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Research Article

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Process Optimization for the Development of Ashwagandha Root Extract Enriched Shrikhand Using Response Surface Methodology

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Abstract

Response surface methodology (RSM)'s central composite rotatable design (CCD) expert tool was used to optimize the "Ashwagandha root extract (ARE) enriched shrikhand" process. Different levels of chakka, powdered sugar and ARE for manufacturing of functional shrikhand has been optimized. The optimized input variables found were 0.742% for ARE, 29.16% for powdered sugar and 69.48% for chakka. Different sensory parameters were optimized by numerical approach methods and found to be 7.74 for color, 7.38 for flavor, 8.15 for sweetness and 7.97 for overall acceptability. The desirability of the selected finished product was found to be approximately 80.1%. The physico-chemical properties of ARE enriched shrikhand was 60.45% moisture, 8.40% protein, 15.92% fat, 60.26% total sugar, 0.79% ash, 1.4% acidity with pH 4.4. The optimized product has 81.40% DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition, 73.25% ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) activity, 53.68 mg/100 g total phenolic content (TPC) as gallic acid equivalent (GAE), 45.85 g firmness, 1000.97 consistency, 30.28% cohesiveness and 66.77 index of viscosity.

Keywords

Ashwagandha, Response surface methodology, Functional foods, Antioxidant activity, Immunomodulation, Health-benefits

Introduction

Animal Husbandry and Dairying Department states that, the amount of milk available per person in the Indian fiscal year 2021-2022 is 427 grams per day, and the total amount of milk produced annually is about 209.96 MT [1]. It is estimated that between 50 and 55 percent of India's milk production is used to make a range of desserts and sweets [2]. A total of 7% of milk produced is used in fermentation, for three primary products, being curd (dahi), concentrated sweetened curd (shrikhand), and stirred curd (lassi) [3].

Shrikhand is a fermented dairy product that is soft, semi-solid, and sweet-sour in taste. In Gujarat, Maharashtra, Karnataka, and some other parts of our country it is very popular among people as dessert dish due to its refreshing sweet taste and nutritional values. Shrikhand can be manufactured by fermenting milk by lactic acid bacteria. Chakka is the base material of finished product, which is curd or strained yogurt that has been carefully combined with sugar and flavorings. It is nutritiously superior to other products made from fermented milk. It is very reviving, especially in the hot season, like dahi (curd). Its distinctive flavor, taste, edible nature, and potential medicinal efficacy make it popular [4].

Ashwagandha, scientific name *Withania somnifera* of family Solanaceae is a medicinal plant that has been used in ayurvedic medicine since long years

back. According to Misra et al. [5], there are numerous health benefits of ashwagandha that includes its anti-inflammatory, anti-tumor, anti-stress, antioxidant, rejuvenating, brain-boosting, immunomodulatory, and anti-aging effects [6]. It contains flavonoids, anolides, and other bioactive substances. The primary causes of the antioxidant activity are the naturally occurring antioxidants glutathione peroxidase, catalase, and superoxide dismutase [7-10].

The research and development in functional and nutraceutical foods is now a days one of the promising areas for scientists working in food product development. Global functional food market is expected to reach approximately USD 597.1 billion by 2032, growing at a registered compound annual growth rate of 6.93% during the forecast period 2023 to 2032, according to data released by precedence research in 2023 [11]. The market was valued at USD 305.4 billion in 2022. The market of functional foods has been prompted due to advancement of scientific knowledge and need of a balanced diet for well-being, technological advancements in the food industry, and growing consumer's demand for food items that promote health [12-14]. Functionality can be introduced by adding probiotic bacteria or other health-promoting food ingredients to the food matrix, or it can be present already in the raw material or that could be added by innovative, novel processing procedures [15].

New food products could be developed, containing functional components to improve consumer desirability, demand, and acceptance. Due to bitter flavor and unattractive appearance, medicinally significant herbs cannot be directly added to any food product. Whole herbs by adding directly into any food products may cause food products to change negatively. Designing nutraceutical foods with the inclusion of herbal extracts that contain active compounds may be a better alternative. There are numerous functionally significant active components in ashwagandha roots that are beneficial for treating tumors, immunomodulation, cognitive enhancement, neuroprotection, etc. [6, 16]. Milk and dairy products act as a vehicle for the functional properties of herbs. Since shrikhand contains a high percentage of fat and sugar, it may be used to incorporate more herbal extracts and has a better sensory quality than other dairy products. The goal of the current project is to make herbal shrikhand by including ARE's while keeping in mind the plant's beneficial and medicinal qualities.

Materials and Methods

The ARE enriched shrikhand was prepared by using the RSM trials with different combinations of ARE, chakka and sugar to optimize the product.

Preparation of herbal extract: The optimized herbal extract possessing highest antioxidant activity and phenol content were dried in vacuum drier were taken [17].

Preparation of curd

Preparation of chakka: Hanging curd in muslin cloth for 14-16 h at 25 ˚C. Collection of chakka.

Preparation of ARE enriched shrikhand [18].

RSM trials were conducted to optimize the levels of ARE, chakka and sugar to prepare the ARE enriched shrikhand.

The optimization has been done based on following responses:

- Antioxidant property (DPPH) [19, 20].
- Sensory attributes (Flavor overall acceptability) [21, 22].
- Textural characteristics (Consistency and cohesiveness).
- Total phenols [23].

Preparation of ARE enriched shrikhand

The process for manufacturing ARE enriched shrikhand has been shown by a flow chart given in figure 1.

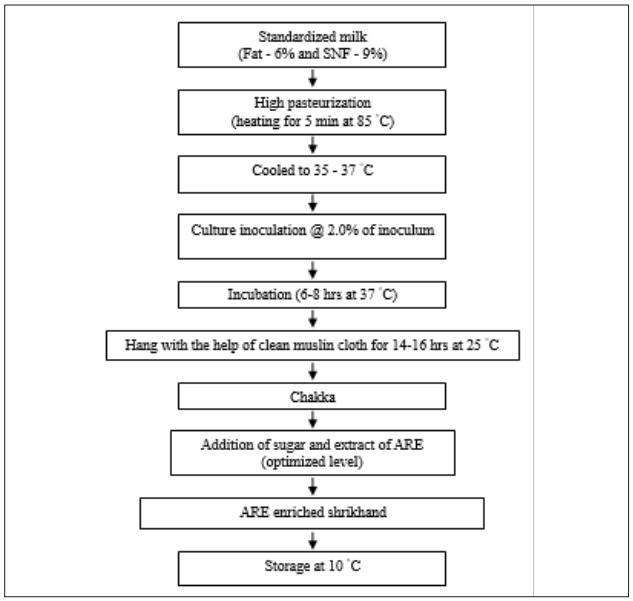


Figure 1: Flow chart of manufacturing of ARE enriched shrikhand.

Design of experiment

A scientific approach to planning is known as statistical design of experiment. This ensures that relevant data are collected and analyzed using statistical methods to produce reliable, meaningful, and objective conclusions.

When the design plan calls for sequential experimentation, CCD is typically advocated since they can incorporate data from a properly designed factorial experiment. The factorial and center points could be used as a first order linear model while demonstrating the significance of a second order contribution. The values of different variables and number of trials are given in table 1.

By adding axial and center points, we then construct the factorial section of the design up into a CCD expert to fit a second-degree model. In addition to making, it simple to achieve the desired design characteristics of orthogonal blocking and rotatability, CCD enable effective estimate of the quadratic terms in the second order model. Table 2 lists the process variables that were employed in the CCD for the ARE, chakka, and sugar percentage variables.

Statistical analysis

Using RSM and CCD, the data obtained during the current study was analysed. Utilizing the following second-order polynomial equation, the response surface regression approach was used to assess the experimental data collected from the design.

$$Y_i = \beta_0 + \sum_i \beta_i x_i + \sum_{ii} \beta_{ii} x_i^2 + \sum_{ij} \beta_{ij} x_i j_i$$

Where, Y_i was the predicted response, $x_i x_j$ were independent variables, β_0 was the offset term, βi was the i^{th} linear coefficient, β_{ii} was the ii^{th} quadratic coefficient and β_{ij} was ij^{th} interaction coefficient. However, in this study, the independent variables were coded as A, B and C. Thus, the second order polynomial equation could be presented as follows:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD$$

The optimized herbal shrikhand was analyzed for the following properties.

- Proximate composition (moisture, sugar, fat, protein, and ash) [24].
- Antioxidant activity (DPPH and ABTS).
- TPC.
- Rheological characteristics (consistency, cohesiveness, and index of viscosity).

Table 1: The CCD for three independent variables.

Std.	Run	A: ARE (%)	B: Chakka (%)	C: Sugar (%)
9	1	0.15	70	30
12	2	1	78.40	30
11	3	1	61.59	30
6	4	1.5	65	35
7	5	0.5	75	35
1	6	0.5	65	25
15	7	1	70	30
19	8	1	70	30
16	9	1	70	30
4	10	1.5	75	25
17	11	1	70	30
3	12	0.5	75	25
13	13	1	70	21.59
2	14	1.5	65	25
5	15	0.5	65	35
20	16	1	70	30
18	17	1	70	30
10	18	1.84	70	30
8	19	1.5	75	35
14	20	1	70	38.40

Table 2: Process variables used in the CCD for three independent variables.

Code	Variables	Unit	Minimum value	Maximum value	Mean	Std. Dev.
A	ARE	%	0.159104	1.8409	1	0.423905
B	Chakka	%	61.591	78.409	70	4.23905
C	Sugar	%	21.591	38.409	30	4.23905

Results and Discussion

Optimization of ARE, chakka and sugar levels in ARE enriched shrikhand by RSM

20 tests for different levels of the variables ARE, chakka, and sugar were chosen using a CCD. Table 3 displays the antioxidant value, textural qualities, and sensory scores as altered by various concentrations of sugar, chakka, and ARE. Table 4 and table 5 reflect the findings from table 3. The data obtained from the experimental results were found fitted to

Table 3: Runs with different variables and responses in CCRD.

Std.	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6
		A: ARE (%)	B: Chakka (%)	C: Sugar (%)	TPC (mg/100 g)	DPPH (%)	Consistency	Cohesiveness	Flavor	Overall acceptability
9	1	0.159104	70	30	45.56	79.05	1000.71	30.507	7	7
12	2	1	78.409	30	45.95	80.35	1050.41	26.022	7	8
11	3	1	61.591	30	50.35	81.85	885.235	26.254	7.5	9
6	4	1.5	65	35	54.48	84.45	1068.34	31.154	8	7.8
7	5	0.5	75	35	69.96	80.35	853.413	32.21	7	7
1	6	0.5	65	25	55.24	83.57	779.621	28.755	7.5	8
15	7	1	70	30	53.96	81.36	1000.61	30.48	7	8.5
19	8	1	70	30	53.96	81.36	1000.61	30.48	7	8.5
16	9	1	70	30	53.96	81.36	1000.61	30.48	7	8.5
4	10	1.5	75	25	32.56	70.75	523.158	26.214	6	7.8
17	11	1	70	30	53.96	81.36	1000.61	30.48	7	8.5
3	12	0.5	75	25	44.03	81.67	918.929	25.313	7.2	7.5
13	13	1	70	21.591	30.25	70.65	638.581	30.791	7	7.8
2	14	1.5	65	25	48.12	80.96	758.612	28.364	8	8
5	15	0.5	65	35	41.25	81.25	965.64	25.338	7	9
20	16	1	70	30	53.96	81.36	1000.61	30.48	7	8.5
18	17	1	70	30	53.96	81.36	1000.61	30.48	7	8.5
10	18	1.8409	70	30	46.35	81.96	1112.22	25.207	7.2	7.8
8	19	1.5	75	35	52.56	84.75	698.509	29.418	7	7
14	20	1	70	38.409	30.25	74.26	835.512	31.643	9	9

Table 4: Scores of the trials of ARE enriched shrikhand by Design Expert 9.0.4.1.

Solutions											
S. No.	ARE	Chakka	Sugar	TPC	DPPH	Consistency	Cohesiveness	Flavor	Overall acceptability	Desirability	Selected
1	0.724	69.480	29.160	53.689	81.436	1001.031	30.281	6.975	8.360	0.807	Selected
2	0.728	69.480	29.160	53.685	81.430	1000.873	30.286	6.976	8.364	0.807	-
3	0.719	69.480	29.160	53.693	81.446	1001.261	30.273	6.974	8.353	0.807	-
4	0.713	69.480	29.160	53.697	81.455	1001.471	30.266	6.973	8.347	0.807	-
5	0.737	69.480	29.165	53.681	81.416	1000.615	30.298	6.977	8.374	0.807	-
6	0.744	69.480	29.160	53.671	81.404	1000.245	30.306	6.978	8.381	0.807	-
7	0.706	69.480	29.160	53.703	81.468	1001.770	30.255	6.972	8.339	0.807	-
8	0.753	69.480	29.160	53.662	81.390	999.900	30.316	6.979	8.389	0.807	-
9	0.699	69.480	29.184	53.722	81.481	1002.418	30.248	6.972	8.333	0.807	-
10	0.671	69.480	29.160	53.722	81.529	1003.140	30.201	6.967	8.296	0.807	-
11	0.577	69.480	29.160	53.730	81.712	1006.841	30.008	6.953	8.159	0.804	-

Table 5: Summary for the formulation of ARE enriched shrikhand by Design Expert 9.0.4.1.

Response	Name	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
R1	TPC (mg/100 g)	20	Polynomial	30.25	69.96	48.5335	9.60575	2.31273	None	Quadratic
R2	DPPH (%)	20		70.65	84.75	80.2015	3.87307	1.19958		
R3	Consistency	20		523.158	1112.22	904.628	156.119	2.12597		
R4	Cohesiveness	20		25.207	32.21	29.0035	2.37033	1.27782		
R5	Flavor	20		6	9	7.22	0.589915	1.5		
R6	Overall acceptability	20		7	9	8.085	0.639305	1.28571		

higher degree of polynomial equations, i.e., quadratic equations, to quantify the curvature effects. The three variables, ARE, chakka, and sugar, are each represented by a coded term in the polynomial equations, as A, B, and C, respectively.

The models in the equations are the ones that remained after the irrelevant variables and their interactions were taken out. According to the statistical analysis, the models had extremely low probability values and were highly significant ($p < 0.01$). At 99% confidence level, it is demonstrated that the models were significant. The coefficient of determination (R^2) was defined as the square of the correlation coefficient for each response. At a 95% confidence level, it is demonstrated highly significance regression.

The adjusted determination coefficient (adjusted R^2) must also have a high value in order to support the model's high significance [25]. For each of the eight responses, the anticipated versus reality plot is also displayed. It demonstrates that the true values are distributed with R^2 values that are quite high and follows the straight line (y = x).

The F-test for lack of fit was used to evaluate the models' suitability [26]. A signal to noise ratio, also known as adequate precision, is a measurement of the range in the projected response in relation to its related error. It should have a value of at least 4 [27]. For all models, it was determined that the value was desirable. Furthermore, as noted by Ahmad et al. [28], low coefficient of variation values signifies improved experiment precision and dependability.

Effect of variables on antioxidant activity of ARE enriched shrikhand

Antioxidant activities in ARE enriched shrikhand are presented in table 3, table 4 and table 5. Details discussed in the following heads:

Effect on TPC

The maximum and minimum values for total phenol were 69.96 mg/100 g of GAE and 30.25 mg/100 g of GAE, respectively (Table 5). The data fits the following quadratic model.

$$TPC = 53.67 - 1.57 \times A - 0.54 \times B + 2.80 \times C - 4.37 \times AB + 1.80 \times AC + 6.70 \times BC - 0.91 \times A^2 - 0.14 \times B2 - 6.47 \times C^2 \quad (1)$$

In equation, the coded terms A, B and C have been used for the three different variables, ARE, chakka and sugar respectively.

For TPC, the quadratic model was determined to be significant ($p < 0.01$). 0.7360 is the coefficient of determination (R^2). There is a fair amount of agreement between the "Adj R-Squared" is 0.49 and the "Pred R-Squared" is 1.07. For an appropriate signal, the "Adeq Precision" is 6.42 and the signal noise ratio is larger than 4. Thus, one may traverse the design space using the model. A three-dimensional response surface plot of TPC as influenced by levels of ARE, chakka and sugar is shown in figure 2.

It was analysed from figure 2a that ARE percentage increases the TPC in the product since the phenolic content in ashwagandha root was high as reported in early part of the research [29, 30]. Increase in chakka percentage also increases the TPC in ARE enriched shrikhand.

Figure 2b depicts that sugar percentage should be optimum near about 30% to get maximum TPC (50%).

Figure 2c shows the actual versus predicted values plot for the TPC of ARE enriched shrikhand. Figure 2 clearly shows

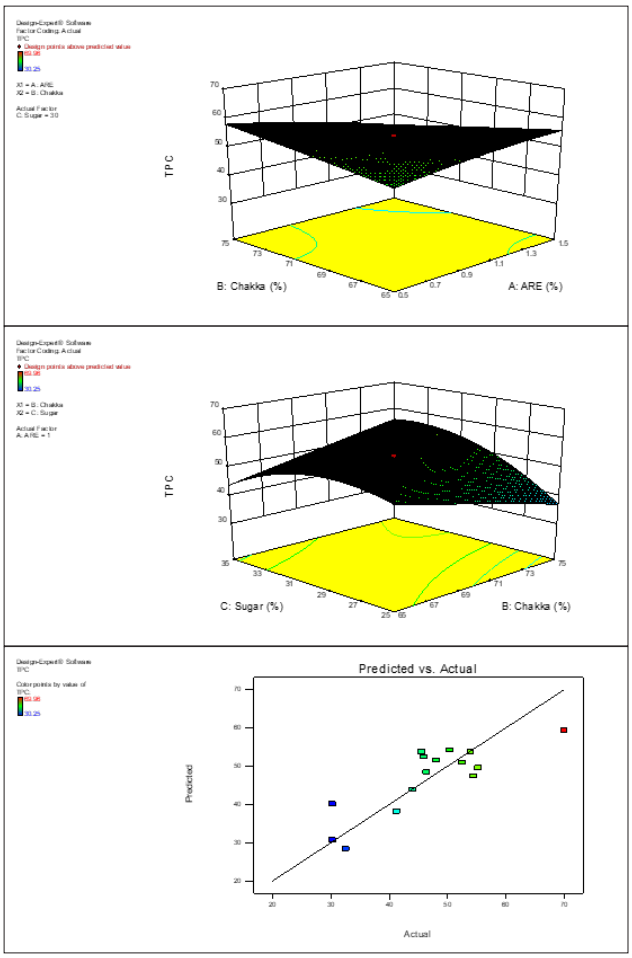


Figure 2: 3D plots showing the effect of levels of ARE, chakka and sugar on TPC of ARE enriched shrikhand. **(a)** ARE and chakka, **(b)** Chakka and sugar, and **(c)** Actual versus predicted values plot for the TPC of ARE enriched shrikhand.

that the predicted values are very much closer to the actual values of TPC. It is clear from the data that the maximum number of observed values are closer to the maximum TPC in ARE enriched shrikhand. Maximum total phenol means product have higher antioxidant value [6, 31].

Effect on DPPH scavenging activity

The maximum and minimum DPPH percentage inhibition obtained were 84.75% and 70.65%, respectively (Table 5). The data fits the following quadratic equation 1.

$$\text{DPPH scavenging activity} = 81.26 - 0.076 \times A - 0.38 \times B + 0.11 \times C + 0.19 \times AB - 0.19 \times AC - 0.26 \times BC - 0.46 \times A^2 - 0.066 \times B^2 - 0.10 \times C^2 \quad (2)$$

It is 0.7988 for the coefficient of determination (R^2). There is a decent agreement between the "Adj R-Squared" of 0.61 and the "Pred R-Squared" of 0.58. A sufficient signal is shown by the "Adeq Precision" of 7.663, which is higher than 4. As a result, one might explore the design space using this approach.

Figure 3 shows the response for DPPH scavenging activity influenced by ARE, chakka and sugar. It can be observed from figure 3a and figure 3b, that with the increased level of ARE (up to 1.5%), the DPPH activity was increased up to 83% [30] whereas with the increase in level of chakka, it was

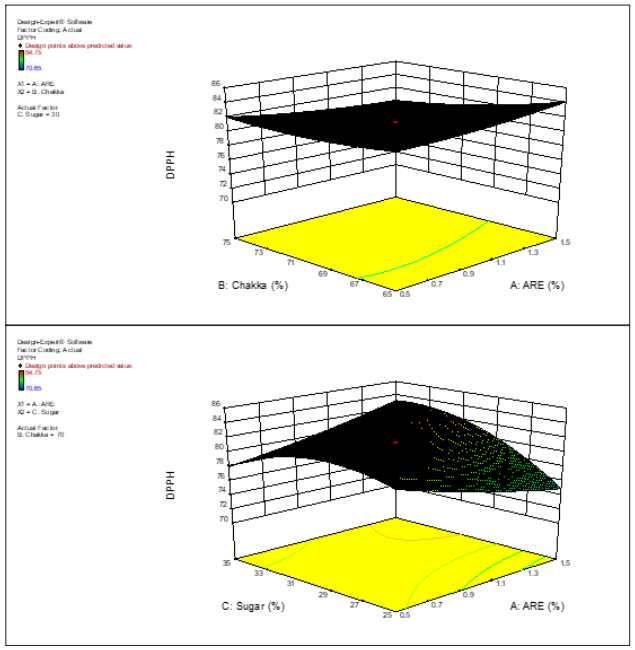


Figure 3: 3D plots showing the effect of levels of ARE, chakka and sugar on DPPH of ARE enriched shrikhand. **(a)** ARE and chakka, and **(b)** ARE and grounded sugar percentage.

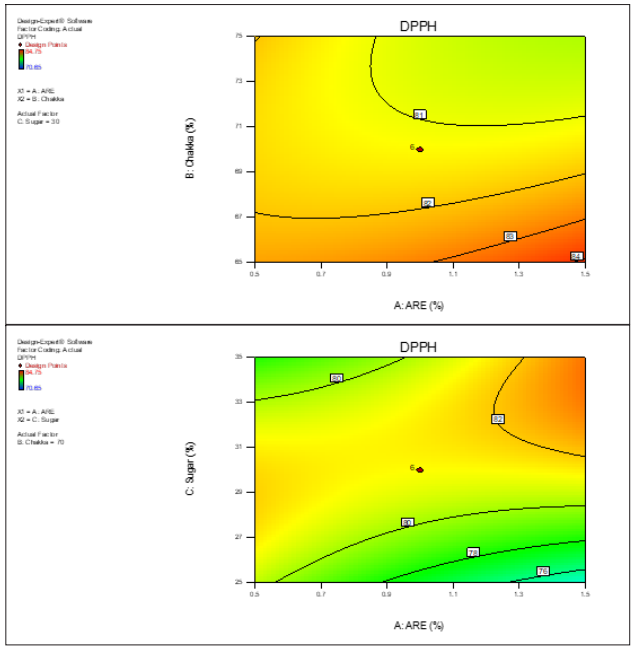


Figure 4: Contour plots indicating the effect of levels of ARE, chakka and sugar on DPPH of ARE enriched shrikhand. **(a)** ARE and chakka, and **(b)** ARE and sugar.

nearly constant. Results are in very much favor with the results observed earlier by Polumackanycz et al. [32]. With the increased percentage of sugar in combination with ARE there was a vast increase in DPPH activity [17].

The two-dimensional contour plots for the aforementioned response, which are based on the quadratic model (equation 2), readily show the impacts of factors on ARE enriched shrikhand (Figure 4). Figure 5 displays the plotted association between actual and expected values.

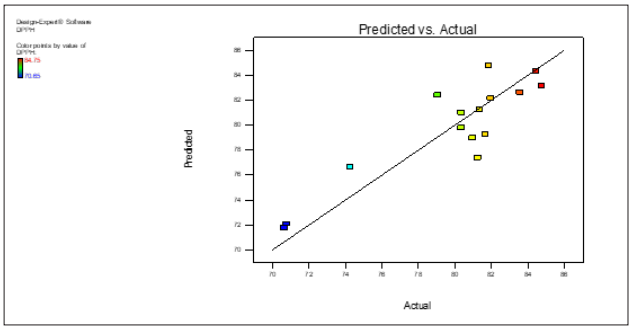


Figure 5: Actual versus predicted values plot for DPPH of ARE enriched shrikhand.

Effect on consistency

The maximum and minimum consistency obtained were 1112.22 and 523.15, respectively (Table 5). The data fits the following quadratic equation:

$$\text{Consistency} = 1003.67 - 20.61 \times A - 22.00 \times B\ 68.59 \times C - 79.05 \times AB + 45.57 \times AC - 48.84 \times BC - 0.26 \times A^2 - 31.60 \times B^2 - 113.19 \times C_2 \quad (3)$$

0.7665 is the coefficient of determination (R^2). There is a fair amount of agreement between the "Adj R-Squared" of 0.5564 and the "Pred R-Squared" of 0.7765. An sufficient signal is indicated by the "Adeq Precision" of 7.253, which is greater than 4. As a result, one might explore the design space using this approach.

Figure 6 shows the graphical response for consistency as influenced by ARE, chakka and sugar. It can be seen from figure 6a that there was increase in consistency by chakka due to its smoothy texture, percentage of ARE does not have any major effect on consistency of the product, may be due to its minute quantity [18, 33].

Figure 6b reveals that sugar has positive response for consistency of the product. There is a direct proportional relation between sugar and consistency of the product [34-36].

The model (equation 3) was used to construct two-dimensional contour plots for the measured responses, which are displayed in figure 6c that aid in understanding the interaction effects of various parameters on ARE enriched shrikhand.

Effect on cohesiveness

The maximum and minimum cohesiveness obtained were 31.64 and 25.20, respectively (Table 5). The data fits the following quadratic equation:

$$\text{Cohesiveness} = 30.48 - 0.39 \times A - 0.062 \times B + 0.80 \times C - 0.91 \times AB + 0.31 \times AC + 1.34 \times BC - 0.91 \times A^2 - 1.52 \times B^2 + 0.27 \times C^2 \quad (4)$$

Where A, B and C used here are for ARE, chakka and sugar respectively.

There is a 0.7318 coefficient of determination (R^2). There is an agreement between "Adj R-Squared" 0.490 and "Pred R-Squared" 1.21. Anadequate signal is shown by the "Adeq Precision" 6.774, which is greater than 4. As a result, one might explore the design space using this approach.

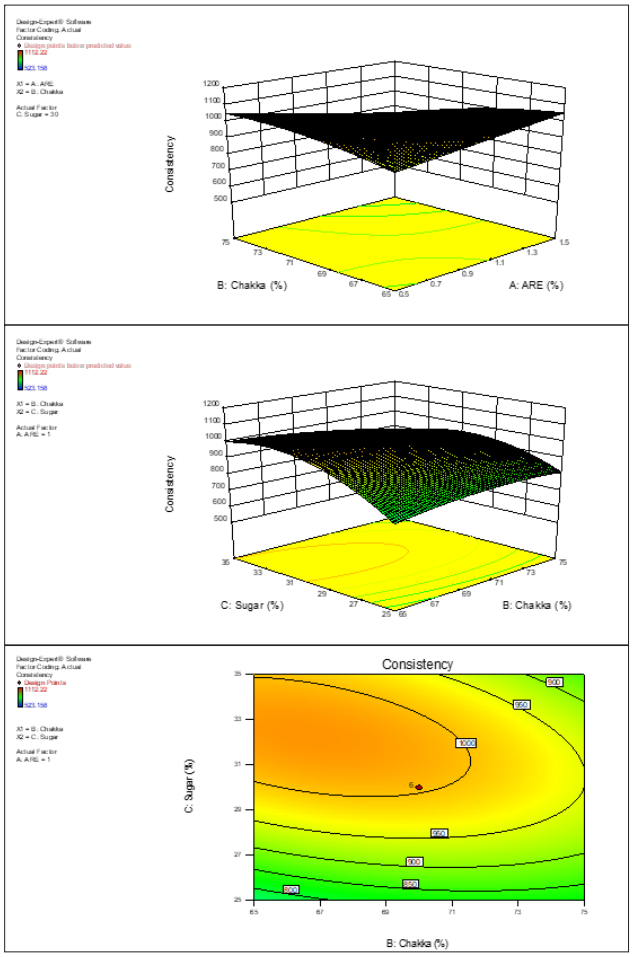


Figure 6: 3D plots suggesting the effect of levels of ARE, chakka and sugar on consistency of ARE enriched shrikhand. **(a)** ARE and chakka, **(b)** Chakka and grounded sugar percentage, and **(c)** Contour plots representing the effect of chakka and sugar on consistency of ARE modified shrikhand.

Figure 7 shows the response for cohesiveness influenced by ARE, chakka and sugar. It can be seen from figure 7a that there was curvature type effect on cohesiveness by chakka percentage and ARE percentage. It means they showed optimum level of cohesiveness at middle levels i.e., 1% of ARE and 70% of chakka [33].

Figure 7b reveals that sugar increases the cohesiveness nature of the product due to its stickiness properties, same pattern was also observed with chakka but only upto 70%, beyond that cohesiveness properties of ARE enriched shrikhand get deteriorated [18]. No major effect on cohesiveness was observed due to the increase in ARE percentage [36]. Figure 7 clearly depicts that increase in ARE percentage does not have any major effect on cohesiveness while there is proportional relation between cohesiveness with sugar and chakka percentage [34, 35].

Effect on flavor

The most crucial element in determining whether a dessert made from fermented dairy will be accepted is flavor (both taste and smell). The following equation can be used to represent the regression equation for the effect of variables (ARE, chakka, and sugar) on the flavor score of ARE enhanced shrikhand.

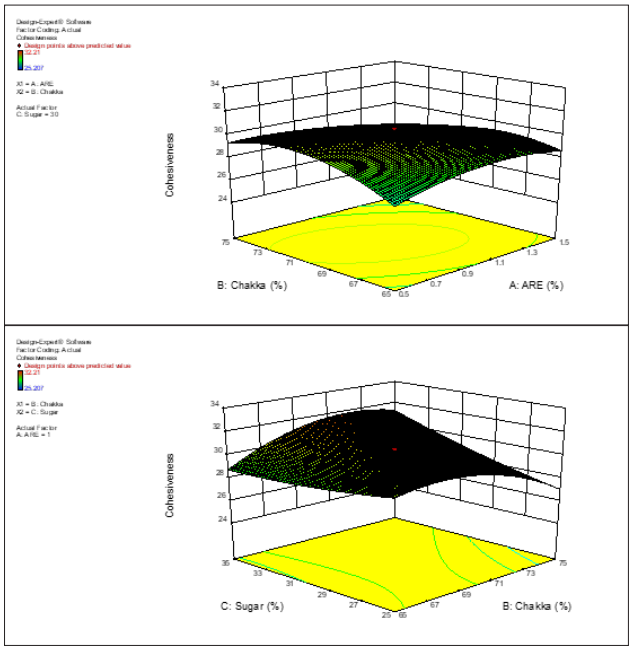


Figure 7: 3D plots representing the effect of levels of ARE, chakka and sugar on cohesiveness of shrikhand formulated with ARE. **(a)** ARE and chakka and **(b)** Chakka and sugar.

$$\text{Flavor} = 7.01 + 0.047 \times A - 0.030 \times B + 0.27 \times C - 0.34 \times AB + 0.21 \times AC + 0.16 \times BC - 0.021 \times A^2 + 0.033 \times B^2 + 0.30 \times C^2 \quad (5)$$

The F-value was used to evaluate the created model's quality of fit. The model of flavor's F-value is significant ($p < 0.01$) at 3.64. Coefficient of determination (R^2) is 0.7662, indicates that the model could be used on account for 76.62% of the variability in the answer. The "Pred. R-Squared" 0.79 and the "Adj R-Squared" 0.55 are reasonably in favor of the model. An sufficient signal is indicated by the "Adeq. Precision" reading of 8.06, which is higher than 4. In terms of flavor, the hedonic scale ranged from 7 to 9 (Table 5).

The flavor score of shrikhand was shown to increase ($p < 0.01$) in tandem with the levels of ARE, which increased from 0.5 to 1.5%. However, the flavor score of shrikhand was not significantly affected by the levels of chakka (65 - 75%) in conjunction with ARE. The flavor score (Figure 8) considerably increased ($p < 0.01$) with an increase in sugar percentage (> 30%) [37]. The flavor score was found to be lower ($p < 0.01$) at the beginning levels of sugar (Figure 8) [18], but opposite pattern was observed when the levels of sugar increased from 25 to 35%. The results likewise closely matched the outcome noted by Landge et al. [38].

Effect on overall acceptability

The following equation has been used to describe the quadratic equation generated by the RSM design expert demonstrating the influence of ARE, chakka, and sugar on ARE enriched shrikhand:

$$\text{Overall acceptability} = 8.51 + 0.033 \times A - 0.38 \times B + 0.11 \times C + 0.19 \times AB - 0.19 \times AC - 0.26 \times BC - 0.46 \times A^2 - 0.066 \times B^2 - 0.10 \times C^2 \quad (6)$$

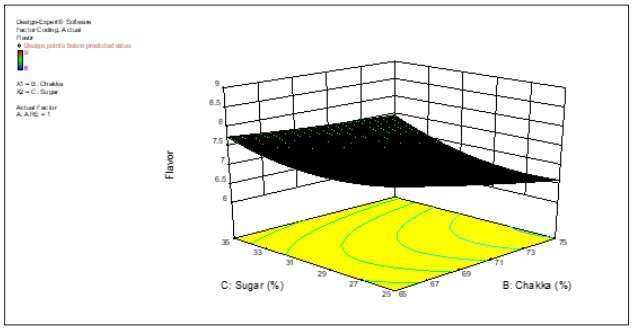


Figure 8: 3D plots showing the effect of levels of chakka and sugar on flavor of ARE enriched.

The F-value was used to evaluate the created model's quality of fit. The texturing model's F-value (4.76) is significant ($p < 0.05$). The coefficient of determination (R^2) is 0.8108, meaning that the model could account for 81.08% of the variation in the answer.

The "Pred. R-Squared" of 0.45 and the "Adj R-Squared" of 0.64 are reasonably in accord. The "Adeq. Precision" value of 6.96, which is greater than 4, denotes a sufficient signal. Consequently, one may utilize this model to explore the design space. According to table 5, the hedonic scale for texture ranged from 7 to 9. Figure 9 shows the response surface plots for overall acceptance.

Drawing from the model, two and three dimensional contour plots were created for the measured response to gain a deeper understanding of the relation between various components on the overall acceptance score of shrikhand supplemented with ARE (Equation 6). The variations in the overall acceptability score of shrikhand enriched with ARE as a function of ARE, chakka, and sugar are displayed in figure 10.

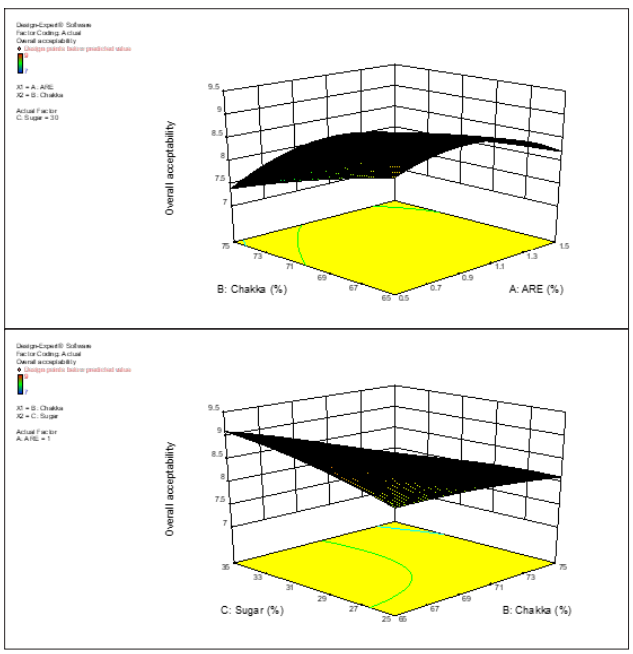


Figure 9: 3D plots producing the effect of levels of ARE, chakka and sugar on overall acceptability of ARE enriched shrikhand. **(a)** ARE and chakka and **(b)** Chakka and sugar.

Figure 9a clearly reveals that overall acceptability gives the best result when the percentage of ARE increased upto a certain limit i.e., upto 1%. After that there is decreasing behavior noticed in the score of overall acceptability. Negative interaction has been shown by chakka percentage because due to addition of more chakka the product was not widely accepted [33]. As the sugar percentage increases linearly, there was a considerable improvement in the overall acceptability of shrikhand (Figure 9b) [18]. Consistent with these results, Akter et al. [39] reported that variances in the total acceptability score [37] were primarily caused by variations in sugar levels. Gelatin could be added to dahi samples during preparation to increase the body and consistency score.

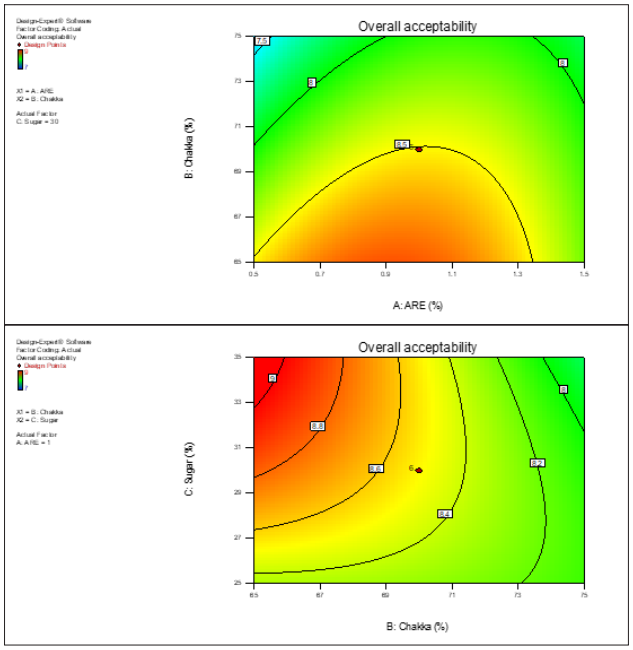


Figure 10: Contour plots representing the effect of levels of ARE, chakka and sugar on overall acceptability of optimized herbal shrikhand. **(a)** ARE and chakka and **(b)** Chakka and sugar.

Validation of optimized solutions

The ideal mixture of the ingredient-based variables was found using the optimization command of the Design Expert 9.0.4.1. The TPC score, DPPH activity, consistency, cohesion, flavor, and overall acceptability were considered for the optimization target.

The method adopted for the process optimization was based on numerical method. The constraints have been listed in table 6.

Table 7 shows the optimized recipe solution as predicted by the RSM software. The solution shows that the concentration of ARE should be 0.74%, chakka should be 69.48% and sugar should be 29.16%.

Chemical composition of optimized ARE enriched shrikhand

Physico-chemical properties of control shrikhand and enriched ARE enriched shrikhand was examined and is tabulated in table 8. These proximate compositions are very much more in line with Dhillon et al. [37] and Sachin et al. [40] findings.

Conclusion

RSM design expert trials were conducted with three vari-

Table 7: Optimized recipe obtained from the solution of RSM trials.

Variable/Responses	Score
A: ARE	0.74%
B: Chakka	69.48%
C: Sugar	29.16%
TPC	53.68 mg/100 g GAE
DPPH	81.43%
Consistency	1000.98
Cohesiveness	30.28
Flavor	6.97
Overall acceptability	8.36

Table 6: Constraints fixed for optimization of levels.

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
A: ARE (%)	Is in range	0.5	1.5	1	1	5
B: Chakka (%)	Is target = 69.48	65	75	1	1	5
C: Sugar (%)	Is target = 29.16	25	35	1	1	3
TPC mg/100 g	Maximize	30.25	69.96	1	1	3
DPPH (%)	Maximize	70.65	84.75	1	1	5
Consistency	Maximize	523.158	1112.22	1	1	5
Cohesiveness	Maximize	25.207	32.21	1	1	5
Flavor	Is in range	6	9	1	1	3
Overall acceptability	Is in range	7	9	1	1	3

Table 8: Compositional properties of control shrikhand and optimized ARE enriched shrikhand.

Treatment	Fat (%)	Protein (%)	Ash (%)	Total sugar (%)	Moisture (%)	pH	Acidity (% lactic acid)
Control shrikhand	8.59 ± 0.0033^{a}	8.51 ± 0.016^{a}	0.78 ± 0^{a}	58.34 ± 0.0058^{a}	42.66 ± 0.0058^{a}	4.68 ± 0.0033^{a}	1.21 ± 0.0033^{a}
ARE enriched shrikhand	15.92 ± 0.017^{a}	8.40 ± 0.01^{b}	0.79 ± 0^{b}	60.26 ± 0.075^{b}	60.45 ± 0.075^{b}	4.49 ± 03^{b}	1.48 ± 0.0033^{b}

ables viz., ARE (0.5-1.5%), sugar (25.00-35.00%) and chakka (65.00-75.00%). The maximum and minimum score for flavor obtained were 9 and 7, respectively. Flavor acceptability increases with increased levels of sugar followed by ARE slightly. Chakka decreases the flavor score of the optimized product. The maximum and minimum overall acceptability obtained were 9 and 7, respectively. Flavor acceptability increases with increased levels of sugar followed by ARE slightly and chakka. The maximum and minimum cohesiveness obtained were 31.64 and 25.20, respectively. With increased level of sugar and increased level of ARE, cohesiveness increased significantly, same pattern was observed with increased level of chakka. The maximum and minimum consistency obtained were 1112.22 and 523.15, respectively. With increased levels of sugar and chakka, the consistency was increased, while no variation was observed with increased percentage of ARE. The maximum and minimum values for DPPH inhibition were 84.75% and 70.65%, respectively. With increased level of ARE, DPPH inhibition increased significantly showing the functional shrikhand developed is rich in antioxidant properties, whereas with the increase in levels of sugar and chakka there was slight DPPH inhibition observed. The maximum and minimum score obtained for TPC were 54.48 mg/100 g GAE and 30.25 mg/100 g GAE in ARE enriched shrikhand, respectively. It was analyzed that with the increase in sugar, total phenol was decreased and with the increase in ARE up to 1.5%, TPC was increased significantly. Increased percentage levels of chakka also decreased the TPC in the product. In RSM solution, 0.742% ARE, 69.48% chakka and 29.16% sugar was found optimum level for incorporation in functional shrikhand to formulate ARE enriched shrikhand with desirability of 80.7%. Physico-chemical properties of optimized ARE enriched shrikhand was 60.45% moisture, 15.92% fat, 60.26% total sugar, 8.40% protein and 0.79% ash. The acidity of the ARE enriched shrikhand was 1.48% as lactic acid with pH value 4.4.

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None.

Conflict of Interest

None.

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