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ORIGINAL ARTICLE

Nutritional properties of green gram germinated in mineral fortified soak water: I. Effect of dehulling on total and bioaccessible nutrients and bioactive components

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Abstract The study aimed at investigating the effect of germinating green gram (*Vigna radiata*, GG) in mineral fortified soak water on total and bioaccessible nutrients and bioactive components in whole and dehulled GG. Whole GG was soaked in water fortified with iron (100 or 200 mg/100 ml) or zinc (50 or 100 mg/100 ml), germinated and a portion was dehulled. Whole and dehulled grains were analyzed for selected total and in vitro digestible/bioaccessible constituents. GG germinated in water served as controls. GG germinated in mineral fortified soak water had high iron and zinc content in whole and dehulled grains. Protein and calcium content did not differ significantly. In vitro digestible starch and protein was higher in dehulled grains. A remarkable increase in bioaccessible iron and zinc was seen in grains germinated in mineral fortified water, the increase was more at lower level of fortification of levels for both minerals. Both total and bioaccessible bioactive components, total phenols, tannins and flavonoids were significantly lesser in grains germinated in fortified water. Germinating pulses in fortified water can be used as a pre-processing technology for fortification of minerals.

Keywords Fortification · Bioaccessible minerals · Starch and protein digestibility · Total phenols · Tannins · Phytates

Introduction

Pulses hold an important place in human nutrition on account of their rich nutritional contribution to diets, particularly for proteins, essential minerals and vitamins, and dietary fiber. They also form a staple part of diets along with cereals as an essential accompaniment. They are of significance in South East Asian dietaries where people are vegetarians or do not have an access to animal sources of proteins due to economic reasons (Egounlety and Aworh 2003). However, these regions are also known for a very high number of malnourished individuals, specially children suffering either with undernutrition or specific micronutrient deficiencies as stated in recent Global Nutrition Report (IFPRI 2016). Continued protein energy and micronutrient malnutrition in children result in lower economic productivity and cognitive abilities in later years (Joffe 2007). Iron deficiency anemia is widespread with nearly 1/3rd of world population having low iron levels. The 6th Report on World Nutrition Situation (UNSCN 2011) states that 40% of women in Asian countries are anemic. Similarly zinc deficiency is also being recognized as a public health problem in many regions. It has been identified as a major risk factor contributing towards DALYs (disability adjusted life years) for nearly 26.7 million population in high mortality developing countries along with iron and vitamin A deficiencies (Stein and Qaim 2007; WHO 2002).

There are many approaches to tackle malnutrition, one of them is to supplement the missing nutrients through foods. Food fortification has been recognized as an effective strategy to overcome micronutrient malnutrition, especially in developing countries. Fortification of wheat flour with iron, folic acid and other micronutrients in countries where wheat is used as a staple, has been

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recommended as a strategy to prevent, control and overcome anemia by WHO and it can be integrated with other strategies over time (WHO and UNICEF 2004; WHO, FAO, UNICEF, GAIN, MI, FFI 2009). Identification of multiple modes of supplement is desirable on account of diverse eating habits of population and realizing the fact that all may not have an access to commercially fortified foods. In such instances, approaching through a dietary practice already in vogue is an easier option.

Pulses are processed in different ways and undergo different types of pre-treatments before cooking. They can be dehulled, germinated or fermented. Each of these processes alters the nutritional quality of grain (Ghavidel and Prakash 2007; Gupta et al. 2015; Oghbaei and Prakash 2016). In addition, the nutritional quality of pulses can also be compromised by the presence of anti-nutritional factors such as oligosaccharides, enzyme inhibitors, saponins, polyphenols, phytates, etc. (Egounlety and Aworh 2003; Gupta et al. 2015). Germinated grains are better in nutritional quality on account of a higher protein and starch digestibility, higher bioavailable minerals, B-complex vitamins, and ascorbic acid and inactivation of many anti-nutritional factors (Luo and Xie 2014). Germination is usually practiced as a household processing technology even in many underdeveloped regions as it is a low cost option to improve the existing dietaries. Among all legumes, green gram or Mung bean (*Vigna radiata*) is used extensively in germinated form in many countries. Present study explored the possibility of using germinated green gram (GGG) as a vehicle for pulse fortification by using mineral fortified soak water for germination. The germinated grains were studied for total and available nutrients and bioactive components.

Materials and methods

Materials

Whole green gram (mung bean, *V. radiata*) was procured in bulk from local market. All experiments were carried out in duplicate/triplicate with analytical grade chemicals and double distilled water. All apparatus were cleaned meticulously to ensure there was no metal contamination at any point of time during the whole analysis. Chemicals were procured from SD Fine Chemicals, India and HiMedia Company, India. The enzymes used for the study were pepsin (Batch No. 3-0060), pancreatin (Batch No. 0-0864), diastase (Batch No. 0695/195/270511) and papain (Batch No. 0993/493/130811). The dialysis tubing was procured from Sigma Aldrich Co. USA with a molecular mass cut off of 8000 kDa.

Processing of green gram

Whole GG (100 g) was washed and soaked in distilled water (control) or in water enriched at two levels of mineral salts (ferrous sulfate or zinc sulfate). To soak the grain, 1000 ml glass beakers were used and germination was done in a grain germinator, a transparent container in which hydration of grains was facilitated through evaporated water. Pulse (100 g) iron (ferrous sulfate equivalent to 100 or 200 mg) and zinc (zinc sulfate equivalent to 50 or 100 mg) were added to 300 ml double distilled water, and mixed well to facilitate complete dissolution. The pulse was added to container and soaked for 7 h. After soaking, water was drained, measured, filtered through ashless filter paper and kept aside for further analysis. The soaked grains were allowed to germinate for 42 h. Germination was carried at room temperature and under normal light. Germinated grains were divided into two batches and one was manually dehulled.

Nutritional composition

The moisture content of samples was estimated by AOAC method (2000). For protein content, nitrogen was determined by Kjeldhal method and multiplied with 6.25. Fat was estimated by repeated distillation in a Soxhlet apparatus (Raghuramulu et al. 2003). For measurement of total starch, starch was degraded to glucose with enzymic hydrolysis followed by determination of glucose (Batey and Ryde 1982; Raghuramulu et al. 2003). Total ash was determined by incineration of sample in a muffle furnace and weighing. This was dissolved in water and used for mineral estimation. Iron by Wong's Method was determined colorimetrically as ferric iron (Raghuramulu et al. 2003). Calcium was precipitated as calcium oxalate, dissolved in hot dilute H_2SO_4 and titrated against standard potassium permanganate (Helrich 1990). Zinc was analyzed by atomic absorption spectrophotometer (AOAC 2000).

Anti-nutrients

Dietary fiber was determined by the method of Asp et al. (1983), Phytic acid was determined according to the method of Thompson and Erdman (1982). Oxalates were estimated by method of Baker 1952.

Bioactive components

Total phenols were determined using Folin–Ciocalteau reagent and concentration was calculated using tannic acid as standard and the results expressed as mg tannic acid equivalents/100 g sample (Matthaus 2002). The flavonoid content was estimated by the Dowd method using quercetin as standard and expressed as mg of quercetin equivalents per 100 g of sample (Arvouet-Grand et al. 1994). Colorimetric estimation of tannins as mg tannic acid equivalent/100 g of sample was determined as discussed earlier (Ranganna 1986).

Digestible/bioaccessible nutrients and bioactive components

In vitro starch digestibility (IVSD) was determined by modification of methods of Kon et al. (1971) and Holm et al. (1985) with sequential enzymic hydrolysis and determination of glucose liberated finally. In vitro protein digestibility (IVPD) was estimated by modified enzymatic method of Akeson and Stahmann (1964). For the measurement of bioaccessible iron, zinc, calcium, total phenols, flavonoids and tannins a simulated gastro-intestinal digestion using pepsin for the gastric stage followed by pancreatin and bile salts for the intestinal stage was used. The constituents diffused through a semi-permeable membrane were estimated to measure dialyzable or bioaccessible nutrients (Luten et al. 1996). The collected dialysate was analyzed for respective constituents through methods that were explained earlier.

Statistical analysis

Data were subjected to statistical analysis using statistical software SPSS 15.0 (SPSS Inc., Chicago, IL). Mean and standard deviation for all values were calculated. Data were analyzed using Students '*T*' test to determine significant differences between whole and dehulled samples for total and bioaccessible constituents. For multiple comparison, data was analyzed using ANOVA and Duncan's multiple range test (Duncan 1995).

Results and discussion

The results of the study are summarized in Tables 1, 2, 3, 4, 5 and 6 for whole and dehulled germinated grains. To facilitate discussion, the variations are designated as—A. control (distilled water), B. distilled water with 100 mg iron, C. distilled water with 200 mg iron, D. distilled water with 50 mg zinc, E. distilled water with 100 mg zinc.

Nutritional composition

The effect of dehulling of GG on nutritional composition is presented in Table 1. The range of moisture content of

Table 1 Nutrients and anti-nutrient content of whole and dehulled germinated green gram (per 100 g db)

Variations	Moisture (%)	Ash (%)	Starch (%)	Protein (%)	Iron (mg)	Zinc (mg)	Calcium (mg)
A. Distilled water (control)							
Whole	56.1 ± 0.50	3.71 ± 0.00	53.76 ± 0.00	27.24 ± 0.00	6.56 ± 0.22	4.00 ± 0.137	72.76 ± 0.00
Dehulled	61.2 ± 0.00	3.22* ± 0.00	58.20*** ± 0.00	26.78 ± 0.49	6.09 ± 0.11	3.94 ± 0.07	48.07* ± 1.68
B. With 100 mg Fe							
Whole	69.0 ± 0.20	4.19 ± 0.32	54.77 ± 0.00	26.94 ± 0.00	94.03 ± 1.32	3.64 ± 0.25	64.55 ± 0.00
Dehulled	63.50 ± 0.50	3.45* ± 0.16	59.29* ± 0.85	27.10 ± 0.60	29.72** ± 0.00	3.86 ± 0.00	37.42* ± 3.70
C. With 200 mg Fe							
Whole	60.6 ± 0.40	4.42 ± 0.15	55.00 ± 0.41	24.42 ± 0.56	149.18 ± 3.55	3.37 ± 0.00	60.18 ± 1.80
Dehulled	61.4 ± 0.40	3.94 ± 0.31	58.11** ± 0.34	25.80 ± 1.19	41.06** ± 0.23	3.36 ± 0.00	40.23* ± 1.99
D. With mg 50 Zn							
Whole	63.1 ± 0.00	3.22 ± 0.00	48.81 ± 0.35	26.91 ± 0.00	6.59 ± 0.19	10.05 ± 0.00	70.89 ± 0.00
Dehulled	64.5 ± 0.50	3.01 ± 0.20	54.14* ± 0.37	25.38* ± 0.00	6.31 ± 0.08	8.37* ± 0.37	46.39** ± 0.70
E. With 100 mg Zn							
Whole	56.1 ± 0.00	3.64 ± 0.16	50.11 ± 0.91	28.29 ± 0.00	6.90 ± 0.00	14.41 ± 0.00	69.41 ± 1.30
Dehulled	61.2 ± 0.20	3.23 ± 0.14	53.89* ± 0.62	27.22 ± 1.19	6.40 ± 0.11	12.93* ± 0.54	45.80* ± 1.68

	Phytic acid (mg)	Insoluble dietary fiber (g)	Soluble dietary fiber (g)	Total fiber (g)
Anti-nutrient content (control)				
Whole	261.62 ± 1.71	18.05 ± 1.29	1.77 ± 0.09	19.82 ± 1.49
Dehulled	200.37* ± 2.05	14.35* ± 1.83	1.86 ± 0.21	16.21 ± 1.11

Significant differences between whole and dehulled grains on application of '*T*' test; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$, values without notation are not significantly different samples was 56.1–69.0%. For better comparison rest of the data are presented on dry weight basis. As expected, ash content was higher in whole grain (range 3.22–4.42%) but differences were negligible, except for control and variation B which showed marginally significant differences. When grains were soaked in mineral fortified water, the ash content increased which indicates that grains absorbed Fe/Zn from water. The highest ash content was found to be 4.42/100 g in variation C for whole GGG. The starch content of all dehulled samples was higher than whole grains. For control sample, the difference was highly significant at lower levels of probability. The range of values for starch content for whole grains was 48.81–55.00/100 g. The protein content of whole and dehulled GGG ranged between 24.42 and 28.29% with slightly higher content of protein in some variations, however, it did not differ significantly between whole or dehulled samples. Compositional difference in whole and dehulled grains arose because of removal of hull which mostly consisted of fiber, mineral content and some vitamins. Blessing and Gregory (2010) reported that on dehulling of germinated mung bean, protein content increased and ash, fiber, and CHO content decreased. The protein content of germinated mung bean was reported as 30% by Mubarak (2005). These findings were similar to our results.

In the control sample of GGG the reduction in iron content due to dehulling by 7.2% was insignificant but when grains were soaked in water with added iron, the Fe content of whole and dehulled grains increased enormously. Most of the iron was adsorbed by husk and a lesser content migrated inside endosperm, so dehulling led to significant differences in Fe content between whole and dehulled grain. When grains were soaked in water with zinc, similar trend was observed. The absorption of zinc was much less than iron, however, some of it moved to endosperm, and the difference in zinc content of whole and dehulled GGG was marginally significant. The high concentrations of iron in soak water could be reason for higher calcium release into soak water. In both whole and dehulled GGG with iron, the calcium content decreased, whereas this trend was not observed for variation E and F which were fortified with zinc. The calcium content of whole GGG varied from 60.18 to 72.76 mg/100 g. All variation of GG lost significant amount of calcium on account of dehulling, the range being 37.42–48.07 mg/100 g. Raghuvanshi et al. (2011) reported a decrease in dietary fiber and mineral content and an increase in fat, protein and CHO in raw mungbean grains on dehusking. Reduction of ash, iron, calcium and phosphorous content after dehulling in selected pulses is attributed to high concentration of these elements in hull portion (Ghavidel and Prakash 2007).

The anti-nutrient content of control sample was also estimated to understand the effect of dehulling on GGG and results showed that on dehulling there was a significant reduction (23.4%) in phytic acid and in insoluble dietary fiber (20.5%). Many authors have reported a decrease in anti-nutritional content of legumes due to soaking, germination or dehulling (Gupta et al. 2015). Abd El-Hady and Habiba (2003) reported varying degree of reduction in phytic acid, tannins, total phenols and trypsin inhibitor activity in faba beans, peas, chickpea and kidney beans. Lestienne et al. (2005) reported a decrease of 4.7% in phytic acid content in mungbean on soaking. Mubarak (2005) reported reduction of calcium content (4%) and anti nutritional factors like tannins and phytic acid by 33.3 and 20.7% respectively after dehulling of mungbean seed.

Mineral and bioactive components in soak water

Leaching of nutrients and bioactive components from cereals and pulses in soak water or due to washing is well documented (Khatoon and Prakash 2006a, b). Factors like ratio of water to grain, temperature of soak water and duration of soaking could affect degree of leaching. The mineral concentration of soak water can affect the leaching of minerals and bioactive components. The result of analysis of soak water for quantity of minerals and bioactive components leached or retained in water following end of soaking period are presented in Table 2. The results are expressed as quantity of mineral/bioactive components leached in water from a known quantity of grain as explained above. The ash content of mineral fortified water was much higher than control (distilled water) and this showed that some amount of added mineral salts was not absorbed by the grains. As level of fortification increased, the ash content also increased. The content of iron and zinc in control and in respective non-fortified variations were in same range but their contents in fortified water with respective salts were much higher than control. The calcium content of different soak water showed that the addition of iron to water increased the calcium leaching significantly, while zinc did not have a significant effect. In case of total phenols and tannins, soak water from control sample contained much lesser amounts indicating that mineral fortification assisted in retention of these constituents. The loss was lesser for iron fortified samples. The loss of flavonoids was more in iron fortified water but significantly lesser in zinc fortified water. Overall, the loss of bioactive constituents was lesser in mineral fortified GG. This could be attributed to binding of these constituents to minerals.

Table 2 Minerals and bioactive components (mg) leached in soak water per 100 g of green gram

Variations	Ash (mg)	Iron (mg)	Zinc (mg)	Calcium (mg)	Total phenols (mg)	Tannin (mg)	Flavonoids (mg)
A. Control	39.62 ± 1.23	0.009 ± 0.00	0.012 ± 0.00	8.52 ± 0.17	49.95 ± 0.85	70.95 ± 1.55	7.95 ± 0.35
B. Fe (100 mg)	345.56*** ± 3.52	4.32*** ± 0.65	0.060* ± 0.00	18.3** ± 0.00	26.75* ± 0.25	35.25** ± 0.95	8.75^{ns} ± 0.15
C. Fe (200 mg)	577.41*** ± 5.67	34.81*** ± 1.15	0.031^{ns} ± 0.00	18.25** ± 0.16	34.46* ± 1.41	34.53** ± 1.4	9.6* ± 0.00
D. Zn (50 mg)	137.48*** ± 4.92	0.008^{ns} ± 0.00	34.92*** ± 0.93	10.49^{ns} ± 0.19	40.34^{ns} ± 1.00	40.32* ± 0.78	1.23** ± 0.1
E. Zn (100 mg)	264.50*** ± 5.53	0.011^{ns} ± 0.00	79.31*** ± 0.05	11.49^{ns} ± 0.24	46.25^{ns} ± 0.15	46.25* ± 0.15	1.04** ± 0.05

Significant differences between control and fortified water on application of ‘*T*’ test; * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$

ns not significant

In vitro availability of selected nutrients

Table 3 presents digestible/bioaccessible nutrients from all variations of whole and dehulled GGG. After dehulling, IVSD increased among all variations of samples significantly. Average increase in IVSD was 5.56/100 g. The IVPD of dehulled grains was higher in comparison to whole grain and marginally significant for variation C, D and E. Dehulling did not affect the bioaccessible iron in control sample as well as samples germinated in zinc fortified water, and was in the range of 0.97–1.11 mg/100 g. GG germinated in iron fortified soak water had a high content of bioaccessible iron, 28.51 and 56.51 mg/100 g for grains soaked in water with 100 and 200 mg iron respectively. On dehulling the values reduced to 8.11 and 14.80 mg/100 g, which were significantly different from their whole counterparts. The bioaccessible zinc in control whole grains was 0.95 mg/100 g and in iron fortified water it reduced to 0.71 and 0.55 mg/100 g in two levels of soaking. Same trend was seen in dehulled samples, though the amount was higher than whole grain. This could be because of competitive inhibition of iron on account on presence of zinc. Samples germinated in zinc water exhibited a concurrent increase in bioaccessible zinc, which was 3–6 times higher than control. Hence it can be seen that when whole GG was soaked in mineral fortified soak water, there was an increase in the mineral content of grain, while most of the mineral was imbibed by the hull portion, some of it, also moved inside and was present in the grain after dehulling. The calcium bioaccessibility of control GGG reduced from 28.55 to 22.78 mg/100 g on dehulling, however, it was not significant. The reduction on dehulling in zinc fortified variations D was marginally significant. As concentration of iron in GG increased, calcium bioaccessibility decreased (variation B and C). Variation C (with 200 mg Fe) of dehulled grains showed higher calcium bioaccessibility than whole grain. The calcium and iron interaction in whole grains reduced the bioaccessible calcium as the iron content was higher.

Table 3 In vitro availability of nutrients in whole and dehulled germinated green gram

Nutrients	Sample	Digestible (g/100 g)		Bioaccessible (mg/100 g)		
		Starch	Protein	Iron	Zinc	Calcium
A. Control	Whole	14.20 ± 0.54	15.73 ± 0.49	1.07 ± 0.03	0.95 ± 0.04	28.55 ± 1.95
	Dehulled	22.44* ± 0.61	16.84 ± 0.30	1.01 ± 0.03	1.02 ± 0.01	22.78 ± 0.57
B. Fe (100 mg)	Whole	19.48 ± 0.26	15.98 ± 1.34	28.51 ± 0.00	0.71 ± 0.04	17.61 ± 0.71
	Dehulled	24.96* ± 0.71	16.38 ± 0.55	8.11** ± 0.26	0.88** ± 0.03	15.04 ± 0.00
C. Fe (200 mg)	Whole	18.11 ± 0.16	11.80 ± 0.43	56.51 ± 1.53	0.55 ± 0.02	13.22 ± 1.12
	Dehulled	22.18 ± 1.14	13.87* ± 0.17	14.80* ± 0.519	0.94* ± 0.01	14.70 ± 0.32
D. Zn (50 mg)	Whole	17.05 ± 0.38	12.32 ± 0.12	1.00 ± 0.05	3.6 ± 0.06	27.60 ± 0.91
	Dehulled	21.97* ± 0.00	13.86* ± 0.31	1.11 ± 0.04	2.87 ± 0.06	20.20* ± 0.68
E. Zn (100 mg)	Whole	17.84 ± 0.02	12.95 ± 0.17	0.97 ± 0.07	6.02 ± 0.19	21.05 ± 0.62
	Dehulled	22.94* ± 0.26	14.86* ± 0.19	1.09 ± 0.01	5.11 ± 0.06	18.83 ± 5.91

Significant differences between whole and dehulled grains on application of ‘*T*’ test; * $P \le 0.05$; ** $P \le 0.01$, values without notation are not significantly different

Ghavidel and Prakash (2007) studied the effect of dehulling and germination on GG, lentil and chickpea. In all pulses the total starch slightly decreased in the range of 0.9–1.9% but IVSD significantly improved (36.3–39.2%) whereas protein and its digestibility increased significantly in range of 2.2–5.1 and 13.2–16.7% indicated the effect of dehulling. Endosperm is a rich source of protein so removing hull portion can increase protein contents, and a reduction in tannin and phytate which bind protein and enzyme required for protein digestion result in higher IVPD. Polyphenols which are concentrated in hull have the potential to bind positively charged proteins, amino acids and/or multivalent cations or minerals such as iron, zinc and calcium (Gilani et al. 2005). They thus reduce the bioavailability of essential minerals and a reduction in their content may result in improved absorption of these nutrients.

Percent in vitro availability of nutrients

Table 4 presents percent in vitro availability of nutrients in whole and dehulled grains. Percent digestibility/bioaccessibility of all nutrients in dehulled control was higher than whole control. Percent digestibility of starch and protein and percent bioaccessible calcium followed same trend among other variations as well. The average increase in IVSD 7.1 among all variations with maximum increase in control variations of GGG (12.1%). Kaur et al. (2015) determined the in vitro characteristics of mung bean subjected to different processing methods and found that germinated legumes had higher amount of digestible starch in comparison to raw and soaked samples. The glycemic index of germinated mung bean was also lower in comparison to heat treated samples. Range of percent IVPD in whole and dehulled GGG was 45.76–57.74 and 53.76–62.99 respectively. Variations A, D and E showed significant increases after dehulling. The iron bioaccessibility of whole and dehulled variations of green gram germinated in normal water ranged between 14.21 and 17.67%, whereas for sample with iron fortification, it was in the range of 27.30–37.90%. Dehulling changed absolute values but did not affect percent iron bioaccessibility of GGG variations. Ghavidel and Prakash (2007) reported that availability of iron (17.4–21.9%) and calcium (13.1–16.6%) improved significantly in dehulled germinated pulses. The significant rise may be attributed to reduction of anti-nutrients which bind mineral and reduce their availability. Zinc bioaccessibility of control GGG (23.75) increased by 2.2% after dehulling. In variations of GGG, bioaccessible zinc was higher in dehulled samples except variation E and differences were significant for variations B and C. Similar trend was observed in whole

Table 4 Percent in vitro availability of nutrients in whole and dehulled germinated green gram

Variations	Sample	Percent digestible		Percent bioaccessible		
		Starch	Protein	Iron	Zinc	Calcium
A. Control	Whole	26.44 ± 1.01	57.74 ± 1.80	16.41 ± 0.47	23.75 ± 1.15	39.26 ± 2.67
	Dehulled	38.57* ± 1.03	62.99* ± 1.11	16.62 ± 0.58	25.95 ± 0.45	47.42* ± 1.18
B. Fe (100 mg)	Whole	35.58 ± 0.47	54.97 ± 3.37	30.32 ± 0.00	19.50 ± 1.10	27.32 ± 1.12
	Dehulled	42.09* ± 1.25	60.46 ± 2.02	27.30 ± 0.90	23.05* ± 0.95	40.24* ± 0.00
C. Fe (200 mg)	Whole	32.95 ± 0.29	48.33 ± 1.76	37.90 ± 1.02	16.51 ± 0.70	21.98 ± 1.85
	Dehulled	38.17 ± 1.96	53.76 ± 0.62	36.07 ± 1.26	28.08* ± 0.43	36.60* ± 0.80
D. Zn (50 mg)	Whole	34.95 ± 0.75	45.76 ± 0.44	15.14 ± 0.8	36.42 ± 0.58	38.93 ± 1.27
	Dehulled	40.59* ± 0.00	54.66* ± 1.25	17.67 ± 0.57	34.38 ± 0.72	43.21 ± 1.11
E. Zn (100 mg)	Whole	35.59 ± 0.05	45.77 ± 0.60	14.21 ± 1.00	41.78 ± 1.34	30.32 ± 0.89
	Dehulled	42.57* ± 0.47	54.59** ± 0.73	17.11 ± 0.23	39.60 ± 0.51	41.11* ± 3.25

Multiple comparisons between different variations for percent *in vitro* availability of nutrients

Variations	W	D	W	D	W	D	W	D	W	D
A. Control	b	a	a	a	c	c	b	cd	a	a
B. Fe (100 mg)	a	a	a	ab	b	b	b	d	b	a
C. Fe (200 mg)	a	a	a	b	a	a	c	c	b	a
D. Zn (50 mg)	a	a	a	b	c	c	a	b	a	a
E. Zn (100 mg)	a	a	a	b	c	c	a	a	ab	a

Different alphabets in a column are statistically different for multiple comparisons

W whole, *D* dehulled

Significant differences between whole and dehulled grains on application of '*T*' test; * $P \leq 0.05$; ** $P \leq 0.01$, values without notation are not significantly different

Table 5 Total and bioaccessible bioactive components in whole and dehulled germinated green gram (mg/100 g db)

Nutrients	Sample	Total phenols		Tannins		Flavonoids	
		Total	Bioaccessible	Total	Bioaccessible	Total	Bioaccessible
A. Control	Whole	235.92 ± 9.29	154.82 ± 1.58	420.99 ± 9.35	260.01 ± 8.51	17.68 ± 0.64	5.10 ± 0.25
	Dehulled	230.10 ± 3.94	155.53 ± 4.94	413.29 ± 7.36	247.09 ± 5.18	10.40* ± 0.09	2.43* ± 0.00
B. Fe (100 mg)	Whole	142.82 ± 12.63	74.34 ± 2.98	294.94 ± 0.97	141.00 ± 2.13	20.32 ± 0.00	5.11 ± 0.15
	Dehulled	143.58 ± 3.30	69.21 ± 6.60	273.70 ± 0.82	120.71 ± 7.84	12.16* ± 0.66	2.63** ± 0.08
C. Fe (200 mg)	Whole	211.87 ± 7.93	94.67 ± 14.14	330.61 ± 6.02	167.73 ± 1.31	18.30 ± 0.58	4.42 ± 0.18
	Dehulled	215.12 ± 5.79	100.01 ± 12.89	338.82 ± 5.45	129.99* ± 3.69	10.85* ± 0.73	2.28* ± 0.03
D. Zn (50 mg)	Whole	167.78 ± 3.71	117.80 ± 2.22	261.41 ± 2.55	176.10 ± 0.00	17.43 ± 0.84	5.26 ± 0.00
	Dehulled	153.52 ± 8.17	116.90 ± 1.41	252.23 ± 14.56	170.18* ± 0.83	9.13 ± 0.51	2.25** ± 0.06
E. Zn (100 mg)	Whole	170.40 ± 1.36	108.47 ± 1.34	341.69 ± 6.13	183.87 ± 3.51	19.32 ± 1.91	5.88 ± 0.23
	Dehulled	189.72 ± 4.61	114.55 ± 2.33	341.89 ± 7.98	187.01 ± 0.41	7.19* ± 0.59	1.77* ± 0.09

Significant differences between whole and dehulled grains on application of '*T*' test; * $P \leq 0.05$; ** $P \leq 0.01$, values without notation are not significantly different

GGG but both variation fortified with zinc (D and E) did not follow similar trend. The changes in variations B and C were significant. Luo and Xie (2014) assessed the changes in phytate content, phytase activity and in vitro availability of iron and zinc in soaked and sprouted green and white faba beans. Soaking and sprouting treatments reduced the contents of iron, zinc and phytate, however, there was a significant increase in phytase activity and an increase was also seen in iron availability (by 19–30%) and zinc availability (by 17.7–25.3%) in sprouted beans in comparison to unsprouted beans. Calcium bioaccessibility of GGG increased after dehulling among all variations and differences were significant for all. In comparison to control, GG germinated in mineral fortified soak water had lesser extent of absorbable calcium, the decrease was more for iron than for zinc. This could have been due to competitive inhibition between minerals. The significance of differences among all variations was analyzed by ANOVA and results compiled in Table 4 show that marginally significant differences were seen in whole and dehulled control sample for IVSD and IVPD respectively, while in fortified samples there was no difference. The bioaccessibility of iron and zinc was significantly higher in variations modified with pre-germination soak water. Calcium bioaccessibility of dehulled variations did not differ, but whole grains samples modified with iron showed significantly lower value.

Total, bioaccessible and percent bioaccessible bioactive components

Outer layers of grains (bran/husk) are rich sources of bioactive components, hence dehulling can change their contents. During pre-germination process especially soaking, the bioactive components lixiviate in water and the water content could affect the rate and kind of bioactive components which move out of the grain hulls. Table 5 presents the total and bioaccessible total phenols, tannins and flavonoids in whole and dehulled GGG. Data on percent bioaccessibility of all bioactive components and multiple comparisons between variations is compiled in Table 6. The total phenol contents of control grain ranged from 230.10 to 235.92 mg/100 g. Addition of minerals reduced the phenolic content to varying extent in all samples, though there were no significant changes on account of dehulling. Bioaccessible phenols were also lesser in samples germinated in mineral water in comparison to control sample. When computed as percent bioaccessibility, in control samples, 65.62–67.59% of total phenols were available, the value being closer to grains germinated in zinc fortified medium. A lesser extent of percent bioaccessibility was seen in iron fortified sample (Table 6). Overall results indicate that addition of iron or zinc to soak water reduced total phenol content and bioaccessibility. This could be due to leaching of phenols in water as well as competitive inhibition between minerals to reduce bioaccessibility. Tannins followed a similar pattern wherein control showed a higher tannin content and bioaccessible tannins than GG germinated in mineral water. The bioaccessible tannins were lesser in iron fortified samples and zinc was similar to control. Dehulling reduced bioaccessible tannin in some of the variants. Tajoddin et al. (2011) studied ten cultivars of mungbean with different seed coat color for polyphenol content in whole seed. The level of polyphenols was in the range of 280–356 mg gallic acid/100 g in whole seeds, with an average of 318 mg/100 g. Lower content in present study could be due to leaching of total phenolics in water during soaking.

All variations of GGG showed reduction in tannin bioaccessibility after dehulling except variation E that

Table 6 Percent bioaccessible bioactive components in whole and dehulled germinated green gram

Variations	Sample	Total Phenols	Tannins	Flavonoids
A. Control	Whole	65.62 ± 0.67	61.76 ± 2.02	28.91 ± 1.39
	Dehulled	67.59 ± 2.14	59.79 ± 1.26	23.40 ± 0.00
B. Fe (100 mg)	Whole	52.05 ± 2.09	47.80 ± 0.72	25.28 ± 0.73
	Dehulled	48.20 ± 4.60	44.12 ± 2.88	21.41** ± 0.69
C. Fe (200 mg)	Whole	44.68 ± 6.67	50.73 ± 0.39	24.20 ± 1.01
	Dehulled	46.49 ± 5.99	38.36* ± 1.09	21.06 ± 0.26
D. Zn (50 mg)	Whole	70.21 ± 1.32	67.36 ± 0.00	30.21 ± 0.00
	Dehulled	76.36* ± 1.14	67.47 ± 0.33	24.83* ± 0.63
E. Zn (100 mg)	Whole	63.66 ± 0.79	53.81 ± 1.03	30.48 ± 1.14
	Dehulled	60.37* ± 1.23	54.70 ± 0.13	24.63** ± 1.19

Multiple comparisons between different variations for percent bioaccessibility of bioactive components

Variations	Whole	Dehulled	Whole	Dehulled	Whole	Dehulled
A. Control	a	ab	a	ab	ab	a
B. Fe 100 (mg)	ab	c	b	c	ab	a
C. Fe 200 (mg)	b	c	b	c	b	a
D. Zn 50 (mg)	a	a	a	a	a	a
E. Zn 100 (mg)	a	b	b	b	a	a

Different alphabets in a column are statistically different for multiple comparisons

Significant differences between whole and dehulled grains on application of '*T*' test; * $P \leq 0.05$; ** $P \leq 0.01$, values without notation are not significantly different

exhibited a negligible increase. The reduction in variation C and D was significant. Dehulled GGG similarly showed lesser percent tannin bioaccessibility in control and variations with iron and changes in variation D and E showed rise which was within 1.5%. Only reduction in variation C (12.37%) was marginally significant and other changes were not significant. Multiple comparisons of percent bioaccessibility of tannin revealed whole GGG with 200 mg Fe was significantly different from other variations and in dehulled grains both variations with iron were significantly lesser than control and variation D. Egounlety and Aworh (2003) stated that the concentration of tannin in hull portion of cowpea, soybean and ground bean was much higher than whole grain. The opposite trend in our result could be due to partial loss of tannin content of hull during soaking.

Total, bioaccessible and percent bioaccessibility of flavonoids reduced in GGG due to dehulling which shows that separated portion could be good source of flavonoids. The flavonoid content ranged from 7.19 to 19.32 mg/100 g in GGG which was markedly lesser than total phenols and tannins. Variation A, B, C and E in GGG exhibited significant reduction, highest loss was found in variation E (12.3 mg/100 g). In all variations of GGG, flavonoid bioaccessibility reduced in dehulled grain significantly. The range of flavonoid bioaccessibility of whole and dehulled GGG was 4.42–5.88 and 1.77–2.63 mg/100 g respectively. Highest percent flavonoid bioaccessibility was found to be 26.21 and 30.21 in variation D. Comparison of all variations of GGG compiled in Table 6 revealed that reduction in percent bioaccessibility of all components was significant in iron fortified samples, except for flavonoids in dehulled GGG. A marginal reduction was also seen in total phenols and tannins in some of the zinc fortified samples. Overall results indicate that dehulling and mineral fortification reduced bioaccessible iron and zinc to a larger extent whereas flavonoids were not affected.

Conclusion

The results of the study show that germinating green gram in mineral fortified soak water significantly increased both iron and zinc content in samples. Though dehulling reduced the extent of increase, the samples still had a higher content as well as bioaccessible minerals in comparison to control. Fortification reduced the content and bioaccessibility of bioactive components to a marginal extent. In conclusion, germinating green gram in mineral fortified soak water can be used as a pre-processing technique to increase the iron and zinc content of grains.

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