Optimization of Fiber Rich Gluten-Free Cookie Formulation by Response Surface Methodology

Bahadur Singh Hathan, B. L. Prassana

Abstract**—**Most of the commercial gluten free products are nutritionally inferior when compared to gluten containing counterparts as manufacturers most often use the refined flours and starches. So it is possible that people on gluten free diet have low intake of fibre content. The foxtail millet flour and copra meal are gluten free and have high fibre and protein contents. The formulation of fibre rich gluten free cookies was optimized by response surface methodology considering independent process variables as proportion of Foxtail millet (*Setaria italica*) flour in mixed flour, fat content and guar gum. The sugar, sodium chloride, sodium bicarbonates and water were added in fixed proportion as 60, 1.0, 0.4 and 20% of mixed flour weight, respectively. Optimum formulation obtained for maximum spread ratio, fibre content, surface L-value, overall acceptability and minimum breaking strength were 80% foxtail millet flour in mixed flour, 42.8 % fat content and 0.05% guar gum.

Keywords**—**Copra meal flour, Fiber rich gluten-free cookies, Foxtail millet flour, Optimization

I. Introduction

THE term biscuit is used in the European countries and cookies in the United States of America. In general, cookies are formulated with high levels of shortening, sugar and low moisture contents as compared to biscuits [1]. Cookies differ from other baked products like bread and cakes because of their low moisture content which ensures that they are free from microbial spoilage and confer a long shelf life on the product [2]. The main ingredients of cookies are wheat flour, sugar, fat, skim milk powder, baking powder and flavour but the health problems associated with gluten limiting the use of wheat flour in such products. The wheat flour gluten should be also to be avoided in case of celiac patients. The celiac disease is an autoimmune disease that is caused by interaction of gluten in genetically predisposed individuals [3]. The only treatment for celiac disease is strict adherence to a gluten-free diet. So there is a need for range of gluten free products as the demand for these products is increasing worldwide with the increase in the number of individuals diagnosed with celiac disease.

Further, gluten-free products are not generally enriched or fortified and are frequently made from refined flour or starch. The nutritive value of the majority of gluten-free flours and products examined was generally lower than that of corresponding conventional products. Further, the persons with celiac disease have a lower intake of fiber as compared to a control group of people on normal diet [4]. Diabetes is on an increase in India, the chief reason being urbanization and life style, besides heredity, race, age, nutritional status, stress, altered immune function, altered physiological and metabolic status, drugs and hormones. Studies have indicated the advantages of inclusion of low Glycemic index (GI) foods in both diabetics and non-diabetics to lower the fasting blood glucose [5]. Improvement in insulin sensitivity has also been reported with the consumption of low GI foods. Salmeron *et al.* [6, 7] and Frost *et al.* [8] have reported that the prolonged absorption of carbohydrate after the consumption of low GI foods help in lowering the blood glucose concentration. The low GI foods reduce hunger and increase satiety [9]. The low GI of millets or millet based foods has also been reported by several investigators. Some of the fiber rich gluten free flours are amaranth, buck wheat, teff, minor millets etc.

Minor millets are nutritionally superior to rice and wheat. The presence of all the required nutrients in millets makes them suitable for industrial scale utilization in the manufacture of food stuffs like baby foods, snack foods and dietary food. Foxtail millet (*Setaria italica*) ranks second in the total world production of millets. It contains 9–14% protein, 70–80% carbohydrates and is a rich source of dietary fiber [10] and contains maximum amount of chromium among all the millets with an account of 0.030 mg per 100 g. Polymers of hexoses, pentoses, cellulose and pectinacious material constitute the major portion of its dietary fiber [11]. Millet is a starchy food with a 25:75 amylose to amylopectin ratio and is a fairly good source of lipids (3–6%), having about 50% of the lipids in the form of polyunsaturated fatty acids [12] Although millet is known to contain amylase inhibitors, the carbohydrate digestibility of millet foods is not affected because of heat-labile nature of the inhibitors [13]. The foxtail millet is beneficial food in obesity-related diseases such as type 2 diabetes due to low GI index and cardiovascular diseases [14]. Foxtail millet also contains antioxidants viz. polyphenols which appears to be beneficial in terms of prevention of cardiovascular disease and cancer [15]. Even though the nutritional qualities of millet have been well recorded [16] but its utilization for food is confined to the traditional consumers in tribal populations mainly due to non-availability of consumer friendly, ready-to-use or ready-to-eat products as are found for rice and wheat. In recent years, millets have received attention, mainly because of their high fiber content and efforts are under way to provide it to consumers in convenient forms.

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The copra meal flour, a by-product obtained after oil extraction of dried coconut (*Cocos nucifera* L.), may play a role in controlling cholesterol and sugar levels in blood and prevention of colon cancer [17] due to the presence of high fiber content. Fortification of gluten free products with coconut flour is advantageous due to the increased nutritional value, as copra meal is rich source of protein and dietary fiber [18].

Further, hydrocolloids can be used as gluten substitutes in the formulation of gluten free products due to their polymeric structure [19]. Commonly used hydrocolloids gluten free baked products are guar gum and xanthan gum. Guar gum has been considered as Generally Recognized As Safe (GRAS) since 1974 in numerous food applications having high dietary fiber content (80-85%).

Therefore, the main objective of this investigation was to optimize the formulation of fiber rich gluten-free cookies using Response Surface Methodology with the purpose of achieving the maximum quality and overall acceptability of produced cookies.

II. Materials And Methods

A. Experimental Procedure for Preparation of Cookies

For the preparation of gluten free fiber rich cookies, refined wheat flour is replaced with mixture having different proportions of foxtail millet flour (FMF) and copra meal flour (CMF). The fat content and guar gum ranges from 30-50% and 0.05-1.50% respectively. The other ingredients used were sugar powder 60%, sodium chloride 1%, sodium bicarbonate 0.4% and water 20% (on 100% weight basis of mixed flour). Guar gum gel was prepared by mixing with water at 40 to 42ºC in the ratio of 1:2 [20]. Shortening and sugar powder were creamed in a Hobart mixer (Hobart mixer, Model N50, Canada) at speed 1 (~61 rpm) for 1 min and continued creaming at speed 3 (~178 rpm) for 240 seconds. Guar gum gel was added to the mixture during fat-sugar creaming. Sodium carbonate and sodium chloride dissolved in about 5ml water were transferred to the above cream and mixed at a speed of 1 (~61rpm) for 120 seconds. Further mixing was done to obtain smooth cream and homogeneous mixture at a speed of 2 (~125rpm) for 120 seconds. Gluten free mixed flour was added to the cream mix and simultaneously remaining water was added at regular interval. At most care was taken to avoid lumps formation. Mixing and kneading of the dough was done at speed of 1 (~61rpm) for 120 seconds. At regular interval, the dough was scraped from the sides of the bowl to avoid an unequal distribution of the ingredients. The dough was sheeted to thickness of 0.6 cm. The sheeted dough was made into circular pieces by circular cookie cutter of 5.5 cm diameter. These circular dough pieces were transferred to aluminum trays and placed in a baking oven and baked at 195°C for 20 min. Baked cookies were allowed to air cool for 15 minutes to room temperature. Cookies were packed in HDPE (150 gauge) bags and sealed air tight.

B. Experimental Design for Optimization of Formulation

In this study the experimental design used was Face-Centered Central Composite Design (CCF). This design is one of the three types of Box-Wilson Central Composite designs. A central composite design contains an imbedded factorial or fractional factorial design with center points that is augmented with a group of 'star points" that allow estimation of curvature. Central composite design is the most popular of the many classes of response surface methodology (RSM) designs [21]. If the distance from the center of the design space to a factorial points is ±1 unit for each factor, the distance from the center of design space to a star points is ± α with |α| >1. In CCF, the star points are at the center of each face of the factorial space, so α = ±1. This design requires three levels for each factor, thus making the total number of experiments equal to 20 instead of 27 with full factorial design.

Total number of experiment = $(2)^{\text{No of Variables}}$ + 2x No. of variables + Central points

For three variables

Total No. of experiments for each solute = $2^3 + 2 \times 3 + 6 = 20$

Three different levels for each experiment in coded form are -1, 0, +1

$$Coded\ \ value = x_i = \frac{2\left(X_i - \bar{X}_i\right)}{R_i} \quad (1)$$

Where Xi is the actual value in the un-coded (original) units of the i[th] factor, $\overline{X_i}$ is the average of the low and high levels for the i[th] factor, and R_i is the difference (spacing) between the low and high levels of X_i. The levels of the input variables in coded (x_i) and un-coded (X_i) form are given in Table 1. The independent variables were percentage of Foxtail millet flour (FMF) in mixed flour, fat content and guar gum**.** The low and high levels of the independent variables were 80% and 90% for percentage proportion of FMF in mixed flour, 30% and 50% for fat content, 0.05% and 1.50% for guar gum, respectively (on weight basis of mixed flour). The ranges of FMF and fat content variables have been selected by conducting preliminary experiments and range of guar gum has been selected on basis of literature [22]. The experiments plan in coded and un-coded form of variables along with responses is as given in Table 2. The experiments were conducted randomly to minimize the effects of unexplained variability in the observed responses because of external factors.

TABLE I
LEVELS OF DIFFERENT INGREDIENT VARIABLES IN CODED AND UN-CODED FORM FOR PREPARATION OF FIBER RICH GLUTEN FREE COOKIES

Independent Variables	Units	Symbol	Levels in Coded form		
			-1	0	+1
Proportion of FMF in mixed flour	%	X_1	80.00	85.00	90.00
Fat content	%	X_2	30.00	40.00	50.00
Hydrocolloid	%	X_3	0.05	0.78	1.50

TABLE II
CENTRAL COMPOSITE FACE CENTERED DESIGN WITH EXPERIMENTAL VALUES OF RESPONSE VARIABLES

Uncoded and coded form of process variables			Responses				
Percentage proportion of FMF (%)	Fat content (%)	guar gum (%)	Spread ratio	Breaking strength (N)	L values	Fiber content (%)	Overall acceptability
80 (-1)	30(-1)	0.05(-1)	5.07	67.9	59.99	5.60	7.45
90 (+1)	30(-1)	0.05(-1)	5.58	50.2	60.39	5.29	6.55
80 (-1)	50(+1)	0.05(-1)	6.55	32.0	58.19	5.68	7.62
90(+1)	50(+1)	0.05(-1)	6.42	24.9	59.72	5.22	6.60
80(-1)	30(-1)	1.50(+1)	4.83	69.2	58.67	5.79	7.45
90(+1)	30(-1)	1.50(+1)	5.05	57.1	60.28	5.22	6.55
80(-1)	50(+1)	1.50(+1)	5.57	34.8	58.14	5.78	7.52
90(+1)	50(+1)	1.50(+1)	6.35	25.1	59.8	5.29	6.62
80 (-1)	40(0)	0.78(0)	5.66	49.5	58.37	5.63	7.42
90(+1)	40(0)	0.78(0)	6.23	37.8	60.08	5.29	6.60
85(0)	30(-1)	0.78(0)	4.98	62.5	59.58	5.37	7.02
85(0)	50(+1)	0.78(0)	6.31	31.5	59.49	5.32	7.10
85(0)	40(0)	0.05(-1)	6.32	44.3	59.72	5.34	6.97
85(0)	40 (0)	1.50(+1)	5.98	45.9	59.76	5.41	7.12
85(0)	40(0)	0.78(0)	6.22	42.5	59.75	5.34	7.10
85(0)	40(0)	0.78(0)	6.17	41.9	59.79	5.36	7.05
85(0)	40(0)	0.78(0)	6.19	42.3	59.77	5.35	7.10
85(0)	40(0)	0.78(0)	6.35	40.7	59.87	5.34	6.97
85(0)	40(0)	0.78(0)	6.14	43.9	59.75	5.36	7.07
85(0)	40(0)	0.78(0)	6.28	44.0	59.85	5.34	7.05

C. Statistical Analysis and Optimization

The second order polynomial equation was fitted to the experimental data of each dependent variable as given below

$$Y_k = \beta_{k0} + \sum_{i=1}^{n} \beta_{ki} x_i + \sum_{i=1}^{n} \beta_{kii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{kij} x_i x_j \quad (2)$$

where Y_k = response variable; Y_1 = Cookie's spread ratio, Y_2 = Cookie's breaking strength (N) ; Y_3 = Surface L-value; Y_4 = Fiber content (%) ; Y_5 = overall acceptability. x_i represent the coded independent variables [x_1 = percentage proportion of FMF in mixed flour (%), x_2 = fat content (%), x_3 = guar gum (%)]; β_{ko} is the value of the fitted response at the center point of the design, β_{ki}, β_{kii} and β_{kij} were the linear, quadratic and cross-product regression coefficients, respectively.

The response surface and contour plots were generated for interaction of any two dependent variables, while holding the value of the third variable as constant (at the central value). Such three-dimensional surfaces could give accurate geometrical representation and provide useful information about the behavior of the system within the experimental design. The optimization of process was aimed at finding the optimum percentage proportion of FMF in mixed flour, fat content and guar gum that would give maximum spread ratio, minimum breaking strength, maximum surface L-value, maximum fiber content and maximum overall acceptability. Response surface methodology was applied to the experimental data using a commercial statistical package, Design-Expert version 6.01 (Trial version, Stat-Ease Inc., Minneapolis, MN). The same software was used for the generation of response surface plots and optimization of fiber rich gluten free cookie formulation.

D. Analysis of Cookie Quality Parameters

1. Cookie Spread Ratio

Diameter (D) and thickness (T) of cookies were measured in centimeter by using digital vernier caliper. Cookie spread ratio was determined from the ratio of diameter and thickness [23].

2. Breaking Strength of Cookies

Breaking strength of cookie was measured using the HDP/BS blade. The individual samples of cookies were placed on the platform such that they were supported at two points and the blade was attached to the crosshead of the instrument. The texture analyzer (TA) setting was kept at: Pre-test speed of 2 mm/s, Test speed of 3 mm/s; Post-test speed of 10 mm/s [24]. This test simulates the evaluation of hardness by consumer holding the cookie in hands and breaking the same by bending.

3. Color Characteristics of the Cookies

Surface L-value of the cookies was determined using the Hunter lab Colour Spectrophotometer (Gretag Macthbeth, I-5, USA). The instrument was standardized each time with a black and a white tile. In the hunter scale, L varies from 0 (darkness) to 100 (Lightness). The chromatic portion of solid is defined by: +a (Red); -a (green); +b (Yellow); -b (blue). Three measurements per cookie were taken and reported as an average.

4. Crude Fiber Content

Digestion of 2g sample (W) was done with 200 ml H_2SO_4 (0.255N) for 30 min. During digestion glass beads were added. Residue was washed with hot distill water. Then digestion was done with 200 ml of NaOH (0.313N) for 30 min. Again washed with hot distill water. Then residue was washed with 15ml ethanol. This residue was kept in hot air oven untill constant weight (W_1) was obtained. Kept in muffle furnace at 550°C for 4-5 hours. Weight (W_2) was taken after it get cooled [25].

$$Crude\ fiber\ content(\%) = \frac{(W_1 - W_2)}{(W)} \times 100$$

5. Sensory Analysis of the Cookies

The cookies were evaluated for appearance, texture, taste, flavor and overall acceptability on a 9-point hedonic scale by a panel of 12 judges according to the method of [26].

III. Results And Discussion

A. Diagnostic Checking of Fitted Model and Surface Plots for Cookie's Spread Ratio

The results of second order response models in the form of Analysis of variance (ANOVA) are given in Table 3. The model F-value of 17.04 implies that the second order model for cookie's spread ratio was significant at 5% level of significance. The value of R2 = 93.88 % indicates that 6.12% of the total variation was not explained by the model (Table 3). The value of adjusted determination coefficient (Adjusted R^2 = 88.37%) was high to advocate a high significance of the model [27] and indicated that second order terms were sufficient and higher order terms were not necessary.

TABLE III
Regression Summary And Anova For Spread Ratio Of Cookies

Source	DF	Coefficient	Sum of squares	F-value	p-value
Model	9	6.168	5.363	18.38	< 0.0001
FMF	1	0.195	0.380	11.73	0.0065
FAT	1	0.569	3.237	99.85	< 0.0001
GUAR	1	-0.216	0.466	14.39	0.0035
FMF^2	1	-0.139	0.053	1.64	0.2291*
FAT^2	1	-0.439	0.530	16.35	0.0023
$GUAR^2$	1	0.066	0.012	0.37	0.5574*
FMF*FAT	1	-0.010	0.001	0.02	0.8783*
FMF*GUAR	1	0.077	0.048	1.48	0.2514*
FAT*GUAR	1	-0.035	0.010	0.30	0.5945*
Residual	10		0.324		
Adjusted R^2		89.17			
R^2		94.30			

*Non-significant at 5 % level

The magnitude of p-value (Table III) indicates that all the variables of linear terms have significant effect at 5% level of significance ($p < 0.05$) on cookie's spread ratio. Further quadratic effect of fat content have significant effect at 5 % level of significance ($p<0.05$). The equation of the model fitted for cookie's spread ratio in the actual form of process variables after eliminating the non-significant terms is

Cookie's spread ratio = $-45.76017+0.97625*X_1+0.42891*X_2-2.11643*X_3-4.39091*10^{-3}*X_2^2$

The magnitude of β coefficients (Table 3) revealed that the linear term of fat content have the maximum positive effect (β= 0.59) followed by FMF (β= 0.19) whereas guar gum (β= -0.22) has negative effect on cookie's spread ratio, which indicates that with increase of fat content and FMF , there will be increase in cookie's spread ratio. The increase in cookie's spread ratio with increase of fat content may be related to increased mobility in the system due to melting of fat during baking. Higher fat levels lead to more oil phase in baking ([28]. The increase in cookie's spread ratio with increase in percentage proportion of FMF may be due to decrease in fiber content in the cookies with increase in proportion of FMF in place of CMF. The spread mechanism in cookies is a function of the total availability of water [29] and the amount of dissolved sugar. The fiber absorbs more water as compared to other constituents of the flour. Lesser the fiber content in cookies more will be the availability of water for dissolving sugar [30] and hence more will be the cookie's spread ratio. Increased levels of guar gum produced cookies with less spread ratio due to high water absorption capacity of guar gum which reduces the volume of the aqueous phase in dough system [31].

The relative effect of ingredient levels on cookie's spread ratio can also be seen from the three dimensional plots Fig. 1(a) and 1(b).

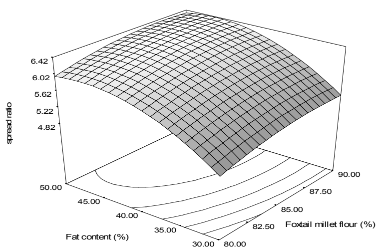

Fig. 1(a) Effect of millet flour and fat content on cookie spread ratio at 0.78% guar gum

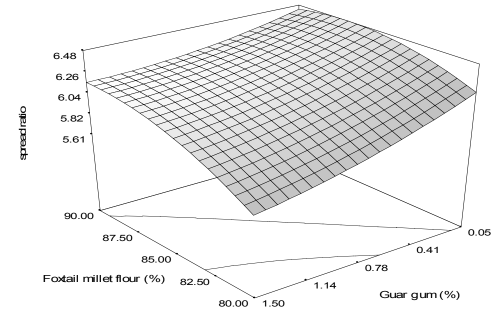

Fig. 1(b) Effect of millet flour and guar gum content on cookie spread ratio at 40% fat content

A. Diagnostic Checking of Fitted Model And Surface Plots For Cookie's Breaking Strength

The model F-value of 107.11 implies that the second order model for cookie's breaking strength is significant at 5% level of significance (Table 4). The value of R^2 is 98.97 % indicates that 1.03% of the total variation was not explained by the model. The value of adjusted determination coefficient (Adjusted R^2 = 98.05%) was high to advocate a high significance of the model which indicates that second order terms were sufficient and higher order terms were not necessary. The magnitude of p-value (Table 4) indicates that linear terms of all variable have significant effect at 5% level of significance ($p < 0.05$) on cookie's breaking strength. Further quadratic effect of fat content and interaction between 'FMF and fat content' have significant effect at 5 % level of significance ($p<0.05$). The equation of the model fitted for cookie's breaking strength in the actual form of process variables after eliminating the non-significant terms is

Cookie's breaking strength (N) $=+98.79555+3.57383*X_1-6.23902*X_2-5.06326*X_3 +0.024500*X_2^2 +0.032500* X_1*X_2$

The magnitude of β coefficients (Table 4) revealed that the linear term of fat content have the maximum negative effect (β= -15.86) followed by FMF (β= -5.83) whereas guar gum (β= 1.28) has positive effect on cookie's breaking strength. With increase of fat content and FMF there will be decrease in cookie's breaking strength. Higher fat levels reduced cookie's breaking strength due to a higher porosity and decrease in strength of the cell walls [28]. Decreased cookie's breaking strength with increase in FMF might be due to decrease in overall fiber content of cookies (i.e. decrease CMF proportion) in formulation. Sudha et al. [32] and Larrea et al. [33] also reported that higher fiber content attributes to the increased toughness in cookies. The increase in breaking strength of cookies with increase in guar gum may be due to the higher water absorption capacity of guar gum which decrease aqueous phase of dough system resulting in increased hardness of cookies [31].

TABLE IV
REGRESSION SUMMARY AND ANOVA FOR BREAKING STRENGTH OF COOKIES

Source		DF	Coefficient	Sum of squares	F-value	p-value
Model		9	43.350	2924.750	107.11	< 0.0001
FMF	x_1	1	-5.830	339.880	112.02	< 0.0001
FAT	x_2	1	-15.860	2515.390	829.04	< 0.0001
GUAR	x_3	1	1.280	16.380	5.40	0.0425
FMF^2	x_1^2	1	-0.90	2.220	0.73	0.4116*
FAT^2	x_2^2	1	2.450	16.500	5.44	0.0419
$GUAR^2$	x_3^2	1	0.550	0.830	0.27	0.6120*
FMF*FAT	x_1*x_2	1	1.625	21.120	6.96	0.0248
FMF*GUAR	x_1*x_3	1	0.375	1.120	0.37	0.5562*
FAT*GUAR	x_2*x_3	1	-0.650	3.380	1.11	0.3160*
Residual		10		30.340		
Adjusted R^2				98.05		
R^2				98.97		

*Non-significant at 5 % level

The relative effect of different ingredient levels on cookie breaking strength can also be seen from the three dimensional plots Fig. 2(a) and 2(b).

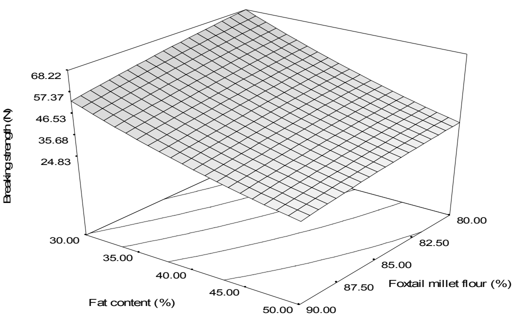

Fig. 2(a) Effect of millet flour and fat content on cookie breaking strength at 0.78% guar gum

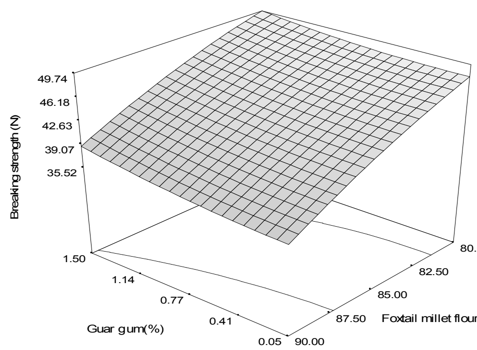

Fig. 2(b) Effect of millet flour and guar gum content on cookie breaking strength at 40% fat content

B. Diagnostic Checking of Fitted Model and Surface Plots for Cookie's Surface L-Value

The model F-value of 13.33 implies that the second order model for cookie's surface L-value is significant at 5% level of significance (Table 5). The value of R^2 is 92.31 % indicates that 7.69% of the total variation was not explained by the model. The value of adjusted determination coefficient (Adjusted R^2 = 85.39%) was high to advocate a high significance of the model and indicated that second order terms were sufficient and higher order terms were not necessary.

The magnitude of p-value (Table 5) indicates that only the linear terms of FMF and fat content have significant effect at 5% level of significance (p <0.05) on cookie's surface L-value. Further quadratic effect of FMF has significant effect at 5 % level of significance (p<0.05). The equation of the model fitted for cookie's surface color in the actual form of process variables after eliminating the non-significant terms is

Cookie's surface L-value = $-51.41538 + 2.65803 * X_1 - 0.23941 * X_2 - 0.015727 * X^2_1$

The magnitude of β coefficients from Table 5 revealed that the linear term of FMF has the maximum positive effect (β= 0.69) whereas fat content has negative effect (β= -0.36) on cookie's surface L-value. With the increase of FMF, there will be increase in cookie's surface. Effect on L-value (i.e. less dark cookies) was due to overall decrease in protein content in mixed flour [34]. The cookies having high protein content have lowest L-values, i.e. the darkest cookies. The darkening of the color of cookies might be due to Maillard browning reactions between proteins and reducing sugars.

TABLE V
REGRESSION SUMMARY AND ANOVA FOR SURFACE L-VALUE OF COOKIES

Source		DF	Coefficient	Sum of squares	p-value	F-value
Model		9	59.725	7.629	13.33	0.0002
FMF	x_1	1	0.691	4.775	75.11	< 0.000
FAT	x_2	1	-0.357	1.274	20.05	0.0012
GUAR	x_3	1	-0.136	0.185	2.91	0.1189*
FMF^2	x_1^2	1	-0.393	0.425	6.69	0.0271
FAT^2	x_2^2	1	-0.083	0.019	0.30	0.5963*
GUAR^2	x_3^2	1	0.122	0.041	0.64	0.4416*
FMF*FAT	x_1*x_2	1	0.147	0.174	2.74	0.1290*
FMF*GUAR	x_1* x_3	1	0.167	0.224	3.53	0.0897*
FAT*GUAR	x_2* x_3	1	0.182	0.266	4.19	0.0678*
Residual		10		0.640		
Adjusted R^2				85.39		
R^2				92.31		

*Non-significant at 5 % level

Huyghebaert [35] also reported the formation of brown polymers/melanoidins as a result of browning reactions due to protein incorporation. As a thumb rule, high fat content decreases the cooking time. There is a decrease in surface L-value (i.e. increase in darkness) with increase in fat content. As in case of cookie's preparation the baking time and temperature were kept constant (as 195°C and 20min); therefore the cookies with higher fat content will get overbaked as compared to cookies having less fat content. This overbaking will result in accelerated caramelization and browning reactions and hence lowering of surface L-value of cookies. Boobier et al. [36] also reported lose in color i.e. decrease in surface L-value with increase in fat content.

The relative effect of different ingredient levels on cookie surface color can also be seen from the three dimensional plots Fig. 3.

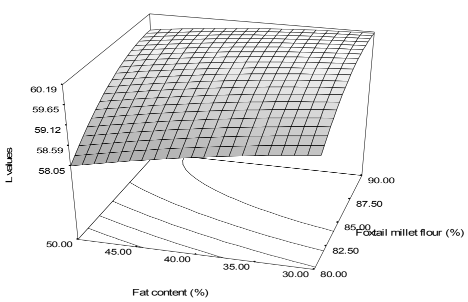

Fig. 3 Effect of millet flour and fat content on surface color at 0.78% guar gum

C. Diagnostic Checking of Fitted Model And Surface Plots for Cookie's Fiber Content

The model F-value of 13.33 implies that the second order model for cookie's fiber content is significant at 5% level of significance. The value of R^2 is 97.56 % indicates that 2.44% of the total variation was not explained by the model (Table 6). The value of adjusted determination coefficient (Adjusted R^2 = 95.36%) was high to advocate a high significance of the model and indicated that second order terms were sufficient and higher order terms were not necessary.

The magnitude of p-value (Table 6) indicates that linear terms of FMF and guar gum have significant effect at 5% level of significance ($p < 0.05$) on cookie's fiber content. Further quadratic effect of FMF and interaction between 'FMF and guar gum' has significant effect at 5 % level of significance ($p < 0.05$) on cookie's fiber content. The equation of the model fitted for cookie's fiber content in the actual form of process variables after eliminating the non-significant terms is

$$\text{Cookie's fiber content (\%)} = +40.05895-0.78901*X_1 +0.786089*X_3 +4.473*10^{-3}*X_1^2 -0.01*X_1*X_3$$

The magnitude of β coefficients revealed that the linear term of FMF has the maximum negative effect (β= -0.22) whereas guar gum (β= 0.03) showed a positive effect on cookie's fiber content. The increase in fiber content with decrease in FMF may be due to the increase in CMF proportion in formulation which has high initial fiber content than FMF. The increase in fiber content with the increase in guar gum may be due to its higher initial fiber content in the range of 1 - 2.5% [37]. The influence of fat content was non-significant on the fiber content of the final cookies may be because fat don't have any fiber content.

TABLE VI
REGRESSION SUMMARY AND ANOVA FOR FIBER CONTENT OF COOKIES

Source		DF	Coefficient	Sum of squares	F-value	p-value
Model		9	5.348	0.580	44.38	< 0.0001
FMF	x_1	1	-0.217	0.470	326.02	< 0.0001
FAT	x_2	1	0.002	$4*10^{-05}$	0.028	0.8711
GUAR	x_3	1	0.036	0.013	8.97	0.0134
FMF^2	x_1^2	1	0.112	0.034	23.81	0.0006
FAT^2	x_2^2	1	-0.003	$2.78*10^{-05}$	0.019	0.8923
$GUAR^2$	x_3^2	1	0.027	$1.98*10^{-03}$	1.37	0.2691
FMF*FAT	x_1*x_2	1	-0.009	$6.13*10^{-04}$	0.42	0.5296
FMF*GUAR	$x_1* x_3$	1	-0.036	0.011	7.28	0.0224
FAT*GUAR	$x_2* x_3$	1	0.006	$3.13*10^{-04}$	0.22	0.6518
Residual		10	95.35			
Adjusted R^2				97.55		
R^2				95.34		

*Non-significant at 5 % level

The relative effect of different ingredient levels on cookie fiber content can also be seen from the three dimensional plot Fig.4.

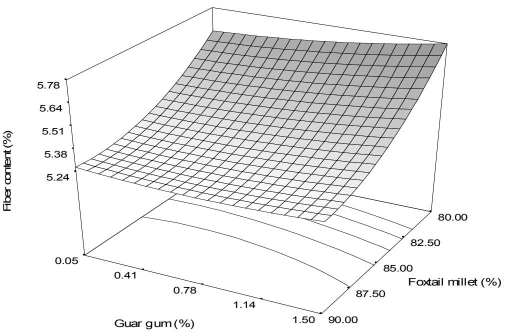

Fig. 4 Effect of millet flour and guar gum content on fiber content of cookies at 40% fat content

D.Diagnostic Checking of Fitted Model and Surface Plots for Cookie's Overall Acceptability

The model F-value of 121.23 implies that the second order model for cookie's overall acceptability content is significant at 5% level of significance. The value of R^2 is 99.09% indicates that 0.91% of the total variation was not explained by the model. The value of adjusted determination coefficient (Adjusted R^2 = 98.27%) was high to advocate a high significance of the model and indicated that second order terms were sufficient and higher order terms were not necessary. The magnitude of p-value (Table 7) indicates that all linear terms have significant effect at 5% level of significance ($p < 0.05$) on cookie's overall acceptability. The effect of all the quadratic terms as well as interaction terms was non-significant on overall acceptability. The equation of the model fitted for cookie's overall acceptability in the actual form of process variables after eliminating the non-significant terms is

Cookie's overall acceptability = $+0.18312 +0.22901*X_1 +0.056439*X_2 -0.17022*X_3$

The magnitude of β coefficients revealed that the linear term of FMF have the maximum negative effect (β= -0.46) followed by guar gum (β= -0.06) whereas fat content has positive effect (β= 0.07) on cookie overall acceptability. The increase in overall acceptability with decrease in FMF may be due to increase in CMF proportion in mixed flour responsible for typical coconut taste and flavor which increases sensory scores for the taste and flavor of the cookie. The increase in overall acceptability with decrease in guar gum may be due to decrease in the bland taste [38]. Overall acceptability increases with increase in fat content, because cookies with high fat content are easier to bite, take less melting time in mouth and have good flavor. The more the shortening, more tender the mouth feel of the finished product [1].

The relative effect of ingredient levels on cookie overall acceptability can also be seen from the three dimensional plots Fig. 5(A) and 5 (B).

E. Optimization of Cookie Formulation

Optimization of cookie's formulation was based on maximum spread ratio, fiber content, surface L-value, overall

TABLE VII
REGRESSION SUMMARY AND ANOVA FOR FOR OVERALL ACCEPTABILITY OF COOKIES

Source		DF	Coefficient	Sum of squares	p-value	F-value
Model		9	7.148	2.270	121.23	< 0.0001
FMF	x_1	1	-0.464	2.150	1034.40	< 0.0001
FAT	x_2	1	0.074	0.055	26.31	0.0004
GUAR	x_3	1	-0.064	0.041	19.68	0.0013
FMF^2	x_1^2	1	-0.046	$5.9x10^{-3}$	2.84	0.1228
FAT^2	x_2^2	1	-0.046	$5.91*10^{-3}$	2.84	0.1228
$GUAR^2$	x_3^2	1	0.034	$3.11*10^{-3}$	1.49	0.2495
FMF*FAT	x_1*x_2	1	-0.007	$4.50*10^{-4}$	0.22	0.6519
FMF*GUAR	$x_1* x_3$	1	-0.002	$5.00*10^{-5}$	0.02	0.8799
FAT*GUAR	$x_2* x_3$	1	0.007	$4.50*10^{-4}$	0.22	0.6519
Residual		10		0.021		
Adjusted R^2					98.27	
R^2					99.09	

*Non-significant at 5 % level

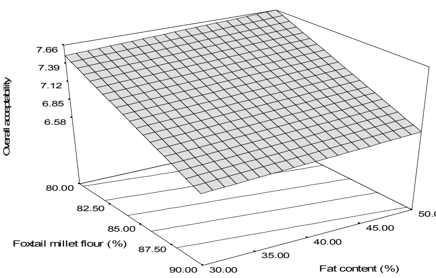

Fig. 5(a) Effect of foxtail millet flour and fat content on overall acceptability of cookies at 0.78% guar gum

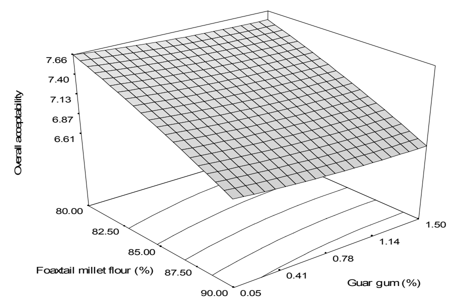

Fig. 5(b) Effect of millet flour and guar gum content on overall acceptability of cookies at 40% fat content

acceptability and minimum breaking strength to achieve the fiber rich gluten free cookies that are comparable to control sample. In order to optimize the formulation for preparation of fiber rich gluten free cookies by numerical optimization technique, equal importance of '3' was given to all the three parameters (viz. percentage proportion of FMF in mixed flour, fat content and guar gum). However, based on their relative contribution to quality of final product, the importance given to different responses was 3, 3, 3, 5 and 5 cookie's spread ratio, breaking strength, surface L-value, fiber content and overall acceptability respectively. Maximum importance was given to the fiber content (%) and overall acceptability, because the aim is to develop gluten-free cookies having high fiber content with maximum overall acceptability. Optimum solution obtained by numerical optimization was 80% proportion of FMF in mixed flour, 42.8% fat content, and 0.05% guar gum to get maximum quality and overall acceptability of fiber rich gluten free cookies.

IV. CONCLUSION

Response surface methodology was effective in optimizing the formulation of fiber rich gluten-free cookies with the ingredients at 80% and 90% levels of foxtail millet flour (FMF) in mixed flour, 30% to 50% fat content and 0.05% to 1.5% guar gum**.** The regression equations obtained in this study can be used for optimum levels for the desired responses within the range of ingredient levels applied in this study. Optimum solution by numerical optimization obtained was 80% proportion of FMF in mixed flour, 42.8 % fat content, and 0.05% guar gum to get maximum quality and overall acceptability of fiber rich gluten free cookies.

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