

Original Research Article

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Nutritional and Biochemical Alterations in *Vigna Radiata* (Mung Bean) Seeds by Germination

Inamul Hasan Madar^{1†*}, Amjad Hussain Asangani^{2†}, Shantkriti Srinivasan^{2†},
Iftikhar Aslam Tayubi³ and Gideon I. Ogu⁴

¹Department of Biotechnology and Genetic Engineering, Bharathidasan University,
Tiruchirappalli-620024, Tamil Nadu, India

²Department of Biotechnology, National College (Autonomous), Dindigul Road,
Tiruchirappalli-620001, Tamil Nadu, India

³Faculty of Computing and Information Technology, King Abdul Aziz University,
Rabigh, Kingdom of Saudi Arabia

⁴Department of Biological Sciences, Novena University, Ogume, Delta State, Nigeria

*Corresponding author; †These author contributed equally to this work

ABSTRACT

Keywords

Mung bean, *Vigna radiata*, Nutrition, Germination, Biochemical composition, Protein, Amino acid, Carbohydrate, Chlorophyll.

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Mung bean (*Vigna radiata*), a rich source of many nutrients with potential health benefits is a commonly used functional food in India. The nutritional composition of *Vigna radiata* seeds were estimated in terms of carbohydrate, free amino acid, protein, chlorophyll and fat content pre and post germination to assess the biochemical changes in seeds. They were found to have slightly higher carbohydrate content (0.021 mg/g) after germination. The amino acid (0.239 mg/g) and protein content (0.207 mg/g) were appreciably increased compared to dry seeds. There was an insignificant difference in the fat, chlorophyll a and b contents of pre and post germinated seeds. These results suggest that the nutritional content and quality of the seeds of *Vigna radiata* improves after germination.

Introduction

Pulses belonging to the *Leguminosae* family have been used as food for thousands of years. Also known as legumes, they have high protein content and are easily digestible (Munoz *et al.*, 1996). Pulses are said to be the poor man's meat and are grown worldwide for consumption due to their high nutrition and low cost aiding in overcoming protein malnutrition in developing countries like

India (Manay and Shadaksharaswamy, 2001). They are also rich in carbohydrate, vitamins and minerals such as calcium, magnesium, zinc, iron, potassium and phosphorus. They restore soil fertility and climate change through their nitrogen-fixing properties. They can be stored in dry and dark conditions for prolonged use. India is the largest producer, consumer and importer of pulses in the world.

Among pulses, Mung bean, known as Green gram, *Vigna radiata* or *Phaseolus aureus*, is cultivated in India, China, Pakistan, Thailand, Indonesia, Burma, Philippines, Bangladesh, Vietnam, Laos, Cambodia and in hot and dry areas of Southern Europe and United States (Verma *et al.*, 2017). In India, it is produced in Karnataka, Andhra Pradesh, Tamil Nadu, Maharashtra, Orissa, Rajasthan, Uttar Pradesh, Gujarat, Madhya Pradesh, West Bengal and Punjab constituting 11.48 % of all pulses (Yadav *et al.*, 2014). It has carbohydrate (51 %), protein (24-26 %), mineral (4 %), vitamins (3 %) and fat (1 %) (Afzal *et al.*, 2008). It has bright green edible legume seeds which ripens uniformly and can be eaten raw or cooked. Mung bean seeds are an invaluable source of digestible protein for vegetarians (AVRDC, 2012).

Along with the nutritional factors, pulses also contain flatulence causing raffinose family oligosaccharides, phytate, heat-stable and heat-labile antinutritional and toxic factors (Salunkhe, 1982). Various pulse processing methods like presoaking, germination or fermentation reduce these factors making legumes as an inexpensive and nutritious human food.

Germination activates biosynthetic enzymes that remove or decrease antinutritional and indigestible factors in legumes thereby enhancing its nutritive value (Bau *et al.*, 1997). Germination was effective in decreasing fat, total carbohydrate, antinutritional factors and total ash contents while increasing crude protein compared to cooked mung bean seeds and higher retaining of all minerals (Mubarak, 2005). Germination of mung beans causes significant changes in metabolite contents, such as breakdown of complex macromolecules like starch, sugars and proteins into smaller and more digestible molecules, decreased concentration of antinutritional factors and increased levels of free amino acids (Chang & Harrold, 1988;

Labaneiah and Luh, 1981; Kataria *et al.*, 1989; Chau and Cheung, 1997).

Biochemical changes due to germination cause synthesis and accumulation of active compounds such as polyphenols, saponins, vitamin C etc. from macromolecular substances supporting absorption and utilization which provides antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antihypertensive, antihypertensive and antitumor activities along with nutritive benefits (Tang *et al.*, 2014). Thus, use of mung bean as a nutrient rich food source is of particular interest. But, research on studying the alterations in its nutritional composition on germination is limited. Thus, the objective of this study was to investigate the effect of germination on biochemical composition and nutritional content of mung bean seeds.

Materials and Methods

All reagents used in this work were of analytical grade. Double distilled water was used for solution preparation.

Seed collection

The seeds of *Vigna radiata* (mung bean) were procured from local retailers in Kumbakonam, cleaned thoroughly and used for further studies.

Seed treatments

Mung bean seeds were used to study interaction between crude extract and treated samples. 250 mg of seeds were soaked in water in 1:3 ratio (w/v) for over 24 h. Soaked grains were then dried at 45 °C and ground to pass a 200 µm screen. Another portion (250 mg) was immersed in water 1:3 (w/v) overnight and grains were spread on trays, bound with cloth at room temperature (25 °C) for one week. It was moistened by water spraying at frequent intervals. After

germination, the seeds were dried separately at 45 °C to constant weight and powdered. One part (250 mg) not subjected to any treatment served as a control (dry or pre-germinated seeds).

Extraction and estimation of total carbohydrates

0.5 g of powdered sample was homogenized with 10 mL of 80 % ethanol. The insoluble residue was removed by centrifuging at 3000 g for 15 min. The precipitate was re-extracted with 2 mL of 80 % ethanol and recentrifuged. The supernatants were pooled and dried under a stream of hot air and residue was resuspended in 5 mL of water.

Total carbohydrates were estimated by Anthrone method (Sadasivam and Manickam, 2008). Seven cleaned test tubes were taken and marked as Blank, S₁ to S₅ and Test. 1 mL water was used as blank. 0.2 to 1 mL of working standard was poured in S₁ to S₅ tubes respectively. The volume was made upto 1 mL with water. Next, the given mung bean solution was made upto 100 mL with water in a standard flask. 1 mL of this diluted mung bean solution was used as the Test sample. The test tubes were kept in ice waterbath for 10 min. Then 4 mL of anthrone reagent was added to each test tube and kept in a boiling waterbath for 15 min. Readings were taken at 640 nm.

Extraction and estimation of protein content

500 mg of the sample was powdered with 5 to 10 mL of buffer, centrifuged and the supernatant was used for protein estimation (Lowry, 1951).

0.2 to 1 mL of working standard and 1 mL of diluted mung bean extract (Test) were poured in a series of test tubes and the volume was made upto 4 mL with distilled water. The

aliquots were incubated with 5.5 mL of alkaline copper sulphate reagent in a water bath for 10 min. Then, 0.5 mL of Folin's reagent was added and incubated at room temperature for 30 min for change to blue colored product. The blank contained 4 mL of distilled water with 5.5 mL alkaline copper sulphate and 0.5 mL Folin's reagent. The optical density of solution was read at 650 nm.

Extraction and estimation of total free amino acids

500 mg of mung bean sample was ground with a small quantity of acid-washed sand in a pestle and mortar. 5 to 10 mL of 80 % ethanol was added to this homogenate, then filtered or centrifuged to obtain clear filtrate or supernatant. The extraction was repeated twice with the residue and all the supernatants were pooled together. The volume was reduced by evaporation and the extract was used for quantitative estimation of total free amino acids.

1 mL ninhydrin solution was added to 0.1 mL extract and the volume was made upto 2 mL with distilled water. Tube was heated in a boiling waterbath for 20 min. 5 mL of the composition was added and contents were mixed. After 15 min Intensity of the purple coloured mixture was measured at 570 nm.

Extraction and estimation of chlorophyll

1 g of sample was ground well with 20 mL of 80 % acetone and centrifuged at 5,000 rpm for 5 min. The supernatant was transferred to a 100 mL volumetric flask. The residue was grounded with 20 mL of 80 % acetone, centrifuged again and the supernatant transferred to the same flask. The procedure was repeated until the residue became colourless. The mortar and pestle was washed thoroughly with 80 % acetone and clear washings were collected in flask. The volume

was made upto 100 mL with 80 % acetone (More and Borkar, 2016).

Chlorophyll content was estimated using method by Arnon (1949). Absorbance of the solution was measured at 663 (chl a) and 645 (chl b) nm with the solvent (80 % acetone) as blank. Absorption coefficients for chlorophyll a and chlorophyll b were used to calculate the chlorophyll concentrations.

Extraction and estimation of fat content

The dry seed sample was weighed accurately in a petridish and extracted with twice volume of anhydrous ether. The extract was filtered and transferred into a weighed conical flask. It was dried in an oven at 80-100 °C, cooled and weighed again.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for triplicates. Biochemical analysis between control and test was subjected to statistical analysis by t-test at 5 % level of significance.

Results and Discussion

Figure 1 showed the dry and germinated seeds of *Vigna radiata* (mung bean).

The nutritional contents of mung bean seeds are depicted in Table. 1-6. the germinated

seeds showed 0.021 mg/g carbohydrate content in the sample while dry seeds had 0.017 mg/g carbohydrates which is comparatively less (Table 1). A smaller amount carbohydrate may be due to lack of raffinose resulting in hydrolysis of sucrose to supply energy (Mubarak, 2005).

The pre-germinated seeds recorded less protein value (0.027 mg/g) whereas post germination, protein content increased manifold to 0.207 mg/g (Table 2). Mung beans contain higher amounts of protein with globulin and albumin as main storage proteins in the seeds (Kirchhoff, 2002). Increase in protein content by germination is attributed to a passive variation.

Dry seeds contained 0.039 mg/g of amino acids while post germination, maximum amino acid content of 0.239 mg/g was observed in the seeds (Table 3). There was a significant difference between the dry and germinated seeds at $P < 0.05$. Thus, a considerable increase in protein and amino acid content was observed post germination in the seeds. They are rich in essential amino acids and proteolytic cleavage of proteins during sprouting further increases the amino acid levels significantly (Tang *et al.*, 2014). The increased amino acid content may be due to synthesis of glutamic acid and other highly active amino acids through the mediation of enzymes in germinating seeds (Sivaramakrishnan and Sarma, 1956).

Table.1 Carbohydrate content in mung bean seeds before and after germination

S. No.	Carbohydrate (mg/g)	
	Dry seed	Germinated seed
1.	0.018	0.02
2.	0.016	0.022
3.	0.018	0.02
Mean ± S.D	0.017 ± 0.001	0.021 ± 0.001

Table.2 Protein content in mung bean seeds before and after germination

S. No.	Protein (mg/g)	
	Dry seed	Germinated seed
1.	0.026	0.206
2.	0.028	0.208
3.	0.026	0.206
Mean ± S.D	0.027 ± 0.001	0.207 ± 0.001

Table.3 Amino acid content in mung bean seeds before and after germination

S. No.	Amino Acid (mg/g)	
	Dry seed	Germinated seed
1.	0.04	0.238
2.	0.036	0.24
3.	0.04	0.24
Mean ± S.D	0.039 ± 0.002	0.239 ± 0.001

Table.4 Variation in fat content in mung bean seeds before and after germination

S. No.	Total Fat (mg/g)	
	Dry seed	Germinated seed
1.	0.072	0.08
2.	0.068	0.082
3.	0.072	0.082
Mean ± S.D	0.071 ± 0.002	0.081 ± 0.001

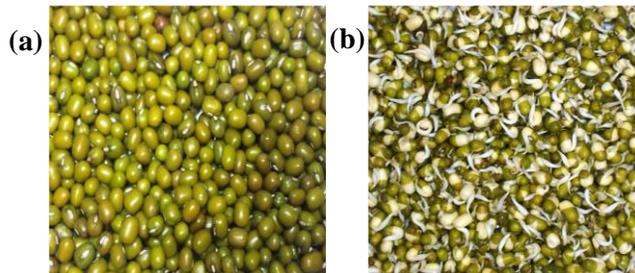
Table.5 Chlorophyll a content in mung bean seeds before and after germination

S. No	Total Chlorophyll a (mg/g)	
	Dry seed	Germinated seed
1.	0.0093	0.01
2.	0.0095	0.011
3.	0.0094	0.01
Mean ± S.D	0.0094 ± 0.0001	0.01 ± 0.0006

Table.6 Chlorophyll b content in mung bean seeds before and after germination

S. No	Total Chlorophyll b (mg/g)	
	Dry seed	Germinated seed
1.	0.0229	0.0237
2.	0.0228	0.0239
3.	0.0227	0.0238
Mean ± S.D	0.0228 ± 0.0001	0.0238 ± 0.0001

Fig.1 Seeds of Mungbean (*Vigna radiata*) (a) pre-germination (b) post-germination



The dry and germinated seeds showed fat content of 0.071 mg/g and 0.081 mg/g respectively (Table 4). Changes in fat levels are minimal displaying no significant difference between the two groups. Legumes are naturally low in fat and are particularly free of saturated fat and cholesterol (Polak *et al.*, 2015). Pulses like beans have a low fat content (Fabbri and Crosby, 2016). The low fat content supplies energy and essential fatty acids (Deraz and Khalil, 2008).

Chlorophyll a content was 0.0094 mg/g and 0.01 mg/g during pre and post germination (Table 5). The chlorophyll b value was recorded as 0.0228 mg/g and 0.0238 mg/g in dry and germinated seeds (Table 6). Chlorophyll b was higher than Chlorophyll a both pre and post germination. However, there was no significant difference in chlorophyll a as well as chlorophyll b content between the germinated and dry seeds. The chl a/b ratio did not vary significantly among the two groups. Seed chlorophyll content is reported to have an inverse relationship with the quality as seeds with lower amounts of chlorophyll show high germination rates in the late phase of seed maturation (Jalink *et al.*, 1998).

Thus, it may be suggested that germination of mung bean seedlings is involved in changes in the nutritive contents, biochemical composition, growth parameters and photosynthetic pigments. There was a dramatic increase in proteins and amino acids involving *de novo* synthesis of new proteins and accumulation of certain existing proteins compared to the dry mung bean seeds. Improved nutritional values of legumes through such techniques have immense importance in alleviation of food crisis. Thus, it is encouraged to increase the consumption of germinated mung

beans for enhanced nutrition uptake and disease prevention.

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