Phytic acid, in vitro protein digestibility, dietary fiber, and minerals of pulses as influenced by processing methods

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Abstract. The objective of this project was to determine the effect of various types of processing on selected nutritional related parameters of commonly consumed Indian pulses and soybean. Germination reduced the phytic acid content of chickpea and pigeonpea seeds by over 60%, and that of mung bean, urd bean, and soybean by about 40%. Fermentation reduced phytic acid contents by 26–39% in all these legumes with the exception of pigeon pea in which it was reduced by less than 10%. Autoclaving and roasting were more effective in reducing phytic acid in chickpea and pigeonpea than in urd bean, mung bean, and soybean. Germination and fermentation greatly increased the in vitro protein digestibility (IVPD). IVPD was only slightly increased by roasting and autoclaving of all legumes. Germination and fermentation also remarkably decreased the total dietary fiber (TDF) in all legumes. Autoclaving and roasting resulted in slight increases in TDF values. All the processing treatments had little effect on calcium, magnesium and iron contents.

Key words: Legumes, Pulses, Phytic acid. In vitro protein digestibility. Germination, Fermentation, Autoclaving, Roasting.

Introduction

Among food legumes, pulses continue to occupy an important place in human nutrition, particularly in the developing countries, as they are good sources of protein, vitamins, and minerals. Besides improvement in productivity, adaptability and yield stability of pulses, the improvement of nutritional quality of pulses has often been emphasized [1]. The supplementation of cereals with protein rich legumes is considered to be one of the best solutions to protein calorie malnutrition in the world. India is the largest producer of pulses in the world with an annual production of about 12 million tons. Pigeonpea, chickpea, mung bean, and urd bean are the main pulse crops grown and consumed in India [2]. In recent years, developing countries have recognized the potential of soybean as a source of proteins that could be used to supplement the traditional cereal staples with protein and energy [3].
Considerable increase in soybean production has been recorded in developing countries, particularly in India [2].

One of the main drawbacks that limits the nutritional quality of legumes is the presence of antinutritional factors. Phytic acid is widely distributed in legume seeds and it accounts for about 78% of the total phosphorus in pulses [4]. Phytates interact with proteins, reducing their solubility and availability [5]. Phytic acid has also been linked to the inhibition of digestive enzymes such as protease [6], alpha amylases [7] and trypsin [8]. Phytic acid in foods of plant origin forms a complex with dietary minerals such as calcium, zinc, iron, and magnesium and makes them biologically unavailable for absorption [9]. Low absorption of minerals has been associated with a high intake of phytic acid and dietary fiber [10].

Some of the deleterious effects of antinutritional factors (including phytic acid) can be reduced by processing. Pulses are consumed in the forms of dhal, which are decorticated dry split cotyledons, and whole seeds. Before use as human food, pulses receive two processing treatments: (1) primary processing or dehulling which converts whole seed into dhal, and (2) secondary processing which includes soaking, autoclaving, germination, roasting and fermentation. Secondary processing varies depending on the type of food and the region of consumption. Since most of the phytate in legumes is present in the cotyledons, dehulling increases the phytic acid content of legumes on a unit weight basis [11]. Among the secondary processing methods, germination and fermentation have been found quite effective in decreasing the phytate concentration in bean seeds [5]. There is voluminous literature on the nutrient composition of legumes. This includes reports on the influence of processing treatments on antinutritional factors, particularly protease inhibitors and oligosaccharides. The present study was undertaken to investigate the effects of processing methods such as wet-heating (autoclaving, which is comparable to a domestic pressure cooker), dry-heating (comparable to roasting), germination and fermentation on phytic acid, in vitro protein digestibility, dietary fiber, and mineral contents of pulse crops and to compare the results with those obtained for soybean processed in similar ways.

### Materials and methods

The seed material for the present study consisted of one genotype each of pigeonpea (*Cajanus cajan* L.), chickpea (*Cicer arietinum* L.), mung bean (*Phaseolus aureus*), urd bean (*Phaseolus mungo*), and soybean (*Glycine max*). Seed samples of pigeonpea (ICP 8094) and chickpea (ICCV 10) were provided by the breeding units of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). They were grown in the 1992/93 postrainy season at ICRISAT Asia Center, Patancheru, India. Mung bean (ML 267) and urd bean (LBD 611) seed samples were obtained from the Andhra Pradesh State Seeds Development Corp., Hyderabad, India. These were grown at Vijayawada, Andhra Pradesh, India, during the postrainy season of 1992/93. Seed samples of soybean (MACS 124) grown during the rainy season of 1992/93 were procured from the University of Agricultural Sciences, Dharwad, Karnataka, India. All the grain samples were cleaned, and stored at 5 °C.

### Dehulling and grinding

A tangential abrasive dehulling device (TADD) was used to prepare dhal samples (decorticated split cotyledons). Seeds were moistened and then dried in an oven at 55 °C overnight and dehulled in a TADD. All dehulled samples were ground in a Udy cyclone mill using a
0.4 mm screen. Samples were defatted in a Soxhlet apparatus using n-hexane. Finely ground raw dhal samples (without processing) of each type of seed were used as the controls.

**Germination.** Seeds were soaked in distilled water for 12 hours at room temperature (25 °C). The soaked seeds were germinated in sterile petri dishes lined with wet filter paper. To obtain uniform sprouts measuring 1.5 cm, seeds of pigeon pea and soybean were germinated for 72 hours and those of chickpea, mung bean, and urd bean were germinated for 48 hours. Seed coats were removed manually from the sprouted seeds and the remainder of the seeds were freeze-dried and ground to a fine powder in a Waring blender to pass through a 0.4 mm sieve. They were then defatted.

**Fermentation.** Dhal samples of pigeon pea, chickpea, and soybean were soaked in distilled water at 25°C, in a seed to water ratio of 1:2 (w/v) for 16 hours; mung bean and urd bean were soaked for 2 hours. The soaked dhal samples were ground to a paste in a Waring blender. The paste was thoroughly mixed with 1.5% (w/v) of inoculum of a natural cured sample containing lactic acid bacteria and fermented for 24 hours in an incubator at 30°C. The fermented paste was freeze-dried and ground to a fine powder in a Waring blender to pass through a 0.4 mm sieve. Finally, samples were defatted.

**Autoclaving (wet-heating).** Dhal samples were autoclaved in a dhal to water ratio of 1:2 (w/v) at 103.5 Kpa for 15 min for pigeon pea, chickpea and soybean, and 10 min for mung bean and urd bean, which are the normal cooking times in a pressure cooker. Different legumes require different times to cook to a desirable softness. In the present study, the legumes were processed for different times to achieve their respective desirable softness. After autoclaving, the contents were freeze-dried and ground to a fine powder in a Waring blender to pass through a 0.4 mm sieve. Samples were then defatted.

**Roasting (dry-heating).** Whole-seed samples were roasted in a sand bath at 200°C for 2 min. The roasted material was separated from the sand by sieving. Seeds were dehulled by TADD, and ground to a fine powder in a Waring blender to pass through a 0.4 mm sieve before being defatted.

**Chemical analysis.** Phyric acid content of processed and control samples was determined according to the method described by Wheeler & Ferrel [12]. Phytate content was calculated from the iron concentration in ferric chloride by assuming a constant Fe-P molecular ratio of 1:1.5 in the precipitate of the extracts. The procedures described earlier for determination of protein [13], in vitro protein digestibility [14] and total dietary fiber [15] were used. For mineral analysis, defatted dhal samples (0.5 g) were weighed, transferred to glass tubes, and digested in a block digestor using a tri-acid mixture which contained nitric acid, perchloric acid and sulphuric acid in the ratio of 20:4:1. After adding 10 ml of tri-acid mixture, the mixture was digested first at 70°C for 30 min, then at 180°C for 30 min and finally at 200°C for 30 min. After digestion, the mixture was cooled, dissolved in glass-distilled water and the volume made up to 50 ml. Suitable aliquots were analyzed for calcium, magnesium, and iron in an atomic absorption spectrophotometer (Varian Tectron Model – 1200) [16].

**Statistical analysis.** For all chemical analyses, two replicates of the same sample were used for the determination of each constituent. Standard error was determined by ‘one way analysis of variance’ [17]. Variance was used to determine the impact of various processing methods of commonly consumed Indian pulses and soybeans on selected nutrition related parameters. Significance was accepted at the p < 0.05 level.

**Results and discussion**

The phytic acid content of the untreated dhal sample (g/kg) was the highest in soybean (39.9) followed by mung bean (14.8), urd bean (13.8), pigeon pea (11.7), and chickpea (9.2) (Tables 1–5). This indicated significant (p < 0.05) differences in the phytic acid content of these grain legumes. A wide variation in phytic acid contents of these legumes has been reported [4]. The decrease in phytic acid content as a result of germination was the highest in pigeon pea (65.8%) followed by chickpea (64.1%), urd bean (40.6%), soybean (38.9%), and mung bean (37.2%). This indicated that the treatment is more effective in chickpea and pigeon pea than in other legumes. These figures are considerably higher than those reported for red kidney bean, mung bean and urd bean [18, 19]. The breakdown of phytic acid during germination could be attributable to an increase in the activity of endogenous phytase as reported for fava bean cultivars [20].

Fermentation also resulted in a significant (p < 0.05) reduction in the phytic acid content of these legumes (Tables 1–5), though it was less effective than germination. In India, urd bean and chickpea are the main ingredients in such fermented steamed food products as ‘idli’ and ‘dohkla’. As a result of fermentation, phytic acid contents of these legumes were reduced by nearly 30% in urd bean (Table 4) and by 40% in chickpea (Table 1). Seventy-two hours of fermentation was reported to substantially reduce the phytic acid content of the legume preparations ranging from 52% to 65% for soybean and cowpea [21]. It has been suggested that the loss of phytate during fermentation might be due to the activity of the enzyme phytase naturally present in legumes...
Table 3. Effect of processing on phytic acid, protein, IVPD, total dietary fiber and mineral contents of dhal of mung bean genotype ML 267a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phytic acid (g/kg)</th>
<th>Protein (g/kg)</th>
<th>IVPDb (g/kg)</th>
<th>TDF (g/kg)</th>
<th>Calcium (g/kg)</th>
<th>Magnesium (g/kg)</th>
<th>Iron (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.8</td>
<td>261.3</td>
<td>70.9</td>
<td>157.2</td>
<td>0.49</td>
<td>1.69</td>
<td>0.05</td>
</tr>
<tr>
<td>Germination</td>
<td>9.3</td>
<td>303.5</td>
<td>82.7</td>
<td>64.5</td>
<td>0.46</td>
<td>1.66</td>
<td>0.04</td>
</tr>
<tr>
<td>Fermentation</td>
<td>10.9</td>
<td>268.1</td>
<td>78.9</td>
<td>59.0</td>
<td>0.47</td>
<td>1.68</td>
<td>0.04</td>
</tr>
<tr>
<td>Autoclaving</td>
<td>12.3</td>
<td>259.3</td>
<td>73.8</td>
<td>218.4</td>
<td>0.51</td>
<td>1.68</td>
<td>0.04</td>
</tr>
<tr>
<td>Roasting</td>
<td>11.6</td>
<td>257.0</td>
<td>75.1</td>
<td>195.1</td>
<td>0.47</td>
<td>1.69</td>
<td>0.05</td>
</tr>
<tr>
<td>SE</td>
<td>±0.22</td>
<td>±2.23</td>
<td>±0.43</td>
<td>±5.32</td>
<td>±0.18</td>
<td>±0.26</td>
<td>±0.01</td>
</tr>
</tbody>
</table>

aAll results are expressed as dry weights.

bIVPD expressed as percent of total protein.

and the fermentative microorganisms in the dough [22]. A low reduction in phytic acid has been reported following 24 hours of fermentation in urd bean [23]. The difference in culture inoculum used for fermentation might produce varying levels of phytase activity and subsequent variation in the extent to which phytic acid is hydrolyzed in the fermented product.

Both wet-heating and dry-heating also significantly (p < 0.05) reduced the phytic acid content of the tested legumes by between 25 and 35% (Tables 1–5). These reductions are slightly higher than those reported for chickpea and urd bean by Duhan et al. [24]. However, in the present study, significant (p < 0.05) differences between the effects of wet and dry heating were observed for all the legumes tested. The heat processing might have reduced the extractability of phytic acid in the present study. Kumar et al. [25] observed that the cooking process decreased both water- and acid-extractability of phytate phosphorus in mung bean, cowpea, and chickpea and attributed the poor extractability of phytate phosphorus with water and HCl to the formation of insoluble complexes between phytate phosphorus and other components during cooking. Endogenous phytase activity is destroyed by heat treatment and exclude any positive effect of this activity in the digestive tract after ingestion. This may influence not only the bioavailability of P but also of Ca, Mg, and Fe (+ other microminerals).

Both germination and fermentation remarkably increased the in vitro protein digestibility (IVPD) in all legumes tested (Tables 1–5). The effects were more pronounced in pigeonpea than in the other legumes. Both germination and fermentation appeared to be equally effective in increasing the IVPD of these legumes. Germination has been reported to increase the protein digestibility of mung bean [26], moth bean [27], soybean [28] and chickpea and urd bean [29]. The hydrolysis of seed proteins, protease inhibitors, phytic acid, and polyphenols during germination may account for considerably increased IVPD in legumes [24]. Boralkar & Reddy [28] reported an improvement in the IVPD of soybean when the fermentation period was increased. Activity of certain proteolytic enzymes by microflora during fermentation may be responsible for the improved IVPD of beans [18]. Germination also significantly (p < 0.05) increased protein content in pigeonpea, urd bean, mung bean, and soybean (Tables 2–5) whereas fermentation was more effective in increasing the protein content of pigeonpea (Table 2) and soybean (Table 5). This could be attributed to a parallel loss of dry matter.

Roasting and autoclaving did not noticeably change the levels of protein in the tested legumes, but resulted in considerable increases in the IVPD of chickpea, pigeonpea, mung bean, and urd bean (Tables 1–4). Heat processing has been reported to increase protein digestibility of grain legumes [27, 29] probably by destroying heat-labile protease inhibitors, and also by denaturing

Table 4. Effect of processing on phytic acid, protein, IVPD, total dietary fiber and mineral contents of dhal of urd bean genotype LBG 611a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phytic acid (g/kg)</th>
<th>Protein (g/kg)</th>
<th>IVPDb (g/kg)</th>
<th>TDF (g/kg)</th>
<th>Calcium (g/kg)</th>
<th>Magnesium (g/kg)</th>
<th>Iron (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.8</td>
<td>276.0</td>
<td>60.4</td>
<td>224.2</td>
<td>0.68</td>
<td>2.13</td>
<td>0.04</td>
</tr>
<tr>
<td>Germination</td>
<td>8.2</td>
<td>301.3</td>
<td>74.2</td>
<td>78.3</td>
<td>0.64</td>
<td>2.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Fermentation</td>
<td>9.6</td>
<td>279.2</td>
<td>71.3</td>
<td>63.0</td>
<td>0.65</td>
<td>2.11</td>
<td>0.04</td>
</tr>
<tr>
<td>Autoclaving</td>
<td>10.8</td>
<td>270.1</td>
<td>64.7</td>
<td>227.1</td>
<td>0.67</td>
<td>2.10</td>
<td>0.03</td>
</tr>
<tr>
<td>Roasting</td>
<td>9.5</td>
<td>270.0</td>
<td>65.9</td>
<td>175.4</td>
<td>0.67</td>
<td>2.08</td>
<td>0.03</td>
</tr>
<tr>
<td>SE</td>
<td>±0.13</td>
<td>±1.75</td>
<td>±0.56</td>
<td>±4.50</td>
<td>±0.11</td>
<td>±0.20</td>
<td>±0.01</td>
</tr>
</tbody>
</table>

aAll results are expressed as dry weights.

bIVPD expressed as percent of total protein.

Table 5. Effect of processing on phytic acid, protein, IVPD, total dietary fiber and mineral contents of dhal of soybean genotype MACS 124a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phytic acid (g/kg)</th>
<th>Protein (g/kg)</th>
<th>IVPDb (g/kg)</th>
<th>TDF (g/kg)</th>
<th>Calcium (g/kg)</th>
<th>Magnesium (g/kg)</th>
<th>Iron (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.9</td>
<td>557.0</td>
<td>63.3</td>
<td>202.2</td>
<td>1.95</td>
<td>4.33</td>
<td>0.08</td>
</tr>
<tr>
<td>Germination</td>
<td>24.4</td>
<td>631.2</td>
<td>73.6</td>
<td>177.3</td>
<td>1.90</td>
<td>4.25</td>
<td>0.06</td>
</tr>
<tr>
<td>Fermentation</td>
<td>27.0</td>
<td>621.4</td>
<td>71.7</td>
<td>80.4</td>
<td>1.90</td>
<td>4.29</td>
<td>0.06</td>
</tr>
<tr>
<td>Autoclaving</td>
<td>27.9</td>
<td>586.3</td>
<td>66.8</td>
<td>179.0</td>
<td>1.92</td>
<td>4.27</td>
<td>0.05</td>
</tr>
<tr>
<td>Roasting</td>
<td>33.1</td>
<td>599.1</td>
<td>65.3</td>
<td>175.1</td>
<td>1.95</td>
<td>4.26</td>
<td>0.06</td>
</tr>
<tr>
<td>SE</td>
<td>±0.62</td>
<td>±4.31</td>
<td>±0.60</td>
<td>±4.71</td>
<td>±0.23</td>
<td>±0.21</td>
<td>±0.002</td>
</tr>
</tbody>
</table>

aAll results are expressed as dry weights.

bIVPD expressed as percent of total protein.
globulin proteins, that are highly resistant to proteases in their native state [31]. In the present study, a decrease in phytic acid content following various processing practices, was associated with an increase in IVPD suggesting that phytic acid interferes with protein digestibility. It has been reported that IVPD was negatively and significantly correlated with phytic acid content in these legumes [4].

Germination and fermentation significantly (p < 0.05) reduced the total dietary fiber (TDF) contents of all the legumes tested (Tables 1–5). Increased alpha-galactosidase activity during germination and fermentation has been reported to cause a decrease in the oligosaccharide content of urd bean and an urd bean/rice blend, leading to reduced levels of dietary fiber [32]. In pigeonpea, chickpea, and mung bean (Tables 1–3) autoclaving and roasting resulted in slight increases in TDF values. Valverde & Frias [33] have reported a considerable increase in the neutral detergent fiber (NDF) content in processed chickpeas and kidney beans.

Fermentation did not bring about any apparent changes in the calcium, magnesium, and iron contents of pigeonpea, chickpea, mung bean, and urd bean (Tables 1–4). Calcium and iron contents were slightly decreased as a result of germination in all the legumes tested. Pressure cooking (autoclaving) did not result in any changes in Ca and Mg contents. This might have happened because cooking water was not discarded. Reddy & Salunkhe [23] did not find a significant decrease in the calcium, magnesium, zinc, and iron contents of urd bean after fermentation. Mineral losses have been reported to occur when legumes are soaked in water which is generally discarded before germination [25]. The heating processes noticeably decreased the iron content of all the legumes tested except mung bean. The levels of calcium and magnesium of the legumes tested were not affected by processing. Meiners et al. [34] reported considerable mineral losses during cooking of different legumes. Such losses were attributed to the leaching of minerals into the cooking water which was then discarded, suggesting that cooking would not reduce mineral levels if the cooking water is not discarded.

Grain legumes are considered to be important sources of minerals. However, the bioavailability of divalent minerals, especially of calcium, magnesium and iron is known to be adversely affected by the presence of phytic acid which is a metal-binding constituent. By breaking down phytate, germination and fermentation minimize the concerns posed by metal chelation brought about by the phytate naturally present in grain legumes.

Among processing methods, germination and fermentation appear to be more effective than autoclaving and roasting in lowering the phytic acid content of legumes, and achieving a corresponding increase in in vitro protein digestibility. These processes appear more beneficial in chickpea and pigeonpea, the most important pulse crops of India, than in soybean. The extent to which the bioavailability of minerals can be enhanced by the processing methods of legumes needs to be further investigated.

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References

Effect of boiled barley-rice-feeding in hypercholesterolemic and normolipemic subjects

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Abstract. Barley contains approximately 10% dietary fiber and is easily cooked with rice, the dominant cereal in Japan, to increase the intake of dietary fiber. This research involved three experiments to examine the influence of barley on blood lipids in human subjects. All subjects received a boiled barley-rice (50/50 w/w mix) supplement two times per day in place of rice for 2 or 4 weeks. In the normolipemic subjects, serum lipids were unaffected by the ingestion of barley for 4 weeks. In twenty hypercholesterolemic men aged 41 ± 5 years, the ingestion of barley was associated with a significant fall in serum total cholesterol, LDL-cholesterol, phospholipids and LDL and VLDL-cholesterol in seven mildly hypercholesterolemic women aged 56 ± 7 years, a significant improvement in serum lipid profiles was observed. The present study suggests the possibility that the ingestion of barley-rice could lower serum lipids in hypercholesterolemic subjects.

Key words: Barley, Cholesterol, Dietary fiber, Hypercholesterolemic subjects, Hypocholes- terolemic effect, Normolipemic subjects

Introduction

Dietary fiber intake in Japan was estimated to be 15.9 g/d per capita (7.9 g/4184 kJ(1000 kcal)) in 1994 according to the National Survey of Nutrition. This value is slightly higher than that estimated as 5.6 g/4184 kJ/d in a simulated American diet [1]. In terms of recommended fiber intake, a report prepared for the US Food and Drug Administration used 25 g/8362 kJ (2000 kcal) as Daily Reference Value in the Nutrition Labeling and Education Act in 1993 [2], while the National Advisory Committee in Great Britain recommends an increase to 25 g/d over the short term and to 30 g/d over the long term [3]. In Japan, the Ministry of Health and Welfare recommends an intake of 20–25 g/d (10 g/4184 kJ) [4]. The current estimated intake of dietary fiber in Japan is below the recommended levels.