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Analysis of minor and toxic elements in puffs prepared from germinated and non-germinated multi-millets using inductively coupled plasma-mass spectrometer (ICP-MS)

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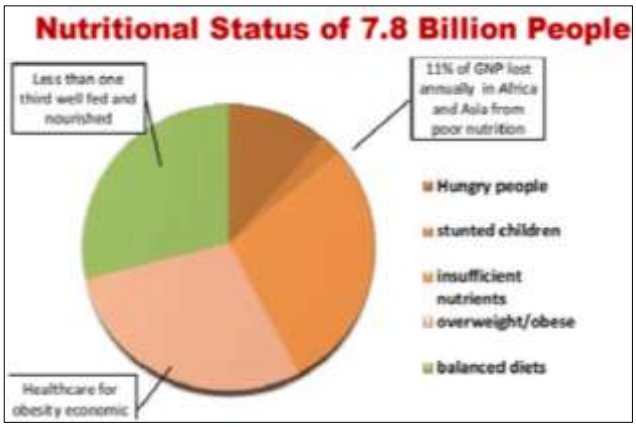
Abstract
The objectives of this study were to measure the concentrations of minor toxic elements in multi-millet puffs using inductively coupled plasma-mass spectrometry (ICP-MS) and to compare concentrations of minor toxic elements in both germinated and non-germinated multi-millet puffs. Principal component analysis of differed germinated and non-germinated multi-millet puffs revealed that though the element concentrations in germinated multi-millet puffs was slightly higher than germinated multi-millet puffs but still it was within permissible limits. The higher concentration of toxic elements in germinated multi-millet puffs may be attributed to the use of water and other ingredients used during preparation. Minor elements included Selenium (Se), Nickel (Ni), Copper (Cu), Chromium (Cr) while toxic trace elements were Arsenic (As), Cadmium (Cd), and Lead (Pb) in non-germinated multi-millet puffs significantly correlated with those in germinated multi-millet puffs (p<0.05). The chemical analysis of elements indicated that germinated (T1-NGP, T2-NGP, and T3-NGP) multi-millet puffs showed higher concentrations of minor elements in germinated multi-millet puffs constituted Se - 286.495-312.689 ng/g, Ni -286.495-312.689 ng/g, Cu - 286.495-312.689 ng/g, Cr - 286.495-312.689 ng/g. The toxic trace elements: including constituted Arsenic (As) 4.3-6.8 ng/g, Cadmium (Cd) 1.2-1.8 ng/g, and Lead (Pb) 5.2-11.3 ng/g were very low and did not pose any threat to consumer as per recommendation of FSSAI as analysed through AOAC, 2015 method.

Keywords: Multi-millets puffs, germinated, minor and toxic elements, ICP-MS

1. Introduction
The present study was designed in view of the malnutrition of 2/3rd population of world (Fig 1) and the declaration of year 2023 as "International Millet Year" by UN to fight with malnutrition and to encourage the cultivation of millets as alternate crops as strategic crops under climate change (Garg, 2023) [9]. Millets are cereal crops generally small-seeded and known for high nutritive value. Millets were indeed one of the oldest foods, but they were discarded in favor of wheat and rice with urbanization and industrialization. The grains form a good source of micronutrients, phytochemical Increasing interest in reviving the consumption of millets across various countries is favoring the growth prospects of this market in recent years. In the present scenario, people are more conscious about the health and nutritional value of food. Nutrient availability of food depends on various factors like the type of food, raw material, and processing of food. Low nutritional value and non-availability of nutrients from a food source is the major cause of malnutrition in underdeveloped countries. WHO/UNICEF (2019) reported inadequate complementary food as a major cause of child malnutrition in many developing countries. In this respect, several nations conduct campaigns/programs to spread awareness among people for maximum use of potential nutritional values of cereals using some cooking or other processing techniques. Germinated multi-millet grains with extrusion cooking have been developed widely for the continuous processing of nutritional-enriched food products development. millets are a convenient meal because of their ease of preparation, low cost, and relatively long shelf life. Changing food habits, increasing population, and urbanization have led to increasing consumption of noodles worldwide (Meherunnahar *et al*. 2023) [13].



Source: FAO

Fig 1: Malnutrition Status of the world Population

Trace elements such as iron (Fe), zinc (Zn), copper (Cu), and selenium (Se) are essential in human metabolism, growth, and development. While toxic elements such as lead (Pb) and cadmium (Cd) induce mental retardation and cardiovascular diseases. Therefore, element concentration in multi-millets products is indicative of their safety and nutritional value. The concentrations of trace and toxic elements in multi-millets puffs vary significantly in multi-milets puffs. Many dangerous elements or compounds such as metals and metalloids accumulate along the food chain due to increase of urban, agricultural, and industrial emissions. The almost ubiquitous presence of some metal pollutants, especially Cd and Pb, facilitates their entry into the food chain and thus increases the possibility of having toxic effects on humans and animals (Zheng *et al*., 2003) [17]. For example, Bajra (*Pennisetum typhoideum)* As- 0.97, Cd-0.003, Cu- 0.54, Pb- 0.008, Sorghum (*Sorghum vulgare)* As -1.53, Cd-0.002, Cu- 0.45, Pb 0.008, Ragi (*Eleusine coracana)* Cd- 0.004, Cr- 0.032, Cu- 0.67, Pb- 0.005, Rice Raw milled Cd- 0.002, Cr- 0.005, Cu- 0.23, Pb- 0.002 (Indian Food Composition Tables, NIN – 2017 and Nutritive value of Indian foods, NIN – 2007), element concentration in millets varies by species and locations. Millets are nutria-cereals grains obtained from different sources like sorghum (*Sorghum bicolor* L.) and available as pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine carocana*), foxtail millet (*Setaria italica*), (become a good source of nutrition in India the millets are cultivated during the Kharif (rainy season) in arid and semi-arid regions, consider a drought-tolerant crop. The pearl millets cultivate in well-drained deep sand to loam soils (Sharma *et al*., 2020) [4]. Jowar is a starchy, gluten-free, high-protein, cholesterol-free source of a variety of essential nutrients, including dietary fiber, iron, phosphorus, and thiamine (Dutta, 2021) [2].

India is the fastest-growing countries in technologically, economically, and educationally. Despite of this Indian suffered malnutrition problems. Malnutrition is the main health crisis in developing countries; people experience both physical and mental problems. According to the Global Hunger Index 2022, India ranks 107 out of 121 countries (GHI rank). Malnutrition is referred to as scarcity, imbalance, or excess intake of food or nutrients (WHO 2022).

However, this (ICP-MS technology is useful to analyze metal contaminants like lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) contents that are usually accumulated in the developing embryos of popular cereals like rice, barley, foxtail millet, sorghum and corn (Choi *et al*., 2018) [7]. ICP-MS but there was no study on multi-millets puffs, therefore, the objective of this study was to determine the minor and trace elements namely Se, Ni, Cr, Cd, Cu, As, and, Pb in non-germinated and germinated multi- millet puffs by using ICP-MS technique, because of its well-known advantage of sensitivity, selectivity and multi-elements analysis. The result obtained were compared to the critical levels specified by compendium of FSSAI, WHO, and, Food and Nutrition Board, for dietary intakes of minor nutritional elements and permissible as per MRL (maximum residue limits) tolerable intakes of toxic trace elements.

The aim of soaking and germination period on the functional properties of flours and the sensorial quality of finger millet porridge. Four finger millet varieties were collected, cleaned, and soaked for 24 h, then germinated at room temperature (20–25 °C) for 24, 48, and 72 h. The germinated samples were oven-dried at 60 °C for 6 h and milled into flour at the size of 1 mm using a cyclomixer. Unsoaked and ungerminated finger millet grains are also milled into flour and used as control. Porridge was prepared with a flour-to-water ratio of 1:12 (weight/ volume), and sensory analysis was done by semi-trained panelists. Germination enhanced the water absorption capacity, solubility, and oil absorption capacity of flour samples significantly (Yenasew *et al*., 2023) [18].

To feed 10 billion people sustainably by 2050. We Need to Close Three Gaps. (a) There exist a 56% food gap between crop calories produced in 2010 and those needed in 2050 under “business as usual” growth. (b) A 593 million-hectare land gap (an area nearly twice the size of India) between global agricultural land area in 2010 and expected agricultural expansion by 2050. (c) An 11-gigaton GHG mitigation gap between expected agricultural emissions in 2050 and the target level needed to hold global warming below 2 °C (3.6°F), the level necessary for preventing the worst climate impacts. The Major Achievements in last 50 Years & Projected Achievements in next 50 years is Life expectancy will further increase to 120-150 years of Health for all No poverty and Quality Nutrition and no hunger Technological War, with little or no use of soldiers Electric Car for everyone Technology based Economical Dominance in the World More awareness towards environment, water and air Life possibilities in Space.

Food security is an ongoing problem, and current staple foods are not sufficient to overcome challenges such as the present COVID-19 pandemic. We propose here that small millets have the potential to become new staple crops, especially in hunger hotspots. Currently, the absence of intensification of millet farming, lack of deployment of genetic tools for trait improvement, and the need for optimization of storage and supply chains limit crop production (Mehanathan Muthamilarasan *et al*. 2021) [12].

2. Materials and Methods

2.1 Instrumentation

The Inductively coupled plasma-mass spectrometry (ICP-MS) Agilent technology analyzed the variety of mineral compositions that were not previously reported and showed the effect of malting processing on minerals, phytic acid, trace elements and physicochemical properties of finger millet varieties. And This method validation protocol applies to the quantitative analytical method for metals, which is used for the determination of metals Arsenic (As), Cadmium (Cd), Copper (Cu), Chromium (Cr), Antimony (Sb), Mercury (Hg),

Manganese (Mn), Lead (Pb), Selenium (Se), Tin (Sn), and Nickel (Ni) in Food in support of regulatory requirements.
However, this (ICP-MS-(7850, Agilent,), technology is useful to analyze metal contaminants like lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) contents that are usually accumulated in the developing embryos of popular cereals like rice, barley, foxtail millet, and sorghum. Which was equipped with a quadrupole hyperboloid, Scott double pass spray chamber, concentric nebulizer, and high matrix introduction (HMI) sample introduction system Operating conditions and measurement parameters are presented in Table 1. The advance microwave digestion (Ethos Easy) with PTFE tubes was used for multi-millets puffs sample digestion; the operating conditions of ICP-MS. The microwave digested using the temperature program as Ramp time 40 (min), Hold time (min) 30, Temperature 200 °C, Power 1500 (W). Ramp time and microwave power settings may vary depending on the number of vessels. Vessels used in the digestion were previously immersed in 20% HNO_3 (v/v) for at least 12 h and rinsed with ultrapure water.

2.2. Reagents

All calibration standard solutions were prepared from 10 mg/L multi-element standard solution (Agilent) by dilution with 19.6% (w/w) HNO3 (the same percentage of acid present in the samples) in ultrapure deionised water, Element Standard solution (each at 1000 mg. L, Inorganic) USA. Analytical reagent grade concentrated Nitric acid (65-69%), Trace metal Grade, Fisher Chemical, Hydrogen peroxide (30-32%), Trace Metal, Fisher Chemical, Hydrochloric acid (35-37%), Trace Metal Grade, Fisher Chemical, Ultrapure deionised water with a resistivity of 18.2 MO cm, was obtained from a Milli-Q Plus water purification system (Milli-Q Ultra-Pure Thermofisher).
All plastic containers were soaked in 10% v/v HNO3 for at least 24 h, and then rinsed extensively with Milli-Q water prior to use. All kinds of glassware were avoided to prevent metal releases. All plastic containers, polypro polypropylene flasks, pipette tips, Pyrex glass digestion tubes and reagents that encountered samples or standards were checked for contamination.

2.3 Preparation of Standard Solutions

The sample is weighed accurately in the digestion vessel, added concentrated Nitric acid, Hydrogen peroxide, Hydrochloric acid of trace metal grade, or an equivalent grade and is digested in a microwave digester using temperature program. The digested sample is made up to the required volume quantitatively. The solution is filtered if required, and aspirated in ICP-MS. The maximum residue limits required concentration for stock preparation as per AOAC 15.01 are presented in Table 2.

2.4 Blank solution

A blank solution will be prepared in the same manner without a sample. Prepared solutions will be aspirated as per the below mentioned instrumental conditions.

2.5. Sample preparation and digestion

The homogenized samples were weighed to approximately 0.5±0.25 g in a pre-cleaned, dry 50 mL capacity microwave digestion vessel. For the spike recovery experiment, a sample will be spiked with all analytes before the addition of any solvent. 200 $\mu g \cdot L^{-1}$ gold will be added to the sample as a final concentration in the sample solution to stabilize mercury. Additionally, 4% nitric acid (HNO_3), 1% hydrogen peroxide (H_2O_2), and 1% hydrochloric acid (HCl) (if required) will be added and the microwave digestion vessels with the sample to be kept in a fume hood for 60 min for pre-digestion.
A total of 18 samples in each sample, the samples belonged to different ratio and multi-millets, in triplicate at different times. For digestion accurately weighed 0.25 g of samples were taken in 300 mm long Pyrex glass digestion tubes (Ethos Easy). Additionally, 4.0 ml concentrated HNO_3 (70%) 1.0 mL H_2O_2 and 1.0 ml HCL. The temperature was increased gradually, starting from 50 °C, and increasing up to 180–20 °C. The digestion was completed in about 70 min, as indicated by the appearance of approximately 6.25 mL colour less solution, just like water. The mixture was left to cool down and the contents of the tubes were transferred to 50 mL self-standing polypropylene volumetric tubes with plug seal caps. The volumes were made to 50 mL with ultrapure deionized water, labelled accurately and used for analysis. At regular intervals during analysis, calibration standards were analysed as samples to monitor instrument drift. Furthermore, ultrapure deionized water blanks were frequently analysed alongside samples to check for any loss or cross contamination. Blanks were prepared by completion of the full analytical procedure without samples.

2.6. Quality Assurance

To demonstrate that the method is suitable for its intended use, validation will be performed for the below parameter. Specificity and Precision is to be performed by spiking the elements to the Sample at the specification level. LOD and LOQ experiments will be performed for all elements by Method blanks. Linearity will be performed minimum from the range of LOQ to 200% specification level. Accuracy, Repeatability and reducibility will be performed at the range of LOQ to 200% of specification level. System suitability parameters will be performed as per the predetermined protocol. Acceptance criteria for the above validation parameters are specified in individual experimental design.
Serval parameters were evaluated for the validation of the analytical method followed, for determine of minor and trace elements in cereals and cereal based products.
The capability of the method as a routine analysis method was estimated through the determination of the limits of every element studied. The limits of detection (LOD) and limits of quantification (LOQ) were calculated with three and ten times the standard deviation of the blank divided by the slope of the analytical curves, respectively (Thompson, Ellison, and Wood, 2002.). The values of LOD were in the range of 0.000-0.756 (ng/g) and the LOQ were 0.000-2.522 (ng/g), as shown in Table 3. As can be seen, the LODs and LOQs allowed the determination of both minor and trace elements at the required levels.
Precision is described as the degree of variability given by the expression of the results, not considering the in influence of the sample (sample variability). Chudzinska and Baralkiewicz (2011), evaluated precision by using relative standard deviation of 10 repeated determination of one sample. Following this method, the percent coefficient of variation (CV%) obtained are shown in Table 3.

2.7. Statistical Analysis

Element concentrations below LOD were replaced by half the value of the respective detection limits. The data were not normally distributed; therefore, non-parametric test was used in the analysis. Data were reported as mean±standard deviation of triplicate measurements. Statistical significance was set at $p<0.05$. Principal component analysis (PCA) was performed with Canoco 5.0. Data analyses were performed using SPSS Statistics Software Version 17.0.

3. Results and discussion

The range of concentration (mean±SD) of analysed minor and toxic teace elements obtained for each type of sample is shown in Table 4. As can be seen from table, elements are categorised into minor elements (with concentration below 10ng/g) and toxic trace elements (with concentration below 10 ng/g). The most significant results are discussed in following sub-sections in detail. The ANOVA for treated and non-treated samples are T_1 (NGP), T_2 (NGP), T_3 (NGP), and T_1 (GP), T_2 (GP), T_3 (GP) of statical for concentration of metal elements in non-germinated and germinated multi-millet puffs, ANOVA two-factor with replication. It was found that minor and toxic elements significantly correlated germinated multi-millet puffs value ($p<0.05$) at dissolution factor 200 are shown in Table 5.

Table 1: Operating conditions and measurement parameters for the ICP-MS Agilent Technologies-7850 Parameters

Parameter	Value (Analysis Mode)
Plasma power (RF)	700 to 1600W
Nebulizer gas	0.7 to 1.2 L/min
Auxiliary gas	0.8 to 1.0 L/min
Cool gas flow (Argon)	14.99 L/min
CCT gas flow (He gas)	4 to 6 L/min
KED bias potential	3 V
Sample uptake/wash time	45 s
Dwell time	0.05 s
Number of readings per sample	Three main runs with 10 sweeps each
Total acquisition time (3 repetitions including rinse)	150 s
Spectrometer	ICPMS- 7850 Agilent Technologies
Nebulizer	Micro Mist
Auto Sampler	Agilent SPS-4
Spray chamber	Cyclonic
Sample Introduction	Peri Pump
Interface	Pt Cones
RF power (kW)	1.35
Isotopes Minor elements	Cr52, Mn55, Ni60, Cu63, Zn66, Se82, Rb85, Sr88
Toxic trace elements	As75, Cd111, Pb208

Table 2: The maximum residue limits required concentration for stock preparation as per AOAC ACT 15.01

Element	MRL		DF	PPB
	(ppm)	(ppb)		100%
Cr	1	1000	200	5
Ni	1	1000	200	5
Cu	30	30000	200	150
As	1.1	1100	200	5.5
Se	5	5000	200	25
Cd	0.1	100	200	0.5
Sn	30	30000	200	150
Pb	0.2	200	200	1

Note. Ppm - per parts million, ppb- per parts billion, DF- dissolution factor, MRL- maximum residue limit

Table 3: LOD, LOQ, percussion and spike recovery for the elements analysed

Element	LOD (ng/g)	LOQ (ng/g)	Precision (CV %)	Recovery (%)
Minor elements				
Se	0.000	0.000	7.599	110.9[a]
Ni	0.205	0.683	2.469	113.9[a]
Cu	0.643	2.145	1.970	99.7[a]
Cr	0.756	2.522	2.764	107.3[a]
Toxic trace elements				
As	0.022	0.072	4.355	114.3
Cd	0.056	0.188	3.181	104.8
Pb	0.505	1.684	1.840	100.7

[a] Elements spiked at 100ng/g. others were spiked at 10 ng/g. LOD- limit of detection, LOQ- limit of quantification

Table 4: Concentrations of metal elements (ng/g) in non-germinated and germinated multi-millet puffs

Elements	Non-Germinated			Germinated		
	T_1 (NGP)	T_2 (NGP)	T_3 (NGP)	T_1 (GP)	T_2 (GP)	T_3 (GP)
Minor elements						
Se	248.06±0.31^e	260.8±0.41^c	281.148±0.31^a	298.250±0.39^b	312.689±0.38^c	286.495±0.29^e
Ni	264.2±0.31^f	226.7±0.52^b	206.4±0.81^c	275.1±0.62^d	259.1±0.49^a	276.7±0.48^f
Cu	10670.7±5.26^e	10534.1±5.47^b	10406.0±6.42^d	11068.1±5.48^e	11149.0±5.21^f	11203.4±5.68^a
Cr	33.1±0.023^c	29.5±0.014^a	27.6±0.024^c	40.0±0.025^a	40.8±0.017^f	36.7±0.03^f
Toxic elements						
As	4.6±0.076^e	4.9±0.0.079^b	4.3±0.068^f	5.6±0.074^a	6.8±0.042^a	5.6±0.071^b
Cd	1.2±0.069^c	1.6±0.058^f	1.3±0.068^a	1.4±0.054^f	1.6±0.035^a	1.8±0.058^c
Pb	9.5±0.001^d	6.4±0.0.003^a	5.2±0.0.002b	11.3±0.003^f	10.5±0.005^e	11.2±0.002^a

Note: T_1, T_2, T_3 (NGP)= Non-Germinated Puffs, T_1, T_2, T_3(GP)= Germinated Puffs a, b, c, d, e, f Values are mean±standard deviations of three (n=3) measurements. Different superscript letters within rows are significantly different (p<0.05).

3.1 Minor elements

The results of minor elements were Se (248.06-312.689), Ni (206.4-276.7), Cu (10406.0-11203.4), and Cr (27.6-40.8 ng/g) (Table 4). The concentration of Se, Ni, Cu, and Cr were varied in NGP and GP multi-millet puffs studied; but Cu was comparatively high in T_3-GP multi-millet puffs. While Cu were very closely similar in T_1 -NGP, T_2 -NGP, and T_3 - NGP multi-millet puffs samples. The minor elements were found higher in germinated puffs in comparison of non-germinated multi-millet puffs. on comparison, the contents of Cu are very close in T_1 -NGP, T_2 -NGP, and T3-NGP non-germinated multi-millet puffs, ranging (10406.0-10670.7 ng/g). Cr was less in T_1 -NGP, T_2 -NGP, and T_3 -NGP non-germinated multi-millet puffs, ranging (27.6-33.1 ng/g). and Cr was little higher in germinated multi-millet puffs, ranging (36.7-40.8 ng/g).

3.2 Toxic trace elements

Toxic trace elements including As, Cd, and Pb are of great concern and their levels especially in germinated multi-millet puffs. In this study the concentrations (ng/g) of these toxic trace elements found were: As (4.3-6.8), Cd (1.2-1.8), and Pb (5.2–11.2), Table 4. Cd was statistically significant (p<0.05) for germinated multi-millet puffs. On comparison, the contents of Cd, are very close in non-germinated multi-millet puffs (T_1 -NGP, T_2 -NGP, and T_3 -NGP) and Germinated multi-millet puffs (T_1 -GP, T_2 -GP, and T_3 -GP). Pb Lead was little higher in germinated multi-millet puffs (T_1 -GP, T_2 -GP, and T_3 -GP), ranging (10.5-11.3 ng/g).

4. Conclusion

In this paper, determination of minor and trace elements in non-germinated and germinated multi-millets puffs using ICP-MS analysis at Regional Public food Analyst Laboratory Meerut. A total of 06 samples of non-germinated and germinated multi-millets puffs were analysed and the results obtained were compared to critical limits values specified by FSSAI/FAO/WHO and Food and Nutrition Board. The results showed that non-germinated and germinated multi-millets puffs are quite safe with no contamination from toxic trace elements, including As, Cd, and Pb. The level of nutritional elements like Se, Cu, Cr and Ni were appropriate and thus non-germinated and germinated multi-millets puffs are making good contribution of these essential elements to daily nutrition of consumers in accordance with RDA.

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