



# EFFECT OF SPROUTING TIME ON ANTINUTRITIONAL FACTORS IN *VIGNA MUNGO* VARIETIES

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## ABSTRACT

In the present study a comparative study was conducted to determine the effect of sprouting time (24 hr, 48 hr, 72 hr and 96 hr) on the antinutritional factors of two *Vigna mungo* varieties (Shekher-2 and Uradsadabahar) was investigated. Results revealed that in the Antinutritional factors viz. Tannin, Total Phenol, Saponin, Oxalate and Trypsin Inhibitors were found to be higher in Uradsadabahar than in Shekher-2. Significant reduction in Antinutritional factors was observed due to sprouting treatments. Among the different sprouting time the maximum reduction in antinutritional was recorded after 96 hr sprouted seeds of both varieties (Shekher-2 and Uradsadabahar) i.e. Tannin (55.56% and 58.74%), Total Phenol (69.80% and 70.50%), Saponin (41.46% and 52.08%), Oxalate (12.07% and 11.61%) and Trypsin Inhibitors (47.63% and 50.65%). The results shows that sprouting improved nutritional worth of the *Vigna mungo* varieties in terms of higher concentration of nutrients, reduced anti-nutritional factors.

**Key words :** *Vigna mungo*, sprouting, antinutritional factors, oxalate.

## Introduction

The anti-nutritional factors (ANFs) may be defined as those substances generated in natural feed stuffs by the normal metabolism of species and by different mechanism (e.g., inactivation of some nutrients, diminution of the digestive process), which exert effects contrary to optimum nutrition (Soetan, 2008). ANFs can be divided into protein and non-protein ANFs. Non-protein ANFs include alkaloids, tannins, phytic acid, saponins and total phenolics, while protein ANFs includes trypsin inhibitors, chymotrypsin inhibitors, lectins and antifungal peptides. Protease inhibitors interfere with digestion by irreversibly binding with trypsin and chymotrypsin in the human digestive tract and in pancreatic hypertrophy (Liener *et al.*, 1996). Trypsin inhibitors are polypeptides that form well characterized stable complexes with trypsin, obstructing the enzymatic action. Protease inhibitors are inactivated by heat especially moist heat, because of even distribution of heat (Bressani and Sosa, 1990). The major concern about the presence of phytate in the human diet is its negative effect on mineral uptake. Phytic acid can bind to several important divalent cations (e.g. iron, zinc, calcium and magnesium) forming insoluble complexes and making them unavailable for absorption and utilization

in the small intestine (Adeyeye *et al.*, 2002). Tanins inhibit enzymes, reducing the digestibility and making black gram astringent.

Black gram (*Vigna mungo*) or urad is one of the important pulse crop in India. It is related to the family leguminoseae. It is reported that Black gram is originated in India and is the largest producer and consumer in the world. It is a rich protein food which contains about 26% protein, almost three times that of cereals. Black gram supplies a major share of protein requirement of vegetarian population of the country.

Pulses are valued for their protein content as well as their low glycemic index and are commonly included in diets in the Indian subcontinent (Khandelwal *et al.*, 2010). Consumption of pulses is highest in India as compared to other pulse growing countries due to low purchasing power and religious restrictions on non-vegetarian diet (Jain *et al.*, 2009). Pulses, when blended with cereal proteins and other foods rich in sulphur containing amino acids and tryptophan, provide a well-balanced essential amino acid profile and may offer a promising alternative source for nutritional and functional proteins.

Black gram *Vigna mungo* is an important pulse crop occupying unique position in Indian agriculture. The seeds

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**Table 1** : Effect of sprouting time on antinutritional factors in *Vigna mungo* varieties (g/100g).

Varieties	Antinutritional factors	Treatments				
		Control	24 hour	48 hour	72 hour	96 hour
Shekher-2	Tannin content (mg/g)	8.37±0.005	6.55±0.013	4.32±0.008	3.85±0.006	3.72±0.006
	Total Phenol content	2.45±0.035	0.88±0.005	0.81±0.006	0.77±0.008	0.74±0.003
	Saponin content	3.37±0.160	2.86±0.045	2.69±0.030	2.53±0.005	2.26±0.012
	Oxalate content	1.74±0.015	1.73±0.008	1.65±0.003	1.63±0.005	1.53±0.008
	Trypsin Inhibitor	21.44±0.0088	16.69±0.032	14.32±0.012	13.23±0.006	11.23±0.012
Urad sadabahar	Tannin content	8.82±1.660	6.97±0.012	4.52±0.011	3.94±0.012	3.64±0.333
	Total Phenol content	2.61±0.006	1.11±0.005	0.89±0.005	0.83±0.010	0.77±0.006
	Saponin content	3.86±0.051	3.15±0.025	2.17±0.005	2.01±0.005	1.85±0.008
	Oxalate content	1.81±0.005	1.79±0.044	1.70±0.003	1.67±0.003	1.60±0.003
	Trypsin Inhibitor	24.74±0.081	17.53±0.008	15.47±0.003	14.79±0.006	12.21±0.012

of black gram contain a moderately high amount of calories (calorific value of 350 cal/100 g), carbohydrates (56.6%), proteins (26.2%) and fat (1.2%). In addition to being an important source of proteins and calories, black gram is rich in minerals:- calcium (185 mg/100 g), iron (8.7 mg/100 g) and phosphorus (345 mg/100 g) and vitamins:- vitamin B<sub>1</sub> (0.42 mg/100 g), vitamin B<sub>2</sub> (0.37 mg/100 g) and niacin 2 mg/100 g (Panwar, 2005).

### Materials and Methods

Seeds of black gram varieties Shekher-2 and Uradsadabahar was obtained from certified seed store of Alopibagh, Allahabad. The seed sample was hand sorted manually to remove all foreign matters. Seeds were rinsed and then soaked in 0.07% sodium hypochlorite solution for 30 minute at ambient temperature (22– 23°C) to remove surface contamination. Seeds were then drained, washed to neutral pH and then soaked in distilled water (1 : 3 w/v) for 12 hrs. Finally, imbibed seeds were transferred to petri dishes lined with wet filter paper and then were placed in a dark, for germination at 20°C. The seeds were germinated for 24 hrs. Dried samples were milled to flour with an electric grinder for the analysis of antinutritional factors.

Analysis for total phenols was carried out calorimetrically by using method Bray and Thorpe (1954) and Tannin contents were measured by Folin-Denis method (1970). Alkaloid content in the sample was determined by the method described by Obadoniand Ochuko (2001), Saponin content in the legume sample was determined by the method of Harbone (1973) and Phytate content in legume meals was determined by procedure elaborated by Mohamed *et al.* (1986). Trypsin inhibitor activities of legume samples were measured

according to the procedure of Roy and Rao (1971).

### Results and Discussion

Tannin content of two *Vigna mungo* varieties as affected by sprouting treatment at different time interval was depicted higher level of tannin content in raw seeds of variety, Uradsadabahar (8.82 mg/g) than Shekher-2 (8.37 mg/g). The wide variation of tannin content recorded in the two varieties of *Vigna mungo* may be due to the variation of pigment in seed coat of Shekher-2 and Uradsadabahar because tannin is mainly concentrated in the seeds coat of the legume, thus preliminary dehulling constitutes the simplest way for their removal. However, among the sprouting seeds of variety Shekher-2 and Uradsadabahar at different times applied, 24 hr (6.55 mg/g and 6.97 mg/g), 48 hr (4.32 mg/g and 4.52 mg/g), 72 hr (3.85 mg/g and 3.94 mg/g) and 96 hr (3.72 mg/g and 3.64 mg/g) were effective in reduction of tannin content. Maximum reduction in tannin content was observed in Shekher-2 and Uradsadabahar variety after 96 hr (55.56% and 58.74%) followed by 72 hr (54.01% and 55.33%) of sprouting treatment. Two fold reduction was recorded during 48 hr sprouted seeds of *Vigna mungo* variety Shekher-2 (48.39%) and Uradsadabahar (48.76%) as compared to raw seeds. Total phenol content was significantly reduced correspondingly with the increase in sprouting time. Phenol content in raw seeds of variety Shekher-2 and Uradsadabahar was observed to be 2.45 mg/g and 2.61 mg/g. Maximum reduction in phenol content was recorded in 96 hr and 72 hr sprouted seeds of variety Shekher-2 (69.8% and 68.58%) and Uradsadabahar (70.5% and 68.2%), respectively. Three fold reduction in total phenol content of variety Shekher-2 was noted after 24 hr (64.09%) and 48 hr (66.94%)

sprouted seeds as compare to raw seed. On the other hand, the Uradsadabahar displayed three fold reduction in phenolics after 48 hr (65.91%) and 72 hr (68.20%) sprouted seeds as compare to raw seeds. The reduction of total phenolics compounds during germination may be attributed to the presence of polyphenol-oxidase and enzymatic hydrolysis (Rao and Deosthale, 1982). Saponin content was observed 3.37 mg/g and 3.86 mg/g in raw seeds of variety Shekher-2 and Uradsadabahar, respectively. Reduction in saponin content of variety Shekher-2 and Uradsadabahar was observed in 24 hr (15.14% and 18.4%), 48 hr (20.18% and 43.79%), 72 hr (24.93% and 47.93%) sprouted seeds as compare to raw seeds and maximum reduction in saponin content was observed in variety Uradsadabahar (52.08%) than Shekher-2 (41.46%) after 96 hr sprouting seeds. Oxalate content in raw seeds of variety Shekher-2 and Uradsadabahar was recorded 1.74 mg/g and 1.81 mg/g. Minor reduction was observed after 24 hr sprouted seeds of Shekher-2 (0.58%) and Uradsadabahr (1.11%). Near about similar results were observed in the reduction of oxalate content after 48 hr and 72 hr sprouted seeds of variety Shekher-2 (1.65 mg/g and 1.63 mg/g) and Uradsadabahar (1.70 mg/g and 1.67 mg/g). Maximum reduction was observed in 96 hr sprouted seeds of variety Shekher-2 (12.07%) and Uradsadabahar (11.61%) as compared to raw seeds. Raw seeds of *Vigna mungo* varieties viz. Shekher-2 and Uradsadabahar recorded the highest level of trypsin inhibitor activity i.e. 21.44 TIU/g and 24.74 TIU/g. Trypsin Inhibitor activity of both varieties Shekher-2 and Uradsadabahar was decreases significantly with the increase in sprouting time that was observed 24 hr (22.16% and 29.15%), 48 hr (33.21% and 37.47%), 72 hr (38.30% and 40.22%) and 96 hr (47.63% and 50.65%), respectively. The variation in trypsin inhibitor activity in the seeds of two variety of *Vigna mungo* may be attributed to their genetical variation causing the synthesis and deposition of this antitrypsic protein to different extent. A decrease in trypsin Inhibitor activity during germination may perhaps be because of mobilization and breakdown of chemical constituents (Kakati *et al.*, 2010).

## References

- A.O.A.C. (2000). *Official method of analysis*, Association of official analytical chemists, 16<sup>th</sup> edition, Washington DC.
- Bishnoi and N. Khetarpoul (1994). Protein digestibility of vegetable field peas (*Pisum sativum*) varietal differences and effect of domestic processing and cooking methods. *Plant Food for Human Nutrition*, **46(1)** : 11-16.
- Bray, H. G. and W. V. Thorpe (1954). Estimation of phenol, Meth. *Biochemical Analysis*, **1** : 27-52.
- Bressani, Mata and Sosa Harborne (1990). Protease inhibitors are polypeptides that stable complex. *Food Chem.*, **78** : 9-14.
- Daur, I., I. A. Khan and M. Jahangir (2008). Nutritional quality of roasted and pressure-cooked Chick Pea compared to raw (*Cicerarietinum* L.) seeds. *Sarhad J. Agric.*, **24** : 1.
- Jain, Sood and Surendra Jha (2009). Consumption of pulses in Indian subcontinent. *Phytochemistry*, **17** : 605-609.
- Khandelwal, A. K. and S. Kumar (2010). Food and feed from legumes and oil seeds *J. Agric. Food Chem.*, **45** : 3138-3142.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). Determination of protein. *J. Biol. Chem.*, **193** : 265-275.
- Liener, I. E. (1996). Effect of processing on antinutritional factors in legumes : the soyabean case. *Arch. Latinoam Nutrition*, **44(4)** : 485-545.
- Mohamed, A. I., P. A. J. Perera and Y. S. Hafez (1986). New chromophore for phytic acid determination. *Cereal Chem.*, **63** : 475-476.
- Panwar, J. D. S., A. K. Jain and S. Kumar (2009). Antinutritional factors and their detoxification in pulses – A review. *Agric. Rev.*, **30(1)** : 64-70.
- Rao, D. S. S. and Y. G. Deosthale (1981). Mineral composition of four Indian food legumes. *J. Food Sci.*, **46** : 1962-1963.
- Rao, P. U. and B. Belavady (1978). Oligosaccharides in pulses: Varietal differences and effects of cooking and germination. *J. Agri. Food Chem.*, **26** : 316-319.
- Soetan, K. O. and O. E. Oyewole (2009). The need for adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds : A review. *African Journal of Food Scienc*, **3(9)** : 223-232.
- Zia-urRehman and W. H. Shah (1996). Quality improvement of legumes through the application of various physico-chemical treatments. *Pak. J. Food. Sci.*, **6(3-4)** : 63-65.