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

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ABSTRACT

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 reduced in soaked, cooked, fermented and germinated, but it was highly influenced by cooking and soaking methods. The results indicated that cooking and soaking enhanced the nutrient contents and drastically reduced the lupin bean alkaloid content.

KEY WORDS: Nutrients, processing, alkaloid, Lupin bean

1. INTRODUCTION

The genus *Lupinus L.* (common name lupine or lupin) belongs to the subfamily Papillionaceae of the Leguminosae family of flowering plants (Belteky B, Kovacs I, 1984). Lupin has been used as a food for humans and livestock for over 2000 years (Moneret et al, 1999). 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 (Gladstones JS, 1970) 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 (Aurelie S, et al, 2017). 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 characterized by the presence of one or two quinolizidine rings in the structure. The development of new food crops from Lupinus, Vicia and Lathyrus species is used to illustrate the problems associated with heat stable low molecular weight anti-nutritional factors (Parul Bora,2014). These substances include proteolytic inhibitors, phytohemaggluttinins, lathyrogens, cynogenetic compounds, compounds causing favism, factors affecting digestibility and saponins. These factors are shown to be widely present in leguminous foods which are important constituents of the diet of a large section of the world's population, and particularly, of people in the developing countries. Knowledge regarding ways and techniques to lower down or reduce the content of anti-nutritional factors in food is needed for health and wellbeing of the population (Considering the processing methods attempted in the present study, dehulling and soaking significantly increased the levels of Protein availability, but they were ineffective for lectin activity. Cooking methods (without combination with other treatments) differently affected the levels of the anti-nutrient. For combination effect, dehulling following soaking, and cooking methods resulted in increase of nutrient availability. However, soaking following cooking methods had different effects on the levels of antinutrients. In vitro protein digestibilty of raw green and white fababean seeds was improved by all processing methods; soaking-dehulling following autoclaving was the most effective for improving protein digestibility. Even though some treatments like dehulling increased the level of ant nutrients, they improved invitro-digestibility of protein (Yu-Wei L and Wei-Hua X, 2014). In traditional households, the beans are soaked for 1–3 days, during which some microbial activities are

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activated, leading to improvement of the nutritional quality of the resulting flour. Recent investigations revealed a positive effect of long-time soaking in reducing the anti-nutrients and the viscosity of maize flour, but this varied with soaking time. In addition, there was a significant interaction of soaking and roasting on the nutritional and pasting properties of maize flour (Agume et al, 2016). However, this has not yet been investigated in soybean flour.
Food and Agricultural Organization of United Nations (FAO) and other organizations have decided to regulate the quinolizidine alkaloid content in lupin flours and foods fixing the maximum limit to 200 mg/kg (FAO, 2014). 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: Local bitter lupin variety (239006) and sweet lupin variety (welela) Samples were taken from the highland pulse breeding program of Holeta Agricultural Research Center. 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 (Aisha M et al, 2015). 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℃ 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 ℃ for 30 minutes. Then after the bean is dried 50ºC 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 continued for three days in an incubator at 25ºC and later lupin bean were dried at 50ºC for further three days. After that the formed roots and test a were rubbed off. Dried, germinated seeds were ground and passed through 1 mm mesh screen to get fine flour (Myrene R. and D'souza, 2013). 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, et al, 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 25ºC for four days. The fermented slurry was dried at 50℃ 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 official method (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 (Food ALYT, Germany) and the percentage nitrogen (%N) obtained was used to calculate the percentage crude protein (% CP) using the relationship: % CP = % N X 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. CHO%= 100-(MC%+CP%+Fat%+Fiber%+Ash %), where CP=crude Protein, CHO=Carbohydrate, MC=Moisture Content.

2.3. Alkaloid content: In its raw form, the mildly toxic lupin alkaloids present in plants causes a bitter taste, and used as defensive mechanism for herbivorous (Harbourne JB, 1973). 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^{o}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 (Harbourne JB, 1989).

2.3. Mineral Content Analysis: For mineral determination dry and ashing method of 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).

2.4. Data analysis: The traditional processing method efficiency as well as 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 level of probability ($p \leq 0.05$). Mean separation was performed by "Each pair values t-test" for multiple comparison of means.

2. RESULT AND DISCUSSION

3.1. Proximate composition: Proximate analysis of unprocessed and processed bean flour Table 1 shows that moisture content was significantly different at ($p<0.05$). Maximum moisture was found in germinated bean while the flour of fermented lupin bean was the lowest moisture contents. The MC totally ranged 9.7 – 11.4%. A similar effect of processing on the moisture content of maize flour has been reported (Aurelie Solange NA, et al, 2017). 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% and it was significantly different at ($p<0.05$). Protein contents of soaked, fermented, germinated and cooked lupin bean were higher than the unprocessed bean. However Baik and Han reported a lower (1%–7%) increase in the protein of processed soybean (Baik, et al, 2012). In this study CP was lower in mean value. This may be due to the natural fermentation involving multiple microorganisms with variable metabolisms could have contributed to the decrease in proteins (Son, S-J,Lee, S-P, 2011, Tchikoua R, 2016). Ash contents unprocessed and germinated sweet lupin flour was found to be considerably higher than those of processed by other methods. However, the ash content of the others was less significantly different at ($p<0.05$). Fat content result shows the decreasing trend for all processed bean and it ranges from 7.17 – 9.29%. The increase in the crude fat content may result from the destruction of cell structure and the efficient release of oil reserve during roasting (Rajon, et al, 2006). But oil content was not increased in this study due to soaking, cooking, fermentation and germination. 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. The protein content was improved by processing as previously reported by literatures (Cuevas-Rodriguez, et al, 2004).

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^b	10.2±0.55^a	17.6±0.90^b	9.1±0.51^a	3.3±0.04^b	34.5±2.63^a
Fermented	24.9±0.70^c	9.5±0.77^a	18.4±0.59^b	8.2±0.46^a	3.1±0.29^b	36.0±1.59^a
Germinated	26.5±0.73^{ab}	11.4±0.61^a	21.4±0.60^a	7.2±0.84^b	3.8±0.19^a	29.7±0.57^b
Soaked	26.7±0.46^a	9.5±2.06^a	15.3±1.04^c	8.9±0.15^a	3.1±0.02^b	36.4±3.07^a
unprocessed	25.2±0.43^{bc}	9.7±1.11^a	18.3±0.75^b	9.3±0.54^a	3.6±0.02^a	33.9±0.39^a

CP: Crude Protein, MC: Moisture Content, CHO: Carbohydrate, a-c: means in the same column with varying superscript letters 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 differs significantly at ($p<0.05$) by different types of processing methods for sweet lupin. Fiber content shows the decreasing trend through all processing methods which range from 14.50% mean value to 11.66%mean value and it was significantly different at ($p<0.05$). 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 and it was significantly different at ($p<0.05$). However, the ash content of different processing method differs significantly at ($p<0.05$). 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 same report, Baik and Han reported a lower (1%–7%) increase in the protein and starch of roasted and fermented soybean (Son, S-J,Lee, S-P, 2011, Tchikoua R, 2016). The results show that the trend of literature reported by other study paper of (El Maki, et al, 2007).

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^{a}	7.6±0.35^{b}	11.7±0.76^{b}	10.4±0.24^{b}	2.9±0.03^{b}	26.8±0.68^{a}
Fermented	35.6±0.71^{c}	10.4±0.69^{a}	13.1±0.66^{b}	11.1±0.43^{ab}	2.9±0.12^{b}	26.9±0.20^{a}
Germinated	40.7±1.00^{a}	9.5±0.41^{a}	11.9±0.45^{b}	9.2±0.73^{c}	3.3±0.04^{a}	25.3±1.25^{a}
Soaked	41.3±0.72^{a}	10.3±0.43^{a}	12.7±0.81^{b}	10.6±0.17^{ab}	2.9±0.10^{b}	22.2±1.62^{b}
unprocessed	39.1±0.76^{b}	10.5±0.62^{a}	14.5±1.00^{a}	11.2±0.10^{a}	3.2±0.01^{a}	21.5±0.93^{b}

CP: Crude Protein, MC: Moisture Content, CHO: Carbohydrate, a-c: means in the same column with varying superscript letters 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. Boiled beans had the lowest iron

extractability possibly because of higher phytate levels. As a divalent cation, iron, is generally associates with phytic acid possibly reducing its extractability (Duhan A, et al, 2002). Soaking reduces phytic acid, freeing iron, and resulting in higher HCl extractability (Duhan A, et al, 2004, Ghavidel R. A and J.Prakash, 2007). Combined processing (sprouting, dehulling followed by either roasting or steaming) of beans resulted in higher iron extractability than it did for Zinc.

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^{c}	5.31±0.64^{a}	117.1±2.31^{b}	89.23±0.65^{b}	4.15±0.33^{c}
Fermented	79.10±0.28^{d}	4.28±0.09^{a}	98.85±0.35^{d}	79.62±0.70^{c}	3.19±0.03^{d}
Germinated	140.41±2.0^{a}	5.43±0.09^{a}	104.37±2.68^{c}	90.48±1.39^{b}	5.34±0.31^{b}
Soaked	103.58±2.61^{b}	4.74±0.65^{a}	139.65±1.72^{a}	91.54±1.29^{b}	4.46±0.24^{c}
unprocessed	142.46±1.18^{a}	5.36±0.33^{a}	102.95±0.45^{cd}	98.76±0.56^{a}	9.06±0.24^{a}

a-c: means in the same column with varying superscript letters 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 unprocessing mean 51.18% highly decreased to 12.42% minimum value differently. But there were significant difference between the means. Boiled beans had the lowest iron extractability possibly because of higher phytate levels. As a divalent cation, iron, is generally associates with phytic acid possibly reducing its extractability (Duhan A, et al, 2002). Soaking reduces phytic acid, freeing iron, and resulting in higher HCl extractability (Duhan A, et al, 2004, Ghavidel R. A and J.Prakash, 2007). Combined processing (sprouting, dehulling followed by either roasting or steaming) of beans resulted in higher iron extractability than it did for Zinc.

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	$126.0±0.44^{a}$	$9.07±0.28^{b}$	$160.20±1.17^{a}$	$77.76±0.33^{a}$	$35.88±1.35^{b}$
Fermented	$63.6±0.64^{c}$	$7.42±0.29^{c}$	$110.70±0.71^{d}$	$69.04±0.43^{b}$	$23.22±0.47^{c}$
Germinated	$111.5±1.66^{b}$	$9.16±0.48^{b}$	$100.65±1.72^{e}$	$63.14±1.15^{c}$	$12.79±0.28^{d}$
Soaked	$56.91±0.36^{d}$	$8.97±0.42^{b}$	$131.56±0.07^{c}$	$67.83±0.75^{b}$	$12.42±0.36^{e}$
unprocessed	$32.47±0.00^{e}$	$10.47±0.79^{a}$	$145.80±1.27^{b}$	$77.18±0.45^{a}$	$51.18±0.65^{a}$

a-d: means in the same column with varying superscript letters 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 (Oke OL, 1966). 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 (S.O. Omoikhoje, 2006). The alkaloid content of the bean was significantly decreased after processing specially for soaking and cooking methods than others.

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

Processing methods	Sweet lupin Alkaloid (%)	Bitter(local) lupin Alkaloid (%)
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	$0.31±0.31^{b}$	$3.78±0.71^{c}$
unprocessed	$1.76±0.36^{a}$	$6.03±0.21^{a}$

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

4. CONCLUSSION

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 fermenting, 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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