Optimization of germination conditions for germinated Mungbean flour by response surface methodology

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Germination of legume seeds is an effective processing treatment to improve the nutritional quality. The aim of this study was to optimize the effect of germination conditions temperature and time. The germinated mungbean seed flour evaluated for the nutritional content changes in mungbean seed during germination. The analysis included determination of ash, carbohydrate, fat, cured fiber, moisture and proteins. The mungbean seeds were germinated at 30-37 ºC for 48-72 hours. The experiments were designed by using the Response Surface Methodology (RSM), with a Central Composite Rotatable Design (CCRD) resulted experimental values ranged for ash 3.29- 3.53% , carbohydrate 58.12-58.37 %, fat 1.41-1.48 %, cured fiber 1.82-2.01% , moisture 7.01-7.31% and proteins 25.0-25.71% and the predicted the optimal values input variables (temperature and time) ranged between 28.55-38.44ºC and 43.02-76.97 hour respectively. The maximum optimal values for basic analyzed food components ash carbohydrate, fat, cured fiber, moisture and proteins from multiple response optimizations were 3.52, 58.40, 1.47, 2.06, 7.43 and 25.68% respectively. It was concluded that germination significantly effect on mungbean seed nutrients. All the derived mathematical models for the various responses were found to be fit significantly to predict the data.

Keywords: Mungean, Germinated Flour, Optimization, Nutrients, Proximate, Analysis.

INTRODUCTION

Food legumes like beans, peas and lentils belong to the family “Leguminosae”, also called “Fabaceae”. They are mainly grown for their edible seeds, and thus also named as grain legumes. They play an important role in human nutrition because they are rich source of protein, calories, certain minerals and vitamins (Deshpande, 1992). However, their role appears to be limited because of several factors including low protein and starch digestibility (Negi and others, 2001), poor mineral bioavailability (Kamchan et al., 2004) the presence of nonnutrient compounds in the seeds which may have adverse effects for human or animal nutrition. Some examples of these compounds are protease inhibitors, lectins, phenolics, phytates, and R-galactosides (Deshpande et al., 1984; Garcia et al., 1997; Trugo and von Baer, 1998).

Consequently, it is desirable to develop transformation processes that could improve the nutritional quality of legumes and also provide new derived products for the consumers.

Germination is considered a potentially beneficial process for legume seed transformation which may decrease undesirable components such as alkaloids and phytates (Muquiz et al., 1998; Oboh et al., 1998; Orue et al., 1998), increase nutrients (Riddoch et al., 1998), and increase protein digestibility (Schulze et al., 1997), consequently improving nutritional quality. Additional advantages of germination are reduction in cooking time and improvement of sensorial attributes of the product (Vanderstoep, 1981; Deshpande et al., 1984).

Germination is a complex metabolic process during which the lipids, carbohydrates, and storage proteins within the
Mungbean seeds were soaked in distilled water (1:5 w/v) procedure described by (Frías and others 2005) 500 g of mungbean seeds were germinated; following the seeds were washed with running water for 3 mints. Broken seeds, dust and other foreign matter mungbean were manually cleaned to remove the seed by weight. The amino acid profile of mungbeans are relatively high in protein, around 25% of the whole seed and in processed form. Whole seeds are sold for use in soup mixes or to produce bean sprouts for soups. Mungbean is used in several food products, both as a whole seed and in processed form. Whole seeds are sold for use in soup mixes or to produce bean sprouts for salads. Mungbeans flour is used for soup bases or for use in soup mixes or to produce bean sprouts for salads. Mungbean is a rich source of protein and amino acid especially lysine and thus can supplement cereal-based human diets. It is low in saturated fat and sodium, and very low in cholesterol. It is also a good source of thiamin, niacin, vitamin B6, pantothenic acid, iron, magnesium, phosphorus and potassium, and a very good source of dietary fiber, vitamin C, vitamin K, riboflavin, folate, copper and manganese (Khalil and Khan, 1994; Mohan and Janardhanan, 1993). 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). Mungbean is used in several food products, both as a whole seed and in processed form. Whole seeds are sold for use in soup mixes or to produce bean sprouts for salads. Mungbeans flour is used for soup bases or sometimes for bean flour. Like most legumes, mungbeans are relatively high in protein, around 25% of the seed by weight. The amino acid profile of mungbeans, similar to other beans, is complementary to cereal grains. The aim of this study was to optimize the germination variables time and temperature on the proximate concentration of mungbean seed flour by using response surface methodology.

**MATERIAL AND METHODS**

Mungbean Seeds (BINA5 variety) were collected from Bangladesh Institute of Nuclear Agricultural Mymensingh, Bangladesh and brought to department of food technology and rural industries and kept in an airtight polyethylene bags at room temperature in a dry place.

**Sample Preparation**

The mungbean seeds were manually cleaned to remove broken seeds, dust and other foreign matter mungbean seeds were washed with running water for 3 mints. Mungbean seeds were germinated; following the procedure described by (Frías and others 2005) 500 g of mungbean seeds were soaked in distilled water (1:5 w/v) for 10 hours at room temperature (30±2). The water was then drained off and imbied 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 2mm in germinating tray. Continuously watered by capillarity in a seed germinator (G-120 Snijders, The Netherlands) Germination was carried out at 30 to 37 ºC for 48 to 72 hours and relative humidity was 99%. The sprouts were washed and dried at 60± 2 ºC for 08 hr in an electric air draught oven (VEB MLW Medizinische Geräte, Berlin Germany). The dried samples were ground to pass through 0.25 mm sieve then packed in kilner jars and kept in a refrigerator at 4 ºC until used for analysis. Proximate compositions of germinated mungbean seed flour were determined according to (AOAC, 2004) Methods moisture content (MC) was determined by oven-dry method. Moisture content (%) was computed by

\[
\text{Moisture (\%) = } \frac{\text{Wt. of original sample - Wt. of dried sample}}{\text{Wt. of original sample}}
\]

(i)\[
W_1 = \text{weight of empty Petri dish; } W_2 = \text{weight of Petri dish \\ & fresh sample; } W_3 = \text{weight of Petri dish \\ & dried sample. Percentage nitrogen (N) was determined following the micro Kjeldahl method (Bremner 1965). Percentage nitrogen was calculated using equation (2) while Crude protein was obtained by multiplying the corresponding total nitrogen content by a conventional factor of 6.25.}

\[
\text{Nitrogen(\%) = } \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Wt. of samples} \times V}
\]

(ii) Where D =Dilution factor; T =Titre value; W = weight of sample; 0.014 = Constant value

Fat content was determined by the soxhlet extraction procedure on 2 grams of the samples with petroleum ether as the solvent at the boiling point range of 60 – 800ºC. Percentage fat content was calculated using equation (3):

\[
\text{Crude Fat (\%) = } \frac{\text{Weight of fat in sample}}{\text{Weight of sample}} \times 100
\]

(iii) Ash content was determined by incineration of the sample at 600ºC according to method No. 08-01 as described in AACC (2000).

\[
\text{Ash content (\%) = } \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100
\]

(iv) Crude fiber content was determined by following the
### 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 (48-72h) and germination temperature (30-37 ºC). Symbols and coded factor levels for these variables are given below ±α.

### Statistical analysis

Statistics 5.0 (Stat soft, USA) was used to determine the effects of the independent variables, to calculate regression coefficients ($R^2$) 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:

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

Where $Y$ is the response variable, $X_1$ and $X_2$, are the coded process variables and $\beta_0, \beta_1, \beta_2, \beta_{11}, \beta_{22}, \beta_{12}$ are the regression coefficients. A stepwise methodology was followed to determine the significant terms in Equation 1. 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 ºC for 48-72 hours because after this time the germinating seeds developed leave. The experimental responses in terms of protein (%), Fat (%), Ash (%), Fiber (%), Carbohydrate (%) and moisture (%) are presented as.

### Optimization of Germination conditions for Ash

Optimization of Germination conditions for Ash

The ash concentration obtained from germinated mungbean seeds varied from 3.29 to 3.53 %. The regression model for this parameter was statistically significant ($p < 0.05$) with $R^2 = 0.91$ which indicates a good adjustment of the model to the experimental data. The 2nd order adjusted model for ash concentration is presented in Eq. (2) and the response surface in (Fig. 1a). The counter plot effect of germination variables (time and temperature) for ash content of mungbean shown in Fig.2a.
Optimization of Germination conditions for Carbohydrate

The carbohydrate concentration obtained from germinated mungbean seeds varied from 58.12 to 58.41 % The regression model for this parameter was statistically significant (p < 0.05) with $R^2 = 0.85$ which indicates a good adjustment of the model to the experimental data. The 2nd order adjusted model for soluble protein concentration is presented in Eq. (2) and the response surface in (Fig.2b). The counter plot effect of germination variables (time and temperature) for carbohydrates of mungbean shown in (Fig.2b).

Carbohydrates (%) = $54.7942 + 0.165692 \times X_1 + 0.00404838 \times X_2 - 0.0003477191 \times X_1^2 + 0.000836164 \times X_1 \times X_2 - 0.000607843 \times X_2^2$

(3)

High values of carbohydrate were observed with low germination times from 60 h (-a) to 48 h (-1) and 33.5°C (-a) of germination temperature. Maintaining the germination time constant at 60h and increase in germination temperature from 28.5 (-1) to 33.5°C (+1) promoted an increase in ash content of mungbean seed. During germination of wheat seed ash content significantly increased this was showed the incensement in mineral content. These results were in agreement with those reported earlier by several workers they reported that ash content increased due to this mineral content. These results were in agreement with finding of some earlier studies they reported that germination produced a small increase in ash content of mungbean seed

Optimization of Germination conditions for Fat

The fat concentration obtained from germinated mungbean seeds varied from 1.41 to 1.48 %. The regression model for this parameter was statistically significant (p < 0.05) with $R^2 = 0.76$ which indicates a good adjustment of the model to the experimental data. The 2nd order adjusted model for fat concentration is presented in Eq. (4) and the response surface in (Fig.1c). The counter plot effect of germination variables (time and temperature) for fat content of mungbean shown in (Fig.2c).

Fat content (%) = $0.306397 + 0.0586478 \times X_1 + 0.00971741 \times X_2 - 0.0011747 \times X_1^2 + 0.000239508 \times X_1 \times X_2 - 0.000152012 \times X_2^2$

(4)

Maximum values of fat were observed with low germination times from 60 h (1) to 48 h (-1) and 33.5°C (1) of germination temperature. Maintaining the germination time constant at 60h and increase in germination temperature from 28.5 (-1) to 33.5°C (+1) promoted a decrease fat. There is significant reduction of fat content on germination of wheat seed the decrease in fat content during germination process can be corroborated with the findings of other workers They observed decrease in the fat contents of the germinated seeds might be due to the increased activities of the lipolytic enzymes during germination(Osman, 2007)..

Similar observation was made for decrease in fat content was observed, which could be due to total solid loss during soaking prior to germination (Wang et al., 1997) or use of fat as an energy source in sprouting process. The results are comparable with findings of (Venderstoep, 1981) for germinated green gram and lentil. This decreased fat content implies an increased shelf-life for the germinated seeds compared to the ungerminated ones Optimum values for fat and independent variables (temperature and time) are 1.47%, 30.68°C and 56.13h results showed in (Table 1).

Optimization of Germination conditions for Fiber

The fiber concentration obtained from germinated mungbean seeds varied from 1.82 to 2.01 % The regression model for this parameter was statistically significant (p < 0.05) with $R^2 = 0.81$ which indicates a good adjustment of the model to the experimental data. The 2nd order adjusted model for soluble protein concentration is presented in Eq. (5) and the response surface in (Figure.1d). The counter plot effect of germination variables (time and temperature) for fiber content of mungbean shown in (Figure.2d).

Fiber content (%) = $6.16873 - 0.186811 \times X_1 - 0.0378637 \times X_2 + 0.00316614 \times X_1^2 - 0.000483004 \times X_1 \times X_2 + 0.000443202 \times X_2^2$

(5)

High values of fiber were observed with low germination times from 43.0 h (-1) to 48 h (-1) and 33.5°C (-a)
Figure 1. Response Surface plot for nutrient in germinated mungbean seed flour (a) ash content (b) carbohydrates (c) fat content (d) fiber content (e) moisture content (f) proteins

of germination temperature. Maintaining the germination time constant at 60h and increase in germination temperature from 28.5 (-1) to 33.5ºC (+1) promoted a decrease fiber content in wheat seed. Fiber content significantly decreased during germination process similar decrease was finding out by many researchers. They reported that the decrease in the crude fiber content can be attributed to the dilution effect on nutrients in processed and cooked samples with the increase in the moisture content. The crude fiber was reduced by germination which confirmed (Mubarak, 2005) for germinated mungbean seeds flour. However Peer and Leeson (1985) stated that sprouting increased the crude fiber contents of seeds. Optimum values independent variables (temperature and time) and proteins are 28.55ºC, 76.97h and 2.06% respectively results are
Optimization of Germination conditions for Moisture

The moisture concentration obtained from germinated mungbean seeds varied from 6.91 to 7.11 %. The regression model for this parameter was statistically significant \((p < 0.05)\) with \(R^2 = 0.79\) which indicates a good adjustment of the model to the experimental data. The 2nd order adjusted model for soluble protein concentration is presented in Eq. (6) and the response surface in (Fig. 1e). The counter plot effect of germination variables (time and temperature) for moisture content of mungbean shown in (Fig. 2e).

\[
\text{Moisture content (\%) = 15.9411 - 0.471752^*X_t - 0.0197007^*X_c + 0.0081318^*X_t^2 - 0.00151077^*X_c^2 + 0.000518141^*X_c^2} \tag{6}
\]

High values of moisture were observed with low germination times from 60 h (1) and 28.5 to 33.5ºC (1) of germination temperature. Maintaining the germination time constant at 60h and increase in germination temperature from 28.5 (-1) to 33.5ºC (+1) promoted a decrease moisture content. Moisture content in wheat flour prepared from processing through germination varied significantly. Similar trends have been reported by several workers in seed (Ghavidel et al., 2007; Khattack et al., 2007). Optimum values for moisture and independent variables (temperature and time) are 7.43%, 38.44ºC and 43.021h results showed in (Table 1).
Optimization of Germination conditions for Protein

The protein concentration obtained from germinated mungbean seeds varied from 25.00 to 25.71 %. The regression model for this parameter was statistically significant (p < 0.05) with $R^2 = 0.90$ which indicates a good adjustment of the model to the experimental data. The 2nd order adjusted model for protein concentration is presented in Eq. (7) and the response surface in Fig. 2f. The counter plot effect of germination variables (time and temperature) for protein of mungbean shown in Fig. 2f.

Proteins (%) = 12.5239 + 0.585427* $X_1$ + 0.139133* $X_2$ - 0.00927447* $X_1^2$ + 0.00016532* $X_1$* $X_2$ -0.00132715* $X_2^2$ (7)

High values of proteins were observed with low germination times from 48 h (-1) to 60 h (-1) and 30.0 to 33.5ºC (1) of germination temperature. Maintaining the germination time constant at 60 and increase in germination temperature from 33.5 (-1) to 37.0 (+1) promoted a decrease protein. The protein content of mungbean flour during germination is shown in Figure. Germination time and temperature influenced the amount of protein present in the germinated mungbean seed. Several researchers reported improvement in the protein quantity as well as quality during germination of mungbean. The protein content trend slight increased during germination of mungbean at different temperatures (Abdus et al., 1989; Sattar et al., 1989) and protein digestibility increased during germination of legumes (Preet and Punia, 2000) similar results was reported by (Mubarak, 2005) for germinated mungbean flour (Fagbemi, 2007) for germinated fluted pumpkin seeds. Optimum values independent variables (temperature and time) and proteins are 32.04ºC, 54.40h and 25.68% respectively results are presented in (Table 1). Germinated mungbean flours, would produce an improvement in the digestive utilization of the mentioned nutrient. Our findings are in agreement with findings of they described a gradual increase in the amount of reducing sugars from day 0 to day 5 in sunflower seeds and with those.

CONCLUSIONS

Response surface methodology was used to establish the optimum process variables (temperature and time) for concentration of ash, carbohydrate, fat, cured fiber, moisture and proteins in 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 basic components of mungbean seed. Therefore, it was recommended that the concentration decrease when the temperature and time increase. It can be inferred that parameters individually had positive effect on increase of concentration of proximate component of mungbean seed. The main effects of parameters are in following order: Main effect of time> temperature. All the derived mathematical models for the various responses were found to be significantly fit to predict the data.

REFERENCES


