Research Article

FORMULATION OF *WITHANIA SOMNIFERA* BASED HERBAL COOKIES USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Herbal based functional foods are extensively used for the prevention and treatment of several health disorders. The present study aims to formulate herbal cookies using *Withania somnifera* leaf powder, an ayurveda component with enhanced the nutraceutical value. Response surface methodology (RSM) was employed to optimize five influencing components in cookies preparation. It includes refined wheat flour, *Withania somnifera* leaf powder, sugar powder, butter and cocoa powder. All the selected five ingredients were choose as the variables and sensory parameters such as appearance, colour, texture, taste and flavour obtained through Nine-point hedonic scale analysis were the responses. After initial trial of experiment, the upper and lower limits for the independent variables were fixed. The relatively high coefficient values were obtained for all the selected responses and hence developed models are statistically accurate. Through three-dimensional response surface plots optimal range independent variables were optimized. The shelf life of the product assessed through microbial analysis and sensory evaluation gave favourable results. Hence, herbal cookies optimized through RSM tool will be novel herbal supplement and could find a prominent position in the both pharmaceutical and food sector

Keywords: *Withania somnifera*, Nutraceutical product, Response surface methodology, Optimization, Nine point hedonic scale.

INTRODUCTION

Nutrition is the provision to cells and organisms and it is a cornerstone that affects and defines the health¹. Good nutrition is essential to prevent not only diseases of deprivation, but also chronic diseases that distress affluent. Nutritional status is the complex interaction between the food we eat, our overall state of health in the environment we live². Exacerbated by poverty, malnutrition combines with disease, both chronic and infectious, which together can be lethal. The consequences include death, disability, stunted mental and physical growth and delayed national development³.

Herbal supplements are obtained from naturally growing plants, fungus, shrubs and trees⁴ and grouped categories of dietary supplements which are. It is unique health care tool and found to be basis of many modern medicines^{5,6} and perhaps these herbal supplements are different from vitamin and mineral supplements. The World Health Organization has estimated that 80% of the world's population continues to use traditional therapies, a major part of which are derived from plants. Worldwide it is projected that 80% of the populace uses herbs in different forms and in the developing country rates could be as high as 95%⁷. *Withania somnifera* is one of the most valued plants in Ayurveda and is commonly used in Indian traditional health care systems⁸. It is also known as Ashwagandha, Indian ginseng, and Winter Cherry and it is a small shrub belonging to the Solanaceae family. It is used in Ayurveda formulations for a variety of health promoting effects. It is used as a general tonic

to increase energy, improve overall health and longevity, and prevent diseases⁹.

W.somnifera contains different natural antioxidants such as superoxide Dismutase, catalyse and glutathione peroxidase which are responsible as a health promoters^{10,11,12}. The main constituents of *W.somnifera* are alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. Due to these beneficial properties, Ashwagandha based herbal formulation can be used as dietary supplements¹³.

Several herbal products containing *W.somnifera* root and leaf powder are available in form of tablets, tonic and capsules and the products are valued by consumers nationally and internationally. Apart from herbal supplements, a wide range of food products like drinks, bakery and confectionary items, can be developed by herbs. Nevertheless, its bitter taste is reflected as a restraint to food products application. Keeping in view of its medicinal properties, the present study was designed to formulate herbal cookies using Response Surface Methodology (RSM).

RSM is a statistical tool which is magnificently used in the development and optimization of food products¹⁴. It is a collection of mathematical and statistical techniques beneficial for evaluating and optimizing the response of multivariate systems¹⁵. RSM model will reduce the number of trials and provide multiple regression approach. The optimized ingredients level accomplished after the numerical and Graphical

optimization serves better for maximum score such as colour, flavour and taste¹⁶. In the present study, herbal cookies is formulated based on optimal level of incorporating all selected ingredients for the acceptable level and it was evaluated based on sensory properties as response variables.

MATERIALS AND METHODS

Ingredients

W.somnifera plant was collected from Tiruppur district, Tamilnadu. It was identified at Department of Biotechnology, K. S. Rangasamy College of Technology. The leaves were washed thoroughly with distilled water and dried under shade condition. Further, dehydrated leaves were pulverised and sieved through a 16 mesh sieve to collect the powder. It was stored under sterile condition for further processing. Other ingredients such as refined wheat flour, butter, sugar powder and cocoa powder were obtained from local market in Erode, Tamil Nadu, India.

Formulation of *W.somnifera* Herbal Cookies

Formulation of *W. somnifera* Herbal Cookies (WHC) was carried out in Baking Unit, K.S.Rangasamy College of Technology, Tiruchengode. For the formulation of WHC all the above mentioned ingredients were measured in appropriate quantity and mixed thoroughly until smooth and homogeneous dough mixture was obtained. It was sheeted to thickness of 0.6cm and made into circular pieces of 5.5 cm diameter¹⁴. The dough pieces were transferred to trays and baked at 145°C for 20 min in baking oven. The baked cookies were allowed to air cool for 20 minutes to room temperature and were packed in airtight plastic container¹⁷.

Experimental Design for Optimization of Formulation

For designing the experimental process, Response Surface Methodology (RSM), a central composite design was applied using a commercial statistical package (Design expert, Trial version 10.0, State Ease Inc., Minneapolis, IN statistical software) for the generation of response surface plot and optimization of process variables¹⁸.

Design and analysis of experiments were carried out by the method outlined by Montgomery¹⁹. Five quantitative controllable factors (independent variables) including level of *W. somnifera* leaf powder (X1), refined wheat flour (X2), sugar powder (X3), butter (X4) and cocoa powder (X5) were used on responses of sensory score parameters such as Appearance (Y1), Colour (Y2), texture (Y3), taste (Y4) and flavour (Y5).

Central levels of chosen parameters were determined from the results obtained by OVAT approach. Each day one parameter was optimised and the optimised value is kept constant while optimising other parameters. The maximised taste acquired while optimising each parameter were taken as central levels²⁰. After initial trial experiment, the upper and lower limits for the independent variables were established and the ranges of values were illustrated in the Table 1.

Five levels of each variable were chosen and fifty baking trials were performed for the evaluation of the optimized formulation. Based on this model construction, the independent and dependent variables were fitted to the second-order model equation and examined for the goodness of fit. A second-order polynomial equation as follows (1):

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1, i < j}^n \sum_{j=2}^n \beta_{ij} X_i X_j \quad (1)$$

Where Y is the response level, β_0 is the intercept term, β_i are linear coefficients, β_{ii} are quadratic coefficient, β_{ij} is interaction coefficient, X_i and X_j are coded independent variables and n is the number of items.

Sensory Evaluation

The sensory evaluation was carried out by an untrained panel of 30 members including 15 males and 15 females) in the age 20 – 45 years involving of both the students and faculty of Department of Food Technology and Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode. The various sensory parameters such as Appearance, Colour, Texture, Taste, and flavour were evaluated following through nine point Hedonic scale²¹

Shelf Life Analysis

The shelf life of the WHC was evaluated by standard plate count method²² using Ethylene blue myosin agar. The number of colonies in the plates was recorded. The sensory evaluation was carried out to get consumer response for shelf-life analysis²³. The evaluation was performed at weekly intervals to assess the changes in sensory parameters for the duration of three weeks.

RESULTS AND DISCUSSION

Central Composite Design

Central composite designs of independent variable X1, X2, X3, X4 and X5 along with the sensory score responses Y1, Y2, Y3, Y4 and Y5 obtained through conducting experiment were illustrated in the Table 2. The optimization variables, responses were selected based on the direct effect on the acceptability and quality of Cookies²⁴. In the present regression model, parameters for explanatory variables were linear, square and quadratic effect. Regression coefficients were evaluated by multiple regression analysis and outcomes were depicted in the Table 3. Values of “prob > F” less than 0.05 indicate that model term is significant and values greater than 0.1 indicate that the model terms are not significant²⁵.

In the present study X2X3 gave significant results for the three different sensory score responses such as appearance, colour and texture. Taste sensory score were favourable for the X1X3 and was found to be significant and whereas, Y5 sensory score response was found to be significant for X1 and X3X4. R^2 value for appearance, colour, texture, taste and flavour was found to be 0.83, 0.88, 0.80, 0.94 and 0.79 respectively. The relatively high coefficient values shows that regression was significant and the developed models are statistically accurate for the system under the given experimental conditions (i.e., at a 99% confidence interval). Hence the present research highly correlate with findings of Das et.al.,²⁶ whereas, R^2 closer to 1.0, shows goodness of fit of the model.

The value of coefficient of variation (CV) was 4.28 % for response. The values are <10%, specified that the results from experiments were reliable and accurate²⁷. The insignificant value of “lack of fit” (>0.05) indicated that the models were statistically appropriate for further use. A polynomial model was proposed for sensory analysis of foods in formulation of WHC. The five outcomes, namely Appearance, Colour, Texture, Taste and Flavour of the product were given as polynomials in equations which are expressed in Table 4.

Table 1: Quantity of Independent variables ingredient for formulation of *W. somnifera* Herbal Cookies

Variables Quantity (gm)	Symbol	Level of codes			
		-1	0	+1	+2
<i>W. somnifera</i> leaf powder	X1	1	1.5	2	2.5
Refined wheat flour	X2	30	35	40	45
Sugar powder	X3	30	40	50	60
Butter	X4	40	50	60	70
Cocoa powder	X5	0	1.5	2	2.5

Table 2: Central Composite Design of Independent variables along with responses of Sensory score parameters

Runs	Independent variables					Responses of Sensory score parameters				
	X1	X2	X3	X4	X5	Y1	Y2	Y3	Y4	Y5
1.	4	35	50	70	2.5	8	6	8	7	8
2.	5	40	40	60	3	6	6	6	5	7
3.	5	40	60	80	2	7	5	6	7	7
4.	4	35	50	70	2.5	6	8	7	9	7
5.	3	30	60	60	2	5	6	7	7	7
6.	4	35	50	70	2.5	6	6	7	8	7
7.	5	30	60	60	3	7	7	8	6	8
8.	5	40	40	80	3	4	4	5	5	5
9.	4	23.10	50	70	2.5	6	7	8	3	7
10.	5	40	60	60	3	4	6	6	4	6
11.	5	40	40	60	2	4	5	5	7	6
12.	4	35	50	70	2.5	5	5	6	6	7
13.	4	35	50	70	2.5	8	8	8	9	8
14.	3	30	40	80	3	3	6	6	5	5
15.	3	40	60	60	3	3	6	5	8	8
16.	5	30	40	60	3	5	6	6	6	5
17.	3	40	60	80	3	5	6	7	7	5
18.	5	30	40	80	3	6	7	6	6	5
19.	1.62	35	50	70	2.5	8	8	8	9	9
20.	5	30	60	80	3	9	8	8	7	8
21.	3	30	40	60	3	9	8	7	8	8
22.	4	35	50	93.78	2.5	9	8	8	6	5
23.	3	30	40	60	2	7	8	7	8	7
24.	4	35	26.21	70	2.5	5	6	4	4	4
25.	5	30	60	40	3	5	6	6	6	5
26.	6.37	35	50	70	2.5	6	5	6	3	5
27.	3	40	60	80	2	9	8	8	8	9
28.	4	35	50	46.21	2.5	6	6	5	8	8
29.	4	35	50	70	1.31	8	7	7	8	8
30.	4	46.89	50	70	2.5	6	8	5	7	6
31.	3	40	60	60	2	6	6	7	7	5
32.	3	40	40	80	3	4	6	5	4	6
33.	3	30	60	80	3	6	8	8	6	8
34.	4	35	50	70	2.5	7	7	7	7	8
35.	3	30	60	60	3	8	7	8	8	8
36.	3	30	60	80	2	9	9	9	7	8
37.	3	40	40	60	3	7	7	6	7	6
38.	5	30	40	60	2	7	7	7	7	5
39.	5	30	60	80	2	8	8	8	7	6
40.	5	40	60	60	2	6	7	8	7	6
41.	5	30	60	60	2	7	8	7	4	5
42.	3	40	40	60	2	7	7	7	6	8
43.	3	30	40	80	2	3	5	5	5	6
44.	3	40	40	80	2	6	5	6	5	6
45.	4	35	50	70	3.68	6	7	7	7	8
46.	4	35	50	70	2.5	7	7	7	9	9
47.	4	35	50	70	2.5	7	7	7	9	9
48.	5	30	40	80	2	4	4	3	3	4
49.	4	35	73.78	70	2.5	6	6	6	8	8
50.	5	40	60	80	3	7	7	7	8	8

X1 *W. somnifera* leaf powder; X2: Sugar powder, X3 Butter, X4 Wheat Flour, X5 Cocoa powder, Y1 Appearance, Y2 Colour, Y3 Texture, Y4 Taste, Y5 Flavour

Table 3: Regression coefficient for the response variables of *W. somnifera* Herbal Cookies

Regression Coefficients	Y1	Y2	Y3	Y4	Y5
Model	6.23	6.61	6.61	6.35	5.92
X1	-0.12	0.014	-0.078	0.36	-0.023*
X2	-0.14	-0.049	-0.049	-0.081	-0.23
X3	-0.35	-0.21	-0.17	-0.18	-0.12
X4	-0.24	-0.17	5.61E-03	0.31	0.18
X5	0.29	0.18	0.13	0.43	0.43
X1X2	-0.16	0.031	0.031	0.062	0.34
X1X3	0.094	-0.16	0.094	0.19**	0.34
X1X4	-0.22	0.094	0.031	0	0.094
X1X5	-0.094	-0.28	-0.34	0.19	0.031
X2X3	-0.16**	-0.094**	-0.094**	-0.063	-0.41
X2X4	-0.094	-0.094	-0.16	0	-0.16
X2X5	-0.22	-0.094	-0.031	-0.063	-0.22
X3X4	0.66	0.47**	0.53	0	-0.031**
X3X5	0.41**	-0.031	0.16	0.062	0.031
X4X5	-0.16	-0.16	0.094	-0.5	-0.34
X1 ²	-0.15	-0.034	0.14	-0.13	0.24
X2 ²	-0.15	-0.21	-0.39	-0.13	-0.11
X3 ²	0.47	0.23	0.14	0.31	0.42
X4 ²	-0.24	-0.034	0.055	-0.04	0.15
X5 ²	0.11	0.055	0.055	0.22	0.24
R ²	0.83	0.88	0.80	0.94	0.79
Adj R ²	-0.0396	0.27	-0.1752	0.68	-0.22
Adeq R ²	5.455	5.63	4.84	7.119	3.45
Std Dev	1.66	0.99	1.33	0.93	1.58

X1 *W. somnifera* leaf powder; X2: Sugar powder, X3 Butter, X4 Wheat Flour, X5 Cocoa powder, Y1 Appearance, Y2 Colour, Y3 Texture, Y4 Taste, Y5 Flavour. ** 1% level of significance.

Table 4: Quadratic Regression equation of response with selected variables

Response	Quadratic Regression equation with five variables
Y1	$6.23 - 0.12 X_1 - 0.14 X_2 - 0.35 X_3 - 0.24 X_4 + 0.29 X_5 - 0.16 X_1 X_2 + 0.094 X_1 X_3 - 0.22 X_1 X_4 - 0.094 X_1 X_5 - 0.16 X_2 X_3 - 0.094 X_2 X_4 - 0.22 X_2 X_5 + 0.66 X_3 X_4 + 0.41 X_3 X_5 - 0.16 X_4 X_5 - 0.15 X_1^2 - 0.15 X_2^2 + 0.47 X_3^2 - 0.24 X_4^2 + 0.11 X_5^2$
Y2	$6.61 + 0.014 X_1 - 0.049 X_2 - 0.21 X_3 - 0.17 X_4 + 0.18 X_5 + 0.031 X_1 X_2 - 0.16 X_1 X_3 + 0.094 X_1 X_4 - 0.28 X_1 X_5 - 0.094 X_2 X_3 - 0.094 X_2 X_4 - 0.094 X_2 X_5 + 0.47 X_3 X_4 - 0.031 X_3 X_5 - 0.16 X_4 X_5 - 0.034 X_1^2 - 0.21 X_2^2 + 0.23 X_3^2 - 0.034 X_4^2 + 0.055 X_5^2$
Y3	$6.61 - 0.078 X_1 - 0.049 X_2 - 0.17 X_3 + 5.61 E - 03 X_4 + 0.13 X_5 + 0.031 X_1 X_2 + 0.094 X_1 X_3 + 0.031 X_1 X_4 - 0.34 X_1 X_5 - 0.094 X_2 X_3 - 0.16 X_2 X_4 - 0.031 X_2 X_5 + 0.53 X_3 X_4 + 0.16 X_3 X_5 + 0.094 X_4 X_5 + 0.14 X_1^2 - 0.39 X_2^2 + 0.14 X_3^2 + 0.055 X_4^2 + 0.055 X_5^2$
Y4	$6.35 + 0.36 X_1 - 0.081 X_2 - 0.18 X_3 + 0.31 X_4 + 0.43 X_5 + 0.062 X_1 X_2 + 0.19 X_1 X_3 + 0 X_1 X_4 + 0.19 X_1 X_5 - 0.063 X_2 X_3 + 0 X_2 X_4 - 0.063 X_2 X_5 + 0 X_3 X_4 + 0.062 X_3 X_5 - 0.5 X_4 X_5 - 0.13 X_1^2 - 0.13 X_2^2 + 0.31 X_3^2 - 0.04 X_4^2 + 0.22 X_5^2$
Y5	$5.92 - 0.023 X_1 - 0.23 X_2 - 0.12 X_3 + 0.18 X_4 + 0.43 X_5 + 0.34 X_1 X_2 + 0.34 X_1 X_3 + 0.094 X_1 X_4 + 0.031 X_1 X_5 - 0.41 X_2 X_3 - 0.16 X_2 X_4 - 0.22 X_2 X_5 - 0.031 X_3 X_4 + 0.031 X_3 X_5 - 0.34 X_4 X_5 + 0.24 X_1^2 - 0.11 X_2^2 + 0.42 X_3^2 + 0.15 X_4^2 + 0.24 X_5^2$

X1 *W. somnifera* leaf powder; X2: Sugar powder, X3 Butter, X4 Wheat Flour, X5 Cocoa powder, Y1 Appearance, Y2 Colour, Y3 Texture, Y4 Taste, Y5 Flavour

Table 5: Optimum value of variables and responses of *W. somnifera* Herbal Cookies

Variables	X1	X2	X3	X4	X6
Optimum value	1.75	37.5	45	55	1.25
Responses	Y1	Y2	Y3	Y4	Y5
Optimum value	6.22	6.61	6.61	6.35	5.92

X1 *W. somnifera* leaf powder; X2: Sugar powder, X3 Butter, X4 Wheat Flour, X 5 Cocoa powders, Y1 Appearance, Y2 Colour, Y3 Texture, Y4 Taste, Y5 Flavour

Table 6: Shelf life analysis of *W. somnifera* Herbal Cookies based overall acceptability

Description	Scale point Assigned Values	No. of responses		
		<i>W. somnifera</i> Herbal Cookies		
		1 st week	2 nd week	3 rd week
Like extremely	9	2	1	1
Like very much	8	6	5	5
Like moderately	7	6	7	5
Like Slightly	6	2	4	3
Neither like or dislike	5	2	1	3
Dislike Slightly	4	1	1	1
Dislike moderately	3	1	1	1
Dislike Very much	2	0	0	1
Dislike extremely	1	0	0	0
No. of respondents		20	20	20
SD±		2.27	2.49	1.85
Like %		80	85	70
Dislike %		10	10	15
Neither like or dislike%		10	5	15

Figure 1: Plot of predicted Vs actual values

Figure 2: Effects of (a) sugar powder and *W. somnifera* powder (b) wheat flour and *W. somnifera* powder (c) wheat flour and butter (d) wheat flour and sugar powder (e) cocoa and *W. somnifera* powder (f) cocoa and sugar powder on appearance of *W. somnifera* herbal cookies

Figure 3: Effects of (a) sugar powder and *W. somnifera* powder (b) Butter and *W. somnifera* powder (c) cocoa and sugar powder (d) cocoa and butter (e) cocoa and wheat flour (f) wheat flour and *W. somnifera* powder on colour of *W. somnifera* herbal cookies

Figure 4: Effects of (a) sugar powder and *W. somnifera* powder Butter and *W. somnifera* powder (c) Cocoa and butter (d) wheat flour and sugar powder (e) cocoa and sugar powder (f) cocoa and wheat flour on Texture of *W. somnifera* herbal cookies

Figure 5: Effects of (a) sugar powder and *W. somnifera* powder (b) cocoa and *W. somnifera* powder (c) wheat flour and sugar powder (d) cocoa and sugar powder (e) cocoa and butter (f) cocoa and wheat flour on taste of *W. somnifera* herbal cookies

Figure 6: Effects of (a) sugar powder and *W. somnifera* powder (b) cocoa and *W. somnifera* powder (c) butter and sugar powder (d) cocoa and sugar powder (e) cocoa and Butter (f) cocoa and wheat flour on the flavour of *W. somnifera* herbal cookies

Figure 7: Microbial analysis of *W. somnifera* herbal cookies

Determination of Model Adequacy

It is significant to confirm that the model provides a realistic approximation to true system. The adequacy of acquired models can be refereed by diagnostic plot, predicted data vs actual data. As seen in the Figure 1, the clustering of points with the diagonal line shows a satisfactory correlation between the experimental data and the predicted values. It also confirms the robustness of the models.

Response Surface Plots

RSM provides a clear understanding of interaction between the different parameters and its optimum significant level¹⁴. Most polynomial models design by RSM was effective in recital the correlation between the studied factors and the responses²⁸. It was investigated by three-dimensional (3D) and two-dimensional (2D) contour plots. Three-dimensional response surface plots illustrating the effects of *W. somnifera* leaf powder, Sugar powder, Butter, Wheat Flour and Cocoa powder on responses such as Appearance, Colour, Texture, Taste and Flavour of WHC is given in Figure 2 – 5. The graphical

representation clearly shows the individual and cumulative effects of the variables and mutual interaction between those variables. Hence, the interaction between all parameters was studied using the graph. Optimum values of variables and responses of *W. somnifera* Herbal Cookies is depicted in the Table 5. Quantity of *W. somnifera* leaf powder was found to be 1.75. Whereas other quantity of other variables was found to be 37.5, 45, 55 and 1.25 for Sugar powder, Butter, Wheat Flour and Cocoa powders respectively.

Shelf Life Study

The newly formulated cookies were diagnosed for its microbiological status by spread plate technique using Eosin Methylene Blue (EMB) agar (Figure 7). The shelf life of the product was analysed to predict the percentage of bacterial growth up to three weeks after packing and storing of final formulated cookies. Reduced viable cell colonies were noticed during storage period and it was due to antimicrobial properties of fortified *W. somnifera* leaf powder. The effect of the spice *Zingiber officinale* in cookies through addition of curry spice has reduced microbial load at significant level²⁹.

Sensory Evaluation for Final Formulated Product

Sensory characteristics are most easily detected, economic and require no instrumentation²⁹ and determine the quality of the product. The sensory scores of the response variables, i.e. appearance, colour, texture, taste and flavour for three weeks is evaluated and shown in the Table 6. From the depicted values in the table, it is clear that the like percentage of herbal cookies is significantly higher than the dislike percentage for all three weeks. Thus, the optimized region for the acceptability of the panel was between the categories of “liked slightly” and “liked moderately”. Texture is the one among predominant factor in determining the overall acceptability and shelf – life of the cookies. The present research reveals that, addition of herbal mix has not interfered in its crispness and taste. It allied with work done by Emmanuel et.al.³⁰ that sorghum flour fortified with wheat flour spiced with curry (*Murraya koenigii*) cookies is highly acceptably in texture and taste.

As a conclusion, nutritional enriched herbal cookie was formulated using RSM with five different ingredients as variables and sensory evaluation scores as responses. All the responses fitted well with the quadratic model. Basic ingredients necessary along with herbal powder was well optimized. Henceforth, the current research clearly visualized the formulation process of herbal fortified nutraceutical product which can be widely accepted by the consumers of all age groups mainly due its health benefits. This could be an alternative approach for supplementing herbal treatment in an easy way to overcome health crises.

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