Quality characteristics of gluten free bread from barnyard millet–soy flour blends

Subir K. Chakraborty¹ · Saumya Gupta² · Nachiket Kotwaliwale¹

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Abstract The effects of formulation of leavened bread by using varying levels (for 50 g base flour) of soy flour–barnyard millet blends (with 5.74, 6.25, 7, 7.75 and 8.26 g of soy flour), yeast (1.83, 2, 2.25, 2.5 and 2.67 g) and salt (0.63, 0.8, 1.05, 1.30 and 1.47 g) on textural, colour and specific volume were determined. A central composite rotatable design of response surface methodology was used to plan the experiments. The second order models obtained were observed to be statistically significant and capable of demonstrating the effects input variables on responses. All the textural properties were affected significantly by amount of soy flour and yeast in the dough. Soy flour had a significant effect on the colour of the bread making it more brown. Interaction of soy flour and yeast affected the specific volume to maximum extent. Two-tailed *t* test established that the efficacy of the models as no significant was observed between the predicted and the actual values.

Keywords Gluten free bread · Millet · Soy flour · Quality characteristics · RSM

Introduction

Bread, a vital constituent of human food basket uses refined wheat flour (RWF) as a key ingredient. Bread made from RWF provides carbohydrate, proteins and certain minerals (magnesium, iron, phosphorus). Addition of new ingredients to dough has been attempted to improve quality in terms of nutritional value of breads. Present times is witnessing a shift in consumer preference for food not only with rich nutritional attributes, but also with characteristics either promising reduced incidence of diseases or at least having the features of delaying the onset of disorders (Riccardi et al. 2005). Jenkins et al. (2007) reported that consumption of fibre rich food results in lowering of glycemic index (GI) leading to a reduced risk of postprandial oxidative stress, thus negating the chance of several chronic diseases. Similar observations were reiterated by Jones and Larzelere (2008), concluding that high fibre consumption assures a healthy gut with comparatively lesser chance of cancer, cardiac diseases, blood pressure and obesity.

The idea behind present research work was to prepare a functional bread, rich in fibre and protein. Fibres or complex carbohydrates are the most sought after food components in the present times. The consumers today are becoming nutritionally aware about the advantages of fibres, particularly soluble dietary fibre (SDF) (Chakraborty et al. 2010). Total dietary fibres (TDF) and SDF have a healing effect upon a large number of diseases. The role of SDF in lowering blood cholesterol in humans (Jenkins et al. 1980) and animals (Ranhotra et al. 1987) is well established. Ergo, food incorporated with fibres will not only have a low calorific value but will also have good medicinal properties. Millets are a group of crops, often referred to as Coarse Cereals. These days millets are

✉ Subir K. Chakraborty
subir8275@gmail.com

¹ Agro Produce Processing Division, ICAR - Central Institute of Agricultural Engineering, Nabibagh, Berasia Road, Bhopal, M.P. 462038, India

² Department of Food Technology, Bundelkhand University, Jhansi, U.P., India

popularly called as *nutricereals* (Desikachar 1977), because of the vast variety of micronutrients, phytochemicals and nutraceuticals that they contain. Among millets and across all cereals, barnyard millet (*Echinochloa frumentacea*) is the richest source of crude fibre, 13.6 g per 100 g edible portion (Hulse et al. 1980). A common way of administering millets to gluten sensitive or diabetic is by using the millet flours to produce regular baked products like, bread, cookies and crackers.

In India, millets are grown by small and marginal farmers in a total area not exceeding 1.3 million hectares (which is a paltry 0.7% of total cropped area) of hills and vast stretches of dry land (IASRI 2006). These farmers do not have access to irrigation water and grow millets for their sustenance and limited economic benefits. Finding a marketable use of millets in the form of breads, perhaps will have a multipronged effect, nutritional products, value added use of millets leading to economic benefits for small farmers and finally, reducing the overall water requirements for agricultural purposes in a broader picture.

The peculiar rheological properties of the RWF dough which makes it suitable for baking and leavening is because of the molecular size and structure of the gluten polymers that are present in it (Chakraborty et al. 2015). The mechanic of dough making process results in entanglement of polymer chains and their branches leading to attainment of required rheological characteristics. In the present study soybean flour was used, with a hypothesis that the soy protein can mimic—to certain extent—the role of gluten as in case of traditional leavened breads.

Incorporation of dietary fibres in food systems has been reported to cause negative effect upon the functional properties of the food (Brennan and Samyue 2004). This is especially because the alteration in the visco elastic properties and water absorption ability of the dough due to the presence of fibre (Mancebo et al. 2015).

Neutral salt like sodium chloride is a key ingredient for bread making plays an important role in the quality of finished product. Right amount of salt results in appropriate dough development during proofing (Preston 1989). Rise in blood pressure and onset of cardiovascular diseases are common health disorders in humans caused by intake dietary salt (He and MacGregor 2007). Processed food contributes to about 70–75% of the total salt intake (James et al. 1997). Salt works as flavour enhancer, affect the volume and control the fermentation process. It is therefore imperative that the quantity of salt used as a bread ingredient needs to be carefully controlled. Too much salt would retard fermentation and proofing, while less salt will make it rapid. Yeast is an important ingredient too; it works as a leavening agent. Optimum amount of yeast ensures bread with perfect texture, volume and structure. In the present

research work, besides blend ratio (soy: millet flour) other variable were salt and yeast.

Response surface methodology (RSM) is a sequence of mathematical and statistical procedures involving first order or second order polynomial equations. It is an effective tool for handling separate variables under designed experimental conditions for predicting the optimum operating conditions (Lenth 2009).

There are a lot of evidence of multiple regression approach of RSM been effectively used to represent relationship between processing parameters and responses where the raw material has been composite flours and product were bakery products (Chakraborty et al. 2010; Rai et al. 2014; Cornejo and Rosell 2015). RSM has been successfully used in determination of optimum conditions for production of wheat bread by Malcolmson et al. (1993) and Ylimaki et al. (1991) has applied RSM to produce and objectively measure properties of non-gluten breads.

This manuscript envisages reporting the quality characteristics of leavened breads from barnyard millet and soy flour blends based on textural, colour and bread volume properties. Further, input variables—soy-millet blend ratio, salt and yeast have been optimized.

Material and method

Material

Dehusked barnyard millet (*Echinochloa frumentacea* L.), compressed yeast, salt (NaCl) and sugar (sucrose) was procured from local market of Bhopal city of India. Dehusked millet was cleaned of all foreign materials before being ground using laboratory attrition mill at Cereal Processing Laboratory and full fat soy (soybean variety: JS 335) flour was obtained from Soybean Processing Pilot Plant of ICAR-Central Institute of Agricultural Engineering (CIAE), Bhopal, India. The millet flour collected as under flow of 140 mesh I.S. sieve was used for the research work. The individual flours were packed in sealed polythene bags till they were used for the experiments. The proximate composition of soy and millet flour in terms of protein, fat, carbohydrate, moisture and ash content were, 38.1, 22.8, 27.2, 7.3, 4.6 and 10.5, 3.6, 75.8, 6.7, 3.4; respectively.

Bread making

The process adopted for soy-millet bread making is straight dough method (Moore et al. 2004). For dough making, all ingredients were taken according to design experiment. The ingredients were used either in fixed (Table 1) or in

Table 1 Gluten free bread making formulation

Ingredients	Remarks
Base flour	Blend of millet and soybean, as per the experimental design (50 g)
Water	Fixed amount, 40 ml
Salt	Variable, as per the experimental design
Yeast	Variable, as per the experimental design
Sugar	Fixed amount, 1.5 g
Butter	For greasing the baking pan for easy lifting of baked bread

Table 2 Plan of experiments and levels of input variables

Actual values			Coded values
Soy flour (g)	Yeast (g)	Salt (g)	
5.74	1.83	0.63	−1.68
6.25	2.00	0.80	−1
7.0	2.25	1.05	0
7.75	2.50	1.30	+1
8.26	2.67	1.47	+1.68
X ₁	X ₂	X ₃	No. of experiments
<i>Experimental design</i>			
0	0	0	6
±1	±1	±1	8
±1.68	0	0	2
0	±1.68	0	2
0	0	±1.68	2
Total experiments			20

X₁ soy flour, X₂ yeast, X₃ salt

variable (Table 2) quantity. Compressed yeast was used in bread making process; it was kept under refrigerated conditions before being used for experiments. The yeast was thawed in lukewarm water at 30 ± 2 °C prior to use in experiments, similar procedure is recommended by Thiessen (1942). All the ingredients were mixed to achieve dough for bread making. Care was observed about the temperature of water as Kim and DeRuiter (1968) have recommended use of water at temperature not more than 30 °C for making dough with soy flour. The proofing thus took place at 100% relative humidity (R_h) and at temperature 35 ± 2 °C for about 4 h. Baking was carried out in a preheated oven at 195 °C (± 5 °C) for 20 min. The baked bread was cooled under controlled conditions before any further test were done.

Bread quality parameters

The quality of the bread was assessed on the basis of its instrumental textural property, colour and specific volume.

Textural property

The textural properties,—hardness, cohesiveness, resilience and springiness of breads were judged on the basis of TPA curve (Kotwaliwale et al. 2007). Texture analysis was carried out with a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, England) equipped with 50 kgf load cell. Tests were conducted by using flat plate probe of 75 mm diameter with trigger force of 0.1 N and 40% strain (Matos and Rosell 2012). The probe speed parameters were: pre-test speed 1 mm/s, test speed 5 mm/s, and posttest speed 5 mm/s.

Colour

The colour and difference in colour (ΔE) was measured using simple digital imaging method. A high resolution digital camera (Nikon L810, 14.0 Mega pixels, 26X Digital zoom) was used to measure the colour by capturing the colour image of bread under D₆₅ illumination. The captured colour images were analysed quantitatively using Photoshop (Adobe Systems 7.0.1, 2002).

Photoshop can display lightness (L), redness (a) and yellowness (b) values in the Info Palette and Histogram Window, but these are not standard colour values. However L, a, and b values can be converted into standard colours, L*, a* and b* by using following expressions (Yam and Papadakis 2004):

$$L^* = \frac{L}{255} \times 100 \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

The recorded L, a and b values were mean of five replications. The colour difference (ΔE) of barnyard millet based bread was quantified by the following equation:

$$\Delta E = \sqrt{(L^* - L_i^*)^2 + (a^* - a_i^*)^2 + (b^* - b_i^*)^2} \quad (4)$$

The variation in colour of the bread was recorded with respect to a reference bread made from RWF in same experimental set up, recorded colour values being, L_i*, a_i* and b_i*.

Specific volume

The expansion of the dough during the baking process was estimated by volume per unit mass. Volume of bread was determined by a rapeseed displacement method (AACC 1998 Standard 10-05) and specific volume was calculated and expressed as cm³/g.

Data analysis

The data obtained from the experiments outlined in Table 2 was analyzed for optimization of input variables (soy flour, yeast, and salt content of the dough) and output variables (textural properties in terms of hardness, cohesiveness, springiness, resilience, colour in terms of L, a, b and ΔE for the breads and specific volume).

Regression analysis and analysis of variance (ANOVA) were conducted for fitting the models (Eq. 5) and to examine the statistical significance of the model terms.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (5)$$

where, Y = dependent variables; X₁, X₂, and X₃ are independent variables.

The adequacy of the models were determined using model F-value, lack-of-fit, coefficient of variation (c.v.) and (coefficient of determination) as outlined by Weng et al. (2001). A model would be statistically sound for

$F_{\text{calculated}} < F_{\text{table}}$, non-significant lack-of-fit, c.v. <10% and $R^2 > 80\%$. The effect of variables at linear, quadratic and interactive level on the response was described using significance at 1, 5 and 10% level of confidence.

Response surfaces and contour plots were generated with the help of trial version of commercial statistical package, Design Expert version 8.0.7.1 (Design Expert 2013). In order to search a solution maximizing multiple responses, the goals are combined into an overall composite function, D(x), called the desirability function (Myers and Montgomery 2002), which is defined as:

$$D(x) = (d_1 \times d_2 \times \dots \times d_n)^{1/n} \quad (6)$$

where, d₁, d₂ ... d_n are the responses and 'n' is the total number of responses in the measure. The numerical optimization finds a point that maximizes the desirability function. The characteristics of a goal may be altered by adjusting the weight or importance (Design Expert 2013).

Results and discussion

Response surface analysis was used for the data generated after the experiments to understand the effect of input variables on textural properties, colour and bread expansion with the help of second order polynomial model. The result of the regression analysis and analysis of variance (ANOVA) for all the models is reported in Table 3.

Table 3 Regression analysis of second order polynomial model for various responses

Predictor	Coefficient (β)								
	Hardness (N)	Cohesiveness	Resilience	Springiness	L*	a*	b*	ΔE	Specific volume (cm ³ /g)
Constant	274.02	0.80	0.60	1.20	21.99	−116.36	−105.83	2.52	2.963
X ₁	15.31**	−0.032***	0.045***	−0.005	−1.202***	1.287***	5.195***	−0.621*	−0.036***
X ₂	−68.21***	−0.012**	0.013**	0.053***	0.497**	1.251***	1.898***	0.047	0.058*
X ₃	5.03	−0.015***	−	0.088***	−0.248	0.305	−0.211	−0.042	−0.130***
X ₁ X ₂	7.65	0.006	0.009	−0.025	−0.525*	−0.431	0.522**	1.144**	0.164***
X ₁ X ₃	−0.86	−0.011**	−	0.069***	−0.309	0.428	−0.575	−0.197	0.034
X ₂ X ₃	8.58**	0.005	0.012	0.006	0.230	1.090***	−0.710	−0.653	−0.050
X ₁ ²	−13.16**	−0.012**	0.001	−0.001	1.150***	−1.090***	−0.230	2.785***	0.016
X ₂ ²	−23.01***	−0.010**	−0.020**	0.003	−0.302	0.112	−1.081***	1.266***	−0.013
X ₃ ²	−20.80***	0.010**	0.007	−0.085***	−0.167	−0.587**	−0.842**	−0.301	0.147***
ANOVA									
F-value	18.69	12.87	12.90	17.13	11.16	11.48	18.05	13.90	6.80
Lack of fit	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
c.v. (%)	9.4	1.91	3.12	3.82	3.13	0.74	1.55	21.70	3.85
R ² (%)	94.4	92.1	92.1	93.9	90.9	91.2	94.2	92.6	86.1

X₁ soy flour, X₂ yeast, X₃ salt, c.v. coefficient of variation, R² coefficient of determination, n.s. not significant

*** $p < 0.01$; ** $p < 0.05$; * $p < 0.1$

Fig. 1 a–h Contour plots depicting the effect of changing two processing parameters within the experimental range (−1.68 to +1.68) on the responses; the third parameter being at ‘Centre Point’ of experimental condition coded as ‘0’

Effect of variables

Quantity of soy flour in the base flour had significant effect on textural properties except ‘springiness’. It has positive effect on ‘hardness’ ($p < 0.05$) and ‘resilience’ ($p < 0.01$). Soy protein acts as a good emulsifier, in addition of the wetting action of lecithin makes the bread crumb more

resilient. Soy flour content in the flour had a negative effect on ‘cohesiveness’ ($p < 0.01$), in other words, increase in the amount of barnyard millet in the bread led to a more cohesive product. Similar observations were made by Dvorakova et al. (2012) with buckwheat flour.

All the colour parameters were significantly ($p < 0.01$, ΔE at $p < 0.1$) affected due to addition of soy

flour. Negative correlation exhibited by L^* shows that more the amount of soy flour in the dough darker would be appearance of bread. Addition of protein source to bread dough has been reported to cause darker bread otherwise gluten free breads usually tend to have lighter colour than wheat breads (Gallagher et al. 2003). This darkening of crumb colour was considered as it was associated with whole grains and wholesomeness of breads (Kim and Yokoyama 2011). Specific volume of the bread decreased significantly ($p < 0.01$) with an increase in the amount of soy flour in bread. This observation was in agreement with the work of Ribotta et al. (2004). Yeast has significant effect on almost all the responses. Amongst the textural features, yeast had a positive effect on resilience ($p < 0.05$) and springiness ($p < 0.01$) while a negative effect on hardness ($p < 0.01$) and cohesiveness ($p < 0.05$). Yeast induces voids in the bread crumb, this may have accounted for its effect on resilience, springiness and hardness. Absence of gluten in the dough system resulted in a challenged protein network, thus the bread was less cohesive at more yeast. This means that this bread will not form a bolus but will disintegrate or crumble upon chewing. The colour indicators, L^* ($p < 0.05$), a^* ($p < 0.01$) and b^* ($p < 0.01$) were all positively affected by yeast i.e. the increased yeast content increased brightness, redness and yellowness of bread. The amount of yeast in the bread and specific volume share a significant ($p < 0.1$) positive relationship. Increase in specific volume reflected enhanced inculcation of air spaces in the bread structure, more the yeast in the dough more shall be such air pockets. Salt has significant ($p < 0.01$) positive effect on springiness, while the effect was negative on cohesiveness and specific volume. The specific volume of bread will be less at higher levels of salt because of the resultant slowed fermentation and proofing.

The yeast-salt interaction was found to be significantly affecting hardness ($p < 0.05$), there was a marked decrease in hardness at maximum amount of yeast in the dough (Fig. 1a). This is because of an increase in the air pockets spread inside the bread dough, rendering softness to bread. Springiness and cohesiveness of the bread was affected at $p < 0.01$ and $p < 0.05$, respectively, by the interaction of soy flour and salt (Fig. 1b, c). Soy flour binds water and salt is hygroscopic in nature, it was observed that springiness and cohesiveness attain highest values at the middle values of soybean over the experimental range. These traits decreased at highest level of salt addition for obvious reasons. There are about 6% non fermentable sugars present in soybean (Eldridge et al. 1979), baking initiates the browning reaction making the crust brown. This was reflected by the interactive effect of soy flour with yeast (Fig. 1d, f, g) on

colour values L^* ($\beta = -0.525$ at $p < 0.1$), b^* ($\beta = 0.522$ at $p < 0.05$) and ΔE ($\beta = 1.144$ at $p < 0.05$).

Specific volume ranged between 2.73 and 3.57 cm³/g (Table 4), these values are in sync with the findings of other researchers (Marco and Rosell 2008) for non-gluten baked products. Specific volume was affected the most ($\beta = 0.164$ at $p < 0.01$) by the interaction of soy flour and yeast. Perusal of Fig. 1h reveals that high yeast and high soybean content in the base flour results in better specific volume. The volume of bread increased because of the water binding ability of soy flour protein; in this case it is enhanced by the presence of the fibre from barnyard millet. However, for low yeast—high soy flour combination the specific volume decreased. This can be attributed to decrease in volume, because of less yeast and increase in weight due to more bound water at higher soy flour content in the bread. It has been reported by Marco and Rosell (2008) that bread enriched with protein do exhibit higher water retention.

Optimization of variables

The process variables were numerically optimized by using Design Expert 8.0.7.1. The optimization was carried with the process variables subjected to certain applied constraints. Under these constraints the software predicted optimum values for the various responses (Table 4) against the corresponding responses as well. Bread was baked under the optimum processing conditions and the responses were recorded (mean of 5 measurements). The veracity of the models was established with the help of a two-tailed, one sample *t* test between the values of responses predicted by the software and those recorded after actual experiments under the optimum conditions (Table 5). The results of the

Table 4 Optimum values of processing parameters and responses

	Lower value	Upper value	Optimum value
<i>Processing parameter</i>			
Soy flour (g)	5.74	8.26	7.75
Yeast (g)	1.83	2.67	2.50
Salt (g)	0.63	1.47	0.8
<i>Responses</i>			
Hardness (N)	108.4	316.3	159.1
Cohesiveness	0.7	0.9	0.77
Resilience	0.5	0.7	0.64
Springiness	0.8	1.3	0.98
L^*	19.3	27.1	21.77
a^*	−121.9	−113.0	−117.54
b^*	−116.4	−98.2	−98.87
ΔE	1.2	11.7	7.73
Specific volume (cm ³ /g)	2.73	3.57	3.44

Table 5 Model validation using two-tailed *t* test

Response	Predicted value (μ_o)	Actual value ^a (μ_1) $\pm$ SD	Standard error	Mean difference	% Variation	T_{cal}
Hardness (N)	159.1	161.68 $\pm$ 7.54	3.370	2.58	1.60	0.77
Cohesiveness	0.77	0.812 $\pm$ 0.09	0.041	0.042	5.17	1.03
Resilience	0.64	0.594 $\pm$ 0.07	0.033	0.046	7.74	1.39
Springiness	0.98	1.0 $\pm$ 0.11	0.047	0.01	1.03	0.21
L^*	21.77	23.98 $\pm$ 4.10	1.833	2.21	9.22	1.21
a^*	−117.54	−116.64 $\pm$ 8.11	3.626	0.9	0.77	0.25
b^*	−98.87	−102.92 $\pm$ 4.98	2.229	4.05	3.94	1.82
ΔE	7.73	7.8 $\pm$ 0.58	0.259	0.07	0.90	0.27
Specific volume (cm ³ /g)	3.44	3.84 $\pm$ 1.14	0.510	0.4	10.42	0.78

h_o : $\mu_o = \mu_1$, h_1 : $\mu_o \neq \mu_1$, $t_{cal} < t_{table}$ at $p < 0.01$, ' h_o ' was accepted

SD Standard deviation

^a Mean of five replications

two tailed *t* test demonstrated no significant difference between the predicted and actual data.

Conclusion

Designed experiments demonstrated that leavened bread can be made from soy-barnyard millet flour blends. Soy flour content in the bread affected texture, colour and specific volume. Textural properties of bread was affected due to change in moisture handling abilities of soy-barnyard millet food matrix as caused by their protein and fibre content. Colour changes may be attributed to Maillard reactions by the protein present in soy flour. The second order models computed can be used to obtain optimized condition for making bread from soy-millet flour blends. Also, the quality characteristics as predicted theoretically by the models exhibited non-significant difference from the actual experimental conditions values.

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