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Full Length Research Paper

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

Imtiaz Hussain* and M. Burhanddin**

*Department of Food Science and Technology, Faculty of Agriculture Rawalakot, The University of Azad Jammu and Kashmir, Pakistan
Tel: + 925824-960007; Fax: +925824-960004
**Department of Food Technology and Rural Industries, Bangladesh Agricultural University Mymensing 2202, Bangladesh

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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

*Corresponding author E-mail: imti_hussain2005@yahoo.com

seed are broken down in order to obtain the energy and amino acids necessary for the plants development (Podesta and Plaxton, 1994; Ferreira *et al.,* 1995; Jachmanian *et al.,* 1995; Ziegler, 1995). Mung bean (*Vigna radiata*), also called green gram is a tropical legume, widely grown in Asia, particularly in Thailand, India, Pakistan and Bangladesh. 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

Munbean 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 (Frıas and others 2005) 500 g f 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 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 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 ◦C 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 ◦C 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 (i)

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

(i)

(ii)

W_1 = weight of empty Petri dish; W_2 = weight of Petri dish & fresh sample; W_3 = 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.

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

(ii)

Crude Proteins (%) = %N × 6.25

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).

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

□□ (iv)□□□

Crude fiber content was determined by following the

Independent variables		Levels				
Coded	Real	-α	-1	0	+1	+α
X_1	Temperature of germination (ºC)	28.5	30	35	37	38.4
X_2	Time of germination (h)	43.0	48	60	72	76.9

Table 1. Optimizes values for independent variables and response

S. #		Independent variables (Optimum Values)				Optimum Value for
	Responses	Temperature (ºC) X_1		Time (h) X_2		Response (%)
	(%)	Uncoded	Coded	Uncoded	Coded	
1	Ash	33.63	1	52.97	-1	1.83
2	Carbohydrate	33.10	1	58.14	-1	70.53
3	Fat	31.30	1	56.72	-1	1.58
4	Fiber	28.55	-1	43.02	-1	1.45
5	Moisture	28.55	-1	56.51	-1	10.44
6	Proteins	34.21	1	53.23	-1	11.46

method No. 32-10 as described in AACC (2000).

$$\text{CrudeFiber}\ (\%)=\frac{\text{Weightof residue- weightof Ash}}{\text{Weightof sample}}\times 100 \quad \text{(v)}$$

Carbohydrate contents (CHO) was determined by the difference method, which was accomplished by following the calculation (CHO = 100 - %MC – CP – CF - fat –ash).

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:

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

Where Y is the response variable, X_1 and X_2, are the coded process variables and β_0, β_1, β_2, β_{11}, β_{22}, β_{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

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.

Ash content (%) = 0.447698 + 0.236108* X_1- 0.023581* X_2 0.00515919* X_1^2 + 0.00155468* X_1* X_2 -0.000213186* X_2^2 (2)

High values of ash content were observed with low germination times from 48 h (-a) to 60 h (-1) and 37ºC (-a) and 33.5ºC of germination temperature. Maintaining the germination time constant at 60h and increase in germination temperature from 28.5 (-1) to 33.5ºC (+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 bioavailability during germination of legumes (Rao and Prabhavathi 1982; Hussein; Savage 1988; Gopalan et al., 1989 and Ghanem 1999). The reduction in ash content was reported by (El- Adawy,2002) germinated for mungbean seeds flour and germinated chickpea flour. Optimum values for ash and independent variables (temperature and time) are 3.52 %, 32.28ºC and 62.42h results showed in (Table 1).

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. (3) and the response surface in (Fig.1b).The counter plot effect of germination variables (time and temperature) for carbohydrates of mungbean shown in (Fig.2b).

Carbohydrates (%) = 54.7942 + 0.165692* X_1+ 0.0404838* X_2 - 0.00347191* X_1^2+ 0.000836164* X_1* X_2 - 0.000607843* X_2^2 (3)

High values of carbohydrate were observed with low germination times from 60 h (-a) to 48 h (-1) and 33.5ºC (-a) of germination temperature. Maintaining the germination temperature constant at 33.5ºC and increase in germination time from 43 (-1) to 60h (+1) promoted an increase carbohydrate. Total free sugar content of wheat flour increased in germinated sample. Degradation of starch in grains during germination led the increase in small dextrin and fermentable sugar. These results are agreement with finding of some earlier studies they reported that germination produced a small increase in carbohydrates levels in legumes utilization. (Donangel, 1995) and starch digestibility increased during germination of legumes. (Kataria *et al.,* 1992). Optimum values independent variables (temperature and time) and proteins are 30.38ºC, 54.19h and 58.40.53 % respectively results are presented in (Table 1).

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* X_1+ 0.00971741* X_2 - 0.0011747* X_1^2 + 0.000239508* X_1* X_2 -0.000152012* X_2^2 (4)

Maximum values of fat were observed with low germination times from 60 h (1) to 48 h (-1) and 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 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%, 30.68ºC 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* X_1 - 0.0378637* X_2 + 0.00316914* X_1^2 - 0.000483004* X_1* X_2+0.000443202* X_2^2 (5)

High values of fiber were observed with low germination times from 43.0 h (-1) to 60 h (1) and 28.5 to 33.5ºC (-a)

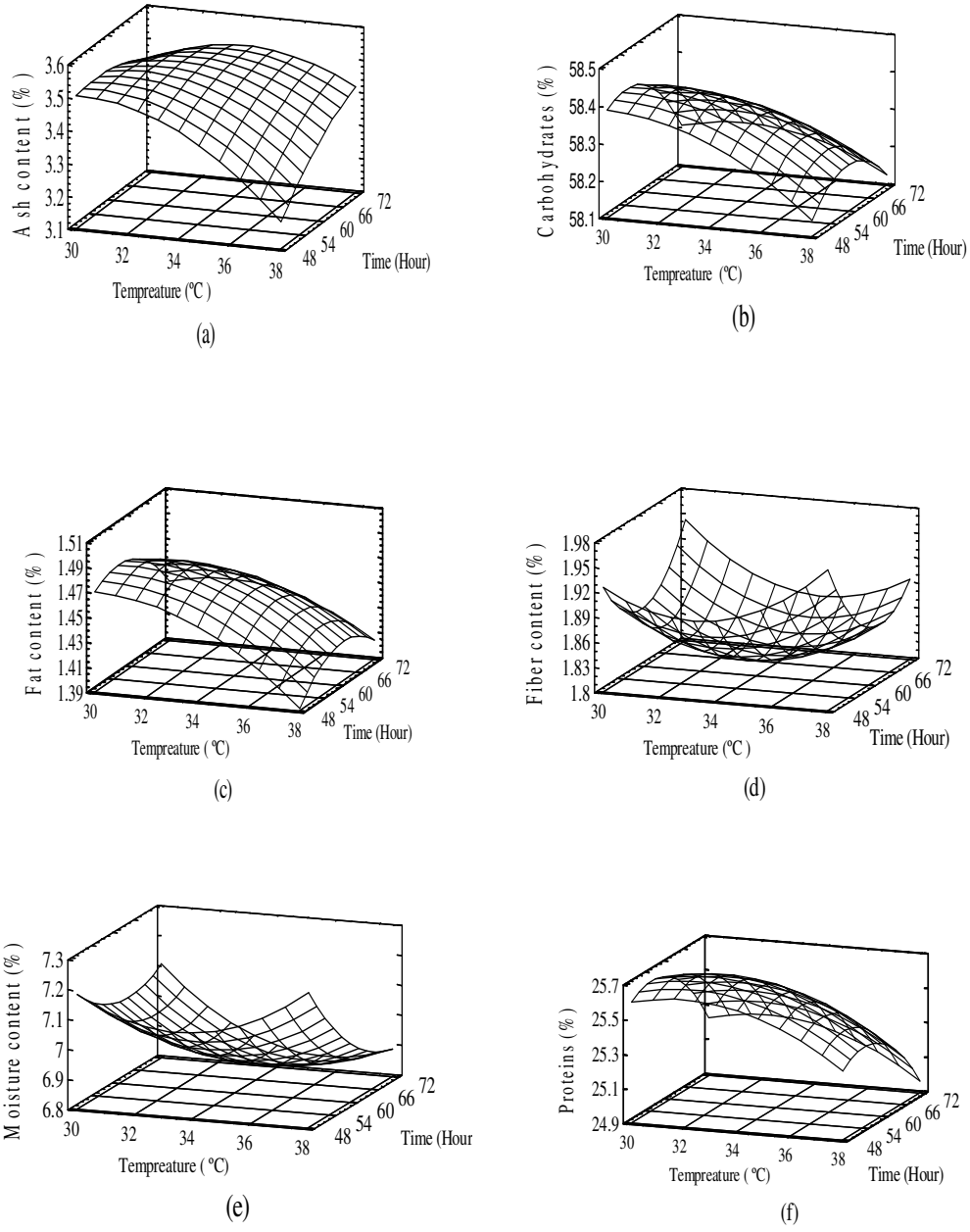

Figure 1. Response Surface plot for nutrient in germinated mungbean seed flour (a) ash content (b) carbohydrates (c) fat content (d) fiber content (e) moisture content (f) proteins

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

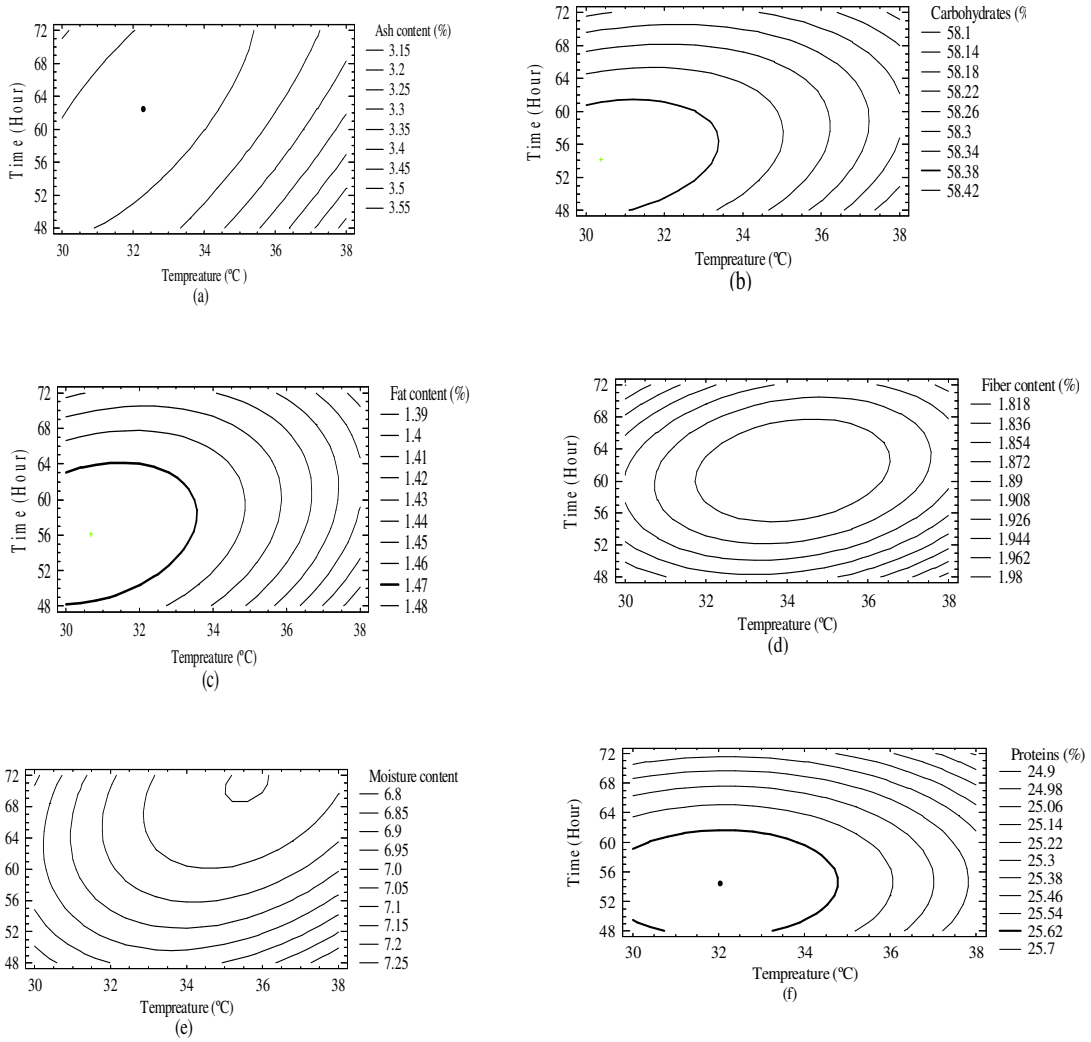

Figure 2. Counter plots for nutrients of germinated mungbean seed flour (a) ash content (b) carbohydrate (c) fat content (d) fiber content (e) moisture content (f) proteins.

presented in (Table 1).

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).

$$\text{Moisture content (\%)} = 15.9411 - 0.471752^* X_1 - 0.0197007^* X_2 + 0.0081318^* X_1^2 - 0.00151077^* X_1^* X_2 + 0.000518141^* X_2^2 \quad (6)$$

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.1f).The counter plot effect of germination variables (time and temperature) for protein of mungbean shown in (Fig.2f).

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

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

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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.

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