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COMMUNICATION

Effect of Processing on Anti-nutritional Factors of Red Kidney Bean *(Phaseolus vulgaris)* Grains

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Abstract The effect of different processing methods (soaking in water or solutions of sodium bicarbonate, citric acid, soaking plus cooking, and germination) on anti-nutritional factors (phytic acid, total polyphenols, tannins, and hydrocyanic acid) of red kidney bean was studied. The anti-nutritional factors were reduced significantly ($P<0.001$) with processing techniques. Cyanide contents were most effectively (25%) reduced by cooking after soaking in sodium bicarbonate solution, followed by germination. The most drastic effect was noted on tannin contents. Cooking after soaking in either citric acid or sodium bicarbonate solutions almost eliminated it. However, simple soaking in water did not result in any reduction in tannin contents. Reduction in total polyphenols was 78.7% with cooking after soaking in sodium bicarbonate solution. Phytic acid contents were reduced only with germination treatment (42.6%), while the other treatments did not bring about any large reduction.

Keywords Red kidney bean · Anti-nutritional factors · Processing · Soaking · Cooking · Germination

Introduction

Kidney bean (*Phaseolus vulgaris*) is an important leguminous human food cultivated in arid and semi-arid areas of Pakistan. Both raw and cooked food legumes are consumed traditionally either alone or combined with cereals and other food groups. It is well established that the proteins of food legumes and cereals are nutritionally complementary in respect of S-containing amino acids and lysine, and a balanced blend or mixture of both grains has a greater nutritional value than either ingredients alone (Kadam and Salunkhe 1985).

Kidney beans are good source of important nutrients with 22.7% protein, 3.5% mineral matter, 1% fat, 5.1% crude fiber, and 57.7% total carbohydrates (Khalil et al. 1986). However, the biological utilization of the nutrients is interfered by various anti-nutritional factors present in legumes. The poor digestibility of proteins and inhibitory effects for absorption and utilization of calcium, iron, and zinc have been attributed to the presence of protease inhibitors (trypsin), *a*-amylase inhibitors, lectins, polyphenolic compounds, tannins, phytic acid, HCN, flatulence causing factors, and allergens in legumes (Leiner 1984).

Besides consumer preferences, the selected cooking method is an important factor affecting not only the food chemical composition but also the intake of bioactive compounds under normal dietary conditions (Rodriguez et al. 2008). At domestic levels, legumes are mostly consumed after ordinary processing. Simple processing methods like soaking, cooking, etc. have been reported to reduce the level of some of the anti-nutritional factors (Despande and Cheruan 1983, Nergiz and Gokgoz 2007, Shimelis and Rakshit 2007). Although the overall effects are well known (Siddhuraju and Becker 2001), optimum processing conditions are not well established, and the mechanisms still need to be understood. It is also generally known that germination markedly improves the nutritional quality of legumes (Camacho et al. 1992, Bakr 1996, Khalil et al. 2007). The addition of germinated soy flour to wheat flour slightly changes the mixing properties of dough but had an improving effect on the overall bread quality (Rosales et al. 2008). A comparison of different appropriate bioprocesses (methods for producing commercially useful biological

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material) for maximum reduction of most of the anti-nutrients in red kidney beans is still needed.

Present studies, therefore, were conducted to study the comparative effects of different processing techniques on the contents of various anti-nutritional factors in kidney beans.

Material and Methods

Samples Red kidney beans (*Phaseolus vulgaris* L.) were purchased at a local market of Peshawar city. The samples were screened to remove broken and shrunken seeds, weed seeds, and foreign matters manually.

Processing Techniques

The following treatments were applied separately to seven different lots of seeds in triplicate: soaking in water, soaking in citric acid solution, soaking in sodium bicarbonate solution, water soaking + cooking, citric acid soaking + cooking, sodium bicarbonate soaking + cooking, and germination.

Soaking Red kidney bean seeds were soaked in the dark at room temperature (25–29 °C) for 9 h in distilled water, 0.1% citric acid (pH 3.4), or 0.07% sodium bicarbonate (pH 8.4). The proportion of seed to soaking solution was 1:5 (*w*/*v*). The soaking solution was drained off after soaking time, seeds air-dried, and then kept in oven at 60 °C for complete drying. Samples were ground to pass through 30-mesh screen. The ground samples were kept in air-tight bottles and stored at 4 °C for subsequent analysis.

Cooking Some of the seeds from the soaking process were boiled in a 1-l conical flask fitted with condenser. Tap water (seed/water ratio, 1:3 *w*/*v*) was added, and soaked seeds were cooked on a hot plate until the seeds were soft when pressed with fingers. The cooking liquid and seeds were separated using a strainer; and the cooked seeds were dried in the same way as after the soaking process.

Germination Red kidney bean seeds were soaked in distilled water (1:5 *w*/*v*) for 6 h at room temperature. The water was drained off, and the seeds transferred to a moisture adherent flax cloth (tarts) to germinate for 4 days in the dark at 22 °C. Every 24 h, the seeds were moistened with distilled water and carefully shaken. After 4 days of germination, the sprouts and the seeds were ground and dried in an air oven at 60 °C for analysis.

Analysis

Analysis of samples was carried out according to the available standard/published methods depending on the availability of lab requirements. Spectrophotometer used in the studies was Hitachi-U 1800 UV-Vis.

Determination of Total Cyanide Contents

Total cyanide contents were determined according to the method (no. 26.151) of AOAC (1984). The sample was cooked in a glass kjeldhal flask (autolysis) with steam distillate collected in NaOH solution and titrated against standard $AgNO_3$ solution in the presence of NH_4OH and KI. The concentration of HCN was calculated from the amount of $AgNO_3$ used for titration.

Determination of Tannin contents

Estimation of tannins was based on the measurement of blue color formed by folin-denis reagent measured at 760 nm (AOAC 1984).

Determination of Total Phenol Contents

Total phenols were determined by the Prussian blue assay (Price and Butler 1977). Defatted sample was extracted twice with acetone/water and centrifuged. Aliquot was diluted with distilled water followed by the addition of standard solutions of FeNH4 $(SO_4)_2$ and $K_3Fe(CN)_6$. The absorbance was read after 20 min at 720 nm. A standard curve was constructed using tannic acid and the results expressed as milligram per gram on dry weight basis.

Determination of Phytic Acid Contents

Simple method for determination of phytic acid by Wheeler and Ferrel (1971) was used to quantify phytic acid in unprocessed and processed red kidney bean samples.

Statistical Analysis

In order to quantify the influence of different processing techniques, data were subjected to analysis of variance. Data were analyzed for analysis of variance using M-Stat-C computer package (MSU 1987). A one-factor randomized complete block design (RCBD), with three replications for each treatment, was applied. Means were separated using Duncon's multiple range (DMR) test.

Results and Discussion

Results on all parameters are given in Table 1.

Table 1 Effect of different processes on anti-nutritional factors of red kidney beans

	Cyanide (mg/g)	Tannin (mg/g)	Total phenols (m/g)	Phytic acid (mg/g)
Unprocessed (raw seeds)	0.20±0.004 A	6.1±0.029 A	21.6±0.144 A	6.1±0.115 AB
Water soaking	0.19±0.003 AB (7.7)	6.3±0.144 A (−3.3)	18.8±0.635 B (12.8)	6.1±0.029 AB (0.8)
Citric acid soaking	0.18±0.004 B (8.7)	2.3±.029 B (62.8)	11.8±0.231 C (45.2)	6.0±0.029 BC (2.5)
Sodium bicarbonate soaking	0.17±0.003 BC (13.9)	2.0±0.029 C (67.8)	10.6±0.173 CD (50.8)	5.9±0.029 BC (4.1)
Water soaking + cooking	0.17±0.002BC (15.3)	1.1±0.115D (81.8)	8.4±0.173EF (61.0)	6.3±0.058A (-3.3)
Citric acid soaking + cooking	0.17±0.006 CD (18.3)	0.4±0.017 E (92.7)	7.1±0.144 F (67.3)	5.8±0.029 C (5.7)
Sodium bicarbonate soaking + Cooking	0.15±0.010 D (25.0)	0.6±0.023 E (90.2)	4.6±0.462 G (78.8)	6.3±0.029 A (−2.5)
Germination (96 h)	0.16±0.005 CD (20.8)	1.9±0.000 C (68.6)	9.8±0.635 DE (54.5)	3.5±0.058 D (42.6)

Values are mean±SE. Values followed by different letters are significantly ($P<0.01$) different from each other. Values in parentheses are percent reduction over control.

Total Cyanide Contents

The level of cyanide content in unprocessed sample was 0.2 mg/kg. Except for soaking in water, all processing techniques significantly ($P<0.001$) reduced its contents. The highest reduction (25%) was achieved with cooking after soaking in sodium bicarbonate solution followed by germination (20.8%), whereas the least reductions were noted with water soaking (7.7%) and soaking in citric acid solution (8.7%). Akindahunsi (2004) reported a value of 3.7 mg/kg in raw African oil beans, which was reduced by soaking and cooking to 2.2 mg/kg. Okafor et al. (2002) reported a range of 5.88 to 28.55 mg/100 g for the cyanogen contents of various legumes. Vijayakumari et al. (1995) noted 87% loss in HCN contents of Indian tribal pulse only with autoclaving treatment, whereas reduction with other treatments like water and NaHCO3 solution etc were much less.

Tannin Contents

Tannin contents were 6.1 mg/g of raw unprocessed red kidney beans and remained almost unchanged during soaking in water. However, all other tested processing techniques significantly ($P<0.001$) reduced its contents. Cooking after soaking in water, citric acid, and sodium bicarbonate solutions almost completely eliminated the tannin contents (82 to 93% reduction), whereas only soaking in citric acid solution (63%), soaking in sodium bicarbonate solution (68%), and germination (69%) resulted in relatively milder reductions.

Although a comparison among different legume species has not been reported, reduction in tannin contents with cooking has been frequently reported in various legumes (Bressani 1993; El Tabey Shehata 1992). DeLeon et al. (1992) noted reduction in tannin contents and cooking time of beans soaked in salt solution. The present findings indicate that, although all the tested anti-nutritional factors were significantly reduced with different processing techniques, tannins proved to be the most labile. Deshpande and Cheryan (1983) observed that the leaching losses during soaking of beans were highest for tannins among the anti-nutritional factor. As much as 66.2% reduction in the tannins by cooking *P. vulgaris* was reported; even though there seems to be a redistribution of tannins from cooked beans to cooking liquid (Bressani et al. 1991). On the other hand, Akindahunsi (2004) reported no reduction in the tannin contents of African oil bean with water soaking and cooking. It is the findings of such studies that necessitate studying processing techniques best suited for individual legumes and preclude the application of "soaking + cooking" technique for all legumes across the board.

Total Phenol Contents

The total polyphenol contents in unprocessed red kidney bean were 21.6 mg/g, which significantly ($P<0.001$) decreased with all processing methods. The order of reduction was: cooking after soaking in bicarbonate solution (79%) > cooking after soaking in citric acid solution (67%) > cooking after soaking in water (61%) > germination (54%) > soaking in sodium bicarbonate solution (51%) > soaking in citric acid solution (45%) > water soaking (13%).

Rehman and Shah (1996) reported a significant reduction in the polyphenol contents of different legumes with various soaking processes. On the average, about 31–37% total phenols decreased on soaking in 1% NaCl solution. Babar et al. (1988) found that germination of bean seed for 40 h decreased the level of polyphenol contents by 35%.

Zeb et al. (2006) observed that dry and moist heating (under pressure) of rapeseed significantly lowered some of the phenolics, such as sinapine, catechin, leucoanthocynidin contents, etc. However, they noted that any single treatment could not be used for detoxification of rapeseed from all these phenolics. They recommended studying combinations of different methods for the removal of undesired phenolics.

Phytic Acid Contents

The level of phytic acid in unprocessed samples was 6.1 mg/g. The only significant ($P<0.001$) reduction in phytic acid content of red kidney beans was noted with cooking after soaking in citric acid solution and germination. However, the reduction with cooking after citric acid soaking was only 5.7%, whereas germination resulted in 43% reduction. The other processing methods did not alter the concentration of phytic acid contents significantly.

It has been reported that cooking beans, particularly after soaking them, will reduce phytic acid in *P. vulgaris* (Iyer et al. 1980). However, Akindahunsi (2004) concluded that the phytic acid contents increased by soaking and cooking processing of African oil beans. Vidal-Valverde et al. (1998) observed that soaking faba beans in either water, acid, or base solutions did not produce significant changes ($P<0.05$) in phytic acid levels. When presoaked faba beans were water-cooked, a larger phytic acid reduction (35%) took place only when the seeds had been soaked in citric acid solution. Phytic acid seems to be soluble in an acidic medium, and cooking leads to a large reduction in its content.

Studies on legumes, cereals, and other oilseeds showed that phytic acid is generally stable under ordinary processing conditions (Thompson 1990). Pure phytic acid in aqueous solution at pH 6.0 was lost (about 50%) after 1 h autoclaving (Kumar et al. 1978), but in biological systems, e.g., in cereals and oilseeds less than 10% loss was observed with autoclaving for 0.5–2.0 h (De Boland et al. 1975, Lease 1966). Boiling of legumes also did not result in a significant breakdown of their phytic acid content (Kumar et al. 1978). Ologhobo and Fetuga (1984) also could not record a significant reduction in phytic acid of soybeans due to cooking, autoclaving, and soaking. Microwave heating of soybean caused a 23% phytic acid reduction after 9 min and 46% after 15 min, while gamma irradiation (1 kGy) reduced the phytic acid content of soybean by only 4% (Hafiz et al. 1989).

On the other hand, reductions in phytic acid contents of cereals and legume seeds with sprouting have been frequently reported (Ibrahim et al. 2002). These reductions are mainly due to an increase in the phytase activity, leading to a solubilization of phytates (Camacho et al. 1992). The simple and inexpensive technique of sprouting has been therefore recommended for both in the home and by industries that produce food products for nutritionally vulnerable persons with high Ca and P requirements (Nestares et al. 1999). Reduction in phytic acid with sprouting among different anti-nutrients has been found more profound than other compounds (El-Adawy 2002; Bakr 1996).

Conclusions

Although all the tested anti-nutritional factors were significantly reduced with different processing techniques, tannins proved to be the most labile, while phytic acid was the most resistant to all processes except sprouting. The highest reductions (25% and 79%) obtained in cyanide and total phenol contents, respectively, were with cooking after sodium bicarbonate soaking. The same treatment caused the second highest reduction in tannin contents. However, for some practically meaningful reduction in phytic acid contents, this bean needs sprouting prior to cooking.

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