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OPTIMISATION OFA READY TO USE "NUTRITIOUS MIX" INCORPORATING INDIAN HERBS USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Indian medicinal herbs are the natural and healthy alternative source of medications possessing side effects for various ailments. Their incorporation in food products can make it both nutritious and healthful. Nutritious mix was formulated as an instant food that can be reconstituted for consumption effortlessly. The purpose of the study was to optimise the amounts of Indian herbs as functional foods for incorporation in the powder to enhance its nutritional and functional properties.RSM (response surface methodology) and CCRD (central composite rotable design) were utilised for optimisation with three process variables (namely, amounts of apple powder, *Rauvolfiaserpentina* and black cumin seeds) and potassium, sodium, fibre and overall acceptability as response variables. The response surface plots along with regression models were produced and regression coefficients and lack of fit tests were used to test the adequacy. The optimum levels that were attained for in range potassium (477.71 mg), minimum sodium (39.85 mg), maximum fibre (4.09 g) and maximum overall acceptability (87.61) were: 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 10.00 g black cumin seeds powder. Optimum recipe was nutritionally adequate and highly acceptable. Nutritious mix can provide beneficial roles to the people in maintaining their health without changing their regular diet patterns.

1. Introduction

Medicinal plants are regaining importance as a result of side effects caused by modern synthetic drugs. However, herbal medicines have sustained to be in demand among the developing countries as a result of being easily accessible, cost effective and culturally acceptable (Sewell and Rafieian-Kopaei, 2014). Herbal medicine remains to be the core of approximately 75 to 80 percent world's population, mostly among the developing world, for primary health care as a result of having enhanced cultural acceptance, being more compatible with the human's body, and possessing fewer side effects (Vidyarthi*et al.,* 2013). India is an immense repository of medicinal plants being traditionally utilised in the treatment of various ailments (Agrawal *et al.,* 2010). Hypertension is a leading public health problem worldwide. Chemical medicines for hypertension generally cause side effects making the usage of medicinal herbs necessary (Pourjabali *et al.,* 2017). There are many scientifically studied and frequently used naturally occurring medicinal plants for the management of hypertension including Black cumin seeds, Rauwolfia and others (Agrawal *et al.,* 2010).

The roots, leaves, seeds, fruits and juice of *Rauvolfia serpentina* having medicinal benefits have drawn the attention of those practicing indigenous therapies. It has been used as a therapy for combating anxiety, epilepsy, excitement, gastrointestinal disorders, hypertension, insanity, mental agitation, traumas, schizophrenia, sedative insomnia(Malviya and Sason, 2016), body aches, burns and skin diseases(Poonam *et al.*, 2013).Current scientific researches on black cumin seeds ((*Nigella sativa* L.) and its oil have shown numerous bioactivities for the plant including anticarcinogenic, antihyperlipidemic, anti-inflammatory, antipyretic and analgesic, antiulcer, antibacterial and antifungal, antihypertensive, hepatoprotective and antioxidant activities as well that includes scavenging the reactive species of oxygen, preventing rheumatoid arthritis in rat models(Toma *et al.*, 2015).

In recent times, the rising health issues have resulted in the transference towards the optimal nutrition diet. Thus, food manufacturers are tended to produce such food products that can satisfy both consumer's appetite and desires for health promotion(Olaiya*et al.*, 2016). Nutraceuticals have come out to be an alternative source of the modern medicines and have shown positive results in decreasing the conventional medicines requirement along with reducing the possibilities of adverse effects (Sharma *et al.*, 2017). Currently, nutraceuticals and functional foods have gained the attention as potential alternative therapies in the hypertension treatment (Chen *et al.,* 2009). Thus, incorporation of medicinal herbs like *Rauvolfia serpentina* and black cumin seeds as nutraceuticals can come out to be a potential alternative source of medicines for hypertension management. Additionally, incorporation of heart healthy food like apple can help in enriching the food product's health benefits.

In spite of having highly beneficial roles in several diseases, incorporation of above mentioned herbs and apples in higher quantities can compromise the overall acceptance of various sensory attributes of developed food product. Therefore, there is a need for such techniques that can help in getting optimum solutions to produce a recipe which is adequate from nutritional point of view and is accepted organoleptically as well. Process optimisation is the one of current techniques being utilised in the formulation of optimum food products with increased nutritional properties. "Response surface methodology (RSM) is a powerful mathematical model with a collection of statistical techniques where in, interactions between numerous process variables can be recognized with fewer experimental trials. It is extensively used to study and optimize the operational variables for experiment designing, model developing and factors and conditions optimization(Karuppaiya *et al.*, 2010)".

Therefore, in the present study, a ready to use nutritious mix incorporating Indian herbs, appropriate for hypertensive people was formulated as instant food with the objective to get the statistically valid optimum combination of amount of apple powder, *Rauvolfia serpentina* and black cumin seeds powder as process variables for their incorporation and in range potassium, minimum sodium, maximum fibre and overall acceptability as response variables through CCRD of response surface methodology.

2. Materials and methods

The present work was done in Banasthali Vidyapith, Rajasthan, India, during the time span of July, 2014 to April, 2015. The raw ingredients and apple as functional food were purchased from Banasthali Vidyapith's local market. Black cumin seeds of brand with a good reputewere acquired from general store of Ghaziabad whereas *Rauvolfiaserpentina* was obtained from a reputed ayurvedic pharmacy.

2.1. Formulation of nutritious mix

Rauvolfia serpentina powder (0.5 g), black cumin seeds powder (5 g), apple powder (10 g), tomato powder (5 g), whole wheat flour (15 g) and roasted bengal gram (15 g) were incorporated for the preparation of nutritious mix. Apples were cut into thin slices, blanched, oven dried at 60°C for 48 hours and then powdered. Black cumin seeds were cleaned and powdered. No specific treatment was given to root powder of *Rauvolfia serpentina* for the purpose of product development. Drying of tomatoes and tamarind was done at 60°C in an oven for 2 days and then were grinded. Roasted bengal gram was grinded to make powder. Black cumin seeds powder and wheat flour were roasted followed by addition of apple powder, *Rauvolfia serpentina* powder, tomato powder and tamarind powder proportionally to make nutritious mix. Auto seal sachets were used for storage of the prepared mix and these were kept in container being air tight. Sensory analysis of reconstituted thick drink was conducted. Reconstitution was done by addition of 100 ml butter milk (*Saras*, plain buttermilk) to the weighed quantity of 25 g nutritious mix and a pinch of powder of cuminseeds after roasting was mixed to it. The formulation of total product of 50g was done which was sufficient for a couple of servings.

2.2. Design of experiments

Developed food product was process optimised through RSM. RSM consists of statistical and mathematical techniques that are beneficial in development, improvement and optimisation procedure(Carley *et al.*, 2004). CCRD comprising 3 independent variables (process variables) at 5 levels was utilised to define the optimum conditions in formulating nutritious mix as presented in table 1. Twenty experimental runs were generated as a result when replication was carried at the center point (0) combination for six times. CCRD comprises of 3 points that are factorial points, centre points and star points and these let to estimate the curvature. The distance in-between the centre of design space and star point is ±α(Singh *et al.*, 2007). Depending upon the one-at-a-time preliminary experiments, the critical factors (process variables), amounts each of apple powder, *Rauvolfia serpentina* and black cumin seeds powder were selected for process optimisation. As per the central composite rotable design, the experiments number in totality is $(2)^n + 2n$ + central points, where n stands for sum total of variables. In present study, there are 3 variables in total for which the experiments' total number for every critical factor will be 20. The codes, -α, -1, 0, 1 and α were given for different 5 levels in every experiment; where $\alpha=2^{n/4}=2^{3/4}=1.682$. Thus, the codes were -1.682 (lowest), 0 (middle) and 1.682 (highest) for process variables. Each ofthe critical factors was analysed for its effect on the dependent variables (response variables)– calculated potassium, calculated sodium, fibre and overall acceptability. 'Design-Expert software (9.0)' (Statease Inc., Minneapolis, MN, USA) came out to generate 20 sample combinations (table 2) by the use of

Table 1. Levels of process factors to optimisenutritious mix

	Name	Units	-1 level	+1 level	-alpha	+alpha
A	Apple powder	G	5.0	15.0	1.591040	18.4090000
B	*Rauvolfiaserpentina*	G	0.3	0.7	0.163641	0.836359
C	Black cumin seeds powder	G	3.0	10.0	0.613725	12.386300

design matrix and combinations of variables in experimental runs. Each one of the sample combinations was produced in food preparation laboratory of Banasthali Vidyapith. The values for dependent variable, fibre were estimated through laboratory analysis whereas calculation of potassium and sodium content was done by the use of values provided in Nutritive Value of Indian Food (Gopalan *et al.*, 2007). The semi trained panelists were asked to score in between 1 to 100 depending upon liking of each combination for overall acceptability. The data sheet of the software was entered with all of these values. Order for carrying out the experiments was random.

Table 2. Experimental designs generated and observed responses of nutritious mix

	Generated			Estimated			
S. No.	**Apple powder (g)**	***Rauvolfia serpentina* (g)**	**Black cumin seeds powder (g)**	**Potassium (mg)**	**Sodium (mg)**	**Fibre (g)**	**Overall Acceptability**
1	10.00	0.50	12.39	535.35	51.17	3.97	86.53
2	10.00	0.50	6.50	431.35	44.32	2.80	88.73
3	5.00	0.30	10.00	492.52	39.90	3.74	85.40
4	10.00	0.84	6.50	431.35	44.32	3.42	86.60
5	10.00	0.50	6.50	431.35	44.32	3.01	85.80
6	10.00	0.50	0.61	327.33	37.80	2.01	86.80
7	15.00	0.30	10.00	493.78	57.02	3.92	88.00
8	18.41	0.50	6.50	432.39	58.70	2.94	88.60
9	10.00	0.50	6.50	431.35	44.32	3.52	87.53
10	15.00	0.30	3.00	370.18	49.08	2.04	89.53
11	10.00	0.50	6.50	431.35	44.32	2.98	88.66
12	10.00	0.50	6.50	431.35	44.32	3.28	86.06
13	5.00	0.70	10.00	492.52	39.90	4.32	87.33
14	5.00	0.70	3.00	368.91	31.96	2.04	88.06
15	10.00	0.16	6.50	431.35	44.32	2.81	86.60
16	1.59	0.50	6.50	430.28	29.92	3.02	88.13
17	15.00	0.70	3.00	470.18	49.08	3.16	85.80
18	15.00	0.70	10.00	493.78	57.02	4.97	87.60
19	10.00	0.50	6.50	431.35	44.32	2.96	87.13
20	5.00	0.30	3.00	368.91	31.96	2.08	88.46

2.3. Data analysis and optimisation

The data obtained by performing experiments on different combinations were then dispensed for a second order polynomial regression analysis by the use ofleast square regression method and the analysis of the significant ($p<0.05$) effect of allthe process variables on the responses was conducted. The second order polynomial equation given below can define the system behaviour:

$$Y= \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x^2 + \sum\sum \beta_{ij} x_i x_j \quad (1)$$

Where Y stands for predicted response, β_0 for the interception coefficients, β_i for the linear term, β_{ii} for the quadratic term, β_{ij} for the interaction term and x_i and x_j are representatives of the levels coded for process variables.

Goodness of fit and the significance of linear, quadratic and interaction effects were calculated through the ANOVA of the regression equation. The independent variables for ANOVA were amounts of apple powder, *Rauvolfia serpentina* and black cumin seeds powder whereas potassium, sodium, fibre and overall acceptability were the dependent ones. Estimation of the validity attained of the models was the function of their coefficients of determination (R^2) values and the lack of fit analysis. A good model should be significant and lack of fit should be insignificant. The value of predicted R^2 should be in reasonable agreement with adjusted R^2. It can be described as the ratio of explained variation which was a degree of fit measure (Chan *et al.,* 2009). The coefficient of variation (CV) can be defined as the dimensionless numeral that measures the degree of variability relative to the mean. Various interactions of any two independent variables along with hold of the third variable's value at the midpoint are depicted through generation of response surfaces and contour plots. Accuracy in geometrical representation as well as useful information accuracy is provided about the system behaviour within the experimental design by the generated contour plots. The aim of optimisation process was to find the levels of process variables that would give potassium, sodium, fibre and overall acceptability as per the set goals. Design-Expert Software's (9.0) numerical optimisation technique was utilised for the concurrent optimisation of these responses. As evident from table 3, desired goals and responses were chosen for each factor in accordance to which the software generated certain optimum solutions. An optimum solution with the highest desirability was chosen as the optimised recipe. This optimised recipe was formulated in food preparation laboratory and further analysis of its nutritional properties was carried out.

Table 3. Optimisation criteria for different process variables and response variables of nutritious mix

Factors/responses	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Apple powder (g)	In range	5.00	15.00	1.00	1.00	3.00
Rauvolfiaserpentina (g)	In range	0.30	0.70	1.00	1.00	3.00
Black cumin seeds powder (g)	In range	3.00	10.00	1.00	1.00	3.00
Potassium (mg)	In range	327.33	535.35	1.00	1.00	3.00
Sodium (mg)	Minimize	29.92	58.70	1.00	1.00	3.00
Fibre (g)	Maximize	2.01	4.97	1.00	1.00	3.00
Overall acceptability	Maximize	85.40	89.53	1.00	1.00	3.00

2.4. Sensory analysis

A selection of semi trained panel of 15 members was done using triangle test to conduct the sensory evaluation (Jellinek, 1985). The overall acceptability (dependent variable of process optimisation) of produced combinations of nutritious mix was evaluated through 100 pointscale. This scale was utilised to acquire fitness of the model for overall acceptability in process optimisation.

2.5. Nutritional analysis

Nutritional evaluation was conducted of the optimised recipe only. Estimations of moisture and ash were done by standard AOAC(2002) procedures. Semiautomatic instrumentation technique was utilised for protein and fat analysis where, assessment of protein was done through microkjeldahl method using Kel Plus (model no. KES06L, manufactured by Pelican, India) and fat was analysed through soxhlet method by the use of Socs Plus (model no SCS6, manufactured by Pelican, India). Carbohydrate was calculated using substraction method and estimation of crude fibre was done through acid alkali digestion method(AOAC, 2002). Iron through Wong's method and vitamin C and calcium using titrametric methods were estimated(NIN, 2003).

3. Results and discussion

3.1. Results

3.1.1. Optimisation of parameters for ANOVA

Selection of a suitable model for a response to compare the models on the basis of p-values was done by fit summary statistics. The model is said to be "significant if the p value comes to be <0.05". ANOVA is importantly used to evaluate whether the regression model and individual model coefficients are significant and the goodness of fit of regression model(Fentie *et al.,* 2014). The results of ANOVA for the independent variables' effect on potassium specified that, the two factor interaction design model (2FI) had a significant ($p<0.05$) effect on potassium (dependent variable). The effect of independent variables on sodium indicated that the quadratic model had significant ($p<0.05$) effect on sodium as a dependent variable. Effect of independent variables on fibre depicted that, the linear model had a significant ($p<0.05$) effect on fibre as a dependent variable. Lack of fit had non-significant($p>0.05$) effect on the model, suggesting that model fits the data well. The model (2FI) had a non-significant effect on overall acceptability, which was a response variable, when observed with respect to the process variables. Lack of fit had non-significant ($p>0.05$) effect on the model for this response, depicting that the model fit the data well.

3.1.2. Optimisation of parameters for regression coefficients (R^2)

Table 4 represents the parameters acquired by fitting of potassium, sodium, fibre and overall acceptability data. It also presents regression coefficients of model's intercept, linear, quadratic and cross product terms. The coefficient of determination was utilised to evaluate if the model is fit and adeuate. The model with the higher order polynomial where the model is significant is said to be a suitable model. The nearer is the R^2 value towards the unity, the better is the empirical model said to fit the actual data(Zaibunnisa *et al.,* 2009). R^2 value for sodium was 1.00 which suggested that the model completely fits the actual data. Gan *et al.* (2007) recommended that to obtain good fit model, value of R^2 should be at least 80% ($R^2 = 0.80$). R^2 value for fibre was 0.84 suggesting a good fit of model. Evidence indicated that generated models were highly adequate if the value of R^2 was > 90% (R^2 >0.90)(Das *et al.,* 2012; Demirel and Kayan, 2012; Seth and Rajamanickam, 2012). R^2 value for potassium was 0.92 suggesting the model to be highly adequate. The model's R^2 value denotes the "proportion of variation in the model rather than random error". The regression model could explain 92% of variations in potassium content, 84% of variations in fibre content, 48% variations in overall acceptability and no variation in sodium content of nutritious mix (table 4). The results of being precise and reliable were depicted by lesser CV values of potassium, sodium and overall acceptability. The greater CV values of fibre revealed the results to be comparatively less precise and reliable.

3.1.3. Effect of process conditions for calculated potassium

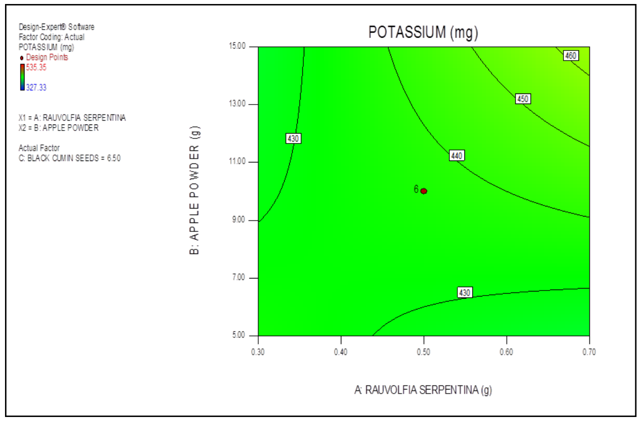
Table 2 depicts the observations for potassium along with the different combination of independent variables. The process variables' effect on potassium as a response of nutritious mix is described by the regression equation given as:

Potassium= 258.95181 + 27.68308 * *Rauvolfia serpentina* - 0.015678 * Apple powder + 31.64362 * Black cumin seeds powder + 12.50000 * *Rauvolfia serpentina* * Apple powder -17.85714 * *Rauvolfia serpentina* * Black cumin seeds powder - 0.71443 * Apple powder * Black cumin seeds powder

Linear curves with *Rauvolfia serpentina* and apple powder are evident from developed response surface (figure 1(a)). The observation was that linear term of *Rauvolfia serpentina* (p=0.125) and cross product of *Rauvolfia serpentina* with black cumin seeds powder (p=0.052) had non-significant effect on the potassium content of nutritious mix. Centre points (6) are depicted through red colour in middle of each graph. Curvilinear plots were observed with *Rauvolfia serpentina* and black cumin seeds powder (figure 1(b)). It was depicted that linear term of black cumin seeds powder (p<0.000) had significant effect and cross product of apple powder with black cumin seeds powder (p=0.052) had non-significant effect on the potassium. The linear curves with apple powder and black cumin seeds powder (figure 1(c)) were developed. The linear term of apple powder (p=0.099) and cross product of *Rauvolfia serpentina* with apple powder (p=0.052) had non-significant effect on the potassium of nutritious mix.

Table 4. Regression coefficients of predicted quadratic polynomial models of nutritious mix (generated by design expert)

Coefficient	Potassium	Sodium	Fibre	Overall acceptability
Intercept	436.350	44.320	3.150	87.370
Linear				
A	7.320	-4.625E-015	0.270	-0.190
B	7.950	8.560	0.130	0.180
C	54.500	3.970	0.800	-0.290
Quadratic				
A^2		0.024		
B^2		0.021		
C^2		0.083		
Cross product				
AB	12.500	5.321E-015		-0.710
AC	-12.500	5.782E-015		0.710
BC	-12.500	6.647E-015		0.510
R^2	0.928	1.000	0.846	0.482
Adjusted R^2	0.894	0.999	0.817	0.243
CV%	3.790	0.150	10.71	1.150



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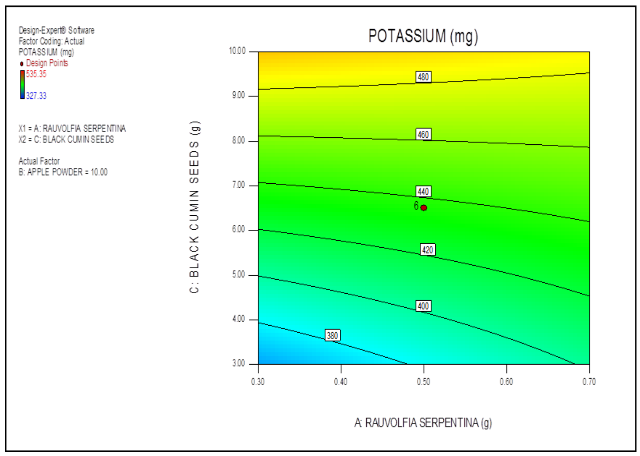

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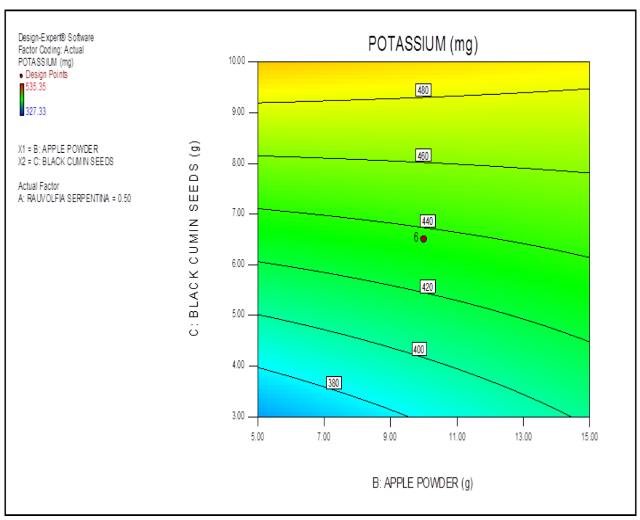


(c)

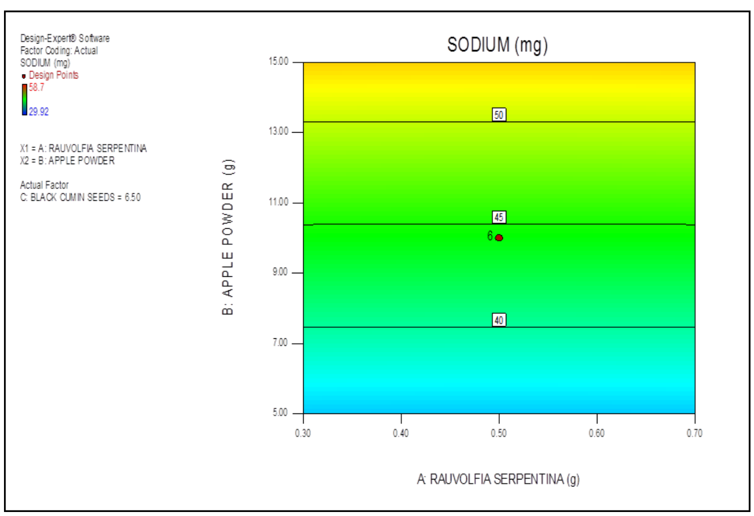
Figure 1. Interactive effect of *Rauvolfia serpentina* and apple powder (a), *Rauvolfia serpentina* and black cumin seeds powder (b) and apple powder and black cumin seeds powder (c) on potassium content of nutritious mix

3.1.4. Effect of process condition for calculated sodium

Table 2 represents the observations for sodium as a response variable with different combination of independent variables. The independent variables' effect on response, sodium of nutritious mix in terms of actual level of variables is described by the regression equation given as:

Sodium= 20.34274 - 0.60775 * *Rauvolfia serpentina* + 1.69508 * Apple powder + 1.04716 * Black cumin seeds powder - 1.50553E-0.15 * *Rauvolfia serpentina* * Apple powder - 1.67980E-015 * *Rauvolfia serpentina* * Black cumin seeds powder + 4.09840E-017 * Apple powder * Black cumin seeds powder + 0.60775 * *Rauvolfia serpentina*^{2} + 8.30971E-004 * Apple powder^2 + 6.74662E-003 * Black cumin seeds powder^2

The response surface developed in figure 2(a) shows linear curves with *Rauvolfia serpentina* and apple powder. The observation was that the linear term of *Rauvolfia serpentina*(p=1.000), cross product of *Rauvolfia serpentina* and black cumin seeds powder (p=1.000) and quadratic term of *Rauvolfia serpentina* (p=0.18) had non-significant effect on sodium (dependent variable). Curvilinear plots were observed with *Rauvolfia serpentina* and black cumin seeds powder (figure 2(b)). Black cumin seeds powder shows significant influence (p<0.000) in terms of linear model, whereas it shows non-significant effect in terms of cross product with apple powder (p=1.000) and quadratic term of black cumin seeds (0.000) shows significant effect on sodium. Linear curves were developed with apple powder and black cumin seeds powder (figure 2(c)). As observed, the linear term of apple powder (p=<0.000) had significant influence whereas cross product of apple powder and *Rauvolfia serpentina* (p=1.000) had non-significant influence and quadratic term of apple powder (p=0.254) had non-significant influence on the response, sodium.



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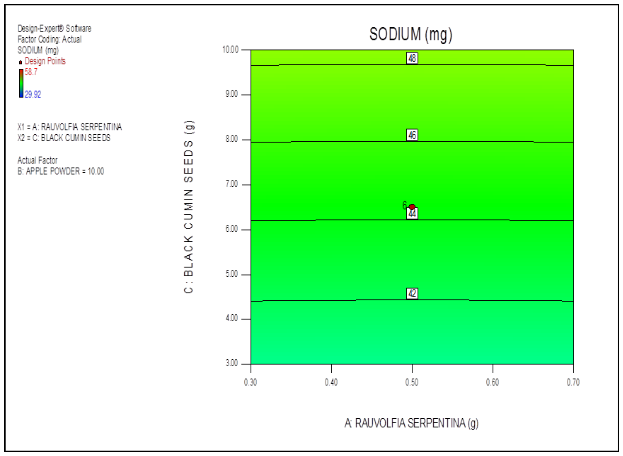

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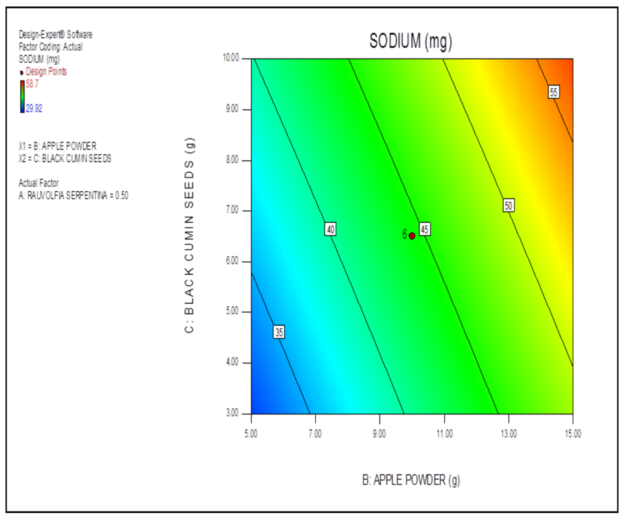


(c)

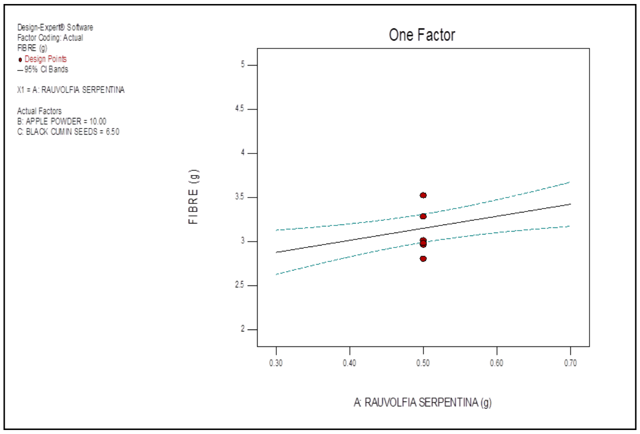
Figure 2. Interactive effect of*Rauvolfia serpentina* and apple powder (a), *Rauvolfia serpentina* and black cumin seeds powder (b) and apple powder and black cumin seeds powder (c) on sodium content of nutritious mix

3.1.5. Effect of process condition for fibre

Table 2 depicts the observations for fibre as a response variable with different combination of independent variables. The effect of the independent variables on response, fibre of nutritious mix in actual level terms of variable is described by regression equation given as:

Fibre= 0.86560 + 1.12247 * *Rauvolfia serpentina* + 0.012182 * Apple powder + 0.23267 * Black cumin seeds powder

Linear plots with *Rauvolfia serpentina* (figure 3(a)), apple powder (figure 3(b)) and black cumin seeds powder (figure 3(c)) are shown on the response plots. The interactive effect of amount of *Rauvolfia serpentina* on independent variable, fibre in figure 3(a) indicates maximum fibre content (3.3 g) obtained at 0.70 g of *Rauvolfia serpentina* and minimum fibre content (2.8 g) attained at 0.3 g of *Rauvolfia serpentina*. The interactive effect of apple powder with fibre in figure 3(b)indicates maximum fibre content (3.2 g) observed at 15.0 g of apple powder and minimum fibre content (3.0 g) obtained at 5.0 g of apple powder. The interactive effect of black cumin seeds powder on fibre (figure 3(c)) specifies the maximum fibre content (3.8 g) observed at 10.0 g black cumin seeds powder and minimum fibre content (2.4 g) was observed at 3.0 g black cumin seeds powder.



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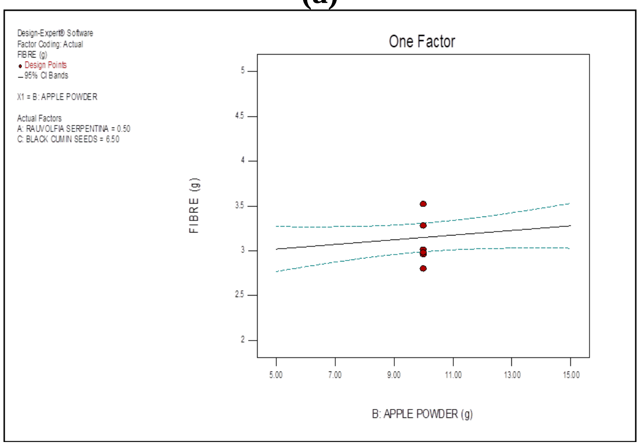

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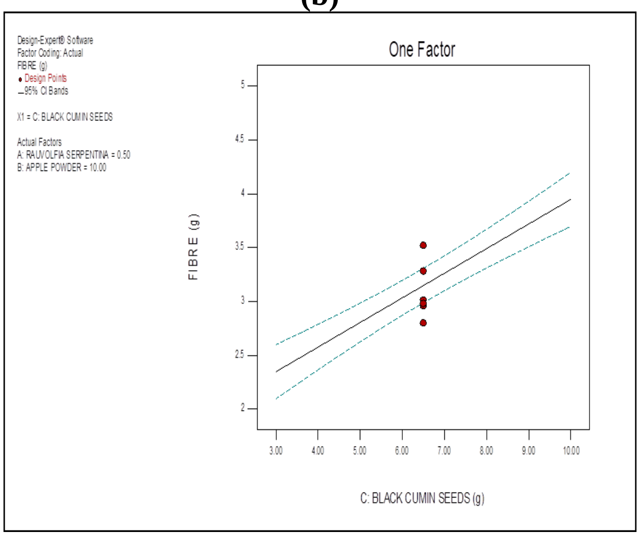


(c)

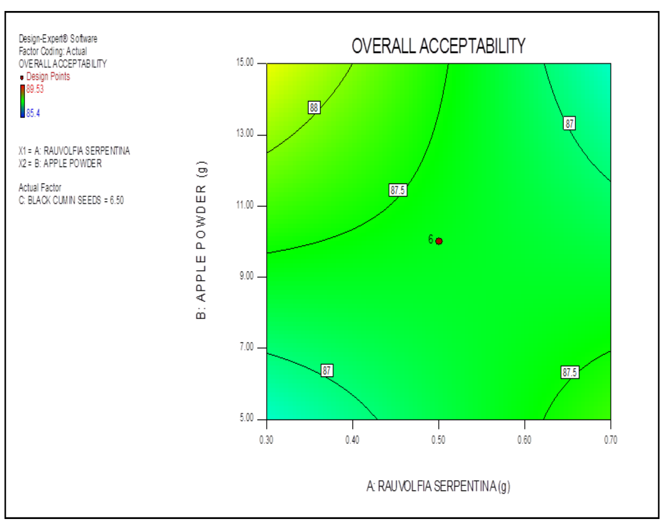
Figure 3. Interactive effect of *Rauvolfia serpentina*(a), apple powder (b) and black cumin seeds powder (c) on fibre content of nutritious mix

3.1.6. Effect of process condition for overall acceptability

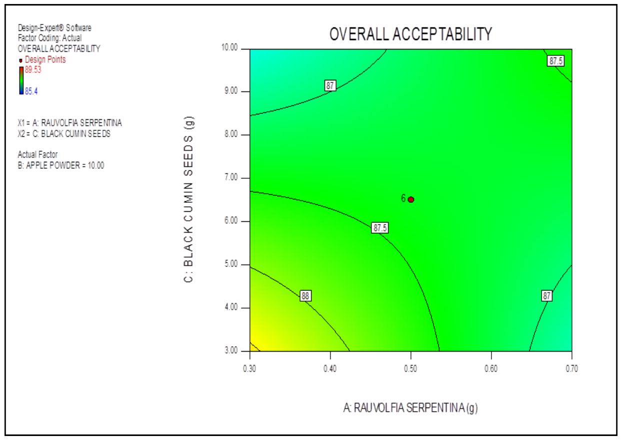
Organoleptic characteristics have a significant importance in modifying, improving, developing and accepting the innovative food products (Yadav *et al.*, 2007). Overall acceptability is a significant factor having direct relation to the likeability of any developed novel food product. Table 2 represents the results observed for overall acceptability with different combination of independent variables. The effect of the independent variables on overall acceptability of nutritious mix in terms of actual level of variables is described by regression equation given as:

Overall acceptability= 89.65441 - 0.44655 * *Rauvolfia serpentina* + 0.20143 * Apple powder -0.87850 * Black cumin seeds powder - 0.70750 * *Rauvolfia serpentina* * Apple powder + 1.01071 * *Rauvolfia serpentina* * Black cumin seeds powder + 0.029000 * Apple powder * Black cumin seeds powder

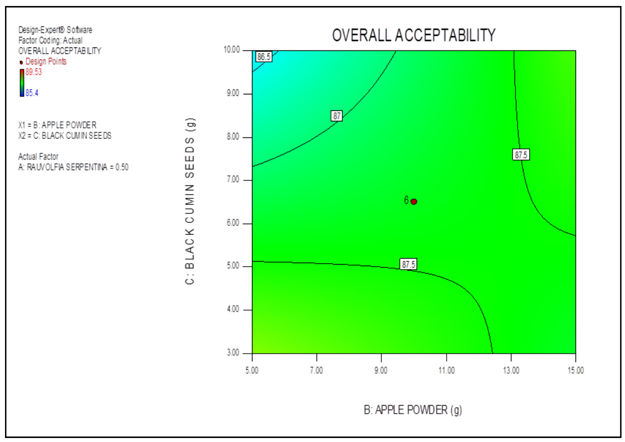
Curvilinear plots were observed with *Rauvolfia serpentina* and apple powder (figure 4(a)). *Rauvolfia serpentina* in its linear term (p=0.494) and in its cross product term with black cumin seeds powder (p=0.067) had non-significant effect on the response, overall acceptability. The response surface developed in figure 4(b) shows linear curves with *Rauvolfia serpentina* and black cumin seeds powder. Linear term of black cumin seeds powder (p=0.302) and cross product term of *Rauvolfia serpentina* and apple powder (p=0.671) had non-significant on the overall acceptability. Linear curves were developed with apple powder and black cumin seeds powder (figure 4(c)). Linear term of apple powder (p=0.516) and its cross product with black cumin seeds powder (p=0.175) as observed had non-significant influence on the response, overall acceptability.



(a)



(b)



(c)

Figure 4. Interactive effect of *Rauvolfia serpentina* and apple powder (a), *Rauvolfia serpentina* and black cumin seeds powder (b) and apple powder and black cumin seeds powder (c) on overall acceptability of nutritious mix

3.1.7. Optimisation of process parameters

The above mentioned results signify the fact that the quality of nutritious mix does not depend on the particular key factor. The properties of the nutritious mix were determined by significant role of all process variables leading to the next step that was to get the best combination of process variables having the ability of producing the expected properties of end product. Thus, numerical optimisation of the process parameters was done to obtain best combination of nutritious mix. Simultaneous optimisation of the multiple response variables took place through the Design Expert (9.0). Table 3 depicts the chosen desired goals for each factor and response. Thirty solutions of independent variables with the predicted responses were generated through the software. The range between 0.569-0.627 was obtained for desirability of optimum solutions. Four optimum solutions were attained depending upon the highest desirability. The optimum recipes consisted (i) 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 10.00 g black cumin seeds powder with 477.71 mg potassium, 39.85 mg sodium, 4.09 g fibre and 87.61 overall acceptability score; (ii) 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 9.96 g black cumin seeds powder with 477.15 mg potassium, 39.81 mg sodium, 4.08 g fibre and 87.61 score of overall acceptability; (iii) 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 9.92 black cumin seeds powder with 476.49 mg potassium, 39.76 mg sodium, 4.07 g fibre and 87.61 score of overall acceptability, (iv) 5.04g apple powder, 0.70 g *Rauvolfia serpentina* and 10.00 g black cumin seeds powder with 477.78 mg potassium, 39.92 mg sodium, 4.09 g fibre and 87.61 overall acceptability score in about 51 g of products. The range of processes which might possibly be contemplated as the optimum range for best quality food product in terms of potassium, sodium, fibre and overall acceptability was provided through these optimum solutions. These were suitable conditions to formulate nutritious mix providing nutritional adequacy without compromising the organoleptic characteristics. Formulation of optimised and enhanced nutritious mix was done using the best solution chosen. Solution 1 with the maximum desirability value of 0.627 in a range of 0.569-0.627, along with in range potassium, minimum sodium, maximum fibre and maximum overall acceptability was chosen for subsequent laboratory estimation.

3.1.8. Nutritional analysis

The nutrients estimation was done as per 100 g quantity. The optimum recipe was adequate in terms of nutrition having 72.46 g carbohydrate, 5.99 g moisture, 0.94 g ash, 15.63 g protein, 3.29 g fat, 1.69 g crude fibre, 9.03 mg iron, 236.29 mg calcium and 1.09 mg vitamin C.

3.2. Discussion

Rapid urbanisation, industrial development and consequential variations in lifestyles of individuals have resulted in progressive formulations of instant dry mixes and ready-to-eat convenient food products(Balasubramanian *et al.*, 2014). These products are gaining popularity as a result of ease of consumption and increased shelf life (Bunkar*et al.*, 2014) along with reducing the time for preparation by eradicating numerous steps of cooking (Balasuramanian *et al.*, 2014). Several researches have been carried out for the development of instant foods including soy-fortified instant *upma* mix (Yadav and Sharma, 2008), *halwa* dry mix (Yadav *et al.*, 2007), pearl millet based *upma* dry mix (Balasubramanian *et al.,* 2014) and instant wheat porridge (*dalia*) mix (Khan *et al.*, 2014).

With convenience, there also comes an increased demand of consumers for value added products with health advantages (Gadhiya *et al.*, 2015). The improvements in the understanding of association between nutrition and health lead to the functional foods development which is a practical and new approach for the achievement of optimum status by promoting the state of being healthy and thus probably decreasing the diseases' risk (Siró *et al.*, 2008). Such products that are claimed to be healthy and have functional and/or heath properties are gaining priority in researches in production of novel foods(de Sousa *et al.*, 2011). Nutraceutical potential of medicinal plants makes them beneficial to be used in medicine and for therapeutic purposes (Harsha and Aarti, 2015). Various herbs possess many therapeutic properties including antioxidative, antihypertensive, anti-inflammatory, antidiabetic, antimicrobial, etc (Oraon *et al.*,2017). Thus incorporation of these herbs asfunctional foods can provide several health benefits to the consumers.Several researches related to development of food products incorporating herbs and other functional foods have been conducted including herbal juice development from traditional Indian plants using *Citrus limetta* as base (Harsha and Aarti, 2015) powdered food developed with addition of *Spirulina* (Santos *et al.*, 2016), development of an apple snack rich in flavonoid (Betoret *et al.*, 2012), development of blended papaya- Aloe veraready to serve beverage(Boghani *et al.,* 2012)to enhance the nutritional properties of the food products. In the present study *Rauvolfia serpentina*, black cumin seeds and apples were incorporated as functional ingredients to enhance the nutritional properties of the food product.

Rauvolfia serpentina and black cumin seeds were found to significantly increase the amount of fibre, whereas apple powder and black cumin seeds significantly decreased the amount of sodium in nutritious mix. This recipe has low sodium content that can be an additional benefit as a result of direct relation of sodium consumption with hypertension in humans (Malviya and Sason, 2016). Apples provide a good source of carbohydrates and vitamins and have less contribution in calories along with no contribution in fat, sodium or cholesterol (Harris *et al.,* 2007). Black cumin seeds constitutes of proteins, minerals, vitamins, enzymes, carbohydrates and fats having about overall fat contained in the form of omega-3 and omega-6 fatty acids in rich amount. They also contain vitamins A, B_1, B_2, B_3 and C as well as calcium, iron, magnesium, zinc and selenium(Hussain and Hussain, 2016). Thus, the incorporation of apples, *Rauvolfia serpentina* and black cumin seeds in nutritious mix have together contributed in obtaining the goal of low sodium and high fibre content.

Optimisation of ingredients in the food formulation is essential for the development of a product. There are number of techniques that are available to draw the best levels of input variables that in turn optimise their responses (Nadeem *et al.,* 2012). RSM is the one which is stated to be an effective measure for optimising a process when the independent variables are hypothesised to possess a dominant or accumulative effect on the desired responses(Martínez *et al.,* 2004). The observations of effect of independent variables on fibre in this study represented the significant effect of linear model on the response. The results for effect of independent variables on sodium showed that the quadratic model had significant effect on response. The 2FI model indicates the non-significant process variables' effecton the response, overall acceptability. Similar studies were conducted, (i) process optimisation for formulating cowpea incorporated instant *kheer* mix by the use ofRSM was conducted in which amounts of cowpea and malted wheat flour and cowpea soaking time and were the process variables and protein, crude fiber and overall acceptability were the responses. Results revealed that the models had non-significant effect on the response, crude fibre and overall acceptability (Gupta *et al.,* 2014); (ii) optimisation of multigrain premix (MGP) to develop high protein and dietary fibre biscuits through RSM was conducted in which levels of MGP and wheat flour concentration were the process variables and protein, soluble, insoluble fibres, biscuit dough hardness, breaking strength and overall acceptability were the response variables. Results revealed that the incorporation of MGP significantly increases the soluble and insoluble fibres content of biscuits(Kumar *et al.,* 2015a); (iii) process optimisation of vegetable cereal mix using RSM was conducted in which amounts of *Trigonella foenum-graecum* and *Gymnema sylvestre*and soaking time of *Trigonella foenum-graecum* were the independent variables and fat, fibre, carbohydrate and overall acceptability were the responses. Results for effect of process variables on the response showed that 2FI model had a significant effect on fibre(dependent variable) whereas 2FI model had non-significant effect of process variables on the response, overall acceptability (Gupta *et al.,* 2016). There are some other similar studies in which food product development of various premixes was conducted using RSM to enhance their nutritional characteristics like optimisation of instant dalia dessert pre-mix formulation by the use of RSM (Jha *et al.,* 2015) and production of multigrain premixes-its effect on rheological, textural, and micro- structural characteristics of dough and quality of biscuits(Kumar *et al.,* 2015b).

The nutritional analysis of optimum recipe resulted in recipe being nutritionally adequate and was rich in iron and good source of calcium. Bhadana *et al.* (2016) carried out a study on product development and nutrients evaluation of value added product incorporated with spirulina powder, soya flour and rice flour. for nutritional analysis revealed that the sample The results containing spirulina powder 20 g, soya flour and rice flour had moisture content of 2.48 percent per 50 g and 1.10 percent per 50 g fat content that were similar to the present study. The instant foods are beneficial in saving very important resources such as time and energy(Lohekar and Arya, 2014) and the value addition of functional ingredients in optimum levels can enhance the nutritional properties of these foods without compromising their acceptability. The nutritious mix owing to low sodium can be of benefit to hypertensive patients as rise in blood pressure is a common disorder in India(Raghupathy *et al.,* 2014) affecting all age groups.

4. Conclusions

RSM came out to be a successful tool to derive the best combination of different processes (amount of apple powder, *Rauvolfiaserpentina* and black cumin seeds) for formulation of nutritious mix. Out of 30 suggested combinations, 4 combinations had highest desirability value (0.627) in comparison to others. Recipe having 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 10.00 g black cumin seeds powder with 477.71 mg potassium, 39.85 mg sodium, 4.09 g fibre and 87.61 overall acceptability score was selected optimum recipe and subjected for further nutritional analysis. Optimum recipe had adequate potassium, low sodium, high fibre and high overall acceptability. It was rich in iron and good source of calcium. Nutritious mix is the instant food which is convenient to be used, affordable and of nutritional importance as well. Incorporation of Indian medicinal herbs into it makes it highly beneficial for various ailments like hypertension.Thus, it can be effortlesslyutilised by the consumers as food add-on devoid of any variation in their regular diets.

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