

bicarbonate (1.22%), water (23%) and vanilla essence (4 drops). Using a response surface methodology, protein-based fat replacers were substituted for 25, 30, 35, 40 or 45% for fat in the formula with different combinations of sugar, ammonium bicarbonate and water (Table 1). Sugar, ammonium bicarbonate and water at 24-36%, 0.5-2.5% and 20-24% on 100 g flour basis was used in combination with protein based fat replacer (Simplese).

Experimental procedure and statistical analysis

The central composite rotatable design (CCRD) was used for optimizing the levels of variable ingredients for the preparation of soft dough low fat biscuits. The variables optimized for low fat biscuits were: sugar (X_1), composite fat (X_2) which consists of fat and Simplese, ammonium bicarbonate (X_3) and water (X_4) based on per cent flour basis, whereas diameter, thickness, spread ratio, hardness, stress-strain ratio and overall acceptability scores (OAA) were taken as responses during the experiments. The coded and actual levels used for independent variables have been given in Table 1. The levels of variables were selected based on the preliminary experiments. Thirty two different experiments were carried out which included eight replications of the centre points using the statistical software package Design Expert® 8.0.6 (Trail version), Stat-Ease Inc., Minneapolis, USA. The following second order polynomial equation was fitted to the data to obtain regression equations for each independent variable as given below.

$$y = \beta_0 + \sum_{i=1}^4 \beta_i x_i + \sum_{i=1}^4 \beta_{ii} x_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} x_i x_j \quad (1)$$

where, y =responses, X_i , X_j =coded processing parameters, β_0 , β_i , β_{ii} , β_{ij} =regression coefficients.

The statistical significance of the terms in the regression equation was examined by analysis of variance (ANOVA).

Manufacturing of biscuits

Soft dough biscuits were prepared according to traditional creamery method as described in [8]. For preparation of low fat biscuits, fat, sugar powder and vanilla were creamed in a mixer at speed 60 rpm for 1 min and continued for creaming for another 3 min. Liquid glucose, skim milk powder and Simplese made into suspensions, sodium carbonate and ammonium bicarbonate dissolved in water and were transferred to the above cream and mixed at speed of 60 rpm for 2min to get smooth cream. Wheat flour was transferred to the above cream and mixed for 2 min at speed 60 rpm to get biscuit dough. The biscuit dough was sheeted to a thickness of 3 mm and cut into round shapes using dough cutter. The cut dough was placed into aluminium trays and placed in baking oven at 190°C for 10 min. and then cooled and packed in LDPE bags.

Simplese was added in gel form during fat-sugar creaming whenever included in the formulation. Water was added to Simplese in 1:1 ratio and was heated at 45-50°C.

Biscuit dimensions

Biscuits were evaluated for diameter, thickness and spread ratio according to the method given in [9]. The diameter of biscuits was measured by laying six biscuits edge to edge and measuring to the nearest mm. The biscuits were rotated to 90° and their diameter was remeasured as a check determination. The average value was reported in cm. Thickness or height of the biscuits was measured by stacking six biscuits one above the other and the average value was expressed as cm. The spread ratio was calculated by dividing the average value of diameter (D) by average value of thickness (T) of biscuits. The reading for thickness, diameter and spread ratio were taken in triplicate and the average value was reported.

Texture evaluation

To obtain samples for measuring textural characteristics, the dough was sheeted using a rolling pin over a rectangular platform and frame with a height of 4 mm to get a sheet of uniform thickness. The sheeted and cut cylindrical discs of 4 mm height were used to assess hardness (N). Hardness of the biscuits was measured in a Stable Micro Systems Texture Analyzer (TAXT 2i). The biscuits were placed under sharp-blade cutting probe, 70 mm long and 0.4 mm thick. A speed of 1 mm/s and a distance of 3 mm were used in the studies. The analyzer was set at a 'return to start' cycle, a speed of 1 mm/s and a distance of 3 mm, pre-test speed 5 mm/s, post test speed 10 mm/s and load cell 500 kg. The force required to break 6 biscuits individually were recorded and the average value reported. Stress was calculated by dividing the maximum force by area of blade and strain was expressed as the maximum distance travelled by probe to break the biscuit. Stress-strain ratio was obtained by dividing stress by strain.

Sensory evaluation

The sensory analysis of biscuits was carried out by a panel of ten semi trained members by assigning a score for each sensory attribute such as colour, texture, taste, flavour and overall acceptability (OAA) on 5-point sensory scale, where scores 1, 2, 3, 4 and 5 represented poor, fair, satisfactory, good and excellent, respectively. Evaluation was done in triplicates and the average value was reported.

Storage study

Moisture, crude protein, crude fat and total ash were determined using standard method [9]. Crude fibre was estimated by method [10]. Carbohydrates were calculated by difference method and calorific value was estimated. Quality evaluation of biscuits at 0, 30, 60 and 90 days of storage (stored at ambient temperature 15°C-25°C) for moisture, free fatty acids (FFA), peroxide value (PV), hardness and sensory properties (taste, flavour, overall acceptability) were carried out. PV was determined method, FFA content was determined according to AOAC method [11]. Hardness of biscuit was determined by using Stable Micro Systems Texture analyzer (TAXT 2i). The biscuits were evaluated for sensory characteristics on 5-point sensory scale for

Ingredient/variable	Code	Coded level				
		-2 ^a	-1	0 ^b	+1	+2 ^a
Sugar	X_1	24	27	30	33	36
Composite fat (Fat, Simplese)	X_2	26.25,8.75	24.5, 10.5	22.75, 12.25	21, 14	19.25,15.75
Ammonium bicarbonate	X_3	0.5	1	1.5	2	2.5
Water	X_4	20	21	22	23	24

^a ± α level, ^bcentre point.

Table 1: Experimental variables for biscuits, their coded and uncoded (actual) values (100 g flour basis).

different parameters like taste, flavor and overall acceptability. All the values were taken in triplicate and average value reported.

Results and Discussion

In this study, soft dough biscuits were prepared using protein-based fat replacers to yield a low fat product. The range of different physical and textural responses namely, diameter, thickness, spread ratio, hardness and stress-strain ratio were found as 5.6 to 5.98 cm, 0.6 to 0.75 cm, 7.89 to 9.45, 24.76 to 84.79 N and 0.44 to 2.17, respectively. Sensory characteristics viz. colour, texture, taste, flavour and overall acceptability (OAA) were found in the range 3.31-4, 2.75-4, 3-4.33, 3-4.33 and 3-4.35, respectively. Response surface analysis was applied to the experimental data and the second order response surface model (Eq. 1) was fitted to all the physical characteristics (viz. diameter, thickness, spread ratio), textural characteristics (hardness and stress-strain ratio) and sensory characteristics (colour, texture, taste, flavour and OAA). The statistical significance of the model terms were examined with the help of regression analysis and analysis of variance (ANOVA). In the present study, for the optimization of independent variables, the responses i.e. spread ratio, hardness, stress-strain ratio and OAA were selected on the basis that these responses had direct effect on the quality and were dependent directly on specific composition of the product. Prinyawiwatkul also reported that texture is a primary attribute which consumers use to judge quality of the snack product [12]. The variability explained by all the models was more than 65 per cent ($R^2 > 0.65$) and the F-values for all the models were significant, implying that the models were accurate enough to predict the responses. Moreover, adequate precision ratio of all the models was more than 4. Therefore, all the models exhibited statistical adequacy and were hence used to study the effect of different parameters on the various responses. The results of the regression analysis and analysis of variance (ANOVA) for all the models are reported in Tables 2-5.

Design Expert (Trial version 8.0.6) of the STAT-EASE software was used for simultaneous numerical optimization of the processing

Variants	Diameter (cm)	Thickness (cm)	Spread ratio	Hardness	Stress-strain ratio
Intercept	5.860	0.656	8.930	56.594	1.279
X1	0.092***	0.024***	-0.168**	7.991***	0.254***
X2	-0.008	0.018***	-0.249***	4.89**	0.056
X3	0.020*	0.014**	-0.147**	0.609	-0.074
X4	-0.005	0.003	-0.056	0.463	-0.022
X1 X2	-0.018	0.013*	-0.187**	2.724	-0.045
X1 X3	0.011	0.006	-0.058	3.251	-0.011
X1 X4	-0.007	-0.004	0.052	3.373	0.163**
X2 X3	0.009	0.006	-0.058	2.101	0.147*
X2 X4	-0.018	-0.004	0.036	1.451	-0.039
X3 X4	-0.004	-0.004	0.040	0.088	0.009
X12	-0.016	0.002	-0.042	-2.955*	-0.075
X22	-0.019*	-0.004	0.032	-3.566**	-0.053
X32	-0.006	0.002	-0.020	-3.272*	0.003
X42	-0.019*	0.003	-0.066	-0.966	0.014
R2 (%)	85.15	70.86	67.82	73.26	66.61
F-value	6.96***	2.95**	2.56**	3.33***	2.42**
Adequate precision	10.49	7.68	7.50	7.74	6.79

Significant at ***1% **5% *10%;
X1: Sugar, X2: Composite Fat, X3: Ammonium Bicarbonate, X4: Water;
X1, X2, X3 and X4 are in coded form.

Table 2: Regression coefficients of full second order model and significant terms for physical and textural properties of low fat biscuits containing Simplese.

Factor	Colour	Texture	Taste	Flavour	Overall acceptability
Intercept	3.709	3.63	4.1	4.079	4.237
X ₁	0.029	0.109 [†]	0.132 [†]	0.159 [†]	0.124 [†]
X ₂	-0.013	-0.084 [†]	-0.074	-0.064	-0.028
X ₃	-0.039 [†]	-0.091 [†]	-0.053	0.004	-0.140 [†]
X ₄	-0.030	-0.101 [†]	-0.082	-0.053	-0.116 [†]
X ₁ X ₂	-0.070 [†]	-0.033	-0.061	-0.038	-0.095 [†]
X ₁ X ₃	0.050 [†]	-0.080	0.031	-0.001	-0.075
X ₁ X ₄	-0.062 [†]	-0.008	-0.014	0.007	0.039
X ₂ X ₃	-0.021	0.114 [†]	0.069	0.147 [†]	0.030
X ₂ X ₄	0.117 [†]	0.093	-0.061	-0.036	0.049
X ₃ X ₄	-0.011	0.002	-0.052	-0.036	0.039
X ₁ ²	0.019	-0.120 [†]	-0.104	-0.118 [†]	-0.189 [†]
X ₂ ²	-0.009	-0.141 [†]	-0.299 [†]	-0.236 [†]	-0.270 [†]
X ₃ ²	-0.053 [†]	-0.023	-0.139 [†]	-0.143 [†]	-0.107 [†]
X ₄ ²	-0.094 [†]	-0.066	-0.107 [†]	-0.134 [†]	-0.192 [†]
R ² (%)	81.65	71.8	73.62	73.52	88.15
F-value	5.40 [†]	3.09 [†]	3.39 [†]	3.37 [†]	9.03 [†]
Adequate precision	9.17	6.84	6.45	5.47	8.55

Significant at [†]1% [†]5% [†]10%;
X₁: Sugar, X₂: Composite Fat, X₃: Ammonium Bicarbonate, X₄: Water;
X₁, X₂, X₃ and X₄ are in coded form.

Table 3: Regression coefficients of full second order model and significant terms for sensory characteristics of low fat biscuits containing Simplese.

Responses	Mean squares				
	Diameter (cm)	Thickness (cm)	Spread ratio	Hardness (N)	Stress-strain ratio
Total individual effect of processing parameters					
Sugar (X ₁)	0.044***	0.003***	0.278*	452.069***	0.435***
Composite fat (X ₂)	0.005***	0.002**	0.430**	234.514**	0.112
Ammonium bicarbonate (X ₃)	0.003	0.001	0.133	113.001	0.096
Water (X ₄)	0.004	0.0002	0.059	49.716	0.094
Combined effect of all processing parameters at					
Linear level	0.054***	0.006***	0.691***	530.109	0.441
Quadratic level	0.008*	0.0002	0.055	244.335	0.064
Interactive level	0.002	0.0007	0.126	95.727	0.138

Significant at ***1% **5% *10%

Table 4: ANOVA for the overall effect of processing parameters on the physical and textural responses.

Responses	Mean squares				
	Diameter (cm)	Thickness (cm)	Spread ratio	Hardness (N)	Stress-strain ratio
Total individual effect of processing parameters					
Sugar (X ₁)	0.0420**	0.1661**	0.1628	0.2097*	0.3361***
Composite fat (X ₂)	0.0617***	0.2258**	0.5926***	0.4258***	0.4738***
Ammonium bicarbonate (X ₃)	0.0338**	0.1050	0.1543	0.1945*	0.188***
Water (X ₄)	0.1131***	0.1033	0.1217	0.1287	0.3005***
Combined effect of all processing parameters at					
Linear level	0.0202	0.2253**	0.1945	0.1941*	0.2954***
Quadratic level	0.0900***	0.2914***	0.9670***	0.7995***	1.1604***
Interactive level	0.0677***	0.0779	0.0426	0.0682	0.0558

Significant at ***1% **5% *10%

Table 5: ANOVA for the overall effect of processing parameters on the sensory responses.

parameters. The optimum conditions obtained for low-fat biscuits were sugar (29.8%), fat (19.25%), Simplese (15.75%), ammonium bicarbonate (2.26%) and water (21.5 ml). The levels were based on

100g flour basis. Actual values for different responses viz. spread ratio, hardness, stress-strain ratio and OAA were 9.37 ± 0.02 , 56.25 ± 3.55 N, 1.15 ± 0.3 and 4.08 ± 0.09 , respectively.

Biscuit dimensions

Measurements of biscuit thickness showed significant variation (Table 2). The effect of sugar, composite fat and ammonium bicarbonate on thickness was significant at $p < 0.01$, $p < 0.01$ and $p < 0.05$, respectively. With increase in sugar and fat replacer level, thickness of biscuits increased significantly ($p < 0.1$) as shown in Figure 1a. Ammonium bicarbonate also significantly ($p < 0.1$) affected the diameter. It is also seen in Figure 1b that spread ratio was significantly affected by sugar ($p < 0.05$), composite fat ($p < 0.01$) and ammonium bicarbonate ($p < 0.05$). A negative correlation was found between the interaction of sugar level and fat level with spread ratio ($p < 0.05$) indicating a decrease in spread ratio with increase in sugar and fat replacer level (Figure 1b) studied the impact of sugar levels on the cookie structure. Cookie diameter increased and its height decreased with increasing sugar or fat levels. The quadratic term of composite fat and ammonium bicarbonate was found significant ($p < 0.1$) for diameter.

Table 4 shows the overall effect of different responses on biscuit dimensions. The overall effect of sugar was found significant on diameter ($p < 0.01$), thickness ($p < 0.01$) and spread ratio ($p < 0.1$). At

linear level all the models of biscuit dimensions were significant at $p < 0.01$, whereas at quadratic level the model of diameter was only found significant ($p < 0.01$).

Biscuit texture

Effect of ingredients on texture profile of the biscuits was studied in this study (Table 2). Sugar significantly affected the hardness and stress-strain ratio ($p < 0.01$). The level of composite fat added to biscuit formulation affected the hardness at $p < 0.05$. Zoulias studied the effect of the type of fat mimetic and of the percentage of fat replacement on textural behaviour of the products by compression tests [13]. Figure 1c showed that with increase in sugar and fat replacer level, the hardness of biscuit increased. It is observed from Figure 1d that the saddle point situation exists due to the interaction of fat and ammonium bicarbonate on stress-strain ratio. The interactive effect of sugar and water showed that stress-strain ratio increased with increase in water level (Figure 1e). The stress-strain ratio is related to brittleness of the biscuits. Cookies that present a high stress-strain ratio are less compressible, more brittle and break easily. Brittleness can be considered a pleasant sensorial characteristic for the cookies as far as it does not become extremely great. The authors also found that the replacement of fat with different mimetics had various effects on textural properties of the biscuits depending on the mimetic and the samples gave maximum stress, as the amount of fat replaced increased [14,15]. The quadratic terms of sugar, fat and ammonium bicarbonate for hardness were significant at $p < 0.1$, $p < 0.05$ and $p < 0.1$, respectively.

The overall effect of different parameters on hardness and stress-strain ratio is shown in Table 4. It was observed that sugar had more significant overall effect on hardness ($p < 0.01$) and stress-strain ratio ($p < 0.01$), whereas fat affected hardness at $p < 0.05$. However, the linear, quadratic and interactive models for hardness and stress-strain ratio were insignificant (Table 4).

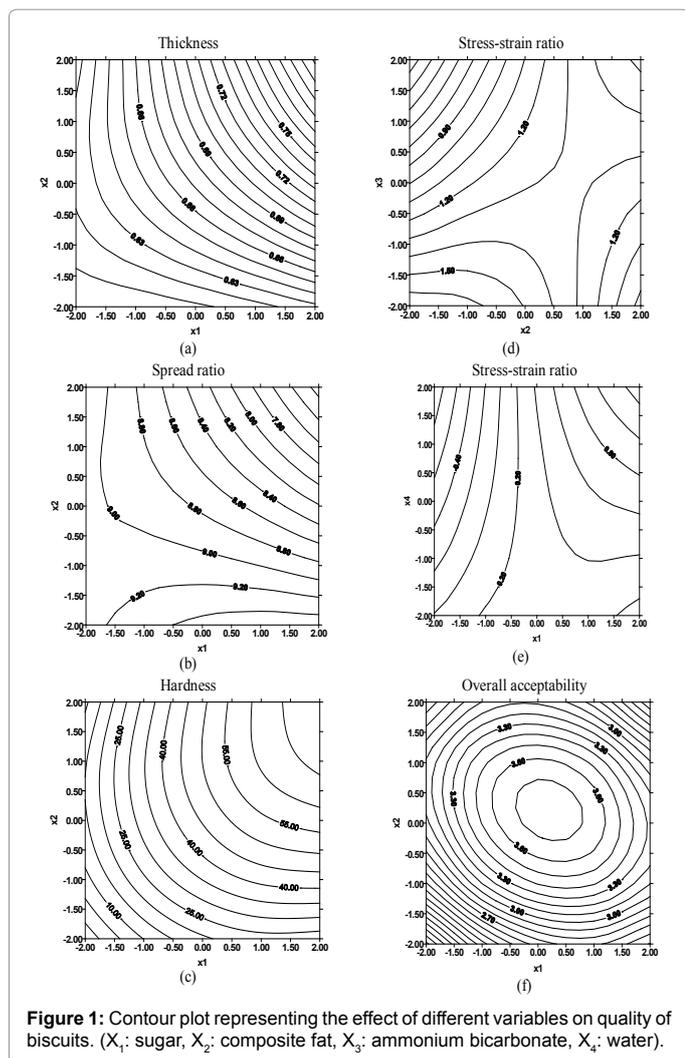
Sensory evaluation of biscuits

The overall acceptability (OAA) score was significantly affected by sugar ($p < 0.01$), ammonium bicarbonate ($p < 0.01$) and water ($p < 0.05$) at linear level (Table 3). OAA score increased with increase in the level of sugar whereas it decreased at higher levels of ammonium bicarbonate and water. The interaction of sugar and composite fat had a significant effect ($p < 0.1$) on OAA. The score of OAA increased significantly at the centre value of sugar and fat replacer (Figure 1f). The quadratic terms of all parameters had insignificant effect on OAA score.

Table 5 shows the combined effect of all independent variables on all the sensory parameters at linear, quadratic and interaction level. At linear level they affected OAA score at $p < 0.01$. At quadratic level, they significantly affected all the parameters at $p < 0.01$ whereas at interactive level, the effect was non-significant on OAA score.

Physical characteristics of optimized low fat biscuit containing Simplesse

After optimization of ingredients as described above, control biscuits containing normal fat content and optimized biscuits having reduced fat level were compared as shown in Table 6. It was observed that the diameter, thickness, spread ratio, hardness and stress-strain ratio of optimized product differed significantly ($p < 0.05$) from the control biscuits. This was attributed to different levels of ingredients (sugar, fat, ammonium bicarbonate and water) in the optimized product. Biscuits made from Simplesse had smaller diameter than control biscuits whereas these low fat biscuits had higher hardness than



Products	Diameter (cm)	Thickness (cm)	Spread ratio	Hardness (N)	Stress-strain ratio
Control biscuit	5.98 ± 0.01 ^a	0.62 ± 0.00 ^a	9.64 ± 0.01 ^a	32.52 ± 1.53 ^a	0.73 ± 0.08 ^a
Low-fat biscuit	5.91 ± 0.01 ^b	0.63 ± 0.001 ^b	9.37 ± 0.02 ^b	56.26 ± 3.55 ^b	1.16 ± 0.3 ^b

*Average of six determinations.

a: ±α level, b: centre point

Table 6: Physical characteristics of different types of biscuits*.

Products	Moisture (%)	Crude protein (%)	Crude fat (%)	Total ash (%)	Crude fibre (%)	Carbohydrates (by difference) (%)	Calorific value (kcal/100 g)
Control biscuit	5.21 ± 0.06 ^a	7.57 ± 0.24 ^a (7.99)	21.47 ± 0.2 ^a (22.65)	0.99 ± 0.00 ^a (1.04)	0.57 ± 0.03 ^a (0.60)	64.19 ± 0.41 ^a (67.72)	480.28 ± 0.83 ^a (506.68)
Low fat biscuit	8.19 ± 0.03 ^b	10.07 ± 0.12 ^b (10.97)	11.98 ± 0.09 ^b (13.05)	1.38 ± 0.00 ^b (1.51)	0.82 ± 0.03 ^b (0.89)	67.54 ± 0.18 ^b (73.56)	418.34 ± 0.46 ^b (455.66)

*Average of three determinations; Values given in parenthesis are on dry weight basis.

a: ±α level, b: centre point

Table 7: Chemical characteristics of different types of biscuits*.

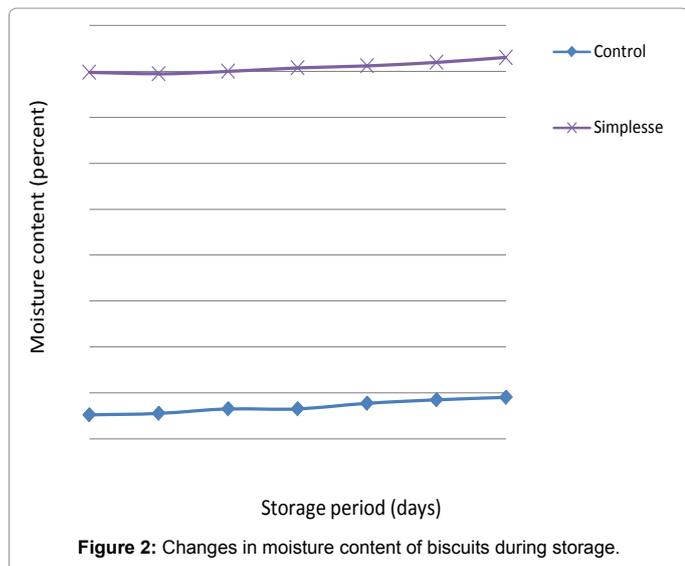


Figure 2: Changes in moisture content of biscuits during storage.

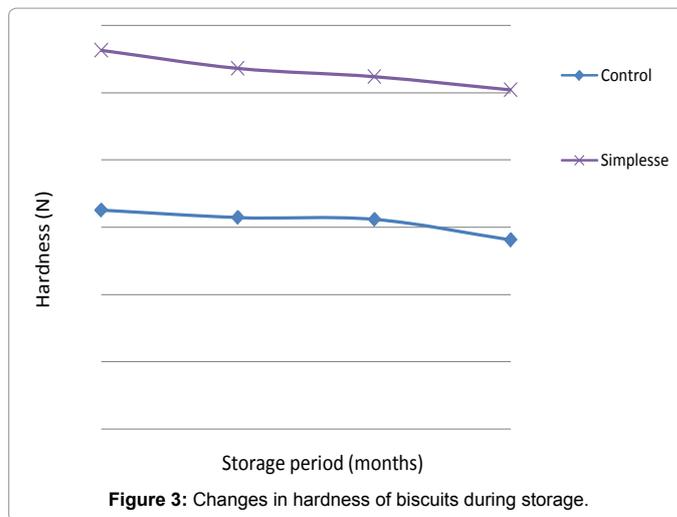


Figure 3: Changes in hardness of biscuits during storage.

control biscuits. This is attributed to the type of fat replacer and level of replacement of fat in biscuit formulation. Similar results were also reported by Zoulias [16].

Chemical characteristics of optimized low fat biscuit Simplese

The chemical composition of control biscuit and low fat biscuit containing Simplese is presented in Table 7. Moisture content (%) of low-fat biscuit containing Simplese was significantly ($p < 0.05$) different from control biscuits (Table 7). Biscuits made using Simplese had significantly higher crude protein content (10.07%) than the control. This was due to the fact that Simplese is derived from whey proteins which increased the protein content of the biscuits. The optimized product had significantly lower amount of fat ($p < 0.05$) than control (Table 7). The amount of fat in control and low fat biscuit containing Simplese was 21.47 and 11.98%, respectively. The percentage replacement found in optimized products made from Simplese was 44%.

Storage study of biscuits

The control and low-fat biscuit (containing protein based fat replacers) were packaged in LDPE bags and evaluated for 90 days of storage (15-25°C) by determining their moisture content, hardness, per cent free fatty acid (FFA), peroxide value (PV) and sensory quality (taste, flavour, OAA scores). There was a significant ($p < 0.05$) increase in moisture content of both control and low fat biscuit during storage as shown in Figure 2. Similar findings were also reported by Rajiv

[17]. It was found that biscuits made from Simplese did not differ significantly in hardness during storage for 3 months (Figure 3). Table 8 shows the changes in peroxide value of the control and low fat biscuit. The low fat biscuit had lower PV as compared to control during storage which might be due to less amount of fat present in biscuits containing protein based fat replacers. A similar trend was seen in case of changes in FFA of biscuits during storage (Table 9).

Changes in taste, flavour and overall acceptability (OAA) scores of biscuits during storage are shown in Figures 4-6. Low fat biscuits made from Simplese showed no significant difference in taste (Figure 4) and flavour scores (Figure 5) throughout storage period but in case of control biscuit the decrease in these scores was observed [18-22]. The OAA score of low fat biscuit containing Simplese was found higher than the control biscuit during storage as shown in Figure 6.

Conclusion

Response surface methodology was effective in optimizing processing parameters for the manufacture of low fat biscuits. The regression analysis yielded models that were used for obtaining optimum conditions for desired responses within the range of conditions applied in this study. Model analysis, which included checking the validity of the model with the help of various relevant statistical ids, such as - F-value, coefficient of determination (R^2) and adequate precision, revealed that all the models were statistically adequate. Sugar and composite fat had a significant effect on all the physical responses whereas overall acceptability was significantly affected by all the parameters. The textural characteristic of low-fat biscuits produced with protein-based

Products ^b	Storage period (days)								SEM	CD at 5%
	0	15	30	45	60	75	90			
A	1.44 ± 0.03	1.70 ± 0.01	1.83 ± 0.01	2.43 ± 0.12	3.20 ± 0.26	4.15 ± 0.15	5.11 ± 0.1	0.07	0.23	
B	0.82 ± 0.02	1.22 ± 0.01	1.44 ± 0.02	2.44 ± 0.03	4.06 ± 0.05	4.20 ± 0.02	4.53 ± 0.06	0.02	0.06	

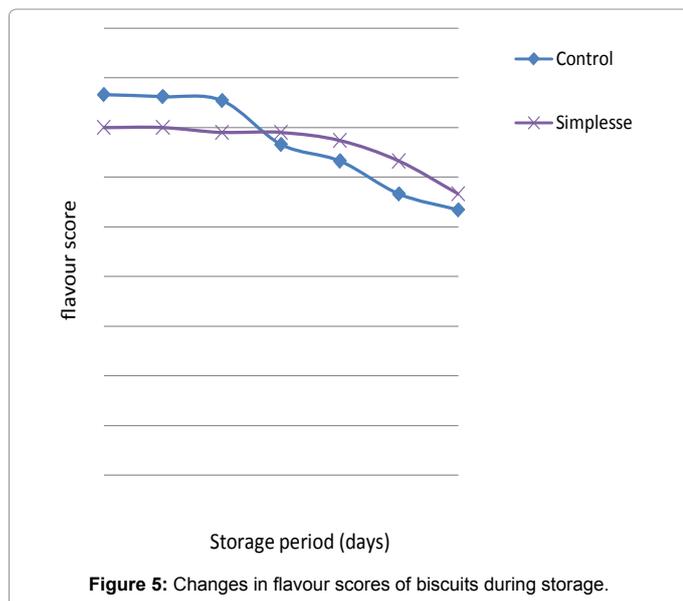
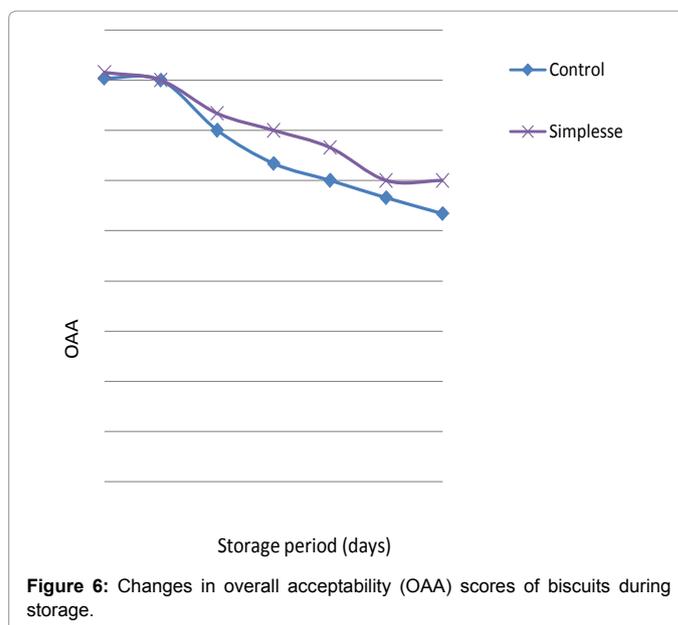
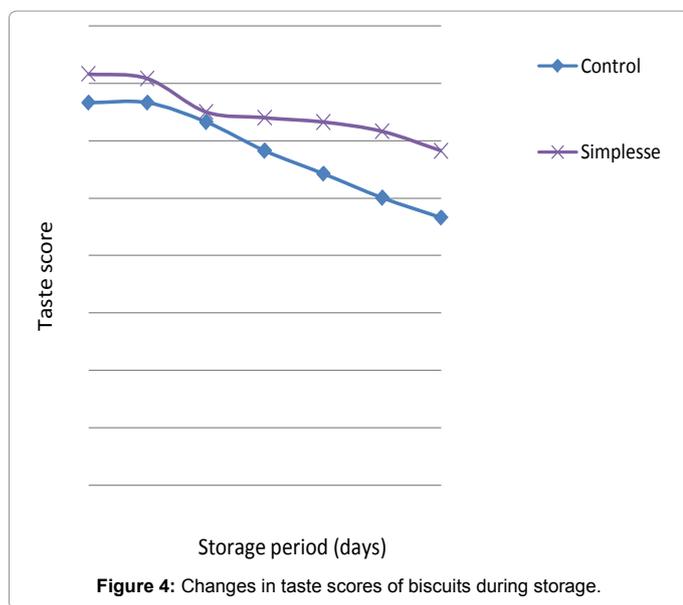
^aAverage of three determinations
^bA: Control biscuits
 B: Low-fat biscuit containing Protein based fat replacers (Simplese)

Table 8: Changes in peroxide value (meq of O₂/kg of fat) of biscuits during storage^a.

Products ^b	Storage period (days)								SEM	CD at 5%
	0	15	30	45	60	75	90			
A	0.031 ± 0.00	0.055 ± 0.00	0.066 ± 0.00	0.073 ± 0.00	0.076 ± 0.00	0.084 ± 0.00	0.220 ± 0.03	0.006	0.018	
B	0.035 ± 0.00	0.046 ± 0.00	0.098 ± 0.00	0.114 ± 0.00	0.120 ± 0.00	0.126 ± 0.00	0.130 ± 0.00	0.001	0.003	

^aAverage of three determinations
^bA: Control biscuits
 B: Low-fat biscuit containing Protein based fat replacers (Simplese)

Table 9: Changes in free-fatty acid content (%) of biscuits during storage^a.



fat replacer was described by two values provided from hardness and stress-strain ratio. These values depend on the fat and fat replacer content of biscuits. A simple regression analysis of full second order model of hardness and stress-strain ratio as functions of the fat replacer content was developed and validated. According to this model a simple reduction of the fat content of biscuits results in an increase of hardness and stress-strain ratio. The brittleness of the low-fat biscuits was also found to increase with the addition of protein-based fat replacer. The optimized low fat product had 44% replacement of fat with Simplese. From storage study it was concluded that the low fat biscuits were more oxidative stable and acceptable than control biscuits.

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