



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, Ogocho, 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

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ere 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

Milling Procedure

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. The flour obtained was stored at 4°C in airtight polyethylene bags until further laboratory analysis.

Mineral Analysis

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 HNO₃ to 1 g of our 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% HClO₄ was added and evaporated to a smaller volume (7 ml) by carefully heating. The resulting solution was transferred into a 50 ml volumetric flask and made up with distilled water. The solution was sprayed 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°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.

$$\text{Metal content (mg/100g)} = (A-B)/10W*V$$

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 HNO₃ (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.

$$P \text{ (ppm)} = (C*V1*V2*mcf)/(S*A) \text{ (equation 25)}$$

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 Tannin Analysis

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.

$$\text{Phytic acid (µg/g)} = ((As-Ab)\text{-intercept})/(\text{slope}*W*3) *10 \text{ (equation 26)}$$

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 our 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;

$$\text{Tannin (mg/g)} = ((A_s - A_b) - \text{intercept}) / (\text{slope} * d * 3 * W) * 10$$

Where, A_s = Sample absorbance A_b = 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 \pm standard error and significant differences were defined at $p < 0.05$.

Result 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 phosphorus [13].

Accordingly, there was a significant difference ($p < 0.05$) in iron

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 anti-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.

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