Influence of grain quality characteristics and basic processing technologies on the mineral and antinutrient contents of iron and zinc biofortified open-pollinated variety and hybrid-type pearl millet

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Summary

To determine whether mineral biofortified pearl millets will maintain significantly higher iron and zinc contents after processing, the effects of decortication, steeping/fermentation and parboiling on mineral, phytate and total phenolic contents in eight types (two biofortified hybrids Dhanashakti and ICMH 1201, five high-iron improved varieties and one traditional variety) was investigated. The hybrids gave higher iron and zinc contents after processing compared to the improved varieties; for example, 17-51% and 10-26% higher iron and zinc after steeping/fermentation followed by decortication compared to the best varieties. Phytate:mineral ratios also indicated that iron bioavailability is higher in the hybrids after processing, but still several times above the critical 1:1 ratio. Across all the types, iron content after processing was positively correlated ($p \leq 0.05$) with high kernel weight, large kernels and high fat content, and zinc with high fat. These kernel characteristics should aid selection of high iron and zinc pearl millet types.

Keywords Biofortification, decortication, fermentation, minerals, pearl millet, parboiling, phenolics, phytate, steeping

Introduction

Iron and zinc are deficient in the diets of many people in developing countries, especially in Africa. Data from nine African countries indicate iron deficiencies among women of childbearing age of 15-51% and among children under 5 years of 11-64% (Mwangi *et al.,* 2017). Concerning zinc, based on the availability in national food supplies and the prevalence of stunting, it has been estimated that the zinc intake of about 25% of the population in sub-Saharan Africa was inadequate during the period 2003-2007 (Wessells and Brown, 2012).

Pearl millet is a major cereal staple in Africa, notably in the more arid parts, where it is processed into a wide variety of products (Taylor, 2016). It accounts for one-third of the cereals crop in the Sahel region (Sahara desert margin) (FAOSTAT, 2016). Overall, the nutrient content of pearl millet grain is appreciable; being a major source of starch, protein, lipids and B-vitamins and a significant source of the essential minerals K, P, Mg, Ca, Fe and Zn in descending content (Taylor, 2016). However, like all cereals, pearl millet is high in phytate, approx. 700-1100 mg/100 g and also relatively high in polyphenols, approx. 500-800 mg ferulic acid equiv./100 g (Krishnan and Meera, 2017). Phytate binds to divalent mineral ions in general and polyphenols to iron in particular and inhibit their uptake and absorption by the body (Fairweather-Tait and Hurrell, 1996). In pearl millet, phytate and polyphenols have generally been found to be associated with reduced iron and zinc bioaccessibility (Krishnan and Meera, 2017).

As a consequence, processing methods such as decortication, soaking, lactic acid fermentation, sprouting and thermal treatment have been investigated with the aim of improving the mineral nutritive value of pearl millet (Lestienne *et al.,* 2005; Hama *et al.,* 2011; Hama *et al.,* 2012; Jha *et al.,* 2015). Additionally, there is intense ongoing research in several countries, which is coordinated by ICRISAT, the International Crops Research

Institute for the Semi-Arid Tropics, to select and develop pearl millet types, including hybrids, with enhanced iron and zinc contents using conventional breeding-type biofortification (Rai *et al.,* 2012; Kumar Are *et al.,* 2019). However, in view of the phytate and polyphenol-type antinutrients in the outer layers of the pearl millet grain and the fact that iron and zinc are also generally concentrated in the outer layers of the kernel (Minnis-Ndimba *et al.,* 2015), a key question is whether these biofortified pearl millets will still deliver significantly enhanced levels of iron and zinc after being processed into foods. To date, this important aspect has received very limited attention. Hama *et al.* (2012) compared the effects of progressive decortication (so-called dehulling) of pearl millet grains on the estimated iron and zinc bioavailability of two biofortified varieties compared to a traditional variety. They concluded that after grain decortication there would be no improvement in iron bioavailability but potentially there would be an improvement in zinc bioavailability.

The objectives of this study were therefore twofold: 1. To determine and compare the effects of the several commonly used basic grain processing technologies: abrasive decortication, steeping/fermentation and parboiling on the essential mineral (focus on iron and zinc) and phytate and phenolic antinutrient compound contents of biofortified pearl millet varieties and hybrids, and 2. To determine whether there were any associations between essential mineral content in processed pearl millet with particular grain quality characteristics.

Materials and methods

Pearl millet varieties

Eight different pearl millet types were used in this study. These comprised:

Six open-pollinated varieties - Mil Souna, a traditional variety from Senegal and five improved varieties with enhanced agronomic characteristics that are also noted to be high in iron - TP 8203, ICRI-TABI, GB 8735 and IBV 8004 (kindly provided by the Institute for Agricultural Research (ISRA) and the Food Technology Institute (ITA) in Senegal), and Kuphanjala-2 (also known as Okashana-2) (kindly provided by ICRISAT, Zimbabwe).

Two mineral biofortified hybrids that have been specifically bred for high iron and zinc contents (Govindaraj *et al.,* 2015) - ICMH 1201 and Dhanashakti (kindly provided by ICRISAT, India).

Grain processing

The grains were cleaned by aspiration using compressed air. Visible foreign material was removed by hand. The cleaned whole grain was processed using three different procedures:

Abrasive decortication for 5 minutes using a Tangential Abrasive Dehulling Device (TADD) (Venables Machine Works, Saskatoon, Canada), according to Gomez *et al.* (1997). The specific decortication time was selected by experimentation as it abraded off up 22% of the kernel by weight, which equates to most of the outside layers. Medium sized pearl millet kernels comprise an average of 24.9% by weight of germ and bran (Abdelrahman *et al.*, 1984).

Steeping followed by abrasive decortication - Whole grain (50 g) was steeped for 8 h at 30^{o}C with a particulate matter-free supernatant (25 mL) from a successful static mixed culture lactic acid bacteria fermentation of sorghum flour (ratio of flour to water 1:1.25 (w/w)), of pH 3.7. During steeping, the grain took up all the liquid. After steeping, the grain was dried at 35^{o}C for 24 h in a forced draught oven.

Parboiling followed by abrasive decortication – Whole grain (50 g) was spread on a 1400 µm mesh opening sieve and then stacked on another sieve on top of the open pan. The second sieve was included to help ensure that water did not splash on the grains. Parboiling was carried out for 20 min using rapidly generated steam.. After parboiling, the grain was dried as described above.

Milling

The processed grain was milled into flour using an IKA MF 10 air-cooled, laboratory mill (Staufen, Germany) fitted with a 500 µm diam. screen opening and stored at $10^{o}C$ for up to one month prior to chemical analyses.

Analyses

Thousand kernel weight (TKW)

This was determined by counting and weighing 1000 sound kernels of a representative sample in triplicate (Chiremba *et al.,* 2011).

Kernel size

This was determined by sieving sound grain through screen opening sizes of 2.36 mm and 1.70 mm, according to Gomez *et al.* (1997) using a vibratory sieve shaker. The test was performed in duplicate. The fractions were divided into three groups: large >2.36 mm, intermediate 1.70-2.36 mm and small <1.70 mm.

Grain hardness estimated by % dehulling loss

This was determined on sound raw grain using the TADD, as described above. The percentage of kernel removed over 5 minutes decortication was calculated as dehulling loss, where:

$$\% \text{ Dehulling loss} = \frac{[\text{Initial weight of the grain} - \text{final weight after decortication}]}{\text{Initial weight of the grain}} \times 100$$

Proximate analyses

Moisture by air-oven method, crude fat by Soxhlet extraction and crude protein (N x 6.25) by Dumas combustion were determined by AACC standard methods 44-15A, 30-25 and 46-30, respectively (AACC, 2000). Crude fibre was determined by a filter bag technique using an Ankom 2000 automated fibre analyzer (Macedon, NY, USA).

Mineral contents

Individual minerals were quantified using approved methods of the AOAC International (2002). Acid digestion was carried out in a heating block according to method 935.13. Accurately weighed samples (0.5 g) were digested using a 5:2 (v/v) ratio of 65% nitric acid and 70% perchloric acid at 240°C. Iron, zinc and magnesium were analysed by atomic absorption spectrophotometry using method 999.10. Calcium was determined using method 935.13 by titration with $KMnO_4$. For phosphorus, a colorimetric method involving reaction with molydovanadate reagent was used, according to method 965.17.

Phytate

Phytate content was determined using the extraction and assay procedure of Frühbeck *et al.* (1995). Dowex1-anion-exchange resin-AG 1 x 4 (4% Cross-linkage, chloride form, 100-200 mesh (74-149 μm) (Sigma-Aldrich, Johannesburg, South Africa) in glass barrel Econo-columns, 7 x 5 mm was used for purification of the extracts. The standard, sodium phytate (P-8810, Sigma-Aldrich) and purified extracts were reacted with Wade reagent (ferric chloride and sulphosalicylic acid), after which absorbance was measured at 500 nm. The phytate contents were used to calculate the phytate to iron and phytate to zinc molar ratios (Hurrell, 2004; Ma *et al.,* 2007).

Total phenolics

Total phenolic compounds were determined using a Folin-Ciocalteu assay, as described by Waterman and Mole (1994) using 0.103 mol/litre HCl in methanol as the extractant at a volume to flour weight ratio of 40:1(Price *et al.*, 1978). Catechin (C-1788, Sigma-Aldrich) was used as a standard and total phenolics were expressed as mg catechin equiv./100 g.

Statistical analyses

All experiments were performed at least twice. Data were analysed using one way and multifactorial analysis of variance (ANOVA) using Statistica v10 (Tulsa, USA) software. Pearson correlations were performed using Microsoft Excel XLSTAT software (Addinsoft, New York, USA).

Results and discussion

Grain physical characteristics and chemical composition

The two biofortified pearl millet hybrids differed in grain physical characteristics from the traditional and the improved high-iron pearl millet varieties. Their kernels were heavier, (TKW >13 g), generally of larger size (>50% larger than 2.36 mm) and generally harder (<12% dehulling loss) (Table 1). In contrast, Mil Souna, the traditional Senegalese pearl millet variety, had the lowest TKW, amongst the smallest small kernel size (<2% larger than 2.36 mm) and was the softest (22% dehulling loss). These data indicate that the biofortified hybrids would be better suited to mechanical decortication than the traditional variety on account of their high kernel weight and low dehulling loss (Chiremba *et al.*, 2011).

The biofortified hybrids also differed somewhat in chemical composition from the open-pollinated varieties, being generally higher in protein (>12%) and fat (≥5.5%). In contrast, they had generally lower crude fibre (branny-type cellulose, pentosan and lignin components) contents (<1.5%). Overall, however, the protein and fat contents of all the pearl

Table 1 Physical characteristics and general chemical composition of the pearl millet types

Type	Thousand kernel weight (g)	Grain size (% >2.36 mm)	Grain hardness (estimated by % dehulling loss by weight after 5 min decortication)	Protein (g/100 g db)	Fat (g/100 g db)	Crude fibre (g/100 g db)
Traditional variety						
Mil Souna	$6.9^{a}\pm0.2$	$1.2^{a}\pm0.0$	$22.0^{d}\pm0.5$	$11.6^{cd}\pm0.1$	$4.52^{b}\pm0.03$	$1.48^{b}\pm0.02$
Improved varieties						
ICRI-TABI	$8.1^{b}\pm0.1$	$2.5^{a}\pm0.1$	$16.7^{b}\pm0.1$	$10.2^{a}\pm0.2$	$5.00^{c}\pm0.00$	$2.24^{c}\pm0.07$
ICTP 8203	$9.5^{c}\pm0.3$	$12.0^{b}\pm0.6$	$15.8^{b}\pm0.3$	$11.3^{c}\pm0.2$	$4.48^{b}\pm0.18$	$1.54^{b}\pm0.07$
IBV 8004	$9.5^{c}\pm0.2$	$10.4^{b}\pm0.2$	$11.8^{a}\pm0.0$	$13.5^{f}\pm0.1$	$5.40^{e}\pm0.07$	$1.48^{b}\pm0.02$
GB 8735	$11.5^{d}\pm0.2$	$35.6^{c}\pm0.3$	$20.6^{c}\pm 0.3$	$10.6^{ab}\pm0.1$	$4.17^{a}\pm0.11$	$2.94^{d}\pm0.07$
Kuphanjala-2	$12.9^{e}\pm0.1$	$58.1^{e}\pm0.3$	$11.5^{a}\pm0.0$	$12.4^{e}\pm0.0$	$5.29^{d}\pm0.07$	$2.24^{c}\pm0.07$
Hybrids						
ICMH 1201	$13.6^{f}\pm0.1$	$51.8^{d}\pm1.0$	$11.9^{a}\pm0.2$	$12.3^{e}\pm0.1$	$5.50^{e}\pm0.14$	$1.23^{a}\pm0.05$
Dhanashakti	$14.7^{g}\pm0.2$	$78.8^{f}\pm0.6$	$11.2^{a}\pm0.1$	$13.4^{f}\pm0.1$	$6.50^{e}\pm0.28$	$1.49^{b}\pm0.03$

Values expressed as means of two independent samples analysed in duplicate (*n*=2) ±1 SD
Values with different superscripts, differ significantly ($p \leq 0.001$)

millet types were within the ranges reported in other studies (Lestienne *et al.,* 2007; Hama *et al.,* 2011).

Concerning mineral composition, the two biofortified hybrids had the highest iron contents, approximately three times higher than the traditional variety, which had the lowest iron content (Table 2). The improved varieties were all intermediate. The iron contents of improved varieties ICRI-TABI and GB 8735 were, however, much lower than those reported by Hama *et al.* (2012) for these two varieties, 4.31 and 5.63 mg/100 g versus 7.29 and 6.73, respectively. The value for ICRI-TABI was, however, similar to that reported by Bashir *et al.* (2014) (3.92 mg/100 g), which was the mean across four cultivation environments in the same season. These differences can be accounted for by the fact that cultivation environment as well as genotype was found to significantly affect ($p < 0.01$) the mineral contents of pearl millet varieties (Bashir *et al.,* 2014).

The two biofortified hybrids also had the highest zinc contents, with the improved variety IBV 8004 having the next highest content and all the other varieties, including Mil Souna, being similarly low in zinc. However, the spread for zinc contents was much lower than iron contents, with the hybrids only having approx. 50% more zinc than the varieties. As with iron, Hama *et al.* (2012) reported much higher contents of zinc in the improved varieties ICRI-TABI and GB 8735, 4.11 and 5.63 mg/100 g versus the 3.17 and 3.61 mg/100 g found in this present work. These differences are presumably also due to the effect of cultivation environment. Regarding other minerals, the two hybrids had among the highest calcium contents but their contents of phosphorus and magnesium where not exceptional.

Concerning antinutrients, the contents of phytate in the two hybrids was intermediate, whereas Mil Souna and ICRI-TABI were low in phytate, <900 mg/100 g (Table 3). The phytate values found here for ICRI-TABI and GB 8735 are quite similar to those reported by Hama *et al.* (2012), 830 ±20 and 1048 ±7 mg/100 g versus 780 and 950 mg/100 g,

Table 2 Effects of pearl millet type, abrasive decortication, steeping/fermentation in back slopped liquor and parboiling on iron, zinc, calcium, phosphorus and magnesium contents (mg/100 g db)

Type	Raw grain		Steeped/fermented grain		Parboiled grain		Means and effect of type (T)
	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated	
				Iron			
Traditional variety							
Mil Souna	3.04^{bcde}±0.15	3.23^{defgh}±0.08	1.33^{a}±0.04(-56)	3.48^{fgh}±0.08(+14)[+161]	2.84^{bcd}±0.11	3.59^{gh}±0.07(+18)[+26]	**2.92^{A}±0.79**
Improved varieties							
ICRI-TABI	4.31^{ij}±0.08	3.09^{bcdef}±0.08(-28)	5.10^{mn}±0.24(+18)	2.74^{bc}±0.08(-36)[-46]	4.96^{lmn}±0.07(+15)	2.73^{b}±0.08(-37)[-45]	**3.82^{B}±1.05**
ICTP 8203	4.50^{ijk}±0.15	3.33^{efgh}±0.08(-26)	5.01^{mn}±0.01(+11)	3.30^{efgh}±0.07(-27)[-34]	5.06^{mn}±0.22(+12)	2.70^{b}±0.15(-40)[-47]	**3.98^{B}±0.96**
IBV 8004	4.72^{jklm}±0.08	3.64^{h}±0.08(-23)	5.22^{no}±0.00(+11)	3.66^{h}±0.14(-22)[-30]	4.92^{klmn}±0.02	3.18^{cdefg}±0.07(-33)[-35]	**4.22^{C}±0.80**
GB 8735	5.63^{opq}±0.07	3.37^{efgh}±0.15(-40)	5.77^{pq}±0.07	4.44^{ij}±0.14(-21)[-23]	5.83^{q}±0.08	3.65^{h}±0.15(-35)[-37]	**4.78^{D}±1.06**
Kuphanjala-2	7.51^{s}±0.07	4.22^{i}±0.07(-44)	8.21^{t}±0.07(+9)	4.28^{ij}±0.15(-43)[-48]	9.59^{vw}±0.07(+28)	4.53^{ijkl}±0.00(-40)[-53]	**6.39^{E}±2.23**
Hybrids							
ICMH 1201	8.84^{u}±0.30	5.26^{no}±0.08(-40)	8.28^{t}±0.35(-6)	5.18^{n}±0.01(-41)[-37]	8.15^{t}±0.09(-9)	5.34^{nop}±0.30(-40)[-35]	**6.84^{F}±1.68**
Dhanashakti	9.55^{vw}±0.01	7.18^{s}±0.08(-25)	9.25^{uv}±0.08	6.70^{r}±0.08(-30)[-28]	9.80^{w}±0.03	6.31^{r}±0.00(-34)[-36]	**8.13^{G}±1.50**
Means and effect of processing (P)	**6.01^{C}±2.27**	**4.17^{B}±1.36 (-31)**	**6.02^{C}±2.45**	**4.22^{B}±1.21(-30)[-30]**	**6.39^{D}±2.42(+6)**	**4.00^{A}±1.25(-33)[-37]**	**PxT p ≤0.001**
				Zinc			
Traditional variety							
Mil Souna	3.37^{ijklmn}±0.08	3.40^{jklmn}±0.00	3.76^{opqr}±0.08(+12)	2.73^{abc}±0.08(-18)[-27]	3.66^{nopq}±0.07	4.18^{tu}±0.00(+24)[+14]	**3.52^{B}±0.46**
Improved varieties							
ICRI-TABI	3.17^{fghijk}±0.00	2.66^{ab}±0.08(-16)	3.38^{jklmn}±0.08	3.71^{opq}±0.07(+17)[+10]	3.25^{ghijkl}±0.07	2.79^{bcd}±0.00(-12)[-14]	**3.16^{A}±0.37**
ICTP 8203	3.18^{fghijk}±0.00	3.06^{defghi}±0.00	3.47^{klmno}±0.07	2.76^{abcd}±0.07(-13)[-20]	3.30^{hijklm}±0.15	2.80^{bcde}±0.15(-11)[-15]	**3.09^{A}±0.28**
IBV 8004	4.12^{stu}±0.00	3.75^{opqr}±0.08(-9)	4.26^{u}±0.15	3.88^{qrst}±0.01[-9]	3.85^{pqrs}±0.01	3.66^{nopq}±0.00(-11)	**3.92^{C}±0.22**
GB 8735	3.61^{mnopq}±0.00	2.88^{bcdef}±0.08(-20)	3.72^{opq}±0.07	3.52^{lmno}±0.08	3.64^{nopq}±0.00	3.54^{lmnop}±0.00	**3.48^{B}±0.29**
Kuphanjala-2	2.95^{bcdefg}±0.08	2.47^{a}±0.08(-16)	3.11^{efghij}±0.00	2.94^{bcdefg}±0.08[-5]	3.00^{cdefgh}±0.00	3.72^{l}±0.38(+26)[+24]	**3.03^{A}±0.40**
Hybrids							
ICMH 1201	4.36^{u}±0.00	4.12^{stu}±0.00	4.22^{u}±0.09	4.26^{u}±0.22	4.21^{u}±0.07	4.06^{rstu}±0.00	**4.21^{D}±0.13**
Dhanashakti	4.83^{v}±0.07	4.97^{v}±0.00	4.81^{v}±0.00	4.88^{v}±0.08	5.06^{v}±0.06	4.76^{v}±0.08	**4.88^{E}±0.12**
Means and effect of processing (P)	**3.70^{C}±0.65**	**3.41^{A}±0.81(-8)**	**3.84^{D}±0.54(+4)**	**3.59^{B}±0.74(-3)[-7]**	**3.75^{CD}±0.63**	**3.69^{BC}±0.66**	**PxT p ≤0.001**

Table 2 Continued

				Calcium			
Traditional variety							
Mil Souna	8.12^{m}±0.23	1.81^{bcde}±0.23(-78)	19.37^{s}±0.43(+139)	0.64^{abc}±0.00(-92)[-97]	19.90st±0.66(+145)	4.99^{hij}±0.23(-39)[-75]	**9.14^{C}±8.15**
Improved varieties							
ICRI-TABI	4.75^{hij}±0.23	7.49^{lm}±0.46(+58)	12.89^{no}±0.02(+171)	1.18^{abcd}±0.30(-75)[-91]	14.56^{p}±0.22(+207)	0.00^{a}(-100)[-100]	**6.81^{B}±5.72**
ICTP 8203	3.95^{ghi}±0.47	0.00^{a}(-100)	15.04^{pq}±0.03(+281)	0.00^{a}(-100)[-100]	16.46^{qr}±0.24(+317)	0.00^{a}(-100)[-100]	**5.91^{A}±7.43**
IBV 8004	13.72^{op}±0.54	6.03^{jkl}±0.24(-56)	23.80^{u}±0.21(+76)	3.72^{fgh}±0.24(-73)[-84]	21.48^{t}±0.84(+57)	2.58^{defg}±0.00(-81)[-88]	**11.89^{D}±8.80**
GB 8735	5.58^{ijk}±0.46	0.00^{a} [-100]	16.35^{qr}±0.24(+193)	0.00^{a}(-100)[-100]	17.17^{m}±0.69(+208)	0.00^{a}(-100)[-100]	**6.52^{AB}±7.85**
Kuphanjala-2	7.51^{lm}±0.71	1.48^{abcde}±0.23(-80)	20.61st±0.48(+174)	3.53^{fgh}±0.45(-53)[-83]	15.11^{pq}±0.45(+101)	6.80^{klm}±0.46(-9)[-55]	**9.17^{C}±6.96**
Hybrids							
ICMH 1201	11.79^{n} ±0.01	3.09^{efg}±0.23(-74)	28.74^{w}±1.56(+144)	0.65^{abc} ±0.00(-94)[-98]	25.99^{v}±1.56(+120)	1.60^{abcde}±0.00(-86)[-94]	**11.98^{D}±12.02**
Dhanashakti	8.30^{m}±0.22	2.27^{cdef}±0.00(-73)	19.42^{s}±0.22(+134)	0.32^{ab}±0.00(-96)[-98]	23.33^{u}±0.97(+181)	1.44^{abcde}±0.23(-83)[-94]	**9.18^{C}±9.46**
Means and effect of processing (P)	**7.96^{D}±3.28**	**2.77 ^{C}±2.62(-65)**	**19.52^{E}±4.90(+145)**	**1.25^{A}±1.47(-84)[-94]**	**19.25^{E}±4.02(+142)**	**2.18^{B}±2.44(-73)[-89]**	**PxT p ≤0.001**
				Phosphorus			
Traditional variety							
Mil Souna	296^{ghijklm}±4	238^{abcdef}±17(-20)	288^{fghijkl}±3	225^{abcd}±7(-24)[-22]	268^{cdefghij}±0	253^{cdefg}±16	**261^{B}±28**
Improved varieties							
ICRI-TABI	287^{fghijkl}±1	216^{abc}±2(-25)	263^{cdefghi}±1	194^{ab}±0(-32)[-26]	251^{cdefg}±0	186^{a}±8(-35)[-26]	**233^{A}±39**
ICTP 8203	343^{mnopq}±1	293^{ghijklm}±2	323^{klmnop}±0	238^{abcdef}±7(-31)[-26]	281^{efghijkl}±1(-18)	195^{ab}±26(-43)[-48]	**279^{B}±53**
IBV 8004	404^{rs}±9	229^{abcde}±167(-43)	363^{opqr}±1	316^{jklmno}±3(-22)[-13]	311^{ijklmno}±2(-23)	262^{cdefghi}±10(-35)	**314^{C}±79**
GB 8735	355^{nopqr}±1	196^{ab}±4(-45)	292^{ghijklm}±1(-18)	265^{cdefghij}±3(-25)	288^{fghijkl}±0(-19)	221^{abc}±18(-38)[-23]	**269^{B}±54**
Kuphanjala-2	416^{s}±10	281^{efghijkl}±29(-32)	389^{qrs}±2	327^{klmnop}±2(-21)	374^{pqrs}±2	307^{hijklmn}±1(-26)[-18]	**349^{D}±51**
Hybrids							
ICMH 1201	358^{nopqr}±4	282^{fghijkl}±3(-21)	276^{defghijk}±5(-23)	246^{bcdefg}±5(-31)	255^{cdefgh}±0(-29)	260^{cdefghi}±8(-27)	**279^{B}±39**
Dhanashakti	370^{pqrs}±7	333^{lmnop}±3	308^{ijklmn}±2(-17)	282^{fghijkl}±1(-24)	323^{klmnop}±4	230^{abcde}±39(-38)[-29]	**308^{C}±47**
Means and effect of processing (P)	**354^{E}±44**	**258^{B}±63(-27)**	**313^{D}±42(-12)**	**262^{B}±44(-26)[-16]**	**294^{C}±40(-17)**	**239^{A}±41(-32)[-19]**	**PxT p ≤0.05**

Table 2 Continued

				Magnesium			
Traditional variety							
Mil Souna	113^{lm}±4	93^{hi}±1(-18)	140^{tuvw}±0(+24)	81^{def}±2(-28)[-42]	131^{qrs}±8(+16)	107^{kl}±2(-5)[-18]	**111^{D}±21**
Improved varieties							
ICRI-TABI	103^{jk}±0	74^{cd}±1(-28)	127^{pqr}±1(+23)	67^{bc}±1(-35)[-47]	114^{lmn}±0(+11)	61^{ab}±1(-41)[-46]	**91^{A}±26**
ICTP 8203	114^{lm}±4	95^{hij}±0(-17)	142^{uvw}±1(+25)	79^{de}±2(-31)[-44]	120^{mnop}±1	66^{bc}±2(-42)[-45]	**103^{C}±27**
IBV 8004	146^{w}±3	123^{opq}±1(-16)	169^{x}±1(+16)	113^{lm}±3(-23)[-33]	138^{stuv}±2	89^{fgh}±2(-39)[-36]	**130^{G}±27**
GB 8735	118^{mno}±2	55^{a}±0(-53)	127^{pqr}±2(+8)	91^{ghi}±3(-23)[-28]	122^{nop}±1	77^{de}±2(-35)[-37]	**98^{B}±27**
Kuphanjala-2	139^{stuvw}±2	89^{fgh}±3(-36)	165^{x}±4(+19)	102^{jk}±3(-27)[-38]	143^{vw}±1	106^{kl}±2(-24)[-26]	**124^{F}±28**
Hybrids							
ICMH 1201	134^{rstu}±2	101^{jk}±0(-25)	138^{stuv}±0	96^{hij}±1(-28)[-30]	122^{nop}±1(-9)	96^{hij}±1(-38)[-21]	**114^{E}±19**
Dhanashakti	132^{rst}±4	116^{mno}±3(-12)	141^{uvw}±2(+7)	99^{ijk}±2(-25)[-30]	139^{tuvw}±0	84^{efg}±1(-36)[-40]	**118^{E}±22**
Means and effect of processing (P)	**125^{C}±15**	**93^{B}±21(-26)**	**144^{E}±15(+15)**	**91^{B}±14(-27)[-37]**	**129^{D}±10(+3)**	**86^{A}±17(-31)[-33]**	**PxT p ≤0.001**

Means of analysis of two independent samples (n=2) ± 1 SD

^{abc}- Values with different superscripts differ significantly (p ≤0.001) for iron, zinc, calcium, magnesium and differ significantly (p ≤0.05) for phosphorus,

^{ABC}- Least Significant Mean values from main effects Factorial ANOVA with different superscripts in the same row/column, differ significantly (p≤0.001) for iron, zinc, calcium, magnesium and differ significantly (p≤0.05) for phosphorus

() – values in brackets are the difference (% change where significant – p ≤0.001) in the mineral content of processed whole grain (steeped or parboiled) and decorticated raw and processed grain (steeped or parboiled) compared to the raw whole grain pearl millet

[] – values in square brackets are the difference (% change where significant – p ≤0.001) in the mineral content of decorticated processed grain (steeped or parboiled) compared to whole grain processed (steeped or parboiled) pearl millet

Table 3 Effects of abrasive decortication, pearl millet type, steeping/fermentation in back slopped liquor and parboiling on phytate and total phenolic contents (mg/100 g, db)

Type	Raw grain		Steeped/fermented grain		Parboiled grain		Means and effect of type (T)
	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated	
				Phytate			
Traditional variety							
Mil Souna	$896^{hijklmn}$±14	816^{fghij}±7	684^{cde}±20(-24)	627^{bcd}±38(-30)	763^{efg}±8(-15)	667^{bcde}±10(-26)	**742^{B}±98**
Improved varieties							
ICRI-TABI	830^{ghijk}±20	571^{b}±15(-31)	762^{efg}±14	457^{a}±11(-45)[-40]	812^{fghi}±13	676^{cde}±18(-19)[-17]	**685^{A}±141**
ICTP 8203	1251^{r}±18	748^{efg}±20(-40)	1228^{r}±28	726^{ef}±6(-42)[-41]	907^{ijklmn}±47(-27)	846^{ghijkl}±7(-32)	**951^{D}±223**
IBV 8004	1395^{s}±8	833^{ghijk}±33(-40)	821^{fghij}±6(-41)	807^{fgh}±41(-42)	1188^{r}±58(-15)	913^{jklmno}±10(-35)[-23]	**1063^{F}±238**
GB 8735	1048^{pq}±7	760^{efg}±8(-27)	1011^{opq}±58	748^{efg}±56(-27)[-26]	971^{nop}±17	724^{def}±43 (-31) [-25]	**877^{C}±144**
Kuphanjala-2	1360^{s}±30	1254^{r}±21(-8)	1008^{opq}±2(-26)	936^{lmno}±35(-30)	1254^{r}±9(-8)	620^{ab}±19 (-54)[-51]	**1002^{E}±260**
Hybrids							
ICMH 1201	1088^{q}±8	943^{lmno}±27(-13)	754^{efg}±29(-31)	698^{cde}±33(-36)	832^{ghijk}±12(-24)	762^{efg}±11(-30)	**846^{C}±140**
Dhanashakti	1221^{r}±43	943^{lmno}±46(-23)	870^{hijklm}±52 (-29)	832^{ghijk}±31(-32)	953^{mnop}±16(-22)	928^{klmno}±40(-24)	**958^{D}±134**
Means and effect of processing (P)	**1136^{E}±200**	**859^{C} ±193 (-24)**	**892^{C}±175 (-21)**	**729^{A}±142 (-36) [-18]**	**960^{D}±172(-15)**	**767^{B}±114 (-32)[-20]**	**P×T p ≤0.001**
				Total phenolics			
Traditional variety							
Mil Souna	293^{opqrst}±13	182^{bcdef}±7(-38)	277^{nopqrs}±22	197^{cdefg}±7(-33)[-29]	268^{mnopqr}±18	$202^{cdefghi}$±14(-31)[-25]	**236^{AB}±47**
Improved varieties							
ICRI-TABI	263^{lmnopq}±18	$219^{efghijkl}$±8	349^{uv}±13(+33)	182^{bcdef}±14(-31)[-48]	186^{cdef}±14(-29)	126^{a}±12(-52)[-32]	**221^{A}±74**
ICTP 8203	297^{pqrst}±14	$233^{ghijklmn}$±13(-22)	246^{ijklmn}±13(-17)	141^{ab}±11(-53)[-43]	$244^{hijklmn}$±8(-18)	169^{abcd}±6(-43)[-31]	**222^{A}±55**
IBV 8004	293^{opqrst}±6	273^{nopqr}±13	319^{stuv}±15	172^{bcd}±15(-41)[-46]	274^{nopqr}±8	189^{cdefg}±6(-35)[-31]	**253^{BC}±57**
GB 8735	251^{jklmno}±19	160^{abc}±15(-36)	253^{klmnop}±7	$209^{defghijk}$±6(-17)[-17]	$242^{hijklmn}$±11	$220^{efghijkl}$±13	**222^{A}±35**
Kuphanjala-2	354^{v}±13	$234^{ghijklmn}$±12 (-34)	307^{qrstu}±6(-13)	$207^{defghij}$±6(-42)[-33]	191^{cdefg}±7(-46)	178^{bc}±10(-50)[-7]	**245^{B}±67**
Hybrid							
ICMH 1201	331^{tuv}±20	276^{nopqrs}±7(-17)	326^{tuv}±6	$226^{fghijkl}$±5(-32)[-31]	247^{ijklmn}±14(-25)	195^{cdefg}±6(-41)[-21]	**267^{C}±53**
Dhanashakti	403^{w}±9	307^{qrstu}±9(-24)	310^{rstuv}±29(-23)	199^{cdefgh}±10(-51)[-36]	275^{nopqrs}±26(-32)	264^{lmnopq}±7(-34)	**293^{D}±65**
Means and effect of processing (P)	**311^{C}±49**	**235^{B}±49(-24)**	**298^{C}±37**	**192^{A}±26(-38)[-36]**	**241^{B}±35**	**193^{A}±39(-38)[-20]**	**P×T p ≤0.001**

Means of analysis of two independent samples (n=2) ± 1 SD

abc- Values with different superscripts differ significantly (p ≤0.05),

ABC- Least Significant Mean values from main effects Factorial ANOVA with different superscripts in the same row/column, differ significantly (p ≤0.05)

() – values in brackets are the difference (% change where significant – p ≤0.001) in the phytate or total phenolic content of processed whole grain (steeped or parboiled) and decorticated raw and processed grain (steeped or parboiled) compared to the raw whole grain pearl millet

[] – values in square brackets are the difference (% change where significant – p ≤0.001) in the phytate and total phenolic content of decorticated processed grain (steeped or parboiled) compared to whole grain processed (steeped or parboiled) pearl millet respectively. The two hybrids had the highest total phenolic contents, 331 ±20 and 403 ±9 mg/100 g in ICMH 1201 and Dhanashakti, respectively. By comparison, the contents of total phenolics in ICRI-TABI and GB 8735, 263 ±18 and 251 ±19 mg/100 g were similar to those reported by Hama *et al.* (2012) 290 and 260 mg/100 g, respectively.

Regarding associations between pearl millet iron and zinc contents and grain quality parameters, iron content was significantly positively correlated ($p \leq 0.05$) with kernel weight, large kernels, and fat content, and inversely with percentage dehulling loss (i.e. positively with grain hardness) (Table 4A.). With cereal grains, fat content is related to the proportion of the kernel that is germ, i.e. high fat content is indicative of a proportionally larger germ. The relationship between iron and zinc and fat content is because the pearl millet germ is rich in these minerals (Minnis-Ndimba *et al.*, 2015). Regarding antinutrients, phytate content was significantly correlated with protein content, presumably because of its high concentration in the protein-rich germ. Total phenolics were significantly correlated with kernel weight, large kernels, fat and protein content, similar to that of iron because they are both concentrated in the outer layers of the grain.

Effects of processing on mineral and antinutrient content of the varieties

Decortication

Across the eight types, decortication reduced the mean iron and zinc contents by 31% and 8%, respectively (Table 2). The difference reflects the fact that the iron in pearl millet kernel is highly concentrated in the outer layers of the kernel, whereas zinc is concentrated in the embryo (Minnis-Ndimba *et al.*, 2015). After decortication, the two biofortified hybrids still had the highest iron and zinc contents, iron 25-70% higher and zinc 10-25% higher than the highest improved varieties. This is despite the fact that the reductions in iron concentration were 25% for Dhananshakti and 40% for ICMH 1201. Their zinc

Table 4 Pearson correlation matrixes of pearl millet grain micronutrient content and quality attributes under differing processing conditions (values in bold are significant at p ≤0.05)

A-Whole Grain

Variables	Kernel weight	Large kernels	% Dehulling loss	Crude fibre	Fat	Protein	Phytate	Total phenolics	Fe	Zn	Mg
Large kernels	**0.976**										
% Dehulling loss	-0.648	-0.593									
Crude fibre	-0.008	-0.008	0.405								
Fat	0.642	0.671	**-0.815**	-0.476							
Protein	0.460	0.482	**-0.728**	-0.607	**0.756**						
Phytate	0.439	0.403	-0.702	-0.203	0.373	**0.723**					
Total phenolics	**0.722**	**0.805**	-0.705	-0.471	**0.845**	**0.729**	0.476				
Fe	**0.977**	**0.958**	**-0.713**	-0.154	**0.753**	0.505	0.371	**0.790**			
Zn	0.576	0.537	-0.461	-0.476	**0.715**	0.647	0.203	0.519	0.629		
Mg	0.564	0.530	**-0.752**	-0.325	0.588	**0.886**	**0.811**	0.556	0.554	0.505	
P	0.612	0.574	-0.705	-0.017	0.413	0.704	**0.924**	0.481	0.532	0.264	**0.903**

B-Decorticated grain

Variables	Kernel weight	Large kernels	% Dehulling loss	Crude fibre	Fat	Protein	Phytate	Total phenolics	Fe	Zn	Mg
Large kernels	**0.976**										
% Dehulling loss	-0.648	-0.593									
Crude fibre	-0.008	-0.008	0.405								
Fat	0.642	0.671	**-0.815**	-0.476							
Protein	0.460	0.482	**-0.728**	-0.607	**0.756**						
Phytate	0.626	0.690	-0.552	-0.109	0.419	0.593					
Total phenolics	0.553	0.517	**-0.901**	-0.664	**0.907**	**0.781**	0.328				
Fe	**0.832**	**0.867**	-0.634	-0.389	**0.877**	0.642	0.458	**0.762**			
Zn	0.479	0.484	-0.421	-0.661	**0.732**	0.683	0.100	**0.723**	**0.820**		
Mg	0.183	0.190	-0.671	**-0.848**	0.705	**0.894**	0.301	**0.837**	0.515	0.698	
P	0.602	0.650	-0.637	-0.586	0.678	0.565	0.516	**0.732**	**0.776**	0.574	0.595

Table 4 Continued

C-Steeped Decorticated Grain

Variables	Kernel weight	Large kernels	% Dehulling loss	Crude fibre	Fat	Protein	Phytate	Total phenolics	Fe	Zn	Mg
Large kernels	**0.976**										
% Dehulling loss	-0.648	-0.593									
Crude fibre	-0.008	-0.008	0.405								
Fat	0.642	0.671	**-0.815**	-0.476							
Protein	0.460	0.482	**-0.728**	-0.607	**0.756**						
Phytate	0.627	0.652	-0.539	-0.022	0.354	0.679					
Total phenolics	0.543	0.565	-0.067	0.168	0.254	0.100	0.145				
Fe	**0.887**	**0.915**	-0.480	-0.205	**0.714**	0.575	0.536	0.532			
Zn	0.630	0.578	-0.547	-0.226	**0.787**	0.459	0.072	0.376	**0.725**		
Mg	0.533	0.504	-0.616	-0.197	0.486	**0.845**	**0.824**	0.273	0.511	0.358	
P	0.508	0.516	-0.588	0.031	0.387	**0.726**	**0.933**	0.149	0.399	0.132	**0.915**

D-Parboiled Decorticated Grain

Variables	Kernel weight	Large kernels	% Dehulling loss	Crude fibre	Fat	Protein	Phytate	Total phenolics	Fe	Zn	Mg
Large Kernels	**0.976**										
% Dehulling loss	-0.648	-0.593									
Crude fibre	-0.008	-0.008	0.405								
Fat	0.642	0.671	**-0.815**	-0.476							
Protein	0.460	0.482	**-0.728**	-0.607	**0.756**						
Phytate	0.252	0.187	-0.452	-0.498	0.492	0.600					
Total phenolics	0.587	0.651	-0.105	0.149	0.414	0.528	0.464				
Fe	**0.863**	**0.914**	-0.521	-0.281	**0.763**	0.579	0.222	**0.731**			
Zn	0.539	0.638	-0.244	-0.373	0.611	0.669	0.238	**0.844**	**0.850**		
Mg	0.197	0.293	-0.132	-0.287	0.190	0.524	-0.275	0.345	0.446	0.669	
P	0.344	0.395	-0.396	-0.146	0.277	0.601	-0.229	0.223	0.416	0.516	**0.911**

concentrations were not significantly reduced, reflecting its different distribution in the kernel. The average (mean) reduction in phytate content and in total phenolic content across the pearl millet types was 24% (Table 3), similarly high to that of iron because of their concentration in the pearl millet kernel peripheral tissues (Hama *et al.,* 2012).

Correlations were also determined between the physico-chemical composition of whole pearl millet kernels and iron and zinc content before and after processing (Table 4). This was done to investigate whether there were any associations which would be useful as early stage selection criteria in breeding programmes for high iron and zinc pearl millet lines that would retain superior mineral levels after processing. After decortication, iron content was still significantly correlated with the raw whole grain kernel weight, large kernels and fat content (Table 4B). Zinc was only significantly correlated with fat content. There were no significant correlations between phytate content and any of the grain quality characteristics. However, total phenolics were significantly negatively correlated with percentage dehulling loss (i.e. positively with grain hardness), reflecting their concentration in the pericarp.

Steeping/fermentation followed by decortication

Across the eight types, steeping/fermentation alone did not affect iron and zinc content (Table 2). This is due to the fact that all the steeping liquor was taken up by the grains so that there were no losses by leaching. However, decortication of the dried steeped kernels reduced the average iron and zinc contents by 30% and 3%, respectively, similar to the levels of reduction as occurred with decortication alone. Furthermore, as with decortication alone the two biofortified hybrids still had by the highest contents of iron and zinc; iron 17-51% and zinc 10-26% higher than the highest improved varieties. This was despite the high losses of iron, 30% for Dhanashakti and 41% for ICMH 1201, caused by decortication following steeping/fermentation. Notably, steeping/fermentation alone significantly ($p \leq 0.05$) reduced the phytate content of the eight pearl millet types, by an average of 21% (Table 3). The well-known reduction in phytate when cereal grains are subjected to lactic acid bacteria fermentation is attributable primarily to the phytase activity of the bacteria (Taylor and Kruger, 2019). Furthermore, steeping/fermentation followed by decortication reduced phytate to a greater extent, average 36% compared to the 24% of decortication alone. However, steeping/fermentation alone did not significantly reduce ($p > 0.05$) total phenolic content, presumably also because all the steeping liquor was taken up by the kernels. Decortication of the steeped kernels reduced the total phenolic content by rather more than decortication alone, by an average of 38% compared to 24%. This is possibly because the acidic conditions of steeping solubilised some of the bound phenolics in the endosperm, leading to a change in their distribution to the outer layers of the kernel so that they were removed by decortication.

After steeping/fermentation followed by decortication, iron content was significantly correlated with raw whole grain kernel weight, large kernels and fat content (Table 4C)., Zinc was only significantly correlated with fat content, as with was the case with just decorticated grain (Table 4B). Also likewise, after decortication there were no significant correlations between phytate content and any of the grain quality characteristics (Table 4C). With total phenolics, there were also no significant correlations with any of the raw whole grain quality characteristics. This is possibly due the acidic conditions of steeping solubilising some of the bound phenolics in the endosperm, as described above.

Parboiling followed by decortication

Across the eight types, parboiling alone did not greatly affect iron and zinc content (Table 2). This would be expected as this treatment involves the use of steam and so there would be no leaching. However, when the dried parboiled kernels were decorticated, there was a slight but significantly greater losses ($p \leq 0.05$) in iron, magnesium and phosphorus compared to kernels that had been solely decorticated and decorticated steeped/fermented kernels. Zinc content, in contrast, was not affected by decortication of the parboiled kernels, again presumably because its concentration in the germ. Nevertheless, the two biofortified hybrids retained their higher iron and zinc contents, iron 18-39% and zinc 9-28% higher than the highest improved varieties.

The generally higher mineral losses with decorticated parboiled pearl millet compared to decortication alone are in agreement data on the effect of parboiling pearl millet by Serna-Saldivar *et al.* (1994) on calcium, magnesium and phosphorus contents. This confirms that parboiling is not an effective technology for pearl millet to redistribute essential minerals from the outer part of the kernel into the endosperm, unlike the situation with rice (Rocha-Villarreal *et al.,* 2018).

With parboiling the mean contents of phytate were substantially lower ($p \leq 0.05$) compared to those in the raw whole and decorticated grain but somewhat higher ($p \leq 0.05$) than in the steeped/fermented and their decorticated kernels (Table 3). A reduction in phytate when pearl millet was steamed was also observed by Jha *et al.* (2015). This reduction can be attributed to partial thermal hydrolysis of phytate to free myo-inositol (Metzler-Zebeli *et al.,* 2014). The total phenolic contents of the whole parboiled kernels were substantially lower ($p \leq 0.05$) than in both the raw and steeped/fermented kernels. However, such observed reductions in phenolics due to thermal treatments may not be due to actual losses but rather due the phenolics being rendered less extractable as a result of increased binding with other kernel components (Taylor and Duodu, 2015).

Overall, with parboiling followed by decortication iron content was again significantly correlated with raw whole grain kernel weight, large kernels and fat content (Table 4D). However, there were no significant correlations between zinc content and any of the raw whole grain quality characteristics. Neither were there any significant correlations between phytate or total phenolics and the raw whole grain quality characteristics.

Effects of pearl millet type and processing on estimated mineral availability (phytate:mineral molar ratios)

Phytate:mineral molar ratios are an indicator for effective mineral absorption and thus serve as a predictor of mineral bioavailability in food (Lazarte *et al.,* 2015). Generally, processing of pearl millet grains had no clear effects on phytate:iron molar ratios. However, the two biofortified hybrids Dhanashakti and ICMH 1201 consistently had the lowest phytate:iron ratios both before and after processing, steeping and parboiling alone resulting the lowest ratios, 7-8:1 (Fig. 1). However, none of the treatments reduced the phytate:iron ratio close to the critical level of 1 where mineral absorption is not seriously inhibited (Hurrell, 2004). This was because whilst a reduction in phytate occurred with the treatments, iron content was also reduced simultaneously.

Concerning zinc, as with iron, the hybrids Dhanashakti and ICMH 1201 generally had somewhat lower phytate:zinc molar ratios (16-25:1) compared to the varieties (12-50:1)(Fig. 2). In contrast to the lack of effect with iron, all processing operations generally reduced the phytate:zinc molar ratios. Dhanashakti and ICMH 1201 when processed by steeping/fermentation alone and when followed by decortication and by parboiling alone and when followed by decortication had phytate:zinc ratios <20:1, approaching the level of 15:1 that enables moderate zinc absorption (Ma *et al.,* 2007).

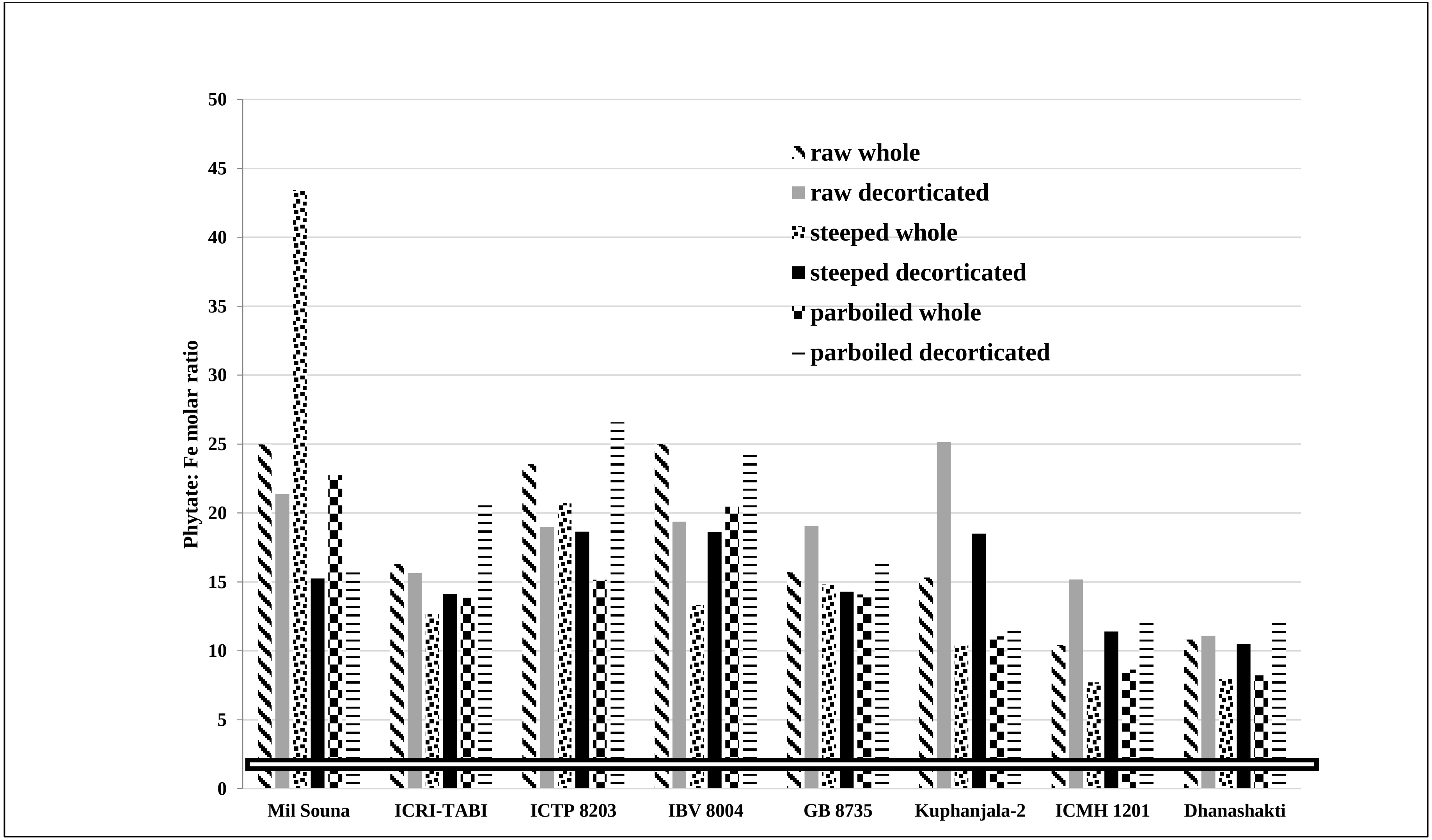

Figure 1 Phytate: iron molar ratio indicating the effects of pearl millet type and processing on iron availability. Critical level above which iron absorption is seriously impaired >1 (Hurrell, 2004)

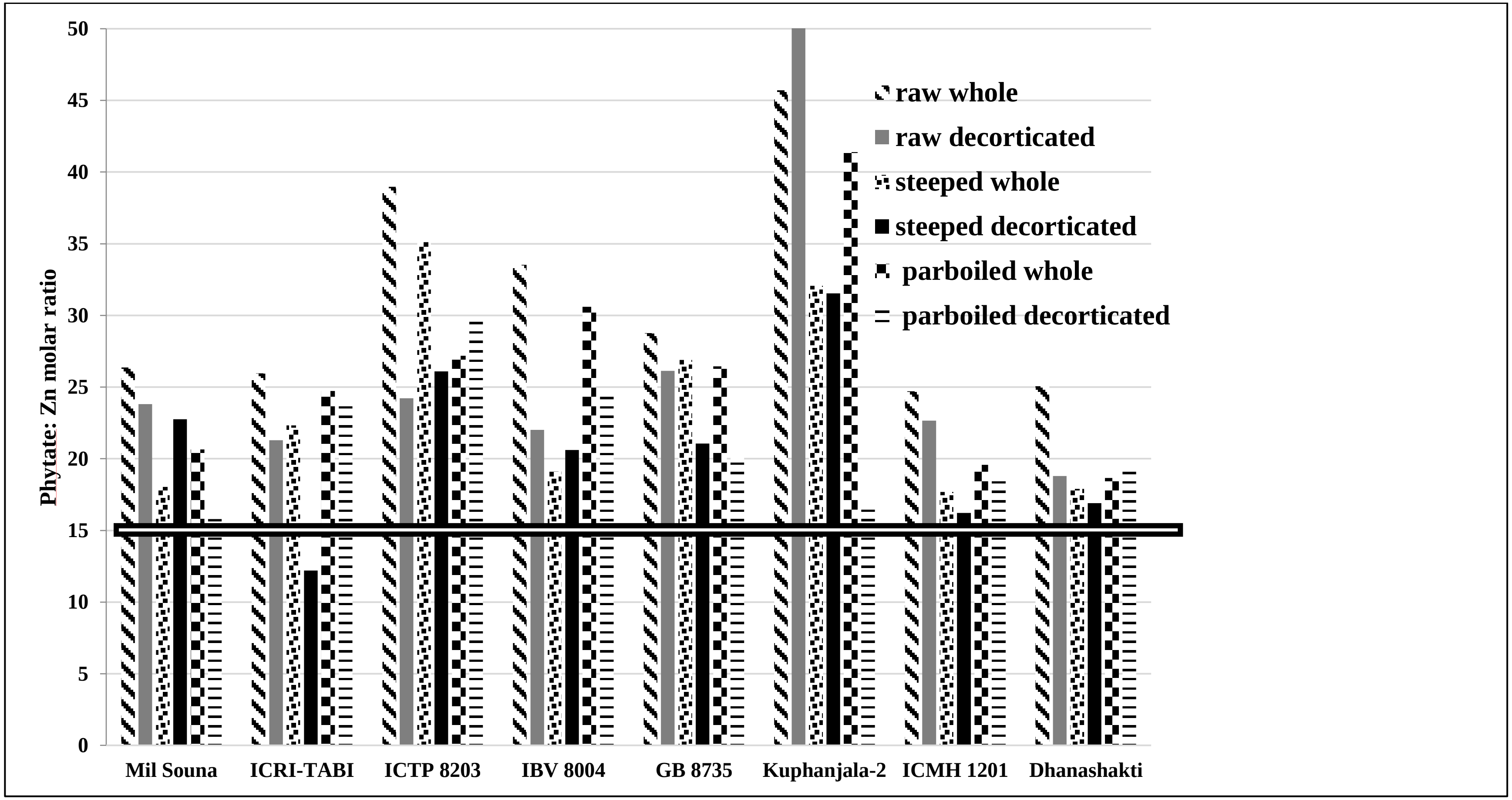

Figure 2 Phytate: zinc molar ratio indicating the effects of pearl millet type and processing on zinc availability. Critical level above which zinc absorption is seriously impaired >15 (Ma *et al.*, 2007)

Conclusions

Mineral biofortified hybrid-type pearl millet kernels have high contents of iron and zinc and generally still have higher contents of these minerals after being subjected to basic grain processing operations when compared to high mineral improved varieties. Furthermore, on the basis of phytate:mineral molar ratios it appears that iron bioavailability is higher in the biofortified hybrids both before and after processing, although processing does not improve the low ratio of iron to phytate. With zinc, the biofortified hybrids also have slightly higher ratios of zinc to phytate than the high mineral improved varieties and processing generally improves the ratio across all pearl millet types.

Although the number of pearl millet types studied was relatively small, there are some clear relationships between kernel characteristics and contents of iron and zinc in the processed grain. Across all the types, iron content of the processed pearl millet is associated with high kernel weight, large kernels and high fat content and zinc with high fat content. These are all valuable grain kernel quality characteristics. Hence, the associations with high levels of iron and zinc before and after processing should be a useful tool as an early stage selection criterion in breeding programmes for mineral biofortified pearl millet lines.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical guidelines

Ethics approval was not required for this research.

Research data

Research data are not shared.

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