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ORIGINAL ARTICLE

Influence of germination on bioaccessible iron and calcium in pearl millet (*Pennisetum typhoideum*)

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Abstract Pearl millet is the staple for economically poorer section of the world's population and improving its mineral bioaccessibility is one of the important approaches to promote its utilization. In the absence of any data on the bioaccessible mineral content from commercially available millet, two varieties namely Kalukombu (native) and Maharastra Rabi Bajra (hybrid) were germinated and its effect on the bioaccessible iron and calcium content was explored using an *in-vitro* method which simulates gastro-intestinal digestion. The millet was germinated for 72 h to facilitate maximum mineral extraction. The bioaccessibility of iron and calcium was considerably enhanced upon sprouting. This higher bioaccessibility could be attributed to decrease in antinutritional factors like phytate and oxalate as a result of germination. Changes in mineral and antinutrient content during sprouting led to significant variations in the antinutrient/mineral molar ratios which had a positive impact on the bioaccessible mineral content. Use of tap water for soaking prior to germination revealed contamination of the millet with iron. Contaminant iron in Kalukombu variety appeared to be less accessible; while the same was potentially bioaccessible in Maharashtra Rabi Bajra variety. Hence bioaccessibility of iron depends on the form in which it is present. The actual bioaccessibility of contaminated iron needs to be further investigated.

Keywords Pearl millet · Commercial varieties · Bioaccessibility · Iron · Calcium · Germination

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Introduction

Pearl millet is a nutritious cereal grown on about 10 million hectares in India, is one of the largest producers of this crop in the world. It ranks third after wheat (*Triticum aestivum*) and rice (*Oryza sativa*) in its production (GOI 2008). Pearl millet (*Pennisetum typhoideum*), also classified as *P. glaucum, P. americanum, or P. spicatum*, and is locally known as bajra in India (Taylor 2004). Its advantage over other cereals is that it can be grown in marginal agricultural areas where annual rainfall is variable, unpredictable and very low (200–500 mm) and where daily temperature reaches 30°C (ICRISAT and FAO 1996). Pearl millet, a lesser known and underutilized crop is a staple food for population below poverty line for economic reasons. Besides being rich in iron, calcium, zinc and high level of fat, it is nutritionally comparable and even superior to major cereals due to the energy and protein value (Fasasi Olufunmilayo Sade 2009; Anu Sehgal and Kwatra 2006; Malik et al. 2002). On the other hand, pearl millet is often rich in fibre - associated antinutrients namely phytate and oxalate which have a negative influence on the bioavailability of minerals. The bioavailability of minerals from foods is defined as the proportion of the minerals that can be absorbed and utilized within the body (Lestienne et al. 2005). Poor absorption of minerals leads to mineral deficiency resulting in conditions like anaemia. The high prevalence of iron deficiencies in developing countries has several adverse effects on the population particularly on women and children (Zimmermann and Hurrell 2007).

Germination is a common household technique carried out at low cost without the use of any sophisticated and expensive equipment. It reduces antinutrients thereby improving nutritional and functional properties of pearl millet and also the mousy odour of damp millet is eliminated. It was observed that decrease in the antinutritional factors of cereal grains was a result of soaking and germination (Gupta and Sehgal 1991). Besides lowering of antinutrients, germination for 72 h significantly increased HCl extractability of minerals which represents mineral bioavailability (Archana et al. 1998; Pawar and Parlikar 1990; Badau et al. 2005; Arora et al. 2003).

Commercially available pearl millet has a lower shelf life compared to those procured from various agricultural institutions. One of the reasons for this reduction in the shelf life could be longer periods of storing due to transportation and some amount of processing before they reach the market. Sufficient studies have substantiated the positive effect of processing on mineral bioavailability of millets (Archana et al. 1998; Pawar and Parlikar 1990; Badau et al. 2005; Arora et al. 2003). In the absence of any data for iron and calcium bioaccessibility of commercially available pearl millet varieties, the effect of germination on commercially available varieties namely Kalukombu (K) and Maharashtra Rabi Bajra (MRB) on the same was investigated. The effects of oxalate and phytic acid on the bioaccessibility of iron and calcium was studied by calculating respective [phytate]/[iron], [oxalate]/ [calcium], [calcium]/[phytate] and [phytate]/ [calcium] molar ratios. In addition, tap water which could be a source of contaminant iron/calcium was used for soaking the grains before germination. Another batch of grains soaked in deionised water prior to germination was used for comparison.

Materials and methods

Two commercially available Pearl millet varieties namely 'Kalukombu' (K) and Maharashtra Rabi Bajra (MRB) were procured from the local market of Mysore, India for the study.

'Kalukombu' (K) is a native variety, traditionally grown by farmers in India (Karnataka, Tamilnadu and Maharashtra). This variety is not improved by the modern plant breeding system. It is considered nutritionally very superior by the local people and is used as food crop to make roti, dumpling and chapattis. The seeds of Kalukombu are small and elongated with persisting glumes/husk.

'Maharashtra Rabi Bajra' (MRB) is a commercially grown hybrid developed by the modern improved plant breeding technique by a commercial seed company. It is basically a winter crop. The seeds are grey/slate coloured, bold and round shaped without a persisting glumes/husk (Anon 2010)

The grain was manually cleaned by sieving out small sand particles, dust, broken seeds and other extraneous materials.

Pepsin, pancreatin, α amylase (porcine origin) and α, α bipiridyl was procured from Sigma chemicals Co., St Louis, MO, USA. Bile extract was procured from Loba Chemie, Mumbai. The dialysis membrane with a molecular mass cut off 12,000 was procured from Himedia, Vadhani Ind. Est, LBS marg, Mumbai. Sodium hydroxide, sodium bicarbonate, potassium ferricyanide and all the other chemicals used were of analytical grade. Deionized water and glassware rinsed in deionized water were used in the entire experiment.

Grain processing Pearl millet varieties (K & MRB) were soaked in tap water (passed through a water purifier) overnight. The water was drained and the grains were tied in a moist muslin cloth and left to sprout at room temperature for 72 h according to Badau et al. (2005). After sprouting the grains were dried in an oven at 50°C and milled into whole flour. While, another batch of grains were soaked in deionized water prior to germination for comparison. Un-germinated pearl millet was used as a control. The processing of the grain was carried out in 2 replicates. The flours obtained from these treatments were kept in air tight polythene bags and stored in a cool and dry place for further use.

Proximate composition and total mineral content Moisture, crude protein (Kjeldhal, N×6.25), fat (Soxhlet, solvent extraction) and ash contents were estimated by the AOAC (2005) standard methods of analysis. Mineral analysis was carried out on samples digested with hydrochloric acid. Total iron was analysed by colorimetric method using α,α bipyridyyl method (AOAC 2005). Total calcium was analysed by Raghuramulu method (2003).

Oxalate and phytate content Oxalate content in the raw and germinated flour was determined by the method of Baker (1952), where oxalate extracted with HCl and precipitated as calcium oxalate from the deproteinized extracts was estimated by subsequent titration with potassium permanganate. Phytate was extracted and determined according to the method of Thompson and Erdman (1982).

Bioaccessibility of iron and calcium The bioaccessibility of iron and calcium was determined using the procedure of Luten et al. (1996). Bioaccessible iron: Free form of iron in the dialysate which reacts with α,α bipyridyl to yield color was determined by AOAC (2005) method. Bioaccessible calcium present in the dialysate was precipitated as oxalates and is titrated against standard potassium permanganate (Raghuramulu et al. 1983).

Statistical analysis The experiment was performed in triplicate of the 2 replicates (n=6). The data was subjected to analysis of variance (ANOVA) test and the differences between the means were compared for their significance ($P<0.05$) using SPSS software v.17.

Results and discussion

Influence of germination on chemical composition As shown in Table 1, the moisture content of the millet ranged from 9.6% (MRB) to 10.1% (K) while, germinated flour had a moisture content of 7.4% (K) and 9.4% (MRB). The moisture in raw and germinated millet was well below the maximum moisture limit (13%) for pearl millet recommended by FAO/WHO (1995). This is the maximum allowable moisture content acceptable for pearl millet flour meant for human consumption. The protein content in both varieties ranged from 9.3% to 10.2%. These values were in accordance with the reported literature (Matilda et al. 1993; Taylor 2004). Germination led to a significant reduction ($P<0.05$) in the protein content of MRB while the same had no effect on K variety grains. The decrease in the protein content could be attributed to leaching of the low molecular weight nitrogen compounds (Alka and Kapoor 1997). The fat and ash content also reduced upon germination. This could be due to losses of total soluble solids during soaking prior to germination (Wang et al. 1997). Total iron content (mg/100 g) varied from 5 (K) to 6.4 (MRB) while the calcium content (mg/100 g) ranged from 41.3 (K) to 49.1 (MRB). Pearl millet was germinated for 72 h based on the literature (Badau et al. 2005) which reported that germination for 72 h significantly increased HCl extractability of minerals which represents mineral bioavailability. Upon germination, iron and calcium content significantly increased ($P<0.05$) which was in accordance with an earlier report on pearl millet (Sushma et al. 2008). This increase in the mineral content could be attributed to the mineral contamination of tap water used for soaking prior to germination.

One of the important approaches to increase mineral bioaccessibility is to decrease the amount of antinutritional factors like phytate and oxalate content. Pearl millet contains considerable amount of phytate which has a negative influence on mineral bioaccessibility. The millet varieties (K and MRB) used in the study contained 0.78 g/100 g and 0.57 g/100 g of phytate respectively, which drastically reduced upon germination (Table 1). The results obtained in this study were in accordance with the reported literature (Archana et al. 1998). Noteworthy was that more than 50% decrease in the phytate content for both varieties that was subjected to germination. The reduction in the phytate content could be attributed to its degradation and phytase synthesized during the process of germination (Pawar and Machewad 2006).

Oxalate content is of nutritional importance because of interference with calcium bioavailability. The oxalate content of the pearl millet varieties employed in this study ranged from 31.6 mg/100 g to 36 mg/100 g. These values were within the range reported in the literature (Isabelle et al. 2005; Abdalla et al.. 1998; Ravindran 1991). The oxalate content obtained in this study was <50 mg/100 g, falling in the range of low oxalate foods. Similar amounts of oxalates were found to occur widely in many vegetables and fruits which do not pose a nutritional problem (Ruth and Hesse 2002). Although the oxalate content was relatively low, it was further reduced by germination. A significant reduction ($P<0.05$) of upto 24% for K variety and as high as 48% for MRB was observed. Leaching of soluble oxalate content during steeping may be accounted for the decrease in total oxalate content.

Influence of germination on bioaccessible iron and calcium content Influence of germination on the bioaccessible iron and calcium content is presented in Fig. 1(a and b). The bioaccessible iron content in K variety grains was 0.16 mg/100 g, while that of MRB was 0.44 mg/100 g; which is nearly 3 times higher than K variety. Similarly, %

Table 1 Chemical composition of raw and germinated pearl millet

	Kalukombu		Maharashtra Rabi Bajra	
	Raw	Germinated	Raw	Germinated
Moisture (g/100 g)	$10.1^{b}\pm1.90$	$7.4^{a}\pm0.10$	$9.6^{a}\pm0.90$	$9.4^{a}\pm0.70$
Protein (g/100 g)	$9.3^{a}\pm1.30$	$9.6^{a}\pm1.20$	$10.2^{b}\pm0.40$	$7.8^{a}\pm1.40$
Fat (g/100 g)	$4.8^{b}\pm0.70$	$3.1^{a}\pm0.10$	$5.4^{b}\pm0.20$	$4.6^{a}\pm0.70$
Ash (g/100 g)	$2.0^{a}\pm0.40$	$1.8^{a}\pm0.00$	$1.5^{b}\pm0.10$	$1.4^{a}\pm0.00$
Iron (Mg/100 g)	$5.0^{a}\pm0.70$	$8.9^{b}\pm0.20$	$6.4^{a}\pm1.50$	$6.5^{a}\pm0.80$
Calcium (Mg/100 g)	$41.3^{a}\pm5.00$	$49.1^{b}\pm1.90$	$40.0^{a}\pm5.00$	$48.8^{b}\pm5.10$
Phytic acid (g/100 g)	$0.78^{b}\pm0.020$	$0.37^{a}\pm0.100$	$0.57^{b}\pm0.100$	$0.26^{a}\pm0.000$
Oxalates (mg/100 g)	$36.0^{b}\pm4.90$	$27.5^{a}\pm2.70$	$31.6^{b}\pm1.10$	$16.4^{a}\pm1.80$

Values are mean ± SD ($n=6$), Means followed by different superscripts (a, b) along the rows are significantly ($P<0.05$) different

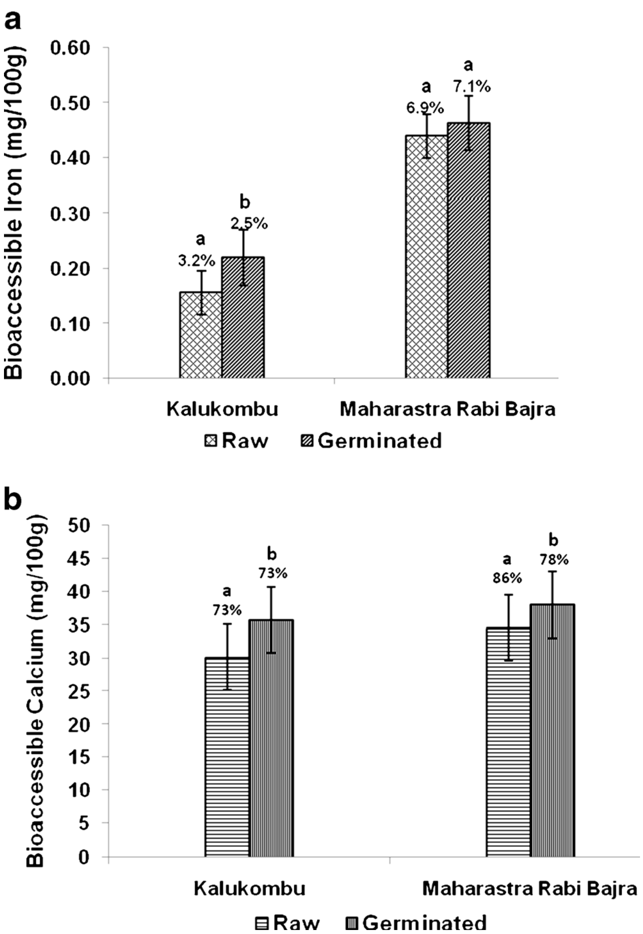

Fig. 1 Influence of germination on bioaccessible (**a**) iron and (**b**) calcium content. Each observation is a mean ± Standard Deviation of triplicate of 2 replicates (n=6),Values on the bars are % bioaccessibile iron, Bars with different alphabets differ significantly ($P \leq 0.05$)

bioaccessibility was higher (7.1%) in MRB while, K variety showed as low as 2.5%. The two varieties were sprouted to see the impact on bioaccessible iron. In the present investigation, sprouting enhanced the bioaccessible iron content (mg/100 g) from 0.16 to 0.22 in K variety grains, while in MRB; an increase from 0.44 to 0.46 was noticed. Sprouting was beneficial in K variety to increase the amount of bioaccessible iron. However this increase was not that high in MRB.

Pearl millet is a fairly good source of calcium (40 mg/100 g) and also contains considerable amounts of antinutrients like phytate which are known to inhibit calcium bioavailability. Figure 1(b) shows the bioaccessible calcium content of raw and germinated flour. The total amount of bioaccessible calcium in the raw flour of MRB (34.56 mg/100 g) was higher than that of K (30.12 mg/100 g). Similarly the % bioaccessibility of calcium was higher in MRB (86%) followed by K variety grains (73%). Germination led to a significant enhancement ($P<0.05$) in the bioaccessible calcium content (from 30.12 to 35.72 mg/100 g in K variety and from 34.56 to 38.01 mg/100 g in MRB). Although germination improved the bioaccessible calcium content in both varieties, the % bioaccessibility was similar (73%) in K variety while MRB showed a significant reduction ($P<0.05$) from 86% to 78%.

Influence of Germination on the antinutrients/mineral molar ratios The bioavailability of minerals depends on the amount of antinutrients and the ratio of antinutrients/minerals. The molar ratios for [Phytate]/[Calcium], [Phytate]/[Iron], [Calcium]/[Phytate] and [Oxalate]/[Calcium] of raw and germinated millet were calculated to study the effect of oxalate and phytate contents on the bioaccessibility of calcium and iron (Table 2). Accordingly, the [Phytate]/[Calcium] molar ratio in K and MRB was 1.15 and 0.86 which decreased to 0.46 and 0.33 respectively, after germination. Germination also resulted in the reduction of [Phytate]/[Iron] molar ratio from 12.75 to 2.87 in K variety and 7.55 to 2.82 in MRB. While, the [Calcium]/[Phytate] and [Oxalate]/[Calcium] molar ratios of raw and germinated flour was well below the recommended critical value of 6.1 and 1.0 respectively (Davis 1979). The molar ratios of [Calcium]/

Table 2 Molar ratios influencing the bioaccessible iron and calcium from raw and germinated pearl millet

Molar ratios	Kalukombu		Maharashtra Rabi Bajra	
	Raw	Germinated	Raw	Germinated
[Phytate]/[Calcium][a]	1.15	0.46	0.86	0.33
[Phytate]/[Iron][b]	12.75	2.87	7.55	2.82
[Calcium]/[Phytate][c]	0.87	2.19	1.16	1.48
[Oxalate]/[Calcium][d]	0.28	0.18	0.25	0.11

[a] Recommended critical value of [Phytate]/[Calcium] is 0.24, Morris and Ellis (1985)

[b] Recommended critical value for[Phytate]/[Iron] is 1, (Hallberg et al. 1989)

[c] Recommended critical value for [Calcium]/[Phytate] is 6.1, (Oladimeji et al. 2000)

[d] Recommended critical value for [Oxalate]/[Calcium] is 1.0, (Davis 1979)

[Phytate] and [Oxalate]/[Calcium] are of significance when they are greater than the recommended critical values. In such cases oxalate and phytate have a potential to complex calcium thus impairing its absorption (Davis 1979; Guansheng et al. 2005).

Effect of the source of water on total iron and calcium content To assess the influence of water (tap and deionized water) on the total iron and calcium content, two pearl millet varieties were soaked in tap water (passed through a water purifier) and deionized water prior to sprouting. Since this study was an approach towards household practice, it was interesting to find out the extent of contamination with extrinsic iron/calcium. Soaking of K variety grains in tap water resulted in high total iron content (mg/100 g) of 8.9, while that soaked in deionized water had 6.6 [Fig. 2(a)] suggesting presence of contaminant iron in tap water. However, in case of MRB the source of water showed no influence on the total iron content. There was a significant decrease in the calcium content of K variety grains, whereas soaking water did not influence the calcium content in MRB. These results suggest that the tap water contributed to extrinsic iron and not extrinsic calcium content.

Effect of the source of water on bioaccessible iron and calcium content The influence of Soaking water (tap/deionized) on the bioaccessible iron and calcium content is presented in Fig. 2(b and c). Although, the total iron content was higher in K variety grains soaked in tap water, it was poorly bioaccessible suggesting that contaminant iron was less bioaccessible than intrinsic iron. Nevertheless, MRB showed higher bioaccessible iron content when soaked in tap water prior to germination. Even though contaminant iron from K variety grains appeared to be less accessible, it was potentially bioaccessible in case of MRB. Contaminant iron may come from soil residues, dust or cooking utensils and its bioaccessibility depends on the source of contamination. Hence, the actual bioaccessibility of contaminated or extrinsic iron needs to be investigated. However, the bioaccessible calcium content did not vary when soaked in tap or deionised water prior germination.

The present study indicated that germination was beneficial in improving the bioaccessible iron and calcium content in two commercial pearl millet varieties. Germination resulted in a significant reduction of phytate and oxalate content which led to an increase in bioaccessible iron and calcium content. The process also resulted in lowering of antinutrients/mineral molar ratios which had a positive impact on the bioaccessible mineral content. Among the varieties used, MRB had a higher total and bioaccessible iron content with lower inherent factors like phytate and oxalate. A comparison of total and bioaccessible iron and calcium content among the grains soaked in tap and deionised water prior to germination revealed that grains soaked in tap water were contaminated with iron which led to a considerable increase in the bioaccessible iron content from MRB however, in K variety grains, the

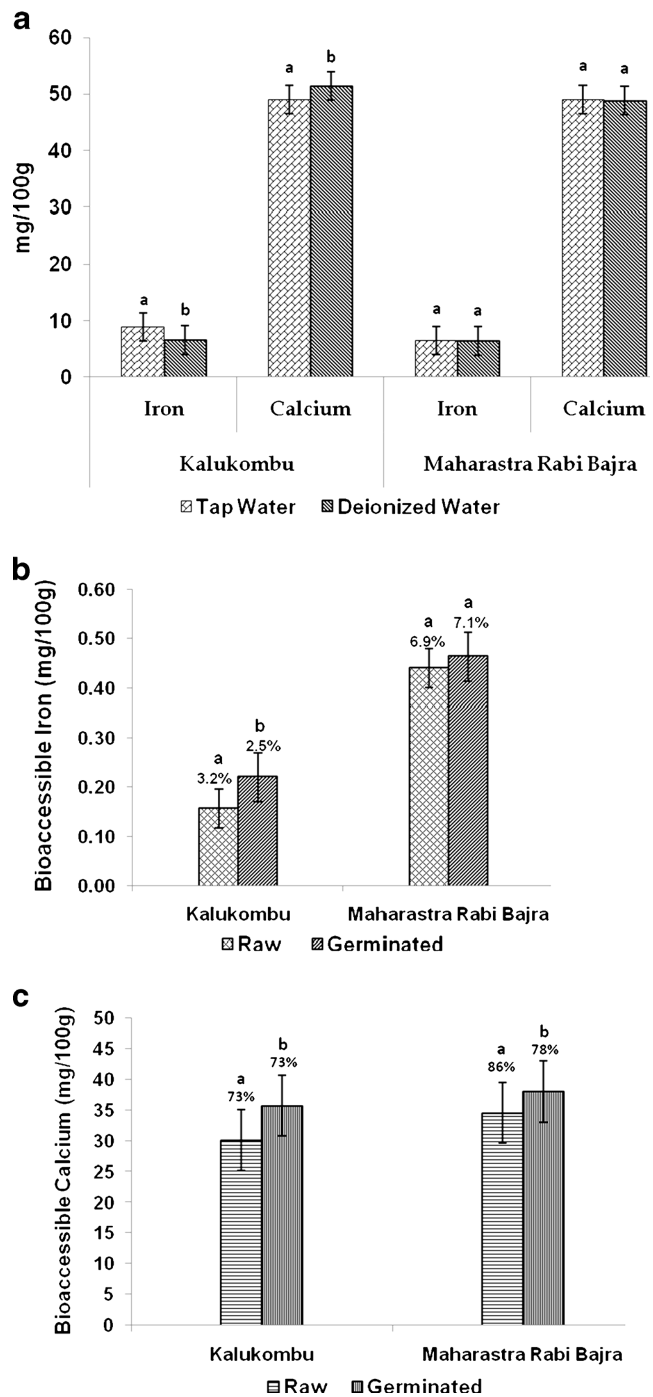

Fig. 2 Influence of the source of water on (**a**) total and bioaccessible (**b**) iron and (**c**) calcium contents. Each observation is a mean ± Standard Deviation of triplicate values of 2 replicates (n=6), Values on the bars are % bioaccessibile iron, Bars with different alphabets differ significantly ($P \leq 0.05$)

contaminant iron was poorly bioaccessible. Hence, the actual bioaccessibility of contaminated or extrinsic iron needs to be investigated since extrinsic iron may increase absorbable iron content in the diet.

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