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Optimization of autoclave pumpkin seed bread using response surface methodology

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ABSTRACT

Bread is one of the major products of baked foods and is consumed worldwide. Autoclave of pumpkin seed help to increase the nutrient content of the bread. The objective of this study was to optimize the refined wheat flour, autoclave pumpkin seed powder and butter with respect to bread-making quality. The optimal Design was employed with the following variables of 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 porosity and specific volume of autoclave pumpkin seed bread. The optimum set of the independent variables was obtained graphically in order to obtain the desired levels of 100g for refined wheat flour, 10g for autoclave pumpkin seed and 6g for butter. Organoleptic evaluation of autoclave pumpkin seed bread shows that variation 4 with 100g refined wheat flour; 10g autoclave pumpkin seed and 6 g of butter had obtained high score for overall acceptability.

Introduction

Bread is a dietary staple diet for the world's population (Ahlborn *et al.*, 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 fibre as well as cereal protein. Apart from that, bread contains the B group vitamins and minerals which are mostly magnesium, calcium and iron. Bread is one of the major products of baked foods and is consumed worldwide (Bakke and Vickers, 2007). Bread products and its production techniques vary from country to country.. It has long been established that both quantity and quality of protein influence the end-use quality of wheat and bread making performance (Wieser *et al.*, 2008). 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).

Pumpkins seeds are a light snack full of energy and health benefits. Pumpkin seeds are nutrient-rich nuts. They contain high amounts of trace minerals such as magnesium, manganese and phosphorus, which are important in brain health and development. Pumpkin seeds are also good sources of iron, copper, zinc and protein. The recommended daily value that you get from pumpkins seeds consists of 46.1 percent magnesium, 16.9 percent protein and 17.1 percent zinc. They will also give you 28.7 percent of your daily iron requirement (Pedersen *et al.*, 2007). Plants contain phytosterols which are lipids similar to cholesterol. Pumpkin seeds are high in phytosterols, containing more than 265 mg per 100 g of the seeds. Recent studies suggest that pumpkin seeds can help those suffering from arthritis or other chronic conditions where inflammation is a problem.

Food preparation at home, in particular, cooking is often the final step in food processing. Processing techniques causes important changes in the biochemical, protein, nutritional and sensory characteristics of seeds. Processing methods, such as soaking, germination, roasting and autoclaving has been reported to improve the nutritional properties of plant seeds (Yagoub and Abdella, 2007). Autoclave can be both beneficial and detrimental to nutrient content of foods. It generally improves the digestibility of foods, making some nutrients more available (Audrey Morris, 2004). Studies have shown that thermally processed foods, especially fruits and vegetables, exhibited higher biological activities due to various chemical changes undergone during heat treatment (Kim *et al*., 2000; Dewanto *et al*., 2002).

The present study was undertaken with the objective to determine the optimal levels of wheat flour, autoclave pumpkin seed and butter for acceptable bread.

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 Autoclave pumpkin seed powder

After removal of hull portion pumpkin seeds were dry heated in an autoclave at 121°C and 15 lb pressure for 15 min and cooled to ambient temperature. The pumpkin seed powder was milled to obtain autoclave pumpkin seed powder.

Experimental design

Response surface methodology was applied to the experimental data using a commercial statistical package (Design expert, Trail version 8.0, 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, 1997). A central composite rotatable design (CCRD) with augmented points in

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three variables refined wheat flour(X1), pumpkin seed powder (X2) and butter(X3).

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).

The coding of the levels was done using the following equations:

X_1 (Refined wheat flour) = (x_1-95)/5

X_2 (Pumpkin seed powder) = (x_2-7.5)/2.5

X_3 (Butter) = (x_3-7)/1

Where X_1, X_2 and X_3 and x_1, x_2 and x_3 are coded and uncoded variables, respectively.

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

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).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 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 mode. 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.

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:

$$\text{Weight loss (\%)} = \frac{W\,i - W\,f \times 100}{Wi}$$

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 (1986) 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

Where SV is the specific volume (cm3/g).

Porosity

Porosity was measured by using the method of Zanoni et al. (1995). Porosity can be defined as the ratio of the volume of the pores to the total volume of the product:

ε = (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. The prepared samples were put inside this apparatus and constant force was applied for 1 min. Since the basement area was constant, porosity can be defined as:

ε = (H0 - Hf)/ H0 (2.9)

Where,

H0 = initial height of the sample (mm),

Hf = final height of the sample (mm) after compression

Optimization and verification

Optimum levels of wheat flour, germinated pumpkin seed power and butter were determined by superimposing the plots for all response variables (Floros and Chinnan, 1988; Henika, 1982; 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

Ten panellists who had completed a graduate course and were familiar with bread were chosen. Instructions were given in full to panellists beforehand. 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 (Figure 1) 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; Saxena and Rao, 1996). Consistency of the panel was checked by subjecting data for the indicated attributes from three replicate rating of bread samples to principal component analysis (Kwan and Kowalski, 1980; Powers, 1984) Samples, selected at random from the different treatments, were removed from polyethylene bags before evaluation. The breads were rated in comparison to regular wheat bread.

Statistical Analysis

Experimental data was analyzed by multiple regressions to fit the quadratic equation to all independent variables. Analysis of variance (ANOVA) was performed with Duncan's Multiple range test 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.

Findings

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, autoclave pumpkin seed and butter shows differences exist among the samples in Weight Loss, porosity and specific volume. Weight loss come the range from 10.00 to 11.91 (%), porosity 42 to 44.43 and Specific Volume 1.43 to 2.00 (cm3/g) .The significance of the F-value depends on the number of Degrees of Freedom (DF) in the model (Cai *et al*., 2008, Qiao, D.L et al., 2009).

Effect of variables on Weight loss

Refined wheat flour, autoclave pumpkin seed powder and butter were found to be there haven't any significant variables on affecting the weight loss of autoclave pumpkin seed bread (Table 3). But as refined wheat flour and pumpkin seed increased, weight loss increased. There had no significant deference in the variation of butter quantity.

The statistical significance of the model was also determined by F-test for analysis of variance (ANOVA). The "Model F-value" of 1.00 implies the model is not significant relative to the noise. The "Lack of Fit F-value" of 10.16 implies the Lack of Fit is significant. There is only a 1.18% chance that a "Lack of Fit F-value" this large could occur due to noise. Ratio greater than 4 is desirable for Adeq Precision. A ratio of 3.83 indicates an inadequate signal and we should not use this model to navigate the design space. The "Pred R-Squared" is -2.7045 . A negative "Pred R-Squared" implies that the overall mean is a better predictor of the response than the current model.

Effect of variables on porosity

Effect of variables on Porosity Autoclave pumpkin seed powder found to be the most significant variables on (p =0.000) and butter shows (p=0.05) affecting the porosity in the bread. But maximum porosity showed in maximum amount of the refined wheat flour and autoclave pumpkin seed powder. (Table 3).

The Model F-value of 3.60 implies the model is significant. The "Lack of Fit F-value" of 8.38 implies the Lack of Fit is significant. the Pred R-Squared is -0.7030.A negative "Pred R-Squared" implies that the overall mean is a better predictor of the response than the current model. A ratio greater than 4 is desirable. Here ratio of 7.463 indicates an adequate signal.

Effect of variables on Specific volume

Refined wheat flour and autoclave pumpkin seed power were not found to be the significant variation on affecting the specific volume of autoclave pumpkin seed bread (table 3). But butter shows (p=0.05) a significant variation on specific volume of the bread.

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 autoclave 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 not highly adequate because responses haven' satisfactory levels of R2, CV and model significance. The porosity showed a rather high CV and could be due to the experimental region covered in the study. However, the model was possesses 47.27 of R^2 for weight loss, and 69.71%for specific volume and 76.44 of R^2 for porosity . Considering the high value of R^2, the model for porosity can be accepted.

Predicative models

Weight loss (%) (Y1) = $-18.886+0.492X1+2.755X2-0.605X3-1.986X1^2-3.107X2^2-0.012X3^2-0.028X1X2+1.00X1X3-0.030X2X3$ …………………………………………………. (1)

In Equation 1, for the predicative model for weight loss, all ingredients showed haven't any significant linear effect on weight loss and refined wheat flour and autoclave pumpkin seed shows positive effect on autoclave pumpkin seed bread. No ingredients showed a quadratic effect, but which was also negative. Interactive effects between refined wheat flour and autoclave pumpkin seed had a negative effect and refined wheat flour with butter had a positive effect on weight loss of the bread.

Porosity (Y2)= $+57.401-0.0123X1-2.863X2-0.895X3-1.854X1^2-2.610X2^2-0.0286X3^2-0.028X1X2+0.015X1X3-0.053X2X3$ ………………………………………………….(2)

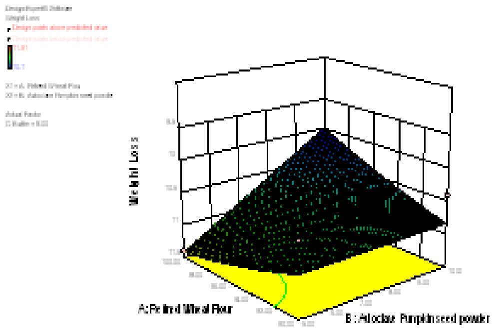
In Equation 2, the predicative model for porosity showed the autoclave pumpkin seed and butter had a linear effect and which was positive. All the three variables showed not significant quadratic, and it was negative effects on porosity. The Interactive effect of refined wheat flour with butter was significant positive effect and other variables had negative effect on porosity of the autoclave pumpkin seed bread.

Sp.Volume (%) (Y3) = $+1.89-0.037X1+8.65X2+8.65X3-0.095X1^2-0.040X2^2+5.69X3^2+0.023X1X2-0.052X1X3+2.50X2X3$ …………….(3)

The relationship between X1, X2, X3 and specific volume is shown by equation 3 and resulted in positive linear effect in refined wheat flour and autoclave pumpkin seed. The variable X3 shows significant linear positive effect. The variable like refined wheat flour showed significant quadratic negative effects on specific volume. The Interactive effect of refined wheat flour with autoclave pumpkin seed had a negative effect and other groups had a positive effect on specific volume of the bread.

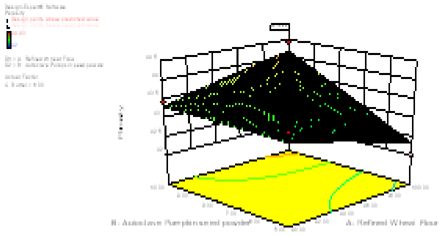
Response Surface Plots

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. 1–3.



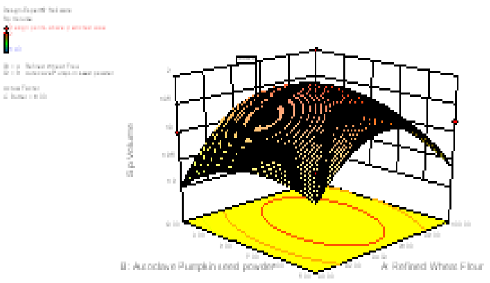
Refined wheat flour – 100g, Autoclave pumpkin seed powder-10 g and Butter-8 g

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



Refined wheat flour –100 g, autoclave seed powder- 10g and Butter-6 g

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



Refined wheat flour – 92.96 g, Autoclave pumpkin seed powder- 8.62 g and Butter-6 g

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

Optimization of autoclave pumpkin seed bread

Design expert software was adopted to determine the workable optimum conditions for the autoclave 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 autoclave pumpkin seed bread were 86.5 to 103.4 g of refined wheat flour, 3.30 to 11.70 g of autoclave pumpkin seed powder, 5.32 to 8.68g of butter. The optimum operating point for refined wheat flour germinated pumpkin seed and butter were, 100, 10 and 6 respectively. Corresponding to these values of process variables, the value of weight loss 9.9727, porosity 44.158 and specific volume 1.9422 (cm3/g).

Organoleptic evaluation of autoclave pumpkin seed bread

The mean organoleptic evaluation of autoclave pumpkin seed bread using Duncan multiple range tests was shown in the table 6. Among the 20 variations, V 10 has scored highest mean Value (4.70^{g}±.483) in Crust Colour. In Crumb Colour, V8 obtained of highest score 4.70^{g}±.483than other variables. Regarding External Appearance and Shape, the highest score 4.40^{e}±.516 is obtained by the variation V5. In Taste and Aroma, V1 scored highest of 4.80^{e}±421 than V2 which the nearest score of 4.60^{g}±.699. Regarding Mouth feel and Texture, the highest score of 5.00^{g}±.000 scored by the variations of V5. Results of the Duncan's test revealed that there was significant difference for all the variations and the high score acquired by V4 which included, 100g refined wheat flour; 10g autoclave pumpkin seed and 6 g of butter. 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).

Agyare et al., 2005, 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., 1990).

Conclusion

Autoclave pumpkin seed powder and butter have a significant effect on the porosity and butter has significant effect of specific volume of autoclaved 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, autoclaved Pumpkin Seed and butter).The optimum set of the independent variables was obtained graphically in order to obtain the desired levels of 100g for refined wheat flour,10g for germinated pumpkin seed and 6g for butter. Organoleptic evaluation of autoclave pumpkin seed bread shows that variation 4 with 100g refined wheat flour, 10g autoclave pumpkin seed and 6 g of butter had a high score for autoclave Pumpkin Seed bread.

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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 are summarized below

Experimental Variables		Code				
		-1.682	-1	0	+1	+1.682
		Uncoded				
Refined wheat flour	X_1	86.5	90	95	100	103.4
Autoclave 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 (X1) (g)	Autoclave Pumpkin seed powder (X2) (g)	Butter (X3)(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

0 represents the centre point; ±1 for factorial points, and ±1.682 for augmented point

Table 2
Results of responses obtained from the quality properties of autoclave Pumpkin seed bread

Variation no.	Refined wheat flour (g)	Pumpkin seed flour (g)	Butter (g)	Weight loss (%)	Porosity	Specific Volume (%)
1	90	5	6	10.3	43.5	1.9
2	100	5	6	11.2	42	1.92
3	90	10	6	10.7	43.44	1.9
4	100	10	6	10.1	44.43	2
5	90	5	8	10.4	42.5	1.8
6	100	5	8	11.4	42	1.6
7	90	10	8	11.4	42.6	1.8
8	100	10	8	10.1	43.2	1.7
9	86.59	7.5	7	11.91	42.7	1.62
10	103.41	7.5	7	10.3	43.4	1.43
11	95	3.30	7	11.85	42.87	1.73
12	95	11.70	7	10.63	43.4	1.63
13	95	7.5	5.32	11.44	43.2	1.9
14	95	7.5	8.68	10.94	43	1.72
15	95	7.5	7	11.27	43	1.9
16	95	7.5	7	10.9	43.2	1.9
17	95	7.5	7	10.6	43.4	1.92
18	95	7.5	7	10.8	43	1.92
19	95	7.5	7	10.75	43	1.8
20	95	7.5	7	10.9	43	1.93

Table 3
Analysis Of Variance (ANOVA) of Second Order Polynomial Model quality properties of autoclave 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	2.54	1.00	0.4978	4.26	3.60*	0.0291*	*0.29*	*2.56*	*0.079*
X_1	0.21	0.75	0.4058	0.043	0.33	0.5795	*0.018*	*1.47*	*0.252*
X_2	1.20	4.24	0.0665	1.52	11.61	0.0067**	*1.023*	*8.248*	*0.97*
X_3	0.44	0.15	0.7024	0.85	6.47	0.0291*	*0.092*	*7.44*	*0.0213**
X_1^2	0.036	0.13	0.7306	0.031	*0.24*	0.6375	*0.13*	*0.33*	*0.0089***
X_2^2	5.436	1.918	0.9892	3.835	*0.029*	0.8677	*0.023*	*1.78*	*0.199*
X_3^2	2.269	8.006	0.9305	0.012	0.13	0.7699	*4.664*	*4.031*	*0.8501*
X_1X_2	0.98	3.46	0.0926	1.61	12.27	0.0057**	*4.05*	*10.50*	*0.580*
X_1X_3	0.020	0.071	0.7959	0.047	*0.35*	0.5649	*0.022*	*1.88*	*0.212*
X_2X_3	0.045	0.16	0.6987	0.14	*1.09*	0.3210	*5.00*	*0.038*	*0.950*
Lack of fit	10.16*			8.38*			9.74*		
Adj R^2	0.0019			0.5523			0.4246		
Pre R^2	-2.745			-0.7030			-1.116		
Adeq precision	3.834			7.463			6.155		

Table 4
Estimated regression coefficients of predicted quadratic model

Predictors	Regression Coefficient		
	Weight loss (%)	porosity	Sp.Volume (cm3/g)
Constant	10.89	43.10*	1.189
Linear			
X_1	-0.13	0.056	-0.037
X_2	-0.30	0.33*	8.656
X_3	-0.057	-0.25*	-0.082*
Quadratic			
X_1^2	-0.050	-0.046	-0.095**
X_2^2	-1.942	-0.016	-0.040
X_3^2	-0.013	-0.029	5.691
Interaction			
X_1X_2	-0.35	0.45	0.023
X_1X_3	0.050	0.076**	-0.095
X_2X_3	-0.075	-0.13	2.500
R^2 (%)	47.27	76.44	69.71

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	is in range	86.5	103.4	100	+1
Autoclave pumpkin Seed	is in range	3.30	11.70	10	+1
Butter	is in range	5.32	8.68	6	-1
				Predicted value	
Weight Loss (%)	minimum	10.1	11.91	9.9727	
Porosity	maximum	42	44.43	44.1589	
specific volume (cm3/g)	maximum	1.43	2.00	1.9422	

Table 6
Organoleptic evaluation of autoclave pumpkin seed bread

Variations	Crust Colour	Crumb Colour	External Appearance and Shape	Taste and Aroma	Mouth feel and Texture
V1	4.00 ±0.666^{ef}	4.00±0.666^{ef}	4.00±0.66^{de}	4.60±0.699 ^{g}	4.60±0 .516^{f}
V2	3.30±0.483^{abc}	3.10 ±0.316^{ab}	3.70±0.823^{abc}	3.30±0.483^{abc}	3.30±0.483^{abc}
V3	3.40±0.516^{abcd}	3.00±0.000^{a}	3.50±0.527^{abcd}	3.70b±0.483^{cdfe}	3.60±0.516^{bcde}
V4	4.60±0.516^{g}	4.60±0.516^{g}	4.00±0.000^{de}	4.00±0.666^{ef}	5.00±0.000^{g}
V5	4.40±0.516^{fg}	4.10±0.567^{f}	4.40±0.516^{e}	3.00±0.000^{a}	3.00±0.00^{a}
V6	3.20±0.421^{ab}	3.40±0.516^{abcd}	3.80±0.421^{cd}	3.30±0.483^{abc}	3.00±0.000^{a}
V7	4.00±0.666^{ef}	3.00±0.000^{a}	4.00±0.666^{de}	3.50±0.707^{abcde}	3.40±0.516^{abc}
V8	3.20±0.421^{ab}	4.70±0.483^{g}	4.00±0.666^{de}	4.20±0.421^{fg}	4.00±0.666^{e}
V9	3.90±0.567^{def}	3.80±0.788^{def}	4.00±0.471^{def}	3.20±0.421^{ab}	3.40±0.516^{abc}
V10	4.70±0.483^{g}	4.00±0.666^{ef}	4.00±0.666^{df}	3.30±0.483^{abc}	3.20±0.421^{ab}
V11	3.90 ±0.737^{def}	3.70±0.483^{def}	4.00±0.666^{de}	3.20±0.421^{abc}	3.30±0.483^{ab}
V12	3.20±0.421^{ab}	4.00±0.471^{ef}	3.00±0.000^{a}	3.90±0.737^{def}	3.20±0.421^{ab}
V13	3.90±0.567^{def}	3.10±0.316^{ab}	3.30±0.483^{abc}	3.80±0.421^{cdef}	3.70±0.483^{cde}
V14	3.70±0.483^{bcde}	3.20±0.421^{abc}	3.80±0.788^{cd}	3.50±0.707^{abcde}	3.70±0.483^{cde}
V15	3.80±0.421^{cde}	3.50 ±0.527^{bcd}	3.80±0.421^{abc}	3.00±0.000^{a}	3.00±0.000^{a}
V16	3.00±0.421^{a}	4.00±0.421^{ef}	3.30±0.000^{abc}	3.20±0.000^{ab}	3.20±0.483^{ab}
V17	3.80±0.421^{cd}	3.10±0.316^{ab}	3.20±0.421^{ab}	3.80±0.421^{cdef}	3.40±0.516^{abc}
V18	3.70±0.674^{bcde}	3.40±0.516^{abcd}	3.50±0.527^{abcd}	3.70±0.483^{bcde}	3.50±0.527^{bcd}
V19	3.70±0.527^{bcde}	3.60±0.483^{cde}	3.70±0.483^{bcd}	3.40±0.516^{abcd}	3.90±0.483^{de}
V20	3.50±0.527^{abcde}	3.70±0.483^{def}	3.30±0.483^{abc}	3.60±0.516^{bcde}	3.70±0.483^{cde}

**-Significant at 0.01% level; *-Significant at 0.05% level; NS-No significant
**-Significant at 0.01% level; *-Significant at 0.05% level; NS-No significant
Values with different superscripts are significantly different from each other on application of Duncan multiple Range test.