

## Anti-Nutritional Factors During Germination in Indian Bean (*Dolichos lablab L.*) Seeds

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**Abstract:** The raw dry Indian bean having a very high trypsin inhibitory activity which progressively decrease by 51% during the 12 h soaking period and further reached to 17% at 32 h germination period. However, the overall fall in polyphenols was 70%, tannins 46%, phytic acids 36%, phytate phosphorus 30 and 40-50% stachyose and raffinose were noticed. The present study also evaluated the changes of anti-nutritional factors of germinating Indian bean by subjecting to boiling, roasting and pressure cooking. Maximum reduction was observed in TIA and phytic acids with roasting, while the boiling and pressure cooking decreases the levels of polyphenols and tannins. Germination was more effective method in reducing trypsin inhibitor activity, tannins, polyphenols and phytic acid than the various cooking treatments.

**Key words:** Dolichos lablab • antinutritional factors • germination • food processing methods • cooking treatments

### INTRODUCTION

Legumes are the most important plant food material, for the outstanding reason that they are the concentrated cheap sources of protein for the vegetarian population. However, they are under-used in various countries because of antinutrient factors, such as enzyme (trypsin, chymotrypsin,  $\alpha$ -amylase) inhibitors, phytic acid, flatulence factors, etc. [1-4]. Indigestible substances include the flatulence producing oligosaccharides, namely, raffinose, stachyose and verbascose which due to the absence of  $\alpha$ -galactosidase in humans, are fermented anaerobically by micro-organisms to produce carbon dioxide, hydrogen and methane [5-7]. Phytic acid binds trace elements and macro-elements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract are making dietary minerals unavailable for absorption and utilization by the body. It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis. Moreover, the phosphorus in phytate has been considered to be largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyse phytate in the small intestine [8]. Phenolic compounds are responsible for the bitterness and astringency of many foods and beverages [9, 10].

The nutritive value of grain legumes depends primarily on their nutrient contents and presence or absence of anti-nutritional and/or toxic factors. Nutritive value is the ability of food to provide a usable form of nutrients: protein, carbohydrates, vitamins and minerals. The food processing methods including soaking, germination, decortications, fermentation and cooking greatly influence their nutritive values. Of these, cooking and germination plays an important role as it influences the bioavailability and utilization of nutrients and also improves palatability which incidentally may result in enhancing the digestibility and nutritive value [11-13]. The presence of anti-nutritional factors in legumes is shown to be reduced at varying degrees based upon the food preparation involved and the properties exhibited by various types of legumes themselves. Therefore, more information is needed about the potential nutritional implications of legume-based diets. Though extensive information is available on the nutritive value of many common legumes, the information available on the nutritive value of Indian bean is limited. Indian bean (*Dolichos lablab L. var lignosus*) is a lesser-known legume, which has not received due attention by biochemists and nutritionists. Thus, the present study was undertaken to study the changes of anti-nutritional factors like trypsin inhibitory activity, phytic acid,

polyphenols and tannins of dried and germinated cotyledons and also evaluated their changes on treatment with various cooking methods.

## MATERIALS AND METHODS

Indian bean (*Dolichos lablab* L. var *lignosus*) seeds were obtained from the Agricultural farm of the Andhra Pradesh Agricultural University, Rekulakunta, Anantapur andhra Pradesh, INDIA. Healthy seeds were sorted; surface sterilized with 0.1% HgCl<sub>2</sub> and rinsed many times thoroughly with sterile distilled water to remove the traces of toxic HgCl<sub>2</sub> left over on seed coats. The seeds were soaked for further 12 h in ten times of their volume of sterile distilled water, following which the water was strained and the seeds were spread on perforated trays lined with wet cloth and covered with another wet cloth. The seeds were allowed to germinate (sprout) at room temperature 27±2°C for a period of 32 h. Sterile conditions were maintained by including 20 ppm of streptomycin sulphate in the incubation medium (sterile distilled water). Seedlings were withdrawn at designated time intervals and the cotyledons were carefully dissected out for analysis.

### Separation and estimation of raffinose and stachyose:

Alcohol extracts was prepared and clarified by the method of AOAC (Sec 6.002 and 6.075) described by Horwitz [14]. Raffinose and stachyose from the clarified alcoholic extract were separated by paper chromatography and estimated by phenol sulphuric acid method [15]. The sugars on the strips were eluted with water and their concentrations estimated colorimetrically by using the modified phenol sulphuric acid method of Dubois *et al.* [16]. The results were expressed as mg/100 g dry wt.

**Determination of trypsin inhibitory activity:** Trypsin inhibitory activity was determined by the modified method of Roy and Rao [17]. The activity of the enzyme trypsin was assayed using casein as substrate and inhibition of this activity was measured in the seed extracts.

Five g of defatted, pulverized seed material was treated with 50 ml of 0.05 M sodium phosphate buffer pH 7.5 and 50 ml of distilled water. The samples were shaken for 3 h and centrifuged at 700 X 4 for 30 min at 15°C. The supernatants were diluted in such a way that there was an inhibition between 40 and 60 per cent of control enzyme activity. One trypsin unit is defined as an increase of 0.01 absorbance units at 280 nm in 20 min for 10 ml reaction mixture under the conditions described and Trypsin

Inhibitory Activity (TIA) as number of Trypsin Units Inhibited (TUI) and expressed as units/100 g dry wt.

**Estimation of tannins:** Tannins were estimated by Vanillin-HCl method of Price *et al.* [18]. Five gram of defatted seed material was used for extraction of tannins by using acidic methanol. One ml of suitably diluted extract was taken in a test tube and 5 ml of freshly prepared vanillin-HCl reagent was added slowly with mixing and colour developed was read at 500 nm. Catechin standards were run simultaneously along with sample. The results were expressed as mg/100 g dry wt.

**Estimation of polyphenols:** Polyphenol substances were estimated by Folin-Denis method [19]. About 200 mg defatted material was taken in a 250 ml round bottomed flask and 100 ml of 1% HCl in methanol was added. The contents were refluxed for 2 h, cooled, filtered and the volume was made up to 100 ml with acid-methanol after few washings. 0.2 ml of extract was taken and 7.5 ml of water and 0.5 ml Folin-Denis reagent were added and mixed. To this, 1 ml of saturated sodium carbonate solution was added and volume made up tot 10 ml with water, mixed and the absorbance was measured at 760 nm after 30 min. The results were calculated as mg tannic acid equivalents/g sample and expressed as mg/100 g dry wt.

**Estimation of phytic acid:** Phytic acid was estimated by the method of Davies and Reid [20]. One g of material was ground and extracted with HNO<sub>3</sub> by continuous shaking, filtered and made up to suitable volume with water. To 1.4 ml of the filtrate, 1 ml of ferric ammonium sulphate solution (21.6 mg in 100 ml water) was added, mixed and placed in a boiling water bath for 20 min. The contents were cooled and 5 ml of isoamyl alcohol was added and mixed. To this, 0.1 ml ammonia solution was added, shaken thoroughly and centrifuged at 3000 rpm for 10 min. The alcoholic layer was separated and the colour intensity was read at 465 nm against amyl alcohol blank after 15 min. Sodium phytate standards were run along with the sample. The results were expressed as mg phytic acid/100 g dry wt.

**Estimation of phytate phosphorus:** Phytate phosphorus was estimated by combination of three methods. Extraction and precipitation was done according to the method of Wheeler and Ferrer [21] and Makower [22] and phosphorus measured by the method of Fiske and Subba Row [23]. The results were expressed as mg/100 g dry wt.

**Statistical analysis:** Each value presented in tables and figure represents mean±SE of 6 individual determinations, unless otherwise stated. The level of significance was calculated by student's t-test. The resulting P values indicated in the tables.

## RESULTS

The dry seed composed of starch is the major component (33%) and protein accounted for 25% of dry weight. However, the fat content was notably lower with only 0.8% and had high dietary fibre constituting 7.2%. Flatulence causing oligosaccharides-raffinose, stachyose (Fig. 1), trypsin inhibitory activity, phytic acid, polyphenols, tannins and phosphorus, phytic acid and phytate phosphorus of Indian bean seeds have been studied during germination (Table 1 and 2). Effect of cooking by applying three methods-roasting, boiling and pressure cooking on the same germinated cotyledons was also been tested (Table 3).

The raw dry Indian bean had a very high trypsin inhibitory activity which progressively decreased by 51% during the 12 h soaking period which decreased gradually to reach a level of 17% of the basal level of dry seeds at 32 h germination. The trypsin inhibitory activity of raw seeds was found to be decreased by all the cooking methods employed. The maximum reduction was caused by roasting. Pressure cooking resulted in increased loss of trypsin inhibitors compared to boiling (Table 1 and 3). Phytic acid and phytate phosphorus decreased during soaking and as well during germination. Phytate phosphorus constituted nearly 56% of total phosphorus in the raw seed and 34% after germinating for 32 h. Roasting reduced phytic acid to 8% followed by boiling and then pressure cooking (Table 2 and 3).

The mean value of polyphenolic compounds in the raw seed was 3.5 mg % which decreased to a marked extent during soaking (42%) and further by germinating for 32 h. The overall fall in polyphenols was by 70% at the end of 32 h germination period. The tannin component was nearly 1/4th of the polyphenolic component in the raw seed which also fell during soaking and germination.

Polyphenols and tannins were reduced to maximum by boiling and pressure cooking. Boiling and pressure cooking reduced polyphenol by 85% in raw seed and roasting brought about only 13% reduction. Similar trend was noticed with tannins. Germination was more effective method in reducing trypsin inhibitor activity, tannins, polyphenols and phytic acid than the various cooking treatments.

## DISCUSSION

Pulses are important sources of protein in the diets of millions of people in the world [24, 25]. However their contribution to the nutrition of the consumer is limited, principally due to poor digestibility and antinutritional factors [2]. Soaking of dry Indian bean seeds for 12 h reduced the trypsin inhibitory activity, phytates, tannins and total polyphenols to the extent of 51, 15, 35 and 43%, respectively (Table 1 and 2). Soaking of mung bean seeds in plain water for 12 h lowered phytic acid and polyphenols of mung bean significantly. This decrease was attributed to leaching out into soaking water along the concentration gradient. Similar losses of phytic acid [26], tannins [27] and polyphenols [28] during soaking have been reported earlier for various legume seeds. The trypsin inhibitor activity had a drop after six day of germination indicating a possible increase on the digestibility of the proteins [29, 30]. A significant decrease in the levels of raffinose and stachyose was noticed during 32 h germination study (Fig. 1).

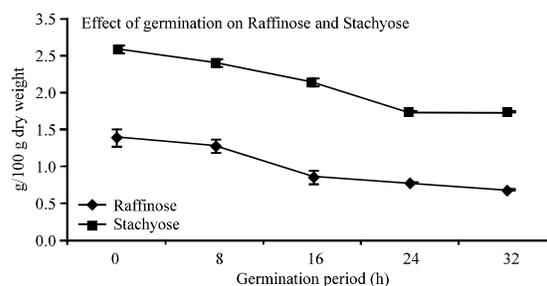


Fig. 1: Changes in the levels of raffinose and stachyose of Indian bean seeds during germination period

Table 1: Changes in trypsin inhibitory activity, tannins and polyphenols in Indian bean during germination

Germination period (h)	TIU/100 g d.wt. (X10 <sup>3</sup> )	Tannins mg/100 g d.wt.	Polyphenols mg/100 g d.wt.
Raw	1916.42±3.97	0.85±0.01	3.50±0.01
0	930.65 <sup>b</sup> ±2.71	0.55 <sup>b</sup> ±0.01	2.00 <sup>b</sup> ±0.01
8	675.18 <sup>b</sup> ±4.95	0.50±0.01	2.00±0.01
16	523.48 <sup>b</sup> ±1.27	0.45±0.01	2.10±0.01
24	390.52 <sup>b</sup> ±1.25	0.39±0.01	1.00 <sup>b</sup> ±0.01
32	318.51 <sup>a</sup> ±1.33	0.40±0.01	1.05±0.01

Table 2: Changes in phosphorus, phytic acid and phytate phosphorus in Indian bean during germination

Germination period (h)	Phosphorus	Phytic acid	Phytate phosphorus
Raw	430±0.52	82±0.33	243±0.52
0	430±0.33	70 <sup>a</sup> ±0.33	230±0.52
8	430±0.33	68±0.33	230±0.33
16	483 <sup>a</sup> ±0.33	60 <sup>a</sup> ±0.33	186 <sup>b</sup> ±0.33
24	483±0.33	57±0.33	184±0.33
32	498±0.52	52±0.33	171±0.33

Significance between 8 h interval, a: p<0.05, b: p<0.001, Indian bean seeds were imbibed in water for 12 h after surface sterilization and germinated in perforated trays as described in materials and methods. At designated intervals of time the seeds were removed and processed as described in materials and methods. Each value is the mean of ±SE of six values

Table 3: Effect of cooking on antinutrient levels of Indian bean seeds

Cooking method	Trypsin Inhibitor activity	Phytic acid	Total polyphenols	Tannins
Raw seeds	1916.42±3.97	82.00±0.33	3.50±0.01	0.85±0.01
Roasting	1061.23 <sup>b</sup> ±2.52	75.80±0.33	3.03±0.02	0.73±0.01
Boiling	1385.78 <sup>b</sup> ±13.57	79.20±0.33	0.52 <sup>b</sup> ±0.01	0.20 <sup>b</sup> ±0.01
Pressure	1172.34±24.77	80.23±0.33	0.48 <sup>b</sup> ±0.01	0.20 <sup>b</sup> ±0.01

Significance difference with raw seeds, a: p<0.05, b: p<0.01; Dry seeds are subjected to cooking and preceded for antinutrients analysis as mentioned in materials and methods. Each value is mean±SE of six values

The residual raffinose was only 50% of the initial and the stachyose also showed similar decrease with an initial value of 3.0 g at 0 h which fell 1.8 g by 32 h during germination. Studies of Alani *et al.* [31] indicated a decrease in the flatus-related sugar content of cow peas, especially stachyose when germinated for 24 h. Germination quantitatively reduces raffinose, stachyose and verbascose while sucrose was increased in all seeds except red lima beans and jack beans [12].

Dehusking, germination, cooking and roasting have been shown to produce beneficial effects on nutritional quality of legumes. The commonest process of preparing pulses for consumption at the household level is to cook them by boiling in water. The levels of antinutrients were reduced on boiling of dry Indian bean (Table 3). Tinsley *et al.* [32] reported 38 and 41% reduction of phytic acid in white and brown tepary beans, respectively and 53, 7 and 16% reduction in fava, cow pea and chick pea, respectively by boiling. The tannin content of Indian bean decreased to 76.47% on boiling. Egbe and Akinyele [33] investigated the effect of boiling on tannin content of lima beans and the level of tannins in raw beans was found to be decreased with increase in cooking time. Pretreatments had a significant effect on the changes in the chemical composition of faba beans and caused a significant decrease in the antinutritional factors, especially soaking followed by dehulling, whereas decortication led to a significant increase in phytic acid content [11]. Egbe and Akinyele [33] demonstrated that total polyphenol of lima

beans decreased from 122 to 55 mg g<sup>-1</sup> when boiled for 60 min and as boiling time increases further significant decrease was noticed. Similar decrease in the polyphenol of Indian bean was observed (Table 2). Fenugreek sprouts had the highest phenolic content of 0.75 mg g<sup>-1</sup> FW on day 3 of germination which was approximately 25% higher. The higher antioxidant activity was observed during early germination, which correlates to higher phenolic content, suggesting that initially phenolics are antioxidant in nature [34]. Soaking for 12 h, dehulling of soaked seeds and germination for different time periods (24, 36 and 48 h) contributed significantly in reducing the phytic acid and polyphenol content of cowpeas. Removal of seed coat (dehulling) of soaked cowpeas reduced the polyphenols by 70-71% [35].

Pressure cooking is popular method of cooking as it saves cooking time and conserves nutrients. The loss of antinutrients was similar either by boiling or pressure cooking except in the case of TIA. A further loss of 11% in the TIA was noticed by pressure cooking compared to boiling. Pressure cooking of pigeon pea seeds completely destroyed the TIA while it was reduced to the extent of 86-88% against the control in 48 h pigeon pea sprouts [36]. Roasting is method classified as dry-heat cookery where hot air is used as a medium of cooking. There was 45% decrease in trypsin inhibitory units when Indian bean was roasted. Similar decrease was observed when groundnut kernels were subjected to roasting [37]. Roasting of Indian bean reduced the levels of phytic acid,

tannins and polyphenols (Table 3). Heat treatment had been proved to be effective to remove phytates from seeds. The results showed by Ibrahim *et al.* [38] that long-time soaking (16 h) caused remarkable reduction in the antinutritional factors.

The level of antinutritional factors-trypsin inhibitory activity, phytates, tannins and total polyphenols reduced considerably with germination. Of the different cooking processes employed with dry seeds, roasting was effective in reducing the phytic acid and TIA. Boiling and pressure cooking were effective in reducing total polyphenols, tannins and TIA. However, the germination was more effective in reducing trypsin inhibitor activity, tannins, polyphenols and phytic acid than the various cooking treatments. The results obtained from this study indicate that the germination is a simple biochemical enrichment tool to enhance the palatability, which incidentally may result in increasing the digestibility and nutritive value through its indispensable food ingredients.

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