DETERMINATION OF LEVELS OF ESSENTIAL AND TOXIC HEAVY METALS IN LENTIL (LENS CULINARIS MEDIK) BY FLAME ATOMIC ABSORPTION SPECTROSCOPY

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ABSTRACT

The concentrations of trace essential metals (Co, Cu, Fe, Mn, Ni and Zn) and toxic heavy metals (Cd and Pb) in lentil samples collected from Dejen (East Gojjam), Boset (East Shewa) and Molale (North Shewa), Ethiopia, were determined by flame atomic absorption spectrometry. A wet digestion procedure, using mixtures of HNO_3, $HClO_4$ and H_2O_2 was developed for the decomposition of powdered lentil samples. The accuracy of the method was checked by the standard addition method. The contents of heavy metals in lentil samples were in the range of 0.009-0.013 for Cd, 0.285-0.360 for Co, 0.226-0.282 for Cu, 9.17-11.91 for Fe, 6.7-8.2 for Mn, 0.120-0.244 for Ni, 0.142-0.176 for lead and 8.62-10.03 for Zn, all in mg/100 g. The results were compared with values reported in the literature. *[AJCE 4(4), July 2014]*

INTRODUCTION

Lentils are becoming increasingly popular and important sources of vegetable protein. High protein content (22 to 34.6%) and 55% starch, low level of anti-nutrients, high fiber content and ability to grow in low water stress conditions are the main attributes that make lentils important legume crops. Lentils also contain significant amounts of mineral elements like Ca, Mg, Fe, Mn, Cu, Co, Ni, B and Se [1, 2].

Lentils accumulate metals at different levels depending upon environmental conditions, metal species and available forms of the heavy metals. Many plants are found to take up large quantities of certain elements from the environment and are said hyperaccumulators of heavy metals [3]. Trace elements play important roles in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living cells of plants, animals and human beings. Cobalt is essential component of vitamin B_{12}; zinc is found in several enzymes and involved in genetic material transcription. Copper is a key component of redox enzymes and nickel has a role in metabolic activities. Iron is vital in oxygen transport and also enables metabolism. Manganese is a component and activator of a number of enzymes. Glycosyl transferase enzymes are specifically activated by manganese [4].

Though required in very small amounts, deficiency of trace elements cause diseases, whereas their presence in excess may result in toxicity to human life by disturbing normal functioning of organs and central nervous system. For instance, anemia, caused by the deficiency of iron, affects more than half of pregnant women and at least one third of children under five years [5]. Trace metals like lead, cadmium and mercury are, on the other hand, known for their detrimental health effects. Cadmium has been considered as an extremely significant pollutant, even in small amounts, affecting all forms of life because of its high toxicity and great solubility

in soil and water. No level of lead in blood should be considered safe for children due to its neurotoxicity [6-7]. The determination of levels of trace and toxic heavy metals in foods, especially in staple foods like lentils, is therefore an important concern of public health studies [2].

Trace metals contents are determined satisfactorily by a variety of methods; with the choice often depending on the precision and sensitivity required. Several spectrometry techniques have been used for macro and trace element determinations in plants or biological materials. The different techniques so far reported for the determination of metals in plant products are: direct current argon plasma optical emission spectroscopy (DCP-OES) [8], flame atomic absorption spectrometry (FAAS) [8-11], graphite furnace atomic absorption (GFAA) [12], inductively coupled argon plasma optical emission spectrometry (ICP-OES) [12, 13] and inductively coupled plasma mass spectrometry (ICP-MS) [12-14]. These methods are most commonly used for the determination of metals in environmental samples because of their inherent selectivity, sensitivity, precision and accuracy.

In this study, we determined the levels of six essential (Co, Cu, Fe, Mn, Ni and Zn) and two toxic (Cd and Pb) heavy metals in lentil samples collected from Dejen, Boset and Molale in Ethiopia. The results are compared with literature values.

EXPERIMENTAL

Sample collection

Whole lentil samples were collected from Dejen area (Gojjam), Boset area (East Shewa) and Molale (North Shewa) Ethiopia. The samples were packed into Polyethylene plastic bags, labeled and transported to laboratory for further treatment.

Sample Preparation

Each of the lentil samples were thoroughly washed with tap water and there after with distilled water to remove surface contaminants like soil, dust and spray residues. The samples were then placed in acid washed clean porcelain crucibles labeled according to the sample and oven dried at 85 ^{0}C for 48 hrs in drying oven (DIGITHEAT, J.P. SELECTA, S.A. SPAIN). At this stage, adequate care was taken to avoid any source of contamination, especially for micronutrients analysis. The dried lentil samples were ground and homogenized into fine powder with a grinding device (MOULINEX, FRANCE) and stored in polyethylene bags for digestion.

A digital analytical balance (Mettler Toleado, Model AG204, Switzerland) with $\pm$ 0.0001g precision was used to weigh the lentil samples. 100 mL round bottom flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest powdered lentil samples. A refrigerator (Hitachi) was used to keep the digested samples till analysis. Atomic absorption spectrophotometer (BUCK SCIENTIFIC MODEL 210 VGP, East Norwalk, USA) equipped with deuterium arc back ground correctors was used for analysis of the metals using air-acetylene flame.

Reagents that were used in the analysis were all analytical grade. HNO_3 (69-72% and $HClO_4$ (70%) [RESEARCH-LAB FINE CHEM INDUSTRIES MUMBAI 400 002 (INDIA)] were used for digestion of lentil samples. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn (BUCK SCIENTIFIC PURO-GRAPHIC^{TM}) were used for preparation of calibration standards and spiking experiments. De-ionized water was used throughout the experiment for sample preparation, dilution and rinsing of apparatuses prior to analysis and during analysis.

Procedure for digestion of lentil samples

Dry ashing [15] and wet digestion [16] are commonly used for analysis of lentil samples by FAAS. Different combinations of mineral acids have been employed for the decomposition of lentil flour by wet digestion. A mixture of HNO_3 and H_2O_2 (2:1 volume ratio) has been used to digest lentil at 350 ^oC for 4 hrs until a colorless solution was obtained [16].

In this study, the optimized parameters were digestion time, volume ratio of reagents and digestion temperature. HNO_3, $HClO_4$ and H_2O_2 in 4:1:1v/v ratio, 300 ^oC and 3 hrs were determined as optimum conditions (Table 1 a – c). 0.5g lentil flour was digested in round bottom flask by 6 mL mixtyre of HNO_3, $HClO_4$ and H_2O_2 in 4:1:1 volume ratio at 300 ^oC for 3 hrs using Kjeldahi apparatus. The digested solution was allowed to cool for 10 minute to room temperature without dismantling the condenser from the flask and for 5 minute after removing the condenser. To the cold solution, de-ionized water was added to dissolve the precipitate formed on cooling. One drawback of wet digestion of organic samples is co-precipitation and formation of insoluble compounds after cooling of the digested filtrate [17]. Addition of some de-ionized water was required to prevent and eliminate any co-precipitation and calcinations on cooling. The solution was then filtered into 50 mL volumetric flask with 125 mm diameter Watman filter paper (pore size 11µm). The volumetric flask was filled to the 50 mL mark with de-ionized water. Blank solutions were prepared following the same digestion procedure as the sample. Triplicate of actual samples and sextet of blanks were digested. The digested samples were kept in the refrigerator until FAAS analysis.

Determinations of the essential and heavy toxic metals in the digested lentil samples

Secondary standard solutions containing 10 mg/L were prepared in 1000 mL volumetric flask from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L (BUCK SCIENTIFIC). Three working standards for each metal of interest were prepared from these secondary standards. These working standards were prepared freshly for each element from the secondary standards by appropriately diluting with de-ionized water for calibration purpose as shown in Table 2. Then, Mn, Co, Cu, Zn, Ni, Cd, Pb and Fe were analyzed by FAAS using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength) were optimized for maximum signal intensity of the instrument. For each element, respective hallow cathode lamp was inserted in to the atomic absorption spectrophotometer, and the solution was successively aspirated into the flame. The acetylene and air flow rates were managed to ensure suitable flame conditions. The elements in the three replicate samples were determined by absorption/concentration mode and then the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in the six digested blank solutions.

RESULTS AND DISCUSSION

Optimization of digestion procedure

Sixteen procedures involving some variations in reagent volume, reagent composition, digestion temperature and time were tested.

Table 1a: Optimized reagents' volume for digestion of 0.5 g of lentil samples

Trials	Reagents	Volume (mL)	Ratio	Observation
1	HNO_3	6		Clear yellow
2	HNO_3:$HClO_4$	5:1		Light yellow
3	HNO_3:$HClO_4$	4:2		Light yellow
4	HNO_3:$HClO_4$	3:3		Clear suspension
5	HNO_3:$HClO_4$:H_2O_2	4:1:1		Clear(optimized)
6	HNO_3:H_2O_2	4:2		Yellowish

The procedures were developed with some modification of a procedure in literature used to determine the levels of trace metal contents in commercial powdered soup samples by atomic absorption spectroscopy [16]. The optimized procedures and conditions indicated in (Tables 1a - c) were used throughout the analysis.

Table 1b: Optimized Time for digestion of 0.5 g lentil samples in HNO_3/$HClO_4$/H_2O_2 mixture

Trials	Time (hr)	Observation
1	2:00	Yellow
2	2:30	Clear yellow
3	3:00	Clear (optimized)
4	3:30	clear

Table 1c: Optimized temperature for digestion of 0.5 g lentil samples in HNO_3/$HClO_4$/H_2O_2 mixture

Trials	Temperature (^oC)	Observation
1	180	Yellow
2	210	Light yellow
3	240	Clear suspension
4	270	Clear suspension
5	300	Clear (optimized)
6	330	Clear

The optimized procedure was selected depending on clarity of digests, minimal reflux (digestion) time, minimal reagent volume consumption, absence of colored undigested lentil samples and simplicity. Based upon these criteria, HNO_3, $HClO_4$ and H_2O_2 (4:1:1 v/v ratio) were selected for complete digestion of 0.5 g lentil flour (Table 1a) at 300 ^oC for 3 hrs. The other tested procedures have some limitation. They require higher reagent volumes, longer digestion time and higher temperature and result in the formation of turbid digests and colored digested solutions. Since wet digestion was used, reagent blanks were also prepared and digested with the same procedure as the sample, and used to correct for impurities present in the acids and de-ionized water.

Instrument calibration

Data obtained from analysis of metals using FAAS are seriously affected by calibration and standard solution preparation procedures. The instrument was calibrated using three series of working standards. The working standard solutions of each metal were prepared fresh by diluting the intermediate standard solutions. Concentrations of the working standards and value of correlation coefficient for each metal is shown in Table 2.

Table 2: Series of working standards and correlation coefficients of the calibration curves for determination of metals in the lentil varieties using FAAS

Metal	Concentration of Standards(mg/L)	Regression Equation (A=mC + b) *	Correlation Coefficient
Cd	0.25, 0.50, 1.00	$A = 0.10C – 0.006$	0.9993
Cu	0.50, 1.00, 1.50	$A = 0.05C – 0.0013$	0.9998
Co	0.25, 0.50, 1.00	$A = 4.28C + 9.1 \times 10^{-5}$	0.9995
Pb	1.20, 2.40, 4.80	$A = 5.63C \times 10^{-4} – 7.73 \times 10^{-6}$	0.9991
Mn	0.25, 0.50, 1.00	$A = 0.02C + 1.13 \times 10^{-4}$	0.9999
Ni	0.25, 0.50, 1.00	$A = 8.8 \times 10^{-3}C + 8.45 \times 10^{-5}$	0.9996
Zn	0.20, 0.40, 0.80	$A = 0.10C + 4.5 \times 10^{-6}$	0.9997
Fe	0.50, 1.00, 1.50	$A = 2.30 \times 10^{-3} + 1.33 \times 10^{-5}$	0.9992

* A = Absorbance, C = Concentration in mg/L

Evaluation of analytical results

Precision

In this study, the precision of the results were evaluated by the pooled standard deviation and relative standard deviation of the results of three samples (n = 3) and triplicate readings for each sample, meaning, nine measurements for a given bulk sample. These parameters are useful in estimating and reporting the probable size of indeterminate error. The results of the present analysis are reported with corresponding pooled standard deviation of nine measurements for a bulk and relative standard deviation as shown in Table 3.

Recovery test of the optimized procedure

The efficiency of the optimized digestion procedure was checked by adding known concentrations of each metal in 0.5 g sample. 100, 50, 50, 50, 50, 50, 50 and 100 □g of Mn, Co, Ni, Cd, Pb, Cu, Zn and Fe respectively were spiked to the samples simultaneously at once, for the recovery analysis. Each recovery test for the sample was performed in triplicates. Standard metals solutions were used to fortify the sample to the specified metal and the percentage recovery were calculated using equation 1.

R = [(Amount after spike – amount before spike)/ Amount added] x 100% ---------- (1)

Recoveries of the metals in the spiked lentil sample were between 89 and 101.5 %. Since the mean percentage recoveries for all analytes were within an acceptable range (75-125%), the laboratory performance for each analyte was in control.

Table 3: Analytical results for Recovery test of the optimized procedure for lentil samples

	Concentration (mg/100g)		
Metal	Added	Found	% Recovery
Cd	0.0	0.012 ± 0.0009	
	5.0	4.63 ± 0.32	92.4
Co	0.0	0.30 ± 0.014	
	5.0	4.75 ± 0.48	89.0
Cu	0.0	0.235 ± 0.0039	
	5.0	5.12 ± 0.53	97.6
Fe	0.0	9.70 ± 0.32	
	10.0	19.85 ± 1.3	101.5
Mn	0.0	7.83 ± 0 .11	
	10.0	17.38 ± 1.7	95.5
Ni	0.0	0.23 ± 0.013	
	5.0	4.93 ± 0.30	94.0
Pb	0.0	0.16 ± 0.008	
	5.0	4.93 ± 0.39	95.4
Zn	0.0	9.97 ± 0.13	
	5.0	14.90 ± 1.1	98.6

Recovery values in the above range are acceptable for both bulk and trace analysis and the digestion procedure is believed to remove metal fractions associated with organic matter.

Levels of metals contents in the analyzed lentil samples

The concentrations of the essential metals Mn, Fe, Ni, Co, Cu and Zn and heavy metals Cd and Pb in the different lentil samples were determined as shown in Table 3 and Figure 1.

Cadmium

In the present investigation, the values of Cd range from 0.009 to 0.013 mg/100 g in various lentil samples. The maximum concentration (0.013 mg/100 g) of Cd was recorded in samples collected from Dejen and Boset, while minimum concentration (0.009 mg/100g) was

obtained in the lentil samples of Molale. The permissible limit for cadmium in foods is 0.05g kg^{-1} on dry basis [15].

Cobalt

The cobalt content in this study varies from 0.285 to 0.360 mg/100g. The lowest concentration (0.285 mg/100g) of cobalt was observed in the lentil samples from Molale and Boset. On the other hand, the lentil from Dejen showed highest concentration of cobalt (0.360 mg/100g). The safety limit for human consumption of cobalt is 0.05 to 1 mg/day for humans [17].

Copper

The acceptable limit for human consumption of copper is 10 ppm [18]. The present investigation revealed that the concentration of copper varied from 0.226 to 0.282 mg/100 g, which lies below the safety limit for copper. The highest concentration of copper was found in Dejen lentil (2.82 mg/100g), while lowest concentration (2.26 mg/100 g) was recorded in lentils of Boset.

Iron

The concentration of iron content was highest in Molale lentils (11.91 mg/100 g), while it was found lowest in lentils of Boset (9.17 mg/100 g). The acceptable limit for human consumption of iron is 8 to 11 mg/day for infants as well as adults [19]. During present investigation, the value of iron was found slightly higher in lentil from Dejen.

Nickel

Nickel is found in soybeans, lentils, nuts, grains and vegetables. Lentil from Boset showed higher content of nickel (0.244 mg/100 g), while lentils of Molale have low concentration of nickel (0.120 mg/100 g). The amount of nickel ranges from 0.120 to 0.244

mg/100 g in the different lentil samples. The prescribed safety limit of nickel is 3 to 7 mg/day in humans [20]. In this study, the contents of nickel are below the safety limit.

Lead

During the present study, the lead content varied from 0.142 to 0.176 mg/100 g, which slightly exceeds the safety limit (1.5 ppm) for human consumption [20]. Lead was not detected in Dejen and Molale lentils while Boset lentil contained 0.155 mg/100 g of lead.

Zinc

The acceptable limit for human consumption of zinc is 150 ppm [19]. In this study, the concentration of zinc was found to be high in lentils of Boset (10.03 mg/100 g), while low concentration of zinc was observed in lentils of Molale (8.62 mg/100 g). The content of zinc ranges from 8.62 to 10.03 mg/100 g, which falls within the range of the recommended daily intake.

Manganese

The recommended intake of Mn from food; water and dietary supplements should not exceed the tolerable daily upper limit of 11 mg/day [21]. In this study, the concentration of manganese ranged between 6.95 and 7.83 mg/100 g. The trend of concentrations of various heavy metals in lentils studied in this work is as follows: Fe > Zn > Mn > Cu > Co > Ni > Pb > Cd.

Table 3: Average levels of essential and heavy toxic metals (mg/100 g) in powdered lentil samples

	Dejen		Molale		Boset	
Metal	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
Cd	0.012 ± 0.0006	4.75	0.010 ± 0.0008	7.26	0.012 ± 0.0009	8.21
Co	0.31 ± 0.013	4.19	0.32 ± 0.012	3.75	0.30 ± 0.014	4.67
Cu	0.272 ± 0.029	1.10	0.253 ± 0.012	0.47	0.235 ± 0.039	1.70
Fe	11.26 ± 0.32	2.84	11.25 ± 0.53	4.71	9.7 ± 0.32	3.30
Mn	6.95 ± 0.09	1.27	6.96 ± 0.20	2.88	7.83 ± 0 .11	1.40
Ni	0.208 ± 0.012	5.76	0.132 ± 0.008	5.82	0.232 ± 0.013	5.62
Pb	BDL	-	BDL	-	0.155 ± 0.008	5.43
Zn	8.79 ± 0.11	1.25	8.73 ± 0.08	0.95	9.79 ± 0.13	1.33

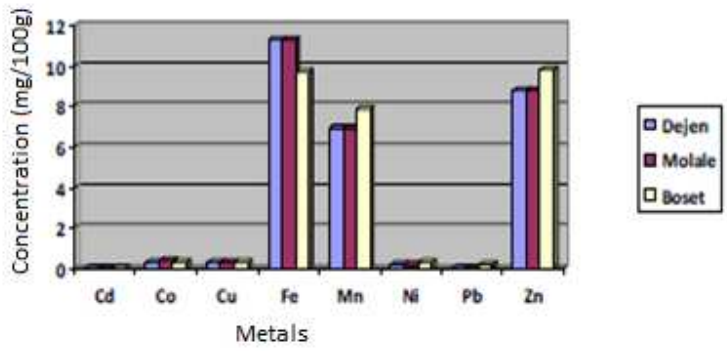

Figure 1: Levels of metals contents (mg/100g) in various lentil samples

Comparison of the analyzed concentration of metals in lentil samples with reported (literature) values

Researchers have reported the concentration of Cd [16, 22,28], Co [27], Cu [16, 22, 26,28], Fe [16, 22, 25, 26,28], Mn [16, 24,28], Ni [22], Pb [16, 23,28] and Zn [16, 27,28] in lentil. The comparison of the metal concentrations determined in this study along with the reported values is presented in Table 4.The concentrations of the heavy metals determined in this study are, in most cases, closer to the lower limits of literature values. The variations between the

results of this study and literature values may be due to sample size, soil type, genetic variation and environmental factors. Environmental factors such as urban waste, fertilizer use, irrigation, pollution as well as climate variation affect the rates of bioaccumulation of metals by plants and their bioavailability [3, 29]. The cadmium contents in literature (0.013-0.024) are relatively in good agreement with the analyzed values in this study [22]. The iron content determined in this work is also comparable with previous reports [26] except that the maximum value in the literature is highest.

Table 4: Comparison of the analyzed concentration of metals in lentil samples with values reported in the literature.

	Concentration (mg/100g)		
Metal	Analyzed	Reported	References
Cd	0.009-0.013	0.009-0.50	16, 23, 28
Co	0 .285-0. 36	82.6 ± 2	25
Cu	0.226-0.282	2.50-7.7	16, 22, 28
Fe	9.17-11.91	0.63-61.7	16, 22, 23, 26, 28
Pb	0.142-0.176	nd*-0.5	16,22,28
Mn	6.70-8.20	7.41-19.0	16, 25, 28
Ni	0.12-0.244	0.24	27
Zn	8.62-10.03	6.11-58	16, 20, 26, 27, 28

* No detection

All the metals analyzed in this work are in close agreement with the lower values in the respective literatures. The higher values in this study are far lower when compared to literature values because of the smaller number of samples analyzed.

STATISTICAL ANALYSES

Differences between the mean values of the various samples obtained in this study were evaluated by student's paired *t*-test. Linear regression statistical test and correlation analysis

were performed for the calculation of slope (m), and correlation coefficient (R) of the regression line as shown in Table 2. Statistical analysis is based on triplicate measurements of all samples.

In pair-wise student's *t*-test, the term on the right side of **equation 2** is computed using *t* values for the 95% confidence level. The number of degrees of freedom for finding the *t* values is $(N_1 + N_2) - 2$, where N_1 and N_2 are number of replicate measurements of sample 1 and sample 2 respectively, and S_d is the pooled standard deviation.

$$x_1 - x_2 = t\, S_d[(N_1 + N_2)/ N_1N_2]^{1/2} \quad \text{---------- (2)}$$

If the experimental mean difference, $x_1 - x_2$, is smaller than the computed value, no significant difference between the two means has been observed. An experimental difference greater than the value computed from *t* indicates that there is a significance difference between the means. As shown in table 5, student's *t*-test at 95% confidence level indicated that there were significant differences between the mean values of Dejen and Molale samples in copper and nickel. Significant differences were observed between Dejen and Boset samples in most metals but cobalt and nickel. Molale and Boset samples showed significant differences in all metals except cobalt.

Table 5: Pair-wise comparison between mean values of various lentil samples by student's *t*-test at the 95 % confidence level

		Metals						
Samples	Parameters	Cd	Co	Cu	Fe	Mn	Ni	Zn
	D (m) *	0.0009	0.016	0.19[a]	0.01	0.012	0.076[a]	0.066
Dejen vs. Molale	t_p	0.002	0.026	0.15	1.04	0.34	0.023	0.18
	D (m)	0.001[a]	0.008	0.38[a]	1.55[a]	0.89[a]	0.023	1.00[a]
Dejen vs. Boset	t_p	0.0007	0.014	0.15	1.00	0.72	0.025	0.41
	D (m)	0.0012[a]	0.024	0.19[a]	1.55[a]	0.88[a]	0.10[a]	1.1[a]
Molale vs. Boset	t_p	0.0008	0.029	0.096	0.39	0.76	0.008	0.18

** D (m): differences between means,* $t_p = tS_d[(N_1+N_2)/N_1N_2]^{1/2}$ *at 95 % confidence level* ($N_1=N_2=3$), *a = significant difference exists*

IMPLICATIONS FOR CHEMICAL EDUCATION

One of the major obstacles in chemical education is the lack of association between what is theoretically taught and what is actually practiced in research. If our teaching is supported and referred to results of research works in chemistry, it will bring greater motivation and interest on students' learning. Bringing such practical works and research experiences, particularly those done on environmental samples, to the chemistry classroom has a large contribution to the quality of chemical education. It could be a practical example of the analysis of real samples using spectroscopic instruments.

CONCLUSIONS AND RECOMMENDATION

An efficient and simple digestion procedure was developed for the analysis of lentil flour and validated by the standard addition method. The optimized digestion procedure allowed the use of mixtures of small volumes of HNO_3, $HClO_4$ and H_2O_2 (4:1:1 v/v) respectively, leading to reduced blank contamination and lower method detection limit. The levels of eight essential and toxic metals were determined in lentil samples by FAAS method. A pair-wise student's *t*-test at the 95% confidence level revealed that there were no significant differences between the mean values of the mineral contents of lentil samples from Dejen and Molale. It also indicated that the random errors associated in sample preparation and measurement steps are not significant, indicating the reliability and applicability of the proposed method for these samples. Research reports indicated that legumes in general and lentils in particular are excellent sources of proteins, carbohydrates, fiber and essential mineral nutrients when used appropriately in our balanced diets [30].

Although the data obtained in this study is small to draw authoritative conclusions about the mineral contents of lentil in Ethiopia, it will provide base line information and initiate further and detailed investigation.

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