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Evaluation of bioaccessibility of some essential elements from wheatgrass (*Triticum aestivum* L.) by *in vitro* digestion method

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Abstract

Bioaccessibility of some essential elements namely K, Mn, Zn, Fe and Na from wheatgrass, consumed as dietary supplement, was measured by *in vitro* gastric and gastro-intestinal digestion methods. Neutron activation analysis was used to determine bioaccessible concentration of these elements. Bioaccessibility of these elements in commercial wheatgrass tablets and wheat grains was also determined. From both the methods, it was found that bioaccessibility of the elements studied was the highest from fresh wheatgrass and the lowest for wheat seeds. The range of values determined by gastric digestion for wheatgrass, wheatgrass tablets and wheat seeds were 37–57%, 17–43% and 9–38% respectively. Corresponding bioaccessibility values determined by gastro-intestinal digestion method were 39–60%, 34–55% and 15–23% respectively. These studies suggested that fresh wheatgrass grown in the laboratory is an effective source of minerals.

Keywords: Bioaccessibility; Wheatgrass; Gastric digestion; Gastro-intestinal digestion; Essential elements; Instrumental neutron activation analysis (INAA)

1. Introduction

Plant based food and food products are the main sources of nutrients such as carbohydrates, proteins, lipids and dietary fiber along with essential elements for human beings particularly for vegetarians. Within agricultural scientific community it is widely accepted that the total micronutrient content of the soil does not indicate the amount of micronutrient 'available' to the plant for growth and other necessary physiological actions. It is also true that all mineral content present in diet may not be available for absorption and/or utilization for normal health and physiological functions of humans. Bioavailability reflects in the efficiency with which the nutrients are absorbed in the body and may be available for further use. 'The proportion of total mineral content in the food utilized for normal body functions' (Fairweather-Tait, 1992) is bioavailability in a more general way. According to them bioavailability reflects in the efficiency with which the nutrients are absorbed from the alimentary tract and are thus available for storage and use.

Bioavailability of various elements is determined by either *in vivo* administration to similar species to humans, e.g., rats or *in vitro* methods by simulating digestive system conditions in the laboratory. In *in vivo* techniques, bioavailable amount of an element of interest is estimated as the difference in the concentration of the element in ingest and excreta, using radiotracers. Most of the *in vivo* studies were carried out on Fe and Zn (McCance & Widdowson, 1937; McCance & Widdowson, 1942). Basic disadvantage of the method is the exposure of ionizing radiations to the sensitive human groups such as pregnant women, infants and young children (Welch & House, 1984). In addition, *in vivo* studies are expensive and laborious with

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certain limitations (Danielsson, Zarpen, & Glynn, 1995). *In vitro* methods were developed which are rapid and inexpensive (Miller, Schricker, Ramussen, & Van Campen, 1981). *In vitro* procedures involve the simulation of the gastric and intestinal digestive conditions in the laboratory. As the experiments are carried out under 'simulated' digestive conditions, the results may not be as accurate as those obtained by *in vivo* studies. The results obtained by *in vitro* methods are based on the formation of digestive products that are soluble or dialyzable. The determined values thus correspond to bioaccessible fractions which represent maximum concentrations of elements soluble in gastro-intestinal media (Ruby et al., 1999). However, these methods are efficient to identify potential food products as nutrient supplements (Van Campen & Glahn, 1999). Both the *in vitro* methods are routinely used to estimate the bioaccesible concentrations of essential elements in the diet. It is shown that the bioaccesible values obtained by these methods can be well co-related with that of human subjects (Menson & Cook, 1979) and many animal models (Forbs et al., 1989). Recently, these methods are applied for bioaccessibility studies in fish (Cabanero, Madrid, & Camara, 2004), in the meals of school going children (Camara, Amaro, Barbera, & Clemente, 2005) and in the medicinal supplements (Lesniewicz, Jaworska, & Zyrnicki, 2006).

Analytical methods such as AAS (Herber & Stoeppler, 1994), ICP-AES (Zang, Zang, Zhou, & Wang, 1997), ICP-MS (Cao, Zhau, Yin, & Li, 1998), EDXRF (Salvador, Lopez, Nascimenta, & Zucchi, 2002) and instrumental neutron activation analysis (INAA) (Balaji et al., 2000a, 2000b; Kulkarni, Acharya, Nair, Rajurkar, & Reddy, 2006a; Rajurkar & Pardeshi, 1997) have been used for identification and estimation of elements. INAA is one of the most popular and widely used analytical techniques for simultaneous multielement determination of elements present at major, minor and trace levels in diverse matrices. In the present studies, INAA was used to determine the concentration of the elements. The technique involves irradiation of the samples with neutrons in a nuclear reactor, leading to the formation of radionuclide whose radioactivity is measured preferably using high-resolution gamma ray spectrometer. It has many advantageous characteristics like high analytical sensitivity, good detection limit (mg/kg to μg/kg) and negligible matrix effect. In our laboratory the INAA methodologies have been routinely used to analyze various biological samples like medicinal leaves, cereals including wheat, legumes, foodstuffs and reference materials for multielement analysis (Acharya, Nair, Reddy, & Manohar, 2002; Balaji et al., 2000a, 2000b; Rajurkar & Pardeshi, 1997).

In Asia and Europe, tender wheatgrass and its juice are consumed for healthy growth of human body although scarce scientific literature is available. We have initiated a systematic study on the fresh wheatgrass. In our previous studies, we have reported that wheatgrass has good antioxidant activity (Kulkarni et al., 2006b). The observed antioxidant activity was attributed to the presence of some biologically active phenolic and flavonoid moiety containing compounds. We have also determined the essential and trace elemental content in the shoots and roots of wheatgrass as a function of growth period ranging from 5 to 20 days under different growth conditions like using (i) only tap water, (ii) tap water with nutrients and (iii) soil with tap water (Kulkarni et al., 2006a). We have observed that, in all the growth conditions wheatgrass reaches a maximum antioxidant potential and optimum elemental content during the period of 8–10 days of its growth. In view of this, it was thought of interest to study the bioaccessibility of essential elements in the shoots of wheatgrass of 8–10 days old. In the present study we have determined the bioaccessibility of elements in the shoots of fresh wheatgrass by subjecting it to *in vitro* gastric and gastro-intestinal incubation. Elemental content in the digest was estimated by INAA. Additionally, for comparison, a set of commercially available wheatgrass tablets and wheat grains (*Triticum aestivum* L.), which were used to grow wheatgrass, were analyzed in a similar way.

2. Materials and methods

2.1. Sample collection

The wheat seeds of crop variety Pbn-51 were procured from Marathwada Agricultural University, Parbhani, Maharashtra State of India. The required amount of seeds (about 25 g) was washed with tap water followed by distilled water. Seeds were soaked for a day and were spread in (i) the perforated plates daily fed by only tap water or (ii) the pots filled with soil (black cotton soil) and daily fed by tap water for plant growth. These two conditions were named as conditions 1 and 2 respectively. The details of the procedure used for plant growth were given elsewhere (Kulkarni et al., 2006a).

The commercially available wheatgrass tablets, used in our studies, were purchased from a local shop in Mumbai. The growth condition of the corresponding wheatgrass is unknown. The average weight of the tablet was 0.5 g and the recommended (by the producer) intake was three tablets per day. These tablets constitute 98% of wheatgrass, 1.5% silica and 0.5% vegetable stearates, as per the specifications.

2.2. Materials

Digestive enzymes, pepsin and pancreatin were procured from SRL Chemicals; Mumbai and bile salts mixture was purchased from SD Fine Chemicals, Mumbai. Other chemicals used in the experiments were of analytical grade NH_4HCO_3 and HCl. All the chemicals were used without further purification. Freshly prepared solutions of digestive enzymes were used in the experiments. All the solutions were prepared in doubly distilled water. Pepsin (1:3000) solution was obtained by dissolving 6 g of pepsin in 0.03 M HCl (pH = 1.75). Pancreatin solution was prepared by dissolving 0.5 g of pancreatin in 100 mL distilled water

containing 0.25 g bile salt mixture. Saturated solution of NH_4HCO_3 was prepared in doubly distilled water to adjust the pH to 7.

2.3. Gastric digestion

Stepwise procedure used for the *in vitro* gastric digestion (Cabanero et al., 2004) is shown in Scheme 1. Accurately weighed amounts (10–12 g) of wheat seeds were transferred to a beaker containing 100 mL of gastric juice solution (6% w/v pepsin 100 mL HCl of pH = 1.75). Initially, the mixture was shaken vigorously for 1–2 min. The beaker was sealed tightly with a parafilm and was placed in a water bath on a magnetic stirrer cum heater. Temperature of the water bath was adjusted such that the temperature of reaction mixture placed in water bath was 37 °C. The reaction mixture was stirred continuously at a low speed for 3 h. This digest was then cold centrifuged (Jouan MR-14.11) at 4 °C for 20 min at 5000 rpm. The supernatant was filtered through 0.45 μm Millipore membrane filter. This solution was labeled and stored in an airtight container at 0–4 °C for further analysis. In the case of fresh wheatgrass, the samples were cut into small pieces (2–3 mm) and then submitted to the gastric digestion. In the case of commercial wheatgrass tablet, it was crushed to powder with the help of pestle and mortar and submitted to gastric digestion.

2.4. Pancreatic digestion of the samples

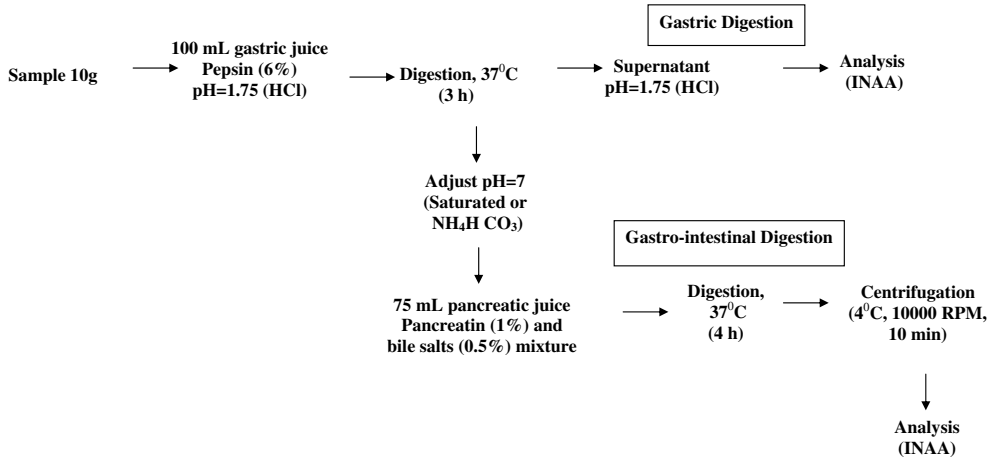
For pancreatic digestion (Scheme 1), the pH of the solution obtained after gastric digestion was adjusted to neutral pH by drop wise addition of saturated solution of NH_4HCO_3. To this mixture, 75 mL of pancreatic digestion solution (mixture of 2% w/v pancreatin and 0.2% w/v bile salts) was added. This mixture was shaken vigorously for 1 min. for degassing. The beaker was placed in a water bath at 37 °C and the reaction mixture was stirred continuously at slow speed for 4 h. This digest was then cold centrifuged at 4 °C for 20 min at 5000 rpm. The supernatant was then filtered through 0.45 μm Millipore membrane filter, was labeled and stored in airtight container at 0–4 °C for further analysis.

2.5. Sample preparation and neutron irradiation

Supernatants of both the digests were clear solutions and when stored beyond 3 days they became turbid. However, sample preparation for INAA was completed within 2 days. For elemental analysis by INAA, 500 μL of either gastric or gastro-intestinal digest was carefully transferred onto a Whatmann filter paper (no. 41) with the help of micropipette of 500 μL capacity and dried. This was repeated 10 times so as to transfer a total of 5 mL. Sample preparation for determination of total elemental concentration of wheatgrass, wheat grain and commercial wheatgrass tablet were described elsewhere (Kulkarni et al., 2006a). The samples, along with the two reference materials ICHTJ-CTA-otl-1 (Oriental tobacco leaves) and NIST SRM-1515 (Apple leaves) were packed in separate polyethene pouches. Elemental standards were prepared using stoichiometric compounds/metal foils. Samples, elemental standards and the reference materials were resealed and co-irradiated in E8 position of APSARA reactor, Bhabha Atomic Research Centre, Trombay, Mumbai, India. The thermal neutron flux at E8 position is in the order of 5×10^{11} cm^{-2} s^{-1} respectively.

2.6. Radioactivity measurements and concentration calculations

After appropriate cooling, the irradiated pouches were unsealed and each irradiated sample was counted using a 40% relative efficiency HPGe detector coupled to a PC based multichannel analyser (8 k MCA) in a fixed sample-to-detector geometry. The detector system had a resolution



Scheme 1. Schematic representation of the gastric and gastro-intestinal digestion of the food samples.

(FWHM) of 1.9 keV at 1332 keV of ^{60}Co. The samples were counted for suitable periods of time in order to obtain good counting statistics. The peak areas under the characteristic gamma rays were determined by using a peak-fit software PHAST developed at BARC (Mukopadhyay, 2001). The peak areas were used for the concentration calculation by the relative method of INAA. Using mass of the element in the standard ($m_{x,\mathrm{std}}$) and count rates (counts per second, cps) of standard ($\mathrm{cps}_{x,\mathrm{std}}$) and sample ($\mathrm{cps}_{x,\mathrm{samp}}$), mass of the element present in the sample ($m_{x,\ \mathrm{samp}}$) was determined by the following equation:

$$m_{x,\mathrm{samp}} = m_{x,\mathrm{std}} \times \frac{\mathrm{cps}_{x,\mathrm{samp}}}{\mathrm{cps}_{x,\mathrm{std}}} \times \frac{D_{\mathrm{std}}}{D_{\mathrm{samp}}}$$

where, D is the decay factor ($\exp(-\lambda t_{\mathrm{d}})$), λ is the decay constant of the radioisotope produced in neutron activation and t_{d} is the decay time. The $m_{x,\mathrm{samp}}$ (μg) was converted to concentration (μg g^{-1}) by dividing with sample mass (g).

From concentration of elements in gastric and pancreatic digests, the % of bioaccessibility (% B) of the element from each food item was calculated by using following the formula:

$$\%\mathrm{B} = \frac{[\mathrm{GD}]\ \text{or}\ [\mathrm{PD}]}{[\mathrm{T}]} \times 100$$

where [GD] = Concentration of a element in gastric digest, [PD] = Concentration of a element in pancreatic digest and [T] = Total elemental content in the food product.

3. Results and discussion

Total elemental concentrations of K, Mn, Zn, Fe and Na in the wheat grains (seeds), wheatgrass grown in conditions 1 and 2, and commercially available wheatgrass tablets were determined by INAA and are given in Table 1. The wheat seed samples and wheatgrass were analyzed in the present studies while data for commercial tablets were taken from our previous work (Kulkarni et al., 2006a). The total concentrations were arrived from three independent experiments. The quoted uncertainties are standard deviations estimated from the triplicate experiments. The elemental concentration values of K, Mn, Zn, Fe and Na in CTA-otl-1 and NIST SRM-1515 are given in Table 2. Good agreement between determined and certified values of concentrations of elements of interest indicated accuracy of the method.

The bioaccessible concentrations and the % of bioaccessibilities of the elements estimated by the gastric digestion method are given in Table 3. In the case of wheat seeds and commercial wheatgrass tablets, the concentrations are expressed as μg g^{-1} and are the mean values arrived from the triplicate experiments. The uncertainties are the standard deviations. In the case of fresh wheatgrass, the concentrations are expressed as μg 100 g^{-1} since 100 g of fresh wheatgrass is the daily consumption in the form of juice for optimum health benefits.

The concentrations and the % of bioaccessibilities of the elements obtained by gastro-intestinal digestion method for

Table 1
Total Elemental concentration (μg g^{-1} unless mg g^{-1} or mg 100 g^{-1} is specified) of wheat seeds, wheatgrass grown under two different conditions and commercial wheatgrass tablets

Samples		Elements				
		K	Mn	Zn	Fe	Na
Wheat grain		3.1 ± 0.03[a]	37.1 ± 0.4	9.2 ± 0.3	143.2 ± 2.6	$(14.7 \pm 4) \times 10^{-3}$[a]
Condition 1	8th day	77.18 ± 4.2[b]	82 ± 6	20 ± 2	142 ± 17	5.3 ± 0.2[b]
	10th day	115.2 ± 2.2[b]	114 ± 10	30 ± 1.6	171 ± 18	7.1 ± 0.3[b]
Condition 2	8th day	150.4 ± 2.8[b]	156.4 ± 14	127 ± 20	632 ± 80	6.7 ± 0.5[b]
	10th day	182.7 ± 2.7[b]	218 ± 18	138 ± 7	1332 ± 64	11.1 ± 0.3[b]
Commercial wheatgrass tablets[c]		50.6 ± 0.5[a]	85.0 ± 6.3	80.1 ± 3.0	1895 ± 77	0.771 ± 0.01[a]

[a] g kg^{-1}.
[b] mg/100 g of fresh wheatgrass.
[c] From Kulkarni et al. (2006a).

Table 2
Comparison of determined and certified concentration values (mg kg^{-1}) of biological reference materials ICHTJ-CTA-otl-1 (oriental tobacco leaves) and NIST SRM 1515 (apple leaves)

Element	ICHTJ-CTA-otl-1		NIST SRM-1515	
	Present work	Certified value (info)	Present work	Certified value (info)
K[a]	153 ± 3	156 ± 5	15.94 ± 0.56	16.10 ± 0.16
Mn	391 ± 22	412 ± 13	59.2 ± 4.3	54 ± 4
Zn	52.9 ± 4.2	49.9 ± 2.5	12.6 ± 0.2	12.5 ± 0.3
Fe	1023 ± 58	(989)	91.6 ± 5.9	(83)
Na	412 ± 32	(345)	25.1 ± 3.2	24.4 ± 1.2

(info) – information value.
[a] g kg^{-1}.

Table 3
Bioaccessible concentrations (μg g^{-1} unless mg g^{-1} or mg 100 g^{-1} is specified) obtained by gastric digestion method for wheat seeds, wheatgrass grown under two different conditions and commercial wheatgrass tablets

Samples		Elements				
		K	Mn	Zn	Fe	Na
Wheat grain		1.14 ± 0.14[a] (37.6)	8.57 ± 0.68 (23.1)	2.13 ± 0.15 (23.2)	12.6 ± 1.3 (8.8)	$(3.7 \pm 0.6) \times 10^{-3}$[a] (25.7)
Condition 1	8th day	27.7 ± 1.2[b] (35.9)	38.8 ± 3.2 (43.3)	11.6 ± 1.6 (59)	66.2 ± 5.1 (45.9)	1.78 ± 0.12[b] (33.5)
	10th day	41.1 ± 2.4[b] (45.1)	47.2 ± 5.1 (41.6)	16.8 ± 2.4 (56.6)	79.2 ± 7.1 (46.5)	2.61 ± 0.25[b] (36.9)
Condition 2	8th day	79.6 ± 2.6[b] (52.9)	78.1 ± 5.9 (50)	55.2 ± 1.6 (43.5)	643 ± 48 (45.7)	1.83 ± 0.20[b] (27.1)
	10th day	105.1 ± 3.3[b] (57.6)	107.2 ± 7.2 (48.7)	76.8 ± 2.4 (55.9)	780 ± 72 (56.8)	2.98 ± 0.17 (27)
Commercial wheatgrass tablets[b]		23.1 ± 1.1[a] (45.7)	23.57 ± 3.41 (27.7)	34.38 ± 2.84 (42.9)	594 ± 16 (31.1)	0.134 ± 0.012[a] (17.4)

Values in the parenthesis are % of bioaccessibility values.
[a] mg/g.
[b] mg 100^{-1} g of fresh wheatgrass.

Table 4
Bioaccessible concentrations (μg g^{-1} unless mg g^{-1} or mg 100 g^{-1} is specified) obtained by gastro-intestinal digestion method for wheat seeds, wheatgrass grown under two different conditions and commercial wheatgrass tablets

Samples		Elements				
		K	Mn	Zn	Fe	Na
Wheat grain		1.28 ± 97[a] (42.4)	13.26 ± 1.0 (37.7)	3.98 ± 0.46 (43.4)	21.8 ± 1.9 (15.2)	$(4.9 \pm 0.4) \times 10^{-3}$[a] (33.9)
Condition 1	8th day	35.2 ± 2.1[b] (45.6)	32.2 ± 3.4 (39.3)	13.6 ± 1.6 (65)	82.4 ± 7.6 (58.1)	2.06 ± 0.18[b] (38.7)
	10th day	50.3 ± 2.5[b] (55.2)	68.4 ± 3.6 (59.8)	18.9 ± 0.2 (60.7)	58.6 ± 14.4 (50.4)	2.54 ± 0.27[b] (37.2)
Condition 2	8th day	94.4 ± 0.9[b] (62.7)	82.1 ± 4.4 (52.6)	59.6 ± 5.7 (46.8)	772 ± 56 (53.1)	1.18 ± 0.37[b] (32.3)
	10th day	124.6 ± 6.8[b] (68.2)	114.4 ± 8.4 (58.7)	62.8 ± 3.2 (51.5)	8480 ± 7.6 (61.7)	3.82 ± 0.37[b] (34.7)
Commercial wheatgrass tablets[b]		27.6 ± 0.9[a] (54.5)	41.3 ± 2.9 (48.6)	38.48 ± 1.98 (48.1)	640 ± 29 (33.8)	0.260 ± 0.002[a] (33.7)

Values in the parenthesis are % of bioaccessibility values.
[a] mg/g.
[b] mg 100^{-1} g of fresh wheatgrass.

all the samples are shown in the Table 4. In the case of laboratory grown wheatgrass, the total concentrations were expressed on fresh wheatgrass weight basis. It should be noted that while determining the total elemental concentrations, dry powders of wheatgrass were neutron irradiated. On an average, 5 g of fresh wheatgrass yielded one gram of dry wheatgrass sample. Accordingly, measured concentrations (μg g^{-1}) were divided by 5 to obtain the concentration of fresh wheatgrass and then expressed as μg 100 g^{-1} of fresh weight. It can be seen that, bioaccessible concentration obtained by the *in vitro* digestions varied with the total concentration of that element in the sample. In all the cases, concentration of an element in the gastric and gastro-intestinal digest linearly depended upon the total concentration of that element in the original sample. However, the % of bioaccessibility of elements did not show linearity with the concentration of the corresponding element in the wheatgrass. It appears that the values depend on factors such as plant growth period and growth condition which might have influenced the bioaccessibility. A brief discussion on each of the element studied and general discussion are given in the following.

3.1. Potassium

Potassium is present in every food material but bioaccessibility of potassium is rarely studied. It was observed that the bioaccessible concentration of K in all the samples is the highest among all the elements studied. The bioaccessible concentrations of K by gastric and gastro-intestinal digestion were found to be 1.14 and 1.28 g kg^{-1} in wheat grain and 23.1 and 27.6 mg g^{-1} in commercial tablet respectively. In the case of fresh wheatgrass, maximum bioaccessible concentration was observed in gastro-intestinal digest of condition 2 (124.6 mg 100 g^{-1}) on 10th day. The concentration of K in wheatgrass grown in condition 2 is almost thrice that of grown in condition 1, which could be attributed to higher uptake of K during the growth of the plant.

3.2. Manganese

Bioaccessible concentrations of manganese by gastric digestion method were found to be 13.3 and 41.3 μg g^{-1} for wheat seeds and commercial wheatgrass tablets respectively corresponding to 37.7% and 48.6% bioaccessibility. The highest bioaccessible concentration by this method was found to be 114.4 μg g^{-1} corresponding to fresh wheatgrass grown in the condition 2 on 10th day of growth. Bioaccessibility of Mn from fresh wheatgrass varied over a small range of 52–69%. This indicated that Mn bioaccessibility is not greatly influenced by growth conditions and growth period of wheatgrass. Better %bioaccessibility of Mn from the fresh wheatgrass indicate potential of wheatgrass as a dietary source of Mn.

3.3. Zinc

Zinc is one of the most widely studied elements in terms of its total elemental content in medicinal and food samples (Balaji et al., 2000a, 2000b; Rajurkar, Shah, & Purushottam, 1990) and bioaccessibility from the plant based food products (House, 1999; Hunt, 2003). Total and bioaccessible Zn concentration varied over a wide range in the studied food samples. This might be due to the composition of the food material under consideration and such as quantity and quality of the proteins, chemical form of the element, nutrient interactions including element–element and element–organic content (House, 1999; Hunt, 2003), presence of compounds such as ascorbic acid, citric acid that facilitate the bioaccessibility and compounds such as oxalates, fibers, polyphenols and phytates that inhibit the bioaccessibility (Harland & Oberleas, 1987; Kies, Young, & Mcendree, 1983). In our studies, bioaccessible concentrations in the gastric and gastro-intestinal digestion of wheat seeds were found to be 2.13 and 3.98 μg g^{-1} respectively corresponding to 23% and 43% bioaccessibility from the total concentration. In the case of commercially available wheatgrass tablets the bioaccessible concentrations of Zn were found to be 34.4 and 38.5 μg g^{-1} with % of bioaccessibilities of 42% and 48% respectively from gastric and gastro-intestinal digests.

Among the essential trace elements studied, the highest % of bioaccessibility was observed for Zn in both the *in vitro* digestion methods for wheatgrass grown in condition 1. In the case of gastric digestion % of bioaccessibility was found to be 59 and 56.6 respectively on 8th and 10th day of growth and 65% and 60.7% on 8th and 10th day respectively in the case of gastro-intestinal digestion. Percent bioaccessibilities for Zn on 10th day are lower as compared to on 8th day during both digestions. With the growth period, Zn may be getting attached to the fibrous plant tissue, which could be inhibiting its bioaccessibility. It is well known that, fiber content of the plant increases rapidly with the growth period during germination.

Recently, Lesniewicz et al. (2006) applied water leaching, HCl extractability and pepsin digestion methods to estimate the bioaccessibility of many elements from many Polish medicinal tablets. They found that % of bioaccessibility of Zn varies in the range of 50–70% by HCl and pepsin leaching method. Our values are also in the same range. However bioaccessibility from meals provided to school going children obtained (Camara et al., 2005) by solubility and digestion methods found to be higher than our values. This may be due to the fact that the meal contained a mixture of different legumes, cereals, vegetables and meat that can act as 'enhancers of bioaccessibility'. In the present studies, we have found that in some of the samples Zn is moderately (25–35%) to highly (>50%) bioaaccessible.

3.4. Iron

Iron is one of the most studied elements for its bioavailability by *in vivo* and *in vitro* methods (House, 1999; Hunt, 2003; Mamatha, Gupta, Lakshmi, & Prakash, 2004; Tuntawiroon, Sritongkul, Pleehachinda, & Suwanik, 1998). Iron was found to be less bioaccessible from wheat seeds (the % of bioaccessibilities are 8.8 and 15.2 by gastric and gastro-intestinal digestion methods respectively) whereas it was moderately available from commercial wheatgrass tablet (32% and 34%). It was found that Fe is present in the highly bioaccessible from the fresh wheatgrass (45–65%) although total concentrations of Fe in fresh wheatgrass grown on the condition 1 (171 μg 100 g^{-1}) and condition 2 (1895 μg 100 g^{-1}) are widely different. In the case of green leafy vegetables, the presence of the compounds such as ascorbic acid and citric acid, known enhancers for Fe bioaccessibility, facilitate bioaccessibility of Fe (Mamatha et al., 2004). Same may be true for fresh wheatgrass.

On the other hand, the lowest bioaccessibility of Fe in wheat seeds might be explained on the basis of its phytate content. Phytates in the grains form complex with divalent atoms such as Fe and Ca, and may co-precipitate as Fe-phytate complex under intestinal digestive conditions (House, 1999).

3.5. Sodium

It can be seen from Table 3 that during the gastric digestion, % of bioaccessible of sodium is <30% for all the samples except for fresh wheatgrass grown in the condition 1. As per WHO (1996), Na is less bioaccessible for most of the plant based food products. In the present studies, it was found that bioaccessibility of Na was found to increase when it was further digested with pancreatic juice (35–40%). Lower bioaccessibility of Na could be due to the competition by other monovalent competing ions such as K.

For most of the elements, we observed that % of bioaccessibilities from laboratory grown fresh wheatgrass are higher as compared to the commercially available wheatgrass tablet. Generally for all the elements studied, it was observed that % of bioaccessibilities obtained by gastro-intestinal digestion were more as compared to gastric digestion in all the samples investigated. It might be due to the fact that food materials further undergo pancreatic digestion in the gastro-intestinal tract at neutral pH resulting in higher bioaccessibilities. Pancreatic digestion is followed by gastric digestion (Scheme 1) during which, an extra digestive enzyme, Pancreatin, is added to the reaction mixture before final incubation. Pancreatin is a mixture of many enzymes including amylase, trypsin, lipase, ribonuclease and protease. These enzymes are capable of breaking starch to carbohydrates, lipids and proteins to simple molecules. During this process, the minerals that are bound to starch, lipids or proteins are set free and hence converted to bioavailable form during this digestive incubation.

We have observed that if the wheatgrass is grown in the soil, the concentration of certain elements such as Fe, Mn and Zn are increased in the wheatgrass. Thus it is feasible to increase the bioaccessibility of essential elements

through controlled growth conditions. Depending on the requirements, one may choose a growth condition to obtain optimum benefits.

4. Conclusion

Instrumental neutron activation analysis was applied to determine the bioaccessibility of some essential elements from wheat seeds, wheatgrass and commercial wheatgrass tablet using *in vitro* gastric and gastro-intestinal digestion methods. Comparatively, bioaccessibilities during gastro-intestinal digestions were higher than gastric digestion indicating higher absorption of minerals in gastro-intestinal tract at neutral pH. It was found that bioaccessibilities of Mn, Zn and Fe were higher from wheatgrass as compared to wheat grain during both the digestions. This indicates that mineral from fresh wheatgrass are easily bioaccessible when grown over a period of 8–10 days even in the simple growth conditions such as tap water and ordinary soil without addition of fertilizers.

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