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Some nutritional attributes of bambara groundnut as influenced by domestic processing

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Abstract

Bambara groundnut (*Vigna subterranean*) seeds were subjected to soaking in distilled water for 14 hours. In order to perform complete processing, the seeds were cooked until soft. The effect of soaking and/or cooking of the seeds on chemical composition, total energy, antinutritional factors, protein digestibility, mineral contents and extractability and amino acid composition were studied. Most of the seeds nutrients were reduced during soaking and cooking but the total energy was increased. Tannin, polyphenols and phytic acid contents were reduced after soaking and cooking of the seeds with a concomitant increase in protein digestibility. Soaking alone and soaking followed by cooking reduced mineral contents of the seed, but HCl-extractability was significantly ($P \leq 0.05$) improved to varying extents. Amino acid composition was slightly increased after soaking and cooking of the seeds. Soaking and/or cooking treatment was thus found to be an effective technique and caused further improvement in the availability of nutrients in bambara seeds.

Introduction

Legumes are important major sources of plant protein and fats in tropical countries. They are good sources of essential amino acids and fats. The industrial application of them depends on the knowledge of nutritional importance and functional properties. Bambara bean [*Vigna subterranea* (L.) *verde*], one of these grain legumes, is widely cultivated in west and central Africa. A high carbohydrate (65%) and relatively high protein (18%) content as well as sufficient quantities of fat (6.5%) make the bambara groundnut rank highly as a complete food. However, lack of adequate processing techniques to overcome the hard-to-cook effect has limited its utilization and hence reduced its production. According to farmers, the decline in bambara groundnut production is due to lack of adequate processing techniques to promote utilization (Christina, 2009).

Bambara groundnut (*Vigna subterranean*) which belongs to family fabaceae is an annual herbaceous, intermediate plant with creeping stems at ground levels. It has a well-developed taproot with profuse geotropic lateral roots. New roots often appear where nodes contact soil. The fibrous lateral roots form nodules for nitrogen fixation. The stems are branched and hairy, with short internodes. The leaves are trifoliate and are borne on long slender petioles. The flowers spread out close to ground level on hairy peduncles, each producing one to three flowers. Most flowers are light yellow in color, although some are deep yellow (especially late in the day). After pollination, each small flower sends down a tendril, or peg, like a long root, which continues to burrow even after it has pierced the soil. Like peanut, the plant then forms pods on, or just beneath, the ground. The pod achieves its mature size about 30 days after fertilization. The seed further develops in the subsequent ten days. It is essentially grown for human consumption. It can be used as an ingredient in cooking, making flour, or eaten as a snack (Goli, 1995). It is very easy to grow because it grows in areas of law rain fall, in poor soils, and without fertilizers (Linnemann, 1990). The seeds contain

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sufficient amount of protein, carbohydrate and fat (Goli, 1995).

Insufficient protein of good quality is a serious problem in many developing countries because of the prohibitive cost of protein from animal sources. Alternative sources of proteins which could alleviate this problem include the proteins from different plants. Several workers have examined the biochemical composition of the seed (Okonkwo and Opara, 2010; Mune *et al.*, 2011) on average, the seeds were found to contain 49.72% carbohydrate, 21.18% protein and 6.38% fat. Lysine and Leucine were the predominant essential amino acids (Mune *et al*., 2011). Soaking followed by cooking is a domestic processing method at household level and used to prepare complementary foods at home (El Maki *et al*., 2007). In this study we would like to investigate the effect of domestic processing on nutritional attributes of bambara groundnut seeds.

Materials and Methods

Materials

Bambara groundnut seeds were purchased from AlGenina market, Western States, 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 14 h at room temperature (24 ± 2ºC) 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 50ºC 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 50ºC for 24 h. All reagents used in this study were of reagent grade.

Chemical composition determination

The chemical composition of raw and processed seeds was determined according to AOAC (1990) methods. Caloric value estimation was done according to Antia *et al.* (2006) by summing the multiplied values for crude protein, oil, and carbohydrate by their respective factors (4, 9, 4).

Tannin content determination

Quantitative determination of tannins was carried out using the modified vanillin-HCl method according to Price *et al.* (1978). A 200 mg sample was extracted with 10 ml 1% (v/v) conc. HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to extract (1 ml) and the absorbance of the colour developed after 20 min at 30°C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e amount of catechin (mg per ml) which gives a colour intensity equivalent to that given by tannins after correcting for blank.

Phytic acid content determination

Phytic acid content was determined according to the method described by Wheeler and Ferrel (1971) using 2.0 gm of dried sample. A standard curve of different $Fe(NO_3)_2$ concentrations was plotted to calculate the ferric ion concentration. Phytate phosphorus was calculated from the standard curve assuming 4:6 iron to phosphorus molar ratio.

Total polyphenols determination

Total polyphenols were determined by spectrophotometric method described by Price and Butler (1977). About 60 mg of the sample were shaken manually for 60 s with 3 ml of methanol in a test tube. The mixture was filtered, then the tube was quickly rinsed with additional 3.0 ml of methanol and the contents were poured at once into a funnel. The filtrate was mixed with 50 ml of water and analyzed within an hour. Three ml of 0.1 M $FeCl_3$ in 0.1 N HCl were added to 1.0 ml of filtrate, followed immediately by timed addition of 3 ml of 0.008 M $K_3Fe(CN)_6$. The absorbance was read at 720 nm after 10 min using spectrophotometer (Jenway 6306 UV/ vis spectrophotometer [London, UK]). Tannic acid was used to prepare a standard curve following the above procedure.

In vitro *protein digestibility determination*

In vitro protein digestibility of treated and untreated samples was measured according to the method of Maliwal (1983) with a minor modification. A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 ml of 0.1 M HCl at 37ºC for 2 h. The reaction was stopped by the addition of 15 ml of 10% trichloroacetic acid (TCA). The mixture was then centrifuged at 630 gm for 5 min. The mixture was then filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method (AOAC, 1990). Digestibility was obtained by using the following equation:

$$\text{Protein digestibility (\%)} = \frac{\text{N in supernatant-N in blank}}{\text{N in sample}} \times 100$$

Total minerals determination

Minerals were extracted from the samples by the dry ashing method described by Chapman and Pratt

(1982). About 2.0 gm of sample was acid-digested with diacid mixture (HNO_3:$HClO_4$, 5:1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was made to 50 ml with double-distilled water and was used for determination of total calcium, phosphorus and iron. Calcium was determined by a titration method. Iron was determined by atomic absorption spectrophotometer (Perkin-Elmer 2380). Phosphorus and other minerals were determined spectrophotometrically using molybdovanadate method.

*HCl extractability of minerals (*in vitro *bioavailability)*

Minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988). About 1.0 gm of the sample was shaken with 10 ml of 0.03 M HCl for 3 h at 37°C and then filtered. The clear extract obtained was oven-dried at 100°C and then diacid-digested. The amount of extractable minerals was determined by the methods described above. HCl extractability (%) was determined as follows:

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0:03N HCl (mg/100g)}}{\text{Total minerals (mg/100g)}} \text{ X100}$$

Determination of amino acid composition

The amino acids composition of the samples was measured on hydrolysates using amino acids analyzer (Sykam-S7130, Tokyo, Japan) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stain (1963). About 200 mg of the sample was taken in a hydrolysis tube. Then 5 ml of 6 N HCl was added to the sample and the tube tightly closed and incubated at 110°C for 24 h. After incubation, the solution was filtered and 200 ml of the filtrate was evaporated to dryness at 140°C for 1 h. The hydrolysates after dryness were diluted with 1.0 ml of 0.12 N citrate buffer (pH 2.2). Aliquot of 150μl of the sample hydrolysate was injected in an action separation column at 130°C. Ninhydrin solution and an eluent buffer (solvent A, pH 3.45 and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 ml/min. The buffer/ninhydrin mixture was heated in the reactor at 130°C for 2 min to accelerate chemical reaction of amino acids with ninhydrin. The products of the reaction mixture were detected at wavelengths of 570 and 440 nm on a dual channel photometer. The amino acids composition was calculated from the areas of standards obtained from the integrator and expressed as gm/100 gm protein.

Statistical analysis

Each determination was carried out on three separate samples and analyzed in triplicate on dry weight basis; the figures were then averaged. Data were assessed by the analysis of variance (Snedecor and Cochran, 1987). Comparisons of means for treatments were made using Duncan's multiple range tests. Significance was accepted at $P \geq 0.05$.

Results and Discussion

Effect of soaking and/or cooking on chemical composition and total energy

Table 1 presents the chemical composition of bambara groundnut seeds flour. The dry matter of raw flour was 93.3% which decreased after soaking of the seeds in water and again increased after cooking. The result obtained was higher than that of Okonkwo and Opara (2010), for bambara groundnut seeds. No significant difference was observed in dry matter after processing of bambara groundnut seeds. Ash content of the seeds was found to be 3.25% which was lower than that obtained by Abdulsalami and Sheriff (2010) and Mune *et al.* (2007) for bambara groundnut seeds. The ash content was slightly decreased after soaking but increased after cooking, which agree with the findings of Abdulsalami and Sheriff (2010). The protein content of bambara seeds was found to be 20.60% which was similar to that reported by Abdulsalami and Sheriff (2010) but higher than that reported by Okonkwo and Opara (2010). The protein content of the seeds was slightly decreased after soaking and even after cooking (19.41%). The results showed that processing of the seeds had no significant effect on protein content. However, an increment in protein content after soaking was also observed by Hassan *et al*. (2005) for Lupin seeds and explained that increment to quantitative reduction of the antinutritional factors (tannin and phytic acid) and other water soluble constituents. The decrease in protein content after cooking possibly due to solublization of protein by heating that lead to loss of protein in the final product as explained by Deman, (1999) or might be attributed to denaturation of it during heating as reported by Bradbury *et al.* (1984). Similar reduction in protein after cooking was observed by Hamed *et al*. (2008) and Hainida *et al.* (2008) for pumpkin and roselle seeds, respectively. Fiber content of bambara seeds was 6.34% which was similar to that obtained by Abdulsalami and Sheriff (2010), and higher than that reported by Mune *et al*. (2007). Fiber content of bambara seeds was slightly decreased after soaking but significantly ($P \leq 0.05$) after cooking (3.83%). Our findings supported the

data that obtained by Abdulsalami and Sheriff (2010) and Hainida *et al.* (2008) for bambara and roselle seeds, respectively. The fat content of bambara seeds was 6.60% which was similar to that reported by Abdulsalami and Sheriff (2010) and higher than that of Mune *et al*. (2007) for bambara seeds. Processing of the seeds had increased fat content after soaking of the seeds and significantly ($P \leq 0.05$) after cooking. These findings agree with those of Abdulsalami and Sheriff (2010). The carbohydrate content of bambara seeds was 56.51% which was similar to that reported by Okonkwo and Opara (2010). The carbohydrate content significantly ($P \leq 0.05$) increased after cooking but slightly decreased after soaking. The changes observed are possibly due to leaching of soluble components into soaking water (Yagoub and Abdalla, 2007). The energy content of bambara seeds significantly ($P \leq 0.05$) increased after soaking and cooking with a maximum value of 412.81 Kcal/100 gm. The calculated metabolizable energy values which ranged between 367.80 and 421.81 Kcal/100 gm showed that bambara seeds have energy concentrations favorable comparable to cereals.

Table 1. Chemical composition (%) and total energy (Kcal/100 gm) of treated and untreated bambara groundnut

Parameters	Treatments		
	Raw	Soaked	Cooked
Dry matter	93.30 (±0.09)^{a}	90.80 (±0.57)^{b}	94.90 (±1.27)^{a}
Protein	20.60 (±1.01)^{a}	19.28 (±1.12)^{a}	19.41 (±1.98)^{a}
Fat	6.60 (±0.07)^{a}	7.29 (±0.98)^{ab}	8.49 (±2.11)^{b}
Fiber	6.34 (±0.02)^{a}	5.29 (±0.00)^{b}	3.83 (±0.00)^{c}
Ash	3.25 (±0.33)^{a}	2.69 (±0.26)^{a}	3.13 (±0.33)^{a}
Carbohydrate	56.51 (±0.33)^{a}	56.25 (±1.83)^{a}	61.01 (±2.31)^{b}
Energy (Kcal/100gm)	367.80 (±1.29)^{a}	375.72 (±5.94)^{a}	421.81 (±17.68)^{b}

Values are mean (± SD) of triplicates. Values not sharing a row superscript in a row for each sample are significantly different at $p \leq 0.05$.

Effect of soaking and/or cooking on antinutrients (tannin, phytate and Polyphenols) and protein digestibility

Table 2 shows the antinutritional factors and *in vitro* protein digestibility of treated and untreated bambara seeds. Tannin content of the seeds was found to be 4.60 mg/100 gm which was higher than that reported by Abiodun and Adepeju (2011) for bambara seeds flour. Soaking and cooking of the seeds significantly ($P \leq 0.05$) decreased tannin content to 3.24 and 2.37 mg/100 gm, respectively. Similar trends was observed by Mubarak (2005), Hassan *et al.* (2005), Abedel Hady *et al.* (2005) for mung bean, lubin, maize and lentil seeds, respectively. Polyphenol content of bambara seeds was found to be 872.35 mg/100 gm which was significantly ($P \leq 0.05$) decreased after soaking to 647.67 mg/100 gm and after cooking to 413.79 mg/100 gm. Those results were in agreement with the findings of Yagoup *et al*. (2004) for roselle seeds. The reduction in polyphenols after soaking may be due to washing out of soluble polyphenols in water and after cooking might be due to interaction with protein during cooking forming poorly extractable protein phenolic complexes. The phytate content of the seeds was 1478.15 mg/100 gm DM. The seeds are rich in protein (20.60%), therefore they had high phytate levels. In legumes, phytates are associated with protein bodies (Sulieman *et al*., 2007) and, therefore, phytate levels should increase with increasing protein content. Depending on the processing method, a significant reduction ($P \leq 0.05$) in phytate content was obtained by soaking whole seeds for 14 hours. Results revealed that soaking could lower the level of this antinutrients below the control value. The loss in phytates during soaking of bambara seeds 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 (Bishnoi *et al*., 1994). Ordinary cooking of bambara 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. According to El Maki *et al*. (2007), 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^{o}C may degrade inositol hexaphosphate to the pentaphosphate or lower molecular weight forms. Further they 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. The *in vitro* protein digestibility of bambara seeds was 70.74%. Soaking of the seeds

Table 2. Antinutritional factors (mg/100 gm) and *in vitro* protein digestibility (IVPD) of treated and untreated bambara groundnut

Antinutreints/IVPD	Treatments		
	Raw	Soaked	Cooked
Antinutreints:			
Tannin	4.60 (±0.60)^{a}	3.24 (± 0.75)^{b}	2.37 ± (0.38)^{c}
Polyphenols	872.35 (±6.33)^{a}	647.67 (±0.00)^{b}	413.79± (12.70)^{c}
Phytic acid	1478.15 (±32.66)^{a}	1230.69 (±32.84)^{b}	1033.31± (16.42)^{c}
IVPD	70.74 (±1.76)^{c}	79.24 (±0.55)^{b}	87.53± (0.61)^{a}

Values are mean (± SD) of triplicates. Values not sharing a row superscript in a row for each sample are significantly different at $p \leq 0.05$.

significantly ($P \leq 0.05$) increased the *in vitro* protein digestibility to 79.24% while cooking increased it to 87.53%. The increment in protein digestibility after soaking and/or cooking of the seeds is likely due to reduction in antinutrients as a result of soaking and cooking of the seeds which are reported to lower the protein digestibility (Babiker and El Tinay, 1993).

Effect of soaking and/or cooking on total and extractable minerals

Mineral contents varied between the treatments. Bambara seeds were found to be rich in calcium. Calcium content of unprocessed seeds was 219.30 mg/100 gm (Table 3) which decreased to 184.00 and 196.90 mg/100 gm after soaking and cooking, respectively. The results indicated that soaking of the seeds, with and without cooking, significantly ($P \leq 0.05$) reduced the calcium content of bambara seeds. The loss of calcium during the treatment may be attributed its leaching out into the discarded water. The results are in close consistence with the results of Duhan *et al.* (2002) who also reported a significant decline in the total calcium content on water soaking. For the cultivar seeds, all other major minerals followed a trend similar to that obtained for calcium (Table 3). The iron content of raw sample was 5.93 mg/100 gm (Table 3). Soaking of the seeds for 14 h reduced iron content to 4.40 and after cooking to 3.87 mg/100 gm (Table 3). The reduction in iron content after soaking and cooking may be due to loss of iron in the soaking medium. The results are in good agreement with those of Lestienne *et al.* (2005), who observed reduction in iron content of the soaked grains as compared to raw ones. For bambara seeds, all other trace minerals followed a trend similar to that obtained for iron (Table 3). HCl-extractability of calcium in control sample was found to be 79.75%. Extractable calcium level, after soaking of the seeds significantly increased to 82.25 (Table 3). Further increase in calcium extractability was observed after cooking the seeds and it was increased to 88.55%. This clearly indicates that a successive increase in the calcium extractability of bambara 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). For bambara seeds, HCl-extractability of all other major minerals followed a trend similar to that obtained for calcium (Table 3). As a result of soaking in water, HCl-extractability of iron increased significantly ($P \leq 0.05$) from the control value of 65.08% to 69.21 within 14 h of soaking of bambara seeds. However, significant increase in HCl-extractability of iron was observed after cooking the seeds and it was increased to 73.61%. For bambara seeds, HCl-extractability of all other trace minerals followed a trend similar to that obtained for iron (Table 3). 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 and other antinutrients as a result of soaking and cooking may explain higher HCl-extractability of iron and other trace minerals (Duhan *et al*., 2002).

Table 3. Total (mg/100 g) and extractable (%) minerals of treated and untreated Bambara groundnut

Minerals	Treatments					
	Raw		Soaked		Cooked	
	Total	Extractable	Total	Extractable	Total	Extractable
Cu	0.28 (±0.04)^{a}	62.89(±0.14)^{c}	0.18 (±0.03)^{b}	65.39(±0.43)^{b}	0.17 (±0.01)^{b}	67.19(±0.33)^{a}
Fe	5.93 (±0.09)^{a}	65.08(±0.34)^{c}	4.40 (±0.07)^{b}	69.21(±0.61)^{b}	3.87 (±0.08)^{b}	73.61(±0.41)^{a}
Mn	2.90 (±0.04)^{a}	76.09(±0.16)^{c}	2.15 (±0.02)^{b}	79.09(±0.21)^{b}	1.88 (±0.30)^{c}	82.39(±0.71)^{a}
Zn	7.90 (±0.02)^{a}	58.87(±0.23)^{c}	5.40 (±0.02)^{b}	63.47(±0.41)^{b}	3.50 (±0.01)^{c}	68.27(±0.47)^{a}
P	266.45(±0.32)^{a}	62.34(±0.53)^{c}	247.45(±0.51)^{b}	65.94(±0.76)^{b}	233.45(±0.64)^{c}	68.09(±0.84)^{a}
Ca	219.30 (±0.02)^{a}	79.75(±0.24)^{c}	184.00 (±0.26)^{b}	82.25(±0.29)^{b}	196.90 (±0.15)^{c}	88.55(±0.74)^{a}
K	50.24 (±0.00)^{a}	64.36(±0.21)^{c}	45.68 (±0.00)^{b}	74.36(±0.31)^{b}	38.70 (±0.05)^{c}	79.30(±0.53)^{a}
Na	11.66 (±0.93)^{a}	71.90(±0.24)^{c}	9.14 (±0.93)^{b}	78.80(±0.14)^{b}	7.20 (±0.93)^{c}	84.89(±0.36)^{a}

Values are mean (± SD) of triplicates. Values not sharing a common superscript in a row for each total and extractable minerals are significantly different at $p \leq 0.05$.

Effect of soaking and/or cooking on amino acids composition

Table 4 shows the amino acid composition of bambara seeds before and after treatments. It is observed that glutamic acid, aspartic acid and leucine are the most abundant amino acids in all the samples. Amino acid contents were slightly increased after soaking and cooking of the seeds. Similar observation has been reported by Olaofe and Akintayo (2000) and Adeyeye and Afolabi (2004) glutamic acid was the most concentrated essential amino acid (17.00%). The increment in amino acid after soaking and cooking of the seeds is likely to be due loss in some nutrients as a result of soaking and cooking. When comparing the essential amino acids in bambara seeds flours with the recommended FAO/WHO provisional pattern, the seeds were superior with respect to aspartic acid, threonine, methionine, leucine, tyrosine, phenylalanine, hstidine and arginine, and while they were adequate in valine and isoleucine. It was only for lysine that supplementation may be required (Table 4).

Table 4. Amino acids composition (mg/100 gm) of treated and untreated bambara groundnut

	Treatments			FAO/WHO (1984)
Amino acids	Raw	Soaked	Cooked	reference protein
Aspartic acid	5.60	5.94	6.20	4.00
Threonine	2.60	2.80	3.00	2.60
Serine	2.70	2.90	3.40	
Glutamic acid	17.00	18.60	18.87	
Glycine	3.38	3.50	3.70	
Alanine	3.90	4.10	4.60	
Cystine	0.7	0.78	0.81	
Valine	4.10	4.45	4.51	4.20
Methionine	2.70	2.80	2.80	2,20
Isoleucine	3.90	3.95	4.07	4.20
Leucine	6.90	6.98	7.60	4.80
Tyrosine	3.40	3.50	3.62	1.40
Phenylalanine	4.80	4.85	4.86	2.80
Histidine	2.40	2.65	2.80	2.40
Lysine	2.80	3.60	4.10	4.20
Ammonia	14.10	8.60	9.00	4.00
Arginine	4.90	5.60	6.10	2.00
Proline	3.75	3.80	4.00	

Values are mean of duplicate samples.

Conclusion

Although soaking and/or cooking brought about a decline in protein and total mineral contents of bambara seeds, the protein digestibility and HCl-extractability of minerals increased significantly after soaking and cooking of the seeds. The losses in protein and mineral contents may be ascribed to leaching of these nutrients 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 protein digestibility and HCl-extractability likey attributed to reduction in antinutrients of the seeds. Thus, cooking of the seeds after soaking can be considered as a beneficial technique for improving bioavailability of nutrients.

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