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CHEMICAL COMPOSITION AND THE EFFECTS OF TRADITIONAL PROCESSING ON NUTRITIONAL COMPOSITION OF GIBTO (*Lupinus albus. L*) GROWN IN, GOJAM AREA

BY

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LIST OF ABBERVATIONS AND ACRONYMS

AAS	Atomic absorption spectrophotometer
ACNFP	Advisory committee on novel foods and processes
ADF	Acid digestible fiber
AME	Apparent metabolizable energy
ANOVA	Analysis of variance
AR	Analytical grade reagent
CS	Chemical score
CSA	Central statistical agency
DE	Digestible energy
DM	Dry mass
EAA	Essential amino acid level
EAAI	Essential amino acid index
EHNRI	Ethiopian health and nutrition research institute
FAO	United nations organization for food and agriculture
FFAs	Free fatty acids
FGD	Focus group discussion
FSNP	Food science and nutrition program
GC	Gas chromatography
GE	Gross energy

gm	Gram
ha	Hectare
HPLC	High performance liquid chromatography
hr	Hour
Kcal	Kilo Calorie
LSD	Least significant difference
ME	Metabolizable energy
mg	Mili gram
MJ	Mega joule
MS	Mass spectrometer
NDF	Neutral detergent fiber
nm	Nano meter
NSP	Non-starch polysaccharides
NVZs	Nitrate vulnerable zones
PER	Protein efficiency ratio
PPM	Parts per million
Qtl	Quintal
RFOs	Raffinose family oligosaccharides
RPM	Revolutions per minute
SD	Standard deviation
SPSS	Statistical product for system solutions

t/a	Ton/ area
TQA	Total quinolizidine alkaloids
TTM	Traditional Turkish method
Vs	Versus
WD	Water deficient
Yr	Year

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ABSTRACT

Lupin seeds (*Lupinus albus*), grown in Ethiopia (Gojam area) were nutritionally investigated. This study was conducted in order to evaluate the effects of some commonly applied traditional processing methods on the nutritional and chemical composition of *Lupinus albus*. Traditional processing methods are observed to be effective in reducing anti-nutritional factors, meanwhile their effects on the nutritional composition is not wel investigated. Accordingly, the current study focused on the effects of traditional processing methods on the nutritional and chemical composition of *Lupinus albus*. Three traditional processing methods (i.e. soaking after roasting for 5 days, soaking after boiling for 5days, and germination for 48 hrs) were taken under investigation. The parameters analysed were proximate composition, anti-nutritional factors, mineral composition and fatty acid profile. The official methods used were AOAC (2000), Osborne and Voogot (1978), Haborne (1973) and Latta and Eskin (1980) for Proximate composition and fatty acid profile analyses, mineral analysis, total alkaloid determination, and phytate content analysis respectively.

The hull size of the two cultivars from Dangla and Tilili were 16.22% and 19.30% respectively. Moisture, protein, fat, crude fiber, ash, utilizable carbohydrates and gross energy for the Dangla sample were 6.94, 37.87, 9.34, 11.08, 2.80, 38.92% and 391.19 (KCal/100 gm) respectively. Similarly, for the Tilili sample the values were 8.04, 39.71, 8.79, 11.07, 2.90, 37.56% and 388.12 (KCal/100 gm). The anti-nutritional factors studied were total alkaloids and phytate. The results for the Dangla sample were 2.46% and 144.33 mg/100 gm for total alkaloids and phytate respectively. Similarly, for the Tilili sample the values were 2.26% and 143.96 mg/100 gm. The mineral composition of the two cultivars was also investigated. Accordingly, the Dangla sample has 6.00, 2.11, 58.43, 8.93 mg/100 gm contents of Fe, Zn, Mn and Mg respectively. The values of the same types of minerals for the Tilili sample were 6.72, 1.81, 63.54, 59.14 and 9.46 mg/100 gm respectively. In the two cultivars an average value of 24.5% saturated and 74.5% unsaturated fatty acid levels were recorded. The un-saturated fatty acids found in the oil are predominantly, Oleic and Linoleic acid, while the saturated ones include Palmitic, Stearic and Eikosanic acids.

Traditional processing methods have shown both an increasing and decreasing effects on the various chemical and nutritional compositions of the raw seed. Among the treatments the commonly used soaking after roasting method was found to reduce the alkaloid content effectively, at same time showing improvement in the nutritional composition. Nutritional compositions like protein and oil have shown an improvement on soaking after roasting treatment. The mineral composition of the raw seed was also affected by the various treatments applied. Except for Zn content all the minerals analyzed have shown a reduction in the treatments. Mn content was found to exceed the safety limit for daily intake. The effects of soaking after roasting and boiling on the fatty acid profile of the oil were insignificant. But germination has reduced the contents of some of the fatty acids significantly. In all the cases the predominant un-saturated fatty acid found on both sample types was oleic acid.

It can be concluded that *Lupinus albus* was an excellent food source with high nutritional value. The total alkaloid content can be reduced effectively after the various traditional processing methods such as soaking after roasting and boiling. These processes including germination also have a potential to enhance the nutritional composition of the raw seed. After some of the treatments (i.e. except germination) the oil content has increased. And having high amounts of un-saturated fatty acids and oil content, it could be a potential oil crop.

Key words: *Lupinus albus*, soaking, roasting, boiling, and germination

1. Introduction

1.1. Background of the Study

Grain legumes are important sources of significant amounts of proteins, carbohydrates, fiber, vitamins and some minerals. They are used in many parts of the world for both animal and human nutrition (Sebasti, *et al*., 2001, Michael, *et al*., 2002, Osman, 2007). As a protein source they are obtained cheaply compared to animal protein sources. Moreover; they are fairly good sources of thiamin, niacin, calcium, and iron (El-Adway, *et al*., 2000 and Asian productivity organization, 2003). Predominantly their consumption is wide in areas like the developing countries of Asia, Africa and South America (Frias, *et al*., 2004). In these countries legumes play a major role as protein source (El-Adway, *et al*., 2000, Asian productivity organization, 2003).

In general, cereals and legumes take a large place of human food consumption. 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 legumes 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).

Proportionally legumes contain 20-25% protein, which is 2-3 times higher than the content in cereals. Therefore, they can be considered as a leading candidate for protein supply to poor areas of the world (Khalil , *et al*., 2006). Especially in areas where there is a pressing need for high energy and protein, their contribution is significant (Osman, 2007).

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

Leguminosae is one of the three largest families of flowering plants, comprising nearly 700 genera and 18,000 species. The legumes used by humans are commonly called food legumes or grain legumes. The food legumes can be divided into two groups, the pulses and the oilseeds. Pulses group consists of dried seeds of cultivated legumes, which have been eaten for a long time (Asian productivity organization, 2003).

Studies on legume seed protein were undertaken for more than a century. Soybean has been for many decades the only legume on which significant research has been undertaken. Besides its traditional uses, an extremely wide area of use has been developed for this crop in animal feeds, human foods and other industrial applications (Jimenez-Martinez, *et al*., 2003). However, soybeans are not adapted to many climatic conditions. On the other hand, the other legume from the same family Leguminosae called lupin, is able to grow under climatic conditions and soils that soybean would never tolerate. In fact in both cases the soil would be improved because legume roots can fix nitrogen and crop rotation can provide better yields (Jimenez-Martinez, *et al*., 2003).

Lupin has been used as a source of protein and oil since ancient times. Currently interest in a wider utilization of this legume seed is rising. This is mainly due to its similarity with soybeans as a high source of protein and to the fact that it can be grown in wider climatic range. Moreover; its adaptation to poor (i.e. leached) soil, makes it economically feasible (Trugo, *et al*, 1992 and Sujak, *et al.*, 2005). Lupin is commonly consumed as a snack in the Middle East and is coming into use as a high-protein soy substitute in the other parts of the world (Kurzbaum, *et al*., 2008).

In general Lupins are used for many purposes. These include pasture improvement, ornamentation, and erosion control and soil stabilization. It has also 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*, 2006). 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).

Out of the many species of lupin, *Lupinus albus* native to Mediterranean area is agriculturally important (Noffsinger and Santenin, 2005 and Kurzbaum, *et al*., 2008). During the past 3000 years, *Lupinus albus* has been used as a minor crop in the old and new world. Human movement and de-centralization has helped *Lupinus albus* to diversify considerably in the primary and secondary centers of its origin. This diversification has helped for the development of interesting characteristics of the plant. These include cold and disease tolerant, having improved leaflet and seed size and shape, flower and seed color, and degree of apical and branch dominance characteristics of the plant (Noffsinger and Santenin, 2005).

Lupin like other legumes has some anti-nutritional factors which inhibits its consumption. Mainly the presence of alkaloids (i.e. quinolizidine alkaloids) hinders its consumption without processing to remove them. Raffinose family oligosaccharides, phytates, tannins are also the other anti-nutritional factors found in the raw seed (Mahamoud, *et al*., 1994, Jimenez-Martinez, *et al*., 2003, Melbas, *et al*, 2004, Sujak, *et al*., 2005).

Nevertheless; the potential of lupin in human nutrition has generally been underestimated worldwide. So there is a need of designing studies worldwide to develop lupin based convenient food products (Joray, *et al*., 2007). In order to make *Lupinus albus* edible various modern and traditional processing methods have been developed. Soaking after roasting and boiling, germination, fermentation, alkaline treatment are some of them (Joray, *et al*., 2007).

The current study focuses on the effects of these processes on the chemical and nutritional composition of *Lupinus albus* seed. In this document there are 6 chapters. Chapter1 discusses a few introductory points, Chapter 2 includes literature review on *Lupinus albus* (i.e. taxonomy and classification, production and utilization, chemical and nutritional composition, toxic substances and nutritional inhibitors, various processes developed, etc), chapter 3 is materials and methods, chapter 4 comprises results and discussion, and in chapter 5 conclusion and recommendations are included.

1.2. Statement of the Problem

In Ethiopia a sizable proportion of the population (those of low income or subsistence status) get less than the FAO recommended averages daily calorie ration of 2200 Calories (world population data sheet, 2007). This could be the result of severe malnutrition in the country. From all possible causes of malnutrition in Ethiopia, protein deficiency is the worst (Schuftan, 1985). Protein deficiency is the result of not getting the correct proportion of protein from diets.

For alleviation of this problem the consumption of vegetable proteins is one option. Accordingly, grain legumes are the main sources of vegetable proteins. Among them *Lupinus albus* is reported to have highest protein content like soybean. It might be a good potential for fortification of different food types with low protein content like enset (*Ensete ventricosum*).

However; it has limitations for continuous consumption by the society. Primarily, anti-nutritional factors like quinolizidine alkaloids limit its direct consumption (Mahamoud, *et al*., 1994, Jimenez-Martinez.C, *et al*., 2003, Melbas, *et al*, 2004, Sujak, *et al*., 2005). The presence of these alkaloids can cause moderate toxicity in vertebrates. Alkaloids also reduce its palatability when used as food by inducing bitter taste (Melbas, *et al*., 2004, Sujak, *et al*., 2005). The second anti-nutritional factors are raffinose family oligosaccharides (RFOs). RFOs can cause flatulence (Martinez-Villaluenga, *et al*., 2005). Other anti-nutritional factors like phytate and tannins will also reduce the minerals bioavailability. Like the other legumes, lupine seeds are rich in lipid components. However, the composition of fatty acid are very important from nutritional and health point of view. So to make the seed edible and get better nutritional benefit there is a need to explore various traditional processing methods, which will reduce the anti-nutritional factors and improve the nutritional composition as wel.

Based on this fact, the aim of this research was to evaluate the various traditional preparation methods used on the chemical and nutritional composition of *Lupinus albus* grown locally. This will let one to choose the effective processing method with less or no damage to the nutritional content of the seed. And also the effective processing which enhances the nutritional status can be identified. Furthermore, information regarding the fatty acid profile of the oil extracted from *Lupinus albus,* which was not reported previously in Ethiopia will be explored.

1.3. Objectives

1.3.1. General Objectives

The general objective of this study was to explore the effects of various traditional methods of processing on the chemical composition and nutritional values of *Lupinus albus*.

1.3.2. Specific Objectives

The specific objectives of this research include: -

1. To assess the effects of soaking on nutritional and chemical composition of *Lupinus albus*
2. To assess the effects of toasting and soaking on the nutritional and chemical composition, anti-nutritional factors (alkaloids and phytate), mineral composition and fatty acid profile of *Lupinus albus*.
3. To have a general information regarding the crop from different parts of the society and agricultural centers (i.e. agricultural features, yield, utilization of the crop as food and feed, local beverages like Araki, price, etc)

1.4. Significance of the Study

1.4.1. Educational

*Researchers, students, teachers, and academicians of the field area can use the findings of the study as a reference material.

*For future research themes to give the next gap to be covered as recommendation.

1.4.2. Social

*To aware the society that *Lupinus albus* is a good nutritious food crop. Instead of considering it as "poor man's food".

*To make *Lupinus albus* flour as an ingredient of fortification for various food items to improve their nutritional and chemical composition

*One remedy for food security problems is food items diversification. Accordingly, *Lupinus albus* contribute in addressing the society's food deficit.

1.4.3. Economic

*If the production increases and farmers are encouraged to produce more, it could be a potential export commodity in the future.

*The non-shattering property of *Lupinus albus* will avoid post harvest economic losses.

1.4.4. Environmental

*Since *Lupinus albus* has a high tendency of growing on marginal lands (i.e. poor soil conditions), it will help to balance the ecology thus could contribute in protecting the degradation of the environment.

*Again the ability of *Lupinus albus* to fix nitrogen, increasing phosphorus, and organic matter content in the soil, will aid in increasing soil fertility. Thus it can be used for crop rotation to increase per hectare yield of cereals and other crop types.

*It is also an ornamental crop having esthetic values to the environment with its different colour spikes of flower.

2. Literature Review

2.1. Taxonomy and Classification

Lupins belong to the genus Lupinus and family of genisteae, which is also called fabaceae or leguminosae (Uzun, *et al*., 2006). This is a large and diverse genus which includes more than 500 species (Kurzbaum, *et al*., 2008 and ARC center of excellence for integrative legume research, 2009). Lupinus is a genus of self or cross-pollinating, consisting of mostly indefinite plant species native to diverse geographic locations (Phan, *et al*, 2007). Second to cereal crops leguminosae is agriculturally important and one of the three largest families of flowering plants. Leguminosae has been divided into three sub-families named as Caesalpinieae, Mimosoideae and Papilionoideae (Phan, *et al*, 2006). Lupin is the common name for members of the genus Lupinus of the legume family (Kurzbaum, *et al*., 2008).

The name lupin 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 center of excellence for integrative legume research, 2009). It is known as lupines in the United States, as turmus in the Middle East and Tawari in Latin America. The plant is characterized by having various flowering spikes in large range of colors (Fig 2.1) (Kurzbaum *et al*., 2008).

L. angustifolius *L. albus* *L. luteus* *L. mutabilis*

Fig 2.1 Flowers of Different lupin species

Commonly, four lupin species are reported as cultigens in the world (Fig 2.1). These include *L.albus L, L.angustifolius L., L.leutus L*and *L.mutabilis L.* (Uzun, *et al*., 2006, Kurzbaum, *et al*., 2008). Trivially, these species are called white lupin, narrow-leafed (blue) lupin, yellow lupin and pearl lupin respectively (ARC center of excellence for integrative legume research, 2009). Out of these four species the focus area of this research is on *Lupinus albus .L.* This species is also called white lupine in most part of the world. In this document we will use its scientific name *Lupinus albus.L* consistently to refer the crop.

The lupin seed is produced in pods which develop on the main stem of the lupin plant (Fig 2.2). Pods contain between three and seven seeds and these seeds vary in size, colour, appearance and composition depending on the species of lupin. Among them the seeds of *Lupinus albus* are the largest. They have a circular flattened shape and are cream in colour (Australian health info center, 2009).

Fig 2.2 Pods of *Lupinus albus* seed

2.2. Centers of Origin

Four different centers of origin have been proposed for the genus lupinus. These include the Mediterranean region (including northern Africa), North America, South America, and East Asia. Today, approximately 90% of the recognized species are found in alpine, temperate and subtropical zones of North and South America, which ranges from Alaska to Southern Argentina and Chile. The remaining species are native to the Mediterranean region and Africa. But due to their larger seeds, most of the economically important species come from the Mediterranean region (Fig 2.3) (ARC center of excellence for integrative legume research, 2009).

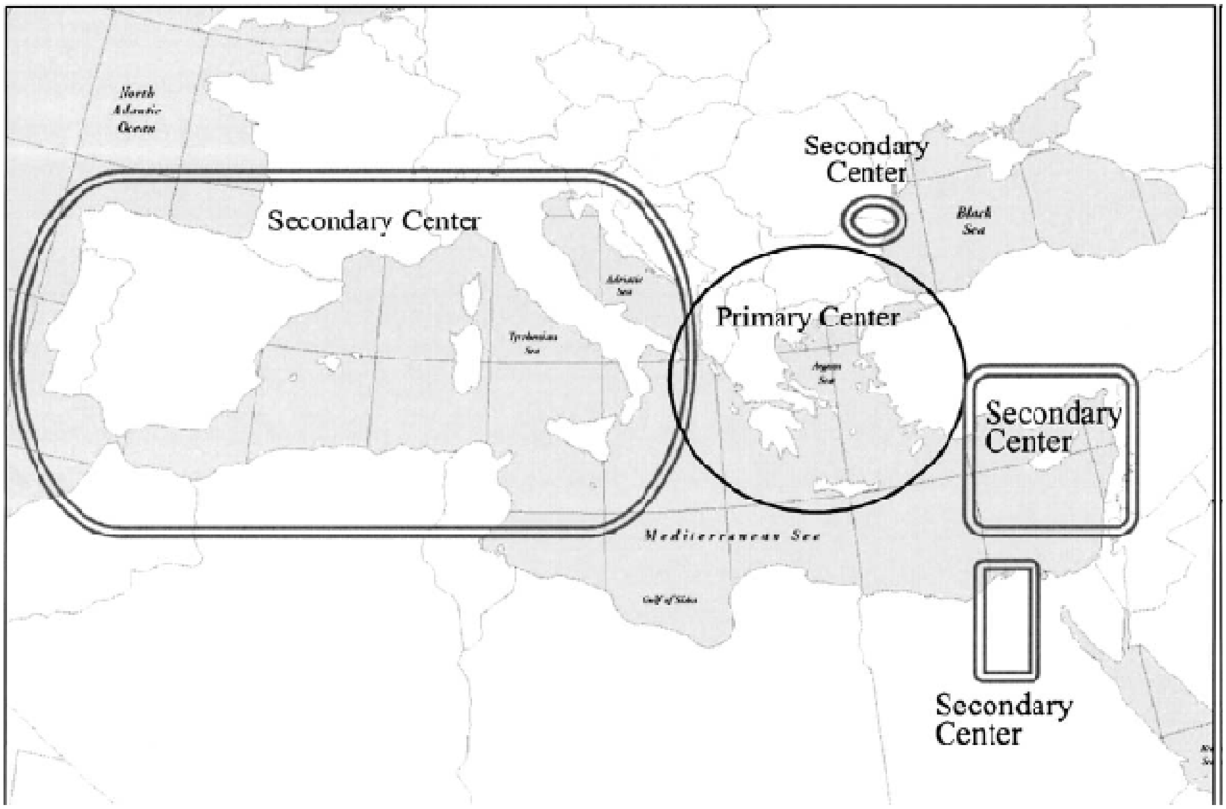


Fig 2.3 Primary (single line circle) and secondary (double line circles) centers of origin for *Lupinus albus* in the Mediterranean Region (Noffsinger and Santen , 2005)

In places where no other crops can be grown profitably, Lupins could be considered as a model for low input plants. Among the common species *Lupinus albus L., Lupinus luteus L.* and *Lupinus angustifolius L.* are Old World species whereas; *Lupinus mutabilis* is a new world species originating from South America (Cowling, *et al,*1998).

2.3. Production and Utilization

2.3.1. History of *Lupinus albus* Utilization

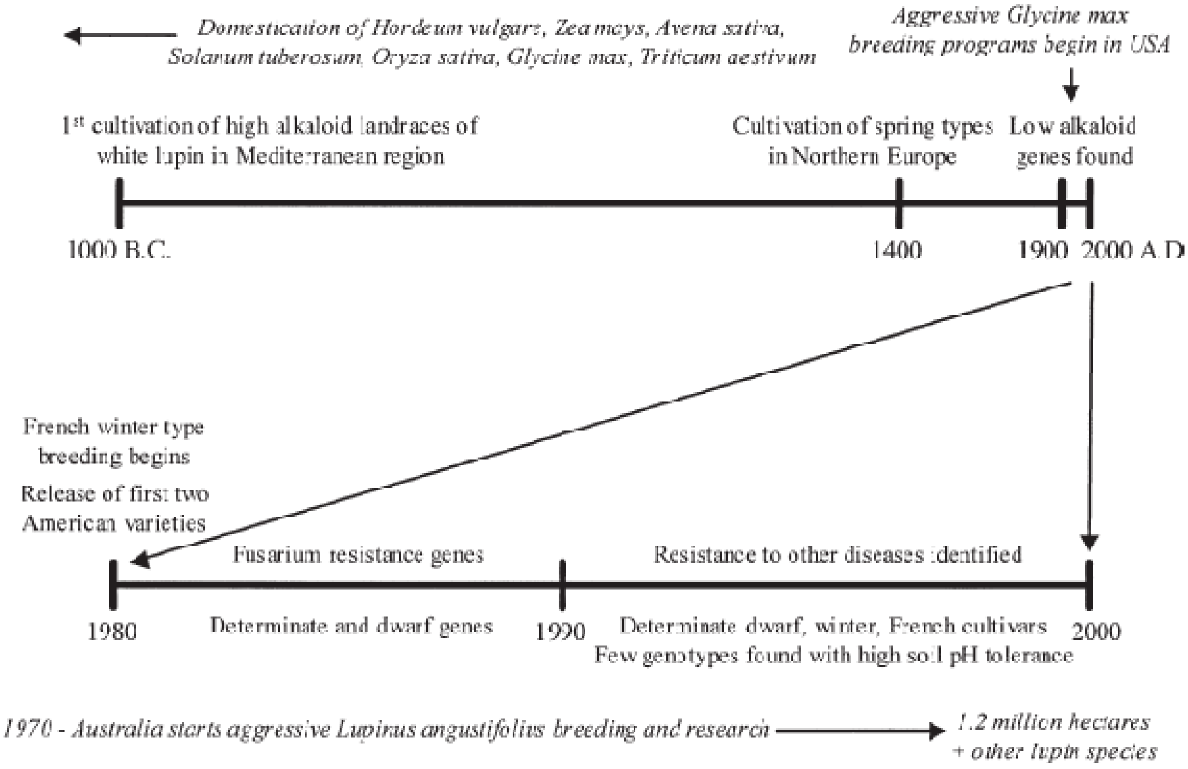


Fig 2.4 Domestication and breeding of *Lupinus albus* vs. other crops (Noffsinger and Santen, 2005)

Legume seeds are protein valuable foods which have been present in the Mediterranean diet since ancient times. Among them, lupins are high protein crops (Frias, *et al*., 2004). Wild and partially domesticated lupin species were grown thousands of years ago both in the Mediterranean region and in the South American Andes before the Incan Empire (Fig2.4). The cultivation of *Lupinus albus* was well known to the ancient Greeks and Romans and its cultivation has been mentioned by early writers including the poet Virgil and Pliny the Elder (Australian health info center, 2009).

It was in the twentieth century that the old bitter types of lupin were replaced by ‘sweet’ low alkaloid types. Before this major development, bitter lupins were spread in southern Europe and North Africa. They were also introduced in northern Europe when Frederick the Great of Prussia sent for lupin seeds from Italy in 1781 to improve the poor soils in north Germany (Frias, *et al.*, 2004**,** Noffsinger and Santenin, 2005, Australian health info center, 2009). Before 1926, lupines had been used as side rates only. The issue of natural existence of low alkaloid lupines was raised by E. Bauer and A. Pryanishnikov. However; works in this field were held back by the absence of reliable and rapid methods of quantifying alkaloids in plants. In 1928, Reinhold Von Sengbusch from the Central German Institute of Genetics proposed a method which was applied to analyze alkaloid in plants (Maknickienė and Asakavičiūtė, 2008).

Lupinus albus is a universal plant with numerous useful properties. It can be used both as fodder and for soil fertilization (Maknickienė, and Asakavičiūtė, 2008). With the development of the sweet varieties, use of *Lupinus albus* for forage and grazing is increasing. In Europe, lupine seeds of all species types have been used for many years to replace cereal grains in flours and pasta. *Lupinus albus* seeds are also sometimes used as a complete or partial substitute for soybeans in the production of milk, milk powder, and tofu (Australian health info. center, 2009). The utilization of this plant can be extended to the production of protein concentrates, which can be added to other food products or fodder can enrich their nutritional values, thus giving functional food (Sujak, *et al.*, 2005).

2.3.2. Agricultural Features of *Lupinus albus*

Lupinus albus is a relatively tall, branching plant, with broad leaves and large, fleshy pods. However, it will take longer time to mature than other lupin species. Furthermore, harvesting and conserving the plant as whole-crop silage removes the need for further processing and storage, and associated equipment and facilities (Fraser, *et al*, 2004).

Lupinus albus is an important grain legume crop that is able to recover from severe water deficit conditions. During the progression of water deficit, the several tissues behave quite distinctly in the amount and rate of water loss. In contrast to the stem stele, which loses only a small amount of water during the stress period, the water content of mature leaves is rapidly and strongly affected by the water shortage (Pinheiro, *et al*., 2004).

Lupinus albus is calcifuges susceptible to iron chlorosis making it grow poorly on calcareous soils. In general cultivation is only possible on acid to neutral soils with moderate calcium content. This has been a major constraint to the development of the lupin crop in Europe and Australia. The growth reduction may be caused by several factors such as high P^H , bicarbonate content, iron deficiency and Calcium toxicity. Thus both shoot and root growth were reduced in Lupinus albus at P^H= 7.5 compared to 6.0 (Raza, *et al*., 2000). The severity of iron chlorosis correlates poorly with shoot growth and seed yield in the field, especially for plants showing iron chlorosis at early growth stages (Raza, *et al*., 2000).

Lupinus albus and other agronomic Lupinus species are relatively undomesticated when compared with most crops (Noffsinger and Santenin, 2005) (Fig 2.4). Cultivation of lupins is also limited worldwide (Jimenez-Martinez, *et al*., 2003). But in the year 2005, the total cultivated area was increased to 1,007,018ha with a yield of 1,086,006 tones. This shows the improvement of the production of the crop from year to year (Uzun, *et al*., 2006). The two types of lupins that are mainly cultivated today are white and blue lupin. And their major sites of production are in Europe and Australia respectively (Yoshie-Stark and Wasche, 2003). Depending on the species, lupins may be either annual or perennial. Most of them are herbaceous, but a few are shrubs or small tree. Western Australia leads the world in lupin exports in recent times (ARC center of excellence for integrative legume research, 2009).

Apart from the high protein content *Lupinus albus* has a strong capability for nitrogen fixation and organic phosphorus release from soil. This let the crop agriculturally to be used in crop rotation during intensive crop production. Especially this feature of *Lupinus albus* is feasible within nitrate vulnerable zones (NVZs) (Sujak, *et al*., 1992, Fraser, *et al*., 2004). For example; in Western Australia, sustained wheat yields are directly dependant on the rotational benefits of *Lupinus albus*. They are also used for weed management and between fields of cereal crops to keep diseases from spreading (ARC center of excellence for integrative legume research, 2009).

Nitrogen is an indispensable element in protein and other nitrogen compounds. It can be taken by plants in NH_4^+ or NO_3^- and under specific conditions in amine form NH_2 (urea). All legumes, including lupins, use the atmospheric nitrogen (N_2) for biological fixation and protein biosynthesis, and synthesis of other nitrogen containing compounds e.g. amino acids, nucleic acids, vitamins, polyamines, alkaloids, etc. In nitrogen fixation, atmospheric nitrogen is converted into ammonia, which is subsequently available for biosynthesis of nitrogen containing molecules (Ciesiolka, *et al*., 2007).

The other advantageous agricultural feature of *Lupinus albus* is being an illuminating model for the study of plant adaptation to extreme phosphorus (P) deficiency. Adaptation to low P is linked to modifications of root development and biochemistry resulting in proteoid (cluster) roots (Phan, *et al*., 2007).

Interest in *Lupinus albus* production is increasing, due to its potential as a source of protein, for pharmaceutical purposes, a green manure and due to the high alkaloid content, as a natural component of plant pesticides (Sujak, *et al*., 2005). For example; *Lupinus albus* is cultivated in the Mediterranean and Egypt for its edible seeds (Mahamoud, *et al*., 1994).

Although *Lupinus albus* has been well- known, widely grown and utilized by people in Mediterranean area and Andean highlands, in Europe its cultivation remains behind that of the other leguminous plants (Sujak, *et al*., 2005). According to FAO the main lupin cultivating countries in Europe are France (24,000 t/ a), Italy (5,000 t/ a) and Spain (9,800 t/ a). In France, the lupin cultivation increased from 6,321 t/ a in 1996 to 24,000 t/ a in 2003, which demonstrates the growing interest for lupin as food and feed source. In Germany the cultivation area of lupins used for food and feed use in 2002 reached 40,000 t/ a (El-Adawy, *et al*., 2000). The amount of lupins used for food applications is estimated to be around 5 to 10% of the total lupin production worldwide (Australian health info center, 2009).

In Ethiopia the information regarding the crop’s agricultural feature is few. Based on the information gathered from the focus group discussion at the beginning of this research the sowing season for *Lupinus albus* is May and June and the maturity time of the seed is five to six months. Usually the farmers use it for intercropping along with teff, wheat, and sometimes with eucalyptus tree. The crop doesn’t need any fertilizer, pesticides, etc for its growth. Regarding the yield there is no information both from the farmers and agricultural centers. In agricultural centers the crop is known by the name “black lupine”, due to the very dark green nature of the whole crop. The crop doesn’t have any soil preferences (i.e. it can grow on marginal lands too) (Annex I).

2.3.3. Some Common Lupin Based Food Types

Lupinus albus seeds meet the requirements as alternative home prepared diets with high nutritional value and reasonable price among leguminous plants (Zraly, *et al*., 2007). Lupines and lupine products have traditionally formed part of the human diet. Food products available on different markets of Europe are lupin snacks, lupin pasta, lupin bread and cookies, lupin coffee and some vegetarian instant meals (Fig 2.5) (Australian health info center, 2009).

Lupinus albus 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 (Sanchez, et a*l*., 2004).

Fig 2.5 Some model foods containing lupin protein

Lupinus albus 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. *Lupinus albus* 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).

In Ethiopia *Lupinus albus* is consumed in few areas of the country. People living in west Gojam are the predominant consumers of the crop. They have consumed the crop primarily as snack and as a raw material for local beverage "Araki" processing. Now a day the local community is also consuming it as "Shiro" like the other common legumes like pea, bean, etc. There is an ancient believe that *Lupinus albus* is a remedy for people having hypertension. And some of the farmers living nearby lakes are using the seed for fisheries, due to its toxic nature of the alkaloids (Annex I).

2.4. Chemical and Nutritional Composition

2.4.1. Proximate Composition

a) Crude Protein

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 *Lupinus albus* have shown that the bio-availability of the constituents is comparable to those of processed soybeans (Joray, *et al*., 2007).

Grain legumes are main sources of vegetable protein, among which *Lupinus albus* is known to have seeds with the highest protein content like soybean (Sujak, *et al.*, 2005 and Martinez-Villaluenga, *et al.*, 2005). Based on this fact *Lupinus albus* seeds have been employed as a protein source for animal and human nutrition in various parts of the world (Sanchez, *et al.*, 2004).

The requirements with regard to chemical composition, nutritional value and product safety were laid down by the Advisory Committee on Novel Foods and Processes (ACNFP) in 1996 for certified lupins (sweet lupins). Based on the strength of this certification, these products were recommended as feedstuffs and food ingredients (e.g. lupin flours for baked goods) (Australian health info center, 2009).

As a member of legume family lupin bean protein is rich in lysine and deficient in sulfur containing amino acids (Jimenez-Martinez, *et al.*, 2003, Sujak, *et al.*, 2005, Phan, *et al.*, 2007). In contrast its arginine content is markedly higher (Zraly, *et al.*, 2007). 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) (Sujak, *et al.*, 2005).

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 (Sujak, *et al.*, 2005).

Currently, there are only few companies in Europe that produce *Lupinus albus* protein ingredients for food use. The products available are toasted and non-toasted lupin flour, grits, granulates, fiber and protein concentrates from the non-defatted seed. Lupins and lupin products were considered to be traditional foods even before the introduction of the Novel Food Decree (1997) (Australian health info center, 2009).

b) Crude Fiber

Lupinus albus species contain very little starch. They usually have lower carbohydrate content when compared to soy bean (Fraser, *et al.*, 2004, Uzun, *et al*, 2006, Joray, *et al.*, 2007, ARC center of excellence for integrative legume research, 2009). Like soybean *Lupinus albus* has high dietary fiber content (mean value30%). It has the lowest Glycaemic Index of any commonly consumed grain (Phan, *et al*., 2007, Zraly, *et al*., 2007).

c) Crude Fat

The fat level in lupin is ranked third after ground nut (*Arachis hypogeae L.*) and soybean (*Glycin max*) among legumes (Uzun, *et al*., 2006, Joray, *et al*., 2007). The lipid contents of *Lupinus albus* are similar to other species of the genus lupinus like *Lupinus campestris* (Jimenez-Martinez, e*t al*., 2003). The mean value of crude fat in *Lupinus albus* grown in different parts of the world is 13% (Jimenez-Martinez, *et al*., 2003, Phan, *et al*., 2006).

The oil extracted from *Lupinus albus* seed consist various types of fatty acids. 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. This means *Lupinus albus* can be a potential source of considerable amount of useful vegetable fat. Among the unsaturated fatty acids, majorly oleic and linolenic acids are found (Uzun, *et al.*, 2006). The high content of unsaturated fatty acids and a desirable ratio of ω

-6 and ω -3 fatty acids, make the crop a healthy alternative edible oil source (Joray, *et al.*, 2007).

Different studies have been undertaken regarding the proximate composition of various cultivars of *Lupinus albus*. In Australia moisture, protein, ash, fat, crude fiber, ADF, NDF, oligosaccharides and lignin contents were reported to be 8.6, 35.8, 3.3, 9.4, 10.6, 14.6, 17.6, 6.6 and 0.6% respectively. This report has shown that *Lupinus albus* has comparable protein content with other legumes like soybean (Cowling, *et al*., 1998). In Spain protein, ash, fat, sucrose, crude fiber contents and starch contents for the crop were found to be 30.6, 3.65, 14.64, 2.58, 39.42 and 3.27% respectively (Melbas, *et al*., 2004). In Turkey similar studies have investigated that *Lupinus albus* has 8.32, 32.2, 2.65, 5.95 and 16.2% of moisture, protein, ash, fat and crude fiber respectively (Sujak, *et al*., 2005).

2.4.2. Anti-nutritional factors and toxic substances

The consumption of grain legumes in human diet is limited due to the presence of certain anti-nutritional factors. These include α-galacto oligosaccharides, phytic acid, condensed tannins, polyphenols, protease inhibitors, α-amylase inhibitors, lectins, etc (Skulinova *et al*., 2002). The most prominent anti-nutritional factors in lupin seeds are the bitter and toxic quinolizidine alkaloids, which occur at concentrations up to 2.5%. RFOs also constitute a considerable amount in the seed (Khalil, *et al*., 2006). In contrast to other leguminous plants such as peas, soy beans, *Lupinus albus* contain extremely low amounts of trypsin inhibitors, lectins, iso-flavones, saponins and cyanogens (Jimenez-Martinez, *et al*., 2003, Joray, *et al*., 2007, Zraly, *et al*., 2007).

a) Raffinose family oligosaccharides

α-galacto oligosaccharides raffinose family oligosaccharides (raffinose, stachyose, verbascose etc.) are characterized by the presence of α(1–6) links between galactose residues and these linkages are not hydrolyzed by the intestinal mucosal enzymes (Skulinova, *et al*., 2002). So the higher levels of soluble non-starch polysaccharides (NSP) and α-galactosides cannot be digested by endogenous enzymes (Zraly, *et al*., 2007).

Flatulence will result due to the action of anaerobic intestinal micro flora of RFOs that cannot be degraded by mammalian digestive enzymes. Though this is the case, there is a benefit associated with the consumption of legumes due to their RFOs. This is related to the slow rate of starch digestion and the high content of resistant starch in legumes (Skulinova, *et al*., 2002).

b) Alkaloids

Lupinus albus are often referred to as either bitter or sweet. Bitter lupins, such as the lupine beans consumed in Europe, have high concentrations of alkaloids. The mean content of alkaloids in these bitter species is 2.5%. Sweet lupins, such as those grown in Western Australia, have low levels of alkaloids (Australia New Zealand food authority, 2001). In comparison with the bitter one, sweet *Lupinus albus* has lesser amount of anti-nutritional factors, particularly quinolizidine alkaloids (Zraly, *et al*., 2007). These sweet varieties have been obtained through breeding programmes. These varieties have advantages of having low alkaloid content but they are also less resistant to disease and herbivore attack (Sanchez, *et al*., 2004). Data indicates that the mean alkaloid content of marketable sweet lupin seed is on average 130-150 mg/kg (Australia New Zealand food authority, 2001).

Although *Lupinus albus* 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. 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).

In plants, alkaloids have been found to be the intermediate forms of nitrogen metabolism, in which these compounds are rendered harmless and accumulate. There are data on the possible participation of alkaloids in the processes of breathing, oxidation of various compounds such as ascorbic and citric acids, hydroquinone, pyrogallol and enzyme synthesis (Maknickene and Asakaviciute, 2008).

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 (Maknickene and Asakaviciute, 2008).

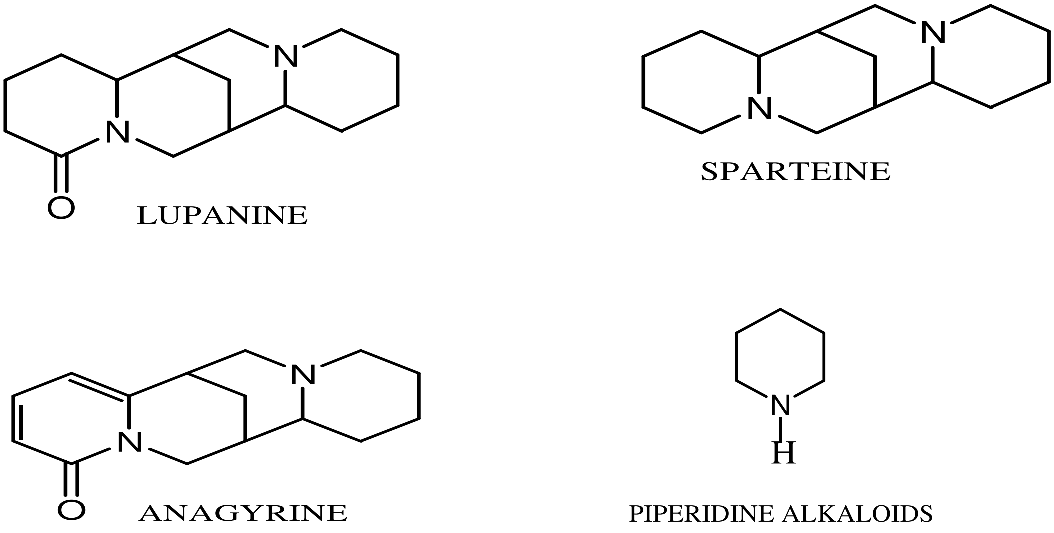
Alkaloid content in *Lupinus albus* depends on numerous factors such as species variety, age (developmental stage), environment and geographical location. At the end of vegetation, alkaloids accumulate in seeds and roots (Maknickene and Asakaviciute, 2008).

Quinolizidine alkaloids occurring in *Lupinus albus* are the largest single group of legume alkaloids with clear ecological functions in the defense of the plant. Quinolizidine alkaloids are used as a nitrogen source for seedlings and they play a defensive role against predators in the plant (Sanchez, *et al*, 2004). 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 (Australia New Zealand food authority, 2001).

Health effects of alkaloids

Alkaloid content in plants has been found to impact the central nervous system of living organisms, with low levels acting as stimulators and higher levels as suppressors (Maknickene and Asakaviciute, 2008). Lupanin and other lupin alkaloids such as cytosine and anagyrine are toxic with agonistic activity on both nicotinic and muscarinic acetyl choline receptors, as Quinolizidine alkaloids display similar agonistic activities as the alkaloid nicotine they also affect Na^+ and K^+ channels, inducing gastrointestinal, nervous and respiratory symptoms in humans and animals (Sanchez, *et al*., 2004). General toxic symptoms include malaise, nausea, respiratory arrest, visual disturbances, ataxia, progressive weakness and coma (Australia New Zealand food authority, 2001). The major potential source of exposure to lupin alkaloids is the use of lupin flour from low alkaloid varieties of lupines to substitute for a small percentage of wheat flour (Australia New Zealand food authority, 2001).

The major alkaloids present in seeds of *Lupinus albus* are Lupanin, hydroxyaphylline, albine, and multiflorine. With respect to the toxicity quinolizidine alkaloids, those belonging to the Spartein and Lupanine types are relatively toxic when injected, but if orally ingested the toxic effect is lower (Fig 2.6). Lupanine has a moderate effect in vertebrates, while alkaloids such as α-pyridine, cytosine, and anagyrinre are highly poisonous (Jimenez-Martinez, *et al,* 2003).



LUPANINE

SPARTEINE

ANAGYRINE

PIPERIDINE ALKALOIDS

Fig 2.6 Chemical structure of some of alkaloids in *Lupinus albus*

2.5. Different Processes Developed Remove Anti-nutritional Factors

Nowadays, there is a growing interest in Western countries in natural, minimally processed and additive-free, nutritional and healthy foods. Traditional food processing such as germination and fermentation of legumes may offer us natural nutritive products, which can be beneficial to health. Furthermore, these processes could enhance the activities of bioactive antioxidant compounds such as vitamins E and C (Frias, *et al.*, 2004).

The prior limitation to the consumption of *Lupinus albus* is the presence of alkaloids. An elaborate cooking process is necessary to remove toxic alkaloids in the seeds (Kurzbaum, *et al.*, 2008). In parallel with the processing methods plant breeders have been trying to develop sweet lupin containing low level of alkaloids. In spite of this effort sweet lupin varieties are not free of alkaloids. In addition to genetic selection for low alkaloid containing lupin seeds, there are some physical and chemical treatments with acids and alkalis for eliminating these anti-nutritional factors (Arslan and Seker, 2002). Beyond removing the unwanted anti-nutritional factors, these processes will improve the nutritive value and digestibility (Khalil, *et al*., 2006).

2.5.1. Soaking (De-bittering process)

In addition to hydrating the seed , de-bittering process helped to eliminate alkaloids, destroy their germinative power, inactivate cell enzymes such as lipase and lipoxygenase, eliminate microorganisms adhered to the seed, and increase the cell wall permeability , facilitating the extraction of alkaloid, oligosaccharides or other anti-nutritional factors (Jimenez-Martinez, *et al*., 2003). Alkaloids are water soluble, but there is little information available on the effect of heating or cooking on the stability of lupin alkaloids (Australia New Zealand food authority, 2001).

In general, soaking of leguminous plants has many advantages. These include to remove or reduce anti-nutritional factors like phytic acid and tannins, to neutralize enzyme inhibitors, to encourage the production of beneficial enzymes, to break down gluten and make digestion easier, to make the proteins more readily available for absorption, to prevent mineral deficiencies and bone loss, to help neutralize toxins in the colon and keep the colon clean, to prevent many diseases causing conditions (Beaty and Foutch, 2006).

The levels of alkaloids in seeds can be reduced through a de-bittering process involving soaking or washing with water. This is commonly practiced in Europe where high alkaloid lupins, so-called ‘bitter lupins’, are grown (Australia New Zealand food authority, 2001). In the aquas de-bittering, the final alkaloid concentration found was wel below that of the toxicity safety limit for animal and human consumption (Jimenez-Martinez, *et al*., 2007).

2.5.2. De-hulling

Different authors likewise demonstrated that one way to increase the energy level of a diet is by de-hulling of seeds. Like supplementation with fat and limiting amino acids can enhance the energy level (Zraly, *et al*., 2007). De-hulling is also an effective method to reduce α-galactosides known to cause flatulence in *Lupinus albus,* due to the hull's cellulostic nature which compromises RFOs too (Joray, *et al*., 2007). *Lupinus albus* seed has high hull weight proportion, which is mainly composed of cellulose and hemi-cellulose materials (Fig 2.7).

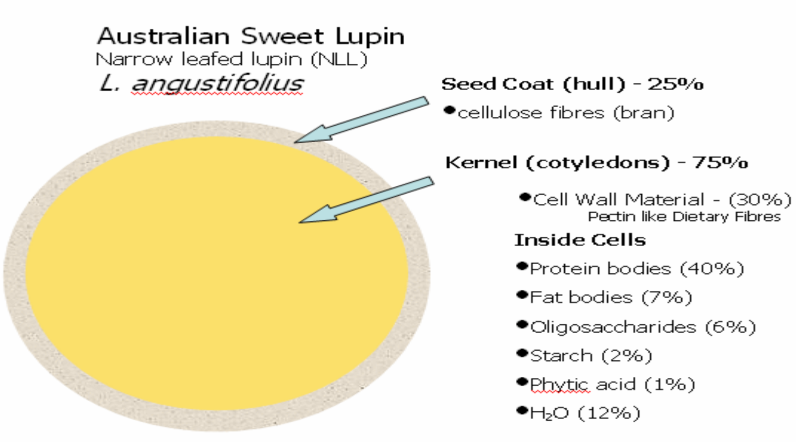


Fig 2.7 Seed coat and cotyledon composition of other species of Lupinus genus (www.lupin.org, 2009)

2.5.3. Germination

Germination of legume seeds is one of the processing methods to increase nutritive value and health qualities as sprouts. This process is simple and inexpensive and different seeds have been germinated for human consumption. These include legumes like (soybean, lentils, beans, chickpeas, peas), cereals (rye, wheat, barley, oats) and, recently, seeds of some other vegetables (alfalfa, radish, mustards, brassica) (Fries, *et al*., 2004). Germination has been suggested as an effective treatment to remove anti-nutritional factors from legumes and mobilizing secondary metabolites (e. g., phytates and α-galactosides) (Uebersax, 2006).

2.5.4. Fermentation

Fermentation also is a very interesting process used in plant foods to increase the nutritional quality and remove undesirable compounds. Fermentation involving lactic acid bacteria offers potential for widespread applications, particularly with respect to the preservation of cereals, legumes and root crops and the provision of safe, low-cost weaning foods for developing countries (Fries, *et al*., 2004).

2.6. Existing information about *Lupinus albus* in Ethiopia

In Ethiopia apart from one native and one cultivated, at least four species of lupines have been introduced (Demissei, 1983). This is attributed for both trials as forage crops or green manure, as well as use for soil and water conservation programs, and as ornamentals. These include *Lupinus luteus*, *Lupinus. mutabilis*, *Lupinus mexicanus* and *Lupinus albus* (locally known as Gibto) (Demissie, 1983) .

According to the plant database from forest gene bank, the currently available species in the country include *Lupinus mutabilis* and *Lupinus albus* (Forest Gene Bank, 2008). But *Lupinus mutabilis* is found in small areas of Oromiya (Shewa), Tigray (Adwa), and SNNP (Gurage) regions. The widely spread species in the country is *Lupinus albus*. This bitter species is widely found in Amhara and SNNP regions. In Amhara region, especially in west Gojam and Gondar areas, it has the highest yield (Table 2.1) (Forest Gene Bank, 200 and CSA, 2007). As it has been observed in the table the largest production is recorded in Amhara regional state. In the region south Gondar and west Gojam have equal annual yield of 7.17 Qtl/ ha (CSA, 2007).

Table 2.1 Report on Area production of crops (private peasant holdings in Mehir season) of pulses and gibto (*Lupinus albus*) (2007/2008).

Areas	**Pulses**			**gibto (*Lupinus albus*)**			
	No of holders	**Area in ha**	**Production in Qt**	**No of holders**	**Area in ha**	**Production in Qt**	**Yield Qt/ ha**
South Gondar	310,364	107,295.76	1,084,606.14	50,813	9,104.75	65,251.33	7.17
East Gojam	291,299	91,096.2	1,227,433	17,391	*	*	*
West Gojam	265,995	74,235.99	970,886.71	39,623	7,283.16	52,222.93	7.17
Awi	69,248	16,287.93	159,377.99	25,536	6,429.58	29,645.95	4.61
Amhara region	2,285,014	703,526.94	8,175,493.73	134,033	26,052.58	162,539.64	6.24
SNNP region	1,364,816	157,092.94	1,627,170.81	2,796	*	-	-
Ethiopia	6,778,640	1,517,661.93	17,827,387.94	138,722	26,398	165,541.58	6.27

Source: - Ethiopian central statistical agency (2007)

N.B:- the area and production designated by (*) indicates the not reported data because of high coefficient of variation, (i.e. they are less reliable). However, they are consolidated in the total estimates.

: - (-) Indicates not reported (Federal democratic Republic of Ethiopia Central statistical agency, 2007).

In Ethiopia *Lupinus albus* is traditionally called ‘gibto’. The local community gives this name; because they thought the origin of the seed is Egypt (Gibtse in Amharic). So they name the crop after Gibtse (Gibt) (Sileshi, 1985). This species has very useful agronomic characteristics. It is non-shattering, disease resistant, high yield giving, growing on marginal soil and so on. This makes the crop better species than the sweet variety growing in Australia (the Australian new crops newsletter, 1999).

The plant characteristic of gibto (*Lupinus albus*) grown in Ethiopia is described as; herbs, rarely as shrubs, leaves. Usually digitate stipules odnate to base of petiole. Flowers in terminal racemes, calyx 2-lipped, divided almost to the base. Corolla variously colored wings connate at apex, keel beaked. Stamens all joined into a closed tube. Pod dehiscent, compressed usually constricted between the seeds (Demissie, 1983).

In Ethiopia, particularly in Gojam and Gondar areas gibto (*Lupinus albus*) is used as food crop. However, few studies were conducted with regard to its chemical composition and nutritional values. The protein quality evaluation of dagussa (*Eleusin coracanal*) and gibto (*Lupinus albus*) and the supplementary value of gibto to dagussa were reported by Sileshi (1985).The results indicated that the addition of gibto (*Lupinus albus*) enhances the digestibility, PER, CS, and BV of dagussa (*Eleusin coracanal*). She has reported the reduction of alkaloids level through selected processes. In her study, she also recommended the following issues to be studied in the future.

1. The need or necessity of removing the alkaloids from the seeds by testing the different methods of food preparations. In addition to this the necessity of avoiding processes that damage the quality of protein is also left as a recommendation.
2. The amino acid contents of the seed as well as the product after processing should be studied.
3. Is there an advantage to use the de-hulled seeds? And how does it compare with Protein supplements of whole seeds?

In the food composition table for use in Ethiopia, the composition of three prepared foods of gibto (*Lupinus albus*) was reported by (EHNRI, 1997). These samples were collected from the market, but information such as the variety, methods of preparation, storage time, cooking time and cooking temperature, were not mentioned (Table 2.2).

Getachew Bolodia had studied the chemical composition of raw Gibto seeds, and reported that the seeds contain adequate amount of protein, carbohydrates, and minerals. Nevertheless, the methods used were crude and qualitative analyses were done.

Table2.2 Composition of Gibto grown in Ethiopia

No **Food items**	**Food energy** (Calories)	**Moisture** (%)	**Nitrogen** (gm)	**Crude Protein** (gm)	**Crude Fat** (gm)	**CHO (inc.fiber)** (gm)	**Crude Fiber** (gm)	**Crude Ash** (gm)	**Ca** (mg)	**P** (mg)	**Fe** (mg)	**β-Carotene equivalent** (mg)	**Thiamine** Mgs	**Riboflavine** (mg)	**Niacin** (mg)	**Ascorbic acid** (mg)
Lupines , boiled	240.1	44.50	3.06	19.10	4.90	29.9	8.5	1.6	117	154	3.9	0.00	0.09	0.15	1.3	2.0
Lupines, roasted	390.7	10.0	4.92	30.80	7.90	49.10	14.40	2.20	211	282	5.70	0.00	0.06	0.26	2.10	4.00
Lupines, raw	394.3	9.30	5.25	32.80	8.30	47.10	12.9	2.50	213	292	8.6	0.20	0.09	0.32	1.8	3.00

Source:-Ethiopian health and nutrition research institute (1997)

3. Materials and Methods

3.1. Materials

Samples of gibto (*Lupinus albus*) were collected from open markets of Dangla and Tilili in west Gojam. Dangla is found 11.25° and 36.60° latitude and longitude respectively. The total population reported in the area is 21,800. Tilili is found in Awi zone with 10.95° and 36.50° latitude and longitude respectively. The total population in the area is 23,800. These sampling sites were chosen because of their high production of the crop (CSA, 2007). The sampling technique used was random sampling. After the sample was bought from the mentioned open markets, it was packed in polyethylene bags and transported to Food Science and Nutrition Laboratory by a vehicle. Then each sample from the sampling sites were mixed individually and packed in polyethylene bags.

3.1.1. Apparatuses, Chemicals and Glass wares

All chemicals used for analysis were AR grade and obtained from Sigma Aldrich Company. Similarly, the glass wares were cleaned and free from any possible contamination prior to analysis. The major equipments used during the analysis are listed below:-

a)	LABCONCO, AJ461A, USA	Freeze dryer
b)	Tecator, CYLOTEC 1093, Sweden	Laboratory mill
c)	U 10, DIN 12880-KI, Memmert, 854 Schwabach, West Germany	Drying oven

d) ARZ 140, N315, SNR=1203290469, USA	Analytical balance
e) Kjeltec*2300 Analyzer unit, FOSS, Sweden	Crude Protein Analyzer
f) 2055 SOXTEC extraction unit, FOSS extractor, Sweden	Crude fat extractor
g) Wagtech, hot plate SH3, UK	Hot plate stirrer
h) Carbolite, Aston Lane, Hope, Sheffield s30 2RR, England	Muffle furnace
i) DYNAC II centrifuge, Clay Adams, division of Becton and Dikinson Company, USA	Centrifuge
j) BECKMAN, Du-64 Japan	Spectrophotometer
k) Maxi mix II M 37610-26 Thrmolyne Dubaque lowa, USA	Vortex mixer
l) DANI, model ALS100, 0305041069, Italy	Gas chromatography

3.1.2. Sample preparation

All the samples were cleaned manually to remove foreign matters, immature and damaged seeds. Then the following main traditional processes were performed.

a) Roasting, Soaking and De-hulling

The cleaned gibto (*Lupinus albus*) seeds from Dangla and Tilili samples were roasted on metal pan for 10 minutes together with pre-cleaned sand. The sand was used to uniform the heating (i.e. to make the roasting uniform). Then it was removed and allowed to cool for about 10 minutes following washing several times and soaking in a bucket of tap water (1:10 ratio). The soaking water was changed every 2 hrs for five days until the bitterness was removed. The removal of the bitterness was checked by tasting the whole seed like in the traditional way. After the bitterness was removed the whole seed was de-hulled and both the whole seed and the kernel were freeze dried at -42 ℃ for 48 hrs. The dried samples were milled using laboratory sample mill with sieve size of 60 meshes and packed in brown glass until analysis.

b) Boiling, Soaking and De-hulling

The raw gibto (*Lupinus albus*) samples from Dangla and Tilili were separately cleaned to remove foreign matter, dust, stone, etc. The cleaned seeds were boiled in water for 4 hrs. The boiled seeds were then soaked in a bucket of water (1:10 ratio). The soaking water was changed every 2 hrs for five days until the bitterness was removed. The removal of the bitterness was checked by tasting the whole seed like in the traditional way. Afterwards, the whole seed was de-hulled and both the whole seed and the kernel were freeze dried at -42 ℃ for 48 hrs. The dried samples were milled using laboratory sample mill with sieve size of 60 meshes and packed in brown glass until analysis.

c) Germination

The raw gibto (*Lupinus albus*) seeds from both sampling sites were cleaned to remove foreign matter, dust, stone, etc. The cleaned seeds were soaked in tap water for 24 hrs. Then the water was drained off and the sample was covered in castor bean leaf and left to germinate at room temperature for 48 hrs. At the end of the germination process, the whole seed was de-hulled and both the whole seed and the kernel were oven dried at 50 ℃ for 24 hrs. The dried samples were milled using laboratory sample mill with sieve size of 60 meshes and packed in brown glass until analysis.

After the two raw samples were processed in the mentioned processing methods, the following codes were given for each sample type in the result tables.

♠=type of sample, 1 and 2 represent the Dangla and Tilili samples respectively.

1. RG……………..Raw Gibto
2. RSW…...............Roasted then soaked whole seed
3. RSK……………Roasted then soaked kernel
4. BSW……………Boiled then soaked whole seed
5. BSK…………….Boiled then soaked kernel
6. GW……………..Germinated whole seed
7. GK……………..Germinated kernel

3.2. Methods

3.2.1. Focus Group Discussion

A technical questioner was prepared for this part of the study. And mainly four groups of the society were selected for the discussion. These are women processing gibto (*Lupinus albus*) for various products, local community consuming different products of the crop, farmers and Amhara agricultural center. After the discussion all the information was taken as a baseline data for the study, specially in choosing the traditional methods of processing.

3.2.2. Proximate Composition Analysis

Moisture Content Determination (AOAC 925.09, 2000)

The aluminum dishes used for the moisture determination were dried at 130 ℃ for 1hr using drying oven. The dishes were removed and kept in a desiccator for about 30 minutes. The mass of empty dishes were measured as M_1. This was regulated until constant weight was obtained. About 5 gm of the sample was weighed using analytical balance in to the dish and recorded as M_2. The sample was mixed thoroughly and dried at 100 ℃ for 6 hrs. And it was taken and kept in a dessicator to cool. After cooling the weight was taken as M_3. Then kept in oven for another 15 minutes. Then it was removed and allowed to cool in a dessicator and again the weight was taken. This process was repeated until constant weight was obtained. Then, the moisture content was calculated using the following formulae:-

$$\text{Moisture } (\%) = \frac{M2 - M_3}{M2 - M_1} \times 100$$

M_1 = Mass of the dish

M_2 = Mass of the dish and the sample before drying

M_3 = Mass of the dish and the sample after drying

Crude Protein Analysis (AOAC 979.09, 2000)

About 0.5 gm of powdered sample was weighed on analytical balance and transferred to the digestion flask. Then 6 mL acid mixture (5:1 Conc. H_3PO_4:H_2SO_4) and 3.5 mL of 30% H_2O_2 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 (0.5:100 Se: K_2SO_4) was added in to the digestion flask. The solution was then digested at 370 ℃ for 1hr. 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.

$$NH_4SO_4 + 2NaOH \Rightarrow 2NH_3 + 2H_2O + Na_2SO_4$$

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 sulphuric acid solution) until the green color changes to pink. The total nitrogen content was calculated using the following formulae:-

$$NH_3 + H_3BO_3 \Rightarrow NH_4^+ + H_2BO_3^-$$

$$\text{Nitrogen } (\%) = (V_{HCl} \times N_{H_2SO_4} \times 14.01)/M \times 100$$

V_{H2SO4} = Volume of H_2SO_4 consumed until the end point of titration

N_{H2SO4} = Normality of H_2SO_4

14.01 = Molecular weight of nitrogen

% Protein = 6.25 × N (%)

M = Weight of sample on dry basis

Crude Fat Determination (AOAC 4.5.01, 2000)

The flasks used for the extraction were washed and then dried in drying oven at 92 ℃ for 1hr and cooled in a desiccator. The masses of the cooled flasks were measured by analytical balance and recorded as M_1. About 2 gm of the powdered sample was weighed in to each thimble lined with cotton at their bottom. The thimbles with its sample content were placed in to the Soxhlet extraction apparatus. Then 70 mL of diethyl ether was added in to each flask and the extraction process was done for about 4 hrs followed by removing this flask with its content from the Soxhlet, it was placed in drying oven at 92 ℃ for 1hr. The flasks with their contents were then placed in a desiccator for 30 minutes. The mass of each flask together with its fat contents was measured as M_2. Then, the total lipid amount was calculated using the following formulae:-

$$\text{Lipid}\ (\%) = (M2 - M1)/M \quad \times 100$$

M_2=mass of flask and lipid extracted

M_1=mass of dried flask

M = weight of sample on dry basis

Crude Fiber Determination (AOAC 962.09, 2000)

1.6 gm of the sample was weighed in 600 mL beaker using the above analytical balqnce. 200 mL of 1.25% H_2SO_4 solution was added to each beaker and allowed to boil for 30 minutes by stirring and rotating it periodically on a hot plate. During boiling the level was kept constant by addition of hot distilled water. After 30 minutes 20 mL of 28% potassium hydroxide was added and again allowed to boil for another 30 minutes. The level was still kept constant by addition of hot distilled water. Then after 30 minutes the solution was filtered through crucibles containing calcite sand by placing it on (Buchner funnel fitted with No.9 rubber stopper). During filtration the sample was washed with hot distilled water followed by 1 % H_2SO_4, hot distilled water, 1 % NaOH and finally with acetone. The crucible with its content was dried for 2 hrs at 130 ± 2 °C in drying oven and cooled in a desiccator and weighed as M_1. Then it was ashed for 30 minutes at 550 °C in muffle furnace and was cooled in a desiccator. Finally it was weighed as M_2. The crude fiber content was calculated using the following formulae:-

$$\text{Crude fiber } (\%) = (M1 - M2)/M \times 100$$

M_1=mass of the crucible, the sand and fiber

M_2=mass of the crucible and the sand

M = Weight of sample on dry basis

Crude Ash Determination (AOAC 923.03, 2000)

The porcelain dishes used for the analysis were washed by dilute hydrochloric acid on boiling and washed with distilled and de-mineralized water respectively. Then dried at 120 °C in an oven and ignited at 550 °C in furnace for 3 hrs. Then the dishes were removed from furnace and cooled in a desiccator. The mass of the dish was measured as M_1. About 2.5 gm of sample powder was being weighed in to the porcelain dish and recorded as M. The sample was charred at120 °C for 4 hrs in 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 8 hrs. The sample was removed from the furnace and placed in a desiccator. Finally the mass was weighed as M_3. And the total ash content was calculated with the following formulae:-

$$\text{Ash } (\%) = (M3 - M1)/(M2 - M1) \times 100$$

M_1=mass of the dried dish

M_2=mass of the dish and the sample on dry basis

M_3=mass of the dish and the ash

Utilizable Carbohydrate Determination

The Utilizable carbohydrate was calculated by difference with the exclusion of crude fiber. This is expressed mathematically as follows.

$$\text{Utilizable Carbohydrate } (\%) = 100 - (Crude\ fiber + Crude\ protein + Crude\ ash + Crude\ fat)$$

Total energy in kilo Calories

The total energy content in each sample was calculated as follows:

$$\text{Total energy } (\%) = (9 \times Crude\ fat + 4 \times Crude\ protein + 4 \times Utilizable\ carbohydrate)$$

3.2.3. Mineral Analysis

The ash was dissolved by 5 mL of 6 M HCl at low temperature on hotplate for about 2 hrs. Then, 7 mL of 3 M HCl was 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 2.5 mL of 10 % Lanthanum chloride solution was added to the flask. Then diluted to 50 mL mark with de-ionized 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 based on the instruction given in the manual. The calibration solutions and the reagent blank solutions were measured first. Them 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 contents of each sample were calculated using the following formulae:-

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

Where; W = weight in gm of the sample V = volume in mL of the extract

a= concentration in ppm of sample solution b= concentration in ppm of blank solution

3.2.4. Anti -nutritional Factor Analyses

3.2.4.1. Phytate

The phytate content in the sample was determined according to the method described by Latta and Eskin (1980), and later modified by Vaintraub and Lapteva (1988). About 0.05 gm of dried sample was extracted with 10 mL 2.4 % HCl in methanol for 1 hr at ambient temperature and centrifuged (3000 rpm) for 30 minutes. The clear supernatant was used for the phytate estimation. 1 mL of Wade reagent (0.03% solution of $FeC1_3.6H_2O$ containing 0.3% sulfosalicylic acid in water) was added to 3 mL 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 (3 mL of water+1 mL 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 100 gm dry weight.

To prepare the phytic acid standard curve, a series of standard solution were prepared containing 5–40 mg/ mL phytic acid in water. 3 mL of the standards were pipetted into 15 mL centrifuge tubes with 3 mL of water used as a zero level. To each tube was added 1mL of the wade reagent, and the solution was mixed on a vortex mixer for 5 s. The mixture was centrifuged for 10 minutes and the supernatant read at 500 nm by using water as a blank.

3.2.4.2.Total alkaloid

The alkaloid content was determined gravimetrically by the method of Haborne, (1973) as cited in (Adeniy, *et al*., 2009). 5 gm 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 60 ℃ for 30 minutes. Then, transferred into 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 (Adeniyi, *et al*., 2009).

3.2.5. Fatty acid analysis

3.2.5.1. Fatty acid profile (AOAC Method 969.3, 2000 and Egan, *et al*., 1981)

To prepare methyl esters by Sodium methoxide Method First using a Pasteur pipette 100 mg (± 5 mg) of sample oil was weighed to the nearest 0.1 mg into a vial or small bottle with a tight sealing cap. Then 5 mL of hexane was added to the vial and the whole content was left to vortex briefly to dissolve the lipid. Then 250 µL of sodium methoxide reagent was added. Afterwards the vial cap was tightened and left to vortex for 1 minute, pausing every 10 s to allow the vortex to collapse. 5 mL of saturated NaCl was added to the solution in the vial. The vial cap was tightened and the whole content was shaked vigorously for 15s and settled for 10 minutes.

Then the hexane layer was removed and transferred to a vial containing a small amount of $NaSO_4$. Any interfacial precipitate or any aquas phase was not allowed to transfer. The hexane phase containing the methyl esters was mixed with $NaSO_4$ for at least 15 minutes prior to analysis. The hexane phase was transferred to a vial or small bottle for subsequent GC analysis. Then standards and samples were injected into the GC. The FFAs are then determined and quantified.

3.2.6. Statistical analysis

Chemical and nutritional composition, mineral composition, anti-nutritional factors and fatty acid profile data of the raw and processed samples of *Lupinus albus* were statistically analyzed using one way analysis of variance (ANOVA) and least significant difference (LSD). The statistical package used was SPSS version 15. Significant differences were determined at the $P< 0.05$ level. Results were expressed as mean $\underline{+}$ SD.

4. Results and Discussion

4.1. Information from Focus Group Discussion (FGD)

A questionnaire was (annex I) used to get information related to the crop. The groups selected for this purpose were farmers, women processing the crop for various products, local community and Amhara agricultural center. Accordingly, the summary of the information indicated that gibto (*Lupinus albus*) is a neglected crop, whose value is ignored as a potential food crop. This neglected crop has been used in the society for long time as snack food majorly. Moreover, agriculturally the farmers have cultivated the crop for crop rotation purpose. They were sowing the seed when they need to make their land fertile. The farmers are not using the crop as forage due to the induced bitterness in the seed. The main limitation of gibto (*Lupinus albus*) to be consumed by the society is the bitterness induced in the seed.

But some processing women used the seed as “shiro” after removing the bitterness from the seed. For that purpose they first roast it on earthen pan and soak it in running water for about of week time followed by processing in the normal way used to prepare “shiro”.

Currently, the predominant usage of the crop is for making “Araki” (local alcoholic drink) processing combined with maize (i.e. other starchy cereals). In the local beverage houses, the roasted then soaked gibto (*Lupinus albus*) is served along with the local alcoholic drinks (i.e. Araki and Tella). The purpose of the snack is to make the customers drink more due to its salty preparation.

The sowing season for the crop is in between May-July like most crops in Ethiopia. Once the crop has got sufficient water on the seedling stage, it can survive water deficient conditions. The full maturity of the seed will take five months. The farmers in Agew area commonly grow it by in inter-cropping with other crops like teff, wheat etc.

According to the information gathered from Amhara agricultural center, gibto (*Lupinus albus*) has not received attention as a crop. It has been difficult to trace the total annual production per hectare. However; it is becoming an export commodity to Egypt. The Egyptians import it for medicinal purposes and one Kg of gibto (*Lupinus albus*) costs 2.00 birr, which was 0.10 cents in earlier times (annex I).

4.2. Hull weight proportion

Table 4.1 Hull weight proportion of raw *Lupinus albus* from Dangla and Tilili sites

Type of sample	**Hull weight proportion (%)^{b}**
RG1♠	16.22 ± .67^{d}
RG2	19.30 ± .41^{c}

^{b} Mean value ± standard deviation, n=3, Means in the same column with different letters are significantly different, c, d -$P < 0.05$ ♠=type of sample, 1 and 2 represent the Dangla and Tilili samples respectively. RG……………..Raw gibto (*Lupinus albus*)

The hull weight proportion of the two raw *Lupinus albus* samples is given in Table 4.1. Accordingly, the Dangla and Tilili samples have 16.22% and 19.30% of hull weight from their whole seed proportion respectively. Sileshi, (1985) has reported that the hull weight proportion of locally grown raw *Lupinus albus* in the range of 20-30% of the total seed weight. The value in the current study is lower than this value.

Table 4.2 Proximate composition of raw and processed *Lupinus albus*

Proximate composition		**Moisture (%)^{b}**	**Crude Fat (%)^{a,b}**	**Crude Protein (%)^{a,b}**	**Crude Fiber (%)^{a,b}**	**Crude Ash (%)^{a,b}**	**Utilizable Carbohydrate* (%)^{a,b}**	**Gross energy (KCal /100gm)^{a,b}**
type of sample	RG1$^{\spadesuit}$	6.94 ± .03^{E}	9.34± .09^{E}	37.87 ± .00^{E}	11.08 ± .161^{E}	2.80 ± .14^{E}	38.91 ± .07^{E}	391.19 ± .56^{E}
	RSW1	6.33 ± .03^{F}	14.01 ± .19^{F}	44.15 ± .03^{F}	11.20 ± 0.205^{F}	1.50 ± .01^{K,F,M}	31.31 ± .02^{F}	427.92 ± 1.78^{H,F}
	RSK1	5.66 ± .13^{G}	16.28 ± .04^{G}	52.10± .03^{G}	2.34 ± .281^{G}	1.35 ± .01^{G}	27.94 ± .28^{G}	466.66 ± .86^{G}
	BSW1	6.38 ± .08^{F}	15.15 ± .14^{H}	47.25 ± .27^{H}	14.66 ± 0.103^{H}	1.60 ± .07^{F,M}	26.09 ± 1.03^{H}	429.75 ± 4.34^{F}
	BSK1	5.43 ± .03^{H}	16.51± .07^{G}	52.25 ± .35^{G}	7.11 ± .928^{I}	1.60 ± .002^{F,M}	22.52 ± 1.35^{I}	447.71 ± 3.34^{I}
	GW1	7.71 ± .14^{I}	7.55 ± .14^{I}	41.87 ± .93^{I}	14.23 ± .256^{J}	2.96 ± .01^{I,J}	33.38 ± 1.05^{J}	368.98 ± 1.73^{J}
	GK1	g5.64 ± .02^{H}	9.00 ± .09^{J}	47.41 ± .24^{H}	3.58 ± .078^{K}	3.11 ± .02^{H}	36.89 ± .24^{K}	418.22 ± .88^{N}
	RG2	8.04 ± .02^{J}	8.79 ± .05^{M,J}	39.71 ± .06^{J}	11.07 ± .06^{E}	2.88 ± .01^{I,E}	37.56 ± .06^{K}	388.12 ± .46^{E}
	RSW2	1.88 ± .09^{K}	11.47 ± .28^{K}	44.27 ± .18^{F}	14.10 ± .385^{I}	1.54 ± .04^{L,M}	28.62 ± .33^{G}	394.79± 3.12^{K}
	RSK2	2.26± .02^{L}	13.71 ± .46^{F}	56.24 ± .03^{K}	2.67 ± .041^{G}	1.47 ± .02^{L,K}	25.90± .49^{L,H}	451.98 ± 2.06^{L}
	BSW2	1.79 ± .08^{K}	11.13 ± .05^{L}	45.58± .15^{L}	14.95 ± .251^{M}	1.70 ± .05^{F}	26.63 ± .40^{H}	389.05 ± 1.49^{E}
	BSK2	2.17 ± .02^{L}	14.28 ± .00^{F}	56.13 ± .21^{K}	2.91 ± .022^{K},^{G}	1.69 ± .01^{F}	24.99 ± .23^{L}	453.01 ± .12^{L}
	GW2	5.44 ± .14^{M,H}	8.59± .19^{M}	41.23 ± .05^{M}	11.74 ± .194^{E}	2.89 ± .06^{I,E}	35.55 ± .12^{M}	384.41± 1.97^{M}
	GK2	6.70 ± .04^{N}	9.89 ± .14^{N}	48.99 ± .09^{N}	2.86 ± .376^{G}	3.01 ± .18^{J,H}	35.25 ± .43^{M}	425.94 ± .09^{H}

*Determined by difference [a] Data are reported on dry basis [b] Mean value ± standard deviation, n=3

Means in the same column with different letters are significantly different, E-N -$P < 0.05$

♠=type of sample, 1 and 2 represent the Dangla and Tilili samples respectively.

1. RG……………..Raw gibto (*Lupinus albus*)
2. RSW…...............Roasted then soaked whole seed
3. RSK……………Roasted then soaked kernel
4. BSW……………Boiled then soaked whole seed
5. BSK…………….Boiled then soaked kernel
6. GW……………..Germinated whole seed
7. GK……………..Germinated kernel

4.3. Proximate composition

The chemical and nutritional composition of the raw and processed samples of the two cultivars of *Lupinus albus* is shown in Table 4.2. Similar results of the proximate composition of the raw and processed samples of *Lupinus albus* grown locally were reported in EHNRI food composition table (EHNRI, 1997). In both cases the results are reported on dry basis.

All the traditional processing methods used in the current study (i.e. soaking after roasting, soaking after boiling and germination) facilitated the manual de-hulling step. The de-hulling is done in order to evaluate the chemical and nutritional composition of the whole seed and the kernel. The upcoming paragraphs will cover the discussion part of the proximate composition of the raw and the processed samples.

4.3.1. Protein

The protein contents of the raw seed from both sampling sites are comparable with soybean. Their protein compositions were 37.86% and 39.71% for Dangla and Tilili samples respectively.

There was a significant protein content difference between the two cultivars (*P*< 0.05). The variation in protein content could be due to the characteristics of the growing conditions and soil type. The protein content of the raw samples was slightly higher than that reported by Jimenez-Martinez, *et al*., (2003) which was 34.4% for the same species *Lupinus albus*. But these values were lower than that reported by Sujak, *et al*., (2005) (i.e. 23.4%). Similarly, El-Adawy, *et al*., (2000) has reported the protein content of the raw *Lupinus albus* to be 42.4%. In the same report the protein content of other legumes like soybean and bean flour were reported as 41.1% and 31.1% respectively. This shows that the protein content of the raw *Lupinus albus* reported in the current study is higher than that of bean flour but less than that of soybean. Similarly, Erbas, *et al*., (2004) has reported that Haricot bean, lentil and soybean contain 28.8%, 26.7% and 40.5% crude protein respectively.

When we compare the results of the current study with that of the previous local researches of the same area the following observations have been recorded. On food composition table for use in Ethiopia (EHNRI, 1997), the protein content of raw *Lupinus albus* was found to be 32.80%. Similarly, Sileshi, (1985) has reported the content to be 38.27%.

When we see the effects of traditional processing methods, in general there was a significant effect on the proximate composition on both sample types (*P*< 0.05). In fact all the treatments have shown an increment in the protein content of the raw seed. Based on this, RSW1, RSK1, BSW1, BSK1, GW1 and GK1 have shown a percentage increment of 14.24%, 27.32%, 19.87%, 27.53%, 9.56% and 20.12% from RG1 respectively. The highest protein content was obtained when the raw *Lupinus albus* was boiled then soaked for five days in water and eaten without the hull. Mean while, the lowest increment was on germinating the seed and served with the hull. Under the same investigation Sileshi, (1985) has reported the increment of protein content, when the seed was roasted and water soaked, roasted and de-corticated and germinated. The observed increments were 12.80%, 37.62%, and 8.4% respectively.

When we see the multiple comparison one way ANOVA of the results of the Dangla sample, all the treatments have a significant effect on the protein content of the raw sample ($P < 0.05$). Comparing the protein content of the whole seed and kernel, RSW1, BSW1 and GW1 have a significant difference in their protein content from their kernels.

Similarly, considering the Tilili sample all the traditional processes have shown an increment trend like the Dangla sample. Accordingly, RSW2, RSK2, BSW2, BSK2, GW2 and GK2 have shown a percentage increment of 10.35%, 29.40%, 12.89%, 29.25%, 3.69% and 18.96% respectively. The highest protein content was observed on the kernel of the boiled then soaked sample like in the Dangla case. Moreover, all the kernels have higher amount of protein from their whole seed.

The protein content increment when the seed was de-hulled indicates that the hull has small amount of protein and was substantially diluted the protein content of the grain. As shown in Table 4.1 above the hull weight is 16-20%, which is significant amount. Sileshi, (1985) has also given the same explanation for the observed protein content increment after similar treatments. The total protein content has increased slightly during germination. This slight increment could rise from a loss of weight through respiration. Thus, the germinated material on a unit basis would contain more seeds and therefore more nitrogen than the un-germinated one (Sileshi, 1985).

Finally when we compare the results of the same treatments on the two samples, protein content is significantly different for treatments RSK1 and RSK2, BSW1 and BSW2, GW1 and GW2 ($P < 0.05$). But the protein percentage between RSW1 and RSW2 is not significantly different ($P > 0.05$). 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 their raw seeds.

Table 4.3 Correlation between total alkaloid and protein percentages

		Protein (%)	Total alkaloid (%)
Protein (%)	Pearson Correlation	1	-.751(**)
	Sig. (2-tailed)		.000
Total alkaloid (%)	Pearson Correlation	-.751(**)	1
	Sig. (2-tailed)	.000	

** Correlation is significant at the 0.05 level (2-tailed).

In general the increments in protein content could be due to the simultaneous removal of nitrogenous compounds such as alkaloids and perhaps non-protein nitrogen (Table 4.3). As we can see from the table, the highest alkaloid removal was on treatments roasting then soaking on both samples. When we look at Table 4.2, the highest protein contents are observed in the same treatments. This correlation was expressed in terms of removal of non-nitrogenous substances (i.e. alkaloids). Statistically a correlation coefficient of -0.751 was calculated for the correlation between total alkaloid and protein content of *Lupinus albus*.

As cited in (Sileshi, 1985), Rabma and Narasinga, (1984) have reported that de-bittering process has increased the protein content of the whole seed. This was due to the loss of water soluble nutrients and hence decreased the percentages of the cell soluble materials. In another study the reason given for the increment of protein content on soaking was the synthesis of enzyme proteins or compositional change following degradation of other constitutes (Bau, *et al*., 1996).

4.3.2. Crude Fat

The fat contents of the raw *Lupinus albus* seed from Dangla and Tilili sites were 9.34 % and 8.79% respectively as shown in the Table 4.2. The fat contents between the two raw samples were significantly different from each other ($P< 0.05$). However, these values were lower than the values reported by Sujak, *et al*., (2005) (i.e. 11.5%), and reported by Jimenez-Martinez, *et al*., (2003) (i.e.10.9%). These observed differences were may be due to the soil type, climatic and harvesting conditions, due to differences in cultivars, etc.

When we compare these values with the fat contents of other legumes, El-Adawy, *et al*., (2000), has reported the oil content of soy flour to be 19.5% and raw bean flour 2%. The oil content of raw *Lupinus albus* from both sampling sites was higher than bean flour, but less than that of soy flour.

On Ethiopian food composition table (EHNRI, 1997) it was reported that the fat content of the raw *Lupinus albus* grown locally to be 8.3%. Similarly, Sileshi, (1985) reported the content to be 9.00%. These values were in agreement with the results of the current study.

All the treatments have significant effect on the fat content of the raw seed. The observed effects were both increasing and decreasing. Treatments like RSW1, RSK1, BSW1 and BSK1 have an increment effect by 33.32%, 42.63%, 38.36% and 43.44% respectively. But treatments GW1 and GK1 have decreased the fat content by 19.13% and 3.61% respectively. Similarly, for the Tilili sample treatments RSW2, RSK2, BSW2 and BSK2 have an increment effect by 23.37%, 35.92%, 21.08% and 38.47% respectively. The process that has a decreasing effect was germination by 2.22%. The maximum oil content was obtained on boiling followed by soaking the raw seed for both sample types. These increments could be due to the inactivation of lipolytic activity enzyme. So there was no breaking down of triacylglycerols, leading to the increment of total fat content.

Similarly, Sileshi, (1985) has investigated the same trend. On her study the fat percentage has increased by 0.55% and 25.56% on roasting and water soaking and roasted and de-corticating and water soaking *Lupinus albus* respectively. But, there was a decrease in the fat content on germinating the seed by 4%. In another investigation the fat content of the raw seed had increased by 37.62% and 40.45% in aquas and alkaline treatments (Jimenez-Martinez, *et al.*, 2003) respectively. In the same reference *Lupinus albus* was recommended as an oil crop, when the seed was left soaked for several hours due to its high oil content.

Similarly, in other legume types this fact holds true. For example, there was a significant decrease in the fat content due to various processing methods (soaking, roasting, germination, etc) of lablab bean seed. Cooked and presoaked beans were more effective than other methods. This can be attributed to high lipolytic activity which, break down the triglyceride to simple fatty acids sterol and polar lipids (Osman, 2007).

On germination, fat content decreased from the raw seed. This could be due to the growth of the seed. As the seed grows it needs protein. For the synthesis of protein the primary energy sources are lipids and carbohydrates. So, as the seed germinates the lipids will be utilized as energy source, making the total content less. But, total protein content increased slightly as mentioned earlier. The same discussion was given by Bau, *et al*., (1996). Accordingly, total lipid content of soybean has shown a decrement as germination proceeds. This might be due to the loss of one half of the triacylglycerols as a major carbon sources for seed growth (i.e. protein requirement). Moreover, in the same investigation it has been reported that after 24 hrs of germination, lipase inhibitor activity will be decreased. This means the lipase activity of the seeds will be improved by the germination process. This could explain a loss of 19% lipid by germination in that study and 2.22% in the current study.

Comparing the oil content of the same treatments of the two samples, RSW1 and RSW2, RSK1 and RSK2, BSW1 and BSW1, BSK1 and BSK2, GW1 and GW2, and GK1 and GK2, there was a statistically significant difference in their fat content ($P< 0.05$).

In another study, the oil content of *Lupinus albus* when soaked in 0.5% $NaHCO_3$ for 8 hrs show an increment of fat content by 18.38% (El-Adawy, *et al*., 2000). This is due to the explanation given earlier.

De-hulling of the seed has a significant effect on the oil content of the whole seed. This could be due to the hull's low oil composition.

4.3.3. Crude Fiber

The other important nutritional component of *Lupinus albus* seed is crude fiber. The Dangla and Tilili samples have crude fiber contents of 11.08% and 11.07% respectively. There was no significant difference between the two samples considering their fiber content ($P > 0.05$). Similarly, El-Adawy, *et al*., (2000) has reported the fiber content of raw *Lupinus albus* seed flour to be 4.5%, which was less than from RG1 and RG2. RG1 and RG2 have higher fiber contents from raw bean and soybean flour, whose values are reported to be 3.9% and 3.6% respectively. In another study, the reported crude fiber content of raw *Lupinus albus* was 11.7% (Jimenez-Martinez, *et al*., 2003). This value was highly comparable with the results of the current study. Similarly, as reported by Erbas, *et al*., (2006) *Lupinus albus* seeds grown in Turkey have a high amount of crude fiber (16.2%). Martinez-Villaluenga, *et al*., (2005) has reported the fiber content of *Lupinus albus* to be varied considerably between the different species and even cultivars, which could explain the results obtained in the present work in cultivars of Dangla and Tilili of the same species *Lupinus albus*.

On Ethiopian food composition table (EHNRI, 1997) the reported fiber content of the raw *Lupinus albus* seed grown locally was 12.9%. In this report the fiber content is highly comparable with the report in the current study.

All the treatments applied in the current study have significant effect on the crude fiber content of the raw sample of Dangla ($P < 0.05$). And the observed effects are both increasing and decreasing ones. Treatments RSK, BSK and GK on the raw sample of Dangla had reduced the fiber content by 78.92%, 35.82% and 67.66% respectively. But treatment GW had increased the fiber content by 22.13%. And treatments RSW and BSW also have increased the content by 1.06% and 24.42% respectively. Similarly, for the Tilili sample the treatments have shown both an increasing and decreasing effects on the fiber content from the raw seed. RSK, BSK, GK, had reduced the fiber content by 75.87%, 73.70% and 74.18% respectively. But RSW, BSW and GW had increased the crude fiber by 21.45%, 25.96% and 5.72% respectively.

In another study, it has been shown that there was an increment of crude fiber content after the raw *Lupinus albus* seed was soaked in 0.5% $NaHCO_3$ solution for 8 hrs (El-Adawy, *et al*., 2000). The increment was by 26.23%, which was similar to the case of GW1 and GW2. The increment in fiber content on germination might be due to the growth of additional plant parts (i.e. shoot, root, etc). This means on soaking the different seed components modify their proportions (Jimenez-Martinez, *et al*., 2003).

But, Bau, *et al*., (1996) has reported the degradation of soybean dietary fiber during germination. The explanation given was the activation of α-L-arabino-furanosidase enzyme which can degrade both high and low molecular weight polysaccharides. During germination the contents of the water soluble fiber in the cotyledons decreases greatly as the seedlings develop, suggesting the use of polysaccharides as energy source or carbon skeleton for macromolecule biosynthesis. Martinez-Villaluenga, *et al.*, (2005) has also reported the reduction of soluble fiber after removing α-galactosides, while there is little or no change on the insoluble one.

The reduction of crude fiber on the RSK, BSK and GK treatments for both sample types is might be due to the removal of some water soluble oligosaccharides such as RFOs in which along with indigestible carbohydrates (i.e. crude fiber) is lost. Moreover, in each treatment there is heat involvement and cell wall damage.

For the Dangla sample there was a significant difference in their fiber content between the whole seed and kernel (P< 0.05). All of the de- hulled seeds have a lower amount of crude fiber than the whole seed (i.e. RSW1 and RSK1, BSW1 and BSK1, GW1 and GK1). The same observation was also obtained for the Tilili sample. The reduction of the crude fiber on de-hulled seed was because of the high fiber content in the hull.

4.3.4. Crude Ash

The crude ash content of *Lupinus albus* from Dangla and Tilili sites were 2.80% and 2. 88% respectively. These observed results were less than that reported by Sujak, *et al.*, (2005) (3.9%), El-Adawy., *et al.*, (2000) (4%) and Jimenez-Martinez, *et al.*, (2003) (3.2%). However; it is higher than the result reported by Erbas, *et al.*, (2006) (2.65%). Sileshi, (1985) has found the ash content of the raw *Lupinus albus* grown locally to be 3.25%. On food composition table for use in Ethiopia (EHNRI, 1997) ash content of raw *Lupinus albus* was reported to be 2.5%. The value in the current study was higher than that reported by Sileshi (1985) but less than the report in Ethiopian food composition table.

The multiple comparison tests show that there was a significant difference between the raw and the processed samples of Dangla in their ash content ($P < 0.05$). For the Dangla sample the ash content decreased from the raw sample by 46.3%, 51.8%, 42.78% and 42.77% on treatments RSW1, RSK1, BSW1 and BSK1 respectively. The reason for this reduction might be due to the internal process of leaching occurred during soaking. Similarly, for the Tilili sample, treatments in RSW2, RSK2, BSW2 and BSK2, showed a significant reduction in crude ash content by 46.51%, 48.82%, 40.94% and 41.44% respectively.

Reduction in the ash content from the raw seed was also investigated by Sileshi, (1985) 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.

4.3.5. Utilizable Carbohydrate

Utilizable carbohydrate content was determined by difference. This means there was no analysis conducted for utilizable carbohydrate determination. Instead it was determined using the formulae stated in the materials and methods part (i.e. utilizable carbohydrate = 100- (crude fat + crude fiber + crude protein + crude ash). The raw samples from Dangla and Tilili have utilizable carbohydrate contents of 38.92% and 37.5% respectively. These values were comparable with the carbohydrate content reported by El-Adawy, *et al*., (2000) (i.e. 38%). The same source has given the values for raw soybean to be 30.9%, which was less than from the contents reported in both samples. The utilizable carbohydrate for raw bean flour was reported to be 59.2% which was higher than from both sample types in the current study. A study by Jimenez-Martinez, *et al*. (2003) has reported the value for utilizable carbohydrate of raw *Lupinus albus* to be 26.8%, which was lower than what was reported for the raw samples in the current study. The multiple comparison test has showed that there was a significant difference between the RG1 and RG2 in their carbohydrate contents ($P < 0.05$).

All the treatments applied on the Dangla sample have a significant effect on the Utilizable carbohydrates. But the effects of all the treatments were reduction in the carbohydrate content. Treatments RSW1, RSK1, BSW1, BSK1, GW1 and GK1 have reduced the utilizable carbohydrate by 19.54%, 28.20%, 32.96%, 42.12%, 14.22% and 5.20 % respectively. Similar treatments on the Tilili sample reduced the utilizable carbohydrate content significantly (P< 0.05). Total carbohydrate content in the samples RSW2, RSK2, BSW2, BSK2, GW2, and GK2 were reduced significantly by 23.78%, 31.05%, 29.09%, 33.44%, 5.35% and 6.14% respectively.

Similarly, Jimenez-Martinez, *et al*., (2003) 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 100 °C 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 $NaHCO_3$ solution for 45 minutes. When we compare the carbohydrate content of the processed samples (i.e. soaked in 0.5% $NaHCO_3$, for 8 hrs), the trend was a decreasing from 38% to 32.6%. This result was similar to the findings of the current study.

The reason behind these observed reductions could be the leaching out of the various components of carbohydrates (i.e. starch and oligosaccharides) (El-Adawy, *et al*., 2000). This fact holds true for other species of the genus Lupinus also. For example, a study on *Lupinus campestris*, has investigated the same trend. During aquas and alkaline treatment in both cases carbohydrate content decreases from 24.7% to 15.6% and 12% respectively.

Bau, *et al*., (1996) has explained that on soaking there will be activation of enzymes which act on polysaccharides. These enzymes can degrade both high and low molecular weight soluble polysaccharides from soybean seed. Like in the case of soybean seed, in *Lupinus albus* there could be activation of these enzymes. So there will be degradation of polysaccharide components, which later on leads to reduction of utilizable carbohydrates.

De-hulling had a significant effect on the carbohydrate content of the whole seed ($P < 0.05$) on Dangla and Tilili samples. The significant effect of de-hulling on treatments RSW and BSW on both sample types has a decreasing trend. This indicates that the hull has a considerable amount of water soluble carbohydrate. The hull was made of cellulose and hemi-cellulose materials (Fig 2.7) (www.lupin.org, 2009). Similarly, on some common types of legumes reduction of utilizable carbohydrates was observed on germination. For example, germination significantly reduced carbohydrate content of lablab bean seed. This, reduction, can be attributed to the use of carbohydrates as source of energy for young seedlings (Osman, 2007).

Jimenez-Martinez, *et al*., (2003) has reported the elimination of 70% and 90% of the original carbohydrate content by aquas and alkaline treatments of *Lupinus albus* seed. This was explained due to the internal process of leaching of less molecular weight oligosaccharides (i.e. water soluble) with the soaking solution.

4.3.6. Gross energy

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

Total energy (KCal) = (9*Fat) + (4*carbohydrate) + (4* protein)

The two raw cultivars of *Lupinus albus* from Dangla and Tilili sites have total energy content of 391.19 KCal/100gm and 388.12 KCal/100gm respectively. All the treatments had significant effect on the total energy contents of the raw samples. But the effects were increasing and decreasing one. Treatments RSW1, RSK1, BSW1, BSK1 and GK1 have increased the total energy content by 8.58%, 16.17%, 8.97%, 12.63% and 6.46% respectively. This increment was in parallel to improvements in protein and fat contents. But GW1 had showed a decrease due to the decrease in fat content of the processed sample.

De-hulling has a significant effect on the total energy content of the whole seed of the Dangla sample. All the treatments on the two cultivars significantly differ considering their total energy content. RSW1 and RSW2, RSK1 and RSK2, BSW1 and BSW2, GW1 and GW2 and GK1 and

GK2 were significantly different in their total energy contents. This might be due to the differences in the other components of the raw samples (i.e. protein, fat, carbohydrate) (Fig 4.1).

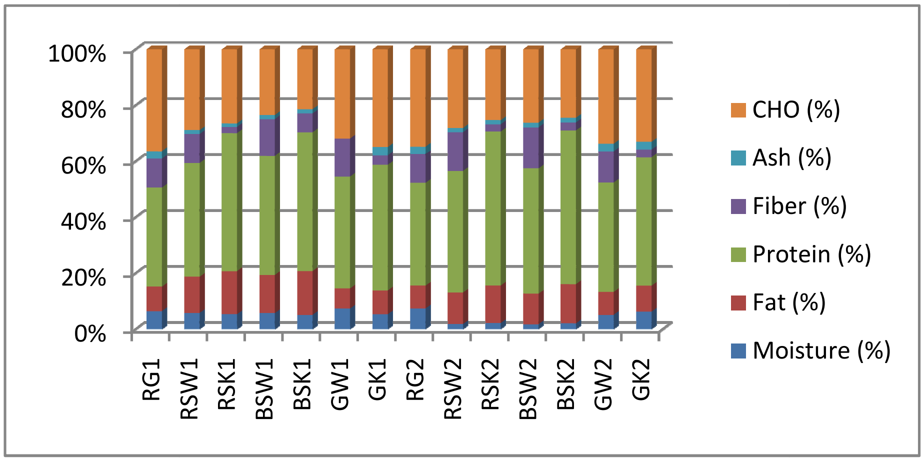


Fig 4.1 Proximate composition of raw and processd *Lupinus albus*

4.4. Anti-nutritional factors

Table 4.4 Concentrations of total alkaloids and phytate in *Lupinus albus* processed in different traditional methods

Type of sample	Total alkaloid (%)[a,b]	Phytate (mg/100gm)[a,b]
RG1♠	2.46 ± .03^{E}	144.33 ± 1.77^{E}
RSW1	.84 ± .01^{F}	133.14 ± .98^{F}
RSK1	.71 ± .00^{G}	129.52 ± 2.14^{G}
BSW1	1.19 ± .02^{H}	117.91 ± 1.73^{H}
BSK1	1.01 ± .04^{I}	112.81 ± 1.14^{I}
GW1	1.65 ± .03^{J}	107.92 ± .24^{J}
GK1	1.48 ± .04^{K}	82.87 ± .54^{K}
RG2	2.26 ± .01^{L}	143.96 ± .40^{L}
RSW2	.79 ± .04^{F}	130.01 ± 2.83^{I}
RSK2	.67 ± .01^{G}	125.94 ± 1.82^{M}
BSW2	1.07 ± .04^{M}	111.66 ± 1.43^{I}
BSK2	.89 ± .03^{N}	105.16 ± .89^{N}
GW2	1.54 ± .04^{O}	92.68 ± .74^{O}
GK2	1.39 ± .01^{P}	78.18 ± .88^{P}

[a] Data are reported on dry basis

[b] Mean value ± standard deviation, n=3

Means in the same column with different letters are significantly different, E-P $P < 0.05$

♠=type of sample, 1 and 2 represent the Dangla and Tilili samples respectively.

1. RG……………..Raw gibto (*Lupinus albus*)
2. RSW…...............Roasted then soaked whole seed
3. RSK……………Roasted then soaked kernel

4. BSW……………Boiled then soaked whole seed
5. BSK…………….Boiled then soaked kernel
6. GW……………..Germinated whole seed
7. GK……………..Germinated kernel

4.4.1. Total alkaloids

The results obtained for the total alkaloids in raw *Lupinus albus* seed from Dangla and Tilili sites were presented in Table 4.4. The total alkaloid content of *Lupinus albus* in this study was similar to the findings of other investigations. The concentration of the raw samples was 2.46% and 2.29% for the Dangla and Tilili samples respectively. There was a significant difference in total alkaloid content of the two raw samples in the current study. These values are comparable with the concentrations of other varieties of the same species (Jimenez-Martinez, *et al*., 2003).

All the treatments on the raw *Lupinus albus* seed from Dangla site have a significant effect on the total alkaloid content. RSW1, RSK1, BSK1, BSW1, BSK1, GW1 and GK1 have reduced the total alkaloid content in the seed by 65.90%, 71.16%, 51.75%, 59.07%, 32.70% and 39.57% respectively. The highest reduction was observed by soaking after roasting the seed and serving it without the hull. Meanwhile, the lowest was through germination process (GW1 and GK1). The same trend has been observed for the Tilili sample. All of the treatments have significant effects on the reduction of the total alkaloid. RSW2, RSK2, BSK2, BSW2, GW2 and GK2 have reduced the total alkaloid content in the raw seed by 68.15%, 70.28%, 60.23%, 52.83%, 31.61% and 38.54% respectively. The highest reduction was observed on RSK treatment.

This gradual decrease in the content of total alkaloids is observed when the seed was submitted to de-bittering process. 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. Hence in order to facilitate the removal of alkaloids the cell wall should be damaged. Accordingly; the order of removal of the alkaloids follow roasting, boiling, and germination (Jimenez-Martinez, *et al*., 2003).

Without soaking the seed for several times, heat treatment alone won't remove the alkaloids effectively. Similarly, Arslan and Esker, (2002) has reported that TTM (traditional Turkish method) is more effective than extrusion technique for the elimination of lupin alkaloids. Extrusion technique resulted in a slight decline in the level of alkaloid components. In this investigation heat and pressure were used for extrusion. If steam had been used for extrusion, it might have resulted in more effective elimination of lupin alkaloids.

The de-hulling process has a significant reduction effect on the total alkaloid content of the raw seed ($P< 0.05$). This might be due to the presence of the alkaloids in the hull also. But in the case of germination, since there was no involvement of heat, the permeability of the cell wall was not as effective as the former case to remove the alkaloids.

According to Sanchez, *et al*., (2004) the total alkaloid content in raw *Lupinus albus* seed was 2.36%. In the same report it was stated that the presence of anti-nutrients such as α-galactosides or phytic acid can be reduced through germination process. But germination reduced the alkaloid content slightly. The reason to this reduction was attributed to the mobilization of alkaloidal nitrogen. The observed total alkaloid reduction was by 22.11% from the raw seed. In the current study the observed reduction by germination was 33.04%.

The less reduction of alkaloids by germination , but higher reductions by RSW, RSK, BSW and BSKis may be due to the improvement of the permeability of the cell wall of the seed. The enhencement of cell wall permeability as explained by Jimenez-Martineze, *et al.*, (2003), has helped the ellimination of alkaloids.

As cited in (Seleshi, 1985) Getachew and Mekibib, (1970) has reported that the roasting and soaking for seven days has reduced the alkaloid content. In a similar reference Gabrial and Marcos, (1977) the alkaloid contents of four *Lupinus albus* seed samples under different treatments (i.e. raw seeds, raw and de-corticated seeds, seed boiled for 1 hr then de-coriticated, seed boiled for half hour then steeped in running water for three days) have been reported, and the alkaloid contents given were1.32, 1.40, 1.16 and 0% respectively.

4.4.2. Phytic acid

RG1 and RG2 have phytic acid contents of 144.33% and 143.96% respectively. There was no significant difference in the phytic acid content between the two cultivars ($P > 0.05$). All the treatments applied on the raw seed affected the phytic acid content significantly. The reduction of phytic acid by treatments RSW1, RSK1, BSW1, BSK1, GW1 and GK1 were by 7.75%, 10.26%, 18.13%, 21.84%, 33.73% and 42.58% respectively. The highest reduction was observed through germination treatments (i.e. GW1 and GK1). The reason behind this reduction might be due to the stable nature of phytic acid on heat treatments. So heat treatment might not be an effective method to reduce its content. Instead, hydration process will facilitate its removal from the raw seed. This is because on hydration there is activation of phytase enzyme. This enzyme can help the breakdown of the phytate molecule. As cited in (Bau, *et al.*, 1996), Reddy, (1982) has reported the reduction of phytic acid during germination of different legume seeds as a result of large increase of phytase activity. 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.

Similarly, for the Tilili sample the observed reductions were 9.69%, 11.25%, 22.44%, 26.95%, 35.62% and 45.69% for the treatments RSW2, RSK2, BSW2, BSK2, GW2 and GK2. In this case a reduction was effective on the germination process.

Similarly, in another investigation soaking process has reduced the anti-nutritional factors in soybean, bean and lupine flours (El-Adawy T.A., *et al.*, 2000). Among them reduction follows the order; hemagglutinin activity, tannins, trypsin inhibitor activity and phytic acid. Accordingly, the decrease of these anti-nutritional factors from legume seeds may be due to internal process of leaching. 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 (El-Adawy, *et al.*, 2000).

Phytic acid contents of *Lupinus albus* seeds have been discussed by Trugo, *et al.*, (1992). Accordingly, the phytic acid content of *Lupinus albus* seed of various cultivars was in the range of 0.4 to 1.2 gm/100gm dry matter. The values found for the raw samples of our study were less than the stated range.

4.5. Mineral Composition

The mineral levels of the two cultivars of Dangla and Tilili are presented on Table 4.5. The analyzed minerals were Fe, Zn, Mn and Mg.

4.5.1. Iron

The iron content of the two cultivars of *Lupinus albus* were 6.01 and 6.72% for Dangla and Tilili samples respectively. There is a significant difference between the iron contents of the two cultivars. Trugo, *et al*., (1992) has reported the iron content of the raw *Lupinus albus* seeds of various cultivars in the range of 3.5-7.7 mg/100 gm. The result of the current study is within the given range. Similarly, EHNRI, (1997) on food composition table use for Ethiopia the iron content of raw *Lupinus albus* was 8.6 mg/100 gm.

In both sample types all the treatments have significant effects on the iron content. Iron content was reduced from the raw sample in all the treatments. In EHNRI, (1997) food composition table is shown reduction of iron content on boiling and roasting treatments. In another study, it was reported that except Na, all the minerals analyzed including Fe were reduced on soaking of raw *Lupinus albus* in $NaHCO_3$ solution (El-Adawy, *et al*., 2000). The reduction of minerals on soaking process could arise because all the water soluble minerals are often lost with the steeping medium and rinsing process. Similar explanation was given by Bau, *et al*, (2000).

Table 4.5 Iron, Zinc, Manganese, and Magnesium contents of raw and processed *Lupinus albus*

Type of sample	Iron (mg/100gm)^{a,b}	Zinc (mg/100gm)^{a,b}	Manganese (mg/100gm)^{a,b}	Magnesium (mg/100gm)^{a,b}
RG1♠	$6.01 \pm .19^{E}$	$2.11 \pm .04^{N,F}$	58.43 ± 1.22^{E}	$8.93 \pm .32^{E}$
RSW1	$5.04 \pm .04^{F}$	$16.92 \pm .13^{F}$	$49.07 \pm .51^{F}$	$8.57 \pm .07^{F}$
RSK1	$4.23 \pm .11^{I,G}$	$17.63 \pm .09^{G}$	$45.51 \pm .11^{G}$	$8.51 \pm .08^{F}$
BSW1	$4.95 \pm .17^{H,F}$	$22.57 \pm .09^{H}$	$55.41 \pm .24^{H}$	$8.04 \pm .16^{G}$
BSK1	$5.17 \pm .19^{F}$	$23.10 \pm .01^{I}$	52.70 ± 1.66^{I}	$7.95 \pm .17^{G}$
GW1	$4.39 \pm .02^{I}$	$2.41 \pm .16^{N,J}$	59.29 ± 1.95^{E}	$8.82 \pm .12^{E}$
GK1	$3.64 \pm .27^{J}$	$2.93 \pm .15^{K}$	$62.54 \pm .59^{L}$	$8.53 \pm .08^{F}$
RG2	$6.73 \pm .02^{K}$	$1.81 \pm .00^{L}$	$59.14 \pm .62^{J,E}$	$9.46 \pm .14^{H}$
RSW2	$5.64 \pm .22^{L}$	$15.59 \pm .56^{M}$	44.62 ± 1.29^{G}	$9.08 \pm .08^{E}$
RSK2	$4.74 \pm .21^{H}$	$2.14 \pm .13^{N,E}$	39.01 ± 1.41^{K}	$8.41 \pm .05^{F}$
BSW2	$2.74 \pm .13^{M}$	$2.59 \pm .02^{O,J}$	$46.36 \pm .26^{G}$	$8.52 \pm .03^{F}$
BSK2	$3.58 \pm .18^{J}$	$2.88 \pm .05^{O,K}$	$46.81 \pm 1.34^{G,F}$	$8.43 \pm .02^{F}$
GW2	$4.92 \pm .28^{H,F}$	$3.13 \pm .12^{K}$	$60.86 \pm .09^{L,E,J}$	$9.34 \pm .00^{H}$
GK2	$4.07 \pm .15^{G}$	$3.08 \pm .09^{K}$	62.12 ± 4.13^{L}	$9.04 \pm .18^{E}$

[a] Data are reported on dry basis [b] Mean value ± standard deviation, n=3

Means in the same column with different letters are significantly different, E-O $P < 0.05$

♠=type of sample, 1 and 2 represent the Dangla and Tilili samples respectively.

1. RG……………..Raw gibto (*Lupinus albus*)
2. RSW…...............Roasted then soaked whole seed
3. RSK……………Roasted then soaked kernel
4. BSW……………Boiled then soaked whole seed
5. BSK…………….Boiled then soaked kernel
6. GW……………..Germinated whole seed
7. GK……………..Germinated kernel

4.5.2. Zinc

Zinc was the other mineral investigated in this study. The Dangla and Tilili cultivars have zinc contents of 2.11 and 1.81mg/100 gm respectively. There was a significant difference between the two samples in their zinc content. Similarly, El-Adawy, *et al*., (2000) reported that raw *Lupinus albus* flour has zinc content of 8.5mg/100 gm. But Trugo, *et al*., (1992) has reported the content to be within the range of 4.5-4.7mg/100 gm for different cultivars of the same species. The findings of these studies were not in agreement with the results of the current study.

All the treatments applied in the current study had shown an increment trend on the zinc content of the raw *Lupinus albus* seed. This is in contrary with the findings of El-Adawy, *et al*., (2000), who reported that the zinc contents of the processed raw *Lupinus albus* seed (i.e. soaked in 0.5% $NaHCO_3$ solution) was observed to decrease.

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 $NaHCO_3$, it was reported that except Na all the analyzed minerals have shown a decrement. This was because of the presence of Na in the soaking solution. In all the treatments of the current study the soaking solution was tap water. The possible explanation for the increment in zinc content on both types of samples might be contamination from tap water. Similarly, sometimes there are unexpected results regarding mineral contents of some samples. This could be attributed to contamination of sample from materials on ashing as wel as the lining of the furnace. Dissolved metal ions present in the distilled water used for analysis of sample preparation can also influence the results obtained (Njiku and Ohia, 2007).

4.5.3. Manganese

The Manganese contents of RG1 and RG2 were recorded as 58.43 and 59.14 mg/100 gm respectively. The range for Mn content of the raw *Lupinus albus* of different cultivars of the same species was reported within 61-327 mg/100 gm (Trugo, *et al.*, 1992). The value in the current study was lower than this range. Similarly, El-Adawy, et al., (2000) has reported the content to be 10.8 mg/100 gm for the raw seed. This value is much less than from both of the previous mentioned reports. The upper limit of safe intake of Mn for human consumption is 5 mg/day (Trugo, *et al.*, 1992). Based on this fact the Mn content found in the current study was higher than the safety limit. So care should be taken while consuming *Lupinus albus* concerning its Mn toxicity.

In both sample types all the treatments had decreased the Mn content. For RG1, treatments RSW1, RSK1, BSW1 and BSK1 has reduced the Mn content by 16.01%, 22.10%, 5.16% and 9.79% respectively. Similarly, for the Tilili sample RSW2, RSK2, BSW2 and BSK2 have reduced the Mn content in the raw seed by 24.55%, 34.03%, 21.62% and 20.84% respectively. A reduction of Mn content after soaking in 0.5% $NaHCO_3$ solution by 54.63% was reported by El-Adawy, *et al*., (2000). These observed reductions might be due to the internal process of leaching with the soaking solution.

4.5.4. Magnesium

The Magnesium contents of the Dangla and Tilili cultivars were 8.93 and 9.46 mg/100 gm respectively. The content of Mg was reported as 73mg/100 gm for raw Lupinus albus seed, and soybean and bean flour to be 172 and 88 mg/100 gm respectively by El-Adawy, *et al*., (2000).

All the treatments on both types of samples have reduced the total Mg content from the raw seed. Treatments RSW1, RSK1, BSW1, BSK1, GW1 and GK1 have reduced the Mg content by 4.05%, 4.74%, 9.97%, 10.91% and 1.21% respectively. Similar trend was observed in treatments of the Tilili sample.

For the analyzed minerals (i.e. Fe, Zn, Mn and Mg) the possible explanation for their reduction on the various treatments might be due to the leaching out process of water soluble minerals on soaking except for zinc (Bau, *et al*., 1996). In fact the crude ash has shown a reduction in most of the treatments. This means most of the inorganic materials (i.e. stones, dust, minerals, etc) have been washed out with the soaking solution.

4.6. Fatty acid profile

Table 4.6 Fatty acid profile of raw and processed *Lupinus albus* seed

Type of sample	Myristic(%)[a]	Palmitic (%)[a]	linoleic (%)[a]	Oleic (%)[a]	Stearic (%)[a]	Eikosanic (%)[a]
RG1♠	.50	6.90	16.20	59.30	2.00	1.20
RSW1	1.90	7.10	15.80	60.41	1.90	5.00
RSK1	.40	7.20	15.90	61.40	2.00	1.20
BSW1	.00	6.30	14.90	60.00	2.00	1.40
BSK1	.00	7.00	16.00	61.40	2.00	1.20
GW1	.00	5.70	22.22	55.70	1.50	1.40
GK1	1.10	5.40	19.60	54.20	1.40	1.10
RG2	.00	6.30	14.60	59.80	2.00	1.40
RSW2	1.84	6.92	15.64	59.67	1.75	4.65
RSK2	.38	6.67	14.76	60.09	1.87	0.98
BSW2	.40	6.60	14.90	61.00	2.00	1.30
BSK2	.00	5.40	12.00	41.50	8.50	.90
GW2	3.00	5.70	19.30	55.10	2.10	1.40
GK2	.00	5.80	20.70	56.70	1.60	1.30

[a] Data are reported on dry basis NB: all the reported results are on singlet analysis, see annexII for the chromatogram of the GC result ♠=type of sample, 1 and 2 represent the Dangla and Tilili samples respectively.

1. RG……………..Raw gibto (*Lupinus albus*)
2. RSW…...............Roasted then soaked whole seed
3. RSK……………Roasted then soaked kernel
4. BSW……………Boiled then soaked whole seed
5. BSK…………….Boiled then soaked kernel
6. GW……………..Germinated whole seed
7. GK……………..Germinated kernel

Table 4.6 shows the fatty acid profile of raw and processed *Lupinus albus* seeds from the two sampling sites. The oil extracted from the raw Dangla sample is composed of 24.55% saturated fatty acids and 75.5% of un-saturated fatty acids. Similarly, the oil extracted from the Tilili sample has 74.4% unsaturated and 25.6% saturated fatty acids. In both cases the amount of un-saturated fatty acids is higher than that of the saturated one.

Oleic acid (59.3%) was the predominant fatty acid in the raw *lupinus albus* seed for both sample types (Fig4.2). Also among the essential fatty acids the oil contained was linoleic acid (16.2%). According to the report by Erbas, *et al*., (2004) the fatty acid composition of *Lupinus albus* oil resembles that of peanut and rapeseed oil, but doesn't involve any erusic acid. Peanut oil contains 6-9% Palmitic acid, 53-71% oleic acid and 13-27% linoleic acid. Rapeseed oil content is 48-55% of oleic acid, 27-31% of linoleic acid and 10-14% of linolenic acid.

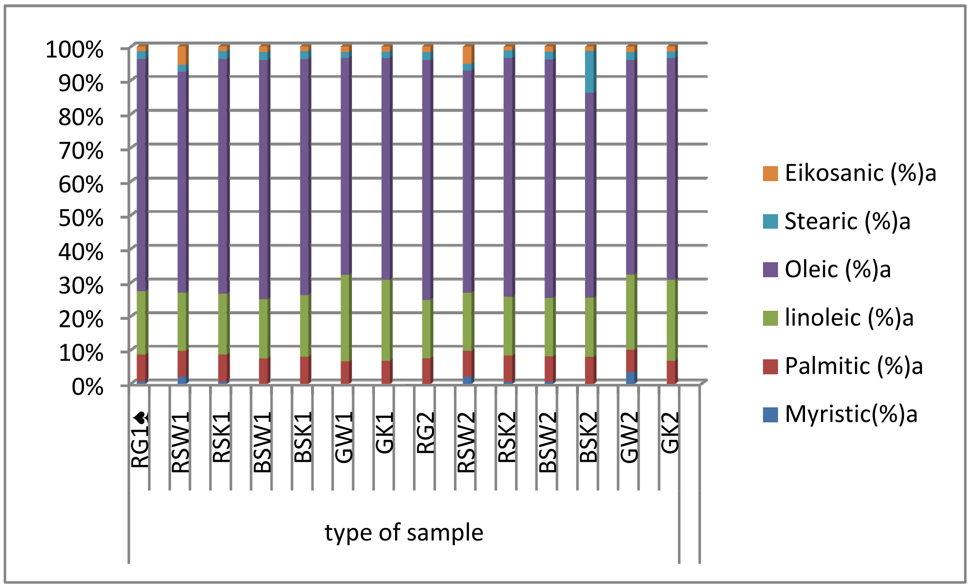


Fig 4.2 Fatty acid composition of raw and processed *Lupinus albus*

The major fatty acids found from the Dangla cultivars were Myristic, palmitic, Linoleic, Oleic, stearic and Eikosanic acids. For the Tilili sample also all the mentioned fatty acids were found except Myristic acid. Among these palmitic and stearic are catagorized under saturated fatty acids, while oleic, linoleic and myristic are un-saturated types of fatty acids (Erbas.M, *et al.*, 2004). In another study by Uzun, *et al*., (2006), the saturated fatty acids found in the oil from the raw *Lupinus albus* seed were arachidic acid (3.5%), palmitic acid (7.6-10%) and stearic acid (1.5%) and the unsaturated types of fatty acids investigated were linoleic (20.3%) and oleic acid (47.65%) predominantly. There is a comparable value between the results of the current study and the report by Uzun, *et al*., (2006) in their Oleic acid content. The amount of linoleic acid in the Dangla and Tilili samples were 20.20 and 28.08% less than the amount reported by Uzun, *et al*., (2006). And the palmitic acid content for the Dangla and Tilili samples was (9.21%, 17.11%) lower than reported by Uzun, *et al*., (2006). But stearic acid level in both types of cultivars was 25% higher in our study than the same report by Uzun, *et al*., (2006).

From all the treatments germination has shown a significant effect on the fatty composition of the raw seed in both types of cultivars. Treatments GW1 reduced Palmitic, Oleic and Stearic fatty acid contents by 17.39, 6.07 and 25% respectively. Similarly, GK1 has shown a reduction of 21.74, 8.60 and 30% for the same fatty acid types. For the Tilili sample, GW2 has reduced the palmitic and oleic acid contents by 9.52% and 7.84% respectively. Similarly, GK2 has shown a reduction of 7.94% and 5.19% on these fatty acids.

This observed reductions in some of the fatty acid types might be due to the reduction of the total fat content on germination. As discussed on 4.3, on germination fat content decreased from the raw seed. This could be due to the growth of the seed. As the seed grows it needs protein. For the synthesis of protein the primary energy sources are lipids and carbohydrates. So, as the seed germinates the lipids will be utilized as energy source, making the total content lower. The same explanation was given by Bau, *et al*., (1996).

5. Conclusion and Recommendations

5.1. Conclusion

In this nutritional study, fourteen samples were analyzed for four parameters. Two cultivars of *Lupinus albus* grown in Ethiopia were taken and subjected to three main treatments. These were roasting then soaking, boiling then soaking and germination. And on each of the treatments the analyses were done on both the whole seed and the kernel. So the effect of de-hulling was also observed. The parameters analyzed were proximate composition, anti-nutritional factors, mineral composition analysis and fatty acid profile.

The proportion of the hull of the raw *Lupinus albus* seed from the two sampling sites was considerable from the total seed weight proportion. So, one can conclude that de-hulling will have an effect on the different composition of the seed.

In all the treatments total protein content has shown an increment with highest and lowest values for BSK and GW treatments respectively. The commonly used traditional processing method on *Lupinus albus* in Ethiopia (i.e. roasting then soaking in running water for five days) has also shown an improvement on the protein content. The crude fat content was increased in the same treatments. In fact in these treatments *Lupinus albus* can be considered as an oil crop. The raw *Lupinus albus* seed has the highest utilizable carbohydrate content from the treated samples. But considering gross energy content the roasted and boiled then soaked samples have shown the highest values.

The total alkaloid contents of the raw *Lupinus albus* samples of the two sites were reduced through all the treatments. Among which treatment roasting then soaking (de-hulled) in both sample types recorded the highest reduction. Therefore this commonly used traditional method of processing in Ethiopia could be an alternative option or way reduce the total alkaloid content within the safety limit (i.e.0.35µg/Kg body weight/day for adult consumption). Germination has the least effect to reduce the alkaloid content, though the de-hulled one is better than the whole seed. But considering the phytate content, germination has shown the highest reduction than the other treatments.

The results presented in this study revealed that raw *Lupinus albus* was enriched with mineral nutrients especially, Fe, Zn and Mg. In the case of Mn, the level was above the safety limit (i.e. 5 mg/100 gm/ day). So care should be taken when the seed is consumed considering the Mn level. Treatments like RSW, RSK, BSW, and BSK have reduced the level of the Mn to some extent, though still the safety limit not satisfied. The adult daily requirement for source of these nutrient is Fe; 13-16 mg and Mg; 13 mg, etc. The values obtained for Fe, Zn and Mg in this study were less than the required daily amount (RDA), but could be augmented by either increasing the quantity of *Lupinus albus* consumption or complementing it with other food sources.

The total oil content of *Lupinus albus* has shown to increase in all the treatments except germination. It can be considered as an oil crop at these levels. But the fatty acid profile should be suitable to human health to be an oil crop. Accordingly, the oil extracted from the raw *Lupinus albus* from both Dangla and Tilili was composed of more of unsaturated fatty acids and less of saturated ones. The dominant unsaturated fatty acids are Oleic acid and Linoleic acid in all the sample types. The saturated ones are Palmitic, Stearic acids and Eikosanic acids. The effects of processing are not significant on the fatty acid profile of the oil in the raw *Lupinus albus* seed except in the case of germination. In general, considering the total fat content and the healthy nature of the fatty acids in the oil, *Lupinus albus* can be recommended as an oil crop.

Combining all the results of this study, the commonly used traditional method of processing on the seed has shown a tremendous reduction in the total alkaloid contents. Moreover, it also enhances the protein, fat, and total energy of the raw sample. But it is better to serve the processed seed after removing the hull. Boiling then soaking has similar effect on the proximate composition and also the total alkaloid contents. But this method has higher energy demand than the roasting process. Germination has the least effect in reducing the total alkaloid, but has shown good enhancement in the proximate and mineral composition. Though this is the case it is difficult to recommend the germinated seed to be eaten due to safety problems.

Lupinus albus have interesting food composition levels with big limitation of alkaloids prior to its consumption. But after removing the alkaloids with the mentioned traditional methods, the crop can be a good source of protein, fat, energy, minerals and also essential fatty acids. It can be used in fortifying various low protein food sources. Besides all this, currently the demand for minimally processed foods has increased because of the enhancement of some nutritional and chemical compositions on those treatments. Moreover; cost wise the traditional ways of processing advisable due to their low energy and power consumptions.

5.2. Recommendations

Now a days, there is a great deal of interest in development of rich protein sources to meet the protein demands of developing country populations. In these countries scarcity of protein dietary intakes has lead to sever malnourishment. The possible remedy for these nations protein malnourishment problem is production of cheap protein sources. These protein sources should be affordable and can improve protein contents of other food products as a supplement.

Among the possible solutions for this problem is diet diversity using legume proteins (i.e. vegetable proteins). Along which *Lupinus albus* could be one of the protein supplements in areas where the consumption of the seed is accepted by the society. The protein content of *Lupinus albus* resembles that of soybean and generally contains more protein than many legumes consumed in Ethiopia. Additionally, *Lupinus albus* can give better yield than other comparable crops like soybean and chickpeas. Its useful agricultural features, like being resistant to water deficit, being diseases resistant, capable of nitrogen fixing, etc and the above mentioned facts can predict the good potential of *Lupinus albus* in alleviating protein malnourishment problems.

The current study with its own limitation has investigated the effects of traditional processes on the nutritional composition of *Lupinus albus*. But the following issues should also be considered in the future based on the outcomes of the current study.

1. The available varieties of the genus Lupinus should be studied.
2. Development of the sweet varieties of the species should be carried out.

3. Though *Lupinus albus* have high protein content, the amino acid composition of the various cultivars grown in Ethiopia has not been studied yet. So amino acid composition of the legume should be studied.
4. The method for total alkaloid determination in the current study is very old (i.e. gravimetric analysis). But recently there are instrumental methods of determination (i.e. GC-MS and HPLC). In the future the total alkaloid and the individual types of alkaloids must be studied with the mentioned recent methods.
5. The total oil content and the fatty acid profile of oil extracted from *Lupinus albus* is very interesting to consider it as potential human edible oil source. But to do so further study should be carried regarding the oil's acid value, peroxide value, iodine value and other edible oil parameters etc prior to consumption.
6. Moreover, the industrial processing of the oil should be also studied. Its extraction rate, application, etc
7. Study should also be undertaken regarding product development using *Lupinus albus* flour. These could be (protein isolates, pastries, milk analogues, etc), like the development of texturized meat from soybean.

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I. Annex-1

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

FOOD SCIENCE AND NUTRITION PROGRAM

TECHNICAL QUESTIONNAIRE FOR FOCUS GROUP DISCUSSION

TECHNICAL QUESTIONNAIRE FOR FOCUS GROUP DISCUSSION

To be completed by agricultural centers, local community, and farmers. Every question should be answered in appropriate way.

1. ADDRESS

Name____

Sex male ☐ female ☐ Age________________

Region____________ Wereda______________ Kebele __________

Occupation Farmer ☐ Agronomist ☐

Others

____

____

____

____

2. INFORMATION ON ORIGIN OF GIBTO

a) What was the geographical origin of Gibto?

____

____

____

b) When was it introduced in the region?

____

3. About gibto

a) What is its scientific name?

Lupinus albus L. ☐ Lupinus angustifolius L. ☐

Lupinus luetus L. ☐ Lupinus mutabilus L. ☐

Others ____

b) What are the existing varieties of Gibto?

____

____

4. INFORMATION ON AGRONOMIC CHARACTERSTICS OF GIBTO

a) What is the farmers sowing season?

Mehir ☐ summer ☐

Spring ☐ Others_____________________________________

b) What is the sowing date (month)?

____

____

b) What altitude range is suitable for its growth?

____

c) What is the total rain fall amount it requires?

____

d) What climatic (weather) conditions are suitable for its harvest?

____

e) What type of soil favors its growth?

____

f) Is any fertilizer enhancement required for its harvest?

Yes ☐ No ☐

g) Does it require pesticide for its harvest?

Yes ☐ No ☐

h) Comparing with other commonly harvested legumes and cereals, does Gibto have an advantage?

Yes ☐ No ☐

i) If the answer for the above question is yes, what are the advantages it has over the others?

____

____

____

____

____

____

____

5. INFORMATION ON ITS YEILD AND COST

a) What is the maximum annual yield per hectare?

____

b) Is there any post harvest loss? If there how much?

____
____

c) If the answer for the above question is yes, what are the main causes of the loss?

____
____
____
____
____
____

d) What is the current market price of Gibto?

____

6. THE CONSUMPTION OF GIBTO IN THE SOCIETY

a) Can Gibto be edible food source?
Yes ☐ No ☐

b) If the answer for the above question is no, what limits its consumption?

____
____
____
____

c) If the answer for the question (6a) is yes, what are the main food
Items that can be prepared from it?

____
____
____
____
____

d) What are the main traditionally used processes to make it edible?

e) What are the effects of these processes on the raw seed? (i.e. palatability, reduction Of bitterness, reducing flatulence, etc)

f) What are the other uses of Gibto for the local community?

Feed ☐ Local beverages like Araki ☐

Others____

g) How is it processed for Araki?

h) Beyond its use as alcohol source, Can the Araki be used for medicinal uses?

Yes ☐ No ☐

i) If the answer for the above question is yes, for what type of diseases can it be a Medicine?

j) Can it really a treat for diseases?

Yes ☐ No ☐

7. In general can gibto be medicinal plant? For what kind of diseases?

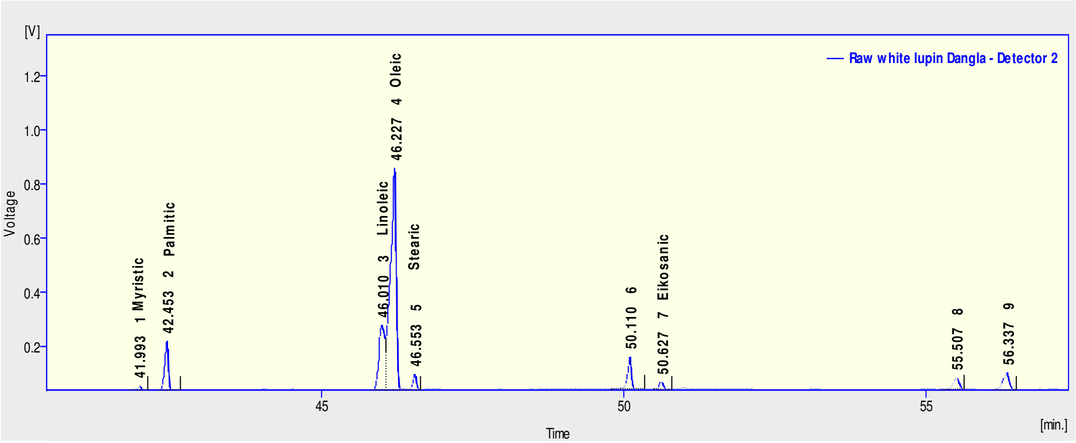
8. What kind of studies have been conducted on the crop so far?

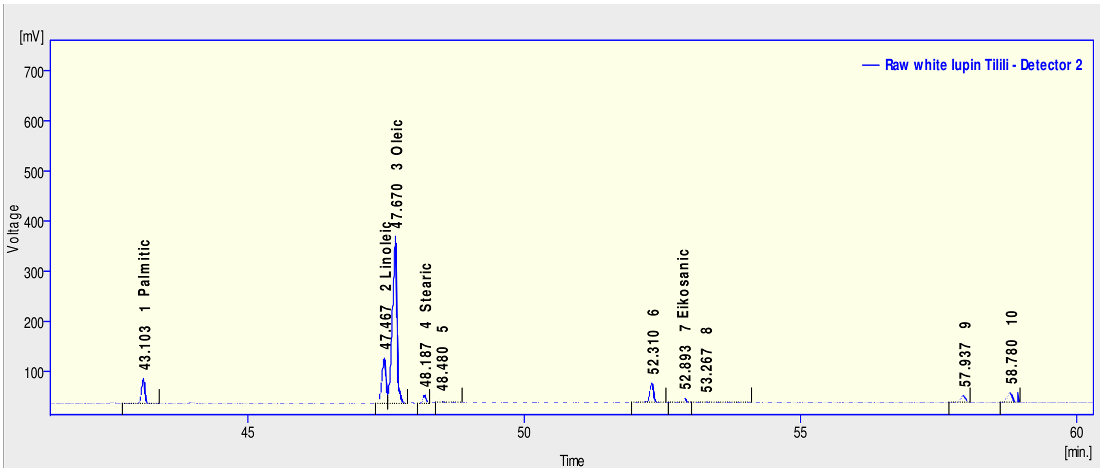
6. ANY INFORMATION THAT YOU WOULD LIKE TO ADD

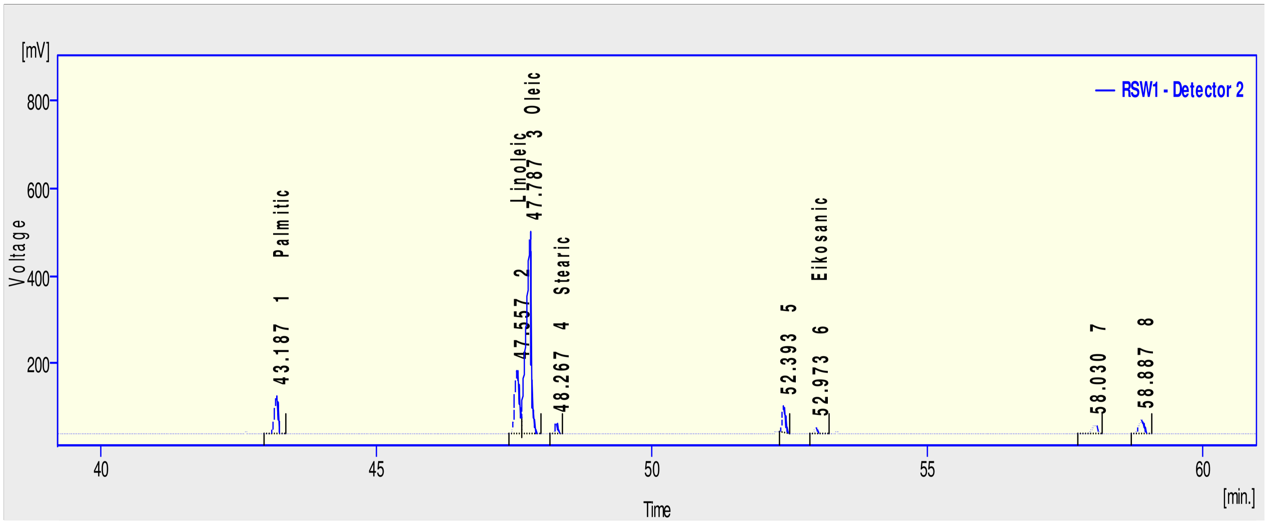
THANK YOU

II. Annex-II

Chromatogram of the GC analysis for fatty acid profile of oil extracted from raw and processed Lupinus albus seed



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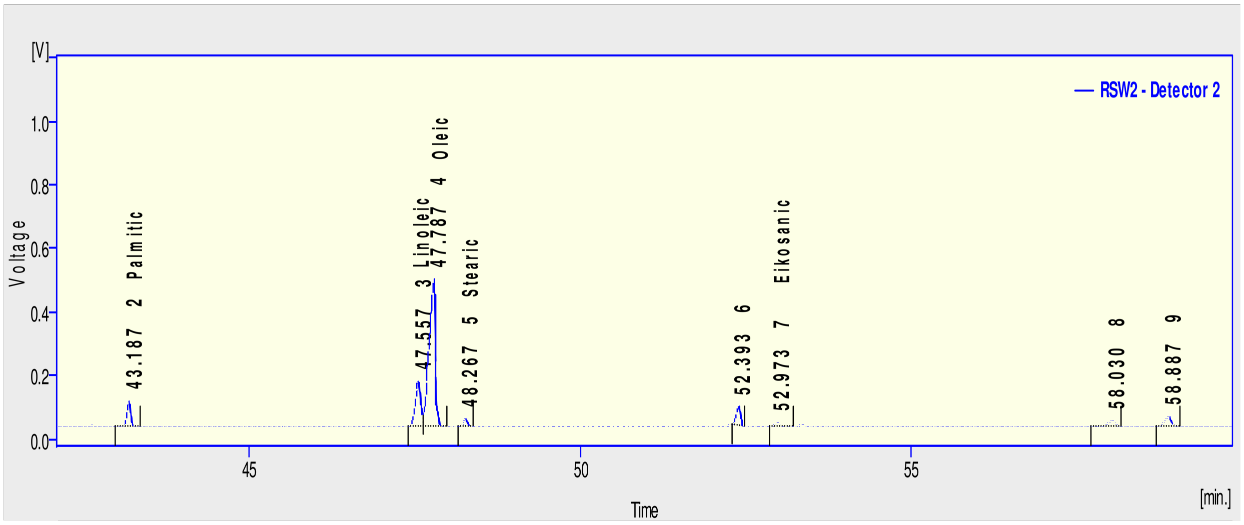
800
600
400
200
Voltage
RSW1 - Detector 2
43.187 1 Palmitic
47.557 2 Linoleic
47.787 3 Oleic
48.267 4 Stearic
52.393 5
52.973 6 Eikosanic
58.030 7
58.887 8
40
45
50
55
60
Time
[min.]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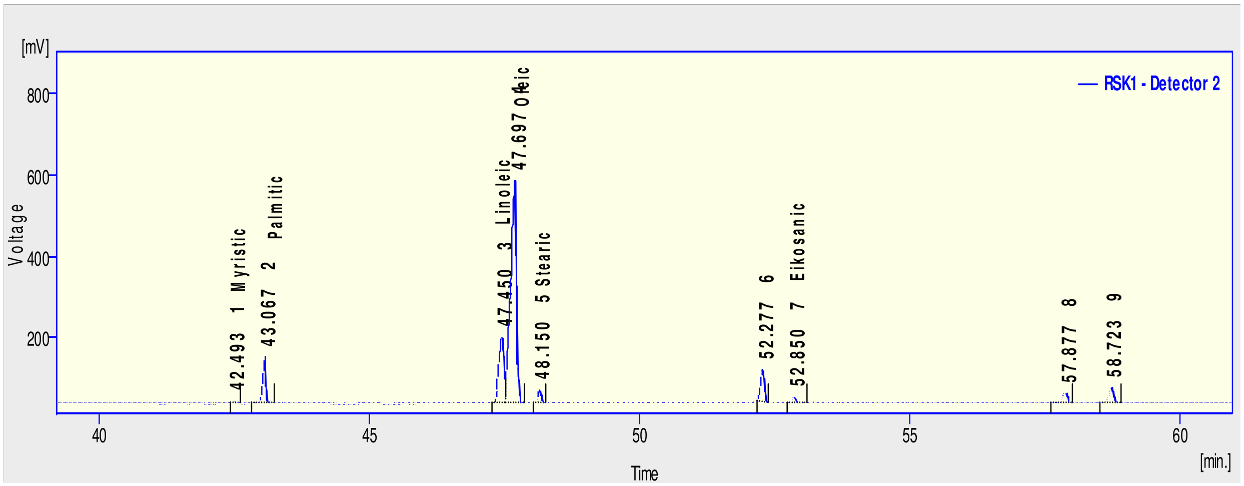
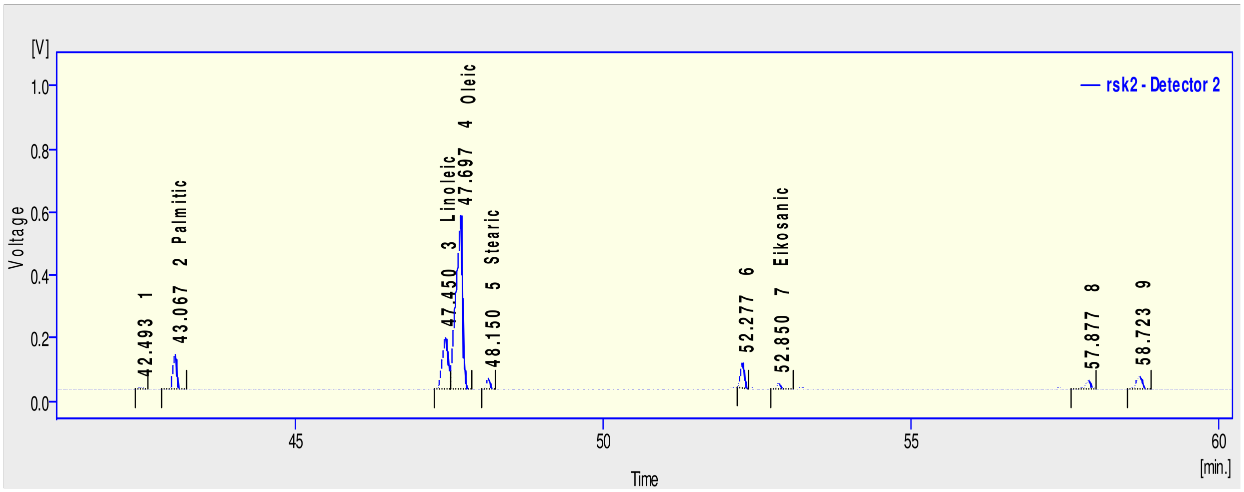
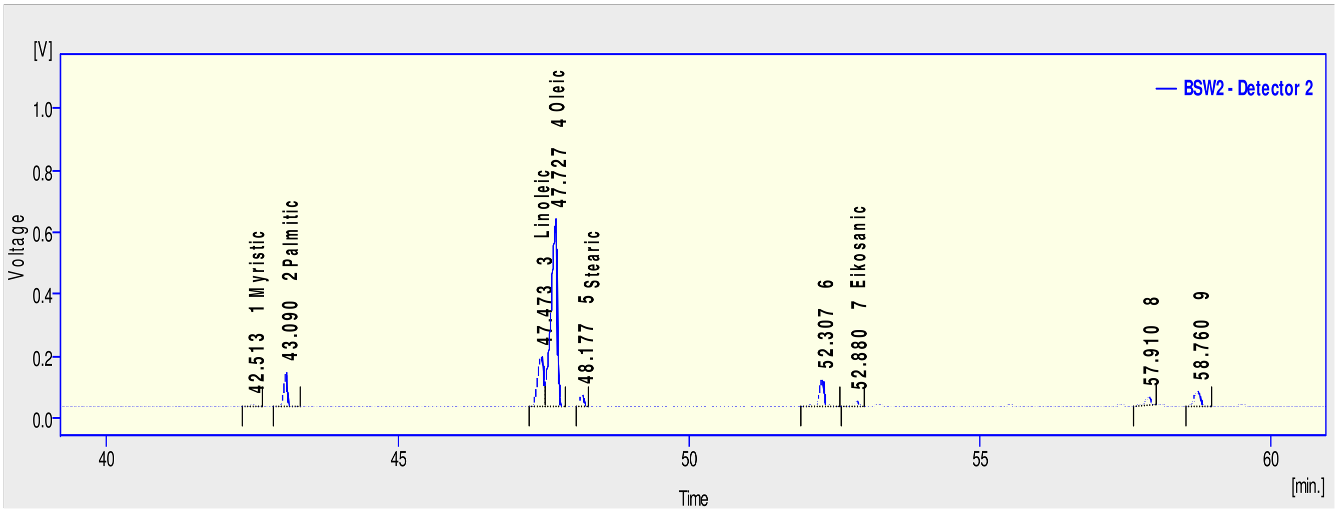
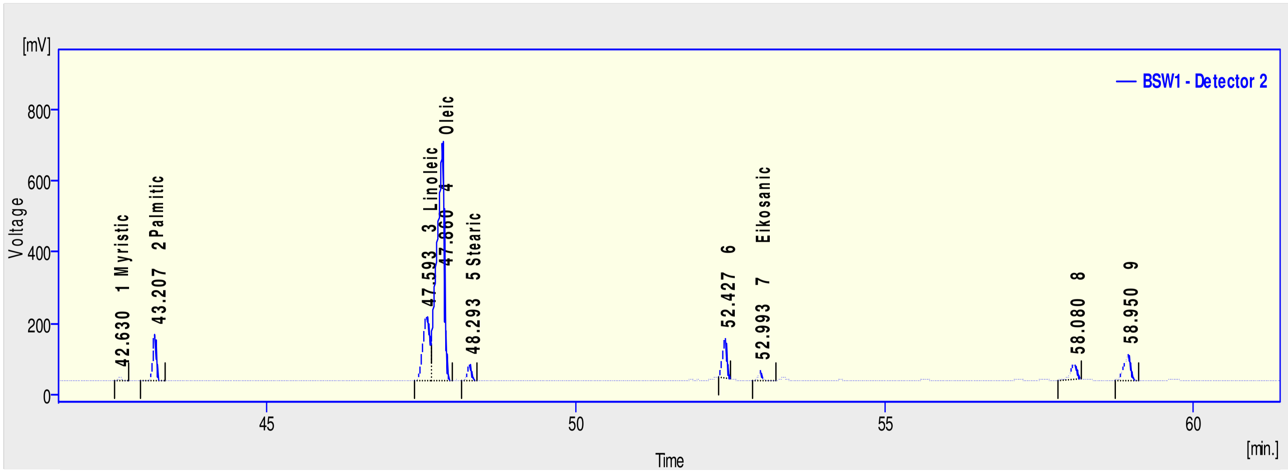
0.6
0.4
0.2
0.0
Voltage
RSW2 - Detector 2
43.187 2 Palmitic
47.557 3 Linoleic
47.787 4 Oleic
48.267 5 Stearic
52.393 6
52.973 7 Eikosanic
58.030 8
58.887 9
45
50
55
Time
[min.]


[mV]
800
600
400
200
Voltage
42.493 1 Myristic
43.067 2 Palmitic
47.450 3 Linoleic
47.697 4 Oleic
48.150 5 Stearic
52.277 6
52.850 7 Eikosanic
57.877 8
58.723 9
— RSK1 - Detector 2
40
45
50
55
60
[min.]
Time

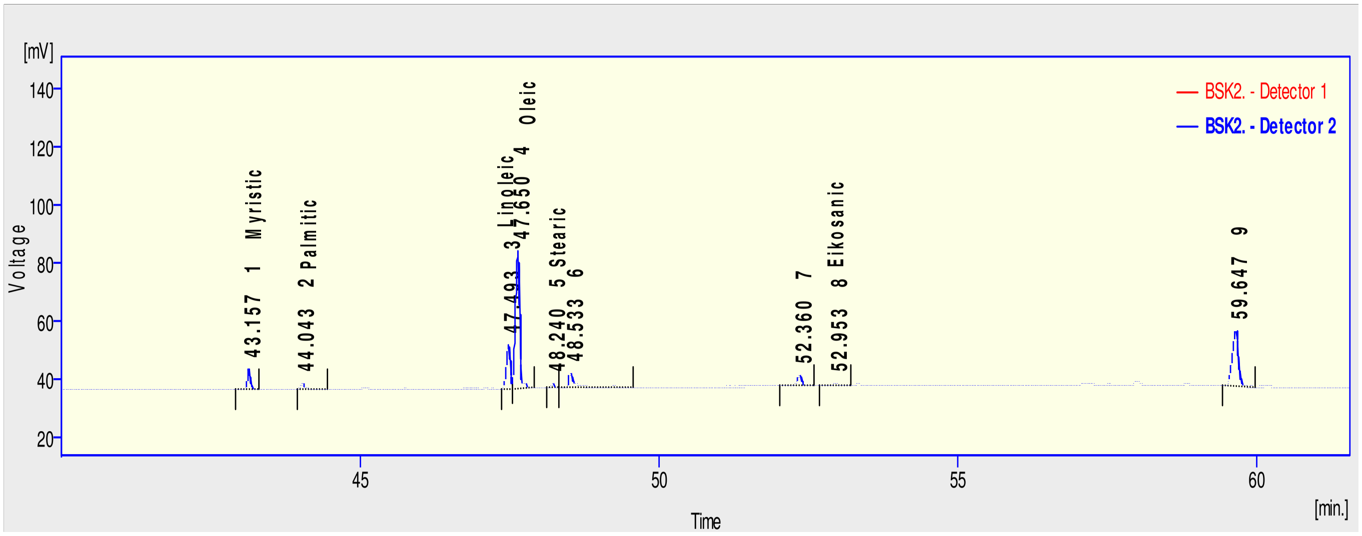

[V]
1.0
0.8
0.6
0.4
0.2
0.0
Voltage
42.493 1
43.067 2 Palmitic
47.450 3 Linoleic
47.697 4 Oleic
48.150 5 Stearic
52.277 6
52.850 7 Eikosanic
57.877 8
58.723 9
— rsk2 - Detector 2
45
50
55
60
[min.]
Time

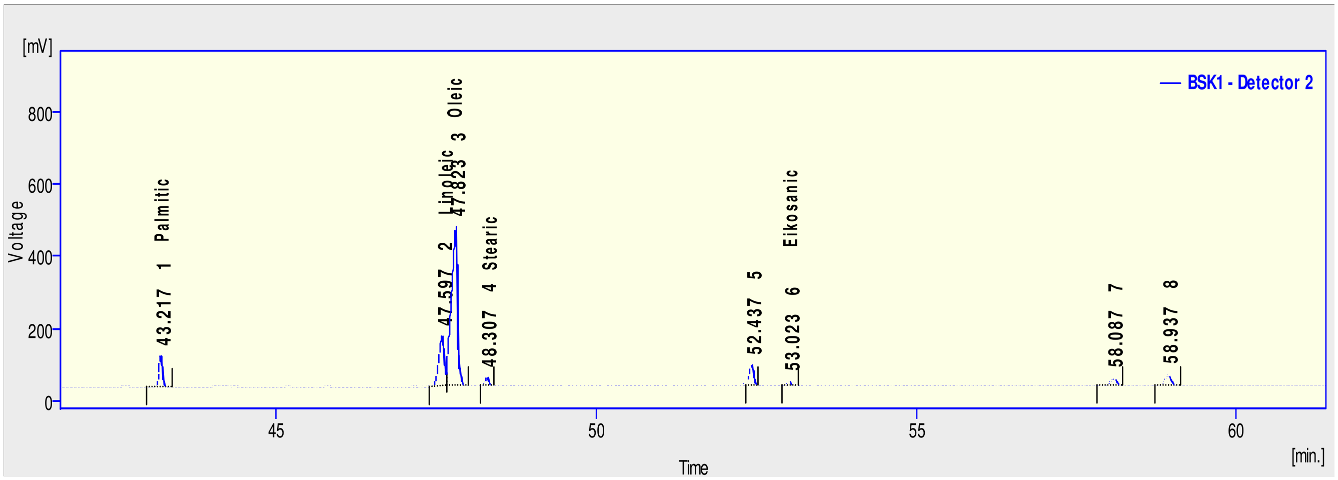


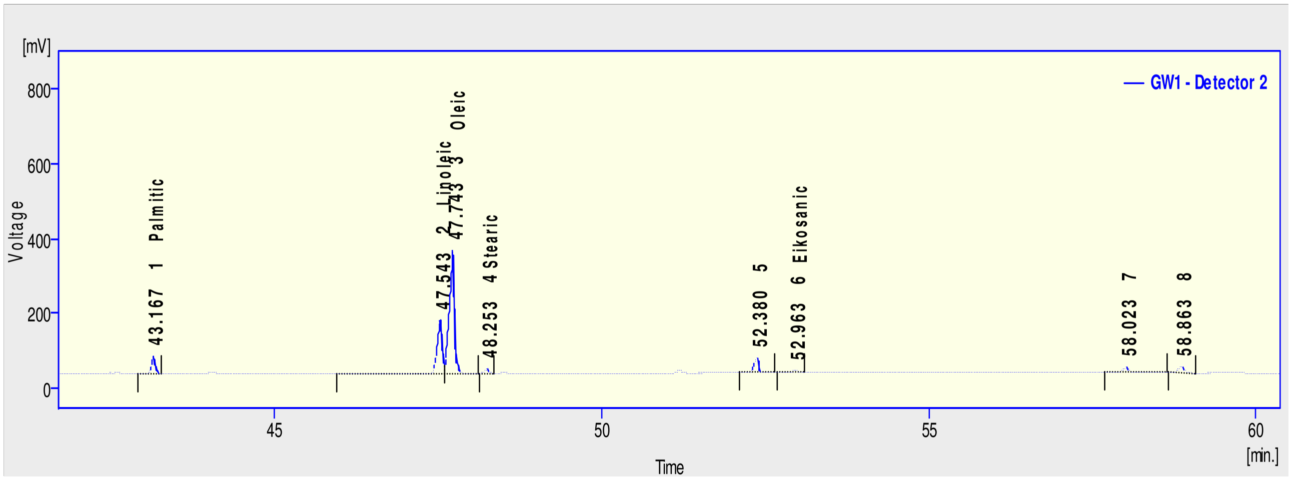
[V]
Voltage
0.0
0.2
0.4
0.6
0.8
1.0
42.513 1 Myristic
43.090 2 Palmitic
47.473 3 Linoleic
47.727 4 Oleic
48.177 5 Stearic
52.307 6
52.880 7 Eikosanic
57.910 8
58.760 9
BSW2 - Detector 2
40
45
50
55
60
[min.]
Time

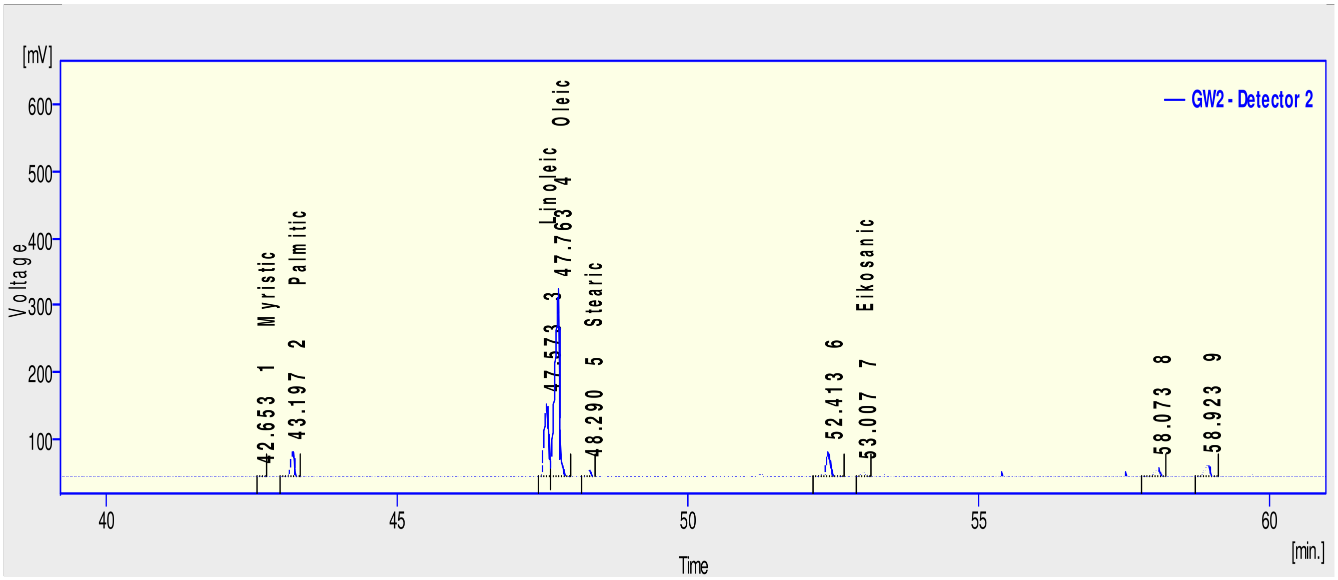


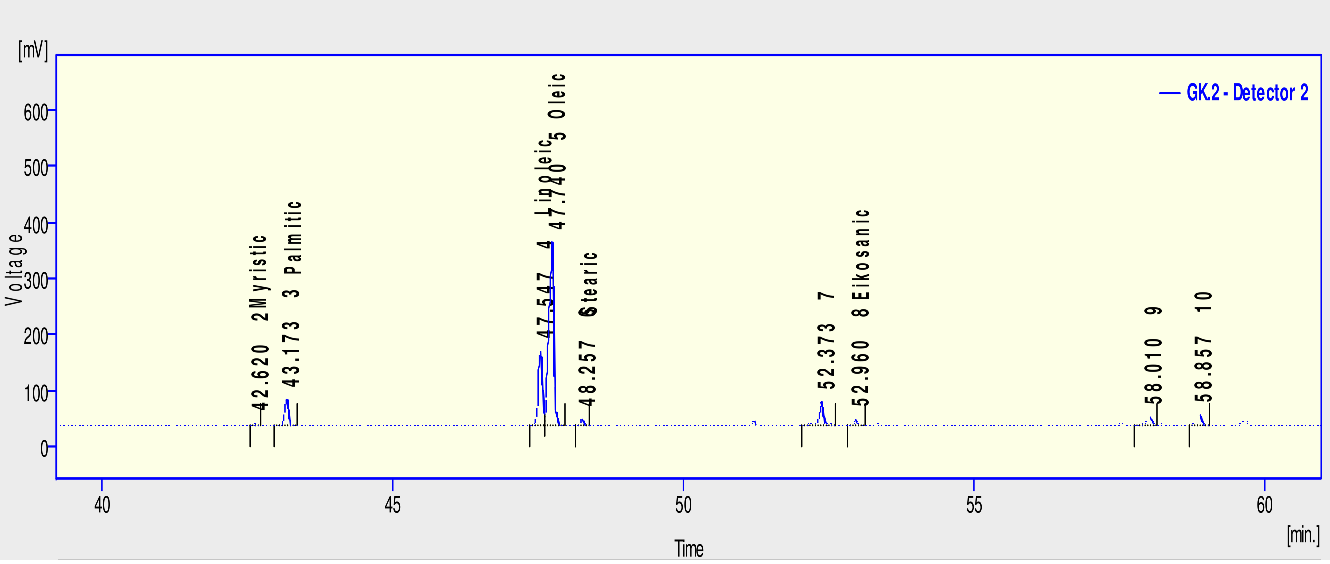
[mV]
Voltage
0
200
400
600
800
42.630 1 Myristic
43.207 2 Palmitic
47.593 3 Linoleic
47.860 4 Oleic
48.293 5 Stearic
52.427 6
52.993 7 Eikosanic
58.080 8
58.950 9
BSW1 - Detector 2
45
50
55
60
[min.]
Time

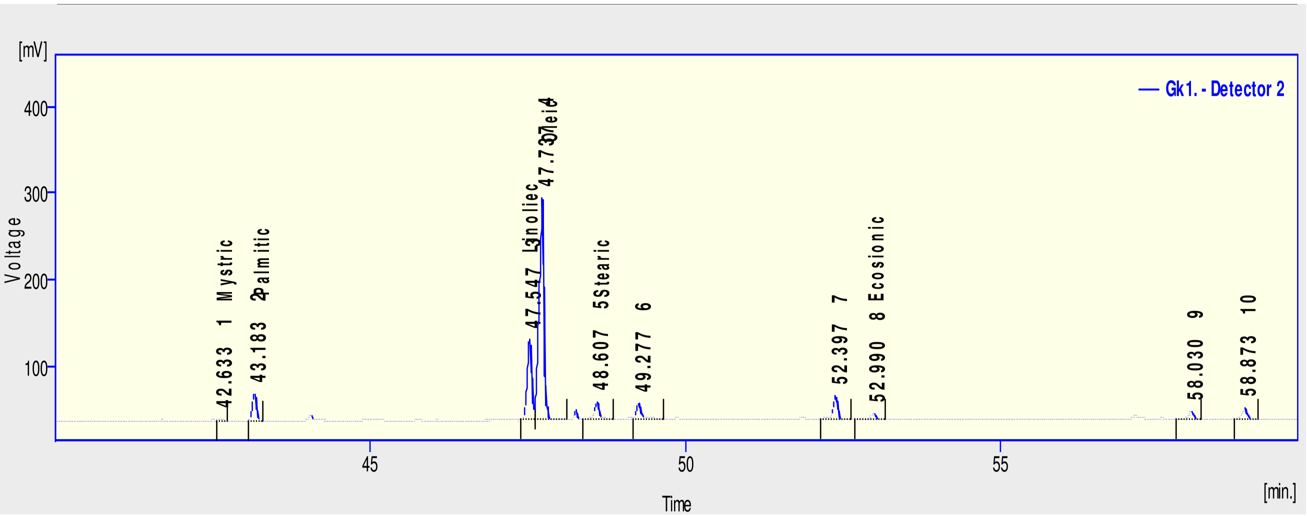

[mV]
140
120
100
80
60
40
20
Voltage
43.157 1 Myristic
44.043 2 Palmitic
47.493 3 Linoleic
47.650 4 Oleic
48.240 5 Stearic
48.533 6
52.360 7
52.953 8 Eikosanic
59.647 9
BSK2. - Detector 1
BSK2. - Detector 2
45
50
55
60
[min.]
Time


[mV]
800
600
400
200
0
Voltage
43.217 1 Palmitic
47.597 2 Linoleic
47.823 3 Oleic
48.307 4 Stearic
52.437 5
53.023 6 Eikosanic
58.087 7
58.937 8
BSK1 - Detector 2
45
50
55
60
[min.]
Time


[mV]
800
600
400
200
0
Voltage
43.167 1 Palmitic
47.543 2 Linoleic
47.743 3 Oleic
48.253 4 Stearic
52.380 5
52.963 6 Eikosanic
58.023 7
58.863 8
— GW1 - Detector 2
45
50
55
60
[min.]
Time


[mV]
600
500
400
300
200
100
Voltage
42.653 1 Myristic
43.197 2 Palmitic
47.573 3 Linoleic
47.763 4 Oleic
48.290 5 Stearic
52.413 6
53.007 7 Eikosanic
58.073 8
58.923 9
— GW2 - Detector 2
40
45
50
55
60
[min.]
Time


[mV]
600
500
400
300
200
100
0
Voltage
42.620 2 Myristic
43.173 3 Palmitic
47.547 4 Linoleic
47.740 5 Oleic
48.257 6 Stearic
52.373 7
52.960 8 Eikosanic
58.010 9
58.857 10
— GK.2 - Detector 2
40
45
50
55
60
[min.]
Time


[mV]
400
300
200
100
Voltage
42.633 1 Mystric
43.183 2 Palmitic
47.547 3 Linoliec
47.737 4 Oleic
48.607 5 Stearic
49.277 6
52.397 7
52.990 8 Ecosionic
58.030 9
58.873 10
— Gk1. - Detector 2
45
50
55
[min.]
Time

DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

Name: Paulos Getachew

Signature: _______________

Date: ________________

The thesis has been submitted with my approval as a supervisor.

Name: Melaku Umeta (PhD) Signature: ________________

Date: _________________

:Nigussei Retta (Professor) Signature: ________________

Date: _________________