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Tetra methyl ammonium hydroxide assisted solubilisation of peanut for trace element determination using atomic absorption spectrometry

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Abstract

Tetra Methyl Ammonium Hydroxide (TMAH) assisted solubilisation of peanut sample preparation was evaluated for the determination of Cr, Cu, Fe, Mn, Ni, Pb and Zn using Graphite Furnace Atomic Absorption Spectrometer (GFAAS) and Na, K, Ca and Mg using Flame Atomic Absorption Spectrometry (FAAS). In the alkaline solubilisation with TMAH, 0.25 g of the sample was heated to 90 ± 3°C for 2h in the presence of 500µL of 25% TMAH solution in aqueous medium. The procedure provided complete solubilisation of the sample. The particle size of the slurry was in the range from 0.2µm to 14 µm with mean size of 1.5 µm, suitable for injection with auto sampler. The LOD and LOQ for peanut slurry samples prepared in TMAH for the respective elements ranged from 0.0002 to 0.0658 and 0.0005 to 0.2343 mg L^{-1}, respectively. The instrumental RSD of the detected elements ranged from 0.11 to 0.81%. The proposed method was compared with complete microwave acid digestion method and the t-values obtained were in the range of 0.08 to 2.03 against critical value t_{10} = 2.23 (P = 0.05), suggesting no significant difference in the values obtained from both the method. SRM- 2387-Peanut butter (NIST) was utilized for validation of the proposed method. The z-scores for values obtained were within ± 1 which indicates accuracy of the TMAH alkaline solubilisation. In comparison to complete acid dissolution method, the proposed TMAH alkaline solubilisation method is simple, requires less reagents and time which in turn minimises contamination risk. The simplicity of the proposed method makes it attractive and an excellent alternative for the preparation of peanut samples for elemental analysis using Atomic Absorption Spectrometry.

Keywords: Peanut; Elemental analysis; Tetra methyl ammonium hydroxide; Slurry; GFAAS; FAAS

Introduction

Peanut (*Arachis hypogaea L.*) is considered as a highly nutritious food stuff. It is cultivated worldwide and utilised for human consumption as a vegetable protein and oil. It is an economic food supplement to battle malnutrition as it contains ~26% protein; 48% of oil; 23% of carbohydrates; 13 essential vitamins; antioxidants and essential minerals [1-3]. The concentration of minerals in peanut may vary from region to region depending upon geological structure of soil. Anthropogenic activities such as mining, smelting, waste disposal, urban effluent, vehicle exhausts, sewage sludge, and use of agrochemical etc. can greatly increase the metal concentrations in agricultural soil and hence in peanut grown in that

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soil. Thus the heavy metals can be transferred to crops, posing a risk to human health [4]. Therefore, it is utmost important to ascertain the metal concentration in peanut to understand its nutritional value as well as contamination due to heavy elements of a particular region. Different analytical techniques have been used in quantifying elements in various kinds of nuts and seeds including Energy Dispersive X-Ray Fluorescence (EDXRF), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Absorption Spectroscopy (AAS) with Flame (FAAS) or Graphite Furnace Atomization (GFAAS) [5-13]. AAS technique is popular among above techniques due to it's the low cost, quickness, and precise and accurate results. FAAS and GFAAS techniques are complementary to each other as they can be used alternatively for trace element analysis depending upon the concentration range. Food stuffs in which dioxins can occur includes meat, fish, eggs, and milk.

FAAS technique is suitable for the higher range of concentration up to Parts Per Million (ppm) suitable for analyzing major elements. GFAAS has better sensitivity in the range of Parts Per Billion (ppb) and superior detection limits with microliter (μL) sample volumes. This is achieved by controlling the furnace temperature program, using appropriate matrix modifiers and reducing environment in the tube so that major matrix is eliminated before atomization. The detection limits of GFAAS are comparable to ICP-MS at lesser running cost and average laboratory facilities [14]. Sample preparation for peanut is a critical step due to the complexity of the matrix as it contains 97% organic matter i.e. fats, proteins and carbohydrates. Generally, Microwave Assisted (MWA) wet digestion method is used for destruction of the organic matrix releasing the metals into the solutions [11-13]. Slurry sampling is an alternative method of sample preparation. This method is advantageous as it is rapid, convenient, use minimal reagents, and minimizes possible contamination or losses compare to other dry and wet digestion methods [15-19]. Dilute nitric acid along with non-ionic surfactant TX-100 or glycerol are commonly used medium for preparation of slurry. Dilute nitric acid concomitantly assists in analytic extraction and the non-ionic surfactant Triton X-100, and glycerol acts as stabilizing agents for slurries [16-21]. Many researchers have found that the recovery of trace elements by slurry prepared using dilute HNO_3 and TritonX-100 depends upon the type of matrix and affected by sedimentation and in-homogenization [16-17]. To overcome this problem magnetic stir bar mixing, vortex mixing, gas bubbling and ultrasonic agitation have been employed [17]. There are also alkaline solubilisation procedures with water-soluble amines such as Tetra Methyl Ammonium Hydroxide (TMAH). TMAH has being employed with success in the solubilisation of biological samples such as milk powder, bovine muscle, muscle tissue, fish muscle, biodiesel samples prior to their analysis [22-28]. Usually, the treatment is very simple and fast. The solubilisation of fat and protein-rich matrices in alkaline solubilisation procedures is performed at lower temperatures, which is an advantage as it prevents the loss of volatile analyses [22-26].

In the present work, a method was developed for analysis of trace elements in peanut samples by FAAS and GFAAS after slurry formation using TMAH. The same peanut samples were also analyzed using the established method of complete MWA dissolution followed by FAAS and GFAAS determination. The accuracy of the developed method was evaluated by comparing the results of developed method with that of established method. Additionally, validation of the TMAH slurry method was carried out using standard reference material SRM 2387-Peanut Butter. The validated method was applied to 20 peanut samples collected from various regions of Maharashtra, India.

The developed slurry sampling method includes the following steps:

1. Crushing and grinding of peanuts.
2. Slurry formation of peanut powder with TMAH.
3. Examination of the stability and homogeneity of the slurry.
4. Optimization of the time-temperature programme for analysis of Cr, Cu, Fe, Mn, Ni, Pb and Zn in the peanut using GFAAS.

5. Optimization of method for determination of Na, K, Ca and Mg using FAAS.

Experimental

Instrumentation

All FAAS measurements were carried out using NovAA-800 and GFAAS measurements were carried out using a ZEEnit 650P atomic absorption spectrometer (Analytik Jena, Germany). GFAAS was equipped with a Transversely Heated Graphite Atomizer (THGA), a longitudinal AC Zeeman background correction system and MPE-60 AS-GF auto sampler. Pyrolytically coated graphite furnace tubes with pin platform were used in all the studies. The equipment was controlled by a computer, using the Aspect LS AAS software program. All measurements were performed using integrated absorbance (peak area). The elemental hollow cathode lamp of Na λ 589.0, K λ 766.5, Ca λ 422.7, Mg λ 285.2, Cr λ 357.9, Cu λ 324.7, Fe λ 248.3, Mn λ 279.5, Ni λ 232.0, Pb λ 253.3, Zn λ 213.9 (Analytik Jena, Germany) were used for the elements under investigation. The optimized temperature program for analytics is shown in **TABLE 1**. Complete acid dissolution was carried out using Ethos one closed Microwave digestion system (Milestone Inc., Italy).

TABLE 1. Optimized temperature program and wavelength used for analysis

Element	Wavelength nm	Slit nm	Pyrolysis temperature ^{0}C	Pyrolysis hold time s	Atomisation temperature ^{0}C	Modifier used
Cr	357.9	0.2	1100	300	2300	none
Cu	324.7	0.5	900	300	2000	none
Fe	248.3	0.2	1100	300	2100	none
Mn	279.5	0.2	900	300	2400	5µL of 0.1% $Pd(NO_3)_2$
Ni	232	0.2	900	300	2200	none
Pb	253.3	1	800	300	2000	5µL of 1% $Pd(NO_3)_2$
Zn	213.9	0.5	800	300	2000	5µL of 0.1% $Pd(NO_3)_2$
Na	589	0.5	Analysed by FAAS with Air- Acetylene flame			1% CsCl
K	766.5	0.5				1% CsCl
Ca	422.7	0.5				0.2M EDTA
Mg	285.2	0.5				0.2M EDTA

Reagents

Supra pure nitric acid 65% and hydrogen peroxide 30% (Merck, India), Tetra methyl ammonium Hydroxide 25% aqueous solution pure(TMAH) (Spectrochem Pvt. Ltd., India), Triton X-100 (BDH, Poole, England) were used for all sample treatments. Standard solutions of elements were prepared by suitable dilution of certified AAS standard solution (1mg mL^{-1}) from Merck, India.0.1% $Pd(NO_3)_2$ solution was prepared by dissolving appropriate quantities of palladium metal (99.999%) in 10 mL conc. nitric acid and making up to volume and used as matrix modifiers in GFAAS analysis as mentioned in table 1. CsCl solution (1% w/v) used as ionization buffer for Na and K was prepared from suprapur CsCl (Merck, India). 1% w/v solution of $La(NO_3)_3$ used as releasing agent for analysis of Ca and Mg was prepared from Analytical Grade $La(NO_3)_3$. 0.2M EDTA used as protective agent for Ca and Mg was prepared using disodium salt of ethylenediaminetetra-acetic acid (EDTA) (Merck, India). Standard reference material, SRM-2387 of NIST was used for method validation. The water used in all procedures was collected from a Milli-Q Direct 8 system (Millipore, SAS, France) and has a resistivity of 18 MΩ cm^{-1}. All glassware, Teflon vessels and polypropylene containers

were decontaminated with 10%v/vHNO_3 and subsequently washed with deionized water.

Collection, Pre-treatment and storage of peanut samples

20 peanut samples were received from various parts of Maharashtra, India. The peanut samples (Kernels) were exposed to sun light for 48 hrs to dry it until constant weight was achieved. 50 grams of dried peanut sample was powdered using agate mortar and pestle to obtain fine powder. The raw powdered peanut samples were filled in polypropylene bottles and kept at -20°C to inhibit growth of microorganisms and retard chemical changes.

Closed MWA complete acid digestion

0.50 g of the powdered peanut samples and peanut butter (SRM-2378) were weighed accurately and transferred to separate teflon vessels of microwave digester. 7 mL of HNO3 (65%, w/w) and 1 mL of H2O2 (30%, w/w) were added. The vessels were closed and subjected to microwave program given in the manual for dissolution of peanut samples. The program was: power 1000 W, 15 minutes' ramp time until 200 ºC, 15 minutes hold at 200 ºC. Two reagent blanks were also run along with every batch of the samples. After cooling, the samples were made up to 50mL in volumetric flask with deionized water. The samples were diluted, as necessary, and the analytic concentrations were determined by GFAAS and FAAS.

Alkaline Solubilisation/ Slurry preparation using TMAH

Accurately weighed peanut powder and peanut butter (SRM-2378) were taken in an acid-decontaminated 15 ml polypropylene tube; an appropriate volume of TMAH was added. Subsequently, the mixture was heated in a water bath (50-90°C) for (10-180) min. Temperature of water bath was observed using thermometer. Sample was made up to 10 ml with deionized water in the polypropylene volumetric flask. Samples were diluted further depending upon the calibration range of the instrument.

Results and Discussion

Trace elements were monitored in the peanut samples to understand its nutritional value and probable health risk due to heavy element contamination, if any owing to anthropogenic activities in the sample collection area. As it contains 97% of organic matter, complete dissolution of sample requires high amount of mineral acid and strong oxidizing agents as hydrogen peroxide. The direct analysis of solids as slurries offers advantages over these conventional sample preparation procedures. The advantages are shorter sample preparation time, reduced sample contamination risk, increased sensitivity (less dilution) and lower analytic loss through volatilization prior to analysis. Among the eleven elements listed in table 1, GFAAS technique was optimized for Cr, Cu, Fe, Mn, Ni, Pb, Zn and FAAS technique was optimized for the major elements viz. Na, K, Ca and Mg.

Closed MWA complete acid digestion followed by AAS analyses

Closed MWA acid digestion was carried out for complete dissolution of the peanut samples as per the procedure mentioned in section 2.4. The solution was then analyzed for Na, K, Ca, Mg using Flame Atomic Absorption Spectrometry and Cr, Cu, Fe, Mn, Ni, Pb and Zn using Graphite Furnace Atomic Absorption Spectrometry. The instrumental parameters are given in table 1. The instrument was calibrated with aqueous standards for each element. For analysis of Na and K, 0.1% CsCl was added as ionization suppressor. For analysis of Ca, Mg, 1 % w/v $La(NO_3)_3$ was added as releasing agent to the calibration standards, sample blank and samples as suggested in the operation manual of the instrument. The obtained results were used as reference values for optimization of the alkaline solubilisation method using TMAH.

Alkaline solubilisation/ slurry preparation followed by AAS analyses

Alkaline solubilisation which transforms the sample into slurry is an alternative method of sample preparation. This method is advantageous as it is rapid, convenient, time efficient and requires minimum reagents.

Selection of medium for slurry preparation: Main challenge in the slurry preparation is to obtain homogeneous and stable slurry, which influence directly to accuracy and precision of analysis. Researchers had used dil. HNO_3 and a non-ionic surfactant like Triton X-100 for preparation of slurry of plant sample 18-21. In present work, slurry using 0.2% HNO_3 and 0.05% Triton X-

100 was prepared. It was observed that the slurry was inhomogeneous with three different phases; the upper phase was of seed coat floating above the solution, middle phase was hazy suspension and there were particles settled at the bottom of the beaker (Fig. 1). Moreover, the microbial growth was observed on the surface of slurry after 24 h at room temperature. The inhomogeneity of the slurry may be due to 48% of oil content in peanuts seeds. Tetra Methyl Ammonium Hydroxide (TMAH) is another versatile reagent used for preparation of slurry, also termed as alkaline solubilisation, of protein rich and fatty biological samples 22-28. Therefore, TMAH was used to prepare slurry of powdered peanut samples. It was observing that the "solution" or slurries formed with the addition of TMAH were viscous, but was sufficiently stable for using a standard auto sampler to pipette them into the graphite tube, and ultrasonic mixing was not necessary as reported by P. Martine et.al 4.

Optimization of amount of TMAH and temperature for slurry preparation: To optimize amount of TMAH different volumes of the 25% m/v TMAH solution viz. 100, 250, 500 and 1000 µL were added to 250 mg of the peanut sample in polypropylene tubes. Incomplete and very slow dissolution of peanut powder was observed at room temperature. Therefore, the mixture was heated on water bath at varying temperatures from 50, 60, 70, 80, 90°C for varying time from 10 min to 180 min. It was observed that, homogeneous slurry was formed by heating the sample at 90±3°C for 2h with 500 µL of TMAH (**FIG.1**). The solution was made up to 10 mL with deionised water and subsequently diluted before analysis depending upon the concentration of elements.

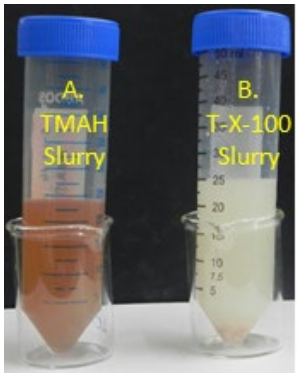

FIG. 1. A. Completely homogenized peanut slurry in 25% m/v TMAH;
B. Peanut slurry in 0.2% $HNO_3$and 0.05% Triton X-100

The particle size of the slurry can influence the stabilization, deposition and atomization efficiency during analysis, which in turn can influence both accuracy and precision. Several researchers have reported the dependency of analytical results on particle size. Ferreira et al in his review article18 confirmed that for successful determination of trace elements using AAS and ICPOES (full form), the particle size diameters of slurry should be <30 µm. The particle size of the sample slurry prepared by grinding with agate mortar and pestle and subsequent solubilisation in TMAH was measured using Nicomp Z3000ZLS (Entegris, USA). It was observed that the particle size was in the range from 0.2µm to 14 µm with mean size of 1.5 µm as shown in **FIG. 2.** This particle size is well within the range given in the literature for accurate and precise analysis using AAS.

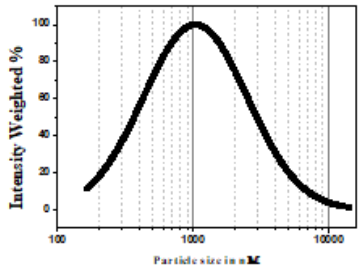

FIG. 2. Particle size distribution of peanut slurry in TMAH

Optimization of Instrumental parameters for GFAAS Analysis: A potential problem with the slurry sample analysis in GFAAS is the interference from matrix due to incomplete pyrolysis, which may have severe effect on sensitivity and repeatability.

The build-up of matrix in the graphite tube may also interfere with the background correction and even hinders the analytic determination because of the partial attenuation of the light beam. Therefore, the optimization of pyrolysis temperature, atomization temperature and suitable chemical modifier is necessary to eliminate any matrix effect and ensure accuracy and precision of the method. To optimize the pyrolysis temperature, the recommended atomization temperature was chosen and the pyrolysis temperature was varied in steps of 100^0C, starting from the recommended temperature till optimum constant absorbance was obtained. Similarly, to optimize atomization temperature, the optimum pyrolysis temperature was kept constant and the atomization temperature was then varied in steps of 100^0C from the recommended temperature till the optimum absorbance was obtained. These optimized pyrolysis and atomization temperatures (Table 1) were used for the analysis of elements in peanut slurry. However, loss of signal was observed for Mn, Pb and Zn at higher pyrolysis temperature and subsequently higher atomization temperature. Hence, 5µL of 0.1% $Pd(NO_3)_2$ was used as modifier for Mn, Pb and Zn.

Optimization of process parameters for FAAS analysis: As per literature, Na, K, Ca and Mg are the major elements present in peanut. The samples were suitably diluted~ 20 to 200 times depending upon the calibration range of FAAS for these elements. The particle size of the slurry was suitable for introduction of sample into air acetylene flame 18. Flow rate of the nebulizer was maintained at 5ml m^{-1}. The flow rate of air and acetylene was optimized to get maximum absorbance. 0.1% CsCl was added to the sample solution as ionization buffer for determination of Na and K. $La(NO_3)_3$ is usually used as releasing agent for Ca, however, due to alkaline nature of TMAH it got precipitated as $La(OH)_3$. Therefore, for Ca and Mg, 0.1M EDTA was used as releasing agent. Standard addition calibration method was applied to observe the matrix interference and compared with linear calibration method using aqueous standards prepared in 0.1% HNO_3. It was observed that the slope of the standard addition calibration curve was better compared to the aqueous calibration curve. This may be due to organic nature of TMAH used for preparing the slurry which provide reducing environment in the flame and ease atom formation. The slope of standard addition calibration curve for Na, K, Ca and Mg were 0.8180, 0.5374, 0.0418 and 1.7945 Abs/mg L^{-1} respectively which are higher in comparison to the slope of aqueous calibration 0.7926, 0.5318, 0.0373, 1.7729 Abs/mg L^{-1} respectively.
Performance characteristics of FAAS and GFAAS for TMAH assisted alkaline solubilisation: The LOD and LOQ were calculated for each element based on process blank measurement. The LOD and LOQ for peanut slurry samples prepared in TMAH for the respective elements ranged from 0.0002 µg L^{-1} to 0.0658mg L^{-1} and 0.001 µg L^{-1} to 0.219 mg L^{-1}, respectively (table2). Regression analysis was used to evaluate the linearity of the calibration curves by using the absorbance readings and concentrations of the calibration standards. Established linearity was good with R^2 values ranging from 0.9928 to 0.9999 and therefore suitable for the quantification of the elements. The performance characteristic of each element is presented in **TABLE 2.**

TABLE.2 Instrumental Performance characteristic of each element

Element	Linear Calibration range	LOD	LOQ	Correlation Coefficient(R^2)	% RSD
	mg L^{-1}	mg L^{-1}	mg L^{-1}		
Cr	0.005-0.050	0.0058	0.019	0.9955	0.32
Cu	0.001-0.020	0.0066	0.022	0.9972	0.27
Fe	0.005-0.050	0.0056	0.019	0.9958	0.81
Mn	0.002-0.020	0.0009	0.003	0.9995	0.26
Ni	0.005-0.050	0.0043	0.014	0.9975	0.6
Pb	0.002-0.010	0.0005	0.002	0.9993	0.33
Zn	0.0005-0.002	0.0002	0.001	0.9984	0.24
Ca	0.5-5	0.0443	0.148	0.9996	0.2
Mg	0.1-1	0.001	0.003	0.9999	0.15

Na	0.1-1	0.0658	0.219	0.9928	0.18
K	0.2-2	0.0356	0.119	0.9979	0.11

Application of the TMAH assisted alkaline solubilisation method

The optimized proposed method was applied for determination of eleven elements in 20 peanut samples received from various parts of Maharashtra, India. Before application to real samples, the developed method was validated by evaluating the performance characteristic outlined in section 3.2.5. Accuracy of the method was established by comparing the mean of measurements (n = 6) obtained from the TMAH alkaline solubilisation method with complete microwave assisted acid dissolution method, the reference method. The t-values obtained were in the range of 0.08 to 2.03 against critical value t_{10} = 2.23 (P = 0.05) suggesting no significant difference in the values obtained from both the method (Table 3). Accuracy was further established by applying the developed method to SRM- 2387-Peanut butter (NIST).

Table 4 shows the comparison of results obtained for the analysis of SRM- 2387, respective z-scores, % recovery and relative standard deviation (RSD) for the elements analysed using the developed alkaline solubilisation process. The z-scores for values obtained were within ± 1 which established the accuracy of the method. The precession was established by calculation the %RSD. The % RSD obtained for the peanut sample (**TABLE 3**) and for the SRM-2387 (**TABLE 4**) were <5 % which established good repeatability of the developed method. Reference values for Cr, Ni and Pb were not mentioned in the certificate of SRM-2387, hence was not listed in the Table 4. However, these elements in peanut butter were below detection limit.

TABLE 3: Comparison of results obtained from peanut powder slurry method and microwave complete acid digestion method.

Element	Slurry method	Acid digestion method	t value 95% CI	RSD
	µg g^{-1}	µg g^{-1}		
Cr	1.27 ± 0.05	1.30 ± 0.04	1.15	3.94
Cu	7.32 ± 0.15	7.02 ± 0.33	2.03	2.05
Fe	23.36 ± 0.32	23.41 ± 0.42	0.23	1.37
Mn	11.32 ± 0.47	11.30 ± 0.20	0.1	4.15
Ni	3.28 ± 0.12	3.24 ± 0.14	1.86	3.66
Pb	1.40 ± 0.05	1.36 ± 0.03	0.69	1.4
Zn	21.78 ± 0.85	21.35 ± 0.95	0.83	3.9
Ca	682.25 ± 0.32	682.28 ± 0.58	0.11	0.05
Mg	2147 ± 27	2151 ± 116	0.08	1.26
Na	18.69 ± 0.08	18.77± 1.32	0.15	0.42
K	8224 ± 268	8237 ± 224	0.09	3.26

TABLE 4: Comparison of reference values of SRM 2370 Peanut butter with the values obtained using proposed method

Element	SRM2370 Peanut butter Reference value µg g^{-1}	Values obtained by slurry method µg g^{-1}	z score	Recovery %	% RSD
Cu	4.93 ± 0.15	5.02 ± 0.25	0.6	102	4.98
Fe	16.4 ± 0.8	16.2 ± 0.6	-0.25	99	3.7
Mn	16.0 ± 0.6	16.4 ± 0.5	0.67	103	3.05
Zn	26.3 ± 1.1	25.9 ± 0.8	-0.36	98	3.09
Ca	411 ± 18	407 ± 13	-0.22	99	3.19
Mg	1680 ± 70	1688 ± 25	0.11	100	1.48

Na	4890 ± 140	4885 ± 76	-0.04	100	1.56
K	6070 ± 200	6128 ± 211	0.29	101	3.44

The result of eleven elements analyzed in 20 peanut samples was summarized in a box and whisker plot and was presented in **FIG. 3**. The descriptive statistics are summarized in **TABLE 5.**

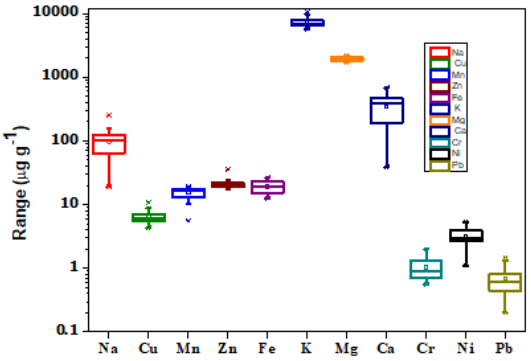

FIG. 3. Box-Whisker plot for concentration of elements in the peanut powder

The concentration of Na, Cu, Mn, Zn, Fe, K, Mg, Ca, Cr, Ni and Pb in the 20 peanut samples collected from various parts of Maharashtra was found in the range 18.8-247.2, 4.3-10.8, 5.6-19.4, 17.6-35.5, 12.5-26.2, 5655-10379, 1673-2196, 38.6-682.3, 0.56-1.99, 1.1-5.3, 0.2-1.5 µg g^{-1} respectively. Large spread of range was observed for Na and Ca, which may be attributed to soil conditions of the area where it had been grown. One outlier was observed for each of Na, Cu, Zn and Mn, which was calculated by the software Origin Lab Ver. 8.5 by setting lower and upper limit from descriptive statistic data. The values compared with literature values indicates that the values are lower than the reported values by B S R Bazie et al and A.H.A. Abugoufa et al and are comparable with L. C. A. GALVAO et al.

TABLE 5. Results of analysis of Peanut powder by proposed slurry method and comparison with literature values.

Element	**Na**	**Cu**	**Mn**	**Zn**	**Fe**	**K**	**Mg**	**Ca**	**Cr**	**Ni**	**Pb**
(n=20)	µg g^{-1}										
	Descriptive statistics										
Mean	98.3	6.4	15.4	21.6	19	7281	1927	342.4	1.2	3.14	0.69
Median	101.5	6.2	16.7	21.3	18.7	6768	1942	395.3	0.9	3.05	0.6
Minimum	18.8	4.3	5.6	17.6	12.5	5655	1673	38.6	0.56	1.1	0.2
Maximum	247.2	10.8	19.4	35.5	26.2	10379	2196	682.3	1.99	5.3	1.5
1st quartile	64.5	5.4	13	18.9	15.3	6438	1815	187.3	0.73	2.68	0.48
3rd quartile	125.2	7.1	17.8	22	22.7	7791	2022	464.8	1.2	3.72	0.8
Literature values											
Ref. 11 Range (median)	-	0.26 - 13.36 (9.12)	4.66 - 34.04 (18.52)	1.45 - 61.37 (31.59)	4.44 - 380.18 (28.92)	-	-	-	1.18–5.40 (2.52)	1.77–8.54 (5.31)	<LD-2.40 (0.6)
Ref. 2 Range (median)	-	9.5-33.8 (24.3)	22.0-46.9 (40.5)	41.9-82.1 (75.6)	18.1-97.8 (65.1)	-	1105-2481 (1799.5)	1031-2536 (1777)	1.1-7.0 (3.4)	11.7-23.7 (18.9)	-
Ref. 12 Mean	83.9	7.5	17	32.9	19.9	6870	1570	480	1.6	-	-

Advantages of TMAH alkaline solubilisation over complete acid dissolution

TMAH alkaline solubilisation method has several advantages over the microwave assisted complete acid dissolution method.

1. There is no constraint on number of samples to be dissolved at a time, which otherwise depends upon the number of microwave vessels available for acid digestion in microwave digester.
2. Very small amount of reagent, TMAH (0.5mL) is required for solubilisation compare to acid mineralization (7 mL HNO_3+1mL H_2O_2).
3. No loss of analytic was observed.
4. Longer life of graphite tube (Twice as compared to acidic sample).
5. TMAH solubilisation process is a greener method which saves both energy and time.

Conclusions

The work demonstrates an alternative sample dissolution method to the conventional acid dissolution method, for protein and oil rich peanut sample. The developed alkaline solubilisation method for peanut sample using TMAH gives stable slurry of the sample with particle size in the range of 0.2 µm to 14 µm with mean size of 1.5 µm which is suitable for GAFFS and FAAS analysis. The developed method was validated with the values obtained from the conventional complete acid dissolution method as well as with a standard reference material SRM 2370. The developed method is time efficient, requires fewer, lesser and mild reagent, does not affects the life of graphite tube. The method gives precise and accurate results. The developed method was applied to 20 peanut samples collected from various parts of Maharashtra. The range of the concentrations of Na, Cu, Mn, Zn, Fe, K, Mg and Ca in the samples was found to be 18.8-247.2, 4.3-10.8, 5.6-19.4, 17.6-35.5, 12.5-26.2, 5655-10379, 1673-2196, 38.6-682.3, 0.56-1.99, 1.1-5.3, 0.2-1.5 µg g-1 respectively. Large spread of range was observed for Na and Ca, which may be attributed to soil conditions of the area where it had been grown. TMAH solubilisation process is a greener method which saves both energy and time.

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