Kasahun, Adv Crop Sci Tech 2022, 10:10

Advances in Crop Science and Technology

ISSN: 2329-8863

Research Article Open Access

Minerals and Anti-Nutritional Factors of Recently Released Ethiopian Bread Wheat (*Triticum aestivum. L*) Varieties

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Abstract

The objective of this study was to investigate the minerals and anti-nutritional factors of recently released bread wheat varieties by the Wheat Improvement Program (WIP) of the Ethiopian Institute of Agricultural Research (EIAR). The Six recently released bread wheat cultivars were, Wane and Daka, Hidasse, Ogolcho, Kingbird, and Lemu, and Pavon earlier released (1982) used as standard check were grown at Kulumsa Agricultural Research Center under the same agronomic practice were evaluated. The standard procedures were used for minerals and anti-nutritional factors analysis using (AAS) Atomic absorption spectrometry and UV-Vis spectrophotometer and the analytical grade standard chemicals and reagents were used. The results minerals content in the wheat flour were significantly different at $p < 0.05$. In this study, the iron concentration in the wheat flour ranged between (0.30-1.94) mg/100gzinc concentration varied significantly among the varieties Kingbird, Lemu, (Ogolcho, Payon), Wane, Daka, Hidasse ($p<0.05$) and zinc content varied from (0.43 to 0.69) mg/100(Wane, Kingbird) respectively respectively. In the present study, tannin and phytate concentrations of wheat flour were below the detection limit (BDL). This might be due to the complete tannin and phytate reduction during the milling process. The nutritional composition of wheat depends on the processing method, therefore, process optimization (milling) should also be needed to get the required nutrients from wheat products.

Keywords: Wheat; Minerals; Anti-nutritional factors

Introduction

There are different species of wheat however; Triticum aestivum (bread wheat) and Triticum durum (macaroni wheat) is the most cultivated worldwide [1] Bread wheat (T. aestivum) is the most common cultivated crop taking a share of up to 95 % [2].Wheat is ideal raw materials for making bread, due to its absolute baking performance in comparison to all other cereals [3]. Among cereals, wheat is the only cereal with sufficient gluten content to make a typical loaf of bread without mixed with other grains [4].

Wheat is a rich source of carbohydrate, it also contains, protein, fat, ash, fiber, and vitamins as well as minerals such as sodium, potassium, calcium, magnesium, iron, phosphorus, copper, zinc, [5].Micronutrient malnutrition, "hidden hunger" affects about two billion people worldwide. It is responsible for escalated morbidity and mortality rates, reduced children's cognitive ability, diminished labor power productivity and higher chronic diseases prevalence. zinc deficiency causes impairment of physical growth, immune system and learning ability, increased risk of infections, DNA damage and cancer development. Meanwhile, iron deficiency results in tissue hypoxia and heart failure, maternal anemia, stunted and unhealthy babies (born from iron deficient mothers), children with poor attention spans, impaired fine motor skills, less capacity for memory and less work productivity. It is difficult to overemphasize the global importance of deficiencies of mineral micronutrients, principally of iron and zinc, in human diets. It has been estimated that globally 43% of children and 29% of women of reproductive age have anemia, and about half of these cases result from iron deficiency [6] Phytates (myo-inositol hexaphosphate) reduces bioavailability of minerals such as Calcium, Iron, Zinc and Magnesium. Most of the inorganic phosphorus (Pi) present in mature cereal seeds (40–80%) is stored as phytate. Micronutrient deficiencies (MNDs) remain widespread among people in sub-Saharan Africa [7].where access to sufficient food from plant and animal sources that is rich in micronutrients (vitamins and minerals) is limited due to socioeconomic and geographical reasons. Here we report the micronutrient composition (calcium, iron, selenium and zinc) of staple cereal grains for most of the cereal production areas in Ethiopia and Malawi [8] reported that some wheat bread sampled from Bahir Dar City did not meet the bread quality standards parameters especially in iron and zinc content therefore, bio fortification of these nutrients or blending the wheat flour with other iron, as well as zinc rich cereal flours are important to satisfy the individual's recommended daily need. Based on the above justification, the aim of this study was to investigate the minerals and anti-nutritional factors of recently released bread wheat varieties by the Wheat Improvement Program (WIP) of the Ethiopian Institute of Agricultural Research (EIAR).

Materials and Methods

Sample Collection and Preparation

Six recently released bread wheat cultivars (Wane and Daka, Hidasse, Lemu, Ogolcho, and Kingbird) and earlier released as standard check (Pavon) grown under uniform condition (in the 2019/20 main cropping season) obtained from Kulumsa Agricultural Research Center were evaluated under this study. The bread wheat samples were made ready for analysis after manually cleaning them by winnowing, sifting, and sorting with handing picking to remove stones, foreign materials (large chaff, dust, and soils) and broken kernels.

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Received: 01-Oct-2022, Manuscript No: acst-22-77330; **Editor assigned:** 06-Oct-2022, Pre-QC No: acst-22-77330 (PQ); **Reviewed:** 20-Oct-2022, QC No: acst-22-77330; **Revised:** 26-Oct-2022, Manuscript No: acst-22-77330 (R); **Published:** 31-Oct-2022, DOI: 10.4172/2329-8863.1000536

Citation: Kasahun C (2022) Minerals and Anti-Nutritional Factors of Recently Released Ethiopian Bread Wheat (*Triticum aestivum. L*) Varieties. Adv Crop Sci Tech 10: 536.

Milling Process

A standard wheat milling procedure was used for the flour extraction method with a standard laboratory milling instrument Chopin technology. The cleaned samples were conditioned to 16.5% of moisture level with distilled water in plastic containers based on the initial moisture content of the grain and left for 24 hours to facilitate tempering situation. The water was added in the blended container which contained 10 kg wheat grain and mixed well for 15 min by using mixers (Chopin Technology, Type: MR 10L, France) and stored in a plastic container and stored for 24 hrs. After tempering, the samples were milled using a laboratory mill (Chopin Moulin CD1 mill, Chopin technology, France). The separation process was done by a centrifugal sifting at 0.8mm sieve size, on each part of the mill. Then wheat flour obtained was stored at 4^{o}C after packing airtight polyethylene bags until further laboratory analysis.

Minerals Analyses

Iron and zinc contents were analyzed as described in [9] using (AAS) Atomic absorption spectrometry (Agilent, AAS 240, USA). The iron content was determined by adding 10 ml of concentrated HNO_3 to 1 g of flour sample and left overnight. The sample was carefully heated until the production of red nitrogen dioxide fumes cease. The sample was cooled and then 4 ml of 70% $HClO_4$ was added and evaporated to a smaller volume (7 ml) by carefully heating. The results solution was transferred into a 50 ml volumetric flask and makeup with distilled water. The solution sprays into the atomic absorption spectrophotometer at 248.3 nm to determine the concentration of iron. The intermediate iron standards were prepared to use with the concentration of 0 ppm, 1ppm, 2ppm, 3ppm, and 4ppm respectively.

The zinc content of wheat flour was determined by weighing 1 g of sample and treating with 7 ml of 6 N HCl to wet it completely. After the sample was ashed by dry ashing at 525^{0}C, then 15 ml of 3 N HCl was added and heated the dish on the hot plate until the solution just boils. The solution was then cooled and filtered into a graduated flask. The solution was sprayed into Atomic absorption spectrophotometer at 213.857 nm to determine the concentration of zinc. The zinc standards used were 0 ppm, 0.5 ppm, 1 ppm, 1.5 ppm, 2 ppm, and 2.5 ppm respectively. Using the atomic absorption spectrophotometer, the calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100g of the sample minerals.

Metal content (mg/100g) = (A-B)/10W*V

Where: W= Weight of sample in (g)

V = Volume of extract (ml)

A = Concentration of sample solution (µg/ml)

Phosphorus amount was determined according to the method by AOAC (2010), protocol 968.08 using UV-Vis Spectrophotometer (Agilent Technologies, Cary 60 UV-Vis, Malaysia)Phosphorus stock solution (50ppm) was prepared by dissolving 0.2197 g dried KH2PO4 into 1 liter distilled water. The standards of concentrations 1, 2, 3, 4, 5, and 6 mg/ml as phosphorus were used. Ammonium molybdate (23 g) and 1.25 g ammonium metavanadate were dissolved into 400 ml and hot 300 ml of distilled water in two beakers, respectively. Concentrated HNO_3 (250 ml) were added into the above-mixed solution and bring to 1 l with distilled water. Then, 5 ml of aliquot was taken from the sample digested by dry ashing into 100 ml volumetric flask, and 10ml of ammonium molybdate and metavanadate solution was added to the sample and standards and make up with distilled water. The resulting solution was shaken for uniform mixing and waited for 30 minutes to develop color. The absorbance of each sample and standard was determined with a UV-Vis Spectrophotometer at 460nm.

P (ppm) = (C*V1*V2*mcf)/(S*A) (equation 25)

Where: C = P concentration in sample digest read from the curve, ppm

V1 = Volume of the digest (100ml)

V2 = Volume of the dilution

S= Weight of the plant material calcinated in g.

A= Aliquot (5 ml)

mcf= moisture correction factor

Phytate and Tanin Analyses

Phytate content was estimated by undertaking phytic acid analysis using the Latta and Eskin method as modified by [10]. UV-Vis spectrophotometer, Labda, 9500) was used and the series standard solution was prepared to contain 0, 5, 9, 27, and 36 µg/g of phytic acid (sodium phytate) weighed 0.1814g in 100 ml of distilled water) in 0.2N HCl. Three ml of each standard was added into 15 ml of centrifuge tubes and 3 ml of 0.2 N HCl was used as a blank. Two ml of Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 15 seconds. The phytate concentration was calculated from the difference between the absorbance of the blank and that of the assayed sample.

About 1 g of dried sample was extracted with 10 ml 0.2 N HCl for 1 hour at ambient temperature and centrifuged at 3000 rpm for 30 min. The clear supernatant was used for phytate estimation. One ml of wade reagent was added to 3 ml of the supernatant sample solution and homogenizes and centrifuged at 3000 rpm for 10 min. The absorbance at 500 nm was measured using a UV-Vis spectrophotometer, Lambda 9500, Malaysia). The phytate concentration was calculated from the difference between the absorbance of the blank (3 ml of 0.2N HCl + 2 ml of wade reagent) and that of the assayed sample. The amount of phytic acid was calculated using a phytic acid standard curve and the result was expressed as phytic acid in µg/g fresh weight.

Phytic acid (µg/g) = ([(As-Ab)-intercept)])/(slope*W*3) *10 (equation 26)

Where; As=Absorbance of sample Ab= Absorbance of blank, W= weight of the sample.

Tannin content was determined by the modified vanillin with HCl assay [11] method. 0.2 grams of flour sample was weighed and then extracted with 10 ml of 1% HCl in methanol screw cap test tube and put on the mechanical shaker (Model: IKA AS130.1, USA) for 24 hours at room temperature. The mixture was centrifuged for 5 min at 3000 rpm and then 1 ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent. About 0.03 g of D-Catechin standard was weighed and dissolved in 100 ml of 1% HCl in methanol (99% concentration).

The standard stock solution 0.0, 0.2, 0.4, 0.6, 0.8, and 1 ml of D-Catechin was taken and adjusted the volume to 1 ml with 1% HCl in methanol and then 5 ml of vanillin - HCl analytical grade reagent was added. After 20 min to complete the reaction, the absorbance of the sample solution and the standard solution was measured at 500 nm by using UV-Vis Spectrophotometer, Labda, 9500). A standard curve was constructed (Absorbance vs Catechin) and the linear portion of the curve was extrapolated to produce the standard curve. The tannin content was calculated using Equation below;

Tannin (mg/g) =((As-Ab)-intercept))/(slope*d*3*W)*10

Where, As= Sample absorbance Ab = Blank absorbance d = Density of solution (0.791 g/ml), and W =Weight of sample.

Statistical Analysis

Statistical comparisons of the mean values were performed by analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS software (SPSS version 20.0 for Windows, SPSS Inc. Illinois, USA). All analyses were conducted in triplicate and the results were expressed as mean ± standard error and significant differences were defined at $p<0.05$.

Results and Discussion

In this study, three minerals iron, zinc, and phosphorus were determined in the seven newly released Ethiopian bread wheat varieties as shown in (Table 1). Because there was a strong genetic component to iron and zinc accumulation in the grain and bran has been shown to have a detrimental effect on the quality of bakery products. [12] reported that higher amounts of iron, phosphorus, zinc in wheat bran, and lower in wheat flour respectively, and deficiency of micronutrients, such as iron and zinc, are a critical and major problem. Also, wheat is one of the cereals which are classified as rich sources of phosphorous [13].

Accordingly, there was a significant difference ($p<0.05$) in iron concentration among the wheat varieties Hidasse, Daka, (Ogolcho, Kingbird), (Lemu, Payon), Wane. Hidasse variety had the highest iron concentration and variation among the varieties might be influenced by the iron concentration. In this study, the iron concentration in the wheat flour ranged between (0.30-1.94) mg/100g, while the previous study, reported in the whole wheat flour was (2.51-3.35) mg/100gm **[13].** Another study reported by indicated that iron concentration in refined wheat flour (1.3) mg/100g which is similar with the finding of present study. The bran and the germ of wheat grain are relatively rich in minerals and the milled products contain lesser content of minerals According to 80% of the total amounts of minerals are concentrated in the aleurone layer of the pericarp (bran), which was removed during the milling process while only 20% minerals are present in the endosperm. As a result of milling, minerals bioavailability, the palatability of the baked products will increase, but the nutritional value of the products will decrease [14].

In the present study, zinc concentration varied significantly among the varieties Kingbird, Lemu, (Ogolcho, Payon), Wane, Daka, Hidasse ($p<0.05$) (Table 3).in the current study, zinc content varied from (0.43 to 0.69) mg/100(Wane, Kingbird) respectively. In a previous study, the white wheat flour contained zinc content in the range of 0.58 to 1.39 mg/100g. As reported in the previous discussion iron part, 80% of the total amounts of minerals are concentrated in the (bran), which was removed during the milling process while only 20% minerals are present in the endosperm.

Table 1: Mineral composition of six released Ethiopian bread wheat varieties.

Varieties	Minerals (mg/100 g flour)		
	Iron	Zinc	Phosphorous
Wane	0.3 ± 0.06^e	0.43 ± 0.01^d	137 ± .0.6^c
Lemu	0.68 ±0.02^d	0.51 ± 0.02^b	172 ± 0.5^c
Ogolcho	0.85 ± 0.01^c	0.47 ± 0.01^c	208.5 ± 0.6^b
Hidase	1.93 ± 0.02^a	0.18 ± 0.01^f	279 ± 0.15^a
Daka	1.25 ± 0.02^b	0.28 ± 0.02^e	305.44 ± 0.7^a
Pavon	0.63 ± 0.01^d	0.47 ± 0.01^c	196.56 ± 0.6^{bc}
Kingbird	0.89 ± 0.04^c	0.69 ± 0.01^a	211 ± 0.42^b

Therefore, such issues would need further evaluation, and the optimum bran content needed for enhancement of nutritional quality of bread, so, it would be critical to compromise all the product qualities in terms of nutritional, minerals bioavailable, sensorial, and processing made from wheat varieties.

The Mineral Content of Bread Baked from Six Recently Released Ethiopian Bread Wheat Varieties

The mineral concentration of the bread baked from the newly released bread wheat varieties is reported in Table 2. Accordingly, the iron concentration was significantly different among all the bread types baked from the varieties Hidasse, Daka, Kingbird, Ogolcho, Lemu, Pavon, Wane, the amount being in the given order form highest to lowest content. Similarly, the bread baked from the wheat varieties Kingbird, (Lemu, Pavon), (Wane, Lemu), Daka, Hidasse had significantly different zinc concentrations ($p<0.05$). Bread baked from these wheat varieties (Daka, Hidasse, (Ogolcho, Kingbird), Pavon, Lemu, Wane had a significant phosphorus content difference ($p<0.05$) (Table 2).

Table 2: Mineral content of bread baked from six recently released Ethiopian wheat varieties.

Varieties	Parameters (mg/100g bread)		
	Iron)	Zinc	Phosphorous
Wane	0.31 ± 0.01^g	0.41 ± 0.01^c	116 ± 0.66^f
Lemu	0.61 ± 0.01^e	0.44 ± 0.04^{bc}	149.33 ± 0.32^e
Ogolcho	0.75 ± 0.01^d	0.45 ± 0.01^b	185.10 ± 0.15^c
Hidasse	1.83 ± 0.02^a	0.17 ± 0.01^e	233.3 ± 0.19^b
Daka	1.25 ± 0.02^b	0.27 ± 0.01^d	302.75 ± 0.24^a
Pavon	0.53 ± 0.01^f	0.45 ± 0.01^b	174.16 ± 0.33^d
Kingbird	0.85 ± 0.03^c	0.66 ± 0.02^a	185.5 ± 0.22^c

All values are expressed means ± SE (n=3). Values followed by different letters within a column indicate a significantly different based on Duncan's multiple range tests ($p< 0.05$).

Anti-Nutritional Factors

In the present study, tannin and phytate concentrations of wheat flour were below the detection limit (BDL).This might be due to the complete tannin and phytate reduction during the milling process. Also, the below detection limit of the anti-nutritional factors might be linked with the formation of insoluble complexes due to thermal degradation and denaturation during the bread baking process [15].

The bioavailability of minerals in wheat bran is under debate because of the presence of the anti-nutrient phytic acid. Phytic acid is a naturally occurring organic compound present in cereals, usually as myoinositol hexaphosphate. It is concentrated in the external covers in the pericarp and aleurone layer of the grain at lower levels, in the germ as well [16] 90% of the phytic acid in the grain is in the aleurone layer with 10% in the embryo [17].Consequently, the amount of phytic acid is greatly determined by the fractions removed during milling: white flour has almost no phytate. Phytate can bind minerals such as iron, calcium, and zinc, and there is some evidence showing decreased absorption of these minerals in the presence of phytate [18-21]. The results of the present study were similar to previous studies since refined wheat flour was used in this study.

Conclusion and Recommendation

The results obtained indicated that the presence of significant variations in the flour minerals content of the bread wheat varieties evaluated. But, lower mineral content in the studied varieties and the ant nutritional factors also removed from white flour.

The nutritional composition of wheat depends on the processing method, therefore, process optimization (milling) should also be needed to get the required nutrients from wheat products.

To get a clear cut for physicochemical and techno-functional properties and baking qualities of the potential genotypes, further researches should be conducted on these varieties at multi-locations, seasonal variation, and processing methods (milling) for better recommendations.

Acknowledgement

The authors gratefully acknowledge the Ethiopia Institute Agricultural Research for giving me full sponsorship for attending his MSc program. I would like to express my greatest appreciation goes to Addis Ababa University center for food science and nutrition and Kulumsa Agricultural research center wheat improvement program and Food Science and nutrition Research team.

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