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Phytate reduction in sorghum grains cultivated in Saudi Arabia

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Abstract

Sorghum grains are the leading cereal crop in Saudi Arabia and act as the principal source of energy, protein, vitamins, minerals, and polyphenols. The flour is mixed with wheat flour to prepare bread. This study was carried out to investigate the effect of soaking sorghum grains on reducing their phytate content and increasing their mineral availability. The results showed that the soaking process in either acidic solution or deionized water reduces phytate contents in sorghum grains during soaking at 20 °C and 50 °C for 72 h. But, soaking at 50 °C reduced phytate more than soaking at 20 °C for all samples. Also, the soaking solution at pH 5 was more effective in reducing phytate than all of the others for both 20 °C and 50 °C. Some minerals, like Fe, P, Zn, and Ca, were determined as indicators of phytate reduction and bioavailability of these minerals in soaking media. The results showed that all minerals were reduced as a function of phytate reduction in soaking media. The highest losses of Fe, P, and Zn were 49.39, 80.0, and 68.55% when grains were soaked in pH 5 solutions at 50 °C for 72 h. While the highest loss of Ca was 58.22% for grains soaked in pH 3 solutions at 50 °C for 72 h. Although after soaking at pH 5 and 50 °C for 72 hours, the losses of soluble minerals are high, but mineral availability is acceptable and within the limits.

Keywords: ; Minerals; Bioavailability; Soaking, Mineral leaching, Anti-nutricional factor

Highlights

- Soaking is very important to reduce phytate, especially in acidic media
- A balance between phytate reduction and mineral losses was proposed
- Fe, Zn, Ca were released in the soaking media and became more available

1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the major crops grown for human consumption in arid and semi-arid regions of Africa and Asia, and it is the fifth most important cereal in world production. It is the king of cereals and is one of the important food crops in dry lands of tropical Africa, India, and China (Shobha et al., 2008). Furthermore, it was used for ethanol production in America and Australia (Patekar et al.,

2017). The nutrient content of sorghum is more or less limited to major entities like carbohydrates, protein, fats, fiber, and polyphenols. Factors such as polyphenols add promising antioxidant capacity to sorghum and, coupled with the fiber content of the cereal, indicate its potential as a functional food (Gopalan et al., 1996). Also, it has other health benefits, such as cholesterol-lowering, anti-inflammatory, slow digestibility, and inhibition of human esophageal and colon cancer cell growth (Awika et al., 2009 and Moraes et al., 2012). Sorghum provides a good basis for gluten-free products and was recommended as safe for celiac patients. Therefore, the future promise of sorghum in the developed world is for wheat substitution for people with celiac disease or allergies to gluten (Bogue & Sorenson, 2008). Minerals are inorganic elements widely distributed in nature and are essential for the growth and proper development of the human organism. The mineral deficiencies in diets may impair mental and physical development, decrease work output, and contribute to morbidity from infections, especially among children, pregnant and lactating women (Hussain et al., 2010; Ng'uni et al., 2011). Phytic acid is known as inositol hexakisphosphate (IP6) inositol polyphosphate, or phytate. Phytic acid has been termed an anti-nutrient due to its ability to bind minerals, proteins, and starches. The effect of phytic acid in human and animal nutrition is related to the interaction of phytic acid with protein, vitamins, and several minerals, thereby restricting their bio-extractability (Svanberg & Lorri, 1997). Phytic acid has a strong ability to chelate multivalent metal ions, especially Zn^{2+}, Mg^{2+}, Ca^{2+}, and $Fe^{2+,}$ to form phytate-mineral complexes, which are insoluble and therefore unavailable for absorption in the intestinal tract (Coulibaly et al., 2011). Sorghum, like other grains, contains non-heme iron, which has a very low bioavailability compared to that of heme iron in meat (Zimmermann et al., 2005). Also, sorghum is commonly consumed as a whole grain. While the bran of sorghum contains the most iron (Mahgoub & Elhag, 1998), it also contains phytate and sometimes tannins, depending on the cultivar, which further decreases the availability of non-heme iron (Hunt, 2003). However, the inhibiting effect of phytate occurs at very low phytate concentrations and to improve iron absorption, the phytate content needs to be degraded to a phytate: iron molar ratio < 1 (Hurrell et al., 2002), and for an improved zinc absorption the phytate: zinc molar ratio needs to be < 5 (Hunt, 2003). Various food processing and preparation techniques, such as decortications, soaking, cooking, germination, and fermentation, are the major efforts made to reduce the amounts of phytate in foods (El Maki et al., 2007; Kumar et al., 2010). Because phytate is water soluble, a significant phytate reduction can be realized by discarding the soaked water. Traditional processes such as soaking, germination, and fermentation can activate native grain phytase, which degrades inositol-6-phosphate to its lower forms (Liu et al., 2019). Soaking media include water, salt, or a combination of salts and alkali (Mulimani & Vadiraj, 1994). Temperature and pH values 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 and 6, which are close to the optimal conditions for phytate dephosphorylation by the intrinsic plant phytases, a significant percentage of phytate (26–100%) can be enzymatically hydrolyzed (Greiner & Konietzny, 1999). This study focused on reducing phytic acid content in whole sorghum grains by activating their natural phytase enzyme through soaking under varying pH levels, durations, and temperatures.

2 Materials and methods

2.1 Materials

Red color sorghum (*Sorghum bicolor* (L.) Moench) grains, Asiri variety, were obtained from the local market at Al Hassa region, Saudi Arabia, Season 2024. All chemicals used in this study were purchased from Alpha Chemicals Company and Sigma–Aldrich Company.

2.2 Methods

2.2.1 Physical characteristics of sorghum grains

The sorghum grains were subjected to physical analysis of geometric mean diameter, thousand kernel weight, thousand kernel volumes, bulk density, and water absorption capacity. It conforms to the quality of sorghum grain. The size of the seed was measured using calipers to the nearest 0.01 mm (Patekar et al., 2017).

The dimensions of sorghum grains were measured as length, thickness, and width. The following equation (Equation 1) was used to determine the Geometric Mean Diameter of grains (Gursoy & Guzel, 2010).

$$Geometric\ Mean\ Diameter\ =\ (Length\ X\ Width\ X\ Thickness) \quad (1)$$

Neat, clean, and sorted 1000 grains weights were measured by an electronic balance, and the average weight was calculated.

The volume of a thousand grams of dry seed was measured by water displacement in milliliters.

The bulk density (BD) of grains was measured by the standard method given by the Association of Official Analytical Chemists (2000).

Water absorption capacity (WAC) is defined as the maximum amount of water that 1 g of material will imbibe and retain under low-speed centrifugation. To estimate water absorption capacity, a five-gram sample was weighed in a 50 ml centrifuge tube, 30 ml of water was added and stirred with a glass rod for 5 min, then allowed to stand for 30 min at ambient conditions, then centrifuged at 4500 rpm for 25 min. The volume of free liquid was measured, and the retained volume was expressed as ml of water absorbed per gram of sample on a dry basis (Oladiran & Emmambux, 2018) (Equation 2).

$$\text{Water absorption capacity } (\%) = \frac{\mathrm{m1}}{\mathrm{m0}} \times 100 \quad (2)$$

Where: m0—the weight of the sample taken into analysis, m1—the weight of the sample after supernatant removal.

2.2.2 Sample preparation

The grains were washed with deionized water to remove surface contaminants, and the grains were air dried at 20 °C overnight. The sample was then milled using a lab hammer mill (Braun, Germany) and then passed through a 60 mesh sieve screen. The powder was stored in a tight container at 5 °C in the dark before further analyses.

2.2.3 Gross chemical composition

Moisture, crude protein, ether extract, ash, and crude fiber contents were determined according to AOAC (Association of Official Analytical Chemists, 2000). Available carbohydrates were calculated by difference.

2.2.4 Soaking process

Control sorghum grains were soaked in deionized water at a grain: liquid ratio of 1:10 (w/w) at room temperature (20 °C) on a mechanical shaker (GFL, 1083, Germany), shaking at a rate of 100 rpm for 12, 24, 36, and 48 h. The soaking water was removed and changed at the end period. While other treated samples were soaked in deionized water at pH 3, 4, and 5 acidified with 1M HCl at room temperature (20 °C) and 50 °C. Soaking water was removed at the end of the soaking period, as in the previous conditions. After soaking, the supernatant was strained off using a cheese cloth. The grains were then dried, milled, and stored as previously described. The soaking process of treated samples was repeated at 50 °C. Phytate and mineral content were determined before and after the soaking process.

2.2.5 Phytate content

Phytate content before and after the soaking process was colorimetrically determined according to the methods outlined by Haug & Lantzsch (1983) and Bhinder et al. (2021). Briefly, 0.5 g of sorghum grain flour was stirred in a 0.2 N solution in 25 mL of HCl for three hours and then filtered. A ferric solution was prepared by dissolving 0.2 g of ammonium iron (III) sulfate in 100 mL of 2N HCl and diluting it to 1000 mL with distilled water. A 2, 20bipyridine solution was prepared by dissolving 10 g of 2, 20bipyridine and 10 mL of thioglycolic acid in 1000 mL of distilled water. A sample extract (0.5 mL) and 1 mL of a ferric solution

were mixed in a boiling water bath for 30 min. A 2,20-bipyridine solution (2 mL) was added after the solution had cooled down, and the absorbance was measured at 519 nm by using an Ultraviolet-visible (UV-Vis) spectrophotometer (Cary 60 Instrument, Agilent, Santa Clara, CA, USA) following a reaction time of 1 min. Samples were also analyzed in triplicate.

2.2.6 Mineral content

Mineral content was determined according to the methods outlined in the Association of Official Analytical Chemists (2000). About 0.5 g sample was supplemented with 30 ml nitric + perchloric acid mixture and subjected to wet digestion until a final volume of 5 ml. Digested samples were cooled and diluted to a 50 mL volumetric flask with deionized water. Minerals were determined by atomic absorption spectrophotometry (Shimadzu AA-7000, Japan). Phosphorus was determined according to the method of Pulliainen & Wallin (1996), the samples were dry ashed with zinc oxide, and the content of phosphorus was then measured colorimetrically as molybdenum blue.

2.2.7 Molar ratios and bioavailability of minerals

The molar ratio of phytate to minerals (Fe, Zn, and Ca) was calculated by dividing the mole of phytate (mass of phytate/660 g/mol) to the mole of minerals (mass of Fe = 56 g/mol; mass of Zn = 65 g/mol; mass of Ca = 40 g/mol) (Food and Agriculture Organization, 2018).

2.2.8 Statistical analysis

The obtained data were statistically analyzed using the General Linear Models Procedure Adapted by Statistical Package for the Social Sciences (Statistical Package for the Social Sciences, 1997) as a user's Guide Duncan Multiple Range Test was used to test the difference among means (Duncan, 1995).

3 Results and discussion

3.1 Physical properties of sorghum grains

Some physical properties of red color sorghum grains, Asiri variety (Figure 1), were tabulated in Table 1. The mean diameter of grains was 4.19 × 2.8 × 2.26 mm, the weight of 1000 grains was 20.1 g, while the volume of 1000 grains recorded 24.13 ml. So, bulk density was 0.71 g/ml. Rooney (2003) found that the milling quality of sorghum is determined by the kernel shape, density, hardness, and structure. Also, Brooker et al. (1992) reported that bulk density is used to determine the capacity of storage and transport. Thus, % of water absorption capacity of sorghum grains was 32.25%, which may be due to the high protein content in the grains, which is both hydrophilic and hydrophobic and therefore can interact with water (Iwe, 2000).

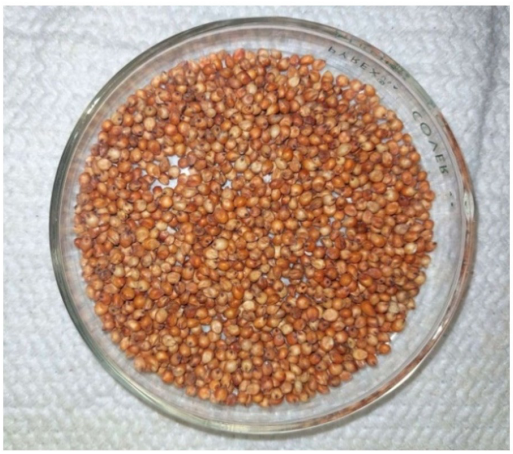

Figure 1. Red color sorghum grains Asiri variety.

Table 1. Some physical properties of sorghum grains.

Sample	Geometric Mean Diameter (mm)	1000 grains weight (g)	1000 grains volume (ml)	Bulk density (BD) g/ml	%Water absorption capacity (WAC)
Sorghum grains	4.19 × 2.8 × 2.26	20.10	24.13	0.71	32.25

3.2 Gross chemical composition

Data in Table 2 shows the chemical composition of sorghum grains from the Al Hassa region. Data shows that the moisture content was 10.95%, so dry matter was 89.05%. Protein, ether extract, ash, and crude fibers recorded 11.97, 4.81, 2.13, and 1.65%, respectively. Our results are in agreement with Abdel Rahman & Osman (2010) who studied the chemical composition of three Saudi sorghum Cultivars named Hamra, Baida, and Shahla, and they found that crude protein ranged between 11.94% and 13.28%, ether extract from 3.84% to 4.03%, ash from 1.98 to 2.01% and total carbohydrate from 80.76 to 82.16%. Besides, El Hag et al. (2015) reported that the chemical composition of three Sudanese sorghum grain cultivars was determined as follows: dry matter was from 91.4% to 91.53%, crude protein from 14.32% to 19.37%, ether extract from 3.74 to 4.16%, ash from 1.71 to 5.15% and crude fiber from 2.07 to 3.39%. Also, these results are close to those reported by Ahmed et al. (2017), who found that the dry matter of Sudanese sorghum grains was 93.83%, crude protein was 14.3%, ether extract was 4.58%, and crude fiber was 2.69%. In addition, Mohapatra et al. (2017) found that sorghum grains contained 12.1% protein, 1.87% ash, 3.32% ether extract, and 67.7% carbohydrate. Eltayeb et al. (2017) reported that the chemical composition of Sudanese sorghum was as follows: dry matter was 89.66%, crude protein 10.24%, ether extract 3.34%, ash 1.6%, crude fiber 2.39%, and carbohydrate 72.09%.

Table 2. Gross chemical composition of sorghum grains (on a dry weight basis).

Component	% of dry weight basis
Moisture	10.95 ± 0.01
Dry matter	89.05 ± 0.11
Crude protein	11.97 ± 0.21
Ether extract	4.81 ± 0.12
Ash	2.13 ± 0.02
Crude fibers	1.65 ± 0.03
Available carbohydrate	79.44 ± 0.12

Values are means ± SD, Available carbohydrate was calculated by difference.

3.3 Phytate contents of sorghum grains as affected by soaking conditions

Data in Table 3 shows phytate contents in sorghum grains as affected by different soaking pH (3, 4, and 5) at two temperatures (20 °C and 50 °C) for 72 h. The results showed that phytate contents of all soaked samples decreased as a function of soaking conditions at different temperatures. Furthermore, samples soaked at pH 3, 4, and 5 had phytate levels lower than those soaked in deionized water. Soaking solution at pH 5 led to a decrease in phytate more than the others. It decreased from 425.6 mg/100 g for the sample without soaking to 310.0 mg/100 g for the sample soaked to 72 hrs at 20 °C, while phytate in the sample soaked in deionized water slowly decreased from 425.6 to 350.1 mg/100 g as a result of an increase in soaking time from zero time to 72 h. Our results for phytate content (425.6 mg/100 g) are lower than those reported by Kruger et al. (2013), who found the phytate contents of whole grain sorghum cultivars varied between 790 ± 0.5 and 1520 ± 2.6 mg/100 g.

Table 3. Phytate (mg/100 g) content as affected by soaking time and pH values at different temperatures.

Temp.	Control (without soaking)	phytate content (mg/100g dry basis)							
		20 ºC				50 ºC			
Time(h)	0	12	24	48	72	12	24	48	72
Soaking in water	$^{N.S}425.6^{a} \pm 0.21$	$^{a}388.3^{b} \pm 0.23$	$^{a}378.6^{c} \pm 0.09$	$^{a}357.4^{d} \pm 0.13$	$^{a}350.1^{e} \pm 0.22$	$^{a}358.3^{d} \pm 0.16$	$^{a}334.9^{f} \pm 0.17$	$^{a}314.3^{g} \pm 0.13$	$^{a}311.1^{g} \pm 0.16$
pH 3	$425.6^{a} \pm 0.21$	$^{b}381.5^{b} \pm 0.3$	$^{b}355.4^{c} \pm 0.11$	$^{a}354.9^{c} \pm 0.13$	$^{b}346.9^{d} \pm 0.23$	$^{b}335.3^{e} \pm 0.21$	$^{a}330.1^{f} \pm 0.16$	$^{b}294.5^{g} \pm 0.09$	$^{b}260.2^{h} \pm 0.15$
pH 4	$425.6^{a} \pm 0.21$	$^{b}383.2^{b} \pm 0.11$	$^{b}351.6^{c} \pm 0.21$	$^{b}330.1^{d} \pm 0.15$	$^{c}323.2^{e} \pm 0.17$	$^{b}330.7^{d} \pm 0.09$	$^{b}312.9^{f} \pm 0.12$	$^{c}275.1^{g} \pm 0.11$	$^{c}198.4^{h} \pm 0.14$
pH 5	$425.6^{a} \pm 0.21$	$^{c}371.3^{b} \pm 0.31$	$^{b}357.4^{c} \pm 0.08$	$^{c}321.7^{d} \pm 0.22$	$^{d}310.0^{e} \pm 0.18$	$^{c}323.6^{d} \pm 0.08$	$^{c}271.5^{f} \pm 0.11$	$^{d}225.4^{g} \pm 0.13$	$^{d}177.1^{h} \pm 0.09$

Means of soaking period having the same right case letter(s) within a column are not significantly different ($p < 0.05$). Means of soaking pH having the same left case letter(s) within a row are not significantly different ($p < 0.05$). N.S = not significant.

Means of soaking time values having the same case letter(s) within a column (right) are not significantly different ($p \leq 0.05$). Means of treatment values having the same case letter(s) within a row (left) are not significantly different ($p \leq 0.05$) as assessed by Duncan's multiple–range test.

Table 4. Percentage of phytate loss as affected by soaking time and pH values at different temperatures.

Time (hrs) Treatment	% of phytate loss							
	20 °C				50 °C			
	12	24	48	72	12	24	48	72
Soaking in water	8.75	11.05	16.03	17.74	15.81	21.31	26.15	26.9
pH 3	10.36	16.5	16.61	18.49	21.22	22.44	30.8	38.86
pH 4	9.96	17.39	22.44	24.06	22.3	26.48	35.36	53.38
pH 5	12.76	16.03	24.41	27.16	23.97	36.2	47.04	58.39

On the other hand, the increase of soaking temperature to 50 °C caused to decrease in phytate of all soaked samples compared to soaking at 20 °C. Also, grains that were soaked in a pH 5 solution had the lowest phytate compared to other soaked samples. It decreased from 425.6 mg/100 g for the sample without soaking to 177.1 mg/100 g after soaking for 72 hrs at 50 °C. It may be due to that used pH and temperature activate phytase that naturally occurs in grains and help to phytate hydrolysis. Greiner & Konietzny (1999) found that when the soaking step is carried out at temperatures between 45 and 65 °C and pH values between 5 and 6, which are close to the optimal conditions for phytate dephosphorylation by the intrinsic plant phytases, a significant percentage of phytate (26% –100%) was enzymatically hydrolyzed.

The percent of calculated phytate losses is presented in Table 4. Data reveal that % of phytic acid losses decreased in all soaked samples as a function of soaking conditions, but the losses of grains that soaked in different pH solutions were higher than those soaked in deionized water. The highest percent of loss was 27.16% when soaked in pH 5 solution compared to 17.74% during soaking in water at 25 °C. Our results for soaking in water were lower than those reported by Afify et al. (2011), who found that after soaking sorghum grains in distilled water for 20 hours at room temperature, there was a 23.59% –32.4% decrease in phytate content. In addition to that, losses of phytic acid in samples that were soaked at 50 °C were higher than those soaked at 20 °C, which recorded the highest loss, 58.39%, at pH 5 compared to 26.9% for soaking in water. Fukushima et al. (2021) found that the enzymatic hydrolysis of phytate by endogenous phytase of sorghum may be the cause of phytate reduction during soaking. The low pH of the soaking solution and temperature may also provide favorable conditions for phytase activity. Also, our results are in agreement with those reported by Fukushima et al. (2021), who found that phytate content was measured in the grains within 48 h

after soaking at two different soaking temperatures, 30 °C and 50 °C. The highest enzyme activity was recorded 36 h after soaking when grains were soaked at 50 °C.

3.4 Effect of soaking conditions on mineral content

Some minerals, such as iron, phosphorus, zinc, and calcium in sorghum grains, were determined before and after the soaking process as affected by pH and temperature, and the results were presented in Tables 5 and 6. All minerals decreased as a result of soaking conditions, either pH or temperature, during 72 hrs.

Iron decreased for both soaked in water and different pH solutions. It decreased from 35.21 to 28.23 mg/100 g when soaked in water from 24 to 72 h at 20 °C (Table 5) compared to the unsoaked sample (35.43 mg/100 g); respectively. The iron content decreased by 20.32% during this period, dropping from 27.38 mg/100 g to 20.83 mg/100g when soaked in a pH 5 solution for 24 to 72 hours at 20 °C. The percent of loss after 72 h reached about 41.2%. Also, when the soaking temperature increased from 20 °C to 50 °C (Table 6) the losses of iron increased. The highest decrement was observed for the sample soaked in pH 5 solution, and the percent of loss was 49.39%, followed by soaked in pH 4 solution, which reached 46.0%, pH 3 solution, which was 42.33%, then soaked in water which recorded 40.92% (Table 6). The loss of iron during soaking may be due to the reduction of phytate (Table 4), which binds some mineral elements. Reduction after soaking may be attributed to the leaching of iron into the soaking media.

As for phosphorus, it decreased for all samples at different pH and temperature during soaking for 72 hrs. This reduction may be related to phytate hydrolysis during soaking. Phytates were found as the main source of inositol and phosphorous (typically accounting for 60% to 90% of total phosphorus (Graf, 1983), as well as an important storage of cations (in the form of phytate salts) and high-energy phosphoryl groups (Chatree et al., 2020). The phosphorus content of the sample soaked in pH 5 solution decreased from 210.22 to 184.12 mg/100 g compared to the sample soaked in water which decreased from 300.04 to 201.34 mg/100 g during soaking time from 24 to 72 h at 20 °C, respectively; while the unsoaked sample was 333.48 mg/100 g (Table 5). The percent of reductions was in the following order pH 5 > pH 4 > pH 3 > soaked in water. On the other hand, when the soaking temperature was increased to 50 °C (Table 6) the percentage of phosphorus reductions was higher than that soaked at 20 °C. Also, other results are in the same trend, the highest loss of phosphorus was found in the sample soaked in the solution at pH 5, which reached about 80%, and the lowest reduction was 30.03% for the sample soaked in water after 72 h.

Concerning zinc, the results show that zinc contents for all soaked samples decreased as a function of pH solution, soaking temperature, and time (Tables 5 and 6). Generally, the losses of zinc during soaking at 50 °C (Table 6) were higher than those soaked at 20 °C (Table 5). It may be due to temperature that activates the phytase enzyme that hydrolyzes phytate and releases zinc and other minerals in the soaking medium.

The greatest zinc losses at 20 °C and 50 °C (Tables 5 and 6) occurred in samples soaked in a pH 5 solution, reaching 42.14% and 68.55%, respectively. This pH level was optimal for phytase activity, facilitating the leaching of zinc ions into the soaking medium. Additionally, zinc appears to be the most impacted by phytate due to its tendency to form the most stable and insoluble complexes.

Regarding calcium, the data in Tables 5 and 6 indicate that the calcium content of all samples soaked at either 20 °C or 50 °C for 72 hours decreased depending on the soaking medium. The calcium losses of all samples soaked at 50 °C (Table 6) were greater than those of samples soaked at 20 °C (Table 5) under the same pH and time conditions. It may be related to losses of phytate during hydrolysis by phytase that is activated at 50 °C. So, the reduction of calcium after soaking may be attributed to leaching into the soaking media. Our results are in disagreement with those reported by Eltayeb et al. (2017), who found that calcium content was not significantly affected by soaking in distilled water and 4% citric acid.

Table 5. Minerals content (mg/100 g dw) and % of loss as affected by soaking conditions at 20 °C for 72 hours and pH values.

Soaking conditions at 20 °C for 72 hours							
Treatment	**0**	**24**	**%loss**	**48**	**%loss**	**72**	**%loss**
Without soaking	35.43 ± 0.32			**Fe**			
Soaking in water		$^{A}35.21^{a} \pm 0.37$	0.62	$^{A}32.67^{b} \pm 0.14$	7.79	$^{A}28.23^{c} \pm 0.12$	20.32
pH 3		$^{B}33.57^{a} \pm 0.23$	5.25	$^{B}27.43^{b} \pm 0.18$	22.58	$^{B}21.17^{c} \pm 0.11$	40.25
pH 4		$^{C}28.17^{a} \pm 0.19$	20.49	$^{B}26.14^{b} \pm 0.17$	26.22	$^{B}21.03^{c} \pm 0.20$	40.64
pH 5		$^{C}27.38^{a} \pm 0.21$	22.72	$^{C}23.63^{b} \pm 0.15$	33.30	$^{C}20.83^{c} \pm 0.12$	41.20
Without soaking	333.48 ±1.19			**P**			
Soaking in water		$^{A}300.04^{a} \pm 0.78$	10.03	$^{A}250.57^{b} \pm 0.77$	24.86	$^{A}201.34^{c} \pm 0.21$	39.62
pH 3		$^{B}266.67^{a} \pm 0.43$	20.03	$^{B}233.34^{b} \pm 0.98$	30.02	$^{B}193.34^{c} \pm 0.45$	42.02
pH 4		$^{C}216.67^{a} \pm 0.26$	35.02	$^{C}211.62^{b} \pm 0.97$	36.54	$^{C}186.67^{c} \pm 0.32$	44.02
pH 5		$^{D}210.22^{a} \pm 0.34$	36.96	$^{D}183.33^{b} \pm 0.58$	45.02	$^{D}184.12^{b} \pm 0.32$	44.79
Without soaking	6.74 ± 0.34			**Zn**			
Soaking in water		$^{NS}6.37^{a} \pm 0.11$	5.49	$^{B}5.22^{b} \pm 0.08$	22.55	$^{B}4.57^{b} \pm 0.11$	32.20
pH 3		$6.03^{N.S} \pm 0.07$	10.53	$^{A}6.0 \pm 0.11$	9.79	$^{A}5.53 \pm 0.08$	17.95
pH 4		$5.97^{a} \pm 0.13$	11.42	$^{C}5.05^{a} \pm 0.06$	25.07	$^{B}4.25^{b} \pm 0.05$	36.94
pH 5		$6.22^{a} \pm 0.15$	7.72	$^{C}4.91^{b} \pm 0.08$	27.15	$^{C}3.90^{c} \pm 0.07$	42.14
Without soaking	312.52 ± 1.03			**Ca**			
Soaking in water		$^{A}307.16^{a} \pm 0.65$	1.72	$^{A}296.40^{b} \pm 0.56$	5.16	$^{A}286.47^{c} \pm 0.89$	8.34
pH 3		$^{A}305.47^{a} \pm 0.29$	2.26	$^{A}285.47^{b} \pm 0.64$	8.66	$^{C}269.90^{c} \pm 0.78$	13.64
pH 4		$^{B}293.87^{a} \pm 0.88$	5.97	$^{A}290.67^{a} \pm 0.86$	7.0	$^{B}273.57^{b} \pm 0.76$	12.46
pH 5		$^{C}281.97^{a} \pm 0.52$	9.77	$^{B}276.1^{b} \pm 0.54$	11.65	$^{D}184.83^{c} \pm 0.67$	40.85

Means of minerals content having the same right case small letter(s) within a column are not significantly different ($p \leq 0.05$) as affected by soaking at 20 °C for 72 hours. Means of minerals content having the same left case capital letter(s) within a row are not significantly different ($p \leq 0.05$) as affected by soaking pH values.

Table 6. Minerals content (mg/100 g dw) and % of loss as affected by soaking conditions at 50 °C for 72 hours and pH values.

Soaking conditions at 50 °C for 72 hours							
Treatment	**0**	**24**	**%loss**	**48**	**%loss**	**72**	**%loss**
Without soaking	35.43 ± 0.32			**Fe**			
Soaking in water		$^{A}27.91^{a} \pm 0.11$	21.22	$^{A}22.23^{b} \pm 0.13$	37.26	$^{A}20.93^{c} \pm 0.06$	40.92
pH 3		$^{A}27.87^{a} \pm 0.09$	21.33	$^{AB}21.33^{b} \pm 0.11$	39.8	$^{A}20.43^{c} \pm 0.11$	42.33
pH 4		$^{B}25.53^{a} \pm 0.12$	27.94	$^{B}20.43^{b} \pm 0.09$	42.33	$^{B}19.13^{c} \pm 0.16$	46.0
pH 5		$^{B}25.32^{a} \pm 0.13$	28.53	$^{A}22.14^{b} \pm 0.16$	37.5	$^{C}17.93^{c} \pm 0.23$	49.39
Without soaking	333.48 ± 1.19			**P**			
Soaking in water		$^{A}266.67^{a} \pm 1.23$	20.03	$^{A}247.43^{b} \pm 1.08$	25.80	$^{A}233.34^{c} \pm 1.76$	30.03
pH 3		$^{C}233.34^{a} \pm 1.21$	30.02	$^{B}133.34^{b} \pm 0.1.23$	60.02	$^{B}100.73^{c} \pm 1.16$	69.79
pH 4		$^{B}258.27^{a} \pm 1.74$	22.55	$^{C}100.24^{b} \pm 1.13$	69.94	$^{C}83.34^{c} \pm 1.05$	75.0
pH 5		$^{D}133.86^{a} \pm 1.27$	59.86	$^{C}100.34^{b} \pm 1.07$	69.91	$^{D}66.67^{c} \pm 1.03$	80.0
Without soaking	6.74 ± 0.34			**Zn**			
Soaking in water		$^{NS}6.11^{a} \pm 0.06$	9.35	$^{A}5.67^{b} \pm 0.05$	15.88	$^{A}4.63^{c} \pm 0.11$	31.31
pH 3		$6.24^{a} \pm 0.11$	7.42	$^{B}4.71^{b} \pm 0.11$	30.12	$^{B}3.85^{c} \pm 0.05$	42.88
pH 4		$6.47^{a} \pm 0.13$	4.0	$^{A}B5.11^{b} \pm 0.07$	24.18	$^{B}3.68^{c} \pm 0.07$	45.40
pH 5		$6.01^{a} \pm 0.09$	19.83	$^{C}4.17^{b} \pm 0.10$	38.13	$^{C}2.12^{c} \pm 0.04$	68.55
Without soaking	312.52 ± 1.03			**Ca**			
Soaking in water		$^{B}291.83^{a} \pm 1.19$	6.62	$^{A}234.37^{b} \pm 1.33$	25.0	$^{A}230.73^{c} \pm 1.43$	26.17
pH 3		$^{A}308.13^{a} \pm 1.34$	1.40	$^{A}230.34^{b} \pm 1.20$	26.3	$^{D}130.57^{c} \pm 1.30$	58.22
pH 4		$^{C}287.93^{a} \pm 1.38$	7.87	$^{B}207.43^{b} \pm 1.13$	33.63	$^{B}151.82^{c} \pm 1.27$	51.42
pH 5		$^{D}175.57^{b} \pm 1.29$	43.82	$^{C}178.5^{a} \pm 1.23$	42.88	$^{C}142.70^{c} \pm 1.42$	54.33

Means of minerals content having the same right case small letter(s) within a column are not significantly different ($p \leq 0.05$) as affected by soaking at 20 °C for 72 hours. Means of minerals content having the same left case capital letter(s) within a row are not significantly different ($p \leq 0.05$) as affected by soaking pH values.

3.5 Molar ratios and bioavailability of minerals

Antinutritional factors (ANFs) like phytate have a negative impact on both the total and bioavailable mineral content. Premilling treatments and processing enhance mineral bioavailability by decreasing the concentration of mineral inhibitors and transforming food components into complex ligands that favorably bind metal ions (Rousseau et al., 2020). Calculated molar ratios between phytate and minerals like Fe, Zn, and Ca before and after soaking at pH 5 and 50 °C for 72 days are presented in Table 7. Soaking process reduced phytate: Fe ratio from 1.02 to 0.84, thus increasing Fe availability.

Table 7. The molar ratio between phytate and iron, zinc, and calcium of sorghum grains as affected by soaking conditions.

Phytate: mineral molar ratio	Phytate:Fe	Phytate:Zn	Phytate:Ca
Control (before soaking)	1.02	6.22	0.08
Soaking (at pH 5, 50 °C for 72 hrs)	0.84	8.23	0.08

Hurrell (2004) clarified that the phytate:Fe molar ratio has to be lower than 1.0 and preferably lower than 0.4 for favorable iron absorption. In contrast, phytate:Zn molar ratio increased as a result of the soaking process from 6.22 to 8.23, although both of them less than 10 and available for nutrition. Morris & Ellis (1989) reported that the phytate to Zn ratio of less than 10 shows adequate availability of Zn, and problems are encountered when the value is greater than 15. Also, molar ratios over 15:1 progressively inhibit Zn absorption and have been associated with suboptimal Zn status in humans (Gibson et al., 1997). Dietary proteins can potentially facilitate Zn absorption even in the presence of phytate. Proteins prevent the precipitation of Zn in the intestinal lumen and amino acids such as cysteine or peptides, which facilitate Zn uptake by the mucosal cells (Sandström et al., 1989). As for Ca, the soaking process did not change the molar ratio between phytate and Ca, and both of them were 0.08 and adequate for nutrition. The phytate: Ca molar ratio less than 0.17 is an indicator of Ca bioavailability (Castro-Alba et al., 2019). To enhance the food and nutritional value of sorghum grains, particularly for children and lactating mothers, it is recommended to soak the grains in acidic media at pH 5 and 50 °C for 48 or 72 hrs before milling. Food acidification increases the solubility of Fe, Ca, Mg, and Zn independently of phytic acid. So, a lactic acid fermentation of PA-rich foods (bread, etc.) allows the release of these minerals from the vegetal matrix and leads to a better bioaccessibility (Lopez et al., 2002).

4 Conclusion

Based on the previous results, soaking sorghum grains is a straightforward and traditional approach to lowering phytate content. A soaking solution at pH 5 and 50 °C proved to be more effective in reducing phytate content during a 72-hour soaking period. However, under these conditions, some minerals such as Fe, P, Zn, and Ca may be lost, as the phytase enzyme catalyzes the hydrolysis of phytate, releasing these minerals into the soaking medium. Although after soaking at pH 5 and 50 °C for 72 hours, the losses of soluble minerals are high, but mineral availability is acceptable and within the limits.

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