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RESEARCH ARTICLE

FORMULATION AND OPTIMIZATION OF FUNCTIONAL BREAD BY EMPLOYING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Bread is considered to be one of the oldest 'processed' foods by the humanity. It is one of the major products of baked foods and is consumed worldwide. Sprouting of pumpkin seed help to increase the nutrient content of the seed. Sprouted grains are seen in a number of baked goods at natural health food stores. The objective of this study was to optimize the refined wheat flour, germinated pumpkin seed flour and butter with respect to bread-making quality. The optimal Design was employed with the following variables of protein, weight loss, porosity and specific volume of the bread. Models developed adequately described the relationships and were confirmed by validation studies. Refined wheat flour showed the greatest effect on models, which effect impaired weight loss and specific volume of germinated pumpkin seed bread. The optimum set of the independent variables was obtained graphically in order to obtain the desired levels of 90g for refined wheat flour, 10g for germinated pumpkin seed flour and 8g for butter. Organoleptic evaluation showed that variation7 with 90g of refined wheat flour; 10g germinated pumpkin seed and 8 g of butter had scored high score for overall acceptability among the variations in the germinated pumpkin seed bread.

INTRODUCTION

Bread is an important ready-to-eat product which is becoming increasingly popular in hotels, restaurants, canteens and in households. The bread is a fast and convenient food based on wheat. Bread is one of the major products of baked foods and is consumed worldwide (Bakke and Vickers, 2007). The bread is an ideal product that can serve as a functional food, since it is daily used by a large population throughout the world. (Martin, 2004; Sluimer, 2005). Bread products are well accepted worldwide because of the low cost, ease of preparation, versatility, sensory attributes and nutritional properties. Bread in human nutrition is not only a source of energy, but also a supplier of irreplaceable nutrients for the human body. It provides little fat, but high quantities of starch and dietary fiber as well as cereal protein. Apart from that, bread contains the B group vitamins and minerals which are mostly magnesium, calcium and iron. The sensory scores for appearance, texture and flavour of breads have been reported to decrease by the incorporation of non wheat flours in wheat flours (Gülçin, 2006).

Incorporation of pumpkin seed powder in bread increase the protein content of the product. The pumpkin is an angiosperm belonging to the *cucurbitaceae* family that is characterized by prostrate or climbing herbaceous vines with tendrils and large, fleshy fruits containing numerous seeds (Acquaah, 2004). Pumpkin of the genus *C. maxima* is also called squash guard. Pumpkin of the genus *C. maxima* is also called squash guard.

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The pumpkin seeds contained 39.25% crude protein, 27.83% crude oil, 4.59% ash, and 16.84% crude fiber; The corresponding values for the kernels were 39.22, 43.69, 5.14, and 2.13%, respectively. The saturated fatty acid content was 27.73% and comprises of 16.41% palmitic acid and 11.14% stearic acid. The unsaturated fatty acid value was 73.03% and consisting mainly of 18.14% oleic acid and 52.69% linoleic acid. The oil obtained from the pumpkin seed kernels had a refractive index of 1.4656, specific gravity of 0.913, iodine value of 105.12 (g I2/100g oil), saponification value of 185.20 (mg KOH/g oil), acid value of 0.53 (mg KOH/g oil), and peroxide value of 0.85 (meq peroxide/kg oil) (Mohammed A. Alfawaz.2004). Considering the lipid and protein content in the kernels, and their fatty and amino acid compositions, the pumpkin seed kernels appear to be quite promising for commercial exploitation. Sprouted grains are seen in a number of baked goods at natural health food stores.

Sprouting is the practice of soaking, draining and leaving seeds or grains until they germinate or begin to sprout. The concept behind the use of such grains and legumes is that the enzymes produced during sprouting convert starch into more digestible maltose and the vitamins and mineral content available for digestion increases. In effect the sprouting process 'predigests' grains (Misty, 2004). It is known that germination induces increase in free limiting amino acid and available vitamins with modified functional properties of seed components (Hallén *et al.,* 2004). Germination also decreases anti-nutritional factors such as trypsin-inhibitor (Uwaegbute *et al.,* 2000).

Sprouting of pumpkin seed help to increase the nutrient content of the bread. Sprouted grains are seen in a number of baked goods at natural health food stores. Spouting is the practice of soaking, draining, and leavening seeds or grains until they germinate or being to sprout. The concept behind the use of such grains is that the enzymes produced during the sprouting convert starch into more digestible maltose and the vitamins and minerals available for digestion increases. Germination induces increasing in free limiting amino acid and available vitamins with modified functional properties of seed components (Hallen *et al.,* 2004). The nutrients released in the spouted pumpkin seeds will play an important role in baked products. Therefore, the objective of this study was to determine the optimized germinated pumpkin seed powder bread, in order to maximize the protein and good baking properties by using RSM.

MATERIALS AND METHODS

The ingredient like refined wheat flour, yeast, butter and sugar were obtained from the local market, Salem, Tamil Nadu, India. The pumpkin seed was collected from matured pumpkin fruit.

Production of Spouted pumpkin seed flour

Pumpkin seeds were soaked in tap water at 1:3 seeds water ratio for overnight in distilled water at room temperature. The seeds were placed on muslin cloth and continuous watering was done for 48 h for the seed to germinate. The sprouted seeds were then dried at 60°C; after hull separation, the material was milled to obtain the germinated seed flour.

Experimental design

Response surface methodology was applied to the experimental data using a commercial statistical package (Design expert, Trail version 7.0, State Ease Inc., Minneapolis, IN statistical software) for the generation of response surface plot and optimization of process variables. The experiments were conducted according to Central Composite Rotatable Design (CCRD) (Khuri, AI and Cornell. JA, 2007. A central composite rotatable design (CCRD) with augmented points in three variables refined wheat flour(X_1), pumpkin seed powder (X_2) and butter(X_3). Each design point consists of the replicates. For the statistical analysis the numerical levels are standardized to-1.682, -1, 0, +1 and +1.682. The experiments were carried out in randomized order (Gacula and Singh, 1984).

$$Y = \alpha_0 + \sum_{i=1}^{n=3} \alpha_i X_i + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \alpha_i X_i X_j + \sum_{i=1}^{n} \alpha_{ii} X_i^2$$

Where, Y is response variable,

α_0 - Constant and coefficient,αi −Linear coefficient ,αii – quadratic coefficient,αij – cross product coefficient ,Xi, Xj – levels of the independent variables, k – Number of the factors tested (k = 3), Xi and Xj are coded independent variables, i.e,

The second order polynomial equation was fitted to the experimental data of each dependent variable as given. The model proposed to each response of Y, (Quality Measurements of bread) weight loss (Y1) porosity (Y2) and specific volume (cm3/g) (Y3).

The analysis of variance (ANOVA) table is generated and the regression coefficients of the individual linear, quadratic, and interaction terms are determined. The significances of all terms in polynomial are judged statistically by computing the F-value at a probability level (P) of 0.01 or 0.05. The model is then submitted to statistical analysis to neglect all terms that are statistically insignificant (P > 0.05). Regression coefficients are used to generate a contour map for the regression model. The model permitted evaluation of quadratic terms of the independent variables on the dependent variable. The response surface and contour plot were generated for different interactions of any two independent variables, where holding the value of third variables as constant at central level. The optimization of the process was aimed at finding the optimum values of independent variables (Agrawal S.R. 2000).

Preparation of Bread Samples

All the dry ingredients (flour, sugar, salt, and butter) were mixed for 1 min by a mixer at 58 rev/min. Then, yeast dissolved in 30ºC water, which is the optimum temperature for the yeast cells to be activated, and melted margarine was added to the dry ingredients. All the ingredients were again mixed for 2.5 min by the help of the same mixer at 85 rev/min and during mixing, water was added to the mixture. After mixing, the dough was fermented in an incubator at 30ºC with 85% relative humidity.

The total fermentation time was 105 min. After the first 70 min, the dough was punched to remove the carbon dioxide and again placed into the incubator. The second punch took place after 35 min. Then, the dough was divided into 50 g pieces and shaped. The shaped samples were placed in greased glass baking pans and again placed into the incubator for 20 min in order to maintain the proofing step, which is defined as the last fermentation. Then, the samples were ready for baking.

Quality Measurements of bread

After baking the breads, in order to determine the optimum baking point, the quality measurements were performed. The quality parameters were the weight loss, specific volume, and porosity of the breads.

Weight Loss

The weight loss of the breads was calculated by measuring the weight of the dough before and bread after the baking process. The following equation was used to express the weight loss:

$$\text{Weight loss (\%)} = \frac{Wi - Wf \times 100}{Wi}$$

Where, *Wi:* weight of the dough before baking, *Wf:* weight of the bread just after baking.

Specific Volume (*Measurement Loaf bread volume*)

The loaf volume expressed in cubic centimeters was determined by the seed displacement method according to Pyler (1973). The loaf was placed in a container of known volume into which millet seeds were run until the container is full. The volume of a seed displaced by the loaf was considered as the loaf volume Loaf bread specific volume: The specific volume of the loaf was calculated according to the AACC (1991) by dividing volume of the loaf (cm3.) by its weight (g). Triplicate measurements were taken.

W seeds = W total – W bread – W container, V seeds = W seeds / ρ seeds

V bread = V container – V seeds

Where, W represents 'weight (g)', V is 'volume (cm3), and ρ is density (g/cm3)'.

The specific volume was calculated by dividing the volume of the bread by its weight;

SV bread = V bread / W bread

Porosity

Porosity was measured by using the method of (Sluimer, 2005). Porosity can be defined as the ratio of the volume of the pores to the total volume of the product:

$\varepsilon = (VT - Vnp)/ Vt$

Where, Vt = total volume of the sample,
Vnp = volume of the non-porous material in the sample.

An apparatus having a constant basement area was designed, which allowed pores to be removed from the bread samples, to measure porosity.

Optimization and verification

Optimum levels of wheat flour, germinated pumpkin seed flour and butter were determined by superimposing the plots for all response variables (Floros and Chinnan, 2008; Henika, 2002; Henika 1972). The optimum levels of ingredients were selected and used for calculating the predicted properties of germinated pumpkin seed bread using the prediction equations derived by RSM. The germinated pumpkin seed bread obtained was experimentally analyzed and the results statistically compared to those predicted by the mathematical model.

Sensory Evaluation

Bread samples were evaluated on a scale of 1 - 5 for five quality parameters: crust colour, crumb colour, external appearance and shape, taste and aroma, and mouth feel and texture. A ballot sheet was prepared to evaluate sensory attributes of breads after modifying parameters and scores of various flat breads to Lavash (Qarooni *et al.,* 1987; Williams, 1988). Consistency of the panel was checked by subjecting data for the indicated attributes from three replicate rating of bread samples to principal component analysis (Khan MN *et al.,* 2005).

Statistical Analysis

Experimental data was analyzed by multiple regressions to fit the quadratic equation to all independent variables. Analysis of variance (ANOVA) was performed to evaluate significant differences between independent variables. To visualize the relationships between the responses and the independent variables, surface response and contour plots of the fitted quadratic regression equations were generated using Design-Expert software version 8.0.

RESULTS AND DISCUSSION

Results of bread quality characteristics used in the optimization procedure are as shown in Table 2 corresponding to the different runs. Bread sample produced with different proportion of refined wheat flour, germinated pumpkin seed flour and butter shows differences exist among the samples in Weight Loss, porosity and specific volume. Weight loss come the range from 10.00 to 11.81 (%), porosity 40.5 to 46 and Specific Volume 1.88 to 2.63(cm3/g). 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 R2 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).

Effect of variables on Weight loss

The statistical significance of the model was also determined by F-test for analysis of variance (ANOVA) .The Model F-value of 20.42 for weight loss implies the model is significant. For weight loss the "Lack of Fit F-value" of 17.53 implies the Lack of Fit is significant. There is only a 0.35% chance that a "Lack of Fit F-value" this large could occur due to noise. Significant lack of fit is bad. Ratio greater than 4 is desirable for Adeq Precision. The ratio of 15.979 indicates an adequate signal. The "Pred R-Squared" of 0.5960 is not as close to the "Adj R-Squared" of 0.9020 as one might normally expect. This model can be used to navigate the design space

Effect of variables on Porosity

There had not significant variation due to refined wheat flour and germinated pumpkin seed flour and butter in the bread. But maximum porosity showed in maximum amount of the three ingredients (Table 3). The Model F-value of 3.14 for porosity implies the model is significant.There is only a 4.45% chance that a "Model F-Value" this large could occur due to noise.The "Lack of Fit F-value" of 15.33 implies the Lack of Fit is significant. A negative "Pred R-Squared" implies that the overall mean is a better predictor of the response than the current model."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. for porosity the ratio of 6.116 indicates an adequate signal. This model can be used to navigate thedesignspace.

Effect of variables on Specific volume

Refined wheat flour was found to be the most significant variables (p =0.000) on affecting the specific volume of

Table 1. The range and the levels of the experimental variables used in the coded and uncoded form for the centre, factorial and augmented point of design

Experimental Variables		Code				
		-1.682	-1	0	+1	+1.682
				Uncoded		
Refined wheat flour	X_1	86.5	90	95	100	103.4
Germinated pumpkin seed powder	X_2	3.30	5	7.5	10	11.3
Butter	X_3	5.32	6	7	8	8.6

Variation no.	Coded			Uncoded		
	x1	x2	x3	Refined wheat flour (X_1) (g)	Germinated Pumpkin seed flour (X_2) (g)	Butter (X_3)(g)
1	-1	-1	-1	90	5	6
2	+1	-1	-1	100	5	6
3	-1	+1	-1	90	10	6
4	+1	+1	-1	100	10	6
5	-1	-1	+1	90	5	8
6	+1	-1	+1	100	5	8
7	-1	+1	+1	90	10	8
8	+1	+1	+1	100	10	8
9	-1.682	0	0	86.59	7.5	7
10	+1.682	0	0	103.41	7.5	7
11	0	-1.682	0	95	3.30	7
12	0	+1.682	0	95	11.70	7
13	0	0	-1.682	95	7.5	5.32
14	0	0	+1.682	95	7.5	8.68
15	0	0	0	95	7.5	7
16	0	0	0	95	7.5	7
17	0	0	0	95	7.5	7
18	0	0	0	95	7.5	7
19	0	0	0	95	7.5	7
20	0	0	0	95	7.5	7

x1,x2,x3- coded form of Refined wheat flour, Germinated Pumpkin seed flour and butter

Table 2. Results of responses obtained from the quality properties of germinated pumpkin seed bread

S.No	X_1	X_2	X_3	Weight loss (%)	Porosity	Sp.Volume (cm3/g)
1	90.00	5.00	6.00	10.2	42.6	2
2	100.00	5.00	6.00	11.21	40.7	2.01
3	90.00	10.00	6.00	10.7	43.0	2.9
4	100.00	10.00	6.00	11.71	43.4	2.03
5	90.00	5.00	8.00	10.4	41.8	1.91
6	100.00	5.00	8.00	11.41	42	2.07
7	90.00	10.00	8.00	10	41	3
8	100.00	10.00	8.00	11.91	46	2.1
9	86.59	7.50	7.00	10.2	40.5	2.02
10	103.41	7.50	7.00	11.81	43	1.98
11	95.00	3.30	7.00	10.64	40.9	2.04
12	95.00	11.70	7.00	11.43	42.2	1.99
13	95.00	7.50	5.32	10.98	41.2	2.00
14	95.00	7.50	8.68	11.22	42.4	1.88
15	95.00	7.50	7.00	10.9	42	2.03
16	95.00	7.50	7.00	10.8	43.5	2.04
17	95.00	7.50	7.00	10.9	43.8	2.04
18	95.00	7.50	7.00	10.8	43.4	2.08
19	95.00	7.50	7.00	10.9	43	2.04
20	95.00	7.50	7.00	10.8	43	2.02

Refined wheat flour (X1), Germinated Pumpkin seed flour (X2), Butter (X3)

Table 3. Analysis of Variance (ANOVA) of Second Order Polynomial Model quality properties of germinated pumpkin seed bread

Source	Weight loss			Porosity			Specific volume		
	Sum of Square	F value	P- value	Sum of Square	F value	P- value	Sum of Square	F value	P- value
Model	15.11	20.42*	<0.001**	*10.22*	1.19	*0.3940*	*1.02*	1.86^{NS}	0.1742
X_1	4.28	154.11	<0.001**	*6.471*	0.31	*0.5881*	*0.20*	*3.34*	*0.0974*
X_2	0.431	15.54	0.0028**	*0.025*	0.025	*0.2959*	*0.281*	*4.60*	*0.0575*
X_3	6.751	0.24	0.6327	*0.024*	0.0241	*0.3100*	*2.798*	*4.598*	*0.9473*
X_1^2	0.013	0.48	0.5059	*5.729*	0.028	*0.8711*	*0.026*	*0.43*	*0.5284*
X_2^2	0.24	0.87	0.3735	*1.942*	0.094	*0.7655*	*0.033*	*0.54*	*0.4794*
X_3^2	0.059	2.12	0,1764	*3.203*	0.15	*0.7021*	*6.495*	*0.11*	*0.4794*
X_1X_2	0.10	3.64	0.0854	*0.041*	1.97	*0.1912*	*0.47*	*7.73*	*0.0194**
X_1X_3	0.10	3.64	0.0854	*0.034*	1.15	*0.2383*	*1.80*	*0.030*	*0.8669*
X_2X_3	0.10	3.64	0.0854	*0.086*	4.17	*0.0685*	*5.000E*	*0.082*	*0.7802*
Lack of fit		17.53*			98.20*			291.09 *	
Adj R^2		0.9020			0.0814			0.2888	
Pre R^2		0.5960			-2.9408			-1.8323	
Adeq precision		15.979			4.690			4.709	

Refined wheat flour (X_1), Germinated Pumpkin seed flour (X_2), Butter (X_3)

Table 4. Estimated regression coefficients of predicted quadratic model

Predictors	Regression Coefficient		
	Weight loss (%)	porosity	Sp.Volume (cm^3/g)
Constant	10.85**	43.29*	2.03
Linear			
x_1	0.56**	-0.99**	-0.12
x_2	0.18**	0.27	0.14
x_3	0.022	0.024	-4.526
Quadratic			
x_1^2	0.030	0.40	0.042
x_2^2	0.041	0.49	0.048
x_3^2	0.064	0.31	0.021
Interaction			
x_1x_2	0.11	0.53	-0.24*
x_1x_3	0.11	0.38	0.015
x_2x_3	0.11	0.20	0.025
R^2 (%)	94.84	73.87	62.57

x1, x2, x3 are the coded form of Refined wheat flour (x_1), Germinated Pumpkin seed flour (x_2), Butter (x_3)

Table 5. Criteria of optimum value for the responses

Optimum values of parameters and responses	Target	Experimental range		Optimum value Uncoded	coded
Refined wheat flour (X1)	is in range	86.5	103.4	90	-1
Germinated Pumpkin Seed (X2)	is in range	3.30	11.70	10	+1
Butter (X3)	is in range	5.32	8.68	8	+1
Weight Loss (%) (Y1)	minimum	10.00	11.91	Predicted value	
				10.284	
Porosity (Y2)	maximum	40.5	43.8	44.484	
specific volume (cm3/g) (Y3)	maximum	1.88	3.00	2.657	

Table 6. Organoleptic evaluation of germinated pumpkin seed bread

Variations	Crust Colour	Crumb Colour	External Appearance and Shape	Taste and Aroma	Mouth feel and Texture
V1	3.20±.421[a]	3.60±.516[b]	3.50±.527[bc]	3.00±.000[a]	3.70±.483[cd]
V2	4.50±.527[d]	4.20±.421[c]	5.00±.000[f]	4.30±.483[d]	4.70±.483[fg]
V3	3.00±.000[a]	3.00±.000[a]	3.00±.000[a]	3.90±.316[c]	3.40±.516[b]
V4	4.80±.42[e]	4.00±.000[c]	4.00±.000[e]	4.00±.000[c]	5.00±.000[g]
V5	5.00±.000[e]	4.00±.000[c]	4.00±.000[e]	4.00±.000[c]	5.00±.000[g]
V6	3.20±.000[a]	3.00±.000[a]	4.00±.000[e]	4.00±.000[c]	3.00±.000[a]
V7	4.90±.316[e]	5.00±.000[d]	4.00±.000[e]	4.80±421[e]	4.90±.316[fg]
V8	3.00±.000[a]	3.30±.483[ab]	3.90±.316[de]	3.30±.483[ab]	3.00±.000[a]
V9	3.00±.000[a]	3.20±.42[a]	4.00±.000[e]	3.20±.421[a]	3.00±.000[a]
V10	4.00±.000[c]	3.20±.42[a]	3.00±.000[a]	3.00±.000[a]	3.00±.000[a]
V11	3.00±.000[a]	3.00±.000[a]	3.00±.000[a]	3.00[a]±.000[a]	4.00±.000[e]
V12	3.00±.000[a]	3.00±.000[a]	3.00±.000[a]	3.00±.000[a]	4.00±.000[c]
V13	4.00±.000[c]	3.00±.000[a]	3.00±.000[a]	3.00±.000[a]	3.00±.000[a]
V14	4.00±.000[c]	3.90±.316[c]	3.00±.000[a]	3.90±.000[dc]	3.50±.527[bc]
V15	4.00±.000[c]	3.00±.000[a]	3.70±.483[cd]	3.00±.000[a]	3.90±.316[cd]
V16	3.00±.000[a]	3.20±.421[a]	4.00±.000[e]	3.00±.000[a]	4.00±.000[e]
V17	3.00±.000[a]	3.20±.421[a]	3.00±.000[a]	3.40±.516[b]	3.70±.483[c]
V18	3.50±.527[b]	3.30±.483[ab]	3.30±.483[b]	3.10±.316[a]	4.00±.000[e]
V19	3.80±.421[c]	3.20±.421 [de]	3.80±.421 [de]	3.30±.483[ab]	3.10±.316[a]
V20	3.50±.527[b]	3.30±.483 [ab]	3.30±.483 [b]	3.10±.316[a]	4.00±.000[e]

Values with different superscripts in a column are significant different ($p<0.05$) from each other on application of Duncan's multiple comparison tests

germinated pumpkin seed bread (table 3). Refined wheat flour increased specific volume increased. The "Model F-value" of 1.19 for specific volume implies the model is not significant. The "Lack of Fit F-value" of 98.20 implies the Lack of Fit is significant . for Adeq Precision ,the ratio of 4.690 for sp.volume indicates an adequate signal. This model can be used to navigate the design space.

Adequacy of the model

The analysis of variance of the effect of germinated pumpkin seed bread as a linear term, quadratic term and interaction on the response variables is shown in Table 4. The results indicated that the model is highly adequate because responses have satisfactory levels of R2 model significance. The weight loss and specific volume, 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 94.84 of R^2 for weight loss and 73.87% for specific volume. Considering the high value of R^2, the model for weight loss and specific volume can be accepted. The R^2 of porosity was less (51.65) compared to other dependent variables.

Predicative models

Weight loss (%) (Y1) $=+32.97-0.343X_1-0.56X_2-2.67X_3+1.21X_1^2+6.54X_2^2+0.063X_3^2+9.00X_1X_2+0.022X_1X_3-0.045X_2X_3$. (1)

In Equation 1 are represented the uncoded value, for the predicative model for weight loss. Two ingredients (refined

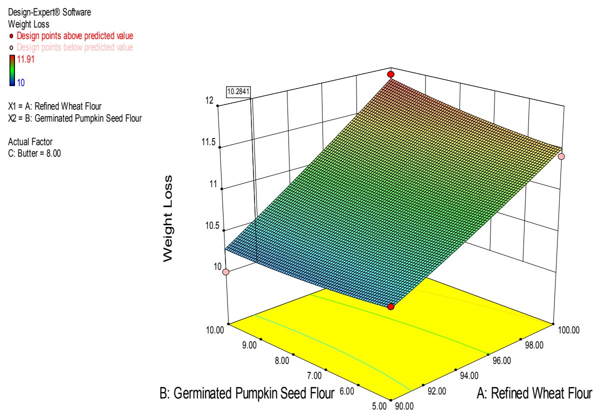

Fig. 1. Response surface plot: effect of refined wheat flour and germinated pumpkin seed powder on weight loss of autoclave pumpkin seed bread

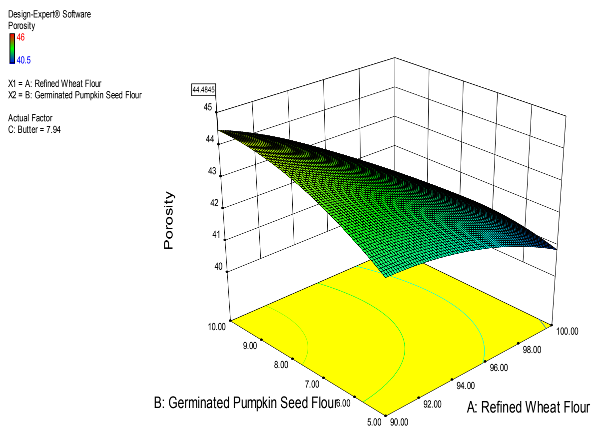

Fig. 2. Response surface plot: Effect of refined wheat flour and Germinated pumpkin seed powder on porosity of germinated pumpkin seed bread

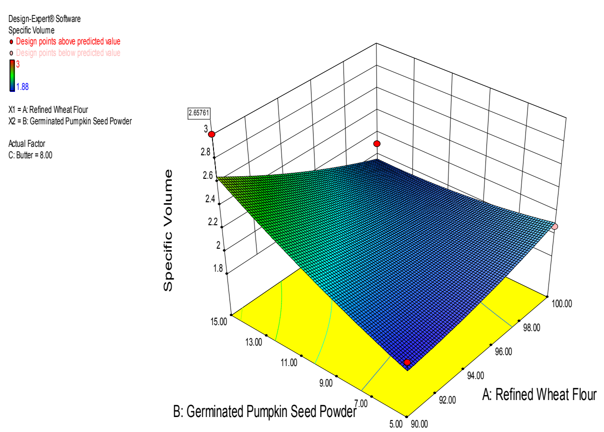

Fig. 3. Response surface plot: Effect of refined wheat flour and germinated pumpkin seed powder on specific volume of germinated pumpkin seed bread

wheat flour and germinated pumpkin seed flour) showed significant negative linear effect on weight loss. No ingredients showed a quadratic effect, but which was positive. Interactive effects between refined wheat flour and germinated pumpkin seed and, flour refined wheat flour and butter were not significant and had a positive effect and on germinated pumpkin seed and butter had a negative effect on weight loss of the bread.

Porosity (Y2)= $177.15+3.65X_1+4.703X_2+10.86X_3-0.015X_1^2-0.07X_2^2-0.30X_3^2-0.042X_1X_2-0.075X_1X_3+0.080X_2X_3$ …….(2)

In Equation 2, represented the uncoded value for the predicative model for porosity showed the three variables hadn't linear effects, but which was positive. All the three variables showed not significant quadratic negative effects on porosity. The Interactive effect of refined wheat flour and germinated pumpkin seed and, flour refined wheat flour and butter were negative and germinated pumpkin seed and butter had a positive effect on porosity of the bread.

Sp.Volume (%) (Y3) = $+13.78088-0.27098X_1+0.87695X_2-0.63674X_3+1.69772X_1^2+1.909X_2^2+0.0212X_3^2-9.700X_1X_2+3.000X_1X_3+5.000X_2X_3$……………………………...…….(3)

The relationship between X1, X2, X3 (the uncoded value for the predicative model)and specific volume is shown by equation 3 and resulted in significant linear negative effect in refined wheat flour and butter. The two variables like refined wheat flour and germinated pumpkin seed showed significant on quadratic and all had a positive effect on specific volume. The Interactive effect of X1X2, X1X3 and X2X3 had a positive effect on specific volume of the bread.

Response Surface Plots

A helpful tool for a better understanding of the link between each factor and response is given by the response surface plots, in which the effect of two factors on one specific response is displayed in 3-D view, keeping the other ones on fixed values. Some selected surfaces are presented in Figs. 1

Optimization of germinated pumpkin seed bread

Design expert software was adopted to determine the workable optimum conditions for the germinated pumpkin seed bread. The contour plots for all the responses were superimposed and regions that best satisfy all the constraints were selected as optimum conditions. The main criteria for constraints optimization were maximum specific volume, porosity and minimum weight loss. These constraints resulted in "feasible zone" of the optimum conditions. The optimum range of process parameters obtained for germinated pumpkin seed bread were 86.5 to 103.4 g of refined wheat flour, 3.30 to 11.70 g of germinated pumpkin seed powder, 5.32 to 8.68g of butter. The optimum operating point for refined wheat flour germinated pumpkin seed and butter were, 90, 10 and 8 respectively. Corresponding to these values of process variables, the value of weight loss 10.284, porosity 44.484 and specific volume 2.657 (cm3/g).

Organoleptic evaluation of germinated pumpkin seed bread

The mean organoleptic evaluation of germinated pumpkin seed bread using Duncan multiple range tests was shown in the table 6. Among the 20 variations, V7 have scored highest mean value (5.00±.000 [e]) in Crust Colour. In Crumb Colour, V7 obtained of highest score 5.00±.000 [d] than other variables.

Regarding External Appearance and Shape, the highest score $5.00^{f}\pm.000$ is obtained by the variation V2. In Taste and Aroma, V7 scored highest of $4.80^{e}\pm421$ than V2 which the nearest score of $4.30\pm.483^{d}$. Regarding Mouth feel and Texture, the highest score of $5.00\pm.000^{g}$ scored by the variations of V4 and V5 and is followed by the V7 with score of $4.90\pm.316^{fg}$. Results of the Duncan's test revealed that there was significant difference for all the variations and the high score acquired by V7 which included, 90g refined wheat flour; 10g germinated pumpkin seed and 8 g of butter. Organoleptic evaluation of germinated pumpkin seed bread shows that variation 6 shows (90g refined wheat flour; 10g germinated pumpkin seed and 8 g of butter) high score for Germinated Pumpkin Seed bread. Erkan *et al.* [2006] had shown that instrumental measurement of baked products' color is an inevitable quality check that could be used in determines the effects of ingredient or product formulation, process variable and storage conditions on baked products. It is shown crumb color characteristics are liable to differ significantly in higher sweet potato paste adding samples. Hardness is commonly used as an index to determine bread quality, as change in hardness is frequently accompanied with loss of resilience during storage (Spices, R., 2000).Bread baking quality is determined by the physical properties of dough, its oxidative potential, flour water absorption, bread volume, and color of crumb and crust (Menkovska *et al.,* 2002). As far as affect of germination on tannins is concerned, germination could make tannins move in the same way as that soaking and cooking do, and the fact that these compounds are heat and light sensitive make them more prone to destruction. As the germination period increased to six days, TIA and the PA contents decreased to a large extend, while amounts of tannins and catechins increased in two lentil cultivars (Vidal-Valverde *et al.,* 1994). Moreover, germination was considered as an effective treatment for destroying trypsin enzyme inhibitors than either phytates or polyphenols in *L. sativus* (Srivastava and Khokhar, 1999).

CONCLUSION

The refined wheat flour and germinated pumpkin seed have a significant effect on the weight loss and refined wheat flour has significant effect of specific volume of germinated pumpkin seed bread. Use of the contour and surface plots in RSM was effective for estimating the effect of three independent variables (Refined wheat flour, Germinated Pumpkin Seed and butter).The optimum set of the independent variables was obtained graphically in order to obtain the desired levels of 90g for refined wheat flour,10g for germinated pumpkin seed and 8g for butter.

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