

# Effect of Processing on Total Iron and Zinc, Ionizable Iron, Extractable Zinc, Phytate and Phytate: Mineral Ratios in Pearl Millet

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**Abstract.** Pearl millet [*Pennisetum glaucum* (L) R. Br] is a good source of essential minerals but their availability is constrained by high amounts of phytate. Hence, this study examined the effect of soaking, germination and malting on phytate content, phytate: mineral molar ratios, ionizability of iron and extractability of zinc in five varieties of pearl millet namely MAHYCO 204, ICTP 8203, H6M33, Mahalakshmi 504, 8203 Nirmal – Mahabeej. Grain samples were soaked for 12 hrs and 24 hrs in grain: water ratios 1:2 and 1:5 and germinated for 48 hrs after soaking for 12 hrs in grain: water ratios 1:2 and 1:5 and germinated grains were roasted for 6 min for malting. All three processing techniques significantly reduced phytate content compared to the raw. Phytate content was significantly and negatively correlated with ionizable iron ( $r = - 0.545$ ), percent ionizable iron ( $r = - 0.653$ ), extractable zinc ( $r = - 0.214$ ), percent extractable zinc ( $r = - 0.749$ ), phytate: iron ( $r = - 0.614$ ) and phytate: zinc ( $r = - 0.631$ ) molar ratios. Mean phytate: iron molar ratio reduced from 15.2 in the raw to 7.6-10.9 on germination and 8.2-8.7 on malting and mean phytate: zinc molar ratio from 19.4 in the raw to 9.4-14.9 on germination and 11.3-11.8 on malting.

**Keywords:** Phytate; ionizable iron; extractable zinc; phytate: mineral molar ratios; germination; malting

## 1 Introduction

Pearl millet [*Pennisetum glaucum* (L.)R. Br] can be cultivated in difficult growing conditions such as drought, low soil fertility and high temperature. Compared to other major cereals it contains higher amounts of calcium, iron, zinc and its protein quality is better [1]. Hence, this millet has the potential for controlling micronutrient deficiencies with specific reference to iron and zinc. However, it contains high amounts of phytate that chelates minerals and reduces their bioavailability.

Several reports in the literature have indicated that phytate content can be reduced by soaking [2, 3] and germination [4, 5]. However, few studies have examined the influence of these processing techniques on the ionizable iron and extractable zinc content as well as the phytate: mineral ratios. The latter is especially relevant to the bioavailability of iron and zinc. Therefore, it is important to determine whether processing can bring the phytate: mineral ratio to the appropriate level as well as improve the ionizability /extractability of iron and zinc.

In Western India, pearl millet is a staple and contributes substantially to the total energy, protein and iron intakes. A survey conducted by this Department on rural adult women in Ahmednagar district, Maharashtra state, India revealed that they consumed about 240 g of pearl millet per day, which contributed 19.1% of daily energy intake, 21.5% of protein intake and about half of the iron and zinc intakes [Dietary Contribution of Pearl Millet in Ahmednagar District, Maharashtra: Consumption and Effect of Traditional Processing on Mineral Bioavailability (2013) Report submitted to Harvest Plus]. In

this context, we analyzed five pearl millet varieties commonly cultivated and consumed within this region. The effect of selected processing techniques on the *in vitro* availability of these minerals and the phytate: mineral ratios were examined.

## 2 Materials and Methods

### 2.1 Sample Collection

Five varieties of pearl millet (MAHYCO 204, ICTP 8203, H6M33, Mahalakshmi 504, 8203 Nirmal - Mahabeej) commonly cultivated in Ahmednagar district in Northwestern region of Maharashtra state, India, were analyzed. Samples were procured directly from farmers. For each variety, 5 to 8 samples were purchased in duplicate from 4-6 villages. In each village, farmers were selected by random sampling from the list of households. All sacks were numbered serially by farmers and from these, two sacks were randomly selected. From each sack three kilograms of pearl millet was purchased by taking one kilogram each from the top, the middle and the bottom portion using an aluminum scoop. The grain was pooled and mixed thoroughly, sealed in plastic Ziplock bags, labeled and transported to the Department in Mumbai city. In the laboratory, the samples were carefully cleaned manually to remove broken grains, stones, chaff and dirt and were stored in fresh clean Ziplock bags.

Sampling of the pearl millet grains was done by quartering method, using stainless steel trays that were washed and rinsed thoroughly with deionized water. Four samples of 300 grams each were taken for each variety. Three samples were kept for studying the effect of processing. The remaining sample was ground to fine flour in a stainless steel mill (mesh size 0.5mm).

### 2.2 Processing Methods

Three processing methods were studied – soaking, germination and malting.

Soaking – 30 g of grains of each sample were accurately weighed in duplicate. The grains were soaked in deionized water, using two grain: water ratios - 1:2 and 1:5 (w/v) and kept at ambient temperature in acid - washed conical flasks for 12 hours and 24 hours, each. After soaking, the excess water was drained and the grains were dried at 60°C to constant weight. The dried seeds were ground to flour (mesh size 0.5mm) in a stainless steel mill.

Germination - 30g of grains were soaked in deionized water using the grain: water ratios mentioned above and kept at ambient temperature (35-40°C) for 12 hours. After soaking, the water was drained and soaked grains were covered with a muslin cloth, allowed to germinate at ambient temperature for 48 hours, were dried to constant weight and ground to obtain the germinated millet flour [2,3,4,5].

Malting- 30g of grains were germinated as described above. The germinated dried grains were roasted for 6 minutes in a thick-bottomed stainless steel pan that had been rinsed with deionized water. After malting, the grains were ground to obtain the malted millet flour.

The flours obtained using the three processing techniques as well as the raw grain flour were packed in plastic bags, sealed and packed in air tight plastic boxes and stored at 4°C until analysis.

### 2.3 Chemicals

All chemicals were used according to analytical grade. Pepsinand thioglycollic acid was purchased from Loba chemie Laboratory reagents and fine chemicals. Hydrochloric acid, perchloric acid, nitric acid, acetic acid glacial, 2,2 -Bipyridyl, sodium acetate anhydrous, hydroxylamine hydrochloride, ammonium ferric sulfate, ammonium ferrous sulfate, iron and zinc standard solutions were procured from Merk Specialities Pvt. Ltd, Mumbai, India. Phytic acidsodium salt hydrate standard (Pcode-100969894) was procured from Sigma-Aldrich Co., St. Louis, USA.

### 2.4 Analytical Methods

For each flour raw and processed, samples were drawn in duplicate and each sample was then analyzed in triplicate. Total zinc and iron: Two grams of each sample was subjected to dry ashing at 550°C in a

muffle furnace as per AOAC method [6] and ash solutions were prepared using deionized water. In these ash solutions, total iron and zinc content were determined by Inductively Coupled Plasma Optical Emission Spectrometry (Perkin Elmer Model Optima 7000 DV, Shelton, CT, USA) in ash solutions.

**Phytate:** Phytate content was estimated Spectrophotometrically (LABINDIA Model UV3000+, Navi Mumbai, India) using the procedure given by Haug and Lantzsch [7] at 519nm.

**Ionizable iron:** Ionizable iron content was estimated at pH 7.5 by the method of Rao and Prabhavathi [8].

**Extractable zinc:** Extractable zinc content was estimated by the method given by Chompreeda and Fields [9].

## 2.5 Data Analysis

Molar ratios of phytate:iron and phytate:zinc were calculated. SPSS (version 17.0) was used for statistical analysis. One – way Analysis of Variance (ANOVA) followed by Bonferroni post - hoc test for multiple comparisons was used to determine whether differences among the raw and processed samples were significant. Pearson's correlation analysis was used to examine the relationship between phytate, ionizable iron and extractable zinc.

## 3 Results

### 3.1 Raw Samples

**Iron and Zinc Content:** Overall mean content of iron and zinc was 5.1 mg% and 4.9 mg%, respectively (Table 1). Significant differences were seen between the five varieties for total iron ( $F=3.072$ ,  $p=0.021$ ) and total zinc ( $F=7.402$ ,  $p=0.000$ ), with ICTP-8203 having the highest content of iron and zinc, among the five varieties. Mahalakshmi 504 and H6M33 had the lowest iron content while zinc content was least in Mahalakshmi 504.

**Phytate Content:** The overall mean phytate content was 878 mg% (Table 1), with significant differences between the five varieties ( $F=4.024$ ,  $p=0.009$ ), H6M33 having the highest phytate content and 8203-Mahabeej/Nirmal the lowest content, followed by ICTP-8203.

**Phytate: mineral ratios:** The phytate:iron molar ratio varied from 8.6 to 20.9, with significant varietal differences ( $F=40.058$ ,  $p=0.000$ ). Similar significant varietal differences were seen in the phytate:zinc molar ratio which ranged from 9.6 to 36.9 (Table 1).

**Ionizable Iron and Extractable Zinc Content:** Mean ionizable iron for all five varieties was  $0.7\pm 0.2$  mg% and mean extractable zinc was  $2.5\pm 0.7$  mg%, with significant varietal differences for both ionizable iron ( $F=31.329$ ,  $p=0.000$ ) and extractable zinc ( $F=4.177$ ,  $p=0.007$ ). ICTP-8203 had the highest whereas H6M33 had the lowest ionizable iron content. Mahalakshmi-504 had the lowest amount of extractable zinc in contrast to ICTP-8203 which had the highest content of extractable zinc. Ionizable iron as a percentage of total iron on average, constituted only  $14.1\pm 4.3\%$ . However, extractable zinc constituted almost half of the total zinc ( $51.2\pm 7.8\%$ ).

**Table 1.** Total and ionizable iron, total and extractable zinc, phytate content and phytate: mineral molar ratios in raw pearl millet (mg/100gm DM)

Raw	Variety					Overall
	204	ICTP 8203	H6M33	8203-Mahabeej/ Nirmal	Mahalakshmi	
<b>Total Iron</b>						
Mean±SD	5.4±1.3	5.7±0.9	4.4±0.1	4.7±0.6	4.4±0.2	5.1±1.0
Min-Max	3.1-8.1	4.9-7.7	4.2-4.5	3.9-5.6	4.1-4.6	3.9-8.1
<b>Total Zinc</b>						
Mean ±SD	5.2±1.0	6.0±0.9	4.0±0.8	4.8±1.4	3.2±0.9	4.9±1.3
Min-Max	4.0-6.8	4.1-6.9	3.4-5.5	3.7-7.0	2.4-4.5	2.4-7.0

Phytate						
Mean $\pm$ SD	9256 $\pm$ 105	833 $\pm$ 137	952 $\pm$ 84	763 $\pm$ 53	868 $\pm$ 64	878 $\pm$ 115
Min-Max	772 -1091	667-1036	828 -1041	676 - 815	789- 950	667 -1091
Phytate:Iron Molar ratio						
Mean $\pm$ SD	15.2 $\pm$ 3.2	12.6 $\pm$ 2.4	18.4 $\pm$ 1.6	13.9 $\pm$ 1.8	16.9 $\pm$ 1.3	15.2 $\pm$ 3.0
Min-Max	9.2-20.9	8.6-16.1	15.9-19.9	11.7-17.3	15.5-18.5	8.6-20.9
Phytate:Zinc Molar ratio						
Mean $\pm$ SD	18.2 $\pm$ 3.5	14.3 $\pm$ 4.7	24.2 $\pm$ 5.3	16.6 $\pm$ 4.4	28.6 $\pm$ 8.6	19.4 $\pm$ 6.7
Min-Max	13.1-24.1	9.6-24.7	14.7-29.8	10.8-21.2	17.4-36.9	9.6-36.9
Ionizable iron						
Mean $\pm$ SD	0.7 $\pm$ 0.1	1.0 $\pm$ 0.1	0.4 $\pm$ 0.1	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.2
Min-Max	0.5-0.8	0.9-1.1	0.3-0.4	0.6-0.9	0.5-0.8	0.3-1.1
Extractable Zinc						
Mean $\pm$ SD	2.4 $\pm$ 0.4	3.1 $\pm$ 0.9	2.2 $\pm$ 0.5	2.7 $\pm$ 0.8	1.8 $\pm$ 0.5	2.5 $\pm$ 0.7
Min-Max	1.9-3.4	1.7-4.1	1.9-3.2	2.0-4.1	1.4-2.5	1.4-4.1

### 3.2 Effect of Processing

**Soaking:** Mean content of total iron and zinc decreased upon soaking with all four treatments i.e. 12 and 24 hours of soaking and grain: water ratios of 1:2 and 1:5 (Table 2). This decrease was statistically significant for iron ( $F=22.435$ ,  $p=0.000$ ) but not for zinc ( $F=0.990$ ,  $p=0.440$ ).

Iron content decreased as the duration of soaking increased for all varieties at both the grain: water ratios. In grains soaked for 12 hrs, iron content was slightly but not significantly higher with grain: water ratio of 1:5 than with a ratio of 1:2. With 24 hours of soaking, there was little difference between the two ratios. Percent reduction in total iron content after 12 hours of soaking was 28.2%, with grain: water ratio of 1:2 and 18.9% with the higher grain: water ratio (1:5). With 24 hours of soaking, about one-third (36.5%) of the iron was lost with grain: water ratio of 1:2 and 39.7% with grain: water ratio of 1:5. In case of zinc, duration of soaking and grain: water ratios did not significantly influence the zinc content. Soaking for 12 hours resulted in 29.8% loss with grain: water ratio of 1:2 and 36.4% with grain: water ratio of 1:5.

The extent of loss in total iron content differed significantly between varieties for all four treatments. Overall, ICTP 8203 showed greater losses than the other varieties, especially after 24 hours of soaking. Similar trends were observed for loss of total zinc.

Phytate content of soaked samples was significantly lower than that of raw samples for all five varieties (Table 2). There was a significant difference between the four treatments i.e. duration of soaking and the two grain: water ratios ( $F=75.057$ ,  $p=0.000$ ). Comparison of the five varieties using post-hoc Bonferroni test indicated significant differences for each of the four treatments ( $p<0.001$ ) with the extent of loss on soaking being 23% for MAHYCO 204, 26.9% for ICTP-8203, 31.4% for H6M33, 32.5% in 8203 - Mahabeej-Nirmal and highest, i.e. 39.9% for Mahalakshmi.

The phytate: iron molar ratio in the soaked samples was similar to the ratio present in the raw grains (Table 3). Significant varietal differences were observed for the phytate: iron ratio ( $F=40.058$ ,  $p=0.000$ ), the lowest being in ICTP 8203 and 8203 - Mahabeej-Nirmal and the highest in MAHYCO 204. Similarly, significant varietal differences were seen in the phytate: zinc molar ratio ( $F=23.539$ ,  $p=0.000$ ), the lowest in 8203 - Mahabeej-Nirmal and the highest in H6M33.

There was no significant difference in the overall ionizable iron content of soaked and raw samples (Table 3). Ionizable iron expressed as a percentage of total iron, was higher in the soaked grains than raw grains, for all varieties, ranging from 20.3 to 25.7% (Figure 1). However, varietal differences were observed. In three varieties, namely, MAHYCO 204, ICTP-8203 and 8203 - Mahabeej-Nirmal, ionizable iron content in the soaked samples was similar to that of the raw samples, regardless of amount of water used and duration of soaking. In contrast, in H6M33 and Mahalakshmi 504, the ionizable iron content was higher in soaked samples than in raw samples (Table 3).

The extractable zinc content decreased slightly after soaking (Table 3). After 12 hours of soaking, with a grain: water ratio of 1:2, mean extractable zinc was slightly but not significantly lower than in

raw samples. When more water was used for soaking (grain:water ratio of 1:5), the mean extractable zinc was similar to the content found with grain: water ratio of 1:2. When the duration of soaking was extended to 24 hours, there was no further decrease at both the ratios of grain to water (Table 3). When the extractable zinc as a percentage of total zinc was examined, the values (58.8% to 66.6%) were higher for soaked samples, with all four treatments, than in the raw samples (Figure 2). Comparison of the four soaking treatments showed that with a grain: water ratio of 1:5 and 24 hours duration of soaking, the extractable zinc constituted about 60% of the total zinc (Figure 2). Varietal differences were not observed for extractable zinc.

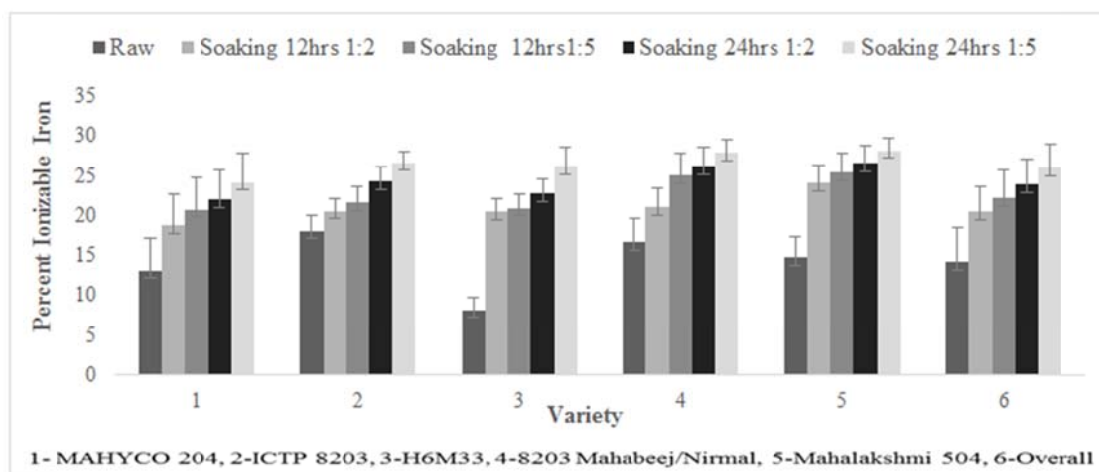


Figure 1. Effect of soaking on percent ionizable iron in five varieties of pearl millet

Table 2. Total iron, total zinc and phytate content in soaked pearl millet (mg/100gm DM)

Parameter	Hours, Grain: Water Ratio	Variety					Overall
		MAHYCO 204	ICTP 8203	H6M33	8203-Mahabeej / Nirmal	Mahalakshmi	
<b>Total Iron - 12 hrs</b>							
Mean ±SD	1:2	3.6±0.5	4.2±0.3	3.2±0.3	3.7±0.3	3.2±0.2	3.6±0.5
Min-Max		3.1-4.8	3.8-4.9	2.8-3.7	3.5-4.2	3.1-3.4	2.8-4.9
Mean ±SD	1:5	4.0±0.6	4.6±0.3	3.8±0.3	4.1±0.7	3.8±0.2	4.0±0.5
Min-Max		3.3-5.6	4.3-5.3	3.4-4.2	3.1-5.0	3.6-4.1	3.1-5.6
<b>Total Iron - 24 hrs</b>							
Mean ±SD	1:2	3.5±0.5	3.3±0.3	2.9±0.4	2.8±0.4	3.0±0.3	3.2±0.5
Min-Max		2.6-4.3	2.8-3.7	2.3-3.6	2.4-3.6	2.7-3.3	2.3-4.3
Mean ±SD	1:5	3.5±0.6	3.0±0.3	2.5±0.3	2.7±0.2	2.9±0.3	3.0±0.6
Min-Max		2.6-4.4	2.41-3.20	2.1-2.9	2.5-3.2	2.6-3.4	2.0-4.4
<b>Total Zinc - 12 hrs</b>							
Mean ±SD	1:2	3.8±0.7	3.4±0.5	2.4±0.3	4.0±0.6	2.9±0.3	3.4±0.8
Min-Max		2.9-5.0	2.7-4.10	2.1-2.8	3.1-4.9	2.5-3.2	2.1-5.0
Mean ±SD	1:5	3.8±0.7	3.4±0.3	3.0 ± 0.3	3.3±0.3	3.0 ±0.5	3.4±0.6
Min-Max		3.0-4.9	2.6-3.7	2.5-3.4	2.9-3.6	2.3-3.5	2.3-4.9
<b>Total Zinc - 24 hrs</b>							
Mean ±SD	1:2	3.8 ± 0.8	2.9±0.7	3.1±0.9	2.8± 0.4	2.4±0.3	3.2±0.9
Min-Max		3.0-5.8	2.1-4.2	2.2-4.5	2.5-3.6	2.1-2.8	2.1-5.8
Mean ±SD	1:5	3.3±0.8	2.9±0.4	2.5±0.3	3.2±0.5	2.8±0.3	3.0±0.6
Min-Max		2.1-4.9	2.5-3.5	2.3-3.0	2.7-3.9	2.3-3.1	2.1-4.9
<b>Phytate - 12 hrs</b>							

Mean $\pm$ SD	1:2	718 $\pm$ 87	657 $\pm$ 105	702 $\pm$ 80	573 $\pm$ 39	548 $\pm$ 79	659 $\pm$ 103
Min-Max		611-898	545-836	549-770	535-636	444 $\pm$ 631	444-898
Mean $\pm$ SD	1:5	676 $\pm$ 94	610 $\pm$ 84	697 $\pm$ 74	539 $\pm$ 43	545 $\pm$ 74	628 $\pm$ 98
Min-Max		563 $\pm$ 857	492-768	625-809	468-588	435-616	435-857
<b>Phytate - 24 hrs</b>							
Mean $\pm$ SD	1:2	702 $\pm$ 67	607 $\pm$ 159	643 $\pm$ 52	489 $\pm$ 85	599 $\pm$ 54	627 $\pm$ 114
Min-Max		537-793	344-804	574-695	333-586	542-674	333-804
Mean $\pm$ SD	1:5	656 $\pm$ 75	562 $\pm$ 59	571 $\pm$ 98	458 $\pm$ 41	393 $\pm$ 66	560 $\pm$ 115
Min-Max		545-786	4667-630	422-714	398-517	322-464	322-786

**Table 3.** Phytate: mineral ratios, ionizable iron and extractable zinc content in soaked pearl millet (mg/100gm DM)

Parameter	Hours, Grain: Water Ratio	Variety					Overall
		MAHYCO 204	ICTP 8203	H6M33	8203-Mahabeej / Nirmal	Mahalakshmi	
<b>Phytate : Iron Molar Ratio - 12 hrs</b>							
Mean $\pm$ SD	1:2	17.0 $\pm$ 3.2	13.5 $\pm$ 2.9	18.8 $\pm$ 2.5	13.1 $\pm$ 0.7	14.5 $\pm$ 2.5	15.7 $\pm$ 3.3
Min-Max		13.1-23.3	8.6-16.1	15.9-23.3	12.4-13.9	12.1-17.4	11.1-23.3
Mean $\pm$ SD	1:5	14.6 $\pm$ 2.9	11.2 $\pm$ 1.7	15.7 $\pm$ 2.4	11.3 $\pm$ 1.6	12.2 $\pm$ 2.2	13.3 $\pm$ 2.9
Min-Max		8.6-19.9	8.8-14.8	12.7-19.9	9.3-13.0	9.4-14.4	8.6-19.9
<b>Phytate : Iron Molar Ratio - 24 hrs</b>							
Mean $\pm$ SD	1:2	17.3 $\pm$ 3.1	16.1 $\pm$ 4.7	19.0 $\pm$ 3.1	15.2 $\pm$ 3.9	16.9 $\pm$ 2.7	16.9 $\pm$ 3.6
Min-Max		12.1-22.3	8.5-21.9	14.6-22.5	7.9-19.1	13.8-21.3	7.9-22.5
Mean $\pm$ SD	1:5	16.3 $\pm$ 3.6	16.3 $\pm$ 2.6	19.2 $\pm$ 2.7	14.4 $\pm$ 1.4	11.6 $\pm$ 2.6	15.9 $\pm$ 3.5
Min-Max		11.9-23.9	12.8-20.3	15.6-23.9	12.8-16.0	8.4-14.5	8.4-23.9
<b>Phytate : Zinc Molar Ratio - 12 hrs</b>							
Mean $\pm$ SD	1:2	19.0 $\pm$ 3.9	19.5 $\pm$ 4.7	28.8 $\pm$ 3.5	14.6 $\pm$ 2.7	19.1 $\pm$ 4.1	19.9 $\pm$ 5.6
Min-Max		13.9-25.6	13.6-26.4	25.1-33.3	11.3-19.3	14.3-23.5	11.3-33.3
Mean $\pm$ SD	1:5	18.2 $\pm$ 4.1	17.9 $\pm$ 3.3	23.6 $\pm$ 4.1	15.9 $\pm$ 0.9	18.5 $\pm$ 3.6	18.6 $\pm$ 4.1
Min-Max		13.7-28.2	13.2-23.8	18.1-30.6	14.5-16.9	14.3-24.2	13.2-30.6
<b>Phytate : Zinc Molar Ratio - 24 hrs</b>							
Mean $\pm$ SD	1:2	18.6 $\pm$ 3.5	22.2 $\pm$ 8.8	21.8 $\pm$ 5.2	17.5 $\pm$ 4.5	24.6 $\pm$ 1.9	20.4 $\pm$ 5.5
Min-Max		11.6-25.1	11.8-34.8	14.7-27.7	9.1-21.6	22.1-27.4	9.1-34.2
Mean $\pm$ SD	1:5	20.8 $\pm$ 5.3	19.1 $\pm$ 2.1	22.4 $\pm$ 3.0	14.1 $\pm$ 1.6	13.9 $\pm$ 2.9	18.8 $\pm$ 4.8
Min-Max		14.9-33.9	15.3-21.8	17.5-26.3	11.5-15.9	10.9-17.2	10.9-33.9
<b>Ionizable Iron - 12 hrs</b>							
Mean $\pm$ SD	1:2	0.7 $\pm$ 0.1	0.9 $\pm$ 0.0	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1
Min-Max		0.5-0.9	0.7-1.0	0.5-0.8	0.7-0.9	0.7-0.8	0.5-1.0
Mean $\pm$ SD	1:5	0.8 $\pm$ 0.2	1.00 $\pm$ 0.1	0.8 $\pm$ 0.1	1.0 $\pm$ 0.1	1.0 $\pm$ 1.0	0.9 $\pm$ 0.2
Min-Max		0.6-1.1	0.9-1.1	0.6-1.0	0.8-1.1	0.9-1.0	0.6-1.1
<b>Ionizable Iron - 24 hrs</b>							
Mean $\pm$ SD	1:2	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1
Min-Max		0.5-1.0	0.6-0.9	0.5-0.8	0.6-0.9	0.7-1.0	0.5-1.0
Mean $\pm$ SD	1:5	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1
Min-Max		0.6-1.0	0.6-0.9	0.6-0.7	0.6-0.9	0.7-0.9	0.6-1.0
<b>Extractable zinc - 12 hrs</b>							
Mean $\pm$ SD	1:2	2.2 $\pm$ 0.3	1.9 $\pm$ 0.3	1.5 $\pm$ 0.2	2.4 $\pm$ 0.4	1.8 $\pm$ 0.2	2.0 $\pm$ 0.4
Min-Max		1.7-2.7	1.4-2.5	1.3-1.8	1.8-3.0	1.5-1.9	1.3-3.0
Mean $\pm$ SD	1:5	2.3 $\pm$ 0.4	2.1 $\pm$ 0.3	1.9 $\pm$ 0.3	2.2 $\pm$ 0.2	1.9 $\pm$ 0.3	2.1 $\pm$ 0.4
Min-Max		1.8-3.1	1.6-2.4	1.6-2.2	1.9-2.4	1.5-2.3	1.5-3.1

Extractable zinc - 24 hrs							
Mean $\pm$ SD	1:2	2.4 $\pm$ 0.4	1.9 $\pm$ 0.5	2.1 $\pm$ 0.6	1.9 $\pm$ 0.2	1.7 $\pm$ 0.1	2.1 $\pm$ 0.5
Min-Max		1.9-3.6	1.3-2.8	1.5-3.1	1.6-2.2	1.6-1.8	1.3-3.6
Mean $\pm$ SD	1:5	2.1 $\pm$ 0.5	2.0 $\pm$ 0.3	1.7 $\pm$ 0.2	2.1 $\pm$ 0.2	1.9 $\pm$ 0.1	2.0 $\pm$ 0.4
Min-Max		1.3-2.9	1.5-2.4	1.5-2.1	1.9-2.5	1.8-2.1	1.3-2.9

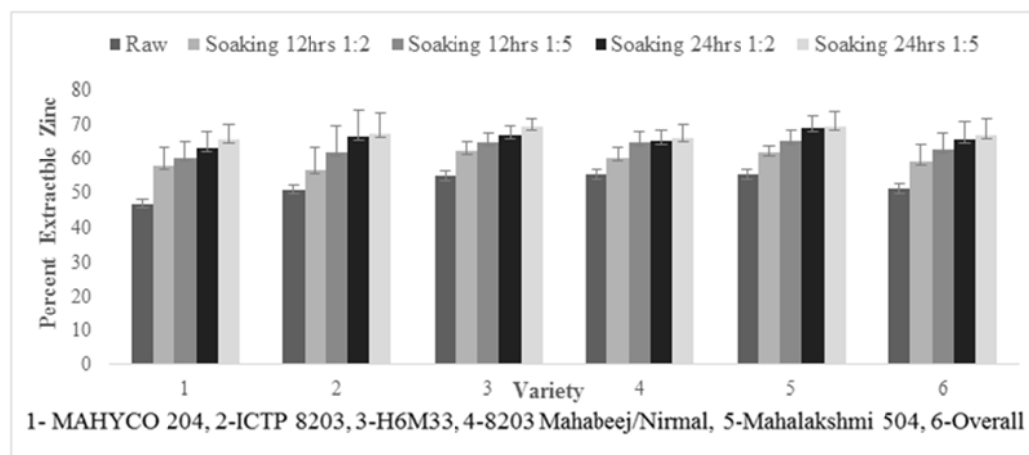


Figure 2. Effect of soaking on percent extractable zinc in five varieties of pearl millet

**Germination:** Germinated grains had a significantly lower iron and zinc content ( $p < 0.001$ ) than the raw grains (Table 4). Percent decrease in total iron was 17.5% when the grain: water ratio was 1:2, whereas when the ratio was 1:5, the decrease was approximately half (8.8%). Similarly there was a decrease in total zinc content with loss being 31.2 % in samples soaked with a grain: water ratio of 1:2 and 41.7 % when grain: water ratio was 1:5. The total zinc and iron content of germinated grains was similar to that in soaked grains.

The mean phytate content of germinated grains was significantly lower ( $p < 0.001$ ) than the raw grains for all the varieties, with the decrease being slightly more than 50% (Table 4). The percent decrease was slightly higher when the grain: water ratio was 1:2 than when it was 1:5. Also, the germinated grains had significantly lower phytate content ( $p = 0.000$ ) than their soaked counterparts.

The phytate: iron molar ratio in germinated grains was significantly lower than in both the raw and soaked samples (Table 4). However, the ratios were slightly higher when the grain: water ratio used for soaking was 1:5 than when it was 1:2. In case of zinc also, the phytate: zinc molar ratio in germinated samples was significantly lower than in the raw and soaked samples. With the 1:5 grain: water ratio, the phytate: zinc molar ratio was higher than that with the 1:2 grain: water ratio.

Ionizable iron content of germinated samples was significantly higher compared to the raw samples, but did not differ significantly from the soaked samples. As a percentage of total iron, the ionizable iron content was approximately 27% (Figure 3). Extractable zinc constituted about 70% of total zinc in the germinated samples (Figure 4). Compared to the raw and soaked grains, extractable zinc content in the germinated samples was only marginally higher.

**Malting:** In contrast to germination, malting did not significantly affect the mean total iron content for both the grain: water ratios (Table 5). Total zinc content of malted samples was significantly lower than in the raw, however, the content was similar to that in the germinated grains. Malting significantly reduced the phytate content when compared to the raw samples, with the percent decrease being about 61%. However, compared to the germinated samples with 1:2 grain: water ratio, the malted samples had 12.9% higher phytate content. In contrast, with 1:5 grain: water ratio, there was 19.6% decrease in phytate content. The phytate: iron molar ratio was about half that observed for the raw and soaked samples, with the difference being statistically significant ( $F = 40.058$ ,  $p = 0.000$ ). However, the malted samples did not differ significantly from germinated samples. A similar trend was observed for the phytate: zinc molar ratio ( $F = 23.539$ ,  $p = 0.000$ ).

As against total iron, the ionizable iron content was significantly higher in the malted than in the raw and germinated grains at both the grain: water ratios. Ionizable iron as a percentage of total iron was 43.3% for the 1:2 grain: water ratio and 52% for the 1:5 ratio (Figure 3). Extractable zinc as a percentage of total zinc was approximately 80%, which is slightly higher than in the raw and soaked grains (Figure 4).

**Correlation between percent ionizable iron, extractable zinc and phytate:** Phytate content was negatively and significantly correlated with ionizable iron content ( $r = -0.545$ ,  $p = 0.000$ ), percent ionizable iron ( $r = -0.653$ ,  $p = 0.000$ ), extractable zinc ( $r = -0.214$ ,  $p = 0.000$ ) and percent extractable zinc ( $r = -0.749$ ,  $p = 0.000$ ). Similarly, a significant negative correlation was observed between phytate: iron molar ratio and ionizable iron content ( $r = -0.614$ ,  $p = 0.000$ ) and between phytate: zinc molar ratio and extractable zinc content ( $r = -0.631$ ,  $p = 0.000$ ).

**Table 4.** Total and ionizable iron, total and extractable zinc and phytate content, phytate: mineral molar ratios in germinated pearl millet (mg/100gm DM)

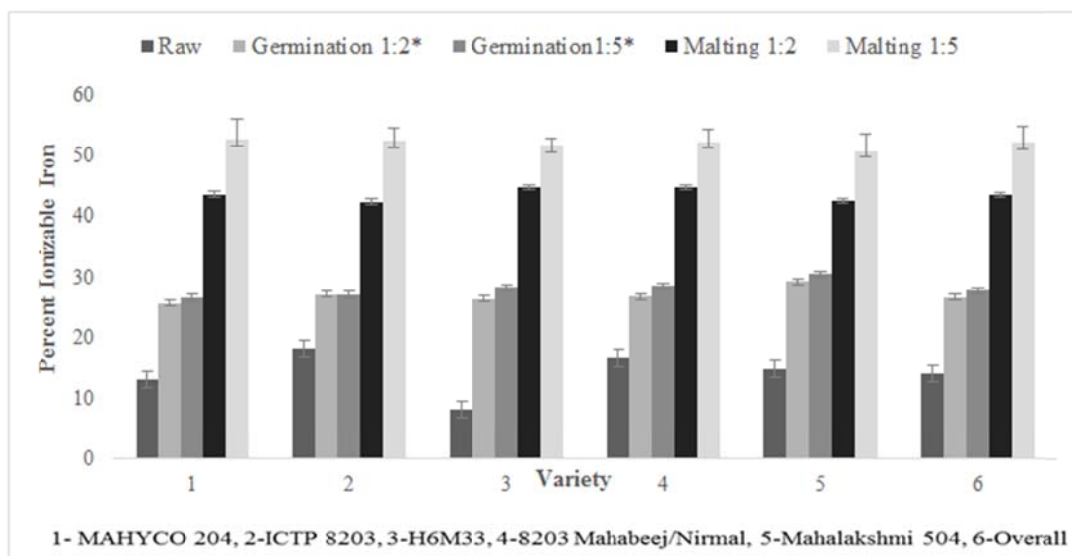
Parameter	Hours, Grain: Water Ratio	Variety					Overall
		MAHYCO 204	ICTP 8203	H6M33	8203-Mahabeej / Nirmal	Mahalakshmi	
<b>Total Iron</b>							
Mean $\pm$ SD	1:2	3.7 $\pm$ 1.0	3.7 $\pm$ 0.3	2.8 $\pm$ 0.2	3.3 $\pm$ 0.2	3.1 $\pm$ 0.3	3.4 $\pm$ 0.7
Min-Max		2.5-5.4	3.2-4.1	2.5-2.9	3.0-3.7	2.7-3.4	2.5-5.4
Mean $\pm$ SD	1:5	4.2 $\pm$ 1.0	3.6 $\pm$ 0.3	3.0 $\pm$ 0.2	3.1 $\pm$ 0.3	2.9 $\pm$ 0.1	3.5 $\pm$ 0.8
Min-Max		2.8-5.8	3.2-3.9	2.7-3.3	2.6-3.4	2.7-3.0	2.6-5.8
<b>Total Zinc</b>							
Mean $\pm$ SD	1:2	3.8 $\pm$ 1.1	3.1 $\pm$ 0.3	2.9 $\pm$ 0.4	3.3 $\pm$ 0.4	2.6 $\pm$ 0.3	3.3 $\pm$ 0.8
Min-Max		2.4-5.4	2.5-3.4	2.5-3.4	2.8-4.0	2.3-3	2.3-5.4
Mean $\pm$ SD	1:5	3.7 $\pm$ 1.0	2.9 $\pm$ 0.5	2.5 $\pm$ 0.4	3.6 $\pm$ 0.6	2.4 $\pm$ 0.3	3.1 $\pm$ 0.8
Min-Max		2.3-5.0	2.5-3.8	1.9-3.6	2.8-4.2	2.1-2.9	1.9-5.0
<b>Phytate</b>							
Mean $\pm$ SD	1:2	239 $\pm$ 102	384 $\pm$ 74	381 $\pm$ 162	251 $\pm$ 8	245 $\pm$ 225	293 $\pm$ 134
Min-Max		99-414	251-465	157-541	238-261	35-600	35-600
Mean $\pm$ SD	1:5	388 $\pm$ 1160	383 $\pm$ 1282	526.1 $\pm$ 87.2	386 $\pm$ 98	544 $\pm$ 113	428 $\pm$ 126
Min-Max		165-524	112-519	361-616	203-461	360-651	112-651
<b>Phytate: Iron Molar Ratio</b>							
Mean $\pm$ SD	1:2	5.9 $\pm$ 3.2	8.7 $\pm$ 1.6	11.6 $\pm$ 4.8	6.5 $\pm$ 0.3	7.1 $\pm$ 6.9	7.6 $\pm$ 4.0
Min-Max		1.7-12.9	5.9-11.1	4.7-16.1	6.1-6.9	0.9-8.6	0.9-18.6
Mean $\pm$ SD	1:5	8.6 $\pm$ 3.6	9 $\pm$ 2.9	15.2 $\pm$ 3.1	10.7 $\pm$ 2.6	16.2 $\pm$ 3.8	10.9 $\pm$ 4.4
Min-Max		2.5-13.2	2.9-12.3	9.4-17.9	6.6-13.7	10.3-20.5	2.5-20.5
<b>Phytate: Zinc Molar Ratio</b>							
Mean $\pm$ SD	1:2	6.8 $\pm$ 3.7	12.3 $\pm$ 1.8	13.5 $\pm$ 6.3	7.6 $\pm$ 0.7	9.7 $\pm$ 9.2	9.4 $\pm$ 5.2
Min-Max		1.9-15.4	9.6-14.9	5.3-20.9	6.5-8.4	1.2-23.9	1.2-23.9
Mean $\pm$ SD	1:5	11.3 $\pm$ 4.9	13.2 $\pm$ 4.9	21.5 $\pm$ 6.5	12.5 $\pm$ 4.1	22.6 $\pm$ 6.1	14.9 $\pm$ 6.7
Min-Max		3.2-12.9	3.5-18.7	11.7-31.4	6.5-16.6	12.4-28.4	3.2-31.4
<b>Ionizable iron</b>							
Mean $\pm$ SD	1:2	0.9 $\pm$ 0.2	1.0 $\pm$ 0.1	0.7 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.2
Min-Max		0.7-1.2	0.9-1.6	0.7-0.9	0.8-1.0	0.8-1.0	0.7-1.2
Mean $\pm$ SD	1:5	1.1 $\pm$ 0.3	1.0 $\pm$ 0.1	0.8 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.2
Min-Max		0.8-1.6	0.9-1.1	0.7-1.0	0.8-0.9	0.6-1.0	0.7-1.6
<b>Extractable Zinc</b>							
Mean $\pm$ SD	1:2	2.8 $\pm$ 0.8	2.1 $\pm$ 0.3	2.6 $\pm$ 0.3	2.3 $\pm$ 0.4	1.9 $\pm$ 0.3	2.3 $\pm$ 0.6
Min-Max		1.8-4.1	1.7-2.5	1.8-2.5	2.1-3.1	1.5-2.1	1.5-4.1



Mean ±SD	1:5	2.9±0.8	2.1±0.4	1.8±0.3	2.3±0.4	1.7±0.2	2.3±0.7
Min-Max		1.7-4.1	1.7-2.8	1.3-2.3	2.0-3.0	1.6-2.0	1.3-4.1

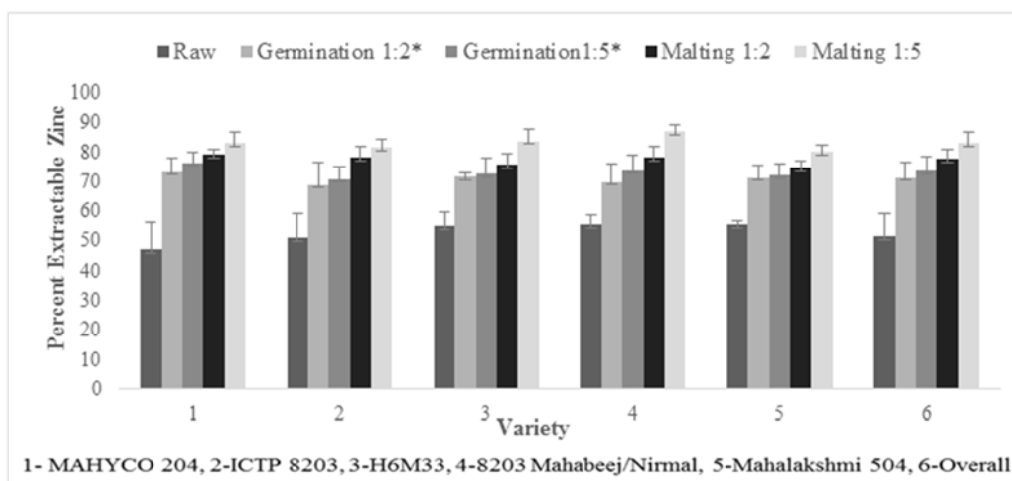
**Table 5.** Total and ionizable iron, total and extractable zinc and phytate content, phytate: mineral molar ratios in malted pearl millet (mg/100gm DM)

Parameter	Hours, Grain: Water Ratio	Variety					Overall
		MAHYCO 204	ICTP 8203	H6M33	8203-Mahabeej / Nirmal	Mahalakshmi	
<b>Total Iron</b>							
Mean ±SD	1:2	4.4±1.3	3.6±0.4	3.1±0.2	3.3±0.4	3.0±0.2	3.7±1.0
Min-Max		2.8-6.3	3.2-4.4	2.8-3.23	2.6-3.7	2.8-3.2	2.6-6.3
Mean ±SD	1:5	4.5±1.3	3.8±0.4	3.0±1.2	3.0±0.2	2.9±0.1	3.7±1.1
Min-Max		2.8-6.4	3.7-4.3	2.8-3.1	2.8-3.3	2.8-3.0	2.6-6.4
<b>Total Zinc</b>							
Mean ±SD	1:2	3.8±1.2	3.0±0.6	2.6±0.3	2.9±0.4	2.6±0.3	3.1±0.9
Min-Max		2.4-5.3	2.5-4.4	2.3-2.9	2.2-3.6	2.1-2.9	2.1-5.3
Mean ±SD	1:5	3.8±1.3	2.1±0.5	2.5±0.5	2.8±0.6	2.2±0.4	3.0±1.0
Min-Max		2.3-5.6	2.2-3.7	1.9-3.1	2.2-3.9	2.0-2.9	1.9-5.6
<b>Phytate</b>							
Mean ±SD	1:2	323±111	404±63	353±106	184±23	385± 89	331±111
Min-Max		160-505	283-478	236-510	139-204	320-536	139-536
Mean ±SD	1:5	294±86	313±68	467±92	271124	474±125	344±121
Min-Max		197-506	209-402	313-566	102- 471	269-562	102-566
<b>Phytate: Iron Molar Ratio</b>							
Mean ±SD	1:2	7.2±3.7	9.7±1.8	9.9±3.6	4.8±0.9	10.8±2.0	8.2±3.4
Min-Max		2.3-13.7	6.7-11.8	6.0-15.6	3.2-6.0	9.0-14.0	2.3-15.6
Mean ±SD	1:5	6.4±3.1	7.5±1.7	12.9±2.4	7.3±4.5	13.6±4.1	8.7±4.2
Min-Max		2.9-13.2	4.9-9.6	8.5-14.9	2.4-15.6	6.9-17.2	2.4-17.2
<b>Phytate: Zinc Molar Ratio</b>							
Mean ±SD	1:2	9.7±5.2	13.6±2.8	13.6±3.9	6.4±1.5	14.8±3.6	11.3±4.8
Min-Max		3.1-18.5	9.9-17.9	9.6-20.8	3.8-8.4	10.9-19.2	3.1-20.8
Mean ±SD	1:5	8.6±3.9	10.6±2.8	18.1±3.4	9.0±4.0	18.4±5.7	11.8±5.6
Min-Max		3.8-17.8	7.9-15.1	13.5-23.0	4.6-16.2	9.4-24.2	3.8-24.2
<b>Ionizable iron</b>							
Mean ±SD	1:2	1.9±0.5	1.5±0.2	1.4±0.1	1.5±0.2	1.3±0.2	1.6±0.4
Min-Max		1.2-2.8	1.3-1.9	1.2-1.6	1.1-1.7	1.1-1.5	1.1-2.8
Mean ±SD	1:5	2.3±0.7	2.0±0.2	1.5±0.1	1.6±0.1	1.4±0.1	1.91±0.6
Min-Max		1.5-3.2	1.7-2.2	1.4-1.7	1.4-1.8	1.4-1.5	1.4-3.2
<b>Extractable Zinc</b>							
Mean ±SD	1:2	3.0±1.0	2.3±0.5	1.9±0.2	2.3±0.4	1.9±0.2	2.4±0.8
Min-Max		1.9-4.4	1.9-3.4	1.7-2.2	1.6-2.7	1.5-2.1	1.5-4.4
Mean ±SD	1:5	3.1±1.0	2.4±0.4	2.1±0.5	2.4±0.5	1.8±0.3	2.5±0.8
Min-Max		2.00-4.6	1.8-3.0	1.5-2.7	2.00-3.2	1.5-2.3	1.5-4.6



\*1:2 and 1:5 represent grain: water ratios used for soaking

**Figure 3.** Percent ionizable iron in five varieties of raw, germinated and malted pearl millet



\*1:2 and 1:5 represent grain: water ratios used for soaking

**Figure 4.** Percent extractable zinc in five varieties of raw, germinated and malted pearl millet

## 4 Discussion

The total iron content of pearl millet observed in the present study was compared well with the range of values reported in literature [1, 10-13]. Velu, Rai and Sahrawat [14] also found that ICTP-8203 had the highest iron content in different varieties. However, values observed in the present study were lower than those reported by researchers from other countries such as Sudan [4,15,16], Nigeria [17,18] and the UAE [19].

The total zinc content observed in the present study was similar to values reported by Velu, Rai and Sahrawat [14], who reported zinc content to range from 2.7 to 5.7 mg per 100 grams in 68 varieties of pearl millet, among which ICTP-8203 had a higher zinc content (4.7 mg%). Similarly, in the present study, the same variety had a slightly higher mean zinc content (5.2 mg%) than the other four varieties examined. However, Dave et al., [20] and Sridevi, Yennagi and Basarkar [21] reported very low contents

(0.022 mg% and 0.73 mg%, respectively). Researchers from other countries (Lestienne et al., [2], Eyzaguirre et al., [22], Ahmed et al., [15] and Abdalla et al., [16]) have reported values in the range of 2.51-7.29 mg/100gm whereas Abdelrahman et al., [4] reported slightly lower values (1.26 - 1.79 mg/100gm). Differences between values observed for both iron and zinc in the present study and those reported by other researchers may be due to different geographic locations and/or different varieties.

Soaking has been reported to result in loss of total iron and zinc [2,15,22]. In the present study, content of these two minerals decreased after soaking, the losses ranging from approximately 20 to 40 percent, with losses being higher as soaking time increased from 12 to 24 hours and in case of iron also, with use of more amount of water for soaking i.e. grain: water ratio of 1:5. Afify et al., [5] reported losses of iron between 28.1% and 40.6% upon soaking sorghum. Loss of these minerals may be attributed to leaching of the ions into the soaking medium due to a concentration gradient [2]. Both iron and zinc are present in the pericarp, aleurone layer and germ of the grain [23]. Lestienne et al., [2] stated that the extent of leaching out of zinc is lower than that of iron and suggested that this may be because iron and zinc are not located in the same part of the seeds and are not linked with the same molecules.

In the present study, germination did not reduce the total iron content to the extent reported by other investigators [5,15,17]. Eyzaguirre et al., [22] observed 50% reduction in total iron content of pearl millet after germination for 4 days. Ahmed et al., [15] found that germination for 3 days reduced the iron content of pearl millet by approximately 2-3 mg. In the present study, the loss upon germination was 8.8% to 17.5%. This may be because the period of germination in the present study was short, i.e. 48 hours, compared to other investigators who studied the effects of germination for longer periods up to 96 hours.

Similarly, germination reduced the total zinc content. However, the extent of loss of zinc was greater than that of iron – 31.2% to 41.7%. The lower content in the germinated grain is largely due to the loss that occurred during soaking, as the content of these two minerals in the germinated samples was not significantly lower than in the soaked samples.

In the present study, malting did not significantly affect iron content compared to the soaked and germinated samples. Abdelrahman et al., [24] reported a minor, non-significant increase in the iron content of malted pearl millet and cereals, whereas other researchers [25,26] have reported decreased total iron content of malted finger millet, wheat and barley. A significant loss of 40% in the total iron content of pearl millet was reported by Rao and Deosthale [27]. In case of zinc, malted samples had lower content compared to raw samples but did not differ from germinated samples, indicating that the last two steps in the process of malting i.e. dehydration and roasting had no effect on the total iron and zinc content.

Phytate content of the raw grains of the five varieties varied considerably. Cheik et al., [28] also observed a wide range of phytic acid content (540-1430 mg/100 gm) in fourteen different cultivars of pearl millet from Burkina Faso. Other researchers, Eltayeb et al., [3], Abdelrahman et al., [4], Sankhala et al., [39], Lestienne et al., [2,40] have reported phytic acid content in the range of values observed in the present study.

Phytate content decreased significantly after each processing. After soaking, the percent reduction was approximately 29%, varying from 23.6% to 32.4%. Similar trends were observed by other investigators [2,3,5,31]. The reduction in phytate content caused by soaking may be due to solubilisation of phytic acid salts in water because it is stored in relatively water soluble form such as sodium and potassium phytate [5,32,33]. Also, phytate is present in the aleurone layer and pericarp and hence may leach out into the water used for soaking [34]. Duhan et al., [35] attributed the loss of phytate in Pigeon pea to leaching out of phytate ions into the soaking water under the influence of a concentration gradient. Further, Bartnik and Szafranska [36] suggested that soaking might activate endogenous phytases depending on the cereal/legume, pH and temperature. Besides activation of intrinsic phytases, Hotz and Gibson [37] suggested that there may be *de novo* synthesis of phytase. The extent of phytate reduction depends on the species, pH, length and conditions of soaking [4,37].

Germination has also been found to reduce phytate [5,38,39]. In the present study, germination resulted in additional loss of approximately 50%, compared to soaking. This decrease is attributable to the increased phytase activity during germination. Hydrolysis of phytate due to phytase activity is a step-wise process, with the phosphate being released through the formation of intermediates –inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol tri (IP3), di (IP2) and monophosphates. The final products are inorganic phosphorus, inositol and its intermediate forms of phytate, some of

which have poor capacity to bind minerals [40,41].

Malting also reduced the phytate content. Malted grains had 60% lower phytate content compared to the soaked samples, but the difference between malted and germinated samples was only 6.5%. As suggested for germination, the decrease in phytate content can be attributed to stimulation of phytase activity, through either *de novo* synthesis and/or activation of intrinsic phytases and formation of intermediate forms of phytate [37,42]. In the present study, malting involved roasting the germinated grains. Embaby [43] reported that roasting resulted in reduction of phytic acid in peanuts and brown/white sesame seeds; that was attributed to formation of insoluble phytate-protein and /or phytate-protein –mineral complexes. The reduction in phytate with heating could also result from the heat labile nature of phytic acid.

In the present study, we analysed the grains for ionizable iron content at pH 7.5, which is close to the pH of human duodenum, the site of iron absorption. The values for percent ionizable iron in raw samples obtained in the present study are in the range of values reported in the literature [4,6,8,10,11,13,17,44]. Rao and Prabhavathi [8] and Das et al., [11] who used the same method as was used in the present study reported that percent ionizable iron was 7.1 % and 13.75%, respectively. Other researchers [4,16,17,44] who used percent HCL extractable iron, found it to be 18.5-55.2%. Agte et al., [10] and Suma and Urooj [13] analyzed the percent dialyzable iron in raw pearl millet to predict iron availability and found it was 3.2% and 6.9% for selected varieties.

Soaking resulted in a slight increase in the ionizable iron content, although the magnitude differed between varieties. Ionizable iron as a percentage of total iron was higher in the soaked than in the raw grains. These findings are in agreement with reports in the literature. Eyzaguirre et al., [22] observed that soaking pearl millet for 24 hours in tap water, increased the percent soluble iron from 30% to 37%. As observed for ionizable iron, extractable zinc as a percentage of total zinc increased to a small extent after soaking.

Ionizable iron content was 23.3% more in the germinated grain than in the soaked grain with a grain: water ratio 1:2. However, when the grain: water ratio was 1:5, the ionizable iron content in the germinated grain was only 7.9% more than the soaked samples. As a percentage of total iron, it constituted about one-fourth. In contrast, the effect on extractability of zinc was less i.e. 17% when the grain: water ratio was 1:2 and 9.5% when it was 1:5. Malting increased the ionizability of iron substantially, the ionizable iron content being about two times more than in the raw and soaked samples and about 75% more than in the germinated samples.

Thus, ionizable iron as a percent of total iron was more than 40% indicating that malting has better potential to improve bioavailability of iron than do soaking and germination. However, malting did not greatly increase the extractability of zinc. The possible reasons for the increase in ionizable iron are the decrease in phytate content as a result of endogenous phytase activity [5,22] and possible reduction in other antinutrients like saponins and phenols [42,45].

Our results on the correlation between the contents of ionizable iron, extractable zinc and phytate support this. We observed strong negative correlation between phytate and ionizable iron, extractable zinc, respectively, indicating that reduction in phytate is an important determinant for improving the ionizability /extractability of these two minerals and thereby their bioavailability. However, the extent of increase in ionizable iron upon malting was more than the reduction in phytate content, suggesting that besides phytate degradation, there may be a positive effect on other inhibitory factors.

There was a significant negative correlation between the ionizable iron content and phytate:iron molar ratio and between the extractable zinc content and phytate:zinc molar ratio. It has been recommended that the phytate: iron molar ratio should be less than one and that of phytate:zinc should be less than 15 [46,47]. In the present study, although germination and malting were effective, they could not lower the ratio below the critical limit. This suggests that there is a need to lower the phytate content further. Greiner [48] reported that if soaking is carried out at 45 to 65°C at pH 5 to 6, as the optimum pH for endogenous phytase in cereals is 5.15, phytate dephosphorylation may be favoured. Thus, the conditions of soaking specifically in terms of the pH and temperature combination need to be optimized.

## 5 Conclusion

Results of this study indicate that availability of iron, zinc can be improved by simple domestic methods

i.e soaking, germination and malting. For both germination and malting, soaking is a preliminary step which decreases phytate and improve ionizable iron and extractable zinc. However, germination and malting improved these beyond the effect of soaking. Hence, these two processing steps may be recommended specially for foods which are prepared for younger children. Optimum time for soaking appears to be 12 hours with grain:water ratio 1:2 being preferable and also germination for 48 hours appears to be suitable. These simple techniques have great potential for scaling up.

**Conflict of Interest.** None

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