OPTIMIZATION OF GERMINATION EFFECT ON FUNCTIONAL PROPERTIES OF MUNGBEAN FLOUR BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT: The present study focused on functional properties of flours derived from germinated mungbean seeds. Germination process of seed has affect on functional properties like bulk density, absorption capacities, foam capacity, swelling power and solubility. The bulk densities of prepared germinated flours were decreased. Germination decreased water absorption capacities and increased oil absorption capacities in germinated flour sample at ambient conditions for mungbean flour. The foaming capacity of all the prepared samples was increased. Swelling power ranged 7.14 to 7.22%.Solubility increased with increase of germination time. Thus, the study indicated that germination improved the functional properties of flour. Germinated mungbean flour can be successfully used for preparation weaning of foods for proteins deficient children.

Keywords: Germination, Mungbean, RSM, Optimization, Functional properties.

INTRODUCTION

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 (Khalil, 2006). It 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. The functional properties of proteins have been defined as those physicochemical properties that affect the processing and behavior of proteins in food systems as judged by the quality attributes of the final product (Udensi, 2006). Determination of the functional properties of proteins is desirable for the utilization of any new protein material. The functionality of protein depends to some extent upon the size and structure of proteins and in part on their interactions with other food components such as carbohydrates and fats, and is modified by various treatments (Hussain, *et al.*, 2005, Prakash and Narasinga, 1986). Modified proteins are known to have entirely different functionality as compared with parent protein and can be added in small amounts to food products for a specific aspect (Yang, *et al.*, 2001). Germinated dry beans are receiving increasing attention due to enhanced flavour and nutritional qualities (Ghorpade and Kadam, 1989; Martin *et al*., 2003).Germination has often been proposed as a simple processing method by which the nutrient composition and certain functional properties of cowpeas might be improved (Hussain, *et al.*, 2012, Giami, 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). The objective of this study was to evaluate the functional properties of mungean and the changes brought about by germination of the beans.

MATERIALS AND METHODS

Preparation of Wheat and Mungbean Flour: Mungbean (MF) flour samples were prepared by taking 300 grams of mungbean seeds. The seeds were sterilized by soaking in ethanol 2% for 1 min. The seeds were and then soaked in tap water for 12 h at room temperature. The soaked seeds were germinated in a plastic tray lined with wet paper towels. Two layers of wet paper towels were used to cover the seeds to prevent rapid moisture loss. They were germinated in the seed germinator at 33.5 $\pm$ 2 ^{0}C for 60 h. The sprouts were washed and dried at 60^{0}C for 12 h in an electric oven (Contherm, Quantherm 200 L, Contherm Scientific, Lower Hutt, New Zealand). The dried sprouts were ground in a hammer mill (Culatti, Model: JKA Werk, Type: DCFH, Germany) and sieved with a 60 mesh screen (Model BS 410, Endeco London, England). The flour was kept, in triplicates, in polyethylene bags and packed in a glass container and stored in a refrigerator at 4^{0}C until analysis (Ayim, *et al.,* 2012).

Functional Properties: The pH was measured by making a 10% (w/v) flour suspension of each sample in distilled water (AOAC, 2005). Each sample was then mixed thoroughly in a plastic beaker, and the pH recorded with an electronic pH meter (Model PHN-850, Villeur-Banne, France).The bulk density (BD) (packed and loosed densities) was determined according to the method described by Okezi and Bello (1988).The water and oil absorption capacities were determined by the method of Adebowal and Lawal (2004). Two grams flour sample was mixed with 20 ml distilled water or refined soybean oil (S.G. 0.9084) and allowed to stand at ambient temperature (32ºC) for 30 min, then centrifuged for 30 min at 2000 x g. Water and oil absorption capacity was expressed as percent water or oil bound per gram flour. The foam capacity (FC) was determined as described by Narayana and Narsinga (1984). Swelling power was determined through the method described by Tester and Morrison (1990) Water solubility was measured according to the method of Anderson (Anderson *et al.*, 1996).

Experimental design: Variation effects in germination time and temperature were analyzed using the response surface methodology (RSM), with a 2 central composite rotational design with the help of statgragh 5.1 software. 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:

Independent variables	Levels
Coded Real	-x -1 0 +1 +x
X_1 Temperature of germination (ºC)	30 35 37
X_2 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 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. 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

The pH values obtained from germinated mungbean seed flour varied from 5.7 to 5.98. The regression model for this parameter was statistically significant ($p < 0.05$). The 2nd order adjusted model for pH is presented in Eq. (01) and the response surface in (Fig.1).The pH values of the flours in water suspension are important since some functional properties such as solubility and emulsion properties are highly affected by pH changes (Narayana and Narasinga, 1982). There was a significant difference ($p < 0.05$) in the pH of the different flours, with the flour of black common bean having the lowest pH. Optimum values for pH and independent variables (temperature and time) are 6.00, 28.55ºC and 43.02h results showed in (Table 1).

pH $= 10.0976 - 0.20884^{*}X_1 - 0.0205147^{*}X_2 - 0.00229597^{*} X_1^2 - 0.000892857^{*} X_1^{*} X_2 -0.000117188^{*} X_2^2$ (02)

The flour prepared from germinated mungbean had the highest bulk density varies from 0.64 to 0.69 (g/cm^3) for packed bulk density and for lose bulk density varied from 0.71 to 0.76 (g/cm^3). The 2nd order adjusted model for pH is presented in Eq. (02 & 03) and the response surface in (Fig.3 & 4).The bulk density of the flour prepared from the mungbean is important with regard to its packaging, while its high emulsion capacity makes it a potential useful ingredient in preparing meat analogs. The time required to reach complete wetness was 2-fold longer for flour of white bean. (Okezi and Bello, 1988; Akubor and Obiegbuna, 1999). Optimum values for pack and lose density and independent variables (temperature and time) are 0.69 g/cm^3, 38.44ºC, 43.02 h, 0. 74 g/cm^3, 36.17ºC and 52.20h respectively results showed in (Table 1).

Pack BD $= 0.796064 - 0.0216271^{*}X_1 + 0.0077564^{*}X_2 + 0.000510221^{*} X_1^2 - 0.000202381^{*} X_1^{*} X_2 - 0.000121528^{*} X_2^2$ (03)

Lose BD $= - 0.02698 + 0.0382135^{*}X_1 + 0.00304746^{*}X_2 - 0.000515316^{*} X_1^2 - 0.0000178571^{*} X_1^{*} X_2 - 0.0000230032^{*} X_2^2$ (04)

Water and oil absorption capacities of the germinate mungbean seed flour varied from 1.90 to 2.4 ml/g and oil absorption capacities (1.90 to 2.0 ml/g) Water and oil absorption capacities of the raw cowpea flours were 2.4 and 2.9 g/g, respectively (Abbey and Ibeh, 1988). The 2nd order adjusted model for water and oil absorption capacities are presented in Eq. (05 & 06) and the response surface in (Fig. 4 & 5).Our results compared favorably with the findings of these investigators and suggest that flours prepared from munbean beans could be used as ingredients in the preparation of comminuted products such as sausages (Padmashree, *et al,* 1987; Bollinger,1999)) showed that these properties enabled bakers to add more water to dough's so as to improve handling characteristics and maintain freshness in the bread. Optimum values for water and oil absorbance capacities and independent variables (temperature and time) are 2.32 ml/g , 34.02ºC, 54.74 h and 2.00 ml/g , 28.55ºC and 76.97h respectively results showed in (Table 1).

Water AC = $- 2.61724 + 0.816183^{*}X_1 + 0.0647991^{*}X_2 - 0.00321444^{*} X_1^2 - 0.0000595238^{*} X_1^{*} X_2 -0.000776908^{*} X_2^2$ (05)

Oil AC = $4.6885 - 0.144221^{*}X_1 + 0.00683023^{*}X_2 + 0.00183676^{*} X_1^2 - 0.000178571^{*} X_1^{*} X_2 -0.000104166^{*} X_2^2$ (06)

Germination increased foam capacity. Values obtained from germinated wheat seeds flour varied from 53.00 % to 53.40 %. The regression model for this parameter was statistically significant ($p < 0.05$).The 2nd order adjusted model for foam capacity is presented in Eq. (07) and the response surface in (Fig.6).Suggested that the superiority of soy flour to cowpea powder in foaming property was due to the high protein content of soy flour(Kinchella, 1981; Diwakar et al., 1996). Optimum values for solubility and independent variables (temperature and time) are 53.43 %, 38.44ºC and 53.02h results showed in (Table 1).

Foam Cap. = $64.1944 - 0.536903^{*}X_1 - 0.0699057^{*}X_2 + 0.00775525^{*} X_1^2 + 0.000357143^{*} X_1^{*} X_2 -0.000451385^{*} X_2^2$ (07)

In this study germinated mungbean flour had swelling power ranged 7.14 to 7.22 %. Therefore, the resulting swelling power indicated that the starch extracts obtained were highly restricted type (Dengate, 1984). The regression model for this parameter was statistically significant ($p < 0.05$). The 2nd order adjusted model for swelling power is presented in Eq. (08) and the response surface in (Fig.07). Optimum values for solubility and independent variables (temperature and time) are 7.22 %, 33.60ºC and 58.91h results showed in (Table 1).

Swelling Cap. = $4.62316 + 0.109589^{*}X_1 + 0.0256561^{*}X_2 - 0.00173473^{*} X_1^2 - 0.000119048^{*} X_1^{*} X_2 - 0.000251735^{*} X_2^2$ (08)

Solubility was pH dependent, and the main difference was in solubility of nitrogen at pH 2. Values obtained from germinated wheat seeds flour varied from 16.32 to 16.64 %. The regression model for this parameter was statistically significant ($p < 0.05$). The 2nd order adjusted model for solubility is presented in Eq. (09) and the response surface in (Fig.8). The minimum nitrogen solubility at pH 4.0 ranged from 18 to 20% for the flours. Winged bean flour had a minimum nitrogen solubility of 23% at pH 4.5 (Shimelis, 2006) cowpea powder had a minimum nitrogen solubility of 19.36% at pH 4.0 (Okaka and Potter, 1979). Optimum values for solubility and independent variables (temperature and time) are 16.4 %, 33.53ºC and 59.95h results showed in (Table 1).

Solubility = $13.3271 + 0.116619^{*}X_1 + 0.037296^{*}X_2 - 0.00163269^{*} X_1^2 -0.000119048^{*} X_1^{*} X_2 -0.000277776^{*} X_2^2$ (09)

Table-1. Table 1.Optimizes values for independent variables and response mungbean flour

S. #	Responses	Independent variables (Optimum Values)				Optimum Value for Response
		Temperature (ºC) X_1		Time (h) X_2		
		Uncoded	Coded	Uncoded	Coded	
1	pH	28.55	-1	43.02	-1	6.00
2	P.Bulk Density (g/cm^3)	38.44	1	43.02	-1	0.695
3	L. Bulk Density (g/cm^3)	36.17	1	52.20	1	0.743
4	Water absorbs. Cap.(ml/g)	34.02	1	54.74	1	2.32
5	Oil absorbs. Capac.(ml/g)	28.55	-1	76.97	1	2.00
6	Foam Capacity (%)	38.44	1	43.02	-1	53.43
7	Swelling Capacity (%)	33.60	1	58.91	1	7.22
8	Solubility (%)	33.53	1	59.95	1	16.4

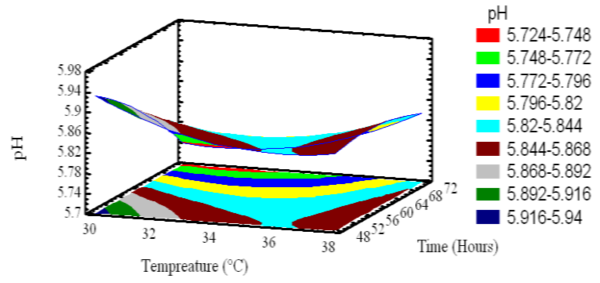

Fig.1: Response Surface plot for effect of germi. on pH of mungbean fllour

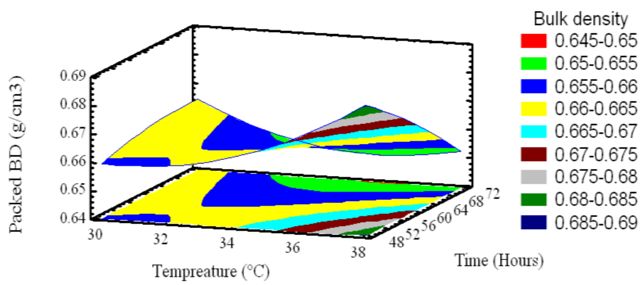

Fig.2:Respo. Surface plot for effect of germi. on packed BD for mungbean flour

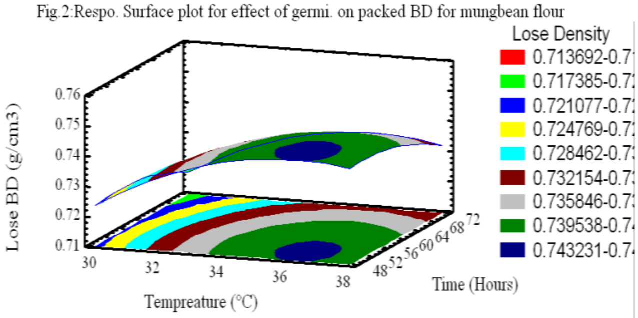

Fig.3: Response Surface plot for effect of germi. on lose BD of mungbean flour

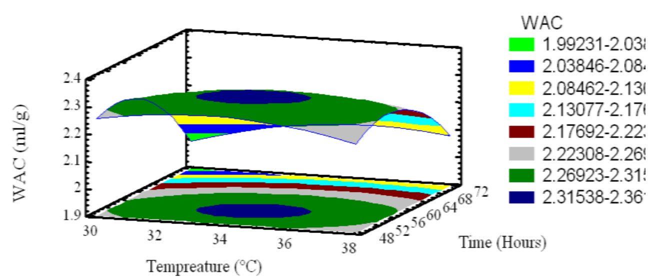

Fig.4:Response Surface plot for effect of germi. on WAC of mungbean flour

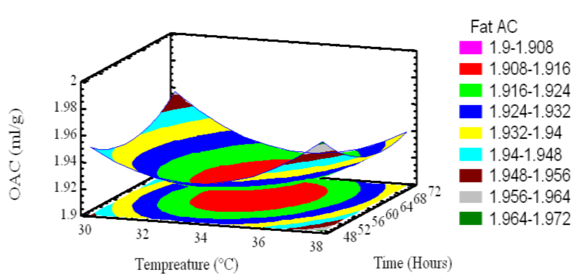

Fig.5:Response Surface plot for effect of germi.on OAC of mungbean flour

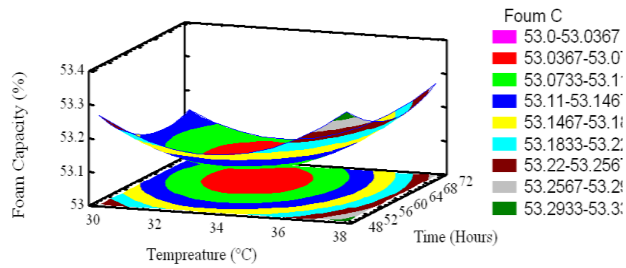

Fig.6:R.Surface plot for effect of germi. on foam capacity of mungbean flour

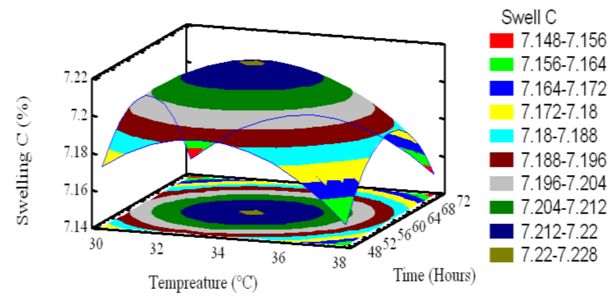

Fig.7:Response Surface plot for effect of germi. on swelling C of wheat flour

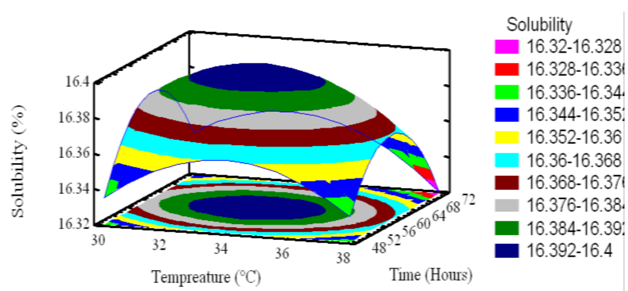

Fig.8:Resp. Surface plot for effect of germi. on solubility of mungbean flour

REFERENCES

Adebowal, K., O. and O. S. Lawal. Comparative study of the functional properties of bambara groundnut (*Voandzeia subterranean*), jack bean (*Cana valiaensiformis*) and mucuna bean (*Mucuna pruriens*) flour. Food Res. Inter. (37): 355-365 (2004).

Akubor, P., I. and J. E. Obiegbuna. Certain chemical and functional properties of ungerminated and germinated millet flour. J Food Sci Technol. (36): 241–243 (1999).

Anderson R.A., Conway H.F., Pfeifer V.F., and E.L. Griffin. Roll and extrusion-cooking of grain sorghum grits Cereal Sci. Today. (14): 372-380. (1996).

AOAC. Official Method of Analysis (18th edn). Washington, DC: Association of Official Analytical Chemistry (2005).

Ayim, I., E.A. Amankwah and K.A Dzisi. Effect of pretreatment and temperature on the air drying of French and False horn plantain slices Journal of Animal & Plant Sciences,. 13: 1771-1780 (2012).

Bollinger, H.. Functional food- use of dietary fiber as a multifunctional component. Food Marketing Technology (13): 10–12 (1999).

Dengate, H.N.,. Swelling, pasting, and gelling of wheat starch. In: Advances in cereal science and technology, American Association of Cereal Chemists (AACC), St. Paul, MN. (Y. Pomeranz, ed.): 49-71 (1984).

Diwakar, P. Kushwah, A. and H.S. Kushwah,. Effect of processing on the functional properties of some local varieties of horse gram (*Dolichos biflorus L.*) in Madhya Pradesh. J Food Sci Technology (33): 150–152 (1996)

Ghorpade, V. M. and S.S. Kadam. Handbook of world Food Legumes: Nutritional Chemistry, Processing Technology and Utilization (3):165–176 (1989).

Giami, S.Y.,. Effect of processing on the proximate composition and functional properties of cowpea (*Vigna unguiculata*) flour Food Chemistry. (47): 153–158 (1993).

Hussain, I. M.S. Anjam. M.B Uddin and M. Hanif. Preparation and Evaluation of Complementary Diets from Germinated Wheat and Lentil for Bangladeshi Children Pakistan Journal of Science Vol. 64: 304-308 (2012).

Hussain, I., I. Shakir and M.Ahamad (2005) Impact of National Support Programme on Development of Rural Areas of Rawalakot A.J&K.Pakistan Journal of Science 57: 109-112.

Irakoze, P.C., Haihua, Z., Qin, L., Zhou K. and Z. Huiming, . Optimization of ultrasonic extraction of olysaccharides from Chinese malted sorghum using response surface methodology. Pakistan Journal Nutrition (4):336-342 (2010).

Khalil., A.A. Nutritional improvement of an Egyptian breed of mung bean by probiotic lactobacilli. African Journal of Biotechnology Vol. 5: 206-212. (2006).

Kinsella, J.E. Functional properties of proteins: possible relationships between tructure and functions in foams. Food Chemistry (7): 273–288 (1981).

Martin-Cabrejas, M. A., Ariza, N., Esteban, R., Molla´, E., Waldron, K. and Lo´pez-Andreu, F. Effect of germination on the carbohydrate composition of the dietary fiber of peas (Pisum sativum L.). Journal of Agriculture and Food Chemistry (51): 1254–1259 (2003).

Narayana, K. and R. M. S. Narasinga. Functional properties of raw and processed winged bean (*Psophocarpus tetragonolobus*) flour. J. Food Science (47): 1534-1538 (1982).

Narayana, K. and R.M.S. Narsinga. Functional properties of raw and heat processed winged bean (*Psophocarpus tetragonolobus*) flour. *J Food Sci*ence (47): 1534– 1538 (1984).

Okezi, B. O. and A.B. Bello. Physicochemical and functional properties of winged bean flour and isolate compared with soy isolate. *J. Food Sci*ence (53): 450-454 (1988).

Padmashree, T.S. Vijayalakshmi, L. and S. Puttaraj. Effect of traditional processing on the functional properties of Cowpea (*Vigna unguiculata*) flour. J Food Sci Technology (24): 221–225 (1987).

Prakash, V. and R.M.S. Narasinga. Physicochemical properties of oilseed proteins. CRC Crit Rev Biochemistry (20): 265–363 (1986).

Shimelis, E. Meaza, M. and S. Rakshit. Physico-chemical Properties, Pasting Behavior and Functional Characteristics of Flours and Starches from Improved Bean (*Phaseolus vulgaris* L.) Varieties Grown in East Africa. Agricultural Engineering International (8):1-19 (2006).

Tester, R.F. and W.R. Morrison. Swelling and gelatinization of cereal starches I. Effects of amylopectin, amylase and lipids. *Cereal Chemistry* (67): 551-557 (1990).

Udensi, E. A. and A.Okoronkok. Effects of fermentation and germination on the physicochemical properties of *Mucuna cochinchinensis* protein isolate. African Journal of Biotechnology Vol. 5: 896-900 (2006).

Yang F, Basu T.K, and B.Ooraikul. Studies on germination and antioxidant contents of wheat grain. J. Food Sci. Nutr. 52: 319-330 (2001).