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Comparison of different digestion methods for analysis of multi element (P, K, Fe, Mn, Zn and Cu) from plant

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Abstract
Microwave is the most reliable sample digestion method. However, it requires expensive microwave digester automation and has relatively low productivity. In this study, three non-automated digestion methods, i.e. wet acid digestion using nitric acid (HNO_3) and hydrogen peroxide ($H2O_2$), wet acid digestion using HNO_3, and dry washing, are compared in order to determine the best approach.

Keywords: Multi element analysis, digestion methods, microwave block digestion, infrared digestion

Introduction
Highly sensitive spectroscopic techniques, such as graphite furnace (GFAAS), hydride generation atomic absorption spectrometry (HGAAS), and flame (FAAS), can accurately measure trace metal elements (TMEs), which are present in a variety of samples (water, soil, plant tissues, etc.) (Uddin *et al.*, 2017) [1]. Since several approaches (Micó *et al.*, 2007) [2] require aqueous samples to measure metal concentrations, solid samples typically need to be transformed into soluble forms employing digestion processes.Plant tissue has been digested using a variety of techniques, including drying and conventional digestion, ignition and microwave digestion, drying and microwave digestion, wet acid digestion, and a combination of concentrated acid digestion, etc., for the study of metals. Certain TMEs in the sample may evaporate during the dry ashing process due to the adsorption of substances on the furnace walls or volatilization, such as arsenic (As), chromium (Cr), and lead (Pb), which may be lost at ashing temperatures of 500–550 °C. (Azcue and Mudroch, 1994) [3]. Typically, acid digestion techniques are utilised before elemental analysis to dissolve samples of herbal products (Güven and Akinci, 1994) [4]. Acid digestion of the sample is, in fact, an essential part of the entire analytical process in spectroscopic elemental analysis. Acid digestion has a sizable impact on the retrieval of many analyte contents in extremely complex matrices like herb and plant materials (Uddin *et al.*, 2017) [1]. Many investigations have demonstrated that, depending on the element under study, acid digestion procedures yield pertinent results that, with the exception of the metals linked with silicates, could reach up to 100% in determining the entire elemental composition. (Andersen and Kisser, 2004, Santoro *et al.*, 2017) [5, 6]. Many acids or acid mixes have been employed for the extraction of metals, with nitric (HNO_3), sulfuric (H_2SO_4), perchloric ($HClO_4$), and hydrochloric acids being the most frequently utilised (HCl). (Hseu, 2004) [7]. The kind of the matrix to be decomposed determines whether a group of acids or a single acid should be used. (Asher *et al.*, 2017) [8]. In addition, there are multifarious methods that require the use of a combination of an acid with an oxidant, such as hydrogen peroxide. With the continuous improvement and modifications in the analytical instrumentation, there needs proper calibration of different methods used for assessing nutrient concentrations from plant/manures/fertilizers with an understanding of acquiring highest precision. It is generally observed that values pertaining to different nutrient concentrations vary with respect to different methodologies and instruments used. Many times an error of minute figure may result in conversion of deficient to moderate category and vice a versa. There rises a question for authenticities of nutrient concentrations obtained wide different digestions accompanied with respective determination methods. Therefore a study is planned to compare digestion methods for plant/manure and to find out the correlation between different digestion methods.

Materials and Methods

This study focused on different plants, castor seeds (S1), groundnut seeds (S2), soybean (S3), rice grain (S4), wheat grain (S5), green gram (S6), pigeon pea (S7), cotton stalk (S8), sugarcane millable cane (S9) and rice leaves (S10). Plant samples (aerial parts) were dried for two weeks (until constant weight) at 60 °C. Then, dry samples were pulverized using a ball mill. The experiment was laid out in a randomized complete block design under factorial concept. The samples were submitted to seven different acid digestion methods: I1, Hot plate at 350 °C (HNO_3:$HClO_4$, 10:4), I2: Infrared digestion at 200 °C (HNO_3:$HClO_4$, 10:4), I3: Infrared digestion at 250 °C (HNO_3:$HClO_4$, 10:4), I4: Infrared digestion at 300 °C (HNO_3:$HClO_4$,10:4), I5: Microwave block digestion (HNO_3:H_2O_2, 4:1), I6: Microwave block digestion (HNO_3:$HClO_4$,10:4), I7: Block digestion (HNO_3:H_2O_2, 4:1). Respective plant and grain edible portions of different crops will be sampled and digested with 10 ml diacid by different methods and analyzed by following standard methods; P by Spectrophotometric Vanadomolybdo phosphoric acid method and K by flame photometer method, Jackson (1979) [9] while atomic absorption spectrophotometric method was adopted for Fe, Mn, Zn and Cu content Elwell and Gridley (1967) [10]. The data collected for various parameters were subjected to statistical analysis. The method of analysis of variance for factorial randomized block design was adopted using the method as described by Panse and Sukhatme (1978) [11].

Results and Discussion

Comparison of different digestion methods:

Data presented in Table 1 indicated that recovery of P, K, Zn, and Cu in castor seed were significantly higher under hot plate method of digestion as compared to other methods of digestion, but it was at par with infrared digestion at 300 °C and microwave digestion (HNO_3:$HClO_4$, 10:4). While in case recovery of Fe and Mn were maximum under microwave digestion (HNO_3:$HClO_4$, 10:4) method but it was at par with hot plate digestion and infrared digestion at 300 °C method.

Data presented in Table 2 indicated that recovery of P, K, Zn, Fe and Zn in groundnut seed were significantly higher under hot plate method of digestion as compared to other methods of digestion, but it was at par with infrared digestion at 300 °C and microwave digestion (HNO_3:$HClO_4$, 10:4). While in case recovery of Cu was maximum under infrared digestion at 300 °C but it was at par with hot plate digestion and microwave digestion (HNO_3:$HClO_4$, 10:4) method.

Data presented in Table 3 indicated that digestion of soybean using hot plate at 300 °C method gives significantly higher recovery of P, K, Fe, Mn, and Cu as compared to other method of digestion while in case of Zn recovery it was maximum under microwave digestion (HNO_3:$HClO_4$, 10:4) method. The P, K, Zn, Fe, Mn and Cu recovery, hot plate method of digestion was statistically at par with microwave digestion (HNO_3:$HClO_4$, 10:4) and microwave digestion (HNO_3:$HClO_4$, 10:4) method. Data presented in Table 4 indicated that digestion of rice grain using hot plate at 300 °C method gives significantly higher recovery of P while, K, Fe, and Cu were higher under infrared digestion at 300 °C method as compared to other methods of digestion. The Mn recovery was found to be non significant, it was maximum under microwave digestion (HNO_3:$HClO_4$, 10:4) method. The K, Fe, and Cu recovery were statistically at par with hot plate method of digestion and microwave digestion (HNO_3:$HClO_4$, 10:4) method. The Zn recovery was found significant, it was maximum under microwave digestion (HNO_3:$HClO_4$, 10:4) method.

Data presented in Table 5 indicated that recovery of P and Cu in wheat grain were significantly higher under microwave digestion (HNO_3:$HClO_4$, 10:4) method as compared to other methods of digestion, but it was at par with hot plate digestion at 350 °C and infrared digestion at 300 °C methods. While in case recovery of K, Fe, and Mn were maximum under infrared digestion at 300 °C but it was at par with hot plate digestion and microwave digestion (HNO_3:$HClO_4$, 10:4) method. The Zn recovery was found to be non significant, it was maximum under infrared digestion at 300 °C method. Data presented in Table 6. indicated that digestion methods were significantly effected in different nutrient recovery of green gram. The P and Mn recovery was significantly higher under using microwave digestion (HNO_3:$HClO_4$, 10:4) method, while, Fe, Zn and Cu were higher under infrared digestion at 300 °C method as compared to other methods of digestion. The K recovery was found significant, it was maximum under Block digestion (HNO_3:H_2O_2, 4:1) method. The recovery of P, K, Fe, Mn, Zn and Cu were statistically at par with hot plate method of digestion.

Data presented in Table 7 indicated that digestion methods were significantly effected in different nutrient content recovery of pigeon pea. The P recovery was significantly higher under using Microwave digestion (HNO_3:$HClO_4$, 10:4) method, while, K, Mn and Zn were higher under Hot plate digestion at 350 °C method as compared to other methods of digestion. The Cu recovery was found to be significant, it was maximum under Infrared digestion at 300 °C method. The recovery of P was statistically at par with Hot plate method of digestion and Infrared digestion at 300 °C method while in case of K recovery was at par with Infrared digestion at 250 °C, microwave digestion (HNO_3:H_2O_2, 4:1) and block digestion (HNO_3:H_2O_2, 4:1). The recovery of Mn and Zn were statistically at par with Infrared digestion at 300 °C and microwave digestion (HNO_3:$HClO_4$, 10:4). The Fe recovery was significantly higher under using infrared digestion at 300 °C method, it was statistically on par with hot plate digestion at 350 °C and microwave digestion (HNO_3:$HClO_4$, 10:4).

Data presented in Table 8 indicated that digestion methods were significantly effected in different nutrient content recovery of cotton stalk except Mn recovery. The P and Cu recovery were significantly higher under using Infrared digestion at 300 °C method, which was at par with Hot plate digestion at 350 °C and microwave digestion (HNO_3:$HClO_4$, 10:4,) while P also at par with Infrared digestion at 250 °C. The K content recovery was significantly higher under microwave digestion (HNO_3:H_2O_2, 4:1) methods it was at par with Hot plate digestion at 350 °C, Infrared digestion at 250 °C and Block digestion (HNO_3:H_2O_2, 4:1). The Fe and Zn was higher under Hot plate digestion at 350 °C method as compared to other methods of digestion but it was at par with infrared digestion at 300 °C and microwave digestion (HNO_3:$HClO_4$, 10:4) method. The Cu recovery was found to be significant; it was higher under Infrared digestion at 300 °C method but it was at par with Hot plate digestion at 350 °C and. Microwave digestion (HNO_3:$HClO_4$, 10:4). The recovery of Mn was to be non significant but it was maximum under Microwave digestion (HNO_3:$HClO_4$, 10:4) method.

Data presented in Table 9 indicated that digestion methods were significantly effected in different nutrient content recovery of sugarcane. The P and Mn recovery were significantly higher under using Microwave digestion (HNO_3:$HClO_4$, 10:4) method, which were at par with Hot plate digestion at 350 °C and Infrared digestion at 300 °C, while P also at par with Block digestion (HNO_3:H_2O_2, 4:1). The K content recovery was significantly higher under Block digestion (HNO_3:H_2O_2, 4:1) methods it was at par with Hot plate digestion at 350 °C, Infrared digestion at 300 °C and Microwave digestion (HNO_3:$HClO_4$, 10:4). The Fe, Zn and Cu recovery were significantly higher under Hot plate digestion at 350 °C method as compared to other methods of digestion but it was at par with Infrared digestion at 300 °C and Microwave digestion (HNO_3:$HClO_4$, 10:4) method. The Mn recovery was found to be significant; it was higher under Microwave digestion (HNO_3:$HClO_4$, 10:4) method but it was at par with Hot plate digestion at 350 °C and. Infrared digestion at 300 °C method.

Data presented in Table 10 indicated that digestion methods were significantly effected in different nutrient content recovery of rice leaves. The recovery of P, Mn, Zn, and Cu in rice leaves was significantly higher under hot plate method of digestion as compared to other methods of digestion, but it was at par with infrared digestion at 300 °C and microwave digestion (HNO_3:$HClO_4$, 10:4). While in case K recovery in rice leaves was significantly higher under Block digestion (HNO_3:H_2O_2, 4:1 method however, it was at par with Hot plate digestion at 350 °C, Infrared digestion at 300 °C and microwave digestion (HNO_3: H_2O_2, 10:4) method. The Fe recovery was significantly higher under infrared digestion at 300 °C method, but it was at par with hot plate digestion and microwave digestion (HNO_3:$HClO_4$, 10:4) method. Data presented in Table 11. indicated that the hot plate digestion at 350 °C, infrared digestion at 300 °C and microwave digestion (HNO_3:$HClO_4$, 10:4) provides higher recovery for P, Fe, Mn, Zn and Cu as compared to other methods of digestion while in case of K, nutrient recovery was significantly higher under infrared digestion at 300 °C, microwave digestion (HNO_3:H_2O_2, 4:1) and block digestion (HNO_3:H_2O_2, 4:1) under investigation. Data presented in Table 12. Indicated that all the digestion methods were highly correlated with each other.

According to Shaibur *et al.* 2010 [12], in the scientific world of plant nutrition, nitric acidperchloric acid mixture is considered to be the prominent digesting reagent. Also, it was shown that the better element recovery from the nitric/perchloric acid technique makes it superior to employing solely nitric acid for digestion. Although the digestion of perchloric acid can result in the loss of K and B by volatilization, it is also necessary to utilise specifically built hoods since perchloric acid poses a risk of explosion (Zarcinas and Cartwright, 1983) [13]. Otherwise, it was demonstrated by Warman and Muizelaar that nitric acid and nitric/perchloric acid methods had the same analytical sensitivity and that there was no benefit to utilising the riskier and more expensive nitric/perchloric acid digestion (Warman and Muizelaar, 1995) [14]. Our findings concur with those of Sahrawat *et al.* 2002 [15], Momen *et al.* 2010 [16], Adamczyk *et al.* 2010 [17], Zheljazkov *et al.* 2010[18], Sastre *et al.* 2010 [19] and Schmid *et al.* 2010 [20].

Economics

Among the digestion methods (Table 13), hot plate digestion method is cheapest one (Rs. 0.30 per sample) followed by infrared digestion (Rs. 1.82 to 2.43 per sample), block digestion (Rs.2.42 per sample) and microwave digestion (Rs. 3.75 per sample).

Table 1: Influence of digestion method on nutrients recovery in castor seed

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at350 °C	0.114	0.762	80.20	44.80	24.60	13.51
2. Infrared digestion at 200 °C	0.076	0.698	72.60	40.80	18.60	12.98
3. Infrared digestion at 250 °C	0.072	0.692	71.40	39.00	17.20	12.69
4. Infrared digestion at 300 °C	0.112	0.754	80.60	46.80	22.80	13.51
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.084	0.760	70.20	39.00	17.60	12.84
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.112	0.680	81.00	47.20	22.80	13.44
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.076	0.758	70.40	37.60	16.20	12.98
S.Em.±	0.007	0.015	1.41	1.50	1.09	0.174
C.D. at 5%	0.021	0.045	4.08	4.35	3.16	0.505
C.V. %	17.28	4.73	4.19	7.97	12.22	2.96

Table 2: Influence of digestion method on nutrients recovery in groundnut seed

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.195	2.004	47.84	33.80	16.20	5.10
2. Infrared digestion at 200 °C	0.171	1.808	41.28	28.40	12.00	3.34
3. Infrared digestion at 250 °C	0.167	1.796	41.72	28.40	12.60	3.14
4. Infrared digestion at 300 °C	0.183	1.994	45.32	32.80	15.40	5.20
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.168	1.700	41.04	27.80	12.40	4.44
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.184	1.888	44.88	29.00	16.00	5.14
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.173	1.764	39.48	25.60	13.00	3.08
S.Em.±	0.006	0.057	1.821	1.599	0.965	0.354
C.D. at 5%	0.017	0.166	5.274	4.632	2.795	1.025
C.V. %	7.52	6.93	9.45	12.16	15.48	18.82

Table 3: Influence of digestion method on nutrients recovery in Soybean

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.312	2.516	92.60	54.00	29.80	15.56
2. Infrared digestion at 200 °C	0.244	2.148	82.60	50.20	25.00	11.18
3. Infrared digestion at 250 °C	0.258	2.168	78.40	45.00	25.80	10.70
4. Infrared digestion at 300 °C	0.254	2.260	88.40	47.60	30.00	11.84
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.228	2.236	83.80	40.80	25.80	10.78
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.284	2.150	84.00	45.40	30.20	12.08
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.248	2.130	77.40	41.20	24.80	10.36
S.Em.±	0.018	0.074	3.015	2.149	1.604	0.895
C.D. at 5%	0.051	0.215	8.733	6.225	4.644	2.592
C.V. %	14.99	7.44	8.04	10.38	13.11	16.98

Table 4: Influence of digestion method on nutrients recovery in rice grain

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.108	1.832	83.20	60.40	26.40	12.72
2. Infrared digestion at 200 °C	0.076	1.544	79.00	59.00	19.80	8.98
3. Infrared digestion at 250 °C	0.078	1.740	77.40	58.60	18.80	9.42
4. Infrared digestion at 300 °C	0.104	1.834	87.20	61.80	29.20	13.18
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.082	1.828	75.40	58.00	17.00	7.70
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.106	1.728	85.40	62.00	30.00	11.96
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.084	1.776	78.40	56.40	18.60	8.50
S.Em.±	0.0061	0.0472	1.782	1.879	1.838	0.967
C.D. at 5%	0.0175	0.1367	5.160	NS	5.32	2.700
C.V. %	16.29	6.01	4.93	7.07	18.00	20.88

Table 5: Influence of digestion method on nutrients recovery in wheat grain

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.318	1.478	67.20	46.40	19.80	10.90
2. Infrared digestion at 200 °C	0.256	1.372	61.80	39.40	19.40	7.34
3. Infrared digestion at 250 °C	0.254	1.426	63.20	38.60	16.20	7.64
4. Infrared digestion at 300 °C	0.316	1.502	70.80	49.60	21.80	11.66
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.250	1.512	60.60	39.60	17.80	8.18
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.326	1.362	69.60	49.60	20.00	11.76
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.270	1.484	61.60	38.60	16.40	8.28
S.Em.±	0.016	0.022	1.658	1.460	1.764	0.640
C.D. at 5%	0.048	0.064	4.802	4.228	NS	1.854
C.V. %	12.92	3.43	5.71	7.57	21.01	15.24

Table 6: Influence of digestion method on nutrients recovery in green gram

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.456	2.31	78.40	69.80	43.00	10.58
2. Infrared digestion at 200 °C	0.400	2.24	70.80	63.80	36.80	7.04
3. Infrared digestion at 250 °C	0.396	2.27	75.60	66.80	36.40	8.66
4. Infrared digestion at 300 °C	0.468	2.32	79.80	72.80	43.20	11.34
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.396	2.33	71.00	65.80	35.20	8.06
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.480	2.23	79.20	73.00	43.00	10.82
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.396	2.36	70.80	63.80	34.20	8.36
S.Em.±	0.016	0.017	1.668	1.861	1.872	0.880
C.D. at 5%	0.047	0.048	4.832	5.390	5.421	2.549
C.V. %	8.43	1.63	4.97	6.12	10.78	21.23

Table 7: Influence of digestion method on nutrients recovery in Pigeon pea

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.276	1.816	78.80	27.40	28.60	10.96
2. Infrared digestion at 200 °C	0.228	1.740	72.60	21.60	19.80	8.78
3. Infrared digestion at 250 °C	0.222	1.728	76.80	20.60	20.40	8.28
4. Infrared digestion at 300 °C	0.282	1.806	79.80	26.20	27.20	11.18
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.228	1.806	68.40	19.20	19.20	9.90
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.302	1.724	79.40	26.00	25.40	10.98
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.206	1.806	73.00	20.00	17.80	9.06
S.Em.±	0.018	0.019	2.011	1.505	1.700	0.930
C.D. at 5%	0.052	0.054	5.824	4.359	4.925	NS
C.V. %	16.06	2.36	5.95	14.63	16.8	21.07

Table 8: Influence of digestion method on nutrients recovery in cotton stalk

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.066	0.198	35.16	20.80	13.20	36.40
2. Infrared digestion at 200 °C	0.048	0.164	27.78	18.00	10.40	31.00
3. Infrared digestion at 250 °C	0.054	0.142	27.12	18.20	9.00	29.80
4. Infrared digestion at 300 °C	0.072	0.194	31.00	20.60	10.80	37.00
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.046	0.204	25.20	18.00	8.80	29.60
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.066	0.148	31.52	23.60	12.20	34.20
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.048	0.202	26.88	17.80	9.00	29.20
S.Em.±	0.0046	0.010	1.455	1.713	0.927	1.278
C.D. at 5%	0.0133	0.028	4.214	NS	2.686	3.703
C.V. %	17.87	12.18	11.13	19.57	19.78	8.81

Table 9: Influence of digestion method on nutrients recovery in sugarcane (millable)

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.216	0.684	135.2	55.00	34.20	8.02
2. Infrared digestion at 200 °C	0.180	0.644	122.0	49.40	29.80	6.50
3. Infrared digestion at 250 °C	0.168	0.650	127.2	51.40	30.60	6.04
4. Infrared digestion at 300 °C	0.216	0.700	134.8	58.20	33.40	7.80
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.168	0.706	129.0	50.00	28.20	6.54
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.220	0.672	133.2	58.80	33.60	8.00
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.196	0.728	121.4	48.40	27.00	5.04
S.Em.±	0.0122	0.014	1.965	1.476	1.630	0.465
C.D. at 5%	0.0353	0.041	5.690	4.276	4.721	1.347
C.V. %	14.03	4.64	3.41	6.23	11.77	15.18

Table 10: Influence of digestion method on nutrients recovery in rice leaves

Treatment (Digestion Methods)	Nutrient Content (%)		Nutrient Content (ppm)			
	P	K	Fe	Mn	Zn	Cu
1. Hot plate digestion at 350 °C	0.126	1.442	150.60	60.40	39.20	11.88
2. Infrared digestion at 200 °C	0.074	1.432	145.00	48.80	29.60	9.96
3. Infrared digestion at 250 °C	0.084	1.410	142.20	48.80	27.40	9.76
4. Infrared digestion at 300 °C	0.116	1.458	151.20	59.40	36.00	11.86
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	0.078	1.490	144.40	46.40	24.80	10.40
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	0.122	1.400	151.00	53.60	24.40	11.26
7. Block digestion (HNO_3:H_2O_2, 4:1)	0.092	1.500	143.60	46.00	25.20	9.78
S.Em.±	0.006	0.020	1.7631	1.7493	2.1381	0.829
C.D. at 5%	0.017	0.057	5.1065	5.0665	6.1926	NS
C.V. %	12.85	3.05	2.68	7.53	16.2	17.31

Table 11: Influence of digestion method on nutrients recovery in different crop material

Treatments		Nutrient Content (%)		Nutrient Content (ppm)			
		P	K	Fe	Mn	Zn	Cu
Digestion Methods (M)							
1. Hot plate digestion at 350 °C		0.212	1.46	84.13	47.24	27.32	13.42
2. Infrared digestion at 200 °C		0.175	1.39	77.75	42.02	22.12	10.71
3. Infrared digestion at 250 °C		0.174	1.42	78.30	42.02	21.44	10.61
4. Infrared digestion at 300 °C		0.218	1.48	84.97	47.54	26.98	13.50
5. Microwave digestion (HNO_3:H_2O_2, 4:1)		0.172	1.48	76.90	40.36	20.68	10.84
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)		0.223	1.40	83.96	47.12	25.96	13.38
7. Block digestion (HNO_3:H_2O_2, 4:1)		0.178	1.48	76.30	39.54	20.22	10.44
S.Em.±		0.004	0.009	0.632	0.559	0.516	0.285
C.D. at 5%		0.019	0.024	1.769	1.565	1.444	0.798
Different crop material (C)							
1	Castor seed	0.093	0.73	75.20	42.06	19.97	13.14
2	Groundnut seed	0.174	1.93	43.47	30.49	13.97	4.46
3	Soybean	0.269	2.20	83.11	46.31	27.34	11.79
4	Rice grain	0.086	1.75	80.86	59.46	22.83	10.35
5	Wheat grain	0.284	1.45	64.97	43.11	18.77	9.39
6	Green gram	0.427	2.30	75.09	67.97	38.83	9.27
7	Pigean pea	0.249	1.78	75.54	23.00	22.63	9.88
8	Cotton stalk	0.057	0.18	29.24	19.57	10.49	32.46
9	Sugarcan millable cane	0.195	0.68	128.97	53.03	30.97	7.01
10	Rice Leaves	0.095	1.45	146.86	51.91	29.51	10.70
S.Em.±		0.005	0.010	0.755	0.668	0.616	0.341
C.D. at 5%		0.014	0.029	2.115	1.871	1.725	0.954
Interaction (M x C)							
S.Em.± MxT		0.013	0.028	1.998	1.768	1.630	0.901
C.D. at 5%		NS	0.077	NS	4.949	4.565	2.524
C.V. %		15.48	4.27	5.56	9.050	15.49	17.01
Z score (-2 to +2)		0.816	0.332	0.993	1.017	1.223	1.068

Table 12:.Correlation between digestion methods in relation to nutrient recovery in different plant materials

	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6	Method 7
P							
Method 1	1.000						
Method 2	0.992**	1.000					
Method 3	0.993**	0.998**	1.000				
Method 4	0.999**	0.995**	0.996**	1.000			
Method 5	0.994**	0.998**	0.997**	0.996**	1.000		
Method 6	0.995**	0.990**	0.985**	0.995**	0.992**	1.000	
Method 7	0.989**	0.995**	0.992**	0.990**	0.991**	0.987**	1.000
K							
Method 1	1.000						
Method 2	0.992**	1.000					
Method 3	0.997**	0.996**	1.000				
Method 4	0.998**	0.995**	0.999**	1.000			
Method 5	0.999**	0.995**	0.999**	0.999**	1.000		
Method 6	0.998**	0.996**	0.999**	0.999**	0.999**	1.000	
Method 7	0.999**	0.997**	0.999**	0.999**	0.999**	0.999**	1.000
Fe							
Method 1	1.000						
Method 2	0.997**	1.000					
Method 3	0.997**	0.995**	1.000				
Method 4	0.998**	0.998**	0.997**	1.000			
Method 5	0.998**	0.997**	0.995**	0.997**	1.000		
Method 6	0.998**	0.997**	0.998**	0.999**	0.995**	1.000	
Method 7	0.997**	0.999**	0.997**	0.998**	0.995**	0.999**	1.000
Mn							
Method 1	1.000						
Method 2	0.984**	1.000					
Method 3	0.974**	0.986**	1.000				
Method 4	0.985**	0.969**	0.976**	1.000			
Method 5	0.968**	0.980**	0.992**	0.984**	1.000		
Method 6	0.962**	0.962**	0.975**	0.991**	0.991**	1.000	
Method 7	0.975**	0.985**	0.990**	0.986**	0.998**	0.990**	1.000

Zn							
Method 1	1.000						
Method 2	0.966**	1.000					
Method 3	0.958**	0.986**	1.000				
Method 4	0.981**	0.969**	0.963**	1.000			
Method 5	0.931**	0.982**	0.990**	0.944**	1.000		
Method 6	0.851*	0.884**	0.916**	0.913**	0.914**	1.000	
Method 7	0.943**	0.985**	0.993**	0.963**	0.991**	0.926**	1.000
Cu							
Method 1	1.000						
Method 2	0.999**	1.000					
Method 3	0.999**	0.999**	1.000				
Method 4	0.999**	0.999**	0.999**	1.000			
Method 5	0.998**	0.999**	0.999**	0.998**	1.000		
Method 6	0.998**	0.999**	0.999**	0.999**	0.999**	1.000	
Method 7	0.999**	0.999**	0.999**	0.999**	0.999**	0.999**	1.000

**significant at 1% level; *Significant at 5% level

Table 13: Analysis cost of different digestion methods

Treatment (Digestion Methods)	Depreciation charge @ 10% per year *	Sample per Batch	Working (hrs) per year **	Digestion time per batch (hr)	Sample digested per year	Cost per sample (Rs./-)
1. Hot plate digestion at 350 °C	4556	25	1200	2.00	15000	0.30
2. Infrared digestion at 200 °C	35000	12	1200	1.00	14400	2.43
3. Infrared digestion at 250 °C	35000	12	1200	0.83	17349	2.02
4. Infrared digestion at 300 °C	35000	12	1200	0.75	19200	1.82
5. Microwave digestion (HNO_3:H_2O_2, 4:1)	108103	12	1200	0.50	28800	3.75
6. Microwave digestion (HNO_3:$HClO_4$, 10:4)	108103	12	1200	0.50	28800	3.75
7. Block digestion (HNO_3:H_2O_2, 4:1)	17455	12	1200	2.00	7200	2.42

* Depreciation charge 10% per year on total cost of Instrument

Conclusion

Among the digestion methods, hot plate digestion method is cheapest and has higher digestion capacity followed by infrared digestion, microwave digestion and block digestion. Hot plate digestion at 350 °C, infrared digestion at 200 °C, 250 °C and 300 °C and microwave digestion (HNO_3:$HClO_4$, 10:4) and (HNO_3:H_2O_2, 4:1) and block digestion (HNO_3:H_2O_2, 4:1) are highly correlated with each other. Microwave digestion method is faster as compared to other methods.

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