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Bread making performance of oat incorporated composite wholegrain flours – a response surface analysis

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Abstract

Bread was prepared using composite wholegrain flour blend comprising wheat, oats, quality protein maize (QPM) and soybean. Central composite rotatable design (CCRD) with three independent variables oat flour, QPM flour and soybean flour at five levels and four dependent variables, i.e. specific volume, loaf height, crumb firmness and overall acceptability scores, were used to conduct the experiments. Highly acceptable composite bread was obtained by combining optimized level of ingredients with whole wheat flour to make a blend of 100 g. The optimized level of ingredients was oat flour 10.00 g, QPM flour 10.00 g and soybean flour 5.375 g per 100 g flour with 81.5% desirability. The resultant bread had high, protein content, crude fibre and overall acceptability. The optimized bread had higher total phenolic content and significantly ($p \leq 0.05$) high anti-oxidant activity as compared to whole wheat (control) bread.

Introduction

Several research reports stress on a strong correlation between diet and health. Increasing incidences of communicable diseases such as diabetes, cancers and heart ailments call for changes in dietary patterns and shift towards foods that provide much more than 'nutrition'. The World Health Organization identifies the need for a shift in nutrient intake towards 'healthier' foods for management of chronic diseases (Malla, 2013). Hence functional foods are gaining popularity the world over. Bread is a popular cereal product that finds its place in the daily diet of millions of people around the world. It is an important source of energy, fibre, cereal proteins, minerals and vitamins. Hence, bread can easily qualify as a functional food (Sluimer, 2005). Functional breads have been prepared from several non-wheat cereals alone or in combination with wheat as composite flours. Breads from whole grains also provide several functional benefits.

Oats are now well acknowledged for their functional attributes because of high total dietary fibre and β-glucan content. These can help lower blood cholesterol, glucose and insulin concentrations (Welch, 1995). Recent studies suggest that oats can be tolerated by people suffering from celiac disease (Thompson, 2005). Bread made from oats has good acceptability due to nutty flavour and pleasant aroma (Flander *et al*., 2007). It possesses excellent moisture retention properties and keeps fresh for long periods of time (McKechnie, 1983). However, oat proteins get denatured by heat treatment (Oomah, 1983) and lead to tight, moist and gummy breads. Addition of dry gluten into the dough can compensate the poor baking performance of oat (Gormley and Morrissey, 1993).

Quality protein maize (QPM) has superior protein quality and protein digestibility over normal maize. QPM protein has 55% more tryptophan, 30% more lysine and 38% less leucine than that of normal maize (Bressani, 1990). Further, quality protein maize can be transformed into edible products without deterioration of its quality or acceptability, and can be used in conventional and new food products. In general maize flour has lower water absorption and lower extensograph parameters, viz., extensibility, resistance to extension and dough energy than wheat flour dough (Yaseen *et al*., 2010).

Soybean *(Glycine max)* has superior food value amongst all plant foods consumed in the world. It has excellent protein content (35-40%), is rich in calcium, iron, phosphorus and vitamins and contains all the essential amino acids (Ihekoronye and Ngoddy, 1985). Soy proteins improve nutritional quality, mechanical behaviour and shelf-life of bread (Moore *et al*., 2006). Several researchers have reported use of soyflour as functional ingredient in production of bread (Dhingra and Jood, 2002).

However, gluten free or low gluten doughs are

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difficult to handle (Schober *et al*., 2005). It is difficult to replace the visco-elastic characteristics and the functional quality of gluten. Without the typical gluten network of gluten bread, the gas holding capacity is low (Lai *et al.*, 1989). Response surface methodology (RSM) is a collection of statistical and mathematical techniques for developing, improving and optimizing product/processes (Myers and Montgomery, 2002). This technique has also been used in the past for optimizing bread quality (Collar *et al*., 2007).

Considering the need to develop healthier foods to meet the threat posed by chronic diseases, whole grains such as wheat, oats, maize and soybean were used for the study. Since there is lack of literature on preparation of functional bread from composite whole grain flours such as oats, soybean and quality protein maize, especially in developing countries such as India, the current study was proposed. The aim of this study was to optimize the level of whole grain flours of oats, quality protein maize and soybean to obtain functional bread of acceptable quality.

Materials and Methods

Selection of ingredients

Wheat kernels (PBW 621), oat grains (OL-9) and soybean (SL 525) grown in the year 2012-13 were procured from Punjab Agricultural University, Ludhiana, Punjab, India. Quality protein maize (QPM) was obtained from Directorate of Maize Research, Karnal, India. Grains were cleaned to remove dust, straw, stalks, stones etc. They were stored at 10±2°C in plastic bins till further use.

Preparation of whole flours

Oat grains were dehulled using Laboratory Impact Oat dehuller (Creative India, Mohali, Punjab). The mixture of groats and husk was separated in laboratory aspirator. Groats were separated from hulled grains by hand sorting and milled using hammer mill (Milcent Magnum, Anand, India) to 100 mesh size. Bran obtained was again fed to the mill until the entire branny mass was ground. The flour obtained was sealed in low density polyethylene bags and stored in refrigerated conditions (4±2°C). QPM and wheat kernels were milled to obtain whole flour and stored in refrigerated conditions.

Soybeans were soaked in tap water (25-30°C) for 4 h. Beans were wrapped in a white muslin cloth and steamed at 1.05 kgm^{-2} for 5 min in autoclave (Equitron, Medica Instrument Manufacturing Company, New Delhi). These were dried in forced air cabinet drier (Narang Scientific Works, New Delhi) at 50±5°C to 7±0.5% moisture (wb) and ground with their hulls in hammer mill to 100 mesh size and stored in low density polyethylene bags in refrigerated conditions (4±2°C).

Chemical analyses

Approved AACC (2000) methods were used to determine crude protein (46-0.01) and ash (08-01). Moisture, fat and crude fibre were determined using AOAC (1995) methods.

Experimental design

Response surface methodology was used to optimize the levels of whole oat, QPM and soyflours to form a composite blend with whole wheat flour. Upper and lower limits of these variables were established after preliminary trials. A central composite rotatable design (CCRD) (Table 1) was prepared to select the level of variables viz. whole oat flour (10-30 g/100g blend), QPM flour (10-20 g/100g blend) and soyflour (5-10 g/100 g blend). Experiments were conducted in randomized manner. For the analysis of experimental design by response surface methodology, it was assumed that n-mathematical functions, f_k (k=1, 2….. n), Y_k in terms of m independent processing factors X_i (i=1,2, ……………., m) existed for each response variable.

$$Y_k = f_k(X_1, X_2, ..., X_m) \quad (1)$$

In this case, n=4, m=3

Full second-order equation was fitted in each response to describe it mathematically and to study the effect of variables. The equation was as follows:

$$Y_K = \beta_0 + \sum_{i=1}^{m} \beta_i X_i + \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} \beta_j X_I X_j + \sum_{i=1}^{m} \beta_i X^2{}_i \quad (2)$$

where, Y_k = response variable, β_0 is the value of the fitted response at the centre point of the design i.e. (0,0) and β_i, β_{ij}, β_{ii} are the linear, quadratic and interactive regression coefficients, respectively. X_i and X_j are the coded independent variables. The magnitude of the coefficients in second order polynomials showed the effect of concerned variable on responses.

Baking

Whole oat, QPM and soybean flours were used in varying amounts as per the experimental design (Table 1). Straight dough method was used for preparation of breads. Weighed amounts of flours were blended with wheat flour using Laboratory mixer (Spar, Taiwan). For each 100 g blend, 3 g compressed yeast, 4 g fat, 2.5 g sugar, 1 g salt, 1 g gluten, ascorbic acid (100 ppm) and potassium bromate (100 ppm) were added. Water was added to optimum level to achieve dough that was easy to

Table 1. Central composite design arrangement with coded variables

Run	Coded value			Actual value (g/100g blend)		
	X_1	X_2	X_3	Oat flour	QPM flour	Soy flour
1	0	0	0	20.00	15.00	7.50
2	-1.68179	0	0	3.18	15.00	7.50
3	0	0	0	20.00	15.00	7.50
4	0	+1.68179	0	20.00	23.41	7.50
5	0	0	+1.68179	20.00	15.00	11.70
6	1	-1	1	30.00	10.00	10.00
7	0	0	0	20.00	15.00	7.50
8	0	0	0	20.00	15.00	7.50
9	-1	-1	1	10.00	10.00	10.00
10	-1	1	-1	10.00	20.00	5.00
11	0	0	-1.68179	20.00	15.00	3.30
12	-1	1	1	10.00	20.00	10.00
13	0	0	0	20.00	15.00	7.50
14	0	-1.68179	0	20.00	6.59	7.50
15	1	1	-1	30.00	20.00	5.00
16	-1	-1	-1	10.00	10.00	5.00
17	1.68179	0	0	36.82	15.00	7.50
18	1	-1	-1	30.00	10.00	5.00
19	0	0	0	20.00	15.00	7.50
20	1	1	1	30.00	20.00	10.00

handle. Mixing of the blend was done for 3 min in laboratory dough mixer (National Manufacturing Company, Colorado, USA). Dough formed was kept for fermentation at 35°C and 80% relative humidity in the proofing cabinet (Narang Scientific Works, New Delhi) for 55 min. Fermented dough was re-mixed and kept again for recovery in the proofing cabinet for 20 min. Sheeting and moulding of the dough was done on Laboratory sheeter (National Manufacturing Company, Colrado, USA) and moulding machine (National Manufacturing Company, Colrado, USA). Moulded dough was kept in labeled and greased pans for proofing for 45 min. The pans were carefully transferred to laboratory baking oven for baking at 230°C for 25 min. Bread loaves were cooled for 2 h and weighed before packing in low density polyethylene bags and sealed. They were stored in plastic containers at room temperature (15-25°C).

Analysis of bread quality

Breads were weighed (g) and their loaf volume (ml) was determined by rapeseed displacement method (Hallen *et al*., 2004). Specific volume (ml/g) was calculated by dividing volume by weight. The bread samples were, thereafter, sliced in the middle using a bread knife to obtain uniform slices of 1 cm thickness. Slice height (cm) was measured using vernier caliper (Mitutoyo Digimatic, Japan). Crumb firmness was evaluated by the Texture Analyzer (TA-XTi2 Stable Microsystems, Surrey, UK). A two cycle crumb compression test was performed using P/75 Aluminum platen probe (test speed 3 mm/s, penetration distance 15 mm). The peak force of compression was reported as firmness in accordance with the AACC method 74-09 (AACC, 2000). All measurements were repeated in triplicate for each bread type.

A semi-trained panel of 10 judges amongst faculty and students of Department of Food Science and Technology evaluated the bread samples in terms of overall acceptability using nine point hedonic scale (Larmond, 1977) from liked extremely (9) to disliked extremely (1). Four samples along with control were presented at a time. Marketed whole wheat bread was used as control in this case.

Total phenolic content and anti-oxidant activity of whole flours and bread

Total phenolic content (TPC) was determined by Folin-Ciocalteu method (Singleton and Rossi, 1965; Das *et al*., 2013). Absorbance was measured at 750 nm in a spectrophotometer (Spectronic 200, Thermo Fisher Scientific, India) using gallic acid as a standard. The results were expressed as mg of gallic acid equivalents (GAE) per g of fresh material.

The DPPH assay was based on the method of Michalska *et al*. (2007). The percentage of inhibition or the percentage of discolouration was calculated as follows:

$$\text{Inhibition\%}=A_{blank}-A_{sample}\times100/A_{blank} \quad (3)$$

where A is the absorbance at 517 nm.

Statistical analysis

Response surface methodology (RSM) was adopted in experimental design and analysis (Khuri and Cornell, 1987). Data were modeled by multiple regression analysis and statistical significance of the terms was examined by analysis of variance for each response. Maximization and minimization of the polynomials thus fitted was done using the numerical optimization technique given in the software package Design expert, version 9.0.2.0, 2014; Minneapolis, MN, USA).

Results and Discussions

Chemical composition

Whole wheat, oat, QPM and soy flours had crude protein contents of 11.279±0.039%, 16.065±0.043%, 9.996±0.306% and 35.636±0.341%, respectively, whereas crude fat was 1.644±0.187%, 6.177±0.022%, 3.057±0.216% and 19.632±0.551, respectively. Soy flour had significantly high crude protein and fat contents. Due to this nutritional dominance of soybean, it is the preferred choice for incorporation into formulated foods. Among the cereals, oats had significantly high crude protein and fat contents. Total ash contents of the flours were 1.526±0.081%, 1.334±0.010%, 1.211±0.196% and 4.427±0.412%, and crude fibre was 1.798±0.23%, 3.548±0.129%, 2.382±0.382% and 5.227±0.204% respectively. Whole grains of wheat, oat maize and soybean contain high content of crude fibre. High fibre content in diet reduces risk of colorectal cancer and cardiovascular disease (Bazzano *et al*., 2003).

Diagnostic checking of the fitted models

The estimated regression coefficients of the fitted quadratic equation as well as the correlation coefficients for each model are given in Table 2. Although according to Henika, the models were considered adequate when the coefficient of correlation (R^2) was more than 80% and the lack of fit test was insignificant (Henika, 1982), Granato *et al.* (2010a, 2010b) established that a $R^2 > 70\%$ was considered good for sensory, colorimetric and physicochemical results. For the models that present a regression coefficient below 70%, it must be considered that there is a failure of the models to represent the data in the experimental domain (Myers and Montgomery, 2002). Moreover, lack of fit was found not significant for all the parameters. The proposed models approximate the response surfaces and can be used suitably for prediction at any values of the parameters within experimental range. The R^2 values for the responses i.e. specific volume, loaf height, crumb firmness and overall acceptability were 93.00%, 76.27%, 85.93% and 95.53%, respectively (Table 2). The calculated F-values (Table 3) were more than the table values (3.02). Based upon these results, all four responses were considered adequate to describe the effect of variables on the quality of bread samples.

Effect of variables on specific volume

Specific volume of the composite whole flour blends varied between 2.205 and 2.703, ml/g, within the combination of variables studied. Negative coefficients of linear terms of oat and QPM flours had highly significant ($p \leq 0.01$) effect on specific volume of bread (Table 2). Krishnan *et al*. (1987) has reported that with increase in addition of oat bran in bread, there was a decrease in loaf volume. This inferior baking quality is principally attributed to lack of gluten proteins and the high content of β-glucan and other dietary fibres. Interaction of oat and maize flours had highly significant negative effect while interaction of QPM and soy flour had highly significant ($p \leq 0.01$) positive effect on bread specific volume. At the quadratic level, negative coefficient of oat flour had highly significant effect ($p \leq 0.01$) on specific volume. The three dimensional response plot (Figure 1a) further depict the effect of the independent variables on the specific volume of the bread. Addition of dry gluten and higher amounts of water has been used to strengthen the protein matrix and enhance specific volume (Flander *et al.,* 2007). However, soy flour had positive influence on specific volume. Kaur *et al*. (1995) attributed increase in specific volume of bread from Punjab wheat varieties with addition of soy flour to increase in water absorption and improved functional properties.

Effect of variables on loaf height

Oat flour had a profound effect on loaf height. The negative linear coefficient of oat flour had highly significant ($p \leq 0.01$) effect on loaf height (Table 2). None of the other variables affected loaf height significantly. Rudel (1990) reported that soluble oat dietary fibre probably inhibited gluten strength leading to unsatisfactory loaves. High amounts of proteins in whole oat flour are also known to interfere with starch by disrupting the uniformity of the starch gel during baking (Flander *et al*., 2007).

Table 2. Estimated coefficients of the fitted quadratic equation for different responses

Factors	Specific volume	Loaf height	Crumb firmness	Overall acceptability
β_0	+2.58	+4.79	+27.03	+8.03
β_1	-0.11**	-0.74**	-1.59*	-0.47**
β_2	-0.067**	+3.633E-003	+3.59**	-0.19**
β_3	-0.047*	+0.084	-0.63	-0.050
β_{12}	-0.089**	+0.032	-0.46	-0.11
β_{13}	+7.384E-003	+0.14	+0.82	+0.015
β_{23}	+0.072**	+0.11	+1.32	+2.500E-003
β_{11}	-0.053**	+0.061	-1.08	-0.24**
β_{22}	-0.037*	-0.098	-0.13	-0.11*
β_{33}	+0.021	-0.17	-0.80	-0.13**
R^2, %	93.00	76.27	85.93	95.53

**Significant at $p \leq 0.01$, *Significant at $p \leq 0.05$

However QPM and soyflours had positive effect on loaf height as evident from positive linear and interactive coefficients and compensated for the negative influence of oat on height. This is also seen in Figure 1(b).

Effect of variables on crumb firmness

Crumb firmness is a textural attribute and represents the resistance of the bread crumb to deformation. It is an important attribute that is correlated with consumers' perception of bread freshness (Ahlborn *et al*., 2005). The observed firmness with different combinations of the ingredients varied from 19.213 N to 33.922 N within the combination of variables studied. Regression coefficients showed that firmness was positively affected ($p \leq 0.01$) by QPM flour at linear level (Table 2). This may be partly due to lack of gluten that affects the overall texture and specifically bread crumb softness. However, linear coefficient of oat flour also affected crumb firmness negatively. This result may be due to the water binding capacity of fibre-rich oat flour. This is probably due to the hydrogen bonding between fibre and starch that also delays starch retrogradation (Sabanis *et al*., 2009). Response surface plots of crumb hardness are shown in Figure 1(c).

Effect of variables on overall acceptability

Overall acceptability represented the sensory quality of bread. Both oat and QPM flours had a significantly negative effect on overall acceptability of bread. This is also evident from the highly significant ($p \leq 0.01$) negative linear coefficients of oat and QPM flours (Table 2 and Figure 1d). In addition, the negative quadratic coefficients of oat and soy flour affected the overall acceptability of bread. The low specific volume and gummy mouth feel provided by oat flour at higher levels were unacceptable to the panelists. Smaller pore size and thicker pore walls

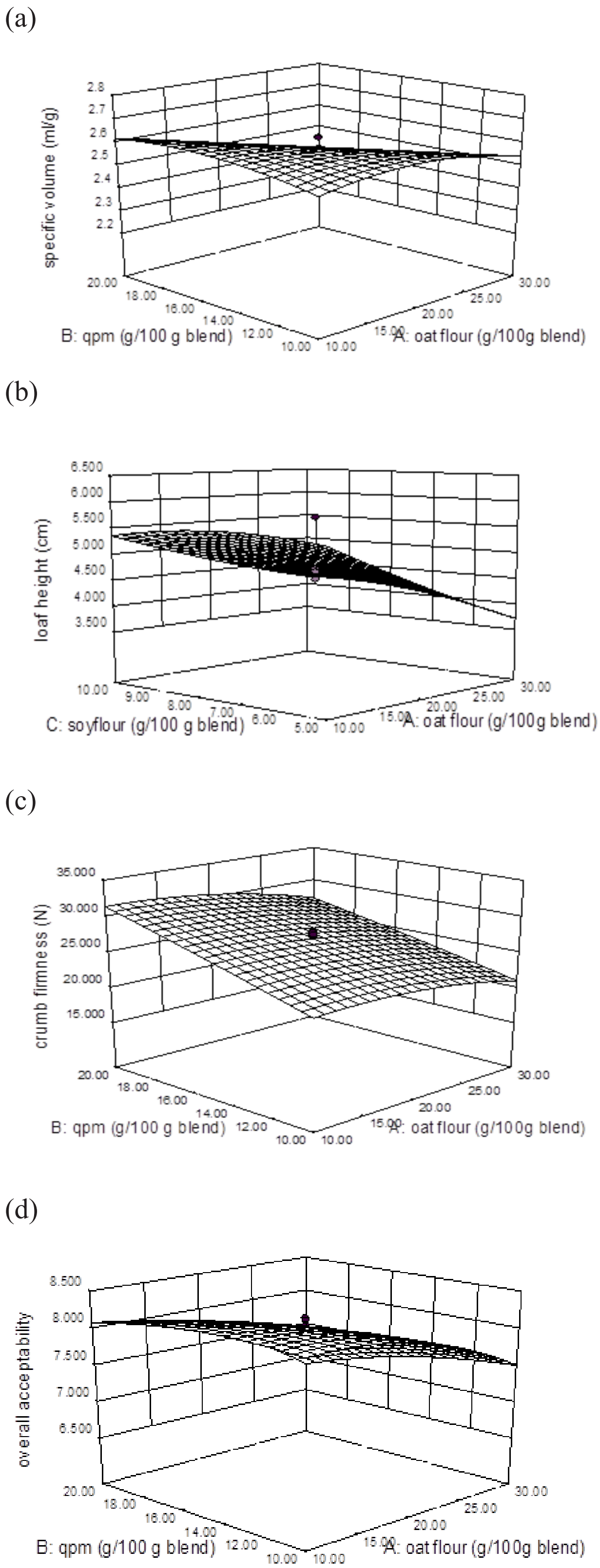

Figure 1(a-d). Response surface plots showing showing the effect of oat flour, QPM flour and soybean flour on various quality parameters of bread

Table 3. Analysis of variance for different models

Response	Source of variation	d.f.	Sum of squares	Mean square	F-value
Specific volume	Model	9	0.42	0.046	14.75**
	Residual	10	0.032	3.151E-003	
	Cor. Total	19	0.45		
	Lack of fit	*5*	*0.025*	*4.982E-003*	*3.78*
Loaf height	Model	9	8.40	0.93	3.57*
	Residual	10	2.61	0.26	
	Cor. Total	19	11.02		
	Lack of fit	*5*	*1.58*	*0.32*	*1.54*
Crumb firmness	Model	9	260.50	28.94	6.78**
	Residual	10	42.66	4.27	
	Cor. Total	19	303.17		
	Lack of fit	5	34.99	7.00	4.56
OA[a]	Model	9	4.78	0.53	23.75**
	Residual	10	0.22	0.022	
	Cor. Total	19	5.01		
	Lack of fit	*5*	*0.19*	*0.037*	*4.90*

[a]Overall acceptability, **Significant at $p \leq 0.01$, *Significant at $p \leq 0.05$.

due to poor gas retention properties of oat dough especially at oat levels greater than 20% may have lead to unacceptable breads. Moreover, maize left a dry mouth feel that was disliked by some of the panel judges. Excessive levels of soy flour were also not acceptable to some panelists.

Analysis of variance

Since F-value for all the responses indicated that all the three variables affected the responses significantly ($p \leq 0.05$) (Table 3), it can be concluded that the selected models adequately represented the data for bread making performance of composite flour blends.

Optimization of the level of independent variables

All the four responses i.e. specific volume, loaf height, crumb firmness and overall acceptability were taken into consideration for optimization of level of variables. These responses had critical role in judging the quality and acceptability of composite whole grain bread as determined by their respective R^2 values. Numerical optimization was carried out for the level of variables to obtain the best product. The desired goals for each factor and response were chosen and different weights were assigned to each goal to adjust the shape of its particular desirability function. Among the solutions obtained, the solution with maximum desirability was selected as optimum ingredients composition. Numerical optimization was done and optimized values were 10.00 g oat flour, 10.00 g QPM flour and 5.375 g soybean flour with 81.5% desirability. These were blend with whole wheat flour to make up the total blend to 100 g. Bread was prepared using the recommended level

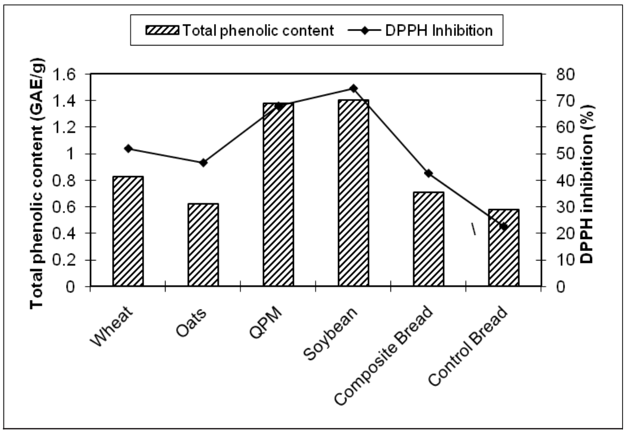

Figure 2. Total phenols and antioxidant activities of individual flours and optimized composite and control bread

of whole flours and the responses were measured. The developed composite bread was also analyzed for crude protein, fat, ash and crude fibre contents and the corresponding values were 12.910±0.059, 2.773±0.078, 1.870±0.107 and 2.575±0.143, g/100 g bread, respectively on dry weight basis. These values were higher than those obtained for control whole wheat bread i.e. 12.54±0.45, 2.241±0.272, 1.436±0.009 and 1.891±0.013, g/100 g, respectively. According to the set conditions, the predicted value of responses was specific volume 2.698 g/ml, loaf height 5.536 cm, crumb firmness 25.136 N, overall acceptability score of 8.20. The corresponding measured or actual values for the optimized composite bread were 2.687±0.125 g/ml, 5.541±0.107 cm, 24.982±0.173 N and 8.23±0.142. Thus, the measured responses had proximity to the predicted ones. The adequacy of the models was thus re-confirmed. Control whole wheat bread had specific volume 2.892±0.048 g/ml, loaf height 6.7±0.1 cm, crumb firmness 19.18±0.147 N and overall acceptability score of 8.2±0.15. Thus, the optimized bread had lower specific volume and loaf height, higher value for crumb firmness and similar overall acceptability as compared to control bread.

Total phenolic and antioxidant activity of whole grain flours and bread

The total phenolic content and antioxidant activities of whole grain flour and the optimized composite bread were analyzed and are presented in Figure 2. The bran and germ in whole grains are rich in antioxidants such as phenolic acids (Miller *et al*., 2000). Soybean had significantly ($p \leq 0.05$) higher total phenolic content and antioxidant activities as compared to wheat, QPM, oat flours and bread samples. Antioxidant activity has been correlated with total phenolic content (Kumar *et al*. 2010). Principal components that show this activity in soybean include dietary flavonoids and isoflavones (Heim *et al*., 2002). Total phenolic content of the composite bread was 0.307 GAE/g (gallic acid equivalents per gram) which was 21.89% higher than control bread. Anti-oxidant activity (in terms of inhibition of DPPH) of the optimized composite bread was significantly higher as compared to control (whole wheat) bread.

Conclusion

Bread making performance of whole grain composite blend comprising whole oat, quality protein maize and soybean flour along with whole wheat flour was analyzed. Optimum level of the flours selected was 10.00 g oat flour, 10.00 g QPM flour, 5.375 g soybean flour per 100 g flour with whole wheat flour with 81.5% desirability. This optimized blend can be used to prepare bread with potential functional benefits due to high protein, crude fibre and phenolic constituents. This utilization of whole grains, viz., oats, quality protein maize and soybean will further help in their value addition. Further scope exists for utilization of additives such as hydrocolloids to further improve acceptability of the whole grain bread.

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