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ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

CENTER FOR FOOD SCIENCE AND NUTRTION

Effect of processing on nutrient composition, antinutritional factors, protein digestibility and bioaccessibility of selected minerals of *Lablab purpureus* (L.) Sweet grown in Ethiopia

A thesis Submitted to the Center for Food Science and Nutrition of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science in Food Science and Nutrition

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Addis Ababa, Ethiopia

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Declaration

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List of Abbreviations

AAS Atomic Absorption Spectrophotometer

ANOVA Analysis of Variance

AOAC Association of Official Analytical Chemists

APO Asian Productivity Organization

CSA Central Statistical Agency

DMRT Duncan's multiple range test

FAO United Nations Organization for Food and Agriculture

FGD Focus Group Discussion

IVPD *In vitro* protein Digestibility

NRC National Research council

PEM Protein Energy Malnutrition

PGRFA Plant Genetic Resources for Food and Agriculture

SNNPR Southern Nations, Nationalities and Peoples Region

SPSS Statistical Package for the Social Science

WPDS World Population Data Sheet

Abstract

Lablab purpureus is one of the underutilized and less studied legumes of arid and semi-arid lands. It is a good source of protein and other nutrients. This study was conducted to determine the effects of some commonly applied traditional processing methods on antinutritional factors, in vitro protein digestibility and mineral bioaccessibility of Lablab purpureus seeds. Proximate composition was determined according to AOAC method. Selected minerals (Ca, Fe, Zn and P) were determined using Atomic Absorption Spectrophotometer (AAS). In vitro protein digestibility and bioaccessibility of minerals were conducted under simulated gastro-intestinal conditions using intestinal enzymes (pepsin and pancreatin). To investigate the effects of traditional processing methods; soaking (for 6, 12 and 18h), dehulling, boiling and germination (for 24, 36 and 48 hrs) were applied on the two varieties of lablab (black and red) seeds. The black variety of lablab raw seeds had a mean of 7.1 % Moisture, 3.4 % ash, 2.5 % fat, and 25.03 % crude protein. There is no significance ($p>0.05$) difference among the two varieties in terms of the above nutritional compositions. The crude fiber content was 13.7 % in black and 10.6 % in red varieties. The total CHO content of raw seeds in black and red varieties was 55.37 % and 58.77 %, respectively. The total energy of 344.1 Kcal in black and 357.32 Kcal in red varieties was recorded. Among the traditional processing methods, dehulled-boiled samples showed higher increment in protein content (22 %). The total CHO and energy contents were higher in dehulled samples (65.75 and 389.01 Kcal, respectively) over their respective controls (55.37 and 344.1 Kcal).The mean of In vitro protein digestibility in raw samples was 70.91% in black and 71.9% in red varieties. All traditional processing methods applied in the current study significantly ($p<0.05$) increased in vitro protein digestibility. Germination showed the higher increment (6.8- 28.6 %). The mineral content of the black variety was 105.59 of Ca, 3.72 of Fe, 3.89 of Zn and 432.75 mg/100g of P in raw sample. There is no significance ($p>0.05$) difference among varieties in Fe and Zn content. The red variety had Ca content of 102.06 mg/100g and P content of 461.15 mg/100g. Dehulling and dehull- boiling samples significantly ($p<0.05$) decreased calcium content (50.49 and 56.03 mg/100g, respectively in black). All traditional processing methods significantly ($p<0.05$) decreased Fe and Zn contents. Dehulling and germination increased phosphorus content up to 21 and 12% respectively. Bioaccessibility of minerals in raw seeds of black variety was 45.39 % for Ca, 34.04 % for Fe and 54.27 % for Zn. There is no significance ($p>0.05$) difference among varieties in bioaccessibility. All traditional processing methods significantly ($p<0.05$) increased bioaccessibility of Ca, Fe and Zn. Dehulling showed the highest increment in Ca bioaccessibility (up to 84 %).The phytic acid content of raw seeds in black and red varieties was 320.83 and 338.33 mg/100g, respectively. The tannin content was 1.32 in black and 1.48mg/100g in red varieties. The total alkaloid content was found to be 1.3% in black and 1.5% in red varieties. All traditional processing methods significantly ($p<0.05$) reduced antinutritional factors (2.16-20 % in phytic acid, 38 %-un detected level in tannin and 13.33-54 % in total alkaloids). Boiling of unsoaked, soaked and dehulled samples showed the highest reduction in phytic acid content (9-20%). The tannin content was reduced to the extent of undetected level due to dehulling and dehulled-boiling. Boiling of soaked and dehulled samples showed the highest reduction in total alkaloids (47-54%). Therefore, soaking, dehulling, boiling and germinating hold a good potential for improving the nutritional value of lablab seeds by reducing antinutritional factors such as phytic acid and tannins thereby enhancing its utilization. Dehulling and germination of seeds are suggested to be promoted for improved protein digestibility and mineral bioaccessibility.

Key words: *Lablab purpureus*, traditional processing methods, nutrient composition, antinutrional factors, protein digestibility, bioaccessibility

1. Introduction

1.1. Background of the study

Food legumes are crops of the family Leguminosae also called Fabacae. They are mainly grown for their edible seeds, and thus are also named grain legumes. They occupy large cropped areas worldwide (Iqbal *et al.*, 2006).

Grain legumes are important sources of significant amounts of proteins, carbohydrates, fiber, vitamins and some minerals. They are used as source of food for animals and human in many parts of the world (Sebastia *et al.*, 2001; Osman, 2007). They are obtained relatively cheap for use as source of proteins in comparison with animal sources. Moreover, they are fairly good sources of thiamin, niacin, calcium and iron (El-Adawy *et al.*, 2000; APO, 2003). Predominantly, their consumption is wider in developing countries in Asia, Africa and South America (Frias *et al.*, 2004). In these countries, legumes play a major role as protein source (El-Adawy *et al.*, 2000; APO, 2003). Besides being a good source of nutrition, there is considerable interest in the relationship between plant-based diets and prevention of certain human diseases, in which increased levels of radicals are implicated. Likewise, legumes seem to be responsible for improving health and can prevent chronic diseases (Frias *et al.*, 2004).

Grain legumes are used as pulse with cereals, grown in both tropical and temperate regions of the globe. They enhance the protein content of cereal-based diets and may improve the nutritional status of the cereal-based diets. Cereal proteins are deficient in certain essential amino acids, particularly lysine. On the other hand, legumes have been reported to contain adequate amounts of lysine, but are deficient in S-containing amino acids (methionine, cystine and cysteine) (Iqbal *et al.*, 2006).

Legumes play an important role in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins (Iqbal *et al.*, 2006).On average, legumes contain 20-25% of protein, which is two to three folds higher than the content in cereals. Therefore, they can be considered as leading candidate for protein supply to poor areas of the world (Mohamed *et al.*, 2006).

However, their role appears to be limited because of several factors including low protein and starch digestibility, poor mineral bioavailability and high antinutritional factors. (Ghavidel and Prakash, 2006).

Legumes are well adapted to a wide range of climates and environmental conditions. Of the thousands known legume species, only few have been extensively promoted and used. Many other potential legumes are still marginally known (Osman, 2007).

Lablab purpureus is one of underutilized and less studied legumes of arid and semi-arid lands (Osman, 2007).

Lablab purpureus (L.) Sweet is a plant species in the family Fabaceae. It is known by several common names across the world as, hyacinth bean (Brazil), dolichos bean, seim bean; and Australian Pea and lab-lab bean (Australia) (Koile, 2014) and Amora guaya, (Ethiopia) (Lost crops of Africa, 2006). Of the two hundred types of lablab recognized, only two varieties, Rongai and highworth, are available commercially (Murphy *et al.*, 1999).

The rongai variety was derived from the Rongai district of Kenya and was released in New South Wales, Australia in 1962. 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 (Murphy *et al.*, 1999).

The highworth variety originated from Coimbatore, South India and is morphologically similar to rongai. Highworth has a purple band near the leaf axil, purple flowers and black seeds. It 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 (Murphy *et al.*, 1999).

Lablab seed is classified as potential source of protein that has not been explored yet. Studies on nutrient composition showed that the seed is good source of protein, carbohydrate and energy (Osman, 2007).

1.2. Statement of the problem

In Ethiopia, a sizable proportion of the population (those of low income or subsistence status) get less than the FAO recommended averages daily calorie ration of 2200 Kcal (WPDS, 2007). This could lead to severe protein energy malnutrition in the country.

Protein energy malnutrition (PEM) is a major nutritional syndrome affecting more than 170 million preschool children and nursing mothers in developing countries and it is the most important documented forms of malnutrition in Ethiopia (Iqbal *et al.*, 2006; Mulugeta *et al.*, 2010).

Provision of adequate proteins from animal sources is difficult and expensive. An alternative for improving nutritional status of the people is to supplement the diet with plant proteins. Attention, therefore, has to be directed to the nutritional evaluation of proteins from plant sources (Iqbal *et al.*, 2006).

Legumes are relatively cheap yet reach source of proteins and are important in alleviating protein energy malnutrition. Though legumes are important sources of dietary proteins for human, their acceptability and utilization have been limited due to presence of relatively higher concentration of antinutritional factors. Nutritional quality is affected by antinutritional factors such as phytate and tannins that interact with the nutrients in the intestinal tract and inhibit their bioavailability (Audu *et al.*, 2011).

In Ethiopia, legume grains are processed and consumed in a variety of forms, depending on cultural and taste preferences. The most common domestic methods of processing include soaking, cooking, germination and fermentation. These processing in legume may bring about changes in the contents of antinutritional factors and improve bioavailability of protein, carbohydrate and minerals and this needs to be investigated. In Ethiopia, different authors wrote about *L.purpureus* intercropping aspect (Alemseged *et al.*, 1996; Hassen *et al.*, 2006; Bekele *et al.*, 2013) but there is no enough data about *L.purpureus* from nutritional point of view. With this perspective in mind, the present study was executed to determine nutritional composition, changes in antinutritional factors, *in vitro* protein digestibility and bioaccessibility of Ca, Fe and Zn during processing of *L. purpureus* local varieties.

1.3. Objectives

1.3.1. General Objectives

To assess the effect of processing on nutrient composition, level of antinutritional factors, protein digestibility and bioaccessibility of selected minerals of *L. purpureus* seeds grown in Ethiopia.

1.3.2. Specific Objectives

- To determine the nutritional and antinutritional compositions of grains of local *L. purpureus* varieties,
- To investigate effects of traditional processing methods on antinutritional factors of *L.purpureus* grains,
- To Assess effects of traditional processing methods on protein digestibility of *L. purpureus* grains,
- Assess effects of traditional processing methods on bioaccessibility of Ca, Fe and Zn of *L. purpureus* seeds.

1.4. Significance of the study

As indicated above, in Ethiopia, there is no enough documented data on nutritional aspects of *Lablab purpureus* seeds. Therefore the findings of this research will;

- Enable people who consume lablab to be aware of their nutritional values and antinutritional factors of the species to make informed choices.
- Helps to suggest the effective processing method to improve nutritional value of the seed
- Helps to promote production and consumption of the seeds in Ethiopia.
- Provide information for future researchers and students who are interested to work on lablab species.

2. Literature Review

2.1. Botanical description of *lablab purpureus*

L. purpureus is a vigorously trailing, twining herbaceous plant. Stems are trailing to upright, reach to 3-6m in length and are robust. Leaves are large and trifoliate with long and slender petioles, the leaflets having a broad ovate-rhomboid shape measuring 7 to 15 cm long, thin and acute at apex. The dorsal side of the leaf is smooth with the underside being hairy. The inflorescence is lax, fascicled, of many-flowered racemes on elongated peduncles. Flowers white (in rongai) and blue or purple (in highworth), on short pedicels. The pods are 4-5 cm long broadly scimitar shaped, smooth and beaked by the persistent style, containing two to four seeds. Seeds in rongai buff or pale brown colored, ovoid, laterally compressed, with a linear white conspicuous hilum, 1.0 cm long x 0.7 cm broad, seeds of highworth black with a linear white hilum (Murphy *et al.*, 1999).

Lablab Purpureus is an annual short-lived perennial vine, which originated from Africa and it's cultivated throughout the tropics. It grows throughout the tropics and subtropics, ranging from 30° South to 30° North Latitude; and altitude ranging from zero to 2100 meters above sea level. Presently, lablab is common in Africa, extending from Cameroon to Swaziland and Zimbabwe, through Sudan, Ethiopia, Uganda, Kenya and Tanzania. As early as 1819 GC, seeds of lablab from Egypt were planted in the Botanical Gardens in Sydney, New South Wales. However, it was not until after the release of the forage cultivar "Rongai" in 1962, that lablab became widely used as forage in Australia. Currently, lablab is one of the major leguminous forage and green manure crop in this area of the world (Murphy *et al.*, 1999).

Lablab has been widely distributed to many tropical and subtropical countries where it has become naturalized. In South and Central America, East and West Indies, Asia, China and India, lablab is grown as an annual or a short-lived perennial. In these areas, the seed and immature pods are used for human food while the herbage is used as green manure, for erosion control, and as a feed supplement for cattle grazing mature pasture in the dry season (Murphy *et al.*, 1999).

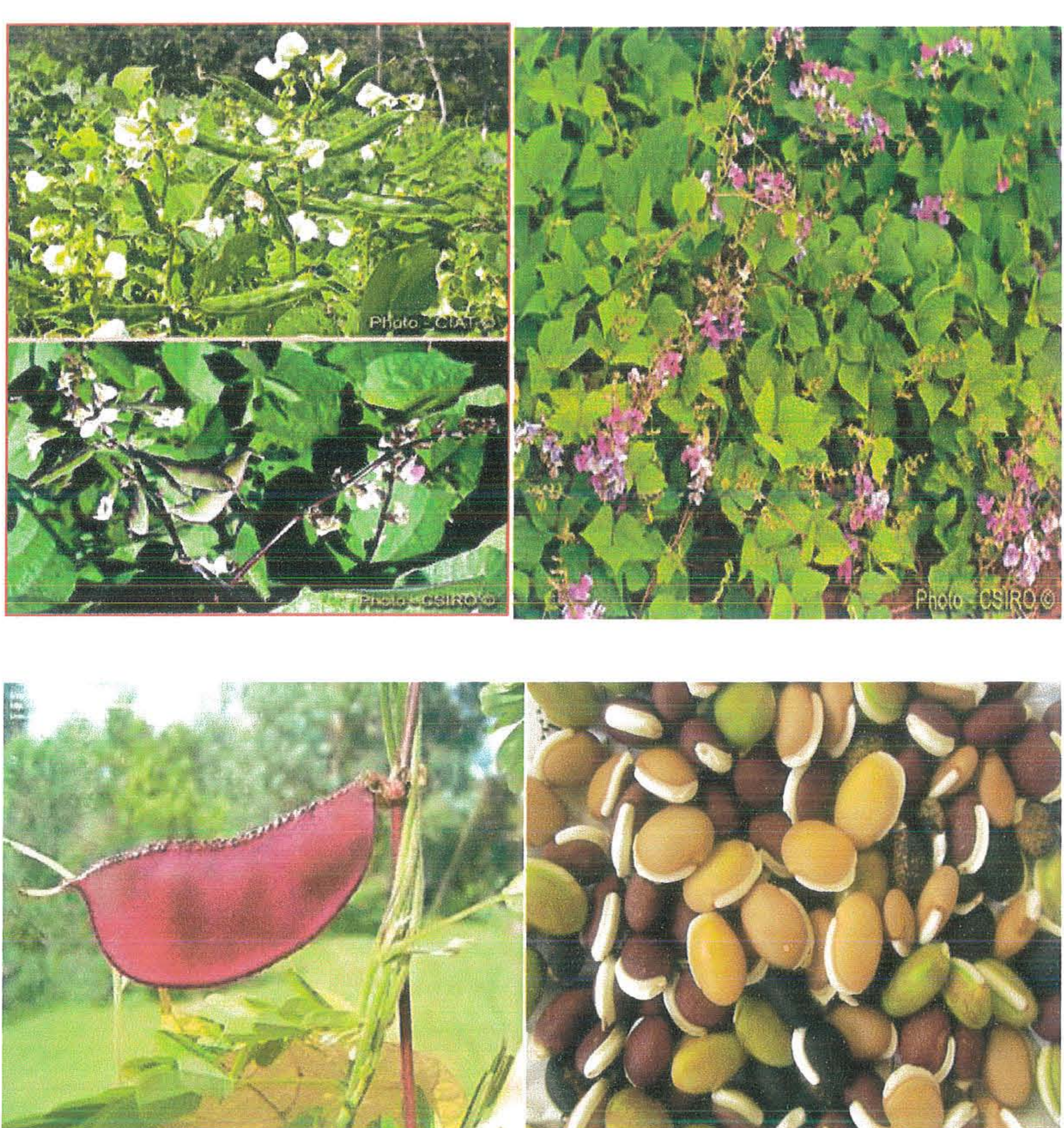

Fig 1. Morphology of leaves, flowers, pod and seeds *of lablab purpureus*

2.2. Agronomic characteristics of lablab purpureus

Lablab purpureus can be cultivated as a pure crop or mixed with crops such as finger millet, groundnut and maize (Koile, 2014). It is a summer growing, twining herbaceous annual or short duration perennial which is generally resistant to disease and insect attack. Lablab is drought

tolerant once established and grows in areas receiving annual rainfall between 200 and 2500mm. It is also tolerant to high temperature ranging between 18 and 30°C while lower temperature reduces plant growth; leaves begin to drop at -2°C but the plant can survive frost for a limited period. Lablab grows in a wide range of soils from deep sands to heavy black clays and can tolerate pH ranges of 4.5-7.5 provided drainage is good. The growth period can vary from approximately 75 to 300 days, with maximum vegetative growth reached 130 days post germination under conducive conditions (Madzonga *et al.,* 2014).

2.3. Uses of *Lablab purpureus*

Lablab is a dual-purpose legume. It is traditionally grown as a pulse crop for human consumption in south and Southeast Asia and Eastern Africa. As food crop; the leaves are eaten raw or cooked as spinach, the green pods are either cooked as vegetable or eaten raw, the flowers are eaten raw or steamed, dried seeds should be boiled in two changes of water before eating since they contain dangerous chemical compounds. In Africa, it is considered as traditional food because of its ability to promote food security and foster development in rural communities, when sold as a food crop like the normal beans (Koile, 2014).

Lablab is also used as an ornamental plant, since they are climbers and they produce flowers of various colors, from purple to white. They have been used as screen on fences or trellis, especially in USA. It is used as forage for livestock, especially the dairy animals to improve birth rates and increase milk production. In Zimbabwe for example, the use of lablab has improved kid birth rates in dairy goats and milk yields. Lablab is a good source of green manure due to its dense foliage which has high nitrogen content. It is also used as cover crop in mixed farming with crops like maize and sorghum as it; reduces moisture loss, soil erosion and supplies nutrients inform of fixing nitrogen into the soil (Koile, 2014).

2.4. Nutritional Composition of *Lablab purpureus*

Different studies have been undertaken regarding nutritional composition of *L. purpureus* cultivars and showed that it is a good source of protein and carbohydrate. The crude protein content of lablab herbage ranges from 10% to 22% on dry matter basis, with a mean of 17%. Leaf crude protein varied from 14.3% to 38.5%, while the stem crude protein content ranges

from 7.0% to 20.1%. Lablab follows familiar growth pattern as protein content drops with maturity of herbage (Murphy *et al.*, 1999).

When they are dry, beans become concentrated sources of nutrients. As they do not contain much water, the energy they provide is higher than in most other vegetables (Hedges *et al.* 2008). The moisture content of lablab seed cultivars ranged from 7.4 to 10.8 g/100g (Mortuza and Tzen, 2009). As indicated by Kalpanadevi and Mohan (2013), the moisture content of *Dolichos lablab* var. *vulgaris* (dark brown and pale brown colored seed coats) was 6.98 and 7.80 g/100g respectively. Osman (2007), reported that the protein content of lablab seed was 26.9 g/100g. In another study, the crude protein content of *L. purpureus* seed was reported to be 24.75g/100g (Arinathan *et al.,* 2009). According to Mortuza and Tzen (2009), crude fat and total energy of lablab seed varieties range from 3.15 to 3.84 g/100g and 1318 to 1394 kJ/100g, respectively in dry matter basis. The caloric values and fat content in dry weight basis of lablab seeds range from 1606.08 to 1609.21 KJ/100g and 5.32 to 5.64 g/100g (Kalpanadevi and Mohan, 2013).

Table 1 Proximate composition of raw *L. purpureus* varieties in dry matter basis

Nutrient	Highworth	Rongai Brown	Rongai White	Mean
Dry matter (g/100g)	90.27	93.77	94.11	92.72
Crude protein (g/100g)	34.33	24.88	28.38	29.20
Crude fiber (g/100g)	7.22	9.73	8.04	8.33
Crude fat (g/100g)	5.87	3.00	2.99	3.95
Ash (g/100g)	4.77	6.85	8.82	6.81
GE (kcal/kg)	3771	3831	3791	3798

Source: Shaahu *et al.*, 2015

Lablab seeds are also rich in different nutrients including minerals such as magnesium, copper, zinc, iron, potassium and phosphorous, and vitamin B1 (thiamine) (Hedges *et al.*, 2008). According to Arinathan *et al.*, (2009), lablab bean contain 520.00 mg/100g of calcium, 268.12 mg/100g of phosphorus, 7.90 mg/100 of Iron and 2.14 mg/100g of Zinc. Shaahu *et al.*, (2015) also showed mineral compositions of different lablab varieties (Table 2).

Table 2 Mineral composition of *L. purpureus* varieties

Mineral	Highworth	Rongai Brown	Rongai White	Mean
Na(%)	0.03	0.03	0.03	0.03
P(%)	0.34	0.28	0.36	0.33
Ca(%)	0.16	0.16	0.16	0.16
Mg(%)	0.09	0.06	0.07	0.07
Fe (mg/kg)	56.00	53.65	53.90	54.52
Mn (mg/kg)	38.45	36.65	38.45	37.85
Cu (mg/kg)	12.45	15.55	16.75	14.92
Zn (mg/kg)	62.65	45.40	50.05	52.70
Cl (mg/kg)	2220	2191.00	2270.50	2227.17

Source Shaahu *et al*, 2015

2.5. Anti-nutritional factors

Anti nutritional factors are chemicals which have been evolved by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value (Habtamu *et al.*, 2014).

The nutritive value of food legumes is compromised by the presence of several antinutritional and toxic substances including oligosaccharides, enzymatic inhibitors, phytates, polyphenols, lectins etc. (Egounlety *et al.*, 2003).

Phytate or Phytic acid, which is also known by other names as inositol hexakisphosphate or inositol polyphosphate is a principal storage form of phosphate, ubiquitously distributed in plants, particularly in cereal grains and in legumes. The effects of phytate in human and animal nutrition are related to the interaction of phytic acid with proteins, vitamins and several minerals, and thereby restrict their bioavailability (Afify *et al.,* 2011). Phytic acid is said to chelate mineral cations and proteins, forming insoluble precipitates, which lead to reduced bioavailability of trace mineral cations and reduced digestibility of proteins (Abdelseed *et al.*, 2011). Phytic acid binds trace elements and macro-elements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract and making the dietary minerals unavailable for absorption and utilization by the body. It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis. Moreover, the phosphorus in phytate has been considered to be largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyse phytate in the small intestine. Ramakrishna *et al.*, (2006) and Osman, (2007) respectively reported phytic acid content of lablab seed as 820 mg/100g and 605.4 mg/100g. However, Kala *et al.*, (2010), reported 314 to 421mg/100g of phytic acid in different lablab varieties.

Tannins are other antinutritional factors. They are heat stable and reduce protein digestibility in animals and humans, either by making protein partially unavailable and/or inhibiting digestive enzymes and increasing fecal nitrogen. Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase and lipase; decrease the protein quality of foods and interfere with dietary iron absorption (Habtamu *et al.*, 2014). Tannins are known to inhibit the activities of digestive enzymes and hence the presence of even a low level of tannin is not desirable from nutritional point of view (Arinathan *et al.*, 2009). There are different reports on the level of tannin in lablab seeds. Ramakrishna *et al.*, (2006) reported 0.85 mg/100g, Osman, (2007) 0.42% and Kala *et al.*, (2010), from 0.23 to 0.40 g/100g in seeds of different lablab verities.

Alkaloids are one of the largest groups of chemical compounds synthesised by plants and generally found as salts of plant acids such as oxalic, malic, tartaric or citric acid. Alkaloids are known to pose negative effect on nervous system and disrupting or inappropriately augmenting electrochemical transmission. For instance, consumption of high tropane alkaloids will cause

rapid heartbeat, paralysis and in worst cases may lead to fatality (Habtamu *et al.*, 2014). Shaahu *et al.*, (2015), showed that the content of total alkaloids in lablab seed varieties ranges from 2.17 to 2.25%.

Trypsin inhibitor and **chymotrypsin inhibitor** are protease inhibitors occurring in raw legume seeds. Trypsin inhibitors that inhibit the activity of the enzymes trypsin and chymotrypsin in the gut, thus preventing protein digestion, are found in many plant species mainly in different grain legumes (Habtamu *et al.*, 2014). Tripsin inhibitors when ingested by human in large quantity disrupt the digestive process and may lead to undesirable physiological reactions (Kalpanadevi and Mohan, 2013). The content of Trypsin inhibitors in lablab seeds was ranged from 29.28 to 31.24 TIU /mg protein (Kalpanadevi and Mohan, 2013).

Cyanogenic glycosides belong to the products of secondary metabolism, to the natural products of plants. They are HCN-producing phytotoxins; HCN is a powerful and a rapidly acting poison (Cho *et al.* 2013). Hydrogen cyanide content of lablab seeds ranged from 0.24 to 0.31 mg/100g (Kalpanadevi and Mohan, 2013).

2.6. Effect of different processing methods on the level of anti-nutritional factors in legumes

The use of some processing methods is known to reduce or eliminate antinutritional factors, which affect the nutritional and food quality of legumes (Udensi *et al.*, 2007).

Soaking: Soaking caused 42.82–48.91% and 10.22–19.85% reduction in phytic acid content and trypsin inhibitor activity (TIA) respectively (Khattab *et al.*, 2009). Duhan *et al.*, (2002), showed that, after 6 hours of soaking of pigeon peas, phytic acid was reduced by 7%. With an increase in the period of soaking, further reductions in phytate content of pigeon peas were noticed. As the period of soaking increases, there was proportionally higher reduction in the level of phytate content (Duhan *et al.*, 2002). After soaking for 12–14 hrs the tannin content was reduced by 54.6%, 72.2% and 30.9% in soybean, cowpea and ground bean respectively (Egounlety *et al.*, 2003).

De-hulling: Dehulling resulted in a decrease of 76% in verbascose, 17% in stachyose and 56% in raffinose of the cowpeas (Wang *et al.*, 1997). There was 26% reduction of phytic acid

observed in pre soaked dehulled pigeon pea over the control (Duhan *et al.*, 2002). According to Egounlety *et al.*, (2003), tannins were not detected in dehulled soybean, cowpea and ground bean.

Boiling: Boiling generally inactivates heat sensitive proteins such as trypsin inhibitors as a result of denaturation (Khattab *et al.*, 2009). Dry heating or deep frying for less than 10 min was found to inactivate 40-50% of trypsin inhibitors. Samples subjected to heat treatment at temperatures above 100°C for longer duration in the presence of water (steaming or boiling) did not show any residual trypsin inhibitor activity (Wang *et al.*, 1997). Pressure cooking of soaked, as well as soaked-dehulled seeds, caused a greater loss in phytic acid, which was reduced by 9, 21 and 38 percent after pressure cooking of unsoaked, soaked and soaked-dehulled pigeon pea seeds, respectively (Duhan *et al.*, 2002). Boiling for 60 min in distilled water resulted in 75% tannin and 81.3% HCN reduction of cowpea (Udensi *et al.*, 2007). Iorgyer *et al.*, (2009), reported that tannin reduction with 30, 40 and 60 minutes boiling of pigeon pea was 15.29, 23.53 and 47.06%, respectively.

Germination: Germination is widely used in legume and cereal grain processing to increase palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytate and protease inhibitors. During germination a marked increase in phytate-degrading activity with a concomitant decline in phytate content was observed (Afify *et al.*, 2011). After 48 hrs of germination, phytic acid content was 39 % less than that of the control pigeon pea seeds (Duhan *et al.*, 2002). According to Ghavidel and Prakash, (2006), there was significant ($P<0.05$) reduction (18–21%) in phytic acid content in germinated samples as compared with raw garins of green gram, cowpea and lentil. Germinating for 24 hrs resulted in 45.9 % reduction in tannin content of lablab seeds (Ramakrishna *et al.*, 2006).

Fermentation: Fermentation is also another food processing technology that is used to increase nutritional quality and reduce or remove undesirable compounds (Fries *et al.*, 2004). Fermentation with *Rhizopus oligosporus* starter culture of soybean (for 36 h), cowpea (30 h) and ground bean (36 h) respectively reduced phytic acid by 30.7%, 32.6% and 29.1% (Egounlety *et al.*, 2003).

Table 3: Effect of processing methods on antinutritional factors of *L. purpureus*

	Trypsin inhibitor (TIU/mg)		Phytic acid (mg/100g)		Tannins (%catechin)
		% Reduction		% Reduction	
Raw	28.96	100	605.39		0.42
Soaking	27.14	6.30	471.07	22.19	0.54
Cooking	9.66	66.66	309.68	44.85	1.08
Roasting	22.23	23.05	237.95	60.69	0.60
Autoclaving	25.46	12.09	288.84	52.29	0.59
Germinated	23.354	19.39	309.10	48.94	1.27

Source: Osman, (2007)

2.7 Effect of different processing methods on in vitro protein digestibility of legumes

Soaking: it increased *in-vitro* protein digestibility of legume seed flours. The *in vitro* protein digestibility of raw soybean seed flour was 60.4% and after 9 hrs soaking in 0.5% $NaHCO_3$, it was improved to 68.5% (El-Adawy *et al.*, 2000). According to Mubarak, (2005), *in vitro* protein digestibility of raw mung bean seeds was 80.2% and after soaking of 12hr, it was increased to 87.4%.

De-hulling: Mubarak, (2005), indicated that *in vitro* protein digestibility of mung bean seeds was improved from 80.2% to 84.3% during dehulling. According to Ghavidel and Prakash, (2006), on dehulling, the percent *in vitro* protein digestibility of samples increased by 27.4%, 21.0%, 20.9% and 20.1% in green gram, cowpea, chickpea and lentil respectively over the control.

Boiling: *In vitro* protein digestibility was improved by boiling; it was 83.61% in raw chick pea and changed to 88.52% after boiling (El-Adawy, 2002). Another study showed that the protein digestibility of raw mung bean seeds was 80.2 % and it was improved to 87.8% after the seeds were boiling (Mubarak, 2005).

Germination: percent of *in vitro* protein digestibility improved following germination at different temperatures; the longer the period of germination, the greater the protein digestibility of the sprouts was observed in pearl millet grain. And it was improved by 35%, 44% and 46% on 36, 42 and 48hr. of germination at 30 ^{0}c respectively (Kumar *et al.*, 1993). In another study *in vitro* protein digestibility of 83.61% and 87.63% in raw and germinated chickpea seeds respectively was observed (El-Adawy, 2002). Germination significantly increased *in-vitro* protein digestibility of *Dolicose lablab* been from 88.17% to 92.27% (Osman, 2007). After 72hr of germination, IVPD of mung bean was improved by 11.1% over the control (Mubarak, 2005).

2.8. Effect of different processing methods on mineral bioaccessibility of legumes

Traditional food processing methods of legumes can affect stability and bioavailability of nutrients and sensory acceptability of processed foods. Furthermore, these processes could enhance the activities of bioactive antioxidant compounds such as vitamins E and C (Frias *et al.*, 2004).

Soaking: six hrs soaking has improved extractability of calcium by 2%, phosphorus 2% and iron 3% as compared with the control. As the period of soaking was prolonged, an enhancement in calcium, phosphorus and iron extractability was observed. After 18 hrs of soaking; calcium, phosphorus and iron extractability respectively improved from 51.5, 33.3 and 36.4 in unprocessed seeds to 54.4, 36.3 and 42.3% in soaked seeds of pigeon peas (Duhan *et al.*, 2002).

Dehuling: on dehulling, the percent bioaccessible iron and calcium increased by more than 100% in chickpea, green gram, cowpea and lentil (Ghavidel and Prakash, 2006).

Boiling: ordinary cooking improved calcium extractability by 9.7% and iron by 15.8%, as compared with the raw pigeon pea grains. On ordinary cooking of soaked and soaked dehulled pigeon pea seeds, an enhancement in calcium (13.6 and 20.8 % respectively) and iron (29.5 and 42.9 % respectively) extractability was observed (Duhan *et al.*, 2002).

Germination: it increased Fe, P and Zn contents by 2.46%, 5.75% and 14.12%, respectively in chickpea (El-Adawy, 2002). Bioaccessibility of P, Ca, Fe and Zn showed improvment following germination of pearl milet. The enhancement was greater at longer duration of germination.

Bioaccessibility of Ca and Fe increased by 100% or more and 50% increase in Zn after 48 hr germination at 30 and 35°c (Kumar *et a.*, 1993). Percent bioaccessible iron of germinated seeds increased significantly ($P<0.05$) by 64.6%, 67.8%, 75.8% and 81.3% in chickpea, green gram, cowpea and lentil respectively over the control samples (Ghavidel and Prakash, 2006).

2.9. Pulse crops (legumes) production in Ethiopia

Although pulses have many desirable characteristics in terms of nutrition and environmental benefits, in most parts of Ethiopia they are considered secondary crops. As a secondary crops category, pulses do not receive investment resources and policy attention from governments as do the cereal crops, which are often, considered food security crops. Studies have shown that pulses on an average contribute 15% of total protein intake, account for 13% of the cultivated land and 8.5% of the total crop production in Ethiopia. In recent years pulse sector shows a steady increase in productivity and total volume of production despite the amount of land allocated show a slight decline (Tewodros, 2013).

Pulses are grown throughout the country. However, the lion share production is concentrated in the Amhara and Oromiya regions, which together account for 92 percent of chickpea production, 85 percent of faba bean production, 79 percent of haricot bean production, and 79 percent of field pea production. The SNNP stands third in overall production of pulses by producing 10% of the faba bean, 18% of the filed pea, 3% of chickpea and 15% of haricot bean (Tewodros, 2013).

Lablab purpureus is known in Konso (southern Ethioipa) and around Gonder and Gojam in central and northern areas. Considerable agro morphological diversity is seen in this species for plant height, leaf size, flower and seed color, number of seeds per pod, seed size and shape and seed yield (Institute of Biodiversity Conservation, 2007).

3. Materials and methods

3.1. Collection of ethnobotanical information on *Lablab purpureus*

Ethnobotanical information on local lablab seeds was collected in Konso Woreda. One Focus Group Discussion (FGD) was conducted in Tachignaw Doketo kebele where lablab is widely grown. FGD participants were represented from four groups of the society. These were agricultural office representatives, women familiar in lablab traditional processing, local community consuming different products of the crop and farmers. Discussion points were prepared for the FGD (annex 1).

3.2. Sample collection and description of the study area

Samples of black and red lablab variety seeds were collected from open markets in Fasha and karatti, Konso. These markets were selected purposely in order to make the sample representatives. Konso Wereda, Southern Nations Nationalities and Peoples (SNNP) region. It is geographically located $5^0 15'$ N and $5^0 30'$ N, and $37^0 15'$ E and $37^0 30'$ E at about 600 km south of Addis Ababa and covers an area of about 2974 km^2 (Zerihun *et al.*, 2006). According to SNNP Regional Health Office (2007), over 250, 4300 people live in Konso Wereda, with less than 5% living in urban centers. Over 96% of the people depend on agriculture while 4% depend on small-scale trade (Zerihun *et al.*, 2006). Konso woreda was selected for the study due to higher production and utilization of the crop in the area (CSA, 2007). After the samples were bought from the open markets, they were packed in polyethylene bags and transported to Food Science and Nutrition Laboratory at Addis Ababa University.

3.3. Sample preparation

All the samples were cleaned manually to remove foreign matters, cracked and broken seeds. The cleaned seeds (0.5Kg) were washed with tap water, rinsed with distilled water and immediately dried in drying oven at 55 °C for 12 hrs (Duhan *et al.*, 2002). The cleaned seeds were later pulverized using electric grinder to pass through 0.05 mm sieve, packed in polyethylene bags and stored at room temperature until analyses.

Fig 2 pictures during sample collection

3.4. Traditional processing methods

3.4.1. Soaking

Seeds were soaked in distilled water for 6, 12 and 18 h at room temperature. The seed to water ratio was 1:5 w/v (Duhan *et al.*, 2002). Following soaking for the specified duration, the seeds were washed and rinsed with distilled water.

3.4.2. Dehulling

After soaking the seeds overnight (12 h) (Duhan *et al.*, 2002), hulls were removed manually.

3.4.3. Boiling

The raw, soaked (12 h) and soaked (12 h) -dehulled seeds were boiled in a stainless steel pot. Volume of water used for boiling was three times the weight of seeds. Seeds were boiled until soft when compressed between fingers (Duhan *et al.*, 2002).

3.4.4. Germination

The soaked seeds (12 h) were washed and rinsed with distilled water. The seeds were rolled in thick layers of cotton cloth and kept in an incubator at 30 °C for 24, 36 and 48 h (Duhan *et al.*, 2002).

All the processed seeds were dried in an oven at 55 °C to a constant weight then ground in an electric grinder using 0.05 mm sieve size. Ground samples were packed in polyethylene bags for further chemical analyses.

Soaked samples

Dehulled samples

Germinated samples

Fig 2 processed samples of lablab seeds

3.5. Laboratory analyses

3.5.1. Proximate Composition

3.5.1.1. Moisture content

Moisture content of the samples was done by using method of AOAC 925.09, 2000. Standard aluminum dishes to be used for moisture determination were washed and dried at 105 ^{0}C for 1hr in a drying oven. The dishes were removed and kept in desiccators for about 15 minutes for cooling. Weights of the empty dishes were measured (M_1). Five gm of each sample was weighed using analytical balance and transferred to an aluminum dish and weighed (M_2). The sample was dried at 105 ^{0}c for 3 hrs and kept in desiccators to cool. Drying for 30 minutes of the sample and cooling was repeated until constant weight was obtained (M_3). The moisture content was then calculated using the following formulae:-

$$\text{Moisture (\%)} = \frac{M_1 - M_3}{M_2 - M_1} \times 100$$

M_1= Weight of the dish

M_2= Weight of the dish and the sample before drying

M_3= Weight of the dish and the sample after drying

3.5.1.2. Ash Determination

Ash content of the samples was determined according to AOAC 923.03, 2000 method. Standard Porcelain dishes used for analysis were washed and dried at 150^{0}C in an oven and ignited at 550^{0}C in a furnace for 30 mins. The dishes were removed from furnace and cooled in desiccators. The weight of the dish was measured (M_1). Sample powder of 2.5 gm was weighed into the porcelain dish (M_2). The sample was charred on a hot plate under a fume hood through gradual increment of temperature until the whole content becomes carbonized. Then, the sample was placed in a furnace at 550^{0}C for five hrs, till it becomes white. The sample was removed

from the furnace and placed in desiccators. Finally, weight of total ash and crucible was measured (M_3) and total ash was calculated using the following formula below:-

$$Ash(\%) = \frac{M_3 - M_1}{M_2 - M_1} \times 100$$

M_1 = Weight of the dried dish

M_2 = Weight of the dish and the sample

M_3 = Weight of the dish and the ash

3.5.1.3. Crude fat

AOAC 4.5.01, 2000 method was used to determine crude fat content of the samples. Extraction cylinders were washed and dried in drying oven at 105^0C for 1hr and cooled in desiccators. The weight of the cooled extraction cylinders were measured using analytical balance (M_1). Two gm of sample was weighed and transferred into thimble, which was lined with cotton at the bottom. The sample containing thimble was placed into Soxhlet extraction apparatus. Fifty ml of petroleum ether was added into each extraction cylinder and exhaustively extracted for 4 hrs. The extract was kept in a drying oven, adjusted to 70^0C for 30 min then removed and placed in desiccators for 30 minutes. The weight of each extraction cylinder together with the extract (crude fat) was measured (M_2). The crude fat was calculated using the following formula:-

$$Lipid\ (\%) = \frac{M_2 - M_1}{M} \times 100$$

M_2 = weight of extraction cylinder and crude fat

M_1 = weight of dry extraction cylinder

M = weight of sample

3.5.1.4. Crude Fiber Determination

Crude fiber content of the samples was determined according to AOAC 962.09, 2000 method. Clean crucible was dried with 1gm celite in oven adjusted at 105 °C for one hour and placed in desiccators. One gm of powdered sample was transferred to the dried crucible (W_1). About 200 ml of 1.25% H_2SO4 was separately transferred to each beaker and allowed to boil for 37 minutes. After 37 minutes, the acid was drained using vacuum pump and the samples were cooled for 5 minutes then, the samples were washed three times using distilled water. For the second step, 1.25%NaOH solution was added to each column and the same step was followed as previous. Crucibles containing residue was dried at 130 ^{O}C for 2 hours in a drying oven and cooled in a desiccator and crucibles were weighed (W_2). Crucibles were transferred to muffle furnace for 3 hours at 525°C and cooled down to 250 ^{O}C before removing them from the furnace. Finally, crucibles were cooled in the desiccators and weighed (W_3). Then, the crude fiber was calculated using the following formula below:-

$$\text{Crude fiber (\%)} = \frac{W_2 - W_3}{W_1} \times 100$$

3.5.1.5. Crude Protein Analysis

The crude protein content of raw and processed samples was done by kjeldahl method according to AOAC 979.09, 2000 method. Powdered sample of 0.5 gm was weighed on analytical balance and transferred to the digestion flask. Then, 6 ml of concentrated sulfuric acid and 3.5 ml of 30% H_2O_2 were subsequently added in to the digestion flask. The tubes were shaken and violent reaction was observed.

After disappearance of the violent reaction, 3 gm of the catalyst mixture (copper sulfate and potassium sulfate) was added in to the digestion flask. The solution was then digested at 370^{0}c for 5hrs. After digestion was completed, the content in the flask was diluted with distilled water, and the solution was adjusted to alkalinity using concentrated sodium hydroxide (40%). Ammonia was then distilled into a receiving flask that consisted solution of excess boric acid (4%). The solution was later titrated with 0.1N HCl. The total nitrogen content was calculated using the following formula:-

Nitrogen (%) $= \frac{V_{HCL} \times N_{HCl} \times 14.01 \times 100}{M \times 100}$

V_{HCl} = Volume of HCl consumed for titration

N_{HCl} = Normality of HCl

14.01 = Molecular weight of nitrogen

M = Weight of sample on

The estimated nitrogen was then converted into protein using the following formula:

$\%\ \text{Protein} = 6.25 \times N(\%)$

3.5.1.6. Total carbohydrate determination

Total carbohydrate content was calculated by difference (AOAC 923.05. 2000) as follows.

Total Carbohydrate (%) = 100 - % of (crude protein + total ash + crude fat+ Crude fiber)

3.5.1.7. Total energy calculation

The gross energy of each samples were estimated (in Kcal/100g) by multiplying the percentage of crude protein, crude fat and total carbohydrate with recommended factors. The total energy content in each sample was calculated as follows:

Gross energy (Kcal/100g) = (9×crude fat + 4 ×crude protein + 4×total carbohydrate)

3.6. *In vitro* protein digestibility (IVPD)

In vitro protein digestibility of samples was measured according to the method described by Malomo *et al.*, (2013). One hundred mg dry sample was incubated in 1.5 mg pepsin in 15 ml of 0.1N HCl at 37 ^{0}C for 3 h, then neutralized with 7.5 ml of 0.2N NaOH. After neutralization 4 mg of pancreatin and 7.5 ml phosphate buffer (pH 8) were added and incubated at 37 ^{0}C for 24 hr. After incubation, 5mg of trichloro acetic acid (TCA) was added and centrifuged at 3000 rpm for 15 min. After that supernatant was discarded and the residue was dried at 50 ^{0}C and carried out

kjeldahl method for protein analyses. The percent IVPD was calculated using the following equation.

$$\text{In vitro protein digestibility (\%)} = \left(\frac{\text{total protein} - \text{residue protein}}{\text{total protein}}\right) \times 100$$

3.7. Mineral Analysis

Total mineral content of lablab seeds were determined according to Osborne and Voogt, (1978) by using Atomic Absorption Spectrophotometer (AAS).

Ash of the respective samples in porcelain dishes were dissolved in seven ml of 6 N HCl at low temperature on hot plate. Then, fifteen ml of 3 N HCl was added and heated on hot plate until the solution boils. The mixture was cooled and filtered through filter paper (42mm, Whatman No. 1) in to 50 ml volumetric flask. Ten ml of 3N HCl was added to the dishes and heated to dissolve the residue then transferred to the volumetric flask. The filter paper was washed thoroughly and the washing was collected in the flask made to the mark. For Ca determination, 2.5 ml of 10% Lanthanum chloride solution was added to the flask. Then diluted to 50 ml mark with distilled water. The blank was prepared by taking the same amount of reagents following all the steps without the sample. The instrument was set and optimized based on the instruction given in the manual. The calibration solutions and the reagent blank solutions were measured first. Then the samples were run following the calibration values. The calibration curve was prepared for the required metal by plotting absorbance versus concentration in ppm. The mineral contents of each sample were calculated using the following formula:-

$$\text{metal content(mg/100gm)} = \frac{(a - b) \times V}{10 \times W}$$

Where; a= concentration in ppm of sample solution

b= concentration in ppm of blank solution

V = volume of extract (ml)

W = weight of sample (gm)

Phosphorus was determined colorimetrically as described by Fiske and Subbarow (1925). The ash obtained after dry ashing at 550^0C was treated with 5 ml of 6N HCl to wet it completely and carefully dried on a low temperature hot plate. About 7.5 ml of 3N HCl was added and the dish was heated on a hot plate until the solution boils. Then, it was cooled and filtered. Again, 7.5 ml of 3N HCl was added to the dish and heated until the solution boils. Finally, cooled and filtered into 50 ml volumetric flask and then filled with distilled water. Five ml of the sample dilution, 0.5 ml molybdate reagent and 0.20 ml aminonaphtholsulphonic acid reagent were added in to a test tube and kept for 10 minutes. Absorbance of the solution was measured at 660 nm inUV spectrophotometer .

The standard (As) was prepared by mixing standard phosphorous solution, molybdate reagent and aminonaphtholsulphonic acid.

The blank (Ab) was prepared by mixing molybdate reagent, aminonaphtholsulphonic acid and deionized water. The concentration of phosphorus was calculated by subtracting the blank from all other reading and by using the following formula:

$$\text{mg P}/100\text{g} = \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Slope} \times \text{sample weight}}$$

3.8. Mineral bioaccessibility

The samples were subjected to in-vitro enzymatic digestion under simulating intestinal conditions with pepsin plus pancreatin according to the method described by Kiin-kabari *et al.*, (2015). Enzyme solution containing 16 mg pepsin, 3.5 ml of 0.06 N HCl and 1.0 g sodium chloride made up to 100 ml with distilled water was prepared. Another solution containing 1.6 g of pancreatin, dissolved in phosphate buffer (pH 7.5) and made up to 100 ml was also prepared. In a test tube, 10 ml of pepsin enzyme solution was added to 0.25 g of the sample and the test tube was closed tightly.

The closed test-tube was shaken and incubated at 37 °C for 3 h. Immediately after peptic digestion, pH was adjusted to 8.0 using phosphate buffer. Toluene was added to the buffer to

prevent the growth of micro organisms. Pancreatin solution (12.5 ml) was then added to the digestion mixtures and samples were subsequently incubated for 20 h at 37 °C. Then clarified by centrifugations at 3,000 rpm for 20 min. The supernatants obtained were subjected to mineral analysis using atomic absorption spectrophotometer (AAS). The results obtained were used in calculating the percentage of minerals in the samples that are bioaccessible in soluble fractions using the formula;

$$\text{minerals in soluble fraction}(\%) = \frac{\text{Minerals in soluble forms after digestion}}{\text{Total minerals}} \times 100$$

3.9. Determination of antinutritional Factors

3.9.1. Phytic acid

Phytic acid was determined according to the method described by Latta and Eskin (1980), which was later modified by Vaintraub and Lapteva (1988) as cited in Adane *et al.*, (2013). About 0.1 gm of dried sample was extracted with 10 ml of 0.2 N HCl for 1 hr at ambient temperature and centrifuged (3000 rpm) for 30 minutes. The clear supernatant was used for phytate estimation. Two ml of wade reagent (0.03% solution of $FeCl_3.6H_2O$ containing 0.3% sulfosalicylic acid in water) was added to 3 ml of the solution. The solution was centrifuged and absorbance was measured at 500 nm using UV spectrophotometer (write the brand name). The phytate concentration was calculated as the difference between the absorbance of the blank (3 ml of 0.2N HCl + 2ml of wade reagent) and that of the assayed sample. The concentration of phytate was calculated using phytic acid standard curve and the result is expressed as Phytic acid in mg per 100 g.

To prepare the phytic acid standard curve, serial dilution of 4-40 ppm (mg/ml) phytic acid in 0.2N HCl was prepared. Three milliliter of the standard solution was pipetted in to centrifuge tubes with 3ml of 0.2N HCl. To each tube 2 ml of wade reagent was added and the solution was mixed on a vortex mixer for 5 seconds. The mixture was centrifuged for 10min and the supernatant was read at 500nm using water as a blank.

Concentration of phytic acid was calculated by using the following formula:-

Phytic acid (µg/g) = $\frac{[(As-Ab)-Intercept]\times10}{Slope\times W\times3}$

Where:As=Sample absorbance   Ab=Blank absorbance   W=weight of sample

3.9.2. Tannin

Tannin was determined using the method of Burns (1971) as cited in Adane *et al.*, (2013). One gm of sample was weighed and transferred in to screw capped test tube. Ten ml of 1% HCl in methanol was then added to each test tube containing the samples. The tubes were kept on mechanical shaker for 24 hrs at room temperature for optimum extraction. After 24 hrs of shaking, the tubes were centrifuged for 5 min. One ml of the clear supernatant was taken and mixed with 3 ml of 1 % HCl in methanol solution (to avoid disturbance of color, the samples were diluted) and it was mixed on a vortex mixer for 5 seconds. One ml of the solution was taken and mixed with 5 ml of vanillin-HCl reagent and allowed to stand for 20 min for complete reaction. Absorbance of the solutions was measured at 500nm using UV spectrophotometer. D-catechin was used as a standard to determine tannin concentration where, 40mg of D-catechin was dissolved in 100 ml of volumetric flask with 1% hydrochloric acid and used as stock solution. A serious of standard were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1ml(0.08, 0.16, 0.24, 0.32 and 0.4 mg/ml) from D-catechin stock solution in a test tube and the volume of each stock taken was adjusted to 1ml with 1%HCl in methanol. Then, 5ml of vanillin-HCl reagent was added to each test tube and waited for 20 minutes to complete the reaction. Finally, absorbance was measured by using UV- Visible spectrometry at 500nm.The concentration of tannin was calculated from D-Catechin standard curve.

Tannin (mg/100g) = $\frac{[(A_s-A_b)-Intercept]\times10}{Slope\ x\ d\ x\ w}\times df$

Where, A_s = Absorbance of sample

A_b = Absorbance of blank

d = Density of the solution (0.791gm/ml)

W = Sample weight

df= dilution factor

3.9.3. Total alkaloid

Total alkaloid content was determined gravimetrically by the method described by Adeniy *et al.*, (2009). Five gm of sample was weighed by using analytical balance. The sample was dispersed into 50 ml of 10 % acetic acid solution in ethanol. The mixture was shaken and allowed to stand for about 4 hrs before it was filtered. The filtrate was then evaporated to one quarter of its original volume on a hot plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter the precipitate. The precipitate was washed with 1% ammonium hydroxide solution and dried in an oven which was adjusted at 70 ^{0}C for 30 minutes. The precipitate was kept in desiccators for cooling and reweighed until a constant weight was obtained. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed.

3.10. Data analysis

All analyses were carried out in duplicates. Data was computed using SPSS (version 20) statistical software packages. One way analysis of variance (ANOVA) was used to study the significant difference between the raw and processed samples with respect to studied parameters. Duncan's multiple range test (DMRT) at $P < 0.05$ was used to determine which means were significantly different. Results were expressed as mean ± SD.

4. Results and Discussion

4.1. Ethnobotany of lablab

Summary of the information found from the focus group discussion indicated that lablab is considered as a potential food crop and it has been used in the society for a long period of time as food and forage for live stocks.

The sowing season for the crop is in between February-April (belg season and locally known as fatana) after most crops are grown. Once the crop has got sufficient water during the seedling stage, it can survive water deficient conditions. Full maturity of the seed takes about five months after sowing. The farmers in Konso area commonly practice mixed-cropping of lablab with other crops mainly maize and sorghum.

There are three different colored seeds available. These are black, red and white. The red is the most common followed by the black. In the present investigation, red and black colored seeds were included.

In Konso, lablab is used as a food after passing through different traditional processing methods like soaking and boiling. Currently, lablab seeds are predominantly used in the preparation of "Hersha" (local food prepared by combining pulses with cereals). For that purpose, the Konso communities first soak the seed in running water for overnight then boil it with maize till it becomes soft.

According to the information gathered from Konso wereda agriculture office, lablab (locally known as okkaalaa) has not received attention as a crop. It has been difficult to trace the total annual production per hectare.

4.2. Proximate composition

Nutritional composition of the raw and processed samples of black and red lablab varieties are shown in Table 4 and 5.

Table 4 Effect of traditional processing methods on nutrient composition of black *L. purpureus* variety

	S.type	Moisture (%)$^{\mu}$	Ash (%)$^{¥,\mu}$	Fat (%)$^{¥,\mu}$	Fiber (%)$^{¥,\mu}$	Protein (%)$^{¥,\mu}$	CHO (%)$^{¥,\mu}$	Gross energy (Kcal/100g)$^{¥,\mu}$
Raw	**BR**	7.1±0.14^{a}	3.4±0.28^{cd}	2.5±0.71^{c}	13.7±0.24^{g}	25.03±0^{a}	55.37±0.18^{b}	344.1±1.38^{abc}
Soaked	**BS6**	8.1±0.14^{b}	3.6±0de	2.25±0.35^{bc}	11.34±0.13^{e}	24.97±0.09^{a}	57.84±0.58^{c}	351.49±1.23^{c}
	BS12	8.3±0.14^{b}	3.2±0^{c}	2.0±0.71^{abc}	11.94±0.08^{f}	24.68±0.25^{a}	58.18±0.88^{c}	349.42±3.85^{b}
	BS18	8.3±0.14^{b}	3.2±0^{c}	2±0^{abc}	6.7±0.19^{c}	25.47±0.12^{b}	62.63±0.31^{d}	370.4±0.76^{d}
Dehulled	**BD**	8.2±0^{b}	3.8±0.28^{e}	1.75±0.35^{abc}	1.05±0.004^{a}	27.57±0.12^{e}	65.83±0.64^{f}	389.35±1.12^{e}
Boiled	**BUB**	9.1±0.14^{c}	3.2±0^{c}	1.5±0^{ab}	13.49±0.07^{g}	26.08±0.25^{c}	55.73±0.32^{b}	340.74±0.28^{ab}
	BSB	9.2±0.28^{c}	2.8±0^{b}	2.0±0^{abc}	11.49±0.05^{e}	25.47±0.12^{b}	58.24±0.17^{c}	352.84±10.88^{abc}
	BDB	7.2±0.28^{a}	2.4±0^{a}	1.25±0.35^{a}	1.95±0.16^{b}	30.46±0.25^{f}	63.94±0.26^{e}	388.85±1.15^{e}
Germinated	**BG24**	7.0±0^{a}	3.6±0de	2.25±0.35^{bc}	9.4±0.17^{d}	26.61±0.25^{d}	58.14±0.43^{c}	359.25±5.28^{d}
	BG36	9.0±0.28^{c}	3.4±0.28^{cd}	1.25±0.35^{a}	11.52±0.06^{e}	25.47±0.13^{b}	58.36±0.11^{c}	346.57±3.13^{b}
	BG48	8.0±0.28^{b}	3.6±0de	1.75±0.35^{abc}	14.65±0.07^{h}	26.34±0.12^{cd}	53.66±0.56^{a}	335.75±1.46^{a}

$^{\mu}$ All such results are Mean ± standard deviation of two independent analyses. $^{¥}$All such results are in dry basis

Means in the same column with different letters are significantly different ($P < 0.05$)

BR=Raw seed, BS6= soaked for 6hrs, BS12=soaked for 12hrs, BS18=soaked for 18hrs, BD=Dehulled seed, BUB=unsoaked boiled, BSB=soaked-boiled, BDB=dehulled-boiled, BG24=germinated for 24hrs, BG36=germinated for 36hrs, BG48=germinated for 48hrs,

The first letter B represents black variety

Table 5 Effect of traditional processing methods on nutrient composition of red *L. purpureus* variety

	S. type	Moisture (%)$^{\mu}$	Ash (%)$^{¥,\mu}$	Fat (%)$^{¥,\mu}$	Fiber (%)$^{¥,\mu}$	Protein (%)$^{¥,\mu}$	CHO (%)$^{¥,\mu}$	Gross energy (Kcal/100g)$^{¥,\mu}$
Raw	**RR**	7.3±0.14^{ab}	3.2±0^{c}	2.5±0^{b}	10.6±0.02^{f}	24.94±0.13^{a}	58.76±0.11^{c}	357.30±0.08^{bc}
Soaked	**RS6**	7.9±0.14^{cd}	4±0^{e}	2.25±0.35^{b}	9.5±0.08^{d}	24.94±0.13^{a}	59.31±0.31^{cd}	357.25±1.43^{bc}
	RS12	8.3±0.14^{de}	3.6±0^{d}	2.25±0.35^{b}	9.56±0.57^{d}	24.68±0.25^{a}	59.91±0.66^{d}	358.61±1.54^{cd}
	RS18	8.5±0.14^{e}	3.6±0^{d}	2.25±0.35^{b}	7.37±0.08^{c}	25.38±0.25^{b}	61.4±0.52^{e}	367.37±2.11^{e}
Dehulled	**RD**	8.4±0^{e}	4±0^{e}	2.25±0.35^{b}	1.05±0.05^{a}	27.74±0.13^{e}	64.96±0.18^{g}	391.05±1.94^{g}
Boiled	**RUB**	9.2±0.28^{f}	4±0^{e}	1.5±0^{a}	10.00±0.07^{e}	26.69±0.13^{d}	57.81±0.21^{b}	351.5±0.31^{a}
	RSB	9.4±0.28^{f}	2.8±0^{b}	2.25±0.35^{b}	9.56±0.33^{d}	25.38±0.25^{b}	60.01±0.23^{d}	361.81±3.1^{d}
	RDB	7.7±0.14^{bc}	2±0^{a}	1.5±0^{a}	3.37±0.16^{b}	30.54±0.13^{f}	62.59±0.29^{f}	386.02±0.65^{f}
Germinated	**RG24**	6.9±0.14^{a}	3.4±0.28^{cd}	2±0^{ab}	7.4±0.09^{c}	26.25±0^{c}	60.95±0.19^{e}	366.8±0.76^{e}
	RG36	9.3±0.14^{f}	3.6±0^{d}	1.5±0^{a}	9.73±0.41^{de}	25.47±0.12^{b}	59.7±0.29^{d}	354.18±1.64^{ab}
	RG48	8.4±0.28^{e}	3.4±0.28^{cd}	2.25±0.35^{b}	11.43±0.13^{g}	26.61±0.25^{cd}	56.31±0.05^{a}	351.93±2.39^{a}

$^{\mu}$ All such results are Mean ± standard deviation of two independent analyses. $^{¥}$All such results are in dry basis

Means in the same column with different letters are significantly different ($P < 0.05$)

RBR=Raw seed, RS6= soaked for 6hrs, RS12=soaked for 12hrs, RS18=soaked for 18hrs, RD=Dehulled seed, RUB=unsoaked boiled, RSB=soaked-boiled, RDB=dehulled-boiled, RG24=germinated for 24hrs, RG36=germinated for 36hrs, RG48=germinated for 48hrs, The first R represents red variety

4.2.1. Moisture

The mean moisture content of raw seeds of black and red varieties of lablab seeds was 7.1 and 7.3 % respectively. This result is comparable with the report of Kala et al., (2010) and Kalpanadevi and Mohan, (2013). There is no significance ($p>0.05$) difference between the two varieties of lablab seeds in terms of their moisture content.

Most of processing methods applied significantly ($p<0.05$) increased moisture content of both black and red varieties as compared to their respective raw seeds. Increment of moisture content due to various processing methods may be due to absorption of water by the seeds during soaking and boiling. Dehulled-boiled and germinated seeds for 24 hrs had no significance ($p>0.05$) effect in moisture content of lablab seeds.

4.2.2. Ash

The mean ash content was found to be 3.4 for black and 3.2 % for red varieties and this result is in agreement with Kala *et al.*, (2010). There is no significance ($p>0.05$) difference among the two varieties in their ash content.

The ash content of soaked-boiled and dehulled-boiled samples significantly ($p<0.05$) decreased when compare to their respective raw seeds in both varieties. The reduction in ash content might be due to the leaching out of minerals into soaking and boiling water. Ash content of dehulled seeds significantly ($p<0.05$) increased as compared with raw while the other processing techniques had no significance effect ($p>0.05$) in both black and red seeds. Increasing the ash content during dehulling might be due to the reduction of other nutrients (crude fiber) while removing the seed coat.

4.2.3. Crude fat

The mean fat content of both black and red lablab seeds was 2.5 g/100g (Table 4 and 5). This is in agreement with Kala *et al.*, (2010), which showed fat content of lablab seeds ranging from 2.28 to 4.17 g/100g. However, Osman, (2007), observed lower result (1.9%) as compared with the present investigation. The mean crude fat content of the two lablab seed varieties in the current study is higher than 1.5 % crude fat content of green gram but lower than 5.2 % fat

content of chick pea and 3.2 % of lentil (Iqbal *et al.*, 2006). There is no significance ($p>0.05$) difference among the two varieties in their crude fat content.

There was a significance ($p<0.05$) reduction in fat content due to all the tested traditional processing methods applied. The highest reduction was observed in dehulled-boiled and germinated seeds for 36 hrs i.e 40 – 50 % in both black and red varieties over the control. The reduction can be attributed to high lipolytic enzyme activity, which breaks down the triglyceride to simple fatty acids, sterol esters and polar lipids, especially with soaked, soaked-boiled and germinated samples Osman, (2007). Bau *et al.*, (1996) reported that after 24 hrs of germination, lipase inhibitor activity will be decreased. This means the lipase activity of the seeds will be improved by the germination process. This could explain the loss of fat by germination.

4.2.4. Crude fiber

The mean crude fiber contents of black and red varieties analyzed in the present study were 13.7 and 10.6% respectively, is higher than 7.22-9.23% reported by Shaahu *et al.*, (2015). This difference might be due to environmental, genetic factors or interaction of the two factors. There is a significance ($p<0.05$) difference between the black and red varieties in their crude fiber content.

Most of the treatments applied in the current study were significantly ($p<0.05$) reduced the crude fiber content of the studied samples. However, germinated seeds for about 48 hrs have increased crude fiber content significantly ($P<0.05$) in both varieties. The increment was 7 % in black and 8 % in red varieties over the control. The dehulled and dehulled-boiled samples exhibited over 90% reduction in crude fiber content. This reduction may be due to accumulation of the crude fiber in the seed coat as compared with the cotyledon.

4.2.5. Crude protein

The mean crude protein content was found to be 25.03 % in black and 24.94 % in red vrieties. Protein content of the present study samples was comparable with the report by Osman, (2007) on lablab seeds. The mean crude protein content of the two varieties of lablab seeds in the present study is similar with the 24.7% crude protein content of cowpea (Iqbal *et al.*, 2006), but

higher than 21% crude protein content of pigeon pea seed (Iorgyer, 2009). There is no significant ($p>0.05$) difference among the two varieties in their crude protein content.

Protein content increased significantly ($p<0.05$) in all traditional processing methods except for soaking. Dehulled-boiled samples showed maximum increment in protein content (22 %) higher than the raw seeds in both varieties. The Protein content increment when the seeds were dehulled indicates that the hull has small amount of protein and was substantially diluted the protein content of the seeds. The decrease in protein content during soaking might be attributed to the leaching out of soluble proteins within the soaking water.

4.2.6. Total carbohydrate

Carbohydrate content was 55.37 % in black and 58.77 % in red varieties. These are lower than that of 67.23 % reported by Osman, (2007). There is a significant ($p<0.05$) difference between the two varieties in their carbohydrate content.

There, was significant ($p<0.05$) increase in carbohydrate content of black varieties in all treatments other than germinated seeds for 48 hrs. Increment in carbohydrate content may be due to reduction in other nutrients since it is calculated by differences. Germinated for 48 hrs significantly ($p<0.05$) reduced carbohydrate content. And in case of red varieties, unsoaked-boiled and germinated for 48 hrs brought a significant ($p<0.05$) decreased carbohydrate content. This reduction could be attributed to the use of carbohydrates as source of energy for young seedlings Osman, (2007).

4.2.7 Gross energy

This study has shown that the mean calorific value of raw seeds were 344.1 and 357.32 Kcal for the black and red varieties, respectively, which is comparable with results indicated by Shaahu *et al.*, (2015). The mean total energy content of the two varieties in the present study is higher than 336 kcal energy content of mung bean (Blessing and Gregory, 2010) but lower than 374 Kcal energy content of cowpea (Hajjagana *et al.*, 2014). There is a significant difference ($p<0.05$) between black and red varieties of lablab seeds.

There was an increasing effect on total energy content of the processed samples over the control of black varieties. Increment of 7.6 % for soaked seeds for 18 hrs, 13.05 % for dehulled seeds, 13 % for dehulled-boiled and 5% for germinated seeds for 24 hrs was observed in black varieties. The other treatments didn't have any significant effect on total energy content of the black variety. In the red variety, treatments unsoaked-boiled and germinated seeds for 48 hrs showed 1.6 and 1.5% reduction in total energy. A significant ($p<0.05$) increment of the total energy was observed in samples of soaked seeds for 18 hrs (2.8 %), dehulled seeds (9.4 %), soaked-boiled seeds (1.26 %), dehulled-boiled seeds (8 %) and germinated seeds for 24 hrs (2.65%) in red varieties of lablab. Increment of the total energy could be attributed to reduction in nutrients other than the energy sources.

4.3.*In vitro* protein digestibility (IVPD)

The table below shows that effect of different processing methods on *in-vitro* protein digestibility (Table 6). The IVPD of raw seeds were found to be 70.91 in black and 71.9% in red varieties. The IVPD of raw seeds in this study was in agreement with that reported by Kalpanadevi and Mohan, (2013) and Kala *et al.* (2010), but, lower than results reported by Osman, (2007). There is no significant ($p>0.05$) difference among the two varieties of lablab seeds in their protein digestibility.

The present study showd that, all traditional processing methods significantly ($p<0.05$) increased IVPD in both varieties except soaking for 6hrs. There was no significance ($p>0.05$) difference in IVPD among raw and soaked for 6hrs samples. However, increasing IVPD was observed when the time of soaking was prolonged. Soaking for 12 hrs of black and red varieties showed 10 and 5% increments, respectively over their respective raw samples. Samples of soaking for 18 hrs brought an increment of about 17 % in black and 12 % in red varieties. Germination was also found to be effective to improve protein digestibility. The improvement was 10 % in germinated for 24 hrs black and 7 % in germinated for 24 hrs red seeds. Improvement of IVPD increased with prolonged germination period. The maximum improvement in IVPD was observed in germination of 48 hrs (i.e 28% in germinated for 48 hrs black and 22% in germinated for 48 hrs red varieties). These results agree well with those reported by Mubarak (2005), Ghavidel and Prakash, (2005) and Osman, (2007).

Table 6 *In vitro* protein digestibility of raw and processed *L. purpureus* varieties (%)

	Black variety of lablab seed		Red variety of lablab seed	
	S.type	Protein digestibility (%) [µ]	**S.type**	Protein digestibility (%) [µ]
Raw	**BR**	70.91±0.16^{a}	**RR**	71.9±0.42^{a}
Soaked	**BS6**	71.24±0.83^{ab}	**RS6**	72.46±0.34^{ab}
	BS12	77.76±0.65^{c}	**RS12**	75.54±0.13^{c}
	BS18	83.04±0.07^{de}	**RS18**	81.01±0.05^{de}
Dehulled	**BD**	84.16±0.57^{e}	**RD**	81.25±0.08^{e}
Boiled	**BUB**	72.2±0.43^{b}	**RUB**	73.8±0.14^{b}
	BSB	88.78±0.59^{f}	**RSB**	84.76±0.86^{f}
	BDB	82.35±0.57^{d}	**RDB**	79.96±0.09^{de}
Germinated	**BG24**	78.22±0.60^{c}	**RG24**	76.76±0.00^{c}
	BG36	82.3±0.43^{d}	**RG36**	79.58±0.10^{d}
	BG48	91.17±0.4^{g}	**RG48**	88.05±1.91^{g}

[µ] All such results are Mean ± standard deviation of two independent analyses
Means in the same column with different letters are significantly different ($P < 0.05$)
R=Raw seed, S6= soaked for 6hrs, S12=soaked for 12hrs, S18=soaked for 18hrs, D=Dehulled seed, UB=unsoaked-boiled, SB=soaked-boiled, DB=dehulled-boiled, G24=germinated for 24hrs, G36=germinated for 36hrs, G48=germinated for 48hrs,
The first letters B and R represent black and red varieties respectively

Improvement in protein digestibility may be attributed to reduced enzyme inhibitory activity, denaturation of protein and hydrolysis of antinutritional factors like phytic acid and tannins during different processing methods (Osman, 2007). Interaction of starch with fiber, phytic acid and tannin, leads to suppression of pepsin activity by dietary fiber and consequent reduction of *in vitro* protein digestibility. Negative correlation between phytic acid and tannin with *in vitro* digestibility of protein support this study observation. Significant negative correlations were observed between in vitro protein digestibility and phytic acid, tannin and total dietary fiber, with least correlation in case of fiber (Ghavidel and Prakash , 2005).

4.4.Mineral content

The mineral content of raw and processed seeds of black and red lablab varieties are presented in Table 7.

Table 7 Mineral content of raw and processed black and red varieties of *L.purpureus* (mg/100

Treatment	Black varieties					Red varieties				
	S.type	Ca [μ]	Fe [μ]	Zn [μ]	P [μ]	S.type	Ca [μ]	Fe [μ]	Zn [μ]	P [μ]
Raw	BR	105.59 ± 0.1^{cd}	3.72 ± 0.05^{f}	3.90 ± 0.44^{g}	432.75 ± 2.5^{e}	RR	102.06 ± 0.5^{c}	3.82 ± 0.02^{h}	3.6 ± 0.28^{e}	461.15 ± 2.1^{e}
	BS6	104.88 ± 1.8^{c}	3.54 ± 0.002^{de}	3.01 ± 0.02^{c}	400.62 ± 1.17^{d}	RS6	102.72 ± 1.9^{c}	3.55 ± 0.06^{h}	3.0 ± 0.002^{b}	338.79 ± 2.5^{d}
Soaked	BS12	106.15 ± 0.6^{cde}	3.47 ± 0.02^{d}	3.17 ± 0.004^{e}	376.12 ± 0.52^{c}	RS12	104.05 ± 0.9^{c}	3.48 ± 0.06^{h}	3.17 ± 0.02^{d}	410.55 ± 1.0^{c}
	BS18	105.30 ± 0.3^{cd}	3.19 ± 0.05^{c}	2.96 ± 0.01^{b}	362.34 ± 2.53^{b}	RS18	104.56 ± 1.2^{c}	3.15 ± 0.03^{cd}	2.95 ± 0.003^{a}	396.07 ± 1.6^{b}
Dehulled	BD	50.49 ± 1.8^{a}	3.02 ± 0.02^{b}	3.21 ± 0.001^{f}	525.66 ± 1.94^{h}	RD	48.06 ± 1.7^{a}	3.08 ± 0.01^{b}	3.11 ± 0.02^{c}	545.16 ± 2.04^{i}
	BUB	108.50 ± 0.1^{e}	3.0 ± 0.03^{b}	2.90 ± 0.01^{a}	373.07 ± 1.53^{c}	RUB	102.95 ± 0.5^{c}	3.01 ± 0.01^{a}	2.94 ± 0.02^{a}	408.6 ± 1.05^{c}
Boiled	BSB	107.81 ± 0.5^{de}	3.61 ± 0.12^{e}	3.0 ± 0.01^{c}	374.22 ± 0.03^{c}	RSB	109.75 ± 1.5^{d}	3.30 ± 0.04^{f}	2.98 ± 0.02^{a}	407.17 ± 1.2^{c}
	BDB	56.03 ± 2.3^{b}	3.07 ± 0.03^{b}	2.96 ± 0.02^{b}	351.24 ± 2.04^{a}	RDB	57.6 ± 1.04^{b}	3.16 ± 0.03^{d}	3.01 ± 0.001^{b}	279.3 ± 2.0^{a}
	BG24	122.0 ± 0.4^{g}	3.26 ± 0.03^{c}	3.10 ± 0.01^{d}	454.43 ± 0.95^{f}	RG24	115.77 ± 2.7^{e}	3.34 ± 0.04^{g}	3.10 ± 0.05^{c}	487.14 ± 2.7^{f}
Germinated	BG36	125.26 ± 0.1^{h}	2.88 ± 0.03^{a}	3.0 ± 0.03^{c}	457.35 ± 1.87^{f}	RG36	125.36 ± 2.8^{f}	3.59 ± 0.02^{g}	3.0 ± 0.01^{b}	491.3 ± 1.0^{g}
	BG48	113.92 ± 0.1^{f}	3.21 ± 0.04^{c}	3.11 ± 0.01^{d}	483.51 ± 2.21^{g}	RG48	113.04 ± 0.13^{de}	3.23 ± 0.03^{e}	3.1 ± 0.18^{c}	510.04 ± 1.5^{f}

[μ] All such results are Mean ± standard deviation of two independent analyses

Means in the same column with different letters are significantly different ($P < 0.05$)

R=Raw seed, S6= soaked for 6hrs, S12=soaked for 12hrs, S18=soaked for 18hrs, D=Dehulled seed, UB=unsoaked boiled, SB=soaked-boiled, DB=dehulled-boiled, G24=germinated for 24hrs, G36=germinated for 36hrs, G48=germinated for 48hrs,

B and R represent black and red varieties respectively

4.4.1. Calcium

The mean value of calcium content of black and red seeds of lablab were 105.59 and 102.06 mg/100g, respectively, which is lower than that reported as 520 mg/100g by Shaahu *et al.*, (2015) and 160 mg/100g by Arinathan *et al.*, (2003). The difference might be due to environmental and genetic factors. There is a significance ($p<0.05$) difference between two varieties in terms of their Ca content.

Traditional processing methods used in the current study showed either significant increment or reduction of Ca content of the samples. Germination significantly ($p<0.05$) increased the Ca content by 15.5 % in germinated seeds for 24hrs, 18.6 % in germinated seeds for 36 hrs and 8 % in germinated seeds for 48 hrs in black varieties as compared to their respective raw seeds. Germination brought 11-23 % increment of Ca in red varieties of lablab. This result is in contrast with result obtained by Duhan *et al.*, (2002) reported as germination for 24, 36 and 48 h resulted in the losses of Ca in pigeon pea seeds. The difference might be due to variation in samples. Loss of Ca by 52 % in Dehulling and 47 % in dehulled-boiling of seeds was observed in both black and red seeds over their respective control. Minerals present in the hulls might have been lost during dehulling. Therefore, contributing to the lower mineral contents in the dehulled lablab seeds. These results agree with that of reported by Duhan *et al.*, (2002).

4.4.2. Iron

The mean value of Fe content of black and red lablab varieties were 3.72 and 3.82 mg/100g, respectively, which are lower than 5.6 reported by Shaahu *et al.*, (2015) and 7.9 mg/100g indicated by Arinathan *et al.*, (2009). The difference might be due to agronomic differences between cultivated places and/or genetic differences among the seeds. There is no significance ($p>0.05$) difference among the two varieties in their Fe content.

All processing methods applied in the current study significantly ($P<0.05$) reduced Fe content in both varieties. With an increase in soaking time, i.e. 6 to 12 and 12 to 18 hrs, the loss in Fe content was noted. The loss was found to be 5-7 % in soaked for 6 hrs, 7-9 % in soaked for 12 hrs and 17-19 % in soaked for 18 hrs samples. Similar results were observed by Duhan *et al.*, (2002). Dehulling also caused significant ($p<0.05$) decrease in Fe content. Reduction of Fe content by 19

% was observed in black and red dehulled samples. This reduction may indicate significant presence of this mineral in the seed coat. Boiling of unsoaked, soaked and dehulled samples and Germination significantly ($p<0.05$) reduced Fe content of lablab seeds. This reduction could be due to leaching of Fe to soaking and boiling water. These results agree with that of reported by Duhan *et al.*, (2002), El-Adawy *et al.*, (2000) and Ghavidel and Praksh, (2007).

4.4.3. Zinc

Zn was the other mineral investigated in this study. The mean values of black and red seeds of lablab varieties were 3.9 and 3.6 mg/100g, respectively. This is lower than reported by Shaahu *et al.*, (2015) but higher than that of indicated by Arinathan *et al.*, (2009) for the same seeds. The differences might be due to environmental and genetic differences among the samples. There is no significance ($p>0.05$) difference between the two varieties in their Zn content.

All treatments used in the current study significantly ($P<0.05$) reduced Zn content of both varieties. Boiling of unsoaked, soaked and dehulled samples caused more reduction in Zn content, which was 23-25% in black and 16-18 % in red lablab seeds, and these results might be due to leaching out of this mineral with boiling and soaking water.

4.4.4 Phosphorus

The mean value of P was 432.75 mg/100g in black and 461 mg/100g in red varieties. This is comparable with results reported by Shaahu *et al.*, (2015) and Ramakrishna, (2008), but higher than that of indicated by Arinathan *et al.*, (2009) and Kalpanadevi and Mohan, (2013). Significant difference ($p<0.05$) among the two varieties in terms of their P content was identified.

Dehulling and germination of black and red lablab seeds has significantly ($P<0.05$) increased the P content. As the period of germination prolonged, further increment of P content was observed. After germination of 48 hrs, increment of P was 12 % in both varieties. These data agreed with that of showed by El-Adway *et al.*, (2002) and Ramakrishna, (2006) but in contrast with results reported by Duhan *et al.*, (2002) and Ghavidel and Prakash, (2007).

4.5. Mineral bioaccessibility

Different domestic processing methods had a significant ($p< 0.05$) positive effect on the bioaccessibility of minerals including Ca, Fe and Zn (Table 8). As a result of soaking, dehulling, boiling and germination, bioaccessibility of minerals improved to varying extents. After 6 hrs of soaking, bioaccessibility of Ca, Fe and Zn improved by 1.2-1.7, 9.9-13 and 6.17-8.8 %, respectively over the controls of the black and red seeds. As the period of soaking prolonged, bioaccessibility of these minerals was enhanced. After 18 hrs of soaking, bioaccessibility increment was 3.8-4.6 % for Ca, 16.5-18.5 % for Fe and 23.7-24.3 % for Zn. Similar results was observed by Duhan *et al.*, (2002) and Kumari *et al.*, (2014). Soaking followed by dehulling further increased bioaccessibility of all minerals studied especially Ca. Bioaccessibility of Ca increased by 75 % in black dehulled and 82 % in red dehulled samples over the raw seeds. After 24 hrs of germination i.e in germinated seeds for 24 hrs black and red samples, increment of 11-15 % Ca, 8-9 % Fe, and 30-32 % Zn were observed. When germination period was prolonged, further improvement in bioaccessibility of minerals was detected. These results are in agreement with previous reports for germinated green gram, cowpea, lentil and chickpea (Ghavidel and Prakash, 2007). There is no significance ($p>0.05$) difference among varieties in mineral bioaccessibility.

Divalent ions, including Ca, Fe and Zn are generally present in association with the antinutrients in plant foods, which may be responsible for lower extractability of these divalent ions. The decrease in the level of antinutrients by various domestic processing methods like soaking, dehulling, boiling and germination, as reported in different studies for other legumes, and in the present study may possibly release these mineral ions in free form and thus, account for their increased in bioaccessibility. The presence of tannin and phytic acid in seed coat as inhibitors was demonstrated to reduce iron absorption by chelating the iron ion. As a divalent cation, Fe, is also generally present in association with phytic acid, and this may be responsible for its lower extractability. However, reduction in phytic acid as a result of soaking and cooking may explain higher bioaccessibility of iron and other minerals. Increase of calcium bioaccessibility after dehulling of samples could be contributed to simultaneous reduction of phytic acid, tannin and dietary fiber. Several reports show the negative correlation of phytic acid and dietary fiber contents of foods with percent of Ca, Fe and Zn bioaccesibility (Ghavidel and Prakash, 2007).

Table 8 Mineral bioaccessibility of raw and processed lablab seed varieties (%)

Treatment	Black varieties				Red varieties			
	S.type	Ca [µ]	Fe [µ]	Zn [µ]	S.type	Ca [µ]	Fe [µ]	Zn [µ]
Raw	BR	45.34±0.31[a]	34.04±1.00[a]	54.27±0.41[a]	RR	46.9±1.09[a]	35.27±0.09[a]	52.30±0.69[a]
Soaked	BS6	45.97±0.92[ab]	38.63±1.05[cd]	57.62±0.86[b]	RS6	47.46±0.66[ab]	38.77±0.93[c]	56.91±1.07[b]
	BS12	46.06±1.02[ab]	38.67±0.11[cd]	64.43±0.93[c]	RS12	48.15±0.62[abc]	40.83±0.88[de]	61.99±0.49[c]
	BS18	46.59±0.01[b]	40.33±0.09[d]	67.12±0.63[d]	RS18	48.70±0.42[bc]	41.07±0.37[de]	65.03±1.36[d]
Dehulled	BD	78.615±0.30[e]	42.98±0.16[e]	68.88±0.4[e]	RD	86.32±1.06[f]	41.72±0.49[de]	67.69±0.39[e]
Boiled	BUB	47.19±0.08[b]	36.14±0.04[ab]	55.32±0.4[a]	RUB	49.05±0.25[c]	36.86±0.28[b]	54.51±1.53[ab]
	BSB	49.06±0.58[c]	34.53±1.24[a]	66.27±0.88[d]	RSB	51.05±0.41[d]	36.46±0.25[ab]	64.1±0.78[cd]
	BDB	49.59±0.18[c]	34.15±1.53[a]	63.62±1.03[c]	RDB	51.60±0.68[d]	35.29±0.99[a]	61.96±0.24[c]
Germinated	BG24	49.96±0.07[c]	37.29±1.6[bc]	70.53±1.07[ef]	RG24	52.01±0.52[d]	38.24±0.53[c]	69.26±2.02[ef]
	BG36	50.16±0.08[c]	40.64±1.17[d]	71.39±1.07[f]	RG36	52.25±0.48[d]	40.40±0.74[d]	70.89±1.44[f]
	BG48	53.82±1.18[d]	50.87±0.25[f]	72.23±0.62[f]	RG48	54.75±0.45[e]	42.26±0.45[e]	71.66±0.52[f]

[µ] All such results are Mean ± standard deviation of two independent analyses
Means in the same column with different letters are significantly different ($P < 0.05$)
R=Raw seed, S6= soaked for 6hrs, S12=soaked for 12hrs, S18=soaked for 18hrs, D=Dehulled seed, UB=unsoaked boiled, SB=soaked-boiled, DB=dehulled-boiled, G24=germinated for 24hrs, G36=germinated for 36hrs, G48=germinated for 48hrs,
B and R represent black and red variety respectively

4.6. Anti nutritional factors

Data on phytic acid, tannins and alkaloids of raw and processed lablab varieties are summarized in Table 9.

Table 9 Anti nutritional factors of raw and processed black and red varieties of *L. purpureus*

Treatment	Black varieties				Red varieties			
	S. type	Phytic acid (mg/100g)	Tannin (mg/100g)	Alkaloids (%)	S. type	Phytic acid (mg/100g)	Tannin (mg/100g)	Alkaloids (%)
Raw	BR	320.83 ± 1.97^{g}	1.32 ± 0.02^{e}	1.3 ± 0.14^{d}	RR	338.33 ± 1.57^{g}	1.48 ± 0.004^{g}	1.5 ± 0.14^{d}
	BS6	303.61 ± 1.97^{e}	0.82 ± 0.07^{d}	1.0 ± 0^{bc}	RS6	320.83 ± 1.97^{f}	0.81 ± 0.00^{f}	1.2 ± 0^{bc}
Soaked	BS12	300.28 ± 1.96^{de}	0.61 ± 0.1^{bc}	0.9 ± 0.14^{bc}	RS12	314.44 ± 0^{e}	0.63 ± 0.1^{de}	1.1 ± 0.14^{bc}
	BS18	296.67 ± 1.57^{cd}	0.51 ± 0.05^{b}	0.8 ± 0^{ab}	RS18	309.44 ± 1.57^{d}	0.54 ± 0.12^{cde}	1 ± 0^{ab}
Dehulled	BD	298.89 ± 0.79^{d}	UN	0.9 ± 0.14^{bc}	RD	312.50 ± 2.75^{de}	UN	1.1 ± 0.14^{bc}
	BUB	293.06 ± 0.39^{c}	0.63 ± 0.06^{bc}	0.9 ± 0.14^{bc}	RUB	304.72 ± 1.18^{c}	0.79 ± 0.08^{f}	1.1 ± 0.14^{bc}
Boiled	BSB	277.78 ± 1.57^{b}	0.61 ± 0^{bc}	0.6 ± 0^{a}	RSB	293.89 ± 1.57^{b}	0.51 ± 0.06^{bcd}	0.8 ± 0^{a}
	BDB	264.45 ± 1.57^{a}	UN	0.6 ± 0^{a}	RDB	270.28 ± 1.18^{a}	UN	0.8 ± 0^{a}
	BG24	313.89 ± 2.36^{f}	0.72 ± 0.02^{cd}	1.1 ± 0.14^{cd}	RG24	313.89 ± 2.36^{e}	0.68 ± 0.07^{ef}	1.3 ± 0.14^{cd}
Germinated	BG36	312.22 ± 1.57^{f}	0.38 ± 0.09^{a}	1.1 ± 0.14^{cd}	RG36	310.28 ± 2.75^{de}	0.43 ± 0.07^{bc}	1.3 ± 0.14^{cd}
	BG48	310.83 ± 1.97^{f}	0.34 ± 0.05^{a}	1.1 ± 0.14^{cd}	RG48	303.89 ± 0.00^{c}	0.38 ± 0.01^{b}	1.3 ± 0.14^{cd}

$^{\mu}$ All such results are Mean ± standard deviation of two independent analyses
Means in the same column with different letters are significantly different ($P < 0.05$)
R=Raw seed, S6= soaked for 6hrs, S12=soaked for 12hrs, S18=soaked for 18hrs, D=Dehulled seed, UB=unsoaked boiled, SB=soaked-boiled, DB=dehulled-boiled, G24=germinated for 24hrs, G36=germinated for 36hrs, G48=germinated for 48hrs,
The first letters B and R represent black and red variety respectively

4.6.1. Phytic acid

The phytic acid content of lablab seed varieties were calculated using standard calibration curve (y=-0.006+0.335, R^2=0.995) (Annex 2-A). The phytic acid content of the raw lablab seeds was 320.83 and 338.33 mg/100g in black and red varieties, respectively. These are lower than that of reported by Ramakrishna *et al.*, (2006) and Osman, (2007), but comparable with those reported by Kala *et al.*, (2010). The mean phytic acid content of the two varieties in the present study is much lower than 886 mg/100g phytic acid content of pigeon pea (Duhan *et al.*, 2002) and 1210 mg/100g phytic acid content of chick pea (El-Adawy, 2002). There is a significance ($p<0.05$) difference among varieties in their phytic acid content.

All traditional processing methods applied in this study significantly ($P < 0.05$) reduced the phytic acid content. Soaking and dehulling contributed significantly towards lowering the phytic acid content (Table 9). After 6 hours of soaking, the percent loss was 5%. With an increase in the period of soaking, i.e. 6–12 and 12–18 hours, further reductions in phytic acid content were noticed. The longer the periods of soaking, the higher the losses of phytic acid content occurred. Dehulled samples had lower levels of phytic acid, i.e. black and red dehulled samples showed 7 % reduction over the control. In the present study, boiling of unsoaked, soaked and dehulled seeds was the most effective treatment in reducing the phytic acid content. Dehulled-boiled samples exhibited 18-20 % reduction in phytic acid followed soaked-boiled samples (13 %) and unsoaked boiled samples (9-10%). This finding, agrees with that of Duhan *et al.*, (2002) and Shaahu *et al.*, (2015). The reduction of phytic acid content by soaking and boiling might be due to leaching out of this compound with soaking and boiling water. Germination also resulted in a significant ($p < 0.05$) reduction of phytic acid. As the period of germination prolonged, i.e. 24–36 and 36–48 hours, increasing reduction in phytic acid content was observed. A loss of 2-7 % occurred during 24 hours germination. After 48 hours of germination, lablab seeds contained phytic acid of 3-10 % less than that of raw seeds. The reduction of phytic acid could have been due to increasing phytase (responsible for breakdown of phytic acid) activity during germination. Germination has been reported earlier by different workers to have a diminishing effect on the phytic acid content of various legumes (Duhan *et al.*, 2002; Ghavidel and Prakash, 2007).

4.6.2. Tannins

The tannin content of lablab seed varieties were calculated using standard calibration curve (y=0.586+0.009, R^2=0.996) (Annex 2-B). Tannin levels in raw black and red varieties were found to be 1.32 and 1.48 mg/100g, respectively (Table 9). These are lower than results reported by Shaahu *et al.*, (2015). The difference might be due to agronomic and/or genetical factors. The mean tannin content of the two varieties of lablab seeds in the current study is much lower than 85 mg/100g tannin content of pigeon pea and 485 mg/100g tannin content of chick pea (Iorgyer *et al.*, 2009; El-Adawy, 2002). There is a significance ($p<0.05$) difference among the two varieties in their tannin content.

All traditional processing methods significantly ($p < 0.05$) reduced the tannin content of the seeds. After 6 hours of soaking, the percent loss was 37 % in black and 45% in red varieties. With an increase in the period of soaking, i.e. 12 and 18 hours, the tannin content was reduced by 54 and 61% in black and 57 and 64% in red lablab seeds, respectively as compared with their raw seeds. No tannins were detected in dehulled and dehulled-boiled samples. This might be due to removal of the seed coat which is known to be much richer in tannins than cotyledon of seeds. Egounlety, (2003) also reported similar results for some decorticated legumes. Black and red soaked-boiled samples showed reduction of tannin content 54 and 66%, respectively followed by black unsoaked boiled (52 %) and red unsoaked-boiled sample (47%). Several investigators have also reported significant ($P<0.05$) reduction in tannin content with boiling of cowpea (Udensi *et al.*, 2007), pigeon pea seeds (Iorgyer *et al.*, 2009) and lablab seeds (Shaahu *et al.*, 2015). The reduction in tannin content may be attributed to leaching out of these compounds into soaking and cooking medium.

Germination significantly ($P<0.05$) reduced the tannin contents of the seeds. After 24hrs of germination, the tannin contents were reduced by 45% in black and 54% in red varieties. As the time of germination prolonged, the reduction of tannin content was further increased i.e 48hrs germination brought 74% reduction in both varieties. This finding, agrees with that of reported by Ramakrishna *et al.*, (2006). Similarly, Ghavidel and Prakash, (2007), reported reduction in tannin content during germination of green gram, cowpea, lentil and chickpea. The reduction of tannin content may be attributed to leaching out of these compounds in to soaking water.

4.6.3. Total Alkaloids

The result obtained for the total alkaloid content of raw lablab seeds was 1.3% in black and 1.5% in red varieties (Table 9). The present result is lower than that reported 2.25 % by Shaahu *et al.*, (2015) for the same seeds. This variation might be due to difference in varieties. There is no significance ($p>0.05$) difference among the two varieties.

Alkaloid content of samples in the present study was significantly ($P<0.05$) affected by different traditional processing methods. Soaked, dehulled and boiled samples had significantly ($P<0.05$) lower content of alkaloids when compare to the raw seeds in both varieties. Loss of alkaloids in 6 hrs soaking was 23 and 20 % in black and red varieties, respectively. When the time of soaking prolonged, percent of loss also increased i.e after 18 hrs of soaking (38 % in black and 33% in red) loss of alkaloids was recorded. Dehulling brought 30 and 27 % reduction of alkaloid content in black and red seeds, respectively. In the present study, Boiling of unsoaked, soaked and dehulled seeds brought the highest reduction in alkaloid content. Boiled samples had 30-54 % and 27-47% reduction of alkaloids in black and red varieties, respectively. These results agree with that of reported by Shaahu *et al.*, (2015). This reduction trend may be due to removal of alkaloids with soaking and boiling water. Since alkaloids are water soluble, soaking in water may have easily removed them from the seed. The other reason for decreasing in total alkaloids may be attributed to removal of the seed coat from the grain which contains certain amount of alkaloids. In this study germination had no significant effect on alkaloid contents.

5. Conclusion and Recommendation

5.1. Conclusion

The present study indicated that *lablab purpureus* is grown as a mixed cropping in Konso, SNNPR and three different colored seeds were identified (black, red and white). Lablab seed is used as one of the ingredients in preparation of "hersha", local food in Konso.

The black and red seeds were included in this study and there is a significant difference ($p<0.05$) among the two varieties in terms of their fiber, CHO, energy, Phytic acid, tannin, Ca and P contents. Red variety of lablab seed contained high amount of CHO, energy, phytic acid and tannin contents when compared with the black seed. Different traditional processing methods applied in this study have a significant ($p<0.05$) effect on nutritional composition, antinutritional factors and nutritional quality of lablab varieties. In most of traditional processing methods applied the total protein content showed a significant ($p<0.05$) increment (1.75-22.45 %) with highest during dehulled-bioling (22.45 %). Dehulled samples had significantly ($p<0.05$) the highest carbohydrate and total energy (65.75 and 389.01 Kcal). All traditional processing methods applied in the current study significantly ($p<0.05$) increase *in vitro* protein digestibility. Germination showed a significantly ($p<0.05$) higher increment (6.8- 28.6 %).

All traditional processing methods significantly ($p<0.05$) decreased Fe and Zn contents. Dehulling and germination increase phosphorus content up to 21 and 12% respectively. Dehulled and dehulled- boiled samples had a significantly ($p<0.05$) decreased calcium content (50.49 and 56.03 mg, respectively in black). All traditional processing methods significantly ($p<0.05$) increased bioaccessibility of Ca, Fe and Zn. Dehulling showed significantly ($p<0.05$) higher increment in Ca bioaccessibility (up to 84 %).

All traditional processing methods significantly ($p<0.05$) reduced antinutritional factors (2.16-20 % in phytic acid, 38 % - un detected level in tannin and 13.33-54 % in total alkaloids). Boiling of unsoaked, soaked and dehulled samples showed the highest reduction ($p<0.05$) in phytic acid content (9-20%). The tannin content was reduced to the extent of undetected level due to dehulling and dehulled-boiling. Boiling of soaked and dehulled samples showed the highest

reduction ($p<0.05$) in total alkaloids (47-54%). Germination had no significant ($p<0.05$) effect on the level of alkaloids.

Overall, *Lablab purpureus* has a good food composition levels with big limitation of antinutrients. But after reducing antinutrients with the mentioned traditional methods, the crop can be a good source of protein, energy and minerals. Therefore, soaking, dehulling, boiling and germinating hold a good potential for improving the nutritional value of lablab seeds by reducing antinutritional factors such phytic acid and tannins thereby enhancing its utilization.

5.2. Recommendations

The current study with its own limitation has investigated the effects of different traditional processing methods on the nutritional composition, antinutritional factors, protein digestibility and mineral bioaccessibility of lablab varieties. The following issues should also be considered in the future based on the outcomes of the current study.

- The production and consumption of lablab should be extended from Konso to other parts of Ethiopia.
- Appropriate processing method must be followed to improve nutritional quality. Accordingly, dehulling and germination of seeds is suggested to be promoted for improved protein digestibility and mineral bioaccessibility.
- Though *Lablab purpureus* have high protein content, the amino acid composition of the seeds has not been studied in this study. So amino acid composition of the seed should be further studied.

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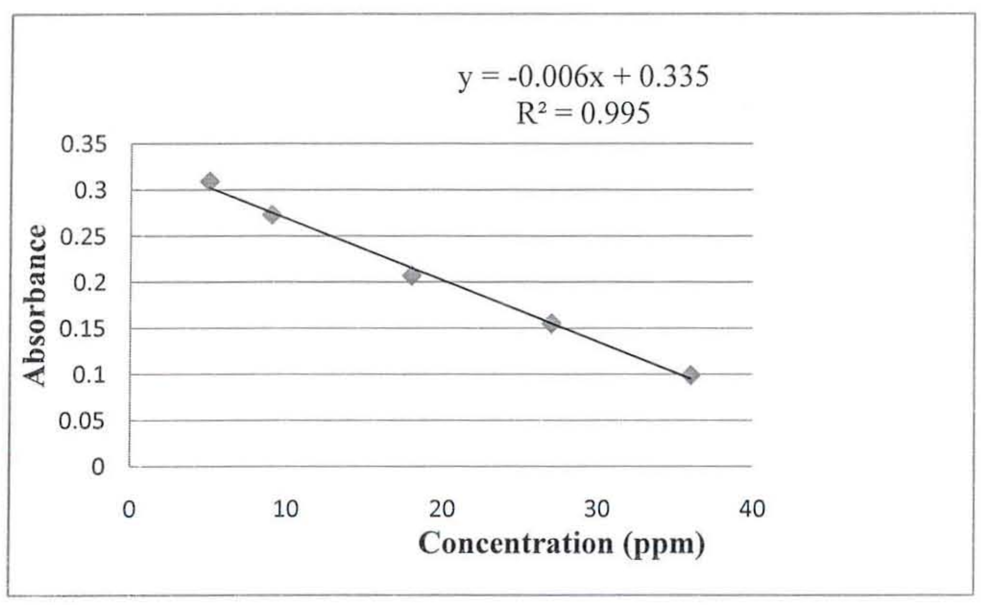
Annex 1-Discussion points for FGD

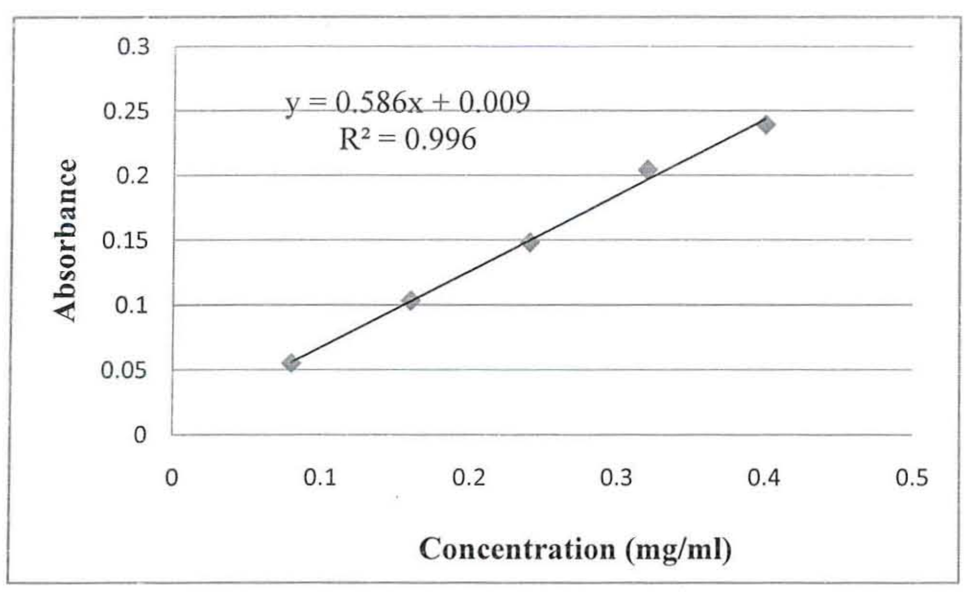
1. What was the geographical origin of lablab?
2. When was it introduced to Konso?
3. What is its local name?
4. Do you know its scientific name?
5. What are the existing varieties of lablab?
6. When is the sowing season of lablab?
7. When is the sowing date (month)?
8. What is the total rain fall amount it requires?
9. What climatic (weather) conditions are suitable for its harvest?
10. What type of soil favors its growth?
11. Is any fertilizer enhancement required for its harvest?
12. Does it require pesticide for its harvest?
13. Comparing with other commonly harvested legumes and cereals, does lablab have an advantage?
14. If the answer for question (13) is yes, what are the advantages it has over the others?
15. What is the maximum annual yield per hectare?
15. Is there any post harvest loss? If there how much?
16. If the answer for the above question is yes, what are the main causes of the loss?
17. Can lablab be edible food source?
18. If the answer for the above question is no, what limits its consumption?
19. If the answer for the question (6a) is yes, what are the main food items that can be prepared from it?
20. What are the main traditionally used processes to make it edible?
21. What are the effects of these processes on the raw seed? (i.e. palatability, reduction Of bitterness, reducing flatulence, etc)
22. What are the other uses of lablab for the local community?
23. What is the current market price of lablab?
24. Is there any studies have been conducted on lablab?
25. Any information that you would like to add

THANK YOU

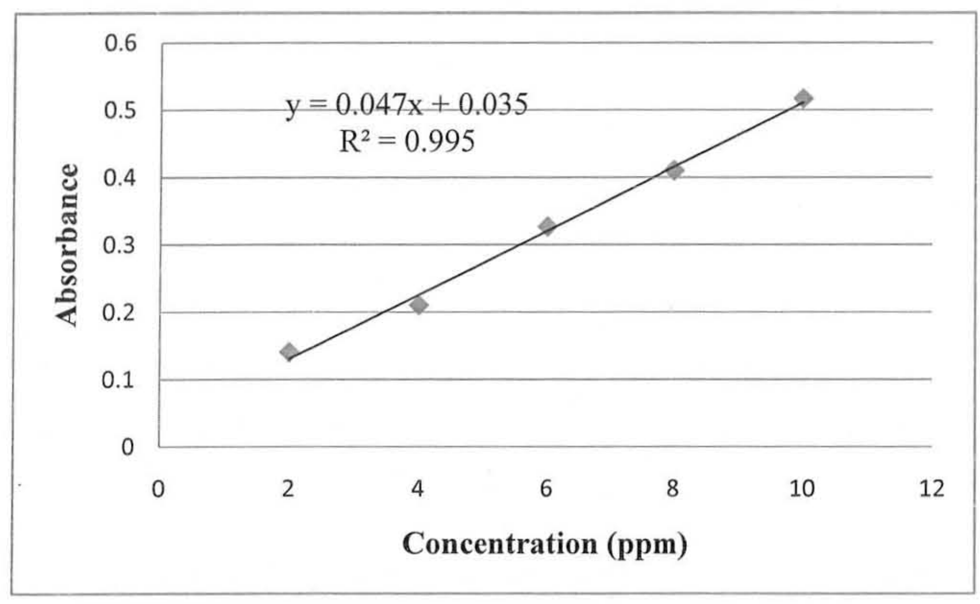
Annex 2 Standard calibration curves of standards



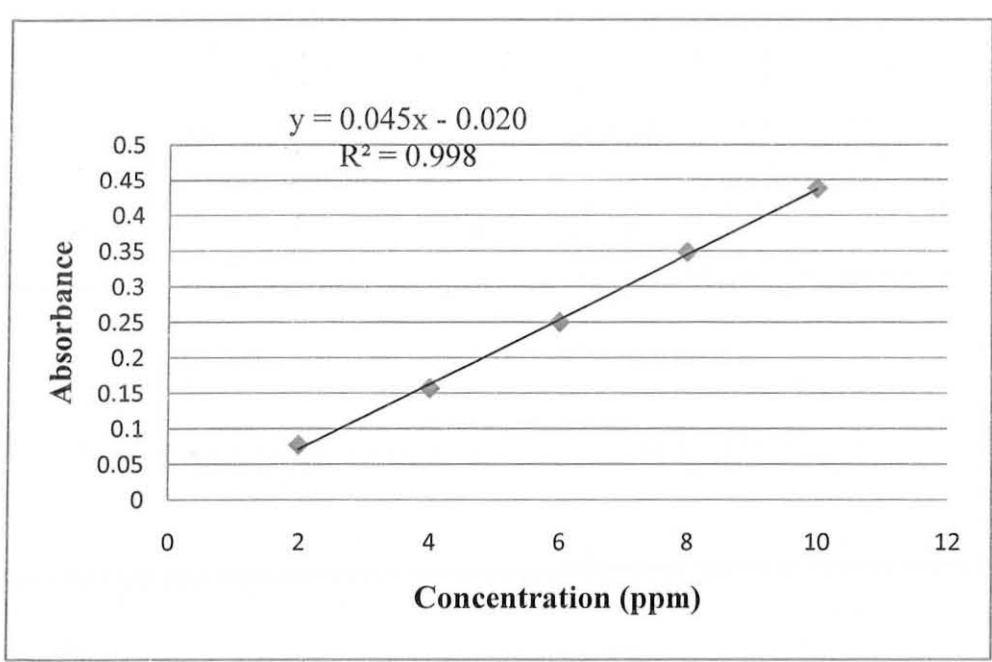
A-Phytic acid



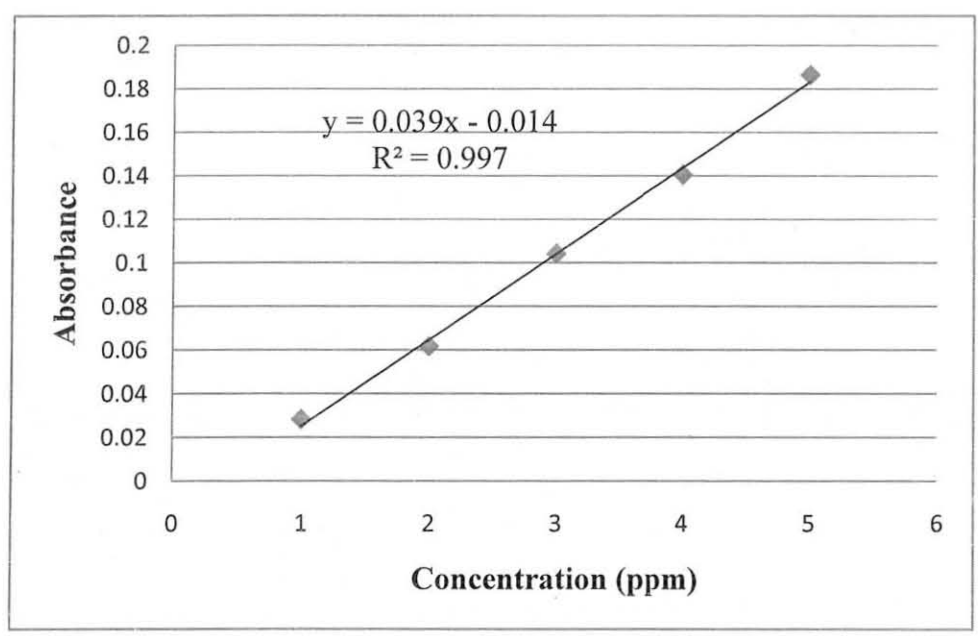
B- Tannin



C- Calcium



D- Ca bioaccessibility



D- Fe bioaccessibility