Botswana Journal of Agriculture & Applied Sciences

Leading Agriculture through Science and Innovation

Please cite this article as: **Getachew, P., Umeta, M., Retta, N., Bekele, T., Molapisi, M. and Haki, G. D. (2014)** Fatty acid profile and mineral composition of traditionally processed gibto (*Lupinus albus* L.) grown in Ethiopia. *Botswana Journal of Agriculture and Applied Sciences* 10 (issue 2): **In press**

The online version of this article is located on the World Wide Web at:

http://www.ub.bw/ojs/index.php/bojaas

BOJAAS

ORIGINAL RESEARCH

Fatty acid profile and mineral composition of traditionally processed gibto (*Lupinus albus* L.) grown in Ethiopia

Getachew, P.[1], Umeta, M.[2], Retta, N.[1], Bekele, T.[1], Molapisi, M.[3] and Haki, G. D[3*]

[1]Center for Food Science and Nutrition, Addis Ababa University,
[2]Medical Faculty, Addis Ababa University,
[3]Department of Food Science & Technology, Botswana College of Agriculture, P/Bag 0027, Gaborone, Botswana

ABSTRACT

White lupin seeds, *Lupinus albus* L. are known for their bitter taste due to the presence of different alkaloids and other related anti-nutritional factors which make the seeds inedible. There are reports on some of processing methods that have the potential to reduce the alkaloids and make it a safe product for consumption. However, studies on the effects of the processing techniques on other biochemical compositions of the seed are lacking. In this study, the effects of commonly used traditional processing methods (roasting followed by soaking for five days, boiling followed by soaking for five days, germination for 48 hrs and dehulling) on the fatty acid profile and mineral composition and total alkaloid contents of *L. albus* seeds grown in Ethiopia are reported. The *L. albus* seeds were collected from two sites named Dangla and Tilili. Analysis of the seeds showed that the contents of iron, zinc, manganese and magnesium in the raw Dangla and Tilili samples were 6.01, 2.11, and 58.43, 8.93 mg/ 100 gm and 6.73, 1.81, 59.14 and 9.46 mg/ 100 gm, respectively. In raw seeds, an average value of 10 % saturated and 75 % unsaturated fatty acids were recorded. The predominant unsaturated fatty acids were C18:1 (n-9) and C18:2 (n-6), while the saturated ones contained C16:0, C18:0 and C20:0. All the traditional processing methods applied have reduced the total alkaloid contents of the raw seed from both sites significantly. Due to these treatments the content of iron in the raw seeds from both sites was reduced by (14-47) % and magnesium by only less than 10 %. Also due to these treatments no loss in the essential fatty acid contents was observed. Therefore, the minimally processed *L. albus* seeds using traditional methods can serve as a potential source of minerals and essential fatty acids after removing the alkaloids.

Keywords. fatty acids, germination, *Lupinus albus* L., minerals, processing, total alkaloids

*Correspondence author
E-mails: hgulelat@bca.bw; Tel: +267 3650100, Fax +267 3928753

Publisher: Botswana College of Agriculture, Gaborone, Botswana.

INTRODUCTION

Lupine belongs to the genus Lupinus and family of genisteae, which is also called fabaceae or leguminosae (Uzun et al., 2006). Commonly, four lupin species are reported worldwide and these include *L. albus* L., *L. angustifolius* L., *L. leutus* L. and *L. mutabilis* L. (Gladstones *et al*., 1998; Cowling *et al*., 1998; Uzun *et al*., 2006 and Kurzbaum *et al*., 2008). It is a legume (similar to soybean) with a high source of protein (38 %) (Getachew *et al*., 2012). Its adaptation to poor soil and wider climatic range makes it an ideal crop for most environments (Hill, 1977; Trugo *et al*.; 1993, Farrell *et al*.; 1999, Gaultier *et al*., 2003; Lampart-Szczapa *et al*., 2003; James *et al*., 2004 and Sujak *et al*., 2006). In addition, *L. albus* has very useful agronomic characteristics; non-shattering, disease resistant, high yield and growing on marginal soil (Australian New Crops Newsletter, 1999).

L. albus seeds are also good dietary sources of macro and micro minerals including calcium, iron, zinc, and copper, when compared to other legumes (Hill, 1977; Sathe *et al.,* 1984 and Donangelo *et al*., 1986). Its crude fat content is ranked third after ground nut (*Arachis hypogeae* L.) and Soybean (Glycine max. L.) (Cowling et al., 1998; VanderMassen and Somaatmadja, 1992; Uzun *et al*., 2006 and Joray *et al*., 2007). The oil extracted from L. albus consists of various types of fatty acids, with higher proportions of unsaturated fatty acids (Uzun *et al*., 2006). The unsaturated fatty acids consists of oleic acid C18:1 (n-9) and linoleic acids C18:2 (n-6) (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 (Uauy *et al.,* 1995 and Joray *et al*., 2007).

Although *L. albus* is a rich source of nutrients, the bitter and toxic quinolizidine alkaloids limit its consumption and

utilization (Calloway *et al*., 1971; Taverner et al., 1983 and Khalil et al., 2006). To reduce or eliminate alkaloids various modern and traditional processing methods have been developed (Getachew et al., 2012). In parallel with the processing methods, plant breeders have also tried to develop sweet lupin containing low level of alkaloids (Sanchez et al., 2005). In spite of this effort, sweet lupin varieties are not free of alkaloids and are less resistant to disease and herbivore attack (Sanchez et al., 2005).

Some physical and chemical treatments have been developed to eliminate the alkaloids (Arslan and Seker, 2002). These include soaking, dehulling and germination (Sripriya *et al.,* 1997), fermentation (Czarnecka *et al*., 1998), cooking (Kaankuka *et al*., 1996), heat treatment (Mulimani and Paramjyothi, 1994) and irradiation (Joseph and Dikshit, 1993). Beyond removing the unwanted anti-nutritional factors, these processes may also improve the nutritive value and digestibility of the seed (Khalil et al., 2006).

In Ethiopia, the available species of lupine include L. mutabilis and *L. albus* (locally called Gibto) (Forest Gene Bank of Ethiopia, 2008). *Lupinus albus* is widely cultivated in the country, especially in the Amhara region (west Gojam (Dangla and Tilili) and Gondar areas). The areas have recorded the highest annual yields of 7.17 Quintal (Central Statistical Agency of Ethiopia (CSA), 2007 and Forest Gene Bank of Ethiopia, 2008). In Gojam and Gondar (northern part of Ethiopia) *L. albus* is used as a food crop. However, only few studies have been conducted with regard to its chemical composition and nutritional values. Some of the previous studies include; proximate composition and anti-nutritional factors of traditionally processed white lupine (*L. albus* L.) fabaceae grown in Ethiopia (Getachew *et al.*, 2014); protein quality evaluation of dagussa (Eleusin coracanal) and *L. albus* and the supplementary value *L. albus* and *Eleusin coracanal* for animal feed (Sileshi, 1985). Ethiopian Health and Nutrition Research Institute (EHNRI) (1997) reported composition of three *L. albus* prepared foods and these appear in the food composition table of Ethiopia. The present study was designed to investigate the effects of the traditional processing methods (roasting followed by soaking for five days, boiling followed by soaking for five days, germination for 48 hrs and dehulling) on the mineral composition and fatty acid profiles of *L. albus* grown and consumed in two areas in Ethiopia.

MATERIALS AND METHODS

Sampling

Samples of L. albus L. were collected from open markets of Dangla and Tilili in west Gojam, Ethiopia. Dangla is located at 11.25o and 36.60o latitude and longitude, respectively. The total population reported in the area is 21,800. Tilili is located at 10.95o and 36.50o latitude and longitude, respectively. The total human population in the area is 23,800. These sites were chosen because of their high production of the crop (CSA, 2007). The sampling technique used was random sampling. The samples were packed in polyethylene bags and transported to the Food Science and Nutrition laboratory of Addis Ababa University. All chemicals used for analysis were Analytical Reagent (AR) grade.

Sample Preparation

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

a) Roasting followed by soaking and de-hulling

The cleaned seeds from both sites were roasted on metal pan for 10 minutes together with pre-cleaned sand, to make the roasting uniform. Then, the roasted seeds were cooled for 10 minutes, washed several times and soaked in a bucket of tap water in (weight to volume) 1:10 ratio. The soaking water was changed every 2 hrs for five days until the bitterness was removed. Lack of bitterness was then determined. Then, the whole roasted and soaked seed (RSW) and subsample was de-hulled (RSK). Both RSW and RSK were freeze dried at -42oC for 48 hrs. The dried samples were grinded to sieve size of 60 meshes (0.25 mm), packed in brown glass and kept at -20^{o}C until analysis.

b) Boiling followed by soaking and de-hulling

The cleaned seeds from both sampling sites were boiled in tap water at 94^{o}C for 4 hrs. The boiled seeds were then soaked in a bucket of tap water (weight to volume) (1:10 ratio). The soaking was carried out as indicated in section above. The removal of the bitterness determined as describe above. The whole boiled and soaked seed (BSW) and a subsample was de-hulled (BSK). Both BSW and BSK were treated as in section above and packed in brown glass and kept at -20^{o}C until analysis.

c) Germination

The cleaned seeds from both sites were soaked in tap water for 24 hrs. Then, the soaking water was removed and the sample was covered in castor bean leaf (similar to the traditional way) and was left to germinate at room temperature for 48 hrs. At the end of germination, the alkaloid content was determined. Then, the whole germinated seed (GW) and a subsample was de-hulled (GK). Both GW and GK were treated as previously described and packed in brown glass and kept at -20^{o}C until analysis.

Analysis

After treatments; roasting, boiling and germination, the elimination of the bitterness was checked by tasting the seed like in the traditional way and also by analysing for alkaloid content according to (Getachew *et al*., 2012).

Fatty acids were determined by gas chromatographic (GC) to quantify its methyl esters (FAMEs), which were prepared using a slightly modified method from AOAC (2000). Total lipid was extracted from the dried samples (0.5gm) using soxhlet extractor. Then, FAMEs were prepared with 5 mL of methylation solution (1 sulfuric acid: 20 methanol: 10 toluene) and heated at 100°C for 1 hr. After cooling, 5 mL of distilled water was added followed by final extraction with 5

mL of diethyl ether. Fatty acid analysis was conducted using GC with FID detector (DANI, ALS100, Italy).

Mineral composition was determined in triplicate by the method of Osborne and Voogot (1978). Briefly, 2.5 gm of powder samples were put into porcelain dish and charred at 120^{o}C for 4 hr, until the whole content became carbonized. Then, the samples were placed in a furnace at 530^{o}C until free from carbon, and the residue appears grayish white after 8 hrs. The crude ash was dissolved with 5 mL of 6M HCl and placed on hot plate for 2 hrs. Then, 7 mL of 3M HCl was added and heated on a hot plate until the solution boils. The digest was cooled and filtered. Then 5 mL of 3M HCl was added to the dishes and heated to dissolve the residue. The minerals were analyzed by using Atomic Absorption Spectroscopy (AAS, Buck Scientific Atomic absorption Spectrophotometer, Ontario, Canada).

Statistical analysis

Data was tested for effects of site and processing using General Linear Model (GLM), followed by Duncan's multiple range test to separate the means using SPSS software version 17.0 (SPSS Inc., Chicago.IL, USA). Results was presented as mean ± SEM.

RESULTS

Total alkaloid

There was a significant difference ($P < 0.01$) in total alkaloid contents of the *L. albus* seeds collected from the two sites. All the traditional processing methods reduced the total alkaloid content of the raw seed from both sampling sites. RSWD, RSKD, BSWD, BSKD, GWD and GKD have reduced the total alkaloid content by 65 %, 71 %, 52 %, 59 %, 33 % and 40 % respectively from the raw seed. Similar reduction was observed in the raw seed from Tilili site with same processing techniques. However, germination showed the least reduction in the total alkaloid contents of the raw seeds from both sites (Table 1, 2 and 3).

Mineral Composition

The mineral contents of the raw *L. albus* L. collected from Dangla and Tilili sites are reported in Table 1. The contents of iron, zinc and magnesium were significantly different in the two sites at $P < 0.01$, $P < 0.01$ and $P < 0.05$ respectively, while there was no significant difference in manganese content. Moreover, the hull size and total alkaloid contents of the seeds from the two sites have shown significant differences at $P < 0.01$. Manganese was the highest in composition compared with the other minerals in the raw seed from both sites.

The effect of the traditional processing on the mineral and total alkaloid content of seeds from the Dangla site is reported in Table 2. The iron content in all the processing methods was reduced ($P < 0.01$) by (14-39) % from the raw seed. The highest reduction was observed by the treatments Germinated Whole seed from Dangla (GWD) and Germinated Kernel from Dangla (GKD) by 27 % and 39 % respectively. Similarly, manganese content in the raw seed was reduced due to treatments Roasted then Soaked Whole seed from Dangla (RSWD), Roasted then Soaked Kernel from Dangla (RSKD), Boiled then Soaked Whole seed from Dangla (BSWD) and Boiled then Soaked Kernel from Dangla (BSKD) by 16 %, 22 %, 5 % and 10 % respectively. In contrast, significant highest increment in zinc content was observed in RSWD, RSKD, BSWD and BSKD by 702 %, 736 %, 1444 % and 994 % respectively. Total alkaloid content was reduced significantly in all the processing methods in order of RSKD, RSWD, BSKD, BSWD, GKD, GWD by 71 %, 65 %, 59 %, 52 %, 40 % and 33 % respectively ($P < 0.01$).

The iron content in the raw seed collected from Tilili site was significantly reduced ($P < 0.01$) due to the treatments Roasted then Soaked Whole seed from Tilili (RSWT), Roasted then Soaked Kernel from Tilili (RSKT), Boiled then Soaked Whole seed from Tilili (BSWT), Boiled then Soaked Kernel from Tilili (BSKT), Germinated Whole seed from Tilili (GWT) and Germinated Kernel from Tilili (GKT) by 16 %, 30 %, 59 %, 47 %, 27 % and 39 % respectively. Upon the same treatments manganese and magnesium contents were reduced from the value in the raw seed. In contrast, zinc content was increased upon all the treatments. The total alkaloid content in the raw seed collected from Tilili site was reduced by 70 %, 65 %, 60 %, 53 %, 40 % and 32 % due to treatments RSKT, RSWT, BSKT, BSWT, GKT and GWT respectively (Table 3).

In this study, there was a significant effect of sampling site in total alkaloids, zinc, manganese and magnesium contents at $P < 0.001$, while for iron content at $P < 0.05$. Similarly due to the processing methods there was a significant difference in the contents of each parameter tested at $P < 0.001$ from the raw seeds. Moreover, there was processing x sampling site interaction effect on total alkaloid, iron, zinc, manganese contents at $P < 0.001$, while for magnesium content at $P < 0.01$ (Table 4).

Fatty Acid Profile

The oil extracted from the raw *L. albus* collected from Dangla site was composed of 10.6 % saturated and 75.5 % of unsaturated fatty acids. Similarly, the oil extracted from the Tilili seed has 75.31 % unsaturated and 15.2 % saturated fatty acids (Table 5). The major fatty acids detected from both sites were myristic acid (C14:0), palmitic acid (C16:0), linoleic acid (C18:2, n-6), oleic acid (C18:1, n-9), C18:0 and C20:0. In both cases the amount of unsaturated fatty acids was higher than that of the saturated

Table 1 Effect of site on iron, zinc, manganese, magnesium, total alkaloid and hull size of raw *Lupinus albus* L. grown in Ethiopia

Minerals	Raw *L. albus* from Dangla	Raw *L. albus* from Tilili	SL
Iron	6.01 ± 0.11[b]	6.73 ± 0.01[a]	**
Zinc	2.11 ± 0.02[a]	1.81 ± 0.00[b]	**
Manganese	58.42 ± 0.70	59.14 ± 0.36	NS
Magnesium	8.93 ± 0.19[b]	9.46 ± 0.08[a]	*
Total alkaloid	2.46 ± 0.02[a]	2.26 ± 0.01[b]	**
Hull size	16.22 ± 0.39[b]	19.30 ± 0.24[a]	**

Table 2 Effect of traditional processing on the contents of iron, zinc, manganese, magnesium and total alkaloid contents of *Lupinus albus* L. collected from Dangla and Tilili sites

Processing	Sampling site	Total alkaloid	Iron	Zinc	Manganese	Magnesium
RG	Dangla site	2.46 ± 0.02[a]	6.01 ± 0.19[b]	2.11 ± 0.04[i]	58.43 ± 1.22[b]	8.93 ± 0.32[b, c]
	Tilili site	2.26 ± 0.01[b]	6.73 ± 0.02[a]	1.81 ± 0.00[j]	59.14 ± 0.62[b]	9.46 ± 0.14[a]
RSW	Dangla site	0.84 ± 0.00[k]	5.04 ± 0.04[d, e]	16.92 ± 0.13[d]	49.07 ± 0.51[e]	8.57± 0.07[d]
	Tilili site	0.79 ± 0.02[k]	5.64 ± 0.22[c]	15.59 ± 0.56[e]	44.62± 1.29[f]	9.08 ± 0.08[b]
RSK	Dangla site	0.71 ± 0.00[l]	4.23 ± 0.11[f, g]	17.63 ± 0.09[c]	45.51 ± 0.11[f]	8.51 ± 0.08[d]
	Tilili site	0.67 ± 0.00[l]	4.74 ± 0.21[e]	2.14 ± 0.13[i]	39.01± 1.41[g]	8.41± 0.05[d]
BSW	Dangla site	1.19 ± 0.01[g]	4.95 ± 0.17[d, e]	22.57± 0.09[b]	55.41 ± 0.24[c]	8.04 ± 0.16[e]
	Tilili site	1.07 ± 0.02[h]	2.74 ± 0.13[i]	2.59± 0.02[g, h]	46.36 ± 0.26[f]	8.52 ± 0.03[d]
BSK	Dangla site	1.00 ± 0.02[i]	5.17 ± 0.19[d]	23.10 ± 0.01[a]	52.70 ± 1.66[d]	7.95± 0.17[e]
	Tilili site	0.89 ± 0.01[j]	3.58 ± 0.18[h]	2.88 ± 0.05[f, g]	46.81 ± 1.34[e, f]	8.43 ± 0.02[d]
GW	Dangla site	1.65 ± 0.02[c]	4.39 ± 0.02[f]	2.41 ± 0.16[h, i]	59.29 ± 1.95[b]	8.82 ± 0.12[c]
	Tilili site	1.54 ± 0.03[d]	4.92 ± 0.28[d, e]	3.13 ± 0.12[f]	60.86 ± 0.09[a, b]	9.34 ± 0.00[a]
GK	Dangla site	1.48 ± 0.02[e]	3.64 ± 0.27[h]	2.93 ± 0.15[f]	62.54± 0.59[a]	8.53 ± 0.08[d]
	Tilili site	1.39 ± 0.01[f]	4.07 ± 0.15[g]	3.08 ± 0.09[f]	62.12 ± 4.13[a]	9.04 ± 0.18[b, c]
Main effects	Site	***	*	***	***	***
	Processing	***	***	***	***	***
Interaction	Processing x Site[1]	***	***	***	***	**

Values are in mg/ 100gm dry weight (DW) for mineral analysis and gm/100gm for the total alkaloid
Means in the same row with different superscripts in differ significantly; * = P < 0.05, ** = P < 0.01; *** = P < 0.001
RGD: Raw Gibto Dangla (*Lupinus albus L.*), RSWD: Roasted then Soaked Whole seed Dangla, RSKD: Roasted then Soaked Kernel Dangla, BSWD: Boiled then Soaked Whole seed Dangla, BSKD: Boiled then Soaked Kernel Dangla, GWD: Germinated Whole seed Dangla, GKD: Germinated Kernel Dangla
RGT: Raw Gibto Tilili (*Lupinus albus L.*), RSWT: Roasted then Soaked Whole seed Tilili, RSKT: Roasted then Soaked Kernel Tilili, BSWT: Boiled then Soaked Whole seed Tilili, BSKT: Boiled then Soaked Kernel Tilili, GWT: Germinated Whole seed GKT: Germinated Kernel Tilili

Table 3 Fatty acid profile of raw and processed *Lupinus albus L*. seeds grown in Ethiopia

Type of sample	C 14:0	C 16:0	C 18:2 (n-6)	C 18:1 (n-9)	C 18:0	C 20:0	Sum SFA	Sum UFA
RGD	0.50	6.90	16.20	59.30	2.00	1.20	10.60	75.50
RSWD	1.90	7.10	15.80	60.41	1.90	5.00	15.90	76.21
RSKD	0.40	7.20	15.90	61.40	2.00	1.20	10.80	77.30
BSWD	0.00	6.30	14.90	60.00	2.00	1.40	9.70	74.90
BSKD	0.00	7.00	16.00	61.40	2.00	1.20	10.20	77.40
GWD	0.00	5.70	22.22	55.70	1.50	1.40	8.60	77.92
GKD	1.10	5.40	19.60	54.20	1.40	1.10	9.00	73.80
RGT	0.00	6.30	14.60	59.80	2.00	1.40	9.70	74.40
RSWT	1.84	6.92	15.64	59.67	1.75	4.65	15.20	75.31
RSKT	0.38	6.67	14.76	60.09	1.87	0.98	9.90	74.85
BSWT	0.40	6.60	14.90	61.00	2.00	1.30	10.30	75.90
BSKT	0.00	5.40	12.00	41.50	8.50	0.90	14.80	53.50
GWT	3.00	5.70	19.30	55.10	2.10	1.40	12.20	74.40
GKT	0.00	5.80	20.70	56.70	1.60	1.30	8.70	77.40

fatty acids as reported in Table 5. In both sampling sites in the raw *L. albus* seed C18:1 (59 %) was the predominant fatty acid. Among the essential fatty acids the seed was consisted of linoleic acid (C18:2, n-6) 16.2 % and 14.60 % in the Dangla and Tilili samples respectively.

DISCUSSION

Total alkaloids

All the treatments on L. albus seeds from both sampling sites reduced the total alkaloid content. Since alkaloids are water-soluble, soaking in water can easily remove it from the whole seed. This depends on the type of soaking solution and permeability of the cell wall (hull) (Jimenez-Martinez *et al*., 2003). Therefore, using traditional ways of processing (roasting and boiling), it is possible to increase permeability of the hull to facilitate alkaloid removal (Getachew *et al.,* 2012). Adewusi and Falade (1996) also reported improvement in the hull permeability of the raw *L. albus* after thermal and soaking treatment. In fact in the present study, de-hulling led to a significant reduction in the total alkaloid than whole seed in all the processing methods. This might indicate the presence of alkaloids in the hulls as well. In roasting and boiling treatments, the hull is damaged, which facilitated the removal of alkaloids as shown in Table 2, 3 and 4. However, in the case of germination since there was no heat treatment, the permeability of the hull was not effective for effective alkaloid removal.

Iron

For different cultivars of raw *L. albus*, iron content was reported to be in the range of 3.5-7.7 mg/ 100 gm (Trugo et al., 1992 and EHNRI, 1997), which is similar to the results of the present study. In both sample types, soaking after roasting and boiling and germination reduced the iron content by (14-29) % (Table 2 and 3). In another study, it was reported that except for sodium, all the minerals analyzed including iron were reduced by soaking of raw seeds in NaHCO3 solution (Abu-Samaha 1983 and El-Adawy *et al*., 2000). The reduction of minerals by soaking might be due to the loss of water soluble minerals by the steeping medium and the rinsing process (Bau et al., 1999).

Zinc

The zinc concentration due to treatments was increased compared to the raw seed (Table 2 and 3). Similar findings were reported by Varriano-Mariston and E-Omana (1979). In addition, El-Adawy *et al*. (2000) has reported an increase in zinc concentration in *L. albus* seeds soaked in 0.5 %

NaHCO3. It should be noted that mineral contents of processed samples depend on the type of soaking solution. For example, El-Adawy *et al*. (2000) revealed that when the soaking solution was NaHCO3, except for sodium, the values of all the analyzed minerals were reduced. The soaking solution in our study was tap water, which is flows through zinc coated pipe lines. Therefore, the observed increment in the zinc content might arise from contamination from the tap water. Hence further investigation using zinc free medium to clarify the increment of zinc due to traditional processing is needed. Meanwhile, among all the treatments, germination showed the least increment in zinc content. This might be an additional indication of less permeability of the seed hull due to germination.

Manganese

There was no significant difference in the manganese content between the two cultivars. The range for manganese content of different cultivars of raw *L. albus* was previously reported to be within 61-327 mg/ 100gm (Trugo *et al.,* 1992), which is higher compared to the values in the present study. In contrast, El-Adawy *et al.* (2000) has reported lower content of manganese (10.8 mg/ 100gm) for raw seeds. The upper limit of safe intake of manganese for human consumption is 5 mg/ day (National Research Council, 1989). If we assume intake of 10 gm lupin *per* day, the manganese content *per* consumption will be 5.8 mg and 5.9 mg from the seeds collected from Dangla and Tilili respectively. However, the traditional processing techniques significantly reduced the manganese content to safe intake level from the raw seed except GW and GK. Similarly, a reduction in manganese content in *L. albus* soaked in NaHCO3 solution was reported by El-Adawy *et al.* (2000). This might be attributed to the increase in permeability of the hull, which might enhanced internal process of leaching in the soaking solution (Jimenez-Martinez *et al.,* 2003).

Magnesium

The magnesium contents of the Dangla and Tilili cultivars were different (Table 1). Compared with the other minerals a slight reduction in the magnesium content was observed due to the traditional processing techniques (Table 2, 3 and 4).

As discussed above most of the minerals studied were reduced due to the processing methods applied. In fact, in our previous study the crude ash content was also reduced due to similar treatments (Getachew *et al*., 2012). This may entail that most of the inorganic materials including minerals have been washed out with the soaking solution. On the other hand, these treatments were effective in reducing the alkaloid contents from the raw seed, which makes it mandatory to apply. Therefore, one can augment the loss of the iron and magnesium to meet the Required Daily Amount (RDA), either by increasing the quantity of *L. albus* consumption or complementing it with other food sources.

Fatty Acid Profile

The major fatty acids detected from the Dangla and Tilili cultivars were C14:0, C16:0, C18:2 (n-6), C18:1 (n-9), C18:0 and C20:0. In Uzun et al. (2006), the major saturated fatty acids in raw *L. albus* seeds were arachidic acid (C20:0, 3.5 %), (C16:0, 7.6-10 %) and (C18:0, 1.5 %) and the unsaturated fatty acids included (C18:2 (n-6), 20.3 %) and (C18:1 (n-9), 47.65 %). The C18:1 (n-9) content in Uzun et al. (2006) is comparable with the values in our study, while the C18:2 (n-6) and C16:0 contents in both sample types were lower. Similar results were reported by Petterson (1998) and Mulayim *et al*. (2002). As cited in Erbas et al. (2005) and Nas *et al*. (1992) the fatty acid composition of L. albus oil resembles that of peanut and rapeseed oil, but does not contain any erusic acid (C22:1, n-9). The effect of each treatment on the content of the fatty acids is discussed below.

Linoleic acid (C18:2, n-6)

There was a significant difference between the two samples in the percentage C18:2 content. Germination increased the C18:2 content by 27 % and 17 %, respectively in GWD and GKD. Also GWT and GKT have increased the percentage of C18:2 by 24 % and 29 %, respectively (Table 5). Similarly, Mital *et al.* (2012) has reported that germination resulted in 48.42 % increase in linoleic acid. The increase in C18:2 content due to germination might be related with the plant lipoxygenases activities. Linolenic acid acts as substrate during the activity of lipoxygenase. Meanwhile C18:2 (Linoleic acid), a polyunsaturated fatty acid is resistant to the attack of lipoxygenase and does not get affected. The more double bonds the chain has in the cis- configuration, the less flexibility it has (Vasishta and Srivastava, 2012).

Oleic acid (C18:1, n-9)

There was no significant difference between the two samples in C18:1 content. In both samples, RSW, apparently which reduced the alkaloid content effectively, increased the C18:1 content in the range 1-3 %. In contrast, germination has reduced the C18:1 content in both cultivars by 5-9 % from the raw seeed. This reduction might be attributed to the reduction of the crude fat due to growth of the seed upon germination. This might also reduce the contents of some of the major fatty acids like C18:1. Also the lipoxygenase activity might target fatty acids like oleic acid, which will reduce the amount (Vasishta and Srivastava, 2012).

Myristic acid (C14:0)

There was no difference between the two raw lupin samples in C14:0 and C14:0 (Table 5).

Palmitic acid (C16:0)

There was no significant difference between the two lupin samples in the percentage of C16:0 content. Treatments GWD and GKD has reduced the C16:0 content by 21 % and

27 %, respectively. In the Tilili cultivar germination reduced the C16:0 content by 10 % and 8 %, respectively in GWT and GKT. Upon germination the reduction in some of the fatty acids might be due to the reduction of the crude fat content on germination from the raw seed. This might be due to the growth of the seed, which 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, which will reduce the crude fat content (Bau *et al*., 1999). Drastic reduction in palmitic acid has also been reported in soybean during later stages of germination (Vasishta and Srivastava, 2012).

Stearic acid (C18:0)

Among all the treatments on Dangla lupin sample, germination has reduced the C18:0 content by 25 % and 30 % in GWD and GKD treatments, respectively. In the Tilili cultivar, BSKT has shown the highest increment in the C18:0 content by 76 %, while GKT reduced it by 25 %. This reduction in stearic acid due to germination might arise from the same reason mentioned for C16:0 and C18:1.

Arachidic acid (C 20:0)

There is no significant difference between the two cultivars in C20:0 content. The effective treatments in reducing the alkaloid, roasting then soaking in both sample types increased the C20:0 content by 76 % and 42 % from raw seed, respectively.

CONCLUSIONS

The traditional ways of soaking after roasting and boiling of *L. albus* seeds are recommended processing methods which results minimal loss of nutrients and effective reduction of alkaloids. Also these processes provided increased amounts of unsaturated fatty acids, while the saturated fatty acids amount was reduced.

CONFLICT OF INTEREST

REFERENCES

Abu-Samaha, O. R. (1983). Chemical, technological and nutritional studies on lentil MSc Thesis. Alexanderia, Egypt.

Adewusi, S. R. and Falade, O. S. (1996). The effects of cooking on extractable tannins, phytate, sugar and mineral solubility in some improved Nigerian legume seeds. *Food Science Technology International,* 2: 231-239.

AOAC. (2000). Official Method of Analysis section 969.3. 18th ed. Association of Official Analytical Chemists, Washington DC. USA.

Arslan, C. and Seker, E. (2002). Effects of processed white lupin seed (Lupinus albus L.) on growth performance of Japanese quail. *Revue de Médecine Vétérinaire,* 153(10):643-646.

Australian New Crops Newsletter. (1999). New crops and oil seeds from Ethiopia and elsewhere. Newsletter. Australia.

Bau, H. M., Villaume, C., Nicolas, J. P. and Mejean, L. (1999). Effect of germination on chemical composition, biochemical constitues and anti-nutritional factors of soybean (Glycine max) seeds. *Journal of the Science of Food and Agriculture*, 73(1):1-9.

Calloway, D., Hickey, C. A. and Murphy, E. L. (1971). Reduction of intestinal gas forming properties of traditional and experimental food processing methods. *Journal of Food Science*, 36(251).

Cowling, W. A., Buirchell, B. J. and Tapia, M. E. (1998). Lupin: Lupinus albus promoting the conservation and use of underutilized and neglected crops. Rome: Institute of Plant Genetics and Crop Plant Research.

Central Statistical Agency of Ethiopia. (2007). Area and production of crops. Statistical Bulletin. Addis Ababa, Ethiopia: Birhanena Selam Printing Agency.

Czarnecka, M., Czarnecki, Z., Nowak, J. and Roszyk, H. (1998). Effect of fermentation and extrusion of bean and pea seeds on nutritional and functional properties. *Nahrung*, 42:7-11.

Donangelo, C. M., Pedersen, B. and Eggum, O. B. (1986). Protein, energy and mineral utilization in rats fed rice: legume diets. *Qualityy of Plant Foods Human Nutrition,* 36:119-37.

Ethiopian Health and Nutrition Research Institute. (1997). Food Composition Table Use in Ethiopia-Part III. Addis Ababa: Ethiopia.

El-Adawy, T. A., Rahma, E. H., El-Bedawy, A. A. and Sobihah, T. Y. (2000). Effects of soaking process on nutritional quality and protein solubility of some legume seeds. *Nahrung*, 44:339-343.

Erbas, M., Certel, M. and Uslu, M. K. (2005). Some chemical properties of white lupin seeds (Lupinus albus L.). *Food Chemistry*, 89:341-345.

Farrell, D. J., Perez-Maldonado, R. A. and Mannion, P. F. (1999). Optimum inclusion of field pea, faba beans, chick peas and sweet lupins in poultry diets.II. Broiler experiments. *British Poultry Science*, 40(5): 674-680.

Forest Gene Bank of Ethiopia. (2008). Plant data base of genus Lupinus in Ethiopia. Plant Data Base. Addis Ababa, Ethiopia.

Gaultier, F., Foucault-Bertaud, A., Lamy, E., Ejeil, A. L., Dridi, S.M. and Piccardi, N. (2003). Effects of vegetable extract from **Lupinus albus** (LU105) on the production of matrix metalloproteinase (MMP1, MMP2, MMP9) and tissue inhibitor of metallo proteinases (TIMP1, TIMP2) by human gigival fibroblasts in culture. Clinical Oral Investigation, 7(4):198-205.

Getachew, P., Umeta, M., Retta, N., Bekele, T. and Haki, D. G. (2012). Proximate composition and anti-nutritional factors of traditionally processed white lupine (*Lupinus albus* L.) fabaceae, grown in

Ethiopia. *Ethiopian Journal of Biological Sciences*, 11(2):133-146.

Gladstones, C. A., Atkins, C. A. and Hamblin, J. (1998). Lupines as a crop plants biology, production and utilization. CAB international, pp1-39.

Hill, G. D. (1977). The composition and nutritive value of lupin seed. *Nutrition Abstracts and Reviews: Livestock Feeds and Feeding*, 47:511-529.

James, L. F., Panter, K. E., Gaffield, W. and Molyneux, R. J. (2004). Biomedical applications of poisnous plant research. *Journal of Agricultural and Food Chemistry*, 52(11): 3211-3230.

Jimenez-Martinez C., Hernandez-Sanchez H. and Davila-Ortiz.G. (2003). Lupines: An alternative for de-bittering and utilization in foods. *Food Science and Food Biotechnology*, 233-252.

Joray, M. L., Rayas-Duarte, P., Mohamed, A. and Vansanten, E. (2007). Coated Lupin Bean Snacks. *Journal of Food Quality*, 30: 267-279.

Joseph, A. and Dikshit, M. (1993). Effect of irradiation on the protease inhibitor activity and digestibility (in vitro) of safflower oil cake. *Journal of American Oil Chemists Society,* 70: 935-937.

Kaankuka, F., Balogun, T. and Tegbe, T. (1996). Effects of duration of cooking of full fat soybeans on proximate analysis, levels of antinutritional factors, and digestibility of weanling pigs. *Animal Feed Science Technology*, 62: 229-237.

Khalil, A. A., Mohamed, S. S., Taha, F. S. and Karlsson, E. N. (2006). Production of functional protein hydrolysates from Egyptian breeds of soybean and lupin seeds. *African Journal of Biotechnology*, 5 (10):907-916.

Kurzbaum, A., Safori, G., Monir, M. and Si-msolo, C. (2008). Anticholinergic syndrome in response to lupin seed toxicity. Israeli *Journal of Emergency Medicine*, 8(2):20-22.

Lampart-Szczapa, E., Siger, A., Trojanows-ka, K., Nogala-Kalucka, M., Malecka, M. and Pacholek, B. (2003). Chemical composition and antibacterial activities of lupin seed extracts. *Nahrung*, 47(5):286-290.

Mittal R., Nagi HPS, Sharma P., Sharma S. (2012). Effect of processing on chemical composition and antinutritional factors in chickpea flour. *Journal of Food Science and Engineering,* 2:180-186.

Mulayim, M., Tamkoc, A. and Babaoglu, M. (2002). Sweet lupins versus local bitter genotype: Agronomic characterstic as affected by different planting densities in the Goller region of Turkey. *European Journal of Agronomy*, 17:181-189.

Mulimani, V. and Paramjyothi, S. (1994). Effect of heat treatment on trypsin/chymotrypsin inhibitor activity of red gram (Cajanus cajan L.). *Plant Foods and Human Nutrition*, 46:103- 107.

Nas, S., Gokalp, H. Y. and Unsal, M. (1992). Bitkisel yag teknolojisi. Erzurum: Ataturk University.

National Research Council. (1989). Recommended Dietary Allowances. National Academy Press. Washington DC, USA.

Njoku, P. C. and Ohia, C. C. (2007). Spectrophotometric estimation of mineral content in three cocoyam cultivars. *Pakistan Journal of Nutrition*, 6(6):616-619.

Osborne D. R and Voogt P. (1978). The Analysis of Nutrients in Foods. Academic Press, London, U.K. 240 pp.

Petterson, D. S. (1998). Composition and food uses of lupines. In: S.G.J., A.A.C. and H. J., Lupines as a crop plant biology, production and utilization (pp. 353-379). South Perth: CAB International.

Reay P. F, Waugh C. (1981). Mineral composition of Lupinus albus and Lupinus Angustifoulius in relation to manganese accumulation. Plant and Soil, 60:435-444.

Sanchez, M. D., Altares, P., Pedrosa, M. M., Burbano, C., Cuadrado, C., Goyoaga, C., Muzquiz, M., Jiménez-Martínez, C. and Dávila-Ortiz, G. (2005). Alkaloid variation during germination in different lupin species. *Food Chemistry*, 90: 347-355.

Sathe, S. K., Deshpande, S. S. and Salunkhe, D. K. (1984). Dry beans of phaseolus A review. part 2. Chemical composition: Carbohydrates, fiber, minerals, vitamins and lipids. *Critical Review in Food Science and Nutrition*, 21(1):41-93

Sileshi, Z. (1985). Protein quality evaluation of dagussa (Eleusin coracanal) and gibto (Lupinus albus L.) and supplementary value of gibto when added to dagussa. Master's Thesis. Addis Ababa University, Addis Ababa, Ethiopia.

Sripriya, G., Antony, U. and Chandra, T. (1997). Changes in carbohydrates, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). *Food Chemistry*, 58:345-350

Sujak, A., Kotlarz, A. and Strobel, W. (2006). Compositional and nutritional evaluation of several lupin seeds. *Food Chemistry*, 98:711-719.

Taverner, M. R., Curic, D. M. and Rayner, C. J. (1983). A comparison of the extent and site of energy and protein digestion of wheat, lupin and meat and bone meal by pigs. *Journal of the Science of Food and Agriculture*, 34:122-128.

Trugo, L. C. (1992). Effect of germination on nutritive value of lupine seeds. VII International Lupin Confrence. Portugal: Evora.

Trugo, L. C., Donangelo, C. M., Duarte, Y. A. and Tavares, C. L. (1993). Phytic acid and selected mineral composition of seed from wild species and cultivated varieties of lupin. *Food Chemistry*, 47: 391-394.

Uauy, R., Gattas, V. and Yanez, E. (1995). Sweet lupins in human nutrition. World Review of Nutrition and Dietetics, 77: 75-88.

Uzun, B., Arslan, C., Karhan, M. and Toker, C. (2006). Fat and fatty acids of white lupin (*Lupinus albus* L.) in comparison to sesame (*Sesamum indicum* L.). *Food Chemistry*, 102: 45-49.

Vander Massen and Somaatmadja, S. (1992). Plant sources of south east Asia. Bogor: Prosea Foundation.

Varriano-Mariston, F. and E-Omana, E. J. (1979). Effects of sodium salt solutions on the chemical composition and morphology of black beans (*Phaseolus vulgaris*). *Journal of Food Science*, 44:531-536.

Vasishta H., Srivastava R. P. (2012). Changes in lipids and fatty acids during soaking and germination of chickpea (*Cicer arietinum*). *Indian Journal of Agriculture and Biochemistry*, 25 (1):14-19.