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Pearl millet: Influence of mineral biofortification and simple processing technologies on minerals and antinutrients

By

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DECLARATION

I declare that the dissertation herewith submitted for the degree MSc Food Science at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

John Gwamba

November 2016

DEDICATION

I would like to dedicate this dissertation to my late Grandfather, Gaopalelwe 'Turuma' Gwamba and my family (Grandmother; Chindi, Father; Paul 'Mpinde', Mother; Bettina, and siblings) for their amazing positive role in my life. Above of all, to God for his continued provision of light and wisdom.

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ABSTRACT

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Supervisor: Prof J. R. N. Taylor

Co-supervisor: Dr J. Kruger

Pearl millet is an important staple food in rural Africa. However, the mineral bioavailability of pearl millet is low due to its high content of antinutrients, particularly phytate. This research investigated the effects of mineral biofortification, steeping/lactic acid fermentation and parboiling alone and in combination with abrasive decortication of pearl millet grain on its mineral and antinutrient contents. Six normal varieties and two mineral biofortified hybrids were investigated.

There was considerable variability in mineral content among the varieties. Iron content ranged from 3.0 to 9.6 mg /100 g and zinc from 3.0 to 4.8 mg /100 g. The mineral biofortified hybrids Dhanashakti and ICMH 1201 had substantially higher iron (21-68%) and zinc (15-39%) contents compared to the normal varieties. Phytate content differed substantially, with levels from 830 to 1360 mg /100 g. There was no definite trend between the phytate content of normal and mineral biofortified types.

Decortication did not cause significant losses in zinc, but resulted considerable iron losses (mean 31%) across the varieties. There were minimal effects of steeping/lactic acid fermentation and parboiling on iron and zinc contents. Mineral biofortified hybrids were associated with high iron and zinc content after all processing treatments. Decortication of raw grain substantially reduced phytate (mean 24%) and by a further 12 percentage points when applied after steeping/lactic acid fermentation. Parboiling plus decortication was less effective in reducing phytate content. The critical phytate: iron molar ratio of <1, above which iron absorption is seriously impaired, was not achieved with any of the processes. However, steeping/lactic acid fermentation plus decortication and parboiling plus

decortication reduced the phytate: zinc molar ratio to below the critical level of <15 in some varieties. Generally, the mineral biofortified hybrids had improved phytate: mineral molar ratios than the normal varieties for both raw and processed grains. Decortication greatly reduced total phenolic content (mean 24%) across the varieties and by an additional 14 percentage points after steeping/lactic fermentation and parboiling.

Abrasive decortication in combination with steeping/lactic acid fermentation is an effective way of reducing phytate content in pearl millet grain, and hence somewhat improving estimated iron and zinc availability. It is recommended that the process is utilised to a greater extent in pearl millet food processing. Parboiling is also effective in phytate reduction, and can be adopted. Because mineral biofortified pearl millet hybrids have much higher iron and zinc contents, their breeding and cultivation should be promoted in rural Africa.

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1. INTRODUCTION

Iron and zinc are two of the most critical micronutrients deficient in developing countries, particularly in rural communities (UNICEF, 2015). Iron deficiency affects an estimated 80% of pregnant women in developing countries (Murray-Kolb and Beard, 2009). Impaired maternal health, gastrointestinal and immune system disorders especially in children and women are among the most devastating effects of zinc deficiency (IZiNCG, 2004). Overall, micronutrient deficiencies, commonly known as "hidden hunger", affect about two billion people mostly in developing countries (FAO, 2013). Due to poverty and low-income status, most communities in developing countries largely consume cereal and inadequate iron foods (Hunt, 2003). Not only does non-haem iron in cereals have low bioavailability compared to haem iron in animal sources, but also absorption is further adversely affected by several dietary components (Hunt, 2003). These dietary components including phytate and polyphenols, which occur in grains like pearl millet, inhibit non-haem iron absorption (Fairweather-Tait and Hurrell, 1996). Kent and Evers (1994) stated that whole grains supply adequate minerals in particular iron for daily required adult intake. However, the presence of endogenous phytate in cereals and legumes leads to reduced mineral absorption.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a drought-tolerant tropical cereal (Taylor, 2004). Poor subsistence farmers in India and African countries (Obilana, 2003) mainly grow it. Pearl millet is cultivated on approx. 31 million hectares and contributes 50% of global millet production (ICRISAT, 2016). It is an important food and income source for over 90 million people worldwide (Mallet and Du Plessis, 2001, ICRISAT, 2016). The primary food use of pearl millet grain is for thin and thick porridges (Obilana, 2003). Large-scale commercial farming of pearl millet is currently limited. Nevertheless, due to improved agricultural practices resulting in improved yields, significant volumes are being made available to commercial food manufacturers (INTSORMIL, 2007). Hence, small-scale food processors in Namibia and the Sahel regions of Africa are currently manufacturing products like fermented sun dried flour, composite flours, and several ready-to-eat products (Taylor et al., 2010). Production of pearl millet products within Africa has great potential but is currently underdeveloped (Taylor et al., 2010). Therefore there is a need for sustainable production of value-added pearl millet products that will penetrate mainstream and lucrative high value food markets.

Pearl millet is energy-dense due to its high starch and fat contents and it is a good source of protein (Taylor, 2004). The two minerals of major public health concern, iron and zinc, can reach up to 5.5 mg/100 g and 3.2 mg/100 g, respectively (Minnis-Ndimba et al., 2015) in pearl millet. However, like all cereals, pearl millet contains phytate, an antinutrient that chelates with minerals forming complexes hence reducing their effective absorption and utilization by humans (Kent and Evers, 1994). The typical ranges of phytate content in pearl millet are 443-1076 mg phytate/100 g (El Hag et al., 2002).

Evidence suggests that food-processing operations can remove significant antinutrients from foods. Established cereal processing methods such as decortication, fermentation, germination, thermal processing, soaking and malting are known to reduce antinutrients levels and hence improve mineral bioavailability in the gastrointestinal tract (GIT) (Sandberg and Andlid, 2002). In addition, there is increasing research done plant breeders to develop micronutrient rich pearl millet varieties, in particular with enhanced iron and zinc contents through biofortification technology (Velu et al., 2011). Concentrating iron and zinc in the edible parts of crops provides an achievable, sustainable and food-based approach to combat micronutrient deficiencies (White and Broadley, 2005).

The objective of this study was to determine the effects of the technologies of abrasive decortication, steeping/fermentation and parboiling on mineral, phytate and phenolic compound contents in normal and biofortified pearl millet types, with the overall aim of improving the mineral bioavailability and thereby address mineral deficiencies among consuming vulnerable groups.

2. LITERATURE REVIEW

2.1 Overview

In this review, the pearl millet grain structure and composition, especially its minerals and antinutrients are described. Then the localisation and role of phytate and phenolic compounds with regard to their interactions with iron and zinc in pearl millet are reviewed. Next, cereal processing technologies and strategies such as biofortification and their potential to reduce the antinutrients that can adversely affect mineral bioavailability in pearl millet are evaluated. Lastly, in vitro assays for evaluating mineral bioavailability are described.

2.2 Pearl millet grain

2.2.1 Grain structure and components

Like most cereal grains, the pearl millet caryopsis (Figure 2.1), comprises a seed tightly adhered to a seed coat or pericarp (Hoseney, 1994). The main components of the caryopsis are the outer pericarp, aleurone layer, starchy endosperm, and germ (Kent and Evers, 1994). Representative data for whole unprocessed pearl millet grain chemical composition are given in Table 2.1.

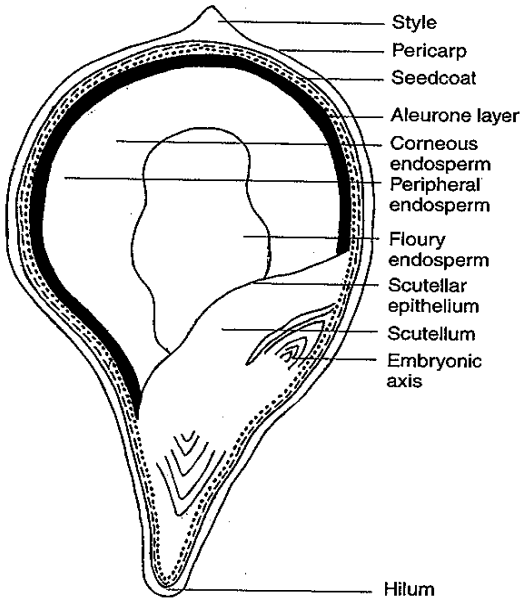


Figure 2. 1 Longitudinal section of a pearl millet grain (Taylor, 2004)

Table 2. 1 Proximate, chemical and other nutrient composition of pearl millet grain

Proximate composition (*db*) (g/100 g) [1,2]		Minerals (*db*) (mg/100 g) [2,3,4]		Vitamins (*db*) (mg/100 g) [1]	
Food Energy (kJ)	1646-1691[1]	Iron	1.9-5.5[2,4]	Vitamin A (*RE)	24[1]
Protein	8.6-19.4[1]	Zinc	2.0-3.2[2,4]	Thiamin	0.3[1]
Starch	63.1-78.5[1]	Calcium	7.2-7.4[4]	Riboflavin	0.2[1]
Lipids	1.5-6.8[1]	Phosphorus	326-373[4]	Niacin	2.9[1]
Crude fibre	2.4-4.0[2]	Magnesium	~137[3]	Vitamin E	1.9[1]
Ash	1.6-3.6[1]				

*Retinol Equivalents

Range and typical values

[1] Adapted from Taylor (2004)

[2] Adapted from Lestienne et al. (2007)

[3] Adapted from Bashir et al. (2014)

[4] Adapted from Minnis-Ndimba et al. (2015).

2.2.2 Mineral composition of pearl millet grain and their anatomical localisation

The mineral components in pearl millet among others comprise the major minerals, phosphorus (326-373 mg/100 g), magnesium (~137 mg/100 g) and calcium (7 mg/100 g) (Table 2.1). Zinc and iron are also present at approximately 1-5 mg/100 g levels. In a study by Hama et al. (2011), minerals as represented by ash content in pearl millet were found to be largely located in the germ (Table 2.2). The germ contained 72.2% of minerals, the bran 13.9% and the endosperm 13.9%.

Proton induced x-ray emission spectrometry (PIXE) has proved to be a useful tool to study important mineral elemental distribution in millets (Kruger et al., 2014; Minnis-Ndimba et al., 2015). The technique is capable of mapping mineral distribution in the grain as well as estimating their quantities (Ryan, 2011). A study on localisation of zinc and iron in pearl millet showed that the scutellum is the grain tissue with the highest levels of iron and zinc (Figure 2.2) (Minnis-Ndimba et al., 2015). The study revealed that the minerals are not actually distributed similarly in the pearl millet grain (i.e. they have different distribution in the grain anatomical components). Zinc is more pronounced in the embryo and iron is

relatively high in the germ and peripheral parts (Table 2.3). Collectively, the germ (scutellum and embryo) tissue has high levels of both iron, zinc and phosphorus (Table 2.3) (Minnis-Ndimba et al., 2015). The endosperm was found to have relatively low levels of iron, zinc and phosphorus.

Table 2. 2 Distribution of different constituents of pearl millet in the kernel (db) (Hama et al., 2011)

	Bran/pericarp	**Germ**	**Endosperm**
% of kernel weight	7.5	17.4	75.1
Ash (minerals) (%)	13.9	72.2	13.9
Lipid (%)	5.9	87.8	6.3
Phytate (%)	25	67	8

Table 2. 3 Concentration of iron, zinc, calcium and phosphorus (mg/100 g) in different major tissues of mature pearl millet grain (Minnis-Ndimba et al., 2015)

Grain tissue	**Fe**	**Zn**	**Ca**	**P**
Scutellum	12	8	7	2183
Embryo	10	13	12	925
Inner endosperm	8	2	8	10
Outer grain layer	19	5	20	77

*Values presented as mean of two representative samples

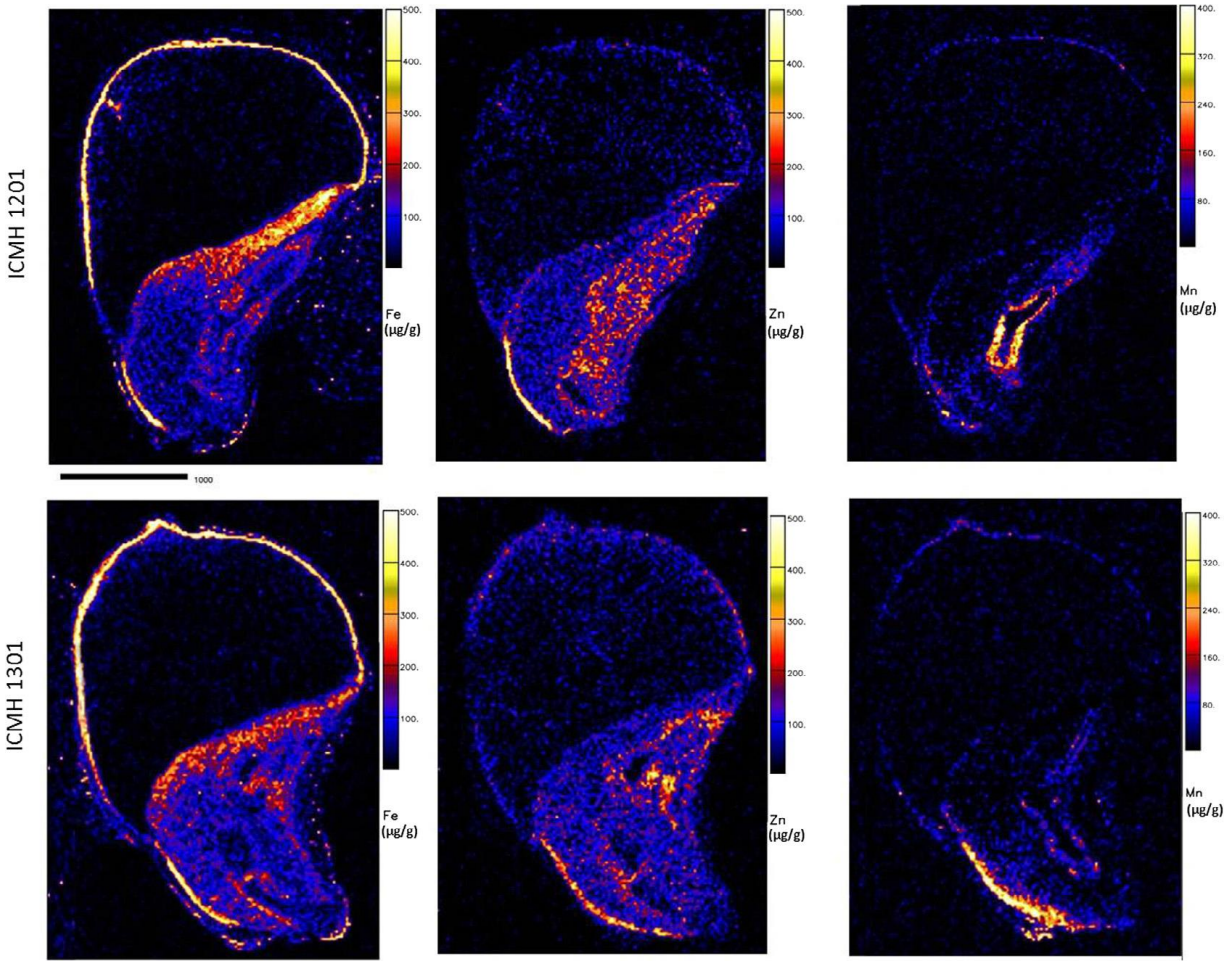


Figure 2. 1 Visual quantitative spatial distribution of minerals (Fe, Zn and Mn) in two pearl millet cultivars (ICMH 1201 and ICMH 1301) and their concentration (Minnis-Ndimba et al., 2015)

2.2.3 Antinutrients and their anatomical localisation in the grain

2.2.3.1 Phytate

Phytate exists in many forms, mainly as *myo*-inositol hexa*kis*phosphate (IP6), although pentaphosphate (IP5), tetraphosphates (IP4) and triphosphates (IP3) have been identified in foods (Lonnerdal, 2000). Phytates are found in salt form and account for approximately 85% of stored phosphorus in cereal grains (Kent and Evers, 1994). Phytic acid (the unstable free form) has negatively charged phosphate sites that strongly bind with cationic elements such as K, Mg, Ca, Fe, Zn and Mn at physiological pH (pH ~7.4) (Bohn et al., 2008). This phytic acid-mineral complex is referred as phytate (Bryant et al., 2005). This binding renders minerals largely unavailable for absorption in the human gastrointestinal tract. Phytate is the

main inhibitor of zinc and iron in cereals, resulting in lowering the cereal nutritional value. Hence, it has been extensively studied. Phytate is important, as it is the predominant form and principal storage of phosphorus (Liu et al., 2004). In pearl millet, phosphorus is mainly located in the scutellum of the grain (Minnis-Ndimba et al., 2015). Thus, it can indicate phytate location in the grain

Several studies have investigated the phytate content of pearl millet grain. El Hag et al. (2002) reported 943 and 1076 mg/100 g in two pearl millet varieties and Elyas et al. (2002) reported 618.4 and 786.2 mg/100 g. Several factors contribute to variations in phytate content, including genetics and environmental conditions of cultivation (Elyas et al., 2002).

2.2.3.2 Phenolic compounds

Phenolic compounds consist of a benzene ring with one or several hydroxyl groups (Dykes and Rooney, 2007). They are basically three types; phenolic acids, flavonoids and tannins (Dykes and Rooney, 2006). Phenolic acids are classified in two groups, hydroxybenzoic acids and hydroxycinnamic acids (Dykes and Rooney, 2006). Lestienne et al. (2005a) identified catechol and galloyl type phenolic compounds as iron binding phenolic compounds through their carboxyl (-COOH) and hydroxyl (-OH) sites. Studies by Jha et al. (2015) confirmed that there are high amounts of flavonoids in the pearl millet bran, mostly c-glycosyl flavones, which are responsible for the distinctive grey colour of pearl millet. The c-glycosyl flavones can act as goitrogens and have been implicated in the high incidences of goitre in areas where pearl millet is a staple food (Taylor and Duodu, 2015).

Phenolic compounds in pearl millet exist both in soluble and insoluble-bound forms (Chandrasekara and Shahidi, 2010). Both forms collectively make up the total phenolic content and subsequently have an effect on both antioxidant and metal chelating capacity. Fermentation and low pH release the bound phenolic compounds (Gabaza et al., 2016, Shahidi and Yeo, 2016,). Pearl millet contain mostly phenolic acids (approx. 168 mg/100 g) and low levels of flavonoids (~ 49 mg/100 g), which are concentrated in the outer layers of the grain (Jha et al., 2015). Polyphenol content of pearl millet is fairly high. El Hag et al. (2002) reported 304 mg/100 g and 444 mg/100 g total polyphenols in two cultivars. The polyphenols are highly concentrated in the bran components of the grain (Jha et al., 2015). Tannins, a group of phenolic compounds of flavone derivatives, are probably absent in pearl millet grain (Dykes and Rooney, 2006).

2.2.3.3 Dietary fibre

Dietary fibre can be defined as cell wall polysaccharides, the phenolic compound lignin and their associated compounds, which are unavailable to digestion by gastrointestinal enzymes (Fuentes-Zaragoza et al., 2010). Dietary fibre is concentrated in the bran part of the pearl millet component grain (Jha et al., 2015). The dietary fibre content of pearl millet ranges between 8 and 9 g/100 g (Taylor, 2004). The crude fibre proportion is considerably lower, ranging between 2.0 and 4.0 g/100 g (Lestienne et al., 2007).

Lonnerdal (2000) considered that fibre has an adverse effect on zinc absorption because high fibre foods mostly contain phytate. In addition, phenolic acid derivatives in cereals are mostly entrapped within fibre components in the cell walls (Liu, 2007).

2.2.4 Mineral bioavailability

Mineral bioavailability is defined as the fraction of ingested mineral that is available for utilization in normal physiological conditions or stored (Hurrell, 1997). Despite the relatively high mineral content of pearl millet grain (Lestienne et al., 2007), antinutrients naturally occurring in the pearl millet grain reduce their bioavailability in the human gut (Elyas et al., 2002). Inhibitors of mineral absorption have been identified as the main cause for poor mineral bioavailability and not the inadequate dietary intake *per se* (Hunt, 2003). This has posed problems in major cereal consuming communities leading to serious micronutrient deficiencies. Many studies have been focused on ways of reducing *myo*-inositol hexa*kis*phosphate, (IP6) chelation with divalent cations Fe^{2+}, Zn^{2+} and Ca^{2+} most commonly by reducing the level of phytate in the grain but maintaining the level of minerals.

2.3 Processing techniques/strategies to improve mineral bioavailability in pearl millet grain products

Several methods or food-processing operations can be used to improve mineral bioavailability in pearl millet. Milling, lactic acid fermentation, steeping/soaking and malting/germination are generally traditional food processing techniques used domestically and in large-scale industrial food processing of cereals (Lonnerdal, 2000; Sandberg and Andid, 2002). These processing techniques tend to free entrapped nutrients (i.e. disruption of mineral and antinutrients complexes) resulting in improved availability of minerals in the lumen (Sharma and Kapoor, 1996).

Here the focus will be on food processing operations and strategies that can be used to reduce antinutrients and improve mineral bioavailability in cereal grain products with emphasis on pearl millet grain.

2.3.1 Abrasive decortication

Taylor and Dewar (2001) described two principles/techniques applied in cereal grain milling. The first is to remove the bran components and then the endosperm is milled to the required particle size. The other technique requires breaking open the whole kennel then separating the endosperm from the bran, a technique mostly applied in wheat and rye. In pearl millet and sorghum milling, a combination of firstly abrasive decortication to remove the bran layers and then hammer milling of the endosperm is usually used (Kebakile et al., 2007). However, this leads to losses of essential nutrients located in the bran layers. Mechanisms to recover and retain essential components from the bran fractions after the decortication process have been devised (Vitaglione et al., 2008).

Many African countries have adopted the use of dehulling machines for decortication of sorghum and pearl millet (Taylor, 2004) Countries such as Botswana and Zimbabwe now manufacturer the machine commercially (Taylor and Dewar, 2001). Dehuller efficiency depends on the time taken for decortication. It is able to remove between 10-30% of the grain depending on the required final colour and fat content (Taylor, 2004). Despite the successful adoption and advantages of dehullers in developing countries, there have been problems associated with the equipment. They do not completely remove the germ, thus leading to high content of fat remaining in the endosperm meal (up to 2-4%) (Taylor and Dewar, 2001). This can lead to limitation in the storage stability of the product. In addition, losses during milling, low quality outputs and low production rates have been observed (Kebakile et al., 2007).

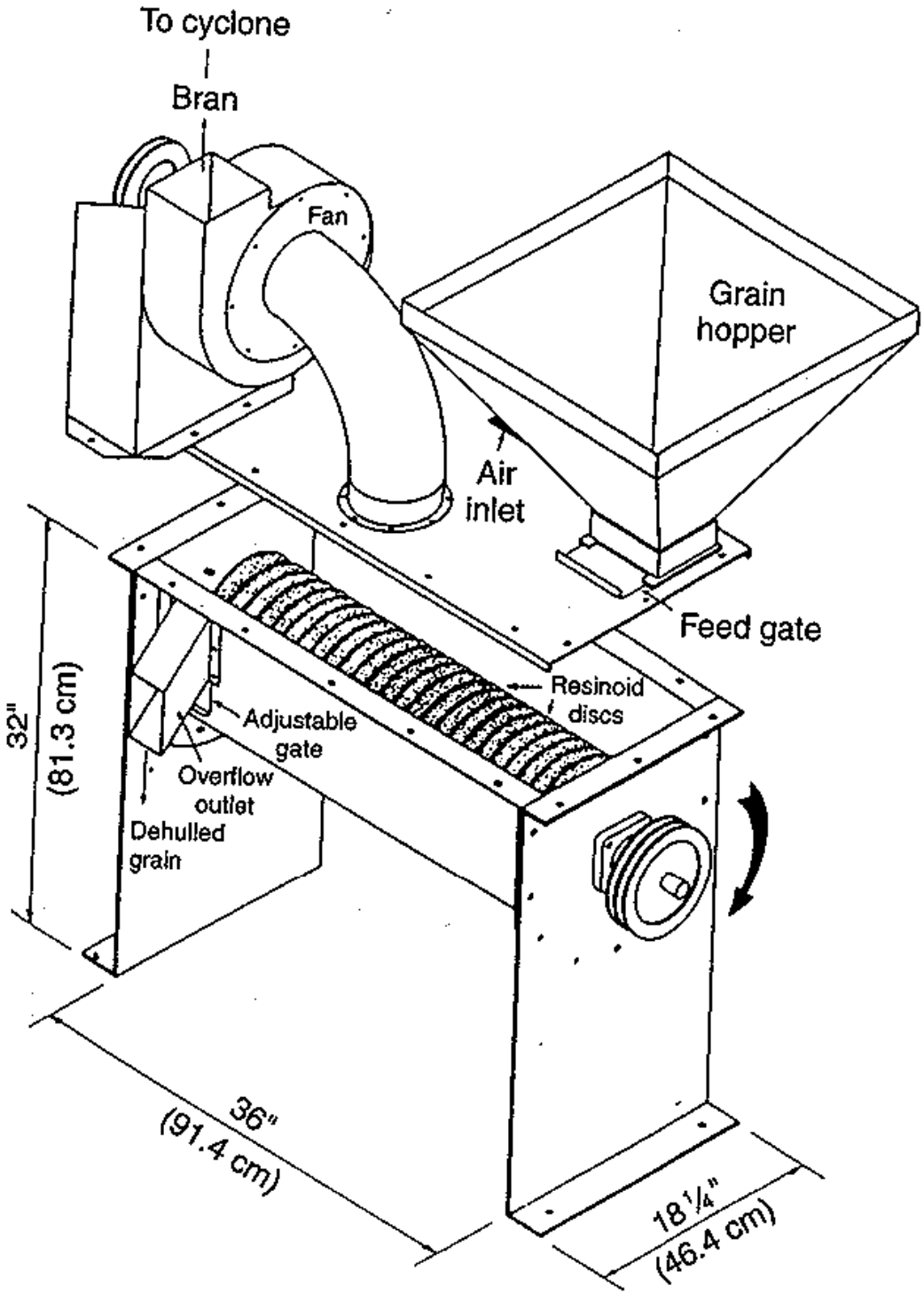


Figure 2. 2 Schematic diagram of PRL-type dehuller (Reichert, 1982)

2.3.1.1 Effect of Abrasive decortication on nutritional value

Several studies have investigated the effect of milling on nutrient and antinutrient losses from pearl millet grain. Lestienne et al. (2007) estimated the losses of nutrients and antinutrients at

an 88% extraction rate by abrasive decortication. There were substantial losses of zinc, iron, fibre and iron binding phenolics (Table 2.4). Lipids and proteins were also reduced but starch losses were minimal. This indicated that the losses were generally based on localization of nutrients in the grain components. The bran, with the high content of fibre (Jha et al., 2015), and significant amounts of minerals (Table 2.2) is mostly affected. Importantly, there were substantial losses of iron binding phenolic compounds, 19% and 51%, respectively.

Table 2. 4 Nutrient and antinutrient factor contents in whole grain of two pearl millet cultivars subjected to 12% decortication and percentage of each compound loss during milling (Lestienne et al., 2007)

Nutrient/antinutrient	**Gampela**		**IKMP-5**	
	Average nutrient content in whole grain	Proportion (%) removed after 12 % grain decortication	Average nutrient content in whole grain	Proportion (%) removed after 12 % grain decortication
Starch (g/100 g DM) [a]	70.13 ± 0.68	3	69.30 ± 0.77	2
Protein (g/100 g DM) [b]	8.73 ± 0.10	13	10.11 ± 0.11	13
Lipids (g/100 g DM) [b]	5.58 ± 0.10	9	5.77 ± 0.09	10
Iron (mg/100 g DM) [b]	1.87 ± 0.04	27	3.41 ± 0.10	32
Zinc (mg/100 g DM) [b]	2.00 ± 0.02	18	2.57 ± 0.04	13
Crude fibre (g/100 g DM) [a]	2.48 ± 0.03	40	4.23 ± 0.10	56
Iron-binding Phenolic compound (g catechin and tannic acid. Eq/100 g DM) [b]	0.67 ± 0.02	51	0.37 ± 0.01	19
Phytate (g/100 g DM) [b]	0.72 ± 0.04	4	0.81 ± 0.02	8
Phytase activity [a]	200 ± 8	42	168 ± 1	11

[a] Means ± standard deviation of 3 determinations. (Phytase activity expressed in mg released H_2PO_4/h per 100 g DM)

[b] Means ±standard deviation of 2 determinations.

It was also found that the phytase activity remained relatively high, however, the percentage phytate content loss was very low (Table 2.4). Therefore this implies that decortication to this degree is not efficient for phytate reduction. This is most probably due to the location of phytate in the grain. As stated, Minnis-Ndimba et al. (2015) found that phytate was largely

located in the scutellum. Since, the scutellum is the inner layer of the germ, little of it will be removed by decortication.

Hama et al. (2011) studied the impact of decortication on the nutrient content of pearl millet. Three methods were used to decorticate the grain: traditional decortication using a wooden pestle and mortar, mechanized decortication using a motorized Engelberg huller and laboratory decortications using a Tangential Abrasive Dehulling Device (TADD). In terms of iron, zinc, phytate and fibre losses, all their levels were reduced after decortication, irrespective of the method used, but starch content increased after decortication (Table 2.5). Both hand pounding and mechanical decortication removed almost half of phytate. However, there were substantial loss of both zinc and iron by the process.

Table 2. 5 Effect of decortication using hand pounding and mechanically on the nutritional composition (dry basis) of Gampela pearl millet cultivar (Hama et al., 2011)

Nutrient/antinutrient	**Gampela millet**		
	Not decorticated	By hand pounding $n = 4$	Mechanically $n = 5$
Iron (mg/100 g)	4.51^{a} ±0.04	2.90^{b} ±0.76	2.52^{b} ±0.37
Zinc (mg/100 g)	2.02^{a} ±0.05	1.59^{b} ±0.17	1.74^{b} ±0.14
Lipid (g/100 g)	4.80^{a} ±0.23	4.78^{a} ±0.67	5.44^{a} ±0.57
Crude fibre (g/100 g)	2.59^{a} ±0.38	1.72^{b} ±0.08	1.75^{b} ±0.48
Phytate (g/100 g)	0.65^{a} ±0.03	0.30^{b} ±0.07	0.23^{b} ±0.05
Starch (g/100 g)	72.2^{a} ±0.4	76.2^{b} ±1.5	76.4^{b} ±2.5

Different superscript letters in the same row are significantly different ($p < 0.05$).

Clearly, there are substantial differences between the results reported by Lestienne et al. (2007) and Hama et al. (2011), particularly with respect to phytate losses. Further studies in relation to optimization of extraction (abrasion decortication) are required to ensure substantial antinutrient removal, while at the same time micronutrient retention to increase the latter's bioaccessibility.

Hama et al. (2012) also investigated the effect of abrasive decortication on estimated mineral bioavailability in iron and zinc biofortified pearl millet grain. In comparison to non-biofortified grain, biofortified varieties had higher zinc and iron content. In addition, upon decortication, higher zinc and iron were retained. However, based on the molar ratios of phytate/minerals to estimate mineral bioavailability, iron bioavailability was found to be low in the biofortified grains. The proposed critical limit of phytate to iron molar ratio is <1, while phytate/zinc is <15 (Hurrell, 2004, Lazarte et al., 2015). Molar ratios of phytate: zinc for biofortified grains ranged between 6 and 18 (before and after decortication) (Hama et al., 2012), indicating some improved bioavailability for those below 15 (Hurrell, 2004, Lazarte et al., 2015). Therefore this implies that abrasive decortication partially removes phytate to an acceptable level in biofortified grain in which it is enough for adequate zinc absorption. As for iron, the molar ratio of less than 1 was not met but in comparison to the non biofortified cultivar, the molar ratio of phytate to iron was much lower in the biofortified cultivars (Hama et al., 2012).

2.3.2 Fermentation

Fermentation is a metabolic process where the microorganism's enzymes act on an organic substrate, usually carbohydrates, leading to the release of energy, and production of acids, gasses or alcohol (Kohajdova and Karovicova, 2007). A common type of food fermentation is lactic acid bacteria (LAB) fermentation (Taylor and Duodu, 2015). Lactobacillus species such as *Lactobaccilus acidophilus* are used in many food fermentation processes and their impact on phytate has been widely studied (Hurrell, 2004). LAB fermentation of cereals produces acidic conditions by production of acidsH, which induces phytase activity. The endogenous phytases from the microflora and the cereal can hydrolyse the phytate destroying the phytate-mineral complex. Furthermore, as phytate is water soluble, it can be solubilized during fermentation hence the substantially reduced if the liquid is removed (Afify et al., 2011).

Reale et al. (2007) found that 24-hour LAB fermentation of cereals resulted in a 100% reduction in phytate for rye, 95-100% for wheat and 39-47% for oats. Studies by El Hag et al. (2002) showed a significant (p<0.05) decrease in phytate and polyphenol content in two pearl millet cultivars. Phytate content in one cultivar decreased from 943 to 380 mg/100 g and from 1076 to 580 mg/100 g in the other cultivar (Table 2.6). The total polyphenols in one cultivar decreased from 304 to 122 mg/100 g and from 444 to 306 mg/100 g in the other. Importantly,

the grain was milled first before fermentation was carried out, thus increasing the surface area for phytase activity. Hurrell (2004) pointed out that the presence of the bran might lead to incomplete action of phytase due to inability to access the substrate.

Table 2. 6 Summary of food processing methods and/ or strategies that can be used to reduce antinutritional compounds to improve iron and zinc bioavailability in pearl millet grain

Processing technology/strategy	**Phytate, TPC, zinc and iron (% reduction)**	**Mechanism**	**References**
Abrasive decortication	Phytate reduction: *(4-8%); *(~ 50%) Iron-binding phenolic compounds: *(19-51%)	Loss of physical structure	Hurrell (2004); Lestienne et al. (2007); Hama et al., (2011)
Fermentation	Phytate reduction: *(46-60%) Polyphenol reduction: * (31-60%)	Enzymatic and microbial activity	El Hag et al. (2002)
Steeping	Phytate reduction: *(28%)	Solubilisation, leaching	Lestienne et al. (2005b); Etcheverry et al. (2012)
Malting/Germination	Phytate reduction *(19-33%) Phenolic compounds loss (goitrogens): *NR*	Enzymatic and microbial activity (solubilisation, leaching)	Hurrell (2004); Onyango et al. (2013); Taylor and Duodu, (2015)
Phytase	Phytate degradation: *(~ 50%) Iron-binding phenolic compounds: *(57-77%)	Phytase activity	Sandberg and Andlid (2002); Lestienne et al. (2005a)
Parboiling	Phytate reduction: *(44%) Mineral increase: *NR*	Phytate autolysis Diffusion/migration of iron and zinc into the endosperm	Hurrell (2004); Messia et al. (2012); Jha et al. (2015)
Biofortification	Iron and zinc increase – Iron to 67-73 mg/kg and zinc to 41-56 mg/kg	Accumulation/uptake of nutrient from soil into the grain	Hama et al. (2012); Saltzman et al. (2014)

* () Represent percent antinutrient reduction and or mineral increase in studies done on pearl millet grain

NR means no representative value was obtained from the literature

2.3.3 Steeping (soaking)

Pearl millet is often soaked in water at ambient temperature during food processing (Mallet and Du Plessis, 2001). The soaking (steeping) of pearl millet serves many purposes. It improves the grain colour and softens the grain, which results in easier milling to flour (Taylor, 2004). Furthermore, the resulting flour organoleptic properties are enhanced due to the acidic flavour resulting from the LAB fermentation that takes place. The steeping water can be inoculated with lactic acid bacteria through a back-slopping process. Furthermore, the aforementioned phenolic compounds responsible for both poor mineral bioavailability and dull grey colour are washed out (Mallet and Du Plessis, 2001, Taylor, 2004). As stated, phytate is a soluble compound, therefore in part it can be dissociated and then discarded with the soaking/steeping medium (Afify et al., 2011).

In a study by Lestienne et al. (2005b), soaking resulted in significant (p<0.05) reduction of phytate in pearl millet (Table 2.6). About 28% phytate was lost from pearl millet after 24 hours soaking at 30^0C of whole grains. However, the phytate: iron molar ratio increased from 5.3 to 6.5, while the phytate: zinc molar ratio decreased slightly from 18.5 to 16.4. The study found that soaking was not effective in improving mineral absorption. In fact, it was observed that soaking lead to leaching out of minerals in particular iron. Therefore steeping for long periods can result in loss of important nutrients from the grain. It might be useful to employ short steeping with the aid of starter culture through back-slopping, as mentioned by Taylor (2004).

The pH of the soaking medium may influence antinutrient losses. Jha et al. (2015) found that an acidic medium reduced phytate by 75-81%, flavonoids by 34-63% in decorticated and recovered bran and endosperm-rich fractions in pearl millet. These was explained as due to solubilisation of phytate and leaching of pH sensitive flavonoids pigments. However, soaking in alkaline conditions did not cause substantial effect on phytate content of bran fraction while resulting in decreasing flavonoids by 50-60% in both bran and endosperm rich fractions (Jha et al., 2015). In the same study, it was found that phytate content was significantly negatively correlated (p<0.05) with zinc bioaccessibility. However, the correlation between phytate and iron bioaccessibility was less significant. Fibre and flavonoids were believed to also be contributing to low iron bioaccessibility.

2.3.4 Malting/Germination

The malting process involves steeping and germination of the grain in moist air under controlled environmental conditions (Taylor and Duodu, 2015). Malting is a common domestic and industrial biotechnological process for cereal processing mainly used to produce beverage and food. However, industrial pearl millet malting is less common, although large scale commercial pearl millet malting in Zimbabwe has been noted (Taylor, 2004). During malting, endogenous enzymes under controlled germination conditions hydrolyze starch and other components of the grain. The primary enzymes involved are amylases, proteases and cell wall degrading enzymes (Kent and Evers, 1994). Malting also enhances the quality of cereals and legumes in terms of nutritional profile, mainly due to hydrolysis of antinutrients and protease inhibitors (Afify et al., 2011). During germination an increase in antinutrient hydrolysing enzyme activity has been observed, particularly phytase activity (Sandberg and Andlid, 2002). Bohn et al., (2008) stated that, for supporting germinating seedlings, during germination endogenous phytase(s) hydrolyse the phytate molecule to release bound micronutrients required by the seedlings. Furthermore, malting has been found to lead to phenolic compounds loss through solubilisation and subsequently leaching out (Taylor and Duodu, 2015).

Hurrell (2004) concluded that malting/germination can be an important strategy to reduce phytate in cereals and improve iron absorption. Phytate can be reduced by about 50% by germination mainly through activation of endogenous phytases. Conditions need to be suitable to activate the phytase, and phytase enzymes must be in contact with the substrate. For example, Onyango et al. (2013) found that three day malting of pearl millet resulted in reduction of between 19-33% phytate (Table 2.6). However, Egli et al. (2002) found that while germination improved phytase activity by 3-5 fold in some cereal grains, generally it had an insignificant effect on phytate reduction.

2.3.5 Phytase addition

Phytase (*myo*-inositol (1,2,3,4,5,6) hexa*kis*phosphate phosphohydrolase; EC No. 3.1.3.8) is an enzyme endogenous to plants or artificially added whose function is to degrade the phytate compound by removing the phosphate groups from phytate (Bohn et al., 2008). This phosphatase thereby releases bound minerals. Sources of dietary phytase are plants and microorganisms such as fungi (i.e. *Aspergillus*) and bacteria (Sandberg and Andlid, 2002). In

food processing, hydrolysis of phytate can be achieved using phytase enzymes or microorganisms producing phytase (Lestienne et al., 2005a). Addition of food grade phytase such as from *Aspergillus* to reduce phytate in cereals or high phytate foods has proved to be useful (Sandberg and Andlid, 2002). However, to ensure iron absorption, significant phytate hydrolysis is required. Oatway et al. (2001) identified two types of phytases, namely 3-phytase that is found in microorganisms and 6-phytase found in plants. Three-phytase is mostly found in the aleurone layer and is inactive in dry cereals. Moisture activates its activity and its activity is pH dependent. Most of the phytate degradation through phytase activity occurs during biological processing techniques such as fermentation, malting and soaking facilitated by suitable environmental conditions (Oatway et al., 2001; Sandberg and Andlid, 2002).

A study by Lestienne et al. (2005a) found pearl millet to have 9.9% available iron *in vitro*. Phytate in pearl millet then was reduced from 592 to 296 mg/100 g upon incubating with exogenous phytase. Treatment with exogenous and endogenous phytases led to significant ($p \leq 0.05$) decreases in the phytate: iron molar ratio from 15.9 to 0.2, thus increasing potential iron bioavailability. In the same study, employing exogenous phytase to degrade phytate resulted in no improvement in zinc bioaccessibility. Another study by Lestienne et al. (2005b) showed that incubation of pearl millet with phytase from wheat and microbial phytase from *Aspergillus ficcum* simultaneously resulted in a significant reduction ($p<0.001$) of phytate from 50% to 99% as compared to not using phytase. Iron binding phenolic compounds were reduced by 57 and 77% (Table 2.6) upon incubation with exogenous phytase. This was explained as being due to both leaching, phytase activity and degradation of phenolics.

2.3.6 Parboiling

Steaming of cereal grains at elevated pressure and temperature is a common practice, particularly for rice (FAO, 1994). The process, commonly known as parboiling, is a hydrothermal process involving steaming and drying (Larmberts et al., 2006). Parboiling has been applied in millet and sorghum. The process serves many purposes mainly increasing milling yield and quality (FAO, 1994). In addition, it has been observed to lead to improved mineral availability. It is assumed that application of heat treatments to cereal grains cause autolysis of antinutrients. However, the loss of phytate due to heat treatments is very low

(Hurrell, 2004). Besides the reduction of antinutrients, parboiling is known to lead to inward diffusion of water-soluble nutrients such as B-vitamins and minerals into the endosperm (Messia et al., 2012). Parboiling also reduces the amount of grains broken during handling (FAO, 1994).

Young et al. (1990) examined the impact of parboiling on the decortication yield of sorghum and pearl millet. Sorghum and pearl millet kernels were soaked overnight and parboiled for 10 minutes. After drying, the grains were assessed for decortication yields. It was found that the treatment resulted in a significant (p<0.01) increase in decortication yield and reduction in kernel breakage in both grains. Serna-Saldivar et al. (1994) adapted the boiling and soaking overnight (12 hours) process described by Young et al. (1990). The effect of parboiling and decortication on the nutritional value of sorghum and pearl millet was investigated. Chemical constituents evaluated in the study included proteins, fibre and the minerals calcium, phosphorus and magnesium. Decortication resulted in a loss of these chemical constituents. It was observed that parboiling enable effective decortication, resulting in less loss of these particular nutrients.

Jha et al. (2015) germinated pearl millet grain for 48 hours and later steamed the grain at atmospheric pressure (97^0C) for 15 min. The purpose of steaming was to inactivate enzymes activated during germination and harden the endosperm. It was reported that germination and steaming led to a 44% phytate loss in the grain. Messia et al. (2012) determined the effect of parboiling on the chemical composition of emmer (a type of wheat). There was significant (p<0.05) increase in ash content after parboiling and decortication. An increase was observed for parboiled-decorticated emmer from 0.92 to 1.18 g/100 g (28% increased mineral content). This was explained to be due to migration of minerals centrally into the endosperm during parboiling. The above studies involved steeping/soaking of the grain overnight. The effect of soaking on leaching out of soluble components of the grain such as minerals and vitamins is well known (Taylor, 2004, Afify et al., 2011). However, there is limited work on parboiling and its effect on mineral retention and loss of antinutrients in pearl millet.

2.3.7 Biofortification

Biofortification is the process by which the bioavailability of important target minerals is increased by concentrating them in the edible parts of plants through agronomic methods and genetic selection (White and Broadley, 2005). The former focuses on developing grain

cultivars capable of absorbing minerals, in particular iron and zinc, from the soil into crop tissues. In micronutrient biofortification, the objective is to insert the traits in crop varieties currently showing preferred agronomic and consumption traits (Saltzman et al., 2014). Uptake and accumulation of significant micronutrients in a crop lead to high density or concentration in the grain (Taylor et al., 2014). One option that has proved feasible using recombinant DNA technology is to develop grains with reduced levels of antinutrients such as phytate. Plant breeders now have the technology to modify the genetic make-up of staple crops resulting in eliminating or reduction of antinutrients (Welch and Graham, 2005).

Advances in increasing micronutrient quantities in staple foods such as cereal grains are in progress with special target to alleviate micronutrient deficiencies in rural cereal consuming populations (Taylor et al., 2014). Biofortification is believed to be a cost-effective and sustainable way of combating micronutrient malnutrition in poor communities (Mayer et al., 2008; Taylor et al., 2014). Pearl millet is one of the crops currently biofortified with iron and zinc (Rai et al., 2012). Velu et al. (2011) reported that several studies conducted on biofortified pearl millet varieties showed some notable relationships. Mineral biofortified pearl millet grain correlated with increased iron and zinc concentration. However, a few indicated negative (r = -0.39 to r = -0.58) relationship between iron content and grain yield. Nevertheless, Saltzman et al. (2014) insisted that iron and zinc biofortification technology in crops is a valuable means of solving malnutrition in rural communities and it will be a success.

Through the international sorghum and millet research institute ICRISAT, (the International Crops Research Institute for the Semi-Arid Tropics), progress has been made in iron and zinc biofortification in pearl millet. It has been found that iron levels can be raised to 67-73 mg/kg, while zinc levels can be improved to 41-56 mg/kg (Table 2.6) in biofortified pearl millet grain (Hama et al., 2012). A study by Kruger et al. (2012) revealed reductions in phytate content by genetic modification of non-tannin (38%) and tannin (36%) sorghum. However, it was concluded that this percent reduction (36-38%) was not adequate to improve iron bioavailability. Nevertheless, it was found that in combination with natural lactic fermentation, a significant increase in iron bioavailability is feasible. In a separate study by Kruger et al. (2013), a 80-86% reduction in phytate was achieved in genetically modified tannin and non-tannin sorghum cultivars. There was also a significant (p<0.05) increase in zinc uptake and absorption by Caco-2 cells. Therefore the reduction of phytate was enough for improved zinc bioavailability.

2.4 Analytical methods to estimate mineral bioavailability

Ethical issues, cost and time implications limites the use of *in vivo* methods for determining bioavailability of nutrients (Carbonell-Capella et al., 2014). However, information on mineral bioavailability is of crucial importance in determination of mineral nutritional value of cereals (Lazarte et al., 2015). Therefore efforts have been devoted to develop *in vitro* methods to estimate mineral bioavailability. Etcheverry et al. (2012) identified four *in vitro* bioaccessibility assays; solubility, dialysability, gastrointestinal model and Caco-2 cell models. In each of these methods, *in vitro* digestion is carried out which mimics human gastrointestinal digestion. This involves the utilization of pepsin, bile salts and pancreatin enzymes under specific conditions like controlled buffer solutions and temperature.

The two assays widely used for iron and zinc bioavailability estimation are dialysability and Caco-2 cell uptake (Etcheverry et al., 2012). Caco-2 cells are diverse human epithelial colorectal adenocarcinoma that were discovered at Sloan-Kettering Institute for Cancer Research in New York (Soto et al., 2014). The Caco-2 cell assay measures how much mineral/nutrient is taken by the Caco-2 cells. They mimic uptake of minerals by epithelial cells in the gastrointestinal tract, hence human gut mineral bioavailability can be estimated. They are also used for studying transport mechanisms of nutrients in the body cells (Fairweather-Tait et al., 2005; Etcheverry et al., 2012). The dialysability is a method developed specifically to estimate iron bioaccessibility in foods (Carbonell-Capella et al., 2014). The *in vitro* dialysability assay involves simulated digestion and a dialysis membrane with a selected molecular weight cut-off to mimic gastrointestinal tract uptake (Fairweather-Tait et al., 2005). Dialysability has also been adapted for zinc bioaccessibility estimation (Etcheverry et al., 2012). Generally, these assays have proved useful in studying mineral absorption and estimation of mineral bioavailability.

Another way of estimating mineral bioavailability of cereals is using simple phytate: mineral molar ratio calculations (Lazarte et al., 2015). The relative molar ratios of phytate to zinc or iron and also phytate × calcium to zinc provide an indication of the interaction and strength of the respective phytate-mineral complexes (Ma et al., 2007, Lazarte et al., 2015). It has been authoritatively stated that phytate: mineral molar ratios are simple, useful and effective (Hurell, 2004, Lazarte et al., 2015, Li et al., 2015). Calcium is a major factor for effective zinc absorption. This is because the phytate-calcium-zinc complex is highly insoluble, hence

rendering zinc unavailable for absorption. However, the calcium level must be high (Ma et al., 2007, Lazarte et al., 2015).

Prediction of iron bioavailability due to interaction of polyphenols and iron can be studied using an algorithm model equation proposed by Hallberg and Hulthen (2000). The strength of the interaction of iron binding polyphenols particularly galloyl groups with iron are expressed as an equation. An absorption ratio which should be less than 1 for effective iron absorption is thus derived from the algorithm.

2.5 Conclusions

The pearl millet grain contains relatively high levels of antinutrients, particularly phytate that have been shown to inhibit mineral absorption. Controlling the levels of mineral binding compounds is of utmost importance. Many researchers have looked at ways of reducing antinutrients in cereals by employing traditional food processing technologies with the aim of improving mineral bioavailability. The results are inconsistent, hence the need to optimize the processes or combine treatments. Abrasive decortication is a useful milling process to reduce the level of antinutrients in pearl millet as it generally applied when the grain is milled in order to improve flour palatability. The degree of decortication can determine the amount of antinutritional factors remaining. However, both the minerals and antinutritional factors are mostly concentrated in the same layers, the scutellum and germ tissues and the bran, hence both are affected by milling similarly. It may be useful to employ several processing techniques such as steeping plus LAB fermentation and decortication or parboiling and decortication in combination to achieve optimal antinutrient reduction and mineral retention. Exploring the use of biofortification to increase the increase the concentration of minerals in the pearl millet grain is also critical, as biofortification is a novel strategy that can be used to overcome mineral deficiencies in the long term.

3. HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

1. Minimal abrasive decortication of steeped/lactic acid fermented pearl millet grain will substantially improve estimated iron and zinc availability compared to abrasive decortication of raw/untreated pearl millet grain.

Phytate, polyphenols, iron and zinc are co-located in the outer layers of pearl millet grain (Jha et al., 2015; Minnis-Ndimba et al., 2015) which is removed during decortication (Hama et al., 2011). The use of spontaneous back slopped liquor of a LAB cereal fermentation with both endogenous cereal and microbial phytase activity (Taylor, 2004) will dephosphorylate the phytate in the aleurone layer of the grain (Hurrell, 2004), and hence release bound minerals which will then relocate towards the endosperm with the movement of the moisture and not be removed during decortication.

2. Abrasive decortication of parboiled (steamed) pearl millet grain will substantially improve estimated iron and zinc availability compared to abrasive decortication of raw/untreated pearl millet grain.

Steaming of cereals forces minerals inwards towards the endosperm (Messia et al., 2012). Therefore iron and zinc that are located in the outer layers of pearl millet grain (Minnis-Ndimba et al., 2015) will be retained upon decortication. In addition, wet steam will degrade mineral binding phenolics (Raes et al., 2014), therefore decreasing their levels in the grain.

3. Abrasive decorticated biofortified pearl millet grain will have substantially improved estimated iron and zinc availability compared to abrasive decorticated normal pearl millet grain.

This is because biofortification increase the density of critical elements in pearl millet (Rai et al., 2012). Abrasive decortication will reduce antinutrients in the biofortified pearl millet grain (Hama et al., 2011) however, high levels of iron and zinc will remain as compared to normal pearl millet grain.

3.2 Objectives

1. To determine the effects of combined abrasive decortication and steeping in lactic acid fermenting liquor of normal and biofortified pearl millet varieties on estimated iron and zinc availability.

2. To determine the effects of combined abrasive decortication and parboiling (steaming) of normal and biofortified pearl millet varieties on estimated iron and zinc availability.

3. To determine the effect of abrasive decortication of mineral biofortified pearl millet grain on estimated iron and zinc availability.

4. RESEARCH

Effects of variety, abrasive decortication, steeping in back-slopped liquor and parboiling of pearl millet grain on proximate composition, antinutritional factors and estimated mineral availability.

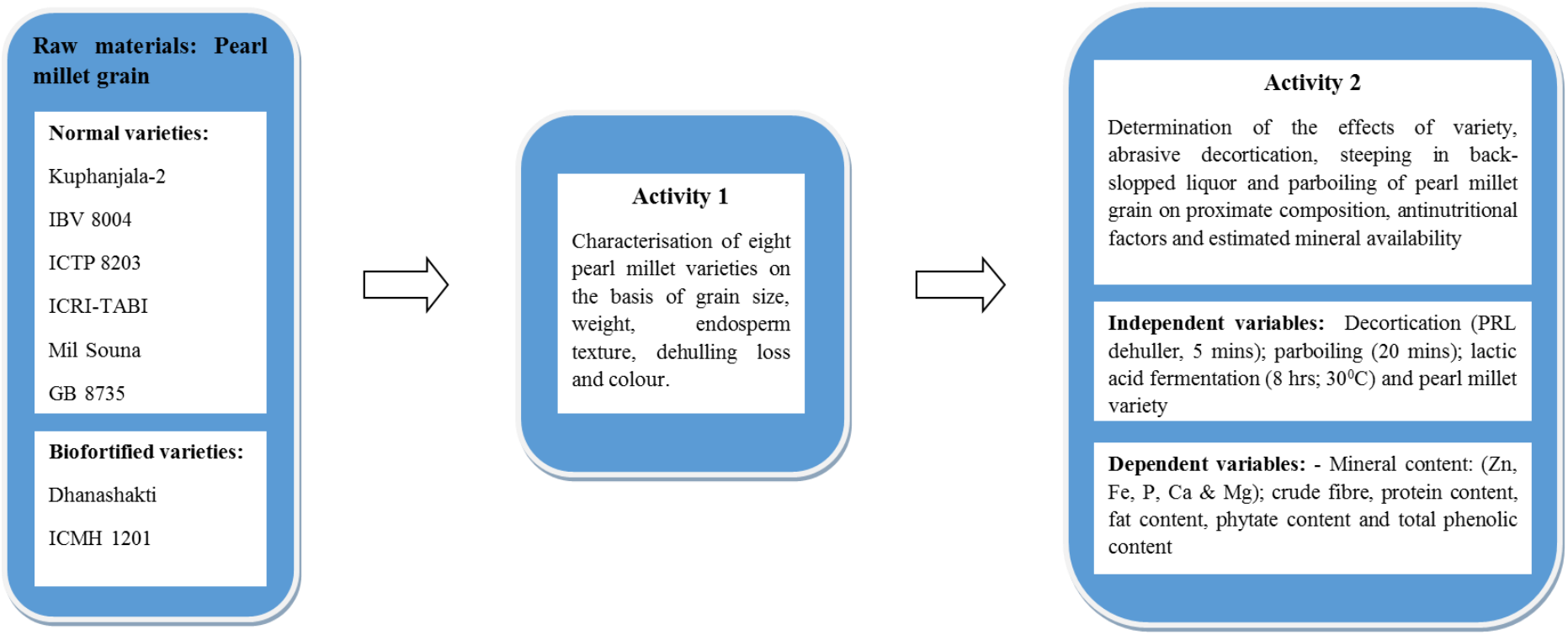


Figure 4. 1 Experimental design used to determine the effects of variety, abrasive decortication, steeping in back-slopped liquor and parboiling of pearl millet grain on proximate composition, antinutritional factors and estimated mineral availability

ABSTRACT

Iron and zinc deficiencies remain a huge burden in pearl millet consuming areas across Africa. Effects of decortication, steeping/lactic acid fermentation and parboiling of six normal pearl millet varieties and two mineral biofortified hybrids on mineral and antinutrient contents were investigated. The biofortified hybrids had far higher iron (21-68%) and zinc (15-39%) contents compared to the normal varieties. Phytate levels ranged between 830 and 1360 mg/100 g. Decortication did not cause substantial zinc losses, but resulted in considerable iron losses (mean 31%). There were minimal effects of steeping/lactic acid fermentation and parboiling on iron and zinc contents. Steeping/lactic acid fermentation of whole pearl millet grain resulted in greater phytate reductions than parboiling. Decortication greatly reduced total phenolic content (TPC) across the varieties (mean 24%), with both steeping/lactic fermentation and parboiling resulting in an additional 14 percentage points TPC reduction. The processing methods did not substantially improve estimated iron availability (phytate: iron ratio). However, steeping/lactic acid fermentation plus decortication and parboiling plus decortication improved estimated zinc availability in some varieties. Generally, the mineral biofortified hybrids had improved estimated mineral availability. Abrasive decortication plus steeping/lactic acid fermentation are viable simple processing methods that reduce phytate in pearl millet and somewhat improve estimated mineral availability. Cultivation of mineral biofortified pearl millet hybrids would be a valuable initiative to help alleviate iron and zinc deficiencies in rural Africa.

4.1 INTRODUCTION

Across Africa, pearl millet grain is processed into various products to meet different consumer needs (Taylor et al., 2010). Overall, the nutritional content of pearl millet grain is appreciable, as a major source of starch, protein, fat and important minerals such as iron and zinc (Taylor, 2004). Iron and zinc deficiencies are major health problem in rural developing African countries (UNICEF, 2015). Limited dietary intake of iron and zinc elements contributes to mineral deficiency (Hunt, 2003), but other factors play a role. Pearl millet, like all grains, also contains mineral binding compounds, in particular phytate and polyphenols (El Hag et al., 2002). Phytate binds with cationic minerals, rendering important minerals such as iron and zinc unavailable for absorption and utilisation in the body (Bohn et al., 2008). Therefore an imbalance of available minerals and high levels of phytate in pearl millet grain could undermine the grain's nutritional value.

Traditional cereal processing methods have been extensively investigated to enhance the nutritive value of pearl millet (Lestienne et al., 2005b, Hama et al., 2011). Decortication and milling as well as steeping and fermentation are important processing technologies (Obilana, 2003). Not only do they improve the nutritional quality and add desirable effects in food products (Taylor and Dewar, 2001), they are also easy to use. Decortication is by far the most important and utilised process in pearl millet grain processing prior to milling to flour (Obilana, 2003). The process primarily improves the palatability and storage quality of the grain (Taylor and Dewar, 2001). In addition, during decortication phytate components are physically removed (Hama et al., 2011). However, minerals co-located with phytate in the pericarp are also removed (Lestienne et al., 2007). Hence, there is a pressing need to determine an appropriate decortication rate for pearl millet grain that will result in high antinutrient loss but improved essential mineral content and bioavailability.

Steeping pearl millet grain in water and consequent lactic acid fermentation is by far the most practiced secondary pearl millet processing technology in Africa (Taylor, 2004). It is seen as a way of preserving and adding value to cereal foods (Obilana, 2003). In fact, steeping/lactic acid fermentation may be the most practical vehicle for antinutrient reduction in many rural community cereal diets (Reale et al., 2007). Upon steeping, phytate and other mineral binding compounds are solubilised and thus leach out (Afify et al., 2011). Lactic acid fermentation also lowers the pH, hence triggering endogenous phytase enzymes which hydrolyse the phytate and release bound minerals (Hurrell, 2004).

Wet steaming processing (parboiling) may cause migration of important soluble minerals into the endosperm of cereal grains (Messia et al., 2012). As decortication results in a significant loss of important minerals (Hama et al., 2011), diffusion of minerals into the endosperm may retain important minerals such as iron and zinc (Messia et al., 2012). Therefore parboiling which is mainly used in rice processing (FAO, 1994), may also find application in pearl millet processing (Young et al., 1990). The limited information suggests parboiling improves mineral retention during decortication (Messia et al., 2012). However, much is not yet known about its efficacy in pearl millet grain processing.

Much research and effort is now focusing on improving the density of critical minerals in pearl millet grain through biofortification (Velu et al., 2011). An ICRISAT programme to produce high iron and zinc content, early-maturing and open pollinated (OPV) varieties and hybrid pearl millet cultivars is yielding results (Rai et al., 2012). Biofortified pearl millet hybrids, Dhanashakti (iron enhanced ICTP 8203) and ICMH 1201 were released in 2012 and 2014, respectively. The iron levels has been raised by up to 77 mg/kg, a 30 mg/kg increment from normal pearl millet levels (ICRISAT, 2016). Biofortification is seen as long-term solution capable of reaching remote and rural areas where there are limited essential minerals in staple crops and lack of diverse diets is common (Saltzman et al., 2014). Therefore, it may eventually improve iron and zinc in diets and help combat mineral malnutrition.

This study investigated the use of the simple processing techniques including steeping/ lactic acid fermentation and parboiling with/without abrasive decortication on normal and biofortified pearl millet grain to improve antinutrient reduction and mineral retention, with the aim of improving mineral bioavailability of pearl millet grain products.

4.2 EXPERIMENTAL

4.2.1 Materials

Eight different pearl millet varieties were used in this study. Five normal varieties, Mil Souna, ICTP 8203, ICRI-TABI, GB 8735 and IBV 8004 were obtained from the Senegal Institute for Agricultural Research (ISRA) and the Food Technology Institute (ITA) in Senegal in June 2015. Another normal variety, Kuphanjala-2 was obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Zimbabwe. Two mineral biofortified hybrids, ICMH 1201 and Dhanashakti were kindly supplied by ICRISAT (India), in July 2015.

4.2.2 Methods

4.2.2.1 Grain processing

Cleaning

Grain was cleaned by blowing with compressed air. Any visible foreign material was handpicked and removed.

Steeping (back-slopped lactic acid fermentation)

Clean whole grain (50 g) was weighed into a 250 mL beaker. A supernatant, (25 mL) of previously LAB fermented sorghum flour was poured into the beaker, which was then covered. The samples were incubated for 8 hours at 30^0C in an incubation cabinet. Steeping/lactic acid fermentation for 8 hours minimised leaching out of soluble minerals from the grain. After incubation, the grain was spread on aluminium foil and dried at 35^0C for 24 hours in a forced draught oven.

Parboiling

Grain (50 g) was spread on a 1400 µm sieve and then stacked on another sieve. Tap water (600 mL) was boiled in a pan, and then immediately upon boiling, the sieves were stacked on top of the open pan (Figure 4.2). Parboiling was carried out for 20 minutes using rapidly generated steam. The shorter parboiling time was done to avoid cooking the pearl millet grain based on preliminary work. After steaming, the grain was spread on an aluminium foil and dried at 35^0C for 24 hours in a forced draught oven.

A B C

Figure 4. 2 Parboiling of pearl millet, A. Sample (50 g) sample spread on a sieve, B. Parboiling setup, C. Dried grain spread on aluminium foil

Decortication

This was performed using a Tangential Abrasive Dehulling Device (TADD, Venables Machine Works, Saskatoon, Canada), according to Gomez et al. (1997). Decortication was carried out on dry raw, steeped and parboiled grains. Grain (10 g) was placed in each of the TADD cups (4 samples in duplicate were decorticated at a time). The TADD cups were closed. Decortication was for 5 minutes. The decorticated grain was removed from the cups using vacuum suction and weighed.

The percentage kernel removed was calculated representing dehulling loss, where

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

Milling

Clean whole grain (raw, steeped and parboiled) was milled into flour using an IKA MF 10 air-cooled coffee mill (MF 10.2) (Staufen, Germany) fitted with a 500 µm sieve screen.

4.2.3 Analyses

4.2.3.1 Grain characterization

The following methods were applied to characterize the pearl millet varieties:

Thousand kernel weight (TKW)

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

Endosperm texture

This was determined by visual estimation according to International Association for Cereal Science and Technology (ICC) Standard 176 (ICC, 2012). Sound grains (20) were cut into half with a scalpel. On the basis of the relative proportion of corneous to floury endosperm, grains were classified into corneous, intermediate and floury. Tests were performed in duplicate. Images of the endosperm texture were also taken using a stereomicroscope (Zeiss Discovery V20, Jena, Germany).

Grain size

Pearl millet kernel size was determined by sieving the grain through screen opening sizes of 2.36 mm and 1.70 mm, according to Gomez et al. (1997). Sieves (2360 µm top, 1700 µm middle and receiving pan bottom) were stacked onto a vibratory sieve shaker (Fritsch Analysette 3 PRO, Idar-Oberstein, Germany). Grain (100 g) was poured over the 2360 µm sieve and the shaker run for 1 min. Grain collected on each sieve and the bottom receiving pan were weighed. The test was performed in duplicate. The fractions were divided into three groups; large > 2.36 mm, medium 1.70-2.36 mm and small < 1.70 mm.

Colour determination

Colour was determined by tri-stimulus colorimetry method using a Minolta colorimeter (Chroma meter- CR-400, Konica Minolta, Osaka, Japan) in Cielab scale L^* a^* b^*values (Oomah et al., 2006). The Konika Minolta Chrome meter CR 400 was calibrated on a white tile. The grains were filled into petri dish. The test was performed in triplicate.

4.2.3.2 Proximate analysis (moisture, crude fat, crude protein and crude fibre)

Moisture, crude fat and crude protein were determined according to approved American Association of Cereal Chemists (AACC International, 2000) and Association of Official Analytical Chemists (AOAC International, 2000) methods. Moisture (was determined by the loss in weight of the samples after drying for 3 hours at 103^0C, following the AACC 30-25 method.

Ether extraction (crude fat) was performed using a Tecator Soxtec System HT 1043 (Apeldoorn, Netherlands) extraction unit following AOCC method 992.23. This was done by extracting 2 g sample using petroleum ether (boiling point; 60^0C). Analyses were performed in duplicate.

Crude protein was determined by the thermal combustion (Dumas) method AOAC 992.23 using a Dumatherm Protein/ Nitrogen Analyzer (Dumatherm N Pro, Königswinter Germany). The methodology is a three phase analysis where the nitrogen in the sample is released through chemical decomposition by heat (combustion at 850^{o} C). The nitrogen content was converted to % protein using a factor 6.25. Protein assays were performed in triplicate.

Crude fibre was determined by a filter bag technique using an automated Fibre Analyser Ankom 2000 (Macedon, New York). Accurately weighed samples (approx. 1 g) were placed on a filter bag, sealed, defatted using petroleum ether and air dried. Digestion was with sulphuric acid (0.255 M) and sodium hydroxide (0.313 M). After extraction, the samples were rinsed with acetone, air dried before complete drying using an oven at 102±2^{0}C for 3 hours. The entire bag and digested samples were then ashed for 2 hours at 600^{0}C in order to subtract inorganic matter. Analyses were performed in duplicate.

4.2.3.3 Mineral content (iron, zinc, phosphorus, calcium and magnesium)

Individual minerals were quantified using approved methods of the AOAC International (2000). Acid digestion was carried out using a heating block according to AOAC method 935.13. An accurately weighed sample (0.5 g) was digested using a combination (5:2, v/v) of 65% nitric acid and 70% perchloric acid at 240^{0}C. After digestion, the samples were neutralised using deionised water and diluted to 50 mL in a volumetric flask. Iron, zinc and magnesium were analysed using AOAC method 999.10. A GBC 905 atomic absorption spectrophotometer (Braeside, Australia) was used to quantify iron, zinc and magnesium.

Calcium was determined following AOAC method 935.13. Calcium interacts with other elements therefore a solution of 1% lanthanum chloride heptahydrate and anhydrous nitric acid was used to inhibit the influence of other elements (Giron, 1973). A Perkin-Elmer, 5100 atomic absorption spectrophotometer (Walluf, Germany) was used.

A colorimetric method was used for phosphorus determination according to AOAC method 965.17. A yellow colour is developed due to ammonium molybdate tetrahydrate and ammonium vanadate reaction with phosphorus in the sample. It is measured against a phosphorus standard. The complex was measured colorimetrically at 400 nm.

4.2.3.4 Phytate content

Phytate content was determined using an indirect quantitative anion-exchange method (Frubeck et al., 1995). The resin used was Dowex 1; anion-exchange resin-AG 1-4, 4% cross-linkage, chloride form, 100-200 mesh (Sigma, Johannesburg, South Africa). The organic phytate phosphate (inositol-1 to 6-phosphate) was reacted with ferric chloride and sulphosalicylic acid and the absorbance of the complex measured at 500 nm. The phytate was expressed as mg phytate equivalents per 100 g.

4.2.3.5 Total phenolic content

Total phenolic compounds were determined using a Folin-Ciocalteu assay according to the method of Singleton and Rossi (1965), as modified by Duodu and Apea-Bah (2016). In this non-specific assay chromogens formed due to the reaction of the Folin-Ciocalteu reagent and phenolic hydroxyl groups are measured. The phenolic compounds were extracted from milled samples (0.5 g) for 2 hours using 1% HCl in methanol. The extracts were centrifuged and the supernatant used for total phenolic analysis. An aliquot of the extract (0.5 mL) was diluted with 39.5 mL water, and reacted with 2.5 mL Folin Ciocalteu reagent and 7.5 mL of 20% (w/v) sodium carbonate solution. The sample was incubated at room at ambient temperature for 2 hours. The absorbance was read at 760 nm. Catechin (Sigma Cat No. C1788) was used as a standard. Total phenols were expressed as mg catechin equivalents per 100 g.

4.2.3.6 Statistical analyses

All experiments were performed at least twice. Data were analysed using one way and multifactorial way analysis of variance (ANOVA) using Statistica v10 (Tulsa, Oklahoma, USA) software. Fisher's Least Significant Difference (LSD) test was used to determine significant differences between the treatments. Pearson's correlation and principal component analysis (PCA) were performed using Statistical Package for the Social Sciences (SPSS) (Armonk, New York) and Microsoft Excel XLSTAT (Addinsoft, New York) software programs, respectively, to determine the relationships between the parameters. Principal component analysis was performed as it emphasises observational correlations/relationships, patterns and variation within and with sets of independent and dependent variables.

4.3 RESULTS AND DISCUSSION

4.3.1 Physical characteristics and chemical composition of eight pearl millet grain varieties

There were significant (p<0.05) and large differences in the quality characteristics among the different pearl millet varieties. Thousand kernel weight (TKW), grain size, endosperm texture, grain colour and dehulling loss (DHL) are important measures that influence processing and end-use attributes of pearl millet (Gomez et al., 1997). TKW, indicating grain size and density (Chiremba et al., 2011), ranged between 6.9 g (Mil Souna) and 14.7 g (Dhanashakti). Varieties Kuphanjala-2, Dhanashakti and ICMH 1201 had the highest 1000 grain weight (TKW), 12.9, 14.7 and 13.6 g, respectively. Most varieties fell within the medium (1.70-2.36 mm) grain size fraction. As with TKW, Kuphanjala-2 (58.1%), Dhanashakti (78.8%) and ICMH 1201 (51.8%) had the highest percentage of large grain size fraction (>2.36 mm). There were highly significant correlations between TKW and grain size (r = -0.866, p<0.01, r = 0.917, p<0.001 for medium and large grain size, respectively) (Table 4.2). Principal component analysis (PCA) illustrates that there was a positive relationship between large kernel size grain (>2.36 mm) and TKW (Figure 4.3). However, TKW was inversely related to small (<1.70 mm) and medium (1.70-2.36 mm) size grains.

Dehulling loss is an important assay with respect to evaluating hardness and flour yield of grain (Chiremba et al., 2011). For pearl millet, dehulling loss ranged between 11.2% (Dhanashakti) and 22.0% (Mil Souna) (Table 4.1). Most of the pearl millet varieties were corneous type with an average of 48.3% corneous, 38.8% intermediate and 12.9% floury. Varieties with dominant corneous endosperm texture were IBV 8004 (76.7%), Kuphanjala-2 (66.7%), ICMH 1201 (51.8%) and Dhanashakti (43.3%). Also, these predominantly corneous endosperm varieties had low dehulling loss, IBV 8004 (11.8%), Kuphanjala-2 (11.5%), ICMH 1201 (11.9%) and Dhanashakti (11.2%). In sorghum, hard kernel grains have been found to have lower dehulling loss and have high flour yield with low bran contamination (Taylor and Dewar, 2001). In relation to floury endosperm proportion, GB 8735 had the highest proportion (28.3%), followed by ICTP 8203 (18.3%) and Mil Souna and ICRI-TABI (13.3%). It is evident that these varieties with a high proportion of floury endosperm also seemed to have high dehulling loss, between 15.8 and 22%. High dehulling loss was positively correlated to floury endosperm (Figure 4.3) (r = 0.741, p<0.05) (Table 4.2).

The grain colour of the pearl millet varieties is given in Table 4.3. L*value, representing grain darkness/lightness ranged from 43.2 (ICRI-TABI) to 49.2 (IBV 8004), a*value (redness-greenness) from 1.7 (ICMH 1201) to 3.7 (ICRI-TABI), and b*value (yellowness- blueness) from 6.1 (ICMH 1201) to 9.6 (IBV 8004). There was no correlation between grain colour and the other parameters.

Crude fibre content ranged between 1.23 g/100 g (ICMH 1201) and 2.94 g/100 g (GB 8735) (Table 4.4). The crude fibre content was lower than reported in previous studies, where values of 2.48 to 4.23 g/100 g (Lestienne et al., 2007, Hama et al., 2011) were found. The difference in crude fibre content can be attributed to the use of different methodologies in the studies. In this study, a filter bag technique using an automated Fibre Analyser Ankom 2000 (Macedon, New York) was used. The crude fat content of the eight varieties ranged between 4.17 and 6.50 g/100 g. Of note was that the mineral biofortified varieties Dhanashakti and ICMH 1201 had the highest fat contents, 6.50 and 5.50 g/100 g, respectively. The fat contents were similar to previous studies of pearl millet. Lestienne et al. (2007) reported 5.58 and 5.77 g/100 g, while Hama et al. (2011) reported 4.80 g/100 g. The fat content of pearl millet grain is substantially higher than of most cereal grains (Taylor, 2004). Crude fibre did not significantly (p>0.05) correlate with any parameter. However, fibre was inversely associated with crude fat (Figure 4.3). With regard to crude fat, it was negatively correlated with dehulling loss (r = -0.815, p<0.05) (Table 4.2). This is because fat is concentrated in the germ (Taylor et al., 2010), hence less affected by decortication.

Concerning protein content, ICRI-TABI had the lowest protein content (10.2 g/100 g), while IBV 8004 had the highest (13.5 g/100 g) (Table 4.5). Taylor (2004) indicated that the protein content in pearl millet grain can vary widely between 8.6 and 19.4 g/100 g, with the largest concentration of protein in the germ. Table 4.2 shows that protein was inversely correlated (r = -0.728, p<0.05) with dehulling loss. Also, there was a significant correlation (r = 0.756, p<0.05) between protein and fat contents.

Mineral contents (Table 4.6) varied substantially among the pearl millet varieties for all the minerals measured. The minerals of major concern, iron and zinc, which are commonly lacking in cereal based diets (Hunt, 2003), varied greatly between the varieties. Iron content ranged between 3.0 (Mil Souna) and 9.6 mg/100 g (Dhanashakti). The zinc levels ranged between 3.0 mg/100 g (Kuphanjala-2) and 4.8 mg/100 g (Dhanashakti). The literature indicates the iron content of normal pearl millet ranges between 1.9 and 5.5 mg/100 g, while

zinc content varies between 2.0 and 3.2 mg/100 g (Lestienne et al., 2007, Minnis-Ndimba et al., 2015). Generally, in the present study, iron and zinc levels for normal pearl millet were within the literature range.

As seen in the present study, the iron content of the two mineral biofortified varieties was double that of most of the normal varieties. In this study, the iron content of Dhanashakti, the first developed ICRISAT biofortified type, was 9.6 mg/100 g, within the reported ICRISAT range of 4.9-10.6 mg/100 g (Rai et al., 2013). The iron content of ICMH 1201 variety was 8.8 mg/100 g. Similarly, a range of 4.7 to 9.7 mg/100 g for this variety has been given (Rai et al., 2013). In this study, the measured zinc contents for Dhanashakti and ICMH 1201 were 4.8 and 4.4 mg/100 g, respectively. Zinc content values of 1.8-7.1 and 2.1-5.8 mg/100 g for Dhanashakti and ICMH 1201 varieties, respectively, have been reported (Rai et al., 2013). Therefore the values found in this study were in good agreement with the ICRISAT studies. Pearson's correlations did not show a significant correlation between iron and zinc contents (Table 4.2). However, PCA (Figures 4.3 and 4.4), shows that ICMH 1201 and Dhanashakti varieties were aligned together in respect of high iron and zinc. Other studies have reported a highly significant and positive correlation between biofortified pearl millet varieties iron and zinc contents, indicating successful simultaneous biofortification of both minerals (Velu et al., 2007, Rai et al., 2012).

The calcium content were very low among the varieties, ranging between 5 and 14 mg /100 g. Abdalla et al. (1998) reported a higher range, between 10 and 80 mg/100 g, similarly Bashir et al. (2014) found an average of 22.9 mg/100 g over 225 accessions of pearl millet. Generally, the calcium content of cereal grains ranges between 100 and 200 mg/100 g (Bock, 2000). Therefore compared to other cereals, pearl millet has low calcium content. The magnesium content for the pearl millet varieties varied between 103 and 146 mg/100 g. This agrees with findings that on average, pearl millet grain magnesium content is approximately 137 mg/100 g (Bashir et al., 2014). Phosphorus levels ranged between 287 mg/100 g (ICRI-TABI) and 416 mg/100 g (Kuphanjala-2). This is similar to the mean value of 402 mg/100 g reported by Bashir et al. (2014). There were significant correlations between calcium and magnesium (r = 0.804, p<0.05) and between magnesium and phosphorus (r = 0.903, p<0.01) (Table 4.2). Phytate is known to bind with cationic elements iron, zinc, magnesium, calcium and phosphorus (Bohn et al., 2008). As stated the major storage molecule for phosphorus is phytate. However, phytate also exists as a mixture of potassium and magnesium salts in the germ tissues (Bryant et al., 2005). Existences of insoluble complexes of calcium, zinc and

phytate have been reported (Lonnerdal, 2000). Therefore the positive correlation and association of calcium, phosphorus, magnesium and phytate might be indicating their co-location in the grain components, particularly in the germ tissues.

As stated, the antinutritional factors in pearl millet grain are of considerable importance as they reduce essential mineral bioavailability (Lestienne et al., 2005b). Phytate (the major antinutrient in grains) (Bryant et al., 2005), varied highly significantly ($p<0.001$) among the pearl millet varieties (Table 4.7). The levels ranged between 830 mg/100 g (ICRI-TABI) and 1360 mg/100 g (Kuphanjala-2). By comparison, El Hag et al. (2002) reported levels of 443 and 1076 mg/100 g in pearl millet grain. Similarly, Elyas et al. (2002) found 618 and 786 mg/100 g. As mentioned, variations in the phytate content in pearl millet grain have been attributed to genetic and ecological factors (Elyas et al., 2002). Phytate content was highly significantly correlated ($r = 0.924$, $p<0.001$) and associated with phosphorus content (Table 4.2, Figure 4.3, respectively). This is expected, as much of the phosphorus is stored as phytate in cereal grains (Bohn et al., 2008).

As stated, phenolic compounds are another component in pearl millet grain that limit mineral bioavailability, in particular iron-binding polyphenols (Lestienne et al., 2005a). The total phenolic content (TPC) of the varieties varied between 251 mg/100 g (GB 8735) and 403 mg/100 g (Dhanashakti) (Table 4.8). In a study of whole pearl millet grain TPC, El Hag et al. (2002) reported similar values of 304 and 444 mg/100 g in two varieties. The major phenolic compounds in pearl millet are phenolic acids and flavonoids of the c-glycosyl flavone type (Jha et al., 2015). PCA shows that high mineral hybrids (Dhanashakti and ICMH 1201) were positively associated with large kernel size (>2.36) and kernel weight (TKW), TPC, fat, iron and zinc contents (Figures 4.3 and 4.4). It appears that large grain size and weight contributes to TPC, fat, zinc and iron contents.

Table 4. 1 Thousand Kernel Weight (TKW), Grain Size, Endosperm Texture and Dehulling Loss (DHL) of Pearl Millet Varieties

Variety	*TKW (g as is basis)	**Grain Size (%)			*Endosperm texture (%)			**DHL (%)
		< 1.70 mm	1.70–2.36 mm	> 2.36 mm	Corneous	Intermediate	Floury	
Kuphanjala-2	12.9^{e} ±0.1	0.8^{a} ±0.1	41.1^{b} ±0.2	58.1^{e} ±0.3	66.7^{ef} ±5.8	28.3^{ab} ±5.8	5.0^{a} ±0.0	11.5^{a} ±0.0
IBV 8004	9.5^{c} ±0.2	2.4^{bc} ±0.0	87.3^{g} ±0.2	10.4^{b} ±0.2	76.7^{f} ±2.9	16.7^{a} ±5.8	6.7^{a} ±2.9	11.8^{a} ±0.0
ICRI-TABI	8.1^{b} ±0.1	3.1^{cd} ±0.1	94.2^{h} ±0.1	2.5^{a} ±0.1	58.3^{de} ±5.8	28.3^{ab} ±2.9	13.3^{ab} ±2.9	16.7^{b} ±0.1
Mil Souna	6.9^{a} ±0.2	25.8^{e} ±0.1	72.8^{e} ±0.3	1.2^{a} ±0.0	38.3^{b} ±2.9	48.3^{c} ±2.9	13.3^{ab} ±2.9	22.0^{d} ±0.5
ICTP 8203	9.5^{c} ±0.3	3.9^{d} ±0.5	84.1^{f} ±0.2	12.0^{b} ±0.6	43.3^{bc} ±2.9	38.3^{bc} ±5.8	18.3^{b} ±2.9	15.8^{b} ±0.3
GB 8735	11.5^{d} ±0.2	0.5^{a} ±0.0	63.9^{d} ±0.4	35.6^{c} ±0.3	8.3^{a} ±2.9	63.3^{d} ±2.9	28.3^{c} ±2.9	20.6^{c} ± 0.3
Dhanashakti	14.7^{g} ±0.2	0.4^{a} ±0.0	20.9^{a} ±0.6	78.8^{f} ±0.6	43.3^{bc} ±2.9	48.3^{c} ±5.8	8.3^{a} ±2.9	11.2^{a} ±0.1
ICMH 1201	13.6^{f} ±0.1	2.6^{ab} ±0.1	46.1^{c} ±1.6	51.8^{d} ±1.0	51.7^{cd} ±2.9	38.3^{bc} ±2.9	10.0^{ab} ±0.0	11.9^{a} ±0.2
Mean	**10.9±2.7[25]**	**4.8±8.3[173]**	**63.9±24.8[39]**	**31.4±28.2[90]**	**48.3±20.0[41]**	**38.8±14.5[37]**	**12.9±7.6[59]**	**15.2±4.2[28]**

Values are mean ± standard deviation (*n = 3; ** n= 2). Mean values in the same column with different superscript letters are significantly different ($p<0.05$)

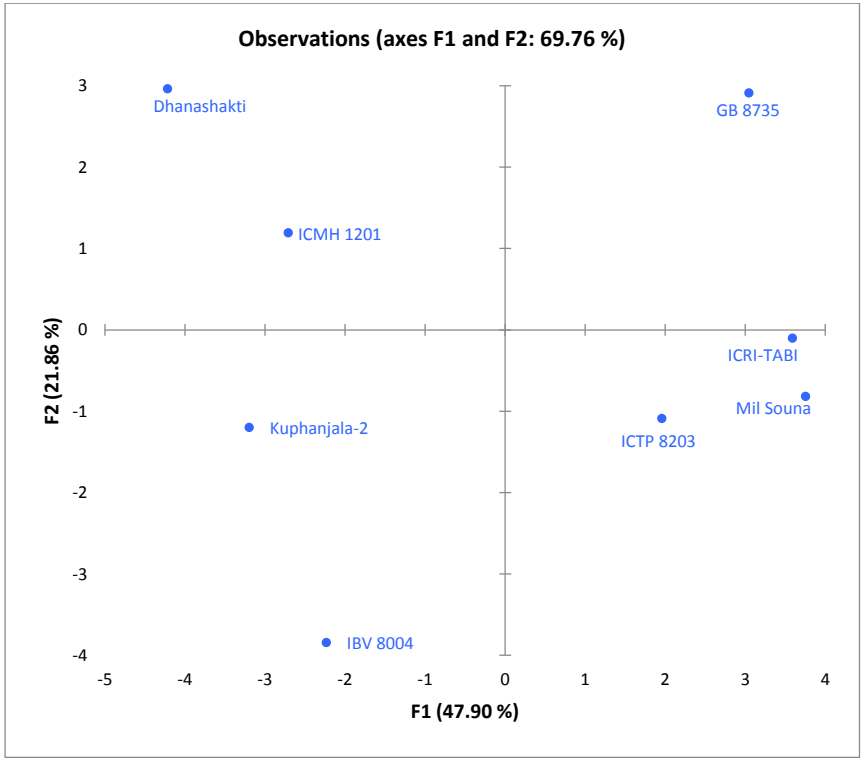
[] – values in square brackets are coefficients of variance

Table 4. 2 **Pearson's Correlation Coefficients Between Phytate, TPC, Fe, Zn, Ca, Mg, P, Phytate:Mineral Molar ratios, Protein, Fibre, Fat Contents and % Dehulling Thousand Kernel Weight, Endosperm Texture and Grain Size for Whole Raw Pearl Millet Varieties**

	Phytate	TPC	Fe	Zn	Ca	Mg	P	Phyt: Fe	Phyt: Zn	PhytCa: Zn	Protein	Fibre	Fat	DHL	TKW	C	I	F	< 1.70	1.70-2.36
TPC	.476																			
Fe	.371	**.790***																		
Zn	.203	.519	.629																	
Ca	.394	.283	.285	.609																
Mg	**.811***	.556	.554	.505	**.804***															
P	**.924*****	.481	.532	.264	.492	**.903****														
Phyt:Fe	.075	-.495	**-.864****	-.435	.000	-.157	-.164													
Phyt:Zn	.676	.098	-.071	-.573	-.161	.300	.582	.292												
PhytCa:Zn	**.784***	.364	.292	-.042	.546	**.814***	**.830***	.060	.697											
Protein	**.723***	**.729***	.505	.647	**.771***	**.886****	.704	-.056	.122	.603										
Fibre	-.203	-.471	-.154	-.476	-.544	-.325	-.017	-.156	.185	-.214	-.607									
Fat	.373	**.845****	**.753***	**.715***	.490	.588	.413	-.537	-.175	.212	**.756***	-.476								
DHL	-.702	-.705	**-.713***	-.461	-.501	**-.752***	-.705	.420	-.276	-.593	**-.728***	.405	**-.815***							
TKW	.465	**.724***	**.975****	.574	.226	.577	.629	**-.820***	.043	.338	.472	-.022	.642	-.665						
C	.439	.284	.053	-.010	.505	.501	.36	.156	.355	.566	.522	-.450	.481	-.683	-.049					
I	-.404	-.062	.109	.137	-.413	-.391	-.306	-.270	-.399	-.509	-.363	.339	-.299	.585	.185	**-.967*****				
F	-.421	-.658	-.357	-.237	-.587	-.616	-.410	.094	-.202	-.569	**-.729***	.580	**-.741***	**.741***	-.225	**-.871****	**.718***			
<1.70 mm	-.486	-.206	-.596	-.256	-.015	-.370	-.566	.563	-.231	-.216	-.146	-.286	-.369	.645	-.665	-.146	.192	.029		
1.70-2.36	-.295	**-.846****	**-.885****	-.521	-.184	-.476	-.461	**.728***	.040	-.260	-.500	.105	-.638	.457	**-.876****	.139	-.352	.300	.257	
>2.36	.403	**.805***	**.958*****	.537	.169	.530	.574	**-.811***	.030	.293	.482	-.008	.671	-.593	**.971*****	-.082	.255	-.271	-.524	**-.958*****

***. Correlation is significant at the 0.001 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
TPC: Total phenolic compounds, DHL: Dehulling loss, TKW: Thousand kernel weight
C: Corneous endosperm, I: Intermediate endosperm, F: Floury endosperm

A



B

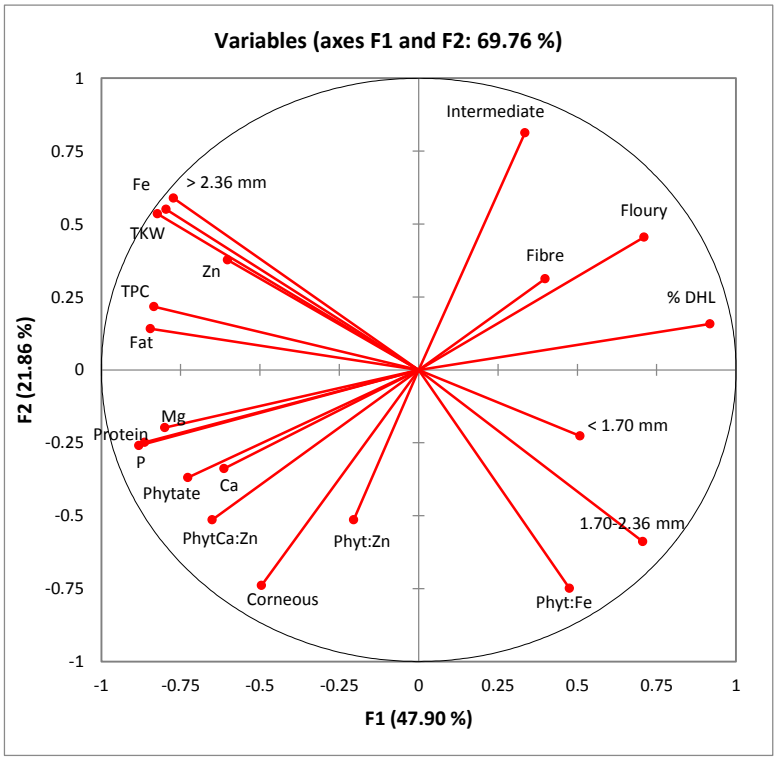


Figure 4. 3 Principal component analysis of the raw whole grain physical characteristics and chemical composition of the eight pearl millet varieties

A. Pearl millet varieties: (Kuphanjala-2, IBV 8004, ICRI-TABI, Mil Souna, ICTP 8203, Dhanashakti, GB 8735 and ICMH 1201)

B. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus, calcium, protein, fat, fibre, phytate: iron, phytate: zinc and phytate × calcium: zinc, % Dehulling loss (% DHL), grain size (<1.70, 1.70-2.36, >2.36 mm) and Endosperm texture (% corneous, intermediate and floury).

Table 4. 3 Lab Colour of the Eight Pearl Millet Varieties

Variety	Colour		
	L (Light-Dark)	**a (Red-Green)**	**b (Yellow-Blue)**
Kuphanjala-2	47.7^{c}±0.3	1.8^{a}±0.2	7.9^{abc}±0.7
IBV 8004	49.2^{c}±1.4	1.8^{a}±0.3	9.6^{c}±0.3
ICRI-TABI	43.2^{a}±1.4	3.7^{c}±0.2	7.6^{abc}±1.1
Mil Souna	48.7^{c}±0.3	2.6^{b}±0.2	9.1^{bc}±0.4
ICTP 8203	48.1^{c}±1.3	2.8^{b}±0.3	9.0^{bc}±0.5
GB 8735	46.8^{bc}±0.7	3.1^{bc}±0.3	8.2^{bc}±0.6
Dhanashakti	43.8^{a}±1.1	2.5^{b}±0.2	7.4^{ab}±0.9
ICMH 1201	44.0^{ab}±2.5	1.7^{a}±0.1	6.1^{a}±0.7
Mean	**46.4±2.4[5]**	**2.5±0.7[28]**	**8.1±1.1[14]**

Values are mean ± standard deviation (n = 3). Mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

[] – values in square brackets are coefficients of variance

Table 4. 4 Effects of Abrasive Decortication and Pearl Millet Variety on Crude Fibre and Fat Content (mg/100 g d.b.)

Variety	Crude fibre			Crude fat		
	Whole	Decorticated	Mean effect of variety (V)	Whole	Decorticated	Mean effect of variety (V)
Kuphanjala-2	2.24^{e} ±0.07	1.34^{bc} ±0.09 (-40)	**1.79^{D} ±0.52**	5.29^{fg} ±0.07	6.60^{j} ±0.15 (+25)	**5.95^{D} ±0.76**
IBV 8004	1.48^{cd} ±0.02	0.98^{a} ±0.02 (-34)	**1.23^{B} ±0.29**	5.40^{fgh} ±0.07	5.76^{hi} ±0.04	**5.58^{C} ±0.21**
ICRI-TABI	2.24^{e} ±0.07	0.78^{a} ±0.01 (-65)	**1.52^{C} ±0.83**	5.00^{ef} ±0.00	3.42^{a} ±0.10 (-32)	**4.21^{AB} ±0.91**
Mil Souna	1.48^{cd} ±0.02	0.80^{a} ±0.00 (-46)	**1.14^{AB} ±0.39**	4.52^{cd} ±0.03	3.85^{ab} ±0.07 (-14)	**4.18^{AB} ±0.39**
ICTP 8203	1.54^{d} ±0.07	0.92^{a} ±0.02 (-40)	**1.23^{B} ±0.36**	4.48^{cd} ±0.18	3.74^{ab} ±0.14 (-17)	**4.11^{A} ±0.44**
GB 8735	2.94^{f} ±0.07	1.19^{b} ±0.02 (-60)	**2.06^{E} ±1.01**	4.17^{bc} ±0.11	4.73^{de} ±0.03 (+13)	**4.45^{B} ±0.33**
Dhanashakti	1.49^{cd} ±0.03	0.91^{a} ±0.01(-39)	**1.20^{B} ±0.34**	6.50^{j} ±0.28	6.15^{ij} ±0.00	**6.32^{E} ±0.26**
ICMH 1201	1.23^{b} ±0.05	0.83^{a} ±0.05 (-33)	**1.03^{A} ±0.23**	5.50^{gh} ±0.14	5.70^{ghi} ±0.07	**5.60^{C} ±0.15**
Mean effect of decortication (D)	**1.83^{B} ±0.56**	**0.97^{A} ±0.19 (-47)**	**V×D *P*≤0.001**	**5.11^{A} ±0.72**	**4.99^{A} ±1.18**	**V×D *P*≤0.001**

Values expressed as means of two independent samples analysed in duplicate (n=2) ± 1 SD

^{abc} - Values with different superscripts, differ significantly (p≤0.001),

^{ABC} - Least Significant Mean values from main effects Factorial ANOVA with different superscripts in the same row/column, differ significantly (p≤0.001)

() – values in brackets are % change (where significant - p≤0.001) in crude fibre and fat content of decorticated grain compared to the whole grain pearl millet

Table 4. 5 Effects of Abrasive Decortication, Pearl Millet Variety, Steeping in Back Slopped Liquor and Parboiling on Crude Protein Content (mg/100 g d.b.)

	Raw grain		Steeped grain		Parboiled grain		Mean effect of variety (V)
Variety	**Whole**	**Decorticated**	**Whole**	**Decorticated**	**Whole**	**Decorticated**	
Kuphanjala-2	12.4^{pq}±0.0	12.0^{nop}±0.1	12.3^{opq}±0.2	11.5^{klmn}±0.0(-7)[-7]	12.3^{opq}±0.1	11.6^{klmn}±0.3(-6)[-6]	**12.0^{E}±0.4**
IBV 8004	13.5^{r}±0.1	12.6^{pq}±0.1(-7)	13.9^{r}±0.1	12.7^{q}±0.0(-6)[-9]	13.6^{r}±0.1	12.5^{pq}±0.0(-8)[-8]	**13.1^{F}±0.6**
ICRI-TABI	10.2^{efg}±0.2	10.2^{defg}±0.0	10.4^{fg}±0.3	8.8^{a}±0.1(-14)[-15]	10.2^{efg}±0.1	8.9^{ab}±0.1(-13)[-13]	**9.8^{A}±0.7**
Mil Souna	11.6^{klmn}±0.1	11.1^{hijk}±0.0	12.0^{nop}±0.1	10.7^{ghi}±0.0(-8)[-11]	11.8^{lmno}±0.0	11.2^{ijklm}±0.7	**11.4^{D}±0.5**
ICTP 8203	11.3^{jklm}±0.2	10.3^{fg}±0.0(-9)	11.4^{klmn}±0.0	10.3^{fg}±0.3(-9)[-10]	11.2^{ijkl}±0.1	9.9^{cdef}±0.0(-12)[-12]	**10.7^{C}±0.6**
GB 8735	10.6^{gh}±0.1	9.6^{cde}±0.1(-9)	10.7^{ghij}±0.2	9.6^{bd}±0.1(-9)[-10]	10.5^{fgh}±0.0	9.5^{bc}±0.0(-10)[-10]	**10.1^{B}±0.6**
Dhanashakti	13.4^{r}±0.1	13.5^{r}±0.1	13.8^{r}±0.4	12.5^{pq}±0.1(-9)[-9]	14.0^{r}±0.1	12.5^{pq}±0.2(-7)[-11]	**13.3^{F}±0.6**
ICMH 1201	12.3^{opq}±0.1	11.4^{klm}±0.1(-7)	12.5^{pq}±0.2	11.8^{mno}±0.2[-6]	12.4^{pq}±0.0	11.7^{lmn}±0.1(-5)[-6]	**12.0^{E}±0.4**
Mean effect of processing (P)	**11.9^{C}±1.2**	**11.3^{B}±1.3(-5)**	**12.2^{D}±1.3(+3)**	**11.0^{A}±1.3(-8)[-10]**	**12.0^{CD}±1.3**	**11.0^{A}±1.4(-8)[-8]**	***P*≤0.001**

Values expressed as means of the analysis of two independent samples (n=2) ± 1 SD

^{abc}- Values with different superscripts, differ significantly (p≤0.001),

^{ABC}- Least Significant Mean values from main effects Factorial ANOVA with different superscripts in the same row/column, differ significantly (p≤0.001)

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

[] – values in square brackets are % change in protein content of decorticated processed (steeped and parboiled) grains compared to whole processed grain pearl millet

Table 4. 6 Effects of Abrasive Decortication, Pearl Millet Variety and Steeping in Back Slopped Liquor on the Iron, Zinc, Calcium, Phosphorus and Magnesium Contents

Variety	Raw grain		Steeped grain		Parboiled grain		Mean effect of variety (V)
	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated	
				Iron (mg/100 g d.b.)			
Kuphanjala-2	$7.51^{s}\pm0.07$	$4.22^{i}\pm0.07(-44)$	$8.21^{t}\pm0.07(+9)$	$4.28^{ij}\pm0.15(-43)[-48]$	$9.59^{vw}\pm0.07(+28)$	$4.53^{ijkl}\pm0.00(-40)[-53]$	**$6.39^{E}\pm2.23$**
IBV 8004	$4.72^{jklm}\pm0.08$	$3.64^{h}\pm0.08(-23)$	$5.22^{no}\pm0.00(+11)$	$3.66^{h}\pm0.14(-22)[-30]$	$4.92^{klmn}\pm0.02$	$3.18^{cdefg}\pm0.07(-33)[-35]$	**$4.22^{C}\pm0.80$**
ICRI-TABI	$4.31^{ij}\pm0.08$	$3.09^{bcdef}\pm0.08(-28)$	$5.10^{mn}\pm0.24(+18)$	$2.74^{bc}\pm0.08(-36)[-46]$	$4.96^{lmn}\pm0.07(+15)$	$2.73^{b}\pm0.08(-37)[-45]$	**$3.82^{B}\pm1.05$**
Mil Souna	$3.04^{bcde}\pm0.15$	$3.23^{defgh}\pm0.08$	$1.33^{a}\pm0.04(-56)$	$3.48^{fgh}\pm0.08(+14)[+161]$	$2.84^{bcd}\pm0.11$	$3.59^{gh}\pm0.07(+18)[+26]$	**$2.92^{A}\pm0.79$**
ICTP 8203	$4.50^{ijk}\pm0.15$	$3.33^{efgh}\pm0.08(-26)$	$5.01^{mn}\pm0.01(+11)$	$3.30^{efgh}\pm0.07(-27)[-34]$	$5.06^{mn}\pm0.22(+12)$	$2.70^{b}\pm0.15(-40)[-47]$	**$3.98^{B}\pm0.96$**
GB 8735	$5.63^{opq}\pm0.07$	$3.37^{efgh}\pm0.15(-40)$	$5.77^{pq}\pm0.07$	$4.44^{ij}\pm0.14(-21)[-23]$	$5.83^{q}\pm0.08$	$3.65^{h}\pm0.15(-35)[-37]$	**$4.78^{D}\pm1.06$**
Dhanashakti	$9.55^{vw}\pm0.01$	$7.18^{s}\pm0.08(-25)$	$9.25^{uv}\pm0.08$	$6.70^{r}\pm0.08(-30)[-28]$	$9.80^{w}\pm0.03$	$6.31^{r}\pm0.00(-34)[-36]$	**$8.13^{G}\pm1.50$**
ICMH 1201	$8.84^{u}\pm0.30$	$5.26^{no}\pm0.08(-40)$	$8.28^{t}\pm0.35(-6)$	$5.18^{n}\pm0.01(-41)[-37]$	$8.15^{t}\pm0.09(-9)$	$5.34^{nop}\pm0.30(-40)[-35]$	**$6.84^{F}\pm1.68$**
Mean effect of processing (P)	**$6.01^{C}\pm2.27$**	**$4.17^{B}\pm1.36$ (-31)**	**$6.02^{C}\pm2.45$**	**$4.22^{B}\pm1.21(-30)[-30]$**	**$6.39^{D}\pm2.42(+6)$**	**$4.00^{A}\pm1.25(-33)[-37]$**	**PxV $P\leq0.001$**
				Zinc (mg/100 g d.b.)			
Kuphanjala-2	$2.95^{bcdefg}\pm0.08$	$2.47^{a}\pm0.08(-16)$	$3.11^{efghij}\pm0.00$	$2.94^{bcdefg}\pm0.08[-5]$	$3.00^{cdefgh}\pm0.00$	$3.72^{l}\pm0.38(+26)[+24]$	**$3.03^{A}\pm0.40$**
IBV 8004	$4.12^{stu}\pm0.00$	$3.75^{opqr}\pm0.08(-9)$	$4.26^{u}\pm0.15$	$3.88^{qrst}\pm0.01[-9]$	$3.85^{pqrs}\pm0.01$	$3.66^{nopq}\pm0.00(-11)$	**$3.92^{C}\pm0.22$**
ICRI-TABI	$3.17^{fghijk}\pm0.00$	$2.66^{ab}\pm0.08(-16)$	$3.38^{jklmn}\pm0.08$	$3.71^{opq}\pm0.07(+17)[+10]$	$3.25^{ghijkl}\pm0.07$	$2.79^{bcd}\pm0.00(-12)[-14]$	**$3.16^{A}\pm0.37$**
Mil Souna	$3.37^{ijklmn}\pm0.08$	$3.40^{jklmn}\pm0.00$	$3.76^{opqr}\pm0.08(+12)$	$2.73^{abc}\pm0.08(-18)[-27]$	$3.66^{nopq}\pm0.07$	$4.18^{tu}\pm0.00(+24)[+14]$	**$3.52^{B}\pm0.46$**
ICTP 8203	$3.18^{fghijk}\pm0.00$	$3.06^{defghi}\pm0.00$	$3.47^{klmno}\pm0.07$	$2.76^{abcd}\pm0.07(-13)[-20]$	$3.30^{hijklm}\pm0.15$	$2.80^{bcde}\pm0.15(-11)[-15]$	**$3.09^{A}\pm0.28$**
GB 8735	$3.61^{mnopq}\pm0.00$	$2.88^{bcdef}\pm0.08(-20)$	$3.72^{opq}\pm0.07$	$3.52^{lmno}\pm0.08$	$3.64^{nopq}\pm0.00$	$3.54^{lmnop}\pm0.00$	**$3.48^{B}\pm0.29$**
Dhanashakti	$4.83^{v}\pm0.07$	$4.97^{v}\pm0.00$	$4.81^{v}\pm0.00$	$4.88^{v}\pm0.08$	$5.06^{v}\pm0.06$	$4.76^{v}\pm0.08$	**$4.88^{E}\pm0.12$**
ICMH 1201	$4.36^{u}\pm0.00$	$4.12^{stu}\pm0.00$	$4.22^{u}\pm0.09$	$4.26^{u}\pm0.22$	$4.21^{u}\pm0.07$	$4.06^{rstu}\pm0.00$	**$4.21^{D}\pm0.13$**
Mean effect of processing (P)	**$3.70^{C}\pm0.65$**	**$3.41^{A}\pm0.81(-8)$**	**$3.84^{D}\pm0.54(+4)$**	**$3.59^{B}\pm0.74(-3)[-7]$**	**$3.75^{CD}\pm0.63$**	**$3.69^{BC}\pm0.66$**	**PxV $P\leq0.001$**
				Calcium (mg/100 g d.b.)			
Kuphanjala-2	$7.51^{lm}\pm0.71$	$1.48^{abcde}\pm0.23(-80)$	$20.61^{st}\pm0.48(+174)$	$3.53^{fgh}\pm0.45(-53)[-83]$	$15.11^{pq}\pm0.45(+101)$	$6.80^{klm}\pm0.46(-9)[-55]$	**$9.17^{C}\pm6.96$**
IBV 8004	$13.72^{op}\pm0.54$	$6.03^{jkl}\pm0.24(-56)$	$23.80^{u}\pm0.21(+76)$	$3.72^{fgh}\pm0.24(-73)[-84]$	$21.48^{t}\pm0.84(+57)$	$2.58^{defg}\pm0.00(-81)[-88]$	**$11.89^{D}\pm8.80$**

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**
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**
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**
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**
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**
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**
Mean 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]**	**PxV *P*≤0.001**
			Phosphorus (mg/100 g d.b.)				
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**
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**
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**
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**
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**
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**
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**
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**
Mean 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]**	**PxV *P*≤0.05**
			Magnesium (mg/100 g d.b.)				
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**
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**
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**
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**
ICTP 8203	114^{lm}±4	95^{hij}±0(-17)	142^{uvw}±1(+25)	79de±2(-31)[-44]	120^{mnop}±1	66^{bc}±2(-42)[-45]	**103^{C}±27**
GB 8735	118^{mno}±2	55^{a}±0(-53)	127^{pqr}±2(+8)	91^{ghi}±3(-23)[-28]	122^{nop}±1	77de±2(-35)[-37]	**98^{B}±27**
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**
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**
Mean 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]**	**PxV *P*≤0.001**

Values expressed as means of the analysis of two independent samples (n=2) ± 1 SD

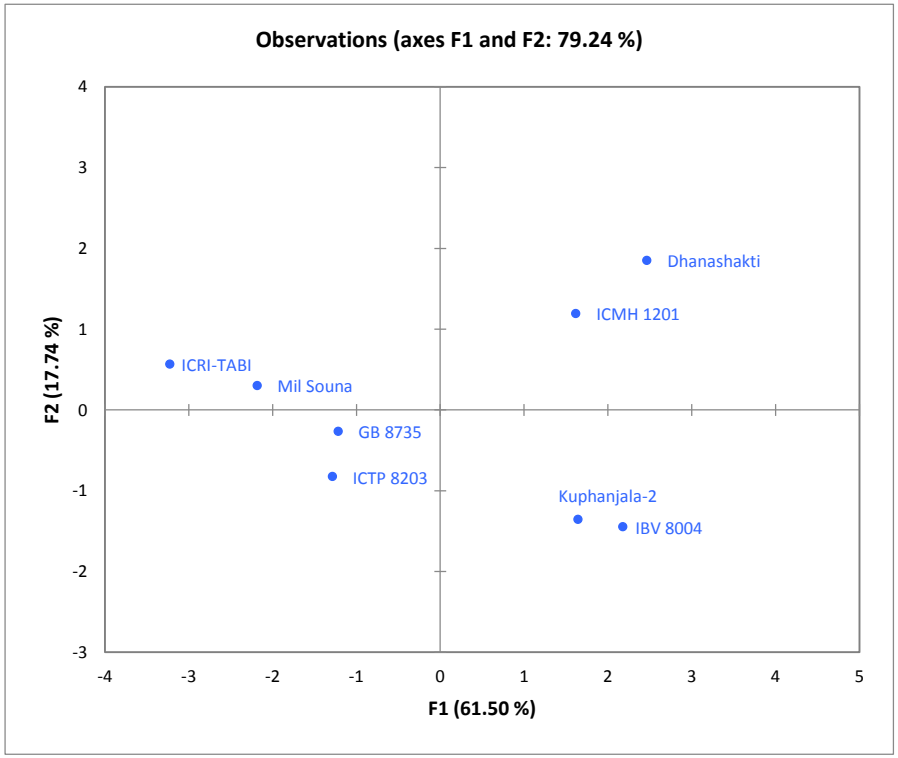
^{abc}- Values with different superscripts, differ significantly (p≤0.001) for iron, zinc, calcium, magnesium and differ significantly (p≤0.05) for phosphorus,

[ABC]- Least Significant Mean values from main effects Factorial ANOVA with different superscripts in the same row/column, differ significantly ($p \leq 0.001$) for iron, zinc, calcium, magnesium and differ significantly ($p \leq 0.05$) for phosphorus

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

[] – values in square brackets are % change in mineral content of decorticated processed (steeped and parboiled) grains compared to whole processed grain pearl millet

A



B

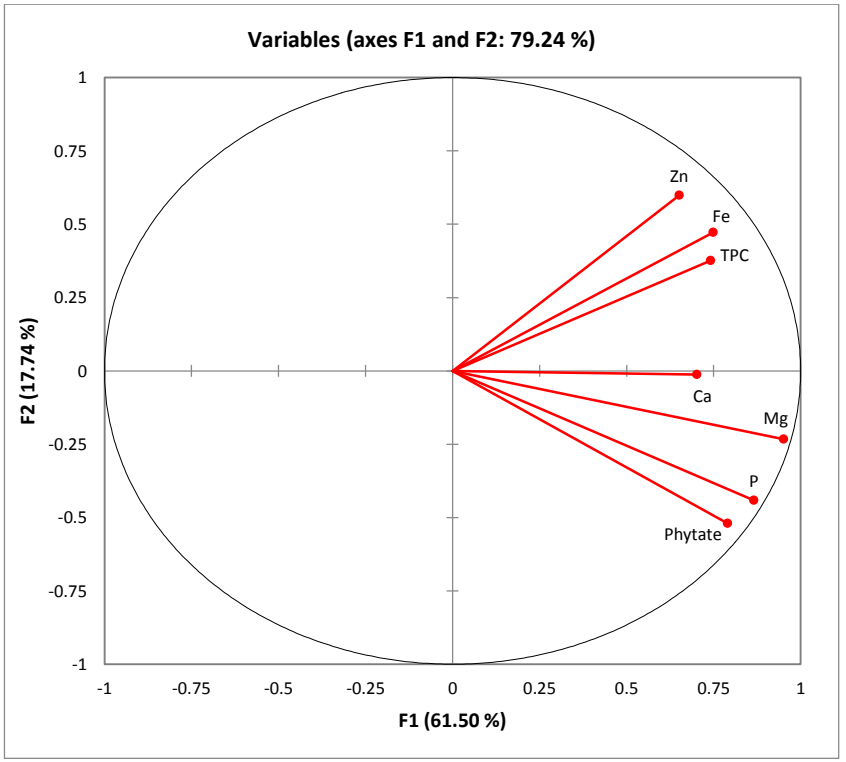


Figure 4. 4 Principal component analysis of the raw whole grain chemical composition (minerals and antinutrients) of the eight pearl millet varieties

A. Pearl millet varieties: (Kuphanjala-2, IBV 8004, ICRI-TABI, Mil Souna, ICTP 8203, Dhanashakti, GB 8735 and ICMH 1201)

B. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus and calcium

Table 4. 7 Effects of Abrasive Decortication, Pearl Millet Variety, Steeping in Back Slopped Liquor, Parboiling on Phytate Content (mg/100 g d.b.)

Variety	Raw grain		Steeped grain		Parboiled grain		Mean effect of variety (V)
	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated	
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**
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**
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**
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**
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**
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**
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**
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**
Mean 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]**	**V×P *P*≤0.001**

Values expressed as means of the analysis of two independent samples analysed in duplicate (n=2) ± 1 SD

^{abc}- Values with different superscripts, differ significantly (p≤0.001),

^{ABC}- Least Significant Mean values from main effects Factorial ANOVA with different superscripts in the same row/column, differ significantly (p≤0.001)

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

[] – values in square brackets are % change in phytate content of decorticated processed (steeped and parboiled) grains compared to whole processed grain pearl millet

Table 4. 8 Effects of Abrasive Decortication, Pearl Millet Variety, Steeping in Back Slopped Liquor, Parboiling on Total Phenolic Content (mg/100 g d.b.)

Variety	Raw grain		Steeped grain		Parboiled grain		Mean effect of variety (V)
	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated	
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**
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**
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**
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**
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**
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**
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**
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**
Mean 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]**	**V×P *P*≤0.001**

Values expressed as means of the analysis of two independent samples analysed in duplicate (n=2) ± 1 SD

^{abc}- Values with different superscripts, differ significantly (p≤0.001),

^{ABC}- Least Significant Mean values from main effects Factorial ANOVA with different superscripts in the same row/column, differ significantly (p≤0.001)

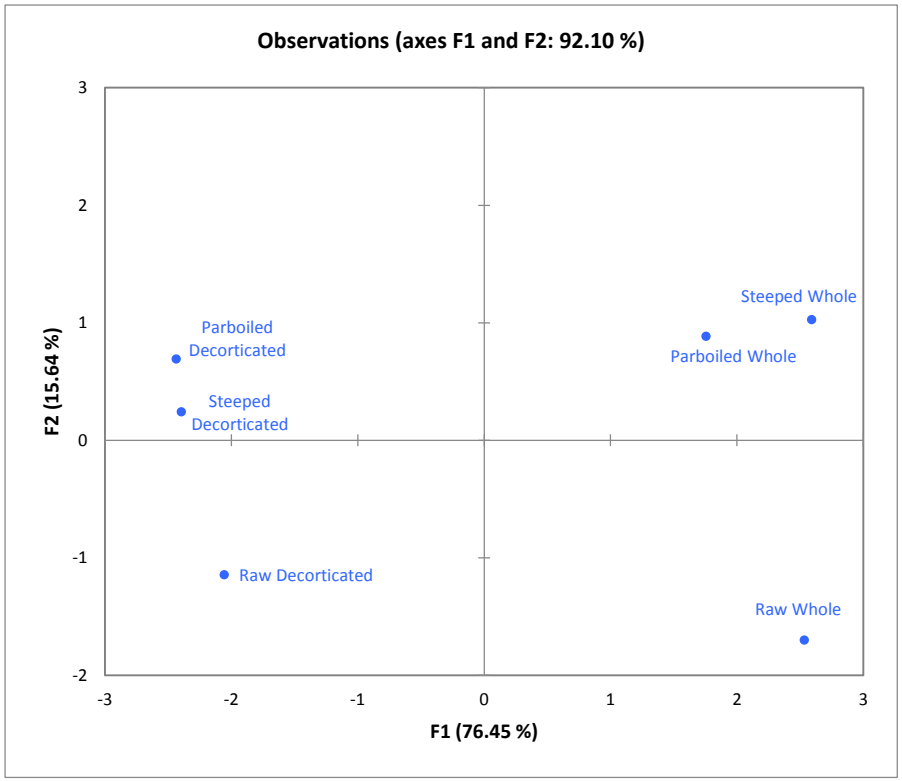
() – values in brackets are difference (% change where significant - p≤0.001) in TPC content of whole processed (steeped and parboiled) grains and decorticated raw and processed grains compared to the whole raw grain pearl millet

[] – values in square brackets are % change in TPC content of decorticated processed (steeped and parboiled) grains compared to whole processed grain pearl millet

4.3.2 Effects of processing (decortication, steeping/lactic acid fermentation and parboiling) of eight pearl millet varieties on nutrients and antinutrients

Principal component analysis shows that decortication had a major impact on pearl millet chemical composition (Figures 4.5 and 4.6). Figure 4.5 shows that both minerals and antinutrients were associated with whole raw, steeped and parboiled grains. On the contrary, decorticated raw, steeped and parboiled grain were inversely associated with mineral and antinutrient contents (Figure 4.6). Clearly, decortication leads to physical removal/loss of nutrients and antinutrients in pearl millet, as was found by Lestienne et al. (2007) and Hama et al. (2011). Similar to Figure 4.5, Figure 4.6 also shows that all nutrients were strongly associated with whole grain no matter which treatment was applied. In addition, it shows that grain physical characteristics (TKW, grain size, DHL and endosperm texture) did not influence the relationship between the grain nutritional composition and processing treatments (decortication, steeping/lactic acid fermentation and parboiling).

A



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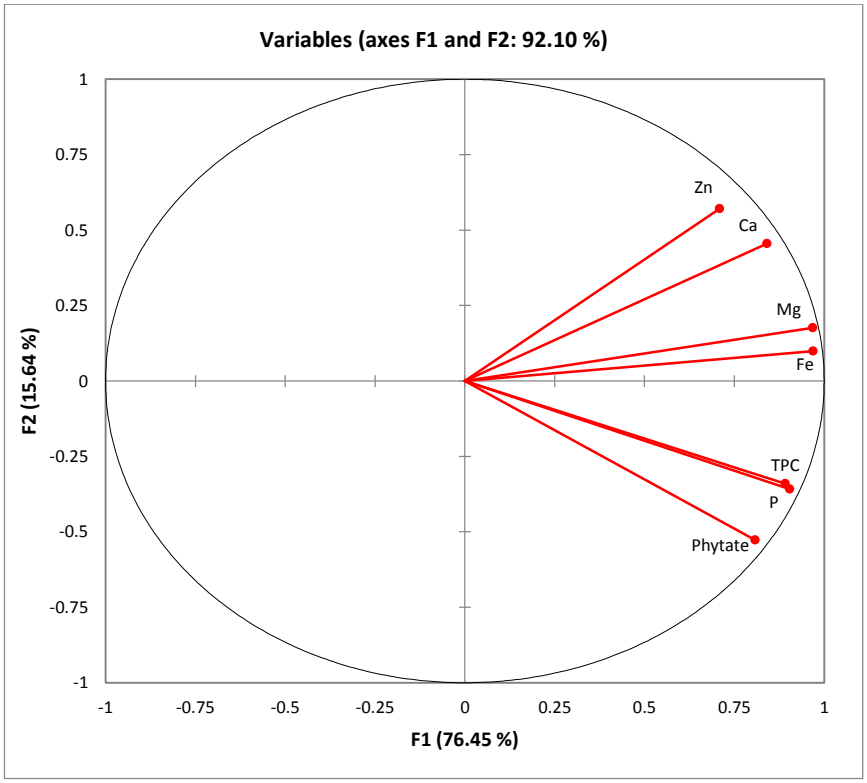
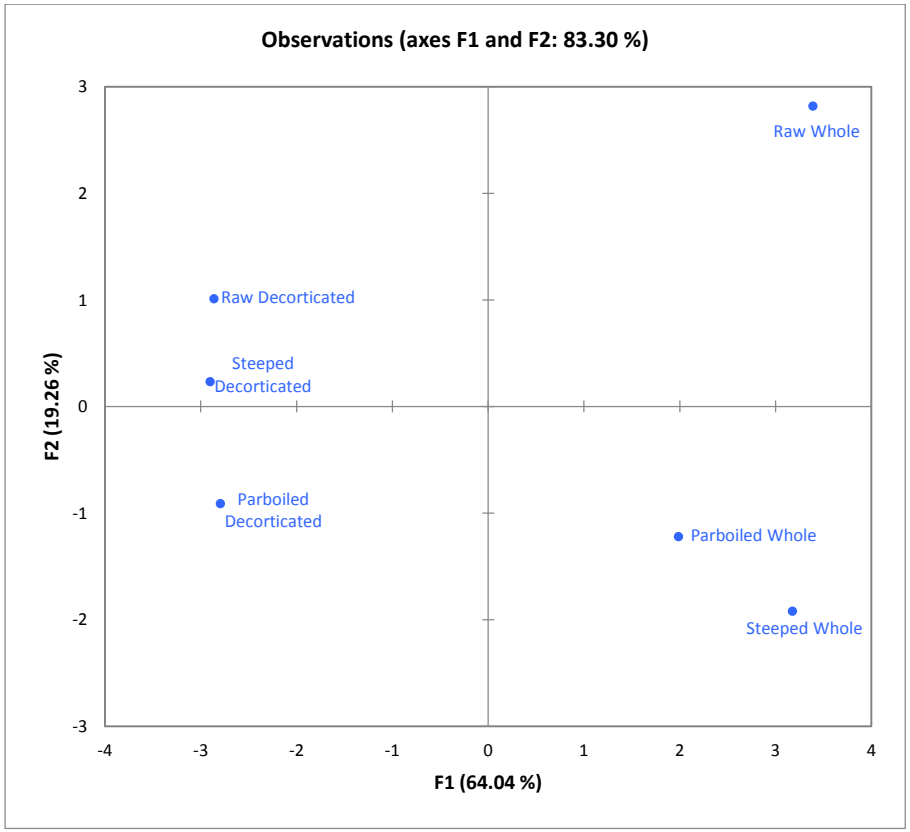


Figure 4. 5 Principal component analysis of unprocessed and processed grain chemical composition (minerals and antinutrients) of the eight pearl millet varieties

A. Processing treatments: Raw Whole, Raw Decorticated, Steeped Whole, Steeped Decorticated, Parboiled Whole and Parboiled Decorticated

B. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus and calcium

A



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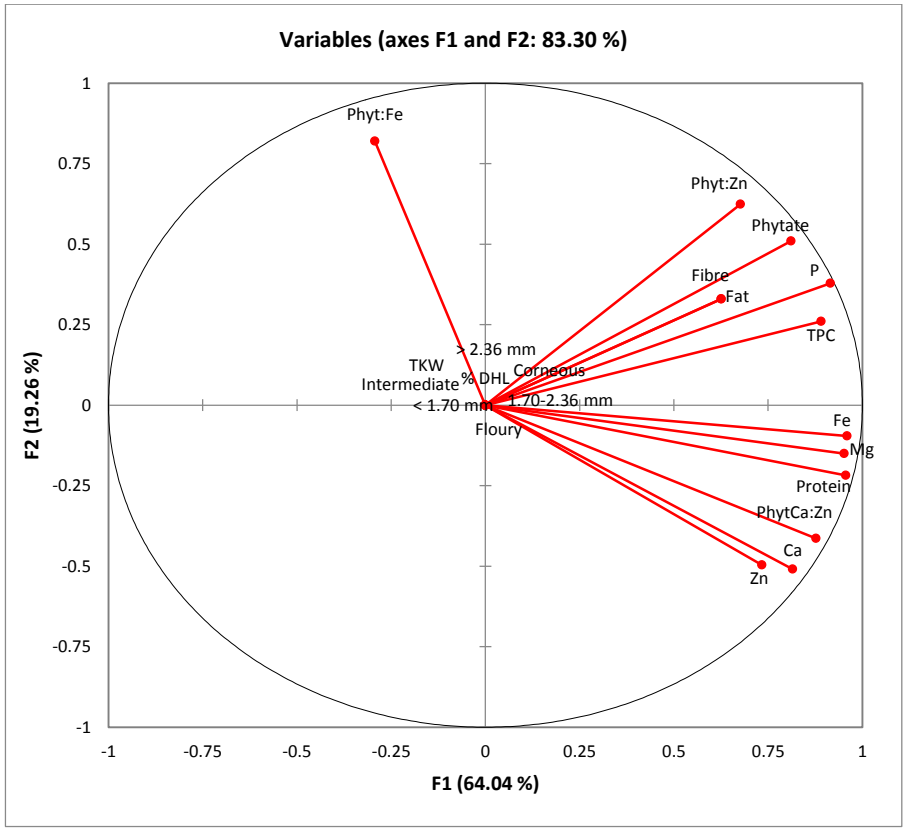


Figure 4. 6 Principal component analysis of the unprocessed and processed grain physical characteristics and chemical composition of the eight pearl millet varieties

A. Processing treatments: Raw Whole, Raw Decorticated, Steeped Whole, Steeped Decorticated, Parboiled Whole and Parboiled Decorticated

B. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus, calcium, protein, fat, fibre, phytate: iron, phytate: zinc and phytate × calcium: zinc, % Dehulling loss (% DHL), grain size (<1.70, 1.70-2.36, >2.36 mm) and Endosperm texture (% corneous, intermediate and floury).

Effects of abrasive decortication on nutritional content

Decortication highly significantly (p<0.001) reduced the crude fibre content in pearl millet (Table 4.4). Overall, decortication resulted in an average reduction of 47% in crude fibre across the varieties ranging between 33 and 60%. These findings were similar to those of Lestienne et al. (2007) where at a 12% decortication rate, 40 and 56% crude fibre loss occurred in two pearl millet varieties. Fibre constituents are mainly concentrated in the bran of the pearl millet grain (Jha et al., 2015) and decortication removes the outer bran rich layers of the grain (Taylor and Dewar, 2001).

With regard to fat, decortication had minimal effect on the overall fat contents across the varieties (Table 4.4). Notwithstanding this, the interaction effects of decortication and variety on crude fat was highly significant (p<0.001). This shows that the effect of decortication on fat content is dependent on the grain variety. Highly significant (p<0.001) reduction (14, 17 and 32%) occurred with Mil Souna, ICTP 8203 and ICRI-TABI, respectively. These particular grains generally had a floury endosperm texture and showed high decortication loss (Table 4.1). Therefore it is probable that germ was removed due to the greater decortication rate. However, in other varieties there was no significant effect of decortication on fat content. This was probably due to minimal germ removal. Furthermore, Hama et al. (2011) stated that pearl millet germ is strongly attached to the endosperm. Thus, decortication removed low levels of fat. The work of Lestienne et al. (2007) on pearl millet decortication also showed a low reduction in fat content (9 and 10%).

Overall, there was a minimal effect of abrasive decortication on protein content (Table 4.5. Only an average 5% reduction occurred in the varieties. However, reduction was highly significant (p<0.001) in four of the varieties, ranging between 7-9%. The work of Lestienne et al. (2007) showed that decortication resulted in a 13% protein reduction in two pearl millet varieties. Low protein loss during abrasive decortication can be attributed to the low protein content in the bran of the pearl millet grain. Protein is mainly concentrated in the germ and endosperm of the pearl millet grain (Taylor, 2004). Therefore depending on decortication level, the inner grain parts are generally not affected by the process.

Overall, there was a highly significant (p<0.001) reduction in all minerals with decortication of raw grain (Table 4.6). The iron content of the raw grains was on average reduced from 6.01 mg/100 g to 4.17 mg/100 g, a 31% decrease. Decortication of raw grain drastically

reduced the iron content for all varieties, with the exception of Mil Souna. Reduction ranged between 23 and 44%. These values are similar to those reported by Lestienne et al. (2007) of 27 and 32% in two pearl millet varieties. The iron content of the Kuphanjala-2 variety was almost halved. A 44% reduction resulting in the iron content decreasing from 7.51 to 4.22 mg/100 g. Notably, large reductions in iron also occurred with the GB 8735 and ICMH 1201 varieties, with both losing 40%. It has been found that the pearl millet bran and aleurone contains relatively high levels of iron, with the germ tissues containing higher concentrations (Minnis-Ndimba et al., 2015).

Overall, zinc reduction was low (8%) across the varieties and was variety dependent (p<0.001) (Table 4.6). Decortication resulted in a small but highly significant (p<0.001) reduction in zinc content in four varieties (9-20%). The small reductions of zinc with decortication were probably due to the zinc location in the grain. Zinc has been found to be mostly located in the scutellum and germ parts of the pearl millet grain (Minnis-Ndimba et al., 2015). Therefore, the effect of decortication on zinc reduction is low. Lestienne et al. (2007) similarly reported a 13 and 18% zinc loss in two pearl millet varieties.

The effect of decortication on calcium reduction was more pronounced. Overall across the varieties, 65% of calcium in the raw grain was lost due to decortication (Table 4.6). In some varieties, apparently up to 100% of the calcium was removed during decortication. In relation to the finding, Minnis-Ndimba et al. (2015) reported high calcium levels in the outer layers of the grain and the germ.

Phosphorus and magnesium losses due to decortication of raw grain were 27 and 26%, respectively (Table 4.6). Phosphorus is mainly located in scutellum and embryo of the pearl millet grain in high concentrations, although the pericarp also contain significant amounts (Minnis-Ndimba et al., 2015). Decortication resulted in a 20 to 45% phosphorus loss across the varieties, while magnesium losses were 12 to 53%.

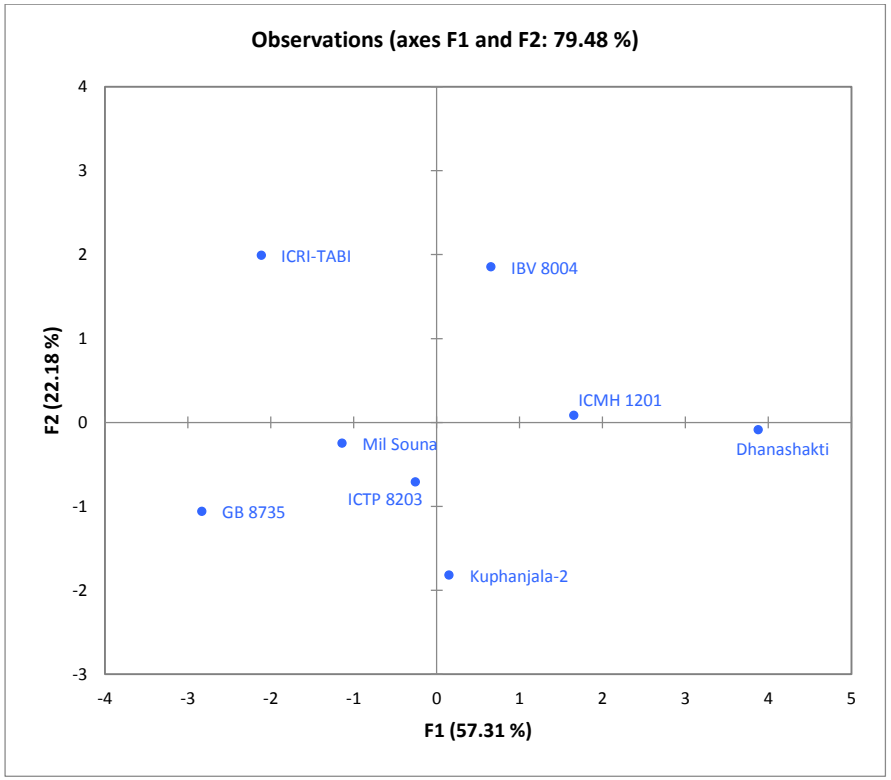
With regard to effect of variety, PCA showed that after decortication the two mineral biofortified hybrids ICMH 1201 and Dhanashakti, as well as the normal variety Kuphanjala-2, were strongly associated with the minerals Fe, Zn, P and Mg (Figure 4.7). This indicates that the minerals were high in all the tissues the biofortified pearl millet and not only in those abraded away.

Decortication of raw pearl millet resulted in a highly significant (p<0.001) reduction in phytate (Table 4.7). Approximately 300 mg phytate (30%) was removed across the varieties.

Kuphanjala-2, the grain with the highest phytate content (1360 mg phytate/100 g), had the lowest percentage phytate reduction, only 8%. The highest phytate reduction after decortication was for ICTP 8203 and IBV 8004, 40%. The mineral biofortified hybrids Dhanashakti and ICMH 1201 had phytate reductions of 23 and 13%, respectively. Principal component analysis illustrates that the ICMH 1201, Dhanashakti, and Kuphanjala-2 varieties were still associated with high phytate content after decortication (Figure 4.7). The extent of decortication can influence the amount of phytate reduction, as observed by Lestienne et al. (2007). These author found that a 12% decortication was not effective in reducing phytate level in two pearl millet varieties, giving a mere 4 and 8% decrease. Localisation of phytate in the grain as well plus external factors such as genetic factors and cultivation conditions can have an effect on the amount of phytate reduced during decortication (Elyas et al., 2002; Lestienne et al., 2007).

Similarly to phytate, TPC reduction was 24% across the pearl millet varieties (Table 4.8). A highly significant (p<0.001) reduction occurred in six varieties, ranging between 17 and 38%. The mineral biofortified hybrids Dhanashakti and ICMH 1201 were among the varieties with high TPC reduction with 24 and 17%, respectively. Jha et al. (2015) stated that the pearl millet bran contains high amounts of polyphenols and flavonoids. Therefore decortication, which removes most of the bran, considerably reduced their content. However, the major phenolic acid in pearl millet ferulic acid, is found mostly in the bound form in cell walls (Chandrasekara and Shahidi, 2010). Therefore substantial amounts can be retained after decortication. As observed by PCA, TPC was strongly associated with fat content (Figure 4.3). Fat is largely found in the pearl millet germ (Taylor et al., 2010), which is probably an indicator of TPC in the germ tissues.

A



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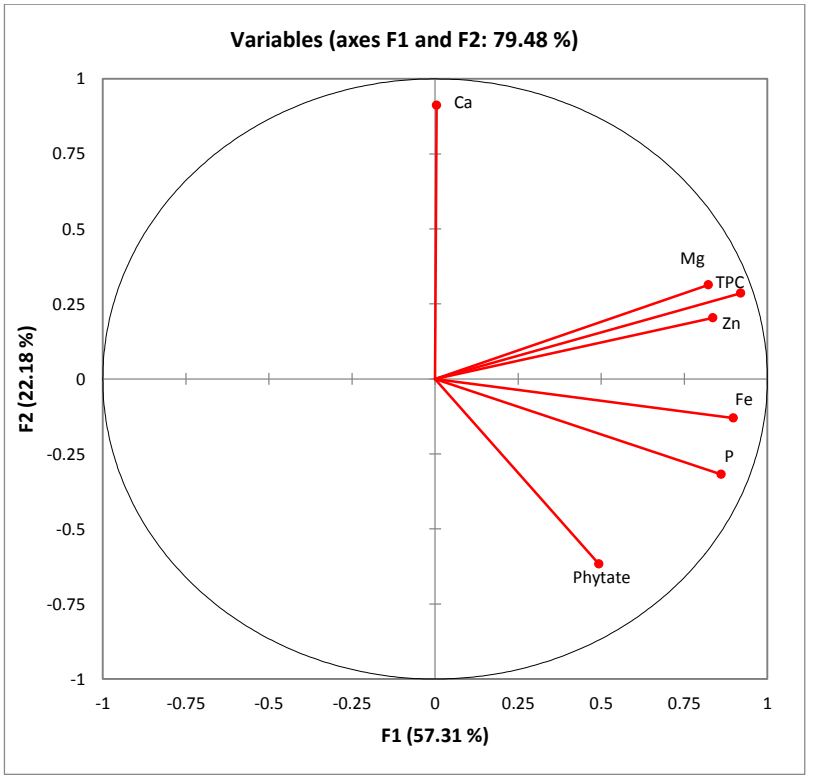

Figure 4. 7 Principal component analysis for raw decorticated grain chemical composition (minerals and antinutrients) of the eight pearl millet varieties

A. Pearl millet varieties: (Kuphanjala-2, IBV 8004, ICRI-TABI, Mil Souna, ICTP 8203, Dhanashakti, GB 8735 and ICMH 1201)

B. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus and calcium

Effects of steeping/lactic acid fermentation

Overall, steeping/lactic acid fermentation whole grain resulted in minimal (5%) grain losses (Table 4.9) across the varieties. However, steeping/lactic acid fermentation in combination with decortication resulted in substantial (24%) grain losses across the varieties. Mil Souna variety had the highest overall grain losses (50%) after steeping/lactic acid fermentation plus abrasive decortication.

Steeping/lactic acid fermentation had little effect on whole grain protein content (Table 4.5). However, steeping/lactic acid fermentation plus decortication resulted in a 10% overall reduction in protein across the varieties, with a range of 6-14%. This low decrease is because protein is concentrated in the germ tissues of the pearl millet grain (Taylor, 2004).

Steeping/lactic acid fermentation of whole pearl millet grain in back-slopped liquor (lactic acid fermentation) yielded varying results with respect to mineral content (Table 4.6). Generally, the effect of steeping on whole steeped grain mineral content was low, with the exception of high calcium increases. In contrast to earlier reports concerning leaching out of minerals (El Hag et al., 2002, Lestienne et al., 2005b), this was not the case in this study. In fact, the mineral content of whole steeped grain remained constant or increased. This is probably because little steeping solution was used (1:2, v/w) and the steeping period (8 hours) was shorter than in other studies. Steeping of whole grains did not have an overall substantial effect on iron content. This differs from the findings of Lestienne et al. (2005b), who recorded substantial reductions (34%). However, in that study, steeping was done for 24 hours. Four varieties, Kuphanjala-2, IBV 8004, ICRI-TABI and ICTP 8203 showed an increase in the iron content of whole steeped grain. The possible reason of this could be due to iron carried forward from the back-slopped liquor.

Steeping/lactic acid fermentation in combination with decortication resulted in a 30% iron reduction across the varieties. There was no overall difference across the varieties in iron reduction between decorticated raw grain and steeped plus decorticated grain. However, the reduction was presumably due to physical removal (Lestienne et al., 2007) of the outer iron rich grain components (pericarp and aleurone) rather than steeping itself. Steeping of whole grains resulted in a minimal zinc increase (4%) across the varieties (Table 4.6). However, a highly significant (p<0.001) increase in zinc was observed in Mil Souna (12%). This may have been zinc carried forward from the steeping liquor. Additionally, the zinc increase may

have been caused by losses of soluble dry matter. Otherwise, no effect occurred in the other varieties. However, steeping/lactic acid fermentation plus decortication resulted in an average 3% significant (p<0.001) zinc decrease across the varieties. The minimal reduction is presumably due to the low levels of zinc in the peripheral parts (pericarp and aleurone) of pearl millet grain (Minnis-Ndimba et al., 2015). Zinc is more pronounced in the germ.

There was great increase in calcium content (145%) across the varieties (Table 4.6). A possible explanation of this unexpected increase could be calcium from the steeping solution. Back-slopped liquor of a three day sorghum ferment was used for steeping the grains. Calcium ions from sorghum may have leached into the liquor. Across all varieties, calcium levels increased by 76-174%. However, upon decorticating the steeped whole grain, a highly significant (p<0.001) decrease in calcium content occurred. Across all varieties, 84% calcium was removed. Apparently, for ICTP 8203 and GB 8735, a 100% calcium reduction occurred. In other varieties, reductions ranged between 52 and 96%.

The effect of steeping/lactic acid fermentation on phosphorus content across the whole grains was significant (p<0.05) but low, a 12% reduction (Table 4.6). However, the phosphorus reductions occurred only with Dhanashakti (17%), GB 8735 (18%) and ICMH 1201 (23%). Two of these, Dhanashakti and ICMH 1201 are mineral biofortified hybrids. Steeping/lactic acid fermentation plus decortication led to 26% phosphorus reduction across the varieties. Interestingly, the reduction did not differ as compared to decortication of raw grains (27%). With regard to magnesium, steeping/lactic acid fermentation significantly (p<0.001) increased magnesium levels (15%) across the varieties. Similarly as calcium, magnesium ions may have been carried forward from previous sorghum ferment steeping solution. However, steeping/lactic acid fermentation plus decortication resulted in highly significant (p<0.001) magnesium reduction (27%) across the varieties. This was similar to the effect of steeping/lactic acid fermentation plus decortication on phosphorus Therefore it can be concluded that decortication was responsible for phosphorus and magnesium losses rather than steeping/lactic acid fermentation.

PCA shows that varieties, Kuphanjala-2, Dhanashakti, ICMH 1201 and IBV 8004 were highly associated with minerals (Fe, Zn, Ca, Mg and P) for steeped/lactic acid fermented and steeped/lactic acid fermented plus decorticated grain (Figures 4.8 and 4.9). Unsurprisingly, the mineral biofortified varieties Dhanashakti and ICMH 1201 were most closely associated with high iron and zinc contents.

A highly significant (p<0.001) phytate reduction (21%) occurred as a result of steeping/ lactic acid fermentation of whole grain (Table 4.7). This was similar to the findings of Lestienne et al. (2005b) of a 28% phytate reduction after 24 hours soaking. The greatest phytate reductions occurred with the two mineral biofortified hybrids Dhanashakti (29%) and ICMH 1201 (31%) and the normal varieties Mil Souna (24%), Kuphanjala-2 (26%) and IBV 8004 (41%). For the others, the reductions were low. Similarly, there was a very large phytate reduction with steeping/lactic acid fermentation plus decortication across the varieties (36%). Thus, compared to decortication of raw grain, steeping/lactic acid fermentation plus decortication increased phytate loss by a further 12 percentage points. Therefore it may be important to combine steeping/lactic acid fermentation and decortication to achieve maximum phytate reduction, as Lestienne et al. (2007) found.

Interestingly, PCA shows that after steeping whole grain, only variety ICTP 8203 was associated with high phytate (Figure 4.8). This confirms the great effect of steeping/lactic acid fermentation on phytate reduction. Phytate is an insoluble compound (Etcheverry et al., 2012). However, steeping/soaking conditions may lead to dissociation of phytate, hence reducing the phytate level due to solubilisation (Afify et al., 2011). Furthermore, activation of endogenous grain phytases results in degradation of phytate in the grain (Hurrell, 2004). However, a large proportion of the phytate reduction during steeping/lactic acid fermentation is caused by microbial phytases and lactic acid bacteria strains from microorganisms in the grain (Elyas et al., 2002, Songre-Ouattara et al., 2008).

Steeping/lactic acid fermentation of whole grain did not have substantial effect on the TPC of whole grain. As stated phenolic compounds exist as soluble and insoluble-bound forms in pearl millet (Chandrasekara and Shahidi, 2010). Therefore the short 8 hours steeping/lactic acid fermentation may have resulted in low solubilisation. However, a highly significant (p<0.001) TPC reduction took place with steeping/lactic acid and decortication. Overall, across the varieties, 38% of TPC was removed. There was a further 14 percentage points TPC loss across the varieties compared to raw grain decortication. PCA revealed that the mineral biofortified hybrids, Dhanashakti and ICMH 1201 remained associated with high TPC after both steeping only and steeping plus decortication treatments (Figures 4.8 and 4.9). Dhanashakti was among the three varieties with a highly significant (p<0.001) TPC loss (23%), while for ICMH 1201 there was no effect of whole grain steeping/lactic acid fermentation. However, steeping/lactic acid fermentation plus decortication resulted in substantial TPC loss in both Dhanashakti (51%) and ICMH 1201 (32%). Although there were

substantial TPC losses on the two mineral biofortified hybrids, they still retained high TPC levels.

Table 4. 9 Treatment Loss (%) of the Eight Pearl Millet Varieties

Variety	Steeping/lactic acid fermentation loss	Decortication plus steeping/lactic acid fermentation loss	Parboiling loss	Decortication plus parboiling loss
Kuphanjala-2	8.9^{ab}±0.5	16.4^{c}±0.4	5.0^{e}±0.5	11.2^{a}±0.1
IBV 8004	2.1^{ab}±0.2	13.2^{a}±0.3	0.4^{a}±0.1	10.5^{a}±0.6
ICRI-TABI	1.1^{a}±0.2	33.8^{f}±0.1	3.6^{cd}±0.2	16.4^{b}±0.1
Mil Souna	6.2^{ab}±7.2	50.4^{g}±0.2	6.5^{f}±0.2	23.5^{c}±2.3
ICTP 8203	1.2^{a}±1.1	25.3^{d}±0.1	1.7^{b}±0.1	16.4^{b}±1.1
GB 8735	5.3^{ab}±0.1	26.8^{e}±0.4	4.4^{de}±0.1	15.3^{b}±0.1
Dhanashakti	4.9^{ab}±0.2	15.3^{bc}±0.1	3.4^{c}±0.0	11.3^{a}±0.3
ICMH 1201	12.0^{c}±0.1	14.5^{b}±0.5	7.4^{f}±0.1	10.8^{a}±0.3
Mean	**5.2±4.2**	**24.4±12.3**	**4.1±2.3**	**14.4±4.4**

Values are mean ± standard deviation (n= 2). Mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

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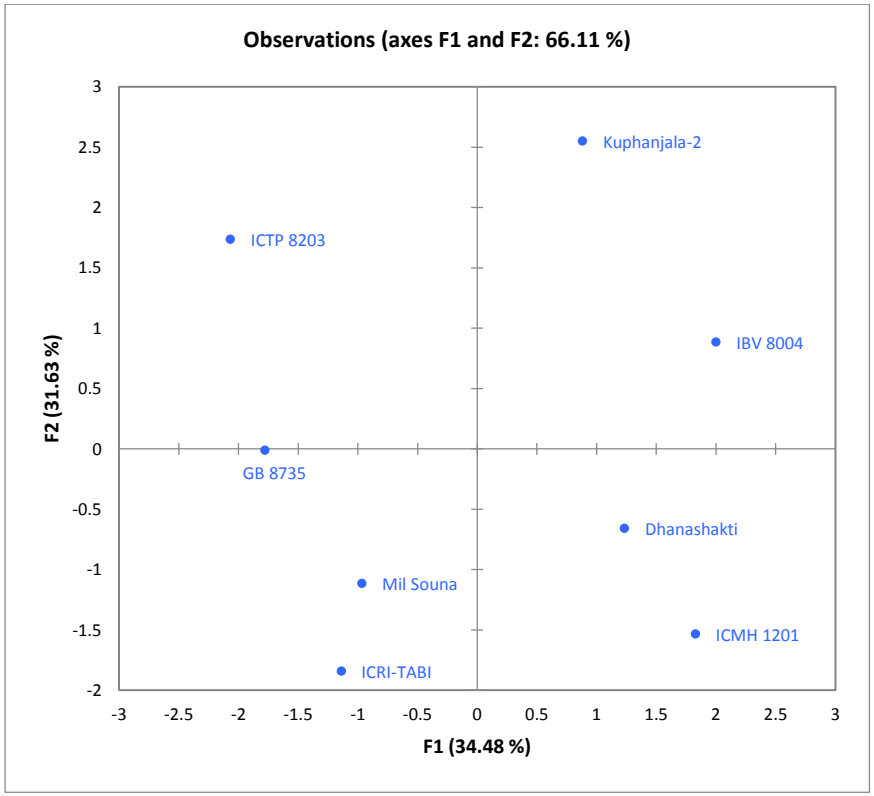

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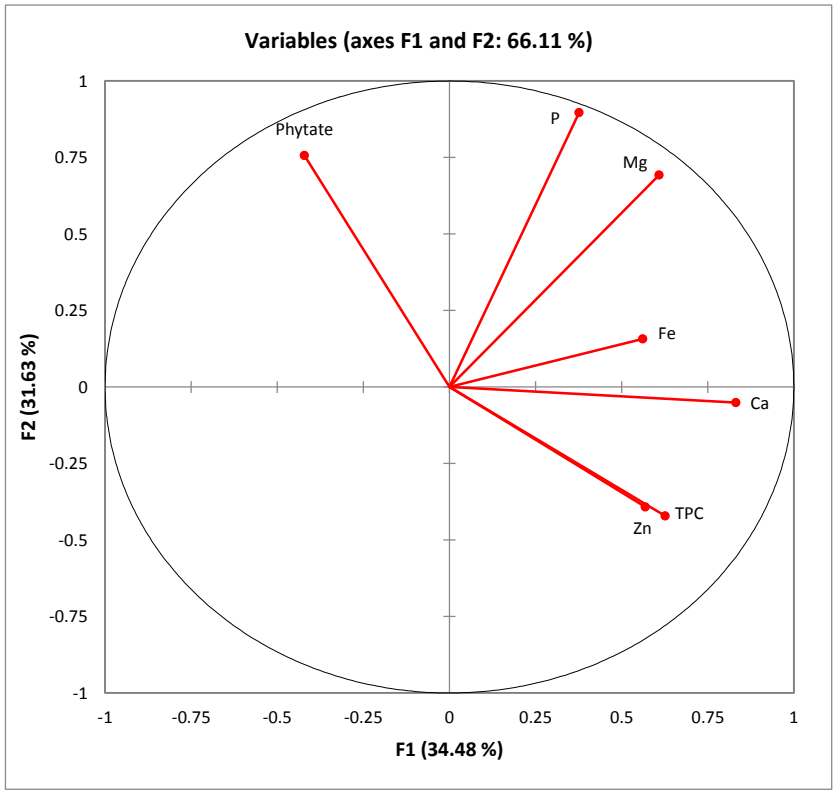
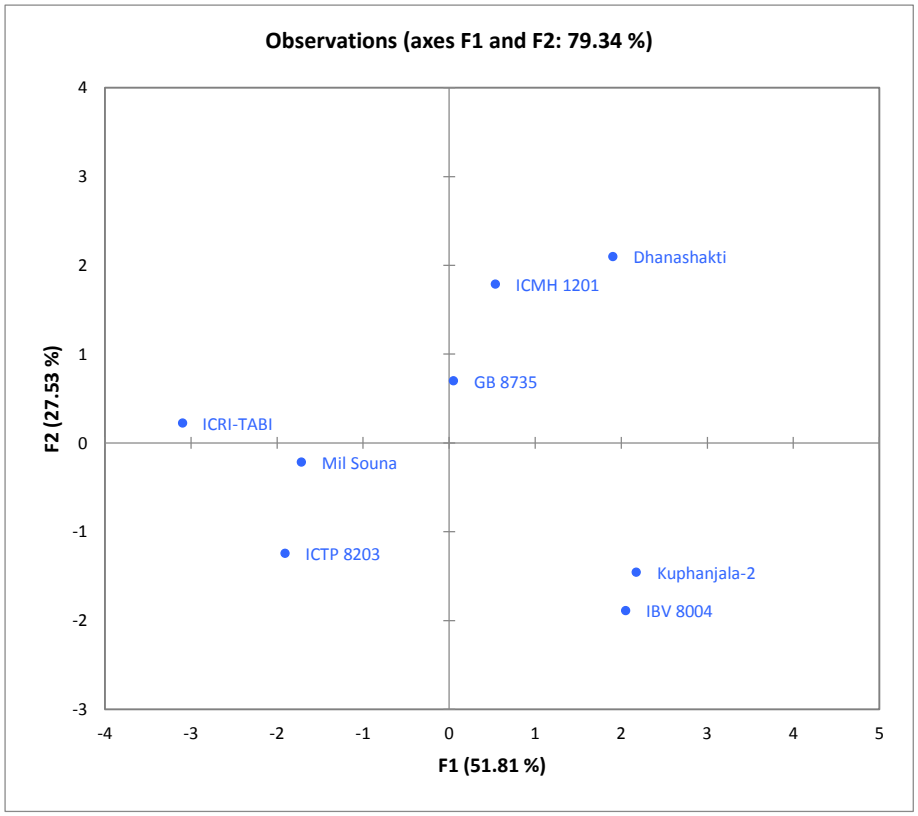


Figure 4. 8 Principal component analysis of steeped whole grain chemical composition (minerals and antinutrients) of the eight pearl millet varieties

A. Pearl millet varieties: (Kuphanjala-2, IBV 8004, ICRI-TABI, Mil Souna, ICTP 8203, Dhanashakti, GB 8735 and ICMH 1201)

B. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus and calcium

A



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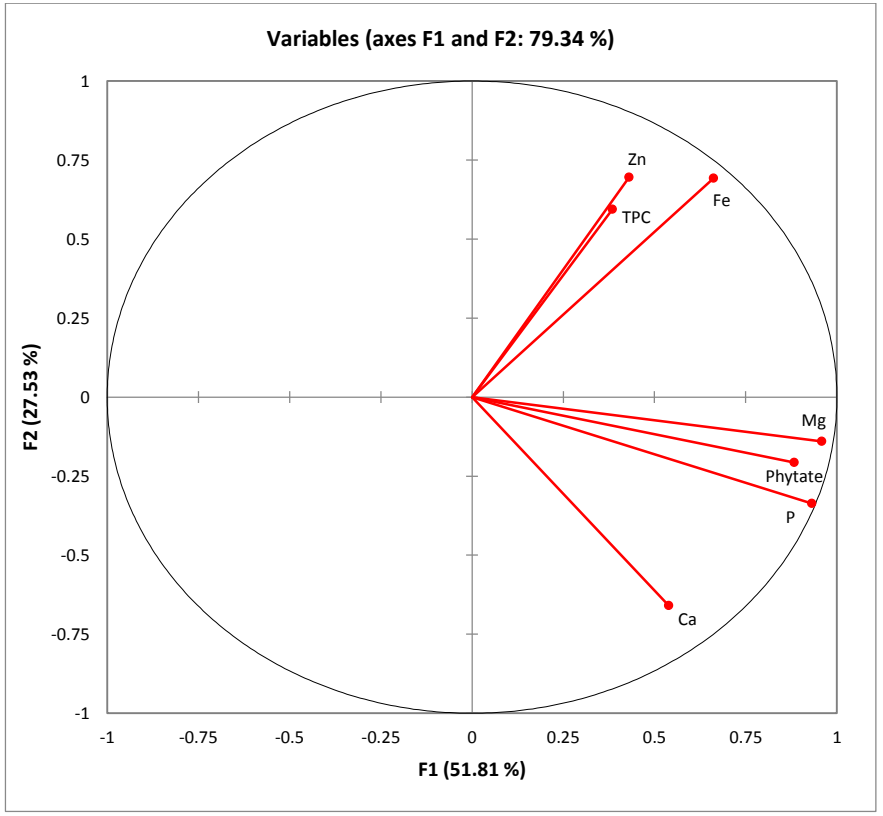


Figure 4. 9 Principal component analysis of steeped decorticated grain chemical composition (minerals and antinutrients) of the eight pearl millet varieties

A. Pearl millet varieties: (Kuphanjala-2, IBV 8004, ICRI-TABI, Mil Souna, ICTP 8203, Dhanashakti, GB 8735 and ICMH 1201)

B. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus and calcium

Effects of parboiling

Parboiling whole grain had little effect on overall grain losses, mean 4% across the varieties (Table 4.9). However, parboiling in combination with abrasive decortication resulted in substantial grain losses across the varieties (14%). Mil Souna had the highest grain losses after parboiling plus abrasive decortication (24%).

As with steeping/lactic acid fermentation, parboiling had little effect on whole grain protein content (Table 4.5). However, parboiling and decortication resulted in an average 8% protein reduction across the varieties, with a range of 6-13%. As stated, decortication has little effect on protein content because there is little protein in the pericarp (Taylor, 2004).

Parboiling yielded varying results with respect to its effect on mineral content (Table 4.6). It was expected that the minerals would move inwards in the germ carried by the moist steam of parboiling (Messia et al., 2012). However, zinc levels were not greatly affected across the varieties after parboiling. Parboiled decorticated grain had highly significantly (p<0.001) lower iron, calcium, phosphorus and magnesium contents compared to raw decorticated and steeped plus decorticated grain. This could be due to greater decortication of the grain caused by parboiling, as observed by Serna-Saldivar et al. (1994).

With whole grain, a highly significant increase (p<0.001) in iron content occurred in the Kuphanjala-2 (28%), ICRI-TABI (15%) and ICTP 8203 (12%) varieties, with an overall 6% increase across the varieties. The increase could be due to iron carried forward from splashing of the tap water during boiling. However, for the mineral biofortified hybrid ICMH 1201, there was 9% iron reduction, while Dhanashakti there was no effect on the iron content. Parboiling plus decortication led to a highly significant reduction (p<0.001) in iron across the varieties. Overall, across the grains, 33% iron losses occurred due to parboiling plus decortication. These reductions were similar to the reduction in iron due to decortication alone. Therefore it can be concluded that the effect of parboiling on iron content was low.

Neither parboiling only nor a combination of parboiling and decortication had an effect on zinc content. The zinc level of mineral biofortified hybrids remained constant after parboiling whole grain and with parboiling plus decortication. However, highly significant reductions (p<0.001) in zinc occurred in ICTP 8203 (11%), ICRI-TABI (12%) and IBV 8004 (11%), and zinc increases in Mil Souna (24%) and Kuphanjala-2 (26%) after parboiling plus decortication. As explained, elemental distribution analysis of pearl millet indicated a high

concentration of zinc in the germ (approx. 21 mg /100 g) and lower concentrations in the bran (pericarp) (approx. 5 mg/100 g) (Minnis-Ndimba et al., 2015). Thus, decortication had little effect on the zinc content of pearl millet grain.

Similar to steeping/lactic acid fermentation, the calcium content of parboiled whole grain increased greatly (142%) with whole grain processing, between 57% and 317% across the varieties (Table 4.6). Tap water was used for parboiling of the pearl millet varieties. In some areas, tap water (drinking water) may have considerable amounts of minerals including calcium (WHO, 2005). In fact, drinking water in Pretoria contains a total of about 80-100 mg/l magnesium and calcium ions, expressed as carbonate (City of Tshwane: Water and Sanitation, 2016). Therefore boiling might have caused splashing of water into the grain, and hence increased calcium levels in the pearl millet. However, a highly significant reduction (p<0.001) in calcium content occurred after parboiling plus decortication. Levels across the varieties were reduced by an average 73%. Apparently for ICRI-TABI, ICTP 8203 and GB 8735, 100% calcium loss occurred after parboiling plus decortication. Parboiling of whole grain caused a smaller, but significant (p<0.05) reduction in phosphorus across the varieties (17%). However, combined parboiling and decortication resulted in increased phosphorus reduction (overall 32%).

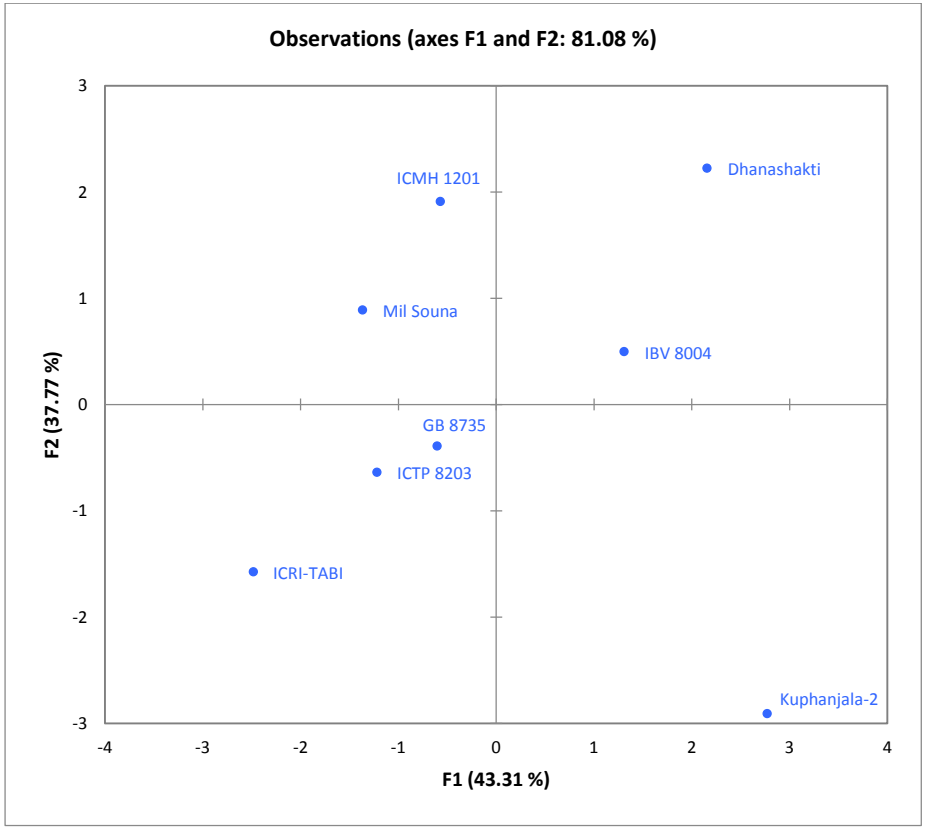
With regard to magnesium, a small increase (3%) occurred across the varieties after parboiling whole grain. Highly significant magnesium increase (p<0.001) occurred on Mil Souna (16%) and ICRI-TABI (11%) after whole grain parboiling. However, a highly significant reduction (p<0.001) occurred in the mineral biofortified hybrid ICMH 1201 (9%), but with the other varieties there was none. PCA showed no clear pattern with respect to association of minerals and pearl millet varieties with respect to parboiling (Figures 4.10 and 4.11).

Concerning phytate, there was a highly significant reduction with parboiling of whole grain (p<0.001) across the varieties (15%) (Table 4.7). The mineral biofortified hybrids Dhanashakti (22%) and ICMH 1201 (24%) along with ICTP 8203 (41%) had the greatest phytate reduction. Parboiling plus decortication proved to be effective in phytate reduction. Phytate loss was increased by a further 8 percentage points across the varieties. Phytate reduction by parboiling can be attributed to the hydrolysis of phytate by heat (Messia et al., 2012). Also, wet steam can activate endogenous grain phytase enzymes depending on the temperature (Raes et al., 2014). Apart of whole grain parboiled Kuphanjala-2, PCA showed

that the varieties were not associated with phytate for either whole parboiled or decorticated parboiled grains (Figures 4.10 and 4.11). Kuphanjala-2 had the highest phytate content (1360 mg/100 g). Parboiling reduced low phytate levels (8%). However, parboiling plus decortication substantially resulted in high phytate loss (54%) for Kuphanjala-2.

Overall, parboiling highly significantly (p<0.001) reduced the TPC across the varieties (23%) (Table 4.8). Heat causes degradation of mineral binding phenolics (Raes et al., 2014). There were highly significant TPC reductions (p<0.001) for Kuphanjala-2 (46%), ICRI-TABI (29%) and ICTP 8203 (18%) and also in mineral biofortified hybrids Dhanashakti (32%) and ICMH 1201 (25%) due to parboiling of whole grain. The application of both parboiling and decortication resulted in highly significant reduction (p<0.001) in TPC across the varieties. On average, 38% of TPC was removed. This was a 14 percentage points increase in TPC loss compared to decortication alone. Only Dhanashakti one of the mineral biofortified hybrids, was associated with TPC after parboiling (Figures 4.10 and 4.11). Dhanashakti had the highest TPC content of all the varieties.

A



B

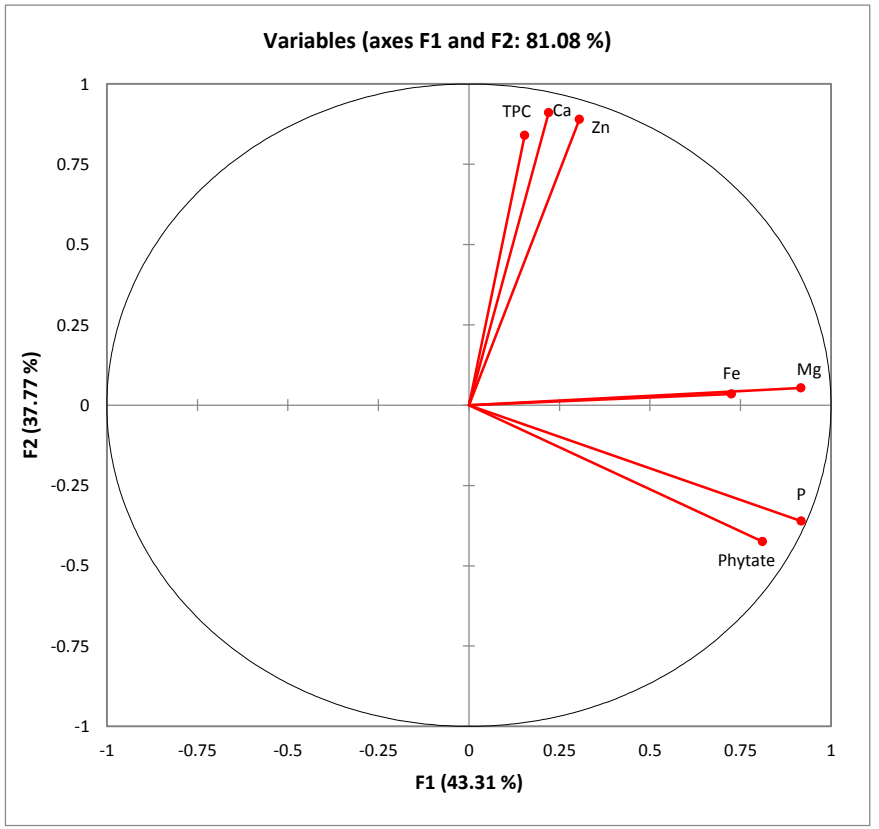
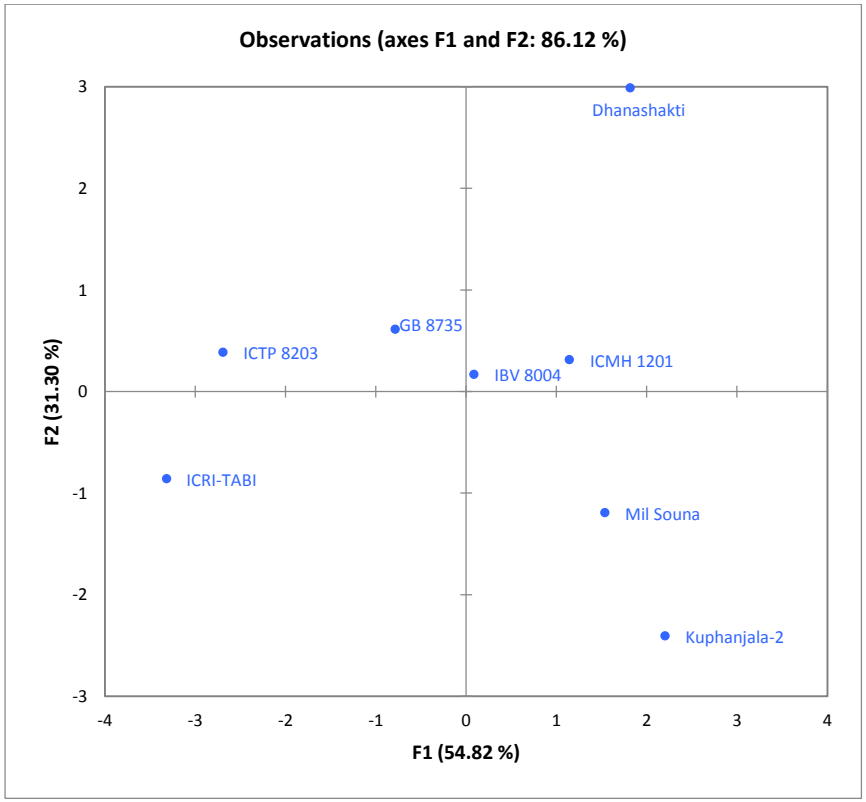


Figure 4. 10 Principal component analysis of parboiled whole grain chemical composition (minerals and antinutrients) of the eight pearl millet varieties

C. Pearl millet varieties: (Kuphanjala-2, IBV 8004, ICRI-TABI, Mil Souna, ICTP 8203, Dhanashakti, GB 8735 and ICMH 1201)

D. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus and calcium

A



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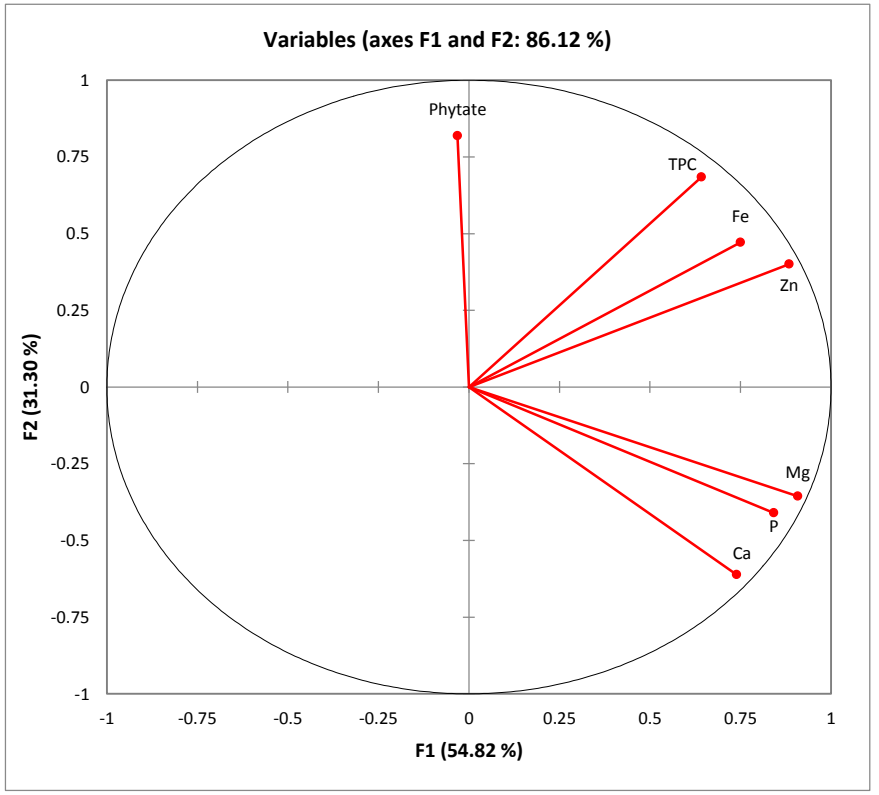

Figure 4. 11 Principal component analysis of parboiled decorticated grain chemical composition (minerals and antinutrients) of the eight pearl millet varieties

A. Pearl millet varieties: (Kuphanjala-2, IBV 8004, ICRI-TABI, Mil Souna, ICTP 8203, Dhanashakti, GB 8735 and ICMH 1201)

B. PCA Loadings: Phytate, total phenolic compounds (TPC), iron, zinc, magnesium, phosphorus and calcium

4.3.3 Effects of abrasive decortication, steeping/lactic acid fermentation and parboiling 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). Decortication, steeping/lactic acid fermentation and parboiling had no clear effect on phytate: iron molar ratios (Figure 4.12). None of the phytate: iron molar ratios were reduced below the proposed critical level of 1 (Hurrell, 2004). Across the varieties, phytate: iron molar ratios for raw grain and after the various processing treatments ranged between 8 and 43. This was probably because whilst a high reduction in phytate occurred with the treatments, iron content was also reduced simultaneously. With respect to the effect of biofortification, it is apparent that the mineral biofortified hybrids ICMH 1201 and Dhanashakti had among the lowest phytate: iron molar ratios (8-15) for raw and processed grains. Among the mineral biofortified hybrids, the lowest phytate: iron molar ratio found was 8 for both mineral biofortified hybrids whole steeped/lactic acid fermented grain. The highest phytate: iron molar ratio was 15 for raw decorticated ICMH 1201 hybrid. Hama et al. (2012) found that mineral biofortified pearl millet hybrids have lower phytate: iron molar ratios than normal varieties. However, similar to this study, the critical level of <1 associated with improved iron absorption was not attained.

Concerning zinc, steeping plus decortication (ICRI-TABI and ICMH 1201) and parboiling plus decortication (Mil Souna) resulted in reduction in the phytate: zinc molar ratio to the proposed <15 (Figure 4.13), required for effective zinc absorption (Ma et al., 2007). Similarly, Lestienne et al. (2005b) found that despite leaching out of zinc, the phytate: zinc molar ratios were improved by 24 hours soaking of pearl millet. Generally, the high mineral hybrids Dhanashakti and ICMH 1201 had lower phytate: zinc molar ratios (16-25) compared to the normal varieties (12-50). This is similar to Hama et al. (2012) who found that phytate: zinc molar ratios for mineral biofortified pearl millet hybrids were lower than normal varieties and were further reduced by decortication.

Zinc absorption is serious impaired by phytate × calcium: zinc molar ratios of above 200 (Ma et al., 2007). All phytate × calcium: zinc molar ratios for raw and processed grains across different varieties were below 16 (Figure 4.14). This could be due to the fact that pearl millet has a lower calcium content compared to other cereal grains (Bashir et al., 2014). Therefore with low levels of calcium, this could reduce the interaction between calcium and zinc, as reviewed by Lonnerdal (2000).

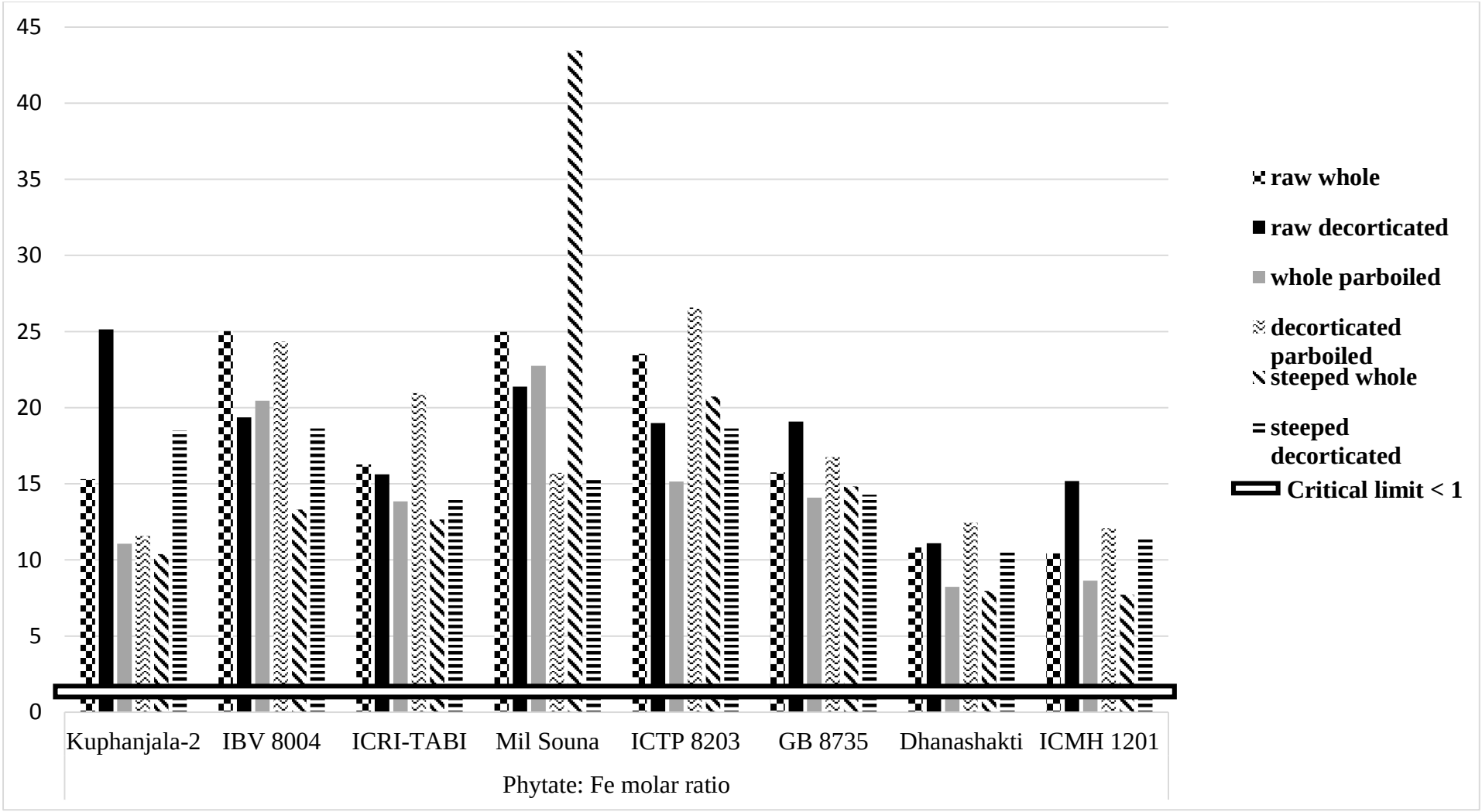

Figure 4. 12 Phytate: iron molar ratio showing effects of processing on iron availability of eight pearl millet varieties. Critical levels above which iron absorption is impaired >1 (Hunt, 2003)

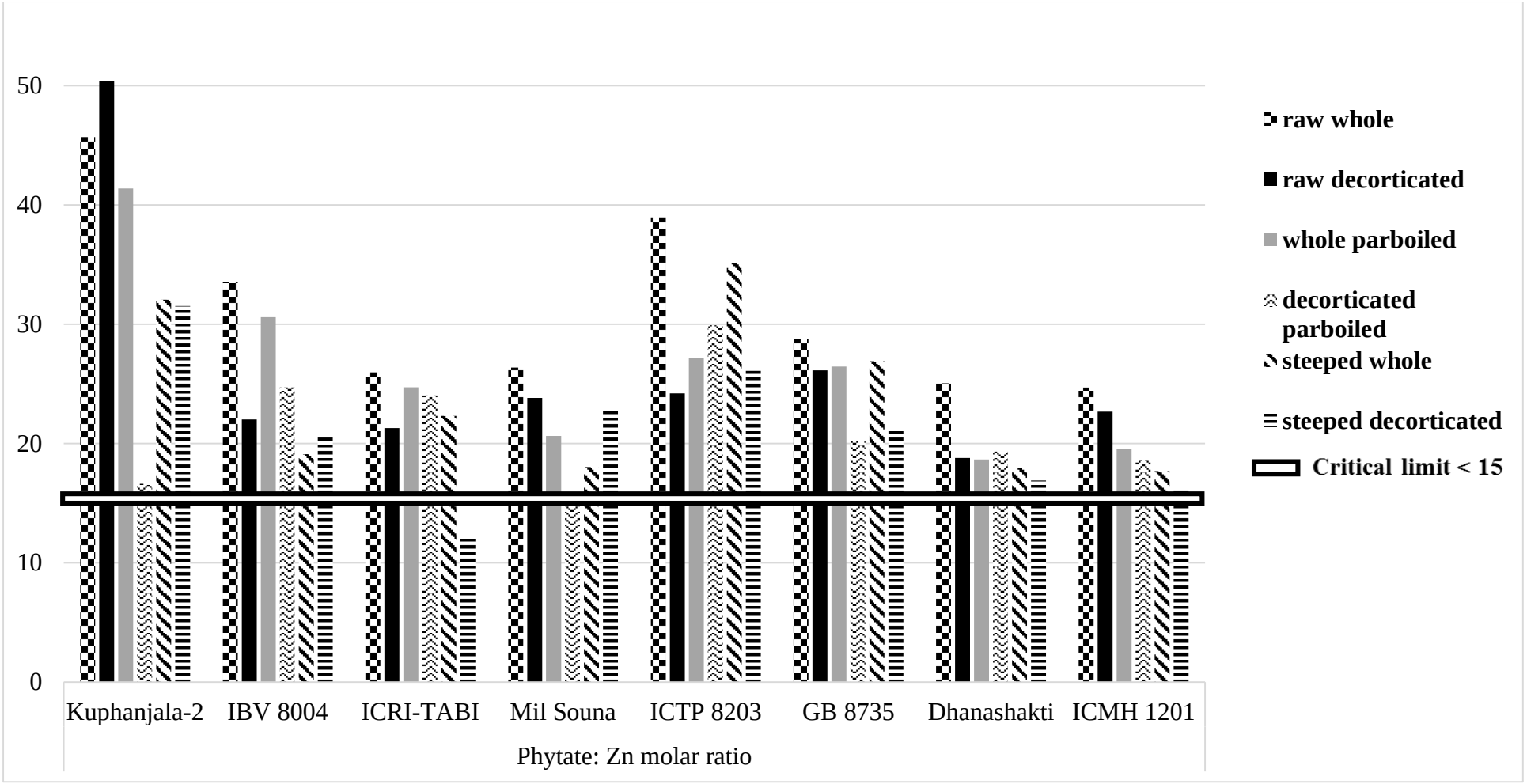

Figure 4. 13 Phytate: zinc molar ratio showing effects of processing on zinc availability of eight pearl millet varieties. Critical levels above which zinc absorption is impaired >15 (Ma et al., 2007)

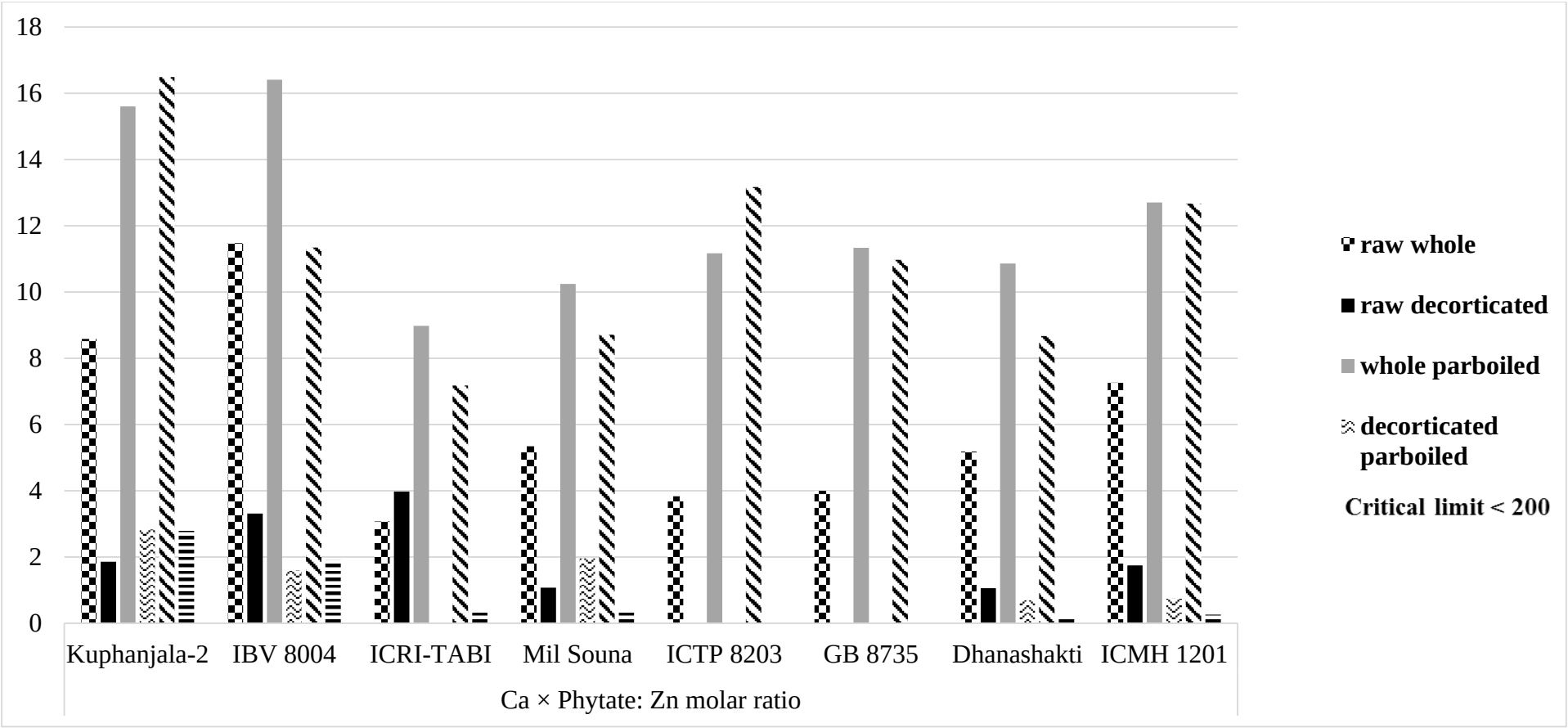

Figure 4. 14 Calcium × phytate: zinc molar ratio showing effects of processing on zinc availability of eight pearl millet varieties. Critical levels above which zinc absorption is impaired >200 (Ma et al., 2007)

4.4 CONCLUSIONS

Abrasive decortication in combination with steeping/lactic acid fermentation is an effective way of reducing phytate content in pearl millet grain, and consequently somewhat improving both estimated iron and zinc availability. It is recommended that abrasive decortication plus steeping/lactic acid fermentation is utilised to a greater extent where pearl millet is a staple food. Mineral biofortified pearl millet hybrids have substantially higher iron and zinc contents compared to normal varieties. Cultivation of mineral biofortified pearl millet hybrids is recommended to help combat iron and zinc deficiencies in pearl millet consuming communities.

5. GENERAL DISCUSSION

This chapter is divided into three sections. The first part of the discussion is an evaluation of the grain processing techniques as applied as models to represent actual processing. The second section deals with major findings from the study and the potential use of food processing techniques (abrasive decortication, steeping/lactic acid fermentation and parboiling) plus mineral biofortification to improve mineral bioavailability in pearl millet grain/products and reduce mineral deficiencies. The third section discusses ways of implementing the recommended technologies.

5.1 Methodological considerations

Cereal processing techniques, abrasive decortication, steeping/lactic acid fermentation and parboiling were studied to evaluate their influence on mineral bioavailability in pearl millet. A laboratory scale dehuller, the Tangential Abrasive Dehulling Device (TADD) equipped with eight sample cups (Reichert et al., 1986) was used to predict mineral and antinutrient loss due to decortication. The TADD is primarily used for the evaluation of milling quality and grain hardness of grains like sorghum and pearl millet (Gomez et al., 1997). The TADD comprises an abrasive disc above which are mounted cups. The disc is operated by an electric motor. The disc rotates and progressively abrades off the outer layers of the grain producing fine grain bran that is sucked underneath by fan air-flow (Gomez et al., 1997).

Traditionally, pearl millet decortication involves tempering the grain (using a suitable quantity of water to wet the pericarp) followed by hand-pounding using a mortar and pestle (Munck, 1995). Pounding is followed by a winnowing process that separate the bran flakes and abraded grain (Kebakile et al., 2007). In some areas, traditional decortication by pounding involves washing the grain with enough water, followed by draining, pounding, then separating the bran from decorticated grain using either excess water, air drying or by winnowing (Bassey and Schmidt, 1989, Munck, 1995). However, unlike traditional decortication, tempering is not done when using the TADD. Clearly, traditional decortication differs with the TADD. Therefore this may lead to varying effects on decorticated grain. However, hand-pounding is rapidly being replaced by mechanised decortication techniques (Kebakile et al., 2007).

The efficiency of the TADD is influenced by grain size and shape (Reichert et al., 1986). The TADD was initially designed for sorghum decortication and later adopted for pearl millet.

However, pearl millet grain differs in size and shape with sorghum. It is smaller, tear shaped, smooth and soft and thus it is susceptible to breakage, high grain losses and uneven decortication (Gomez et al., 1997). This may pose a problem in producing uniformly decorticated grain. Another grain dehulling device, the Kett Pearlest rice pearler has been suggested as being more suitable than the TADD for pearl millet, despite both using the same principle (Gomez et al., 1997).

Low cost small industrial scale dehullers have been developed that have benefited subsistence farmers and small scale commercial millers in Africa (Taylor et al., 2010). The most common is the Canadian developed PRL (Prairie Regional Laboratory) dehuller (Taylor and Dewar, 2001). The RIIC (Rural Industries Innovation Centre) in Botswana modified the PRL dehuller to suit rural needs with the addition of a few components such as a trap door to release the decorticated grain when the dehuller is operated in batch mode (Bassey and Schmidt, 1989). The PRL/RIIC dehuller comprises a barrel fitted with carborundum abrasive discs on an axle. The bran is removed from the grain by means of the rotating abrading discs. Similarly to the TADD, abraded bran is removed by a suction fan. Unlike the TADD, abraded grain in PRL/RIIC dehuller barrel is channelled out by an outlet chute at the end of the barrel when operated in the continuous mode (Taylor and Dewar, 2001). The PRL/RIIC dehuller can decorticate a batch of about 5 kg every 3 minutes with throughputs of about 800 kg/hour (Bassey and Schmidt, 1989). Although the TADD is a laboratory instrument (usually working with approximately 30 g or less sample size) for research purposes, it is considered reliable and has nearly the same principle as the PRL/RIIC dehuller (Reichert et al., 1986). Therefore, the reproducibility of pearl millet decortication with the PRL/RIIC dehuller and the TADD is likely to be similar.

Steeping of pearl millet grain with back-slopped liquor to induce lactic acid fermentation is widely practiced domestically in African rural households (Taylor, 2004). However, unlike in this study steeping/lactic acid fermentation is usually done with decorticated grain. Also, the decorticated grain is usually left to ferment for about 1-3 days at ambient temperature (Taylor et al., 2010). In this study, whole grain was steeped/lactic acid fermented for 8 hours at 30^0C.

The limited literature indicates that parboiling of pearl millet grain is not common in Africa. However, across Francophone regions, a steamed pearl millet granulated flour product, couscous, is produced (Obilana, 2003). Parboiling is very widely used for rice processing (FAO, 1994) and appears to be used for sorghum processing (Young et al., 1990) in rural

communities. In North Africa, a traditional steamer (Figure 5.1) is widely used to prepare couscous, including pearl millet couscous (Dicko et al., 2006; Taylor et al., 2010). The steamer (Figure 5.1) resembles the one used in this study, as shown in section 4.2.2.1. Therefore they are likely to cause similar effects on the parboiled product.

Figure 5. 1 Traditional steamer used for preparing couscous (steamed cooked product) in North Africa https://en.wikipedia.org/wiki/Couscous#/media/File:Couscoussier.jpg

5.2 Major findings and potential of technologies

Mineral biofortified pearl millet hybrids have substantially higher iron and zinc contents compared to normal varieties (Table 5.1). The study has indicated that the estimated mineral availability in pearl millet is influenced by mineral and phytate loss during processing. The estimated iron availability worryingly remained low as abrasive decortication removed high levels of iron across the varieties. This is presumably because iron is concentrated in the pearl millet pericarp (Minnis-Ndimba et al., 2015), which is removed by decortication. Nevertheless, phytate was considerably reduced by abrasive decortication alone and in combination with steeping/lactic acid fermentation or parboiling. Therefore although the critical phytate: iron molar ratio of <1, associated with improved iron absorption (Lazarte et al., 2015) was not attained, processing involving steeping/lactic acid fermentation or parboiling could improve iron bioavailability somewhat as a result of the reduction in phytate levels.

Zinc was less affected by abrasive decortication and its level in the pearl millet grain remained fairly constant (Table 5.1). This is because zinc is concentrated on the scutellum part of the germ in the pearl millet grain (Minnis-Ndimba et al., 2015), and hence less affected by decortication. The estimated zinc availability improved considerably with processing due to high phytate loss and minimal zinc loss during processing.

Phytate content varied considerably in both normal pearl millet varieties and the biofortified hybrids (Table 5.1). Abrasive decortication in combination with steeping/lactic acid fermentation was the most effective way of reducing phytate content in pearl millet. To a lesser extent, parboiling plus abrasive decortication also reduced phytate levels across the varieties and hybrids. Both these processes somewhat improved both iron and zinc estimated availability. Therefore abrasive decortication plus steeping/lactic acid fermentation and also parboiling plus abrasive decortication can benefit pearl millet consumers in rural Africa who suffer from iron and zinc deficiencies.

Total phenolic content (TPC) varied substantially in normal pearl millet varieties and the biofortified hybrids (Table 5.1). However, the biofortified hybrids had amongst the highest TPC levels. Decortication greatly reduced TPC (mean 24% reduction) across the varieties. Steeping/lactic fermentation and parboiling of whole pearl millet grains alone did not have substantial effect on TPC. However, decortication of steeped/lactic fermented and parboiled similarly resulted in an additional 14 percentage points TPC reduction across the varieties as

compared to decortication of raw pearl millet grain. Therefore abrasive decortication in combination with either steeping/lactic acid fermentation or parboiling can effectively reduce the levels of specifically iron-binding phenolics (Lestienne et al., 2007), thereby presumably improving iron availability.

5.3 Implementation of recommended technologies

As underlined by this work, mineral biofortification, abrasive decortication, steeping/lactic acid fermentation and parboiling are feasible technologies that can improve pearl millet grain iron and zinc availability. Therefore they have the potential to address iron and zinc deficiencies in rural Africa. Thus, how these technologies should be implemented is of particular importance.

The existence of elevated iron and zinc pearl millet varieties has been recognised in this study and other related work (Velu et al., 2007, Rai et al., 2012). However, putting pearl millet mineral biofortification into effect depends on several factors. As stated, iron and zinc biofortification is a rural-based intervention aimed for implementation in specific African rural areas, which are characterised with diverse and adverse environmental conditions (Bouis et al., 2011). Therefore the varieties have to show the capacity to perform in dry, semi-arid drought-prone areas and show high yielding capacity (Velu et al., 2007, Rai et al., 2012). Rai et al. (2012) reported a negative correlation between micronutrients (iron and zinc) and grain yield. This implies that varieties rich in these micronutrients need to be bred without affecting grain production capacity.

Information on iron and zinc biofortified pearl millet grain yield stability across varying African environments is scarce. However, Velu et al. (2007) found that the crop performs better during the rainy season than the dry season when soils have high iron and zinc levels. The iron and zinc contents in the grain produced during the rainy season can be up to 19-20% more as compared to the dry season (Velu et al., 2007). This is important because across rural Africa, pearl millet is cultivated during the rainy season. Pearl millet biofortified seeds must be disseminated to target areas, so that they can be adopted by small-holder farmers for cultivation and consumption (Bouis et al., 2011, Saltzman et al., 2014). Small-holder farmers in rural African areas should be assisted with modern agricultural practices and how to consume these mineral biofortified varieties (Bouis et al., 2011). Follow up studies to evaluate the efficacy of the biofortified varieties in terms of yield capacity, stability in

different environments and impact on the iron and zinc status of consumers will assist the prospects of breeding and adoption of iron and zinc biofortified pearl millet.

Steeping/lactic acid fermentation of pearl millet grain may not require new introduction and adoption across rural Africa. The process is simple and well-known across Africa in cereal processing including optimum pearl millet grain (Obilana, 2003). Rural women make use of their experiential skills to determine the steeping/lactic acid fermentation conditions and period. Hot/sunny conditions allow drying of the steeped/lactic acid fermented grain (Taylor, 2004, Taylor et al., 2010). At present, effort needs to be devoted towards communicating the value of steeping/lactic acid fermentation where pearl millet is a staple.

To ensure palatability the dried steeped/lactic acid fermented pearl millet grain is decorticated to remove the bran (Taylor and Dewar, 2001). Importantly, this study has shown that abrasive decortication plus steeping/lactic acid fermentation is the best processes combination for effective phytate reduction (Table 5.1). Similarly to steeping/lactic acid fermentation, abrasive decortication using a wooden mortar and pestle is well known and practiced by woman across rural Africa (Taylor et al., 2010). However, mechanised pearl millet decortication equipment such as the PRL/RIIC dehuller (Reichert et al., 1986) exists. This may ensure effective pearl millet decortication and provide a viable alternative for small-holder farmers to the labour intensive traditional manual decortication.

As for parboiling, the process may require familiarising in other parts of Africa. As stated, in certain parts of Africa particularly in the North and West Africa, steamed cereal products including pearl millet are consumed (Obilana, 2003, Dicko et al., 2006). Therefore, parboiling in other parts of Africa where it is less common may be introduced and implemented based on the current study findings as well as adapting what is currently practiced in North and Francophone African regions. Dandedjrohoun et al. (2012) devised a strategy to ensure that parboiling of rice technology was diffused, implemented and adopted in Benin. Emphasis was put on support, training, knowledge/technology transfer and access to the equipment by women. It was found that a good portion of people (67%) adopted the technology. Therefore such a strategy should also work for pearl millet parboiling technology implementation in rural Africa. In essence, parboiling may eventually gain popularity over steeping/lactic acid fermentation. This is because the grain is cooked and Maillard reactions are induced during processing (Lamberts et al., 2006). Hence, a quality enhanced and ready-to-eat product for consumption is produced.

Table 5. 1 Summary of major findings showing the effects of food processing methods and biofortification on iron, zinc, phytate and TPC across the pearl millet varieties and the impact on estimated iron and zinc availability

Processing technology/strategy	Iron and zinc	Phytate	Total phenolic content (TPC)	Estimated iron and zinc availability
Mineral biofortification	Iron: biofortified *(8.8-9.6) higher than normal grain *(3.0-7.5) Zinc: biofortified *(4.4-4.8) higher than normal grain *(3.0-4.1)	No definite trend in phytate content (between biofortified hybrids and normal varieties)	Mineral biofortified hybrids among those with high TPC	Biofortified hybrids have improved phytate: mineral ratios than normal varieties
Abrasive decortication (whole grain)	High iron loss (31%) Minimal zinc loss (8%)	24% phytate reduction	24% TPC reduction	Low iron availability Zinc availability generally improved
Steeping/lactic acid fermentation (whole grain)	No significant effect on iron Minimal zinc increase (4%)	21% phytate reduction	No significant effect on TPC	Iron availability remained low Zinc availability substantially improved compared to raw grain
Parboiling (whole grain)	Minimal iron increase (6%) No significant effect on zinc	15% phytate reduction	No significant effect on TPC	Iron availability remained low Zinc availability substantially improved compared to raw grain
Abrasive decortication plus steeping/lactic acid fermentation	High iron loss (30%) Minimal zinc loss (3%)	High phytate reduction (36%) **12% points phytate reduction	High TPC reduction (38%) **14% points TPC reduction	Iron availability remained low Critical level of <15 for Zn availability was attained in some varieties
Abrasive decortication plus parboiling	High iron loss (33%) No significant effect on zinc	High phytate reduction (32%) **8% points phytate reduction	High TPC reduction (38%) **14% points TPC reduction	Iron availability remained low Critical level of <15 for Zn availability was attained in some varieties

% represents the overall mineral, phytate or TPC reduction/increase across all pearl millet varieties

* Mineral content in mg/100 g, ** Percentage points phytate and TPC reduction from the initial raw grain percent phytate reduction

6. CONCLUSIONS AND RECOMMENDATIONS

Mineral biofortified pearl millet hybrids have considerably improved iron and zinc contents compared to normal varieties. The livelihood of people in rural communities in Africa can be improved by cultivation and consumption of mineral biofortified pearl millet. Efforts must be devoted to expanding production of biofortified pearl millet grain by small-holder farmers. This can be attained with enough support/assistance from relevant stakeholders (i.e. ICRISAT, private seed companies, NGOs, research institutions and government policy makers).

Abrasive decortication does not cause substantial losses in zinc, but results in considerable iron losses (mean 31% reduction) across the pearl millet varieties. High iron loss through abrasive decortication remains a major concern. Steeping/lactic acid fermentation and parboiling do not greatly affect pearl millet iron and zinc contents.

Phytate levels vary considerable among pearl millet varieties and hybrids, but the levels are not substantially different in normal and biofortified hybrids. Abrasive decortication greatly reduces phytate (mean 24% reduction) across the varieties. Importantly, abrasive decortication in combination with steeping/lactic acid fermentation additionally reduce phytate (mean 36% reduction) across the varieties. Abrasive decortication in combination with parboiling also somewhat reduces the level of phytate in pearl millet grain.

Total phenolic content (TPC) varies substantially among pearl millet varieties and hybrids. The mineral biofortified pearl millet hybrids have among the highest levels of TPC. Abrasive decortication effectively reduces the TPC level (mean 24% reduction) across the pearl millet varieties. Abrasive decortication of steeped/lactic fermented and parboiled pearl millet grain greatly reduces TPC (mean 38% reduction), thereby potentially reducing iron-binding phenolics.

Abrasive decortication plus steeping/lactic acid fermentation is the most effective way of phytate reduction in pearl millet grain. It results in the estimated iron and zinc availability of pearl millet being somewhat improved. Abrasive decortication in combination with steeping/lactic acid fermentation therefore seems to be a feasible method to help address iron and zinc deficiencies in rural African communities where plant-based food diets high in phytate and phenolics are the norm.

It is recommended that abrasive decortication in combination with steeping/lactic acid fermentation of pearl millet is utilised to a greater extent across rural Africa. Future studies should involve dietary intervention trials to determine whether consumption of biofortified pearl millet with increased iron and zinc levels with reduction of phytate and phenolics leads to improved iron and zinc status in pearl millet consuming populations.

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