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LEVELS OF ESSENTIAL AND NON-ESSENTIAL ELEMENTS IN RAW AND PROCESSED *LUPINUS ALBUS* L. (WHITE LUPIN, GIBTO) CULTIVATED IN ETHIOPIA

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ABSTRACT

White lupin (*Lupinus albus* L.) cultivated in Ethiopia is locally known as 'Gibto'. Its seed is used as a snack, for the preparation of local alcoholic drink, 'Areqi' and as 'Shiro' flour for the people living in the north western part of Ethiopia. It is also used for maintaining soil fertility and as a food. Certain elements are essential for plant growth, for animal and human health. However, if present in excessive concentrations they become toxic. Other elements are non-essential and toxic to human health even at trace level. Even though the levels of four of the elements are reported, there is no report in literature on the levels of other essential and non-essential elements in white lupin cultivated in Ethiopia. Therefore, this study was carried out to determine the levels of essential (K, Na, Mg, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn) and non-essential elements (Pb and Cd) in raw and processed grains *Lupinus albus* L. (Gibto) cultivated in Ethiopia (particularly Debretabor, Dembecha and Kosober). The micro and macro-nutrient levels of the raw and processed white lupin grains were determined by flame atomic absorption spectrometry. Known weight of dried raw and processed samples were wet digested using 2 mL of HNO_3, 1 mL of $HClO_4$ and 1 mL of H_2O_2 at temperature round 270 ^{0}C with 3 hours total time. The mean metal concentration (µg/g dry weight basis) ranges in raw and processed white lupin samples, respectively, were: K (5142-6215, 1100-1222), Na (31.9-50.1, 15.1-31.1), Mg (1739-2159, 629-759), Ca (502-967, 709-1284), Cr (11.3-17.6, 9.40-10.3), Mn (1657-4095, 1075-2265), Fe (77.9-92.8, 70.7-83.8), Co (16.2-16.6, 16.7-17.2), Ni (12.0-15.6, 6.90-11.7), Cu (4.80-9.90, 5.10-12.2), Zn (40.3-53.6, 55.6-64.6) and Pb (10.8-16.4, 9.90-13.1). The level of Cd was below method detection limit in both the raw and processed samples from all the sample sites. The concentrations of all the nutrients were higher in raw as compared to processed white lupin samples except for Ca, Co, Cu and Zn samples from all of the sampling sites. Analysis of variance (ANOVA) at 95% confidence level indicated that there is significant difference between the mineral contents of raw and processed samples from the three sample sites. Pearson correlation indicated positive correlation between most elements in both the raw and processed samples. In general, white lupin is rich in essential elements and safe to consume and could be an alternative source of the essential elements to the individual daily intake.

Key words: *Lupinus albus*, legumes, food, elements, Ethiopia

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INTRODUCTION

Legumes have been widely grown seeds for human food consumption. Grain legumes including beans, lentils, lupins, peas and peanuts are used for human and animal consumption. They are important foodstuffs in most of the tropical and subtropical countries [1, 2]. They are rich in protein (average 12-40%) and can be considered as a natural supplement to cereals [3-8]. Although they are usually deficient in the essential amino acids methionine and cystine, they contain adequate amounts of lysine, whereas cereals are deficient in lysine, but contain adequate amounts of methionine and cysteine [1]. They are important sources of significant amounts of proteins, carbohydrates, fiber, vitamins and some minerals [1].

Legumes are inexpensive, nutrient-dense sources of protein that can be substituted for dietary animal protein [2]. The small quantities of fats in legumes are mostly unsaturated fats and excellent sources of essential minerals [9].

The chemical composition of food legumes vary and it is controlled by the cultivar, geographical location and growth conditions [2-9]. Legumes are good sources of dietary fiber and minerals. Legumes also contain some anti-nutritional factors (ANFs) like trypsin inhibitor and others. These are chemical substances which, although non-toxic generate adverse physiological responses and interfere with the utilization of nutrients. Besides, most of the legumes in raw form contain a wide variety of anti-nutritional factors or toxic principles. In addition, many of the grain legumes cause flatulence [1]. Fortunately, most of the anti-nutritional factors are heat labile and are destroyed during cooking [1].

White lupin is one of the four lupins (*Lupinus albus*, *Lupinus angustifolius*, *Lupinus luteu* L., *Lupinus mutablis*) widely known commercially and agriculturally important, large seeded annual legume crop for human consumption and animal feed in some countries, particularly in Australia and New Zealand [10]. The composition of the seed and especially the high protein content makes white lupin highly suitable for livestock diets. Its adaptation to poor soil makes it economically feasible [10].

The presence of quinolizidine alkaloids and some anti-nutritional factors hinders its consumption in the form of raw seed. To make the lupin edible for human consumption different modern and traditional processing methods have been developed. Among the methods, soaking after roasting, boiling, germination, fermentation and alkaline treatments can be mentioned [11].

White lupin cultivated in Ethiopia is locally known as ‘Gibto’ [12]. Its seed is used as a snack, for the preparation of local alcoholic drink, ‘Areqi’ and as ‘Shiro’ flour for the people living in the north western part of Ethiopia especially West Gojam, Awi zone and South Gondar [13]. The white lupin variety grown in the targeted areas is bitter variety due to its high alkaloid content [13]. Before consumption, the seeds are roasted and soaked up to 3-7 days in running water to remove the bitter and toxic alkaloids [13]. In Ethiopia, it is used for maintaining soil fertility and as a food

security crop. In Ethiopian case, among the four agriculturally important lupin species *Lupinus albus* L. is found mainly in the Amhara region especially in West Gojam, Awi and South Gondar zones [14].

Lupin seed is high in protein (30-40%) like soybean but is significantly higher in dietary fibre (30%) and lower in oil (6%) and contains minimal starch [15]. There is supportive scientific evidence that consuming lupin enriched foods may have an advantage on food appetite and energy balance [15]; beneficially influence glycaemic control [16] and improve blood lipids [17]. Lupin flour can be used in production of different products. It can be added to pasta, bread and emulsified meat products to increase nutritional value, aroma as well as modify the texture of the end products. In the Middle East, lupin seeds are consumed as a snack after they are soaked in water, scalded and dehulled. Additionally, in some European counties, pickle is produced from lupin seeds [18].

Among the major reasons that preclude a greater inclusion of lupin in human or animal diets are the production of flatulence, which results from the content of R-galactoside oligosaccharides, and the presence of phytic acid. Phytic acid decreases the availability of important dietary elements because it forms insoluble complexes with di- and trivalent cations at the physiological pH of the small intestine of monogastric animals [19-21]. With the aim of removing non-nutritional factors such as R-galactosides and phytic acid from legume seeds, treatments such as soaking in different pH solutions and cooking, germination, and fermentation have been developed [19-21]. Prolonged soaking in different pH and temperature conditions may lead to important element losses as a result of leaching [22, 23].

Some element contents (Fe, Zn, Mn and Mg) in raw white lupin have been determined in the two specific areas of Ethiopia. The Dangla sample has 60.0, 21.1, 584.3 and 89.3 µg/g contents of Fe, Zn, Mn and Mg, respectively, where as that of Tilili sample were 67.2, 18.1, 591.4 and 94.6 µg/g for the same types of elements, respectively [13]. Seeds of wild lupin species and several cultivars were analyzed in Brazil for Ca, Zn, Fe, Cu, and Mn composition. Accordingly the results were 610-3270, 24-108, 29-176, 04-14, and 1340-3500 µg/g dry matter of Mn, Fe, Zn, Cu, and Ca, respectively [24]. Some other elements have also been reported: 1760, 1980, 4400, 44, 48 µg/g dry matter of Ca, Mg, P, Fe, Zn, respectively [10, 25, 26].

Elements are prevalent in the environment. Certain elements are essential for plant growth, animal and human health but if present in excessive concentrations they become toxic [27]. The recommended daily allowance of Ca for adults is 2000 mg including for pregnant and nursing women. Recommended dietary allowance of Na for adults is 500 mg and that of Mg for females 31-50 years is 320 mg. It is recommended that adults between the ages of nineteen and fifty need 1000 mg of Ca/day, while adults aged fifty one and over need to increase their Ca intake to 1200 mg/day. Micro elements required in a typical adult are between 1-100 mg/day [28].

Since some of the people in Ethiopia under estimate this crop as a food with the exception of the people in the study area, this study can provide information regarding the levels of elements in both raw and processed white lupin samples.

Even though there are some literatures about white lupin and its few elements (Fe, Zn, Mn and Mg) contents there is no report on the levels of essential and non-essential elements in white lupin cultivated in Ethiopia. Furthermore, there is no literature report on the element contents of processed seeds of white lupin which are more commonly used by humans. Therefore, the objectives of this research were to determine the concentration of major (K, Na, Mg, Ca), trace (Cr, Mn, Fe, Co, Ni, Cu, Zn), and toxic (Pb, Cd) elements in dry (raw) and processed white lupin samples using Flame Atomic Absorption Spectroscopy (FAAS), to compare the levels of elements found in raw with the processed *Lupinus albus* and to correlate the levels of the identified elements in raw commercially available white lupin and processed white lupin for multipurpose case.

MATERIALS AND METHODS

Equipments

An electronic blending device (Moulinex, France) and Kjeldahl apparatus were used. Buck Scientific Model 210 VGP (East Norwalk, USA) atomic absorption spectrophotometer was used for the determination of elements (K, Na, Mg, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb, and Cd) using air-C_2H_2 flame.

Reagents and chemicals

All the reagents used were of analytical grade. Nitric acid (69-72%, Spectrosol BDH, England), perchloric acid (70%, Analar BDH, England), and extra pure hydrogen peroxide, 30% H_2O_2, (Scharlau, European Union) were used for digestion of the dried (raw) and processed white lupin samples. A 1% w/v lanthanum chloride hydrate (Aldrich, USA) was used to prevent the chemical interference on Ca and Mg during the analysis of the dried (raw) and processed white lupin samples. Stock standard solutions containing 1000 mg element/L, in 2% HNO_3, of the elements K, Ca, Mg, Fe, Zn, Na, Mn, Cu, Co, Cr, Ni, Pb, and Cd (Spectrosol BDH, England) were used for preparation of calibration standards and in the spiking experiments. Deionized water was used throughout the experiment.

Description of sampling sites

The samples were collected from three areas, Debretabor (11^{o}51’N, 38^{o}1’E, 2706 m) located in South Gondar zone of Amhara region, Kosober (10^{o}57’N, 36^{o}56’E, 2560 m) in Awi zone of Amhara region and Dembecha (10^{o}33’N, 37^{o}29’E, 2083 m) in West Gojam zone of Amhara region. These areas were selected to represent the area where white lupin is dominantly cultivated and consumed in the country.

Collection and preparation of white lupin samples

Both raw (dry) and processed white lupin samples were collected from three similar sites for comparison. About 1.5 kg for both raw (dried) and processed white lupin

samples were bought from farmers from each site separately. From a specific main site, three sub-sites were taken for the purpose of random sampling. About 500 g of the sample from five farmers per each sub site (for example, a total of fifteen white lupin samples from fifteen farmers, 100 g from each farmer) were taken and then mixed in to a single polyethylene plastic bag to get 1.5 kg of one bulk sample. Then the collected samples were packaged into polyethylene plastic bags, labeled and transported to laboratory for further treatment.

Raw white lupin seeds were washed and dried using sunlight. The dried raw white lupin seeds were split into two parts for de-hulling purposes using traditional equipment (grinder stone). After de-hulling, the raw samples were rinsed with deionized water, dried, ground and powdered with a metal grinder with sieve size of 0.425 mm and packed in polyethylene plastics until analysis.

The seeds of white lupin were roasted on electric oven at 110 ^{0}C for 12 min and it was removed and allowed to cool for about 12 min for each sample followed by soaking in tap water. The soaking water was changed in 4 h intervals for six days until the bitterness was removed. The bitterness of the lupin was checked by de-hulling and tasting it with tongue to establish if there is any bitterness left. After the bitterness was removed, the whole seeds were de-hulled and the kernels without seed coat (hull) were dried in sun light. The dried samples were ground and powdered with metal grinder with sieve size of 0.425 mm and packed in polyethylene plastics until analysis.

Optimization of digestion procedure

For both raw (dried) and processed white lupin samples different digestion procedures were assessed based on reagent volume, digestion time and digestion temperature. The optimized procedure was selected depending upon: clarity of digests, minimal reflux time/digestion time, minimal reagent volume consumption, absence of undigested lupin samples and simplicity. The optimizations of digestion procedure were done by varying one variable and keeping the other constant. A mixture of nitric acid, hydrogen peroxide and perchloric were used for both samples studied.

Wet digestion of white lupin samples

Known amounts of 0.5 g of powdered and homogenized raw (dry) and processed white lupin samples were weighed and transferred into a 100 mL round bottom flask. To this, 2 mL concentrated HNO_3 (69-72%), 1 mL of H_2O_2 (30%) and 1 mL of $HClO_4$ (70%) were added based on the optimized procedure. The mixture was then digested in Kjeldahl digestion apparatus (Gallenkamp, England) fitting the flask to a reflux condenser by setting the temperature to dial at 4 (120 ^{0}C) for 30 min followed by dialing at 9 (270 ^{0}C) for 2 hour and 30 min until a clear solution was obtained following the optimized digestion procedure. After a total of 3 hours, the digested solutions were allowed to cool for 30 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution, 10 mL portions of distilled-deionized water were added to dissolve the precipitate formed on cooling and gently swirled to reduce dissolution of the filter paper by digest residue.

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The cooled digested samples were filtered into a 50 mL volumetric flask with a Whatman filter paper (110 mm) to remove any suspended or turbid matter. After filtration, the solution was clear and colorless. To each sample 1% w/v 'matrix modifier' lanthanum nitrate hydrate was added so that lanthanum may bind the phosphate and liberate calcium and magnesium in case large phosphate exist in the sample. The solution was filled to the mark (50 mL) with deionized water. Triplicate digestions were carried out for each bulk sample. Six blank solutions were prepared following the same digestion procedure as the sample.

Instrument calibration and method detection limit

The calibration curves were drawn for each of the studied elements from five standard solutions. The correlation coefficients of all the calibration curves were > 0.999 which showed that there is good correlation (relationship) between concentration and absorbance. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank (S_{blank}) by three (LOD = 3 x S_{blank}, n = 18). The method detection limits (LOD) of all the elements analyzed in (µg/g) were: (Na, 2.0), (K, 2.0), (Mg, 0.9), (Ca, 8), (Cr, 6), (Fe, 7), (Mn, 6), (Co, 6), (Ni, 5), (Cu, 4), (Zn, 0.6), (Pb, 8) and (Cd, 3). The method detection limits are low enough (≤ 8 µg/g) to detect the presence of elements of interest at trace levels in both the raw and processed samples.

Validation of optimized procedure

Spiking experiments were performed to ascertain the reliability and efficiency of the developed optimized procedure. The spiked samples were prepared by adding a small known quantity of the element standard solutions. From the stock solution of (1000 mg/L), 385.7 µL of K, 130 µL of Mg, 38 µL of Ca and 307 µL of Mn solutions were added to 0.5 g of raw white lupin samples. For the rest of the elements an intermediate standard solution (100 mg/L) was prepared with the exception of Cd (10 mg/L) and 48.0 µL of Na, 32.0 µL of Cr, 58.0 µL of Fe, 36.0 µL of Co, 24.0 µL of Ni, 22.0 µL of Cu, 40.0 µL of Zn, 24.0 µL of Pb and 25.0 µL of Cd solutions were added to 0.5 g of the raw (dried) white lupin samples. Similarly, 110.0 µL of K, 79.0 µL of Mg, 89.0 µL of Ca and 226 µL of Mn solutions were taken from 1000 mg/L stock solution. An intermediate standard solution of 10 mg/L for Cd and 100 mg/L for the rest of the elements was prepared from the stock solution of (1000 mg/L) and 57.0 µL of Na, 38.0 µL of Cr, 194.0 µL of Fe, 63.0 µL of Co, 28.0 µL of Ni, 40.0 µL of Cu, 167.0 µL of Zn, 40.0 µL of Pb and 15.0 µL of Cd solutions were added to 0.5 g of the processed white lupin samples.

For raw white lupin samples, Na, Mg, Ca, Cr, Fe, Co, Cu and Pb were spiked in to one of the digestion sample in triplicate while K in the second set of samples in triplicate and that of Mn, Ni, Zn and Cd were spiked in the third set of samples in triplicate. Similarly for the processed white lupin samples, Na, K, Mg, Ca, Cr and Cd were spiked in to one of the digestion sample in triplicate while Mn and Zn in the second set of samples in triplicate and the rest elements, Fe, Co, Ni, Cu and Pb were spiked in the third set of samples in triplicate. Then the samples were digested with the optimized procedures for both raw and processed white lupin samples. As used for original samples triplicate spiked samples were prepared and triplicate readings were

recorded. The spiked and non-spiked samples were digested and analyzed in the same condition.

Validation of analytical method

The percentage recoveries for the studied elements in both raw and processed samples were between 94% and 108%, which are within the acceptable range (100 ± 10%) for all the elements. These results verify that the optimized digestion procedures are valid for the analysis of both the raw and processed white lupin samples. The relative standard deviations (RSD) were < 10%, which indicates that the precision of the method is good and within the acceptable range.

Analysis of variance

In this study, white lupin samples were collected from three different areas and the element levels of each sample was determined by FAAS. The variation in sample mean of the analyte was tested by using analysis of variance (ANOVA), whether the source for variation was from experimental procedure or from heterogeneity among the samples (for example, difference in mineral contents of soil, pH of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides, etc or other variations in cultivation procedures). The ANOVA results clearly indicated that there exist statistically significant differences at 95% confidence level in mean concentration of all the twelve elements except Cd. The source for this significant difference between sample means may be the difference in element contents of soil or pH of soil, element content of the white lupin, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides, or other variations in cultivation procedures).

RESULTS

Composition of major, trace and toxic elements in raw (dried) *Lupinus albus* L. samples

Tables 1 and 2 show the results of the mean concentration (n = 9) of major, trace and toxic elements with the corresponding standard deviation (SD) of the white lupin samples. The overall error (resulting from sample inhomogeneity and from analytical error) is within the acceptable range (RSD ≤ 10%).

As can be seen from Tables 1 and 2, there is a wide variation in mean concentration of macro- and microelements in the white lupin samples (both in the raw and processed seeds) and there is also some variation in most of the elements along with geographical location. Element uptake in plants is a function of element concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants, and age of the plant [29]. Thus, a wide variation in mean concentration of elements might be related to the difference in element uptake in plant due to the above factors.

Table 1 shows the ability of white lupin to accumulate high amounts of both macro- and micronutrient elements. The most abundant element among the macroelements

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determined was K followed by Mg, Ca and Na. Whereas Mn content of the white lupin seeds was the predominant among the tested micronutrient heavy elements followed by Fe, Zn, Co, Cr, Ni and Cu. On the other hand, the content of the toxic non-essential heavy element Pb was found at lower concentration while Cd was not detected in all the investigated samples. It can be deduced from the levels of all the elements in the studied white lupin samples from all the sampling sites that the concentrations of the macro- and the micronutrient elements followed non-uniform patter for all the samples. In general, ranges of concentrations of the studied macronutrient, micronutrient and toxic elements could be arranged according to their levels in the white lupin samples from all the sampling sites in the following order in dry weight basis in µg/g: K (5,142–6,215) > Mn (1,657–4,095 > Mg (1,739–2,159) > Ca (502–967) > Fe (78–93) > Zn (40.3–53.6) > Na (31.9–50.1) > Co (16.2–16.6) > Cr (11.3–17.6) > Ni (12.0–15.6) > Pb (10.8–16.4) > Cu (4.80–9.90).

Table 2 shows the ability of the processed white lupin to retain considerable amounts of both macro- and micronutrient elements. While there was a decrease in the concentration of K, Mn, Mg, Zn, Co and Cu there was an increase in the concentration of Ca, Fe, Na, Cr, Ni and Pb in the processed white lupin seeds. Cadmium was not detected in all the investigated samples. The processed white lupin samples also showed non-uniform patter of the levels of all the elements in the samples from all the sampling sites. In general, ranges of concentrations of the studied macronutrient, micronutrient and toxic elements could be arranged according to their levels in the processed white lupin samples from all the sampling sites in the following order in dry weight basis in µg/g: Mn (1075–2265) > K (1100–1220) > Ca (709–1284) > Mg (629–759) > Fe (70.7–83.8) > Zn (55.6–64.6) > Na (15.1–31.1) > Co (16.7–17.2) > Pb (9.9–13.1) > Ni (6.9–11.7) > Cr (9.4–10.3) > Cu (5.1–12.2).

Figures 1 and 2 show the comparative results of the mean concentration of the studied elements in the raw and processed white lupin samples

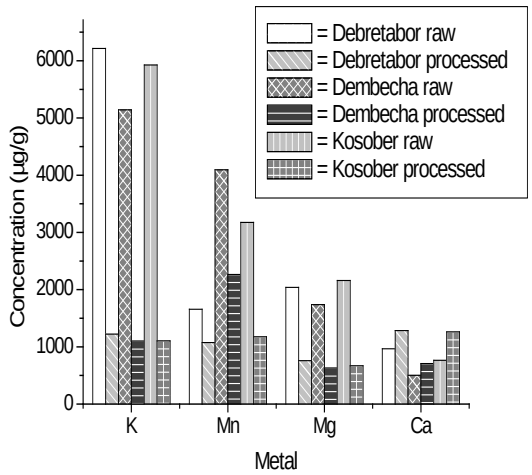

Figure 1: Comparative study of mean concentrations of K, Mn, Mg and Ca in raw and processed raw and processed white lupin food samples

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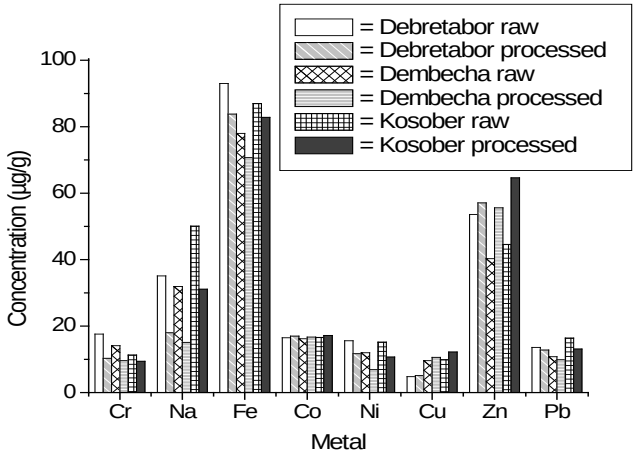

Figure 2: Comparative study of mean concentrations of Cr, Na, Fe, Co, Ni, Cu, Zn and Pb in raw and processed white lupin food samples

DISCUSSION

Distribution pattern of the elements in raw (dried) *Lupinus albus* samples

As it can be seen from Table 1, there is a large difference in concentration of different elements within raw white lupin samples and slight variation in elements of the same type along with the study area. Among the macro elements distribution of K in all the raw samples was the highest followed by Mg and Ca throughout the three sample sites. Na was the least among the macro elements. The higher level of K and Mg in the white lupin sample is probably due to the fact that nutrient elements such as N, P, K, S, and Mg are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue [30]. If the soil used for cultivating the plant is highly fertilized with manure and organic residues, there is higher availability of K, Ca and Mg [30]. Thus, this might be the other probable reason for higher levels of K, Mg and Ca.

Table 2 indicates that Mn was the highest followed by Fe among the trace elements in the raw white lupin samples from all sample sites. Higher Mn levels in white lupin might be attributed to the availability of this micronutrient in relatively acidic soils of the farmland. The availability of Mn present in soil is known to depend on soil pH [31]. The solubility of Mn becomes high at low pH due to this reason its concentration might be high in acidic soil [31]. The high concentration of Zn and Fe from trace metals next to Mn in white lupin may be because of the fact that these ions are readily transferred from the soil to plants, and accumulate in plants [29, 32, 33]. The levels of other essential trace elements detected in raw white lupin were Cr, Co, Ni. Cu had the least distribution within the samples. This might be due to either the intake of this element by the plant may be low or the bioavailability of the element is very small in the plant [29, 32, 33]. The non-essential heavy element, Cd, was found to be below

the method detection limit. Thus Cd has low exposure to the farm lands as well as to the plant.

The difference in concentration of each element from sample site to sample sites might be because of a variation in at least one of the following factors: competition of uptake of the elements by the plant, bioavailability, physical property of the soil, soil pH, and elements content of the soil. [28, 31, 32].

Distribution pattern of the elements in processed *Lupinus albus* L. samples

The average concentrations of the essential and toxic elements in the processed *Lupinus albus* L. samples are shown in Table 2. Among the macro elements Na was the least in all the samples. Among the micro nutrients Mn concentration was the highest followed by Fe in all the samples.

The concentration of Cu in sample from Debretabor, Ni in sample from Dembecha and Cr in sample from Kosober were the least in processed samples. From the concentration of the toxic elements, Pb was more than Cd, which was below method detection limit. In general the distribution pattern of the elements in the *Lupinus albus* processed samples from the three sample sites were slightly varied. In relation to the raw lupin, samples from Debretabor and Kosober had the highest contents of Cr, Na, Fe, Ni and Pb, respectively while samples from Debretabor and Dembecha had the least contents of Co, Cu and Zn, respectively. The Kosober processed sample had the highest contents of Co, Cu and Zn while Cr, Na, Fe, Ni and Pb were the least amongst the processed samples from Kosober and Dembecha, respectively. From this trend it is possible to conclude that raw white lupin contains higher concentration of Cr, Na, Fe, Ni and Pb as compared to processed white lupin samples.

Comparison of element levels between raw and processed *Lupinus albus* samples

K, Mn, Mg and Ca

Figure 1 shows that the concentrations of K, Mn, Mg and Ca were higher in raw compared to processed white lupin samples from all of the sampling sites except in case of Ca, in which the processed samples was higher than the raw white lupin samples. The reduction of elements on soaking process could arise because all the water soluble elements are often lost with the steeping medium and rinsing process. The element contents of processed samples depend on the type of the soaking solution. The results of a previous study showed that when $NaHCO_3$ is used as a soaking solution, all the analyzed elements have shown a decrement except Na [22]. This was because of the presence of Na in the soaking solution. In all the treatments of the current study the soaking solution was tap water. The possible explanation for the increment in Ca content of the processed white lupin samples in all sample sites might be contamination from tap water [22, 23].

The raw lupin samples collected from Debretabor, Dembecha and Kosober were the highest in K content, Mn content and Mg content respectively among all analyzed elements. The processed sample from Debretabor was found to have the highest in the contents of Ca while that of the raw samples from Dembecha was the least among the

six samples. The processed sample from Dembecha and Debretabor had the least in the K, Mn and Mg contents. From this trend it is possible to conclude that raw white lupin contains higher concentration of K, Mn and Mg as compared to processed white lupin samples with the exception of Ca in which the processed samples were higher than raw.

Cr, Na, Fe, Co, Ni, Cu, Zn and Pb

Figure 2 shows that the concentrations of Cr, Na, Fe, Ni, Cu, Zn and Pb were higher in raw samples compared to processed white lupin samples except in case of Co, Cu and Zn in which the concentrations of the processed samples were higher than the raw white lupin samples from all of the sampling sites. There is a possibility that the soluble elements could have dissolved in water and eliminated while discarding the soaking solution. The reduction of elements on soaking process could arise because all the water soluble minerals are often lost with the soaked tap water and rinsing process [22, 23]. The element contents of processed samples depend on the type of the soaking solution [22, 23]. The possible explanation for the increment in Ca, Co, Cu and Zn content of the processed white lupin samples from all sample sites might be contamination during processing with tap water [22, 23].

Pearson correlation of elements

Pearson correlation of elements within raw (dried) white lupin samples

Table 3 indicate that there is high positive correlation of K with (Mg, Ca, Fe, Co, Ni, Zn and Pb), Na with (Mg, Co and Pb), Mg with (Ca, Fe, Co, Ni and Pb), Ca with (Fe, Co, Ni and Zn), Cr with (Zn), Mn with (Cu), Fe with (Co, Ni and Zn), Co with (Ni and Pb) and Ni with (Zn and Pb). The high association between elements, evidenced by high positive correlation coefficient, can arise from common anthropogenic or natural sources as well as from similarity in chemical properties [34]. Moderate positive correlation in elements: Na with Ni, Mg with Zn and, Ca with Pb, Fe with Pb and Co with Zn and moderate negative correlation in elements: K with Cu, Mg with Mn, Cr with Mn, Mn with Co and Ni with Cu was shown in above table. The high negative correlation of K with Mn, Na with Cr, Ca with Mn and Cu, Cr with Cu, Mn with Fe, Ni and Zn, Fe with Cu and Cu with Zn indicate the large absorption of one elements may affect the absorption of the others elements in raw white lupin plant. The other elements have weak negative or positive correlation indicating that the presence or absence of one element affect in lesser extent to the other. This poor relationship might be due to different size of seeds of the species, soil type, environmental conditions and capacity of the plant to accumulate specific element [34].

Pearson correlation of elements within processed white lupin samples

From Table 4, high positive correlation of the elements within processed white lupin samples can be seen between the elements: K with (Mg and Cr), Na with (Co, Zn and Pb), Ca with (Mg, Fe, Co, Ni and Pb), Mg with (Cr, Fe and Ni), Fe with (Co, Ni and Pb), Co with (Ni, Zn and Pb) and Ni with Pb and high negative correlation of the elements can be also seen in elements: K with Cu, Ca with Mn, Mg with (Mn and

Cu), Cr with Cu and Mn with (Fe, Co, Ni and Pb). K with (Ca, Fe and Ni), Na with (Ca, Fe and Cu), Ca with Zn, Mg with Pb, Fe with Zn, Cu with Zn and Zn with Pb were shown moderate positive correlation and that of K with Mn, Na with (Cr and Mn), Cr with Zn and Mn with Zn were also exhibited moderate negative correlation within the processed white lupin samples. The other elements have weak negative or positive correlation indicating that the presence or absence of one element affect in lesser extent to the other. This poor relationship might be due to slight variation of time during soaking, contamination of the seed with soaking solution, slight variation during roasting of the seeds of white lupin samples extra [34].

Pearson correlation of elements between raw white lupin with processed white lupin samples

As it can be seen from Table 5, most of the elements analyzed exhibited high positive correlation with the exception of Mg (moderate positive correlation), and Zn (weak negative correlation) between the raw and processed white lupin samples. The high positive correlation indicates that the more accumulation of the elements in raw white lupin, the more the level of elements in the processed white lupin samples [34].

CONCLUSION

Among the macro elements, distribution of K in all the raw samples was found at the highest level followed by Mg and Ca throughout the three sample sites. Manganese was the highest followed by Fe and Zn from the trace elements in all sample sites. Copper was found in the least amount in all the three raw white lupin samples. In the processed white lupin samples Ca was found at highest level in all samples except in sample from Dembecha with mean concentration of Mn greater than Ca. In both raw and processed white lupin samples, Na was found the least from the macro elements in all the samples. Among the micro elements, Mn concentration was the highest followed by Fe, Zn, Na and Co in all sample sites and the concentration of Cu in Debretabor, Ni in Dembecha and Cr in Kosober sample sites were the least in the processed samples. The amount of Cd was below method detection limit and was not detected. The concentrations of all analyzed elements were higher in raw compared to processed white lupin samples except in case of Ca (macro), Co, Cu and Zn (micro) elements in which the processed samples were higher than the raw white lupin samples from all of the sampling sites. *Lupinus albus* raw (dried) and processed white lupin can be a good source of major, minor and trace elements which are essential to human in addition to its food flavoring purpose. The levels of most of the elements in the studied raw samples found positive correlation with the levels found in the processed samples.

In general, in accordance with the daily allowance of elements, the levels of most of the elements analyzed in white lupin samples are suitable for human and animal consumption. Further studies are necessary (i) to determine the optimum soaking time required for removal of unwanted alkaloids and (ii) to determine the levels of elements in the white lupin cultivated in different climatic conditions and geographical locations.

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Table 1: Average concentration (mean ± SD, n = 9, µg/g dry weight basis) of major, minor and toxic elements in raw (dried) white lupin samples from Debretabor, Dembecha, and Kosober sites

Element	Sample from Debretabor Mean ± SD (µg/g)	Sample from Dembecha Mean ± SD (µg/g)	Sample from Kosober Mean ± SD (µg/g)	Mean conc. range (µg/g)
K	6215 ± 73	5142 ± 150	5926 ± 100	5142 – 6215
Na	35.1 ± 0.8	31.9 ± 0.8	50.1 ± 1.0	31.9 – 50.1
Mg	2039 ± 71	1739 ± 53	2159 ± 60	1739 – 2159
Ca	967 ± 18	502 ± 6	764 ± 10	502 – 967
Cr	17.6 ± 0.7	14.1 ± 1.0	11.3 ± 0.7	11.3 – 17.6
Mn	1657 ± 67	4095 ± 87	3175 ± 78	1657 – 4095
Fe	93 ± 1.8	78 ± 2.1	87 ± 1.7	78 – 93
Co	16.5 ± 0.1	16.2 ± 0.1	16.6 ± 0.1	16.2 – 16.6
Ni	15.6 ± 0.7	12.0 ± 0.4	15.2 ± 0.5	12.0 – 15.6
Cu	4.80 ± 0.33	9.60 ± 0.50	9.90 ± 0.71	4.80 – 9.90
Zn	53.6 ± 2.0	40.3 ± 0.1	44.6 ± 0.6	40.3 – 53.6
Pb	13.6 ± 0.9	10.8 ± 0.5	16.4 ± 0.6	10.8 – 16.4
Cd	ND[a]	ND[a]	ND[a]	–

[a]Concentration of the tested heavy element was below the method detection limit

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Table 2: Average concentration (mean ± SD, n = 9, µg/g dry weight basis) and relative standard deviation (% RSD) of major, minor and toxic elements in processed white lupin samples from Debretabor, Dembecha, and Kosober sites

Element	Sample from Debretabor Mean ± SD (µg/g)	Sample from Dembecha Mean ± SD (µg/g)	Sample from Kosober Mean ± SD (µg/g)	Mean conc. range (µg/g)
K	1222 ± 20	1100 ± 18	1104 ± 17	1104 – 1222
Na	18.0 ± 0.9	15.1 ± 1.1	31.1 ± 1.5	15.1 – 18.0
Mg	1284 ± 7	709 ± 4	1261 ± 3	709 – 1284
Ca	759± 21	629 ± 19	669 ± 18	629 – 759
Cr	10.3 ± 0.1	9.60 ± 0.10	9.40 ± 0.11	9.40 – 10.3
Mn	1075 ± 93	2265 ± 120	1175 ± 83	1075 – 2265
Fe	83.8 ± 1.3	70.7 ± 1.5	82.8 ± 2.7	70.7 – 83.8
Co	17.0 ± 0.1	16.7 ± 0.1	17.2 ± 0.1	16.7 – 17.2
Ni	11.7 ± 0.7	6.90 ± 0.53	10.7 ±0.7	6.90 – 11.7
Cu	5.1 ± 0.4	10.6 ± 0.9	12.2 ± 0.5	5.1 – 12.2
Zn	57.1 ± 0.3	55.6 ± 0.1	64.6 ±1	55.6 – 64.6
Pb	12.8 ± 0.5	9.9 ± 0.7	13.1 ± 0.7	9.9 – 13.1
Cd	ND[a]	ND[a]	ND[a]	–

[a]Concentration of the tested heavy element was below the method detection limit

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Table 3: Correlation matrices for elements in raw (dried) white lupin samples (n = 9)

	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Pb
K	1											
Na	0.413	1										
Mg	0.855	0.825	1									
Ca	0.983	0.236	0.744	1								
Cr	0.322	-0.730	-0.220	0.492	1							
Mn	-0.920	-0.020	-0.590	-0.977	-0.670	1						
Fe	0.989	0.277	0.771	0.999	0.455	-0.968	1					
Co	0.875	0.803	0.999	0.769	-0.180	-0.616	0.795	1				
Ni	0.987	0.554	0.928	0.940	0.165	-0.846	0.953	0.941	1			
Cu	-0.670	0.399	-0.190	-0.797	-0.920	0.9067	-0.770	-0.230	-0.540	1		
Zn	0.895	-0.040	0.535	0.963	0.710	-0.998	0.950	0.567	0.812	-0.931	1	
Pb	0.706	0.937	0.971	0.562	-0.440	-0.374	0.596	0.961	0.811	0.052	0.317	1

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Table 4: Correlation matrices for elements in processed white lupin samples (n = 9)

	K	**Na**	**Ca**	**Mg**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Pb**
K	1											
Na	-0.318	1										
Ca	0.555	0.612	1									
Mg	0.962	-0.05	0.76	1								
Cr	0.971	-0.54	0.339	0.869	1							
Mn	-0.588	-0.58	-0.999	-0.79	-0.377	1						
Fe	0.582	0.586	0.999	0.782	0.37	-0.999	1					
Co	0.143	0.893	0.903	0.408	-0.098	-0.885	0.888	1				
Ni	0.683	0.476	0.987	0.856	0.487	-0.992	0.992	0.821	1			
Cu	-0.97	0.539	-0.34	-0.87	-0.999	0.374	0.37	0.101	-0.48	1		
Zn	-0.332	0.999	0.601	-0.06	-0.549	-0.568	0.574	0.886	0.463	0.551	1	
Pb	0.451	0.703	0.993	0.677	0.224	-0.987	0.988	0.948	0.96	-0.22	0.692	1

Table 5: Pearson correlation coefficient for elements in raw white lupin with processed white lupin sample (n = 9)

Metals	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Pb
r	0.728	0.999	0.521	0.915	0.97	0.832	0.943	0.986	0.995	0.987	-0.045	0.905

Where, r is the Pearson correlation coefficient between element level in raw white lupin and processed white lupin samples

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