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Estimated Zinc Bioavailability in Milling Fractions of Biofortified Wheat Grains and in Flours of Different Extraction Rates

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Abstract

In present study, control and Zn-biofortified grains were milled to estimate Zn bioavailability in various grain milling fractions and flours of various extraction rates. In all tissues of wheat grains, soil Zn application increased Zn concentration and bioavailability, while decreased phytate concentration and [phytate]:[Zn] ratio. Compared with control grains, trivariate model of Zn absorption based estimated Zn bioavailability in various flour fractions of biofortified grains was greater by 50% or more. On average, bran had higher concentration of Zn and phytate as compared to whole grain and other milling fractions. Therefore, a large decrease was observed in concentration and estimated bioavailability of Zn from both control and biofortified wheat flour at lower flour extraction rates (80 and 65% extraction) when compared to flour of 100% extraction rate. Compared with four commercial wheat flours of similar flour extraction rate (80%), Zn bioavailability was significantly lower in flour from the control grains, while significantly greater in flour from the biofortified grains. Conclusively, only biofortified whole grain flour can ensure optimum Zn bioavailability (≈3 mg Zn per 300 g flour) for the human population groups reliant on wheat grains.

Keywords: Bioavailability; Biofortification; Grain milling; Wheat; Zinc

Introduction

Prevalence of Zn deficiency is estimated to be more than 20% globally; however, Zn deficiency is more common in developing countries where up to 80% the population is living under the risk of Zn deficiency (WHO, 2002; Bouis *et al.*, 2012). The human population with severe Zn deficiency is reliant on a diet based on cereal grains produced on Zn-deficient soils, for example in India, Pakistan, China, Iran and Turkey (Hotz and Brown, 2004; Alloway, 2008, 2009).

Wheat grains are major source of calorie and mineral intakes in Pakistan and many other countries of the world (FAO, 2012). Therefore, Zn biofortification of wheat grain by genetic and agronomic approaches is generally recommended to solve human Zn deficiency (Bouis and Welch, 2010; Bouis *et al.*, 2011). However, the largest part of grain (endosperm) has low Zn concentration, with the most of seed-Zn being located in the embryo and the aleurone layer (Ozturk *et al.*, 2006). Zinc fertilization may increase Zn concentration in grain, but it is mostly accumulated in aleurone layer that is removed during grain milling (Cakmak *et al.*, 2010; Stomph *et al.*, 2011).

Phytate, present in cereal grains in large quantities, binds with Zn and other metal cations to form insoluble complexes that hinder Zn absorption in the human intestine. Therefore, the [phytate]:[Zn] ratio has been generally employed to categorize the Zn bioavailability of food (Brown *et al.*, 2001). Recent advances in human nutrition allow quantitative estimation of Zn bioavailability in our daily diets by using mathematical models of Zn absorption (Bouis and Welch, 2010). A trivariate model of Zn absorption as a function of dietary Zn and phytate (Miller *et al.*, 2007) was successfully tested in a labeled Zn investigation of absorption in adult women (Rosado *et al.*, 2009). The model accounts for 80% of variability in the quantity of Zn absorbed (Hambidge *et al.*, 2010) and therefore can be employed for categorization of human diet for estimated Zn bioavailability.

In Pakistan and some other countries, wheat is either stone milled or roller milled. The flour obtained from stone milled grains is either consumed as a whole or sieved to remove larger particles of bran. However, bran and germ are variably removed from endosperm in commercial mills (roller mills). The different fractions obtained from different

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milling streams of roller mills are mixed in different proportions to have flour of various extraction rates. Mostly, the Zn-rich parts of wheat grains are removed during commercial grain milling, thus resulting in a marked reduction in flour Zn concentrations (Slavin *et al.,* 2000). The removed Zn-rich grain parts are also rich in phytate that also influences bioavailability of Zn to humans (Liang *et al.,* 2008; Peng *et al.,* 2010). Thus, selecting a suitable milling procedure to ensure good Zn bioavailability in flours seems a complex task.

Zinc biofortification through Zn application is generally suggested to increase grain Zn concentration (Rengel *et al.,* 1999; Cakmak, 2008) and Zn bioavailability (Hussain *et al.,* 2012a, b). Various studies have also reported differential localization of Zn and phytate in various grain parts (Ozturk *et al.,* 2006; Cakmak *et al.,* 2010) and their removal with various milling streams (Liang *et al.,* 2008; Peng *et al.,* 2010). However, losses of Zn, phytate and especially Zn bioavailability in flours under various millings of standard (control) and biofortified wheat grains are rarely reported. Therefore, the objectives of the study were: (i) to measure Zn concentration and estimated Zn bioavailability in different grain milling fractions of control and biofortified wheat grains; (ii) to measure Zn concentration and estimated Zn bioavailability in flours extracted at different rates from control and biofortified wheat grains; and (iii) to compare the commercially available flours with control and biofortified flour for Zn concentration and estimated Zn bioavailability to humans.

Materials and Methods

Control and Biofortified Grains

Control and biofortified wheat (cv. Shafaq-2006) grains for the study were produced in the field. Duplicates plots received basal application of 0 or 18 kg Zn ha^{-1} in the form of zinc sulphate. Wheat was sown (400 seeds m^{-2}) in six-row plots, 5 m long and 0.2 cm between rows.

Before sowing, randomized soil samples (0–15 cm depth) were used to determine soil physicochemical properties. Soil texture, a loam, was analyzed by hydrometer method (Gee and Bauder, 1986). The soil had a pH of 7.9 (measured in saturated soil paste by a Calomel glass electrode). Electric conductivity of saturated soil paste extract was 2.6 dS m^{-1}. Organic matter was 7 g kg^{-1} soil (determined according to Walkley-Black method, Nelson and Sommers, 1982). Free lime ($CaCO_3$), estimated by acid dissolution (Allison and Moodie, 1965), was 46 g kg^{-1} soil. Plant-available soil Zn, extracted by 0.005 M DTPA (Lindsay and Norvell, 1978), was 0.71 mg kg^{-1} [determined by an atomic absorption spectrophotometer (PerkinElmer, 100 AAnalyst, Waltham, USA)]. Basal uniform rates (in kg ha^{-1}) of 60 N, 90 P and 50 K were applied as urea, di-ammonium hydrogen phosphate and potassium sulphate. Another dose of 60 kg N ha^{-1} was applied 45 days after sowing. Plants were harvested at maturity and threshed to separate grains. Grains from duplicate plots were combined and stored at –20°C until processing.

Grain Milling Fractions and Flour Samples

Part (1): One kg of each control and biofortified grains, in triplicates batches, was milled in a Buhler Laboratory Mill (*Model MLV-202*, Switzerland) to have different milling fractions (reduction flour, break flour, bran and shorts).

Part (2): Different grain milling fractions were mixed to prepare flour of three different extraction rates (Vetrimani *et al.,* 2005): 100% (whole grain flour), 65% (straight-run flour comprising reduction flour and break flour) and 80% (prepared by proportionate mixing of shorts and bran with straight-run flour).

Part (3): Zinc concentration and bioavailability in control and biofortified wheat flours (obtained at 80% flour extraction rate) were compared with that in four different commercial wheat flours. The collected commercial wheat flours were also of 80% flour extraction rate and were collected from retail shops in triplicates.

Determination of Zn, Phytate and Zn Bioavailability

Grain and flour samples of wheat were dried in an air forced oven at 60°C for 48 h (Liu *et al.,* 2006). Dried samples were finely ground with a mill (*IKA Werke, MF 10 Basic*, Staufen, Germany) fitted with a stainless steel chamber and blades. Finely-ground 1.0 g samples of wheat flour were digested in a di-acid (HNO_3:$HClO_4$ ratio of 2:1) mixture (Jones and Case, 1990). The Zn concentration in the digest was estimated by atomic absorption spectrophotometry (PerkinElmer, 100 AAnalyst, Waltham, USA). For phytate determination, 60 mg finely-ground samples were extracted with 10 mL of 0.2 N HCl at room temperature for 2 h under continuous shaking. Phytate in the extract was determined by indirect method that uses absorption of pink color developed by un-reacted Fe (III) with 2,2′-bi-pyridine (Haug and Lantzsch, 1983) at 519 nm with a spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan). All samples for Zn and phytate determinations were prepared and analyzed in duplicates.

Molar concentrations of phytate and Zn in wheat grains were used to calculate [phytate]:[Zn] ratio. Zinc bioavailability was also quantitatively estimated by using trivariate model of Zn absorption (Miller *et al.,* 2007). The model is based on Zn homeostasis in human intestine and is given below:

$$\mathbf{TAZ} = 0.5 \cdot \left(A_{MAX} + TDZ + K_R \cdot \left(1 + \frac{TDP}{K_P}\right) - \sqrt{\left(A_{MAX} + TDZ + K_R \cdot \left(1 + \frac{TDP}{K_P}\right)\right)^2 - 4 \cdot A_{MAX} + TDZ} \right)$$

Where, A_{MAX}, maximum absorption; K_P, equilibrium dissociation constant of Zn-phytate binding reaction; K_R, equilibrium dissociation constant of Zn-receptor binding reaction; TAZ, total daily absorbed Zn (mg Zn d^{-1}); TDP, total daily dietary phytate (mmol phytate d^{-1}); TDZ, total

daily dietary Zn (mmol Zn d^{-1}). The model has independent (predictor) variables, TDZ and TDP, with TAZ being the dependent (response) variable. The parameters, A_{MAX}, K_R, and K_P, relate to Zn homeostasis in human intestine and have constant values of 0.091, 0.680 and 0.033, respectively (Hambidge *et al.*, 2010).

The Zn absorption in human intestine is a saturable process; therefore, trivariate model predicts TAZ not only on the concentrations of both Zn and phytate in daily diet, but also on their daily intake levels. The per capita consumption of wheat in Pakistan is about 300 g d^{-1} (FAO, 2012). Therefore, TAZ was estimated for 300 g of wheat flour and was termed *estimated Zn bioavailability*. However, estimated Zn bioavailability results are based on adults consuming 300 g wheat flour as a sole daily diet (Rosado *et al.*, 2009).

Statistical Analysis

The data obtained for Zn, phytate, [phytate]:[Zn] ratio and estimated Zn bioavailability were subjected to analysis of variance using *Statistix 9® for Windows* (*Analytical Software*, Tallahassee, USA). Significantly different treatment means were separated using least significant difference (LSD) test (Steel *et al.*, 1997). The level of significance (α) used was 0.05 ($P \leq 0.05$).

Results

Grain Milling Fractions and Zn Bioavailability

Main and interaction effects of grain type (control or biofortified grains) and milling fraction (reduction flour, break flour, bran and shorts) significantly ($P \leq 0.05$) influenced Zn concentration in various fractions of wheat grains (Table 1). Zinc concentration in various milling fractions ranged from 4 to 61 µg Zn g^{-1} for control grains and from 5 to 126 µg Zn g^{-1} for biofortified grains. On average, Zn concentration was 2-fold higher in biofortified than control grains. Zinc concentration in different milling fractions of both types of wheat grains ranked: bran > shorts > whole grain flour > break flour > reduction flour.

Compared to control grains, Zn concentration was 25 (reduction flour) and 33% (break flour) greater in milling fractions of biofortified grains (Table 1). However, this difference was non-significant at $P \leq 0.05$. Zinc concentration was 123% greater in shorts and 107% greater in bran of biofortified grains when compared with control grains.

There were significant ($P \leq 0.05$) main and interaction effects of grain type and milling fraction on phytate concentration in different grain milling fractions (Table 1). On average, phytate concentration in various grain fractions ranked: bran > shorts > whole grain flour > reduction flour = break flour. Compared to milling fractions of control grains, biofortified grains had significantly lower (19–28%) phytate concentration in various milling fractions.

Similar to Zn and phytate concentrations, [phytate]:[Zn] ratio was also significantly ($P \leq 0.05$) influenced by grain type and milling fraction (Table 1). Biofortified grains had significantly lower [phytate]:[Zn] ratio in various milling fractions as compared to control grains. The [phytate]:[Zn] ratio was highest in reduction flour (132) followed by break flour (92) of control grains. On the other hand, the [phytate]:[Zn] ratio was lowest in shorts (11) and break flour (14) of biofortified grains.

There were significant ($P \leq 0.05$) main and interaction effects of grain type and milling fraction on trivariate model of Zn absorption based estimated Zn bioavailability in various grain milling fractions (Table 1). Compared to various milling fractions of control grains, estimated Zn bioavailability was 50, 56, 69, 82 and 84% greater in, respectively, reduction flour, break flour, bran, shorts and whole-grain flour of biofortified grains. Maximum estimated Zn bioavailability (≥3.33 mg Zn per 300 g) was in bran and shorts of biofortified grains while minimum (0.44 mg Zn per 300 g) was in reduction flour of control grains.

Flour Extraction Rates and Zn Bioavailability

There were significant ($P \leq 0.05$) effects of grain type, flour extraction rate and their interaction on Zn and phytate concentrations in wheat flour (Table 2). Zinc concentration in flour of various extraction rates was significantly greater for biofortified than control grains. Zinc concentration in prepared wheat flours increased progressively with extraction rates and it was maximum (41 µg Zn g^{-1}) in the flour from biofortified wheat grains when extracted at 100% rate. Conversely, phytate concentration decreased progressively with flour extraction rates (minimum of 4.5 mg phytate g^{-1} at 65% extraction of biofortified grains). Moreover, phytate concentration was significantly greater in different flours of various extraction rates from control grains when compared to flours of respective extraction rates from biofortified grains.

There were also significant ($P \leq 0.05$) effects of grain type, flour extraction rate and their interaction on [phytate]:[Zn] ratio and estimated Zn bioavailability in prepared wheat flours (Table 2). The [phytate]:[Zn] ratio ranged from 18 (at 100% extraction of biofortified flour) to 120 (at 65% extraction of control flour). Estimated Zn availability was significantly increased with extraction rate of flour from both control and biofortified grains. Compared to various extraction rates of control grains, increase in estimated Zn bioavailability was 84, 85 and 52% in respectively, flour of 100, 80 and 65% extraction rate from biofortified grains.

Zinc Bioavailability in Commercial Flours

The tested flours (four commercial flours, one control flour and one biofortified flour; all extract at same rate of 80%) significantly ($P \leq 0.05$) differed in Zn concentration (Fi. 1a). Zinc concentration in biofortified flour (27 µg Zn g^{-1}) was

Table 1: Zinc, phytate, phytate-to-Zn molar ratio ([phytate]:[Zn]) and estimated Zn bioavailability in various milling fractions of control and biofortified wheat (cv. Shafaq-2006) grains

Fraction	Control (−Zn)	Biofortified (+Zn)	Control (−Zn)	Biofortified (+Zn)
	Zn (µg g^{-1})		Phytate (mg g^{-1})	
Whole grain	21.6 ± 1.0	40.8 ± 0.8	11.1 ± 0.3	8.4 ± 0.3
Reduction flour	3.9 ± 0.3	5.4 ± 0.3	5.8 ± 0.3	4.5 ± 0.2
Break flour	5.6 ± 0.3	7.7 ± 0.5	5.8 ± 0.4	4.2 ± 0.1
Bran	60.7 ± 2.0	126.1 ± 2.5	23.9 ± 0.2	19.4 ± 0.4
Shorts	31.2 ± 0.8	68.8 ± 2.1	11.5 ± 0.1	8.8 ± 0.3
$LSD_{(0.05)}$	2.3		0.5	
	[phytate]:[Zn] ratio		Estimated Zn bioavailability (mg per 300 g)	
Whole grain	47 ± 1	18 ± 1	1.41 ± 0.04	2.59 ± 0.03
Reduction flour	132 ± 4	75 ± 5	0.44 ± 0.03	0.66 ± 0.02
Break flour	92 ± 3	48 ± 4	0.61 ± 0.05	0.95 ± 0.03
Bran	35 ± 0	14 ± 1	1.97 ± 0.04	3.33 ± 0.08
Shorts	33 ± 0	11 ± 1	1.85 ± 0.02	3.36 ± 0.03
$LSD_{(0.05)}$	6		0.06	

Table 2: Zinc, phytate, [phytate]:[Zn] ratio and estimated Zn bioavailability in wheat (cv. Shafaq-2006) flours extracted at various rates from control and biofortified grains

Flour extraction (%)	Control (−Zn)	Biofortified (+Zn)	Control (−Zn)	Biofortified (+Zn)
	Zn (µg g^{-1})		Phytate (mg g^{-1})	
100	21.2 ± 1.0	40.8 ± 0.8	11.1 ± 0.3	8.4 ± 0.3
80	14.0 ± 0.3	26.9 ± 0.3	8.7 ± 0.2	6.8 ± 0.2
65	4.3 ± 0.1	5.9 ± 0.1	5.8 ± 0.3	4.5 ± 0.1
$LSD_{(0.05)}$	1.0		0.4	
	[phytate]:[Zn] ratio		Estimated Zn bioavailability (mg per 300 g)	
100	47 ± 1	18 ± 1	1.41 ± 0.03	2.59 ± 0.04
80	55 ± 1	23 ± 1	1.13 ± 0.01	2.13 ± 0.03
65	120 ± 6	67 ± 3	0.48 ± 0.02	0.73 ± 0.02
$LSD_{(0.05)}$	5		0.04	

Values are means of three replications ± standard deviations; LSD values are for the interaction effect; Control (−Zn) and biofortified (+Zn) wheat grains were produced by applying 0 or 18 kg Zn ha^{-1} during crop growth

significantly greater than in the tested commercial flours. Zinc concentration was minimum in control flour (14 µg Zn g^{-1}) while it ranged from 16 to 22 µg Zn g^{-1} in the tested commercial flours.

The tested commercial flours differed significantly in phytate concentration that ranged from 6.3 to 7.3 mg g^{-1} (Fig. 1a). Phytate concentration in the tested commercial flours was 17 to 28% lower than in control flours. The resultant [phytate]:[Zn] ratio in commercial wheat flours ranged from 29 to 37 (Fig. 1b). Compared to the tested commercial flours of similar extraction rate (80%), the control flour had significant greater and the biofortified flour had significantly lower [phytate]:[Zn] ratio.

Estimated Zn bioavailability in commercial flours ranged from 1.44 to 1.75 mg Zn in 300 g flour (Fig. 1b). The biofortified flour had significantly greater (2.13 mg Zn per 300 g flour) and the control flour had significantly lower Zn bioavailability (1.14 mg Zn in 300 g flour) than the commercial flours of similar flour extraction rates.

Discussion

Zinc in wheat grains is not equally distributed in various tissues; bran and germ have greater Zn concentration than starchy endosperm (Ozturk *et al.*, 2006; Tang *et al.*, 2008; Cakmak *et al.*, 2010). Therefore, the reduction flour, which comprises mainly of endosperm, had lower Zn concentration (Table 1). Zinc application to cereal crops significantly increased grain Zn concentration (Ahmad *et al.*, 2012; Ghaffar *et al.*, 2011; Hussain *et al.*, 2012*a*). However, most of the added Zn was deposited in the aleurone layer of wheat grain (Stomph *et al.*, 2011). In biofortified whole grains used in the study, Zn application to soil resulted in a greater increase in Zn concentration in bran and shorts when compared to reduction and break flours (Table 1). Therefore, maximizing bran in flour by adjustment of milling improves Zn concentration (Slavin *et al.*, 2000; Hemery *et al.*, 2007) and this could be particularly important for the flour obtained from biofortified grains.

The minerals, including Zn, are complexed with phytate in wheat grains (Weaver and Kannan, 2002). Therefore, the phytate-rich parts of grains e.g. bran, are also rich in Zn (Guttieri *et al.*, 2006). As optimum bioavailability of Zn to humans requires high Zn and low phytate concentrations in flour, selection of suitable grain milling for the better Zn nutrition of human population appears to be a complex task. The [phytate]:[Zn] ratio is considered a measure of Zn bioavailability to humans (Turnlund *et al.*, 1984). Compared with control grains, [phytate]:[Zn] ratio was significantly lower in milling fractions of biofortified grains. The trivariate model of Zn absorption based estimated Zn bioavailability in reduction flour and break flour was 3- to 5-fold lower than in shorts and bran (Table 1). Compared with other grain fractions, bran actually

possessed relatively greater Zn concentration; therefore, it had lowest [phytate]:[Zn] ratio and highest estimated Zn bioavailability.

The Zn- and phytate-rich parts of the wheat grains are separated during commercial milling. Wheat flours available in market usually have ≤80% flour extraction rate (Poutanen, 2012) and very little to no portion of total bran and germ is included in the final flour (Dewettinck *et al.*, 2008). Although both Zn and phytate concentration decreased at lower extraction rates, the [phytate]:[Zn] ratio increased progressively with decreasing flour extraction rate from 100 to 65% (Table 2). Therefore, 100% extraction (whole-grain flour) is most suitable for human consumption based on Zn concentration (Doblado-Maldonado *et al.*, 2012), [phytate]:[Zn] ratio and estimated Zn bioavailability (Table 2). These results confirmed the importance of whole grain consumption for improved Zn nutrition of human population.

The commercially available flours tested in this study had lower phytate concentration than control flour of similar flour extraction rate (Fig. 1a). Moreover, Zn concentration in these commercial wheat flours was lower than generally reported in wheat grains. This is due the fact that commercially available flours are composed of grain fractions obtained from various streams of grain milling and have high extraction of Zn- and phytate-rich parts of wheat grains (Liang *et al.*, 2008; Tang *et al.*, 2008; Peng *et al.*, 2010). Moreover, wheat grains processed for grain milling also have inherently low Zn concentration (Hussain *et al.*, 2012b, c). This is particulary true when soils are not supplied with Zn (Hussain *et al.*, 2013). Net intestinal absorption of about 3 mg Zn is required daily bases to ensure appropriate functioning of human organisms (Institute of Medicine, 2001; Hotz and Brown, 2004). However, the tested control and commercial flours of 80% extraction rate had lower Zn bioavailability than the required level. Biofortified flour of a similar flour extraction rate had significant greater Zn bioavailability than control and commercial flours of similar extraction rates. However, bioavailability from biofortified flour of 80% extraction rate was only 2.13 mg Zn per 300 wheat flour. Only biofortified flour of whole wheat grains (100% flour extraction rate) ensured optimum Zn bioavailability (≈3 mg Zn per 300 wheat flour) for a reference adult (Table 2). Therefore, the study characterized the importance of biofortified whole grains for enhanced Zn nutrition of humans.

In conclusion, as compared to other grain milling fractions, concentration of Zn and phytate was significantly greater in bran. Bioavailability of Zn in bran and shorts was also greater than in other grain milling fractions. There was a large decrease in concentration and bioavailability of Zn in lower flour extraction rates from both control and biofortified wheat grains. Zn bioavailability was greater in the biofortified compared to control flour and commercially available flours of similar flour extraction rates. Only biofortified whole grain flour can ensure optimum Zn bioavailability for the human population groups reliant on wheat grains.

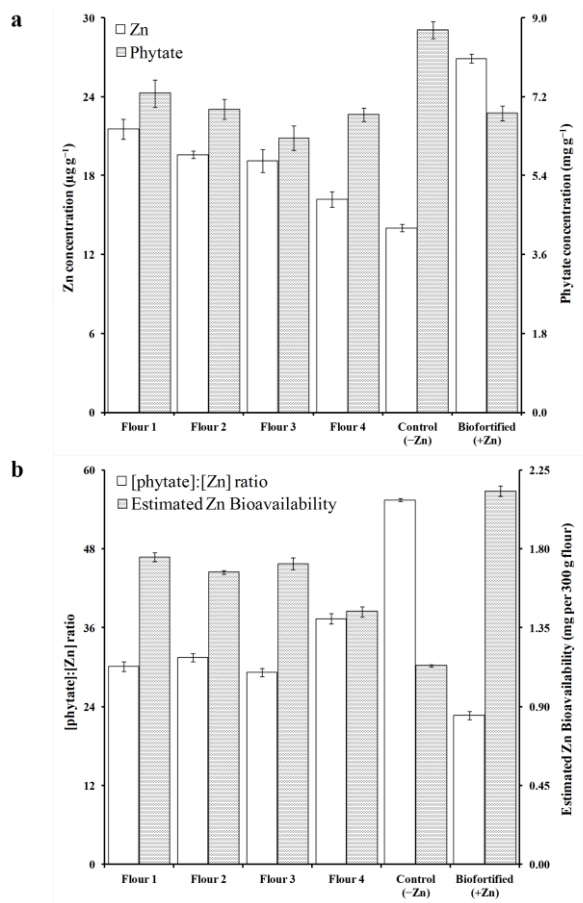

Fig. 1: (a) Zinc concentration, phytate concentrations, **(b)** [phytate]:[Zn] ratio and estimated Zn bioavailability in commercially available flours (Flour 1 to 4) in comparison with control and biofortified flours extracted at similar (80%) flour extraction rate

Control (−Zn) and biofortified (+Zn) wheat grains were produced by applying 0 or 18 kg Zn ha^{-1} during crop growth. Values are means of three replications ± standard deviations. LSD $_{(0.05)}$: 1.0 (Zn concentration); 0.41 (phytate concentration); 1 ([phytate]:[Zn] ratio), 0.04 (estimated Zn bioavailability)

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References

Ahmad, H.R., T. Aziz, S. Hussain, M. Akraam, M. Sabir, S.U.R. Khashif and M.M. Hanafi, 2012. Zinc-enriched farm yard manure improves grain yield and grain zinc concentration in rice grown on a saline-sodic soil. *Int. J. Agric. Biol.*, 14: 787–792

Alloway, B.J., 2008. *Zinc in Soils and Crop Nutrition*, 2nd edition. International Fertilizer Industry Association, Paris, France

Alloway, B.J., 2009. Soil factors associated with zinc deficiency in crops and humans. *Environ. Geochem. Health*, 31: 537–548

Bouis, H.E. and R.M. Welch, 2010. Biofortification: A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Sci.*, 50: S20–S32

Bouis, H.E., C. Hotz, B. McClafferty, J.V. Meenakshi and W.H. Pfeiffer, 2011. Biofortification: A new tool to reduce micronutrient malnutrition. *Food Nutr. Bull.*, 32: S31–S40

Bouis, H.E., E. Boy-Gallego and J.V. Meenakshi, 2012. Micronutrient malnutrition: Causes, prevalence, consequences, and interventions. *In*: *Fertilizing Crops to Improve Human Health: A Scientific Review, Volume 1: Food and Nutrition Security*, pp: 29–63. Bruulsema, T.W., P. Heffer, R.M. Welch, I. Cakmak and K. Moran (eds.). International Plant Nutrition Institute, Norcross, GA, USA; International Fertilizer Industry Association, Paris, France

Brown, K.H., S.E. Wuehler and J.M. Peerson, 2001. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food Nutr. Bull.*, 22: 113–125

Cakmak, I., 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil*, 302: 1–17

Cakmak, I., M. Kalayci, Y. Kaya, A.A. Torun, N. Aydin, Y. Wang, Z. Arisoy, H. Erdem, A. Yazici, O. Gokmen, L. Ozturk and W.J. Horst, 2010. Biofortification and localization of zinc in wheat grain. *J. Agric. Food Chem.*, 58: 9092–9102

Dewettinck, K., F. Van Bockstaele, B. Kühne, D. Van de Walle, T.M. Courtens and X. Gellynck, 2008. Nutritional value of bread: Influence of processing, food interaction and consumer perception. *J. Cereal Sci.*, 48: 243–257

Doblado-Maldonado, A.F., O.A. Pike, J.C. Sweley and D.J. Rose, 2012. Key issues and challenges in whole wheat flour milling and storage. *J. Cereal Sci.*, 56: 119–126

FAO, 2012. *Food Supply Database 2007 of Food and Agriculture Organization*. Online at http://faostat.fao.org/site/609/default.aspx#ancor on October 15, 2012

Ghaffar, A., E.N. Akbar and S.H. Khan, 2011. Influence of zinc and iron on yield and quality of sugarcane planted under various trench spacings. *Pak. J. Agric. Sci*., 48: 25–33

Gee, G.W. and J.W. Bauder, 1986. Particle-size analysis. *In*: *Methods of Soil Analysis. Part 1: Physical and Mineralogical Methods*, 2nd edition, pp: 383–411. Klute A. (ed.). American Society of Agronomy, Madison, USA

Guttieri, M.J., K.M. Peterson and E.J. Souza, 2006. Mineral distributions in milling fractions of low phytic acid wheat. *Crop Sci.*, 46: 2692–2698

Hambidge, K.M., L.V. Miller, J.E. Westcott, X. Sheng and N.F. Krebs, 2010. Zinc bioavailability and homeostasis. *Amer. J. Clin. Nutr.*, 91: 1478S–1483S

Haug, W. and H. Lantzsch, 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J. Sci. Food Agric.*, 34: 1423–1424

Hemery, Y., X. Rouau, V. Lullien-Pellerin, C. Barron and J. Abecassis, 2007. Dry processes to develop wheat fractions and products with enhanced nutritional quality. *J. Cereal Sci.*, 46: 327–347

Hotz, C. and K.H. Brown, 2004. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull.*, 25: S91–S204

Hussain, S., M.A. Maqsood and L.V. Miller, 2012b. Bioavailable zinc in grains of bread wheat varieties of Pakistan. *Cereal Res. Commun.*, 40: 62–73

Hussain, S., M.A. Maqsood, Z. Rengel and T. Aziz, 2012a. Biofortification and estimated human bioavailability of zinc in wheat grains as influenced by methods of zinc application. *Plant Soil*, 361: 279-290

Hussain, S., M.A. Maqsood, Z. Rengel and M.K. Khan, 2012c. Mineral bioavailability in grains of Pakistani bread wheat declines from old to current cultivars. *Euphytica*, 186: 153–163

Hussain, S., M.A. Maqsood, T. Aziz and S.M.A. Basra, 2013. Zinc bioavailability response curvature in wheat grains under incremental zinc applications. *Arch. Agron. Soil Sci.*, 59: 1001–1016

Institute of Medicine, 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, pp: 442–501. National Academy Press, Washington, USA

Jones, J.R.J. and V.W. Case, 1990. Sampling, handling, and analysing plant tissue samples. *In*: *Soil Testing and Plant Analysis*, pp: 389–428. Westerman R.L. (ed.). Soil Science Society of America, Madison

Liang, J., Z. Li, K. Tsuji, K. Nakano, M.J.R. Nout and R.J. Hamer, 2008. Milling characteristics and distribution of phytic acid and zinc in long-, medium- and short-grain rice. *J. Cereal Sci.*, 48: 83–91

Lindsay, W.L. and W.A. Norvell, 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Amer. J.*, 42: 421–428

Liu, Z.H., H.Y. Wang, X.E. Wang, G.P. Zhang, P.D. Chen and D.J. Liu, 2006. Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (*Triticum aestivum* L.). *J. Cereal Sci.*, 44: 212–219

Miller, L.V., N.F. Krebs and K.M. Hambidge, 2007. A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. *J. Nutr.*, 137: 135–141

Nelson, D.W. and L.E. Sommers, 1982. Total carbon, organic carbon and organic matter. *In*: *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*, pp: 570–571. Klute, A. (ed.). Soil Science Society of America, Madison, USA

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

Peng, W.U., Z.H.A.O. Tao and T.I.A.N. Ji-chun, 2010. Phytic acid contents of wheat flours from different mill streams. *Agric. Sci. Chin.*, 9: 1684–1688

Poutanen, K., 2012. Past and future of cereal grains as food for health. *Trends Food Sci. Technol.*, 25: 58–62

Rengel, Z., G.D. Batten and D.E. Crowley, 1999. Agronomic approaches for improving the micronutrient concentration in edible portions of field crops. *Field Crops Res.*, 60: 27–40

Rosado, J.L., K.M. Hambidge, L.V. Miller, O.P. Garcia, J. Westcott, K. Gonzalez, J. Conde, C. Hotz, W. Pfeiffer, I. Ortiz-Monasterio and N.F. Krebs, 2009. The quantity of zinc absorbed from wheat in adult women is enhanced by biofortification. *J. Nutr.*, 139: 1920–1925

Slavin, J.L., D. Jacobs and L. Marquart, 2000. Grain processing and nutrition. *Crit. Rev. Food Sci. Nutr.*, 40: 309–326

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Bio-Metrical Approach*, 3rd edition. McGraw Hill Book Co., Inc., New York, USA

Stomph, T.J., E.Y. Choi and J.C.R. Stangoulis, 2011. Temporal dynamics in wheat grain zinc distribution: Is sink limitation the key? *Ann. Bot.*, 107: 927–937

Tang, J., C. Zou, Z. He, R. Shi, I. Ortiz-Monasterio, Y. Qu and Y. Zhang, 2008. Mineral element distributions in milling fractions of Chinese wheats. *J. Cereal Sci.*, 48: 821–828

Turnlund, J.R., J.C. King, W.R. Keyes, B. Gong and M.C. Michel, 1984. A stable isotope study of zinc absorption in young men: Effects on phytate and α-cellulose. *Amer. J. Clin. Nutr.*, 40: 1071–1077

Vetrimani, R., M.L. Sudha and P.H. Rao, 2005. Effect of extraction rate of wheat flour on the quality of vermicelli. *Food Res. Int.*, 38: 411–416

Weaver, C.M. and S. Kannan, 2002. Phytate and mineral bioavailability. *In*: *Food Phytate*, pp: 211–223. Reddy, N.R. and S.K. Sathe (eds.). CRC Press, Boca Raton, USA

WHO, 2002. *World Health Report 2002: Reducing Risks, Promoting Healthy Life*. World Health Organization, Geneva, Switzerland

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