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# Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties

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## Abstract

The changes in phytate, phytase activity and *in vitro* bioavailability of iron and zinc during soaking and germination of three white sorghum varieties (*Sorghum bicolor* L. Moench), named Dorado, Shandweel-6 and Giza-15 were investigated. Sorghum varieties were soaked for 20h and germinated for 72h after soaking for 20h to reduce phytate content and increase iron and zinc *in vitro* bioavailability. The results revealed that iron and zinc content was significantly reduced from 28.16 to 32.16% and 13.78 to 26.69% for soaking treatment and 38.43 to 39.18% and 21.80 to 31.27% for germination treatments, respectively. Phytate content was significantly reduced from 23.59 to 32.40% for soaking treatment and 24.92 to 35.27% for germination treatments, respectively. The *in vitro* bioavailability of iron and zinc were significantly improved as a result of soaking and germination treatments.

Keywords: Sorghum; Soaking; Germination; Phytate; Phytase activity; In *vitro* bioavailability of iron and zinc.

## Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a crop that is widely grown all over the world for food and feed. It is one of the main staples for the world's poorest and most insecure people in many parts of the developing world, especially in the drier and more marginal areas of the semi-tropics [1]. In these areas sorghum serves as the principal form of protein and energy for several hundred million people [2].

The nutrient composition of sorghum indicates that it is a good source of energy, proteins, carbohydrates, vitamins and minerals including the trace elements. Sorghum grain contains 1.3 to 3.3% of ash and minerals such as phosphorus, potassium and magnesium in varying quantifies. Sorghum and millets are important sources of some minerals, particularly iron and zinc, but all except finger millet is low in calcium [3].

Iron and zinc deficiencies are major public health threats worldwide. Iron (Fe) and zinc (Zn) are essential trace elements in human nutrition. Among the micronutrient malnutrition situations afflicting the human population, Fe and Zn deficiencies are of major concern not only because of the serious health consequences they may have, but also because of the number of people affected worldwide particularly in Africa [4].

Sorghum nutritional quality is dictated mainly by its chemical composition and the presence of anti-nutritional factors, such as phytic acid. Phytic acid and/or phytate is a principal storage form of phosphate, ubiquitously distributed in plants, particularly in cereal grains and in legumes. The effects of phytic acid in human and animal nutrition are related to the interaction of phytic acid with proteins, vitamins and it several minerals, and thereby restricts their bioavailability [5]. In view of the anti-nutritional effects of phytic acid, many attempts to reduce phytate have been made. Other attempts to reduce the phytate content such as fertilisation [6] and

activation of the indigenous enzyme phytase and/or addition of microbial phytase have been tried [7]. Because phytate is water soluble, a significant phytate reduction can be realised by discarding the soak water. Soaking usually forms an integral part of processing methods such as germination, fermentation, cooking and the toasting. Soaking media include water, salt or combination of salts and alkali [8]. In addition, action of endogenous phytases contributes to phytate reduction. Temperature and pH value have been shown to have a significant effect on enzymatic phytate hydrolysis during soaking. If the soaking step is carried out at temperatures between 45 and 65 °C and pH values between pH=5.0 and 6.0, which are close to the optimal conditions for phytate dephosphorylation by the intrinsic plant phytases, a significant percentage of phytate (26–100 %) was enzymatically hydrolysed [9].

Germination is a process widely used in legumes and cereals to increase their palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytate and protease inhibitors. In non-germinated legume grains and cereal seeds, with the exception of rye and to some extent wheat, triticale and barley, only little intrinsic phytate-degrading activity is found [10,11], but during germination a marked increase in phytate-degrading activity with a concomitant decline in phytate content was observed [12,13]. Long time of germination periods are needed to improve mineral bioavailability through germination. The objective of this study was to eliminate the anti-nutritional factors associated with sorghum grain and improve iron and zinc bioavailability by using simple methods.

## Materials and methods

### Materials

### Samples and chemicals

Pepsin, pancreatin, lipase and Cetylpyridinium bromide were purchased from Sigma– Aldrich Chemical Co. (St. Louis, USA) and bile extracts from Win Lab Laboratory chemicals reagents. All other chemicals used were of analytical reagent grade.

Three white sorghum varieties (*Sorghum bicolor* L. Moench), named were grown during the 2007 season were obtained from Crops Research Institute, Agricultural Research Center for Shandweel-6, and from Central Administration for Seed Certification (CASC), Ministry of Agriculture and Land Reclamation, Giza, Egypt for Dorado and Giza-15. The grains were carefully cleaned and freed from broken seeds and extraneous matter.

**Soaking of grains**

Sorghum seeds were soaked in distilled water for 20 hours with a ratio 1:5 w/v and the soaked water changed twice times. At the end of soaking period, the soaked water was discarded. The seeds were rinsed twice in distilled water and the grains were dried at 45±5ºC. The grains were milled in a Laboratory mill to obtain fine flour and kept at -20ºC until analysis.

**Germination of grains**

Soaked seeds were germinated for 72 hours at room temperature, and then the grains were dried. The root portions were manually removed. The grains were milled into fine flour and kept at -20ºC until analysis.

**Chemical analysis**

**Iron and zinc determination**

Total Iron and zinc content were determined according to the method outlined in A.O.A.C [14] by using the Perkin Elmer (Model 3300, USA) Atomic Absorption

Spectrophotometer. Approximately 2 g were weighed of sample after dry mineralization at 550 ºC. Then the ashes were digested with hydcloric acid 1M.

**Phosphorus and Phytate determination**

Total phosphorus (TP) was determined by the method of Trough and Mayer [15]. Phytate was extracted according to the procedure described by Mohammed *et al*., [16]. Sample (1.0 g) was extracted with tri-chloro acetic acid (3% w/v) at 37°C for 45 min. with simple shaking. The extracted phytate (0.2ml) was mixed with 4.6 ml of distilled water and 0.2ml of chromogenic solution and the tubes were heated in a water bath at 95°C for 30min, and then were allowed to cool. The developed color was read at 830 nm against blank. Standard phytate solution was prepared by dissolving sodium phytate in distilled water to prepare different phytate concentrations as described above in the tested samples. The amount of phytate in the tested samples was expressed as mg phytate/100g sample.

**Phytase activity assay**

**Extraction of phytase**

Phytase activity assayed according to the procedure described by Barrientos *et al*. [17] and modified by Jog *et al*. [18]. Sample (2 g) was added to ice cold Buffer (16ml of 10mM Tris–HCl, pH 7.0, containing reduced glutathione, 0.5mM). The suspension was stirred with a glass rod. Solid cetylpyridinium bromide (80mg, finalconcentration 0.5% w/v) was added to the suspension. The suspension was homogenized with homogenizer at 27,000 rpm for 2x1 min. with a 1min delay in-between. The resulting crude homogenate was centrifuged at 10,000*g* for 30min. The supernatant containing phytase activity was collected.

**Alkaline phytase assay**

Alkaline phytase activity was assayed by measuring the inorganic phosphate (Pi) released by the enzyme. The assay mixture contained Tris–HCl buffer (100mM, pH 8.0), NaCl (0.5M), $CaCl_2$ (1mM), sodium phytate (1mM), NaF (10mM), and an aliquot of enzyme solution in a total volume of 250µl. The assay mixture was incubated at 37 °C for 1h and the reaction was stopped by the addition of 50µl of 50% TCA. In brief, ammonium molybdate solution (700µl of a 1:6 solution of 10% w/v ascorbic acid and 0.42% ammonium molybdate (w/v) in 0.5M $H_2SO_4$) was added and the solution was incubated at 37°C for 1h. Absorbance at 820nm was measured and the inorganic phosphate concentration was determined from a calibration curve using $KH_2PO_4$ as the standard. One unit of enzyme is defined as the amount of enzyme that releases 1µmol of Pi from sodium phytate per minute under these conditions.

**Acid phytase assay**

Acid phytase activity was assayed in a solution containing sodium acetate buffer (100mM, pH 5.0), sodium phytate (1mM), and $CaCl_2$ (1mM). NaF was not added to this assay mixture. The assay mixture was incubated at 37 °C for 1 h and the reaction was stopped by the addition of 50µl of 50% TCA. Pi released in the reaction was quantitated as described above. Soluble protein was determined according to Lowry *et al* [19].

***In vitro* availability of iron and zinc**

The enzymatic degradation of the *in vitro* digestion method described by Kiers *et al*., [20] was used. Triplicate samples of sorghum whole meal (5g) were suspended in 30ml distilled water and digested under simulated gastro-intestinal conditions, subsequently using α-amylase solution, stomach medium consisting of lipase and pepsin, and pancreatic solution consisting of pancreatin and bile. After digestion, the suspension was centrifuged at 3600g for 15min. The supernatant was decanted and

the pellet was discarded. The supernatants were pooled and filtered through a 0.45mm pore filter. A blank was included consisting of 30 ml distilled water digested and filtered as described above. Both filtered supernatants from sample and blank were analyzed for Fe and Zn. Samples were corrected for added reagents/water by subtracting Fe and Zn content of blank from that of supernatants from samples. Iron and zinc content were by using the Perkin Elmer (Model 3300, USA) Atomic Absorption Spectrophotometer. The amounts of Fe and Zn (in supernatant were regarded as soluble minerals. Percentage of soluble mineral was calculated as: solubility ratio % [(Fe or Zn in supernatant—Fe or Zn in blank)/ (Fe or Zn in undigested sample)] x 100.

**Statistic analysis**

For the analytical data, mean values and standard deviation are reported. The data were analyzed using the one-way ANOVA model was used applying the LSD test to evaluate significant difference among means at $P<0.05$.

## Results and discussion

**Changes in iron and zinc during soaking and germination of whole grains**.

From Table 1, it could be noticed that the Fe content ranged between 5.54-7.65 mg/100g raw sorghum, while the Zn content ranged between 3.99-5.02 mg/100g raw sorghum, these finding are in agreement with the findings of Jambunathan [21] who reported that Fe content ranged between 2.6–9.6 mg/100g in samples of about 100 varieties of sorghum. The same result was observed by Kayodé [4] who reported that Fe concentration of the sorghum grains ranged from 3.0 to 11.3 mg/100g. The Zn concentration ranged from 1.1 to 4.4 mg/100g. In general, cereals high in phytate tend to have higher iron content. Low extraction (white) flour contains less phytate and iron, while high extraction (brown) flour has both more phytate and more iron.

After soaking, the Fe content of the sorghum was significantly lower than raw sorghum. The reduction after soaking was reduced by 28.16 to 40.06%, these finding are in contrast with the findings of Lestienne *et al*. [22] who reported that up to 40% of Fe content of sorghum grain may be lost as a result of soaking. As for germination, the Fe content of the sorghum was significantly reduced by 38.43 to 39.18%.

Lestienne *et al*. [22] found that the zinc content also decreased significantly, but the reduction did not exceed 30% except on Zn content of Shandweel-6. Reduction after soaking may be attributed to leaching of iron and zinc ions into the soaking medium [23]. The leaching of zinc was lower than iron and this phenomenon may be due to the fact that zinc and iron are not located in the same place in the seeds nor are they linked with the same molecules. Indeed, zinc is found in a large number of enzymes and other proteins, where it plays an important structural role.

**Changes in phytate content and phosphorus during soaking and germination of whole grains**

The content before and after treatments are shown in Table 2. Phytate content varied from 556.52 to 606.07 mg/100 g DW of raw sorghum. These values are close to those reviewed by Greiner and Konietzny [24] and Kayodé [4] whom found that sorghum phytate ranged from 590 to 1180 and from 400 to 3500 mg/100 g DW. A positive correlation existed between phytic acid and total phosphorus. Phytate is therefore a common constituent of plant-derived foods. Depending on the amount of plant derived foods in the diet and the grade of food processing, the daily intake of phytate can be as high as 4500 mg. On average, daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries and 150–1400 mg for mixed diets [25].

Weaning foods in developing countries are usually based on cereals, which contain phytate, a known inhibitor of iron and zinc absorption [26]. These phytate-containing foods may therefore be a strong contributing factor to poor iron and zinc status, which is commonly seen after 6 months of age, primarily in low-income countries but also in high-income countries [27]. In a study from Malawi, a high intake of phytate was correlated with poor iron and zinc status in preschool children [28].

After soaking and germination the phytate content was decreased (23.59 -32.4% and 24.92-35.27%). These findings are in range of the findings by Allen and Ahluwalia [29] and Mahgoub and Elhag [30] whom found that soaking, germination, mashing, boiling and fermentation strongly reduced the phytate content and is more effective if whole grains are used. The magnitude of reduction induced by soaking in this study can be explained by the leaching in soaking medium or by partial hydrolysis by endogenous phytase.

The reduction in phytic acid caused by soaking may be due to water solubilization of some phytic acid salts. Also, Towo *et al*. [31] found that phytate content in the sorghum flour was significantly ($P$<0.05) reduced in all processed samples, eg soaking and boiling and fermentation. Also, germination activates endogenous grain phytase which can degrade phytate [32, 33]. The grain-phytate correlated with the ash content of the grain, which could be expected as elements like phosphorus, iron and other minerals that account for the ash, are also part of the phytate and phytate-mineral complex structure [34]. Most seed InsP6 is deposited as mixed ‘phytin’ salts of mineral cations such as potassium, magnesium, iron and zinc2. During germination, phytins are broken down by endogenous phytase enzymes, releasing

their P, myo-inositol (hereafter referred to as ‘inositol’) and mineral contents for use by the growing seedling.

The same Table revealed that the values of total phosphorus of raw sorghum ranged from 334.46 to 381.37 mg/100 g DW. After soaking and germination the total phosphorus content was decreased (275.75 to 358.65 and 203.14 to 275.55 mg/100 g DW). Phytate phosporus ranged from 159.79 to 174.01 mg/100 g DW. These findings are in range of the findings by Radhakrishnan and Sivaprasad [35] and Godoy *et al*. [36].

**Effect of soaking and germination of whole grains on phytate iron and zinc molar ratios**

The phyt/Fe and phyt/Zn molar ratio**s** of raw sorghum (Fig.1) which associated with iron and zinc absorbtion capacity. It could be noticed that the phyt/Fe molar ratio**s** ranged from 6.66 to 8.68 for raw sorghum. While the phyt/Zn ratio ranged from 12.16 to 14.08 in raw sorghum. As after soaking, the phyt/Fe molar ratio increased (7.06 – 9.23) while the phyt/Zn molar ratio decreased (11.29-12.38). In fact there was an increase in Phy/Fe molar ratios after soaking, because of the decrease in the iron content. After soaking the Phy/Zn molar ratios decreased slightly in almost all sorghum varieties [22]. These data approved by Kayodé [4] who showed a phytate/Fe ratio lower than 14, which is the critical value above which Fe availability is strongly impaired. Our results reinforce previous results by Ferguson *et al*. [37] and Adeyeye *et al*. [38] whom showing that the bioavailability of zinc in cereals and legumes would be lower than that in vegetables and in some roots and tubers whose Phy/Zn molar ratios are generally less than 20. Kayode *et al*. [39] calculated the phytate/Fe and phytate/Zn molar ratios as an index for the potential mineral bioavailability. Also, sorghum phytate was hydrolyzed during germination, so that iron solubility under

simulated physiological conditions was greatly increased. It is somewhat difficult to predict the overall impact of soaking or germination on iron solubility. Soaking, or germination might be effective in reducing the phytate content of white sorghum, and showed more efficient if whole grains are used [40].

**Effect of soaking and germination of whole grains on phytases (acid and alkaline) activity**

The activity of phytases (acid and alkaline) before and after treatments is shown in Table 3. The data showed significant differences between activity of acid and alkaline phytase and non significant increase in acid and alkaline phytase activities after soaking and germination. These finding are in agreement with the findings of Marero *et al*. [41] who reported that phytase, enzyme degrades phytate, to improve mineral availability in plant foods. Phytate has been degraded in cereal foods by adding phytases or by activating endogenous phytase by a combination of soaking, germination and fermentation which is of a similar order of magnitude as observed by us. Also, humans have negligible intestinal phytase activity [42], even if they usually consume high phytate diets [43]. Cereals, however, contain an endogenous phytase. Because the endogenous cereal phytase has a pH optimum of 5.15, it is probably inactivated in the low pH of the stomach. Thus, there has been some interest in reducing the phytate content of cereals by soaking or germination (which activate endogenous phytase), or by adding a commercial phytase enzyme. Soaking under optimal conditions activates naturally occurring phytases in cereals and results in varying degrees of phytate hydrolysis depending on the kind of cereals [44].

Most plant grains and seeds exhibit phytate-degrading activity over a wide pH range (pH=3–10) [24] with maximal activity at pH values from pH=5–5.5 [45]. Compared to legumes, cereals_exhibit, in general, a significantly higher phytate-

degrading activity in the pH range from pH=5–5.5 [10, 11], whereas phytate-degrading activity at pH=8.0 was slightly lower in cereals compared to legumes [24]. Performing activity assays by incubation of flours of grains and seeds at pH=5.5 and defining 1 phytase unit (U) as equivalent to the enzymatic activity liberating 1 μmol of phosphate per minute, in cereal seeds from 0.10 to 7.0 U/g in [11]. To understand phytate hydrolysis it is important to recognize and account not only for phytase activity, but also for activities of further phosphatases present in the plant material. Per definition all enzymes capable of dephosphorylating phytate are classified as phytases. However, myo-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates, the products of phytase action on phytate, might be further dephosphorylated during food processing by phytases as well as phosphatases which do not accept phytate as a substrate. The same Table revealed that the specific activity (unite/mg protein). The data showed significant differences between activity of acid and alkaline phytase and non significant increase in acid and alkaline phytase activities after soaking and germination.

**Effect of soaking and germination of whole seeds on *in vitro* iron and zinc bioavailability**

*In vitro* iron and zinc bioavailability before and after soaking and germination are shown in Table 4. It could be noticed that the *In vitro* iron and zinc availability ranged between 8.02-13.60 and 7.35-9.73% of raw sorghum, respectively. While the *In vitro* iron and zinc bioavailability after soaking and germination increased (14.62-20.75 and 9.07-10.72 for soaking treatment and 16.67-20.63 and 12.06-18.30 for germination treatment), these finding are in agreement with the findings of Henriksen *et al*. [46] who reported that Food processing such as heat treatment, baking, fermentation, soaking, and milling may enhance or reduce iron availability. As well as phytase

enzymes will break down inositol hexa and penta phosphates, which inhibit iron absorption to smaller inositol phosphates and inorganic phosphate, which do not affect iron absorption. Hexa and penta phosphates remaining after treatments that involve phytase, such as germination or soaking has therefore been used as an indicator of phytase activity and is a good predictor of remaining phytate and its ability to impair iron absorption [47]. Soaking of wheat bran increased the soluble iron content from less than 5 percent to over 50 percent by destroy practically all their phytate and enhance *in vitro* iron availability [44]. Two common inhibitors of Fe absorption are tannins and phytate. These components form complexes with Fe within the intestinal lumen, reducing Fe bioavailability. Antinutritional factors chelate dietary mineral in the gastrointestinal tract reducing their bioaccessibility and bioavailability [48]. Processing techniques such as soaking, cooking, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing [49]. Iron bioavailability is low due to high levels of dietary phytates and fibers in vegetarians [50]. Vegetarian meals have a poor bioavailability of zinc, and these diets may or may not have low zinc content [51]. At low zinc intakes and with an absence of inhibitors, zinc absorption can be greater than 50% [52] Further, in Indian cooking processes, the main inhibitory factor of zinc bioavailability, phytate, gets partially degraded and may not remain as a strong inhibitor [53].

## Conclusion

Soaking and germination of sorghum whole grains is a traditional process for preparations of beverages and food which significantly reduce the amount of phytate and improve the *in vitro* bioavailability of iron and zinc. The effect was enhanced by increasing phytase activity during the soaking and germination process (at pH 5-8).

Phy/Fe and phy/Zn molar ratios could be used as indicator of the bioavailability of phytate as well as iron and zinc.

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**Figure**
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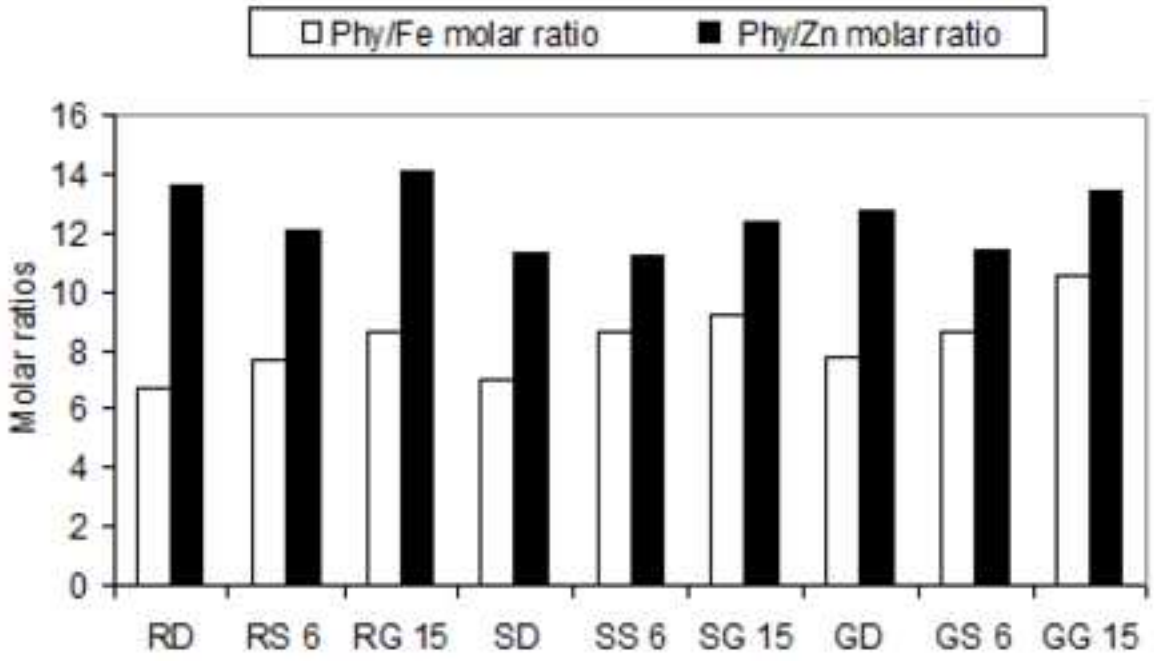


**Fig.1. Effect of soaking and germination of whole seeds on phytate iron and zinc molar ratios**

RD: Raw Dorado; RS 6: Raw Shandaweel-6; RG 16: Raw Giza-15; SD: Soaking Dorado; SS 6: Soaking Shandaweel-6; SG 15: Soaking Giza-15; GD: Germination Dorado; RS 6: Germination Shandaweel-6; GG 15: Germination Giza-15.

**Table**

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**Table 1. Changes in iron and zinc during soaking and germination of whole grains (mg/100 g DW)*.**

| Treatments | Fe | % Fe loss | Zn | % Zn loss |
|---|---|---|---|---|
| **Raw** | | | | |
| Dorado | 7.65±0.71$^{a}$ | - | 4.43±0.05$^{ab}$ | - |
| Shandaweel-6 | 6.84±0.32$^{ab}$ | - | 5.02±0.25$^{a}$ | - |
| Giza-15 | 5.54±1.82$^{bc}$ | - | 3.99±0.49$^{bc}$ | - |
| **Soaking** | | | | |
| Dorado | 5.19±0.08$^{cd}$ | 32.16 | 3.78±0.33$^{bcd}$ | 14.67 |
| Shandaweel-6 | 4.10±0.17$^{cde}$ | 40.06 | 3.68±0.48$^{bcd}$ | 26.69 |
| Giza -15 | 3.98±0.60$^{cde}$ | 28.16 | 3.44±0.02$^{cd}$ | 13.78 |
| **Germination** | | | | |
| Dorado | 4.71±0.40$^{cde}$ | 38.43 | 3.34±0.03$^{cd}$ | 24.60 |
| Shandaweel-6 | 4.16±0.87$^{cde}$ | 39.18 | 3.45±0.32$^{cd}$ | 31.27 |
| Giza-15 | 3.41±0.39$^{e}$ | 38.45 | 3.12±0.59$^{d}$ | 21.80 |
| **L.S.D** | 1.3281 | | 0.7412 | |

*Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at $p<0.05$.

**Table 2. Changes in phytate content, total phosphorus (TP) and phytate phosphorus (PP) during soaking and germination of whole grains*.**

| Treatments | Phytate content mg/100 g dw | % Phytate loss | Total phosphorus mg/100 g dw | Phytate phosphorus mg/100 g dw | Percentage PP/TP |
|---|---|---|---|---|---|
| **Raw** | | | | | |
| Dorado | 591.00±14.45$^{ab}$ | - | 376.09±12.33$^{a}$ | 169.69±4.14$^{ab}$ | 45.12 |
| Shandaweel-6 | 606.07±34.64$^{a}$ | - | 334.46±1.89$^{b}$ | 174.01±9.94$^{a}$ | 52.03 |
| Giza-15 | 556.52±15.83$^{b}$ | - | 381.37±23.02$^{a}$ | 159.79±4.54$^{b}$ | 41.90 |
| **Soaking** | | | | | |
| Dorado | 425.86±4.30$^{c}$ | 27.94 | 358.65±12.84$^{a}$ | 122.43±1.26$^{c}$ | 34.14 |
| Shandaweel-6 | 409.71±15.92$^{c}$ | 32.40 | 275.75±5.39$^{d}$ | 117.63±4.57$^{c}$ | 42.66 |
| Giza-15 | 425.26±13.83$^{c}$ | 23.59 | 300.73±20.42$^{c}$ | 122.10±3.97$^{c}$ | 40.60 |
| **Germination** | | | | | |
| Dorado | 421.21±13.85$^{c}$ | 28.73 | 235.50±18.62$^{e}$ | 120.94±3.98$^{c}$ | 51.35 |
| Shandaweel-6 | 392.31±33.83$^{c}$ | 35.27 | 203.14±4.43$^{f}$ | 112.64±9.71$^{c}$ | 55.45 |
| Giza-15 | 417.85±13.56$^{c}$ | 24.92 | 275.55±7.80$^{d}$ | 119.97±3.89$^{c}$ | 43.54 |
| **L.S.D** | 34.5136 | | 23.7418 | 9.9096 | |

*Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at $p<0.05$.

**Table 3. Effect of soaking and germination of whole grains on acid and alkaline phytase activity (unit / g DW)*.**

| Treatments | Acid Phytase activity | Alkaline Phytase activity | L.S.D | Acid Phytase activity unit /mg protein | Alkaline Phytase activity unit /mg protein | L.S.D |
|---|---|---|---|---|---|---|
| **Raw** | | | | | | |
| Dorado | 1.005±0.045[a] | 0.777±0.071 [b] | 0.1353 | 0.141±0.006[a] | 0.110±0.01[b] | 0.01914 |
| Shandawee1-6 | 1.016±0.005[a] | 0.781±0.006[b] | 0.0530 | 0.116±0.001[a] | 0.090±0.001[b] | 0.00183 |
| Giza-15 | 1.011±0.011[a] | 0.797±0.005[b] | 0.0733 | 0.125±0.001[a] | 0.10±0.001[b] | 0.0028 |
| **Soaking** | | | | | | |
| Dorado | 1.020±0.03[a] | 0.80±0.019[b] | 0.0120 | 0.124±0.002[a] | 0.096±0.002[b] | 0.0044 |
| Shandaweel-6 | 1.023±0.007[a] | 0.788±0.071[b] | 0.1136 | 0.113±0.001[a] | 0.088±0.007 [b] | 0.01097 |
| Giza-15 | 1.021±0.033[a] | 0.798±0.003[b] | 0.0141 | 0.126±0.004[a] | 0.098±0.004[b] | 0.0088 |
| **Germination** | | | | | | |
| Dorado | 1.040±0.05[a] | 0.825±0.005[b] | 0.0187 | 0.080±0.003[a] | 0.063±0.001[b] | 0.0054 |
| Shandaweel-6 | 1.020±0.006[a] | 0.784±0.007[b] | 0.0526 | 0.061±0.001[a] | 0.050±0.001[b] | 0.0024 |
| Giza-15 | 1.030±0.040[a] | 0.798±0.006[b] | 0.0646 | 0.072±0.001[a] | 0.055±0.001[b] | 0.0022 |

*****Values are mean of three replicates ±SD, number in the same column or raw followed by the same letter are not significantly different at $P<0.05$.

**Table 4. Effect of soaking and germination of whole seeds on *in vitro* iron and zinc bioavailability*.**

| Treatments | *In vitro* iron availability % | *In vitro* zinc availability % |
|---|---|---|
| **Raw** | | |
| Dorado | 9.07±0.92$^{cd}$ | 7.35±1.37$^{c}$ |
| Shandaweel-6 | 8.02±1.12$^{d}$ | 8.87±0.09$^{bc}$ |
| Giza-15 | 13.16±0.73$^{bc}$ | 9.73±2.87$^{bc}$ |
| **Soaking** | | |
| Dorado | 15.50±5.70$^{b}$ | 10.23±4.19$^{bc}$ |
| Shandaweel-6 | 14.62±0.94$^{b}$ | 9.07±0.52$^{bc}$ |
| Giza-15 | 20.75±1.20$^{a}$ | 10.72±1.11$^{bc}$ |
| **Germination** | | |
| Dorado | 17.38±0.37$^{ab}$ | 12.06±0.81$^{b}$ |
| Shandaweel-6 | 16.67±4.39$^{ab}$ | 18.30±1.07$^{a}$ |
| Giza-15 | 20.63±2.84$^{a}$ | 16.94±0.33$^{a}$ |
| **L.S.D** | 4.6263 | 3.1928 |

*****Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at $p<0.05$.