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Optimized Conditions of Steeping and Germination and Their Effect on Sorghum [*Sorghum bicolor (L.) Moench*] Composition

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Abstract: The work consisted in optimizing steeping and germination condition and their effect on sorghum grain in term of malt loss, Soluble Solid (SS) yield, cold paste viscosity, amylase activity, tannin and protein content. The factors studied included steeping time and temperature with temperature and time of germination. Germination significantly affected the increase in malt loss, SS yield, amylase activity and protein content with a decrease in cold paste viscosity and tannin content of sorghum. Optimum conditions for sorghum were: steeping time for 24 h at 31°C and 4.5 d of germination at 30°C. Values predicted at optimum conditions by the response surface model for all responses were experimentally tested and close agreement between experimental and predicted values was observed.

Key words: RSM, sorghum, steeping, malting

INTRODUCTION

Recently, there has been increased interest in sorghum as a gluten-free cereal to substitute the gluten-rich cereals in the diet of people suffering from celiac disease (Elkhalifa *et al*., 2005). The physiological maturity of sorghum grain generally occurs 50 days after anthesis and marks the end of nutrient delivery and the beginning of senescence and caryopsis desiccation (Waniska, 2000). The mature grain is then harvested and stored. In a dormant stage, it is characterized by dehydration and a dramatic decrease of metabolic activity. Germination is induced by rehydration of the seed, which increases both respiration and metabolic activity thus allowing the mobilization of primary and secondary metabolites (Limami *et al*., 2002). Germination induces the synthesis of hydrolytic enzymes. Significant changes occur in seed during germination in biochemical and physical aspects (Obatolu, 2002) and the total nutrition value is improved (Badau *et al*., 2005; Jingjun *et al*., 2010).

Germination is a process used to make malt for the brewing industry, although germinated or malted flour can also be used in bakery products (Selvaraj *et al*., 1986), nonalcoholic drinks and weaning food formulations (Wahed *et al*., 1994; Malleshi *et al*., 1989; Malleshi *et al*., 1986; Marero *et al*., 1988). Flour from germinated seeds has been reported to have better nutritional properties than flours from nongerminated cereals (Finney, 1983; Lorenz, 1980). 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 (Wahed *et al*., 1994; Malleshi *et al*., 1989; Malleshi *et al*., 1986; Marero *et al*., 1988).

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; Qiao *et al*., 2009).

The objective of this study was to determine the optimum condition of steeping and germination and their effect on sorghum composition. Since very few reports have been carried out in this area, the present investigation was further conducted by using RSM and the model could develop an equation that could predict the quality of the germinated sorghum flour. This work highlighted the different biochemical modifications that occurred in sorghum grain during steeping and germination and presented an improvement of the nutritional properties of sorghum flour acceptable to brewers and infant foods.

MATERIALS AND METHODS

Sorghum (Sorghum bicolor (L.) Moench) was grown in Shandong, a coastal province East of China and known to have average January temperature of 0°C and July

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28°C. The average annual rainfall is about 500 mm, most of which falls in the summer. Red sorghum was obtained from 2008 and 2009 harvest. The length/breadth ratio of sorghum kernel was 1.12/1.23 and the density (g/l) was 691.40. The average weight of 1000 kernels was 26.80 g.

All the chemicals used were of analytical grade and purchased from Sinopharm Chemicals Reagent Company (SCRC), Shanghai, China.

Germination and preparation of sorghum flour: After removing chaff and unviable grain, sorghum grains (1000 g) were thoroughly cleaned by washing with tap water and then soaked in 0.20 ppm wooden ash water (1:2, w/v) for 24 h at ambient temperature with the soaking water being changed at 8 h interval. After soaking, the grains were evenly spread on jute bags and covered with the same material, in a secluded and dark area and allowed to germinate. The grains were wetted with water at regular interval of 24 h. The Steeping and germination conditions are stated in Table 1. The withered rootless were gently brushed off and dried grain were milled using a bench-top attrition mill (Dade, DFT-600, 25000 rpm, Zhejiang Linda Mechanic Ltd Co, China). The resultant flour was sieved into a particle size of 70-mesh. The flour was then packaged in a low density polyethylene bag and stored using plastic containers with lids in a refrigerator at 4°C for later use.

Analysis of sorghum composition: Malt loss was calculated as the total of leaching, metabolic and vegetative losses as described by Malleshi and Desikachar (1986a,b). Percentage yield or recovery was the proportion of soluble solids in malted sorghum flour. The flour slurry (15 g/L) was prepared by mixing flour with distilled water in a glass beaker, heating to 95°C within 7-10 min, holding at 95°C for 5-10 min then cooling to 30°C. The viscosity of the gruel was measured after cooling the hot porridge to 50°C using a Rapid Visco Analyser (RVA, Brabender, Duisburg, Germany) (Mosha and Svanberg, 1983). Amylase activity was analyzed following the method of Bernfeld (1987). The flour sample was extracted with acetate buffer (pH 4.8) for 1 h at ambient temperature (about 20°C). Amylase activity of the extract was expressed as maltose units and defined as the amount of maltose (mg) released by the action of malt enzyme extracted from 1 g malt flour in acetate buffer (pH 4.8) on soluble starch at 37°C for 30 min (Malleshi and Desikachar, 1986a,b).

Quantitative estimation of tannin, as catechin equivalent, was carried out using the modified vanillin-HCl method of Price *et al.* (1978). Total protein content was measured using AOAC standard methods (AOAC, 1984) and % N was multiplied by a factor of 6.25. The moisture content was determined following the AACC (1991) method (Price, 1991). All chemical and physical analyses were carried out in duplicate and expressed on a dry weight basis unless otherwise stated.

Experimental design and statistical analysis: A central composite second-order design was chosen to determine the influence of four independent variables and the optimum steeping and germination conditions. The effect of the variables, steeping time (X1), steeping temperature (X2), germination time (X3) and germination temperature (X4) in sorghum composition was investigated. Each variable was coded at five levels: -2, -1, 0, 1, 2 (Table 1). The process variables and the responses were defined from published data (Mizubuti *et al.*, 2000; Moure *et al.*, 2002; Quanhang and Caili, 2005).

The quality of the malted sorghum flour needed for lowbulk, high-energy supplementary food formulations was chosen as the dependent variables, namely: malt loss (y1), SS yield (y2), viscosity (y3), amylase activity (y4), tannin (y5) and protein (y6) contents. These variables were expressed individually as a function of the independent variables.

The data were fitted to a Taylor second-order approximating function:

$$Y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X_1^2 + \sum_{\substack{i=1 \\ i<j}}^{k} B_{ij} X_i X_j \qquad (1)$$

Where Y is the response function, *B*o the centre point of the system, Bi, Bii and Bij represent the coefficients of the linear, quadratic and interactive effects, respectively, and Xi, Xii and XiXj represent the linear, quadratic and interactive effects of the independent variables (steeping time, steeping temperature, germination time and germination temperature), respectively.

Optimization and verification: Optimum processing conditions for steeping time and temperature and germination time and temperature were determined by superimposing the plots for all response variables

Table 1: Process variables and their levels in the four-factor, five-level response surface design

		Coded variables levels				
Variable	Symbol	-2	-1	0	1	2
Steeping time (St) (h)	X1	0	8	16	24	32
Steeping temperatures (ST) (c)	X2	5	15	25	35	45
Germination time (Gt) (d)	X3	0	1.5	3	4.5	6
Germination temperature (GT) (c)	X4	10	20	30	40	50

(Floros and Chinnan, 1988; Henika, 1982; Henika, 1972). The region that fulfils the requirements for formulating low-bulk, high-energy supplementary food, such as low viscosity and tannin and high protein content, was considered and shaded. The optimum germination conditions were selected and used for calculating the predicted properties of germinated sorghum flour using the prediction equations derived by RSM.

Verification of the optimum conditions for germinating sorghum was performed by germinating the seed. The germinated sorghum flour obtained was experimentally analyzed and the results statistically compared to those predicted by the mathematical model.

RESULTS AND DISCUSSION

Fitting the models: The effects of steeping and germination on viscosity, malt loss, enzyme activity, SS yield, tannin and protein contents are shown in Table 2. The independent and dependent variables were fitted to the second order model equation and examined for the goodness of fit. In general, exploration and optimization of a fitted response surface may produce poor or misleading results, unless the model exhibits a good fit, which makes checking of the model adequacy (Liyana-Pathirana and Shahidi, 2005). Several indicators were used to evaluate the adequacy of the fitted model. A test for the lack of fit was used wherein a low F-value indicates that the model equation is an adequate approximation to the data. The R^2 values, Coefficients of Variation (CV) and model significance (F-value) were also used to judge the adequacy of the model. The significance of the F-value depends on the number of Degrees of Freedom (DF) in the model (Cai *et al*., 2008; Qiao *et al*., 2009).

The analysis of variance of the effect of germination conditions as a linear term, quadratic term and interaction on the response variables is shown in Table 3. The results indicated that the model is highly adequate because responses have satisfactory levels of R^2, CV and model significance. The SS yield and protein, however, showed a rather high CV and could be due to the experimental region covered in the study. However, the model was highly significant and possesses 88.03% of R^2 for SS yield and 88.57% for protein. Considering the high value of R^2, the model for SS yield and protein can be accepted.

Table 2: Central composite design arrangement and responses for sorghum seed germination process

	Variable levels[b]				Responses[c]					
Run[a]	X1	X2	X3	X4	Y1	Y2	Y3	Y4	Y5	Y6
1	-1(8)	-1(15)	-1(1.5)	-1(20)	2.04	8.55	4.87	2.6	1.39	8.98
2	1(24)	-1(15)	-1(1.5)	-1(20)	3.48	8.56	5.40	3.5	1.4	8.68
3	-1(8)	1(35)	-1(1.5)	-1(20)	2.12	8.44	4.34	2.4	1.43	8.81
4	1(24)	1(35)	-1(1.5)	-1(20)	4.06	16.41	8.45	4.2	1.22	16.80
5	-1(8)	-1(15)	1(4.5)	-1(20)	2.45	9.39	3.50	2.6	1.17	9.63
6	1(24)	-1(15)	1(4.5)	-1(20)	4.23	16.70	4.60	4.2	1.24	16.48
7	-1(8)	1(35)	1(4.5)	-1(20)	5.24	21.80	12.76	5.7	1.36	21.27
8	1(24)	1(35)	1(4.5)	-1(20)	6.69	26.10	12.94	6.8	1.22	26.38
9	-1(8)	-1(15)	-1(1.5)	1(40)	2.52	9.50	4.60	2.1	1.37	9.82
10	1(24)	-1(15)	-1(1.5)	1(40)	2.57	8.81	4.20	2.5	1.44	8.77
11	-1(8)	1(15)	-1(1.5)	1(40)	2.56	8.75	4.10	2.9	1.32	8.55
12	1(24)	1(35)	-1(1.5)	1(40)	4.56	17.86	8.80	4.7	1.15	17.65
13	-1(8)	-1(15)	1(4.5)	1(40)	5.67	23.90	20.55	5.2	1.09	23.51
14	1(24)	-1(15)	1(4.5)	1(40)	8.25	32.50	20.85	8.8	1.25	32.70
15	-1(8)	1(35)	1(4.5)	1(40)	9.69	39.30	22.24	9.3	1.26	39.69
16	1(24)	1(35)	1(4.5)	1(40)	14.46	56.80	34.34	14.3	1.15	56.93
17	-2(0)	0(25)	0(3)	0(30)	2.67	10.16	4.40	2.3	1.29	10.37
18	2(32)	0(25)	0(3)	0(30)	4.68	18.40	8.56	4.7	1.24	18.31
19	0(16)	-2(5)	0(3)	0(30)	8.93	34.40	12.56	8.5	1.23	34.07
20	0(16)	2(45)	0(3)	0(30)	10.37	41.20	20.65	10.7	1.22	41.4
21	0(16)	0(25)	-2(0)	0(30)	1.47	6.80	4.40	1.6	1.46	6.53
22	0(16)	0(25)	2(6)	0(30)	8.52	33.20	36.78	8.5	1.2	33.16
23	0(16)	0(25)	0(3)	-2(10)	2.35	9.60	4.43	2.6	1.37	9.57
24	0(16)	0(25)	0(3)	2(50)	4.47	20.70	11.42	4.5	1.21	19.52
25	0(16)	0(25)	0(3)	0(30)	2.56	9.10	4.50	2.4	1.39	9.64
26	0(16)	0(25)	0(3)	0(30)	2.22	8.40	4.80	2.2	1.41	8.34
27	0(16)	0(25)	0(3)	0(30)	2.68	10.81	4.50	2.4	1.4	10.61
28	0(16)	0(25)	0(3)	0(30)	5.47	25.83	8.50	5.5	1.41	25.36
29	0(16)	0(25)	0(3)	0(30)	5.76	22.84	9.21	5.4	1.39	22.56
30	0(16)	0(25)	0(3)	0(30)	5.93	23.75	8.56	5.4	1.41	23.60

[a]Experimental runs were performed in random order. [b]Based on coded variables. [c]y_1 = malt loss (%); y_2 = SS yield (%); y_3 = cold paste viscosity (poise); y_4 = amylase activity (maltose unit); y_5 = tannin (µg/g); y_6 = protein (%)

Table 3: Analyses of variance on the effect of germination conditions (Xk) as linear, quadratic and interaction (cross product) terms on the response variables (Yk)

		Sum of squares					
		Malt loss	SS Yield	Viscosity	Amylase	Tannin	Protein
Source	Degree of freedom	Y1	Y2	Y3	Y4	Y5	Y6
Model	14	0.49***	3908.79***	12.61***	244.14**	0.31***	3922.60***
Linear	4	0.37***	2544.18***	8.62***	147.64***	0.13**	2511.60***
Quadratic	4	0.09**	780.11***	1.79***	58.12***	0.085*	795.07***
Cross product	8	0.026	166.59	0.55	8.73	0.00348	168.60
Residual	7	0.073	364.72	0.76	15.09	0.0045	337.99
Lack of fit	10	0.026	199.26	0.68	9.37	0.0035	192.23
Pure error	5	0.073	332.05	0.63	14.45	0.00483	313.77
R squares		83.14	88.03	90.63	93.89	98.71	88.57
CV (%)		19.97	30.34	13.98	25.46	1.26	29.65

*Significant at 10% level: **Significant at 5% level; ***Significant at 1% level.

$Y_1 = 1/y1^{1/2}$; $Y_2 = y2$; $Y_3 = \ln y3$; $Y_4 = \ln y4$; $Y_5 = y5$; $Y_6 = y6$

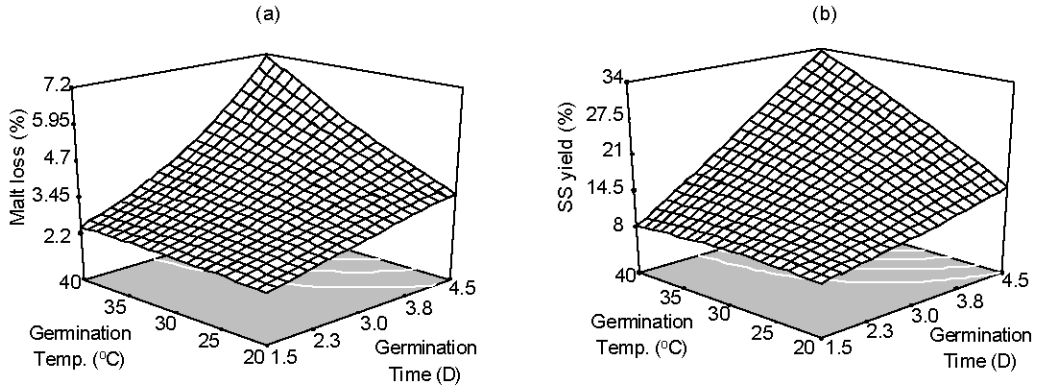

Fig. 1: Response surface plots of the effect of germination conditions on malting loss and SS yield of sorghum

Malting loss and SS yield: The viability of germination can be realized if high SS yield and low malting loss can be maintained. Malting loss includes losses due to leaching of solids during steeping and losses due to increased metabolic growth during germination. Increase in malting loss may subsequently decrease the level of water soluble nutrients in the germinated flour. However, during seed germination, adequate moisture must be attained to hasten metabolic development of the roots and shoots.

The seed should attain suitable moisture content by steeping for the optimum time and temperature. The effects of steeping and germination conditions on malting loss (Y1) are shown in Table 4 and 5. The mathematical model clearly suggests that any increase in germination time and temperature will significantly increase malting loss (Fig. 1a).

The germination conditions that affected the SS yield (Y2) are presented in Table 4 and 5. Steeping time and temperature, as well as germination time and temperature, significantly influenced the SS yield. There was no significant interaction between the independent variables.

The regression coefficient of the model suggested that any increase in germination time (bk3 = 8.02) will increase the SS yield. The significant quadratic and linear effect of germination time (bk33 = 4.73) further accelerated the increase in SS yield. These findings clearly support the hypothesis that the development of roots and shoots contributes soluble sugars from starch and protein. In general, during malting the reserves of nutrients like starch and protein are respectively degraded to soluble sugars and amino acids to meet the seedling requirements during the germination process. Depression of starch and protein degradations indicated the interference with metabolic systems operating on reserve starch and protein, mainly enzymes such as amylases and proteases acting in malting system (Dalvi, 1974). Fig. 1b illustrates the influence of the process variables on the SS yield of malted sorghum flour.

Table 4: Regression coefficients of the second-order polynomials showing the relationship between response variables (Yk) and independent variables (Xk)

	Malt loss	SS Yield	Viscosity	Amylase	Tannin	Protein
Coefficients	Y1	Y2	Y3	Y4	Y5	Y6
Bk0	0.53***	16.7883***	1.8473***	3.8833***	1.4016***	16.685***
Bk1	-0.048**	2.9412**	0.1462*	0.875***	-0.0175***	2.9170**
Bk2	-0.031*	3.7979***	0.2023***	0.9666***	-0.0108***	3.8404***
Bk3	-0.1***	8.0170***	0.4755***	1.9083***	-0.0625***	7.9912***
Bk4	-0.043**	4.3195***	0.2659***	0.9***	-0.03***	4.1870***
Bk11	-0.0038	1.4781	0.0986	0.2	-0.0587***	1.5468
Bk21	0.012	1.3318	-0.0441	0.4	0.0175***	1.4156
Bk22	0.017	0.9331	0.0040	0.3375	0.0137***	0.9268
Bk31	-0.017	2.8418*	0.1217	0.7375**	0.045***	2.8993*
Bk32	0.0046	1.1531	-0.0911	0.4	-0.0187***	1.1581
Bk33	-0.033	4.7218***	0.3211***	1.175***	-0.005***	4.8468***
Bk41	0.0029	-0.9974	-0.0331	-0.1479	-0.0339***	-0.9113
Bk42	-0.051***	4.8826***	0.2080***	1.3770***	-0.0439***	4.9373***
Bk43	0.0150	0.4326	0.1490**	0.2395	-0.0177***	0.4648
Bk44	0.0093	-0.7799	0.0037	-0.1354	-0.0277***	-0.8601

*Significant at 10% level: **Significant at 5% level; ***Significant at 1% level. Y1 = 1/y1 ½; Y2 = y2; Y3 = ln y3; Y4 = ln y4; Y5 = y5; Y6 = y6.

Table 5: Analysis of variance showing the significance of the overall effect of germination conditions (Xk) on each of the response variables (Yk)

			Sum of squares					
			Malt loss	SS Yield	Viscosity	Amylase	Tannin	Protein
Germination condition	Code	DF	Y1	Y2	Y3	Y4	Y5	Y6
Steeping time (St) (h)	X1	14	0.0550**	207.6228**	0.5132**	18.375***	0.0073***	204.225**
Steeping temperatures (ST) (c)	X2	14	0.0232*	346.1801***	0.9830***	22.4266***	0.0028***	353.9712***
Germination time (d)	X3	14	0.2459***	1542.567***	5.4265***	87.4016***	0.0937***	1532.642***
Germination temperature (Gt) (GT) (c)	X4	14	0.0439**	447.8112***	1.6972***	19.44***	0.0216***	420.76****

Significant at 10% level: **Significant at 5% level; ***Significant at 1% level. $Y_1 = 1/y1^{1/2}$; $Y_2 = y2$; $Y_3 = \ln y3$; $Y_4 = \ln y4$; $Y_5 = y5$; $Y_6 = y6$. DF = Degree of Freedom

Viscosity and amylase activity: Germination is one of the methods that can be used to reduce the viscosity of the grain extracts (Wahed *et al*., 1994; Malleshi *et al*., 1989; Malleshi *et al*., 1986; Marero *et al*., 1988). During germination, the starch is degraded by the action of enzymes present in the seed. Amylases break down the amylose and amylopectin components of the starch producing smaller dextrins, maltose and glucose (Bewley and Black, 1985; Allen and Spradlin, 1974), thus reducing the viscosity. A good correlation between viscosity or falling number and amylase activities in cereals has been reported (Raschke *et al*., 1995). The factors affecting the viscosity of malted sorghum flour during germination were investigated and are presented in a model equation (Y3) in Table 4. The coefficients of determination indicated a strong dependence of viscosity to the linear and quadratic terms. The viscosity decreased significantly by time and temperature of germination increased. Likewise, increase in steeping time contributed to a decrease in cold paste viscosity. Significant linear effects were contributed by germination time (bk33 = 0.3211). Analysis of variance shown in Table 5 further validates this result and can be clearly seen in Fig. 2a.

The relationship between amylase activity and the factors that contribute to its change during germination are presented in Table 4. The Ln transformation of the model equation (Y4) revealed that not only the time and temperature of steeping with germination contributed significantly to the increase in enzyme activity, but also the linear terms of germination time (bk33 = 1.175). Thus enzyme activity increased dramatically and alpha- with ß-amylase require sufficient moisture and temperature to hydrolyze starch in the sorghum grain. The ranges of steeping and germination temperatures to which the sorghum seeds have been subjected favorably increase their enzyme activity at different rates. Similar observations were reported in germinated millets (Malleshi and Desikachar, 1986a,b), kaffircorn (Morrall *et al*., 1986, Novellie, 1962), barley (Pal *et al*., 1976) and wheat (Reddy *et al*., 1984). The relationship of the independent variables to amylase activity is shown in Fig. 2b.

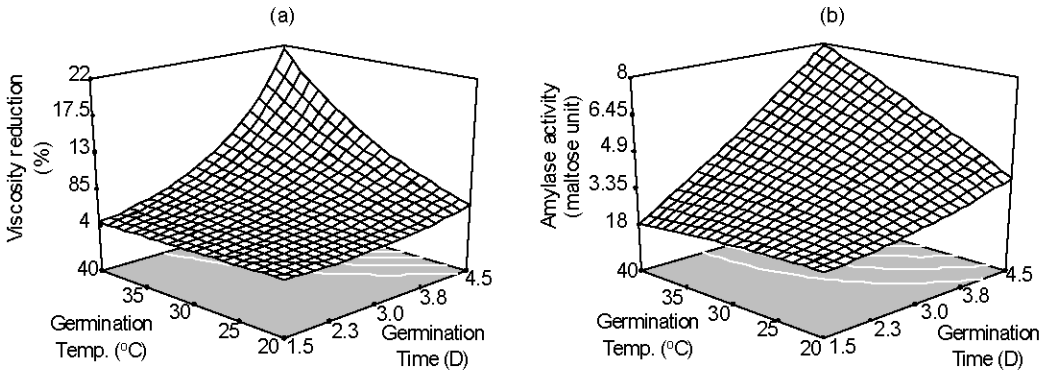

Fig. 2: Response surface plots of the effect of germination conditions on cold paste viscosity and amylase activity of sorghum

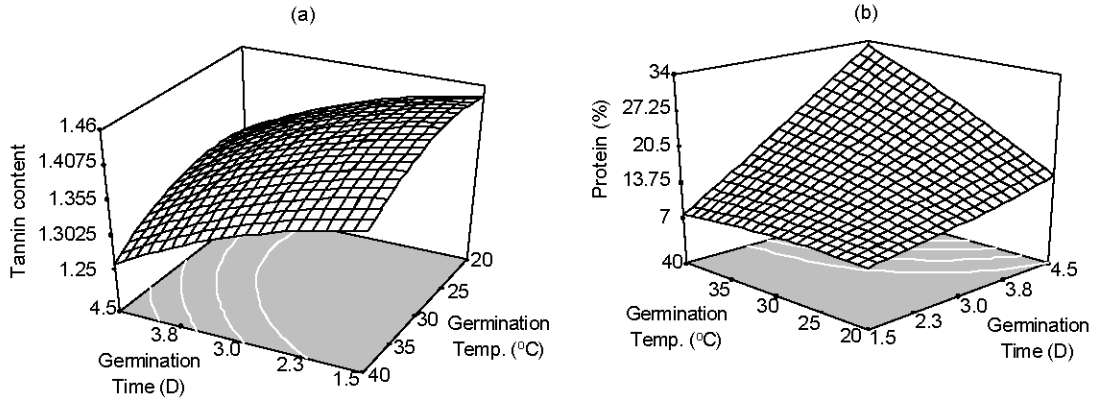

Fig. 3: Response surface plots of the effect of germination conditions on tannin (µm/g) and protein content of sorghum

Tannin and protein contents: Tannins are located in the seed coat (Jambunathan and Mertz, 1973) and are reported to form complexes with hydrolytic enzymes and to inactivate them (Milic *et al*., 1972). The changes in the seed coat permeability may be much greater and rapid thus allowing higher solid losses. Part of the tannins may enter into the endosperm along with the imbibed water. Such tannins are likely to form complexes with reserve seed protein and enzymes and to inactivate them (Price *et al*., 1978). The loss of tannins, therefore, can be attributed to leaching of tannins into the growth medium during malting (Capanzana and Malleshi, 1989). During germination sorghum seed is steeped in water which may decrease some water soluble nutrients, including tannin. The influence of this operation was investigated and the result of the mathematical model and analysis of variance showing the effect of process variables are presented in Table 4 and 5, respectively.

The regression coefficients (Y5) indicated that increasing in germination time (bk3 = -0.0625) and temperature (bk4 = -0.03) subsequently decreased the tannin content. Steeping time and temperature showed also a significant effect (Table 5). Interaction effects between independent variables were noted and implied that the effect of one variable depends on the specific levels of other variables. From these findings it can be concluded that steeping and germination can be used as a process for reducing the level of tannin. The decrease in viscosity and tannin content after stepping and germination of sorghum flour can be considered as an advantage, particularly if the flour is to be used for formulating supplementary weaning food. Fig. 3a illustrates the effect of the independent variables on the tannin content of sorghum flour.

The protein content of sorghum flour during germination is shown in Fig. 3b. Germination time and temperature and steeping temperature influenced the amount of protein present in the germinated sorghum. This is further confirmed in the analysis of variance presented in Table 5. Several researchers reported improvement in the protein quantity as well as quality during germination of maize (Tsai *et al*., 1975), millet (Malleshi *et al*., 1986), sorghum (Obizoba, 1988) and wheat (Dalby and Tsai, 1976).

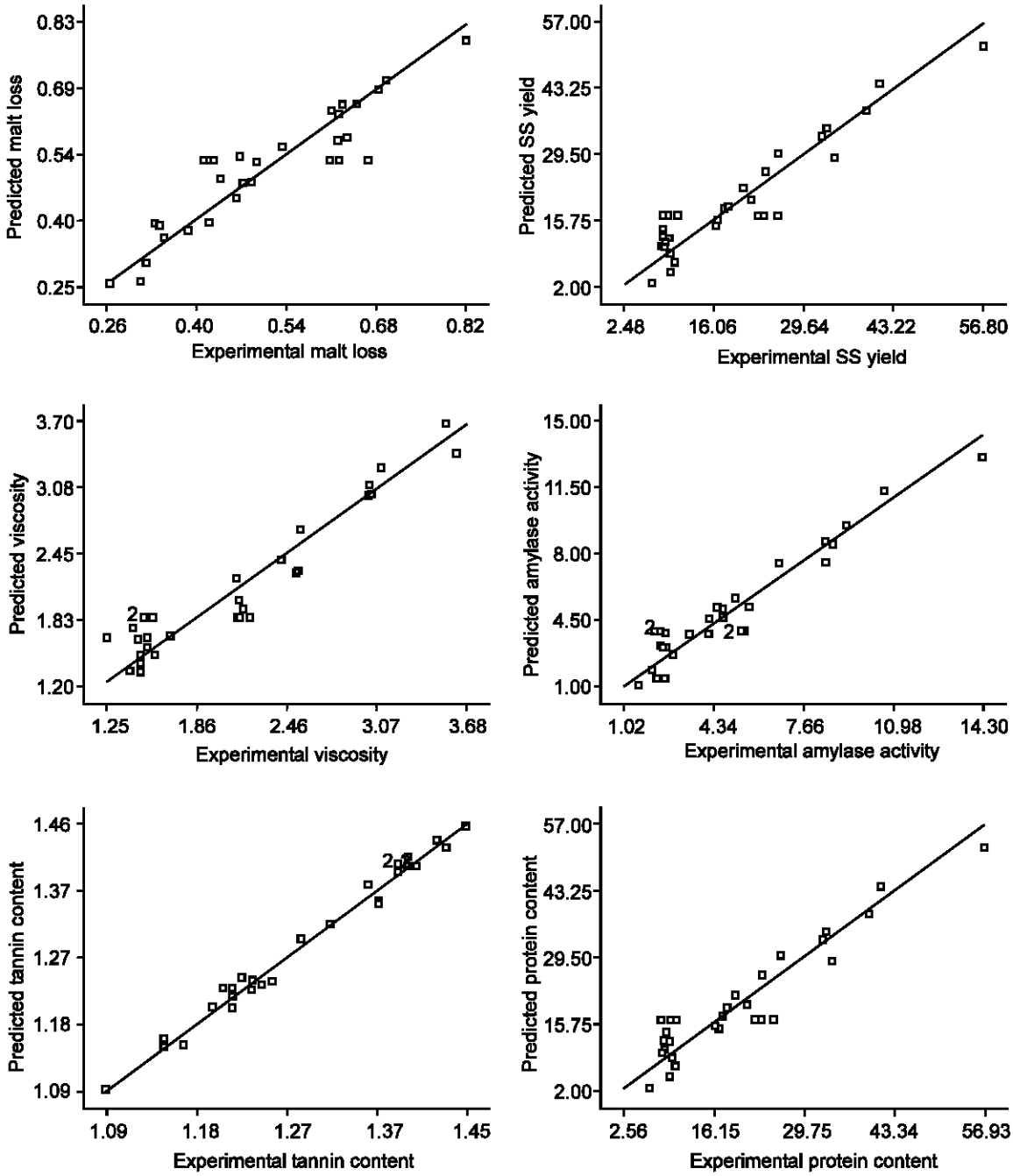

Fig. 4: Response trace plots of experimental data against predicted values of malt loss; SS yield; cold paste viscosity; amylase activity; tannin content and protein

Adequacy of the model: The coefficient of determination (R^2) was calculated to examine the amount of the variation in the response that is explained by the model. A relatively high R^2 value is a sign that the regression model can be used with confidence for the purpose of the predicting response values (Hu, 1999). RSM optimization approach was also used to validate experimentally and predict the values of the responses using the model equation. The experimental and predicted values were within the range and were found to be not statistically different at 5% level of significance (Table 6).

Furthermore, we plotted the experimental data against the predicted values by the model (Fig. 4). Overall, the points are scattered favorably around the straight line, which indicates that the model fits the data. Thus, the

Table 6: Predicted and experimental values of the responses at optimum conditions

		Experimental value[a]		
Response variable	Code	Predicted value	Mean	Range
Malt loss (%)	Y1	6.84	7.96±0.79	1.47-14.46
Yield (%)	Y2	34.28	31.8±1.75	6.8-56.8
Viscosity (poise)	Y3	14.03	17.26±2.28	33.11-15.9
Amylase activity (maltose unit)	Y4	8.59	7.95±0.45	1.6-14.3
Tannin (%)	Y5	1.17	1.27±0.07	1.09-1.46
Protein	Y6	34.19	31.73±1.73	6.53-56.93

[a]Mean value of five determinations. Optimum conditions are: steeping time, 24 h; steeping temperature, 35°C; germination time 30 h germination temperature, 40°C. $Y_1 = 1/y1^{1/2}$; $Y_2 = y2$; $Y_3 = \ln y3$; $Y_4 = \ln y4$; $Y_5 = y5$; $Y_6 = y6$

model can be used to predict the quality of the germinated sorghum flour and can be applied between a steeping time of 0-32 h, steeping temperatures of 5-45°C, germination time of 0-6 d and germination temperature of 10-50°C.

Conclusion: Introduction of germination in the processing of sorghum brought significant improvements in the nutritional quality and functional properties of malted sorghum flour. Germination induces important desirable nutritional modifications, and the low level of tannin achieved would make the flour suitable as a carrier for micronutrient fortification. The model equation developed can be used for predicting the quality of malted sorghum flour. The optimum germination conditions for sorghum suitable for supplementary food formulations with low bulk but with high nutrient density were established to be steeping for 24 h at 31°C with 4.5 d of germination at 30°C.

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