100 kernel weight, it was in the range of 15.75g-32.00g for K-Awash mittin and while F-Awash 2 respectively. Significant difference was observed in most of the samples with 100 kernel eight parameter if one-to-one and general comparison is considered at P<0.05 except some few samples. Significant difference was observed in 100 kernel weight for same sample but collected from different sources at P<0.05. With respect to the bulk density, Sample with code D-Seri 119 has the lowest in bulk density 0.6785g/ml) while F-Awash 2 has the highest in bulk density which is 0.8265g/ml. Significant difference was observed in most of the samples with bulk density parameter if one-to-one and general comparison is considered at P<0.05 except some few samples. Relatively minor difference was observed in bulk density for

same sample but collected from different sources or research center. The difference both in 100 kernel weight and bulk density is may be due to varietal difference.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The research was done on six haricot bean varieties with fifteen total samples collected from three research center under Somali Region Pastoral and Agro-Pastoral research institute (SoRPARI). From the data obtained we conclude that, the ash content of all sorghum varieties is high which an indication for their high mineral content. The moisture content of all varieties below 12% which is an indication for their better shelf stability. The fiber, and carbohydrate is also up to the standard which indicates that, haricot bean is good sources these components. The protein content of all haricot bean varieties is very high which indicate haricot bean is the goof source protein which in turn help in fighting against protein energy malnutrition. But fat content of all haricot bean varieties is very low. When it comes to minerals content, all varieties of sorghum have high amount calcium, iron, zinc and potassium. Regarding the anti-nutritional content of haricot bean, especially sample with varieties name Seri 119 and Seri 125 is high both tannins and phytate content.

Generally, haricot bean with variety name Awash mittin is good in almost all parameter such as protein, ash, fiber, mineral and anti-nutritional content. Significant different were observed in all varieties for both macronutrient, micronutrients and Anti nutritional content of different haricot bean varieties where agro-ecology, varietal difference, soil nature, farming management are the main expected factors for their difference.

5.2. Recommendation

Based on the data obtained from this research we forward the following recommendation;

- The data shows that, all haricot bean varieties have high amount of protein, ash and fiber, therefore, we recommend possibility of using haricot bean by mixing with other staple cereals such maize, sorghum in the preparation meal so that the nutritional content can be enhanced.
- We specifically recommend, haricot bean with varieties name *Awash mittin* to be used in the preparation different food product such as complementary food, weaning food as it has high amount of protein, ash, minerals and less amount of anti-nutritional factors.

- We recommend also, intercropping of haricot bean especially, *Awash mittin* as part of fighting against malnutrition.
- Further research should be done to identify the exact ration of mix and to identify the nutritional content of prepared products for different age group.
- Further research should be done by including more nutritional parameters such as more minerals, vitamins and also to see the impact of soil nature on haricot bean mineral and other nutritional content.
- Further research and then technologies should be introduced on mean to minimize anti-nutritional content especially when it comes to haricot bean with *Seri 119* and *Seri 125* varieties.
- Government and other actors on haricot bean and other different grain should do more on the promotion of haricot bean
- Platform should be established which put all haricot bean and other grains actors like researchers, investors, government bodies and NGOs.

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Annex: Photo during sample preparation and Laboratory demonstration













