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Evaluation of various preparatory operations for analytical recovery of selected plant nutrients in grains of wheat (*Triticum aestivum*), lentil (*Lens culinaris*) and rapeseed (*Brassica napus*)

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Abstract

Laboratory techniques involved in analytical procedure poses many complications on the results that influence the efficacy of research work. An experiment was conducted to study the effect of washing, grinding and digestion methods on K, Zn and Fe concentrations in grains of wheat, lentil and rapeseed. The investigation was undertaken at the laboratory of National Agricultural Research Centre (NARC), Islamabad. The samples were divided into two sets; one was washed with distilled water and other was processed unwashed. Four different grinders namely Kinematica Model PX–MFC, J 200 GE 247–A, Cyclotec 1093 and mortar & pestle were used for sample grinding. The samples were subjected to dry ashing as well as wet digestion (HNO_3 + $HClO_4$). The results showed that K, Zn and Fe concentrations affected significantly with different washing methods, grinding machines and digestion procedures. Washing as well as dry ashing decreased K, Zn and Fe contents.

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Introduction

Many techniques being used for determination of nutrient requirement for crop fertilization. These include soil and plant tissue analysis, pot experiment and field eperimentation. The plant tissue analysis technique used to determine the plant nutrient requirements for crop fertilization and considered to be more authentic tool in this respect. This technique needs utmost care and precision to obtain accurate results and make right decision in crop fertilization program.

Prior to the laboratory analysis, there are several procedural steps to prepare soil and plant tissue samples. There are chances that samples may be contaminated at any step of its preparatory operation i.e., sampling, transportation of samples from field to laboratory, handling, grinding, etc. For example, one of the sources through which contamination may occur is sample container. The soil samples were collected and placed into plastic air-tight screw-top containers which were placed in the back of a truck for transportion to laboratory and be analyzed for fertilizer recommendations. The truck had previously been used for fertilizer transportation, the result will be that all sample containers will be coated with a fine dust of fertilizer, proper analysis of the soil samples will become almost impossible (Jones and Case, 1990; McCrimmon, 2008).

The proper cleanliness and sanitation of equipment may lead to précised and accurate results that will curtail in making right decision for crop fertilization program. Utmost care is needed in analyzing the nutrient contents in the sample; for example a machine used for grinding the samples was not properly cleaned and used can affect the nutrient concentration being analysed that ultimately affects the quality of analysis.

Different types of analysis also need specific care and glasswares that must be taken in to account while analyzing nutrients in crop fertilization program. For example using ferrous grinders or grinders containing ferrous grinding surfaces should be avoided when analyzing for iron. Similarly, for determination of boron, plastic ware should be used to avoid contamination of boron. Choice of proper analytical procedures like dry ashing and wet digestion can also affect or cause loss of the analyte through volatilization. The nutrients may be lost with washing like K and Fe, etc (Frank, 2005; McCrimmon, 2008). Moreover, the grinding machines being used and the digestion methods adopted, may affect nutrient concentration being analysed in the samples and this whole process affects the quality of analysis. The preparatory operations caused a significant losses or addition in the contents of analyzed elements (Lisiewska, 2006). Contamination in samples can come from poorly washed laboratory glassware, especially from pipettes which may have been previously filled with concentrated reagents. Consider contamination from a pipette which was used to transfer phosphoric acid, improperly washed and subsequently used to take an aliquot of a sample for determination of phosphorus can effect P concentration (Winkleman *et al.*, 1990; Labanauskas, 1966; Munson and Nelson, 1990).

In the developing countries like Pakistan there is a need to investigate the causes and sources of contamination that may affect quality of analysis to obtain precised results from analytical techniques being used. In Pakistan no doubt a lot of research has been undertaken on this aspect but there is still need to do a lot. The government is taking steps in this regard and facilitating different organizations in getting recognized their laboratories by ISO certification. A countless flase and ignorances do exist which need fine tuning. Keeping in view the above facts, a study was conducted to find out the causes and sources of contamination or losses of some metal elements that affect the quality of analysis during the preparatory operations.

Materials and methods

A laboratory experiment was conducted in Land Resources Research Institute, National Agricultural Research Centre, Islamabad to study the quality of analyses as affected by preparatory operations like washing, grinding, digestion methods etc. It is assumed that relatively more nutrients are lost during dry ashing as compared to that of wet digestion and washing of samples also affects the quality of analyte.

For this purpose, initially seeds of three crops; wheat (*Triticum aestivum*), lentil (*Lens culinaris*) and rapeseed (*Brassica napus*) were selected for the study.

Determination of *K, Zn and Fe*

Three nutrients one macro and two micro i.e., K, Zn and Fe were chosen for study.

The seeds of three selected crops; wheat, lentil and rapeseed (one kilogram each) were taken and each of them was divided into two halves, one half was washed with distilled water while the other was kept unwashed. The washed and unwashed samples were air and then oven dried at 65 ^{o}C and then ground by four types of grinders namely; *Kinematica Model PX–MFC, J 200 GE 247–A, Cyclotec 1093* and *marble mortar & pestle*. The plant grain samples were digested by two different techniques namely:

Dry ashing

One gram of ground plant grin sample was taken in sequentially numbered crucible and placed in muffle furnace at temperature round 550^{o}C for 2 hours. Then furnace was turned off and allowed to cool for one hour, then opened the furnace door gently and cooled for an hour. Then the samples were removed from furnace and 10mL 0.7 N H_2SO_4 was added to it and the material was swirled strongly and allowed to stand for one hour with occasional mixing. The samples were filtered into 50mL volumetric flask. Then the volume was made with distilled water and K concentration was determined by flame photometer (Winkleman *et al.,* 1990) and Zn, Fe, by using atomic absorption spectroscopy.

Wet Digestion

In this method 0.25 gm ground plant grin samples were taken in 100ml flasksnd add 10 ml mixed acid solution (HNO3: HClO4) in 2:1 ratio (Winkleman *et al.,* 1990) and determined K by flame photometer and Zn, Fe, by using atomic absorption spectroscopy.

Results

Wheat Grain K, Zn and Fe Contents as Affected by Sample Preparatory Operation

The wheat grain K, Zn and Fe concentration as affected by different preparatory operations is presented in Table 1. The results revealed that K contents were less affected by different grinders used for sample grinding under wet digestion and dry ashing. The difference between K contents of samples wet digested and dry ashed was non-significant. The dry ashing reduced grain K contents by 3.3% vis-a-vis wet digested samples. As far as grinders are concerned, more decrease of K content was recorded in samples grinded with Kinemetica PX–MFC (6.2%) followed by J200 GE and Cyclotec 1039 grinders. The highest K contents were observed in samples ground with mortar and pestle. Washing decreased K by 7.2as compared to samples processed without washing on overall basis. Similarly, the zinc (Zn) contents of wheat grain were affected by sample grinders, digestion methods and washing. Different grinders affected Zn contents but Zn was found significantly higher in samples ground with J 200 GE 247–A as compared to other grinders. The samples ground in marble mortar & pestle and Cyclotec 1093 grinder and subjected to dry ashing showed the maximum Zn reduction. Contrarily, maximum Zn loss was observed in samples ground by Kinematica PX–MFC where minimum Zn in wheat samples was recorded. On overall basis, Zn contents in dry ashed as well as wet digested samples were at par with each other (Table 1). Washing of the samples reduced Zn contents and the difference between washed and unwashed samples was 12.6% on overall basis.

Quite interesting results were found regarding Fe contents of wheat grain when samples ground by different grinders and subjected to wet digestion and dry ashing. The sample ground by the pestle & mortar and wet digested, had significantly higher Fe concents as compared to that of other grinders. It was followed by Kinematica PX–MFC which was at par to J 200 GE 247–A grinder (Table 1). The Fe in the samples ground by Cyclotec 1093 grinder was lowest. On overall basis, wet digestion had relatively more amount of Fe in samples as compared to those of dry ashed ones, showing loss of Fe from samples by ashing about 60% (Table 1). Washing of samples improved the precision of the results which is clear from data that Fe content affected significantly by washing and the difference between samples, run unwashed and washed was 19.6% on overall basis.

Table 1. Wheat grain Zn, Fe and K contents as affected by sample preparatory peration.

	Wet Digestion			Dry Ashing			
Zn contents (mg kg^{-1})	Unwashed	Washed		Unwashed	Washed		Grinder
Grinders			Mean			Mean	Mean
KINEMATICA PX-MFC	24.9 ^{bcde}	24.1 ^{cde}	24.5 ^{bc}	23.5 ^{cde}	22.9 ^{de}	23.2 ^{c}	23.8^{B}
J 200 GE 247 A	30.4 ^{abc}	28.1 ^{abcd}	29.2 ^{ab}	34.8 ^{a}	30.8 ^{ab}	32.8 ^{a}	31.1^{A}
Cyclotec 1093	27.0 ^{bcde}	26.7 ^{bcde}	26.8 ^{bc}	24.1 ^{bcde}	21.5 ^{de}	22.8 ^{c}	25.1^{B}
Pestle and Marter	28.6 ^{abcd}	21.3 ^{e}	24.9 ^{bc}	30.7 ^{abc}	22.9 ^{de}	26.8 ^{bc}	25.7^{B}
Mean washing	27.7 ^{ab}	25.0 ^{ab}		28.2 ^{a}	24.5 ^{b}		
Mean digestion	26.50 ^{a}			26.38^{a}			
LSD Digestion methods NS							
Washing 2.44							
Grinders 3.45							
Digestion methods X Grinders X Washing =NS							

Fe contents (mg kg^{-1})

	Wet Digestion			Dry Ashing			Grinder
Grinders	Unwashed	Washed	Mean	Unwashed	Washed	Mean	Mean
KINEMATICA PX-MFC	73.75^{c}	69.17 ^{c}	71.46^{a}	30.25g	28.70 ^{g}	30.25 ^{e}	50.4^{C}
J 200 GE 247 A	80.83 ^{b}	59.67 ^{e}	70.25 ^{a}	67.30 ^{cd}	62.45 ^{de}	64.88 ^{b}	67.3^{A}
Cyclotec 1093	58.48 ^{ef}	53.45 ^{f}	55.96 ^{c}	30.55 ^{g}	27.90 ^{g}	29.23 ^{e}	42.5^{D}
Pestle and Marter	88.61 ^{a}	58.13 ^{ef}	73.37 ^{a}	59.75 ^{ef}	33.65 ^{g}	46.70 ^{d}	59.9^{B}
Mean washing	75.41 ^{a}	60.10 ^{b}		46.96 c	38.18 d		
Mean digestion	67.75 ^{a}			42.27^{b}			
LSD Digestion methods 3.49							
Washing 2.47							
Grinders 3.45							
Digestion methods X Grinders X Washing 6.98							

K Contents (g 100 $^{g-1}$)

	Wet Digestion			Dry Ashing			Grinder
Grinders	Unwashed	Washed	Mean	Unwashed	Washed	Mean	Mean
KINEMATICA PX-MFC	0.44 ^{abcd}	0.41 ^{cd}	0.43 ^{ab}	0.41 ^{cd}	0.40 ^{cd}	0.41 ^{b}	0.42^{B}
J 200 GE 247 A	0.47 ^{ab}	0.40 ^{d}	0.44 ^{ab}	0.43 ^{abcd}	0.41^{bcd}	0.42 ^{ab}	0.43^{AB}
Cyclotec 1093	0.45 ^{abcd}	0.42 ^{abcd}	0.44 ^{ab}	0.43^{abcd}	0.43 ^{abcd}	0.43 ^{ab}	0.44^{B}
Pestle and Marter	0.48 ^{a}	0.43 ^{abcd}	0.46 ^{a}	0.46^{abc}	0.42 ^{abcd}	0.44 ^{ab}	0.45^{A}
Mean Washing	0.46 ^{a}	0.42 ^{b}		0.43 ^{a}	0.41 ^{b}		
Mean Digestion	0.438 ^{a}			0.424^{a}			
LSD Digestion methods NS							
Washing 0.0216							
Grinders 0.030							
Digestion methods X Grinders X Washing 0.061							

Lentil Grain K, Zn and Fe Contents as Affected by Sample Preparatory Operations

The data pertaining K content of lentil grain sample as affected by digestion methods and grinders used and washing is presented in Table 2. The K contents variably affected by preparatory operations.

The difference between the K contents of samples pulverized by different grinders was significant in both wet digestion and dry ashing methods. The K was lost by washing of samples as the difference between the K content of washed and unwashed samples was about 3% showing more K in unwashed samples. The Zn was less affected by different grinders under wet digestion, and small variations was observed in samples ground by different grinders (Table 2). The lowest value of Zn was recorded in samples ground by pestle & mortar and the highest was in J 200 GE 247–A, which showed significantly higher Zn, followed by Kinematica PX–MFC grinder. Similar trend of Zn content was observed in samples

subjected to dry ashing. On overall basis, the difference in Zn between wet digested and dry ashed samples was about 19.7% (Table 2) showing more Zn loss from samples by ashing. The washing also reduced Zn in the samples as the difference between the washed and unwashed samples was more than 6%.

The lentil grain sample preparation techniques affected by grinders, digestion method and washing had variable effect on Fe contents analyzed. In wet digestion, the sample ground by J 200 GE 247–A had significantly higher Fe content followed by Kinematica PX–MFC and mortar & pestle (Table 2) and the lowest Fe contents in samples ground by Cyclotec 1093. On overall basis, about 68% Fe was lost from samples when subjected to dry ashing compared to wet digestion. The washing of the samples also reduced Fe contents as the difference between the washed and unwashed samples was significant and about 60% more Fe was recorded in samples run unwashed.

Table 2. Lentil grain Zn, Fe and K contents as affected by sample preparatory operation**.**

	Wet Digestion			Dry Ashing			
Zn contents (mg kg^{-1})	Unwashed	Washed		Unwashed	Washed		Grinder
Grinders			Mean			Mean	Mean
KINEMATICA PX-MFC	46 ^{a}	47 ^{a}	46.8 ^{ab}	45 ^{a}	36 ^{a}	40.8 ^{ab}	43.5
J 200 GE 247 A	47 ^{a}	47 ^{a}	47.4 ^{a}	45 ^{a}	35 ^{a}	55.6 ^{ab}	43.7
Cyclotec 1093	44 ^{a}	45 ^{a}	44.5 ^{ab}	35 ^{a}	36 ^{a}	36.3 ^{b}	40.1
Pestle and Marter	43 ^{a}	45 ^{a}	44.2 ^{ab}	38 ^{a}	33 ^{a}	35.9 ^{b}	39.3
Mean washing	45 ^{a}	46 ^{a}		40.8 ^{ab}	35.1 ^{b}		ns
Mean digestion	45.5 ^{a}			38.0 ^{b}			

LSD Digestion methods 5.64

Washing 5.64
Grinders 7.97
Digestion methods X Grinders X Washing 13.89

Fe contents (mg kg^{-1})

	Wet Digestion			Dry Ashing			Grinder
Grinders	Unwashed	Washed	Mean	Unwashed	Washed	Mean	Mean
KINEMATICA PX-MFC	123 ^{b}	100 ^{c}	112 ^{b}	68 ^{a}	51 ^{b}	59 ^{ab}	85.5^{B}
J 200 GE 247 A	169 ^{a}	95 ^{cd}	132 ^{a}	73 ^{a}	57 ^{b}	65 ^{a}	98.5^{A}
Cyclotec 1093	77 ^{def}	56 ^{ghi}	67 ^{d}	65 ^{a}	49 ^{b}	57 ^{ab}	62.0^{C}
Pestle and Marter	101 ^{e}	92 ^{cde}	96 ^{c}	70 ^{a}	53 ^{b}	62 ^{a}	79.0^{B}
Mean washing	117.5 ^{a}	85.8 ^{b}		69 ^{c}	52.5 ^{d}		
Mean digestion	101.6			60.88			

LSD Digestion methods 6.94

Washing 6.94
Grinders 9.82
Digestion methods X Grinders X Washing 13.89

K Contents (g 100 $^{g-1}$)

	Wet Digestion			Dry Ashing			Grinder
Grinders	Unwashed	Washed	Mean	Unwashed	Washed	Mean	Mean
KINEMATICA PX-MFC	1.05^{abc}	1.09 ^{a}	1.09 ^{a}	0.96 ^{efg}	0.96 ^{ef}	0.95 ^{bc}	1.05^{A}
J 200 GE 247 A	0.94 ^{efg}	0.92 ^{efg}	0.92 ^{bc}	0.94 ^{efg}	0.91 ^{fg}	0.92 ^{c}	0.93^{B}
Cyclotec 1093	1.08 ^{ab}	1.04 ^{abcd}	1.03 ^{a}	0.97 ^{def}	0.93 ^{efg}	0.95 ^{bc}	1.01^{A}
Pestle and Marter	1.00 ^{bcde}	0.97 ^{def}	0.98 ^{b}	0.99 ^{cdef}	0.88 ^{g}	0.94 ^{bc}	0.96^{B}
Mean Wshing	1.02 ^{a}	1.01 ^{a}		0.97 ^{b}	0.92 ^{c}		
Mean Digestion	1.01 ^{a}			0.94 ^{b}			

LSD Digestion methods 0.027
Washing 0.027
Grinders 0.038
Digestion methods X Grinders X Washing 0.077

Rapeseed Grain K, Zn and Fe Contents as Affected by Sample Preparatory Operations

The K content of rapeseed least affected by different grinders used and digestion methods as well as washing adopted in the study. The K content in rapeseed samples ground by different grinders ranged from 1 to 7% (Table 3).. The K contents in samples pulverized with different grinders varied and the maximum value was recorded in samples ground with Kinematica AG MFC and the minimum was in J200 GE–247–A in case of wet digestion whereas in dry ashing the maximum was in Cyclotec 1093 and minimum in Kinematica AG MFC and J200 GE–247–A. On overall basis, 12.5% K was lost from samples by washing showing the contamination through dust that was removed by washing (Table 3). The Zn contents were greatly affected by different grinders, washing and digestion methods. The Zn content of the samples ground by the Kinematica PX – MFC caused minimum reduction as compared to other grinders that showed higher Zn content followed by J200 GE 247–A and Cyclotec 1093 under wet digestion (Table 3). The samples ground by pestle and mortar had minimum Zn contents. In dry ashing, the above mentioned trend was also observed. On overall basis, the difference in Zn content between wet digested and dry ashed samples was 20% (Table 3) and washing of sample reduced Zn by 7% on overall basis. The Fe content in rapeseed samples affected by different preparatory operations. In case of wet digestion, the J200 GE 247–A and Kinematica PX–MFC showed more Fe as compared to other two grinders used. The Fe contents of samples ground by mortar & pestle and and Cyclotec 1093 were significantly different from each other. While in dry ashing, Fe was greatly lost from samples by J200 GE 247–A followed by Kinematica PX–MFC and least Fe loss by Cyclotec 1093 and mortar & pestle grinders. It meant that Cyclotec 1093 and mortar & pestle grinders caused less Fe contaminaation during the grinding the samples and helped in yielding précised results. On overall basis, the difference of Fe content between wet digested and dry ashed samples was more than 100% (Table 3). The washing improved quality of analysis and reduced Fe concentration in the samples by 14% on overall basis.

Table 3. Grain Zn, Fe and K contents of Rapeseed as affected by preparatory operations.

	Wet Digestion			Dry Ashing			
Zn contents (mg kg^{-1})	Unwashed	Washed		Unwashed	Washed		Grinder
Grinders			Mean			Mean	Mean
KINEMATICA PX-MFC	45.6 ^{ab}	42.2 ^{abc}	43.9 ^{a}	38.4 ^{cdefg}	37.7 ^{cdefg}	38.1 ^{bc}	41.0^{A}
J 200 GE 247 A	47.5 ^{a}	38.4 ^{cdefg}	42. 7 ^{a}	34.6 ^{defg}	32.5 ^{g}	33.5 ^{cd}	38.0^{AB}
Cyclotec 1093	41.0 ^{bcd}	39.5 ^{bcdef}	40.3 ^{ab}	34.1 ^{efg}	33.2 ^{fg}	33.6 ^{cd}	36.8^{B}
Pestle and Marter	40.3 ^{bcde}	39.5 ^{bcdef}	39.9 ^{ab}	33.9 ^{fg}	32.8 ^{g}	33.3 ^{d}	36.6^{B}
Mean washing	3.6 ^{a}	39.9 ^{b}		35.28 ^{c}	34.05 ^{b}		
Mean digestion	41.65 ^{a}			34.66^{b}			

LSD Digestion methods 2.25
Washing 3.18
Grinders 3.18
Digestion methods X Grinders X Washing 6.36

Fe contents (mg kg^{-1})

	Wet Digestion			Dry Ashing			Grinder
Grinders	Unwashed	Washed	Mean	Unwashed	Washed	Mean	Mean
KINEMATICA PX-MFC	117.74 ^{ab}	109.29 ^{b}	113.4 ^{a}	47.00 ^{e}	43.85 ^{e}	45.50 ^{e}	79.5^{A}
J 200 GE 247 A	119.56 ^{a}	120.37 ^{a}	119.7 ^{a}	42.55 ^{e}	41.90 ^{e}	41.92 ^{e}	80.8^{A}
Cyclotec 1093	91.35 ^{c}	91.26 ^{c}	91.3 ^{c}	67.00 ^{d}	47.95 ^{e}	57.42 ^{d}	74.4^{B}
Pestle and Marter	115.19 ^{ab}	85.66 ^{c}	100.0 ^{b}	72.15 ^{d}	48.95 ^{e}	60.48 ^{d}	80.2^{A}
Mean washing	110.71 ^{a}	101.50 ^{b}		57.18 ^{c}	45.58 ^{d}		
Mean digestion	106.10 ^{a}			51.33^{b}			

LSD Digestion methods 3.31

Washing 3.301
Grinders 4.67
Digestion methods X Grinders X Washing 9.33

K Contents (g 100 g^{-1})

	Wet Digestion			Dry Ashing			Grinder
Grinders	Unwashed	Washed	Mean	Unwashed	Washed	Mean	Mean
KINEMATICA PX-MFC	1.05 a	0.99 abcd	1.02 a	0.91 def	0.89 ef	0.90 d	0.96 AB
J 200 GE 247 A	0.95 bcde	0.91 def	0.93 bcd	0.94 b-f	0.86 f	0.90 cd	0.91 B
Cyclotec 1093	1.02 ab	0.93 b-f	0.97 ab	0.98 abcd	0.94 b-f	0.96 abc	0.97 A
Pestle and Marter	1.01 abc	0.87 ef	0.94 bcd	0.98 abcd	0.93 c-f	0.95 bcd	0.95 AB
Mean Wshing	1.01 a	0.92 bc		0.95 b	0.90 c		
Mean Digestion	0.966 a			0.928 b			

LSD Digestion methods 0.030
Washing 0.03
Grinders 0.043
Digestion methods X Grinders X Washing 0.087

Discussion

The samples preparatory operations like washing, grinding and digestion methods affect the quality of analysis. The results of study revealed that nutrient concentration in samples greatly affected by preparatory operations like washing caused a considerable reduction in K, Zn and Fe contents in samples as compared to those analyzed without washing. Decrease in K, Zn and Fe contents from wheat samples by washing was 9.8%, 12.6% and 24.8% as compared to samples processed without washing, respectively which indicated that increase in nutrient concentration in samples might have come through dust that affected the quality of analyte. Similarly, in lentil the K, Zn and Fe contents differed significantly in washed samples while in unwashed samples more K, Zn and Fe concentration i.e., 3%, 5.8% and 35% was recorded, respectively. By the washing of rapeseed samples, the respective concentration of K, Zn and Fe recorded was 7.6%, 6.6% and 14.1% (Tables 1, 2 and 3). It meant that there might be interference of nutrient through dust and during preparatory operations which could be reduced by washing and handling samples with care. So proper washing of samples can reduce this contamination and crucial in improving the quality of analysis. Because the dust that contaminate the samples to be analysed, was washed away by washing from surface as well as from the crevices of shriveled grains samples and contamination was lessened consequently affect the quality of analysis (Markert, 1995).

Mc Crimmon, 2008 compared washed and unwashed plant tissue samples of bentgrass washed with deionized water while the remainder of the sample was not washed and analyzed for macro and micronutrient composition.

He reported great difference between nutrients concentration between washed and unwashed samples. This indicates the possibility of contamination in the unwashed samples. Nutrient analyses of the washed plant tissue samples gave a more consistent and reliable status of the nutrient content. Frank, 2005 reported a reduction in Zn and Fe content by washing Golden Delicious apple leaf samples processed after washing compared to unwashed samples. Adding a 0.1M HCl for washing step further reduced leaf Zn concentration but had no additional effect on Fe concentrations. These findings indicate that washing is crucial for eliminating Fe contamination introduced by dust (Mc Crimmon, 2008).

Dry ashing of samples also caused considerable nutrient losses from the samples because K, Zn and Fe contents were relatively greater in the samples subjected to wet digestion. The difference in K, Zn and Fe contents of samples processed through dry ashing and wet digestion was 5.6, 15.2 and 78%, respectively. Results also indicated that samples subjected to dry ashing caused more nutrients losses as compared to wet digestion (Table 1, 2 & 3). The losses of Fe from lentil and rapeseed by dry ashing were more pronounced as compared to wheat.

The low K losses are because of its stability and tolerance to high temperature and of Zn are due to the presence of silica that helped in avoiding losses (Rashid and Fox, 1992; Banuelos *et al.*, 2008). The samples were subjected to high temperature directly in the furnace at 550°C while in wet digestion the samples were subjected to high temperature but were first treated with acid mixture and heated openly on hot plates at 350°C. The other reason might be that dry ashed samples were burnt in crucible and when they were completely converted into ash, a fraction of sample might have been lost during handling or remained adsorbed with walls of crucible that affected the nutrient contents and reduced their contents during dry ashing. Banuelos *et al.*, 2008 compared the effects of dry ashing and wet acid digestion of selected plant tissues for determination of boron (B) using the Azomethine H method.

They reported lowest recovery of B from samples treated with sulfuric acid in dry ashing. Boron concentrations in tissues oxidized in wet acid digestion were at least 40% higher than the results obtained for any dry ashing technique. Srivastava *et al.*, 2008 studied the effect of sample digestion methods on analytical value of macro and micro nutrient using different instruments and reported that both wet digestion and dry ashing procedures produced statistically similar analytical values for B, Ca, Fe, and Mo. However, the mean coefficients of variation were higher with the wet digestion procedure (6.19 to 9.64%) as compared to the dry ashing procedure (2.14 to 3.45%), results are in line with those reported by Akio and Lehmann, 2012.

The different grinders used for sample grinding affected the nutrient content to variable extent. The K concentration was least affected by different grinders used for grinding (Table 1, 2 & 3). The Zn and Fe contents in samples varied and minimum values was recorded in the samples pulverized with Cyclotec grinder which indicated that Cyclotec grinder caused less contamination of nutrients in the samples during the preparatory operation as compared to other grinders used for the study, similar results have been reported by Allan *et al.*, 1999.

So the contamination during the preparatory operations can be checked by choosing appropriate grinder to be used for grinding the samples. If some one is interested to determine the mineral composition of the commodities to be used for human nutrition, they should be careful about the selection of digestion methods and grinder to be used for sample preparation. Similarly, washing should be done for preventing the contamination from the environment. Excessive washing should be avoided because it causes leaching of the nutrients from the samples especially in case of leaf samples as K may be leached by excessive washing. Rosolem *et al.*, 2007 conducted an experiment on pearl millet (*Penisetum glaucum*) to evaluate K leaching from straw and reported a considerable K leaching right after plant desiccation and it was correlated with the residue nutrient content that can be as high as 64 kg ha^{-1}. For undertaking quality analysis, utmost care should be taken to assess the accurate mineral composition of the commodity being analyzed.

Analysis of Fe and Zn showed that amount of metal contamination from each mill was related to the abrasiveness of the plant material and the metal composition of the internal components of the mill. Least contamination was achieved using the Newport Scientific 6200 mill fitted with a stainless steel impeller and an abrasive steel strap with industrial diamonds set in pure nickel. The Newport mill ground samples in less time and reduced plant dry matter to finer particles, but impeller wear caused more variation in the distribution of particle size, than the Cyclotec mill.

Conclusions

The quality of analysis is based on proper washing, selection of digestion method and suitable grinder to minimize nutrient contamination from dust and other sources. Hence, proper washing with wet digestion method proves a reliable combination in order to obtain the more précised results. Cyclotec type of grinder can be helpful in reducing contamination of Zn, K and Fe during processing the samples.

The analyst must be extremely cautious while collecting, preparing and analyzing sample and have complete control over the reagent blank to ensure good quality control over all laboratory techniques and reducing all possible sources of contamination.

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