Optimization of ingredients for formulating a diabetic dietary supplement

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Abstract A diabetic dietary supplement comprising of multiple ingredients was designed based on recommendation of Indian Council of Medical Research for a diabetic adult. Central composite rotatable design using three variables (ingredient source) and five responses comprising of sensory and physico-chemical attributes were used for computation of an optimized solution. All the responses fitted well into quadratic equation with $R^2 > 0.80$. The optimum levels of ingredient combinations recommended with 93% desirability were obtained. A total of 27 combinations were prepared and evaluated. The dietary supplement comprising of milk fat and groundnut oil (90:10), whey protein concentrate and sodium caseinate (50:50) and resistant starch and maltodextrin (70:30) were selected on 100 point sensory scale. The prepared supplement using recommended levels of ingredients contained 4.37 moisture, 15.93 protein, 10.15 fat, 66.15 carbohydrate and 3.39% ash.

Keywords Diabetic · Dietary supplement · Resistant starch · Response Surface Methodology

Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion or insulin action or both (ICMR 2005). It is growing at an alarming rate all over the world particularly in India, where it is estimated that around 30 million personnel are diabetic which is estimated to increase to 60 million by the year

2017 (Kutty and Raju 2010). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels (ICMR 2005).

The present therapy for diabetes is based on lifestyle (diet and exercise) and pharmacological interventions (Florkowski 2002; Capriotti 2005). Antidiabetic drugs act either on the β -cells to increase insulin secretion, or on peripheral tissues to decrease insulin resistance, or decrease intestinal glucose uptake (α -glucosidase inhibitors such as acarbose) or inhibition of gluconeogenesis (Abuissa et al. 2005; Marchetti 2005). Although insulin deficiency has been prevented through medical interventions, various life threatening side effects are also associated with medication (Spiller and Sawyer 2006; Tuomilehto and Wareham 2006).

As an alternative to medicine, oral nutritional supplements are in vogue recently, particularly to treat chronic rather than acute life-threatening disease states (Dham et al. 2006). Moreso, the diabetes patients frequently face nutrient intake imbalances due to food restrictions. Thus, these patients may benefit from nutritional support. In recent past, the area of special dietary products and their consumption have received considerable attention due to increased consumer awareness towards health improvements. Nutraceuticals are also gaining importance as a dietary adjunct for preventing various diseases (Mandal 2007). Presently very few dietary supplements are available for diabetic patients in the market and most of these formulations contain constituents that are found to metabolize rapidly and thus could result in rapid rise of blood glucose levels.

An attempt was therefore, made to standardize formulation of a dietary supplement using multiple ingredient approach through Central composite rotatable design (CCRD) for optimizing the levels of different ingredients that would

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provide an acceptable dietary supplement in relation to its physico-chemical and sensory attributes.

Material and methods

Fibersym® RW resistant wheat starch was procured from MGP, Ingredients Inc. Kansas (U.S.A) while the Maltodextrin (DE- 22–25) was supplied from M/s Rai Agro Industries Ltd., Sangrur (Punjab). Whey protein concentrate (WPC-70) and skim milk powder were purchased from Modern Dairy Private Ltd., Karnal (Haryana). Sodium caseinate was purchased from Avani Food Products, Mehsana (Gujarat). Ground nut oil (Amrit Banaspati Co. Ltd, Rajpura) was obtained from the local market. All the chemicals used in the analysis were of analytical grade from Central Drug house, New Delhi.

Experimental design In order to arrive at the optimum ingredient combination required for formulation of an anti diabetic supplement confirming to RDA requirements for an adult diabetic patient, the central composite rotatable design (CCRD) was used. The experimental plan consisting of 3 variables (fat, protein and carbohydrate) at 5 different levels have been drawn as shown in Table 1. The response functions (y) used were: flavor (FL), appearance (for both dried [AP (D)] and reconstituted [AP (R)] supplement), color (CL) and overall acceptability (OA), free fat (FF) (%), dispersibility (DIS) (%) and sedimentation (SED) (ml). These responses were related with the coded factors by a second-degree polynomial (Eq. 1) using the method of least squares (Sendecor and Cochran 1968).

$$Y = b_0 + b_1A + b_2B + b_3C + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 + b_{12}AB + b_{13}AC + b_{23}BC + \varepsilon \quad (1)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1 , b_2 , b_3 (linear terms), b_{11} , b_{22} , b_{33} (quadratic terms) and b_{12} , b_{13} , b_{23} (interactive terms) and ε (random error). The variables were standardized for computation and deduce the relative effect of variables on

the response. A polynomial equation was used to obtain regression equation.

Preparation of fat blend Predetermined amounts of milk fat and groundnut oil were mixed and heated to 40–45 °C to which calculated amount of emulsifier was added and mixed properly in a slow speed mixer.

Preparation of dry mix To the freshly prepared fat blend, calculated amounts of sodium caseinate, whey protein concentrate, skim milk powder, maltodextrin and resistant starch were dry blended using Hobart mixer. The sequence of ingredients mixing was followed by 10 min interval.

Sensory evaluation The dry mix as well as reconstituted dietary supplement (15 g of dry mix dissolved in 100 ml of warm water (40 °C) by continuous stirring) was subjected to sensory evaluation using 100 point scale (Bodyfelt et al. 1988), by a panel of 10 semi trained panelists drawn from faculty of Dairy Technology Division, National Dairy Research Institute, Karnal.

Physical properties Dispersibility of the supplement was determined using method of ADMI (1965), and sedimentation was determined using IS: SP: 18 (Part XI) (1981) method.

Chemical analysis The samples were analysed for proximate composition. Moisture and ash were determined using methods given in AOAC (1995). Protein and fat were determined by IS: SP: 18(Part XI) (1981). Carbohydrate was calculated by difference. Free fat was estimated by the method delineated by Hall and Hedrick (1971).

Statistical analysis The experimental data obtained was subjected to RSM using statistical package design expert version 8.0.1 and response surface plots were generated. The statistical significance of the terms in the regression equation was examined by analysis of variance (ANOVA). While three replications of the different variables has been statistically analysed.

Table 1 Coded and Actual levels of variables in RSM experiment

Level	Coded			Actual		
	Factor-1	Factor-2	Factor-3	Fat	Carbohydrate	Protein
−1.682	−1.682	−1.682	−1.682	8.64	56.64	25.64
−1.0	−1	−1	−1	10	58	27
0	0	0	0	12	60	29
+1.0	+1	+1	+1	14	62	31
+1.682	+1.682	+1.682	+1.682	15.36	63.36	32.36

Results and discussion

Basis of formulation The dietary supplement was designed to meet the requirement of a diabetic adult as per ICMR recommendations (1800 Kcal/day). Different sources of fat were chosen to supply maximum MUFA, adequate ratios of MUFA: PUFA and minimum saturated fat. Suitable protein sources to supply all essential amino acids and carbohydrate sources for controlled release of glucose (a pre-requisite for diabetes) and to makeup the calorific density of the formulation were used. The basic calculations were done based on energy supplied by various components as per ICMR recommendations. Different ratios of source ingredients were used, as variables, for optimization purpose applying RSM of CCRD. Sensory and physico-chemical attributes (essential for acceptability of dried products) were the response function (Henika 1982) for computation. The optimized product thus obtained was subjected to sensory evaluation to assess its suitability/acceptability both in dried as well as reconstituted form.

Optimization Based on preliminary trials, various levels of sodium caseinate, whey protein concentrate, skim milk powder, resistant starch, maltodextrin, and milk fat-groundnut oil mixture were integrated into RSM to determine

optimum levels. CCRD for three factors as independent variables was adopted to determine their optimum levels to elucidate effect of these variables on sensory and physical properties of the resultant product. CCRD design constituted of 20 experiments which were carried out in randomized order. The results are presented in Table 2. The data generated was analysed using Design Expert Software (8.0.1 version) and a present polynomial equation was obtained for each response. The second order equation was built to describe the response.

$$y = b_0 + \sum_i b_i x_i + \sum_i b_{ii} x_i^2 + \sum_{ij} b_{ij} x_i x_j$$

where y is the response, x is the factor, x_i are regression coefficient for linear effect, x_i^2 is the regression coefficient for quadratic effect, $x_i x_j$ is the regression coefficient for interaction effect and b is the coefficient for each term calculated by multiple regression analysis lack of fit was calculated. Model considered adequate when F (adequacy ratio) was more than Table F value and the coefficient of determination (R^2) that reflects the proportion of variability in data explained or accounted for by the model and a larger R^2 values suggest a better fit of model data. 3D plots and contour graphs were developed using 2nd order polynomial models. These were used to determine interaction between 2

Table 2 Experimental design for the optimization experiments as computed using RSM

Std. No	Coded values			Actual values		
	Factor 1 A: Fat (g)	Factor 2 B: Carbohydrate (g)	Factor 3 C: Protein (g)	Factor 1 A: Fat (g)	Factor 2 B: Carbohydrate (g)	Factor 3 C: Protein (g)
1	−1	−1	−1	10.00	58.00	27.00
2	−1	−1	−1	14.00	58.00	27.00
3	−1	1	−1	10.00	62.00	27.00
4	1	1	−1	14.00	62.00	27.00
5	−1	−1	1	10.00	58.00	31.00
6	1	−1	1	14.00	58.00	31.00
7	−1	1	1	10.00	62.00	31.00
8	1	1	1	14.00	62.00	31.00
9	−1.682	0	0	8.64	60.00	29.00
10	1.682	0	0	15.36	60.00	29.00
11	0	−1.682	0	12.00	56.64	29.00
12	0	1.682	0	12.00	63.36	29.00
13	0	0	−1.682	12.00	60.00	25.64
14	0	0	1.682	12.00	60.00	32.36
15	0	0	0	12.00	60.00	29.00
16	0	0	0	12.00	60.00	29.00
17	0	0	0	12.00	60.00	29.00
18	0	0	0	12.00	60.00	29.00
19	0	0	0	12.00	60.00	29.00
20	0	0	0	12.00	60.00	29.00

variables with regard to their effect on sensory and physical attributes. The effects of different variables on sensory and physical responses of the diabetic dietary supplement prepared using different combinations as per experimental designed are presented in Table 3.

Effect on flavor The average flavor scores of dietary supplement varied from 52.71 to 56.71. The minimum flavor score obtained for the combination 14, whereas the combination 6 had the highest flavor score as revealed in Table 3. The regression analysis of the data presented in Table 4 reveals that the coefficient of determination (R^2) was 0.8453 indicating that the model was significant. The ANOVA of quadratic model indicates that model F value of 6.07 was more than the tabulated F value. Furthermore the adequate precision value (APV) was found to be 8.486, which was higher than the minimum desirable (4.00) for high prediction ability. The statistically analysis therefore, proves that the model can be used successfully to describe the effect of variables on flavor of developed product. The coefficient estimates of flavor model showed that the level of fat had a highly significant ($p \leq 0.01$) positive effect and increasing the fat content enhanced the flavor score of the dietary supplement. High fat content is a desirable feature in many foods (Drewnowski 1990). The positive effect of

increasing fat content on pleasantness of strawberry yoghurt was reported by Tuorila et al. (1993). Kahkonen et al. (1995) also observed that higher the fat content, more intense was the flavor perceived in the cheese soup. The interaction between carbohydrate and protein level also had significant negative effect ($p \leq 0.05$) and thus higher flavor score was observed when the carbohydrate content was increased from 58 to 62 g and the protein content decreased from 31 to 27 g as depicted in Fig. 1a. The squared terms of carbohydrate had a positive significant effect ($p \leq 0.05$) and increasing the carbohydrate content enhanced the flavor attribute of the supplement. This may be due to the use of resistant starches as food ingredient which improves sensory properties such as a better mouthfeel, color, and flavor (Sajilata et al. 2006).

Effect on appearance

Dry supplement The average score for appearance of the dried dietary supplement ranged from 12.14 to 13.57. The maximum value of appearance score was found in combination 3 whereas, the combination 11 had the lowest appearance score as revealed in Table 3. The regression analysis of the data presented in Table 4 reveals that the coefficient of determination (R^2) was 0.9298 which indicates that the model was significant. Further model F-value (14.71) was

Table 3 Sensory and physical characteristics of dietary supplement made with different levels of fat, carbohydrate and protein sources

Standard	Flavour	Appearance (Dry)	Appearance (Reconstituted)	Color	Overall acceptability	Free fat (%)	Dispersibility (%)	Sedimentation (ml)
1	54.3	12.4	12.1	4.1	82.9	7.2	80.6	0.6
2	55.7	12.2	11.8	3.9	83.4	11.0	80.6	0.7
3	54.4	13.6	13.0	4.2	85.2	6.4	80.4	0.7
4	56.6	13.3	12.4	4.3	85.2	11.0	81.3	0.7
5	54.7	13.3	12.5	4.3	84.8	9.4	71.8	0.7
6	56.7	12.9	12.3	3.9	84.9	10.3	77.7	0.8
7	53.3	13.4	12.3	4.1	82.4	7.0	79.6	0.7
8	53.6	12.8	12.1	4.3	81.9	9.4	82.6	0.7
9	54.1	13.3	12.6	4.2	84.2	6.4	79.6	0.7
10	55.1	12.7	11.7	4.1	83.4	10.1	80.3	0.8
11	56.1	12.1	12.1	4.0	84.9	9.8	79.0	0.7
12	54.7	13.1	12.0	4.3	84.4	9.8	80.8	0.7
13	54.1	13.1	12.9	4.1	83.9	12.1	81.3	0.7
14	52.7	13.4	12.3	4.1	82.6	10.5	71.0	0.7
15	53.9	12.8	12.0	4.1	83.9	9.1	75.5	0.7
16	54.9	12.9	12.4	4.1	84.2	7.7	79.9	0.7
17	54.1	12.8	12.4	4.2	84.5	9.6	77.9	0.7
18	54.3	12.7	12.0	3.9	82.9	9.1	77.0	0.7
19	53.7	12.3	12.1	4.1	85.3	10.1	79.9	0.8
20	54.9	12.9	12.4	4.1	84.2	7.7	79.9	0.7

Figures are average of three Replications

Table 4 Regression coefficients and ANOVA of fitted quadratic model for sensory and physical characteristics of diabetic dietary supplement

Partial coefficients	Flavor	Appearance		Color	OA	Free fat	Dispersibility	Sedimentation
		(Dry)	(Reconstituted)					
Intercept	54.31	12.71	12.22	4.07	84.16	8.90	78.32	0.70
A-Fat	0.55**	−0.20**	−0.20**	−0.040 ^{ns}	−0.091 ^{ns}	1.30**	0.80 ^{ns}	0.017**
B-Carbohydrate	−0.44*	0.28**	0.075 ^{ns}	0.088**	−0.15 ^{ns}	−0.29 ^{ns}	1.19*	8.088E-003 ^{ns}
C-Protein	−0.38*	0.11*	−0.071 ^{ns}	0.014 ^{ns}	−0.36*	−0.16 ^{ns}	−2.07 **	9.785E-003 ^{ns}
A ²	0.20 ^{ns}	0.098*	−0.017 ^{ns}	0.032 ^{ns}	−0.13 ^{ns}	−0.41 ^{ns}	0.71 ^{ns}	2.333E-003 ^{ns}
B ²	0.46*	−0.031 ^{ns}	−0.056 ^{ns}	0.023 ^{ns}	0.16 ^{ns}	0.13 ^{ns}	0.69 ^{ns}	−0.014*
C ²	−0.23 ^{ns}	0.19**	0.14*	0.018 ^{ns}	−0.32 ^{ns}	0.68*	−0.65 ^{ns}	4.738E-003 ^{ns}
AB	−0.12 ^{ns}	−0.034 ^{ns}	−0.036 ^{ns}	0.099*	−0.15 ^{ns}	0.29 ^{ns}	−0.25 ^{ns}	−0.030**
AC	−0.16 ^{ns}	−0.066 ^{ns}	0.071 ^{ns}	−0.011 ^{ns}	−0.11 ^{ns}	−0.62 ^{ns}	1.00 ^{ns}	0.000 ^{ns}
BC	−0.70**	−0.28**	−0.23**	−0.026 ^{ns}	−1.18**	−0.30 ^{ns}	1.53*	−5.000E-003 ^{ns}
LACK OF FIT	ns	ns	ns	ns	ns	ns	ns	ns
Model F value	6.07**	14.71**	4.14*	3.32*	5.13**	4.58*	4.03*	4.89*
R ²	0.8453	0.9298	0.7883	0.7494	0.8220	0.8049	0.7837	0.8148
Press	17.69	0.43	2.09	0.19	6.64	42.86	177.23	0.013
Adequate Precision Value	8.486	13.204	8.089	6.747	8.595	8.884	7.336	8.997

* $p \leq 0.05$, ** $p \leq 0.01$ and *ns* non significant

more than the tabulated F value. The Adequate precision value (APV) was found to be higher 13.204 than the required (4.00) for high prediction ability. All this indicates the significance of quadratic regression model. The coefficient estimates of the appearance model of the dried dietary supplement showed that the fat and carbohydrate had a highly significant effect ($p \leq 0.01$) on the appearance of the dried product. It could be seen from Fig. 2a that the increase in the fat content decreased the appearance score while the increase in the carbohydrate content increase the appearance of the dried supplement. Onwulata et al. (1993) also reported that powders with higher levels of unencapsulated fat on the surface tended to stick together and form lumps, thus affecting the appearance of powdered formulations. The protein content also had a positive significant ($p \leq 0.05$) effect at the linear level. The quadratic effect of protein had a highly significant positive effect ($p \leq 0.01$) on the appearance score; the fat level also had a significant effect ($p \leq 0.05$) while the carbohydrate effect was non significant. However, the interactive effect of the carbohydrate and protein had a negative significant ($p \leq 0.01$) effect and the higher appearance score of dried product was observed, when the level of one variable was increased with subsequent decrease in the level of the other variable (Fig. 2b).

Reconstituted supplement The average score for appearance of the reconstituted dietary supplement ranged from 11.71 to 13.00. The maximum value of appearance score of reconstituted dietary supplement was found in combination 3 whereas, the combination 10 had the lowest appearance

score as revealed in Table 3. The regression analysis of the data presented in Table 4 reveals that the coefficient of determination (R^2) was 0.7883 indicating that the model was significant. Further model F-value (4.14) was more than the tabulated F value. The APV was found to be higher 8.089 than the required (4.00) for high prediction ability. All this indicates the significance of quadratic regression model. The fat content had a statistically negative significant ($p \leq 0.01$) effect on the appearance of the reconstituted supplement at the linear level. Thus decrease in the fat content increased the appearance score of the reconstituted supplement as shown in Fig. 3a. The presence of fat on the surface of milk powder increases the hydrophobicity of the surface, decreasing the wettability of the powders (Faldt and Bergenstahl 1996). This may result in the appearance of fat globules on the surface of the reconstituted supplement causing the lesser score. The quadratic effect of protein on the appearance score had a significant ($p \leq 0.05$) effect while no significant effect was observed in case of fat and carbohydrate levels. The interactive effect of carbohydrate and protein levels had a negative significant ($p \leq 0.01$) effect on the appearance i.e. the higher appearance score of reconstituted product was observed, when the level of one variable had to be increased with subsequent decrease in the level of the other variable (Fig. 3b).

Effect on colour The average score for color of the diabetic dietary supplement ranged from 3.86 to 4.29. The maximum value of color score of dietary supplement was observed in the following combinations namely 4, 5, 8 and 12 while the

Fig. 1 Effect of carbohydrate, protein and fat on flavor, color, overall acceptability scores, free fat content and dispersibility of the diabetic dietary supplement

combination 18 had the lowest color score as revealed in Table 3. The regression analysis of the data presented in Table 4 reveals that the coefficient of determination (R^2) was 0.7494 indicating that the model was significant. The APV was found to be higher 6.747 than the required (4.00) for high prediction ability. All this indicates the significance of

quadratic regression model. Among the ingredients, carbohydrate level had a most significant ($p \leq 0.01$) positive effect on color of the diabetic dietary supplement in linear terms. Interaction response curve showed that the color of the supplement increased by increasing the carbohydrate content from 58 to 62%. As it could be seen from Fig. 1b both the fat

Fig. 2 Effect of fat and carbohydrate and protein on appearance score of dried dietary supplement

and carbohydrate levels had a significant ($p \leq 0.05$) positive effect on the color at the interactive level.

Effect on overall acceptability The overall acceptability scores obtained for the complementary food were between 81.93 and 85.29. The combination 8 had lowest overall acceptability, while the highest overall acceptability was found in combination 19 as revealed in Table 3. The regression analysis of the data presented in Table 4 reveals that the coefficient of determination (R^2) was 0.8220 and a model F value (5.13) in comparison to tabulated F value was also higher. Moreover APV of 8.595, which was more than the required value i.e. 4.00, indicates that quadratic regression model could be used to describe the effect of ingredients on overall acceptability of developed product. Decreasing the protein content in formulation significantly improved the overall acceptability of dietary supplement in linear terms ($p \leq 0.05$). However, the other two ingredients had insignificant effect on overall acceptability of diabetic supplement. The quadratic effect of all the three ingredients did not exert any significant effect on the overall acceptability.

Fig. 3 Effect of fat and carbohydrate and protein on appearance score of reconstituted dietary supplement

Response surface 3-D curves (Fig. 1c) show that increasing the carbohydrate content from 58 to 62% with a decrease in protein below the 31% resulted in enhanced overall acceptability of the dietary supplement. The other interactive levels did not exert the significant effect on the overall acceptability. Resistant starch which was added as carbohydrate source in the dietary formulation, acts as a functional ingredient which tends to impart white appearance, bland flavor in the final product (Sajilata et al. 2006). In addition, RS also provides good handling and improves texture in the product (Yue and Waring 1998) and thus has shown increased overall acceptability as the carbohydrate content was increased in the formulation.

Effect on free fat The free fat per cent of the dietary supplement obtained ranged from 6.40 to 12.10. The combination 3 and combination 9 had lowest free fat per cent, while the highest level was found in combination 13 as revealed in Table 3. The regression analysis of the data presented in

Table 4 reveals that the coefficient of determination (R^2) was 0.8049 and a model F value (4.58) in comparison to tabulated F value was also higher. Moreover APV of 8.884, which was more than the required value i.e. 4.00, indicates that quadratic regression model could be used to describe the effect of ingredients on free fat content of the developed product. The coefficient estimates of free fat model shows that the levels of fat had a highly significant ($p \leq 0.01$) positive effect and increasing the fat content from 10 to 14% enhanced the free fat content of the dietary supplement (Fig. 1d). This result is supported by the findings of Esther et al. (2002) which showed that as the fat content of powder was increased, there was a sharp increase in the surface fat coverage (free fat) and the fat seemed to cover the whole particles. The results presented by Kim et al. (2002) suggested that the amount of protein on the surface of the milk powder was largely determined by the amount of fat content. Thus the square terms of protein level also showed the significant positive effect on the dietary supplement ($p \leq 0.05$).

Effect on dispersibility The dispersibility of the product obtained ranged from 71.00 to 82.59. The combination 14 had lowest dispersibility, while the highest dispersibility was found in the combination 8 as revealed in Table 3. The regression analysis of the data as presented in Table 4 reveals that the coefficient of determination (R^2) was 0.7837 and a model F value (4.03) in comparison to tabulated F value was also found to be higher. Moreover APV of 7.336, which was more than the required value i.e. 4.00 indicates that quadratic regression model, could be used to describe the effect of ingredients on dispersibility of the diabetic supplement. The protein level in the formulation exhibited most significant ($p \leq 0.01$) negative effect along with the carbohydrate which had a positive significant effect ($p \leq 0.05$) on the dispersibility of the dietary supplement. Interactive terms of carbohydrate and protein had significant effect ($p \leq 0.05$) on the dispersibility. The response surface 3-D curves showed that increasing the protein content resulted in the decrease in the dispersibility of the product. While the increase in the carbohydrate level from 58 to 62% exhibited improved dispersibility of the product (Fig. 1e). The formulation was composed of resistant starch and maltodextrin as the carbohydrate sources. Setser and Racette (1992) observed that maltodextrins are known to bind flavours and fats and act as oxygen barriers and render excellent dispersibility and solubility and resist to caking.

Effect on sedimentation The sedimentation of the dietary supplement obtained ranged from 0.63 to 0.75. The combination 1 had lowest sedimentation value, while the combinations 6, 10 and 19 were encountered with the highest sedimentation values as revealed in Table 3. The regression analysis of the data presented in Table 4 reveals that the

coefficient of determination (R^2) was 0.8148 and a model F value (4.89) in comparison to tabulated F value was also higher. Moreover APV of 8.997, which was more than the required value i.e. 4.00 indicates that quadratic regression model, could be used to describe the effect of ingredients on protein content of developed product. In linear terms, the level of fat in the formulation had a positive significant ($p \leq 0.05$) effect on the sedimentation whereas the other two variables carbohydrate and protein had no significant effect. The square term of protein showed significant negative effect ($p \leq 0.01$) on sedimentation value. It means higher sedimentation values were obtained at or near centre value of protein. Interactive terms of fat and carbohydrate sources had negative significant effect on sedimentation ($p \leq 0.01$), whereas interactive terms of fat and protein as well as carbohydrate and protein were found to be non significant.

Conclusion

Response Surface Methodology could be successfully used to optimize the level of various ingredients sources like fat, carbohydrate and protein for the formulation of a suitable diabetic dietary supplement. The optimum levels of ingredients used during formulation resulted in an acceptable dietary supplement within the limits of recommended dietary allowance for an adult diabetic patient. The diabetic formulation thus obtained was also adjusted sensorily acceptable.

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