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RESEARCH PAPER

Development and Evaluation of Sugar Free Biscuit Prepared by Fenugreek Seed Powder and Natural Sweetener Stevia and Process Optimization by Response Surface Methodology

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ABSTRACT

Sugar free biscuit were developed by fortifing with fenugreek seed powder and with sugar replaced with natural sweetener stevia. The functional component of fenugreek seed powder is trigonelline which is helpful in the production of insulin. In the present research work, 31 trials were performed by taking four factors viz; skimmed milk powder (SMP), stevia, butter, and fenugreek seed powder and the responses were analysed by RSM for optimization of developed sugar free biscuits. The ingredients used to prepare sugar free biscuits were 0.5 g to 6.5g SMP, 0.5g to 5.5 g stevia, 10 to 50 g butter and fenugreek seed powder 0.5 to 6.5%. Taking into account, these four factors optimization was done by RSM. The optimized parameters for developed sugar free biscuits includes SMP (1.7727 %), Stevia (4.3485 %), butter (37.8788 %) and fenugreek seed powder (0.5 %).After optimization of sugar free biscuit, diameter, thickness and spread ratio was recorded as 5.04 cm, 0.82 cm, and 4.92 cm, respectively. The final optimized product contained carbohydrate (76.75 %), protein (5.90 %), fat (14.85), ash (~ 1%), moisture (4%) and crude fibre (1.5 %).

Keywords: SMP, fenugreek seed powder, stevia, sugar free biscuit, optimization

Biscuit manufacturing is India's largest industry amongst food industries, with an estimated annual turnover of about ₹ 3000 billion. The bakery industries have been established in organized and unorganized sectors. Biscuit is a diverse group of bakery product available in different varieties such as high and low in fat, high and low in sugar and more or less combinations. These can be made in different shapes, sizes, and textures (Saghir and Mushir, 2014).

Diabetes is a very serious problem all over the world in general, particularly in India. The sugar free biscuits are not easily available in Indian market. Fenugreek seed powders have a functional component "trigonelline" which helps in the insulin production. *Trigonella* is most widely cultivated species. Various portions of fenugreek plants, including seeds, leaves, and extracts, have been extensively used as anti-diabetics. Sharma (1986) reported that *in-vivo* experiments showed possible hypoglycemic and hypolipidemic properties of fenugreek seed powder when taken orally. From the ancient time, fenugreek has been utilized as a traditional medicine to treat a wide range of diseases and result have shown that fenugreek seed powder decreased body fats and was effective in obesity (Ptropoulos 2012). Trigonelline content is the active pharmacological constituent of fenugreek and constitutes approximately 0.1-0.15%

of the seed weight (Souvair *et al.* 1998). Talwalkar (1962) and, Atal *et al.* (1964) observed the other components of fenugreek viz; steroids, alkaloid, polyphenolic substances, volatile constituents, and amino acids. Trigonelline is a plant hormone that is widely distributed in plants within the subclass Dicotyledonae (Allred *et al.* 2009). Trigonelline has been reported to have hypoglycemic, hypolipidemic, sedative, anti-migraine, antibacterial, antiviral, and anti-tumour effects, and helps to improve memory retention and inhibit platelet aggregation (Zhou *et al.* 2012).

Stevia (*Stevia rebaudiana*) is a small shrub native to subtropical and tropical South America and Central America (North to Mexico, Paraguay and Brazil). Native Indians of the Guarani tribe appear to have used the leaves of this herb as a sweetener since pre-Columbian times. It is also called as sweet leaf or sugar leaf and is a genus of about 150 species of herbs and shrubs. In 1887, a South American natural scientist named Antonio Bertoni first discovered it. Different glycosides extracted from the stevia were named steviosides, rebaudiosides and dulcoside (Pomaret and Lavieille, 1931). It is having many of the therapeutic properties to prevent of diabetes. RSM is collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (out variable) which is influenced by several independent variables (input variables).

In the bakery industry it can be used to minimize the number of baking trials while gathering all information relating to ingredient interaction and quality characteristics (Lorezen *et al.* 1993).

MATERIALS AND METHODS

The ingredients used were purchased from the local market of Varanasi India viz., Stevia, Fenugreek seed powder, SMP, (Skimmed milk powder), Wheat flour (Maida), Butter, Pastry Roller, Pastry Board, Biscuit Cutter, others.

Statistical Optimization

Response surface Methodology which involves designing of experiments, selection of levels of variables in experiments runs, fitting mathematical models and finally selection levels of variables by optimization the response was used in the study (Khalil *et al.* 1999). Central Composite Rotable Design (CCRD) was used to design the experiments comprising three independent processing parameters or factors (Lorezen *et al.* 1993) in all, 31 trials were conducted for the optimization of different composition of sugar free biscuits. The experiments were conducted in randomized order to minimize the effect of unexpected variability in the observed responses because of extraneous factors. The experimental design and the codes for the processing parameter or factor are detailed here. Response surface analysis required coding of the values of the processing parameters are fenugreek seed powder, stevia, SMP and Butter. There Lower and Upper Limits has been shown below:

Table 1: Factors of RSM for development of sugar free biscuits

Factor	Name	Low	High
A	Fenugreek seed powder	0.5	6.5
B	Stevia	1	4
C	SMP	0.5	6.5
D	Butter	10	50

The rest of the ingredient used in manufacturing of sugar free biscuit were kept constant and their quantities are as given below**:**

Baking powder- 1gram**,** Maida- 100 gram, Water – As per requirements

Optimization

The processing parameters or factors were optimized with respect to the responses viz. Colour and appearance (C and A), Flavour, Overall Acceptability (OA), Hardness and Fracturability of the biscuit. Numerical optimization technique of the Minitab 17 software was used for simultaneous optimization of the multiple responses. The desired goals for each processing parameter or factor and response were chosen. The goals may be applicable to either

processing parameter or factor and responses. The possible goals or constraints are; Maximize, Minimize Target and within Range and set to an exact value (for processing parameters or factors only). In order to search a solution, called the desirability function; the maximum value of the function is unity. The response surfaces help to understand the effect of varying the processing parameter or factors upon response, i.e. in which direction the response is increasing or decreasing. Response surfaces were generated with the help of statistical package (Minitab 17).

Table 2: Experimental design for manufacturing of Sugar Free Biscuit by using RSM

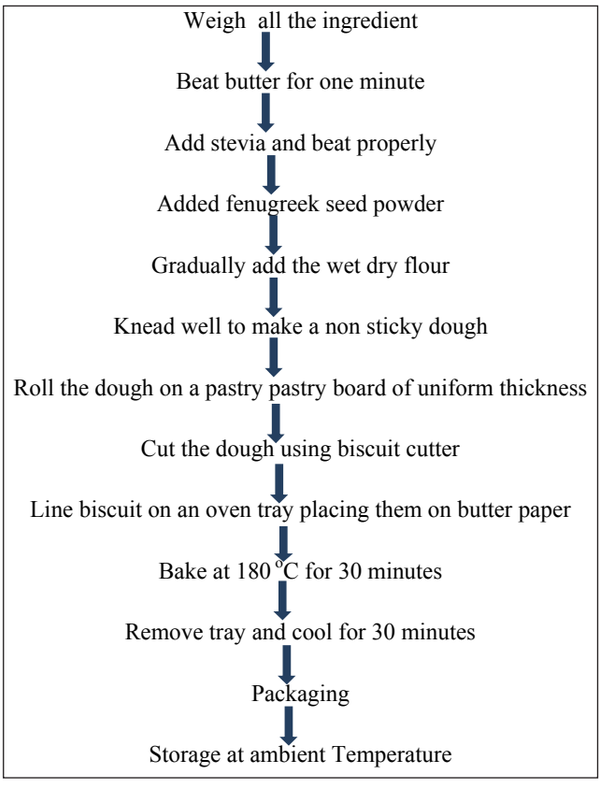
Factor 1		Factor 2	Factor 3	Factor 4
Run	**SMP (%)**	**Stevia (%)**	**Butter (%)**	**F. S .P (%)**
1	2.0	1.0	40	2.0
2	3.5	2.5	30	3.5
3	2.0	4.0	40	5.0
4	0.5	2.5	30	3.5
5	5.0	1.0	20	5.0
6	3.5	2.5	50	3.5
7	3.5	2.5	30	3.5
8	5.0	1.0	40	2.0
9	5.0	1.0	40	5.0
10	3.5	2.5	30	0.5
11	2.0	1.0	40	5.0
12	3.5	5.5	30	3.5
13	3.5	0.5	30	3.5
14	6.5	2.5	30	3.5
15	5.0	4.0	40	5.0
16	3.5	2.5	30	3.5
17	5.0	4.0	40	2.0
18	2.0	4.0	20	5.0
19	3.5	2.5	30	3.5
20	2.0	1.0	20	2.0
21	3.5	2.5	30	6.5
22	2.0	4.0	20	2.0
23	5.0	4.0	20	2.0
24	3.5	2.5	30	3.5
25	3.5	2.5	30	3.5
26	2.0	1.0	20	5.0
27	5.0	1.0	20	2.0
28	5.0	4.0	20	5.0
29	3.5	2.5	30	3.5
30	2.0	4.0	40	2.0
31	3.5	2.5	10	3.5

Abbreviation: *Skimmed milk Powder (SMP); Fenugreek Seed powder (FSP).*

Preparation of Sugar Free Biscuit

An Electric Beater was used to beat the butter for about a minute, after which the stevia gradually added and beating was done till it was light fluffy for 5 minutes. The fenugreek seed powder, SMP, and baking powder were then added and beating was done for 30 second until a smooth wet mixture was formed.

Flow Diagram of manufacturing Sugar free biscuit in reached with Stevia and FSP



The wheat flour was weighed, sieved and mixed well. Gradually the wet mixture was added to the dry

flour mixture in a bowl and kneaded gently to make soft non sticky dough. The dough was then cut into equal round shapes with uniform thickness using a biscuit cutter and placed prior preheated oven to bake at 180 °Ċ for 30 minutes. After baking the biscuit were taken out of the oven and allowed to cool. For manufacturing of sugar free biscuit step by step given in the above flow chart.

Fig. 1: Ready for baking sugar free biscuit

Fig. 2: Final Baked

Physico-chemical and Sensory Analysis

Textural Profile analysis

Hardness and fracturability of sugar free biscuit was measured by Texture Analyser (TA.XT Plus texture profile analyser stable Micro System, UK). The peak force (g) and force mean distance at (mm) were recorded.

Table 3: Parameter used for measurement of hardness and fracturability by Texture Analyser

Sl. No.	T.A. Setting	Measure Force in Compression
1	Pre-test speed	1.50mm/sec
2	Test speed	5mm/sec
3	Post-test speed	10.00mm/sec
4	Target mode	Distance
5	Distance	5.00 mm
6	Trigger type	Auto force
7	Trigger force	50.0g
8	Break	Off
9	Stop plot	Start position
10	Tare mode	Auto
11	Advance option	On

Crude protein (AOAC, 2004): wo grams of biscuit sample was weighed in Kjeldahl digestion flask and 15g digestion mixture (Na_2SO_4/K_2SO_4 + 1g $CuSO_4$) was added. 25 ml conc. $H_2SO_2$was also added. The content was boiled vigorously, until the appeared clear or transparent. Heating was continued for 2-3 hours.

The digested sample was taken in to a conical flask filled with 25ml 4% Boric acid (neutralized with a mixture of methyl red and in ratio of (5:7) and then flask was placed in distillation chamber. After that, the sample was diluted and alkali was added till the sample change the colour to brown, then the distillation chamber was allowed to run for 10 minutes. After completion of 10 minutes, the conical flask was taken out from the distillation chamber and titrated against 0.1N HCL.

Fat Analysis (AACC, 2000): Five gram of biscuit sample was taken in a thimble and then placed in previously weighed soxhlet beaker. The beaker was then placed in the extractor. After that extractor was filled with petroleum ether and their top were covered with cotton plugs. The soxhlet apparatus was then switched on with a set temperature of 70 °C for half an hour .after completion of extraction, temperature was increased up to 130 °C for 10 minute. For the complete removal of moisture. The cooled beaker were then removed from the apparatus and cooled in dessicator. The beakere were then weighed.

Ash Content (AACC, 2004): Five gram biscuit sample was completely homogenized and then taken in a silica crucible. The crucibles were then placed on a hot plate at 130 °C till smoke disappeared. The crucibles were then placed in to muffle furnace at 550 °C (2-3 hours). Weight of the cooled crucibles was then noted down and calculated as per the routine practices.

Moisture (AACC, 2004): In washed, preheated, cooled and weighed empty silica crucible, 2 gram of samples were weighted in duplicate. The crucibles were then placed in preheated, hot air oven at 100±5 °C for 24 hours. After drying, the crucible were cooled in the desiccator and weighed.

Crude Fibre (AACC, 2004): It was carried out by taking 3 g of each fat free flour sample and digested first with 1.25% H_2SO_4, washed with distilled water and filtered. Then ignited the sample residue by placing the digested sample in a muffle furnace maintained for 3-5 hour at temperature of 550-650 °C till or white ash was obtained. The percentage of crude was calculated after igniting the sample.

Carbohydrate (AOAC, 1995): The carbohydrate content was determined by difference method that is by subtracting the measure protein, fat, ash, moisture and Crude fibre from 100 g of food.

Free Radical Scavenging activity by DPPH inhibition, For DPPH inhibition, Brand (1995). Biscuit sample was dried in an oven (40 °C) for 24 hrs. The dried material as grounded and sieved through a 60 mesh-screen to obtain in powdered biscuit. The powdered biscuit was extracted with 80% aqueous ethanol (1 gm per 10 gm) for 2 hrs of shaking at 37 °C. The samples were then centrifuged at 10000 rpm for 15 min. The supernatant collected was used in the essay. DPPH radical solution was prepared by dissolving 10 mg of DPPH in 25 ml of 80 % ethanol blank was prepared by 250µl ethanolic DPPH solution and 2.1 ml of 80% ethanol. 100µl of biscuit extracts were taken and to it 250 µl of DPPH solution and 2.0ml of 80% ethanol were added. Mixture was shaken vigorously and allowed to stand in the room temperature for 20 minutes. The decrease in absorbance of the resulting solution was monitored spectrophotometrically at 517 nm.

Sensory Evaluation: The sensory quality of Sugar free biscuits sample was judge by a panel of five judges. The sample of each trial was evaluated for sensory attributed viz. colour, appearance and Overall acceptability by an experienced panel comprising of five judges selected from the Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi with the help of 9 point Hedonic scale.

RESULTS AND DISCUSSION

Optimization of sugar free biscuits fortified with fenugreek seed powder and natural sweetener stevia by using RSM, (Minitab 17, e-academy software) was applied for the optimization of sugar free biscuits and the parameters taken into consideration were Stevia, SMP, Fenugreek seed powder, and Butter. The equation used was second order polynomial models. Table 4 shows the central composite rotable design (CCRD) for the optimization of sugar free biscuit.

Effect of Ingredient on DPPH Inhibition of Sugar Free Biscuit

The average DPPH inhibition of sugar free biscuit varied from 2.7845 to 64.5278 (Table 4). The minimum and maximum DPPH inhibition was obtained for experiment number 4 and 21, respectively. In experiment number 4, the level of SMP, stevia, butter and fenugreek seed powder 0.5, 2.5, 30, 3.5, respectively while in experiment number 21 the level of SMP, Stevia, butter and fenugreek seed powder

Table 4: Central composite Design (CCRD) by RSM for the optimization of sugar free biscuits

Run Order	SMP (%)	Stevia (%)	Butter (%)	FSP (%)	C&A	Flavor	OA	Hardness (Kg/cm^2)	Fracturability (mm)	% DPPH Inhibition
1	2	1	40	2	7.6	6.6	7	3.0165	43.9065	35.7142
2	3.5	2.5	30	3.5	7.4	7	7.5	2.655	41.4915	3.1476
3	2	4	40	5	7.8	7.8	8	4.2525	41.8485	10.8958
4	0.5	2.5	30	3.5	7	7.2	7.6	1.159	42.3765	2.7845
5	5	1	20	5	7.2	7	7.8	7.378	44.053	19.6125
6	3.5	2.5	50	3.5	6.2	7.4	7	0.593	42.21	6.4164
7	3.5	2.5	30	3.5	7.8	8	8	4.316	43.8925	54.2372
8	5	1	40	2	7.6	7.3	7	5.0725	44.1305	37.4092
9	5	1	40	5	8	7.2	7	1.795	42.0575	28.6924
10	3.5	2.5	30	0.5	7.6	7.6	7.4	2.7395	41.5265	21.0653
11	2	1	40	5	8.5	8	8	2.1745	43.1655	23.7288
12	3.5	5.5	30	3.5	7.8	7.4	7.5	3.2745	43.5265	30.6295
13	3.5	-0.5	30	3.5	8	8	8	3.6295	44.4765	41.7719
14	6.5	2.5	30	3.5	7.8	7.2	7	6.3955	42.48	36.8038
15	5	4	40	5	8	7	7	1.656	43.7465	33.6561
16	3.5	2.5	30	3.5	7.6	7	7	1.622	43.0375	37.5302
17	5	4	40	2	8	7.5	7.4	1.5145	43.6165	46.0048
18	2	4	20	5	7.8	7.4	7.6	1.7555	43.603	36.8038
19	3.5	2.5	30	3.5	8	7.4	8	3.394	44.8695	51.937
20	2	1	20	2	7.6	7	7.2	7.628	43.8575	34.0193
21	3.5	2.5	30	6.5	8	7.7	8	4.074	41.1045	64.5278
22	2	4	20	2	8	8	7.8	4.4055	42.6185	28.2082
23	5	4	20	2	8	7.4	7.3	7.831	43.062	38.0145
24	3.5	2.5	30	3.5	7	6	6	4.5675	41.6825	36.5617
25	3.5	2.5	30	3.5	7.5	7.2	7	5.059	41.653	19.7336
26	2	1	20	5	7	6	6.5	6.5915	41.529	1.6949
27	5	1	20	2	6.8	6.5	6.8	4.6335	43.9815	1.5738
28	5	4	20	5	8	7.6	7	5.5805	41.7925	41.888
29	3.5	2.5	30	3.5	8	7.8	8	7.535	42.663	33.8983
30	2	4	40	2	8.5	8	8	1.417	42.29	45.6416
31	3.5	2.5	10	3.5	6.5	6.4	6	6.869	42.5315	29.1767

was 3.5, 2.5, 30, 6.5 respectively. The data fitted the following Quadratic Model.

–31.3 - 0.0 SMP (%) + 6.1 Steviia (%) + 3.83 Butter (%) – 5.1 Fenugreek Powder (%) – 1.58 SMP (%) *SMP (%) + 0.25 Steviia (%) *Steviia (%) 0.0405 Butter (%) *Butter (%) + 0.98 Fenugreek Powder (%) *Fenugreek Powder (%) + 1.27 SMP (%) *Steviia (%) + 0.123 SMP (%) *Butter (%) + 1.98 SMP (%) *Fenugreek Powder (%) 0.322 Steviia (%) *Butter (%) + 0.01 Steviia (%) *Fenugreek Powder (%) 0.275 Butter (%) *Fenugreek Powder (%).

Fig. 3 show the response surface plot for DPPH as influenced by the level of butter and skimmed milk powder (SMP). From the figure it can be observed that with increase in SMP there is an increase in the DPPH. There is a little effect on DPPH due to butter.

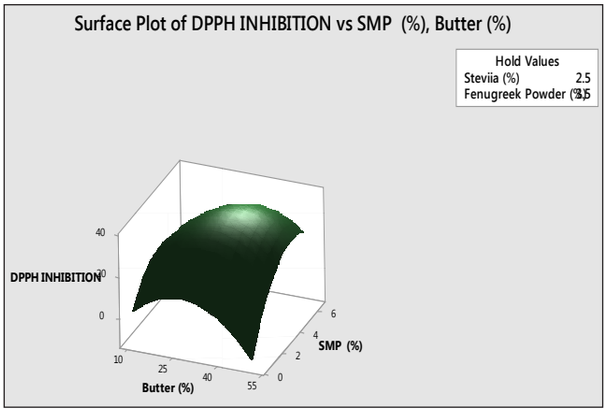


Fig: 3: Effect of butter and skimmed milk powder

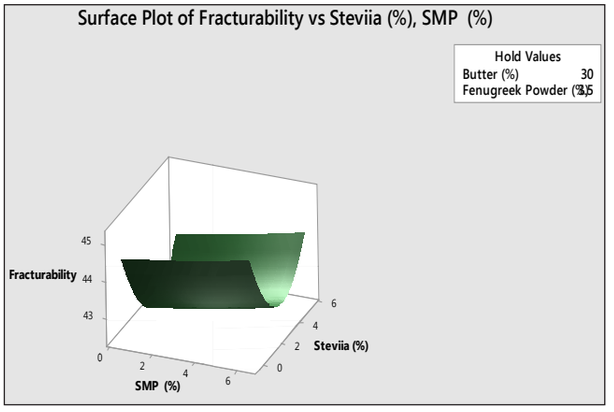


Fig. 4: Effect of SMP and Stevia

Effect of ingredient on fracturability of sugar free biscuit

The average Fracturability of sugar free biscuit varied from 41.1045 to 44.1305 (Table 4). The minimum and maximum Fracturability was obtained for experiment number 21 and 8 respectively. In experiment number 21, the level of SMP, stevia, butter and fenugreek seed powder 3.5, 2.5, 30, 6.5 respectively while in experiment number 8 the level of SMP, Stevia, butter and fenugreek seed powder was 5, 1, 40, 2 respectively. The data fitted the following Quadratic Model

Fracturability = 44.44 – 0.00 SMP (%) – 1.580 Steviia (%) – 0.012 Butter (%) + 0.48 Fenugreek Powder (%) + 0.0043 SMP (%) *SMP (%) + 0.1791 Steviia (%) *Steviia (%) 0.00005 Butter (%) *Butter (%) – 0.1193 Fenugreek Powder (%) *Fenugreek Powder (%) + 0.003 SMP (%) *Steviia (%)+ 0.0044 SMP (%) *Butter (%) 0.017 SMP (%) *Fenugreek Powder (%) + 0.0024 Steviia (%) *Butter (%)+ 0.124 Steviia (%) *Fenugreek Powder (%) - 0.0024 Butter (%) *Fenugreek Powder (%).

Fig. 4 shows the response surface plot for fracturability as influenced by the level of Skimmed milk powder SMP and stevia. From figure it can observed that with increase in SMP there is an increase in the fracturability and there is very little effect on fracturability due to stevia.

Effect of ingredients on hardness of sugar free biscuit

The average hardness of sugar free biscuit varied from 0.593 kg/cm^2 to 7.831kg/cm^2 (Table 4). The minimum and maximum hardness was obtained for experiment number 6 and 23 respectively. In experiment number 6, the level of SMP, stevia, butter and fenugreek seed powder 3.5, 2.5, 50, 3.5, respectively while in experiment number 23 the level of SMP, Stevia, butter and fenugreek seed powder was 5, 4, 20, 2, respectively. The data fitted the following Quadratic Model.

Hardness

7.95 + 0.87 SMP (%) – 1.10 Steviia (%) – 0.129 Butter (%) + 0.05 Fenugreek Powder (%) – 0.000 SMP (%) *SMP (%) – 0.036 Steviia (%) *Steviia (%) – 0.00012 Butter (%) *Butter (%) – 0.041 Fenugreek Powder (%) *Fenugreek Powder (%) + 0.147 SMP (%) *Steviia (%) – 0.0244 SMP (%) *Butter (%) – 0.026 SMP (%) *Fenugreek Powder (%) + 0.0143 Steviia (%) *Butter (%) + 0.014 Steviia (%) *Fenugreek Powder (%) + 0.0085 Butter (%) *Fenugreek Powder (%).

Fig. 5 shows the response surface plot for hardness as influenced by the level of Skimmed milk powder (SMP) and stevia. From figure it can observed that with increase of SMP there is increase in hardness and there is little effect on hardness due to stevia.

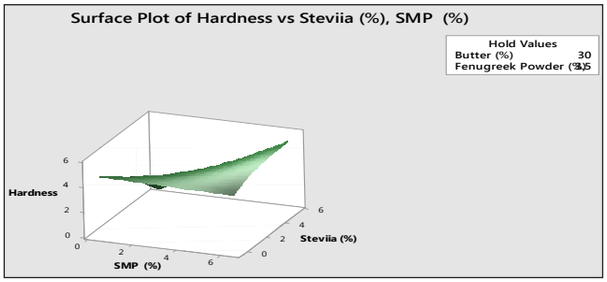


Fig. 5: Effect of SMP and Stevia

Effect of ingredient on overall acceptability of sugar free biscuit

The average overall acceptability of sugar free biscuit varied from to 6.5 to 8 (Table 4). The minimum and maximum overall acceptability was obtained for experiment number 6 and 30 respectively. In experiment number 6, the level of SMP, stevia, butter and fenugreek seed powder 3.5, 2.5, 50, 3.5, respectively while in experiment number 30 the level of SMP, Stevia, butter and fenugreek seed powder was 2, 4, 40, 2, respectively. The data fitted the following Quadratic Model.

Overall acceptability = 4.07 + 0.381 SMP (%) + 0.289 Stevia (%) + 0.1636 Butter (%) – 0.202 Fenugreek Powder (%) – 0.0045 SMP (%) *SMP (%) + 0.0455 Steviia (%) *Steviia (%) – 0.00210 Butter (%) *Butter (%) + 0.0399 Fenugreek Powder (%) *Fenugreek Powder (%) – 0.0722 SMP (%) *Steviia (%) – 0.01000 SMP (%) *Butter (%) + 0.0056 SMP (%) *Fenugreek Powder (%) – 0.00000 Steviia (%) *Butter (%) – 0.0611 Steviia (%) *Fenugreek Powder (%) + 0.00333 Butter (%) *Fenugreek Powder (%)

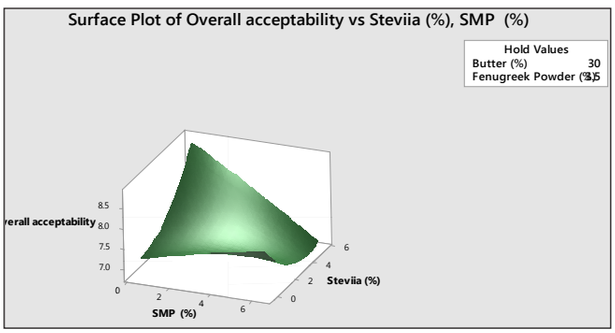


Fig. 6: Effect of SMP and Stevia

Fig. 6 show the response surface plot for overall acceptability as influenced by the level of SMP and stevia. From the figure it can observed that with increase SMP there is increase in the overall acceptability and little effect on the overall acceptability due to stevia.

Sensory Evaluation: The sensory quality of final optimized Sugar free biscuits were evaluated by judges on the 0-9 point hedonic rating scale viz; colour and appearance (7.6), flavour (8.4197), overall acceptability (8.6465) which is given in the Table 5 which was acceptable to consume.

Table 5: Optimized parameter are given below by RSM for the development of sugar free biscuits

Factors		Responses							
SMP	Stevia	Butter	FSP	C and A	Flavour	OA	H	F	DPPH Inhibition
1.7727	4.3485	37.8788	0.50	7.6	8.4197	8.6465	0.9788	41.8247	40.8247

Abbreviation: *OA = Overall Acceptability, C and A = Colour and Appearance, F = Fracturability, H = Hardness, FSP = Fenugreek seed powder.*

Physical characteristics of developed sugar free biscuit

Data for physical analysis of sugar free biscuit is given in the Table 6 spread ratio, thickness and diameter.

Table 6: Physical characteristics of sugar free biscuit are given below

Sl. No	Physical characteristics	Value (cm)
1	Diameter	5.04
2	Thickness	0.82
3	Spread ratio	4.92

Frcturability of sugar free biscuit

The Fracturability of final optimized sugar free biscuit was found 39.20 mm by Texture profile analyser using Biscuit cutting set knife Probe.

Hardness of sugar free biscuit

The hardness of final optimized sugar free biscuit was found 3.04 Kg by Texture profile analyser using Biscuit cutting set knife Probe.

DPPH inhibition

The DPPH inhibition of final optimized sugar free biscuit was found 38% and similar finding was reported Rao *et al.* (2017) where the plain biscuits have DPPH inhibition 36 %. It can be concluded that in the sugar free biscuit antioxidant properties is more than other normal biscuit which is available in the market.

Proximate analysis of sugar free biscuit

The proximate analysis of sugar free biscuits was performed for the composition of carbohydrate, protein, fat, ash, moisture and fibre (Table 7). During analysis of sugar free biscuits carbohydrate (76.75%), protein (5.90%), fat (14.85%), ash (~1%), fibre (1.5%) and moisture (4%). Carbohydrate percentage is very high in the sugar free biscuit due to refined wheat flour. And less in the protein due to use of butter and other ingredients Similar finding were also reported by Kamal *et al*. (2013) fortified biscuit carbohydrate (73%), fat (14%), protein (7.65%) and moisture (3%).

Table 7: Proximate composition of Optimized sugar free biscuit

Sl. No.	Constituents	Amount (g/100)
1	Protein	5.90
2	Fat	14.85
3	Ash	~1.00
4	Moisture	4
5	Carbohydrate	76.75
6	Fibre	1.5

CONCLUSION

The present research work "Development and Evaluation of sugar free biscuit prepared by fenugreek seed powder and natural sweetener stevia and process optimization by Response surface methodology" was carried out and 31 trials were performed with taking four factors Viz; Skimmed milk powder (SMP), stevia, butter, and fenugreek seed powder responses and analysed by RSM for optimization of developed sugar free biscuits. After optimizing, proximate analysis, textural properties, were studied of the developed sugar free biscuits. The ranges were 0.5 g to 6.5g SMP, 0.5g to 5.5 g stevia, 10 to 50g butter and fenugreek seed powder 0.5 to 6.5. Taking into account, these four factors were optimized by using RSM design sets of experiments CCRD (Central Composite Rotable Design). The average score of the sensory characteristics colour and appearance varied from 6.2 to 8, flavour 6 to 8 and overall acceptability varied from 6 to 8. It is concluded from the sensory evaluation score that butter plays the major role in acceptability of the sugar free biscuit, stevia and fenugreek the major effect was seen in the flavour. The proximate analysis of optimized sugar free biscuits was performed and it was found that the final optimized product contain carbohydrate, protein, fat, ash, moisture and crude fibre. On the basis of proximate composition of prepared sugar free biscuit it could be concluded this product was fortified with fenugreek seed powder, non-nutritive natural sweetener stevia so recommend for diabetic patients.

REFERENCES

AACC. 2000. Approved methods of analysis. American Association of cereal chemist, Minnesota, USA.

AAOC. 2004. Approved laboratory methods, American Association of cereal chemist, Minnesota, USA.

AOAC. 1995. Official method of analysis of AOAC, international vol. II 6th edition, Verginia, USA.

Atal, C.K. and Sood, S.P. 1964. A phytochemical investigation of *Trigonella corniculata Linn*. *Journal of Pharmacy and Pharmacology*, **16:** 627-629.

Allred K.F, Yackley, K.M., Vanamala, J. and Allred, C.D. 2009. Trigonelline is a novel phytoestrogen in coffee beans. *Journal of Nutrition,* **139**(10): 1833-1838.

Khalil, A.H., Mansour, E.H. and Dawood, F.M. 1999. Influence of malt on rheological and baking properties of wheat flour LTW- *Journal of Food Science & Technology*, **30**(3): 159-164.

Mehruz Kamal *et al*. 2013. The College at Brockport State University of New York, Investigating the Effects of Multitasking with Technology.

Petropoulos, GA. 2012. Fenugreek, The genus *Trigonella*. Taylor and Francis, London and New York, pp. 255.

Pomaret, M.M. and Lavieille, R. 1931. Le principle a saveursucree du kaa-hee-e (*Stevia rebaudiana Bertoni*). IV. Qualques proprietes physiologiques du stevioside. *Bull Society of Chemical Biology,* **13**: 1248-1253.

Rao, T.S.S., Ramanuja, M.N., Ashok and Vibhakar, H.S 1995. Storage properties of whole egg powder incorporated biscuits. *Journal of Food Science and Technology,* **32**(6): 470-476.

Saghir, Ahmad and Mushir Ahmed 2014. Department of Post-Harvest Engineering and Technology, Aligarh. Muslim University, Aligarh-202002.

Sharma, R. 1986. Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses in human subjects. *Nutrition Research*, **6**(12): 1353-1364.

Sauvaire, Y., Petit, P., Broca, C., Manteghetti, M., Baissac, Y., Fernandez- Alvarez, J. Gross, R., Roye, M. Leconte, A. Gomis, R. and Ribes, G. 1998. 4- Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. *Diabetes*, **47**(2): 206-210.

Talwalkar, R.T. and Patel, S.M. 1962. Nutritive value of some leaf-proteins. I. Aminoacid composition of (*Trigonella foenum-graecum)* and (*Hibiscus cannabinus*. *Ann. Biochem). Journal of Experimental Medicine*, **22**: 289-294.

Zhou, J. Zhou., S. and Zeng, S. 2012. Experimental diabetes treated with trigonelline: effect on beta cell and pancreatic oxidative parameters. *Fundam. Clin. Pharmacol.,* DOI: 10.1111/j.1472-8206.2011.01022.x.