Optimization of Gluten Free Bread Formulation by Adding Xanthan Gum, Potato Starch and Sorbitol Using Response Surface Methodology

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Abstract - Gluten is a major component of some cereal and bakery product. Gluten can cause intestinal absorption problem especially to people who have celiac diseases. The aim of this research was to optimize a formulation of gluten free bread (GFB) based on rice flour. The target is to achieve high in volume and weight, but has minimum firmness value. A response surface methodology was used to analyze the amount of xanthan gum, potato starch and sorbitol based on physical properties of GFB. A Box-Behnken design with three independent variables and three levels was used to develop models for the different responses. The optimal formulation was 1.5g/100g xanthan gum, 40g/100g potato starch and 4.1/100g sorbitol, in rice flour basis. Three of breads were baked at the same time at optimum condition to validate the model. The optimize bread was found to be the best ranking of overall acceptance based on hedonic scale compared to the control bread at significant level $p \leq 0.05$, according to sensory evaluation. The results are useful in understanding the limitations amount of xanthan gum, potato starch, and sorbitol in order to produce a better quality of GFB.

Keywords *– Gluten Free Bread, Optimize Formulation, Sorbitol, Potato Starch*

1. INTRODUCTION

Celiac Disease is related to the intake of foods containing gluten and cause damage to the surface of the intestinal mucosa, which leads to an inability to use nutrients. Intolerance to wheat, rye, barley, oats and malt in celiac people make it impossible the consumption of baked foods, pasta, biscuits, cookies, ice creams, and many other foods .The celiac diet must be maintained for life since the insistent uses of gluten where can lead to lymphoma or others types of cancer [1].The most difficult product to be replace for celiacs is bread, a basic food for everyday life, and this could be the interested subject to study in this research. In recent years, several researcher have been conducted in development of gluten free products have been obtained using starches, dairy product, gums, hydrocolloids, probiotics and other combinations as alternative to gluten in order to improve the structure, taste, acceptability, and product shelf life [2]. Elimination of gluten increases the role of starch in providing the structure and texture to the gluten free bread. The most important starch sources are based on corn, rice, tapioca and potato. Starch could be divided into three groups based on water absorption abilities. Potato starch has the highest swelling effect [3]. The effect of replacement of corn or potato starch in the formulation of Gluten Free Bread was also been studies due to the cause an increase in total dietary fibre [4]. Bread with supplemented with resistant starch was characterized with softer crumb,

improves bread volume and its elasticity [5] [6].Hydrocolloid is one of the additives that usually added in the formulation of free gluten product. This compound is commonly name gums are capable of controlling both the rheology and texture of aqueous systems throughout the stabilization of emulsions, suspension and foams. It is also have to mimic the visco-elastic properties of gluten. Xanthan gum is polysaccharides that may form complex aggregates resulting high viscosity at low shear rates. It is also soluble in cold water [7].Sorbitol is a sugar alcohol, a group of reduced calorie sweeteners, natural and nutritive sweeteners. It is a digestible carbohydrate which can be used instead of sucrose. The versatile and unique properties of sorbitol which exhibits over the others polyols are it gives up water very slowly to dry atmosphere and takes up water very slowly from a humid atmosphere. Polyols provide the functional to bakery goods when the sugar used are replaced with polyols [8].The primary focus of this research was to optimize a formulation based on rice flour by adding xanthan gum, potato starch and sorbitol in order to improve the quality of gluten free bread (GFB).

2. MATERIALS AND METHOD

A. Materials

Rice flour with the commercial name CAP DUA GAJAH (7.1% protein,0.3% ash,0.2% crude fiber,85% carbohydrate; dry basis), potato starch from (Bake Hut), instant yeast (Mauripan), xanthan gum and sorbitol liquid as a food grade from (Eugene Sdn Bhd) , bread improver, milk powder, shortening, sugar and salt was purchased from local market.

B. Bread formulation

The ingredients for gluten free rice bread on flour basis; rice flour(100 g), milk powder (5 g),shorthening (12 g), Salt (2 g),Yeast (3 g),bread improver (1 g),Sugar (7 g) and water (90 g) for the control sample and (120 g) for the optimize formulation. There is no sugar added in the optimize formulation. The amount of xanthan gum, potato starch and sorbitol were added at three substitutions levels based on rice flour weight (Table 1).

C. Bread making process

The main solid ingredients were mixed together for 5 min at minimum speed using Kitchen Aids (model KSM150PSER) in the stainless steel bowl with a flat beater. Yeast was dissolved in the water and sorbitol liquid was added at the same time. The batters were mixed for 15 minutes. A 200g of batter were place in the aluminium baking pans (measuring 20 x 11 x 6.5 cm^3), and rested in the proofer at 35^oC for 45 minutes. The baking process was carried out for 30 minutes at 200^oC top and bottom in the laboratory oven (Hanabishi, model HA6180). All the bread was cooled at room temperature for 2 hours. The loaves were packed in a clip-on polyethylene bag and store at room temperature for quality, texture and sensory analysis.

D. Specific volume measurement

The bread loaf volume is determined using a modified standard rapeseed displacement method [15], but using sesame seed instead of rapeseeds. Each loaf was weight and the specific loaf volume was obtained from the ratio of volume per weight.

E. Moisture content

The moisture content of bread crumb was measured by weight difference before and after drying in a hot air oven at 105^oC [15].

F. Crumb texture analysis

Crumb firmness was evaluated by Texture Analyzer (TA-XT plus, Stable Microsystems, UK).Texture profile analysis (TPA) was carried out equipped with 25 kg load cell. The bread sample was slices in the middle of the loaf to obtain uniform slice of 20 mm thickness. A two cycle of crumb compression test was performed using the series of P/75 aluminium platen probe (test speed 3 mm/s and penetration distance 15 mm).The peak force of compression was reported as firmness [15].

Table 1 : Independent variables and their coded and actual value used for optimization.

Independent variables	Units	Symbols	Coded levels		
			-1	0	1
Xanthan gum	g/100g	X_1	0.5	1	1.5
Potato starch	g/100g	X_2	20	30	40
Sorbitol	g/100g	X_3	3	5	7

G. Color measurement

Bread crumb and bread crust were measured for colour in the L*,a*,b* system by using a Minolta colorimeter CR-400 (Konica Minolta Business Technologies, Inc., Japan) which was calibrated with a white standard porcelain plate (L*=97.10, a*=-0.07,b*=+1.97), where L* represent the lightness, a* and b* is colours coordinate.

H. Sensory evaluation

A semi-consumer panel of 30 members (including staff and students in Food Technologies Department, Polytechnic Sultan Haji Ahmad Shah) with nine point hedonic scales has to evaluate the sample individually in the sensory booths at room temperature. During the evaluation, the samples were presented in three digit coded with 1.5cm thick taken from the centre of the loaf and used scale from 1 (Dislike extremely) to 9 (Like extremely). Each respondents was asked to assess the breads for overall acceptance based on overall texture, fluffiness, colour of bread crumb and crust, taste and aroma. Sensory profiles of optimize formulation were compare to control formulation.

I. Experimental design and statically analysis

Response surface methodology was used to optimize the formulation of gluten free bread that contains of xanthan gum, potato starch and sorbitol. A Box-Behnken design with three factors and three levels was chosen to evaluate the combined effect. After the preliminary baking trial the lower and the upper limit of the independents variables were established. The 17 baking trials (Table 2) were performed to evaluate the optimized formulation.The Physical properties data of bread were analyzed using one way analysis of variance (ANOVA).The sensory data were analyzed using independent t-test, at significant value at $p \leq 0.05$.All the data were analyzed using SPSS statistical package (SPSS v.17,SPSS Inc, USA).

3. RESULTS AND DISCUSSION

A. Effect of xanthan gum, potato starch and sorbitol on crumb texture

The baked bread was evaluated for firmness as shown in Table 4b, the highest value of firmness is for control bread. The optimized bread has lowest firmness value, 173.126g which is four times smaller than the control bread. It is also proved that the xanthan gum, potato starch and sorbitol with the correct amount can significantly decrease the value of firmness. The reason for softness, hydrocolloids have a weakening effect on starch structure, leading to a better water distribution and decrease in crumb stiffness [10]. The optimized bread have higher amount of potato starch and xanthan gum, where the amount and rate of starch retro gradation is controlled by the moisture contents which, as it increase the hardness will be decrease [11]. It is also proved that xanthan gum incorporation also has a softening influence on the gluten free bread [12].

B. Effect of xanthan gum, potato starch and sorbitol on specific volume

In all formulation, the present of xanthan gum, potato starch and sorbitol showed statistically significant variations regarding to loaf weight, loaf volume and specific volume, at $p \leq 0.05$. This might have been due to increase water absorption capacity of polyols and addition of polyols improved the gas retention properties [9]. The highest weight of loaf is produced when the amount of xanthan gum and potato starch is at maximum level, respectively in Run 6. The optimized bread is more dense than the control bread, significantly difference due to added of xanthan gum, potato starch and sorbitol. The volume of the control bread is two times smaller than optimized bread. The present of hydrocolloids, starch and polyols improve the volume of bread. The higher the specific volume, the bread is much acceptance in term of appearance. It showed that the optimized bread has significantly difference in term of volume and specific volume. The objective of these research is achieved due to the optimized bread has approved with high value of volume and specific volume.

C. Effect of xanthan gum, potato starch and sorbitol on crumb and crust color

The addition of sorbitol affected the colour of crumb and crust of gluten free bread. In most cases there were a significant differences was observed between control and optimize formulation as well as all others formulations. Regarding the crust colour, the highest value of lightness L was observed in optimized bread. This is because the present of sorbitol in the formulation. Sorbitol is a sugar alcohol which means that it does not go for Maillard reaction to become browning instead of sucrose. The colour of crust for L value for control bread is 64.93 which are darker than optimize bread. The present of sucrose in the formulation tends to have Maillard reaction. The browning process is happened in the crust of the bread. In all analyzed samples the a and b value were positive (red hue and yellow hue).The incorporation of potato starch, xanthan gum and sorbitol affected a significant decrease in redness and the yellowness. Crumb colour was differently influenced by potato starch, xanthan gum and sorbitol. The optimized bread was characterized by higher lightest value. The crumb a value for both bread is negative (green hue) tends to slightly greenness. The crumb b value is positive for both bread (yellow hue) but control bread is more yellowness than the optimize bread. Colour is an important attribute in the baked product and it's depend on the formulation and baking condition [13]. The present of xanthan gum in the formulation of optimized bread simply increase in crumb lightness [10].

Table 2 : The Box-Behnken experimental design and responses

RUN	block	Factor 1 Xanthan gum	Factor 2 Potato starch	Factor 3 Sorbitol	Moisture %	Firmness (g)	loaf weight (g)	loaf volume (cm^3)	Specific volume (cm^3/g)
1	Block 1	-1	-1	0	48.89	109.235	417.78	1105	2.65
2	Block 1	1	-1	0	48.23	200.435	398.48	1360	3.41
3	Block 1	-1	1	0	49.37	490.393	342.71	561	1.64
4	Block 1	1	1	0	47.31	467.61	467.61	1156	2.47
5	Block 1	-1	0	-1	48.96	329.61	329.61	782	2.37
6	Block 1	1	0	-1	47.68	406.437	398.23	884	2.22
7	Block 1	-1	0	1	49.91	348.58	388.26	798	2.06
8	Block 1	1	0	1	49.98	1324.567	384.98	799	2.08
9	Block 1	0	-1	-1	47.21	513.27	402.26	1275	3.17
10	Block 1	0	1	-1	47.57	409.04	409.04	655	1.60
11	Block 1	0	-1	1	49.49	2590.647	348.58	629	1.84
12	Block 1	0	1	1	49.38	962.085	381.44	476	1.25
13	Block 1	0	0	0	45.29	1431.37	357.91	748	2.09
14	Block 1	0	0	0	45.31	1398.57	359.07	746	2.08
15	Block 1	0	0	0	45.34	1405.66	359.23	750	2.09
16	Block 1	0	0	0	45.29	1412.12	359.21	748	2.08
17	Block 1	0	0	0	45.27	1421.78	360.81	744	2.06

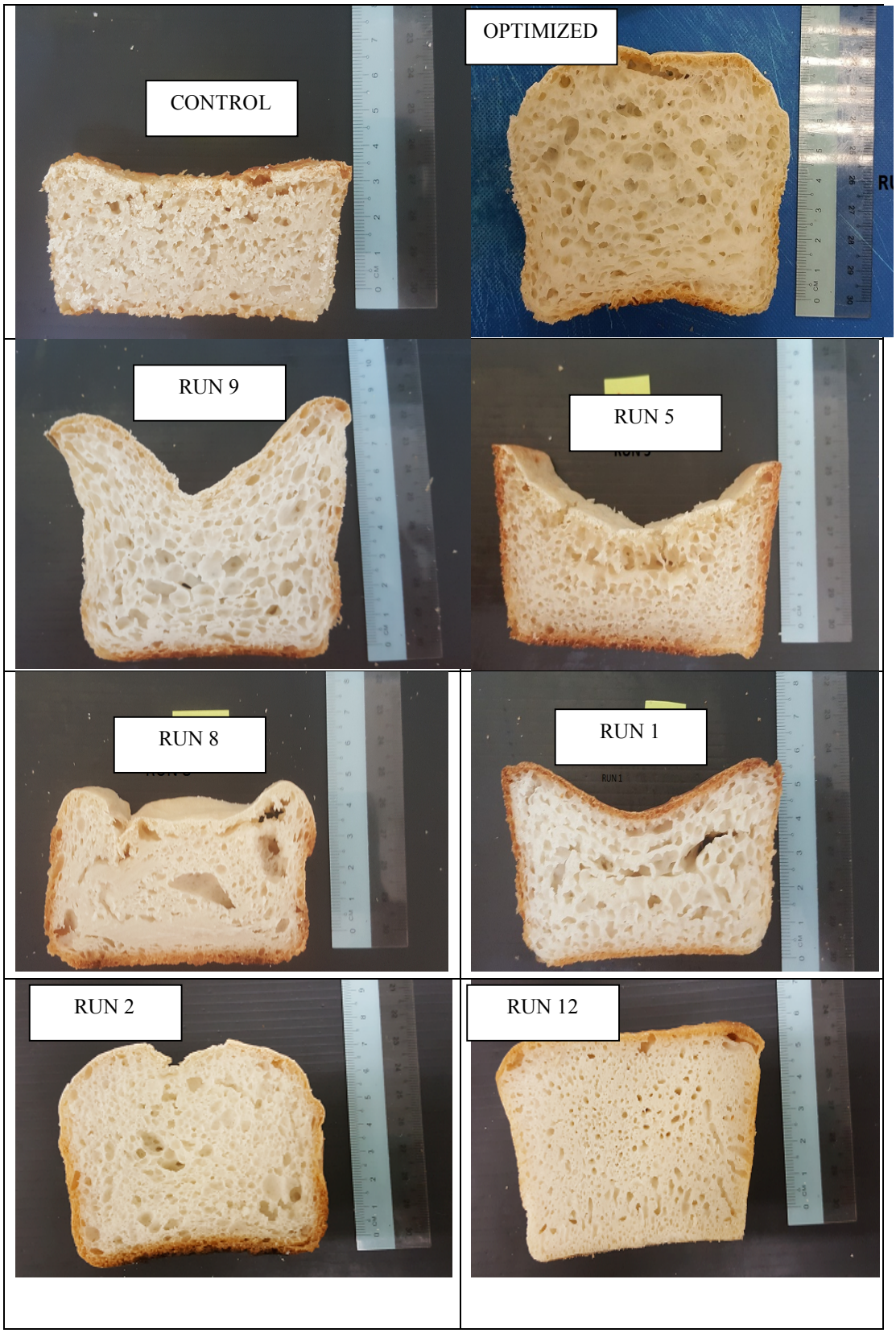


Figure 1: Picture of the appearance of cross section of some selected gluten free bread

Table 3: Colour data for optimize and control bread

Sample	Colour of crumb			Colour of crust		
	L	a	b	L	a	b
Optimized	80.45	-1.66	10.65	69.47	5.71	25.59
Control	74.75	-0.99	15.54	64.93	9.22	29.11

D. Optimization of gluten free bread formulation

Based on table 4a the regression equation for crumb firmness, bread weight, and bread volume are significantly affected by quadratic equation. Crumb firmness is crucial parameter that affects consumer acceptance. Consumer demands a springy and the softness of crumb. To fit the response function and experimental data, the quadratic effects of the independent variables, as well as their interactions on the response variables were evaluated by analysis of variance (ANOVA).The ANOVA of regression model showed that the model was significant due to very low probability, P value. The coefficient of determination, R-squared value which was defined as the ratio of the variation explained by the model to the total variation was used to measure of the degree of fit. The R-squared value for the model of crumb firmness is 0.8325 and the value of bread weight is 0.8648 and follow by bread volume model, the R-squared is 0.8544. It is showed the addition xanthan gum, potato starch and sorbitol affected the value of bread volume.

Moisture content for optimized bread is much higher than the control bread. High amounts of water are needed in the optimized bread due to the present of hydrocolloids, starch and polyols. Hydrocolloids like xanthan gum had more impact in order to increase moisture in the bread and had the lowest firmness value [16].Besides, the present of potato starch also have increase the moisture content in the optimized bread. There was a significant difference of moisture content percentage, between optimized and control bread. The gumminess and chewiness of both breads are significantly different at $p \leq 0.05$, the value of control is 4 times higher than optimized.

E. Verification of result

A verification experiment was performed with three replicate. The results were compared with the predicted responses from the model equations. The experimental and predicted value of optimize GFB were found not significantly different ($p \leq 0.05$).Thus, the RSM optimization approach can be used to develop an optimize GFB rice bread formulation [14].

F. Sensory characteristics

The sensory evaluation of control and optimize bread was performed by untrained panelist using a hedonic scale of nine points for overall acceptability (Table 6).The highest score for overall acceptability is optimize bread with is 8.3 (like very much), where the control bread score is 3.5 (dislike slightly). Its show that the addition of xanthan gums, potato starch and sorbitol have improved the texture, fluffiness, taste, aroma and overall acceptability. There were a significant difference between optimize and control bread. Even thou there is no sucrose added in the optimize formulation; the sweetness of the bread is still acceptable. Sorbitol can be used as sweetener by replacing sucrose in order to have low carbohydrate and low calories value [9].

Table 4a: Box Behnken design- regression equations.

Regression equation	Degree of freedom, F value	P value
Crumb firmness = $1413.90 + 140.15A - 135.56B + 445.95C - 28.50AB + 224.79AC - 381.08BC - 806.72A^2 - 290.26B^2 - 4.88C^2$	3.86	0.0442
Bread weight = $359.25 + 21.37A + 4.21B - 4.49C + 36.05AB - 17.97AC + 6.25BC + 18.67A^2 + 28.73B^2 - 2.65C^2$	4.97	0.0230
Bread volume = $747.20 + 119.13A - 190.12B - 111.75C + 85AB - 25.25AC + 116.75BC + 177.65A^2 + 120.65B^2 - 105.10C^2$	4.56	0.0289

Where A = Xanthan gum, B = Potato starch, C=Sorbitol

Table 4b: Data for quality of bread

Sample	Moisture %	Firmness (g)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience	loaf weight (g)	loaf volume (cm^3)	Spesific volume (cm^3/g)
Optimize	48.99	173.126	0.976	0.91	161.494	157.966	0.429	474.01	1047	2.21
Control	45.23	784.491	0.993	0.894	734.789	730.061	0.419	344.56	570	1.48

Table 5: Comparison of predicted and experimental values for optimized formulation of GFB.

Response / Sample	Experimental value: first duplicate	second duplicate	third duplicate	Predicted value: Optimize GFB
Firmness (g)	173.126 ± 0.372a	173.621 ± 0.442a	173.38 ± 0.536a	173.036a
Loaf weight (g)	474.01 ± 0.55a	473.99 ± 0.12a	474.8 ± 0.19a	474.39a
Loaf volume (cm3)	1047 ± 1.52a	1048 ± 1.52a	1046 ± 1.52a	1047a

a Assay were perform in three replicates. Mean and standard deviation value in the same row followed by different letters are significantly difference at ($p \leq 0.05$).

Table 6 : Sensory evaluation scores for optimize and control gluten free bread.

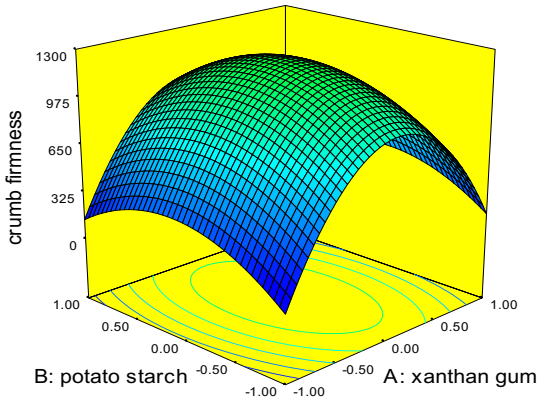
Type of Bread	Texture	Fluffiness	Color of crumb	Color of crust	Taste	Aroma	Overall acceptability
Optimize	8.43±0.56	8.07±0.74	7.53±0.94	7.70±0.75	8.10±0.92	8.0±0.74	8.3±0.71
Control	3.43±1.77	2.93±1.48	3.87±1.67	3.83±1.58	3.53±1.01	3.83±1.18	3.5±1.33

*Significant at ($p \leq 0.05$). Evaluation was made at nine point hedonic scale from 1 (dislike extremely) to nine (like extremely).

4. CONCLUSIONS

The addition of xanthan gum, potato starch and sorbitol has a great influence on the quality of gluten free bread. The moisture content is increase, the bread weight and volume can be increase, and the same time the firmness is at the lowest value. The sensory data also showed the optimized bread are more acceptable by the scale of 8.3 (Like very much) for overall acceptance. The texture of bread score was 8.43 (Like very much), since the fluffiness also have score more than 8. The present day consumer looks for better appeal, sugar free, healthy and convenience from bakery product. Potato starch cannot be added more than 50% in the formulations due to the technological limitations. Sorbitol has good bulking agents and can be used as sweeteners in the gluten free bread. At the optimum formulation, 40g of potato starch, 1.5g xanthum gum, 4.1g sorbitol have produced acceptable gluten free bread.

a)



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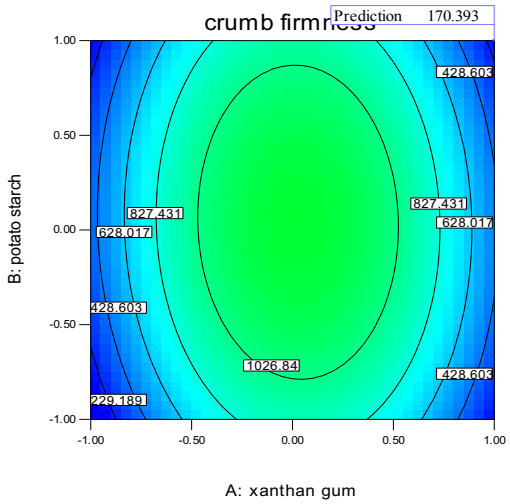

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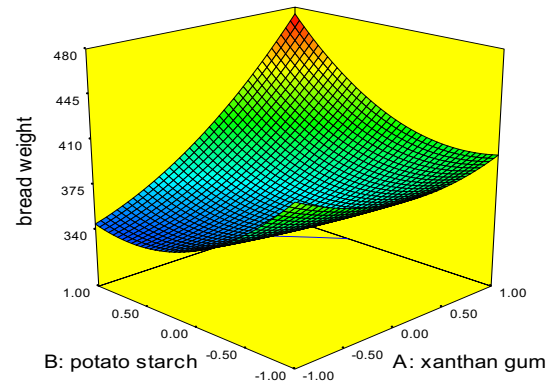

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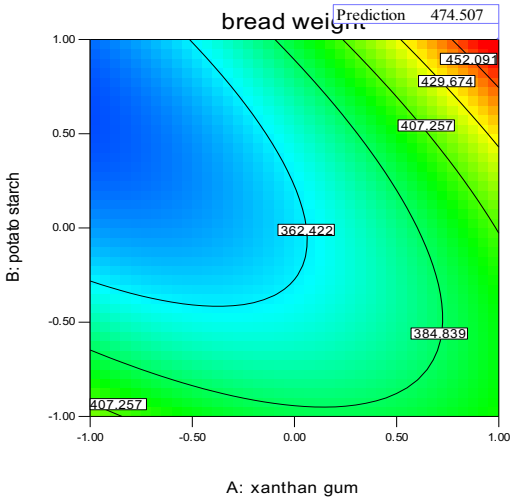

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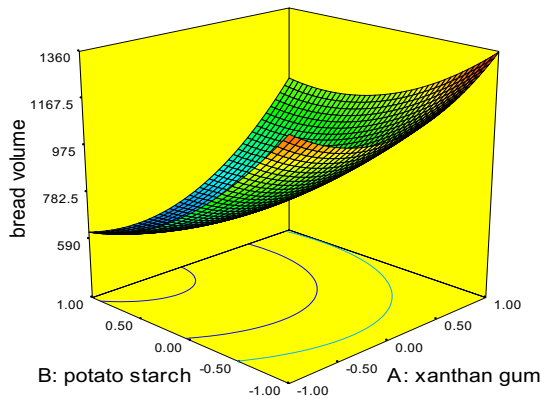

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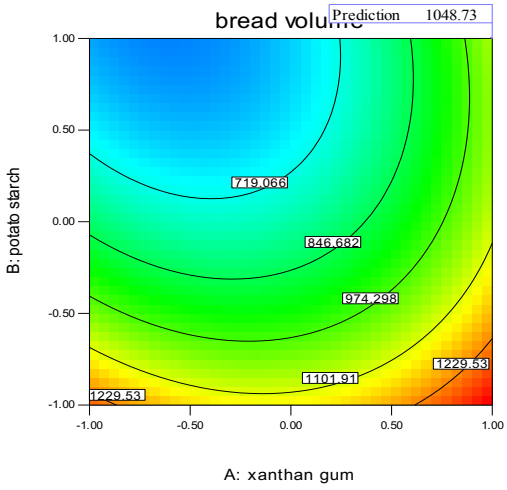

Figure 2: The 3D surface a),b) c) and contour plots d),e),f) of firmness, weight and volume of gluten free bread as affected by the percentage of xanthum gum and potato starch incorporated. The percentange of sorbitol is kept constant at medium level of 4.1g/100g.

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