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Antinutritional Factors Content and Minerals Availability in Faba Bean as Affected by Cultivar and Domestic Processing

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Abstract: Two faba bean cultivars (Hudieba 72 and Bsabir) were used in this study. Chemical investigation of the cultivars showed that they contained about 30% proteins and 3.2 ash. Minerals content (mg/100 g) varied between the cultivars with a maximum value of 35.9, 1037.5, 427.2, 288.6, 207.9, 6.5, 2.7, 0.86, 1.7 and 2.8 for Na, K, Ca, Mg, P, Fe, Mn, Cu, Co and Zn, respectively. Phtic acid and polyphenols as antinutrients ranged from 291.2 to 298.9 and from 322.1 to 388.6 mg/100 g, respectively. Minerals availability (%) varied between the cultivars with a maximum value ranged from 74.3, 12.8, 50.0, 24.2, 8.2, 18.5, 8.3, 21.2, 40.3 and 2.8, for the minerals, respectively. Soaking of the seeds in distilled water for 1, 2 and 3 days significantly (p=0.01) reduced total minerals, phytic acid and polyphenols with a maximum reduction observed after 3 days soaking. However minerals availability was significantly (p=0.01) improved after soaking. Cooking significantly (p=0.01) reduced total minerals, phytic acid and polyphenols contents. However, mineral availability was increased significantly (p=0.01) after cooking.

Key words: Faba bean, cultivars, minerals, domestic, processing, antinutrients

INTRODUCTION

It is well recognized that food grain legumes such as common beans, lentils and kidney beans, represent the main supplementary protein source in cereal starchy food-based diets consumed by large sectors of the population living in the developing countries. The nutritional value of food grain legumes, is not always fully understood and appreciated by consumers; it is composed of two large groups, positive and negative factors. The positive factors include high protein and lysine content, which allows legumes to serve as excellent protein supplements to cereal grains[1]. The health related value of beans include: their positive effect on blood cholesterol and glucose levels[2], possibly through the dietary fiber present in beans. The negative factors fall into two groups: antinutritional factors include enzyme inhibitors, hemagglutinin, flatulence factors, polyphenols, tannin and phytic acid, which inhibit the proteolytic activity of the digestive enzymes such as pepsin and trypsin which reduced the availability of amino acids [3]. Condensed tannins have been reported to occur in appreciable amounts in legumes, they can cross-link with protein by reacting with lysine or methonine, making them unavailable during digestion (Davis, 1981). The degree of polymerization of these polyphenolic compounds plays an important role in both the effect of protein digestibility and the availability of vitamins and minerals[5]. Phytate content in legumes has been involved in reducing bioavailability of minerals[5] and inhibiting the activity of several enzymes[6]. Phytate is widely distributed in plants, especially in seeds; with high concentration in mature legumes, cereal grains and oil seeds[7,8]. In legumes phytate is uniformly distributed and integrated with protein bodies[9]. The ability of phytic acid to complex with metals is well known and is one of the nutritional concerns; it and its derivatives can complex with essential dietary minerals, thus making them unavailable or only partially available for absorption[10]. Phytate thus combines with Ca, Zn, Fe and other divalent metals to form complexes with low solubility[10]. Magnesium as an essential element in human and animal nutrition, greatly affected by phytate content [11]. Polyphenols on the other hand can form complexes with metal cations through carboxylic and hydroxylic groups and thus interfere with the intestinal absorption of minerals[12,14]. To reduce the harmful effect of antinutrients, soaking could be one of the processes to solublize such factors, however, some metabolic reactions will take place during soaking, affecting the content of some compounds[15]. Cooking

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generally inactivates heat sensitive factors such as trypsin and chymotrypsin inhibitors and volatile compounds. Therefore, in the present work we would like to investigate the effect of local processing methods on antinutrional factors and availability of minrals of faba bean cultivars.

MATERIALS AND METHODS

Two faba bean (Vicia faba L.) cultivars; Bsabir, Hudieba-72 were obtained from Hudieba Agricultural Research Station, Sudan. The seeds were carefully cleaned and freed from foreign materials and the grains were ground to pass 0.4 mm screen. All reagents used in this study were of ragent grade.

Processing

Soaking: Seeds were soaked in water for 24, 48 and 72 h at room temperature (30±2°C) with seed to water ratio of 1:5 (w/v). Thereafter, the soaked seeds were washed twice with ordinary water followed by rinsing with distilled water and then dried in a hot air oven at 50°C for 24 h.

Cooking: Seeds were soaked for 12 h and then rinsed with distilled water and placed in round-mouthed tall beakers fitted with condensers. The content of the beaker were boiled to soft as felt between fingers. Cooked seeds, along with cooking water, were dried at 50°C for 24 h. Unsoaked seeds were also cooked in the same manner, using a seed to water ratio of 1:7 (w/v) and then dried.

Analytical methods

Total minerals: Minerals were determined according to Chapman and Pratt (1961) method. About 2.0 g of sample was placed in a muffle furnace at 550°C for 4 h, then the samples were cooled and 10 mL of 3N HCl was added, covered with watch glass and boiled gently for 10 min. The content was cooled and diluted to 100 mL with distilled water. Fe, Mn, Cu, Zn, Co were determined by atomic absorption spectrophotometry (Perkin Elmer 2380), Ca and Mg were determined by titration[16], K and Na were determined by flame photometry (Cornig EEL) and P was determined spectrophotometrically [17].

Hcl extractable minerals: Hydrochloric acid extractability of minerals was performed according to[18] method. About 1.0 g was extracted using 10 mL of 0.03N HCl with shaking at 37°C for 3 h. The clear extract obtained was dried at 100°C and then placed in a muffle furnace at 550°C for 4 h. Thereafter, the samples were cooled and about 5 mL of 5N HCl were added and boiled gently for 10 min and then cooled, diluted to 100 mL with distilled water. Minerals were determined as described above.

Minerals extractability (%)=

$$\frac{\text{Mineral extracted with 0.03N Hcl}}{\text{Total minerals}} \times 100$$

Potassium and sodium determination: Potassium and sodium contents were determined according to[17] method.

Phosphorus determination: Phosphorus was determined using the molybdovanadate method as described by Chapman and Pratt [19] using spectrophotometer (Model 6305 - England).

Calcium and magnesium determination: Calcium and magnesium were determined according to Champan and Pratt [19] method.

Phytic acid determination: Phytic acid content was determined by the method described by Wheeler and Ferrel [20] using 2.0 g of a dried sample. A standard curve was prepared expressing the results as $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming 4:6 iron to phosphorus molar ratio.

Total polyphenols determination: Total polyphenols were determined according to Purssion Blue spectrophotometric method (Price and Butler, 1977) with a minor modification. Sixty milligrams of ground sample were shaken manually for 1 min in 3.0 mL methanol. The mixture was filtered. The filtrate was mixed with 50 mL of distilled water and analyzed within an hour. About 3.0 mL of 0.1 M $FeCl_3$ in 0.1 M HCl were added to 1 mL of the filtrate followed immediately by timed addition of 3.0 mL of freshly prepared $K_3Fe(CN)_6$. The absorbance was monitor on a spectrophotometer (Pye Unicam SP6-550 UV) at 720 nm after 10 min from the addition of 3.0 mL of 0.1 M $FeCl_3$ and 3.0 mL of 0.008 M $K_3Fe(CN)_6$. A standard curve was prepared expressing the result as tannic acid equivalent i.e. amount of tannic acid (mg/100 g) which gives a color intensity equivalent to that given by polyphenols after correction for blank.

Statistical analysis: Each sample was analyzed in triplicate and the values were then averaged. Data were assessed by the analysis of variance (ANOVA) described by Snedecor and Cochran [21] and by Duncan'multiple range test [22] with a probability P = 0.01.

RESULTS AND DISCUSSION

Total and available major and trace minerals: Total and available minerals of faba bean cultivars is shown in Table 1. Na content and availability was found to be significantly (p=0.01) higher for the cultivar Bsabir compared to Hudieba-72. El Tinay *et al.* [23] reported similar results but Khalil [24] reported lower ones for Egyptian variety. Both cultivars contained similar amount of K and Ca. However, the available K and Ca for Bsabir was significantly (p=0.01) higher. Bsabir contained higher amount of significantly (P=0.01) Mg compared to Hudieba-72 with no significant difference in available Mg. Results obtained in this study were lower than those reported by Khalil [24] but higher than those reported by El Tinay et al. [23]. P content for the two cultivars was differ slightly and its avalability was very low for both cultivars. Higher availability of Ca and Na was observed in Bsabir cultivar despite that it contained higher level of phytic acid and total polyphenols. This observation is a departure from an otherwise opposing relationship between phytate and available Ca and Na. The explanation for this is not clear, but c ould be due to qualitative differences between phytate of the cultivars. These variations could lead to differences in response to the low concentration of phytate which is not observable under the more powerful influence of higher concentrations. Both Fe content and availability were observed to be higher for the cultivar Bsabir (Table 1) compared to Hudieba-72. Other trace minerals follow similar trend. Similar results were observed by Khalil [24] and El Tinay *et al.* [23]

Effect of soaking in water on phytic acid and polyphenols: Effect of soaking on antinutrients and total minerals of faba bean cultivars is shown in Table 2. The cultivar Bsabir contained higher amount of phytic acid and polyphenols compared to Hudieba-72. Soaking for 1, 2 and 3 days significantly (p=0.01) reduced phytic acid and polypenols contents for the two cultivars. The longer the time of soaking, the greater the loss of phytic acid and polyphenols. Phytic acid for both Hudieba-72 and Bsabir cultivars was reduced by 10.4 and 5.7% after soaking for 3 days, respectively. Hudieba-72 showed the highest reduction in phytic acid compared to Bsabir. Total polyphenols of cultivar Hudieba-72 was reduced by 9.5% while that of Basbir reduced by 24.8% after soaking for 3 days.

Table 1: Content (mg/100 g) and availability (%) of minerals of faba bean cultivars

	Cultivars			
	Hudieba-72		Bsabir	
Minerals	Content	Availability	Cotent	Availability
Na	26.60(±0.18)	70.22(±0.02)	35.94(±10.39)	74.30(±0.02)
K	1037.45(±6.93)	7.80(±7.80)	1030.66(±1.89)	12.83(±0.02)
Ca	427.17(±1.14)	33.74(±1.90)	422.92(±0.70)	50.04(±0.10)
Mg	255.32(±2.54)	22.12(±0.44)	288.58(±2.73)	24.18(±0.26)
P	196.63(±0.68)	8.21(±0.60)	207.99(±0.16)	2.43(±0.11)
Fe	5.97(±0.06)	13.38(±0.48)	6.47(±0.02)	18.45(±0.79)
Mn	2.69(±0.04)	7.06(±0.05)	2.01(±0.05)	8.34(±0.97)
Cu	0.72(±0.01)	21.17(±1.58)	0.86(±0.05)	18.02(±1.02)
Co	1.21(±0.01)	21.07(±0.18)	1.70(±0.10)	40.25(±0.84)
Zn	2.22(±0.02)	8.24(±0.60)	2.78(±0.02)	14.45(±1.27)

Values are means(±SD)

Table 2: Effect of soaking in water on phytic acid and polyphenols contents (mg/100 g) of faba bean cultivars

		Phytic acid		Total polyphenols	
Cultivars	Soaking time (days)	Total	% reduction	Total	% reduction
Hudieba-72	0	291.15(±0.13)	-	322.08(±0.13)	-
	1	290.22(±0.01)	0.32	311.68(±0.01)	3.23
	2	281.83(±0.03)	3.69	308.23(±0.06)	4.30
	3	260.92(±0.27)	10.38	291.41(±1.00)	9.50
Bsabir	0	298.91(±6.02)	-	338.64(±0.06)	-
	1	295.17(±0.02)	1.27	333.52(±0.01)	1.51
	2	288.29(±0.01)	3.55	321.48(±0.01)	5.07
	3	281.92(±0.03)	5.68	292.25(±0.01)	13.70

Table 6: Effect of soakin and/or cooking on minerals content (mg/100 g) of faba bean cultivars

Cultivars	Processing	Na	K	Ca	Mg	P	Fe	Mn	Cu	Co	Zn
Hudieba-72	Untreated	26.60 (±0.18)^{b}	1037.45 (±6.94)^{b}	427.17 (±1.14)^{b}	255.32 (±2.54)^{b}	196.63 (±0.68)^{b}	5.97 (±0.06)^{b}	2.69 (±0.04)^{b}	0.72 (±0.01)^{b}	1.21 (±0.01)^{b}	2.22 (±0.02)^{b}
	Cooking	16.61 (±0.29)^{c}	916.19 (±1.42)^{c}	356.28 (±0.54)^{c}	198.51 (±0.31)^{c}	167.55 (±1.01)^{c}	4.24 (±0.03)^{c}	1.48 (±0.02)^{c}	0.53 (±0.01)^{c}	0.87 (±0.02)^{c}	1.5 (±0.18)^{c}
	Saoking & cooking	14.29 (±0.01)^{d}	727.29 (±0.86)^{d}	306.23 (±0.36)^{d}	189.18 (±1.4)^{d}	159.58 (±1.75)^{d}	3.77 (±0.03)^{d}	1.37 (±0.08)^{d}	0.44 (±0.01)^{d}	0.78 (±0.78)^{d}	1.2 (±0.01)^{d}
Bsabir	Untreated	35.94 (±10.39)^{b}	1030.66 (±1.89)^{b}	422.92 (±0.70)^{b}	288.58 (±2.73)^{b}	207.99 (±0.38)^{b}	6.47 (±0.02)^{b}	2.01 (±0.10)^{b}	0.86 (±0.05)^{b}	1.70 (±0.01)^{b}	2.78 (±0.02)^{b}
	Cooking	28.62 (±0.06)^{c}	656.26 (±40.58)^{c}	321.43 (±0.53)^{c}	202.50 (±3.54)^{c}	178.11 (±1.80)^{c}	4.96 (±0.23)^{c}	1.31 (±0.2)^{c}	0.53 (±0.03)^{c}	1.47 (±0.03)^{c}	2.42 (±0.02)^{c}
	Saoking &	26.43 (±0.0)^{c}	644.79 (±15.14)^{c}	276.35 (±0.47)^{d}	172.44 (±0.29)^{d}	170.99 (±0.29)^{d}	4.63 (±0.16)^{c}	1.04 (±0.1)^{d}	0.43 (±0.01)^{d}	1.38 (±0.02)^{d}	2.29 (±0.08)^{d}

(Values are means±SD), Means in a column not sharing a common superscript letter are significantly($P \leq 0.01$) different as assessed by Duncan's multiple range test

Table 7. Effect of soaking and/or cooking on minerals availability(%) of faba bean cultivars

Cultivars	Processing	Na	K	Ca	Mg	P	Fe	Mn	Cu	Co	Zn
Hudieba-72	Untreated	70.22 (±0.02)^{d}	7.80 (±0.62)^{d}	33.74 (±1.00)^{d}	22.12 (±0.44)^{c}	8.21 (±0.6)^{d}	13.38 (±0.48)^{d}	7.06 (±0.05)^{d}	21.17 (±1.58)^{d}	21.07 (±0.18)^{d}	8.23 (±0.6)^{d}
	Cooking	77.37 (±0.12)^{c}	33.59 (±1.00)^{c}	60.68 (±0.09)^{c}	44.10 (±1.78)^{b}	15.04 (±0.11)^{c}	36.68 (±0.7)^{c}	26.71 (±0.35)^{c}	51.09 (±1.64)^{c}	66.82 (±1.52)^{c}	22.20 (±1.3)^{c}
	Soaking & cooking	77.58 (±0.09)^{b}	52.63 (±0.001)^{b}	63.41 (±5.7)^{b}	44.24 (±0.1)^{b}	15.83 (±0.19)^{b}	45.47 (±2.04)^{b}	31.22 (±1.7)^{b}	68.48 (±4.94)^{b}	79.03 (±2.82)^{b}	33.7 (±1.82)^{b}
Bsabir	Untreated	74.28 (±0.04)^{c}	12.83 (±0.02)^{d}	50.04 (±0.10)^{c}	24.18 (±0.26)^{c}	2.43 (±0.11)^{d}	18.45 (±0.79)^{c}	8.34 (±0.97)^{d}	18.02 (±1.02)^{d}	40.25 (±0.84)^{d}	14.45 (±1.27)^{d}
	Cooking	78.00 (±0.73)^{b}	40.92 (±2.51)^{c}	70.72 (±1.19)^{b}	44.45 (±0.71)^{b}	7.94 (±0.31)^{c}	44.34 (±1.58)^{b}	33.11 (±0.6)^{c}	38.75 (±2.47)^{c}	63.95 (±1.11)^{c}	39.79 (±3.33)^{c}
	Soaking & cooking	78.41 (±0.4)^{b}	53.55 (±1.27)^{b}	71.85 (±0.12)^{b}	64.08 (±0.29)^{a}	9.39 (±0.37)^{b}	47.95 (±3.88)^{b}	36.85 (±0.7)^{b}	50.97 (±1.6)^{b}	69.92 (±0.99)^{b}	45.62 (±0.96)^{b}

Values are means(±SD), Means in a column not sharing a common superscript letter are significantly($P \leq 0.01$) different as assessed by Duncan's multiple range test

the soaking water. Similar observation was reported by Tinay *et al.*, [23] and Khalil [24]. Incontrast, soaking of faba bean seeds in water significantly (p=0.01) improved the availability of both major and trace minerals for both cultivars (Table 4). The rate of increment depend on soaking time, cultivar and type of mineral. It was observed that available Na of the cultivar Hudieba-72 was increased from 70.22 to 76.38% while that of Bsabir increased from 74.28 to 76.96% after soaking for 3 days. The available Fe increased from 13.38 to 33.02% for Hudieba-72 while for Bsabir increased from 18.45 to 36.14%. All other minerals followed similar trend for both cultivars. Reduction in total minerals is likely due to washing out in the soaking water while increase in availability is likely due to reduction of antinutrional factors due to soaking of seeds in water. Duhan *et al.*, [25] reported that availability of phosphorus was significantly improved when pigeon pea seeds were soaked in distilled water. The results obtained in this study disagree with Hassan *et al.*,who reported that soaking of lupin in distilled water for 3 days decreased potassium availability. Reduction was attributed to complexation of potassium with other food constituents.

Effect of soaking and/or cooking on phytic acid and polyphenols: Effect of soaking and/or cooking in water on phytic acid and polyphenols content of faba bean cultivars is shown in Table 5. The cultivar Bsabir contained higher amount of phytic acid and polyphenols compared to Hudieba-72. Cooking of the seeds significantly (p=0.01) reduced both phytic acid and polypenols contents for the two cultivars. Further reduction in phytic acid and polypenols contents was observed when the seeds were cooked after being soaked in distilled water. Many researchers[23-25] reported similar result.

Effect of soaking and /or cooking on total and available minerals: The effect of soaking and/or cooking of faba bean seeds in water on total minerals is shown in Table 6. Cooking of both cultivars seeds significantly (p=0.01) decreased both major and trace minerals contents. However, for both cultivars cooking after soaking was observed to cause greater rduction compared to cooking alone. It was observed that Na content of the cultivar Hudieba-72 was reduced from 26.6 to 14.29 mg/100 g when the seeds were cooked after being soaked in water while that of Bsabir reduced from 35.94 to 26.43 mg/100 g. Fe reduced from 5.97 to 3.77 mg/100 g for Hudieba-72 while for Bsabir reduced from 6.47 to 4.63. All other minerals followed similar trend for both cultivars. Reduction in total minerals is likely due to heat treatment during cooking which may cause complexation of minerals with other food constituents. Similar observation was reported by El Tinay et al. [23] and Khalil [24]. Incontrast, cooking of the seeds after soaking soaking of the cultivars in water significantly (P = 0.01) improved the availability of both major and trace minerals for both cultivars (Table 7). It was observed that available Na of the cultivar Hudieba-72 was increased from 70.22 to 77.58% while that of Bsabir increased from 74.28 to 78.41% when the seeds were cooked after soaking. The available Fe increased from 13.38 to 45.47% for Hudieba-72 while for Bsabir increased from 18.45 to 47.95%. All other minerals followed similar trend for both cultivars. Reduction in total minerals was likely due to leaching out with water as well as water vapor. The improvement in minerals availability may be due to reduction in phytic acid and polyphenols contents as due to soaking and heating. Duhan et al. [25] reported that availability of minerals was significantly improved when pigeon pea seeds were soaked in distilled water.

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