

Elemental Characterization of Indigenous Food Cereal, Hordeum vulgare, Using Neutron Activation Analysis

Technique and Gamma Ray Spectrometry

B. Ayele. Getaneh¹, A.K. Chaubey², B. Akile. Teshager³, M. HailuTamene³,

¹Department of physics, Addis Ababa University, Ethiopia

Abstract

*This study intended to assess the elemental characterization of plant cereal *Hordeum vulgare*, that has served as staple food by all Ethiopian women during the period of post child delivery for the first three months, and also monitoring for some toxic elements, if any. The study identified essential and trace elements, in the sample, that support health and enhance nutritional value.*

Neutron activation analysis of indigenous white barely was done on

sample collected directly from shola which is one of largest indigenous cereals markets in Addis Ababa, Ethiopia. The experiment was carried out at the Czech Republic, using LVR-15 research reactor at the CANAM infrastructure of NPICAS, Rez. The irradiated samples were analyzed and more than 40 elements most of which are essential elements for human health, in major, minor and trace levels were identified.

Key words: *Hordeum vulgare NAA(INAA),cereal sample, major, minor and trace elements*

1. Introduction

The white barley (*Hordeum vulgare* L.) is the fourth most produced cereal in the world after wheat, maize and rice [6]. It is mainly used as animal feed but there is a growing interest in it for human food. In general, barley has been used in the feeding of adult monogastrics and ruminants, all of which have an important digestible capacity (Bergh et al., 1999). Barley grain is mainly energetic feed, is important source of protein for the nutrition of animals, but is deficient in certain essential amino acids when used as food for monogastrics animals. The major components of barley are starch, dietary fiber, and crude protein, constituting: 60, 20, and 12% of dry matter, respectively (Åman and Newman, 1986; Oscars son et al., 1996).[1-2] Plant foods can contribute significantly to human nutrition and health, because they contain almost all essential human nutrients. Cereal food plants in Ethiopia, take the largest part of food components due to their availability, ease of preservation, and relatively low cost than other components, particularly, cheaper than animal origin foods. Thus their elemental analysis is essential to determine the composition in elemental level of the diet usually consumed

by the community. Minerals and water diffuse to part of a plant from the soil through the roots of the plant or absorbed by leaves of the plant from the atmosphere. Using sun light, plants produce starch and sugar by a process called photosynthesis. Plants absorb essential elements in major and minor quantities from the soil in which they grow and most trace elements in plants are produced by metabolic processes of plant cells (Dekker, New York). [3]

Plants consumed minerals, such as potassium (K), sulfur(S), Nitrogen (N), Magnesium (Mg), Calcium (Ca), phosphorus (P), Aluminum (Al) and Tillicum (Ti) in large amount from the soil, depending on their availability in the soil. Other elements such as Molybdenum (Mo), iron (Fe), Boron (B), Zinc (Zn), chlorine (Cl), Copper (Cu), manganese(Mn), etc. are needed by plants in small amount. Different parts of a plant (root, leaf, stem, seed /fruit) may have different concentration of different elements [4]

Barley originated in Ethiopia and Southeast Asia, where it has been cultivated for more than 10,000 years. Barley was used by ancient civilizations as a food for humans and animals, as well as to make alcoholic beverages; the first known recipe for barley

wine dates back to 2800 BC in Babylonia. Barley played an important role in as a staple bread-making grain as well as an important food for athletes, who attributed much of their strength to their barley-containing training diets.[5] Most Ethiopian athletes are from the cereal cultivation region (Arsi and Bale).

Barley was one of the first domesticated grains in the fertile Crescent, an area of relatively abundant water in Western Asia, and near the Nile river, horn of Africa (Ethiopia), 8500BC.[6-7]. In a 100g serving, raw barley, above 352Calories and is a rich source (20%) or more of the daily value of essential nutrients, including protein, dietary fiber, B-vitamins, niacin(31%)and B6(20%) and several minerals. Raw barley has 78% Carbohydrates, 1% fat, 10% protein, and 10% water.[8] Ethiopian

indigenous barley/*Hordeum vulgare*/ grows in the highlands of the country with altitude from 1000 to 3000m above sea level⁷. Most of the regions of cultivation of the plant, *Hordeum vulgare*, use no fertilizers or very small amount of fertilizers. The soil fertility within the altitude mentioned, is suitable for the cereal growth and yield of the cereal.

Considering the importance of nutritional values and maintenance capability of macro and micronutrients, in various human metabolic processes, the present study; elemental analysis of white barley /*Hordeum vulgare*/using instrumental neutron activation analysis, create scientific awareness about the cereal content and change its mode of cultivation based on the findings.

Theory of Neutron activation Analysis

Most naturally occurring isotopes can be transformed into radioactive ones when exposed to neutron radiation in a research reactor, and the activity of the produced radioactive products can be measured by means of appropriate counter system. The activity is affected by the neutron flux in the point of irradiation, the number of target atoms and by the activation cross section of the target material (detector), provided the activation cross section is known, the neutron flux can be determined by measurement of the activity of the sample irradiated.

When a neutron is captured by a stable target nucleus, in a sample, radioactive nuclides in excited states (the compound nucleus) is produced. The compound nucleus formed in the activation process usually decays by emission of a beta particle and characteristics gamma ray(s) with a unique half-life which are fingerprints of elements in the sample.

Delayed gamma (γ -) rays is a function of the half-life time of the radioactive nucleus, which could be in ranges from fractions of a second to several years. It is by analyzing

these delayed gamma-rays that the identity and concentrations of the elements in a given samples determined. A high-resolution gamma-ray spectrometer is used to detect these ‘delayed’ gamma rays in the presence of the artificially induced radioactivity in the sample for both qualitative and quantitative analysis.

For NAA, in which neutrons are used, several nuclear reactions are possible depending on the target nucleus and the neutron energy (n,γ), (n,n), (n,α), (n,p). Other more limited neutron reactions used in NAA are induced fission, (n,f), for fissionable elements (U, Pu, Th) and inelastic neutron scattering, ($n,n'\gamma$), in which a radioactive isomeric state of the target nuclide is measured (e.g., ^{77m}Se , ^{111m}Cd , ^{204m}Pb). Most applications of NAA to determine trace elements in biological materials utilize the (n,γ) reaction because of the generally higher sensitivity achieved compared to other reactions. All (n,γ) reactions with stable isotopes, with the exception of $^4\text{He}(n,\gamma)^5\text{He}$, are exoergic and thus have zero neutron threshold energies[9] The activity of induced

radionuclide in the irradiated sample is given by the expression

$$A = \varepsilon \sigma I_{\gamma} \varphi (1 - e^{-\lambda t_i}) e^{-\lambda t_d} (1 - e^{-\lambda t_c}) / \lambda t_c \quad (1)$$

Where A the activity of induced radionuclide, λ the decay constant of the radionuclide, σ is the reaction cross section, I_{γ} - the gamma ray emission probability, φ - the average neutron flux, ε – detector efficiency and the terms in brackets are, in sequence, the saturation, the decay and the counting correction factors, respectively and the suscripts for time terms account for irradiation(i), decay(d) and counting(c) interval.

In the relative standardization method of INAA, employed in this work, the concentration of an element in the unknown sample was calculated by irradiating the unknown sample and a standard containing a known amount of the element of interest together in the reactor. If the unknown sample and the standard are both measured on the same detector and the decay and irradiation times between the two are corrected, the mass of the element in the unknown sample can be calculated by using the half-life of the measured radio isotope. The equation commonly used to calculate the

mass of an element in the unknown sample relative to the standard is

$$\frac{A_{sa}}{A_{st}} = \frac{M_{sa}}{M_{st}} \frac{(1 - e^{-\lambda t_d})_{sa}}{(1 - e^{-\lambda t_d})_{st}} \quad (2)$$

Where, A is induced activity of the sample (sa) and standard (st), M_{sa} is mass of the element and M_{st} is that of standard, λ is decay constant for the induced radioisotope, and t_d is the decay time.

For a stable reactor flux and when performing short irradiations, the irradiation, decay, and counting times are normally fixed for all samples and standards such that the time-dependent factors canceled out. Thus, the concentration of the element of interest in the sample is (simplifying (1) and (2))

$$C_{sa} = C_{st} \frac{W_{sa} A_{sa}}{W_{st} A_{st}} \quad (3)$$

Where, C_{sa} and C_{st} are concentration of the element and standard and W_{sa} and W_{st} are weight of the sample and standard, respectively. Thus measuring the weights of sample and standard, and their corresponding activities of known concentration one can deduce the concentration of the element of interest in the sample.

2. Material and Method

2.1. Sample preparation

The sample, collected from shola market that is found in Addis Ababa, Yeka sub-City, washed with non- ionized water to avoid contaminant and dried in oven at 50⁰c for 12Hrs, in homeland, in Addis Ababa University Polymer laboratory. Then the dried sample was crushed into fine powder using a clean Agate mortar and pestle. The powdered sample was sieved and homogenized with a 600mesh sieve packed in a clean polyethylene bag with a total mass of 100g.

The sample is taken to the Physics Institute of the Czech Academic Science (NPICAS), research center, Rez for further preparation and irradiation. The dried sample, taken to NPICAS), Rez, was further dried to avoid moisture content in an oven until weight stability was maintained. A weight of 100mg of the powdered sample was wrapped in a polyethylene film and this in turn sealed in a clean rabbit capsules that were soaked for three days in 1:1 concentrated HNO₃ and then heat sealed.

Preparation was done for short INAA, short epithermal NAA and long irradiation regime; each sample was fed to the nuclear reactor

port by means of pneumatic transfer system with the aid of rabbit capsule and retrieved with the same system for counting. For short and medium lived radioisotopes, the wrapped films were packaged into a 7ml polyethylene vial (i.e. one sample in one polyethylene vial), which were in turn air tight and heat sealed for irradiation. Standard reference materials namely NIST SRM 1547(Peach leave) was prepared and packed similarly as the samples. However, for the long lived radioisotopes, the standard reference material and the sample were sealed together into one polyethylene vial for irradiation.

2.2. Quality assurance

The precision and accuracy of the experimental work was confirmed by analysis of NIST SRM 1547 (Peach leaves) and comparing the result with the NIST values of the elements composed in the standards, The results were fit well with the NIST value for almost all the available elements(Table 1) with the deviations not exceeded 5%.

2.3. Sample irradiation and counting

No. LM2011019, the LVR-15 experimental

reactor of the research center Rez was operated at a power of 9.7MW and was providing $3.2 \times 10^{13} \text{ncm}^{-2} \text{s}^{-1}$ neutron fluence rate for short thermal irradiation, $1.1 \times 10^{13} \text{ncm}^{-2} \text{s}^{-1}$ neutron fluence rate for short epithermal and fast irradiation and $3.6 \times 10^{13} \text{ncm}^{-2} \text{s}^{-1}$ fluence rate for long thermal irradiation. To irradiate the prepared sample, the reactor transport system has a pneumatic facility within a transport time of 3.5s. The samples were irradiated in different mode of irradiation depending on the half life time of the kind of elements of interest to be identified. The γ -ray product radionuclides were qualitatively identified by the energies emitted and the quantitative analysis was done by converting the counts as area under the photo peaks using the relative method of analysis. Through appropriate choice of cooling time, the detector's dead time was controlled to be less than 10 %. Samples were irradiated for short time thermal irradiation (t_i) 1min, decay time(t_d) 10min and counting time (T_c) 10min, to determine specific amount of short lived radionuclides in the sample.

For epithermal irradiation, time group for each (irradiation, decay and counting), respectively, was 0.75min, 8min and 15min.

The long regime irradiation, decay and counting time group was, respectively, 2hrs, 4-7days, 25min and 2Hrs, 30days, & 3hrs in two regions of long irradiation. The effective geometry of detection for short was 5cm intermediate 2cm, long 7cm & 1cm(for 30 days decay time). The gamma radiations emitted by the induced radionuclides of the sample were detected and evaluated using high pure germanium (HPGe) coaxial detector. Long irradiated samples were counted twice within two decay-time intervals: first long within 4 to 7days, and second long within 30 to 35 days and short epithermally irradiated samples were counted twice within two decay time intervals: 10min and 10min by using Canberra (CAN coaxial, 78% efficient and resolution of 1.9keV at FWHM for 1.33 MeV γ - line of ^{60}Co) and ORTEC (ORT coaxial, 53% efficiency and 1.8KeV, at FWHM for 122 KeV γ - line of ^{57}Co) detectors. Iron monitors were inserted between each set of samples and/or standards to determine the axial neutron flux gradient.

The HPGe detectors were interfaced to Canberra Genie 2000 gamma-spectroscopy computer controlled analyzer through a chain of associated linear electronics. A Canberra

599 Loss Free Counting module was used to correct variable count-rate and dead time [10]

2.4. Data analysis

The results represent mean \pm standard deviation (Stdev) the statistical determinations. An ANOVA test (SPSS 7.0 statistical software, SPSS Inc.) was used to compare the mean value of each treatment. Significant differences between the means were determined by using the Tukey HSD test ($P < 0.05$).

3.0. Results and Discussion

Of the 45 elements identified, Na, Mg, Al, Fe, K, Ti, Si are major elements detected in this sample, with Si(32.7 ± 0.7) and Al(8.53 ± 0.14) exceptionally of high concentrations may be arise from the soil in which the plant grew. Cu, La, Ba and Ce are in minor levels and the rest are found in trace. Elements in trace levels include: Cl, Sc, Ca, V, Cr, Mn, Co, Ni, Zn, Ga, As, Se, Br, Rb, Sr, Ag, Cd, Sb, Cs, La, Pr, Sm, Eu, Tb, Dy, Ho, Tm, Yb, Lu, Hf, W, Ir, U, Th, while Ta(< 0.0007 ppm) and Au(0.011) is found in ultra-trace level.

The presence of elements such as Mn, Mg, Se, K, Na, Zn, Cu, I, and Fe in appropriate amounts made the cereal food sample as the healthiest food of choice. Fe(1.73 ± 0.03), Ca(0.088 ± 0.012), Na(2.87 ± 0.04),

K(2.66 ± 0.06), Zn(17.2 ± 1.0) are important elements with concentration in percentage by weight available for biological metabolic system as well as human health, and were observed in the cereal sample in appropriate and safe amounts/concentration because the high concentration of Na and K and have no adverse effect upon the physiological system [11-12]

Iron is an essential element in man and plays a vital role in the formation of hemoglobin, oxygen and electron transport in human body (Kalagbor and Diri, 2014). The result found showed that its concentration is below the maximum intake set by FAO/WHO (425mg/kg).

Zinc is essential for metabolism, growth, development, and is co-factor for a large number of enzymes in the body.

The level of Zn in the sample is below the maximum intake set by FAO/WHO (99.4ppm).

Manganese is essential for normal bone structure, reproduction and normal functioning of the nervous system.

Cobalt is an integral component of Vitamin B-12 and is required in the manufacture of red blood cells but excessive intake causes overproduction of red blood cell (Kalagbor, et.al, 2014).

Nickel is essential for enzyme functioning.

Chromium(III) is used for normal functioning of sugar and fat metabolism and co-factor of insulin and its value in the

investigated sample is below the maximum permissible level(2.3ppm, FAO/WHO, 2001).

Cadmium is non-essential and toxic heavy metal and its poisoning causes in man, anemia, renal damage, bone disorder, and cancer of the lung (Edward, et.al,2013). But its value in the sample is in ppm level. Iodine is required in large amount (macro mineral) for normal functioning of hormones^[21]

Toxicity

INAA was observed in this work, also as a method of elemental analysis of concentration in the order of part per Billion (Au, Ir). The presence of some naturally assumed toxic elements such as Cd, As, Hg Cd, Pb, Hg, Sn, Pu, Am, Sr, Ru, I, ²³⁵U-, S, ⁶⁰Co, ^{134,137}Cs, Ce, Ir, Tc, ¹⁴C-have been identified as shown in the result and they were having concentration below their toxic levels and some were even not present(Pb, Hg) [13-14]

The Na ion is responsible for maintaining normal hydration and osmotic pressure and the K ion is necessary for cell growth and function. [15]

The continuous intake of diets that are excessively high in a particular element can cause changes in the functioning, forms, and activities of some organs or concentrations of such element in the body tissue and fluids above the permissible limit can cause malfunction of cells, organs, or body tissues. So deficiency or overdose of, even essential elements can produce toxicity in human body [16]. But the indigenous barley is found to be a very good source of molybdenum, manganese, dietary fiber and selenium. It also serves as a good source of the copper, vitamin B1, chromium, phosphorus, magnesium and niacin.[17-19].

Table 1: INAA results (mass fraction in mg/kg, or wt%)were stated for quality control materials of NIST SRM 1547

Element	This work	NIST Value	Element	This work	NIST Value
Na(wt%)	0.0032±0.0001	0.0024±0.0002	Ag	<30	-
Mg(wt%)	0.432±0.018	0.432±0.008	Cd	<0.6	(0.03)
Al(wt%)	0.0264±0.0010	0.0249±0.0008	Sb	0.019±0.005	(0.02)
Si(wt%)	ND	-	I	0.285±0.08	(0.3)
Cl	337±14	360±19	Cs	0.068±0.005	-
K(wt%)	2.42±0.10	2.43±0.03	Ba	111±8	124±4

Ca(wt%)	1.52±0.06	1.56±0.02	La	9.8±0.3	(9)
Sc	0.041±0.002	(0.040)	Ce	9.4±0.6	(10)
Ti(wt%)	0.0028±0.0010	-	Nd	6.3±0.6	(7)
V	0.36±0.08	0.37±0.03	Sm	1.13±0.04	(1)
Cr	0.96±0.10	(1)	Eu	0.19±0.01	(0.17)
Mn	100±4	98±3	Tb	0.11±0.06	(0.1)
Fe(wt%)	0.021±0.001	(0.0220)	Dy	0.62±0.04	-
Co	0.067±0.006	(0.070)	Ho	0.09±0.01	-
Ni	<1.2	0.69±0.09	Tm	0.025±0.004	-
Cu	4.2±0.3	3.7±0.4	Yb	0.14±0.02	(0.2)
Zn	17.2±1.0	17.9±0.4	Lu	0.017±0.004	-
Ga	<0.4	-	Hf	0.079±0.006	-
As	0.086±0.014	0.060±0.018	Ta	0.005±0.002	-
Se	0.123±0.004	0.120±0.009	W	<0.04	-
Br	10.1±0.3	(11)	Th	0.053±0.004	(0.05)
Rb	17.6±1.0	(19)	U	0.030±0.010	(0.015)
Sr	54±3	53±4			

Table2: Identified elements of interest in the irradiated sample, *Hordeum vulgare* (mass fraction in mg/kg, or wt%)were stated).

Identified Elements	Concentration	Identified Elements	Concentration
Na (wt. %)	2.87±0.04	Cd (ppm)	<9
Mg (wt. %)	0.16±0.06	Sb (ppm)	0.52±0.02
Al (wt. %)	8.53±0.14	I(ppm)	1.2±0.3
Si (wt. %)	32.7±0.7	Cs(ppm)	30.329±0.023
Cl (ppm)	<70	Ba(ppm)	933±24
K (wt. %)	2.66±0.06	La(ppm)	59.3±0.9
Sc (ppm)	5.05±0.08	Ce(ppm)	124.9±1.9
Ca (wt. %)	0.088±0.012	Pr(ppm)	<22
Ti (wt. %)	0.46±0.01	Nd (ppm)	61.9±1

V (ppm)	6.3±0.9	Sm(ppm)	14.2±0.33
Cr (ppm)	6.4±0.4	Eu(ppm)	3.58±0.06
Mn (ppm)	76±3	Tb(ppm)	2.61±0.04
Fe (wt. %)	1.73±0.03	Dy (ppm)	21.1±0.4
Co (ppm)	0.670±0.015	Ho(ppm)	3.95±0.12
Cu (ppm)	<130	Tm(ppm)	2.82±0.05
Ni (ppm)	<13	Yb (ppm)	12.34±0.21
Zn (ppm)	39.4±0.7	Lu(ppm)	2.08±0.04
Ga (ppm)	34±10	Hf(ppm)	17.4±0.3
As (ppm)	1.12±0.13	Ta(ppm)	4.85±0.08
Se (ppm)	<0.4	W(ppm)	1.77±0.22
Br (ppm)	<0.8	Ir (ppm)	<0.0007
Rb (ppm)	37.8±1.2	Au(ppm)	<0.011
Ag (ppm)	<0.3	Th(ppm)	8.01±0.13
Sr (ppm)	<24	U(ppm)	2.31±005

Conclusions:

The result of the study showed that minerals and metals content in the sample were found at different levels. The cereal food sample is rich in essential minerals for body building, maintenance, cures of some diseases and is a source of different variety (more than 40, major, minor and trace elements contained in the studied sample), which make our body healthiest. The presence of essential major, minor and trace elements in the cereal food sample plays an important role in the building up and restoration phenomenon of health and protection against disease of human body and is an indication of the cereal's preference for the staple special food cereal as one capable of maintaining and repairing cells and tissues as exercised by the community. Its composition of more than 40 elements in considerable amount and its lowest limit of toxic elements concentration

had given it a particular attention than other cereals for the intended purposes. Progress of investigation of the composition of the cereal in compound forms has occurred in the area of health sciences during the last few years and has found that the cereal needs to be reconsidered further as a major source of our healthiest nutrition [7-8]

Acknowledgements

The authors wish to express our appreciation to the Physics Institute of the Czech Academic Science (NPICAS) experimental Nuclear Reactors Research Centre Rez for providing the full facility of the center to obtain such remarkable results and close and friendly supports the staff provides us during and post experimental activities; without which such scientific analysis was not possible.

References

- [1] Oser, B. L., 1951. Methods for the integrating essential amino content in the nutritional evaluation of protein. *Journal of the American Dietetic Association*, 27: 399-404.
- [2] Tallberg, A. and B. O. Eggum, 1981. The nutritional value of highlysine barley genotypes. *Plant Foods for Human Nutrition*, 31: 151-161
- [3] M. Abdulla, S.B. Vohora, M. Athar (Eds.), *Trace and Toxic Elements in Nutrition and Health*, Wiley Eastern Ltd., New Delhi, 1993).
- [4] Mark Sutton, Nov. 2003. *Trace Elements for Organic Science Direct*, pp: 818-822. *Vegetables, Organic Farming technical Summary*

- [5] www.whfoods.com/genpage.php?p?name=foodspice&dbid=127, ESHA Research, Salem, Oregon, USA.
- [6] Badr, A.; M, K.; Sch, R.; Rabey, H.E.; Effgen, S.; Ibrahim, H.H.; Pozzi, C.; Rohde, W.; Salamini, F. (2000). "On the Origin and Domestication History of Barley (*Hordeum vulgare*)". *Molecular Biology and Evolution*. **17** (4): 499–510. doi:10.1093/oxfordjournals.molbev.a026330. PMID 10742042
- [7] Zohary, Daniel; Maria Hopf (2000). Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe, and the Nile Valley (3rd ed.). Oxford University Press. pp. 59–69. ISBN 0-19-850357-1.
- [8] "FAOSTAT". Food and Agriculture Organization of the United Nations. Archived from the original on July 3, 2008. Retrieved 2009-05-18.]
- [9] . Nuclear activation analysis.
 I. Alfassi, Zeev B. D606.
 A252 1990 5431.0882--dc20
- [10] Kemanic J., Amsil H., Kucera J., (2015); "Determination of elemental impurities in phosphoric Acid by INAA employing a novel method of phosphate precipitation", *J Rational Nucl Chem*, 304:157-162.
- [11] Block, J. H, Roche EB, Soine, T. O., Wilson C. O., *Inorganic Medicinal and Pharmaceutical Chemistry*, Indian Edn, Varghese Publishing House, Bombay, pp.181-183, 1986.
- [12]. Tietz, N.W., Pruden E. L. and Siggaard O. In: *Fundamentals of Clinical Chemistry* (Tietz NW, Ed), 3rd Edn, Saunders, Philadelphia, pp 614-618 , 1987 .
- [13]) Khan KY, Khan MA, Niamat R, Munir M, Fazal H, Mazari P, et al. Element content analysis of plants of genus *Ficus* using atomic absorption spectrometer. *Afr J Pharm Pharmacol* 2011; 5: 317-21.].
- [14] Achudume AC, Owoye D. Quantitative assessment of heavy metals in some tea marketed in Nigeria- bioaccumulation of heavy metals in tea. *Health* 2010; 2: 1097-100
- [15] Manzoor Iqbal Khattak ,Determination of trace elements in some medicinal plants in Balochistan , Department of Chemistry , Balochistan University, Quetta, Pakistan , *Sci.Int(Lahore)*,25(3),599-602,2013
 ISSN 1013-5316; CODEN: SINTE 8
 and the reference in it

- [16] Biajunwa EI, Adebajo AC, Omobuwajo OR. Essential and trace element contents of some Nigerian medicinal plants. *J Radioanal Nucl Chem* 2002; 252: 473-6.].
- [17] Bansal HC, Strivastava KN, Eggum BO, Mehta SL. Nutritional evaluation of high protein genotypes of barley. *J Sci Food Agric* 1977 Feb;28(2):157-60. 1977. PMID:16310.
- [18] Ensminger AH, Ensminger M. K. J. e. al. *Food for Health: A Nutrition Encyclopedia*. Clovis, California: Pegus Press; 1986. 1986. PMID:15210.
- [19]. Bárány E, Bergdahl IA, Schüttz A, Skerfving S, Oskarsson A: Inductively coupled plasma mass spectrometry for direct multi-element analysis of diluted human blood and serum. *J Anal At Spectrom* 1997;12:1005–9.)