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RESEARCH ARTICLE

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, Addis Ababa, Ethiopia
[2]Medical Faculty, Addis Ababa University, Addis Ababa, Ethiopia
[3]Department of Food Science & Technology, Botswana University of Agriculture and natural Resources, P/Bag 0027, Gaborone, Botswana.

PG, conceived idea, designed study, collected data, laboratory analysis, preparation of manuscript; MU, conceived idea, designed study; NR, conceived idea, designed study, TB, conceived idea, designed study; MM, substantial revision of manuscript; GDH, conceived idea, designed study, statistical analysis, preparation of manuscript, revision of manuscript

ABSTRACT

White lupin seeds, *Lupinus albus* L. are known for their bitter taste due to the presence of various alkaloids and other related anti-nutritional factors which make the seeds inedible. There are some traditional processing methods that have the potential to reduce the alkaloid content and make the product safe for human consumption. However, studies on the effects of these processing techniques on other biochemical compositions of the seed are lacking. In this study, the effects of three commonly used traditional processing methods on the fatty acid profile, mineral composition and total alkaloid contents of *L. albus* seeds grown in Ethiopia are reported. The processing methods comprised boiling followed by soaking for five days, roasting followed by soaking for five days and germination for 48 hours, each combined with dehulling by hand, at the end of the processing treatment. 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, 58.43 and 8.93 mg/100 g and 6.73, 1.81, 59.14 and 9.46 mg/100 g, respectively. In raw seeds, an average value of 10% saturated and 75% unsaturated fatty acids were recorded. The predominant saturated fatty acids were C16:0, C18:0 and C20:0 while the unsaturated fatty acids were C18:1 (n-9) and C18:2 (n-6). All the traditional processing methods applied reduced the total alkaloid content of the seed from both sites significantly. Due to these treatments the content of iron in the seeds from both sites was reduced by between 14 and 47% and magnesium by less than 10%. However, no reduction in essential fatty acid contents was observed. Therefore, minimal processing of *L. albus* seeds using traditional methods can serve as a means of reducing alkaloid content and thus allow the product, which is a potential source of minerals and essential fatty acids, to be palatable to humans.

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

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

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INTRODUCTION

Lupins belong to the genus Lupinus and family genisteae, which is also called fabaceae or leguminosae (Uzun *et al*., 2006). Worldwide, four lupin species are common; these are *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). Lupins are legumes, with a high protein content (38%) according to Getachew *et al*., (2012). Their adaptation to poor soil and their wide climatic range make them an ideal crop for many 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 resistance, high yield and it grows on marginal soils (Francis, C.M., 1999).

Compared to other legumes, *L. albus* seeds are also good dietary sources of macro and micro minerals including calcium, iron, zinc, and copper, (Hill, 1977; Sathe *et al.,* 1984 and Donangelo *et al*., 1986). Its crude fat content is ranked third after groundnut (*Arachis hypogaea* L.) and soybean (Glycine max. L.) (Cowling *et al*., 1998; Van der Massen 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 consist of oleic acid C18:1 (n-9) and linoleic acid C18:2 (n-6) (Uzun *et al.,* 2006). The high content of unsaturated fatty acids and a desirable ratio of ω-6 to ω-3 fatty acids make the crop an alternative source of a healthy edible oil (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 a low level of alkaloids. 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 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).

Two species of lupin are endemic to Ethiopia; these are L. mutabilis and *L. albus* (locally called “gibto”) (Forest Gene Bank of Ethiopia, 2008). *L. albus* is widely cultivated in the country as a food crop, especially in northern Ethiopia, in the Amhara region (west Gojam (Dangla and Tilili) and Gondar areas). These areas have recorded the highest annual yields of 7.17 quintal/ha (about 720 kg/ha) (Central Statistical Agency of Ethiopia (CSA), 2007 and Forest Gene Bank of Ethiopia, 2008). However, only a few studies have been conducted with regard to its chemical composition and nutritional value. Previous studies include; proximate composition and anti-nutritional factors of traditionally processed white lupin (*L. albus* L.) fabaceae grown in Ethiopia (Getachew *et al.*, 2014) and an evaluation of the protein quality of dagussa (finger millet; *Eleusine coracana*) and *L. albus* as animal feeds (Sileshi, 1985). The Ethiopian Health and Nutrition Research Institute (1997) reported the composition of three food products prepared using *L. albus* and these appear in the food composition table of Ethiopia. The present study was designed to investigate the effects of three traditional processing methods (roasting followed by soaking for five days, boiling followed by soaking for five days, germination for 48 hours), each combined with dehulling by hand, at the end of the processing treatment, on the mineral composition and fatty acid profiles of *L. albus* grown and consumed in two areas in Ethiopia. The study also investigated the effects of processing on the total alkaloid content of the seeds.

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 11.25ºN and 36.60ºE and the total human population reported in the area is 21,800. Tilili is located at 10.95ºN and 36.50ºE and the total 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 matter, dust, immature and damaged seeds. Then the following common traditional processes were performed.

a) Roasting followed by soaking and de-hulling

The cleaned seeds from both sites were mixed with pre-cleaned sand and then roasted on a metal pan for 10 minutes. The sand ensured that the roasting was uniform. The roasted seeds were then cooled for 10 minutes, washed several times and soaked in a bucket of tap water at a ratio of 1:10 (weight to volume). The soaking water was changed every two hours for five days until the bitterness was removed. Lack of bitterness was determined by the traditional method which is tasting the seed. A portion of the roasted and soaked whole seed (RSW) was then de-hulled using a mortar and pestle and an air blower to give the roasted and soaked kernel (RSK) sample. Both RSW and RSK were freeze dried at -42ºC for 48 hours. The dried samples were then ground and passed through a 60 mesh size sieve (0.25 mm), before being packed in brown glass and kept at -20ºC until analysis.

b) Boiling followed by soaking and de-hulling

The cleaned seeds from both sampling sites were boiled in tap water at 94ºC for four hours. The boiled seeds were then soaked in a bucket of tap water (weight to volume 1:10 ratio). The soaking was carried out as described above (a), as well as testing for presence of bitterness. Again, a subsample of the boiled and soaked whole seed (BSW) was de-hulled (BSK). Both BSW and BSK were treated as described above and packed in brown glass and kept at -20ºC until analysis.

c) Germination followed by de-hulling

The cleaned seeds from both sites were soaked in tap water for 24 hours. Then, the soaking water was removed and the sample was covered with a castor bean leaf (as is the traditional method) and was left to germinate at room temperature for 48 hours. After germination, the alkaloid

content was determined. Then a subsample of the germinated whole seed (GW) 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. The elimination of bitterness was also checked by analysing for alkaloid content according to the method described by Getachew *et al*., 2012.

Mineral composition was determined in triplicate according to the method of Osborne and Voogt (1978). Briefly, 2.5 g of pulverized samples were put into a porcelain dish and charred at 120^{o}C for four hours, until the entire sample was carbonized. Then, the samples were placed in a furnace at 530^{o}C for eight hours until free from carbon, when the residue appeared grayish white. The crude ash was then dissolved in 5 mL of 6M HCl and left on a hotplate for two hours, after which 7 mL of 3M HCl was added and heating continued until the solution boiled. The digest was cooled and filtered. Then 5 mL of 3M HCl was added to the dishes and heated to dissolve the residue. Mineral content was determined using a Buck Scientific Atomic Absorption Spectrophotometer, (210VGP, 4-555 Wentworth Street East Oshawa, Ontario, L1H 3V8, Canada).

The content of each of six fatty acids were determined by gas chromatography (GC). These were myristic acid (C14:0), palmitic acid (C16:0), linoleic acid (C18:2, n-6), oleic acid (C18:1, n-9), stearic acid (C18:0) and arachidic acid (C20:0). To quantify the methyl esters (FAMEs) present in each sample, a slightly modified method from that given in AOAC (2000) was used. Total lipid was extracted from 0.5 g dried samples 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 one hour. 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).

Statistical analysis

Data was tested for effects of site and processing on total alkaloid content, mineral composition, and fatty acid content using a General Linear Model (GLM) and the means were separated by Duncan's multiple range test using SPSS software version 17.0 (SPSS Inc., Chicago, USA). Results are presented as mean value plus or minus the standard error of the mean. For each of six fatty acids, content as a percentage of the total fatty acid present was used in analysis.

RESULTS

Total alkaloids

There was a significant difference (P<0.01) in total alkaloid content of the raw *L. albus* seeds collected from the two sites (Table 1). The total alkaloid content of the raw seed from both sampling sites was reduced significantly (P<0.001) by each of the traditional processing methods. With seeds from Dangla this reduction was equivalent to -71%, -65%, -59%, -52%, -40% and -33% for treatments RSK, RSW, BSK, BSW, GK and GW, respectively, compared to raw seeds (Table 2). Similar reductions were observed in seeds from Tilili with the same processing techniques; treatments RSK, RSW, BSK, BSW, GK and GW reduced total alkaloid of the raw seed by -70%, -65%, -60%, -53%, -40% and -32%, respectively (Table 2). At both sites germination showed the least reduction in the total alkaloid content (Table 2).

Mineral Composition

The mineral contents of the raw *L. albus* L. seeds collected from Dangla and Tilili are reported in Table 1. The contents of iron, zinc and magnesium were significantly different in seeds from the two sites at P<0.001, P<0.01 and P<0.05, respectively. However, there was no significant difference in manganese content, which was much higher than the content of other minerals in seed from both sites. Hull size of seeds from the two sites showed significant differences at P<0.01, with the larger seed being that from Tilili.

The effect of traditional processing methods on the mineral content of seeds from both sites is reported in Table 2. With each of the processing methods iron content was reduced (P<0.01) by between -14% and -39% compared with the raw seed. In the seed from Dangla the highest reduction was observed in germinated whole seed and germinated kernel (treatments GW and GK); -27% and -39%, respectively. Similarly, manganese content in the raw seed was reduced by roasting then soaking and by boiling then soaking; contents in both whole seed and kernels were reduced by -16%, -22%, -5% and -10% for treatments RSW, RSK, BSW and BSK, respectively. In contrast, a significant (P<0.001) and large increase in zinc content was observed in RSW, RSK, BSW and BSK (702%, 736%, 1444% and 994%) respectively. A much smaller increase occurred with treatments GW and GK.

The iron content in the raw seed collected from Tilili was significantly reduced (P<0.01) by all of the processing treatments; RSW, RSK, BSW, BSK, GW and GK produced reductions of -16%, -30%, -59%, -47%, -27% and -39%, respectively. Manganese and magnesium contents were also reduced from the value in the raw seed by the same treatments.

As shown in Table 2, the effect of sampling site on total alkaloid, zinc, manganese and magnesium contents was significant at P<0.001, while for iron content this was at P<0.05. Similarly, the processing method significantly affected the content of each parameter tested at P<0.001 when compared with the raw seeds. Moreover, there was a processing by sampling site interaction which was significant at P<0.001 for total alkaloid, iron, zinc and manganese contents, while for magnesium content this was at P<0.01.

Fatty Acid Profile

At both sampling sites unsaturated fatty acid content was higher than that of saturated fatty acids; the oil extracted from the raw *L. albus* seeds collected from Dangla was composed of 10.6% saturated and 75.5% unsaturated fatty acids. Similarly, the oil extracted from the Tilili seed had

Table 1 Content of iron, zinc, manganese, magnesium, total alkaloids and hull size of raw *Lupinus albus* L. seeds from two sites in Ethiopia

Mineral	Raw *L. albus* from Dangla	Raw *L. albus* from Tilili	Level of significance
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	Not significant
Magnesium	8.93 ± 0.19[b]	9.46 ± 0.08[a]	*
Total alkaloids	2.46 ± 0.02[a]	2.26 ± 0.01[b]	**
Hull size (mm)	16.22 ± 0.39[b]	19.30 ± 0.24[a]	**

Values are in mg/100g dry weight for mineral analysis and g/100g for total alkaloids
Means in the same row with different superscripts differ significantly; * = P<0.05, ** = P<0.01; *** = P<0.001

Table 2 Effect of traditional processing on the content of total alkaloids (g/100g) and iron, zinc, manganese and magnesium (mg/100g) in *Lupinus albus* L. from two sites in Ethiopia

Processing method	Sampling site	Total alkaloids	Iron	Zinc	Manganese	Magnesium
None (raw seed)	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	***	***	***	***	**

RSW: Roasted then Soaked Whole seed
RSK: Roasted then Soaked Kernel
BSW: Boiled then Soaked Whole seed
BSK: Boiled then Soaked Kernel
GW: Germinated Whole seed
GK: Germinated Kernel
* = P<0.05, ** = P<0.01; *** = P<0.001

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

Processing method	Sampling site	C 14:0	C 16:0	C 18:2 (n-6)	C 18:1 (n-9)	C 18:0	C 20:0	Saturated fatty acids	Unsaturated fatty acids
None (raw seed)	Dangla site	0.50	6.90	16.20	59.30	2.00	1.20	10.60	75.50
	Tilili site	0.00	6.30	14.60	59.80	2.00	1.40	9.70	74.40
RSW	Dangla site	1.90	7.10	15.80	60.41	1.90	5.00	15.90	76.21
	Tilili site	1.84	6.92	15.64	59.67	1.75	4.65	15.20	75.31
RSK	Dangla site	0.40	7.20	15.90	61.40	2.00	1.20	10.80	77.30
	Tilili site	0.38	6.67	14.76	60.09	1.87	0.98	9.90	74.85
BSW	Dangla site	0.00	6.30	14.90	60.00	2.00	1.40	9.70	74.90
	Tilili site	0.40	6.60	14.90	61.00	2.00	1.30	10.30	75.90
BSK	Dangla site	0.00	7.00	16.00	61.40	2.00	1.20	10.20	77.40
	Tilili site	0.00	5.40	12.00	41.50	8.50	0.90	14.80	53.50
GW	Dangla site	0.00	5.70	22.22	55.70	1.50	1.40	8.60	77.92
	Tilili site	3.00	5.70	19.30	55.10	2.10	1.40	12.20	74.40
GK	Dangla site	1.10	5.40	19.60	54.20	1.40	1.10	9.00	73.80
	Tilili site	0.00	5.80	20.70	56.70	1.60	1.30	8.70	77.40
Main effects	Site	***	***	***	***	***	***	***	***
	Processing	***	***	***	***	***	***	***	***
Interaction	Processing x Site	***	***	***	***	***	***	***	***

For the fatty acid analysis, composition as a percentage of total lipids was used for comparison.
RSW: Roasted then Soaked Whole seed RSK: Roasted then Soaked Kernel
BSW: Boiled then Soaked Whole seed BSK: Boiled then Soaked Kernel
GW: Germinated Whole seed GK: Germinated Kernel
*** = P<0.001

9.7% saturated and 74.4% unsaturated fatty acids (Table 3). Also at both sites oleic acid (C18:1, n-9) was the predominant fatty acid, accounting for 59-60% of total lipids while the essential fatty acid linoleic acid (C18:2, n-6) accounted for 16.2% and 14.6% of total lipids in the Dangla and Tilili samples respectively.

DISCUSSION

Total alkaloids

The alkaloid content of the seeds from Dangla and Tilili was significantly different. All of the treatments reduced the total alkaloid content of seeds from both sampling sites. Since alkaloids are water-soluble, they are easily removed by soaking in water. However, the extent of removal depends on the type of soaking solution and the permeability of the hull's cell wall (Jimenez-Martinez *et al*., 2003). The traditional methods of processing which involve roasting and boiling, increase permeability of the hull and facilitate alkaloid removal (Getachew *et al.,* 2012). Adewusi and Falade (1996) also reported improvement in

hull permeability of *L. albus* after thermal and soaking treatments. In fact in the present study, with all the processing methods there was a significantly greater reduction in total alkaloid content with de-hulling than in whole seeds. This may indicate the presence of alkaloids in the hull as well as in the kernel. With roasting and boiling treatments, the hull is damaged, which facilitated the removal of alkaloids as shown in Table 2. However, in the case of germination where no heat treatment was applied, the permeability of the hull was not altered resulting in higher content of alkaloid.

Iron

Iron content of the Dangla and Tilili cultivars was significantly different. For different cultivars of raw *L. albus*, iron content has been reported to be in the range of 3.5 to 7.7mg/100g (Trugo *et al*., 1993 and EHNRI, 1997), while in the present study they were around 6 mg/100g. With seeds from both sites, soaking after roasting or boiling and also germination reduced the iron content by 14-29% (Table 2). In other studies, it has been reported that except for sodium, all the minerals analyzed including iron were reduced by soaking of raw seeds in $NaHCO_3$ solution (Abu-Samaha 1983 and El-Adawy *et al*., 2000). Soaking probably reduces mineral content due to the leaching of water-soluble minerals into the steeping medium and that used in the rinsing process (Bau *et al*., 1999).

Zinc

Again, the zinc content of the seed from the two sites was significantly different (Table 1). Compared to the raw seed, the zinc content of seeds increased with all treatments (Table 2). 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% $NaHCO_3$. It would appear that the mineral content of processed samples depends on the type of soaking solution. As noted above, El-Adawy *et al*. (2000) found that when the soaking solution was $NaHCO_3$, the values of all minerals analyzed were reduced, except for sodium. The soaking solution in this study was tap water, which flows through zinc-coated (galvanized) pipes. Therefore, the observed increase in zinc content may be due to contamination from tap water. Further investigation using zinc-free water (distilled water) is needed to clarify the cause of the rise in zinc content with traditional processing. Meanwhile, among all the treatments, germination showed the least increment in zinc content. This might be an another indication of the lower permeability of the seed hull when germination is used to treat *L. albus*, compared to those involving boiling or roasting with subsequent soaking of the seed.

Manganese

There was no significant difference in the manganese content between the two cultivars. Manganese content of different cultivars of raw *L. albus* has previously been reported to be in the range of 61 to 327mg/100g (Trugo *et al.,*1993), which is high compared to the values in the present study (just less than 60mg/100g). In contrast, El-Adawy *et al.* (2000) has reported an even lower content of manganese (10.8 mg/100g) for raw seeds. The upper limit of safe intake of manganese for human consumption is 5mg/day (National Research Council, 1989). Assuming an intake of 10g lupin per day, the manganese content per day would be 5.8mg and 5.9mg from the raw seeds collected from Dangla and Tilili, respectively. However, except for the germination treatment, the traditional processing techniques significantly reduced the manganese content to a safe intake level. A reduction in manganese content in *L. albus* soaked in $NaHCO_3$ solution was also reported by El-Adawy *et al.* (2000). This might be attributed to an increase in permeability of the hull, which may have enhanced leaching of the mineral into the soaking solution (Jimenez-Martinez *et al.,* 2003).

Magnesium

Compared with the other minerals,a slight reduction in the magnesium content was observed with the traditional processing techniques. Again, the content of magnesium in the raw seed from Dangla and Tilili was significantly different. As with alkaloids, iron and zinc, this difference may be due to various environmental factors including soil pH, drought, soil nutrient composition, heat, etc. Jansen *et al*. (2012) have reported that alkaloid content varies in different lupin cultivars due to the factors mentioned above and in fact, Dangla and Tilili are located in two different temperature zones, Tilili being hotter.

The processing methods applied reduced the content of most of the minerals studied and in fact, in a previous study the crude ash content was also reduced with similar treatments (Getachew *et al*., 2012). This implies that most of the inorganic materials including minerals have been leached out of seeds by the soaking solution. On the other hand, these treatments were effective in reducing the alkaloid contents from the raw seed, which makes them mandatory to apply. The recommended daily amount (RDA) of iron and magnesium could still be met by either increasing the quantity of *L. albus* consumption or complementing it with other food sources.

Fatty Acid Profile

Uzun *et al*. (2006) reported major saturated fatty acids in raw *L. albus* seeds to be arachidic acid (C20:0) at 3.5%, palmitic acid (C16:0), 7.6-10% and stearic acid (C18:0), 1.5%. The same authors found that unsaturated fatty acids included linoleic acid (C18:2 n-6) at 20.3% and oleic acid (C18:1 n-9), 47.65%. The content of oleic acid and C18.0 given by these authors are lower than the values found in the current study, while the C16:0, C18:2 n-6 and C20:0 contents from their study are higher than those found in seeds from either site in the present study. Similar results to those found here 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 groundnut and rapeseed oil, but does

not contain any erucic 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 sampling sites in C18:2 content (P<0.05). Germination increased the C18:2 content of whole seeds and kernels from Dangala by 27% and 17%, respectively. With seeds from Tilili, C18:2 content increased with the germination treatment by 24% in whole seeds and by 29% in kernels. Mittal *et al.* (2012) reported that germination resulted in a 48.4% increase in linoleic acid. The increase in C18:2 content due to germination might be related to lipoxygenases activity in the plant. Linolenic acid acts as a substrate during the activity of lipoxygenase but it is a polyunsaturated fatty acid and is resistant to the attack of the enzyme. According to Vasishta and Srivastava (2012), the more double bonds a fatty acid chain has in the cis-configuration, the less flexibility it has.

Oleic acid (C18:1, n-9)

There was no significant difference in C18:1 content between the two sampling sites. In whole seed from both sites, roasting and soaking (RSW), which effectively reduced the alkaloid content, increased the C18:1 content in the range of 1-3%. In contrast, germination reduced the C18:1 content of the raw seed of both cultivars by 5-9%. This reduction may be attributed to a reduction in crude fat content due to growth of the seed on germination. This might also reduce the content of other major fatty acids such as C18:1.

Myristic acid (C14:0))

In raw seeds from both sampling sites the percentage C14:0 content was the lowest of the six fatty acids analysed but there was no significant difference between sites. In addition, in neither cultivar did the different treatments show any significant difference in myristic acid content. This fact needs further investigation.

Palmitic acid (C16:0)

There was no significant difference between the two lupin cultivars in the percentage of C16:0 content. With seed from Dangla the germination treatment reduced the C16:0 content by 21% and 27% in whole seeds and in kernels respectively while with the cultivar from Tilili germination reduced the C16:0 content by 10% and 8% in whole seeds and in kernels. Again, germination of the raw seed may cause a reduction in some fatty acids due to the reduction of crude fat content associated with the production of proteins, required for growth; the synthesis of protein require energy sources such as lipids and carbohydrates. Thus, as a seed germinates, lipids are utilized as a source of energy which reduces the crude fat content (Bau *et al*., 1999). A drastic reduction in palmitic acid levels has also been reported in soybean during later stages of germination (Vasishta and Srivastava, 2012).

Stearic acid (C18:0)

There was no significant difference between the two sampling sites in C18:0 content (P<0.05). Germination reduced the C18:0 content by 25% in whole seeds and by 30% in kernels of seeds from Dangla. In the Tilili cultivar, germination reduced kernel stearic acid content by 25% but it increased slightly in whole seeds. The reduction in stearic acid due to germination probably arises for the same reason mentioned for C16:0, above. The boiling and soaking treatment showed a 76% increase in C18:0 content of kernels, but with whole seeds it was unchanged.

Arachidic acid (C20:0)

There was no significant difference in C20:0 content between the two cultivars. The roasting then soaking treatment which was found to be effective in reducing alkaloid content, increased the C20:0 content of whole seeds by 76% (Dangla) and 42% (Tilili), respectively.

CONCLUSIONS

The traditional processing methods of soaking *L. albus* seeds after roasting or boiling result in minimal loss of nutrients and effective reduction of alkaloids. Also these processes provide increased amounts of unsaturated fatty acids, while the amount of saturated fatty acids are reduced. These methods are therefore recommended as effective processing methods.

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Conflict of interest **None**

REFERENCES

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

Francis, C.M. (1999). New crops and oilseeds from Ethiopia and elsewhere. *The Australian New Crops Newsletter*, 11 (17).

Bau, H. M., Villaume, C., Nicolas, J. P. and Mejean, L. (1999). Effect of germination on chemical composition, biochemical constitutes 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*, 36:251-255

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. *Quality of Plant Foods Human Nutrition,* 36:119-137.

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 plant. 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 poisonous plant research. *Journal of Agricultural and Food Chemistry*, 52(11): 3211-3230.

Jansen G., Jürgens H. U., Schliephake E., Ordon F. (2012) Effect of the Soil pH on the Alkaloid Content of *Lupinus angustifolius*. *International Journal of Agronomy* doi:10.1155/2012/269878

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 anti-nutritional 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, H.P.S., Sharma, P., Sharma, S. (2012). Effect of processing on chemical composition and anti-nutritional 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 characteristics 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). Bitkiselyagteknolojisi. Erzurum: Ataturk University, Turkey.

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

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.

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 (*Eleusine coracana*) 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 one meal by pigs. *Journal of the Science of Food and Agriculture*, 34:122-128.

Trugo, L. C. (1993). Effect of germination on nutritive value of lupine seeds. VII International Lupin Conference. 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.

Van der M. and Somaatmadja, S. (1992). Plant resources 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. and 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.