Assessment of inhibitory factors on bioaccessibility of iron and zinc in pearl millet (*Pennisetum glaucum* (L.) R. Br.) cultivars

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Abstract The variations in iron and zinc bioaccessibility as influenced by inhibitory factors in 13 pearl millet cultivars were evaluated. The results indicated that iron and zinc contents ranged between 5.59–13.41 and 2.11–5.19 mg/100 g. Polyphenols, flavonoids and phytic acid were highest in GHB744 (781 mg/100 g), HHB223 (116 mg/100 g) and HHB226 (1.080 g/100 g) respectively. Insoluble fiber content range from 9.36 to 12.89 g/100 g. Iron and zinc bioaccessibility was the highest in local Anantapur (17.95%) and GHB744 (15.19%) cultivar with low phytic acid. HHB226 exhibited high β -carotene and phytase activity. In this study, the cultivars with high iron and zinc content also possessed high inhibitory factors which affected bioaccessibility. However, the bioaccessibility of iron did not seem to depend on the phytic acid: iron ratio alone. Further, a trend was observed in cultivars with low iron: zinc ratio had increased iron bioaccessibility. On the contrary, phytic acid: zinc ratio appears to play a significant role in zinc bioaccessibility. Certain cultivars with high iron content also had high phytase activity and β -carotene content which could be exploited for further technological treatments to enhance the bioaccessibility of iron and zinc.

Keywords Pearl millet cultivars · Bioaccessibility · Inhibitory factors · Iron · Zinc · β -carotene

Introduction

Millets, are warm-season small cereals grains valued for their food, feed and fodder in various parts of the world and is an indispensable part of human life for ages. These crops also serve as cover crop and also used in crop rotation. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the most widely grown millets followed by foxtail millet, and finger millet (Kaur et al. 2014). Pearl millet is well adapted to growing areas characterized by drought, high temperature, low soil fertility, and high soil salinity. Pearl millet is a major staple crop in sub-Saharan regions and India. In certain Indian regions, it accounts for 50% of the total grain consumption and is a primary source of vitamins, minerals, and energy (Rao et al. 2006). The food value of pearl millet is better than most other cereals as it has high levels of iron, zinc, lipids and quality proteins (Rai et al. 2013; Rao et al. 2006). Nutritionally pearl millet is at least equivalent to maize and superior to sorghum in protein content and metabolizable energy levels (Agtei et al. 1999). Consumption of pearl millet is in the form of porridge; steamed products like couscous or as roti (flat pancakes) which utilises the whole grain flour for their preparation.

Malnutrition due to deficiency of micronutrients especially iron and zinc is a severe problem in the developing countries. To tackle this issue plant cultivars are being biofortified to increase the mineral densities. Pearl millet is one of the important crops that are being targeted for biofortification. Biofortified pearl millet has been shown to improve the iron status in the Beninese woman (Cerca-mondi et al. 2013). However, pearl millet cultivars are known to vary widely in the shape, size color, etc. Hence it can be assumed that the pearl millet cultivars may have variation in the inhibitory factors and the effect on bioaccessibility may vary. Moreover, it has been demonstrated

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that an increase in iron concentration in pearl millet might not necessarily increase iron absorption since genotypes with high iron levels may also have increased concentrations of iron absorption inhibitors (Tako et al. 2015). Therefore, it is necessary to increase the amount of bioavailable iron and zinc rather than increasing the amount of minerals. Apart from phytic acid, polyphenols and fiber are known to influence bioaccessibility of iron and zinc (Camara and Amaro 2003). The impact of fiber on mineral availability has been explained through cation exchange mechanism, which explains how fiber matrix binds and holds ions (Gupta and Premavalli 2011). It was also shown that the cation exchange capacity increases with a decrease in acidity. Although phytic acid has been reported to be the major inhibitory factor, insoluble fiber also forms fiber-phytate-mineral complexes (Lestienne et al. 2005). Polyphenols also form insoluble complexes with iron and cause inhibition of iron absorption (Brune et al. 1991). The high concentration of the phenolic compound in the food-to-food fortified meal was reported to reduce the bioavailability of iron (Cercamondi et al. 2014). Studies also pointed out that certain grain components like vitamin A and β -carotene acted as enhancers by binding to iron, keeping it in the soluble form in the intestinal lumen, thus preventing the phytic acid and polyphenols inhibiting iron absorption (Garcia-Casal et al. 1998). Most studies focus on mineral and phytic acid contents in pearl millet cultivars (Abadalla et al. 1998; Abdelrahman et al. 2005). The variations in phytic acid, polyphenols, oxalic acid, iron and zinc bioaccessibility in two pearl millet cultivars were reported (Pushparaj and Urooj 2014). Studies concerning the effect of enhancers and inhibitory factors on iron and zinc bioaccessibility in pearl millet cultivars differing in mineral content are limited.

In the present study, pearl millet cultivars, including hybrids and open pollinated cultivars with varying iron and zinc contents, were evaluated for variation in the content of various inhibitory factors and their relationship with bioaccessibility of iron and zinc.

Materials and methods

Pearl millet cultivars

Thirteen pearl millet (*P. glaucum* (L.) R. Br.) cultivars (Table 1) procured from different locations: University of Agricultural Science, Dharwad (Karnataka, India)—ICTP8203, ICMV221 (open pollinated cultivars), GHB558 (hybrid); All India Coordinated Pearl Millet Improvement Project, Jodhpur (Rajasthan, India)—ICTP8203J, ICMV221J, JBV2 (open pollinated cultivars), GHB744, RHB173, HHB67 improved, HHB223, HHB226 (hybrids);

Mysore local market (Karnataka, India)—Mysore local cultivar and Anantapur local market (Andhra Pradesh, India)—Anantapur local cultivar were used for the study.

Chemicals

Pepsin, pancreatin, amyloglucosidase, ferulic acid, catechin, β -carotene and dialysis membrane (MW cut-off 8–12 kDa, Sigma Chemical Co., St. Louis, MO, USA), phytic acid/total phosphorus kit (K-PHYT, Megazyme International Ireland Ltd, Bray, Ireland) and reference standard mineral solutions for atomic absorption spectrometry (AAS) (Merck Specialities Pvt. Ltd, Mumbai, India) were procured. All other chemicals used were of analytical grade.

Preparation of sample

Grains of different cultivars of pearl millet were cleaned to separate out damaged grains and foreign materials. The cleaned grains were milled to a fine powder (250 μ m mesh), and powdered samples were stored in sealed bottles at 5 °C in a refrigerator for further analysis.

Iron and zinc content

The ash was dissolved in concentrated hydrochloric acid, and the mineral contents (iron and zinc) were determined according to the AOAC method (No. 965.09) by atomic absorption spectrometry (Shimadzu AAF-6701, Japan).

Determination of inhibitory factors

The total dietary fiber content was determined by enzymatic–gravimetric method (Asp et al. 1983). The total polyphenolic content was determined by Folin-Ciocalteu colorimetric method (Singleton et al. 1995) where ferulic acid was used as the standard. Flavonoids were determined by Zhishen et al. (1999) with catechin as the standard. The phytic acid content was evaluated using the Megazyme kit (K-PHYT, Megazyme, Bray, Ireland).

Phytase activity assay

The sample (0.1 g) was extracted in 5 mL sodium acetate buffer (0.1 mol/L, pH 5) at 4 °C for one hour and centrifuged at 4000 rpm for 10 min. 350 μ L of 0.1 mol/L sodium acetate buffer and 100 μ L of 2 mol/L sodium phytate were mixed and incubated at 40 °C for 10 min. To this assay solution, 100 μ L of the extracted supernatant was added and incubated for 30 min at 40 °C. After incubation, 1.5 mL of color reagent was added, and the absorbance was measured at 355 nm (Azeke et al. 2011).

Table 1 Polyphenols, phytic acid, flavonoids, and dietary fiber content in pearl millet cultivars

Cultivars	Polyphenols (mg/100 g)	Phytic acid (g/100 g)	Flavonoids (mg/100 g)	Soluble dietary fiber (g/100 g)	Insoluble dietary fiber (g/100 g)	Phytase activity (μmol/g)	β-carotene (μg/g)
ICMV221	624.05 ± 7.54 ^b	0.682 ± 0.02 ^l	55.38 ± 1.49 ^g	0.99 ± 0.092 ^h	10.25 ± 0.57 ^{bc}	0.053 ± 0.007 ^h	1.65 ± 0.02 ^e
ICMV221J	595.72 ± 5.61 ^c	0.957 ± 0.02 ^d	97.86 ± 2.42 ^c	1.21 ± 0.049 ^f	10.88 ± 0.63 ^b	0.235 ± 0.048 ^d	2.04 ± 0.03 ^d
ICTP8203	521.95 ± 6.14 ^d	0.827 ± 0.01 ^h	54.49 ± 1.51 ^g	1.20 ± 0.056 ^f	10.21 ± 0.70 ^{bc}	0.111 ± 0.015 ^g	1.60 ± 0.01 ^e
ICTP8203J	628.32 ± 6.80 ^b	0.889 ± 0.01 ^f	99.32 ± 2.11 ^c	1.53 ± 0.092 ^c	11.70 ± 0.71 ^b	0.174 ± 0.022 ^e	2.21 ± 0.01 ^c
JBV2	591.97 ± 9.98 ^c	0.880 ± 0.02 ^g	97.92 ± 1.19 ^c	0.89 ± 0.092 ^h	11.71 ± 0.69 ^b	0.145 ± 0.019 ^f	2.83 ± 0.04 ^c
Mysore local	691.56 ± 7.54 ^{ab}	0.681 ± 0.01 ^l	104.18 ± 2.39 ^b	1.09 ± 0.042 ^g	9.82 ± 0.61 ^{bc}	0.229 ± 0.019 ^d	2.31 ± 0.03 ^c
Anantapur local	630.51 ± 8.65 ^b	0.729 ± 0.04 ^k	101.97 ± 1.35 ^b	1.25 ± 0.071 ^f	9.36 ± 0.42 ^{bc}	0.311 ± 0.007 ^c	2.03 ± 0.01 ^d
GHB558	655.52 ± 7.87 ^b	0.785 ± 0.05 ⁱ	78.68 ± 2.29 ^c	1.11 ± 0.021 ^g	9.85 ± 0.93 ^{bc}	0.477 ± 0.011 ^b	2.20 ± 0.01 ^d
GHB744	781.14 ± 8.03 ^a	0.735 ± 0.02 ^j	87.29 ± 1.24 ^d	1.68 ± 0.064 ^b	10.95 ± 0.87 ^b	0.214 ± 0.034 ^d	2.78 ± 0.01 ^c
RHB173	637.22 ± 8.99 ^b	0.958 ± 0.03 ^d	105.93 ± 2.17 ^b	1.86 ± 0.057 ^a	10.24 ± 0.51 ^{bc}	0.148 ± 0.007 ^f	3.71 ± 0.08 ^b
HHB67 improved	605.26 ± 6.78 ^{bc}	1.007 ± 0.04 ^c	58.55 ± 2.36 ^g	1.39 ± 0.035 ^e	11.69 ± 0.54 ^b	0.185 ± 0.029 ^e	1.63 ± 0.04 ^e
HHB223	772.02 ± 6.99 ^a	1.051 ± 0.07 ^b	116.51 ± 1.67 ^a	1.49 ± 0.057 ^d	10.37 ± 0.57 ^{bc}	0.572 ± 0.024 ^a	3.73 ± 0.06 ^b
HHB226	670.49 ± 7.31 ^b	1.080 ± 0.03 ^a	89.37 ± 1.28 ^d	1.37 ± 0.064 ^c	12.89 ± 0.94 ^a	0.582 ± 0.014 ^a	6.97 ± 0.12 ^a

Values are mean ± SD of three independent determinations (dry weight basis)

Mean values with different superscripts in a particular column vary significantly ($p < 0.05$)

β-carotene

The β-carotene was determined using the modified method of Santra et al. (2003). The sample (2 g) was added to a mixture of n-butanol and water (8:2, v/v) and mixed by vortexing, and kept in the dark for 16–18 h for extraction of β-carotene. Samples were centrifuged at 7000 rpm, and the absorbance of the collected supernatant was measured at 440 nm (Spectrophotometer, Hitachi U-2900). The β-carotene was used to prepare the standard curve.

In vitro bioaccessibility of iron and zinc

The bioaccessibility of iron and zinc was analyzed deploying the method of equilibrium dialysis. All samples were subjected to the simulated gastrointestinal digestion by adjusting the pH to 2.0, followed by the addition of pepsin (3 mL of 16% pepsin in 0.2 M HCl) and incubated in a shaker water bath (37 °C for 2 h). Titratable acidity was tested by adding 5 mL of pancreatin-bile extract mixture (4 g of pancreatin and 25 g bile extract in 1 L of 0.1 M NaHCO₃) to an aliquot of the digest (20 mL) and titrated against 0.2 M NaOH until a pH 7.5 was attained.

The digest (20 mL) was subjected to simulated intestinal digestion. Accordingly, dialysis tubes (molecular cut-off 8–12 kDa) containing 25 mL NaHCO₃ (equivalent to moles of NaOH determined by titratable acidity) were dialyzed. 5 mL of pancreatin-bile extract mixture was added to flasks containing tubes, were incubated in a shaker

water bath at 37 °C for another 2 h (until the pH reaches to 7.0) (Luten et al. 1996). The iron and zinc present in the dialysate were analyzed by Atomic Absorption Spectrometry (Shimadzu AAF-6701, Japan) (AOAC 2000).

Statistical analysis

Values obtained were presented as per 100 g of dry matter. Experiments were performed in three independent trials, and data were presented as means ± SD (standard deviation). Data of treatments were assessed for statistical significance (ANOVA, $p \leq 0.05$). The multiple regression coefficient between variables was determined (Microsoft Excel 2007, Microsoft Corp., Redmond, WA, USA), and the principal component analysis was done (XLSTAT, Addinsoft, New York, USA).

Results and discussion

Inhibitory factors

The inhibitory factors in pearl millet grains varied amongst cultivars (Table 1). The polyphenol content was found to be the highest (781.14 mg/100 g) in GHB744, while it was the lowest (521.95 mg/100 g) in ICTP8203. A similar observation was also made in previous reports (Abdelrahman et al. 2005). Cultivars like ICMV221 and ICTP8203J, Anantapur local cultivar, and hybrids GHB588 and

HHB226 showed no differences ($p > 0.05$) in polyphenol content. According Brune et al. (1991) polyphenols form an insoluble complex in the gastrointestinal lumen, making the iron less available for absorption.

Hybrid series (HHB226, HHB223, HHB67 improved and RHB173) had the high phytic acid content (1.024 g/100 g), while the open pollinated cultivars (JBV2, ICMV221, and ICTP8203 series), contained 0.682–0.957 g/100 g. Certain cultivars like ICMV221 and Mysore local; ICMV221J and RHB173 had similar phytic acid contents that were not significantly different ($p > 0.05$). The present results were in accordance with the findings of Hama et al. (2011) and Abdelrahman et al. (2005) who reported phytic acid content in the range of 0.78 to 1.10 g/100 g. Phytic acid a known major inhibitory factor is often used to predict the bioaccessibility, as bioaccessibility is dependent on the ratio of phytic acid to iron and zinc.

The flavonoid content was the lowest in ICTP8203 (54.49 mg/100 g), ICMV221 (55.38 mg/100 g), and HHB67 improved (58.55 mg/100 g) with no significant differences. However, it was high in HHB223 (116.51 mg/100 g), followed by RHB173 (105.93 mg/100 g), Mysore local (104.18 mg/100 g) and Anantapur local (101.97 mg/100 g).

The insoluble dietary fiber content ranged between 9.36 and 12.89 g/100 g, with Anantapur local recording the least, while HHB226 recording significantly high ($p < 0.05$) (Table 1). Reichert and Youngs (1977) reported that the fiber content was the highest in the bran portion of the pearl millet. As far as the soluble fiber content was concerned, RHB173 contained maximum (1.86 g/100 g) followed by GHB744 (1.68 g/100 g) and HHB series (1.37–1.49 g/100 g), while JBV2 and ICMV221 contained minimum soluble fiber content (0.89 and 0.99 g/100 g). Soluble fiber content was non-significant ($p > 0.05$) among ICMV221J, ICTP8203, and Anantapur local cultivar. The insoluble dietary fiber content of 12.6 g/100 g and soluble dietary fiber content of 0.70 g/100 g was reported previously in certain pearl millet cultivars (Pushparaj and Urooj 2011).

Phytase activity

The phytase activity in pearl millet cultivars ranged from 0.053 to 0.582 $\mu\text{mol/g}$, with cultivars HHB226 and HHB223 recording the highest activity (Table 1). Phytase activity in cultivar ICMV221 was the lowest (0.053 $\mu\text{mol/g}$). A Nigerian pearl millet cultivar showed the phytase activity of 0.37 $\mu\text{mol/g}$ (Azeke et al. 2011). In the present investigation, cultivars (HHB223 and HHB226) with high phytic acid content also showed high phytase activity, while cultivar like ICMV221 with low phytic acid had

decreased phytase activity; other cultivars deviated from this pattern. The study suggested that depending on the phytase activity; cultivars could be chosen for further processing since phytases are known to get activated during soaking, germination, and cooking, and are capable of hydrolyzing hexa- and pentaphosphates. Hence, the phytase activity could be considered as a promoter of both iron and zinc bioaccessibility on the processing of pearl millet.

β -carotene

The β -carotene content in the pearl millet cultivars ranged from 1.60 to 6.97 $\mu\text{g/g}$ (Table 1). Hash et al. (1996) reported β -carotene up to 137 $\mu\text{g}/100\text{ g}$ in certain pearl millet breeding line. In the present study, ICTP8203 had low β -carotene while HHB226 showed significantly ($p < 0.05$) high content. The β -carotene has been shown to enhance the bioaccessibility of iron since it keeps the mineral in a solubilized form (Garcia-Casal et al. 1998). β -carotene at pH 6 binds iron liberated during digestion and form complex that acts as a chelating agent preventing the inhibitory effect of phytic acid and polyphenols on non-heme iron absorption. Although β -carotene was high in HHB226, decreased bioaccessibility of iron was noticed, which is mainly due to the presence of high quantity of other inhibitory factors. However, certain varieties like ICMV221, ICTP8203 and HHB67 improved showed less β -carotene content and the bioaccessibility of iron in these cultivars was also the lowest but other cultivars deviated from this pattern.

Fe/Zn content and bioaccessibility

The iron content varied among the pearl millet cultivars (Table 2). The iron content was the minimum in Anantapur local cultivar (5.59 mg/100 g), while it was the maximum in HHB226 (13.41 mg/100 g), which is slightly higher than the findings of Abdelrahman et al. (2005) and Abadalla et al. (1998) in Sudanese cultivars (7.5–11.2 mg/100 g). On the contrary, the cultivar HHB67 improved had high zinc content (5.19 mg/100 g), while GHB558 had the low zinc content (2.11 mg/100 g). The zinc content ranged between 5.3 and 7.1 mg/100 g, for Sudanese cultivars (Abadalla et al. 1998). Most hybrid cultivars with high zinc content (with the exception of GHB558) varied significantly ($p < 0.05$) from open pollinated cultivars (except ICTP8203J). Considering that pearl millet crop is cultivated in different soil conditions with varying levels of fertility in India, the micronutrient content of grain in cultivars might also vary. The iron content was stable in ICTP8203 and ICMV221 obtained from two different locations.

Table 2 Bioaccessibility of iron and zinc, and molar ratio (phytic acid: iron; phytic acid: zinc) and (iron: zinc) in pearl millet cultivars

Cultivars	Iron (mg/100 g)			Zinc (mg/100 g)			
	Total	Bioaccessible	Phytic acid: iron ratio	Total	Bioaccessible	Phytic acid: zinc ratio	Iron: zinc ratio
ICMV221	8.39 ± 0.47 ^c	0.1526 ± 0.012 ⁱ	8.5	3.04 ± 0.91 ^c	0.3837 ± 0.32 ^c	22.2	3.23
ICMV221J	8.79 ± 0.23 ^c	0.7479 ± 0.019 ^c	8.8	3.23 ± 0.14 ^c	0.4201 ± 0.12 ^c	29.4	3.19
ICTP8203	9.01 ± 0.80 ^b	0.2377 ± 0.066 ^b	6.1	3.48 ± 0.41 ^c	0.5268 ± 0.51 ^b	23.5	3.03
ICTP8203J	9.10 ± 0.72 ^b	0.6449 ± 0.039 ^d	7.8	4.15 ± 0.97 ^b	0.5343 ± 0.15 ^b	21.2	2.57
JBV2	7.02 ± 0.97 ^d	0.9126 ± 0.019 ^b	10.6	3.21 ± 0.72 ^c	0.3679 ± 0.43 ^f	27.2	2.56
Mysore local	5.92 ± 0.66 ^f	0.9629 ± 0.070 ^a	9.7	3.60 ± 0.29 ^b	0.3934 ± 0.40 ^d	18.7	1.93
Anantapur local	5.59 ± 0.63 ^g	0.9783 ± 0.036 ^a	11.0	3.59 ± 0.37 ^b	0.3419 ± 0.19 ^g	20.1	1.82
GHB558	6.06 ± 0.86 ^f	0.3357 ± 0.017 ^g	10.9	2.11 ± 0.43 ^d	0.2393 ± 0.42 ⁱ	36.9	3.36
GHB744	6.83 ± 0.46 ^e	0.4614 ± 0.060 ^f	9.1	4.68 ± 0.98 ^a	0.7111 ± 0.18 ^a	15.5	1.71
RHB173	6.87 ± 0.57 ^e	0.7901 ± 0.034 ^c	11.8	5.08 ± 0.82 ^a	0.3740 ± 0.45 ^e	18.7	1.58
HHB67 improved	9.34 ± 0.93 ^b	0.3574 ± 0.061 ^g	9.1	5.19 ± 0.36 ^a	0.3908 ± 0.22 ^d	19.2	2.11
HHB223	5.78 ± 0.36 ^g	0.5133 ± 0.032 ^c	15.4	5.04 ± 0.64 ^a	0.2802 ± 0.34 ^h	20.7	1.34
HHB226	13.41 ± 0.95 ^a	0.6941 ± 0.048 ^d	6.8	4.86 ± 0.38 ^a	0.7207 ± 0.36 ^a	22.0	3.23

Values are mean ± SD of three independent determinations (dry weight basis)

Mean values with different superscripts in a particular column vary significantly ($p < 0.05$)

The present study indicated that increasing the iron and zinc contents in pearl millet by breeding might not necessarily increase bioaccessible iron and zinc content proportionately. However, it is necessary to identify and quantify the potential inhibitors of iron and zinc bioaccessibility in the mineral enriched crops. In spite of the low iron content in Mysore and Anantapur local cultivars (5.92 and 5.59 mg/100 g), high bioaccessibility (16.27% and 17.95%) (Fig. 1) was documented. It is mainly due to low phytic acid (0.681 and 0.729 g/100 g) and insoluble fiber (9.82 and 9.36 g/100 g) content followed by low iron to zinc ratio (1.93 and 1.82). Although HHB226 had the highest iron content (13.41 mg/100 g), its bioaccessibility (5.18%) (Fig. 1) was low, which is attributed to the synergistic effect of increased quantities of phytic acid (1.08 g/100 g), insoluble fibers (12.9 g/100 g), polyphenol (670.49 mg/100 g) and iron to zinc ratio 3.23. Variation in bioaccessibility of iron and zinc has been documented in pearl millet grain (Hama et al. 2012).

The bioaccessible zinc, in the present study, ranged from 0.23 to 0.72 mg/100 g, the highest in HHB67 improved and the lowest in GHB558 (Table 3). The results are in corroboration with that of a previous report of Tripathi et al. (2010) which documented 0.70 mg/100 g bioaccessible zinc in pearl millet. GHB744 showed highest zinc bioaccessibility (15.19%) due to low phytic acid (0.735 g/100 g) and phytic acid to zinc ratio (15.5), while the other cultivars with higher phytic acid to zinc ratio deviated from

this pattern (Table 1, Fig. 1). In the case of zinc absorption in diets, based on unrefined cereals, if the phytate/zinc ratios are higher than 18, zinc absorption is estimated to be 18–28% (Brown et al. 2004). Cultivars ICMV221 and ICMV221J had similar zinc bioaccessibility (13%) (Fig. 1). Zinc present in plant foods exists in only one valency state (divalent), which could be explained for its higher bioaccessibility than that of iron, which is present in both divalent and trivalent forms (Garrow et al. 2000). In case iron absorption first the Fe^{3+} has to be reduced to Fe^{2+} form, which is not in the case of zinc which is absorbed naturally in existing form. The present study identified that certain cultivars with high iron bioaccessibility had low zinc bioaccessibility and vice versa (Fig. 1). A similar observation was made by Cilla et al. (2009) in fruit beverages fortified with iron and zinc. Regression coefficient analysis too showed a negative correlation between iron and zinc bioaccessibility (Table 3).

The molar ratio of phytic acid: iron ranged from 6.1 to 15.4 in pearl millet cultivars (Table 2). Although local cultivars, GHB558, HHB223, RHB173, and JBV2 had high phytic acid to iron molar ratio, these cultivars had relatively high iron bioaccessibility when compared to the other cultivars. Therefore phytic: iron ratio alone can't explain the adverse effect on iron bioaccessibility. The phytate-to-mineral ratios have been shown to be critical for mineral absorption and should be preferably $< 0.4:1$ to $< 1:1$ for iron in grain based meals (Hurrell 2004). The

Fig. 1 The iron and zinc bioaccessibility in grains of pearl millet cultivars

molar ratio of phytic acid: zinc ranged from 15.5 to 36.9 in pearl millet cultivars; maximum in cultivar GHB558 and minimum in cultivar GHB744 (Table 2). In the present study, phytic acid to zinc ratio could be used to predict the zinc bioaccessibility. Cultivars with a high ratio like GHB558 (36.9), ICMV221J (29.4), JBV2 (27.2), ICMV221(22.2), HHB226 (22) showed lower zinc bioaccessibility with exception to ICTP8201J (23.5). On the other hand cultivar GHB744 with the least phytic acid: zinc (15.5) ratio showed the highest zinc bioaccessibility (15.19%) (Fig. 1).

Correlation between bioaccessibility of minerals and inhibitory factors

The multiple regression analysis revealed that polyphenols had a significant negative relationship with iron and zinc bioaccessibility (Table 3). Krishnan et al. (2012) reported a strong effect of polyphenols on zinc bioaccessibility in finger millet. Tako et al. (2015) stated that polyphenols play a vital role in iron absorption in pearl millet. Phytic acid also showed strong negative correlation with iron and zinc bioaccessibility (Table 3). However, the adverse effect of flavonoids on iron and zinc bioaccessibility was not evident. Among the dietary fibers, soluble fiber showed

a negative correlation with iron and zinc bioaccessibility. A study conducted Hemalatha et al. (2007) on cereals and pulses revealed that both soluble and insoluble dietary fibers had an adverse effect on zinc bioaccessibility in cereals, while in pulses only insoluble fiber showed the negative effect. Iron bioaccessibility was negatively correlated with zinc bioaccessibility and vice versa, which indicated that an increase in bioaccessible iron decreases the bioaccessible zinc, which in turn might correspond to the iron/zinc ratio (Cilla et al. 2009; Garrow et al. 2000). The multiple regression model is a goodness-of-fit model ($p < 0.05$) for iron ($R^2 = 0.938$) and zinc ($R^2 = 0.886$) bioaccessibility as it could explain 93 and 88% of variables, respectively.

Principal component analysis (PCA)

Using the PCA model, the relationship of iron and zinc bioaccessibility in different cultivars to inhibitory factors was explored (Fig. 2). The relationships among cultivars with respect to β -carotene and phytase activity were also assessed. Principle axis 1 explained for 35.65% while, principle axis 2 explained for 25.14%, and together 60.80% of the variation was explained. Cultivar HHB226, located in the first quadrant was closely associated with inhibitory

Table 3 Effect of inhibitory factors on bioaccessibility of iron and zinc in pearl millet cultivars

Inhibitory factors	Regression Iron	coefficient Zinc
Constant	17.16	10.11
Polyphenols	− 0.0189	− 0.0100
Flavonoids	0.231	0.109
Phytic acid	− 29.15	− 27.01
Dietary fiber soluble	− 1.61	− 0.963
Insoluble	1.79	2.51
Zinc	− 0.777	−
Iron	−	− 0.521
R ²	0.938	0.886

Regression model

Iron bioaccessibility $y = 17.16 - 0.0189x_1 + 0.231x_2 - 29.15x_3 - 1.61x_4 + 1.79x_5 - 0.777x_6$

where x_1 = polyphenols, x_2 = flavonoids, x_3 = phytic acid, x_4 = soluble dietary fiber and x_5 = insoluble dietary fiber, x_6 = zinc

Zinc bioaccessibility: $y = 10.11 - 0.0100x_1 + 0.109x_2 - 27.01x_3 - 0.963x_4 + 2.51x_5 - 0.521x_6$

where x_1 = polyphenols, x_2 = flavonoids, x_3 = phytic acid, x_4 = soluble dietary fiber and x_5 = insoluble dietary fiber, x_6 = iron

Significant at $p < 0.05$

factors phytic acid and insoluble fiber in the same axis which might account for the lower iron bioaccessibility. This cultivar was also associated with enhancers, phytase,

and β -carotene which could be exploited during processing to improve the bioaccessibility. Local cultivars from Mysore and Anantapur districts were closely associated with high iron bioaccessibility. Iron bioaccessibility appears to be associated with flavonoids, polyphenols, and soluble dietary fiber in cultivars RHB173, and HHB223. Varieties like ICMV221, ICTP8203, and HHB67 improved with high zinc bioaccessibility were located in the opposite quadrant of iron bioaccessibility indicating that iron and zinc bioaccessibility are negatively correlated.

Conclusion

The study clearly showed that pearl millet cultivars which contained increased iron and zinc contents invariably contained increased quantities of certain inhibitory factors that might be detrimental to iron and zinc bioaccessibility, thus reducing the nutritional potential of pearl millet grains. Most hybrids had high zinc content when compared to the open pollinated cultivars. In the present study, the molar ratio of phytic acid to iron could not explain for iron bioaccessibility, while the bioaccessibility of zinc was dependent on phytic acid to zinc ratio. Furthermore, iron bioaccessibility appeared to be influenced by iron: zinc ratio. The study throws light on the possibility of selecting cultivars like HHB223 and HHB226 with high iron and zinc contents, as well as high phytase activity. The phytase

Fig. 2 Principal Component Analysis biplot showing variation in mineral bioaccessibility, inhibitory factors, β -carotene and phytase activity in pearl millet cultivars

activity in such cultivars could be suitably activated by the process of germination, soaking or fermentation thereby hydrolyzing the phytic acid and improving bioaccessibility of iron and zinc. Although pearl millet cultivars with high iron contents are available, the nature of its utilisation may play a vital role in improving iron bioaccessibility. Hence, from this study, it can be seen that although HHB226 had high iron content, it also had high phytic acid and phytase activity. The grains of this cultivar can be subjected to fermentation or germination to increase the enzyme activity before consumption and improve the iron and zinc absorption.

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