

Addis Ababa University
School of Graduate Studies
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Comparative Study on the Nutritional Composition, Ant-nutritional Factors and Functional Properties of Newly Introduced (*Lupinus angustifolius*) and locally Grown (*Lupinus albus*) Lupin (Gibto) Varieties in Ethiopia.

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A thesis submitted to the School of Graduate studies 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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LIST OF ABBREVIATIONS AND ACRONOMYS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CS	Chemical Score
DAFWA	Department of Agriculture Western Australia
EAA	Essential Amino Acid
EAAI	Essential Amino Acid Index
EHNRI	Ethiopian Health and Nutrition Research Institute
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
g	gram
Ha	Hectare
Kg	Kilo Gram
Mg	Mill Gram
MPC	Maximum Permitted Concentration
N	Nitrogen
NSP	Non-starch Polysaccharide
NVRC	National Variety Release Committee
PA	Phytic Acid
QAs	Quinolizidone Alkaloids
UK,	United Kingdom
WFP	World Food Program

ABSTRACT

*Inadequate availability and consumption of protein foods in developing countries are a major concern as large segments of population of these countries suffer from protein-energy malnutrition. Exploitation of underutilized locally available varieties, introducing and adaptation of new types of legume crops with better nutritional profiles and yielding capacity is considered as an important approach to combat the protein-malnutrition. A comparative study on proximate composition, mineral composition, antinutritional factors and functional properties for both raw and processed seeds of newly introduced Australian Sweet Lupin (*Lupinus angustifolius*) and locally grown white lupin (*Lupinus albus*) varieties obtained from Holeta Agricultural Research Institute Center was conducted. The mean crude protein, crude ash, crude fat and crude fiber contents of the raw new and local lupin varieties were 25.55, 2.92, 6.42, 14.89g/100g and 35.17, 2.75, 6.59, 14.79g/100g, respectively. The local lupin variety had higher crude protein content than the new lupin variety. The mineral, Zn, Fe and Ca contents were 2.74, 2.48, 112.62mg/100g and 3.46, 2.94, 44mg/100g, respectively for the new and local lupin varieties. The new lupin variety had higher Ca content than the local lupin variety. The new lupin variety (*Lupinus angustifolius*) had lower total alkaloid and higher phytic acid content than the local lupin (*Lupinus albus*) variety. The new lupin variety showed comparable functional properties and sensory acceptability with the local lupin variety. The new lupin variety had higher water absorption capacity, emulsion activity and foaming capacity than the local lupin variety. Except the minerals content, both traditional processing methods applied in the present study significantly ($p < 0.05$) increased the protein, fat and fiber content and reduced antinutritional factors. Although there were variations between the two varieties in terms of their nutritional compositions, the lower alkaloid content, possibility to be used in food formulations without longer pre-treatment practices coupled with relatively better crude protein and fiber content gives the new variety an advantage over the local lupin variety for further production and use in human food and animal feed.*

Key Words: Lupin, Chemical composition, Antinutritional factors, Functional properties

1. INTRODUCTION

1.1 Background of the Study

Inadequate availability and consumption of protein foods in developing countries are a major concern as large segments of population of these countries suffer from protein-energy malnutrition (Maikhuri *et al.*, 1991). It is well documented that developing countries do not produce enough food and of the right nutritional quality to meet daily needs (Aletor and Aladetimi, 1989). The prevalence of hunger and protein malnutrition in the tropical and subtropical areas of the world is well recognized and appreciated (FAO, 1994). This can be attributed mainly to the ever-increasing population pressure, enhanced dependence on a cereal – based diet, poverty, scarcity of fertile land, fast depletion of natural resources and low agricultural production (Steiner, 1996).

The growth rate of food production continues to lag behind population growth rate with adverse consequences for food security. Protein foods and particularly animal protein have continued to be in short supply. Most experts consider protein deficiency as the commonest form of malnutrition in the developing countries, especially in regions where diets are mainly based on roots and tuber crops (Pelletier *et al.*, 1995). The production of protein-rich foods (leguminous seeds and particularly animal products) has been much less efficient.

The increasing pressure of population and predicted food shortages are creating a demand for new food sources either: for direct use as a food or animal feed ingredient or as a raw material for seed protein and oil extraction (Ikechukwu and Madu, 2010).

Accordingly, in an attempt to widen the narrow food base, food and agricultural scientists are screening lesser known and under-exploited native plants and introducing new crop varieties for possible potential sources of food (Ezeagu and Ologhobo, 1999). Recent reports have revealed that quite a large number of lesser-known native crop species are high in nutrients and many could possibly become advanced crops in the future if given priority and research resources (Lalas and Tsaknis, 2002).

Grain legumes or pulse crops share the larger proportion and could make a useful contribution in this regard because of their well adaptation to adverse environmental conditions, exhibition of resistance to pests and pathogens and unique nutritional compositions (Arinathan *et al.*, 2009). Legumes have been an important source of protein, oil, starch, mineral, vitamins and health protecting compounds from the beginning of human history. Their seeds play a basic role in the traditional diet of many peoples of the world and are a valuable basic material for the food and animal feed industries.

Legumes are relatively a rich source of protein as the seeds contain 200-250g protein/kg. Legume seeds are rich in lysine and sulfur containing amino acids (methionine and cysteine) compared to cereals. Lysine is the first limiting amino acids so it is important that legumes complement cereals in lysine balance. The immature pods, green and mature seeds are used for human consumption according to traditional recipes and the mature seeds are the basic material for producing flour concentrates and isolates. These products are used in the food industries and for animal feeds. The dietary fiber content of seeds is a very important factor from the nutritional point of view.

Animal proteins being more expensive, especially people in developing countries depend largely on plant to fulfill their protein requirements. Grain legumes alone contribute to about 33% of the dietary protein nitrogen needs of humans. Moreover, it is also a good source of minerals (Kirmizi and Guleryuz, 2007).

Besides being a good source of nutrition, there is a considerable interest in the relationship between plant-based diets and the 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). Cholesterol-free legume (legumes low in fat content) in combination with their low sodium content form a good food stuff not only for people living in developing countries, but also for those living in industrialized nations (Sebastia, *et al.*, 2001).

Legumes are well adapted to a wide range of climates and environmental conditions. This is mainly because of their high prosperity to grow in depleted soils thereby serving as a medium of fertilizing succeeding crops through their unique symbiotic capability with nitrogen-fixing

rhizobium bacteria which are inhabited in root nodules of the legumes, and the nitrogen balance in the soil is thereby preserved (Okara *et al.*, 2002).

Of the thousands known legume species, only few have been extensively promoted and used. Many other potential legumes are still marginally known (Osman, 2007). This may be attributed to several factors such as deficiencies in sulfur containing amino acids (methionine and cysteine), inducing flatulence factors (raffinose, stachyose and verbacose) and presence of enzyme inhibitors (trypsin, chymotrypsin, α -amylase inhibitors and toxics like phytohemagglutinins) (El Adaway, *et al.*, 2000; Khalil, *et al.*, 2006).

Among underutilized legumes, the genus *Lupinus* is widespread in tropical and sub-tropical regions of world and is considered as an alternative protein source. Lupin is an under-utilized legume species grown predominantly in Europe, North and South America, Mediterranean and in parts of Africa including Ethiopia. Traditionally in Ethiopia, the mature seeds of lupin are consumed by Northern communities of Gojam and Gondar areas after repeated processing (roasting and soaking) to remove alkaloids. It mainly grows in these parts of the country because the communities in the area have adapted and use the crop as the raw material for some locally preparing food products such as 'Arake'(oral discussion).

Lupins can be divided into sweet lupins, which contain low levels of alkaloids, and bitter lupins, which contain higher levels of alkaloids. Debittering, which is an ancient procedure, involves the elimination of anti-nutritional factors to improve the nutritive value, and it is widely used to wash out the bitter components of seeds. Due to the fact that the alkaloids of lupin are water-soluble, the alkaloid level in bitter lupins (0.05–4 g/kg) can be easily decreased to levels that are safe for human consumption, by boiling the seeds and then soaking them in water (Sanchez *et al.*, 2005).

Lupin seeds have been part of normal food intake since ancient times and are consumed as snacks in several European countries. Since the introduction of lupin flour as an ingredient in wheat flour in the 1990s for its nutritional and food processing qualities, lupin consumption became more widespread in Europe.

Lupin flour contains comparatively higher protein (about 40 % by weight) and dietary fibre (type of carbohydrate which is not digestible by human digestive enzymes) (30%) contents (both soluble and insoluble). Lupin flour also found to provide a wide range of phytochemicals including antioxidants and phytosterols which are beneficial to health (Pettersson, 1998).

Out of the many species of lupin, white lupin (*Lupinus albus*), Australian Sweet Lupin or narrow leafed lupin (*Lupinus angustifolus*), yellow lupin (*Lupinus leutus*) and pearl lupin (*Lupinus mutabilis*) are agriculturally important (Kurzbaum, *et al.*, 2008). Currently, interest in a wider utilization of these legume seed is rising. This is mainly due to its similarity with soybean as a high source of protein and to the fact that it can be grown in wider climatic range (Sujak, *et al.*, 2005). Moreover; its adaptation to poor (nutrient deficient and acidic) soil, contribute to reducing or eliminating nitrogen fertilizer input and high potential to provide a rotation yield response to proceeding summer grain crops makes it economically feasible (Henderson, 1989). It has been used as a green manure and for fixing atmospheric nitrogen to the soil. Furthermore, it can be mixed in the soil during the flowering period in green houses to control some pests due to its alkaloids (Uzun *et al.*, 2007) as cited by Zerihun (2012).

The most commonly used part of lupin is the seed. Lupin seeds are highly valuable both for human food and animal feed (Uzun, *et al.*, 2006).

Considering its nutritional and functional properties, it has a high potential to be used in different foods such as fermented foods, pasta, crisps, bread, biscuits and cakes (Dervas, *et.al* 1999). Lupin flour is lower in cost compared with other similar legume flours such as soybean (Jayasena, and Quarl, 2004).

Hence the exploitation, introduction and characterization of such, hitherto, little known grain legumes, like *lupinus* species including wild legumes consumed by different tribal sects throughout the tropical regions as well as their genetic improvement of the quality and quantity of protein would be a great contribution to combat the protein-malnutrition in developing countries (Kala *et al.*, 2010). Nevertheless, prior to utilization of such novel resources, data indicating the nutrient composition, ant-nutritional factors and toxicants and the methods of processing that will enhance their utility as food or feed ingredient are all necessary in order to achieve optimal utilization and human health and safety concerns.

This study therefore seeks to explore further the nutrient composition, ant-nutrient factors and functional properties of newly introduced lupine variety, Australian Sweet Lupin (*Lupinus angustifolius*) and to compare it to that of commonly consumed locally grown lupine variety, white lupin (*Lupinus albus*) with a view to assessing their nutritional potential.

1.2 Statement of the Problem

While good nutrition is being the foundation for human health and well-being, physical and cognitive development, and economic productivity, malnutrition particularly protein malnutrition remains one of the major obstacles to human well-being and economic prosperity in developing countries (Foster *et al.*, 2002).

FAO's most recent estimates indicate that 12.5 percent of the world's population (868 million people) are undernourished in terms of energy intake, yet these figures represent only a fraction of the global burden of malnutrition (FAO, 2012).

While the nature and causes of malnutrition including protein energy malnutrition is complex, the common denominator among all types of malnutrition is a nutritionally inappropriate diet.

In Ethiopia a sizable proportion of the population (those of low income or subsistence status) get less than the FAO recommended average daily calorie ration of 2200 Calories. This could be the result of severe malnutrition in the country. From all possible causes of malnutrition in Ethiopia, protein deficiency, which is the result of not getting the correct proportion of protein from diets, is the worst.

Exploitation of underutilized locally available varieties and introducing and adaptation of new types of legume crops with better nutritional profiles and yielding capacity is considered as an important approach to combat the protein-malnutrition. However, to assure this, an evaluation and characterization of newly introduced cultivars for their agricultural and nutritional values before releasing to the producers and consumers by concerning bodies is mandatory and primary requirement. With these facts and considerations in mind, the current research seeks to investigate the nutrient composition, ant-nutrient factors and functional properties of newly introduced lupine variety and to compare it to that of commonly consumed locally grown lupine variety with a view of assessing their nutritional potential.

1.3 Significance of the study

Result of the research will significantly help in understanding the nutritional potential of the two lupine varieties.

It will significantly contribute in promoting the production and consumption of the crop by rural farmers and thereby help in ensuring food security of the poor socio-economic groups of the rural communities, increased income from high production, improved nutritional status of individuals and communities as well in economical point of view.

The research result will have a particular significance in reducing energy; time and cost need to process the local lupin (*Lupinus albus*) variety since it has a bitter test due to higher alkaloid contents which limits its use as a potential ingredient in food formulations.

In social benefit viewpoint, the results also greatly help to enhance the awareness of the community about the nutritional value of the lupin cultivars and to make their flour as an ingredient of fortification for various food items to improve nutritional and chemical composition.

Educationally, findings of the study can serve as reference material for researchers, students, teachers, and academicians in the same field area and for future research themes to give gap to be carried out.

Will provide a clue how to process and use the lupin cultivars (particularly the new lupin variety, *Lupinus angustifolius*) in food production and preparation process.

1.4 Objectives

1.4.1 General objective

To investigate the nutritional composition, ant-nutritional factors, and functional properties of the new (*Lupinus angustifolius*) and to compare with locally grown (*Lupinus albus*) lupine (Gibto) variety with a view to assessing their nutritional potential.

1.4.2 Specific objectives

To analyze and compare the proximate chemical composition of the new (*Lupinus angustifolius*) and locally grown (*Lupinus albus*) lupine varieties.

To determine the ant-nutritional factors of the new lupine Variety and to compare it with that of local lupine varieties.

To investigate the functional properties of the flour samples and evaluate sensory characteristic of product prepared from the two cultivars.

To evaluate the effect of commonly used traditional processing methods on the nutritional compositions and ant-nutritional factors of the cultivars.

To identify and recommend the cultivar with better nutritional profile for further development and dissemination for the producers and consumers.

2. LITERATURE REVIEW

2.1 Description of the genus *Lupinus*

Lupinus is a diverse genus in the legume families which contains both annual and perennial species, mostly herbaceous and but also rarely contains shrubby and tree species. Originally the name lupine is derived from the Latin word *Lupus*, meaning 'wolf'. The Romans believed that lupins robbed the soil nutrients in the same way that wolf would steal domestic animal (ARC, 2009). Lupin is known by different vernacular names such as 'Gibto' in Ethiopia (Shimelis and Tizazu, 2010; Habtie, *et. al.*, 2009), Lupine in the United States, Turmus in Arab countries and India, and Tawari in Latin America. The local community in the North -Western Ethiopia gave the name 'Gibto' because they thought the seed originated and introduced from Egypt ('Gibtse' in Amharic). So, they name the crop after 'Gibtse' (Gibt) (Habtie *et al.*, 2009).



Lupinus angustifolius



Lupinus albus

Fig 1. Flowers of Australian sweet lupin (*Lupinus angustifolius*) and white lupin (*Lupinus albus*)

2.2 Centers of origin and domestication of lupinus

This diverse genus exists in both the eastern and western hemispheres. Centers of origin is believed to be the Mediterranean, North America, South America and East Asia (Kurlovich *et a.,l* 2002b). Molecular evolution studies suggest that the center of origin is the Mediterranean and Northern and Eastern African, North America and South America (Wolko *et al.*, 2011).

Today, approximately 90 % of the recognized species are found in alpine, temperate and subtropical zones of North and South America, which ranges from Washington states of the USA to Southern Argentina and Chile. The remaining species are distributed in the Mediterranean region and Africa, with some populations extending to highland and mountains regions of tropical east Africa and subarctic climate of Alaska and Iceland (Gladstone 1998; Wilko *et al.*, 2011). Domestication occurred first in the Mediterranean region and the American continent, but the real breakthrough that made lupin a modern agricultural crop occurred in Europe and Australia. The history of lupin domestication may be outlined as follows (Clements *et al.*, 2005a)

- ✓ Before 2000 BC. Primary domestication of *L. albus* in ancient Greece and Egypt to produce grain for human and animal consumption, as well as for cosmetics and medicine
- ✓ 1000-800 BC. Utilization of *L. albus* as green manure in ancient Rome and , subsequently, in other Mediterranean countries
- ✓ 700-600 BC. Primary domestication of Andean pearl lupin (*L. mutabilis*) on the American continent
- ✓ 1860s. Domestication of *L. leutus* and *L. angustifolius* for green manure production in Baltic countries and after wards in Germany
- ✓ 1927-1928 methods for selecting low alkaloid lupin mutants developed in Germany
- ✓ 1930s-1970s. Sweet lupin varieties with permeable seeds were developed from *L. leutus*, *L. albus*, *L. angustifolius* and *L. mutabilis* in Germany, Sweden and Russia
- ✓ 1980s-1990s Fully domesticated *L. cosentinii* and further domestication of other potential lupin species (*L. atlanticus* and *L. pilosus* and *L. polyphllus* Lindl.) in Australia and Russia.

In Ethiopia two types of cultivated lupin plants are found: a large-seeded type as grown in Egypt and Sudan, but also small-seeded type with small seeded (Jansen *et al.*, 2006) as cited by Zerihun (2012). The local community in the North-Western Ethiopia gives the name 'Gibto' because they thought the seed is originated and introduced from Egypt ('Gibste' in Amharic) (Habtie *et al.*, 2009). White lupin is a traditional crop in Ethiopia which growth at 1500- 3000m altitude.

2.3 Taxonomy and classification

Lupinus is a large and diverse genus in the legume families (Fabaceae). *Lupinus* is a genus of self or cross-pollinating, consisting of mostly indefinite plant species native to diverse geographic

locations (Phan, *et al.*, 2007). Its common name used in Europe and Australia is lupin for both native and domesticated species, while the common name for native *lupinus* in North America is lupine (Information portal for lupins 2010a).

Taxonomically, lupins are classified in the order Fabales, Family Fabaceae, tribe Genisteae and genus *lupinus* L. (Clements *et al.*, 2005a). The number of species in this genus is not well defined and it was thought to be over 1000 (Kurlovich *et al.*, 2002b). However, the commonly agreed number of the existing lupin species is around 280. Out of the recognized species only four: (Reinhard *et al.*, 2006): (*L. albus* L.: white lupin, *L. angustifolius* L.: blue or narrow-leafed lupin, *L. luteus* L.: yellow lupin and *L. mutabilis* L.: pearl or Tarrwi lupin) are of agronomic interest (Reinhard *et al.*, 2006; Mulayim *et al.*, 2002; Uzun *et al.*, 2007).

2.4 Production and distribution of lupin

Australia has been the dominant lupin producer in the world since 1990, and produces 1.4 million tones of lupins a year, making it the world's largest producer and the only significant exporter. It accounted for around 85% of global lupin production over the ten years to 2006 (Lawrance 2007).

According to the 2012 figure from the Australia bureau of statistics, in terms of growing area lupins is the fifth largest crop grown in Australia after wheat, barley, canola and oats.

An averaged 41% of annual Australian lupin was exported during the five years to 2005-06 to destining South Korea, European Union, Japan, Chinese and Taipei with each export destination taking around 50, 27, 12 and 3%, respectively (Lawrance 2007). From 2006, Australia lupin production reduced dramatically due to drought. In 2008, the global lupin production was around 774,000 tonnes, of which 63% was produced in Australia (FAO 2008). Other major lupin producing countries are Belarus, Poland, Germany, Chile and Russia.

According to Erbas *et al.*, (2005), a total of 1,387,660 t of lupin seed was produced worldwide in 2001. A recent figure shows that South Korea, Japan, Neatherland, Malaysia and Germany were the top five western Australia lupin export markets in 2010-11 (DAFWA 2012). Lupin has a long history in the United States as a green-manure nitrogen source. At one time, over one million

hectares of blue, yellow, and white lupin were grown in the southern United States as green manure for cotton (Bhardwa, 1998).

The area of lupins in the former USSR is estimated to have reached 600,000 ha for grain and up to 2 million ha for green forage and green manure, of which more than 90% was yellow lupin (Takunov *et al.*, 1993). The major regions for yellow lupin cultivation are the Belarus, Lithuania, Latvia, the forest-steppe zone of Zhitomir and Chernigov Provinces in Ukraine and the Bryansk Province of European Russia (Kurlovich, 2002). Fusarium wilt spread through the former USSR in the 1960s and caused a decline in lupin area which was not reversed until the introduction of resistant cultivars in the late 1970s. There has been a steady decline in both *L. luteus* and *L. albus* production in Europe since the 1980s for two main reasons. Firstly, anthracnose which began to spread through Europe in the early 1980s, affected these species more than narrow-leafed lupins. Secondly, the freeing up of imports by East Germany, Poland and Russia in the 1990s has seen greater use of soybean meal for feed and more available nitrogen fertilizers. An increasing interest in growing lupins in France and the UK occurred in the 1990s in response to a desire by the local animal industries to source a 'home-grown' non-GM source of vegetable protein (LISA, 2007).

L. angustifolius is becoming more popular in the UK, Germany and Poland due to greater anthracnose resistance compared to the other lupin species. In Portugal, Spain, Morocco and Tunisia bitter and shattering *L. luteus* landraces have been selected over the centuries for forage. In these regions sheep and goat grazing is practised, and often the lupins are a regenerating cover crop under cork oak plantations (Gladstones, 1998).

South African lupin production reached 60,000 tonnes in the 1960s (FAOSTAT, 2008). *L. angustifolius* and *L. luteus* have been grown in rotation with wheat in the Western Cape which has a typical Mediterranean winter rainfall climate. *L. albus* has been grown more in the northern summer rainfall zone, but weed control has been a major constraint in these areas (Van der Mey, 1996). Disease problems including powdery mildew, phomopsis and more recently anthracnose have limited the expansion of the crop throughout the country (Koch, 1996). Van der Mey (1996) estimated that there were about 80,000 ha of lupins in South Africa at that time, grown for grain, 'standing hay' and silage.

Currently, Chile is the only country in the world where lupin production is increasing with annual production of 70,000 t in 2006 in South America (FAOSTAT, 2008). Most of this production is *L. albus* which is grown in Region IX and has benefited from a strong local breeding effort. However, there is increasing interest in *L. angustifolius* and *L. luteus*. A significant proportion of lupin is being utilized by the large Chilean salmon aquaculture industry.

In the Andean highlands a small quantity of bitter *L. mutabilis* is still cultivated using traditional methods by farmers in Ecuador, Peru and Bolivia. FAO production data indicates production in Peru is consistently in the order of 9,000 t (FAOSTAT, 2008).

Current production in Argentina is insignificant, but trial plots' data matched to climate and soil-type analysis indicate that substantial areas of the Pampas where wheat is currently grown are suitable for *L. albus* production (Ravelo and Planchuelo, 2005).

2.5 Agricultural importance of lupin

The role of lupins in agricultural systems has changed dramatically over the millennia and particularly over the last century since they have been domesticated as a grain crop for modern agriculture. The major cultivated species of *Lupinus* (lupins) genus belonging to Fabaceae are *L. albus* L. (white lupin), *L. angustifolius* L. (blue lupin), *L. luteus* L. (yellow lupin) and *L. mutabilis* (Andean lupin or tarwi) (Mulayim *et al.*, 2002).

Lupin crops are often grown in rotation with other crops, usually cereals. Throughout the history of use of lupins their role as a nitrogen fixer and soil health improver has remained central. Incorporating lupin in to rotations confers benefits to the farming system that includes the reduced disease in the subsequent cereal crop, increased supply of organic nitrogen, increased supply of high quality sheep feed and more options to control weeds (Harries and Peek 2008). Wheat: lupin rotations widely used in Australia is contributed directly to increased wheat yield and effective control of weeds.

Lupins play a complementary or alternative role as sources of organic fertilizer to replenish nitrogen depleted soils in farming systems. Like other legumes, lupins obtain their nitrogen requirement through symbiotic association with N- fixing rhizobium. Annual nitrogen fixation or assimilation by lupinus species is estimated to range from 145 to 208 kg /ha/year and has

reported by Jansen (2006) an atmospheric nitrogen fixation rates up to 400kg N/ha/year have been observed in Europe and Australia. The high nitrogen yield of lupins can be exploited by using them in rotation with grasses or cereal crops.

Lupin has high above ground biomass and deep taproots (often going down to 2m (Eastwood *et al.*, 2008), which make it highly useful in aerating soil, or supplying it with oxygen and water. It helps create a better environment for growth and survival of other plants. It makes it excellent to be used as green manure. Lupin is an undervalued grain legume that can be grown under marginal agricultural conditions (Mann and Truswell 2002).

Since lupin has a high tendency of growing on marginal lands, it will help to balance the ecology, thus could contribute in protecting in the degradation of the environment and also helps in stabilizing soil (Uzun,*et al.*, 2007). Lupin can be mixed in the soil during the flowering period in green houses to control some pests due to its alkaloid contents. Yildiz (2011) as cited in Yorgancilar *et al.*, (2009) reported that waste of boiled lupin water which contain bitter alkaloid compound might be used in organic farming systems against pests. Moreover, lupin extract has suppressive effect on plant parasitic nematodes. Lupin has a characteristics of non shattering, disease resistant, high yield giving, growing on marginal soil with poor soil nutrient and minimum or low agricultural practice on acidic type soils which in turn requires

The *Lupinus* species mostly have habitats range from desert valleys to tropical highlands; from high mountain regions to coastal plains and in general the species seems well adapted to a number of climatic environments and there is considerable variation within this species (Wolko *et al.*, 2011).

2.6 Australian Sweet Lupin (*Lupinus angustifolius* L.)

Australian sweet lupin' refers to the legume crop of *Lupinus angustifolius* (narrow-leafed lupin) cultivated in Australia (Wolko *et al.*, 2011).

During the 1960s, Australian scientists domesticated this species to create the Australian sweet lupin of today - an earlier flowering, sweet seeded version of its wild relative with nonshattering pods and a permeable seed coat. Food Standards Australia New Zealand has recognized

Australian sweet lupin as fit for human consumption since 1987.its wild type is characterized by bitter seeds, shattering pods, an impermeable seed-coat and is late flowering, making it unsuited to modern farming and human consumption. In Europe, lupins are used to replace cereal grains or soy in food products such as baked goods, small goods and noodles and pasta. In fact it is estimated that 500,000 tonnes of food products in Europe contain European white lupin and Australian sweet lupin as an ingredient. Australia is the world's largest producer of Australian sweet lupin with Australian farmers producing about one million tones of the grain each year.

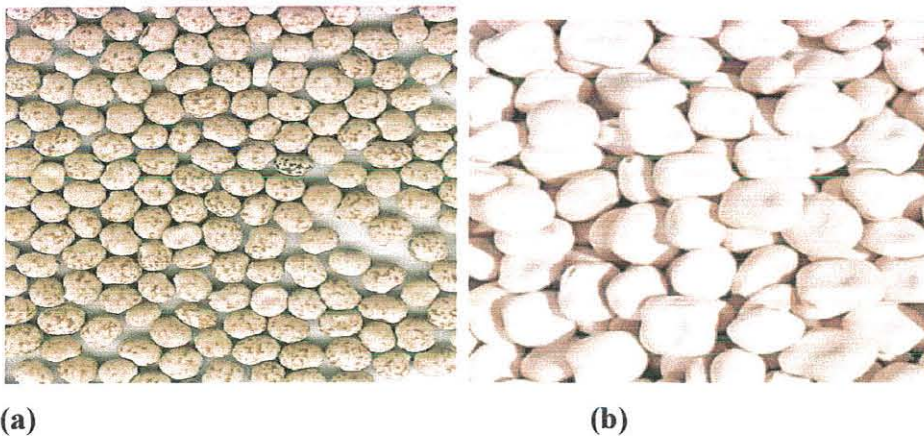


Fig 2. Seeds of Australian sweet lupin (*Lupinus angustifolius*) (a) and white lupin (*Lupinus albus*) (b)

Australian sweet lupin has a typical dicotyledonous structure. The seeds coat comprises 25 per cent of the total seed weight and is mostly cellulose and hemi cellulose. It is the cotyledons (kernels) that are most suitable for use in food product development (Batterham and Egan 1987). The protein content of the lupin kernel is very high and can constitute more than half the kernel's total weight. Most of the proteins consist of globulin type storage proteins called conglutins; these make up about 85 per cent of the total protein and have similar size and physical properties to the storage proteins of other grain legume species. The remaining 15 per cent of proteins are albumins, which are soluble at pH 5 and vary in size from about 6,000 to 117,000 daltons.

The lipid content of Australian sweet lupin is made up of triglycerides (71%), phospholipids (15%), free sterols (5%), glycolipids (3.5%) sterol and wax esters (Ahmed 2014). (0.5%), free

alcohols (0.4%) and unidentified waxy material (4%). The sterols present in the non-saponifiable fraction of the oil are mainly sitosterol and campesterol with smaller amounts of stigmasterol and avenasterol.

The mineral (ash) content of Australian sweet lupin varieties fluctuates between 3.2 and 4.6g/100g dry matter. Typical mineral contents in mg/g are: calcium between 15 and 29; magnesium 11 – 20; sodium 3 - 11 and; potassium 66 - 90. Typical trace element contents in mg/kg are: iron 31 – 150; zinc 24 - 45 and; copper 2.5 – 6.8(Cowling *et al.* 1998).

Australian sweet lupin has a very low content of proteinaceous antinutritional factors. Typically, trypsin inhibitor activity is < 0.1 mg/kg, and chymotrypsin inhibitor activity < 0.1 mg/kg. Lectin activity is virtually non-existent in Australian sweet lupin. The grain of modern domesticated Australian sweet lupin cultivars typically contains less than 200 mg/kg alkaloids. In contrast, grain from 'bitter' wild lupin types can contain from 5,000 to 40,000 mg/kg alkaloids (Cowling *et al.* 1998).

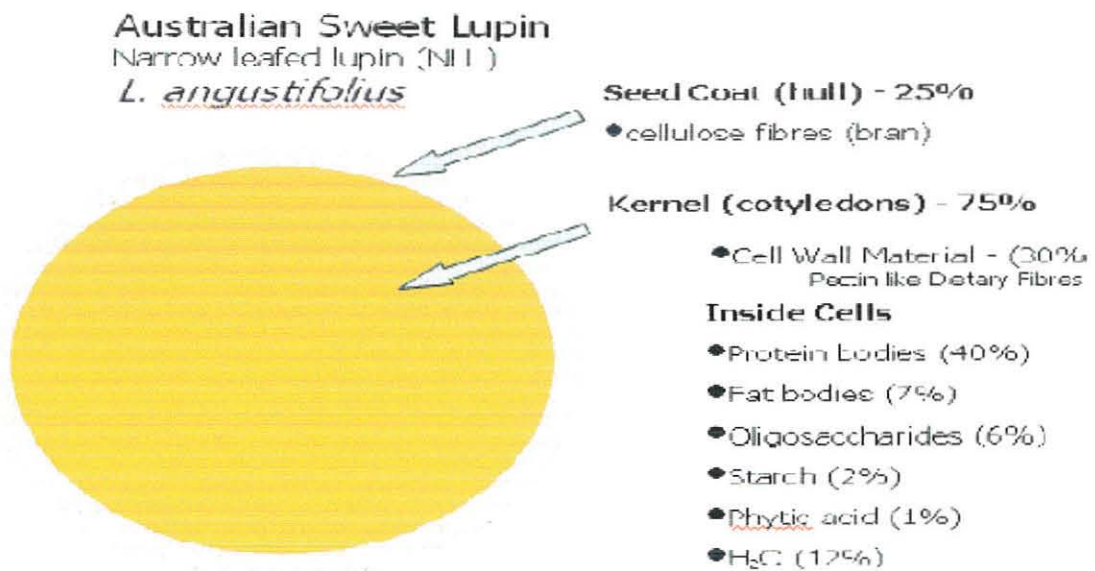


Fig.3. Seed coat and cotyledon composition of Australian Sweet lupin (*Lupinus angustifolius*)

2.7 Chemical composition and nutritional value of lupin

2.7.1 Proximate composition

2.7.1.1 Crude protein

The major biochemical feature of lupin is the capability to synthesize high proportion of protein due to its coexistence with nodule bacteria, which provides the lupin with the ability to fix the atmospheric nitrogen and change it into usable form (protein and other nitrogen substances) (Kurlovich *et al.*, 2002).

Lupin, like other legumes, is a source of high quality protein, essential amino acids, oil and other nutritive substances. Protein content of white lupin seed (33–47%) is higher than other legumes, and close to the soy protein content. Lupin seed storage protein is made up of a large proportion (85%) of globulins and small proportion (15%) of albumins (Pettersen, 1998).

The globulins fraction contains three major proteins: α -, β -, and γ - conglutins. Of which, γ -conglutin is a lupin specific globulin (Salmanowicz 1995) and has recently attracted more attention due to its unique glucose controlling- properties.

Compared to other grain legumes such as peas, soyabean, and string bean, lupins appear to contain the least amount of protein having antinutritious properties: inhibitors of proteinase and hemagglutinins (lectins). They are practically absent in the main cultivated species and cultivars (Kurlovich *et al.*, 2002).

The amino acid profile of lupin seed proteins is high in arginine, lysine, leucine and phenylalanine when compared to soyabean. The notable difference is methionine and cysteine (Glencross, 2001).

Contrary to cereals, lupin proteins contain high amount of lysine and low amount of sulphur containing amino acids (Dervas *et al.*, 1999). As a member of legume family lupin bean protein is rich in lysine and deficient in sulfur containing amino acids (Sujak, *et al.*, 2005). In contrast its arginine content is markedly higher and also the value of leucine is satisfactory for most of the species of lupinus. Apart from the highest level of amino acids within the crude protein, it was found to have a better and nutritionally more beneficial amino acid composition and the highest essential amino acid level (EAA). It is also characterized by a higher essential amino acid index

(EAAI) as well as chemical score (CS) of restrictive amino acids, and the highest protein efficiency ratio (PER), expressed in terms of the availability of leucine and tyrosine as compared to blue and yellow lupine varieties (Zrally, *et al.*, 2007). Based on this fact *Lupinus* seeds have been employed as a protein source for animal and human nutrition in various parts of the world (Sanchez, *et al.*, 2004). Generally plant proteins are increasingly used as food ingredients because they improve nutritional profile, stabilize the texture and optimize recipe costs. Analyses of nutritional values of different *Lupinus species* have shown that the bio-availability of the constituents is comparable to those of processed soybeans (Joray, *et al.*, 2007).

Currently, there are only few companies in Europe that produce *Lupinus* protein ingredients for food use. The products available are toasted and non-toasted lupin flour, grits, granulates, fiber and protein concentrates the non-defatted seed.

2.7.1.2 Carbohydrates

Lupins are typically low in starch and most species contain less than 1.5 % in the seeds. therefore the non-starch polysaccharides (NSP) constitute the major proportion of the carbohydrate fraction of all lupin species, typically being about 40% (Glencross, 2001). Lupin seed hull and cotyledon contain different types of carbohydrates. The hull is predominantly composed of NSP such as cellulose and in contrast, the main NSP in cotyledons are non-structural polysaccharides of the cell wall, with the main constituent sugars of being galactose, arabinos and uronic acids (Pettersson, 1998).

2.7.1.3 Lipids

The contents of lipids vary considerably among different lupin species. The composition of total lipids with the whole seed of *L. angustifolius* as example is: triacylglycerols (or triglycerides 71.1%, phospholipids (14.9%), free sterols (5.2%), glycolipids (3.5%), sterols and wax esters (0.5%), free alcohols (0.4%), hydrocarbons (0.4%), and unidentified wax materials (0.4%) (Glencross 2001).

Lupin has considerable amount of oil (5–20%) in the whole seed although it is not an oilseed crop (Mohamed and Rayas-Duarte 1995). The fat level in lupin is ranked third after ground nut (*Arachis hypogaeae*) and soybean (*Glycin amx*) among legumes (Joray, *et al.*, 2007).

The fatty acids of the oil from the raw seed are composed of more of unsaturated fatty acid and small percentage of saturated fatty acids. Among the unsaturated fatty acids, mainly oleic and linolenic acids are found (Uzun, *et al.*, 2006).

2.7.1.4 Fiber

The hull constitutes considerable part of the lupin seeds (20%) with a high content of dietary fibre and other valuable source of health promoting ingredients especially antioxidants (Gorecka *et al.*, 2000). Lupin seeds with 34.44– 39.42% dietary fiber content (3.64–5.21% soluble and 30.80–34.22% insoluble) may also be a potential source for the production of dietetic food (Martinez-Villaluenga *et al.*, 2006).

2.7.2 Toxins

Most wild lupin species are considered to be toxic due to their high content of quinolizidine alkaloids (Keeler, 1989). Lupines are also associated with a mycotoxicosis called lupinosis caused by phomopsins (Allen 1998). However, toxins in commercially grown lupins have generally been reduced to manageable levels as a result of domestication and breeding (Cowling *et al.*, 1998). Alkaloid content is a dominant trait determined by four genes in yellow lupine, by five genes in narrow-leaved and by eight genes in white lupine (Phan *et al.*, 2007). Through plant breeding programme, the level of alkaloids could be reduced to acceptable level for human and animal consumption. In Australia, lupin varieties with low alkaloid content, so-called ‘sweet lupins’, have been developed through plant-breeding programs. Data indicates that the mean alkaloid content of marketable sweet lupin seed is on average 130-150 mg/kg.

2.7.3 Antinutritional factors

2.7.3.1 Alkaloids

The most prominent anti-nutritional factors in lupin seeds are the bitter and toxic quinolizidine alkaloids, which occur at concentrations up to 2.5%. Although *Lupin* seeds are currently gaining global popularity as a good protein source, their high alkaloid content is a significant limiting factor to their more widespread consumption.

There are many toxic alkaloids present in the *genus Lupinus*, including pyrrolizidine and piperidine alkaloids (Panter *et al.*, 1998). The lupin alkaloids in the species of agricultural

importance are usually derivatives of quinilizidine and, therefore, called quinolizidine alkaloids (QAs). Over 100 QAs have been reported in the genus *Lupinus* (Wink *et al.*, 1995).

The main role of alkaloids is to provide the plant a chemical defense against herbivores. Some of the alkaloids may also display antimicrobial activities and confer resistance to bacterial and fungal pathogens and are used as a nitrogen source for seedlings (Erdemoglu *et al.*, 2007). However, alkaloids make lupin seeds bitter and are toxic when ingested by humans or animals. Another theory proclaims alkaloids to be useless products of protein metabolism (Clements *et al.*, 1996). Yet another opinion is that alkaloids, accumulated in the underground parts of a plant, participate in metabolic processes, induce root growth and, on leaching into the soil, become a barrier to microorganisms (Peneva, 2006). However, none of the above theories comprehensively explains the significance of alkaloids to plants, because some plants accumulate alkaloids while others do not.

Different lupin species have different profiles of alkaloids, and within species there are usually four or five major alkaloids and several minor ones (Allen, 1998). Lupanine and sparteine are the most common QAs that show acute oral toxicity due to neurological effects leading to the loss of motor co-ordination and muscular control (Resta *et al.*, 2008a). Wild lupins have more complex alkaloid profiles than the domesticated lupin cultivars. However, total alkaloid concentrations range from 0.01 to 4% depending on the species, plant part and growing conditions (Allen, 1998).

The alkaloids confer a bitter taste to the seed and are toxic when ingested. To eliminate them, an elaborate cooking process is necessary or a breeding work should be done to develop a sweet variety (Kurzbaum, *et al.*, 2008). Lupin alkaloids are characterized by toxic as well as pharmacological properties. In yellow fodder lupine, the alkaloid concentration may range from 0.005% to 1.7% and in narrow-leaved from 0.005% to 3.0%. In lupines, the alkaloid levels varying between 0.025- 0.099% is considered low. Breeding alkaloid-free lupin varieties or varieties with low amounts of alkaloids offers new possibilities for lupine application not only in forage production but also in the food industry.

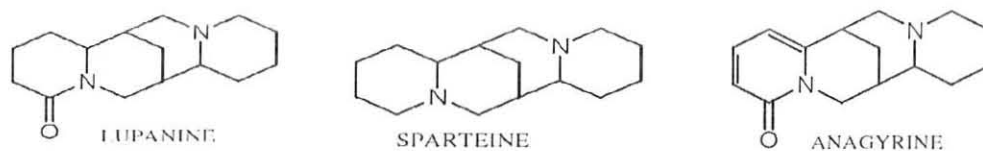


Fig. 4. Chemical structure of some of alkaloids in *Lupinus* species

Alkaloids show an uneven distribution in plant organs. Some plants accumulate them mostly in seeds and others in leaves, roots or cortex. The same plant may accumulate both similar and different alkaloids. Alkaloid content changes during vegetation period. It will reach maximum level on flowering while its concentration is lower at the stage of budding and lowest at the stage of full ripeness (Resta *et al.*, 2008a). Alkaloid content in *Lupin* depends on numerous factors such as species variety, age (developmental stage), environment and geographical location. At the end of vegetation, alkaloids accumulate in seeds and roots. These alkaloids may be found in any derivative of the seed or plant, including flours and meal that can be used to prepare pastas, pastries and dairy product substitutes.

2.7.3.1.1 Health effects of alkaloids

Lupin alkaloids can impact the central nervous systems of the mammals, with low levels acting as stimulators and higher levels as suppressors (Kurzbaum *et al.*, 2008). QA intoxication is characterized by trembling, shaking excitation, and convulsion, and can lead to anticholinergic syndrome with blurred vision, dry mouth, nervousness and malaise (Kurzbaum *et al.*, 2008). Lupanine and sparteine are the most common QAs that show acute oral toxicity due to neurological effects leading to the loss of motor co-ordination and muscular control (resta *et al.*, 2008a). The food safety and health authorities of some countries, including France, UK, Australia and New Zealand have set the maximum limit of alkaloid content in lupin flours and food at 200mg/kg (Resta *et al.*, 2008a). Quinolizidine alkaloids also affect Na⁺ and K⁺ channels, inducing gastrointestinal, nervous and respiratory symptoms in humans and animals.

2.7.3.2 Phytates

The consumption of grain legumes in human diet is limited due to the ant-nutritional factors. These include, α -galactoligosaccharides, phytic acid, condensed tannins, polyphenols, protease

inhibitors, α - amylase inhibitors, lectins, etc (Joray, *et al.*, 2007). Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis-dihydrogen phosphate) is a compound found in cereal grains, legumes, nuts and oilseeds, it is known as an antinutrient because it decreases bioavailability of minerals and protein in animal and humans. Mineral ions may bind with one or more phosphate groups in one or more PA molecules and result in reduced bioavailability of Ca, Zn, Fe and Mg (Torres *et al.* 2005).

Unlike other leguminous plants such as peas, soy beans, Lupins contain extremely low amounts of trypsin inhibitors, lectins, iso-flavone, saponins, and cyanogens and phytates. Trugo *et al* (1993) reported that lupin varieties grown in South America contain 0.4-1.2% phytic acid. These values were similar to those of lentil beans and peas (Donangelo *et al* 1986), but lower than soybeans. The phytic acid content in cultivated lupin is below 1 %, which is less than barley, wheat and soya bean (Allen 1998).

During food processing some applications such as dehulling, grinding, soaking, cooking, fermentation and phytase addition reduce the PA content of the end product at variable degree depending on process condition (Bilgiçli *et al.* 2006). In legume seeds, long soaking process at suitable pH and cooking processes under atmospheric condition/pressure or by using microwave are effective methods for lowering the PA content of raw material.

2.7.4 Traditional processing methods used to remove ant-nutritional factors

Attempts to increase the utilization of legumes have employed a wide range of processing techniques, such as roasting, soaking, boiling, de-hulling, germination, cooking, fermentation and recently extrusion cooking. De-hulling, soaking and germination were found to increase the biological value of legumes. A brief description of the major methods which are used today to process lupins is provided below.

2.7.4.1 Roasting

Heat treatment is the most common method used to reduce or remove any anti-nutritional factors in grain legumes including lupins. Subjecting grains to heat for varying periods of time, namely, toasting and roasting, is widely practiced as a method of decortication. These practices are basically techniques for drying the grains. Initially, either presoaked moistened legumes or those

to which no additional water has been added are used. The addition of heat to legumes by roasting, toasting, or parching renders the husks easier to remove since they become brittle and subsequently crack. When these methods are applied to moistened grains, the cotyledons have a tendency to shrink more than the husk, resulting in the husk being loosened from the cotyledon (Kurien and Parpia 1968). In addition to facilitating husk removal, heating can be effective in destroying toxic factors present in legumes (Kurien *et al.* 1972). Liener (1962) has reported that most antinutritional or toxic effects of legumes can be partially or wholly eliminated by the proper application of heat. Such factors include trypsin inhibitors, haemagglutinins, goiterogenic agents, cyanogenic glucosides, alkaloids, and saponins. Roasting as a legume processing method also improves the flavour, texture, and nutritive value of the grain. It also can serve as a preliminary step in facilitating husk removal during wet or dry grinding.

2.7.4.2 Soaking

Soaking is among the most noteworthy methods that do not rely on the use of heat but are successful in improving the nutritional value of grain legumes. Legumes and some cereal grains are soaked before further processing in order to make them soften, to shorten the cooking time and remove the bitter taste and toxic substances of seeds. Previous studies have shown that cooking and soaking increase the nutritional properties such as ash, mineral and protein digestibility, and decrease antinutrients such as trypsin inhibitor, oligosaccharides and PA (Mubarak 2005). These processes also decrease the total phenols, flavonoids, soluble minerals, vitamin E and beta-carotene contents due to the leaching of these components into soaking/cooking water (Bilgiçli 2009). Many varieties of lupin have high levels of alkaloids which give bitter and undesirable taste to seeds. These compounds are removed from the seeds by boiling and then washing under running water for several days (Rahma and Narasinga 1984).

The effects of soaking and de-hulling on changes of nutritional composition of legumes were found to be significant in different studies which have which have been conducted (Youssef *et al.*, 1987). Soaking the seeds in distilled water caused maximum reduction in phytic acid content, whereas soaking in NaHCO₃ solution reduced phenolics and tannins by significant levels (72% and 78%, respectively). Soaking legume seeds in water generally helps to reduce anti-nutritional factors like phytic acid and tannins, to prevent mineral deficiencies and make the proteins more

readily available for absorption. The alkaloid levels in lupin seeds can be reduced to the optimum levels through soaking and de-hulling practices.

The soaking process of legume seeds was also thought to change texture characteristics and facilitate protein extraction process from the seed such as soy protein (Pan and Tangratanavalee 2003). It was reported that textural changes of legume seeds resulted from water absorption during soaking process. Similarly, in our country, soaking lupin seeds in the running water is commonly practiced traditional processing methods to remove alkaloid contents by lupin producing and consuming communities (northern parts of Ethiopia) (Paulos, 2009).

2.7.4.3 Germination

Germination is a natural process that causes important changes in the biochemical, nutritional and sensory characteristics of legumes. It is one of the methods that do not rely on use of heat for improving the nutritive value and decreasing the level of antinutritional factors. It is considered to be a suitable procedure to improve the nutritional value of legume seeds by reducing level of ant-nutritional factors. Germination is considered a potentially beneficial process for legume seed transformation which may decrease undesirable components such as alkaloids and phytates

During germination, some grade of transformation of alkaloids to other more bioactive compounds, such as esters, occurs. Cuadra *et al.*, (1994) found a slight increase in alkaloids during germination of *L. albus*, *L. angustifolius*, and *L. campestris*, and no α -pyridone alkaloids, such as the highly toxic anagyrine and cytosine, were detected in any of these species. Germination also increases nutrients such as vitamin C and increase protein digestibility (Kuo *et al.*, 2004), consequently improving nutritional quality. Additional advantages of germination are reduction in cooking time and improvement of the product sensorial attributes. Germination has been shown to decrease the level of α -galactosides of different legume seeds including soybean, black bean, and lupin seed, with the corresponding decrease in carbohydrates available for fermentation in the large human intestine. The content of trypsin inhibitors and phytates is also decreased, but considerable amounts of these factors are still present after germination (Trugo *et al.*, 1990).

2.7.4.4 De-hulling

De-hulling which is detaching or removal of the outer coat/ husk of legume beans is widely used traditional processing methods to eliminate ant-nutritional factors and enhance nutritional value of legume seeds. De-hulling is also an effective method to reduce α -galactosides known to cause flatulence. The processing also noted to increase the contents of copper, zinc and potassium in beans, while while it significantly decreased the amounts of iron, manganese, phosphorus, calcium, magnesium and sodium (Youssef *et al.*, 1987).

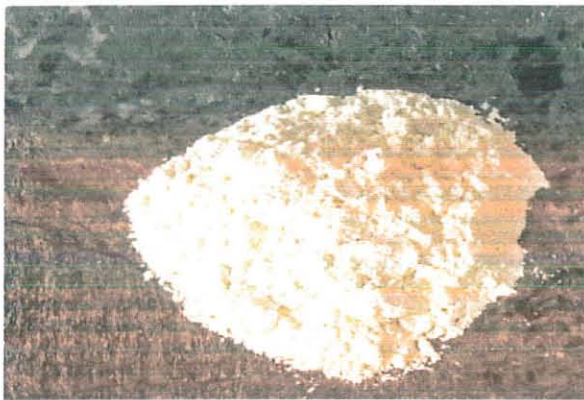
2.7.4.5 Fermentation

Fermentation is widely used in food detoxification process (Salih *et al.*, 1991) and a variety of fermented foods are eaten around the world. The further development of fermented foods has been advocated by nutritionists because of the nutritional benefits of such products. Fermentation is also an effective means of food preservation (Nouts and Robouts 1992). Fermented foods can be prepared on an industrial or household scale. Indeed, many fermented food products are prepared by very simple techniques a fact which facilitates their adoption in underdeveloped countries for the detoxification of alternative food sources such as lupin alkaloids. Incorporation of fermentation process in in to simple food technologies offers good prospects for detoxification of food sources while simultaneously giving flexibility in the manipulation of flavor, texture and color of the raw material. In practice, the fermentation process breaks down carbohydrate (starch) to acid as the final end product by the action of microorganisms (bacteria, moulds, and yeast). In the household practice, such microorganisms in the atmosphere are the fermenting organisms. This is also true in village-scale operations. Conversely, controlled fermentation, using specific moulds and bacteria, is followed in large-scale commercial operations.

2.7.4 Commonly consumed lupin based food products

Food products available on different markets of Europe are lupin snacks, lupin pasta, lupin bread and cookies, lupin coffee and some vegetarian instant meals. Lupines and lupine products have traditionally formed part of the human diet (Sanchez, *et al.*, 2004). Lupin seed flour can substitute part of the wheat flour used for bread, cake and pasta. Lupin flour is added for nutritive value and also provides functional properties in bakery and pastry products, protein concentrates and other industrial products, as well as the elaboration of lactose free milk and

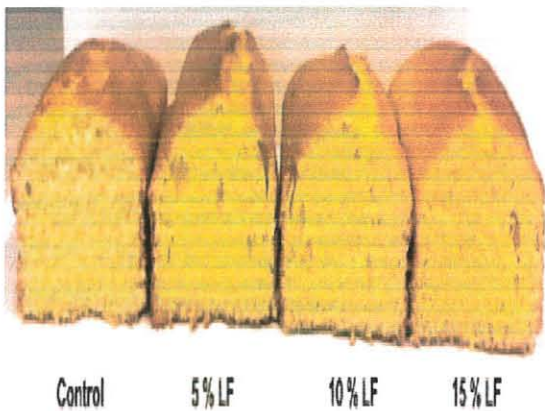
yoghurt analogues. Lupin flour has characteristics of improving the micro distribution of water in dough and mixtures. Products could then resist freezing and thawing better, the preparation of bread dough could be easier, shrinking could be limited, and emulsifying power will be good, for a yellow color development, to change some of rheological parameters, like crispness and smoothness. Lupin flours are largely used as eggs substitute, for example in cakes, pancakes, and biscuit. The flour can also be used as a butter substitute in cakes (Lacana, 1999).



(a)



(b)



(c)



(d)

Fig. 5. Some of lupin flour incorporated food product (lupin kerneyl flour (a), lupin pasta(b), lupin protein product (d) and lupin supplemented bread(c).

2.7.5 Production and consumption information of lupin in Ethiopia

Of the four agriculturally important lupin species, white lupin (*Lupinus albus*) is widely produced and consumed in Ethiopia. This bitter lupin is widely found in Northern, particularly Gojam and Gonder areas of Amhara region and Southern (Guraghe) areas of the country. In these areas lupin (*Lupinus albus*) is used as a food crop (CSA, 2007) as cited by Paulos (2009). This species has very useful agronomic characteristics. It is non-shattering, disease resistant, high yield giving (1.2t/ha), growing on marginal soil and so on. But, regarding its good agricultural and nutritional value, only few researches have been done to efficiently use the crop to its maximum full potential. Shimelis Emire (2010) studied the chemical composition, physico-chemical and functional properties of lupin (*Lupinus albus*) seeds, and reported that the seeds contain adequate amount of protein, carbohydrates, and minerals. People living in west Gojam are the predominant consumers of the crop (oral communication) as cited by Paulos (2009). People in that area consume the crop primarily as snack and as a raw material for local beverage “Araki” processing. Now days the local community consumes it as “Shiro” similar to other common legumes like pea, bean, etc. There is an ancient belief that *Lupinus albus* is a remedy for people having hypertension. And some of the farmers living nearby lakes are using the seed for fisheries as a feed, due to its toxic nature of the alkaloids.

3. MATERIALS AND MEHODS

3.1 Sample collection and preparation

The samples of new (*Lupinus angustifolius*) and local (*Lupinus albus*) lupin seeds were obtained from Holetta Agricultural Research Center. The samples were packed in polyethylene bags and transported to Food Science and Nutrition Laboratory, Addis Ababa University. The samples were thoroughly cleaned manually by picking all broken kernels, stones, together with other foreign particles. The cleaned seeds were ground in 60-mesh flour, packed in polyethylene bags and stored at room temperature (25⁰C) until analysis. The seed flour obtained was analyzed for proximate chemical composition, mineral compositions, anti-nutritional factors and functional properties. The cleaned seeds of both samples were further subjected to two main traditional processing methods for purpose of product development and sensory analyses. These methods were roasting followed by soaking and boiling followed by soaking.

3.1.1 Roasting and soaking

The cleaned seeds of each lupin samples (*Lupinus angustifolius* and *Lupinus albus*) were roasted on metal pane for about 10 minutes (Paulos, 2009) together with pre-cleaned sand. The sand was used for uniform heat flow (conduction) during roasting. Then the sand was removed followed by washing and allowed to cool for 15 minutes. The washed seeds were soaked for 114h at lupin to water ratio of 1:10(w/v) and the soaking water was changed every 12h interval to wash out alkaloid according to the same author. The soaking times were used as exactly applied in traditionally and this variation of soaking time between the two lupins is related to the level of alkaloids. The removal of the bitterness was checked by tasting the whole seed similar to the traditional processing (tasting the seed while processing). After soaking, the seeds were oven dried at 50⁰C for 24 hrs. The oven dried samples were ground in 60-mesh flour, packed in polyethylene bags and stored at room temperature until analyses. The seed flour obtained was analyzed for proximate chemical composition, mineral contents, ant-nutritional factors and functional properties.

3.1.2 Boiling and soaking

The cleaned lupin seeds were boiled in water for 75 minutes according to Erbas (2010) to destroy thermo labile anti-nutritional factors, such as trypsin inhibitors and particularly to soften the

seed. The boiled seeds were then soaked for 144h at lupin to water ratio of 1:10(w/v) and the soaking water was changed every 12 hrs interval. The removal of the bitterness was checked by tasting the whole seed as explained in the section 3.1.1 above. After soaking, the seeds were oven dried at 50⁰C for 24h. The oven dried samples were then milled using laboratory sample mill with sieve size of 60 meshes and packed in polyethylene bags. The packed lupin flour was stored at room temperature until analysis.

In addition, 24 hour soaking was used for the new lupin variety in each case which was not used in the case of the local variety. This is because the new variety is assumed to have lower alkaloid content since it is sweet lupin type. Where the local lupin variety is considered to have high total alkaloid content and usually soaking for at least 5 to 7 days is recommended to de-bitter the seeds (Paulos, 2009).

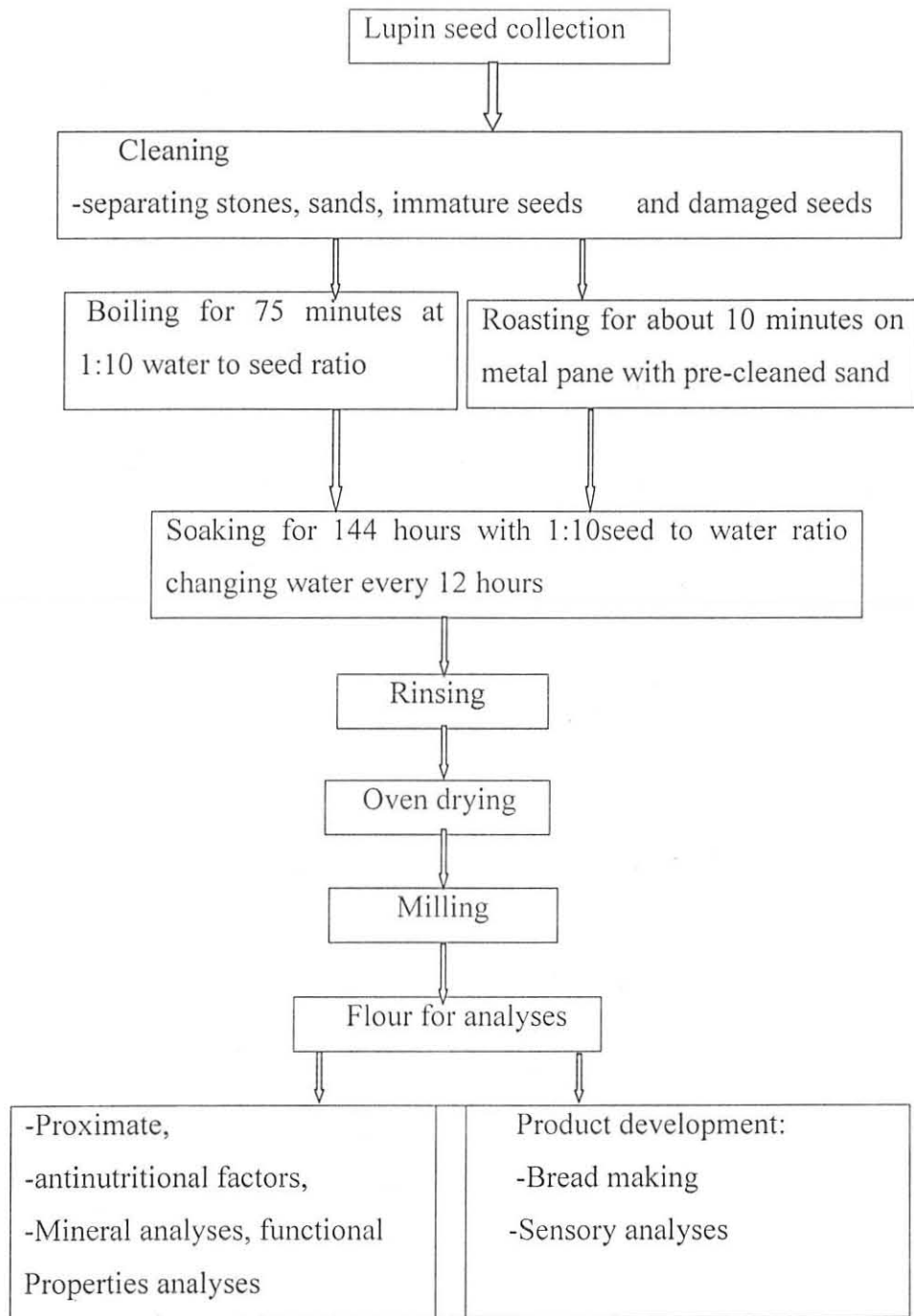


Fig.6.Flow chart for lupin flour preparation

3.2 Proximate analysis

Proximate composition of the seed flour including moisture, crude fat, crude protein, total ash and crude fiber were determined using AOAC official methods of 925.09, 4.5.01, 979.09, 923.03 and 962.09 of 2000, respectively. Total carbohydrates and utilizable carbohydrate content in the lupin samples were calculated by difference. The total energy content of each sample was estimated (in kcal/g) by multiplying the percentages of crude protein, crude lipid and available carbohydrate with the recommended factors.

Total energy (g/100g) = (9 x crud fat + 4x crud protein + 4x utilizable carbohydrate).

Seed contents of Ca (macro) and Fe and Zn (microelements) were determined using Atomic Absorption Spectrometric methods.

3.2.1 Moisture content analysis

Triplicate samples were used for moisture content in a hot-air circulating oven (Galenkamp) (method No. 925.09 (AOAC, 2000). A five gram of sample was weighed using analytical balance in to the dish and mixed thoroughly and dried at 105⁰C for 3 hrs. Then it was cooled in desiccators and kept in oven for 15 min. This process was repeated until constant weight was obtained. Then, the moisture content was calculated using the following formulac;

$$\text{Moisture (g/100g)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100, \text{ where}$$

M₁= Mass of the dish

M₂= Mass of the dish and the sample before drying

M₃ = Mass of the dish and the sample after drying

3.2.2 Crude ash analysis

Ash was determined by incineration (550⁰C) of 2.5g weights of the samples in a muffle furnace (Method No 923.03, 2000).

The porcelain dishes used for the analysis were washed by dilute hydrochloric acid on boiling and washed with distilled water respectively. Then dried at 120⁰C in an oven and ignited at 550

$^{\circ}\text{C}$ in furnace for 3 hrs. Then the dishes were removed from furnace and cooled in a desiccator. The mass of the dish will be measured as M_1 . A 2.5 gm of sample powder were being weighed in to the porcelain dish which is washed, dried and measured and recorded as M . The sample was charred at 120°C for 4 hrs on a hot plate, until the whole content becomes carbonized. Then the sample was placed in a furnace at 550°C until free from carbon and the residue appears grayish white after 5 hrs. The sample was removed from the furnace and placed in desiccator.

$$\text{Ash (g/100g)} = \frac{M_3 - M_1}{(M_2 - M_1)} \times 100 \text{ where,}$$

M_1 =mass of the dried dish

M_2 =mass of the dish and the sample

M_3 =mass of the dish and the ash

3.2.3 Crude fat analysis

Crude fat was determined by exhaustively extracting sample in petroleum ether (boiling point, 40 to 60°C) in a Soxhlet extractor (Method No 4.5.01) (AOAC, 2000). A 2g of the sample was weighed in a thimble lined with cotton at their bottom and these thimbles were placed in to soxhlet extraction apparatus. Then 50 mL of diethyl ether was added in to and the extraction process continue for 4 hrs and then this flask with its content is removed from the soxhlet and placed in to drying oven at 92°C for 30 and was placed in to desiccators for 30 min. and finally the mass of each flask together with its fat contents was measured as M_2 . Then, the total lipid amount was calculated using given formula.

$$\text{Fat (g/100g)} = \frac{M_2 - M_1}{M} \times 100, \text{ where}$$

M_2 = mass of flask and lipid extracted

M_1 = mass of dried flask

M = weight of sample on dry basis

3.2.4 Crude protein analysis

Protein ($\text{N} \times 6.25$) was determined by the Kjeldahl method (Method No 979.09A) ((AOAC, 2000).

A 0.5 gm of powdered sample was weighed on analytical balance and transferred to the digestion flask. Then 6 mL H₂SO₄ and 3.5 mL of 30% H₂O₂ was added in to the digestion flask step by step. The tubes were shaken observing a violent reaction. After this violent reaction disappeared 3 gm of the catalyst mixture (1:10 Cu: K₂SO₄) was added in to the digestion flask. The solution was then digested at 370 C° for 4hrs. After digestion was completed, the content in the flask was diluted by water and concentrated sodium hydroxide (40 %) was added to neutralize the acid and to make the solution slightly alkaline.



The ammonia was then distilled into a receiving flask that consisted solution of excess boric acid (4%). The borate ion was formed as a result of the reaction of the boric acid and the ammonia and this was titrated with standard acid(0.1N hydrochloric acid) until the green color changes to pink. The total nitrogen content was calculated using the following formulae;



$$\text{Nitrogen (\%)} = (\text{VHCl} \times \text{NH}_2\text{SO}_4 \times 14.01) / \text{M} \times 100$$

VH₂SO₄= Volume of H₂SO₄ consumed until the end point of titration

N H₂SO₄= Normality of H₂SO₄

14.01 = Molecular weight of nitrogen

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

M = Weight of sample on dry basis

3.2.5 Crude fiber analysis

Crude fiber was determined using fibertec after digesting a known weight of sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (Method No 962.09) (AOAC, 2000). About one gram of sample is weighed in to a pre-dried crucible which contain one gram of celite sand for the purpose of simplifying filtration (W_1). Then the crucible with its content was placed in the fibertec and the sample was digested with 1.25% of sulphuric acid for 30 minutes and followed by digestion with 1.25% of sodium hydroxide for 30 minutes. Afterward, the crucibles were take-off and dried in oven at 130 °C for 2 hrs. Then cooled in desiccator and weighed as (W_2). Finally the crucibles were placed in muffle-furnace and the sample was ashed at 525 °C for 3hrs. Cooled to room temperature in desiccator and weighed again as (W_3). The crude fiber content was calculated by using the following formula.

$$\text{Crude fiber (g/100g)} = \frac{W_2 - W_3}{W_1} \times 100, \text{ Where}$$

W_1 = Weight of sample

W_2 = Weight of crucible- residue

W_3 = Weight of crucible- ash

3.2.6 Total carbohydrate determination

The total and available carbohydrate content was determined by difference. Addition of the percentages of crude fat, crude protein and ash was subtracted from 100.

$$\text{Carbohydrate (g/100gm)} = [100 - (\text{Fat} + \text{Ash} + \text{Crude protein}) \text{ in grams}].$$

$$\text{Available carbohydrate (g/100gm)} = [100 - (\text{Fat} + \text{Protein} + \text{Ash} + \text{Fiber}) \text{ in grams}].$$

The total energy content of each sample was estimated (in kcal/g) by multiplying the percentages of crude protein, crude fat and carbohydrate with the recommended factors.

$$\text{Total energy (in kcal/100g)} = (9 \times \text{crud fat} + 4 \times \text{crud protein} + 4 \times \text{utilizable carbohydrate}).$$

3.3 Ant-nutritional factors analysis

3.3.1 Phytic acid

The phytate content in the sample was determined according to the method described by (Adeniyi *et al.* 2009). A 0.08 gm of dried sample was extracted with 10 mL 0.2 NHCl for 1 hr at ambient temperature and centrifuged (3000 rpm) for 30 minutes. The clear supernatant was used for the phytate estimation. 2 mL of Wade reagent (0.03% solution of $\text{FeCl}_{3.6}\text{H}_2\text{O}$ containing 0.3% sulfosalicylic acid in distilled water) was added to 3 of the sample solution and the mixture was centrifuged. The absorbance at 500 nm was measured using spectrophotometer. The phytate concentration was calculated from the difference between the absorbance of the control (3mL of 0.2NHCl and 2 mL of Wade reagent) and that of the assayed sample. The concentration of phytate was calculated using phytic acid standard curve and the results were expressed as of phytic acids in mg per 100gm dry weight.

To prepare the phytic acid standard curve, a series of standard solution was prepared containing 4–45 mg/ mL phytic acid in 0.2 N HCl. 3 mL of the standards was pipette in to 15mL centrifuge tubes with 3 mL of 0.2 N HCl as blank. To each tube 1mL of the wade reagent was added and the solution was mixed on a vortex mixer for 5 s. The mixture was centrifuged for 10minutes and the supernatant read at 500 nm by using water as a blank.

$$\text{Mathematical formula; phytic acid in } \mu\text{g/g} = \frac{[(Ab - As) - \text{Intercept}] \times 10}{\text{slope} \times W \times 3}$$

Wher, A_s = sample Absorbance

A_b = blank absorbance

W = Weight of sample

3.3.2 Tannin

Tannin content was determined by the method described by Embaby (2010). About 1gm of the sample was dispersed in to 10 mL of 1% of concentrated HCl in methanol solution and put on mechanical shaker for 24 hrs at room temperature.

The extracts were centrifuged at 3,000×g for 20 min and the supernatant was analyzed for tannins. In a test tube 1mL of the supernatant mixed with 5 mL of Vanillin-HCl reagent was added. After 20 min the tannins content was measured at 500 nm against experimental blank adjusted to zero absorbance. Tannic acid was used as a standard compound and the results were expressed as mg/g (dry matter) by the following formula;

$$\text{Tannin in mg/g} = \frac{(A_s - A_b) - \text{Intercept}}{\text{Slope} \times d \times W}$$

Where, A_s = sample Absorbance

A_b = blank Absorbance

D = Density of solution (0.791g/ml)

W = Weight of sample in gram

3.3.3 Total Alkaloid

The alkaloid content was determined gravimetrically by the method of Haborne, (1973) as cited in (Adeniy, *et al.*, 2009). 5 g of each sample was weighed using weighing analytical balance. Then the sample was dispersed into 50 mL of 10 % acetic acid solution in ethanol. The mixture was well shaken and then 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 off the precipitate and the precipitate was washed with 1 % ammonium hydroxide solution followed by drying in an oven at 60⁰c for 30 minutes. Then was transferred in to a desiccator to cool and then 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. The experiment was repeated three times for each sample type and the reading recorded as the average of the triplicates.

3.4 Mineral

Minerals analyzed in the present study were Zinc, Iron and Calcium.

The mineral (Zn, Fe and Ca) content of the samples were determined by the method described by (Dickman and Bray, 1940). The ash was dissolved by 7 mL of 6 NHCl at low temperature on hotplate for about 2 h. 15Ml of 3 NHCl was added and the crucibles were heated on the hot plate

until the solution just boiled. Then, 10 of 3 N HCl were added and heated on a hot plate until the solution boils. The digest was cooled and filtered through a filter paper (42 mm, Whatmann) in to a 50 mL volumetric flask. Then 5 mL 3 M HCl was added to the dishes and heated to dissolve the residue in the dishes and then transferred to the volumetric flask. Then the filter paper was washed thoroughly and the washing was collected in the flask made to the mark. Afterwards the mineral concentration was determined by AAS. For calcium determination 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 through the steps all of the above 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 the absorption values against the metal concentration in ppm. The mineral content of each sample was calculated using given formula (Carvalho 2005).

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

W = weight in gm of the sample

a= concentration in ppm of sample solution

V = volume in mL of the extract

b= concentration in ppm of blank solution

3.4 Functional properties of the lupin flours

3.4.1 Water and oil absorption capacity

The centrifugal Beuchat method was employed to determine the water and oil absorption capacity of the lupin flour. Precisely weighed 1 g of the sample was mixed with 10 ml distilled water and or oil for 30 seconds in a mixer. The samples were then allowed to stand at room temperature for 30 min, and were centrifuged at 5000 x g for 30min. The volume of the supernatant was noted in a 10ml graduated cylinder. Density of water was assumed to be 1g/ml and that of the oil is 0.98 g/ml. The results were expressed on a dry weight basis.

3.4.2 Bulk density and dispersibility of flours

Bulk density of flour was determined according to the method described by Narayana and Narasinga (1984). An empty calibrated tube was weighed and filled with a sample to 5 ml by

constant tapping until there was no further change in volume. Bulk density was calculated as the mean of the triplicate determination of the weight determined by difference per unit volume of the sample. Dispersibility of lupin flour in water was also determined using the described by Kulkarni *et al.* (1991). Accurately weighed 10 g of each flour sample was placed in a 100 ml measuring cylinder followed by addition of distilled water up to the 100 ml mark. The sample was vigorously stirred, mixed and next allowed to settle for 3 h. The volume of the settled particles was recorded and subtracted from 100 to give a difference which is considered as percentage dispersibility.

3.4.3 Foaming capacity

The capacity and stability of foam were determined following method described by Lin *et al.* (1974). Exactly 50 ml of 3% (w/v) dispersion of material in distilled water was homogenized for 5 min at speed of 1600 rpm. The mixture was immediately transferred into a 250 ml graduated cylinder and the foam volume was measured. The foaming capacity was expressed as the percentage volume increase (v/v, %).

3.4.4 Emulsion activity

The emulsion activity and stability were both determined using the methods described by Yasumatsu *et al.* (1972). The emulsion (2 g sample, 20 ml distilled water and 20 ml soybean oil) was prepared in a calibrated centrifuge tube. The emulsion was centrifuged at 2000 x g for 10 min. The emulsion activity (%), were calculated as the ratio of the height of the emulsion layer to the total height of the mixture.

3.5 Bread making procedures

Five bread samples were prepared by using lupin to wheat flour in the proportions 5%, 10%, and 15% for the new variety and 10% for the local lupin variety. Additional 5, 10 and 15% were used for the new variety to evaluate if there was difference in terms of their sensory attribute. But this was not the case in the local variety since it has been evaluated for its 5, 10, and 15 and 20% and 10 per cent was recommended as appropriate proportion for lupin-wheat blend to make bread (Alemayehu, 2011). Wheat flour (100%) was used as control. The wheat flour was obtained from Misrak Flour and Bread Factory. The level of lupin flours (10%) used was based on the

recommended level for the local lupin variety to replace wheat flour for bread making (Alemayehu, 2011). The blends were homogenized with a mixer robot (model R6VV, Robot-Coupe) for 3 min.

In bread making process, each blend was kept in different containers. 1.6% salt and 2.4% yeast were added to each lupin-wheat blends and homogenized well. In the mixing steps, flour, water, salt and leavening were first mixed together and water was added gradually to the appropriate amount. Then, kneading (gluten formation) begins. The resulting dough was allowed to rest for 10 minutes in a cabinet at room temperature. The dough were moulded and put in to baking tins and proofed at 30 °C for another 10 minutes. The loaves were baked at 200±5°C in an electric oven for 20 minutes and then cooled at room temperature and then served for sensory analyses.

3.6 Sensory evaluation

The bread samples were evaluated by randomly selected untrained 13 consumer panelists who are familiar to the product for their sensory attributes (appearance, color, odor, taste and overall acceptance). The panelists were in good health and familiar with the color, taste, odor and other attributes of the product. The panelists were well instructed about the aim of the test. The breads were prepared and served in coded plastic sensory evaluation plates. Each panelist was presented with a glass of water after each testing session to rinse their mouths so as to prevent carryover effect. The assessment was conducted in a well lit room designed for sensory evaluation.

The sensory quality characteristics of breads (appearance, color, odor, taste, texture and overall acceptability) were evaluated on a 1-9 scale where 1 represented “dislike extremely”, 5 “neither like nor dislike” and 9 represented “like extremely”.

3.7 Statistical analyses

The nutritional and antinutritional factors results were statistically analyzed. The data was analyzed using SPSS version 15.0 and completely randomized design (CRD) was used. The mean and standard deviations of the triplicate analyses of the samples was calculated. One-way analysis of variance (ANOVA) was performed to determine significant differences between the means of proximate composition, minerals and antinutritional factors; while the means were

separated using the Tukey multiple range test judged significantly different at 95% confidence level ($p < 0.05$).

4. RESULTS AND DISCUSSION

4.1 Proximate Composition

The chemical and nutritional composition of both raw and processed new and local lupin varieties is shown in Table 1.

Both of the traditional processing methods used in the current study (i.e. soaking after roasting, soaking after boiling) had significant effect on the nutritional composition and ant-nutritional factors.

4.1.1 Crude protein

The protein contents of the raw seed from both varieties are comparable with soybean. Their protein compositions were 25.54g/100g and 35.17g/100g for new and local samples respectively.

There was a significant difference in protein content between the two cultivars ($P < 0.05$). The protein content of the new lupin cultivar was lower than that of local cultivar. Mostafa and Haiam (2013) have reported the protein content of the same variety narrow-leafed lupin (*Lupinus angustifolius*) was 29.1% which is slightly higher than the result of current study. However, in another investigation, Hill (1977) as reported by Batterham and Egan reported that the protein content of *Lupinus angustifolius* and *Lupinus albus* to be in the ranges of 25-35 and 35-45g/100g of dry mater. The results of the two lupins in the present study were in agreement with the reported protein values.

The variation in protein content could be due to growing condition, soil type and genetic makeup. The protein content of the raw local lupin cultivar was slightly higher than the value reported by Jimenez Martinez, *et al.*, (2003) which was 34.4g/100g, highly comparative to the result reported by Hill (1977) as reported by Batterham and Egan (35-45g/100g) and Petterson (1999) (35.76g/100g). However, it is slightly lower than protein content reported by Mostafa and Haiam (2013) (38.10g/100g). But these values were higher than protein content of the new variety. Similarly, Petterson (1999) has reported the protein content of the raw *Lupinus angustifolius* to be 44.1g/100g where as that of *Lupinus albus* was 38.10g/100g.

Table 4.1: Proximate composition (g/100g) of both processed and unprocessed new and local lupin varieties.

Proximate composition	Sample							
	RNLV	BSNLV	RSNLV	BSNLV2	RSNLV2	RLLV	BSLLV	RSLLV
Moisture	7.76±0.26 ^a	6.20± 0.33 ^b	6.02±0.41 ^b	5.48±1.41 ^c	4.84±0.89 ^c	6.59±0.22 ^b	5.58± 0.1 ^b	6.52±0.59 ^b
Total ash	2.92±0.5 ^a	1.69±1.31 ^a	1.94±1.04 ^b	1.37±0.21 ^c	1.41±0.01 ^d	2.75± 0.55 ^a	1.56± 0.95 ^a	2.17± 0.83 ^a
Crude protein	25.55±0.25 ^a	29.4± 0.18 ^b	27.48±0.35 ^c	31.15±0.62 ^d	30.8±0.89 ^d	35.17±0.4 ^f	41.13± 0.80 ^g	36.63± 4.01 ^g
Crude fat	6.42± 0.90 ^a	9.19± 1.54 ^b	5.99±2.26 ^{ab}	10.57±1.4 ^c	8.87±0.69 ^c	6.59±1.00 ^a	11.97±0.45 ^c	9.01± 0.53 ^c
Crude fiber	17.89± 0.43 ^a	17.67± 4.42 ^a	16.72±4.10 ^a	15.47±0.71 ^a	16.66±0.24 ^a	14.79± 0.03 ^{ab}	15.4± 8.0 ^{ab}	15.39±3.24 ^a
Total carbohydrate	65.12± 2.27 ^a	59.72± 1.59 ^b	64.63± 2.59 ^a	67.91±1.21 ^a	67.72±1.03 ^{ab}	55.54±1.18 ^c	45.39±0.87 ^d	52.24±3.94 ^c
Available carbohydrate	50.33±0.61 ^a	39.05±1.59 ^b	47.91±2.59 ^{ac}	54.44±0.67 ^d	54.72±1.45 ^d	41.02± 0.67 ^b	29.99± 0.87 ^c	36.85±3.94 ^b
Gross energy (in Kcal)	361.74±7.28 ^a	356.5±7.62 ^a	355.41±11.24 ^b	369.56±2.05 ^{ac}	372.71±1.25 ^c	362.52± 6.36 ^a	392.22±1.62 ^d	375.02±2.38 ^c

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

Sample types

RNLV \iff Raw New Lupin Variety

BSNLV \iff Boiled and Soaked (24hr) New Lupin Variety

RSNLV \iff Roasted and Soaked (24hr) New Lupin Variety

BSNLV2 \iff Boiled and Soaked (144hr) New Lupin Variety

RSNLV2 \iff Roasted and Soaked New Lupin Variety

RLLV \iff Raw Local Lupin Variety

BSLLV \iff Boiled and Soaked Local Lupin Variety

RSLLV \iff Roasted and Soaked Local Lupin Variety

The protein content of raw *Lupinus albus* was found to be 32.80g/100g on Food Composition Table for use in Ethiopia (EHNRI, 1997) which is slightly lower than the result of the current study for the same species.

The traditional processing methods used in the current study generally had a significant effect on the proximate composition on both sample types ($P < 0.05$). In fact both the treatments have shown an increment in the protein content of the raw seed. Accordingly, BSNLV2, RSNLV2, BSLLV and RSLLV have shown a percentage increment of 17.97%, 17.05%, 14.49%, and 3.40%, respectively. The highest protein content was obtained when the raw lupin was boiled then soaked for six days in water for the two cultivars. This could be the relatively higher loss of soluble component in boiling and soaking than roasting and soaking.

When we compare the effect of the same treatments on the two samples, protein content is significantly different ($P < 0.05$) in each treatment with various increments. Consequently, BSNLV2 showed higher protein increment than BSLLV. Similarly, in treatment RSNLV2 there was higher protein increment than RSLLV. These observed differences in protein contents between the same treatments of the two samples were may be due to the significant difference in protein content of the respective raw seeds.

The increase in the protein content of processed seed flours compared to the protein content in the raw lupin flour could be increased rate of protein releasing during soaking after roasting and/boiling. It also may be a result, at least in part, of the loss or removal of non protein nitrogenous compounds such as alkaloid during the boiling or roasting and then soaking process. The increase in protein content in the present study was higher than that found by Dagnia *et al.* (1992) who reported that boiling, then soaking for 24 hours of *L. angustifolius* increased the protein content by about 10%. The increase in protein content after soaking was also found in other legumes such as soybean (Mostafa and Rahma 1997), mungbean (Mubarak 2005), fenugreek (El-Mahdy and El-Sebaiy, 1982) and dry bean, lentils, faba beans (Hsu *et al.*, 1980). This could be due to the loss of water soluble nutrients, the synthesis of enzyme proteins.

The treatments BSNLV and RSNLV increased the protein content by 13.11 and 7.58% respectively which is lower when compared to the treatments BSNLV2 and RSNLV2.

4.1.2 Crude fat

Another major chemical component of lupin is crude fat, which is a source of nutritional components and bioactive compounds such as mono- and polyunsaturated fatty acids, tocopherols and phytosterols.

The fat contents of the raw lupin seeds of the new and local varieties were 6.42g/100g and 6.54g/100g respectively as shown in the Table 1. The fat contents between the two raw samples were not significantly different from each other ($P > 0.05$). The fat content of the new variety in this study is in agreement to the result of fat content reported by Rumiya *et al.*, (2012) for the same variety, (6.4g/100g). However, the value in the current study for fat content for the same variety was slightly higher than fat content reported by petterson (1999), (5.9g/100g) and it was lower than the value stated by Abreu *et al.*, (1998) (7.2g/100g). Similarly, the value for fat content of the local lupin variety (*Lupinus albu*) was lower than the value reported by Petterson (1999) and Mostafa and Haiam (2013) which were 9.49.g/100g and 9.94g/100g respectively, and the value reported by Brand *et al.*, (2004), (9.32g/100g). Similarly, Ahmend (2014) reported the fat content of *Lupinus albus* to be 9.94g/100g which was higher than the result of fat content in the present study. The oil content of raw Lupin samples was higher than bean flour, but less than that of soy flour.

When we compare effect of processing methods used, both treatments have significant effect on the fat content of the raw seed. The observed effects were increasing. Treatments like BSNLV2, RSNLV2, BSLLV and RSLLV have an increment effect by 39.26%, 27.62%, 45.36% and 27.41% respectively. The maximum fat content was observed on boiling followed by soaking the raw seed for local lupin variety sample. The possible explanation for fat content increment could be inactivation of lipolytic activity enzyme.

Lupin oils are characterized by a balanced fatty acid composition with total saturated fatty acids of about 10 % and total unsaturated fatty acids around 90 % (Bhardwaj *et al.*, 1998; Petterson, 2000). This can be attributed to high lipolytic activity which, break down the triglyceride to simple fatty acids sterol and polar lipids (Osman, 2007).

When we compare the effect of the same treatment on each sample, there was a significant difference ($p < 0.05$) in their fat content increment. Consequently, BSNLV2 showed lower fat

increment (39.26%) than BSLLV (45.36%). In contrast, treatment RSNLV2 resulted in higher (27.62%) increment in fat content when compared to RSLLV (27.41%). Treatments BSNLV and RSNLV increased the fat content by 30.14% and .70% respectively, and which compared to the treatments BSNLV2 and RSNLV2, they had lower increment in fat content.

4.1.3 Crude fiber

Lupin contains a high content of dietary fiber and this plays an important role in its hypocholesterolemic properties. The new and local lupin samples in the current study had crude fiber contents of 14.89 g/100g and 14.79g/100g respectively. There was no significant difference between the two samples considering their fiber content ($P > 0.05$) in the current study.

The crude fiber content of the new variety in this study was highly comparable with the result reported Mostafa and Haiam (2013) (14.4 g/100g) and it was slightly higher than the value reported by Batterham and Egan (1987), (i.e. 13.00g/100g) for the same variety (*Lupinus angustifolius*).

Petterson *et al.*, (1997) similarly has reported the fiber content of raw *Lupinus albus* seed flour to be 4.5g/100g, which was less than from results in current study. BSLLV and BSNLV have higher fiber contents from raw bean and soybean flour, whose values are reported to be 5.9g/100g and 9.6g/100g respectively. In another study, the reported crude fiber content of raw *Lupinus albus* was 12.7g/100g (Davila-Ortiz *et al.*, 2002). This value was slightly lower than the results of the current study.

On Ethiopian Food Composition Table (EHNRI, 1997) the reported fiber content of the raw *Lupinus albus* seed grown locally was 12.9g/100g. This value is slightly lower than the value obtained in the current study.

Both the treatments applied in the current study have significant effect on the crude fiber content of the raw samples ($P < 0.05$) with increasing effects in both cases. Treatments BSNLV2 and RSNLV2 had increased the fiber content by 3.75% and 7.11% respectively. But the same treatment (BSLLV and RSLLV) had increased the fiber content of local lupin variety by 3.96% and 3.90% respectively.

In another study, it has been shown that there was an increment of crude fiber content after the raw lupins (*Lupinus albus*) seed was soaked in 0.5% NaHCO₃ solution for 8 hrs (El-Adawy, *et al.*, 2000) as reported by Paulos (2009). This could be due to modification of seed components on soaking. Fibers have many desirable properties, including white color, high water-holding capacity and beneficial effects on human health. Therefore, lupin flour can be incorporated into a wide range of foods to make dietary fiber rich products. On the other hand, the treatments BSNLV and RSNLV increased the fiber content of the flour sample by 15.73 and 10.95% respectively which was higher than the value observed in the treatments above. The possible reason for this might be losses of some husks with soaking process.

Comparing the effect of each treatment on crude fiber content the two samples, BSNLV2 showed lower (3.75%) increment than BSLLV (3.96%). In contrast, treatment RSNLV2 resulted in higher increment in fiber content when compared to RSLLV whose values respectively were 7.11% and 3.90%.

4.1.4 Crude ash

The total ash, which is an indirect indicator of the mineral content of foodstuffs, was found to be 2.92 g/100g for the new and 2.75 g/100g for the local lupin varieties. There was no a significant difference ($p>0.05$) between the two lupin varieties in their crude ash content. New lupin variety has higher crude ash content than the local lupin variety. The ash level of lupins was somewhat variable and has been reported to be dependent on the soil type where the plant was grown.

The result of ash content in the current study was slightly higher than results reported in pulse Australia (2006) (2.00g/100g) for the new variety (*Lupinus angustifolius*). But, it is lower than the value reported by Mohamed and Rayas-Duarte, (1995). Similarly, the ash content of the local variety in the current study was lower than the result stated by Porres *et al.*, (2007) (3.52g/100g). Conversely, its content in the current study was lower than the value reported by (Mohamed and Rayas-Duarte, 1995; Ahmed 2014).

However, the ash content of the local lupin (*Lupinus albus*) in this study was highly comparable with the result reported by Batterham and Egan (1987), (i.e. 2.8mg/100g). The variation in ash content may be due to growing environment, soil type and pH of the growing soil. On Food Composition Table for use in Ethiopia (EHNRI, 1997) ash content of raw *Lupinus albus* was

reported to be 2.5g/100g. Comparing with this result, the new variety in the current study had higher (2.92g/100g) total ash content.

When we compare the effects of both traditional processing methods applied in this study, there was a significant difference on the raw and processed lupin seeds in terms of their total ash content. For the new lupin cultivar, the ash content decreased from the raw sample by 53.08 and 51.71% on treatments BSNLV2 and RSNLV2 respectively. The reason may be leaching out of minerals during soaking. Similarly, for the local lupin cultivar, treatments BSLLV and RSLLV showed a significant reduction in crude ash content by 43.27 and 21.09% respectively.

Reduction in the ash content from the raw seed was also investigated by Sileshi, (1985) as reported by Paulos (2009) on treatments roasting and water soaking, roasting and de-corticating and germinating *Lupinus albus* seed. The respective decrements for the mentioned treatments were by 22.15%, 31.69% and 0.92%. The explanation for these observed reductions was given by Arslan and Esker, (2002) who has reported the decline of ash content can be related to washing and filtration of lupin seeds during processing. Thus, sand, stone and soil like substances were separated from the seed.

Treatments BSNLV2, BSLLV and RSNLV2 and RSLLV affected ash content differently. Accordingly, BSNLV2 decreased total ash content by 53.08% which was lower when compared to decrease in ash content on treatment BSLLV on the other variety (43.27%). In the same way, treatment RSNLV2 showed lower (51.71%) total ash decrement than RSLLV (21.09%).

On the other hand, the treatments BSNLV and RSNKV reduced the ash content by 42.12 and 33.56% respectively which were lower when compared to decrease in total ash contents on treatments BSNLV2 and RSNLV2 respectively. The difference in total ash content between the same treatments with different soaking time could be more leaching out of solid matter during soaking process with increased soaking time. Erbas (2010) reported reduction in ash content lupin seeds during soaking as a result of the decrease in crude fiber content and the removal of minerals by the soaking process.

4.1.5 Total and available carbohydrate

The total carbohydrates content of raw lupin seeds used in this study were 65.12 and 55.54g/100g for the new and local lupins respectively as shown in table 1 above. There was a significant difference ($p < 0.05$) between the two varieties in terms of their total carbohydrate contents. Accordingly, total carbohydrate of the new variety (65.12g/100g) was higher when compared to the local variety (55.54g/100g).

Mohamed and Rayas-Duarte (1995) has reported total carbohydrate contents of *Lupinus angustifolius* and *Lupinus albus* to be 46.0 and 48.0% respectively. The results of total carbohydrate contents in the current study for each variety were slightly higher than the reported values. The possible reason could difference between two lupin varieties in their crude protein, crude fat and ash content of which total and available carbohydrates calculated by difference methods. Available carbohydrate which is a portion of carbohydrate that can be digested by human enzymes, is absorbed and enters into intermediary metabolism is calculated by difference method. The raw samples of new and local lupin varieties have utilizable carbohydrate contents of 41.02 and 50.33g/100g respectively. There was a significant difference ($p < 0.05$) between the two cultivars in terms of their available carbohydrate content. The available carbohydrate content of the new lupin variety (*Lupinus angustifolius*) was lower than the available carbohydrate content of the local lupin variety (*Lupinus albus*). The value of available carbohydrate for the new variety was slightly lower than the result reported by Rayas-Duarte (1995)) (i.e. 48g/100g). But, the result of the local cultivar was higher than the stated available carbohydrate content by the same reporter.

Treatments used in this study have a significant effect on the Utilizable carbohydrate content of the new variety. But the effects of both the two treatments were reduction in the carbohydrate content.

Treatments BSNLV2 and RSNLV2 have reduced the utilizable carbohydrate by 22.45 and 4.89% respectively. Similar treatments on the local lupin sample reduced the utilizable carbohydrate content significantly ($P < 0.05$). Total carbohydrate content in the samples BSNLV2, RSNLV2, BSLLV and RSLLV were reduced significantly by 8.32%, 0.82%, 18.26% and 5.96% respectively. Highest reduction was in the BSLLV and RSNLV2 showed the lowest reduction.

Similarly, Jimenez-Martinez, *et al.*, (2003) as reported by Paulos (2009) has reported that boiling for 60 minute reduced total carbohydrate content in beans by 20-45%. In the same study, it has been reported an increment trend in carbohydrate contents after boiling for 15 minutes in the case of red gram, chickpea, black gram and green gram. In another report Jimenez-Martinez, *et al.*, (2003) a reduction of 26% in utilizable carbohydrate contents present in soybeans using acid treatment and boiling temperature of 100⁰C for 5 minutes was observed. In the same paper it was reported that elimination of 70% of carbohydrates in different legumes by soaking for 6 hrs and boiling in NaHCO₃ solution for 45 minutes. When we compare the carbohydrate content of the processed samples (i.e. soaked in 0.5% NaHCO₃ for 8 hrs), the trend was a decreasing from 38% to 32.6%. The reason behind these observed reductions could be the leaching out of the various components of carbohydrates (i.e. starch and oligosaccharides).

The total energy in (KCal) is estimated using the equation:-

$$\text{Total energy (KCal)} = (9 \times \text{Fat}) + (4 \times \text{carbohydrate}) + (4 \times \text{protein})$$

The two raw varieties have total energy content of 361.74 KCal/100gm and 362.52 KCal/100gm for the new and local varieties respectively. There was no a significant difference ($p > 0.05$) between the two lupin varieties in terms of their total gross energy. These values are lower than the value reported by Porres *et al.*, (2007) (501.9kcal/100g). Both the treatments had significant effect on the total energy contents of the raw samples. But the effects were increasing in the local variety and decreasing one in the new variety. Treatments BSNLV2 and RSNLV2 have decreased the total energy content by 1.49% and 1.78% respectively. Similarly, the same treatments have increased the total gross energy by 8.21% and 4.05% for the local lupin variety.

4.2 Ant-nutritional factors

4.2.1 Phytic acid

The new and local lupin cultivars have phytic acid contents of 434.88mg/100g and 402.95mg/100g respectively. There was no significant difference in the phytic acid content between the two cultivars ($P > 0.05$). Accordingly, the new lupin variety has higher phytic acid content (434.88mg/100g) than the local lupin variety.

The phytic acid content of the two cultivars in this study was higher than the value reported by pettersson (1997). But, Trugo, *et al.*, (1993) has reported the phytate content ranging from 400 to 1200mg/100g dry matter for the same varieties between which the result of phytate content in the current study fails. In another investigation, Ertaş and Bilgiçli (2012) have reported the phytic acid content of the raw *Lupinus albus* (the local lupin variety) in this case to be 1486.8mg/100g. The result of phytic acid content in our current study was much less than this reported value. Phytic acid forms an insoluble compound with minerals, especially with zinc. Both the treatments applied on the raw seed affected the phytic acid content significantly.

Yorgancılar and Bilgiçli (2010) reported PA loss (18.8 %) during bulgur process which contains cooking and dehulling of lupin seeds.

The reduction of phytic acid by treatments was BSNLV2 and RSNLV2 were by 41.71 and 40.34% respectively. The highest reduction was observed through boiling then soaking treatments which could be due to activation of phytase enzyme. This enzyme can help the breakdown of the phytate molecules. The increment in phytase activity in the range of 800-2000% has been reported for several varieties of legumes during the first five days of germination. As the number of germination days increased, the higher will be the reduction in phytate content.

Likewise, for the local lupin sample the observed reductions were (19.21%) and (35.73%) for the treatments BSLLV and RSLLV. These PA losses are compatible with previous studies on mung bean (soaking, 26.7 %) (Mubarak 2005), breadnut, cashew nut and fluted pumpkin seeds (boiling, 32.1–64.8 %) (Fagbemi *et al.*, 2005).

In another investigation, soaking process has reduced the anti-nutritional factors in soybean, bean and lupine flours cooking (Manez *et al.*, 2002). When a high phytic acid pigeon pea was released by the Department of Plant Breeding in India, they tested it for different methods of reducing phytic acid. They found that germination (sprouting) was the best method of reducing phytic acid (35% to 39%), while dehulling, soaking and then pressure cooking also had good results. All in all, soaking, soaking and dehulling, and sprouting and cooking pigeon peas all were effective in decreasing phytic acid and increasing the availability of minerals such as calcium, phosphorus and iron (Duhan *et al.*, 2002). Accordingly, the decrease of these anti-nutritional factors from

legume seeds may be due to internal process of leaching and activation of phytase enzyme. The removal of phytate depends on the nature of phytin (i.e. it may be in the form of K, Ca or Mg salts), and the phytase activity and diffusion.

Another study found that soaking cowpeas for 12 hours, dehulling of soaked seeds and germinating cowpeas contributed significantly to reducing phytic acid and tannin levels (Preet and Punia 2000).

Similarly in another investigation, Preet and Punia 2000) has reported soaking Mung Bean (*Phaseolus aureus* L) for 12 hrs reduced the phytic acid content by 13- 41% while soaking for 12 hrs followed by germination for 48 hrs reduced 60-73% of phytic acid. These significant ($p < 0.05$) decreases of phytic acid during soaking treatment might be leaching out of phytic acid into the soaking or cooking medium due to increased permeability of the seed coat in soaking water.

4.2.2 Total alkaloid

The seed alkaloid concentration of the raw samples was 1.52 and 3.12g/100g for the new and local samples respectively. There was a significant difference in total alkaloid content of the two raw samples in the current study. Accordingly, the local variety showed higher total alkaloid contents.

The values of total alkaloid content of the lupin seeds used in this study were higher than the result of total alkaloid content reported by Butler *et al.*, (1996) and Robbins *et al.*, (1996) whose reported values were 0.015g/100g and 0.013g/100g respectively for the same varieties. However, these values are below the current maximum permitted concentration (MPC) of 0.02g/100g. But, the total alkaloid content of *Lupinus angustifolius* (1.92g/100g) was slightly higher than the result reported by Gisela *et al.*, (2012) for the same variety, (i.e. 1.12g/100g). In another investigation Mostafa and Elkatry (2013) reported the total alkaloid concentration of the same species to be 1.47g/100g, and on the other hand Muzquiz *et al.*, (1994) reported the value to be 1.91g/100g.

The quinolizidine alkaloids are likely to be found in any derivative of the seed, including: flour, meal, pastas and pastries, dairy product substitutes, and coffee substitutes based on lupin seeds

(Lowen *et al.*, 1995). Alkaloid concentration in lupines depends on many factors – plant variety, age (developmental stage), environment and geographical location. The seed alkaloid content of lupins (*Lupinus angustifolius*) is influenced by different environmental factors such as fertilizers (Gremigni *et al.*, 2003), ambient temperature during initiation of flowering up to pod ripening (Wink *et al.*, 1995), and drought stress (Christiansen *et al.*, 1997) and pH level of growing soil. Jansen *et al.*, (2009) reported that the grain yield at pH = 7.2 is lower than at pH = 5.8. On the other hand the alkaloid content of *Lupinus angustifolius* decreases significantly at a higher pH.

Both the treatments on the raw Lupin seeds of the new and local varieties have a significant effect on the total alkaloid content. BSNLV2 and RSNLV2, BSLLV and RSLLV for the new and local variety respectively reduced alkaloid by 80.26% and 77.63%, 44.55% and 52.56%. The highest reduction was observed by soaking after boiling in the new variety and soak after roasting in the case of local lupin variety. Decrease in total alkaloid contents during soaking is due to removal of alkaloids with soaking water. Since alkaloids are water soluble, soaking in water can easily remove them from the whole seed. However; this depends on the type of soaking solution and permeability of the cell wall of the hull.

Oladele *et al.*, (2009) has reported reduction in alkaloid contents of tigernut (*Cyperus esculentus* L.). Accordingly, soaking reduced the alkaloid content by 3-13% tannin by 15-61%, polyphenol by 15-48%, phytate by 27-44%, and oxalate by 37-58% while toasting reduced tannin by 36-71%, polyphenol by 25-65%, phytate by 22-40%, oxalate by 57-77% and alkaloid by 13-27%.

Table 4. 2: Ant-nutritional factors of both processed and raw lupin cultivars

Sample	Parameter		
	Phytic acid(mg/100g)	Tannin(mg/100g)	Alkaloid(g/100g)
RNLV	434.88± 23.97 ^a	0.20 ± 0.10 ^a	1.52 ±0.08 ^a
BSNLV	309.04± 7.47 ^b	0.09 ± 0.04 ^a	0.57 ±0.04 ^b
RSNLV	304.83± 22.39 ^c	BDL ± 0.00 ^b	0.37 ±0.18 ^b
BSNLV2	253.49 ± 3.85 ^c	0.16 ± 0.00 ^{ac}	0.30 ±0.03 ^b
RSNLV2	259.44 ± 11.94 ^c	0.13 ± 0.02 ^{ae}	0.34 ±0.06 ^b
RLLV	402.95 ±38.84 ^a	0.3 ± 0.02 ^{af}	3.12 ±0.02 ^c
BSLLV	325.55 ± 21.68 ^c	0.19 ± 0.05 ^{ag}	1.73 ±0.10 ^d
RSLLV	258.99 ±20.90 ^d	0.13 ± 0.00 ^e	1.48 ±0.11 ^c

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

4.2.3 Condensed tannin

The tannin contents of the two cultivars are presented in table 3. The tannin content of the new and local lupin cultivars were 0.20 and 0.31mg/100g, respectively. There was a significant difference between the two lupin cultivars in terms of their tannin content. Accordingly, the tannin content of the new lupin cultivar (0.02mg/100g) was lower than the tannin content of the local lupin cultivar (0.31mg/100g). The tannin levels in both processed and unprocessed lupin varieties are very low. This is in agreement with statement reported by Petterson (1993). Accordingly, the tannin content of lupins is so low (\square 0.01%) that it is unlikely to impair protein utilization by humans and animals.

Comparing to other legumes such as soya beans in terms of their tannin content, Lupins in general contain a lower concentration of tannins and saponins, lectns (Petterson *et al.*, 1997) than soybean and need no heat treatment to deactivate substances such as the lectins and protease inhibitors that reduce protein digestion and availability (Wolko *et al.*, 2011).

Treatments BSNLV2 and RSNLV2 used in this study reduced the tannin content by 20.0% and 35% respectively. Similarly, the same treatments reduced tannin content of the local lupin

variety (*Lupinus anlbis*) by 7.23 and 14.16% respectively for BSLLV and RSLLV treatments which are lower when compared to percentage of reduction by the treatments BSNLV2 and RSNLV2.

El-Hady and Habiba (2003) reported that total tannin contents were reduced significantly by a 16 hour soaking of kidney beans. The reduction was as much as 17%. Boateng *et al.* (2008) also observed as slight decrease in total tannin content levels in soaked kidney and pinto beans. The same author also reported the combination of soaking followed by extrusion reduced tannin content of the raw kidney bean flour by 23%. In another investigation, soaking soyabean seeds in distilled water caused a maximum reduction in the phytic acid, where as soaking in NaHCO₃ tannins and phenolics by significant levels (78% and 72%) respectively. This reduction in tannin content is attributed to the tannin activity during soaking (Mubarak 2005) that leads to the formation of hydrophobic association of tannins with seed proteins and enzymes. Some losses of tannins during soaking may be due to leaching of tannins in to soaking water. Reddy *et al.*, (1955) found that overnight soaking of garins followed by germination for two days significantly reduced tannin content by 50% in wide variety of legumes. Treatments BSNLV and RSNLV reduced the tannin content by 34.67 and 30.29% respectively.

4.3 Mineral analyses

The results of mineral analyses (calcium (Ca), Zinc (Zn), and Iron (Fe)) of the new and local lupin varieties are presented on Table 3 below.

Table 4. 3: Mineral (Zn, Fe and Ca) composition of both processed and raw new and local lupin varieties.

Samples	Mineral composition (mg/100g)		
	Zn	Fe	Ca
RNLV	2.74± 0.29 ^a	2.48± 0.24 ^a	112.62± 6.83 ^a
BSNLV	1.79± 0.04 ^b	1.54± 0.79 ^b	101.3± 2.03 ^b
RSNLV	1.91± 0.65 ^b	2.43± 1.01 ^b	100.57± 0.32 ^b
BSLV2	1.65± 0.43 ^{cb}	2.41± 0.60 ^b	97.80± 3.05 ^b
RSLV2	1.84± 0.25 ^{db}	2.30± 0.12 ^b	90.32± 0.55 ^c
RLLV	3.46± 0.14 ^e	2.94± 0.24 ^{bc}	44.00± 1.93 ^d
BSLLV	3.21± 1.31 ^f	1.98± 1.03 ^{bd}	38.20± 0.97 ^e
RSLL	2.97± 1.06 ^f	2.31± 0.36 ^{be}	35.71± 2.02 ^e

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

4.3.1 Zinc

The zinc content of the two cultivars was 2.74mg/100gm and 3.46mg/100gm for the new and local variety respectively.

There was a significant difference between the two samples in their zinc content.

Porres *et al.* (2007) reported that raw *Lupinus angustifolius* flour has zinc content of 3.65mg/100 gm. The Zn content in the current study for the same species (*Lupinus angustifolius*) is not in agreement with the stated value. Accordingly, the zinc content was slightly lower than the result reported by porres *et al.*, (2007). However, in another investigation, Trugo, *et al.*, (1993) has reported the content to be within the range of 2.9-17.6mg/100 gm for different cultivars of the same species. Conversely, the zinc content of the local cultivar in the present study was 3.46mg/100g of dry mater which is higher than the zinc content of the new cultivar in this study, But lower than the value reported by Porres *et al.*, (2007) for the same species (*Lupinus albus*), (i.e. 4.3mg/100g). The difference in the zinc content between the two cultivars may be due to genetic makeup variation since cultivated in the same environmental conditions.

In another investigation, petterson (1993) explained the range of values for various minerals of lupins and factors that determine the mineral contents of lupins. Accordingly, the range of values of calcium, magnesium, phosphorus, potassium and sulphur for each of the main lupin species is up to about 30% of the mean and sodium content is more variable, depending on soil type.

The range for minor elements is greater with variations in the content of iron (3.1-15.0mg/100g) and manganese (0.6-7.6mg/100g) in Australian sweet lupin (*Lupinus angustifolius*) in related to rainfall zone, with seed from low rain fall areas generally having a higher content. The concentration of zinc (Zn) in plant-based foods generally varies from 0.05 to 11.8 mg/100 g. The lower levels of Zn are found in fresh fruits (0.02–0.61 mg/100 g).

Both the treatments used in the current study have shown decreasing effect on the zinc content of the raw samples. Accordingly, the treatments BSNLV2 and RSNLV2 reduced the Zn content by 39.78% and 32.85% respectively. Similarly, Ertas and Bilgiçli (2012) reported decrease of Zn content by 46.7% and 23.2% at different soaking times and medium respectively.

The mineral contents of processed samples depend on the type of the soaking solution. For example; on the report by El-Adawy, *et al.*, (2000), when the soaking solution was NaHCO₃, it was reported that except Na all the analyzed minerals have shown a decrement. The decrease in Zn content may be due to leaching in to soaking water when the soaking water is changed.

4.3.2 Iron

Iron was another other mineral analyzed in this study. The iron contents of the new and local cultivars in this study were 2.48 and 2.94 mg/100gm respectively. There is a significant difference between the iron contents of the two cultivars. Porres *et al.*, (2007) has reported the iron content of the raw Lupin of the same cultivar (*Lupinus angustifolius*) to be 4.15mg/100g on dry mater base. The finding of the current research was not in agreement with this specified result for the same species. The possible explanation could be growing geographical conditions soil type, rain fall, pH of the soil. However, in another finding, Trugo, *et al.*, (1993) has reported the iron content to be in the range of 2.4–10.8 mg/100 gm. The result of the current study is within the given range.

In both sample types both the treatments have significant effects on the iron content. Iron content was reduced from the raw sample in all the treatments. The treatments BSNLV2, RSNLV2, BSLLV and RSLLV have reduced the iron content by 37.9%, 14.11%, 32.65% and 20.4% respectively. In another study, it was reported that except Na, all the minerals analyzed including Fe were reduced on soaking of raw lupin bean (*Lupinus albus* and *Lupinus angustifolius*). The reduction of minerals on soaking process possibly could be leaching of all the water soluble minerals with the steeping medium and rinsing process. Ertaş and Bilgiçli (2012) reported that after debittering process, significant ($p < 0.05$) decreases (between % 5.7 and 75.7) were observed in calcium, phosphorus, zinc, iron, magnesium and manganese contents of the lupin seeds. The seed iron content was decreased by 11.8% during different soaking time (48, 96 and 144hrs).

The level of minerals in vegetables depends on a number of factors including genetic properties of the crop species, climatic conditions, soil characteristics and the degree of maturity of the plant at the moment of harvesting.

4.3.3 Calcium

The calcium content of the two cultivars was 112.62 and 44mg/100gm for the new and local lupins respectively. There is a significant difference between the calcium contents of the two cultivars. The calcium content of the new variety was higher than the local variety.

The values for calcium content in the current study for the new lupin cultivar (*Lupinus angustifolius*) is slightly lower than the value reported by pores *et al.*, (2007) (i.e. 143mg/100g).

The result of calcium content for the local variety was not in agreement with the value reported by the same author (139.0mg/100g). Similarly, in another study the calcium content of the same species is reported to be in the range of 150-290 and 120-250mg/100g for *Lpinus angustifolius* and *Lupinus albus* respectively, (Petterson 1997). The reason probably may be genetic difference and environmental conditions where the lupins grown. The concentration of calcium (Ca) in foods of plant origin shows a wide range of variation. The lower values belong to apples (*Malus domestica*), green pepper (*Capsicum annuum*) and potatoes (*Solanum tuberosum*) (< 8.7 mg/100 g) and higher values are present in broccoli (*Brassica oleracea* L. var.italica) (100 mg/100 g) and spinach (*Spinacia oleracea*) (600 mg/100 g). Treatments applied in the present study had a significant effect on the calcium content of the two varieties. Consequently, treatments, BSNLV2

and RSNLV2 reduced the calcium content by 13.16 and 19.80% respectively. Similarly, the treatments BSLLV and RSLLV reduced the calcium content by 13.18 and 18.84%. When compared to the treatments BSNLV2 and RSNLV2, the treatments BSLLV and RSLLV had higher reduction percentage.

4.4 Functional properties

The most important functional properties of the lupine seed flour in food formulations were characterized in Table 4.4 below.

4.4.1 Water absorption capacity

Water absorption capacity of the new and local lupin varieties was 2.85 and 2.65g/g respectively. There was a significant difference ($p>0.05$) between the two lupins in terms of their water absorption capacity. The water absorption capacity of the new variety (2.85g/g) was higher when compared with local variety (2.65g/g).

Both treatments applied showed increasing effect on the water absorption capacity of the flours. The treatments BSNLV and RSNLV increased the water absorption capacity by 14.04 and 17.54% respectively. Similarly, the treatments BSLLV and RSLLV increased water absorption capacity by 11.32 and 13.21% respectively.

The higher water absorption of flours from processed seeds might have been due to their protein content which was higher than that in the corresponding raw seed flour. Increase in water absorption in soy products (flour, concentrate and isolate) with increase in protein content was reported by Flemming *et al.*, (1974). A number of factors such as hydrophilic-hydrophobic balance of amino acids molecular size and shape influence the water absorption of flours. According to Butt and Batool (2010), protein has both hydrophilic and hydrophobic properties, and so can interact with water in foods.

Treatments BSNLV2 and RSNLV2 have showed water absorption capacity of 2.80 and 2.82 g/g respectively. There is no significance difference between the two treatments in terms of their water absorption capacity. However, when compared to the flour from raw lupin varieties, there was a significant difference ($p<0.05$) between the two treatments.

Table 4. 4: Functional properties (Mean \pm SD) of both raw and processed lupin flours of the new and local lupin varieties

Parameter	Sample							
	RNLV	BSNLV	RSNLV	BSNLV2	RSNLV2	RLLV	BSLLV	RSLV
Bulk density (g/ml)	0.86 \pm 0.00 ^a	0.86 \pm 0.01 ^a	0.84 \pm 0.00 ^a	0.85 \pm 0.03 ^a	0.81 \pm 0.01 ^a	0.84 \pm 0.03 ^a	0.83 \pm 0.01 ^a	0.76 \pm 0.0 ^b
Dispersibility (%)	44.00 \pm 2.83 ^a	47.51 \pm 1.79 ^a	45.78 \pm 1.63 ^{ab}	46.45 \pm 0.4 ^{ab}	46.4 \pm 0.2 ^b	43.02 \pm 2.03 ^c	51.50 \pm 1.43 ^d	49.87 \pm 2.0 ^d
Water absorption (g/g)	2.85 \pm 0.07 ^a	3.25 \pm 0.35 ^b	3.35 \pm 0.21 ^b	2.80 \pm 0.00 ^c	2.82 \pm 0.21 ^d	2.65 \pm 0.07 ^d	2.95 \pm 0.07 ^a	3 \pm 0.00 ^b
Oil absorption (g/g)	0.98 \pm 0.69 ^a	1.23 \pm 0.21 ^b	1.52 \pm 0.15 ^b	1.08 \pm 0.14 ^c	1.17 \pm 0.14 ^c	1.02 \pm 0.07 ^c	1.42 \pm 0.07 ^d	1.57 \pm 0.13 ^d
Emulsion activity (%)	53.2 \pm 0.67 ^a	53.3 \pm 0.54 ^a	54.01 \pm 0.77 ^a	51.03 \pm 0.1 ^b	53.8 \pm 0.4 ^c	48.47 \pm 0.87 ^d	53.25 \pm 0.99 ^e	52.7 \pm 1.01 ^e
Foaming capacity (%)	67.02 \pm 0.3 ^a	66.3 \pm 0.88 ^a	71.01 \pm 0.79 ^b	67.2 \pm 0.21 ^a	68.9 \pm 0.32 ^c	65.4 \pm 0.07 ^a	65.7 \pm 2.12 ^a	65.8 \pm 0.76 ^a

Means in the same raw with different letters are significantly different ($p < 0.05$)

The treatments showed lower water absorption capacity than the treatments BSNLV and RSNLV for the same variety. This decrease in the water absorption capacity for the treatments BSNLV2 and RSNLV2 could be the reason explained earlier.

There are economic benefits in adding water to a product which is priced according to its weight, and a positive impact on the shelf life, hence food manufacturers prefer to incorporate food ingredients with high water absorption capacities in their formula.

4.4.2 Bulk density

There was no significant difference between the two lupis in their bulk density. The bulk density of the new and local lupins respectively was 0.86 and 0.84g/ml.

The result in the present study for bulk density was comparable with the finding reported in Technical Data Sheet for Australia Sweet Lupin for the new variety (*Lupinus angustifolius*), (i.e. 0.56g/ml).

The bulk density of the local lupin cultivar was slightly higher than the result reported by Shimelis (2010) (i.e. 0.76g/ml) for the same cultivar.

Both treatments applied in the present study had no significant effect on the bulk density of the lupins except RSLLV which had significant effect on the bulk density (0.76g/ml).

Bulk density of flour is related to the textural characteristics and eases of rehydration. Bulk density is an indication of the porosity of a product which influences packages design which could be used in determining the type of packaging material required for the product. Bulk density has been found to be a function of flour wettability (Solsuki, 1962). It is also important in infant feeding where less bulk density is desirable (Iwe and Onalope, 2001). According to Padmasshree *et al.*, (1987), higher bulk density is desirable for greater ease of dispersibility of flours. In contrast, however, low bulk density would be an advantage in the formulation of complementary foods.

4.4.3 Dispersibility of the flours.

Dispersibility of flour in water shows the ease of breakup of agglomerates which allow particles to sink below the surface and disperse rapidly in a liquid. Flour obtained from the new lupin variety was found to be superior to that of the local variety in terms of dispersibility.

Dispersibility of the two lupins was 44.00 and 43.02% for the new and local varieties respectively. There was a significant difference ($p < 0.05$) between the lupin varieties in their dispersibility. The new lupin variety showed higher dispersibility than the local lupin variety. The dispersibility value for both new and local cultivars in the present study was lower than the value reported by Shimelis Emire (2010) (i.e. 56.7%). The difference in dispersibility could be due to growing condition, soil type, rain fall and pH of the soil where the lupins grown.

Both the treatments applied had a significant effect on the dispersibility of lupin flours. Accordingly, the treatments BSNLV2 and RSNLV2 increased dispersibility of flours by 5.56 and

5.45% respectively. Similarly, the treatments BSLLV and RSLLV increased dispersibility by 19.71 and 15.93% respectively.

Dispersibility of flour in water shows the ease of break-up of agglomerates which allow particles to sink below the surface and disperse rapidly in a liquid.

4.4.4 Oil absorption capacity

The oil absorption capacity of the new and local lupin cultivars was 0.98 and 1.02g/g respectively. There was no significant difference ($p>0.05$) between the two varieties in their oil absorption capacity.

When we compare the effect of treatments used, there was a significant effect on the oil absorption capacity which was increasing in both samples. Accordingly, treatments BSNLV and RSNLV increased oil absorption capacity by 25.51 and 55.10% respectively. Similarly, the treatments BSLLV and RSLLV increased the oil absorption capacity by 39.22 and 53.92% respectively. Sosulski *et al.*, (1976) reported increase in oil absorption capacity of lentil during soaking and germinating for hours.

Treatments BSNLV2 and RSNLV2 have showed oil absorption capacity of 1.08 and 1.17 g/g respectively. There was a significance difference ($p<0.05$) between the two treatments in terms of their oil absorption capacity. when compared to the flour from raw lupin varieties, there was a significant difference ($p<0.05$) between the two treatments. The treatments showed higher oil absorption capacity than the treatments RNLV and lower oil absorption value than the treatments BSNLV and RSNLV for the same variety. Mubarak (2005) studied the effect of some home traditional processes on the nutritional composition and antinutritional factors in mung bean seeds and reported that soaking for 12 h resulted in significant reductions in crude ash (12 %) and crude protein (2 %).

When we see water and oil absorption trends of the two samples, water absorption trends 2.85, 3.25, 3.35 and 2.65, 2.95, 3 g/g for RNLV, BSNLV and RSNLV and RLLV, BSLLV, RSLLV flours, respectively, were higher than oil absorption trends 1.02, 1.42, 1.57 and 0.98, 1.23, 1.52 g/g for RNLV, BSNLV, RSNLV and RLLV, BSLLV, RSLLV flours, respectively.

4.4.5 Emulsion activity

The emulsion activity, which reflects the ability of the proteins to aid formation and stabilization of newly created emulsion, were found to be 53.21, 52.1, 54.01, and 48.47, 53.25, 52.7 in the RNLV, BNLV, RNLV and RLLV, BLLV, RLLV flours, respectively. The emulsion activity of the two lupins flour was 53.21 and 48.47% for the new and local lupin varieties respectively. There was a significant difference between the two lupins in terms of their emulsion activity. All treatments used in this study have significant effect on the emulsion activity of the flours. Treatments had both increasing and decreasing effects. Consequently, BSNLV decreased emulsion activity by 2.09% and whereas treatment RSNLV increased the emulsion activity by 1.50%. However, both treatments showed increase in emulsion activity for the local lupin variety. Accordingly, the treatments BSLLV and RSLLV increased emulsion activity by 9.86 and 8.73% respectively. The treatments BSNLV2 and RSNLV2 have showed lower emulsion activity than the treatments BSNLV and RSNLV whose respective values were 53.01, 51.03, 54.2, and 52.1 %.

4.4.6 Foaming capacity

Foaming capacity of the flour samples were 67.2, 66.3, 71.01 and 65.4, 65.7, 65.8 for RNLV, BSNLV, RSNLV and RLLV, BSLLV, RSLLV respectively. There was a significant difference between the two cultivars in terms of their foaming capacity. The foaming capacity of the new variety (67.2%) was slightly higher than foaming capacity of the local variety (65.4%). The value for foaming capacity for the local lupin variety (65.4%) in the present study was highly agrees with result reported by Shimelis Emire (65.0 %) for the same lupin species.

Both treatments have showed a significant effect on the foaming capacity of the flour samples. Accordingly, BSNLV and RSNLV decreased the foaming capacity by 1.36% and conversely RSNLV increased the foaming capacity by 5.67%. Similarly, the treatments BSLLV and RSLLV increased the foaming capacity by 0.46 and 0.61 respectively. Ahmadzadeh *et al.*, (2006) reported increase in foaming capacity for soaked, germinated and de-hulled cowpea by 8%. The treatments BSNLV2 and RSNLV2 have foaming capacity of 67.02 and 68.9 % respectively which was slightly higher than the foaming capacity value of the raw lupin flour (67.2) of the same variety. However, when compared to foaming capacity value of the treatments BSNLV

(66.3%) and RSNLV (71.01%), higher foaming capacity was observed for the treatment BSNLV2 and it was lower for the treatment RSNLV respectively for the same treatments.

Some food proteins are capable of forming good foams, and their capacity to form and stabilize foams depends on the type of protein, degree of denaturation, pH, temperature and whipping methods. The egg white is one of the most commonly used food ingredients for foaming properties. Since foam capacity appears to be due to solubilized protein, higher values will enhance its functionality in its uses for the production of cakes and whipping toppings where foaming is an important property.

4.5 Sensory evaluation

Table 4. 5: Sensory evaluation of lupin-wheat blend bread

Lupin types	Blend proportion	New Lupin variety				Local Lupin variety
		Control (100%) Wheat (B0)	5% lupin (B1)	10% (raw) (B2)	10% lupin (B3)	15%lupin (B4)
Appearance	8.39± 1.05 ^a	7.32±1.19 ^a	7.54±0.79 ^b	6.85±0.54 ^c	6.39±1.57 ^{ab}	7.08±1.12 ^{ac}
Color	8.31± 0.63 ^a	7.60± 1.12 ^a	7.69±0.52 ^{ab}	7.08±1.01 ^b	7.08±1.29 ^{ab}	8.08±1.51 ^b
Odor	7.77± 1.68 ^a	7.70± 0.92 ^a	7.47±0.75 ^a	7.51±0.68 ^b	7.31±1.60 ^c	7.16±1.36 ^c
Taste	7.16± 0.83 ^a	7.53±0.75 ^a	6.93±0.94 ^b	6.93±0.74 ^b	6.39±1.47 ^c	5.47±0.60 ^d
Texture	7.31± 1.00 ^a	7.40±0.56 ^b	7.62±0.60 ^b	6.77±0.43 ^b	6.66±1.59 ^c	6.00±1.02 ^d
Over all acceptance	6.50± 0.41 ^a	6.78±0.82 ^b	7.16±0.91 ^b	6.47±0.31 ^c	5.59±1.56 ^d	4.93±1.06 ^e

Means in the same raw with different letters are significantly different (p<0.05).

B0 ⇒ control (100%) wheat flour

B1 ⇒ 5% processed new lupin flour

B2 ⇒ 10% raw new lupin variety

B3 ⇒ 10% processed new lupin variety

B4 ⇒ 15% processed new lupin variety

B5 ⇒ 10% processed local lupin variety

Sensory evaluation was done for the bread made from five different products from lupin to wheat flour proportions and one product from wheat alone flour (control). These were 5%, 10% and 15% of processed new lupin variety flour, 10% of raw new variety lupin flour and 10% local lupin flour. Wheat flour (100%) was used as a control. Sensory analyses was done to identify if there was a significant difference between (for bread made from the new and local lupin variety flours with the same lupin to wheat proportion level) and within sensory analyses (for breads made from processed and unprocessed new lupin variety flours) if significant difference exists in their sensory characteristics. Sensory attributes for which analyses done were appearance, color, odor, taste, texture and overall acceptance of the products.

The results sensory analyses of products for appearance were 8.39, 7.32, 7.54, 6.85, 6.39 and 7.08. When we compare B3 (bread made from 10% of new lupin variety flour) with B5 (bread made from 10% of local lupin variety flour), there was a significant difference ($p < 0.05$) between the two products in terms of their appearance sensory attribute. The results for their sensory evaluation of appearance were 6.85 and 7.08 respectively. Accordingly, B5 showed higher sensory value than B3. The possible explanation could be dark yellowish seed color of the new lupin variety when compared to white yellowish color of the local lupin variety seed which has a direct effect on the appearance of the product.

Compared to B1, B2 and B4, there was a significant difference ($p < 0.05$) between B1, B2, B3 and B4 in terms of their appearance attribute. Accordingly, B3 (6.85) showed higher appearance value than B4 (6.39) and lower value than B1 (7.32) and B2 (7.54). At this juncture, one can conclude that it is possible to use 10% of raw new lupin variety flour in similar way like processed lupin flour is used at the same proportion to replace wheat flour.

When compared to control, B0 (100%) wheat flour, B3 was inferior in terms of its appearance sensory attribute. In general, all products were acceptable in terms of their appearance sensory attribute and scored above five grades.

Color is another sensory attribute together with appearance investigated in this study that determines the product quality and acceptance by consumers. The result of sensory analyses of color for B3 and B5 were 7.08 and 8.08 respectively. B5 showed significantly higher ($p < 0.05$) value when compared to B3 for its color attribute. However, both products were above unacceptable level in terms of their color attribute.

The sensory evaluation results of the products B1, B2, B3 and B4 for their color attribute were 7.6, 7.69, 7.08 and 7.08 respectively. Compared to B1 and B2, there was significant difference ($p < 0.05$) with B3. Accordingly, B1 and B2 showed higher sensory results in terms of their color attribute. However, compared to B4, there was no significant difference ($p > 0.05$).

When compared to B0 (control), B3 showed significantly lower ($p < 0.05$) results in terms of its color attribute.

Odor is another important sensory attribute investigated in the present study. The results of sensory analyses of products B0, B1, B2, B3, B4 and B5 are shown in table 5. Compared to B5, B3 showed higher sensory results in terms of its odor attribute. Similarly, B3 showed significantly higher ($p < 0.05$) sensory value when compared to products B1, B2, B4 for its odor attribute. However, it showed no significant difference ($p > 0.05$) when compared with B0 (control).

Taste is very important and unforgettable parameter in sensory study of food products. The results of sensory analyses of products B0, B1, B2, B3, B4 and B5 for taste attribute are shown in table 5. As the results of sensory analyses for taste indicated, there was a significant difference ($p < 0.05$) between B3 and B5 in terms of their taste attributes. Consequently, B3 showed a higher value than B5 whose values respectively were 6.93 and 5.47. This variation between the two products in terms of their taste attribute could be the bitterness taste in the local lupin flour due to alkaloids. When compared to products B1, B2 and B4, there was no significant difference ($p > 0.05$) between B3 and B2 in terms of their taste attributes. However, there was a significant difference ($p < 0.05$) between B2, B4 and B3 in terms of their taste attributes. Accordingly, B3 showed lower sensory results than B2 and B4 in its taste attribute. There is a significant difference ($p < 0.05$) between B3 and B0 indicating B0 has higher sensory score. In fact, all products were accepted by the panelist.

The results of sensory analyses of each product for texture are shown in the table 5. There was a significant difference ($p < 0.05$) between B3 and B5 in terms of their texture attribute. B3 showed higher value than B5 in its texture attribute. When compared to B0, B1, B2 and B4, there was no significant difference ($p > 0.05$) between B3 (6.77) and B2 (7.62). However, it was significantly different ($p < 0.05$) from B1 (7.40) and B4 (6.66). Accordingly, B1 showed higher sensory value in terms of its texture attribute where B4 was lower value. Similarly, B0 was significantly different ($p < 0.05$) from B3 indicating higher sensory result for its texture.

Overall acceptance is another sensory attribute tested in the present study which determines the market value of a given product. The sensory analyses results of products in the current study for overall acceptable were 6.50, 6.78, 7.16, 6.47, 5.59 and 4.93 for B0, B1, B2, B3, B4 and B5 respectively.

There was a significant difference ($p < 0.05$) between the B3 and B5 in terms of their overall acceptance. Accordingly, B3 showed higher overall acceptance than B5 whose values respectively were 6.47 and 4.93. B5 scored below ac

ceptable level in terms of its overall acceptability. Compared to B1, B2 and B4, B3 was significantly different ($p < 0.05$) from B2 and B4. Consequently, B3 showed higher overall acceptable value than B4 and lower overall acceptable value than B1 and B2. This indicates decreasing value of overall sensory acceptability of the product with increasing proportion of lupin flour up to and above 15% level.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

According to the results obtained, the new lupin variety (*Lupinus angustifolius*) investigated in this study had good nutritional value. Except in its protein content in which it had lower value, the new variety revealed comparable nutritional composition with locally grown lupin (*Lupinus albus*) variety.

The results also revealed that the new variety (*Lupinus angustifolius*) was rich with minerals especially, Fe, Zn and Ca and it has higher calcium content when compared to the local lupin variety. The new variety revealed lower alkaloid content from which one can conclude that it can be consumed without extra treatment like other commonly consumed grain legumes.

The traditional methods used in the present study increased the nutrient levels in both varieties except minerals and reduced the alkaloid and phytate contents significantly. The new variety showed similar trends in each case with the local variety.

The results of functional properties indicated that the new variety can be used for various food products manufacturing analogous to the local lupin variety in the same way the local variety is processed and used. Therefore, the research findings can be used by food companies in recipe development of lupin-based processed foods, including fortified food products to combat the protein-energy malnutrition problem in Ethiopia.

The new lupin flour can equally be used in food preparation as the local lupin variety. Incorporation of 10% lupin flour from the raw and processed flour of the new lupin variety showed better sensory acceptance than the processed local lupin variety. This may indicate the possibility of the new variety to be used in food production.

The successful growth and showing comparable nutritional value with the local lupin variety under existing environmental condition indicates the wide adaptation potential of the new variety and the possibility to use this crop as a cheap home-grown protein source in Ethiopia.

Although there were variations between the two varieties in terms of their nutritional compositions, the lower alkaloid content, possibility to be used in food formulations without longer pre-treatment practices together with relatively better crude protein and fiber content gives the new variety an advantage over the local lupin variety for further production and use in human food and animal feed.

5.2 Recommendations

1. Further study should be conducted to evaluate amino acids and fatty acids profiles of the new crop under Ethiopian condition.
2. For reliable information, laboratory results need to be verified by animal and human evaluations.
3. Protein digestibility and efficiency ratio should be investigated.
4. Effect of traditional processing methods not mentioned in this study such as sprouting, fermentation should be evaluated.
5. Specific alkaloid contents, types, distribution in different parts of the crop (leaves, seeds, roots) in the crop should be investigated.
6. Industrial lupin flour based product such as lupin protein isolates, lupin milk and yoghurt should be developed and sensory acceptability should be evaluated.

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DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in other University, and that all sources of materials used for the thesis have been duly acknowledged.

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