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Content of antinutritional factors and HCl-extractability of minerals from white bean (Phaseolus vulgaris) cultivars: Influence of soaking and/or cooking

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Abstract

White bean seeds were subjected to soaking in distilled water for 1, 2 and 3 days. In order to perform complete processing, the seeds were cooked until soft. Effects of soaking and/or cooking of white bean seeds on antinutritional factors, mineral contents and HClextractability were studied. Phytic acid and polyphenol contents of all cultivars were reduced. Soaking alone and soaking, followed by cooking, reduced mineral contents of the cultivars, but HCl-extractability was significantly ($P \le 0.01$) improved to varying extents, depending on cultivar type. Soaking and/or cooking treatment was thus found to be an effective technique and caused further improvement in the availability of both major and trace minerals in white bean. $© 2005 Elsevier Ltd. All rights reserved.$

Keywords: White bean; Cultivars; Minerals; Soaking; Cooking; Antinutrients

1. Introduction

Legumes play an important role in human nutrition since they are rich source of protein, calories, certain minerals and vitamins [\(Deshpande, 1992\)](#page-5-0). In African diets, legumes are the major contributors of protein and calories for economic and cultural reasons. Antinutrients, especially phytate, a powerful chelating agent, reduce the bioavailability of divalent cations by the formation of insoluble phytates [\(Reddy & Salunkhe, 1981](#page-6-0)). Hence, it is imperative to lower the amount of phytate, as well as other antinutrients, by processing methods, so as to improve the nutritional quality of the pulses. Various processing methods, namely soaking, dehulling, sprouting, have been reported to lower the level of antinutrients, and especially phytic acid [\(Rani & Hira, 1993\)](#page-6-0). The most effective treatments are fermentation ([Marfo, Simpson, Idowu, & Oke,](#page-5-0)

Corresponding author. E-mail address: elfadilbabiker@yahoo.com (E.E. Babiker). [1990\)](#page-5-0) and germination ([Honke, Kozlowska, Vidal-Valv](#page-5-0)[erde, & Gorecki, 1998](#page-5-0)) but their application remains limited because of the additional workload they imply or the particular organoleptic properties they induce. Soaking is a domestic technological treatment that is often used by mothers to prepare complementary foods at home. Moreover, it can be a simple prolongation of the obligatory washing of the seeds and can also have other advantages, such as facilitating dehulling or swelling of seeds. Previous studies ([Duhan, Khetarpaul, & Bishnoi, 2002;Sandberg &](#page-5-0) [Svanberg, 1991\)](#page-5-0) have shown that a long soaking period before fermentation or germination reduced phytate content and enhanced mineral HCl-extractability (Index of mineral bioavailability). Processing namely, soaking, dehulling and germination, improved the extractability of Ca, Fe and P to varying extents but sprouting was found to be the best among different processing methods for enhancing the extractability of Ca, Fe and P ([Saharan,](#page-6-0) [Khetarpaul, & Bishnoi, 2001](#page-6-0)). In Sudan, legume grains are generally processed before consumption, depending

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upon cultural and taste preferences. The most commonly used domestic methods for processing of legumes include soaking for different time periods and ordinary and pressure-cooking and sometimes germination; and these have been reported to be beneficial for enhancing the nutritive value of some food legumes, including white bean, peas, faba bean, and pigeon bean. Therefore, this study was conducted to find the effects of soaking and cooking on contents of antinutrients and HCl-extractability of minerals of white bean cultivars.

2. Materials and methods

2.1. Source, soaking and cooking of seeds

Three white bean *(Phaseolus vulgaris)* cultivars *(Serege,* Giza and RO21) were obtained from Hudieba Agricultural Research Station, Sudan. The seeds were carefully cleaned and freed of foreign materials and the seeds were ground to pass a 0.4 mm screen. Seeds were soaked in water for 1, 2 and 3 days at room temperature $(30 \pm 2 \degree C)$ with a 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. Seeds, before and after soaking, were placed in round-mouthed tall beakers fitted with condensers. The contents of the beaker were cooked until they felt soft between fingers. Cooked seeds, along with cooking water, were dried at 50 \degree C for 24 h. All reagents used in this study were of reagent grade.

2.2. Total minerals determination

Minerals were determined in the samples by the dry-ashing method described by [Chapman and Pratt \(1961\).](#page-5-0) The amounts of iron, zinc, manganese, cobalt and copper were determined using atomic absorption spectroscopy (Perkin– Elmer 2380). Ammonium vanadate was used to determine phosphorus, by the ammonium molybdate method of [Chapman and Pratt \(1982\)](#page-5-0). Calcium and magnesium were determined by a titration method described by [Chapman](#page-5-0) [and Pratt \(1961\).](#page-5-0) Sodium and potassium were determined by flame photometer (CORNIG EEL), according to [AOAC \(1984\).](#page-5-0)

2.3. HCl-extractable minerals

Hydrochloric acid extractability of minerals was performed according to the [Chauhan and Mahjan \(1988\)](#page-5-0) method. About 1.0 g was extracted using 10 ml of 0.03 N HCl with shaking at 37° C for 3 h. Thereafter, the extract was filtered and the clear filtrate 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 5 N HCl were added and boiled gently for 10 min and then cooled and diluted to 100 ml with distilled water. Minerals were determined as described above.

Mineral extractability %

$$
= \frac{\text{Mineral extraction in 0.03 N HCl}}{\text{Total mineral content}} \times 100
$$

2.4. Phytic acid determination

Phytic acid content was determined by the method of [Wheeler and Ferrel \(1971\)](#page-6-0) using 2.0 g of a dried sample. A standard curve was prepared, expressing the results as $Fe(NO₃)₃$ equivalents. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

2.5. Total polyphenols determination

Total polyphenols were determined according to the Prussian Blue spectrophotometric method [\(Price & Butler,](#page-6-0) [1977](#page-6-0)) with a minor modification. Sixty milligrammes of ground sample was shaken manually for 1 min in 3.0 ml of methanol. The mixture was filtered. The filtrate was mixed with 50 ml of distilled water and analyzed within 1 h. About 3.0 ml of 0.1 M FeCl₃ in 0.1 M HCl was 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 monitored in a spectrophotometer (Pye Unicam SP6 – 550 UV) at 720 nm 10 min after the addition of 3.0 ml of 0.1 M FeCl₃ and 3.0 ml of 0.008 M $K_3Fe(CN)_6$. A standard curve was prepared, expressing the result as tannic acid equivalents, i.e. amount of tannic acid (mg/100 g) which gives a colour intensity equivalent to that given by polyphenols after correction for blank.

2.6. Statistical analysis

Each sample was analyzed in triplicate and the values were then averaged. Data were assessed by the analysis of variance (ANOVA) as described by [Snedecor and Coch](#page-6-0)[ran \(1987\)](#page-6-0) and by Duncan' [multiple range test \(1955\)](#page-5-0) with a probability $P \leqslant 0.01$.

3. Results and discussion

3.1. Effect of soaking and/or cooking on phytate and polyphenols

The phytate contents of the cultivars varied from 352 to 457 mg/100 g DM. The cultivars are rich in protein (21.3– 26.8%), therefore they had high phytate levels. In legumes, phytates are associated with protein bodies [\(Reddy, Sathe,](#page-6-0) [& Salunkhe, 1982](#page-6-0)) and, therefore, phytate levels should increase with increasing protein content. Depending on the type of cultivar, a significant reduction ($P \le 0.01$) in phytate content (between 4% and 16%) was obtained by soaking whole seeds for 3 days at 30 $\rm{°C}$ ([Table 1](#page-2-0)). Results revealed that soaking for different time periods could lower the level of this antinutrient below the control value.

Table 1

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

RO 21 0 396 (± 0.07)^a – 219 (± 0.01)^a –

1 345 $(\pm 0.02)^b$ 2 671 $(\pm 0.01)^b$ 0.8 2 343 $(\pm 0.02)^c$ 3 611 $(\pm 0.02)^c$ 9 3 340 $(\pm 0.01)^d$ 4 589 $(\pm 0.02)^d$ 13

1 382 $(\pm 0.06)^b$ 4 216 $(\pm 0.03)^b$ 2 2 375 $(\pm 0.03)^c$ 6 205 $(\pm 0.06)^c$ 6 3 328 $(\pm 0.01)^d$ 15 183 $(\pm 0.02)^d$ 16

Longer the periods of soaking caused greater losses in the phytic acid content. The loss in phytates during soaking of white bean may be due to leaching of phytate ions into the soaking water under the influence of a concentration gradient (difference in chemical potential), which governs the rate of diffusion. Similar results for reduction in phytic acid in the soaked bean have been earlier reported ([Bish](#page-5-0)[noi, Khetarpaul, & Yadav, 1994](#page-5-0)). Ordinary cooking of unsoaked and soaked white bean seeds brought about a significant decrease in phytic acid content when compared to the control (Table 2). A reduction in phytic acid content was noticed after ordinary cooking of unsoaked seeds but this loss appeared to be less than that in seeds, which were cooked after soaking. The percent loss in phytic acid content of soaked and cooked seeds was slightly higher than that when the seeds were cooked without soaking. According to ([de Boland, Garner, & O](#page-5-0)'dell, 1975), the differences in the loss of phytic acid contents during cooking could probably be explained on the basis that phytase activity at a temperature of $40-55$ °C may degrade inositol hexaphosphate to the pentaphosphate or lower molecular weight forms. [Kumar, Venkataraman, Jaya, and Krishna-](#page-5-0) [murthy \(1978\)](#page-5-0) observed that phytic acid content decreased during cooking because insoluble complexes between phytate and other components were formed and, accordingly, the amount of free phytate was reduced. Polyphenol contents of the different raw materials varied from 219 to 676 mg/100 g DM. For all cultivars, results obtained for polyphenols had a trend similar to those reported for phytate after soaking in water (Table 1) or soaking and cooking of seeds (Table 2).

% Reduction

3.2. Effect of soaking and/or cooking on total and HClextractable minerals

Mineral contents varied between the cultivars. White bean was found to be rich in calcium. Calcium contents of unprocessed cultivars were 377, 346 and 321 mg/100 g for Serege, Giza-3 and ROH21 cultivars, respectively [\(Table 3\)](#page-3-0) which decreased to 191, 240 and 188 mg/100 g for the cultivars, respectively, after 3 days of soaking [\(Table 3](#page-3-0)). They further decreased to 162, 201 and 148 mg/100 g, for the cultivars, respectively, when the soaked seeds were cooked ([Table 4](#page-3-0)). Therefore, the results

Table 2

Effect of soaking and/or cooking on phytic acid and polyphenol contents (mg/100 g) DM of faba bean cultivars

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

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

Table 4

Effects of soaking and/or cooking on total minerals(mg/100 g) of white bean cultivars

Cultivars	Treatment	Na	K	Ca	Mg	P	Fe	Mn	Cu	Co	Zn
Serage	Untreated	23.6 $(\pm 0.001)^{b}$	1291 $(\pm 1.06)^b$	377 $(\pm 1.38)^b$	289 $(\pm 1.87)^b$	204 $(\pm 3.63)^b$	10.7 $(\pm 0.10)^{\circ}$	2.86 $(\pm 0.05)^{b}$	$0.68~(\pm 0.02)^b$	1.64 (± 0.02)	2.75 $(\pm 0.03)^{b}$
	Cooked	17.3 $(\pm 0.001)^c$	965 $(\pm 4.5)^{\circ}$	175 $(\pm 0.33)^{\circ}$	201 $(\pm 9.06)^{\circ}$	176 $(\pm 1.92)^{\circ}$	7.36 $(\pm 0.20)^{\circ}$	1.64 $(\pm 0.21)^{\circ}$	$0.27~(\pm 0.01)^c$	1.19 $(\pm 0.01)^{\circ}$	2.24 $(\pm 0.06)^{\circ}$
	Soaked and cooked	16.8 $(\pm 0.001)^d$	880 (± 1.43) ^d	162 $(\pm 0.31)^c$	193 $(\pm 3.47)^{\circ}$	162 $(\pm 3.73)^d$	6.35 $(\pm 0.11)^{a}$	1.32 $(\pm 0.08)^d$	$0.23~(\pm 0.010)^{\alpha}$	1.12 $(\pm 0.01)^{\circ}$	2.08 $(\pm 0.06)^{\circ}$
Giza-3	Untreated	22.8 $(\pm 0.001)^b$	1151 $(\pm 13.2)^b$	346 $(\pm 3.19)^b$	312 $(\pm 2.71)^b$	$210 \ (\pm 1.63)^b$	9.40 $(\pm 0.14)^b$	2.88 $(\pm 0.07)^{b}$	$0.64~(\pm 0.01)^b$	1.52 $(\pm 0.05)^k$	2.62 $(\pm 0.01)^{b}$
	Cooked	11.1 $(\pm 0.01)^c$	744 $(\pm 17.0)^{\circ}$	218 $(\pm 0.57)^{\circ}$	263 $(\pm 2.47)^{\circ}$	154 $(\pm 3.84)^c$	6.91 $(\pm 0.18)^c$	2.19 $(\pm 0.07)^{\circ}$	0.49 $(\pm 0.02)^{\circ}$	$1.15~(\pm 0.05)^{\circ}$	2.36 $(\pm 0.07)^{\circ}$
	Soaked and cooked	10.1 $(\pm 0.005)^{\circ}$	$703(\pm 20.4)^{\circ}$	201 $(\pm 0.81)^d$	243 $(\pm 6.42)^d$	141 $(\pm 3.86)^d$	6.65 $(\pm 0.12)^{\circ}$	1.81 $(\pm 0.11)^{a}$	0.45 $(\pm 0.01)^{\circ}$	$1.05~(\pm 0.03)^{\circ}$	2.27 $(\pm 0.050)^{\circ}$
R ₀₂₁	Untreated	27.1 $(\pm 0.001)^{\circ}$	1319 $(\pm 9.77)^b$	321 $(\pm 0.52)^b$	300 $(\pm 0.89)^a$	185 $(\pm 3.87)^b$	8.60 $(\pm 0.07)^{b}$	2.22 $(\pm 0.03)^b$	$0.52~(\pm 0.001)^{\circ}$	$1.16~(\pm 0.02)^{1}$	2.46 $(\pm 0.04)^{b}$
	Cooked	18.1 $(\pm 0.002)^c$	853 $(\pm 16.6)^{\circ}$	161 $(\pm 1.17)^c$	250 $(\pm 3.17)^{\circ}$	166 $(\pm 4.86)^{\circ}$	6.35 $(\pm 0.26)^{\circ}$	1.74 $(\pm 0.003)^{\circ}$	0.39 $(\pm 0.01)^c$	$0.80~(\pm 0.01)^{\circ}$	2.12 $(\pm 0.04)^{\circ}$
	Soaked and cooked	17.6 $(\pm 0.003)^{\circ}$	838 (± 8.54)	148 $(\pm 0.16)^d$	213 $(\pm 0.23)^c$	144 $(\pm 4.39)^d$	5.83 $(\pm 0.21)^c$	1.55 $(\pm 0.050)^{\alpha}$	$0.35~(\pm 0.02)^d$	$0.73~(\pm 0.02)^{\circ}$	2.10 $(\pm 0.02)^{\circ}$

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

indicated that soaking of the seeds, with and without cooking, significantly ($P \le 0.01$) reduced the calcium content of white bean cultivars. The loss of calcium during the treatment may be attributed its leaching out in to the discarded water. The results are in close consistence with the results of [Duhan et al. \(2002\)](#page-5-0) who also reported a significant decline in the total calcium content on water soaking. For all cultivars, all other major minerals followed a trend similar to that obtained for calcium. The iron contents of raw samples were 10.7, 9.4 and 8.59 mg/100 g for Serege, Giza-3 and ROH21 cultivars, respectively. Soaking of the seeds for 3 days reduced iron content to 8.42, 7.39 and 7.09 ([Table 3](#page-3-0)) and, with soaking followed by cooking ([Table 4\)](#page-3-0), they were further reduced to 6.35, 6.65 and 5.83 for the cultivars, respectively. This may be due to loss of iron in the soaking medium. The results are in good agreement with those of [Lestienne,](#page-5-0) Icard-Vernie`Claire, Mouquet, Picq, and Treche (2005), who observed reduction in iron content of the soaked grains as compared to raw ones. For all cultivars, all other trace minerals followed a trend similar to that obtained for iron. HCl-extractabilities of calcium in control samples were found to be 44%, 32.4% and 24.4% for Serge, Giza-3 and ROH21 cultivars, respectively (Table 5). Extractable calcium level, after soaking of the seeds for 3 days, significantly increased to 55.4%, 53.8% and 49.5% for the cultivars, respectively (Table 5). Further increase in calcium extractability was observed after cooking the soaked seeds and it was increased to 62.2%, 66.5% and 67.1% for the cultivars, respectively ([Table](#page-5-0) [6\)](#page-5-0). This clearly indicates that a successive increase in the calcium extractability of white bean occurred with increase in the soaking period and cooking of the soaked seeds. Divalent cations, such as Ca, are generally present in association with phytic acid, this may be responsible for its lower extractability. However, reduction in phytic acid as a result of soaking and cooking may explain higher HCl-extractability of calcium and other minerals [\(Duhan et al., 2002\)](#page-5-0). For all cultivars, HCl-extractability of all other major minerals followed a trend similar to that obtained for calcium [\(Table 6\)](#page-5-0). As a result of soaking in water, HCl-extractability of iron increased significantly $(P \le 0.01)$ from the control values of 15.7%, 11.7% and 7.74% for the cultivars Serge, Giza-3 and RO21, respectively, to 32.0% , 23.5% and 19.2% for the cultivars, respectively, within 3 days of soaking of white bean seeds (Table 5). However, significant increase in HCl-extractability of iron was observed after cooking the seeds and it was increased to 47.3, 49.4 and 45.3 for the cultivars, respectively ([Table 6](#page-5-0)). For all cultivars, HCl-extractability of all other trace minerals followed a trend similar to that obtained for iron [\(Table 6\)](#page-5-0). As a divalent cation, Fe, is also generally present in association with phytic acid, and this may be responsible for its lower extractability. However, reduction in phytic acid as a result of soaking and cooking may explain higher HCl-extractability of iron and other trace minerals ([Duhan et al., 2002](#page-5-0)).

4. Conclusion

Although soaking and/or cooking brought about a decline in total mineral content of white bean cultivars, the HCl-extractability of both major and trace minerals increased significantly as the period of soaking was prolonged. The losses in mineral contents may be ascribed to leaching of these minerals into the soaking medium. Dietary essential minerals, such as phosphorus, calcium and iron, are present in association with antinutrients and this may be the reason for their lower HCl-extractabilities. Improvement in HCl-extractability, which is an index of the bioavailability of minerals, may be explained by the soaking and cooking which release these minerals in free form, thereby increasing their HCl-extractability. Thus, cooking of the seeds after soaking can be considered as a beneficial technique for improving the bioavailability of minerals.

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