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The Impact of Different Soaking Solutions on Minerals Availability and *In Vitro* Protein Digestibility of Faba Bean (*Vicia faba)*

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Abstract: ***This study was carried out to investigate the effect of soaking of faba bean (Agabat) on total minerals and its availability, phytic acid, in vitro protein digestibility, phytate phosphorus and non-phytate phosphorus content. Sample was purchased from local market in Khartoum. Soaked samples were prepared by soaking in distilled water and 4% citric acid at room temperature then all samples were sun dried, milled and kept in polyethylene bags at 4^0C for evaluating. All data were subjected to statistical analysis using (SPSS). The results showed that Mineral content significantly (P≤0.05) not affected by Soaking in distilled water and 4% citric acid except zinc content which was significantly (P≤0.05) increased. Minerals availability significantly (P≤0.05) increased by soaking in 4% citric acid except iron availability which was not affected, on the other hand soaking in distilled water significantly (P≤0.05) decreased the magnesium and zinc availability and increased calcium availability while potassium and iron availability were not affected. The anti-nutritional factor phytic acid was also affected by soaking showing significant (P≤0.05) reduction from 196.1(mg/100g) in the control to 116.6 and144.3 in the sample soaked in distilled water and 4% citric acid respectively. In vitro protein digestibility significantly(P≤0.05) improved by soaking. Phytate phosphorous decreased significantly(P≤0.05). In conclusion, soaking of faba bean enhanced their nutritive value, using 4%citric acid resulted in higher nutritive value compared with distilled water.***

Keywords: Faba bean, mineral, availability, protein digestibility, phytate

1. Introduction

Legumes play an important role in agriculture sector and contribute a lot for diet and a major source of important nutrients for many people both in developed and developing countries [1,2]. Among the different legumes, faba bean which is considered as the fourth most important pulse crop in the world grown mainly for its seeds or as green vegetable. Faba bean is important food legume in most urban parts of Sudan and its high protein content makes it a good source of plant protein [3]. Also, Faba bean has dense with vitamins, minerals, and Thiamin. Legumes are rich in minerals, but availability of these minerals is influenced directly or indirectly by other components of the legume such as protein [4], phytic acid [5], anti-nutritional factors [6], other minerals [7], and fiber [8]. Hence elimination or in activation of such anti-nutritional compounds is necessary to improve nutritional quality of Faba bean effectively utilize its full potential as human food. It is evident that the nutritional importance of given food or feedstuff depend not only on nutrient composition of raw stuff, but also on the amount utilized [9].

One way of solving this problem through food processing techniques such as soaking [10,11], germination[12], cooking[13], fermentation[14,15], heat treatment[16] and mechanical methods such as dehulling and milling .Soaking in water or some other salt solutions is one of the processing used to remove soluble anti-nutritional factors, breaking down several components into simpler components which alter the texture, flavor, aroma and taste[17]. Citric acid has been reported to increased utilization efficiency of dietary phytate-bound phosphorus and protein as reported by Wikramasinghe *et al.* [18], and would not be expected to have a large effect on intestinal pH, because it's an organic acid that is metabolic rapidly [19].

The present study was designed to evaluate how the pH of the soaking solution, a processing technique applied before the legume is cooked, affects the nutritive quality of the main minerals and protein in a common bean grown in Sudan

2. Materials and Methods

Materials

- Faba bean (Agabat) was purchased from the local market of Khartoum.
- Chemical and reagent
- Citric acid 4% prepared by adding 4g citric acid to 100ml distilled water.

Methods

Preparation of Faba bean and treatments

Faba bean was cleaned and sorted by removing broken kernels and extraneous matter, part of the grains from each are milled separately and passed through 60 mesh sieves then kept at 4°C for subsequent chemical analysis. The remaining part was used for soaking in 4% citric acid and distilled water for 24hs at room temperature and then the grains were sun dried for 24hs, milled and kept at 4°C for analysis.

Determination of total minerals

Total minerals solution which includes K, Ca, Mg, Fe, Zn and P was prepaired according to the method of Chapman and Pratt,[20] as described below:

Two grams of sample were weighed in clean dry crucible the crucible was placed in a muffle furnace for 4 hours at 550°C, sample were cooled and 10 ml of 3NHCl was added, covered watch glass and boiled gently for 10 minutes, then cooled. The contents were filtered through what man filter paper No 4 and the volumes was made to 50 ml with distilled water and was taken for mineral determination either by flame photometry for potassium (K) determination or by atomic absorption spectrophotometer (Perkin-Elmer 2380).

Determination of minerals availability

One gram of sample was extracted in 10ml (0.03N) HCL by shaking the content at 37°C for 3h. the clear extract obtained after filtration through what man filter paper No4 was oven dried 100°C then placed in a muffle furnace at 550°C for 3h, cooled and 3ml of (0.03N) HCl were added and the volume was made to 50ml with distilled water and were taken for mineral determination [21] .

$$\text{Mineral extractability} = \frac{\text{extracted mineral in 0.03N)Hcl}}{\text{Total minerals (mg/100g)}} \times 100$$

Determination of Phytic acid

The Phytic acid content was determined according to the method of Wheeler and Ferrel[22].

Phytate phosphorus

Phytate phosphorus determined according to the formula as follow:

$$phytate\ phosphorus\ mg/100 = \frac{A \times 28.18}{100}$$

Where:

A: Phytic acid content.

Non phytate phosphorus

Non phytate phosphorus was calculated by difference between the total phosphorus and phytate phosphorus content.

Determination of *in vitro* protein digestibility

In vitro protein digestibility was determined for samples by using pepsin enzyme the method described by Maliwal [23] was used as modified by Monjula and Jon [24]

Two g of the sample containing 16mg nitrogen taken in triplicates and hydrolyzed with one mg pepsin in 15ml of (0.1N) HCl at 37°C for 2hours; the reaction was terminated by addition of 15ml of 10% (W/V) trichloroacetic acid (TCA). The mixture was filtered quantitatively through what man filter paper NO1. The TCA soluble fraction was assayed for nitrogen using the micro – kjeldhyl method. Digestibility was calculated using the following equation:

$$Protein\ digestbility = \frac{\text{N in Supernatant} - \text{N in blank}}{N\ in\ sample} \times 100$$

Where

N in blank = N in pepsin enzyme.

3.2.9 Statistical analysis

All data were subjected to statistical analysis using *SPSS Vito* means tested by analysis of variance Factorial design. Value of probability of 5% was used to indicate significance according to DMRT [25].

3. Results and Discussions

As shown in table (1) calcium content of Faba bean was 324.36mg/100g. This result was lower than the range of 422.92 to 427.17mg/100g reported by Balla [26] and higher than the range of 77.00- 222.00mg/100g that reported by Ghavidel and Parakash [27] (2007) for calcium content of legume cultivars. Calcium content was not affected by soaking in distilled water while soaking in 4%citric acid significantly (P≤0.05) decreased calcium content from324.36mg/100g to 317.30mg/100g. This resulted agreement with Nestares *et al.* [10] who reported that Calcium content of soaking Phasulas decreased from 165.5mg/100g to139mg/100g and Desalegn [12] who mentioned that Calcium content of soaked Chickpea was 140 mg/100g.Soaking in distilled water and 4%citric acid significantly (P≤0.05) increased calcium availability of Faba bean from 31.31% to 38.92% and 46.31%, respectively. This result was higher than the range 15.7-29.3% that reported by Ghavidel and Parakash [27], and lower than 32.83% that reported by Luo and Xie [11] for Calcium available of Faba bean grains.

Magnesium content of Faba bean was 1.96mg/100g. Out of this amount about 50.81% was found to be available. This result was disagreement with 28-Osman [28] who reported that Magnesium content of Faba bean cultivars ranged from 222.90-225.72 mg/100g. Soaking in distilled water and 4% citric acid significantly (P≤0.05) not affected magnesium content, while availability was significantly (P≤0.05) decreased by soaking in distilled water and increased by soaking in 4% citric acid.

Potassium content of Faba bean was 524.82mg/100g only 38.29% of this amount was available. These results were lower than the range 957.4-1025.7 mg/100g reported by Osman [28]. Soaking in distilled water and 4% citric acid significantly (P≤0.05) not affected potassium content, while availability was significantly (P≤0.05) increased by soaking in 4% citric acid and not affected by soaking in distilled water.

Iron content of raw Faba bean was 3.74mg/100g, out of these amounts about 6. 9%was found to be available. These results were found to be lower than the range5.25-6.25mg/100g that reported by Osman [28], and 6.04mg/100g reported by12- Desalegn [12] (2015) for soaked Chickpea. Though the amount of iron was decreased, and its availability increased after soaking taken place, but it was not statistically different (p<0.05) from the control

Zinc content of Faba bean was 0.309mg/100g and its availability was 49.8% This result was higher than 15.20% that reported by Luo and Xie [11] for available Zinc of Faba bean grains. Soaking in distilled water and 4% citric acid was significantly (P≤0.05) increased zinc content from 0.309mg/100g to 0.325 and 0.319mg/100g, respectively. These results obtained were disagreement with that reported by Desalegn [12] who found Zinc content of soaked Chickpea was 2.53 mg/100g. Zinc availability was significantly (P≤0.05) decreased by soaking in distilled water and increased by soaking 4% citric acid.

Improvement of minerals availability was attributed to reduction of phytic acid which has a strong binding affinity to the dietary minerals, calcium, iron, and zinc, inhibiting their absorption [29,30] . When iron and zinc bind to phytic acid, they form insoluble precipitates and are far less absorbable in the intestines. This process can therefore contribute to iron and zinc deficiencies in people whose diets rely on these foods for their mineral intake, such as those in developing countries[31,32] . Mineral availabilities were increased as the pH of the soaking solution reduced.

Table 1: Total and available of minerals content of Faba bean as affected by soaking in distilled water and 4% citric acid

Treatments	Ca		Mg		K		Fe		Zn	
	Total (mg/100g)	Available (%)	Total (mg/100g)	Available (%)	Total (mg/100g)	Available (%)	Total (mg/100g)	Available (%)	Total (mg/100g)	Available (%)
A	324.36^{a} ±6.69	31.31^{c} ±1.01	1.958^{a} ±0.08	50.81^{b} ±0.74	524.82^{a} ±10.56	38.29^{b} ±0.96	3.742^{a} ±0.25	6.93^{ab} ±0.91	0.309^{c} ±0.02	49.80^{b} ±1.33
B	318.75^{a} ±5.26	38.92^{b} ±0.52	1.927^{a} ±0.05	46.98^{c} ±2.08	522.95^{a} ±9.07	39.10^{b} ±0.46	3.810^{a} ±0.01	6.371^{b} ±0.13	0.325^{a} ±0.00	47.30^{c} ±0.97
C	317.30^{ab} ±2.44	46.31^{a} ±1.73	1.817^{a} ±0.03	54.42^{a} ±2.57	514.62^{a} ±0.24	52.62^{a} ±1.15	3.793^{a} ±0.02	7.689^{a} ±0.37	0.319^{b} ±0.01	59.37^{a} ±0.14

Mean (±SD) values bearing different superscript (s) in the same column are significantly different (P≤0.05) according to DMRT.

Key:
A ≡ Faba bean
B ≡ Faba bean soaked in distilled water
C ≡ Faba bean soaked in 4% citric acid

Phytic Acid (PA): As shown in table (2) Phytic acid content of raw Faba bean was 196.06 mg/100g. This result was higher than the range 192.71-186.08 mg/100g and 94.76mg/100g that reported by Osman [28] and Desalegn [12] for Faba bean and Chickpea, respectively. Soaking in distilled water and 4%citric acid of Faba bean significantly (P≤0.05) decreased Phytic acid content. Higher reduction was noted (116.67mg/100g) when distilled water was used for soaking and value 144.35mg/100g was obtained when Faba bean soaked in 4% citric acid. These results were found to be higher than the range 4.10-5.35mg/100g that reported by Luo *et al.* [33]. Abd Elhady and Habiba [34] reported that Phytic acid content of soaked Legumes cultivars decreased to the range 2.6-9.8%. Alnose *et al.* [35] reported that Phytic acid content of soaked Faba bean reduced by 32.7%. Reduction of phytic acid was due to activation of the endogenous phytase and the diffusion of the products by soaking [36,37]

***In vitro* protein digestibility (IVPD):** *In vitro* protein digestibility content of Faba bean control was 61.31% (table 2). These results were higher than 61.00- 65.6% that reported by Ghavidel and Parakash [27]and disagreed with Abdelrahim [38] who mentioned that *in vitro* protein digestibility of Faba bean cultivars varied from 69.45-75.09%. These results obtained in this study were lower than the range 76-87.5% that reported by Khattab *et al*. [39] for soaked Legumes cultivars. In addition, Abdelrahim [38] reported range of 73.23-77.68% for soaked faba bean cultivars. Soaking in distilled water and 4% citric acid significantly (P≤0.05) increase the *in vitro* protein digestibility of faba bean. Improvement of protein digestibility after soaking could be attributed to the reduction or elimination of different ant-nutrients. Thus, PA as well as condensed tannins and polyphenols are known to interact with protein to form complexes. These interactions could increase the degree of cross-linking, decreasing the solubility of proteins making protein complexes which impair protease access to labile peptide bonds. Despite the rate of reduction of phytic acid produced by soaking in distilled water was higher than that of soaking in 4% citric acid , the rate of increment of in vitro protein digestibility produced by soaking in4% citric acid is higher than that of soaking in distilled water, these results indicate that phytic acid is not the only responsible factor for lowering IVPD and many other factors may have a role in this process. Other factors such as other anti-nutritional factor and soaking media influence the protein digestibility, and we can conclude that soaking faba bean in acidic media might improve protein better than soaking in neutral media.

Table 2: Phytic acid (mg/100g) and *in vitro* protein digestibility of Faba bean as affected by soaking in distilled water and 4% citric acid

Treatments	Phtyic acid (mg/100g)	IVPD (%)
A	196.10^{a}±3.18	61.31^{c}±0.85
B	116.67^{c}±3.88	67.13^{b}±1.87
C	144.35^{b}±7.84	71.20^{a}±2.21

Mean (±SD) values bearing different superscript(s) in the same column are significantly different (P≤0.05) according to DMRT.

Key:
A ≡ Faba bean whole grains
B ≡ Faba bean soaked in distilled water
C ≡ Faba bean soaked in 4% citric acid

Phosphorus Content (PC): PC of Faba bean was 87.49 mg/100g (table 3). This result was lower than the values obtained in the previous studies [27,28]. However, the content of Phytate phosphorus was 56.34 mg/100g that accounted about 64.39% of the total P in faba bean seeds. These results are in close agreement with those reported by [40, 41], and lower than 243 mg/100g that reported by Ramakrishna *et al.* [42] for Phytate phosphorus content of Indian bean. There was no loss of total phosphorus during soaking, but only of phytic phosphorus which was significantly decreased by about 26.2% and 40.36%when faba bean soaked in distilled water and 4% citric acid, thus the phytates were probably hydrolyzed to inorganic P, which remained in the grain, Similar trend were observed in soaked soybeans [43]. On the other hand, non-phytate phosphorus content was significantly (P≤0.05) increased by soaking. The increment may be due to the reduction of phytic acid content which chalet phosphorus element

Table14: Total Phosphorus, Phytate and Non-phytate phosphorus (mg/100g) of Faba bean as affected by soaking in distilled water and 4% citric acid

Treatments	Total Phosphorus (mg/100g)	Phytate phosphorus (mg/100g)	Non-phytate Phosphorus (mg/100g)
A	87.49a ±0.76	56.34^{a} ±1.13	31.03^{c} ±1.61
B	87.29a ±0.70	41.57^{b} ±2.26	45.72^{a} ±2.95
C	85.69a ±0.22	33.60^{c} ±1.12	52.09^{b} ±1.14

Mean (±SD) values bearing different superscript(s) in the same column are significantly different (P≤0.05) according to DMRT.

Key:
A ≡ Faba bean whole grain
B ≡ Faba bean soaked in distilled water
C ≡ Faba bean soaked in 4% citric acid

4. Conclusion

1) Availability of calcium, magnesium, potassium and zinc increased by soaking in 4% citric acid while soaking in distilled water improved availability of calcium, but magnesium and potassium were not affected and availability of zinc decreased. Mineral availabilities were increased as the pH of the soaking solution reduced.
2) Soaking Faba bean in distilled water and 4%citric acid significantly (P≤0.05) decreased Phytic acid content but the rate of reduction is more in neutral media.
3) Soaking Faba bean in distilled water and 4% citric acid significantly (P≤0.05) increase the *in vitro* protein digestibility of faba bean but the rate of increment is more in acidic media.

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