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Development of functional food formulation; Cardiacare-DM by using Response Surface Methodology

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Abstract:

India's T2DM population is increasing rapidly, with cardiovascular disease complications being the leading cause of morbidity and mortality, which can be prevented by incorporating bioactive compounds into diets. The study aims to enhance Nutricare-DM, a functional food formulation, by adding *nigella sativa* and *zingiber officinale* extracts (NSE and ZOE) to "Cardiacare-DM" to reduce CVD risk, using Response Surface Methodology with reference to sensory analysis and glucose adsorption capacity. Among the 13 combinations, taste score ranged from 14.96 to 15.71, flavor 14.91 to 15.74, foldability 16.69 to 17.4, texture 16.68 to 17.46, breakability 16.81 to 17.67, overall acceptability 16.53 to 17.09; GAC in 5mmol @ 1% -0.51 to -0.14 mmol/dL, @ 2% 0.02 to 0.35 mmol/dL, in 10mmol @ 1% 0.9 to 1.96 mmol/dL, @ 2% 0.8 to 2.4 mmol/dL, in 25mmol @ 1% -0.31 to 0.86 mmol/dL, @ 2% 0.4 to 2.33 mmol/dL, and in 50mmol @ 1% 5.65 to 8.57 mmol/dL, @ 2% 8.17 to 12.86 mmol/dL. The study found that 500mg of NSE and 900mg of ZOE were the optimal solutions, with a best-fit desirability of 0.854 and a good overall acceptability score of 17.08, effectively adsorbing glucose at various concentrations.

Key words: Cardiacare-DM, Response Surface Methodology, Nigella Sativa Extract, Zingiber Officinale Extract, Glucose adsorption capacity, Sensory analysis

Introduction:

Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes in adults, accounting for 90-95% of all diabetes worldwide. India has the second-largest number of adults with diabetes globally, with 463 million people worldwide affected. The prevalence of diabetes in Indian adults was 77 million in 2019, and it is projected to be 101 million in 2030 and 134 million in 2045, with low and middle-income countries carrying almost 80% of the diabetes burden.[1]

Diabetes is a chronic metabolic disease causing both acute and chronic complications, with cardiovascular disease (CVD) being the leading cause of morbidity and mortality. CVD

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is 2-4 times more likely to occur in people with diabetes compared to people without diabetes, and it tends to occur at an earlier age. Over 50% of patients with T2DM die of coronary artery disease (CAD), stroke, and peripheral vascular disease (PVD).[2] Hyperglycemia can trigger beta cell dysfunction and insulin resistance[3], leading to glucose buildup in the blood.[1] As low-density lipoprotein cholesterol (LDL-C) contributes to cholesterol buildup on the walls of blood vessels and hence facilitates atherosclerosis, elevated serum cholesterol, particularly cholesterol associated with LDL-C, increases oxidative stress, which is responsible for the development of atherosclerosis and CVD.[4,5]

The identification of functional foods is required for dietary management in order to treat the disease. Due to their bioactive components, which have positive health effects, potential spices are regarded as nutraceuticals and have therapeutic properties. There is a growing interest in natural antioxidants from a safety perspective. They are generally used in small quantities, typically <1%, in combination.[4] A wide variety of phenolic compounds present in them are extensively used as food adjuncts and possess potent anti-diabetic, antioxidant, anti-inflammatory,[5] hypocholesterolemic, and hypotriglyceridemic activities.[4]

Thus, it is necessary to look for an immediate and efficient way to prevent the acute and chronic complications of the treatment regime as a holistic approach, which includes the development of a functional food formulation to reduce the risk of developing CVD in T2DM subjects by using potential bioactive compounds with dietary fiber in the product.

Nutricare-DM (N-DM), a potential anti-hyperglycemic functional food formulation, was developed by Sairam[6] for T2DM patients in order to fulfill their nutritional needs and manage their diabetes effectively. Which has a lower glycemic index (GI) than wheat because it contains primarily insoluble dietary fiber and is rich in cereal (barley, oats) β-glucans, which are dietary fibers with water-holding and binding properties. These compounds are also more effective at lowering blood sugar, insulin responses, and serum cholesterol levels. Psyllium husk-soluble fiber, which creates a thick gel in aqueous solution and slows carbohydrate access to digestive enzymes, is one mechanism of action for lowering blood sugar in T2DM patients. Thus, N-DM exerts its hypoglycemic action, which in turn aids in reducing or maintaining blood sugar levels.

The most popular daily staple meal for those with type 2 diabetes is ‘chapathi’, the traditional Indian flatbread made with wheat flour.[7,8] It was prepared from the N-DM[6] and value-added with the extracts of ginger (gingerol)[5] and black cumin (thymoquinone).[9] NSE and ZOE have been extensively studied and documented as a spice, flavoring agent, and conventional treatment for diabetes[7] in India, and they possess much lower potential for side effects than synthetic drugs; they have low adverse reactions compared to synthetic drugs,[10] which have been extensively studied and documented in both preclinical and clinical studies for their anti-diabetic potential and cardio-protective activity.[9,5-11]

The combinations of NSE and ZOE in chapathi have not been clinically evaluated for their sensory analysis and *in-vitro* glucose adsorption capacity. Hence, an attempt is made to develop an anti-hyperglycemic, cardio-protective functional food formulation that is nutritionally rich, cost-effective and made with locally available food ingredients. In the present study, the raw materials were known for their organoleptic characteristics, such as

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phenolic flavor and bitter taste. A statistical design using RSM, an effective tool for optimization, has been used in an attempt to bring out a highly acceptable functional food. The optimization of the C-DM with overall acceptability based on sensory attributes and *in-vitro* glucose adsorption capacity to determine the percent of glucose adsorbed by the food formulation with more emphasis on nutritional and functional considerations.

Materials and methods:

Materials:

Food ingredients such as wheat (*Tricum aestivum L*), jowar (*Sorghum bicolour L*), barley (*Hordeum vulgare*), and soya (*Glycine max*) flour were procured from local markets on a fresh basis; oats (*Avena sativa*), salt, and cooking oil were procured from supermarkets; psyllium husk (*Plantago ovata*), cinnamon (*Cinnamomum zeylanicum*) extract, black cumin (*Nigella sativa)* extract, and ginger (*Zingiber officinale*) extracts were procured from ISO-certified Bhagavathi herbal and healthcare private limited, Gujarat, India. Analytical-grade chemicals and diagnostic kits were used, such as anhydrous D-Glucose (Qaligen's) and glucose estimation kit (GOD-POD) (Agappe Diagnostic Ltd.).

Raw materials processing:

Food ingredients such as wheat, jowar, barley, soya, oats, psyllium husk flour, cinnamon, ginger, and black cumin extract were mixed well and sieved through a 60-mesh sieve for uniform mixing. The above functional food formulation was named 'Cardiacare-DM' (C-DM). Fresh chapathi (unleavened Indian flat bread) were made using C-DM and subjected to sensory analysis and *in vitro* hypoglycemic potential by glucose adsorption capacity using standard protocols.[12,13]

Methods:

The composition of 'Nutricare-DM' (N-DM) functional food formulation[6] was slightly modified and improvised by incorporating extracts of black cumin and ginger. Chapathi was prepared by using C-DM, water, cooking oil, and a pinch of salt (optional). It was prepared as per the method described in an earlier study.[14]

Experimental Design

The percentage of *Nigella sativa* extract (NSE) and *Zingiber officinale* extract (ZOE) was as per the runs obtained by design expert statistical software. A Central Composite Rotatable Design was used without blocking. The number of design points was obtained on basis of the number of independent variables decided. The statistical software package design expert 7.1.5, Stat -Ease Inc., Minneapolis, MN, was used to construct the experimental design as well as analyze the data. The parameters that influence the product's quality and acceptability were taken as responses. Since overall acceptability and glucose adsorption capacity are important criteria for product development and acceptance, they have been considered as one of the responses. Variables NSE and ZOE were selected as independent variables and taste, flavour, foldability, texture, breakability, and overall acceptability were scored; glucose adsorption capacity at 1% and 2% of sample concentration in 5, 10, 25, and 50 mmol/dL of glucose solution concentration were selected as the responses. The design considered 4 factorial points, 4 axial points, and 5 central points, leading to 13 sets of experiments.[15,16-17] Each independent variable investigated in this experiment had five levels,

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which were -1.4142, -1, 0, +1, and +1.4142 (**Table 1**). A total of 13 combinations (design points) were generated for the two independent variables, and the alpha values in the design outside the ranges were selected for the rotatability of the design for Cardiacare-DM. The center point (the level combination in which the value of each coded variable was 0) was repeated five times for the two-variable design and was selected while keeping the ingredients at levels expected to yield, at least, satisfactory experimental results.

The regression analysis of the responses was conducted by fitting suitable models represented by equations 1 and 2.

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i x_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i\neq j=1}^{n} \beta_{ij} x_i x_{ij} \quad \ldots\ldots\ldots(2)$$

where, β_o was the value of the fitted response at the center point of the design, i.e., point (0, 0, 0); β_i, β_{ii} and β_{ij} were the linear, quadratic, and cross-product (interaction effect) regression terms, respectively, and 'n' denoted the number of independent variables i.e. in this case n is 2, and x_i, x_{ij} are independent variables in coded values represented by X_1 and X_2 in Table 2a and b.

Table.1 Experimental design for Cardiacare-DM with process variables and their levels

Process variables	**-1.414 (α point)**	**-1 (factorial point)**	**0 (centre point)**	**+1 (factorial point)**	**+1.414 (α point)**
NSE (mg)	189.33	500	1250	2000	2310.66
ZOE (mg)	672.18	900	1450	2000	2227.82

Note: NSE- Nigella Sativa Extract, ZOE- Zingiber Officinale Extract, mg- Milli Gram and X- Variable

X_1	**X_2**	**Runs**
± 1	± 1	4
± 1.414	0	2
0	± 1.414	2
0	0	5

Sensory analysis:

Sensory evaluation involves a dilution test to assess the intensity of sensory responses to food components in a product. The test detects the smallest unknown amount when mixed with a standard material. A series of 13 variations were analyzed using the arithmetic progression of the RSM. The composite scoring test was used to compare products in overall quality grading for product acceptability.[18]

The optimized variable was freshly prepared and C-DM (Chapathi) were subjected to sensory analysis carried out in sensory booths and evaluated by semi-trained panellists (n = 30) recruited from the department of studies in Food Science and Nutrition, University of

Mysore, Mysuru, using a descriptive composite score card. The formulated products with 13 variations were coded and evaluated for sensory quality attributes such as taste, flavor, foldability, texture, breakability, and overall acceptability,[12] and the composite score card scale rating was in the order: 1–4: poor; 5–8: fair; 9–12: satisfactory; 13–16: good; 17–20: excellent.[18,19]

Determination of glucose adsorption capacity:

The glucose adsorption capacity of the samples was determined according to the method of Ou et al.[13] with slight modifications. 1% (250 mg) and 2% (500 mg) samples were added to 25 ml of glucose solution at concentrations 5, 10, 25, and 50 mm/dL in duplicate, vortexed, and incubated in a shaker water bath for 6 h at 37 °C. The samples were then centrifuged at 4000 × g for 20 minutes, and the glucose content in the supernatant (10 microliters) was analyzed using the glucose oxidase peroxidase (GOD-POD) enzymatic kit (1 ml) and incubated at 37 °C for 10 minutes. Readings were taken by spectrophotometer against a standard. The glucose bound was calculated by using the following formula:

$$\text{Glucose(mg/dl)} = \frac{\text{OD of sample}}{\text{OD of standardx 100}}$$

$$\text{Glucose (mmol)} = \frac{\text{Glucose(mg/dl)}}{\text{Weight of glucose}} \text{ x Glucose concentration}$$

$$\text{Glucose adsorbed} = \text{Glucose concentration (mmol)} - \text{Glucose (mmol)}$$

Results and Discussion:

The central composite rotatable design for Cardiacare-DM with independent variables and responses is given in **Table 2a and b**. Among the 13 combinations, taste score ranged from 14.96 to 15.71, flavor 14.91 to 15.74, foldability 16.69 to 17.4, texture 16.68 to 17.46, breakability 16.81 to 17.67, overall acceptability 16.53 to 17.09; glucose adsorption capacity in 5mmol @ 1% -0.51 to -0.14 mmol/dL, @ 2% 0.02 to 0.35 mmol/dL, in 10mmol @ 1% 0.9 to 1.96 mmol/dL, @ 2% 0.8 to 2.4 mmol/dL, in 25mmol @ 1% -0.31 to 0.86 mmol/dL, @ 2% 0.4 to 2.33 mmol/dL, and in 50mmol @ 1% 5.65 to 8.57 mmol/dL, @ 2% 8.17 to 12.86 mmol/dL.

The analysis of variance was calculated for each selected model, and the effects of the independent variables using NSE and ZOE on the response at linear, quadratic, and interactive levels are presented in **Table 3a and b**. The sign and magnitude of the coefficients indicate the effect of the variable on the responses. The negative sign of a coefficient at the linear level indicates a decrease in response with an increase in the level of the variable, whereas at the interactive level; level of one variable could be increased while that of the other decreased to obtain the same response. All linear, quadratic, and interactive effects were calculated for each model. Quadratic response surface models were selected for all the responses. The adequacy was calculated by the F-ratio, mean, standard deviation, coefficient correlation, and lack of fit test. The R^2 value was > 98%, and the lack of fit was highly non-

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significant for all the responses. The regression coefficient and correlation coefficients for the responses were 0.99 for all the sensory scores and glucose adsorption capacity at 1% and 2% in 5 mmol, 10 mmol, and 2% in 50 mmol, whereas at 1% and 2% glucose adsorption in 25 mmol and 1% in 50 mmol, they had values of 0.98. The closer the value of R^2 to unity, the better the empirical model fits the actual data. On the other hand, the smaller the value of R^2 the less relevance the dependent variables in the model have in explaining the behavior of variations.[20]

Table 2a: Experimental design for Cardiacare-DM on Sensory attributes.

Run	Variable levels (uncoded)		Responses					
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
	NSE	ZOE	Taste	Flavour	Foldability	Texture	Breakability	OAA*
	(mg)	(mg)	(Score)	(Score)	(Score)	(Score)	(Score)	(Score)
1	1250	1450	15	15.01	16.76	16.91	17.24	16.53
2	1250	1450	15.12	15.02	16.75	16.96	17.2	16.53
3	1250	672.18	15.61	15.58	17.28	17.07	17.35	17.06
4	1250	1450	15.02	15.03	16.76	16.88	17.23	16.58
5	2310.66	1450	14.96	14.91	16.69	16.72	16.81	16.55
6	500	900	15.68	15.65	16.89	16.95	17.15	17.09
7	1250	2227.82	15.71	15.74	17.3	17.2	17.42	16.75
8	500	2000	15.37	15.25	17.4	17.46	17.67	16.54
9	189.34	1450	15.33	15.18	16.95	17.15	17.33	16.67
10	2000	900	15	14.95	17.2	17.06	17.26	16.68
11	1250	1450	15.05	15.03	16.75	16.93	17.23	16.56
12	1250	1450	15.03	15.03	16.76	16.9	17.2	16.53
13	2000	2000	15.52	15.58	16.72	16.68	16.82	16.77

Note: NSE- Nigella Sativa Extract, ZOE- Zingiber Officinale Extract, mg- Milli Gram, X- Variable, Y- responses, OAA- Overall acceptability and ***-** Sensory evaluation by using composite score card scale.

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Table 2b: Experimental design for Cardiacare-DM on Glucose adsorption capacity

Run	Variable levels (uncoded)		Responses							
	X_1	X_2	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}	Y_{13}	Y_{14}
	NSE	ZOE	GAC_1	GAC_2	GAC _3	GAC _4	GAC _5	GAC _6	GAC _7	GAC _8
	(mg)	(mg)	1% in 5mmol (mmol/dL)	2% in 5mmol (mmol/dL)	1% in 10mmol (mmol/dL)	2% in 10mmol (mmol/dL)	1% in 25mmol (mmol/dL)	2% in 25mmol (mmol/dL)	1% in 50mmol (mmol/dL)	2% in 50mmol (mmol/dL)
1	1250	1450	-0.24	0.35	1.46	2.37	0.34	0.92	6.23	12.72
2	1250	1450	-0.25	0.34	1.42	2.35	0.4	1.1	6.66	12.75
3	1250	672.18	-0.14	0.3	1.35	0.8	0.51	2.33	7.87	8.17
4	1250	1450	-0.25	0.34	1.42	2.35	0.46	0.93	6.23	12.71
5	2310.66	1450	-0.51	0.1	1.48	2.29	0.86	0.46	8.01	11.5
6	500	900	-0.25	0.27	1.67	1.15	0.64	1.71	7.89	10.5
7	1250	2227.82	-0 .31	0.14	1.5	1.67	-0.31	0.92	7.01	12.42
8	500	2000	-0.25	0.02	0.9	1.57	0.07	1.13	5.65	12.49
9	189.34	1450	-0.31	0.076	1.18	1.68	0.6	1.06	6.61	12.51
10	2000	900	-0.27	0.15	0.98	1.4	0.79	1.58	7.26	8.75
11	1250	1450	-0.24	0.35	1.46	2.4	0.34	0.98	6.35	12.76
12	1250	1450	-0.25	0.35	1.42	2.25	0.38	1.06	6.33	12.7
13	2000	2000	-0.51	0.18	1.96	2.33	0.16	0.4	8.57	12.86

Note: NSE- Nigella Sativa extract, ZOE- Zingiber Officinale Extract, mg- Milli Gram, mmol/dL- Milli Molar/Deci Liter, %-Percentage, X- Variable, Y- responses and GAC-Glucose adsorption capacity

Table 3a: Coefficient of second order polynomial regression models Cardiacare-DM on Sensory attributes

Coefficient	Taste	Flavour	Foldability	Texture	Breakability	OAA
	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
β_0	15.044	15.024	16.756	16.916	17.22	16.546
β_1	-0.132[a]	-0.094[a]	-0.092[a]	-0.159[a]	-0.184[c]	-0.044[b]
β_2	0.044[d]	0.057[a]	0.007[c]	0.039[c]	0.0224	-0.112[a]
β_{11}	0.048[c]	0.012[c]	0.0314[a]	0.010	-0.076	0.035[c]
β_{22}	0.306[a]	0.319[a]	0.266[a]	0.110[a]	0.082[c]	0.183[a]
β_{12}	0.208[a]	0.258[a]	-0.248[a]	-0.223[a]	-0.24[d]	0.16[a]
R^2 %	99.06	99.96	99.88	99.09	99.75	99.45
p%	<0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Mean	15.26	15.23	16.94	16.99	17.23	16.68
SD	0.037	0.008	0.004	0.026	0.014	0.019
F-value	146.72	3874.46	8462.57	152.75	580.85	252.43

Note: # Values with different superscripts are significant at the level, a: p<0.0001, b: p<0.001, c: p<0.01, d: p<0.05, OAA- overall acceptability, X- Variable, Y- responses

Table 3b: Coefficient of second order polynomial regression models Cardiacare-DM on Glucose adsorption capacity

Coefficient	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}	Y_{13}	Y_{14}
	GAC_1	GAC _2	GAC _3	GAC _4	GAC _5	GAC _6	GAC _7	GAC _8
β_0	-0.246	0.346	1.436	2.344	0.384	0.998	6.36	12.73
β_1	-0.070[a]	0.009[b]	0.099[a]	0.234 [a]	0.076[c]	-0.214[a]	0.534[a]	-0.351[a]
β_2	-0.060[a]	-0.056[a]	0.053[a]	0.322[a]	-0.295[a]	-0.469[a]	-0.238[c]	1.513[a]
β_{11}	-0.083[a]	-0.129[a]	-0.053[b]	-0.178[a]	0.173[a]	-0.116[c]	0.467[a]	-0.362[a]
β_{22}	0.009[b]	-0.063[a]	-0.005	-0.553[a]	-0.142[a]	0.317[a]	0.532[a]	-1.217[a]
β_{12}	-0.06[a]	0.07[a]	0.438[a]	0.322[c]	-0.015	-0.15[1]	-0.268[a]	0.53[a]
R^2 %	99.90	99.93	99.74	99.95	98.95	98.96	98.39	99.99
p%	<0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Mean	-0.291	0.228	1.4	1.89	0.403	1.12	6.98	11.76

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SD	0.004	0.004	0.018	0.049	0.042	0.068	0.146	0.024
F-value	1398.19	1867.67	542.17	288.39	131.57	133.23	85.32	10926.4

Note: # Values with different superscripts are significant at the level, a: $p<0.0001$, b: $p<0.001$, c: $p<0.01$, d: $p<0.05$, Y- responses and GAC- Glucose adsorption capacity

Effect of variables on Sensory parameters:

Taste, flavor, foldability, texture, breakability, and overall acceptability were considered sensory parameters for the independent variables analyzed by the semi-trained sensory panelists. In the present study, optimization of the functional food formulation Cardiacare-DM with NSE and ZOE had good sensory scores for taste (14.96 to 15.71) and flavor (14.91 to 15.74) of the product are due to the presence of bioactive compounds (phenolic and pungent flavors) of the extract.[5,11] The foldability (16.69 to 17.4), texture (16.68 to 17.46), breakability (16.81 to 17.67), and overall acceptability (16.53 to 17.09) had scored excellent for the easy foldability, soft in texture, easy in breakability, and appetizing by overall acceptability of the product has been ranked due to the functional ingredients (oats, barley, and psyllium husk) for their soluble and insoluble dietary fiber, which absorbs water and holds with it of the product chapathi.[6] The overall acceptability of the sensory attributes showed an excellent acceptability, which indicates the compatible for regular human consumption. Considering the statistical analysis for optimization of ingredients, the model obtained from the regression can be written as follows for sensory parameters:

Taste $= 15.044 - 0.1317 X_1 + 0.044 X_2 + 0.2075 X_1^2 + 0.3055 X_2^2 + 0.2075 X_1 X_2$ $\quad R^2 = 0.99$

Flavor $= 15.0240 - 0.094 X_1 + 0.050 X_2 + 0.012 X_1^2 + 0.319 X_2^2 + 0.2575 X_1 X_2$ $\quad R^2 = 0.99$

Foldability $= 16.756 - 0.092 X_1 + 0.007 X_2 + 0.0313 X_1^2 + 0.2664 X_2^2 - 0.248 X_1 X_2$ $\quad R^2 = 0.99$

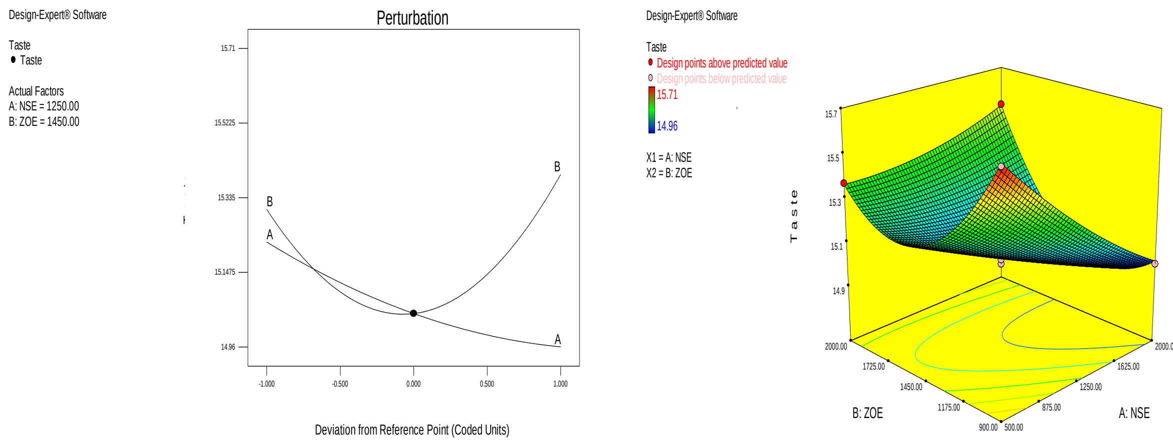
Texture $= 16.916 - 0.159 X_1 + 0.039 X_2 + 0.010 X_1^2 + 0.110 X_2^2 - 0.222 X_1 X_2$ $\quad R^2 = 0.99$

Breakability $= 17.22 - 0.184 X_1 + 0.022 X_2 - 0.075 X_1^2 + 0.082 X_2^2 - 0.24 X_1 X_2$ $\quad R^2 = 0.99$

OAA $= 16.546 - 0.043 X_1 - 0.112 X_2$

$$+ 0.035 X_1^2 + 0.183 X_2^2$$
$$+ 0.16 X_1 X_2 \qquad R^2 = 0.99$$

From the above equation, it can be concluded that at the linear level, ZOE had a positive effect on all the sensory parameters except the OAA score, in which flavor had a significant effect at the level of $p<0.0001$; foldability, texture, and taste at the level of $p<0.01$ and taste at $p<0.05$. NSE had a significant negative effect on all the responses at the levels $p<0.0001$ for taste, flavor, foldability, and texture, $p<0.001$ for overall acceptability, and $p<0.01$ for breakability. At the quadratic level, variables had positive effects on all sensory parameters except NSE, which showed a negative effect on breakability. It can be clearly observed from **Table 3a** that NSE affected foldability significantly at $p<0.0001$; taste, flavor, and OAA at $p<0.01$ and ZOE had a significant effect at $p<0.0001$ for all sensory parameters except breakability. At the interactive level, variables had a positive effect on taste, flavor, and OAA at the level of $p<0.0001$, while foldability and texture were affected negatively at the level of $p<0.0001$. It is clear from **Figs. 1a–1f** that both variables had an effect on sensory scores. As there was a decrease in NSE concentration, there was a decrease in all the sensory parameters; the breakability score decreased drastically, while overall acceptability was affected slightly. As ZOE concentration decreased, there was a drastic increase in taste, flavor, and foldability, while a slight increase was observed in texture, breakability, and OAA. It is noteworthy to mention that both variables, viz., NSE and ZOE, had a great influence on sensory parameters.



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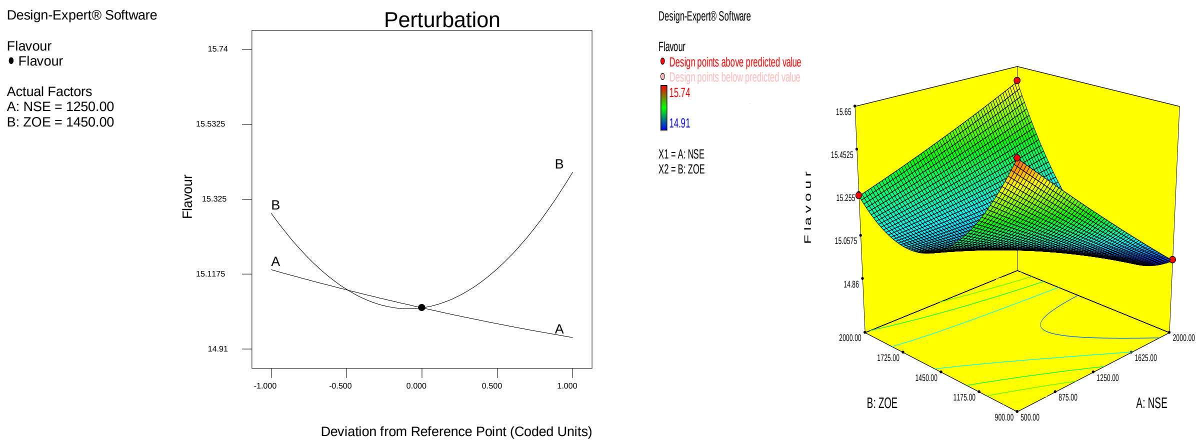
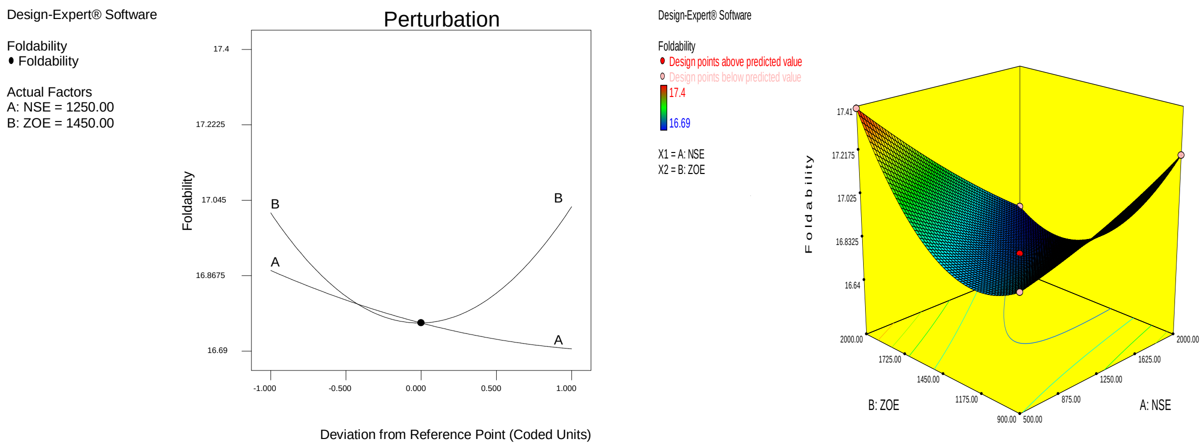


1b



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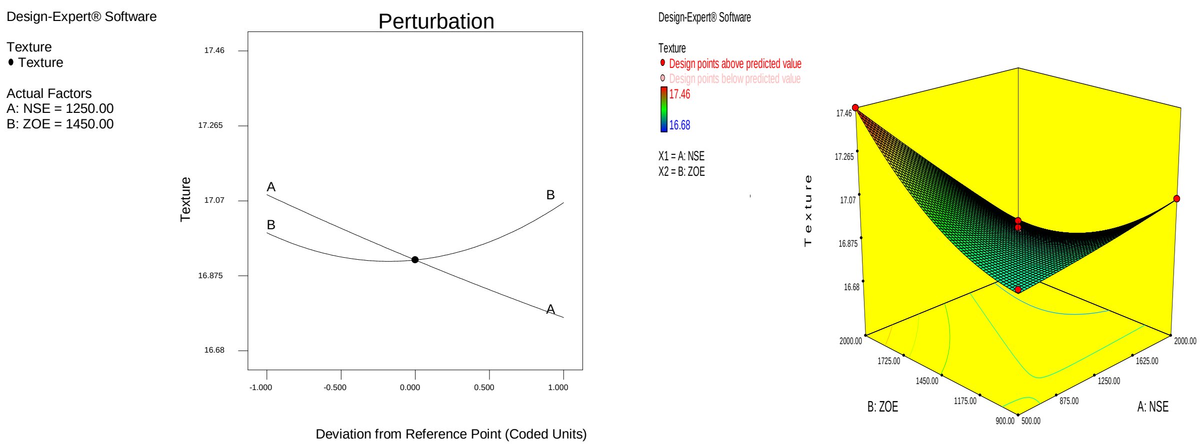

1d

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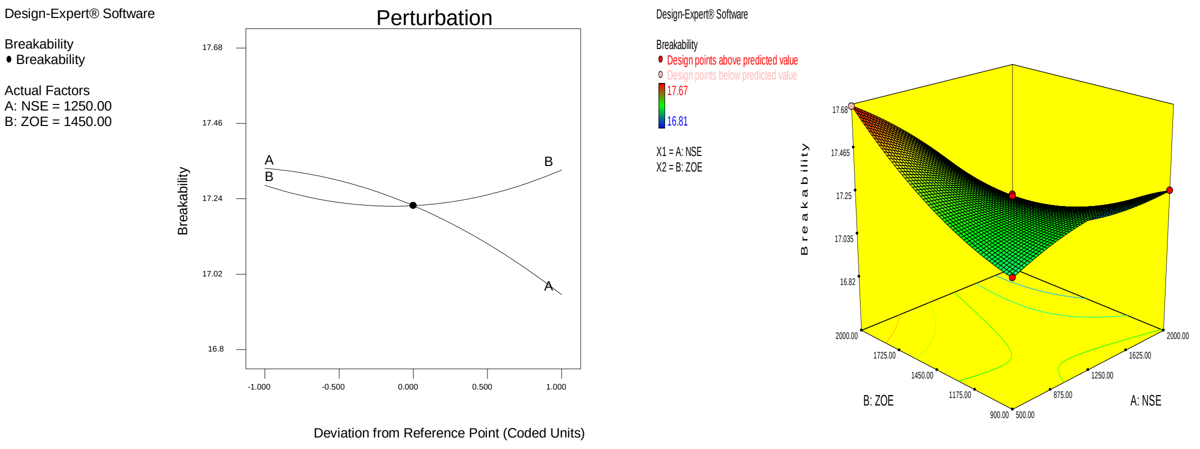
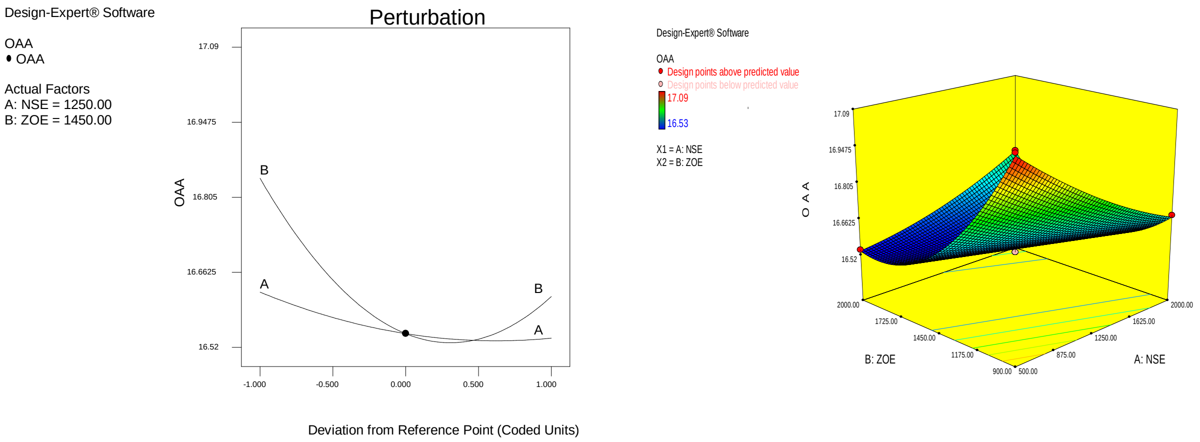


1e



1f

Fig.1 Perturbation and 3D graph for Sensory parameters; 1a Taste, 1b Flavour, 1c Foldability 1d Texture, 1e Breakability and 1f OAA of Cardiacare-DM.

Effect of variables on Glucose adsorption capacity:

The *in vitro* assay which mimics the physiological environment, plays an important role in the evaluation of hyperglycemic potential of a functional food formulation for improving blood glucose control and preventing long-term complications in T2DM.[10] The present study was undertaken to assess the glucose adsorption capacity of nigella sativa and ginger extracts. In comparison to various sample concentrations (1% and 2%), the ability of the chosen extracts to bind glucose at various glucose concentrations (5, 10, 25, and 50 mmol) was examined. It was observed that the glucose adsorption capacity of C-DM was directly proportional to the molar concentration of glucose, and higher amounts of glucose were bound at an increased glucose concentration. The functional ingredients are rich in soluble and insoluble dietary fiber from oats, β-glucan from barley, and viscous polysaccharides from psyllium husk,[14,21] respectively. were effective in adsorbing glucose at both low and higher concentrations of glucose used in the study by the action of water holding capacity, swelling, diffusion suppressing ability (viscous, gel formation), binding properties by dietary fiber,[22] and extracts of NSE and ZOE could bind glucose effectively by

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its bioactive compounds, and the glucose-binding capacity was directly proportional to the glucose concentration.[10,23] As **Fig. 2**. Studies also suggest that the inhibition of glucose diffusion in the small intestine is due to the adsorption or inclusion of the smaller sugar molecules within the structure of the fiber particles. Functional foods with a nutritional profile are appropriate for diabetics since the adsorption of glucose is delayed, resulting in reduced glycemic reactions and, as a result, a reduction in postprandial glycemia.[6]

In the present study, optimization of Cardiacare-DM with NSE and ZOE had glucose adsorption capacity in 5 mmol @ 1% -0.51 to -0.14 mmol/dL, @ 2% 0.02 to 0.35 mmol/dL, in 10 mmol @ 1% 0.9 to 1.96 mmol/dL, @ 2% 0.8 to 2.4 mmol/dL, in 25 mmol @ 1% -0.31 to 0.86 mmol/dL, @ 2% 0.4 to 2.33 mmol/dL, in 50 mmol @ 1% 5.65 to 8.57 mmol/dL, and @ 2% 8.17 to 12.86 mmol/dL. Considering the statistical analysis for optimization of ingredients in C-DM, the model obtained from the regression can be written as follows for glucose adsorption capacity:

$$\text{Glucose adsorption capacity\_1} = -0.246 - 0.0704\,X_1 - 0.060\,X_2 - 0.0826\,X_1^2 + 0.0099\,X_2^2 - 0.06\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{Glucose adsorption capacity\_2} = 0.346 + 0.009X_1 - 0.056\,X_2 - 0.129\,X_1^2 - 0.063\,X_2^2 + 0.07\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{Glucose adsorption capacity\_3} = 1.436 + 0.099\,X_1 + 0.053\,X_2 - 0.053\,X_1^2 - 0.006\,X_2^2 + 0.438\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{Glucose adsorption capacity\_4} = 2.344 + 0.234\,X_1 + 0.323\,X_2 - 0.179\,X_1^2 - 0.5534\,X_2^2 + 0.1275\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{Glucose adsorption capacity\_5} = 0.384 + 0.076\,X_1 - 0.295\,X_2 + 0.173\,X_1^2 - 0.142\,X_2^2 - 0.015\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{Glucose adsorption capacity\_6} = 0.998 - 0.214\,X_1 - 0.469\,X_2 - 0.116\,X_1^2 + 0.317\,X_2^2 - 0.15\,X_1X_2 \qquad R^2 = 0.99$$

$$\text{Glucose adsorption capacity\_7} = 6.36 + 0.534\,X_1 - 0.268\,X_2 + 0.467\,X_1^2 + 0.539\,X_2^2 + 0.888\,X_1X_2 \qquad R^2 = 0.98$$

$$\text{Glucose adsorption capacity\_8} = 12.73 - 0.351\,X_1 + 1.514\,X_2 - 0.362\,X_1^2 - 1.217\,X_2^2$$

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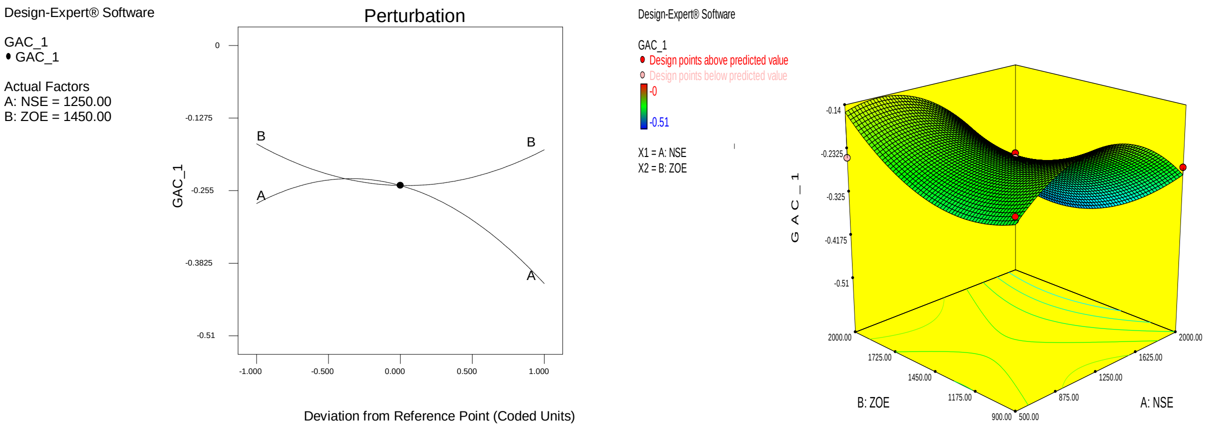
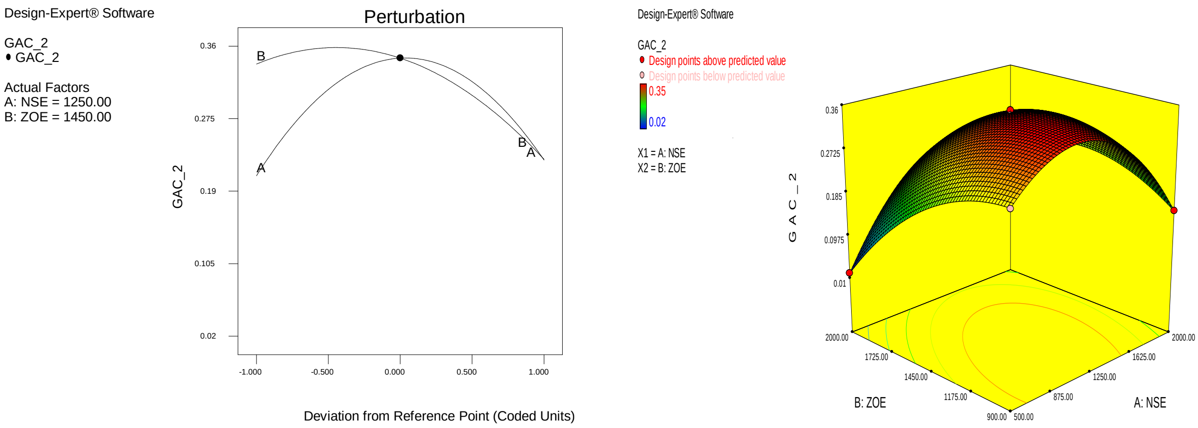
$$+ 0.53 X_1 X_2 \qquad R^2 = 0.99$$

As seen in **Table 3b** at the linear level, both NSE and ZOE had positive effects on GAC at 1% and 2% in 10 mmol (mmol/dL), while at 2% in 25 mmol (mmol/dL) they had a negative effect. NSE had a negative significant effect ($p<0.0001$) on GAC at 2% in 25 mmol and 2% in 50 mmol (mmol/dL), whereas ZOE showed a positive significant effect at 2% in 50 mmol (mmol/dL) ($p<0.0001$). At the quadratic level, for GAC, NSE was positively significant at the level of $p<0.0001$ for 1% in 25 mmol (mmol/dL) and 1% in 50 mmol (mmol/dL), and was negatively significant at the level of $p<0.0001$ for 1%, 2% in 5 mmol (mmol/dL); 2% in 10 mmol and 50 mmol (mmol/dL). ZOE affected significantly at quadratic level for 2% in 5 mmol (mmol/dL), 2% in 10 mmol (mmol/dL), 1% in 25 mmol (mmol/dL), 2% in 25 mmol (mmol/dL), 1% in 50 mmol (mmol/dL), and 2% in 50 mmol (mmol/dL) ($p<0.0001$), 1% in 5 mmol (mmol/dL) ($p<0.001$), while it was insignificant for 1% in 10 mmol (mmol/dL). From the above equation, it can be concluded that at the interactive level, NSE and ZOE affect GAC positively at 2% in 5 mmol (mmol/dL), 1% in 10 mmol (mmol/dL), 2% in 10 mmol (mmol/dL), and 2% in 50 mmol (mmol/dL), while negatively at 1% in 5 mmol (mmol/dL), 1% in 25 mmol (mmol/dL), 2% in 5 mmol (mmol/dL), and 1% in 50 mmol (mmol/dL).

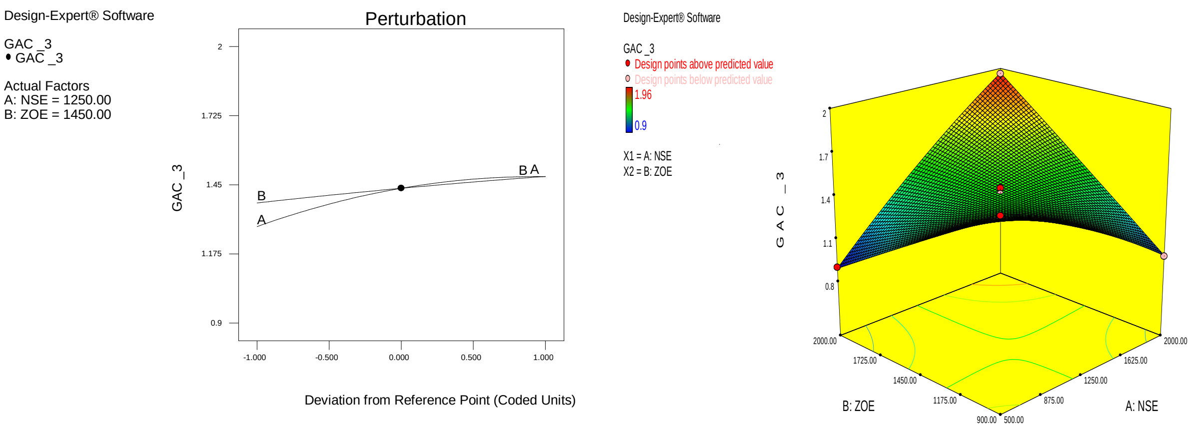
The surface plots for GAC in relation to NSE and ZOE are shown in **Fig 2a** and **2h,** respectively. From the plots, it can be observed that as there was an increase in NSE, there was an increase in the GAC at concentrations of 1% in 10 mmol (mmol/dL), 2% in 10 mmol (mmol/dL), and 1% in 50 mmol (mmol/dL), and when the concentration of the variable was reduced, there was a decrease at the levels of 1% in 5 mmol (mmol/dL), 2% in 25 mmol (mmol/dL), and 2% in 50 mmol (mmol/dL). When there was a slight decrease in the concentration of NSE, it showed that the values of 1% in 25 mmol (mmol/dL) increased and 2% in 50 mmol (mmol/dL) decreased. ZOE had no much effect on glucose adsorption at concentrations of 2% in 25 mmol (mmol/dL) and 1% in 50 mmol (mmol/dL), while it influenced a lot at the concentration of 2% in 50 mmol (mmol/dL). It was observed that as there was an increase in NSE, the values at the concentration increased by 1% in 10 mmol (mmol/dL) and 2% in 50 mmol (mmol/dL). In the present study, with the decrease in ZOE, there was a decrease in GAC at a level of 1% in 5 mmol (mmol/dL). Therefore, both variables, viz., NSE and ZOE, had a great influence on GAC.


Design-Expert® Software
GAC_1
● GAC_1
Actual Factors
A: NSE = 1250.00
B: ZOE = 1450.00
Perturbation
GAC_1
Deviation from Reference Point (Coded Units)
Design points above predicted value
Design points below predicted value
-0
-0.51
X1 = A: NSE
X2 = B: ZOE
B: ZOE
A: NSE

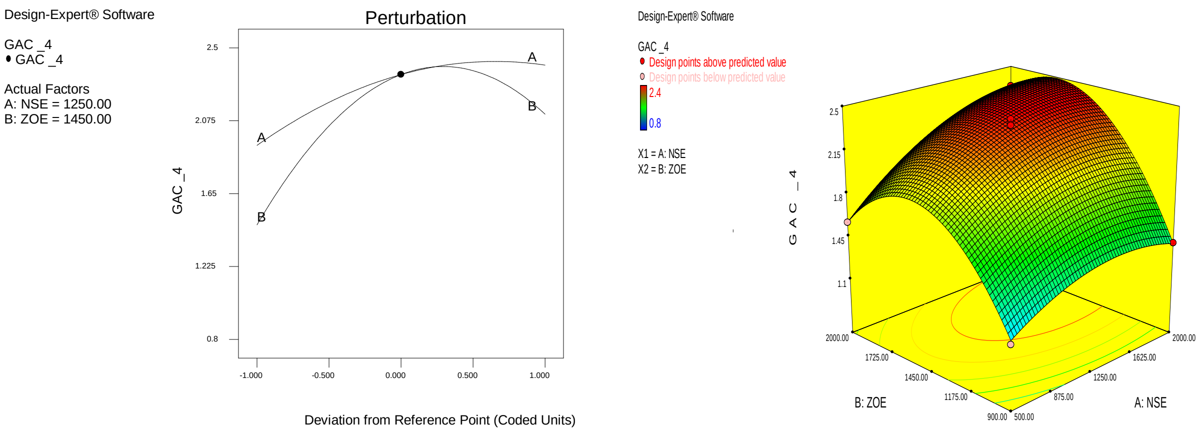
2a


Design-Expert® Software
GAC_2
● GAC_2
Actual Factors
A: NSE = 1250.00
B: ZOE = 1450.00
Perturbation
GAC_2
Deviation from Reference Point (Coded Units)
Design points above predicted value
Design points below predicted value
0.35
0.02
X1 = A: NSE
X2 = B: ZOE
B: ZOE
A: NSE

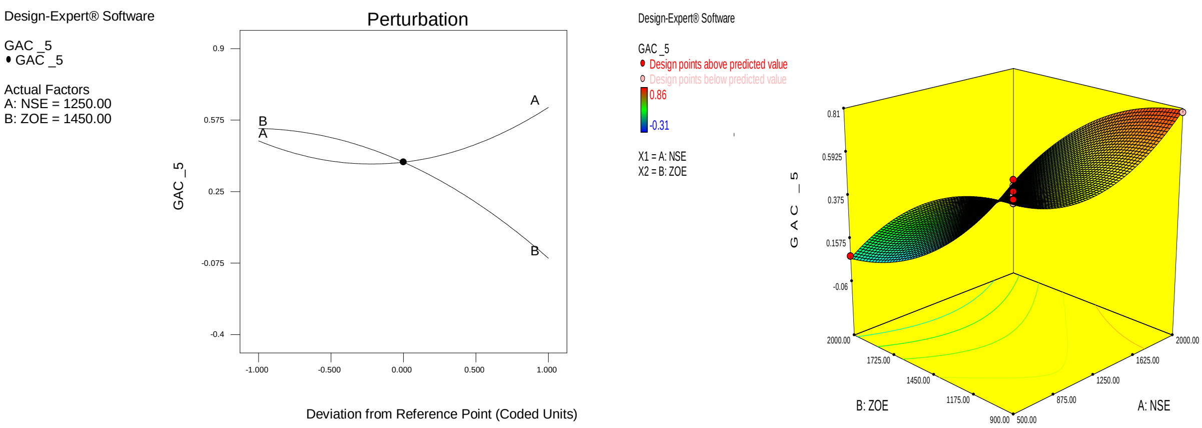
2b


Design-Expert® Software
GAC _3
● GAC _3
Actual Factors
A: NSE = 1250.00
B: ZOE = 1450.00
Perturbation
GAC _3
Deviation from Reference Point (Coded Units)
Design points above predicted value
Design points below predicted value
1.96
0.9
X1 = A: NSE
X2 = B: ZOE
B: ZOE
A: NSE

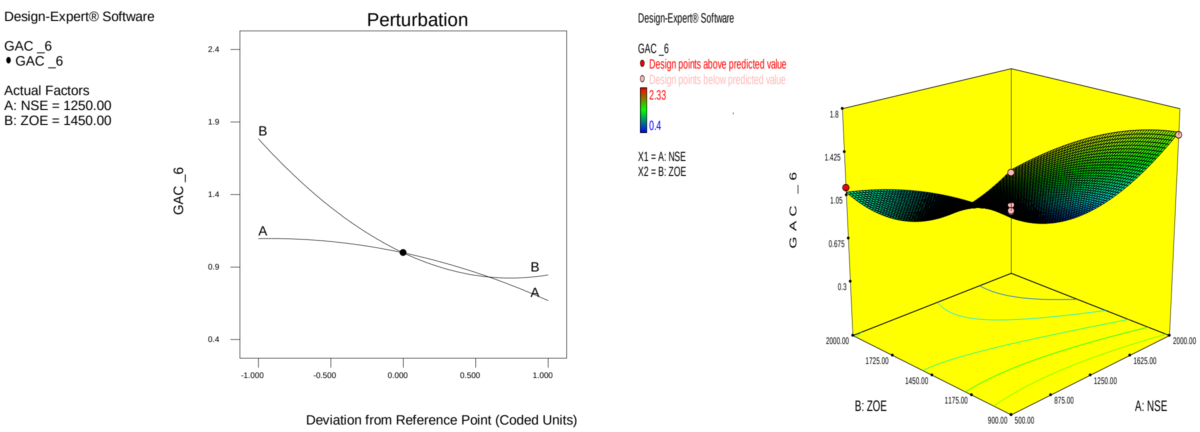
2c


Design-Expert® Software
GAC _4
GAC _4
Actual Factors
A: NSE = 1250.00
B: ZOE = 1450.00
Perturbation
GAC _4
Deviation from Reference Point (Coded Units)
Design-Expert® Software
GAC _4
Design points above predicted value
Design points below predicted value
2.4
0.8
X1 = A: NSE
X2 = B: ZOE
B: ZOE
A: NSE

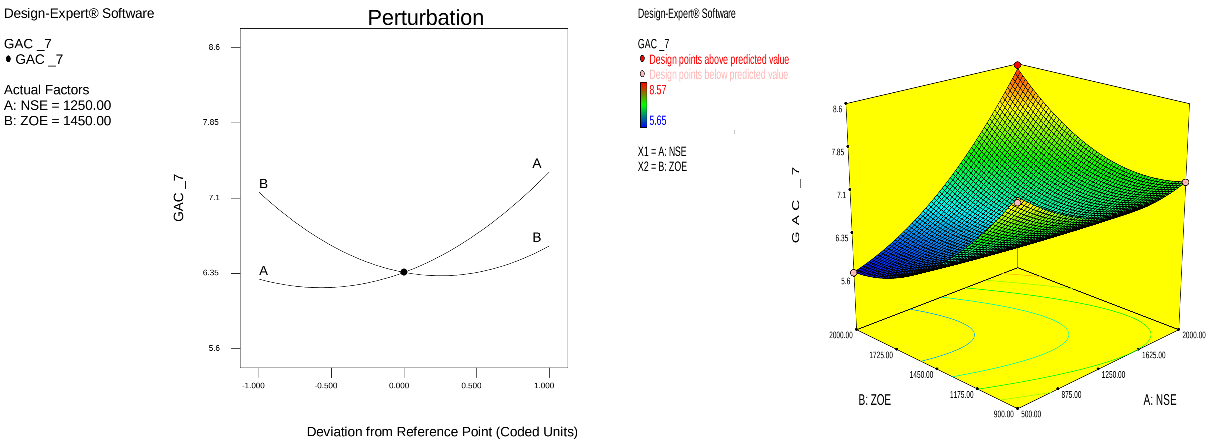
2d


Design-Expert® Software
GAC _5
GAC _5
Actual Factors
A: NSE = 1250.00
B: ZOE = 1450.00
Perturbation
GAC _5
Deviation from Reference Point (Coded Units)
Design-Expert® Software
GAC _5
Design points above predicted value
Design points below predicted value
0.86
-0.31
X1 = A: NSE
X2 = B: ZOE
B: ZOE
A: NSE

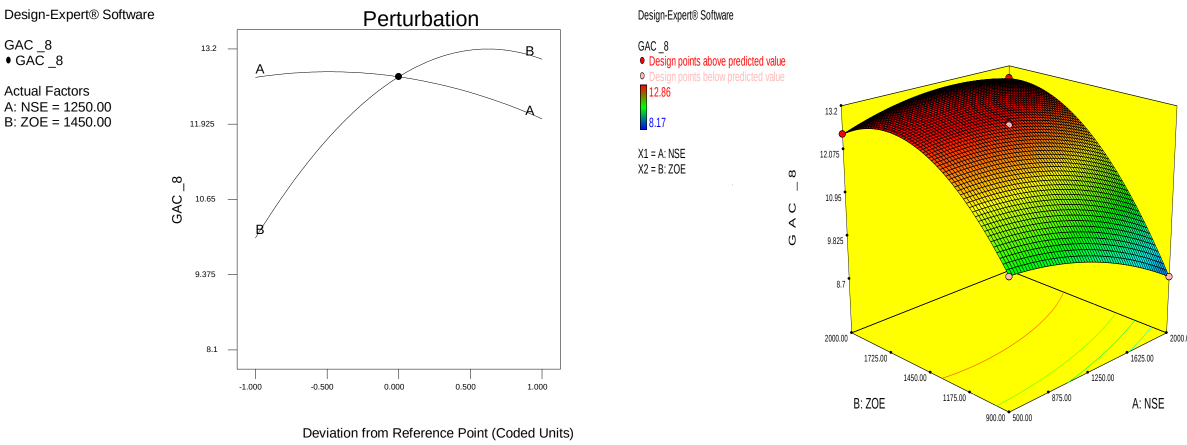
2e


Design-Expert® Software
GAC _6
GAC _6
Actual Factors
A: NSE = 1250.00
B: ZOE = 1450.00
Perturbation
GAC _6
Deviation from Reference Point (Coded Units)
Design-Expert® Software
GAC _6
Design points above predicted value
Design points below predicted value
2.33
0.4
X1 = A: NSE
X2 = B: ZOE
B: ZOE
A: NSE

2f



2g



2h

Fig 2: Perturbation and 3D graph for glucose adsorption capacity (GAC) @ 2a: 1% in 5mmol, 2b: 2% in 5mmol, 2c: 1% in 10mmol, 2d: 2 % in 10mmol, 2e:1% in 25mmol, 2f: 2% in 25mmol, 2g: 1% in 50mmol and 2h: 2% in 50mmol **of Cardiacare-DM**

Optimization of independent variables:

Numerical optimization of independent variables, NSE and ZOE, was achieved using design expert software. The predicted and actual values of the response are given in **Table 4**. The aim of the experiment was to increase the overall acceptability score and glucose adsorption capacity. The optimal solution was 500mg NSE and 900mg ZOE, with a best fit desirability of 0.854 (**Fig. 3**). The optimized results in score were taste 15.69, flavor 15.64, foldability 16.89, texture 16.93, breakability 17.14, and an overall acceptability 17.07; glucose adsorption capacity in 5 mmol at 1% -0.24, 2% 0.27, in 10 mmol at 1% 1.66, 2% 1.18, in 25 mmol at 1% 0.61, 2% 1.73, in 50 mmol at 1% 7.98, 2% 10.10 mmol/dL. The predicted response value as compared to the actual value for responses, as shown in **Table 4,** were in concurrence with each other; hence, the fitted models are suitable for predicting the responses. RSM showed that quadratic response surface models were fitted. F values were

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significant in all selected responses, and a high R^2 value showed the fitness of the polynomial regression models for describing the effect of variables.

Table 4: Predicted and actual response values for the optimized composition Cardiacare-DM

Responses	Predicted	Actual (n=3)
NSE (mg)	500	500
ZOE (mg)	900	900
Taste (Score)	15.70	15.23 ± 1.90
Flavor (Score)	15.70	15.60 ± 2.04
Foldability (Score)	16.89	17.23 ± 1.94
Texture (Score))	16.93	17.00 ± 1.90
Breakability (Score)	17.15	17.66 ± 1.01
OAA * (Score)	17.08	17.40 ± 1.91
GAC[1% in 5mmol (mmol/dL)]	-0.24	-0.24 ± 0.04
GAC [2% in 5mmol (mmol/dL)]	0.27	0.29 ± 0.03
GAC [1% in 10mmol (mmol/dL)]	1.66	1.66 ± 0.09
GAC [2% in 10mmol (mmol/dL)]	1.18	1.21 ± 0.31
GAC [1% in 25mmol (mmol/dL)]	0.61	1.4 ± 0.21
GAC [2% in 25mmol (mmol/dL)]	1.73	1.08 ± 0.25
GAC [1% in 50mmol (mmol/dL)]	7.98	7.84 ± 0.56
GAC [2% in 50mmol (mmol/dL)]	10.51	10.1 ± 1.24

Note: * Over all acceptability (OAA) scored on Composite score card scale, GAC-Glucose adsorption capacity.

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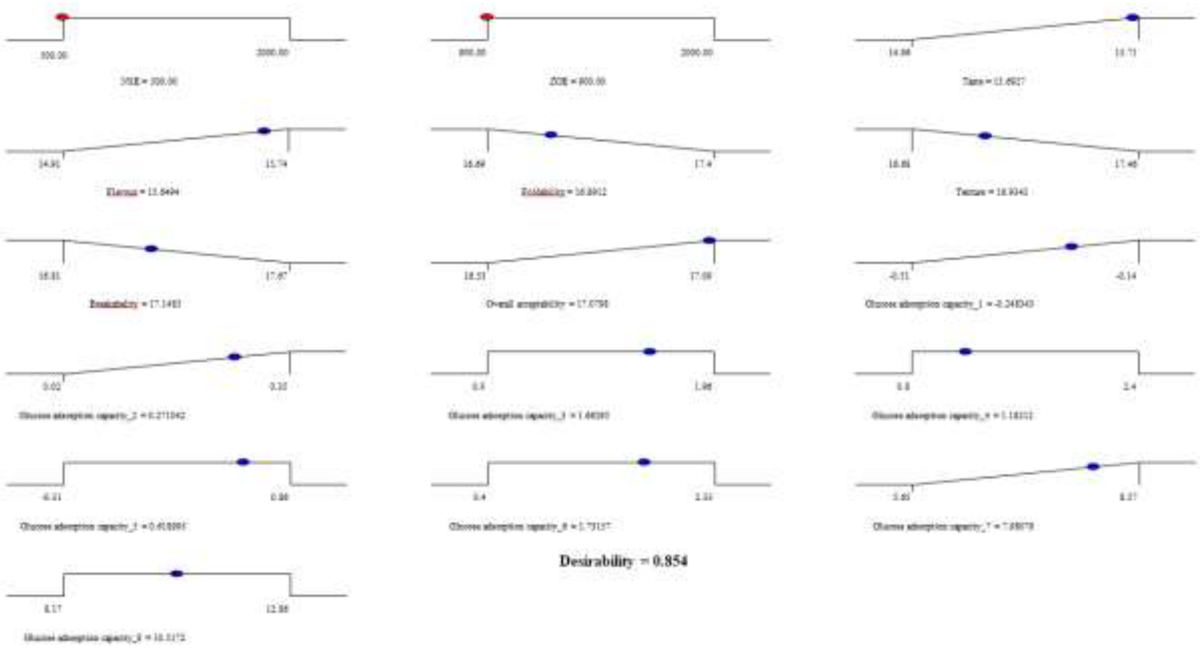

Fig 3: Optimized levels of variable and their desirability plot for Cardiacare-DM

Conclusion:

The application of RSM for optimizing the ingredients and process showed that the quadratic response surface model was fitted. F values were significant in all selected responses, and a high R^2 value of >98% showed the fitness of the polynomial regression models for describing the effects of variables. The results of the study indicated that the effect of NSE and ZOE concentrations was significant for all the selected responses, and RSM could be useful in optimizing the NSE and ZOE to get a highly acceptable product. Using the optimized conditions, chapathi from C-DM was prepared for sensory evaluation and glucose adsorption capacity. The optimal composition was 500mg of NSE and 900mg of ZOE, with a best-fit desirability of 0.854. The product has a good overall acceptability score of 17.08 on the 1-20 composite score card scale and can be consumed on a daily basis. With better glucose adsorption capacity at increased glucose concentrations in the *in-vitro* model at 50 mmol at 1 % 7.98 mmol/dL and at 2 % 10.51 mmol/dL, will help in the management of blood glucose levels. According to the findings, the improved C-DM was nutritionally and functionally robust and highly accepted.

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Author's contributions: Puneetha P. contributed to the creation of the work's design as well as the analysis, interpretation, and design analysis. Conceptualization, important intellectual contributions, and submission approval were all handled by Asna Urooj. Devaki C. S. contributed significantly to the design as well as the statistical analysis for this publication and the findings write-up.

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