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EVALUATION OF GRAIN NUTRITIONAL QUALITY AND RESISTANT STARCH CONTENT IN KENYAN BREAD WHEAT VARIETIES

S.N. KARIITHI[1], S.I. ABWAO[1], P.N. NJAU[2] and J.N. NDUNG'U[3]
[1]Egerton University, Biochemistry and Molecular Biology Department, P. O. Box 536-20115, Egerton, Kenya
[2]Highland Agri-consult Services Limited, P. O. Box 470-20107, Njoro, Kenya
[3]Kenya Agricultural and Livestock Research Organisation, Food Crops Research Centre Njoro, Kenya
Corresponding author: sammy_re@yahoo.com

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ABSTRACT

Micronutrient deficiency is a major problem worldwide, particularly in developing countries that rely heavily on cereal rich diets for sustenance. Wheat (*Triticum aestivum* L.), a popular staple cereal crop contains substantial levels of micronutrients, thus could be used for alleviating dietary micronutrient deficiencies. The objective of this study was to evaluate the effect of wheat genotype and selected agro-ecological zones on kernel accumulation of zinc, iron, resistant starch and phytic acid. In whole meal flour prepared from nine Kenyan bread wheat varieties, Zn and Fe levels ranged from 111 to 305 µg g^{-1}, and 26 to 91 µg g^{-1}. Phytic acid and resistant starch levels ranged from 2.66 to 5.05 µg g^{-1} and 0.37 to 6.00 g 100 g^{-1}, respectively across three sites. Variety × site influenced iron and resistant starch content significantly ($P<0.05$), whereas zinc and phytic acid were influenced by variety and interaction. Njoro BWII variety had the highest Zn and phytic acid levels; while Fe and resistant starch were obtained on K. Korongo and K. Tai varieties, respectively. Overall, Eldoret site had optimal conditions that enhanced accumulation of preferred nutrition values. In conclusion, whole meal flour prepared from all 9 varieties contained considerable levels zinc, iron, resistant starch and low levels of the undesirable phytic acid.

Key Words: Iron, phytic acid, *Triticum aestivum,* zinc

RÉSUMÉ

La déficience en micronutrients est un problème majeur de par le monde, surtout dans les pays en voie de développement, qui dépendent fortement de mets à base de céréales. Le blé (*Triticum aestivum* L.), un aliment de base céréalier très populaire, contient un taux très élevé de micronutrients, il peut ainsi être utilisé pour corriger les régimes alimentaires carents en micronutriments. L'objectif de l'étude était d'évaluer l'effet des génotypes de blé et de zones agro écologiques ciblées sur l'accumulation de zinc, fer, amidon resistant, et acide phytique dans les grains de blé. Les teneurs en zinc et en fer des des repas à base de blé entier préparés à partir des variétés de blé Kényan variaient de 111 à 305 µg g^{-1}, et 26 à 91 µg g^{-1}. Les teneurs en acide phytique et en amidon résistant allaient de 2,66 to 5,05 µg g^{-1}et 0,37 à 6,00 g 100 g^{-1}, respectivement dans trois sites d'expérimentation. L'interaction variety-site d'expérimentation a influencé les teneurs en fer et en amidon résistant de façon significative ($P<0.05$), tandis que les teneurs en zinc et en acide phytique ont été influencées par la variété et l'interaction. La variété Njoro BWII avait la teneur en zinc et en acide phytique la plus élevée, tandis que fer et amidon résistant avaient été respectivement obtenus avec les variétés K. Korongo et K. Tai. Au total, le site de Eldoret présentait les conditions optimales qui favorise l'accumulation des nutriments préférés. Il convient de conclure que des repas entiers préparés à partir de 9 variétés de blé Kényan contenaient des niveaux considérables de zinc, fer, amidon et de faibles niveaux d'acide phytique non desirable.

Mots Clés: Fer, acide phytique, *Triticum aestivum,* zinc

INTRODUCTION

Human beings get nutritional requirements mostly through consumption of edible plants and animal or their products. The nutrition quality of edible plant parts is highly influenced by crop genotypes, soil composition and environmental factors, which greatly influence nutrient uptake and storage in plants (Marschner, 2012). Environmental changes, especially in temperature and precipitation, affect plant physiological processes, which in turn influence nutrient uptake and utilisation by plants (Olesen *et al.,* 2011). On the other hand, soil composition has great influence on the concentrations of micro-nutrients such as zinc and iron in kernels of cereals like wheat (Abrar *et al.,* 2010). Similarly, the anti-nutritive elements, particularly phytates in cereal crops, are influenced by soil composition, environmental factor, stage of maturity and genotype. For instance, it is well demonstrated that high temperatures and water-deficit conditions lead to increased phytic acid content in the wheat grains (Singh *et al*., 2012; Gordana *et al*., 2015).

Majority of cereal based diets, especially maize and wheat, are inherently low Zn and Fe content (Polleti *et al.,* 2004) yet the little present is lost during milling and polishing of these cereals. Most of the seed Zn and Fe are located in the embryo and aleurone layer; whereas the endosperm is very low in Zn and Fe (Ozturk *et al*., 2006). Although Zn and Fe are important micronutrients, their concentrations in the kernels, apart from the edaphic factors are influenced by variety. Biofortification is, therefore, necessary for some varieties in order to improve their Zn and Fe content. Biofortification of cereal crops has been achieved through over-expression of the transcription factor *NAM-B1* (Uauy *et al*., 2006). The *NAM-B1* gene was initially used to increase protein levels, but has also been used to increase Zn and Fe levels in cereals (Distelfeld *et al.,* 2007). Varieties with improved levels of these micronutrients could be used to make an important contribution towards improving the health of millions of people worldwide. Iron and Zinc malnutrition, affect over 25 % of the global population (Philippa *et al*., 2014).

Wheat also contains substantial amounts of resistant starch, which has been found to improve the absorption of micronutrients in the gut (Katharina *et al.,* 2007). Classified as a prebiotic, it stimulates growth and activity of a number of bacteria in the gastrointestinal tract exerting a health-promoting effect (Schrezenmeir and de Vrese, 2001). Resistant starch has been used as a nutrition approach to reduce the number of people who are at risk of developing type II diabetes (Mindy *et al.,* 2013). Its fermentation in rodent studies resulted in a healthier gut, demonstrated by increased amounts of short chain fatty acids, positive change in the microbiota, and increased gene expression for gene products involved in normal healthy proliferation and apoptosis of potential cancer cells (Michael *et al.,* 2015). Additionally, consumption of resistant starch has been associated with reduced abdominal fat and improved insulin sensitivity.

Phytic acid (PA) which is an anti-nutrient with respect to micro-elements, is the main storage form of phosphorous in seeds, accounting for 60 to 90 % of the total seed phosphorous and 1.5 % of the seed dry weight (Bohn *et al.,* 2008).The negatively charged phosphates in phytic acid strongly bind to metallic cations (such as K^{+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Ca^{2+}, and Zn^{+}) to form a mixed salt called phytin or phytate. In seeds, phytate is predominantly found in the protein bodies of embryo and aleurone layers, where there is a high deposition of mineral nutrients as well (Steadman *et al.,* 2001). The chelation of zinc and iron with phytic acid has a strong negative effect on absorption of these minerals in humans and other monogastric animals that largely lack the phytase enzyme, which is required to degrade phytate. The objective of this study, therefore, was to examine the influence of AEZs in Kenya where commercial wheat growing is practiced, and selected Kenyan bread wheat varieties, on the concentrations of zinc and iron, resistant starch and phytic acid in whole meal flour preparation.

MATERIALS AND METHODS

Nine Kenyan bread wheat varieties were planted in three study sites, namely, Eldoret, Mau-Narok and Naivasha, with different edaphic and

environmental factors summarised in Table 1. These sites were selected since they represented the key wheat growing regions in Kenya. Nine wheat varieties recently released in Kenya were selected, namely, Robin, Eagle 10, Kenya Tai, Kenya Sunbird, Kenya Wren, Kenya Korongo, Kenya Hawk 12, Kenya Kingbird and Njoro BWII were obtained from the Kenya Agricultural and Livestock Research Organisation, Food Crops Research Centre located at Njoro in Nakuru county, Kenya. Variety Njoro BWII was used as the control due to its superior milling and baking properties, especially high protein and gluten levels.

The main characteristics of the selected wheat varieties are presented in Table 2. Prior to planting, the seeds were coated with copper oxychloride ($Cu_2(OH)_3Cl$) a broad spectrum fungicide that controls both fungal and bacterial diseases. The wheat varieties were planted in a completely randomised block design with plots measuring 6 by 1.5 metres.

The land was treated with di-Ammonium phosphate (DAP) fertiliser at the rate of 50 kg/ ha before planting. The varieties were then allocated randomly to the plots within the three blocks to give rise to three replicate plots for each variety, using the Random Number Table. No irrigation was done during planting and growing of the wheat crops.

Dry wheat heads were harvested at maturity (kernels filled and turn golden brown in colour) in each of the study sites, using a Hege Machine model 125C; and the grains were later threshed

TABLE 1. Geographical location and edaphic features of the three study sites

Site	Altitude (m)	Longitude	Latitude	$_P$H	Zinc (μg g^{-1})	Iron (μg g^{-1})	Precipitation (mm)
Eldoret	2073	35°17'E	0°31'N	5.4-6.0	5	20	1103
Mau-Narok	2830	36°0'E	0°36'S	4.8-5.6	25	100	980
Naivasha	1884	36°26'E	0°43'S	6.2-7.4	25-65	110	677

TABLE 2. Kenyan bread wheat varieties and their characteristics

Variety	Year of release	Description	Yield (t ha^{-1})
Robin	2011	Red, hard grain, resistant to stem rust especially Ug99 strain and spring growth habits	8.1
Eagle 10	2011	Red, hard grain, resistant to stem rust especially Ug99, spring growth habits and matures in 100-110 days	6.5
Kenya Tai	2012	Red, hard grain, resistant to stem and yellow rust, spring growth habits and matures in 100-110 days	6.5
Kenya Sunbird	2012	Red, hard grain, resistant to stem and yellow rust, high biomass and matures in 100-110 days	6.5
Kenya Wren	2012	Red, hard grain, resistant to stem rust strain Ug99, spring growth habits and matures in 120-130 days	8.5
Kenya Korongo	2012	White hard grain, resistant to stem rust strain Ug99, spring growth habits and matures in 120-130 days	8.5
Kenya Hawk 12	2012	Hard, red grain, resistant to stem rust strain Ug99, spring growth habits and matures in 120-130 days	8.5
Kenya Kingbird	2012	White, hard grain, resistant to stem rust strain Ug99, spring growth habit and matures in 90-110 days	6.0
Njoro BWII	2001	Red, hard grain, resistant to sprouting, spring growth characteristics	8.0

using a single plant wheat thresher (Almaco thresher model, SVPT). The grain was further dried to reduce moisture to about 12-13 %, which was confirmed using the NIR Spectrophotometer Grain Analyzer machine. At this moisture level, the grains can stay for long without risk of germination but still remain viable to be used as seed.

For each variety, a sample of about 10 g from the harvested crop was separately milled using a chromium ball mill (Retsch mill model, MM 400), whose milling compartment was coated with Teflon a plastic material which prevents contamination from the milling chamber. To analyse Zn and Fe content in the kernels, 0.3 g of the finely ground whole meal flour per sample was taken and digested with 4 mls of the selenium-sulphuric acid mixture until the mixture was clear or pale yellow. The digest was then transferred into a 100 ml volumetric flask and filled up to the mark with de-ionised water. After cooling, the digests were then analysed for iron and zinc, by measuring their absorbance at 248.33 nm for iron and 213.86 nm for zinc using the Atomic Absorption Spectrophotometer (Shimadzu Model AA-6300, Tokyo-Japan).

Standards at concentrations of 2.5, 5.0, 7.5 and 10 µg g^{-1} were prepared from a 1000 µg g^{-1} standard stock solutions of $Zn(NO_3)_2$ and $Fe(NO_3)_3$ for Zn and Fe, respectively; and their absorbance was determined. The results were then used to construct a calibration curve with absorbance against concentration whose trend line had an R^2 value of 0.999 and whose graph equation was Y=0.34X for zinc and Y=0.004X for iron: where Y=absorbance and X=actual concentration.

From the milled whole meal flour, 1 g sample was taken and transferred into a 1.5 ml microfuge tubes to which 1 ml of 0.4 M hydrochloric acid was added and incubated at 4 °C for 12 hours to extract phytic acid present in the samples. The samples and prepared standards were then vortexed briefly and 20 µl aliquots were transferred into microtitre plates and supplemented with 180 µl of Chen's reagent (1 volume 6 N H_2SO_4, 1 volume 2.5 % ammonium molybdate; 1 volume 10 % ascorbic acid, 2 volumes H_20). Addition of Chen's reagent in a sample containing phytic acid, induced the formation of phospho-molybdate compound, which has blue colouration. The intensity of colour formed is dependent on phytic acid concentration in the sample. The assay was then allowed to develop upto 2 hr at room temperature, after addition of Chen's reagent. An image of the microtitre plates was then taken after 1 and 2 hours and comparison was made between colour intensity of the samples and the standards.

The standards were prepared by dissolving 0.174 grams of di-Potassium hydrogen phosphate (K_2HPO_4) in 1 litre distilled water to give a concentration of 1 Mm K_2HPO_4. Eight standards were then prepared in the order of increasing concentrations by pipetting 15, 30, 45, 60, 75, 105 and 120 µl and supplementing it with 100 µl of Chen's reagent, 10 µl of 0.4 M HCl and distilled water in the order of reducing volumes. The protocol used for determining phytic acid content in wheat flour was adopted from a study by Raboy *et al.* (2000).

Resistant starch was analysed using the megazyme resistant starch assay procedure (11/ 02 AOAC Method 2002.02, AACC Method 32-40). Grain samples (approximately 50 g) were ground and whole meal flour passed through a 1.0 mm sieve. A sample of 100 mg was then digested with 4 ml of pancreatic á-amylase, with amyloglucosidase (AMG) in tubes by incubating it in a shaking water bath at 37 °C for 16 hr: Non-resistant starch was hydrolysed to D-glucose by the combined action of the two enzymes. The reaction was then terminated by addition of an equal volume of ethanol, and RS recovered as a pellet upon centrifugation at 3000 revolutions per minute (rpm). The pellet was then washed twice by re-suspending it in 50 % ethanol v/v (50 % ethanol dissolved in 50 % distilled water), followed by centrifugation and decanting of the supernatant (ethanol). The RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice water bath, using a magnetic stirrer. The solution was later neutralised with 8 ml of 1.2 M sodium acetate buffer and starch was quantitatively hydrolysed to glucose with amyloglucosidase (AMG). D-Glucose was then measured with glucose oxidase/peroxidase reagent (GOPOD). Absorbance of the samples was determined at 510 nm, using a UV/ Visible light spectrophotometer and converted to actual

RS content using a formula provided with the Megazyme resistant starch assay kit manufactured by Megazyme International Ireland.

Data were collected in triplicates per variety for each experimental site and then subjected to Analysis of variance (ANOVA) at $P<0.05$ level of significance using SAS software Version9.1.3 (SAS Institute, Inc., 2004). The means were further analysed using MINITAB software to cluster the varieties with highest similarity based nutritional qualities assessed.

RESULTS

Iron concentration in the wheat kernel ranged between 111 and 305 μg g^{-1} irrespective of the variety tested (Table 3). Its concentration was significantly ($P<0.05$) influenced by variety, cropping site and the interaction of variety × site. For varieties grown in the Eldoret site, iron concentrations ranged between 138 and 305 μg g^{-1} while those grown in Mau-Narok and Naivasha it ranged between 111 and 222 μg g^{-1}. Overall, the highest iron concentrations were recorded in varieties K.Korongo and K.Hawk 12, for the crop grown in the Eldoret (Table 3). The control variety Njoro BWII generally recoded the lowest iron concentration for crop grown in the Naivasha and Eldoret sites, while K.Wren and K.Korongo recorded the lowest concentrations for the crop grown in Mau-Narok site (Table 3). Zinc levels ranged from 26 to 91 μg g^{-1} irrespective of variety and experimental site (Table 3). The concentration of zinc in the whole meal flour samples was significantly ($P<0.05$) influenced by variety and variety × cropping site. For the Naivasha crop, zinc concentrations ranged from 32 to 91 μg g^{-1}, while for Eldoret and Mau-Narok crop it ranged from 26 to 88 μg g^{-1}. Generally, the highest concentration of zinc was obtained on the control variety, Njoro BWII for wheat crop grown in Naivasha and Eldoret. On the other hand, varieties K.Wren and K.Korongo had the lowest zinc concentrations across the three study sites (Table 3). However, relatively higher concentration of zinc were obtained on varieties Eagle10 and Robin across the three study sites (Table 3). Overall, the highest concentrations were recorded in the control Njoro BWII, Eagle10, Robin and K.Kingbird; whereas lowest concentrations were recorded in K.Wren and K.Korongo (Table 3).

Variety and interactions (site × variety) significantly ($P<0.05$) influenced the level of phytic acid in whole meal flour, prepared from the wheat varieties tested. Irrespective of wheat variety, the level of phytic acid concentration for the crop grown in the Eldoret site ranged from 2.66 to 4.79 μg g^{-1}, whereas for Mau-Narok and Naivasha sites the range was 2.66 to 5.05 μg g^{-1}. Generally, the highest phytic acid content was observed in flour obtained from wheat grown in Naivasha, while those grown in Mau-Narok had the lowest content, except for the Njoro BWII. In addition Robin, Eagle10, K.wren and K.Tai had the lowest phytic acid irrespective of the experimental site (Table 3). For Eldoret site highest phytic acid content was obtained on K.Sunbird, K.Kingbird and Njoro BWII. None the less, a general trend was observed where highest phytic acid was obtained with Njoro BWII variety across all study sites, while the lowest phytic acid values were obtained with K.Wren, Eagle10 and Robin varieties (Table 3).

Resistant starch, glucose and total starch concentrations, irrespective of the experimental site and wheat variety ranged from 0.37 to 6.00 g 100 g^{-1}, 20 to 33 g 100 g^{-1} and 22 to 37 g 100 g^{-1}, respectively (Table 4). ANOVA revealed that RS, soluble glucose and total starch were significantly ($P<0.05$) influenced by variety, site and, variety × site. For the Eldoret crop, the highest concentration of RS was obtained on Kenya Hawk 12, Kenya Tai and Kenya Wren, with 6.0, 5.9 and 4.7 g 100 g^{-1}, respectively; which were significantly ($P<0.05$) higher than the control Njoro BWII variety, which had RS concentration of 2.0 g 100 g^{-1}. However, for Naivasha and Mau-Narok sites, significantly ($P<0.05$) higher concentration of RS were obtained on Njoro BWII variety than the 8 varieties tested (Table 4).

On the other hand, highest concentration of glucose for wheat crop grown in Eldoret site was obtained on K.Sunbird, the levels were significantly ($P<0.05$) higher than that obtained for Njoro BWII. For Naivasha and Mau-Narok sites, however, varieties with highest glucose concentrations were not significantly ($P<0.05$) different when compared to the control variety, except for K.Kingbird grown in Mau-Narok site

TABLE 3. Determination of iron, zinc and phytic acid concentration in 9 Kenyan wheat-bread varieties grown in three sites located in Eldoret, Mau-Narok and Naivasha.

Variety	Iron			Zinc			Phytic acid		
	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha
Robin	194.44±11.56	138.89±11.55	194.44±11.45	65.35±3.27	62.09±3.27	84.97±3.27	2.71±0.05	2.66±0.05	2.66±0.05
Eagle 10	194.44±11.50	222.22±11.55	138.89±11.45	52.29±3.27	75.16±3.27	71.89±3.27	2.66±0.05	2.66±0.05	2.71±0.05
K.Tai	194.44±11.45	194.44±11.55	111.11±11.60	42.48±3.27	35.95±3.27	65.36±8.65	3.04±0.22	2.71±0.05	5.05±0.17
K.Sunbird	194.44±11.56	222.22±11.50	194.44±11.56	26.14±3.27	75.16±3.27	45.75±6.54	4.79±0.43	3.48±0.22	4.62±0.05
K.Wren	222.22±11.56	111.11±11.43	138.89±11.60	35.95±3.27	26.14±3.27	42.48±3.27	2.66±0.05	2.71±0.05	2.66±0.05
K.Korongo	305.55±11.55	111.11±11.60	138.89±11.45	45.75±3.27	45.75±3.27	32.68±6.54	3.04±0.02	3.26±0.38	2.71±0.05
K.Hawk12	305.55±11.43	138.89±11.45	138.89±11.50	42.48±6.54	42.48±3.27	65.36±3.27	3.70±0.02	2.83±0.22	5.05±0.17
K.Kingbird	194.44±11.43	222.22±11.45	194.44±11.60	68.63±5.66	68.63±5.66	32.68±3.27	4.35±0.43	2.83±0.22	5.05±0.22
Njoro BWII	138.89±11.60	222.22±11.50	111.11±11.60	88.23±5.66	62.09±3.27	91.5±3.27	4.79±0.43	5.05±0.17	5.05±0.17

*Values = Means ± SEM, all values are in μg g^{-1}. N=3

TABLE 4. Determination of resistant starch, glucose and total starch concentration in 9 Kenyan wheat-bread varieties grown in three sites located in Eldoret, Mau-Narok and Naivasha

Variety	Resistant starch			Glucose			Starch		
	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha	Eldoret	Mau-Narok	Naivasha
Robin	1.17±0.07	1.47±0.18	0.37±0.01	26.16±0.72	24.88±2.47	27.92±0.87	27.33±0.65	26.35±2.57	28.29±0.88
Eagle 10	1.42±0.11	1.16±0.04	0.45±0.05	31.73±1.21	29.11±0.44	26.09±1.18	33.14±1.11	30.26±0.41	26.54±1.14
K.Tai	5.90±0.07	2.65±0.18	1.30±0.03	21.9±0.94	28.38±1.10	29.07±1.59	27.81±0.87	31.03±0.92	30.38±1.59
K.Sunbird	1.47±0.03	0.48±0.03	0.37±0.05	32.91±1.05	20.16±1.12	33.09±1.65	34.38±1.07	20.65±1.10	33.46±1.67
K.Wren	4.66±0.14	0.83±0.07	0.98±0.06	22.43±1.22	30.34±0.89	25.28±1.13	27.09±1.07	31.17±0.94	26.26±1.19
K.Korongo	2.27±0.14	0.78±0.09	0.68±0.16	28.36±1.02	21.86±0.97	26.03±0.11	30.63±0.48	22.64±0.90	26.72±0.26
K.Hawk12	6.03±0.59	0.91±0.16	0.68±0.20	31.10±1.02	21.97±0.92	24.15±1.26	37.13±1.19	22.88±0.86	24.84±1.42
K.Kingbird	2.35±0.13	2.98±0.14	0.92±0.25	22.90±1.39	33.41±0.69	30.96±0.74	25.25±1.32	36.39±0.64	31.88±0.74
Njoro BWII	2.01±0.05	2.04±0.05	2.01±0.05	29.30±0.45	29.77±0.46	29.24±0.45	31.31±0.50	31.81±0.48	31.25±0.48

*Values = Means ± SEM, all values are in g $100g^{-1}$. N=3

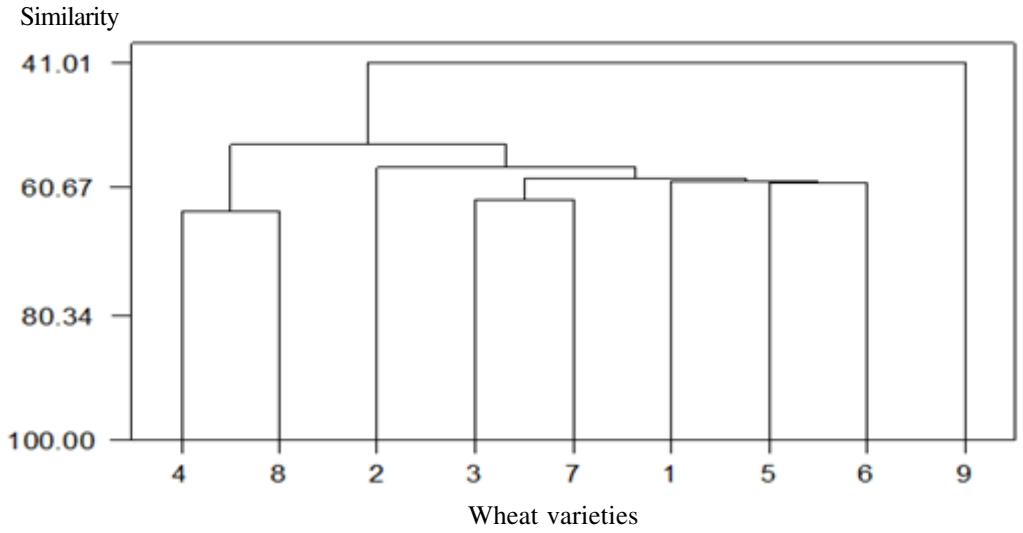

Figure 1. Differential clustering of Kenyan bread wheat varieties based on their nutritional qualities. Legend 1-9 represents variety 1 to variety 9 where 1-Robin, 2-Eagle10, 3-K.Tai, 4-K.Sunbird, 5-K.Wren, 6-K.Korongo, 7-K.Hawk12, 8-K.Kingbird and 9-Njoro BWII

(Table 4). For total starch, highest concentration for the crop grown in Eldoret was obtained on K.Sunbird, and K.Hawk 12, the starch concentration in these two varieties were significantly ($P<0.05$) higher than in the control variety. Naivasha and Mau-Narok sites, however, the varieties with highest total starch concentrations were not significantly ($P<0.05$) different when compared to the control variety, except for K.Kingbird grown in Mau-Narok site (Table 4).

Cluster analysis of the varieties based on the means of the all the nutritional qualities analysed, namely zinc, iron, resistant starch, glucose, total starch and phytic acid revealed three distinct clusters (Fig. 1). The clustering was as in the order; K. Sunbird and K. Kingbird, K.Tai and K. Hawk12, Robin, K.Wren and K. Korongo with 64, 62 and 58% similarity, respectively. The varieties Eagle 10 and the control Njoro BWII formed isolated clusters different from the rest.

DISCUSSION

The concentrations of iron for the nine Kenyan wheat bread varieties in the study reported herein this paper were all above the target with the lowest concentrations, being almost two folds more than the targeted level of 60 µg g^{-1}, while the highest was five times more. Micronutrient concentrations especially zinc and iron are relatively low in kernels of most cereals crops. Furthermore variation in the kernel concentration of micronutrient is influenced by genotype; for instance studies on wild relatives and landraces of wheat have established that considerable variations exist in grain zinc and iron concentration (Genc *et al*., 2005; Gomez-Becerra *et al*., 2010a, b). The targets for zinc and iron biofortification in wheat grain have been set at 40 µg g^{-1} and 60 µg g^{-1}, respectively (Ortiz-Monasterio *et al*., 2007). However it has been suggested that grain zinc and iron concentrations should be increased by at least 10 and 25 µg g^{-1} in order to have a measureable biological impact on human health, (Ortiz-Monasterio *et al.*, 2007; Chatzav *et al.*, 2010). Plant tissue zinc and iron concentrations are highly influenced by soil conditions especially soil *p*H and microelement composition (Alloway, 2004). The wheat varieties grown in areas where soils generally have a higher *p*H value and low Fe concentrations, such as Eldoret accumulated more Fe in their kernels compared to the varieties grown in soils with adequate Fe composition and slightly lower *p*H such as Mau-Narok and Naivasha. Uptake of Fe

usually occurs through direct transport using ZRT-, IRT-like proteins (ZIPs) or *via* secretion of phytosiderophores (PS), which chelate Fe cations and are subsequently taken up by yellow stripe like (YSL) transporters (Sperotto *et al*., 2012). Monocots plants such as wheat prefer the chelation strategy for Fe uptake. Among the chelators is nicotineamine (NA) which in Fe deficient soils, plants over-express the nicotine amine synthase (NA) gene, producing more NA which binds with the iron present in the soil and transports it into the plant sinks increasing their concentration (Gyana and Sunita, 2015). In a study conducted on transgenic soy beans which were genetically engineered to over-express the NA synthase gene, the plants accumulated 2 to 4 fold more Fe compared to the non-transgenic plants (Tomoko *et al*., 2014). Thus future research in enhancing microelement uptake from the soil should largely focus on manipulating the NA synthase gene.

Plants that accumulate more than 20 μg g^{-1} of zinc in their kernels are classified as Zn sufficient; while those with less than 10 μg g^{-1} are deficient (Bhupinder *et al*., 2005). Concentrations recorded in all the test varieties across the three sites revealed that all kernels were zinc sufficient (Table 3). Comparison between soil zinc levels across the three sites and those accumulated in the wheat grains (Table 3) revealed that crops grown in areas with high levels of soil zinc had higher accumulation in their grains, for instance Mau-Narok and Naivasha crop. Interestingly, despite having relatively low level of soil zinc, wheat crop from Eldoret site had also higher concentration of grain Zn. This observation might be a result of enhanced release of phytosiderophores especially 2'-Deoxy-mugineic acid by wheat in soil to enhance uptake of zinc and its ultimate storage in grains (Dotaniya *et al*., 2013a). Once released, phytosiderophores chelate Zn ions in the soil and transport them to various tissues first through the xylem and the phloem in the later stages of maturity.

Varietal differences in grain zinc and iron concentrations in cultivated wheat have also been attributed, in part to allelic variation at a chromosomal locus that promotes early senescence and remobilisation of protein, iron and zinc from senescing leaves to seeds (Distelfeld *et al*., 2007). This may explain the reason some varieties recorded overall high levels irrespective of site. During senescence, cytoplasmic components such as organelles in the leaves are gradually dismantled and degraded; and their components such as zinc and iron translocated to the sinks especially grains where they form a reservoir for future generations (Mathieu *et al*., 2014).

Phytic acid content in plant seeds and grains ranges from 0.5-5 % and reaches upto 90 % in dormant seeds (Loewus, 2002). In flour, concentrations of 3.77, 2.96 and 8.50 μg g^{-1}, have been reported for hand-made refined flours, factory refined flours and for the whole grain flours, respectively (Febles *et al*., 2002). Depending on the amount of plant-derived foods in diets and level of processing, daily intake can be as high as 4500 mg (Reddy, 2002). In comparison with phytic acid results obtained from a study carried out by Febles *et al*. (2002), levels obtained in this study for whole meal flour were all below 6 μg g^{-1}, irrespective of site or variety grown and were below previously reported values. The results also indicated that site and varietal factors had significant influence on phytic acid content in wheat grains, but site was a predominant factor in influencing the phytic acid content. Similar results have been reported in studies done on barley and rice, which concluded environmental effects as the main contributors to phytic acid content (Liu *et al*., 2005b; Fei *et al.,* 2007). The effect of site, and especially the prevailing weather conditions such as temperature and rainfall have a great effect on phytic acid content. High temperatures and low rainfall increases the anti-nutrients such as phytic acid in cereals (Sondeep *et al*., 2012). This was observed in Naivasha site, which experiences lower rainfall and high temperatures than the other two sites. Mau-Narok, which experiences low temperatures and high rainfall recorded lowest phytic acid levels. The effects of temperature and rainfall influence phytic acid synthesis, which starts with a rate limiting step involving the enzyme *myo*-inositol-3-phosphate synthase (MIPS). MIPS is highly affected by temperature and *p*H, with its optimum temperature in plants being 35 ^{o}C, while the *p*H is around 7.0 to 7.5 (Chhetri *et al*., 2006). The high levels of

phytic acid content for varieties grown in Naivasha may be attributed to the high temperatures (25 to 29 °C) experienced in the area throughout the year. In addition, the levels of phytate in seed has also been associated with the amount of phosphorus concentration in the soil (Kim *et al*., 2002). Crops require phosphorus in large amounts and once internalised, it is redistributed to various plant cells; while some of the portion is stored in form of phytates.

In our experiment, each cropping site was treated with the same amount of fertiliser (DAP); however, soils conditions in Naivasha, especially *p*H may have favoured phosphate uptake by the varieties, compared to the other study sites. Mobility of phosphorus is enhanced at *p*H range of 6.0 to 7.0 since at this range, the inorganic phosphate (P*i*) is free compared to lower ranges observed in Eldoret and Mau-Narok which provides an environment for binding of inorganic phosphorous to metal ions making it unavailable for plant uptake (Rastija *et al*., 2014).

Resistant starch (RS), soluble glucose and starch concentration in cereals vary depending mostly on site and genotype. However, for RS, environmental variation component is difficult to predict and control compared to genetic variation (Birt *et al*., 2013). Genetic variation occurs within botanical sources due to allelic variation in the starch biosynthetic genes as is the case in commercial maize varieties, which exhibit little variation in resistant starch levels (Rohlfing *et al*., 2010; Pollack *et al*., 2011). This variation in RS content was observed in our study genotypes with some recording upto 5 folds more RS than others when grown in the same site (Table 4). Wheat varieties contain the same synthetic genes for RS, but some genotypes are able to over express them than in other varieties leading to enhanced RS levels.

Environmental effect, particularly rainfall and temperature affect RS levels by influencing enzymes involved in its synthesis (Keeling *et al*., 1994). Eldoret and Mau-Narok sites experience much higher rainfall and cooler temperatures, and this may have favoured enzyme activities involved in enhancing RS synthesis, leading to higher concentrations in kernel for wheat grown in these two sites. On the other hand, warm weather conditions and low rainfall generally experienced in Naivasha could have affected the enzyme activities leading to low RS levels in the kernels for crop grown in this site.

The starch content in wheat constitutes 65 to 75 % of the total grain dry weight and out of this portion; amylose contributes 20 to 30 %; while amylopectin contributes the remaining 70 to 80 %.The amylose portion is positively correlated with RS levels (Shrestha *et al.,* 2010). The amylose portion is slowly degraded by enzymes which have to remove the terminal glucosyl residues each at a time compared to the amylopectin portion which is easily degraded due to its branching. The results for starch observed in this study were below previously reported values by almost by half (Cornel, 2003). The low levels might have been contributed by size of grain and also presence of bran particles in the sample, which might have reduced percentage content of starch. The amount of soluble glucose was highly correlated with starch content with varieties having more starch also recording high levels of soluble glucose. In a study conducted by Maryke *et al*. (2007), variety × site interactions influenced starch levels and this was observed in the varieties K.Korongo and K.Hawk12 when grown in Eldoret site compared to the other two sites i.e. while a variety may contain high levels when grown in one location, the levels are not certain and may differ when the same variety is grown under different environmental conditions.

The clusters obtained revealed that the control Njoro BWII and Eagle 10 did not cluster with any of the other varieties. Njoro BWII and Eagle 10 are the oldest among the other test varieties used in the experiment, and the results obtained suggest their nutritional content based on Fe, Zn, RS, glucose and starch levels differ significantly from all the others. K.Sunbird and K.Kingbird formed their own distinct clusters and it could be as a result of growth conditions and maturity time (100 to 110 days).

In a study conducted on phytic acid in rice and pearl millet, varieties which matured early and were grown in areas which experienced high rainfall had less of the anti-nutrient due to inadequate time to concentrate it in the grain and phosphorous leaching to deeper levels that is inaccessible to plant roots (Pelig-Ba, 2009). In the present study, genotypes used are spring

varieties which do well in moderate temperatures. The varieties which clustered together have similar growth characteristics as described (Table 2) especially the maturity time and yields. This observation suggests that maturity duration for wheat varieties greatly influences the levels of the evaluated parameters.

CONCLUSION

Microelements Zn and Fe, anti-nutrient phytic acid and prebiotic resistant starch content in whole meal flour prepared from kernels of the 9 selected Kenyan bread wheat varieties varies with respect to genotype and experimental site. However, overall site analysis for the nutrients analysed indicate that Eldoret site had the optimal edaphic and environment factors, which would give the preferred nutrition values since all the parameters were within the desired range, i.e., high RS, high iron concentrations and low phytic acid levels. The only drawback for the crop grown in Eldoret site was relatively low levels of zinc micronutrient. Mau-Narok site recorded moderate nutritional values, and in comparison to the other two sites, Naivasha did not provide the desired nutritional quality except for Zn concentrations. Despite the influence of site, generally higher levels of Zn and phytic acid was obtained on Njoro BWII, whereas for Fe and resistant starch relatively higher levels were obtained on the varieties K. Korongo and K. Tai, respectively. In end, whole meal flour from all the 9 Kenyan bread wheat varieties contained significant levels zinc, iron, resistant starch and low levels of the undesirable phytic acid. Hence, the results obtained in this study suggest that consumption of products made using whole meal flour from the 9 varieties can provide sufficient Zn and Fe for human uptake, thus contribute in alleviation of micronutrient deficiency.

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REFERENCES

Abrar, H., Hans, L., Ramune, K. and Eva, J. 2010. Mineral composition of organically grown wheat genotypes: contribution to daily minerals intake. *International Journal of Environmental Research and Public Health* 7:3442-3456.

Alloway, B.J. 2004. Zinc in soil and crop nutrition. *International Zinc Association. Brussels, Belgium.* pp.130.

Bhupinder, S., Senthil, K.A.N., Singh B.K. and Usha, K. 2005. Improving zinc efficiency of cereals under zinc deficiency. *Journal of Current Science* 88(1):36-44.

Birt, D.F., Terri, B., Suzanne, H., Jay-Lin, J., James, H., Li, L., John, M., Samuel, M., Gregory, J.P., Matthew, R., Kevin, S., Paul, S. and Elizabeth, M. 2013. Resistant starch: Promise for improving human health. *Journal of Advances in Nutrition* 4:587-601.

Bohn, L., Meyer, A.S. and Rasmussen, S.K. 2008. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University Science* 9:165-191.

Chatzav, M., Peleg, Z., Ozturk, L., Yazici, A., Fahima, T., Cakmak, I. and Saranga, Y. 2010. Genetic diversity of grain nutrients in wild emmer wheat: potential for wheat improvement. *Annals of Botany* 105 (7):1211-1220.

Chhetri, D.R., Mukherjee, A.K. and Adhikari, J. 2006. Myo-inositol content in pteridophytes, the isolation and characterization of L-myo-inositol-1-phosphate synthase from *Diplopterygium glaucum. Brazillian Journal of Plant Physiology* 18(2):291-298.

Cornell, H. 2003. The chemistry and biochemistry of wheat: Bread making; improving quality. Woodhead Publishing Limited Cambridge, United Kingdom. 70pp.

Distelfeld, A., Cakmak, I., Peleg, Z., Ozturk, L., Yazici, A.M. Budak, H. 2007. Multiple QTL-

effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. *Journal of Plant Physiology* 129:635-643.

Dotaniya, M.L., Meena, H., Lata, M. and Kumar, K. 2013. Role of phytosiderophores in iron uptake by plants. *Journal of Agricultural Science Digest* 33(1):73-76.

Febles, C.I., Arias, A., Hardisson, A., Rodrýguez-Alvare, C. and Sierra, A. 2002. Phytic acid level in wheat flours. *Journal of Cereal Science* 36:19-23.

Fei,D., Junmei, W., Saihua, Z., Zhenzhen, X. and Guoping, Z. 2007. Genotypic and environmental variation in phytic acid content and its relation to protein content and malt quality in barley. *Journal of Food Chemistry* 105:606-611.

Genc, Y., Humphries, J.M., Lyons, G.H. and Graham, R.D. 2005. Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. *Journal of Trace Elements and Medical Biology* 18:319-324.

Gomez-Becerra, H.F., Erdem, H., Yazici A., Tutus, Y., Torun, B., Ozturk, L. and Cakmak, I. 2010a. Grain concentrations of protein and mineral nutrients in a large collection of spelt wheat grown under different environments. *Journal of Cereal Science* 52:342-349.

Gomez-Becerra, H.F., Yazici, A., Ozturk, L., Budak, H., Peleg, Z., Morgounov, A., Fahima, T., Saranga, Y. and Cakmak, I. 2010b. Genetic variation and environmental stability of grain mineral nutrient concentrations in *Triticum dicoccoides* under five environments. *Euphytica* 171:39-52.

Gordana, B., Vesna, D., Dejan, D., Miroslav, Z., Desimir, K., Sladana, Z., Srbislav, D. and Gordana, S. 2015. Genotype and Environment interaction for antioxidants and phytic acid contents in bread and durum wheat as influenced by climate. *Chilean Journal of Agricultural Research* 75(2):139-146.

Gyana, R. R. and Sunita, S. 2015. **Role of iron in plant growth and metabolism.** ***Journal of*** *Reviews in Agricultural Science* 3:1-24.

Katharina, E.S., Peter, A., Berit, M., Petra, W., Wolfram, T., Yahya, A., Claus-C, G. and Schrezenmeir, J. 2007. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *The Journal of Nutrition* 137: 838S-846S.

Keeling, P.L., Banisadr, R., Barone, L., Wasserman, B.P. and Singletary, G.W. 1994. Effect of temperature on enzymes in the pathway of starch biosynthesis in developing wheat and maize grain. *Australian Journal of Plant Physiology* 21:807-827.

Kim, J.C., Mulla, B.P., Selle, P.H. and Pluske, J.R. 2002. Levels of total phosphorus and phytate in three varieties of Western Australian wheat in response to growing region and growing season. *Australian Journal of Agricultural Research* 53:1361-1366.

Liu, Z.H., Cheng, F.M. and Zhang, G.P. 2005b. Grain phytic acid content in japonica rice as affected by cultivar and environment and its relation to protein content. *Journal of Food Chemistry* 89:49-52.

Loewus, F.A. 2002. Biosynthesis of phytate in food grains and seeds. In: Reddy, N.R., Sather, S.K Eds. *Food Phytate*. CRC Press, Boca Raton Florida, United States of America. pp. 53-61.

Marschner, P. 2012. Marschner's mineral nutrition of higher plants 3rd edition. Elsevier Publishers, Oxford, United Kingdom. pp. 672.

Maryke, T., Labuschagne, N.G. and Garry, O. 2007. The influence of environment on starch content and amylose to amylopectin ratio in wheat. *Journal of Starch* 59:234-238.

Mathieu, P., Celine, M., Kohki, Y. and Sebastien, T. 2014. Autophagy as a possible mechanism for micronutrient remobilisation from leaves to seeds. *Frontiers in Plant Science* 5(11):1-8.

Michael, J.K., June, Z., Maren, H., Christine, P., Holiday, A.D., Diana, B.C., and Roy, J.M. 2015. Role of resistant starch in improving gut health, adiposity and insulin resistance. *Journal of Advances in Nutrition* 6:198-205.

Mindy, P., and Maziarz, M.S. 2013. Role of Fructans and Resistant Starch in Diabetes Care. *Journal of Diabetes Spectrum* 26(1):35-39.

Ortiz-Monasterio, J.I., Palacios-Rojas, E., Meng, E., Pixley, K., Trethowan, R. and Pena, R.J. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant

breeding. *Journal of Cereal Science* 46:293-307.

Ozturk, L., Yazici, M.A., Yucel, C., Torun, A., Cekic, C., Bagci, A., Ozkan, H., Braun, H.J., Sayers, Z. and Cakmak, I. 2006. Concentration and localisation of zinc during seed development and germination in wheat. *Journal of plant physiology* 128:144-152.

Pelig-Ba, K.B. 2009. Assessment of phytic acid levels in some local cereal grains in two districts in the upper east region of Ghana. *Pakistan Journal of Nutrition* 8 (10):1540-1547.

Philippa, B., James, M.C., Janneke, B., Anthony, J.M., Dale, S. and Cristobal, U. 2014. Biofortification of wheat grain with iron and zinc: integrating novel genomic resources and knowledge from model crops. *Journal of Plant Science* 5:53doi: 10.3389/fpls.2014.00053. Accessed on 11-01-2016.

Poletti, S., Gruissem, W. and Sautter, C. 2004. The nutritional fortification of cereals. *Journal of Current Opinions in Biotechnology* 15:1-4.

Pollak, L.M., Scott, M.P. and Duvick, S.A. 2011. Resistant starch and starch thermal characteristics in exotic corn lines grown in temperate and tropical environments. *Journal of Cereal Chemists* 88:435-440.

Rastija, M., Jurica, J., Dario, I., Vlado, K. and Domagoj, R. 2014. Response of winter wheat to ameliorative phosphorus fertilisation. *49th Croatian and 9th International Symposium on Agriculture*, Dubrovnik Croatia. pp. 412-415.

Reddy, N.R. 2002. Occurrence, distribution, content and dietary intake of phytate. In: Reddy, N.R. and Sathe S.K. (Eds.). *Food phytates*. CRC Press, Boca Raton Florida, United States of America. pp. 25-51.

Rohlfing, K.A., Pollak, L.M. and White, P.J. 2010. Exotic corn lines with increased resistant starch and impact on starch thermal characteristics. *Journal of Cereal Chemists* 87:190-193.

Schrezenmeir, J. and de Vrese, M. 2001. Probiotics, prebiotics and synbiotics: Approaching a definition. *American Journal of Clinical Nutrition* 73: 2361S-2364S.

Shrestha, A.K., Lopez-Rubio, A., Blazek, J., Gilbert, E.P. and Gidley, M.J. 2010. Enzyme resistance and structural organisation in extruded high amylose maize starch. *Journal of Carbohydrate Polymers* 80:699-710.

Singh, S., Gupta, A.K. and Kaur, N. 2012. Influence of drought and sowing time on protein composition, anti-nutrients and mineral contents of wheat. *The Scientific World Journal Article* 485751. doi:10.1100/2012/485751. Accessed on 08-01-2016.

Sondeep, S., Anil, K.G. and Narinder, K. 2012. Influence of drought and sowing time on protein composition, anti-nutrients, and mineral contents of wheat. *The Scientific World Journal*, doi:10.1100/2012/485751. Accessed on 08-01-2016.

Sperotto, R.A., Ricachenevsky, F.K., Waldow, V.D. and Fett, J.P. 2012. Iron biofortification in rice: it's a long way to the top. *Journal of Plant Science* 190:24-39.

Steadman, K.J., Burgoon, M.S., Lewis, B.A., Edwardson, S.E. and Obendorf, R.L. 2001. Minerals, phytic acid, tannin and rutin in buckwheat seed milling fractions. *Journal of Science Food and Agriculture* 81:1094-1100.

Tomoko, N., Suyoen, K., Yusuke, K., Michiko, T., Hiromi, N. and Naoko, K.N. 2014. Enhanced levels of nicotineamine promote iron accumulation and tolerance to calcareous soil in soybean. *Journal of Bioscience, Biotechnology and Biochemistry* 78(10):1677-1684.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., and Dubcovsky, J. 2006. A NAC gene regulating senescence improves grain protein, zinc and iron content in wheat. *Journal of Science* 314:1298-1301.

Welch, R.M. and Graham, R.D. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* 55:353-364.