

# Effect of Some Traditional Processing Methods on Nutritional Composition and Alkaloid Content of Lupin Bean

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## Abstract

The sweet and bitter lupin bean were processed by traditional common processing methods soaking, cooking, fermenting and germinating techniques. The proximate, mineral and alkaloid content of unprocessed, soaked, fermented, germinated and cooked sweet as well as bitter lupin were determined. According to the results crude protein and carbohydrate were significantly highest in soaked and cooked than in fermented and germinated lupin bean. Fiber content, fat content and total ash were significantly reduced in cooked, soaked and fermented bean, but fiber and total ash significantly increased for the germinated sweet and bitter lupin. In the sweet lupin K, Zn, Fe levels were significantly reduced in soaked, fermented and cooked bean, but Na level was significantly highest in germinated, soaked and cooked except in fermented lupin bean. For the bitter lupin K level was significantly increased in soaked, cooked, fermented and germinated bean. But Ca and Na level significantly increased in cooked bean only. Fe and Zn significantly reduced in, cooked, soaked, fermented and germinated. Alkaloid content of the bean was significantly ( $p < 0.05$ ) reduced in soaked, cooked, fermented and germinated, but it was highly influenced by cooking and soaking methods. The results indicate that cooking and soaking enhanced the nutrient contents and drastically reduced the main anti-nutrient of lupin bean alkaloid content.

**Keywords:** Nutrients, processing, alkaloid, Lupin bean

## 1. INTRODUCTION

The genus *Lupinus L.* (common name lupine or lupin) belongs to the subfamily Papilionaceae of the Leguminosae family of flowering plants (Belteky B, Koracs, 1984). There are 450 species (Moneret-Vautrin D-A, 1999), which incorporates 874 named members of the genus *Lupinus L.* Four species have been cultivated for animal and human consumption, which includes both "bitter" and "sweet" varieties of *L. angulstifolia* (blue or narrow-leaf lupin), *L. albus* (white lupin) and *L. luteus* (yellow lupin) from Europe, and *L. mutabilis* (pearl or tarwi lupin) from South America. Lupin has been used as a food for humans and livestock for over 2000 years (Gladstone's JS, 1970). In recent years lupin seed appears particularly promising as a source of innovative ingredients having high protein content (34-43% of dry matter) and an acceptable composition of essential amino acids. Moreover lupin protein concentrates and isolates exhibit useful techno-functional properties (D'Agostina et al, 2006) allowing their use in the production of several food products, such as biscuits, pasta, and beverages.

There are many toxic alkaloids present in *lupinus spp*, including pyrrolizidine and piperidine alkaloids (Panter KE et al, 1998). However, in the species of agricultural interest the toxic compounds of general concern, the quinolizidine alkaloids are commonly referred to as "lupin alkaloids". This class of molecules is characterised by the presence of one or two quinolizidine rings in the structure. There have been approximately seventy different quinolizidine alkaloids identified in *lupinus spp*. Quinolizidine alkaloids are not limited to *lupinus spp* and have been found in many genres of the pea (*Fabaceae*) family as well as several other families (Keeler RF, 1989). The frequency and distribution of lupin alkaloids varies according to species. The major alkaloids in *L. albus* are lupanine, 13-hydroxylupanine, and some European varieties contain sparteine. The major alkaloids in *L. angustifolius* are lupanine, 13-hydroxylupanine, and angustifoline (Pettersen DS, 1995).

Heath Authorities of some countries (Great Britain, France, Australia and New Zealand) have decided to regulate the quinolizidine alkaloid content in lupin flours and foods fixing the maximum limit to 200 mg/kg (ANZFA, 2001). The study totally aimed at the effect of cooking, soaking, fermenting and germinating on the alkaloid content and nutritional composition of bitter and sweet lupin bean.

## 2. MATERIALS AND METHODS

**2.1. Material collection and preparation:** One local bitter lupin variety (239056) and sweet lupin variety (welela) Samples were taken from the highland pulse breeding program of Holeta Agricultural Research Center of Ethiopian Institute of Agricultural Research. These samples were graded, sorted and cleaned manually and tagged for further treatment. They were treated under processing methods: soaking, Boiling (cooking) and germinating and untreated sample also used as control from both sample. 200g of the bitter and sweet lupin bean samples were

used for each the treatments of processing methods.

**2.1.1. Soaking:** Soaking is one of traditional processing method which influences the product positively and negatively as described by (Akinyele, I.O and A.Akinolostu, 1991). The dried beans placed into the pot and the entire pot was filled with fresh, clean, cold water. The more water over the beans the better. The beans were soaked for at least 24 hours. At the end of 24 hours, the beans were fully replaced by fresh water. Again for the next morning the water was drained from the beans and rinsed thoroughly with cold, clean water. The beans were placed back into your soaking pot and the entire pot filled with fresh, clean water. The water was changed and the beans rinsed again in the evening. Rinsing process was repeated “twice a day” (once in the morning and once in the evening) for six days or until the beans were no longer bitter. Then the beans were washed and dried for three days at 50°C in oven. After that the dried sample was milled into fine by passing through 0.5mm sieve size with cyclone sample miller.

**2.1.2. Cooking:** As (Khokhar, S. and Chauhan, B.M., 1986) cooking has significant effect on nutritional and anti-nutrition of legume beans. The 200g of cleaned lupin bean was boiled into Philips dish cooker by adding 1500ml of water in which the cooker is adjusted 150 °C for 30 minutes. Then after the bean is dried 50°C for three days and milled into fine flour by passing through 1mm sieve size cyclone miller. Then flour was labeled for further analysis.

**2.1.3. Germinating:** Lupin bean were cleaned and soaked in water for 24 hours at room temperature. The hydrated seeds were spread on trays and covered with clean polyethylene sheet. Germination went on for three days in an incubator at 25°C and later lupin bean were dried at 50°C for further three days. After that the formed roots and testa were rubbed off. Dried, germinated seeds were ground and passed through 1 mm mesh screen to get fine flour (Dagnia S.G, 1992). Then the flour was made ready for another further analysis.

**2.1.4. Fermenting:** This method is one of the traditional processing method by which we can improve our food products. Fermented lupin bean flour was produced by subjecting the both the sweet and bitter bean to natural lactic fermentation as described by (Hallen E, 2004). Lupin bean were cleaned and ground and passed through a 1 mm mesh screen. The flour was then mixed with water (1:4) to form slurry followed by addition of 5% salt by weight of flour. The slurry was left to ferment in incubator at 25°C for four days. The fermented slurry was dried at 50°C and ground to get fermented lupin bean flour. And the flour is subjected to nutrient, alkaloid and mineral analysis.

**2.2. Proximate Composition:** Proximate composition of the whole lupin bean and the processed bean samples were determined using the (AOAC, 2005). The moisture content (MC) was determined by drying samples in an oven at 105°C for 24 hours to obtain %MC. Crude protein percentage was determined using the Kjeldahl method with the SBS 2000 analyzer unit (FoodALYT, Germany) and the percentage nitrogen (%N) obtained was used to calculate the percentage crude protein (% CP) using the relationship:  $\% CP = \% N \times 6.25$ . Ether extract percentage was determined using Soxhlet system Tecator-1050 extractor technique. The percentage ash (%) was determined by incinerating the samples in a muffle furnace at 550°C for 4hrs. The ash was cooled in a desiccator and weighed. Crude fiber percentage (% CF) was determined by dilute acid and alkali hydrolysis. Carbohydrate was calculated by difference including fiber.

**2.3. Alkaloid content:** Over 500 species of the genus *Lupinus* are known. In its raw form, the mildly toxic lupin alkaloids present in plants causes a bitter taste, and used as defensive mechanism for herbivorous (Hebourn JB, 1973). Alkaloids were commonly removed (or reduced) by soaking the raw seeds in water prior to use. Alkaloid content was determined by weighing 5g of the lupin bean flour on balance and dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 h before it was filtered. The filtrate was then evaporated to one quarter of its original volume on 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 it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried in an oven at 60°C for 30 min, transferred into desiccators to cool and then reweighed until a constant weight was obtained. The constant weight was recorded. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed (Hebourn JB, 1989).

**2.4. Mineral Content Analysis:** For mineral determination, dry and ashing method of the all samples were carried out according to the method (Jones JR et al, 1990). Calcium, magnesium, sodium potassium, Zink and iron were determined by atomic absorption spectrophotometer of (Agilent AAS series 200, USA).

**2.5. Data analysis:** The traditional processing method efficiency as well as the the bean flour nutritional composition test results of treatments were analyzed by one way ANOVA (Analysis of Variance) using statistical tools of SAS version 20 (SAS. Statistical Analysis System, 2004). Significance was accepted at 0.05 level of

probability ( $p \leq 0.05$ ). Mean separation was performed by “Each pair student’s t-test” for multiple comparison of means.

### 3. RESULTS AND DISCUSSION

**3.1. Proximate composition:** proximate analysis of composite flour Table 1 shows that moisture content of composite flours did not differ significantly by different types of sweet lupin bean flours. Maximum moisture was found in control while the flour of fermented lupin bean flour gives the lowest moisture contents. Unprocessed bean flour used in this study had protein contents of 25.24% and the protein contents for processed bean flours ranged from 25.45 to 26.73%. Protein contents of soaked, fermented, germinated and cooked lupin bean were higher than the unprocessed bean raw lupin bean. Ash contents unprocessed and germinated sweet lupin flour were found to be considerably higher than those of processed by other methods. However, the ash content of the others have no significant difference. Fat content result shows the decreasing trend for all processed bean and it ranges from 7.17 – 9.29%. Fiber content shows an increasing trend except for cooked and soaked bean. The CHO content of unprocessed and processed sweet lupin bean was not significantly different except for germination method which was 29.69% mean value.

**Table 1: Proximate compositions of processed and unprocessed of bitter (local) lupin bean.**

Processing methods	Proximate analysis parameters for sweet lupin					
	CP	MC	Fiber	Fat	Ash	CHO
Cooked	25.5±1.14 <sup>b</sup>	10.2±0.55 <sup>a</sup>	17.6±0.90 <sup>b</sup>	9.1±0.51 <sup>a</sup>	3.3±0.04 <sup>b</sup>	34.5±2.63 <sup>a</sup>
Fermented	24.9±0.70 <sup>c</sup>	9.5±0.77 <sup>a</sup>	18.4±0.59 <sup>b</sup>	8.2±0.46 <sup>a</sup>	3.1±0.29 <sup>b</sup>	36.0±1.59 <sup>a</sup>
Germinated	26.5±0.73 <sup>ab</sup>	11.4±0.61 <sup>a</sup>	21.4±0.60 <sup>a</sup>	7.2±0.84 <sup>b</sup>	3.8±0.19 <sup>a</sup>	29.7±0.57 <sup>b</sup>
Soaked	26.7±0.46 <sup>a</sup>	9.5±2.06 <sup>a</sup>	15.3±1.04 <sup>c</sup>	8.9±0.15 <sup>a</sup>	3.1±0.02 <sup>b</sup>	36.4±3.07 <sup>a</sup>
unprocessed	25.2±0.43 <sup>bc</sup>	9.7±1.11 <sup>a</sup>	18.3±0.75 <sup>b</sup>	9.3±0.54 <sup>a</sup>	3.6±0.02 <sup>a</sup>	33.9±0.39 <sup>a</sup>

CP: Crude Protein, MC: Moisture Content, CHO: Carbohydrate, a-c: means in the same column with varying superscripts differs significantly at ( $p < 0.05$ ).

Proximate analysis of bitter (local) lupin bean Table 2 shows that CP content of cooked, soaked, and germinated bitter bean increasing trend except for fermented bean flour. Which 41.25% mean is higher value of CP and 35.62% is the lowest mean value. MC did not differ significantly by different types of processing methods. Maximum moisture was found in control while the cooked bitter bean gives the lowest moisture contents. Fiber content shows the decreasing trend through all processing methods which range from 14.50% mean value to 11.66% mean value. But fermented bean shows the most fiber content improvement. Fat content results show the decreasing trend for all processed bitter beans that ranges from 11.22% unprocessed means to 9.24% germinated bean mean value. However, the ash content of different processing method differs significantly. But in terms of germination method ash content has higher mean value (3.32%) than the others. The fermented bean was low in ash content 2.90% mean value. In case of CHO content there was no significant difference for cooking, fermenting and germination methods with 26.82%, 26.85%, 25.26% mean values respectively. However the soaked bean mean value (22.15%) is significantly different from the others. In general CHO shows increasing trend in all processing methods. The results show that the trend of literature reported by other study paper of (S.O.Omoikhoje, 2006).

**Table 2: Proximate compositions of processed and unprocessed of bitter (local) lupin bean.**

Processing methods	Proximate analysis parameters for bitter (local) lupin					
	CP	MC	Fiber	Fat	Ash	CHO
Cooked	40.6±0.37 <sup>a</sup>	7.6±0.35 <sup>b</sup>	11.7±0.76 <sup>b</sup>	10.4±0.24 <sup>b</sup>	2.9±0.03 <sup>b</sup>	26.8±0.68 <sup>a</sup>
Fermented	35.6±0.71 <sup>c</sup>	10.4±0.69 <sup>a</sup>	13.1±0.66 <sup>b</sup>	11.1±0.43 <sup>ab</sup>	2.9±0.12 <sup>b</sup>	26.9±0.20 <sup>a</sup>
Germinated	40.7±1.00 <sup>a</sup>	9.5±0.41 <sup>a</sup>	11.9±0.45 <sup>b</sup>	9.2±0.73 <sup>c</sup>	3.3±0.04 <sup>a</sup>	25.3±1.25 <sup>a</sup>
Soaked	41.3±0.72 <sup>a</sup>	10.3±0.43 <sup>a</sup>	12.7±0.81 <sup>b</sup>	10.6±0.17 <sup>ab</sup>	2.9±0.10 <sup>b</sup>	22.2±1.62 <sup>b</sup>
unprocessed	39.1±0.76 <sup>b</sup>	10.5±0.62 <sup>a</sup>	14.5±1.00 <sup>a</sup>	11.2±0.10 <sup>a</sup>	3.2±0.01 <sup>a</sup>	21.5±0.93 <sup>b</sup>

CP: Crude Protein, MC: Moisture Content, CHO: Carbohydrate, a-c: means in the same column with varying superscripts differs significantly at ( $p < 0.05$ ).

**3.2. Mineral analysis:** The mineral contents of various processing methods for sweet lupin bean are shown in Table 3. The unprocessed value of K was 142% which decreased gradually by processing treatment. But the processing methods were significantly different in their efficiency. Zn mean value shows the non-significant difference between the processing methods. Na content mean reflects the increasing trend in mean value except for the fermented bean. Therefore there was a significant difference among the methods. The unprocessed mean value of Ca content was 98.76% which decreased after the four processing methods. However there was no

significant difference between the processing methods except for fermenting method. The Fe content mean value shows the decreasing mean for the different methods of processing that the unprocessed mean was 9.06% but decreased to 3.19% after processed. But soaking and cooking methods were not significantly different except the other two.

**Table 3: Mineral content of processed and unprocessed sweet lupin bean**

Processing Methods	Mineral analysis parameters for sweet lupin				
	K	Zn	Na	Ca	Fe
Cooked	84.40±0.74 <sup>c</sup>	5.31±0.64 <sup>a</sup>	117.1±2.31 <sup>b</sup>	89.23±0.65 <sup>b</sup>	4.15±0.33 <sup>c</sup>
Fermented	79.10±0.28 <sup>d</sup>	4.28±0.09 <sup>a</sup>	98.85±0.35 <sup>d</sup>	79.62±0.70 <sup>c</sup>	3.19±0.03 <sup>d</sup>
Germinated	140.41±2.0 <sup>a</sup>	5.43±0.09 <sup>a</sup>	104.37±2.68 <sup>c</sup>	90.48±1.39 <sup>b</sup>	5.34±0.31 <sup>b</sup>
Soaked	103.58±2.61 <sup>b</sup>	4.74±0.65 <sup>a</sup>	139.65±1.72 <sup>a</sup>	91.54±1.29 <sup>b</sup>	4.46±0.24 <sup>c</sup>
unprocessed	142.46±1.18 <sup>a</sup>	5.36±0.33 <sup>a</sup>	102.95±0.45 <sup>cd</sup>	98.76±0.56 <sup>a</sup>	9.06±0.24 <sup>a</sup>

a-c: means in the same column with varying superscripts differs significantly at (p<0.05).

The mineral contents of various processing methods for local lupin bean are shown in Table 4. The processing methods highly influenced the mineral content of bitter lupin. Because the unprocessed bean K content mean was 2.47% which gradually increased to 126.0% mean value after processed. The Zn content shows decreasing trend as processed by different methods. But there was no significant difference between the processing methods except fermenting method in which the minimum Zn content mean recorded. The Na content mean value shows higher value for cooking methods but shows decreased trend for the other methods. This means cooking method was most effective than others in improving Na content of the bean. Ca content mean was decreased through processing except for cooking method in which the unprocessed mean 77.18% increased to 77.76%. Cooking also improves Ca content of the lupin bean. Fe result shows decreased trend in all methods during processing. This reflects that the processing methods have negative impact on the Fe content that zero processing mean 51.18% highly decreased to 12.42% minimum value differently. But there were significant difference between the means.

**Table 4: Mineral content of processed and unprocessed bitter (local) lupin bean.**

Processing Methods	Mineral analysis parameters for bitter (local) lupin bean				
	K	Zn	Na	Ca	Fe
Cooked	126.0±0.44 <sup>a</sup>	9.07±0.28 <sup>b</sup>	160.20±1.17 <sup>a</sup>	77.76±0.33 <sup>a</sup>	35.88±1.35 <sup>b</sup>
Fermented	63.6±0.64 <sup>c</sup>	7.42±0.29 <sup>c</sup>	110.70±0.71 <sup>d</sup>	69.04±0.43 <sup>b</sup>	23.22±0.47 <sup>c</sup>
Germinated	111.5±1.66 <sup>b</sup>	9.16±0.48 <sup>b</sup>	100.65±1.72 <sup>c</sup>	63.14±1.15 <sup>c</sup>	12.79±0.28 <sup>d</sup>
Soaked	56.91±0.36 <sup>d</sup>	8.97±0.42 <sup>b</sup>	131.56±0.07 <sup>c</sup>	67.83±0.75 <sup>b</sup>	12.42±0.36 <sup>c</sup>
unprocessed	32.47±0.00 <sup>e</sup>	10.47±0.79 <sup>a</sup>	145.80±1.27 <sup>b</sup>	77.18±0.45 <sup>a</sup>	51.18±0.65 <sup>a</sup>

a-d: means in the same column with varying superscripts differs significantly at (p<0.05).

**3.3. Alkaloid content:** The determination of alkaloids in the lupin bean samples were carried out by employing previously reported techniques (Hebourn JB, 1973). The results which were the mean values of three replicate determinations are presented in Table 5. The range of the percentage alkaloids present in the unprocessed and processed sweet bean was from 1.76 – 0.31%. This result shows that the alkaloid content of the bean decreased by more than half after processing treatments. The efficiency of cooking, fermenting and soaking were almost no significant difference except germination method in which higher alkaloid content mean was recorded. The alkaloid content of bitter lupin bean also shows decreasing trend to each processing methods which ranges from 6.03% to 3.78%. But we could saw that the cooking and soaking methods were more effective than the others in decreasing alkaloid content and improving the nutritional quality of the bean. The result was in agreement with previous literature report that tubers and plant leaves contain a substantial proportion of alkaloids (Oke OL, 1966).

**Table 5: Alkaloid content of processed and unprocessed lupin bean.**

Processing methods	Sweet lupin	Bitter(local) lupin
	Alkaloid	Alkaloid
Cooked	0.76±0.36 <sup>b</sup>	4.60±0.22 <sup>bc</sup>
Fermented	0.59±0.43 <sup>b</sup>	4.66±0.48 <sup>b</sup>
Germinated	1.51±0.24 <sup>a</sup>	5.99±0.59 <sup>a</sup>
Soaked	0.31±0.31 <sup>b</sup>	3.78±0.71 <sup>c</sup>
unprocessed	1.76±0.36 <sup>a</sup>	6.03±0.21 <sup>a</sup>

a-c: means in the same column with varying superscripts differs significantly at (p<0.05).

#### 4. CONCLUSION

The sweet and bitter lupin bean were processed by traditional common processing methods soaking, cooking,

fermenting and germinating techniques. Results obtained from these treatments were significantly compared to conclude the overall study. The results from the study indicate that soaking and cooking processing methods were highly efficient in improving nutritional quality and reducing alkaloid contents of lupin bean. These processing were highly important for bitter bean than sweet to make palatable it for food. Therefore after processing it was good to consume the lupin bean food products for human consumption.

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