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Determination of the level of Essential and Non-essential Metals in Lupinus Albus (Gibto) Grain Cultivated in Amhara Region, Ethiopia

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Abstract: White lupin, the local name of "Gibto" is one of the common pulse crops grown in Ethiopia. In this study, the concentration of some metals in raw and processed white lupin cultivated in different parts of Amhara region, Ethiopia were determined using flam atomic absorption spectroscopy. The concentration of metals in raw lupin was found; Ca (94.02 to 115.96 mg/100 g), Fe (24.19 to 27.72 mg100 g), Zn (14.29 to 22.11 mg/100 g), Pb (5.16 to 6.34 mg/100 g) and Cd (1.32 to 1.38 mg/100 g). After processing the seed, the concentration of metals was; Ca (61.12 to 77.98 mg/100 g), Fe (24.00 to 27.6 mg/100 g), Zn (12.12 to 19.94 mg/100 g), Cd (1.18 to 1.25 mg/100 g) and Pb (7.05 to 7.82 mg/100 g). The effect of geographical location on Ca, Zn, Cd, and Pb were significantly different while the Fe content was insignificant. While processing has significant impact on the content of Ca, Cd and Pb. But there is no significant difference among Fe and Zn content after and before processing. In general, the content of metals in white lupin were found; Ca > Fe > Zn > Pb> Cd. The result of this study showed that white lupin has good source of Ca, Fe, and Zn for human beings. However; the concentration of Pb was found to be higher in all the studied samples as compared to the WHO standards. Thus, further studies should be required so as to re-confirm the data.

Keywords; White lupin, Essential elements, Non-essential elements, FAAS, Concentration

1. Introduction

Plants are the most important sources of food for human beings. Among plant sources, legumes products are important sources of protein and minerals in the diets of millions of people in the world [1]. The legumes used by humans are commonly called food legumes or grain legumes. The food legumes can be divided into two groups, the pulses and the oilseeds. Pulses group consists of dried seeds of cultivated legumes, which have been eaten for a long time. Grain legume is important source of significant amounts of proteins, carbohydrates, fibber, vitamins and minerals. They are used in many parts of the world for both animal and human nutrition [1].

The other legume from the same family Leguminosae called lupin, it is one of the traditional pulse crops in Ethiopia especially in Amhara regional state. It is used as human food and animal feed since early Roman times. It is an economically and agriculturally valuable Plant and its seeds are employed as a protein source for animal and human nutrition in various parts of the world, not only for their nutritional value, but also for their adaptability to soils and climates. Lupin seeds have a high protein and fat content but their use for human nutrition is very limited due to the presence of high alkaloid content requires large processing steps [2].

Different studies have been under taken regarding the metal contents of different cultivars of white lupin. In Spain, the effect of removal of α-galactosides from Lupinus albus L. Var. maltoilupa on the chemical composition of prepared flour and the daily availability of N, total P, Ca, Mg, Fe, Zn and Mn was studied. From those study the content of Mn, Cu, Fe, Zn, P, Mg and K were 63.9 ± 1.30, 6.62 ± 0.28, 63.3 ± 0.14, 55.0 ± 1.11 (μg/g), 565.8 ± 0.90, 189.61 ± 5.81 and 600 ± 23.7 mg/100 g of dry matter respectively [3].

Similarly in Portugal the lead and copper amount also studied in white lupin root and leaves using anodic striping voltametry. From these study toxicity symptom of lead is observed in leaves and roots of white lupin. Likewise in Europe, the amount of some macronutrients of lupin was analysed. These study established that the presence of K, P, Ca, Mg, Mn, and low level of Na. The manganese level of white lupin was found 896 mg/kg which can approaches toxicity concentration and the level of Ca, P, Mg, K, Na, and Mn was found 2.1 to 4.66, 4.29 to 7.20, 1.20 to 2.25, 8.6 to 11.1 and 0.1 to 0.2 g/kg respectively [4].

Tizazu *et al* [5] studied that chemical composition, physical and chemical properties of Gibto seed grown in Ethiopia. Up on his study the analysed metal content of lupin in two cultivars of white lupin (Debretabor and Dembecha) has been reported. From this study the P and Ca level of Debretabor lupin are 248.77 and 97.98 mg/100 g respectively and in Dembecha lupin the P and Ca level are 249.02 and 67.13 mg/100 g respectively. In this study the content of P, Fe, Zn and Ca are 248.90, 12.51, 4.68 and 82.56 mg/100 g respectively.

Getachew [1] studied that the chemical composition and the effect of traditional processing on nutritional composition of Gibto (Lupinus albus L.) grown in Gojam area. His observation conducted in two areas; Dangla and Tilili. The mineral composition of the two cultivars was reported that, the Dangla sample had 6.00, 2.11, 58.43, and 8.93 mg/100 gm contents of Fe, Zn, Mn and Mg respectively. The values of the same types of minerals for the Tilili sample were 6.72, 1.81, 63.54, 59.14 and 9.46 mg/100 gm respectively.

White lupin is mainly consumed in different parts of Ethiopia. The concentration of mineral in white lupin is determined depending on the choice of cultivars and environment like soil type and climate conditions. Appropriate conditions together with selection of lupin cultivars that have a high metal concentration can be used to promote high metal bioavailability. According to Ethiopian central statistical agency, white lupin is highly cultivated in Amhara region, Ethiopia. For this reason, in order to know their health effect, it is necessary to determine the level of minerals in white lupin growing in three selected areas of Amhara region.

Since trace metals have role in disturbing at high levels in human tissue and body fluids. Therefore; knowing the content of metals in white lupin grain is a universal interest from a nutritional point of view. There are no other studies carried out in determining the level of metals in Ethiopian white lupin commonly known as “Gibto”. Therefore; the main objective of this study is to determine the concentrations of metals in white lupin (Lupinus albus) grain grown in different parts of Ethiopia.

2. Material and Method

Study Area

Samples of white lupin were collected from open markets of sampling area. The sampling sites are Debretabor, Merawi and Kossober. The geographic locations of the sampling sites are found in Amhara region. Debretabor is found in South Gondar, Merawi is found in west Gojam (Bahirdar zuria) and Kossober is found in Awi zone. The sampling techniques were random sampling.

Sample collection and processing

White lupin (Lupinus albus) sample from each sampling site were collected randomly from the mentioned market and processed. The seeds were cleaned and roasted on a metal until a black mark was seen at the centre of the seeds. The roasted seed is allowed to cool for about 10 minutes and soaked with distilled water in a clean erlymineary flask. The soaking water was changed every 12 hrs for 5 days until the bitterns was removed and rinsed with deionised water. After the bitterness was removed the seed was de-hulled and the kernel was dried with oven drying for 48 hrs at 50 °C. The dried kernel seed was grinded and sieved. The milled sample was stored with clean dried plastic bottles in a refrigerator [1]. The raw lupin sample was washed with tap water and as well with distilled water. Finally it is rinsed with deionised water and air dried. The dried whole seed was powdered and sieved. The powder was collected and stored with clean and dried plastic bottles in a refrigerator [6, 7]**.**

Digestion of white lupin sample

Sample digestion was carried out under optimum conditions of Nitric acid-perchloric acid mixture (7:3 v/v), digestion temperature (250 °C) and a total digestion time of 3:30 hours. Through applying the optimized procedure, 7 mL concentrated HNO_3 (69-72 %) acid and 3 mL of $HClO_4$ (60-62 %) (7:3) was added to 0.5 g of each separately sieved raw and processed white lupin powdered samples. The mixture was digested under a hot plate by covering the beaker by watch glass until the entire sample was digested and 1-2 mL colourless solution was remained. After digestion the raw and processed sample separately was allowed to cool for 10 minutes at room temperature followed by addition of 10 mL deionised. The solution was filtered and diluted with deionised water. On the same way the blank solution was prepared from 10 mL of reagents (i.e. a mixture of 7 mL of HNO_3 and 3 mL of $HClO_4$), boil the mixture as the same time and temperature used for the sample digestion procedure [8]. For Ca determination lanthanum nitrate hydrated was added to 50 ml flask containing the sample then placed in a freezer to avoid any decomposition until analysis [9].

Reagents and chemicals

All reagents and chemicals used in the study were analytical grade. HNO_3 (69-72%) $HClO_4$ (60-62 %) both from (SD Fine Chem Industries Mumbai, India), and H2O2 (30%, Scharlau, European Union), Lanthanum nitrate trihydrate (99.9%,Aldrich, USA) and standard stock solutions containing 1000 mg/L, in 2 % HNO3, of the metals

Ca, Fe, Zn, Cd and Pb (BUCK SCIENTIFIC GRAPHIC tm) were used.

Statistical analysis

Statistical Analysis of data was carried out using SPSS statistical package programs. A one-way analysis of variance (ANOVA) was performed by Origin (Version 6.1) software for the source of statistically significant difference.

Sample preparation

Working standard solutions were prepared by dilution from FAAS standard solutions containing 1000 mg/L using de-ionised water. All working standard solutions of each metal used for analysis was prepared fresh on the same day of the analysis made by diluting the intermediate standard solution with de-ionized water. Since the instrument is old the metal could not detect from the prepared calibrated standard near to the detection limit of the instrument. Therefore; working standards of all metal was prepared above the detection limit of the instrument. And finally all solutions were labelled to keep track of them [7].

Measurements were made using the hollow cathode lamps for Ca, Fe, Zn, Cd and Pb at the proper wavelength and the slit width using air acetylene flam. For determination, three replicate measurements were carried out for each sample. The concentration of each metal was calculated (Microsoft excel- 2007 and SPSS soft ware version 20) and the results were reported as mean standard deviation [7]. The concentration (ppm) of metals in lupin samples were calculated from the regression equation of the calibration curve and concentration in ppm is converted to mg/100 g. The metal contents of each sample were calculated as;

$$\text{Metal content (mg/100 g)} = \frac{(a-b)\times v}{10\times W}$$

Where; W = weight in gm of the sample a = concentration in ppm of sample solution

V = volume in ml of the extract b = concentration in ppm of blank solution [1]

Table 1: *Instrumental operating conditions for determination of major, minor and toxic metals in white lupin samples using FAAS.*

No.	Element	Flame Type	Wave length(nm)	Detection limit (mg/L)
1	Ca	Acetylene gas	422.7	0.05
2	Fe	Acetylene gas	372.0	0.05
3	Zn	Acetylene gas	213.9	0.005
4	Pb	Acetylene gas	217.0	0.08
5	Cd	Acetylene gas	228.9	0.001

Result and Discussion

Precision

To ensure the precision of the analysis, each sample was digested in triplicate and triplicate reading was carried out. The precision of the results were evaluated by relative standard deviation (RSD) of each metals analyzed for each sample. For Ca, Fe, Zn, Cd and Pb analysis the relative standard deviations of the measurements (Table 5) were found in the acceptable range. For Cd analysis in Debretabor raw, Merawi raw, kossober raw and kossober processed lupin and Pb analysis in all sample (excluding kossober raw lupin), the % RSD was found smaller value (% RSD < 5). This shows that the result is highly precise. But for Pb analysis in kossober raw lupin sample was 11.41 % and the % RSD of Fe in Debretabor lupin sample was 10.2 %. Since the % RSD is greater than 10, the lead concentration in kossober raw lupin and Fe content in Debretabor lupin is less agreement with that of the other samples.

Concentration of major, minor and toxic metals

Before analysis, FAAS was calibrated using standard solutions of each element. The concentration of selected metals (Ca, Fe, Zn, Cd and Pb) was determined using Flame Atomic Absorption Spectroscopy (FAAS). All raw and processed samples of white lupin contain the metal mentioned above. The level and variation of analyzed metals in three study areas and the effect of processing on metal content of each sample above (Table 2).

Values in the same column that are followed by a different litters (a-k) are significantly different at P < 0.05 by using one way ANOVA Duncan’s multiple-range test

When plants grow under different climatic condition, its chemical composition is different from place to place. Because, the geographical location, environmental condition, chemical and physical property of the soil, type of fertilizer used and PH of the soil are the main parameters affecting variation in chemical composition of all plants [10]. As a result the factors mentioned above can influence the content of metals in lupine grains.

Level of metals in raw white lupin

As indicated from table 2, Ca was found in maximum concentration compared to other metals. Additionally, the Ca content of Merawi lupin is higher (109.63 ± 6.33 mg/100 g) than Debretabor lupin (107.5 ± 9.668 mg/100 g) and kossober lupin

(90.646 ± 6.33 mg/100 g). This increasment might be due to the presence of higher amount of Ca in the soil. According to Atlabachew [11], Ca is found in a broad range in soil and water (especially ground and sea) and it is highly mobile to plant tissue. Due to this reason it may found in higher amount in lupin grains. This is can also explained as Ca was the most important macronutrient for lupin growth.

Table 2: *The metal contents (mean ±SD, N = 3) (mg/100 g) of raw and processed white lupin using Flame Atomic absorption spectrophotometer (FAAS).*

Metals	**Ca (mg/100 g)**	**Fe (mg/100 g)**	**Zn (mg/100 g)**	**Cd (mg/100 g)**	**Pb (mg/100g)**
Debretabor (raw)	107.5 ± 9.67a	24.52 ± 0.25e	15.3 ± 1.25f	1.38 ± 0.02i	5.091± 0.148j
Merawi (raw)	109.63 ± 6.33a	27.399 ± .46e	24.7 ± 2.169g	1.34 ± 0.01hi	6.597± 0.115j
Kossober (raw)	90.65 ± 6.33b	25.964 ± 2.5e	14.587± 1.25f	1.32 ± 0.04h	6.585 ± 0.75k
Debretabor (processed)	82.21 ± 7.31d	24.52 ± 0.25e	13.14 ± 1.25f	1.25 ± 0.01h	6.88 ± 0.337j
Merawi (processed)	61.11 ± 3.65c	26.9 ± 2.215e	22.54 ± 2.169g	1.19 ± 0.06h	7.54 ± 0.137k
kossober (processed)	65.33 ± 6.33c	25.96 ± 2.5e	12.418 ±1.25f	1.187± 0.01h	7.89±0.2998k

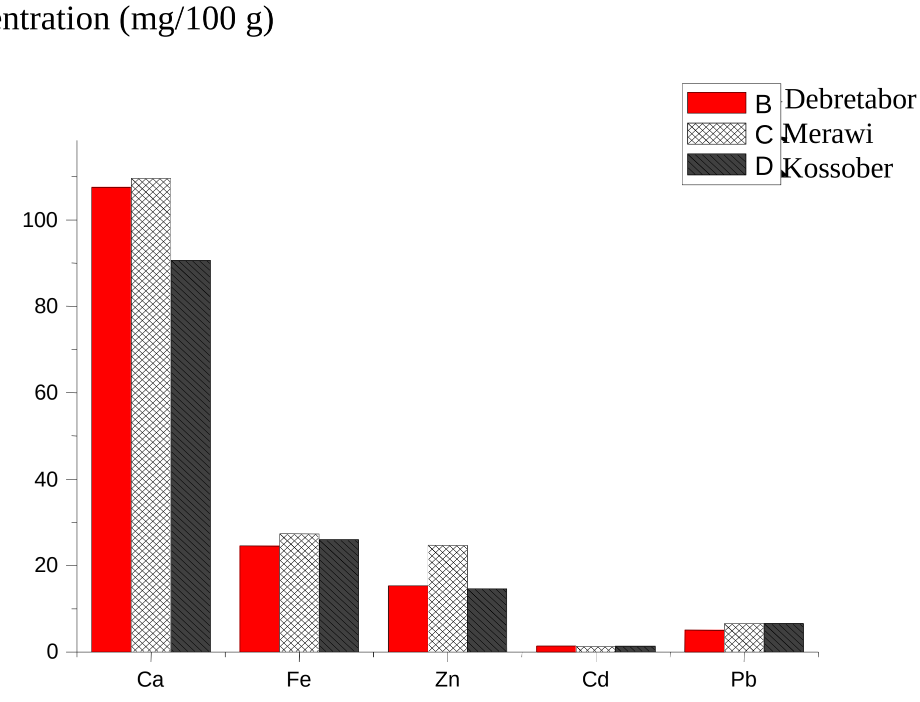

Fig. 1: Distribution of metals in raw lupin

Using one way ANOVA Duncan's multiple-range test, pair wise statical analysis of the result was done to verify whether there was a significant difference in metal content between lupin sample of the current study or not. This was done using SPSS, considering effect of geographical location as independent variable and concentration of the metals as dependent variable at stated confidence level. But no significant difference was observed on Ca content of Debretabor and Merawi lupin, while kossober raw lupin has significantly different ($P < 0.05$). The presence of significant difference on Ca content might be due to the variation in all the above mentioned factors.

Fe was found in relatively higher amount in lupin grain and found in the range from 24.515 ± 0.25 to 27.399 ± 1.46 mg/100 g. The Fe content of Merawi lupin is higher (27.399 ± 1.46 mg/100 g) than kossober lupin (25.964 ± 2.5 mg/100 g) and Debretabor lupin (24.515 ± 0.25 mg/100 g. But no significant difference was observed on Fe content in three study sites. The absence of significant different on Fe might be due to the fact that the climate condition, the physical and chemical property of the soil, the Fe content of the soil and the Fe absorbing capacity of the lupin plant might be similar.

The Zn content of Merawi lupin was higher than Debretabor and kossober lupin with a dry weight of 24.71 ± 2.169, 15.31 ± 1.25 and 14.587 ± 1.25 mg/100 g respectively. No statically significant difference observed on Debretabor and kossober lupin, while the Zn content of Merawi lupin was significantly different from other lupin samples. The probable explanation of presence of significant difference on Zn content might be due to difference in geographical location, soil metal content, PH of the soil and metal absorbing capacity of plants from the soil. Thus, highest concentration of Fe and Zn in lupin grain is considered as they are most important nutrient for plant growth like, protein synthesis, DNA synthesis, cell division etc.

In general; in the current study Ca, Fe, and Zn were detected relatively higher amount in lupin grain, one of the factor of this result might be PH of the soil and soil metal content. Atlabachew [11] result shows that soil PH is one of the most influencing parameter which controls the conversion of metals from immobile solid phase to the more mobile and bio available solution phase forms. Thus the low PH of soil results a more transfer of metals from the soil to plants. In Ethiopia, white lupin was grown in acidic soil (pH ranging between 4 and 5) [12]. Among this acidity of the soil, the metal can easily absorbed by lupin plants from the soil and lupin grain contains higher amount of major and minor metals.

Also, toxic metals Cd and Pb were found in the range 1.320 ± 0.0442 to 1.38 ± 0.015 and 5.091 ± 0.148 to 6.597 ± 0.115 mg/100 g respectively. As shown in table 5, the order of Cd present in three sampling area of raw lupin was found to be; Debretabor > Merawi > kossober. But no statically significant difference was observed at Merawi and kossober lupin, while between Debretabor and kossober lupin significant difference was observed. On the other hand the order of Pb content was found to be kossober > Merawi > Debretabor. Statically the Pb content of Merawi and Debretabor lupin were insignificant, while kossober lupin was significantly different from other lupin samples on Pb content. The presence of toxic metals Cd and Pb can be due to contamination of the plant during growth, storage of the seed after farming and transportation of the seed [13]. Atmospheric input and the use of phosphate fertilizer are the major source of Cd metal [14].

In Ethiopia, there is no enough industry to cause atmospheric pollution. So the major source of contamination of the plant with toxic metals might be comes from agricultural activities, such as the use of animal wastes, fertilizers and pesticides and irrigation with a contaminated msludge. And also storage place of the seed after harvesting, use of contaminated material for transportation of the seed might be the main source of contamination of lupin seed with toxic metals. The increasing trend of metals in the three area of raw lupin was found to be Ca > Fe > Zn > Pb > Cd and their distribution was given in Fig 1.

Level of metals in processed white lupin

The mean concentration of selected metals in processed white lupin was given in table 2. After processing the seed, the amount of Ca, Fe, and Zn were found in relatively higher amount in the range of Ca (61.11 ± 3.654 to 82.207 ± 7.31), Fe (24.515 ± 0.25 to 26.92 ± 2.215) and Zn (12.418 ± 1.25 to 22.54 ± 2.169 mg/100 g). Similarly the toxic metals Cd and Pb were found in the range of 1.187 ± 0.0115 to 1.250 ± 0.010 and 6.88 ± 0.337 to 7.89 ± 0.2998 mg /100 g respectively. The distribution of analysed metals in processed lupin is shown at Fig. 2. After processing the raw lupin seed, the mean concentration Ca was found in maximum value (82.2075 ± 7.3 mg/100 g) than other metals while Cd was found in lower value (1.187 ± 0.01 mg/100 g). In general, the presence of major and minor element in processed lupin and a decrease in Cd content after processing considered as white lupin can be one of the sources of minerals like other food legumes. Therefore; appropriate processing procedure must be implemented to use white lupin for feeding porpoise.

Comparison of levels of metals in raw and processed samples of white lupin

The concentration of metals in raw and processed white lupin samples is to some extent different since roasting, soaking and de-hulling process. Using one way ANOVA the variation in concentration of metals between raw and processed lupin studied lies within the overall range summarized in table 3. As indicated in table 3, Ca was detected in the range of 94.02 - 115.96 mg/100 g in the three varieties of raw lupin while it becomes 61.12 - 77.98 mg/100 g after processing of the seed. Thus, after processing the seed, a significant decrease in Ca content was observed.

Fe content was found in the range of 24.19 to 27.72 mg/100 g in raw lupin and 24.00 - 27.6 mg/100 g in processed lupin. While no significant difference was observed on Fe content between raw and processed lupin. This showed that processing of lupin seed could not affect the Fe content. While according to Getachew [1], the Fe content was reduced during soaking, boiling and germinating of raw lupin. And also according to Suliburska et al. [15] report, the removal of hull from the whole grain of white lupin resulted in a significant reduction of Fe content. Thus the result of these studies does not agree with those found by Getachew [1] and Suliburska *et al.* [15] on effect of processing in Fe content.

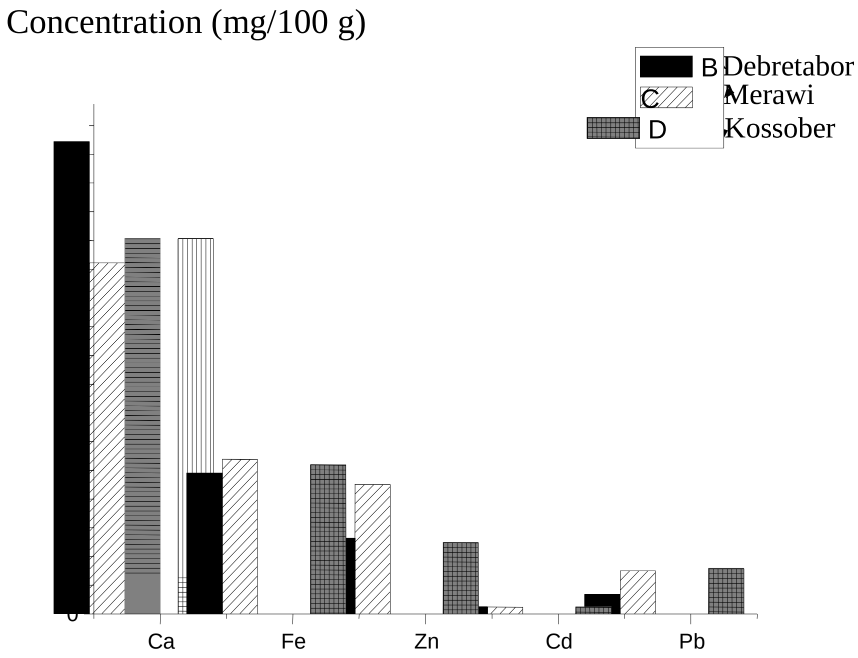

Fig.2: Distrbution of metals in processed lupin

Table 3: *Rang of metal concentration in raw and processed lupin sample (mg/100 g) at 95 % confidence level.*

Metals	Sample type of lupin	Range (mg/100 g)
Ca	Raw	94.02 - 115.96
	Processed	61.12 - 77.98
Fe	Raw	24.19 - 27.72
	Processed	24.00 - 27.6
Zn	Raw	14.29 - 22.11
	Processed	12.12 - 19.94
Cd	Raw	1.32 - 1.38
	Processed	1.18 - 1.25
Pb	Raw	5.16 - 6.34
	Processed	7.05 - 7.82

Zn content in raw lupin was detected in the range of 14.29 to 22.11 mg/100 g while it becomes 12.12 to 19.94 mg/100 g after processing the seed. Therefore; Zn is the second accumulated minor metal next to Fe in all sample types of lupin. But one way ANOVA result shows that processing of lupin has no significant difference on Zn content.

In general, except iron, the concentration of metals in lupin sample was decrease after processing. Because all the water soluble metals might be lost with roasting and washing process and some amount of metal might be removed to the husk of the grain. As a result a removal of hull from the whole grain probably results a reduction of Ca, Zn, and Cd content. The amount of Pb in white lupin was significantly increased after processing the seed. The possible explanation for this might be contamination during processing of raw lupin sample i.e roasting, soaking and as well digestion process. In general the pattern of concentration of metals in the six varieties of lupin samples was: Ca > Fe > Zn > Pb > Cd and the distribution is given in Fig. 3.

Comparison of levels of metals

The metal content of the three cultivars of Debretabor, Merawi and kossober of raw and processed white lupin were listed at table 5. The concentration of Ca found in this study from the raw lupin was higher than the value reported by Tizazu *et al.* [5] (82.56 mg/100 g) while the Ca content of processed lupin is comparable. On the other hand the amount of Ca in this finding is higher than the value reported by Bohumila *et al.*[6] (2.1 to 4.66 g/ kg) [4] and Jesus (138.92 ± 1.21 µg /g).

According to Getachew [1] finding, the concentration of Fe in two different cultivars (Dangla and Tilili) was 6.00 and 2.11 mg/ 100 g respectively. Thus the Fe content of this study was much higher than the value reported by Getachew[1]. Similarly the Fe content of the current study was higher than the value reported by Tizazu *et al.*[5] (12.51 mg/ 100 g) and Jesus *et al.* [3] (63.3 ± 0.14 µg/ g). Thus the result of this study does not agreed with the literature values. This might be due to difference in geographical location and soil type where lupin is growing. In the same way the Zn content of this study was higher than the value reported by Getachew[1] (1.81 - 2.11 mg/100 g), Tizazu *et al.*[5] (4.68 mg/100 g) and Jesus *et al.* [3] (55.0 ± 1.11 µg/g). According to *Maria et al* [16], lead was found in white lupin leaves and roots. But there is no other study found for determination of lead and Cd in white lupin. In this study the lead content of raw and processed lupin was found in the range of 5.16 to 6.34 and 7.05 to 7.82 mg/100 g respectively.

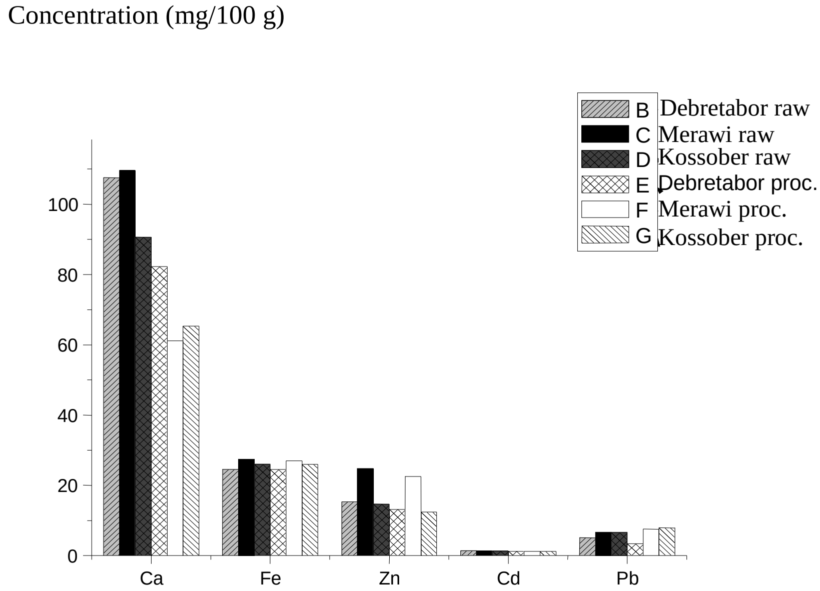

Fig. 3: Compariation of level of metals in raw and processed lupin

In general difference in metal content in this study with other study might be affected by several factors such as different in geographical location, soil metal content, the instrument used, the analyst, and sample preparation technique.

5. Conclusion and recommendation

The content of metals in raw and processed sample with similar trained across the verities is in the order of Ca > Fe > Zn > Pb > Cd. Additionally the concentration of different metals detected in the three sampling site was found as; Ca and Zn: Merawi > Debretabor > kossober lupin, Fe : Merawi > kossober > Debretabor lupin, Cd: Debretabor > Merawi > kossober and Pb: kossober > Merawi > Debretabor. This observation helps to conclude that geographical location have an effect on the metal content of the seed. Processing of the lupin seed reduced the metal content of each element except lead. The concentration of Pb was found to be higher in all the studied samples as compared to the WHO standards. Thus, this study was limited to conclude the lead content of white lupin seeds. Based on the findings of this study the following recommendations are forwarded. In order to aware users about the metal composition and to keep users safe from health risk, further study should be carried out by collecting samples from all major lupin growing areas of the country. In this study the amount of lead was found in higher proportion. Therefore; further analysis of its content is recommended. Additionally, analysis of the soil metal content where white lupin is growing and validating the method of analysis by characterizing using another instruments (ICP-MS, XRF) is very important.

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