Effect of Processing Methods on Nutritional and Alkaloid Content of Lupine Varieties

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Abstract

Beans of sweet and bitter lupine varieties were processed using soaking, cooking, fermentation and germination techniques and their effect on nutritional composition and alkaloid contents were evaluated. Standard methods of analysis were used to determine proximate composition, mineral and alkaloid contents. According to the results, all processing methods, cooking, fermentation, germination and soaking significantly affected nutritional compositions and alkaloid contents of sweet and bitter lupine bean varieties ($p<0.05$). From the study, it can be concluded that soaking, cooking and fermentation methods were highly efficient in improving nutritional quality and reducing alkaloid contents. These processing techniques were particularly recommended to improve taste of bitter lupine bean varieties, where bitterness is one of the major challenges affecting consumption of the crop.

Introduction

Lupine is a member of the genus Lupinus, family Leguminosae, subfamily Papilioniodeae (Gladstones, 1998). Lupinus is a large and diverse genus comprising 200 500 annual and perennial herbaceous species, mostly with a height of 0.3 1.5 m and attractive long flowers (Ainouche and Bayer, 1999). Having high protein content, well-balanced amino acids, good source of dietary fiber and good techno-functional properties, lupine seed appears to be promising source of innovative food ingredients in recent years. It is also cholesterol free and proved to contain negligible amounts of trypsin inhibitors. The development of new food crops from Lupinus is used to illustrate the problems associated with heat stable low molecular weight anti-nutritional factors. These substances include proteolytic inhibitors, phytohemagglutinins, lathyrogens, cynogenetic compounds, compounds causing favism, factors affecting digestibility and saponins. These factors are shown to be widely present in leguminous

population, and particularly, of people in the developing countries (Paurl *et.al*. 2014).

There are many toxic alkaloids present in Lupinus spp, including pyrrolizidine and piperidine alkaloids (Aurelie *et. al*., 2017). In the species of agricultural interest, the toxic compounds of general concern, the quinolizidine alkaloids are commonly referred to as lupine alkaloids. This class of molecules is characterized by the presence of one or two quinolizidine rings in the structure. In its raw form, the mildly toxic lupin alkaloids present in plants causes a bitter taste, and used as defensive mechanism for herbivorous (Harborne *et.al*.,1973).

Traditionally, households soak the beans for 1 3 days, during which some microbial activities place, leading to improvement of the nutritional quality of the resulting flour. An investigation by Agume *et al*. (2016) revealed a positive effect of long-time soaking in reducing the anti-nutrients and the viscosity of maize flour. The nutritional and pasting properties of maize flours were also significantly affected by the interaction of soaking and roasting. In Ethiopia, lupin is not common as a food and the major limitation is its bitterness. There are many local processing methods believed to reduce its bitterness and deserve scientific validations. The objective of this study was, therefore, to evaluate the effect of soaking, cooking, germinating and fermenting on the nutritional and alkaloid content of bitter and sweet lupine varieties in Ethiopia.

Materials and Methods

Sample collection

A 200 g lupin bean samples of each bitter *(239006)* and sweet *(welela)* varieties were collected from Holetta Agricultural Research Center. The samples were sorted and cleaned manually to make ready for different processing methods.

Processing methods

Soaking: The beans were placed into pot filled with clean water and soaked for 24 hours. Then, the water was drained from the beans and rinsed thoroughly after 24 hrs. The beans were placed back into the soaking pot and the entire pot filled with fresh water. The water was changed and the beans rinsed again in the evening. Rinsing process was repeated in the morning and evening for six days, until the beans were no longer bitter. Then, the beans were washed and dried for three days in an oven at 50°C. Cyclone sample miller milled the dried sample by passing through 0.5 mm sieve size.

Cooking*:* The 200g cleaned lupin beans were boiled into dish cooker by adding 1.5 litter of water at 150°C. After 30 minutes, the beans were dried at 50°C for three days and milled into fine flour by passing through 1mm sieve cyclone miller.

Germinating: Cleaned lupin beans were soaked in water for 24 hours at room temperature. The hydrated seeds were spread on trays and covered with clean polyethylene sheet and germination continued for three days in an incubator at 25°C. Then, the beans were dried at 50°C for further three days. The formed roots and testa were rubbed off; dried and germinated seeds were milled to pass through 1 mm mesh screen.

Fermenting: This processing method was undertaken as described by Hallen *et al*. (2004). Clean lupin beans were ground and passed through a 1 mm mesh screen. The flour was then mixed with water (in 1:4 ratio) 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℃ and ground to get fermented lupin bean flour.

Proximate composition

Methods developed by Association of Official Analytical Chemists (AOAC, 2005) were used to determine crude protein, crude fat, crude fiber, and moisture and ash contents of whole lupin bean and flour samples. Moisture content was determined gravimetrically by drying samples in an oven at 105°C for 24 hrs. Crude protein content was determined by the Kjeldahl method with the SBS 2000 analyzer unit (Food ALYT, Germany) using N conversion factor of 6.25. Soxhlet method was used to estimate crude fat. Ash was determined using the combustion method in a muffle furnace at 550 ^{o}C. Carbohydrate content was estimated using the difference method by calculating the value as 100 minus all other proximate components.

Mineral analysis

Samples were prepared using dry and ashing method as described by Jones *et al.* (1990). Then, the content of minerals including, calcium, magnesium, sodium, potassium, zinc and iron were determined by Atomic Absorption Spectrophotometer of Agilent AAS series 200, USA.

Alkaloid content

The procedure developed by Harborne *et.al*. (1989) was followed to estimate alkaloid content of lupin bean flours. A 5g flour was dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture was well shaken and, then, allowed to stand for about 4 hrs. before it was filtered. The filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated ammonium hydroxide was added drop by drop 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 alkaloid content was determined by weight difference of the filter paper. Besides, it is expressed as a percentage of the sample weight analyzed.

Statistical analysis

Data collected to see the effect of different processing methods on nutritional and alkaloid compositions of lupine varieties were analyzed by one way ANOVA (Analysis of Variance) using statistical tools of SAS (SAS, 2004). Significance was

each pair values using t-test for multiple comparison of means.

Results and Discussions

Proximate composition

All processing methods such as cooking, fermentation, germination and soaking are significantly ($p<0.05$) affected proximate compositions of beans of sweet and bitter lupin varieties (Table 1 and 2). Protein and fat contents of bitter lupin variety were higher than the sweet one, while the later was better in fiber, ash and carbohydrate contents. Moisture content was in the range of 7.6-11.4 g/100g and was significantly affected by processing methods only for bitter lupin. A similar effect of processing method on moisture content of maize flour has been reported by Aurelie *et.al*. (2017). Protein contents of soaked, germinated and cooked lupin beans were significantly higher than for the raw beans ($P<0.05$), whereas fermentation resulted in lowest values for both varieties. In agreement with these results, Baik and Han (2012) have reported an increase in protein content of soybean from 1 to 7% due to processing. On the other hand, germination resulted in significantly lower crude fat content than did other processing methods and unprocessed treatment. Ash content of flours from raw and germinated sweet lupin beans was significantly higher than for soaked, fermented and cooked treatments ($P < 0.05$).

Table 1. Effect of processing methods on proximate composition of beans of sweet lupin variety (g/100g).

Processing method	Moisture	Protein	Fiber	Fat	Ash	Carbohydrate
Cooked	$10.2±0.55^{a}$	$25.5±1.14^{b}$	$17.6±0.90^{b}$	$9.1±0.51^{a}$	$3.3±0.04^{b}$	$34.5±2.63^{a}$
Fermented	$9.5±0.77^{a}$	$24.9±0.70^{c}$	$18.4±0.59^{b}$	$8.2±0.46^{a}$	$3.1±0.29^{b}$	$36.0±1.59^{a}$
Germinated	$11.4±0.61^{a}$	$26.5±0.73^{ab}$	$21.4±0.60^{a}$	$7.2±0.84^{b}$	$3.8±0.19^{a}$	$29.7±0.57^{b}$
Soaked	$9.5±2.06^{a}$	$26.7±0.46^{a}$	$15.3±1.04^{c}$	$8.9±0.15^{a}$	$3.1±0.02^{b}$	$36.4±3.07^{a}$
Raw (Unprocessed)	$9.7±1.11^{a}$	$25.2±0.43^{bc}$	$18.3±0.75^{b}$	$9.3±0.54^{a}$	$3.6±0.02^{a}$	$33.9±0.39^{a}$

Means with the same letter in a column are not significantly different (p>0.05)

Table 2. Effect of processing methods on proximate composition of beans of bitter lupin variety (g/100g).

Processing method	Moisture	Protein	Fiber	Fat	Ash	Carbohydrate
Cooked	$7.6±0.35^{b}$	$40.6±0.37^{a}$	$11.7±0.76^{b}$	$10.4±0.24^{b}$	$2.9±0.03^{b}$	$26.8±0.68^{a}$
Fermented	$10.4±0.69^{a}$	$35.6±0.71^{c}$	$13.1±0.66^{b}$	$11.1±0.43^{ab}$	$2.9±0.12^{b}$	$26.9±0.20^{a}$
Germinated	$9.5±0.41^{a}$	$40.7±1.00^{a}$	$11.9±0.45^{b}$	$9.2±0.73^{c}$	$3.3±0.04^{a}$	$25.3±1.25^{a}$
Soaked	$10.3±0.43^{a}$	$41.3±0.72^{a}$	$12.7±0.81^{b}$	$10.6±0.17^{ab}$	$2.9±0.10^{b}$	$22.2±1.62^{b}$
Raw (Unprocessed)	$10.5±0.62^{a}$	$39.1±0.76^{b}$	$14.5±1.00^{a}$	$11.2±0.10^{a}$	$3.2±0.01^{a}$	$21.5±0.93^{b}$

Means with the same letter in a column are not significantly different (p>0.05)

Minerals content

The effect of various processing methods on mineral content of beans of sweet and bitter lupin varieties is shown in Table 3 and 4. It was observed that the mineral contents of both sweet and bitter lupin beans were significantly influenced by different processing treatments. The content of K for unprocessed sweet lupin was 142 mg/100gm and reduced to 140, 103, 84 and 79 due to germination, soaking, cooking and fermentation, respectively (Table 3). In contrast, the value of K for bitter lupin variety increased from 32 mg/100g (unprocessed) to 57, 64, 112 and 126 by soaking, fermentation, germination and cooking methods, respectively (Table 4). The content of Zn for sweet lupin showed non-significant difference due to processing methods, while a slight reduction was observed for the bitter variety. On the other hand, Na and Ca contents of both bean varieties were significantly affected by processing methods, but with inconsistent trends. However, Fe content of beans of both varieties was significantly reduced due to processing treatments, where unprocessed beans, followed by cooking, had higher values than did soaked ones especially for the bitter variety (Table 4). In line with this, Duhan *et al*. (2002) have indicated that the low iron extractability of cooked beans is because of higher phytate levels. The authors further explained that a divalent cation, iron, is generally associates with phytic acid possibly reducing its extractability. Soaking reduces phytic acid, freeing iron, and resulting in higher HCl extractability (Duhan *et al*., 2004).

Table 3. Effect of processing methods

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Table 5. Effect of processing methods on alkaloid content of sweet and bitter lupin bean varieties (%)

Processing method	Sweet lupin	Bitter lupin (%)
Cooked	0.76±0.36[b]	4.60±0.22[bc]
Fermented	0.59±0.43[b]	4.66±0.48[b]
Germinated	1.51±0.24[a]	5.99±0.59[a]
Soaked	0.31±0.31[b]	3.78±0.71[c]
Raw	1.76±0.36[a]	6.03±0.21[a]

Means with the same letter in a column are not significantly different (p>0.05)

Conclusion

Nutritional composition, mineral and alkaloid contents of beans of sweet and bitter lupine varieties processed using soaking, cooking, fermentation and germination techniques were evaluated. Based on the results of this study, it was concluded that soaking, cooking and fermentation methods were highly efficient in improving nutritional quality and reducing alkaloid contents. These processing techniques were particularly recommended to improve the taste of bitter lupin varieties, where bitterness is one of the major challenges affecting consumption of the crop.

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