

## Optimization of antinutritional factors from germinated wheat and mungbean by Response Surface Methodology

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**Abstract:** Cereals and legumes are commonly used as a source of protein and carbohydrates in the human diet in Bangladesh as well as in many other developing countries. Legumes and cereals are also contain antnutritional factors like phytic acid and tannins Phytic acid lowers the utilization of elements like calcium, magnesium, zinc and iron due to its ability to form insoluble salts with their ions. Germination of legume and cereals seeds is an effective processing treatment to reduce anti-nutritional factors and improve the nutritional quality by increasing the level of some amino acids, vitamins and minerals. In the present study was to optimize the effect germination temperature and time on concentration of phytic acid and tannin wheat and mungbean seed. The effect of germination temperature and time on phytic acid and tannin were analyzed by using the Response Surface Methodology (RSM), with a Central Composite Rotatable Design (CCRD).The germination wheat and mungbean seeds at 30-37°C for 48-72 hours resulted experimental values for phytic acid from 533-380 and tannin from 353-296 mg/100 g in wheat seed and 635-482 and 452-396 mg/100 g in mungbean seed phytic acid and tannin respectively. The optimal values input variables (time and temperature) 33.4°C and 60.6 h for phytic acid, 33.5°C and 60.3 h for tannin respectively. The minimum optimal values from multiple response optimizations were 379.9 and 481.9 mg/100 g phytic acid content for wheat and mungbean seeds, 295.7 and 395.8 tannin content for wheat and mungbean seeds respectively. It was concluded that germination of seeds significantly decreased phytic acid and tannin content in wheat and mungbean. All the derived mathematical models for the various responses were found to be fit significantly to predict the data.

**Keywords:** Phytic acid, tannin, estimation, germination effect, optimization, response variable

### Introduction

Legumes and cereals are commonly used as a source of protein and carbohydrates in the human diet in Bangladesh as well as in many other countries. Poor nutritive values of the food legumes, because of the presence of some antinutritional substances (Morrow, 1991). Anti-nutrients are chemical substances in food that do not offer nourishment to the body e.g. phytic acid and tannins. The effect of these anti nutrients in the body depends on the type and the concentration in which it is present in the food material. However, the presence of antinutritional factors (tannins and phytates) limits the utilization of the legumes as a main source of protein (Alonso *et al.*, 1998). Mung bean is an excellent source of protein (27%), and its essential amino acid. However, anti-nutritional factors limit the food applications of mung bean (El-Adawy, 1996). Germination is one of the most common techniques used to reduce most of the antinutritional factors in legumes (Abu-Samaha, 1983). Moisture, total ash and total protein contents have reportedly increased with increased germination time while the antinutritional factors, particularly phytic acid and hemagglutinin activity, decreased

(El-Adawy, 1986; El-Beltagy, 1996). It indicated that antinutritional factors were decreased gradually during germination while water absorption capacity, protein solubility index, emulsification capacity and in vitro protein digestibility improved. Phytic acid (inositol hexaphosphate) is an organic acid found in plant materials (Heldt, 1997). Phytic acid combines with some essential elements such as iron, calcium, zinc and phosphorus to form insoluble salts called phytate, which are not absorbed by the body thereby reducing the bioavailability of these elements. Anemia and other mineral deficiency disorders are common in regions where the diet is primarily a vegetarian (Erdman, 1979). Phytic acid lowers the utilization of elements like calcium, magnesium, zinc and iron due to its ability to form insoluble salts with their ions. Tannins inhibit the digestibility of protein and phytic acid reduces the bioavailability of some essential minerals (Duhan *et al.*, 1989; Vander, 1990). Tannins have the ability to precipitate certain proteins they combine with digestive enzymes thereby making them unavailable for digestion (Abara, 2003). Tannins form insoluble complexes with proteins, carbohydrates and lipids leading to a reduction in digestibility of these nutrients (Binita and Khetapaul,

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1997). Germination conditions for a high amylose rice cultivar were optimized using response surface methodology. Germination time and temperature significantly affected the amylase activity and thiamin content of the subsequent flour. All germination conditions investigated significantly affected the malting loss (Capanzana and Buckle, 1997). Certain tannins and other polyphenols in legumes (e.g., *Vicia faba*) and red sorghum may also be reduced during germination as a result of the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides (Camacho *et al.*, 1992). A number of anti-nutritional factors occur in wheat and in extreme cases may have a toxic effect. Phytic acid may reduce the bioavailability of trace elements in animal diets through chelation of minerals such as iron, zinc, phosphate, calcium, potassium and magnesium (OECD, 2003). Flour from germinated seeds has been reported to have better nutritional properties than flours from non germinated cereals (Lorenz, 1980; Finney, 1983). Supplementary foods made from germinated flours have low viscosity and high nutrient density (Wahed *et al.*, 1994) and have acceptable properties to weaning and infants foods in developing countries (Malleshi *et al.*, 1986; Marero *et al.*, 1988; Malleshi *et al.*, 1989).

Response surface methodology (RSM) is an effective statistical technique for optimizing complex processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions with less laborious and time-consuming (Irakoze *et al.*, 2010). RSM is widely used in optimizing the extraction process variables, such as polysaccharides, anthocyanins, vitamin E, phenolic compounds and protein from varied materials (Chandrika and Fereidoon, 2005; Lee *et al.*, 2005; Li and Fu, 2005). The objective of this research work was to optimize the germination time and temperature in reducing the anti-nutritional (phytic acid and tannin) factors in wheat and mungbean by using response surface methodology and this flour will be used for the supplementary foods preparation.

## Materials and Methods

### Materials

Wheat Seeds (Sofabdy variety) were collected from Bangladesh Agricultural Development Corporation Balashpur Mymensingh. Mungbean seeds (BINA-5 variety) were collected from Bangladesh Institute of Nuclear Agriculture Mymensingh and brought to department of food technology and rural industries and kept in an airtight

polyethylene bags at room temperature in a dry place. Amount of phytic acid per 100 g in the raw seeds tested were  $533.0 \pm 5.000$  (wheat) and  $635.0 \pm 5.000$  (mungbean) and tannin  $353.0 \pm 5.000$  (wheat) and  $452.66 \pm 7.637$  (mungbean).

### Sample preparation

The wheat and mungbean seeds were manually cleaned to remove broken seeds, dust and other foreign matter. Wheat and mungbean seeds were washed with running water for 3 mins. Wheat and mungbean seeds were germinated; following the procedure described by (Frias *et al.*, 2005) 500 g f wheat and mungbean seeds were soaked in distilled water (1:5 w/v) for 10 hours at room temperature ( $30 \pm 2$ ). The water was then drained off and imbibed seeds were germinated by layering them over a moistened filter paper to keep moisture constant in a single layer of seed, thickness of layer was 2 mm in germinating tray. Continuously watered by capillarity in a seed germinator (G-120 Snijders, The Netherlands) Germination was carried out at 30 to 37°C for 48 to 72 hours and relative humidity was 99%. The sprouts were washed and dried at 60°C for 8 h in an electric oven (Contherm, Quantherm 200L, Contherm Scientific, Lower Hutt, New Zealand). Germinated sprouts were grinded with hummer mill at 80 mm particle size for analysis of phytic acid tannin.

### Assay of anti-nutritional factors

Anti-nutritional factors phytic acid and tannins were determined by following methods.

**Estimation of phytic acid:** Phytic acid was determined by the according to method (Sadasivam and Manickam, 1992). One g of material was ground and extracted with HNO 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 standard curve of phytic acid was prepared according to method (AOAC, 2004) for measurement the concentration of phytic acid in our samples (plotting the concentration of different Fe (NO) (mg) against the corresponding reading of Spectrophotometer in Absorbance), the phytates phosphorus was calculated from the concentration of

ferric iron assuming 4: 6 (irons: phosphorus molar ratio).

The results were expressed as mg phytic acid/100 g dry weight with the help of following equation.

$$\text{Phytic acid mg/100g} = \frac{1.5 \times A \times C \times 20 \times 10 \times 50 \times 100}{1000 \times S}$$

Where A= optical density, C= concentration corresponding to optical density, S= weight of sample.

**Estimation of tannins:** Tannins were estimated by Vanillin- HCl method described (Price and Butler, 1987) as modified method (Chang *et al.*, 1994). 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. The standard curve of tannic acid was prepared according to (AOAC, 2004) for measurement the concentration of tannin in our samples (plotting the concentration of tannin acid (mg) against the corresponding reading of Spectrophotometer in Absorbance). The results were expressed as mg/100 g dry weight basis with the help of following equation.

$$\text{Tannin mg/100 g} = \frac{C \times 10 \times 100}{200}$$

Where C = Concentration corresponding to the optical density. 10 = Volume of the extract (ml), 200 = Sample weight (mg).

*Experimental design*

Variation effects in germination time and temperature were analyzed using the response surface methodology (RSM), with a 2 central composite rotational design. The independent variables studied were germination time (48-72 h) and germination temperature (30- 37°C). Symbols and coded factor levels for these variables are given in Table 1.

**Table 1.** Symbols and coded factor levels for the variables

Independent variables		Levels				
Coded	Real	-x	-1	0	+1	+x
X <sub>1</sub>	Temperature of germination (°C)		30	35	37	
X <sub>2</sub>	Time of germination (h)		48	60	72	

*Statistical analysis*

Statistics 5.0 (Stat soft, USA) was used to determine the effects of the independent variables, to calculate regression coefficients (R<sub>2</sub>) carry out

analysis of variance (ANOVA) and build the response surface, at a 5% significance level. The following second order polynomial model was fitted to the data:

$$Y = \beta_0 - \beta_1 X_1 - \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

Where Y is the response variable, X<sub>1</sub> and X<sub>2</sub>, are the coded process variables and β<sub>0</sub>, β<sub>1</sub>, β<sub>2</sub>, β<sub>11</sub>, β<sub>22</sub>, β<sub>12</sub> are the regression coefficients. A stepwise methodology was followed to determine the significant terms in Equation 1. Experimental data were expressed as mean ± standard deviation of triplicate measurements of replicate extraction. One-way analysis of variance with Tukey’s test was used to determine the significant differences (p<0.05) between the means. The experimental data were fitted to the second order polynomial model instead of second order polynomial was fitted to the data. Regression coefficients were obtained from the second order polynomial model.

**Results and Discussion**

Germination mobilizes reserve nutrients required for growth and therefore may help in the removal of some of the unwanted components of dry legumes which are thought to function as reserve nutrients (Sathe and Salunkhe, 1989). The germination conditions adopted in this study were selected after several trial runs, in order to clearly distinguish their influence. The germination was studied at 30-37°C for 48-72 hours because after this time the germinating seeds developed leaves and increased photosynthetic activity was apparent in illuminated samples. In the assay, catechin was used as a standard for tannin assay and sodium phytate as a standard for phytic acid in developing a standard curve equation for the estimation purposes. Hence, the data should be expressed in mg catechin / 100 g dry weight and mg sodium phytate / 100 g dry weight.

*Effect of germination on wheat Seed*

Phytic acid content of raw wheat was 533 mg. (Rambach *et al.*, 1994) reported that cereal contain considerable amounts of phytic acid (Reddy *et al.*, 1989) reported that phytic acid content is present in the range of 1- 5% of many cereals, legumes and oil seeds. The flour of the wheat varieties contained 869.2-869.4 mg/100 g phytic acid content (Ihsan *et al.*, 2003). This declined 380-421 mg/100 g during 72 hours of germination. The of phytic acid content can also be reduced during baking (Faqir *et al.*, 2002). With the mixing of locally available cereals (wheat and barley) and pulses flour phytic acid content reduced in the processed weaning food (Ponam and Salil, 1993). The loss of phytic acid

during germination may have been due to hydrolytic activity of the enzyme phytase, which is reported to be present in various plant foods. In earlier studies, germination has been reported to have a diminishing effect on phytic acid content of various legumes like cowpeas, soybeans and lima beans (Sinha and Kawatra, 2003). Phytase activity during germination, resulting in hydrolysis of phytate phosphorus down to inositol monophosphate, contributes to the decrease in phytic acid. The liberated phosphorus is possibly transported to the embryo for further synthesis of organic phosphates (Reddy *et al.*, 1982). The rise in phytase activity during seed germination could be due to activation of the pre-existing enzyme or de novo synthesis of phytase (Gibson and Ullah, 1988). The maximum reduction for experimental values was recorded for 60 hour for 33.5°C (380 mg/100 g) minimum for 48 hour and 30°C (421 mg/100 g). Our results with wet processes, showing that they could significantly decrease phytic acid in wheat grain, agree well with reports about similar treatments on other cereals, such as fermentation of white rice flour (Reddy and Salunkhe, 1980), and steeping and sprouting of oats or corn (Larsson and Sandberg, 1995; Fageer *et al.*, 2004). We found that soaking, germination and fermentation have different efficacies in reducing the content of phytic acid. Germination and fermentation were observed to reduce the antinutritional content (phytic acid and tannin) of sorghum as reported by many researchers (El Khalifa *et al.*, 1994; Uрга *et al.*, 1997; Ibrahim *et al.*, 2005; Idris *et al.*, 2005). The fermentation of various Bengal gram dhal blends prepared by mixing them in different proportions, brought about significant decrease in phytic acid content (Anusho and Neelam, 1995). The phytic acid concentration for experimental values and the response surface are presented in (Figure 1). This reduction was more extreme than those previously reported (El-Madhy *et al.*, 1985; Gorospe *et al.*, 1992).

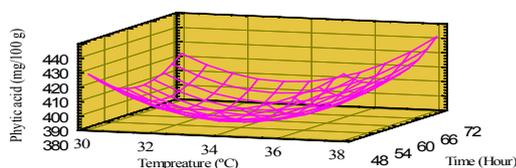


Figure 1: Response Surface plot for phytic acid in wheat seed

The results for tannin content of wheat seed was showed that tannin content was reduced after germination the similar results reported (Tabera *et al.*, 1995). The trend observed in 72 hour germinated seeds. Reductions in tannins contents were observed to various extents depending upon the germination

conditions. Maximum reductions for experimental values for tannins (296 mg/100 g) which observed for 60 hours and 33.5°C and minimum (338 mg/100 g) for 60 hours and 28.5°C were obtained by the germination method. The tannin concentration presented in the response surface (Figure 2). This was generally due to effect of a significant decrease in tannin content which could indicate a degradation of tannins during these periods of germination procedure, although these hypotheses needs further conformation.

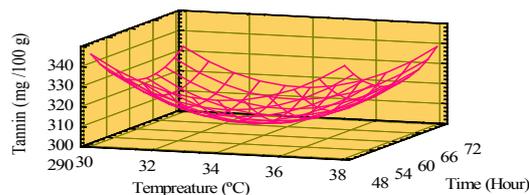


Figure 2: Response Surface plot for tannin in wheat seed

#### Effect of germination on mungbean Seed

Tannins and phytic acid in mungbean seeds were significantly ( $p < 0.05$ ) reduced by germination. Phytic acid content of raw mungbean was 635 mg, which declined 522-482 mg/100 g during 72 hours of germination the maximum reduction was recorded for 60 hours for 33.5°C (380 mg/100 g) minimum for 48 hours and 30°C (421 mg/100 g) for experimental values. Several legumes are known to contain phytase enzyme and its activity varies widely. In a study 13 different legumes and the authors found the rate of phytic acid hydrolysed ranging from 20-16 to 77-44% after germination for 5 days (Reddy *et al.*, 1982). In legumes, complete hydrolysis of phytate did not take place during 5 days of germination (Lu *et al.*, 1987) have demonstrated that considerable variation on the levels of phytase activity during germination exists among different cultivars of the same species. The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. Similar losses of phytic acid during soaking and germination have been reported (Grewal *et al.*, 2006; Khattab *et al.*, 2009) reported that soaking caused a 42.82-48.91% reduction in phytic acid content (Rehman and Shah, 2005) who stated that tannin content of black grams, red kidney bean and white kidney bean significantly reduced (Rakic *et al.*, 2007). The phytic acid concentration are presented in the response surface (Figure 3). Reductions in tannins contents were observed to various extents depending upon the germination conditions. Maximum (396 mg/100 g) reductions for tannins observed for 60 hour and 33.5°C and minimum (522 mg/100 g) for 60 hour and 38.44°C were obtained by the germination in mungbean seed. The tannin concentrations are

presented in the response surface (Figure 4). Similar results were obtained (Vijayakumari *et al.*, 1998) for *Vigna aconitifolia* and *Vigna sinensis*. Dehulling and soaking processes were less effective than germination processes in reducing phytic acid and tannins. The higher reduction of tannin could have been due to the tannin activity during germination. These results agree well with those reported (El-Adawy, 2002) for germinated chickpea. Another study reported reduction of antinutritional factor (tannins) in faba bean seeds germination. (Sharma and Sehgal, 1992)

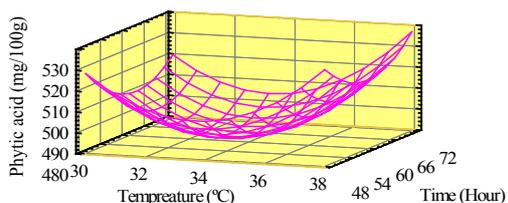


Figure 3: Response Surface plot for phytic acid in mungbean seed

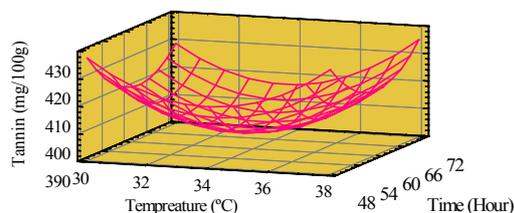


Figure 4: Response Surface plot for tannin in mungbean seed

*Optimization of parameters*

To minimize the experimental runs and time for optimization of germination conditions of wheat and mungbean seed, a two-factor central composite design (CCD) was adopted on the basis of coded level from two independent variables temperature and time resulting in ten simplified experimental set (Figure,1-4). The phytic acid and tannin concentration, in the wheat and mungbean germinated flour were investigated in the ranges of 380-421, 296-338 mg/100 g and 482-522, and 396-428 mg/100 g, respectively. The uncoded form of input variables i.e. temperature and time was optimally found to be 33.4°C and 60.6 h for phytic acid , 33.5°C and 60.3 h for tannin respectively. In practice, however, it is difficult to maintain the recommended conditions during germination and some deviation is expected. Therefore, optimum conditions were varied as temperature and time. The minimum optimal values from multiple response optimizations were 379.9 and 481.9 mg/100 g phytic acid content for wheat and mungbean seeds 295.7 and 395.8 tannin content for wheat and mungbean seeds respectively. Which were predicted by using second order polynomial the optimum responses summarized Table 2. The optimal

conditions to reduce some antinutritional factors (tannins and phytic acid) in lentils were 6 days of seed germination (Ayet *et al.*, 1997).

**Table 2.** Optimizes responses of phytic acid and tannin

Independent variables	Optimum Value		Responses	Optimum Value	
	Uncoded	Coded		Wheat	Mungbean
Temperature (°C)	33.4	1.00	Phytic acid (mg/100 g)	379.9	481.9
Time (h)	60.6	1.00			
Temperature (°C)	33.5	1.00	Tannin (mg/100 g)	295.7	395.8
Time (h)	60.3	1.00			

**Conclusions**

Response surface methodology was used to establish the optimum process variables (temperature and time) for concentration of phytic acid and tannin in wheat and mungbean seeds. By using response surface the optimum set of operating variables can be obtained graphically, in order to achieve the desired pretreatment levels for the phytic acid and tannin. Therefore, it was recommended that the concentration increase when the temperature and time decreased. It can be inferred that parameters individually had positive effect on decrease of concentration of phytic acid and tannin. The main effects of parameters are in following order: Main effect of temperature> time. All the derived mathematical models for the various responses were found to be significantly fit to predict the data.

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