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Optimization of formulation of gluten-free biscuit based on rice flour and soybean flour by RSM

Afsaneh Morshedi[1], Zahra Poursharif[1], Azar haghighatasiabar[2], Mina Akbarian[3*], Saber Khazani[4]

[1]*Department of Food Science and Technology- Ferdowsi University of Mashhad-Iran*

[2]*Department of food science, Sarab branch, Islamic Azad University, Sarab, Iran*

[3]*Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*

[4]*Research and development manager of Aysuda company, Iran*

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Key words: Biscuit, Gluten Free, Soybean flour, Rice flour, Response surface methodology.

Abstract

This study used responece surface methodology (RSM) based on a central composite design to investigate the influence of rice flour, soybean flour, lecithin and their combination on gluten-free biscuit. Biscuits with different formulations of gluten free mixes were prepared according to RSM. 30 panelists (students) evaluated samples on acceptability of colour, appearance, texture and taste using a five point Hedonic. Acceptability of texture of biscuit (by panelist) was mainly affected quadraticly by soybean flour and lecithin whereas the linear and quadratic effect of rice flour was not significant at 5% level. The interaction effects of rice flour and soybean flour were significant at 5% on acceptability of texture of gluten free biscuit by panelist. The panelist acceptability for appearance shape of samples, is decreasing with increasing of soybean flour. The purpose of this study was to compare preference and acceptability of different formulations of gluten free biscuit for consumer and a formulation was optimised based on acceptability of appearance shape, coloure, textur and taste of samples.

***Corresponding Author:** Mina Akbarian ✉ mina.akbariyan65@yahoo.com

Introduction

Celiac disease is a genetically acquired autoimmune disease in which the presence of gluten, found in foods containing wheat, rye and barley, causes atrophy of intestinal cells. Therefore, strict compliance to a gluten free (GF) diet is necessary to maintain optimum health in individuals with celiac disease. Dietetic professionals need to be aware of the availability and quality of GF products and mixes for their clients (Hauenstein *et al*,. 2010). Currently, the only effective treatment for coeliac disease is the complete avoidance of gluten, a protein found in wheat, rye and barley. The production of high-quality leavened baked goods made from ingredients other than wheat flour represents a major technological challenge, due to the absence of the visco-elastic gluten compound. To tackle these problems, hydrocolloids such as xanthan gum and hydroxyl propyl methylcellulose (HPMC) are often incorporated into gluten-free formulations (Hager and Arendt, 2013). Increasing numbers of diagnosed cases and growing awareness makes the availability of gluten-free foods an important socioeconomic issue. The production of high-quality leavened baked goods made from ingredients other than wheat flour represents a major technological challenge. The absence of the visco-elastic gluten compound results in reduced gas.

retention and structure formation. Hence, breads based solely on gluten-free flours are usually characterised by significantly lower volumes and a firmer crumb when compared to wheat counterparts (Hager *et al.*, 2012). To tackle these problems, hydrocolloids such as xanthan gum and hydroxypropylmethylcellulose (HPMC) are often incorporated into gluten-free formulations (Hager and Arendt, 2013). Among the components of bread, cake and biscuit dough, gluten is unique in exhibiting viscoelastic networks that are responsible for the elastic and extensible properties which assists to retain gas produced from yeast fermentation and oven rise (Demirkesen *et al.*, 2014).The keeping quality of baked foods such as crackers, cookies and biscuits is of great economic importance since these products are widely used and are often stored for extended periods before consumption (Reddy *et al.*, 2005). But, product formulated without gluten are of poor quality with low volume, poor texture, and flavor and fast staling. Since these products are not enriched and fortified, they do not contain adequate amount of vitamins, minerals, and fiber to meet the nutritional needs of celiac sufferers. Thus, gluten replacement remains to be one of the most challenging tasks for cereal technologist and scientists (Demirkesen *et al.*, 2010). To ensure acceptability of gluten free products, modifications in formulations by replacing wheat flour by alternative flours and by using ingredients such as hydrocolloids, emulsifiers, sugars, shortening, enzymes and fibers have long been established by the gluten-free baking industry (Demirkesen *et al.*, 2010; Demirkesen *et al.*, 2013; Demirkesen *et al.*, 2011; Purhagen *et al*, 2012).

Arendt *et al.*(2002) studied the effects of rice, corn, soya, millet, buckwheat and potato starches, in combination with different fat sources (palm oil, cream powder, microencapsulated high fat powder and low fat dairy powders) on the formulation of gluten-free biscuits. Rice, corn, potato and soya with high fat powders produced biscuit doughs, which were sheetable, and biscuits of comparable quality to wheat biscuits. The same authors found that cornstarch, guar gum and high fat powder produced acceptable gluten-free pizza bases (Gallaghera, 2004). Gan, and Small (2002) found that fine white and ground rice flours gave gluten-free breads of good quality when used in combination with CMC (0.8%) and HPMC (3.3%). Ylimaki *et al.,* (1991) used RSM to produce and objectively measure gluten-free breads based on three types of rice flour (varying in grain size and grinding method). Amongst their results, they found that optimal loaves were formulated with medium grain, finely ground rice flour, low levels of HPMC and low levels of CMC. These beads were the most similar to wheat flour breads, based on crust and crumb colour, Instron firmness and loaf moisture. RSM is currently being employed at the authors' laboratory at The National Food Centre to develop and optimize a gluten-free bread formulation based on rice flour, potato starch, skim milk powder and hydroxyl propyl methyl cellulose (HPMC). A central composite design with two variables (water; 70–95% flour weight and HPMC; 0.5–2.5% flour weight) was prepared, and a formulation was optimised based on loaf weight, specific volume, texture profile analysis and image analysis measurements. Optimization was based on the generation of the best results for specific volume, crumb hardness and image analysis data. The effects of rice, corn, soya, millet, buckwheat and potato starches, in combination with different fat sources (palm oil, cream powder, microencapsulated high fat powder and low fat dairy powders) on the formulation of gluten-free biscuits was studied by Arendt *et al.* (2002). Rice, corn, potato and soya with high fat powders produced biscuit doughs, which were sheetable, and biscuits of comparable quality to wheat biscuits.

This study used central composite design to optimization of formulation of gluten free biscuit include of rice flour, soybean flour, lecithin and their combination on gluten-free model systems. Response surface methodology was used as it does not only allow the evaluation of the relative effect of predictor variables (e.g. rice flour, soybean flour, lecithin levels) on response variables (e.g. appearance shape, colour, texture and taste) but also allows the determination of optimum ingredient levels.

Materials and methods

Rice flour (Podrineh, Iran), soybean flour (Behtaam, Iran), lecithin (Argentinien), oil (Ladan, Iran), invert syrup (Arian glucose, Iran), dried milk powder (Guigoz), sodium bicarbonate, citric acid (Kaselcit, China), suger, vanille and salt were purchased.

Preparation of biscuit

Bake trials were conducted under laboratory conditions. Dough mixing, processing and baking were performed on laboratory-scale equipment. The ingredients were weighed according to the proportions listed in Table 1. Biscuits were prepared as per the following method (Sai Manohar & Haridas Rao, 1999, Reddy *et al.*, 2005). Sugar, invert syrup, fat and lecithin were creamed for 3–4 min in a Hobart mixer. Rice flour, soybean flour, sodium bicarbonate, dried milk powder, citric acid, vanille and salt were mixed and sieved and then added to the above cream and mixed (and added water) for 5 min to obtain a homogenous dough. Dough were wrapped in polyethylene bags and left to rest at room temperature for an hour. Then the dough was sheeted to a thickness of 3.5 mm and cut into circular shapes using 45mm cutter and placed on an aluminium tray, baked at 160 ºC for 10 min and then allowed to cool. The biscuits were stored in air-tight containers at ambient temperature (Reddy *et al.*, 2005; Ghanbarzadeh).

experimental design

Response surface methodology was used to evaluate the effect of the independent variables (level of rice flour, soybean flour and licethin) on the dependent variables (sensory evulation include appearance shape, texture, taste and colour). Hereupon, optimum ingredient levels could be determined. A circumscribed, two-dimensional central composite design was developed featuring variations in the addition levels of rice flour (ranging from 40 to 60%), soybean flour (3 to 6%) and lecithin (ranging from 0.01 to 1%). The upper and lower limits of these levels were selected based on previous our research. Acceptiblity of resulting biscuts were evaluated (Hager *et al.*, 2012). The different formulation of rice flour, soybean flour and lecithin on the experimental design were shown in Tables 2.

The response of each of the investigated parameters was analysed by fitting different models to the data in order to identify significant ($p < 0.05$) effects of the variations in ingredient levels on the responses. Three dimensional graphs for the models were used to visualise overall trends. Significance of the lack-of-fit error term, R^2 value, coefficient of variation, and model significance were used to judge adequacy of model. The multiple regression coefficient R^2 represents the power of fit and is a measure of how well the regression model fits the raw data. It ranges from 0 to 1, where 1 is the perfect model. For optimization of rice flour, soybean flour and lecithin levels, a multiple response method called desirability was applied. The following responses were used: acceptibilty of appearance shape (maximise), colour (maximise), texture (maximise) and taste (maximise) of gluten free biscuit by panelists.

Sensory studies

Sensory evaluation of gluten free biscuits was conducted to determine the acceptability of the product prepared. 30 panelists were selected among the students in the Department of Food science, on the basis of their willingness to participate. Five differently coded samples were served to the panelists. Sensory scores for different attributes like appearance shape, color, texture and taste were obtained (Reddy *et al.*, 2005). (The codes of samples include: 1=very bad, 2= bad, 3=middling, 4=good and 5= very good).

Statistical analysis

Design Expert Version 7 (Stat-Ease, U.S.A.) was used for experimental design and to generate surface response plots that permitted evaluation of effects of independent variables on the selected dependent variables and to optimise ingredient levels. The linear, quadratic and interaction terms of independent variables in the response surface models were predicted. For evaluation the relationship between the response and independent variables the generalized polynomial model was usedas below :

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j$$

In this model, Y is a calculated response (i.e.,) X_i and X_j are factors (i.e., %) β_i ،β_{ii} and β_{ij} are linear, quadratic and interaction coefficients, respectively and β_0 is a constant (Akbarian *et al.*, 2013).

Results and discution

The results of experimental data obtained by the response variables were shown in table 3. Response surface methodology has the ability to determine main, quadratic and interaction effects of two components on each studied response variable. Table 3 shows acceptiblity of appearance shape varied from 2.25 to 4.75.

Table 1. The formulation of gluten free biscuit.

Ingradients	Percentage in formulation
Rice flour	According to RSM
Soy bean flour	According to RSM
Lecithin	According to RSM
oil	4.6
Suger	11.4
Invert syrup	3.3
Dried milk powder	1.64
sodium bicarbonate and salt	0.15
citric acid and vanille	0.2
water	Variable according to dough

RSM suggested response surface models to show the relationship between independent variables and dependent variables. According to table 4 the model of 2F1 can show the effect of variables on acceptiblity of appearance shape of gluten free biscuit by panelist better other than the other models.

Relatively high correlation coefficients (i.e. R^2= 0.87) (Table 5) was obtained for acceptiblity of appearance shape indicating good fit of experimental data to Eq. (1). Obtained summarized model to predict the effects of rice flour (X_1), soybean flour (X_2) and lecithin (X_3) on panelist appearance shape of samples (Y), after excluding non-significant factors, is as follows:

$$Y= -0.25 X_2 + 0.37 X_3 + 0.17 X_1^2 - 0.7 X_2^2 + 0.42 X_3^2 + 0.28 X_1X_2 + 0.41 X_1X_3 + 0.16 X_2X_3 \quad (1)$$

Table 2. Experimental design used for of rice flour, soybean flour and lecithin for formulation of gluten free biscuit.

Independent variables			
Formule	Rice flour (%)	Soy bean flour (%)	Lecithin (%)
1	50.00	4.50	0.51
2	50.00	3.00	0.51
3	60.00	6.00	1.00
4	40.00	3.00	1.00
5	40.00	6.00	1.00
6	60.00	4.50	0.51
7	50.00	4.50	0.51
8	40.00	4.50	0.51
9	60.00	6.00	0.01
10	40.00	6.00	0.01
11	50.00	4.50	0.51
12	50.00	4.50	0.51
13	50.00	4.50	1.00
14	50.00	4.50	0.51
15	60.00	3.00	1.00
16	40.00	3.00	0.01
17	50.00	6.00	0.51
18	50.00	4.50	0.51
19	60.00	3.00	0.01
20	50.00	4.50	0.01

According to table 5 the lack of fit (0.73) was not significant for appearance shape of samples at P = 5% level. The analysis of variance for final reduced models (Table 4) showed that acceptability of appearance shape of biscuit (by panelist) was mainly affected linearly by soybean flour and lecithin whereas the linear effect of rice flour was not significant at 5% level. The quadratic and interaction effects of rice flour, soybean flour and lecithin were significant at 5% on panelist acceptability of appearance shape of gluten free biscuit (Table 5).

To visualize the combined effect of the two factors on the response, the response surface and contour plots were generated for each of the models in the function of two independent variables, while keeping the remaining independent variable at the central value (Figure 1, 2, 3 and 4) (Chin and Law, 2012; akbarian *et al.*, 2013). The effect of changing percentage of rice flour and soybean flour on the panelist acceptability for appearance shape of gluten free biscuit is given in Fig. 1.a. The panelist acceptability for appearance shape of samples, is decreasing with increasing of soybean flour (Fig. 1. a). According this figure the effects of rice flour and soybean flour on panelist acceptability for appearance shape of biscuit is quadratic. Fig. 1. b showd that panelist acceptability for appearance shape of biscuit is increased with increasing of rice flour and decreasing of lecithin percentage in formulation of biscuit.

Table 3. Responses for different formulations of biscuit containing rice flour, soybean flour and lecithin.

	dependent variables			
Formule	Appearace shape	Colour	Texture	Taste
1	3.75±0.5	4.5±0.57	3.25±0.5	3±0.81
2	3.5±0.57	3.75±0.5	2.5±0.57	3.25±0.5
3	4.5±0.57	4±0.81	2.5±0.57	2.5±0.57
4	3.75±0.5	3.25±0.5	3±0.81	3±0.81
5	2.25±0.5	3.5±0.57	3.5±0.57	2.25±0.5
6	4.25±0.5	4.75±0.5	4±0.81	3.75±0.95
7	4.5±0.57	4.25±0.5	4.25±0.5	3±0.81
8	4±0.81	4.25±0.5	3.25±0.5	3.25±0.5
9	3.25±0.5	3±0.81	3±0.81	3±0.81
10	3.75±0.5	4.75±0.5	3.25±0.5	4.5±0.57
11	4±0.81	4±0.81	3.5±0.57	4.25±0.5
12	3.75±0.5	4.75±0.5	3.5±0.57	4.25±0.5
13	4.75±0.5	4±0.81	4.25±0.5	3±0.81
14	4±0.81	4.5±0.57	4.25±0.5	3.75±0.5
15	3.75±0.5	4.75±0.5	4±0.81	3.25±0.5
16	4.75±0.5	3±0.81	3.5±0.57	3.75±0.95
17	3±0.81	3±0.81	3.25±0.5	2.25±0.5
18	3.25±0.5	3.75±0.95	3±0.81	2.5±0.57
19	4.25±0.5	3.75±0.5	4±0.81	3.5±0.57
20	4 ±0.81	3.5±0.57	3.25±0.5	2.25±0.5

As shown in Table 3, panelist acceptiblity for colour of gluten free biscuit varied from 3 to 4.75. According to table 4 the model of quadratic can show the effect of variables on acceptiblity of colour for gluten free biscuit better other than the other models. According to table 5 relatively low correlation coefficients (i.e. R^2) was obtained for panelist acceptiblity for colour of gluten free biscuit, don't indicate good fit of experimental data to Eq. (2). Obtained Summarized model to predict the effects of rice flour (X_1), soybean flour (X_2) and lecithin (X_3) on panelist appearance colour of samples (Y), after excluding non-significant factors, is as follows:

$$Y= 0.17 X_1^2-0.07 X_2^2+ 0.28X_1X_2+0.41 X_1X_3 \quad (2)$$

Table 5 showed the lack of fit for parameter of panelist acceptiblity for colour of gluten free biscuit was not significant at P = 5% level. The quadratic effects of rice flour and soybean flour on panelist acceptiblity for colour of gluten free biscuit were significant at 95%.

Fig 2. Indicated the profile of response surface for colour of gluten free biscuit containing two independent variables, while keeping the remaining independent variable at the central value. As can be seen from figure 2.a, maximum panelist acceptiblity for colour of gluten free biscuit was shown in mean values of rice flour. Fig 2. c showed panelist acceptiblity for colour of samples increased with increasing of soyebean flour and lecithin.

Table 3 shows acceptiblity of texture varied from 2. 5 to 4.25. According to table 6 the model of cubic can show the effect of variables on acceptiblity of texture of gluten free biscuit by panelist better other than the other models.

Table 4. Sequential Model Sum of Squares.

		Appearance shape		Colour	
Source		Sum of Squares	Pr>F	Sum of	Pr>F
	DF	296.45		Squares	
Mean	1	1.38	0.3089	312.05	
Linear	3	2.15	0.0931	0.46	0.7773
2FI	3	1.44	0.1376	2.94	0.0482
Quadratic	3	1.21	0.1984	2.15	0.0274
Cubic	4	0.86		0.64	0.4482
Residual	6	303.50		0.90	
Total	20			319.13	

Relatively high correlation coefficients (i.e. R^2= 0. 74) (Table 5) was obtained for acceptiblity of texture indicating Relatively good fit of experimental data to Eq. (1). Obtained summarized model to predict the effects of rice flour (X_1), soybean flour (X_2) and lecithin (X_3) on panelist texture of samples (Y), after excluding non-significant factors, is as follows:

$$Y= -0.62\ X_2^2 + 0.25\ X_3^2 -0.34\ X_1X_2 \qquad (3)$$

Table 5. Analysis of variance table (Responses: appearance shape and colour.

	Appearance shape			Colour		
Source	Coefficient	SS	p	Coefficient	SS	p
model	-	6.19	0.07	-	5.54	0.02*
Intercept	3.91	-	-	4.19	-	-
X1	0.13	0.03	0.65	0.15	0.22	0.25
X2	-0.25	1.2	0.02*	-0.02	6.250E-003	0.80.84
X3	0.37	1.2	0.02*	0.15	0.22	0.25
X12	0.17	1.28	0.02*	0.47	0.06	0.07
X22	-0.7	2.56	0.005**	-0.66	1.19	0.01**
X32	0.42	1.68	0.01**	-0.28	0.22	0.25
X1X2	0.28	1.83	0.01**	-0.44	1.83	0.01**
X1X3	0.41	2.52	0.005**	0.38	1.13	0.02*
X2X3	0.16	1.39	0.02*	-0.19	0.28	0.2
Residual	-	0.86	-	-	1.53	-
Lack of Fit	-	0.021	0.73	-	0.86	0.4
Pure Error	-	0.84	-	-	0.68	-
Cor Total	-	7.05	-	-	7.08	-
R2	0.87	-	-	0.78	-	-
CV	9.86	-	-	9.91	-	-

*, **: significant at P < 5% and P < 1%, respectively.

According to table 6 the lack of fit (0.67) was not significant for texture of samples at P = 5% level. The analysis of variance for final reduced models (Table 7) showed that acceptability of texture of biscuit (by panelist) was mainly affected quadraticly by soybean flour and lecithin whereas the linear and quadratic effect of rice flour was not significant at 5% level. The interaction effects of rice flour, soybean flour were significant at 5% on acceptability of texture of gluten free biscuit by panelist (Table 6).

The effect of changing of rice flour and soybean flour on the panelist acceptability for texture of gluten free biscuit is given in Fig. 3. The panelist acceptability for texture of samples, is increasing with increasing of rice flour (Fig. 3. a). According this figure the effects of soybean flour and lecithin on panelist acceptability for texture of biscuit is quadratic.

Table 6. Sequential Model Sum of Squares.

		Texture		Taste	
Source		Sum of Squares	Pr>F	Sum of Squares	Pr>F
	DF	238.05		206.40	
Mean	1	0.33	0.7931	1.46	0.0391
Linear	3	0.96	0.4232	1.21	0.4869
2FI	3	1.13	0.3473	0.95	0.6202
Quadratic	3	1.64	0.0255	1.79	0.5691
Cubic	4	1.40		3.37	
Residual	6	243.50		215.19	
Total	20				

Table 7. Analysis of variance table (Response: texture shape and taste).

	Texture			Taste		
Source	Coefficient	SS	p	Coefficient	SS	p
model	-	4.05	0.37	-	5.41	0.69
Intercept	-	-	3.27	-	-	3.46
						3.46
X1	0.28	0.31	0.25	0.12	0.65	0.25
X2	0.38	0.95	0.09	0.27	1.6	0.14
X3	0.5	0.95	0.09	0.27	1.6	0.14
X12	0.13	0.99	0.08	0.5	2.29	0.09
X22	-0.62	2.02	0.02*	-0.25	1.77	0.012**
X32	0.25	1.12	0.07	-0.38	1.99	0.01**
X1X2	-0.34	1.89	0.02*	-0.16	1.79	0.12
X1X3	-0.03	0.96	0.08	0.28	2.23	0.09
X2X3	-0.03	0.96	0.08	-0.22	1.98	0.01**
Residual	-	1.4	-	-	3.37	-
Lack of Fit	-	0.05	0.67	-	0.69	0.3
Pure Error	-	1.34	-	-	2.68	-
Cor Total	-	5.45	=	-	8.78	-
R^2	0.74	-	-	0.61	-	-
CV	13.98	-	-	23.33	-	-

As shown in Table 3, panelist acceptiblity for taste of gluten free biscuit varied from 2.25 to 4.5. According to table 6 the model of linear can show the effect of variables on acceptiblity of taste for gluten free biscuit better other than the other models. According to table 7 relatively low correlation coefficients (i.e. R^2) was obtained for panelist acceptiblity for colour of gluten free biscuit, don't indicate good fit of experimental data to Eq. (4). Obtained Summarized model to predict the effects of rice flour (X_1), soybean flour (X_2) and lecithin (X_3) on panelist appearance taste of samples (Y), after excluding non-significant factors, is as follows:

$$Y = -0.25 X_2^2 - 0.38 X_3^2 - 0.22 X_2X_3 \quad (4)$$

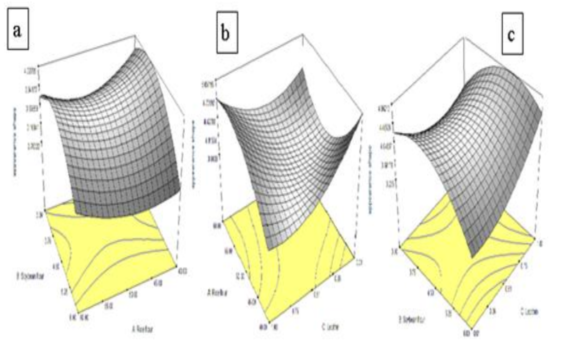

Fig. 1. Profile of response surface for appearance shape of gluten free biscuit containing (a) rice flour and soybean flour (%) (b) rice flour and lecithin (%) (c) soybean flour and lecithin (%).

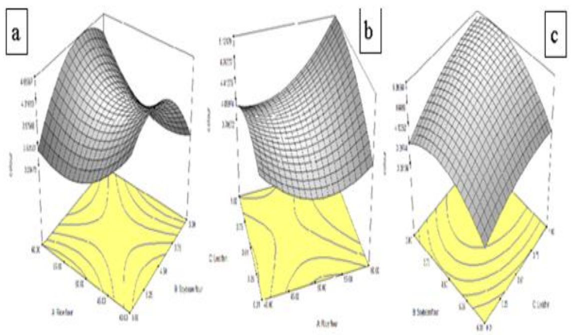

Fig. 2. Profile of response surface for colour of gluten free biscuit containing (a) rice flour and soybean flour (%) (b) rice flour and lecithin (%) (c) soybean flour and lecithin (%).

According to table 6 the lack of fit (0.3) was not significant for taste of samples at P = 5% level. The analysis of variance (Table 6) showed that acceptability for taste of biscuit (by panelist) was mainly affected quadraticly by soybean flour and lecithin whereas the linear and quadratic effect of rice flour was not significant at 5% level. The interaction effects of rice flour, lecithin were significant at 5% on acceptability for taste of gluten free biscuit by panelist (Table 7).

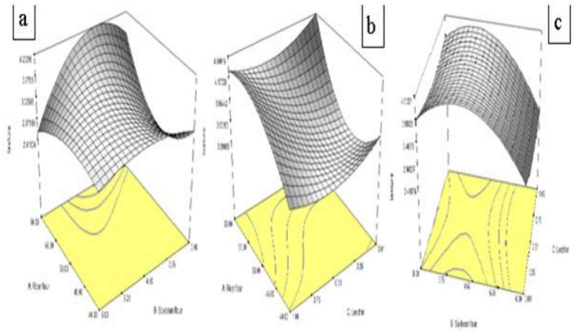

Fig. 3. Profile of response surface for texture of gluten free biscuit containing (a) rice flour and soybean flour (%) (b) rice flour and lecithin (%) (c) soybean flour and lecithin (%).

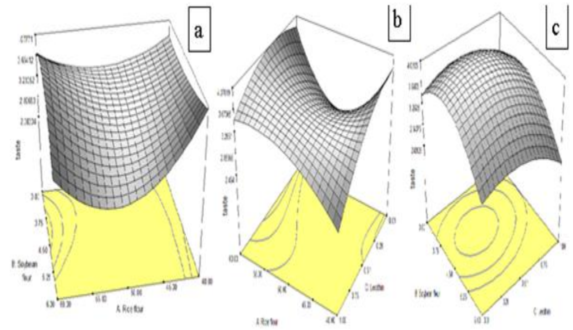

Fig. 4. Profile of response surface for texture of gluten free biscuit containing (a) rice flour and soybean flour (%) (b) rice flour and lecithin (%) (c) soybean flour and lecithin (%).

The effect of changing of rice flour, soybean flour and lecithin on the panelist acceptability for taste of gluten free biscuit is given in Fig. 4. The panelist acceptability for taste of samples, is increasing with decreasing of soybean flour and lecithin (Fig. 4. c). According this figure the effects of soybean flour and lecithin on panelist acceptability for taste of biscuit is quadratic.

Finally, the optimum formulation of this study for gluten free biscuit was obtained: Rice flour (60%), soy bean flour (4.30%) and lecithin (0.34%).

Conclusion

A greater awareness, and improved reliability of diagnostic procedures has recently highlighted the prevalence of coeliac disease. Lifelong adherence to a gluten- free diet remains the cornerstone treatment for the disease. Gluten replacement in bakery products remains a significant technological challenge. Rice flour, soybean flour and lecithin have studied in formulation of gluten-free biscuit. Acceptiblity of appearance shape of biscuit (by panelist) was mainly affected linearly by soybean flour and lecithin whereas the linear effect of rice flour was not significant at 5% level. At mean values of rice flour, maximum panelist acceptiblity for colour of gluten free biscuit was shown. Panelist acceptiblity for colour of samples increased with increasing of soyebean flour and lecithin. The panelist acceptability for texture of samples, is increasing with increasing of rice flour. The effects of soybean flour and lecithin on panelist acceptability for texture of biscuit is quadratic. Final optimum formulation of gluten free biscuit was obtained: Rice flour (60%), soy bean flour (4.30%) and lecithin (0.34%).

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