

# Development of Bittergourd Fermented Beverage Using Response Surface Methodology

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**Abstract:** Bittergourd (*Momordica charantia*) is used as a vegetable by the Asian community and is commonly used as an antidiabetic and antihyperglycemic agent. The aim of the present study was to develop nutritionally strengthful, highly acceptable, shelf stable bittergourd beverage by fermented process using statistical software on Response Surface Methodology (RSM). The optimization of the fermentation process with reference to curd concentration and the period has been attempted by using RSM with more emphasis on nutritional and functional considerations. The statistical design gave 13 formulations, where the fermentation process period varied from 6 to 34 hours and curd concentration was from 6.6 to 16.4%. The standard methodology was followed for the analysis of all the parameters studied. The product varied formulations had quinine range of 119 to 327.5 mg%, antioxidants 40.2 – 64.5%, total phenols 30.5 to 42.5 mg%, water soluble vitamins; B<sub>1</sub> – 0.07 to 0.81 mg%, B<sub>2</sub> – 0.02 to 0.28 mg%, B<sub>3</sub> – 0.4 to 1.08 mg%, B<sub>6</sub> – 0.04 to 0.32 mg% and vitamin-C – 17.2 to 34.6 mg%. Fermentation time for 30 hours and curd concentration of 15% was the optimized composition with the best fit of desirability 0.80. The product with the good taste and flavour was acceptable with 6.7 score on 9-point hedonic scale. The fermented bittergourd beverage was preferred to fresh bittergourd juice.

**Keywords:** Bittergourd, Curd, Fermentation, RSM, Quinine, Vitamins.

## 1. INTRODUCTION

Vegetables are strongly recommended in the human diet since they are rich in antioxidants, vitamins, dietary fibres and minerals. The major part of the vegetables consumed in the human diet are fresh, minimally processed, pasteurized or cooked by boiling in water or microwaving. Minimally processed and especially, fresh vegetables have a very short shelf life since subjected to rapid microbial spoilage and the above cooking processes would bring about a number of not always desirable changes in physical characteristics and chemical composition of vegetables [1, 2]. Among the various technological options, lactic acid fermentation may be considered as a simple and valuable biotechnology for maintaining and/or improving the safety, nutritional, sensory and shelf life properties of vegetables [3, 4]. Lactic acid fermentation of vegetables has nowadays an industrial significance only for cucumbers, cabbages, red beet, carrot, celery, tomato and olives [5, 6]. However, the studies on nutrient profile have not received much attention. *Momordica charantia* L also known as bittermelon, bittergourd, balsam pear or karela is widely cultivated as a vegetable and medicinal herb in many Asian countries and has been shown to exert hypoglycemic effects in animal models and humans. Bittergourd fruit can be preserved by dehydration, steeping

preservation and pickling but no work has been done to prepare fermented beverage from bittergourd in India. Studies on the use of lactic acid bacteria starters to get reliable and controlled fermentation processes should be warranted [7]. Although a large number of lactic acid bacteria starters are routinely used in dairy, meat and baked good fermentations, only a few cultures have been used for vegetable fermentations. Keeping this in view an attempt is made to develop nutritionally strengthful, highly acceptable, shelf stable bittergourd fermented beverage using natural mixed culture of curd by using statistical software by RSM for persons suffering from diabetes. The optimization of the fermentation process with reference to curd concentration and the period has been attempted by using RSM with more emphasis on nutritional and functional considerations. However, the studies on nutrient profile has not received much attention thus the fermentation conditions followed for vegetable juices and the changes in their nutrients still needs a radical approach to achieve the best quality product. Therefore in the present study, the raw material bittergourd, statistical design using RSM, an effective tool for optimization have been used with an attempt to bring out the nutritional strength of the fermentation process.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Good quality raw materials *i.e.* bittergourd (*Momordica charantia*) - light green variety, salt, lemon

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were procured from local market and milk, curd were procured from Nandini milk parlour, Mysore. All the chemical and reagents used for the analysis were AR grade. For HPLC analyses HPLC grade solvents and standards were procured from M/s SK Chemicals, Burdick and Jackson, HPLC certified solvents and M/s Sigma Aldrich, St.Louis, USA.

## 2.2. Raw Material Processing

Bittergourd were sorted, washed thoroughly in running water to remove adhering foreign materials and cut horizontally, removed seeds, cleaned in salt water and cut into pieces, blanched at 90°C for 5 mins with 0.1% Potassium metabisulphite and 0.05% Magnesium oxide and extracted the juice using a juice extractor (RayLons Metal Works, Bombay, India). Curd was added (6.6 to 16.4gm/100ml) and fermented at room temperature for 6 to 34 hours period by adding 2% salt. Good quality milk was seeded with curd (2%) and kept at 37°C for 10 hours and was homogenized and used for fermentation.

## 2.3. Experimental Design

The percentage of curd concentration and time of fermentation was as per the runs obtained by design expert statistical software. A Central Composite Rotatable Design was used without blocking. The number of design points were obtained on basis of the number of independent variables decided. The statistical software package design expert 8.0.2, Stat - Ease Inc., Minneapolis, MN, was used to construct the experimental design as well as analyze the data. The parameters that influence the product quality, acceptability and functionality were taken as responses. Variables curd concentration and fermentation period were selected as independent variables and quinine (mg%), total phenols (mg%),

antioxidants (%), Water soluble vitamins (mg%)- B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub> and vitamin-C were selected as the responses. The factorial design considered 4 factorial points, 4 axial points and 5 central points leading to 13 sets of experiments [8]. Optimized ranges of the variables are shown in Table 1. Each independent variable investigated in this experiment had five levels which were -1.4142, -1, 0, +1 and +1.4142. A total of 13 level combinations (design points) were generated for the two independent variables and the alpha values in the design outside the ranges were selected for rotatability of the design. The center point (the level combination in which the value of each coded variable was 0) was repeated five times for the two-variable design and was selected keeping the ingredients at levels expected to yield, at least, satisfactory experimental results.

The regression analysis of the responses was conducted by fitting to the suitable model represented by the following equation

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 + \sum_{i \neq j=1}^n \beta_{ij} x_i x_j$$

where,  $\beta_0$  was the value of the fitted response at the center point of the design, while  $\beta_i$ ,  $\beta_{ii}$ , and  $\beta_{ij}$  were the linear, quadratic and interactive-effect regression terms, respectively, and  $n$  denoted the number of independent variables i.e. in this case  $n$  is 2, and  $x_i$ ,  $x_{ij}$  are independent variables in coded values represented by  $X_1$  and  $X_2$  in Table 2.

After fermentation for varied periods the respective samples were filtered in muslin cloth. Then the bottles were sealed and pasteurized for 15mins at 90°C. The samples were analysed immediately after the completion of the fermentation process.

**Table 1: Process Variables Their Levels and Experimental Design**

Process variables	-1.414 (augmented form)	-1 (factorial point)	0 (centre point)	+1 (factorial point)	+1.414 (augmented form)
Fermentation (Hours)	6	10	20	30	34
Curd (gm/100ml)	6.6	8	11.5	15	16.4

X <sub>1</sub>	X <sub>2</sub>	Runs
± 1	± 1	4
± 1.414	0	2
0	± 1.414	2
0	0	5

**Table 2: Actual Experimental Combinations and Response Values**

Exp. No	X <sub>1</sub>	X <sub>2</sub>	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6	Response 7	Response 8
Run	A:Time	B:Curd	Quinine	Antioxidants	Total Phenols	Thiamine	Riboflavin	Niacin	Pyridoxine	Vitamin C
	Hours	%	mg%	%	mg%	mg%	mg%	mg%	mg%	mg%
			Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>	Y <sub>7</sub>	Y <sub>8</sub>
1	10	8.0	299.9	42.5	36.6	0.81	0.019	0.67	0.092	17.2
2	30	8.0	245.3	51	39.1	0.33	0.098	0.76	0.3	27.6
3	10	15.0	158.6	64.5	42.5	0.11	0.2	0.86	0.081	21.7
4	30	15.0	286	54.1	41.2	0.32	0.28	0.7	0.27	33.4
5	6	11.5	172.5	56.5	41.35	0.6	0.075	1.08	0.042	18
6	34	11.5	237.1	55	38.5	0.4	0.22	1.02	0.32	34.6
7	20	6.6	327.5	40.2	36.35	0.5	0.035	0.4	0.2	20.5
8	20	16.4	253.5	59.3	39.8	0.068	0.24	0.4	0.17	27.2
9	20	11.5	132.3	54.2	30.5	0.4	0.19	0.86	0.15	24.9
10	20	11.5	119	55.2	34.63	0.35	0.19	0.89	0.16	26
11	20	11.5	127.7	54.5	34.05	0.4	0.24	0.8	0.15	25.1
12	20	11.5	128.9	52.6	34.5	0.4	0.25	0.9	0.16	25
13	20	11.5	134	53.9	35.6	0.35	0.24	0.9	0.149	25.8

#### 2.4. Analytical Evaluation

The fermented bittergourd juice was analysed for quinine [9], total phenols and free radical scavenging capacity (DPPH activity) [10], and water soluble vitamins *i.e.*, thiamine, riboflavin, niacin, pyridoxine and vitamin C analysis were performed using HPLC on reverse phase C<sub>18</sub> analytical column [11].

The analysis for all the parameters was carried out in duplicate and the average value has been reported.

#### 2.5. Organoleptic Evaluation

A semi trained panel evaluated the samples for bitterness, colour, aroma, taste and overall acceptability using a nine point hedonic scale [12]. The samples were subjected to microbiological analysis for the study of TPC, Coli, Yeast and Moulds. Fresh bittergourd juice, fresh bittergourd beverage with dilution and optimised fermented bittergourd beverage and fermented diluted beverage were served to the panel members at room temperature. Samples were randomly drawn for each experimental block, coded and served to the panelists.

### 3. RESULTS AND DISCUSSIONS

The experimental central composite rotatable design with independent variables and responses is

given in Table 2. Over the 13 combinations quinine ranged from 119 to 327.5mg%, antioxidants 40.2 to 64.5%, total phenols 30.5 to 42.5 mg%, water soluble vitamins; B<sub>1</sub> – 0.07 to 0.81 mg%, B<sub>2</sub> – 0.02 to 0.28 mg%, B<sub>3</sub> – 0.4 to 1.08 mg%, B<sub>6</sub> – 0.04 to 0.32 mg% and vitamin-C – 17.2 to 34.6 mg%.

The effects of the independent variables curds and fermentation time on the response at linear, quadratic and interactive levels are presented in Table 3. The sign and magnitude of the coefficients indicate the effect of the variable on the responses. Negative sign of a coefficient at linear level indicates decrease in response with an increase in level of the variable where as at interactive level, level of one variable could be increased while that of the other decreased to obtain the same response.

All main, linear, quadratic and interactive effects were calculated for each model. Quadratic response surface models were selected for all the responses. The adequacy was calculated by F-ratio, mean, standard deviation, coefficient correlation and lack of fit test. R<sup>2</sup> value was more than 90% and lack of fit was highly non-significant. The regression coefficient, correlation coefficient for the responses were 0.99 for quinine, antioxidant activity, B<sub>1</sub>, B<sub>6</sub>, Vitamin C and 0.98 for B<sub>3</sub>, 0.95 for B<sub>2</sub> respectively indicating that all the values were more than 90%. The R<sup>2</sup> value for total phenols was 0.85, but because of the subjective nature

**Table 3: Coefficient of Second Order Polynomial Regression Models**

Coefficient	Quinine	Antioxidants	Total Phenols	Thiamine	Riboflavin	Niacin	Pyridoxine	Vitamin-C
	mg%	mg%	%	mg%	mg%	mg%	mg%	mg%
	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>	Y <sub>7</sub>	Y <sub>8</sub>
β <sub>0</sub>	128.38	54.08	33.87	0.38	0.22	0.87	0.154	25.36
β <sub>1</sub>	20.51****	- 0.502	- 0.354	-0.069****	0.0455**	-0.019	0.099****	5.69****
β <sub>2</sub>	-25.65****	6.51****	1.609*	-0.165****	0.0816****	0.0163	-0.010***	2.47****
β <sub>11</sub>	38.16****	0.904	3.247**	0.06***	-0.035**	0.096***	0.014****	0.445*
β <sub>22</sub>	81.01****	-2.096***	3.23**	-0.0478**	-0.040**	-0.229****	0.016****	-0.78**
β <sub>12</sub>	45.5****	-4.725****	- 0.95	0.1725****	0.00025	-0.0625*	-0.0048	0.325
R <sup>2</sup> %	99.74	99.09	85.27	99.00	95.11	97.96	99.82	99.57
p%	<0.0001	<0.0001	<0.01	<0.0001	<0.001	<0.0001	<0.0001	<0.0001
Mean	201.72	53.34	37.28	0.387	0.175	0.788	0.173	25.15
SD	5.08	0.782	1.75	0.024	0.025	0.038	0.0046	0.43
F-value	526.06	154.03	8.11	139.27	27.28	67.38	785.13	326.08

Note: Significance: \*\*\*\* p>0.0001, \*\*\* p<0.001, \*\* p<0.01, \* p<0.05.

of the response the model is considered significant (Table 3).

### 3.1. Effect of Variables on Functional Parameters

Quinine, total phenols and antioxidant activity were considered as functional parameters for the independent variables. Phenolics or polyphenols have received considerable attention because of their physiological function, including antioxidant, antimutagenic and antitumour activities [13]. Plant phenolics present in fruit and vegetables have received considerable attention because of their potential antioxidant activity [14]. Phenolic compounds are widely distributed in plants [15], which have gained much attention due to their antioxidant activities and free radical scavenging abilities, which potentially have beneficial implications for human health [15, 16]. Total phenol content was determined in comparison with standard Gallic acid and the results are expressed in mg/100ml of sample. Wu and Ng [17] found that extracts of wild bittermelon, possessed potent antioxidant and free radical scavenging activities. Some researchers have also found that Thai bittergourd fruit contained anticarcinogens or chemopreventive agent [18, 19]. However no report exists on fermentation effect on total phenols and antioxidant activity on bittergourd. Table 3 reveals that at linear level time had a significant (p<0.0001) positive effect on quinine content, while it had a negative effect on total phenols and antioxidant activity. Curd had a significant (p<0.0001) negative effect on quinine

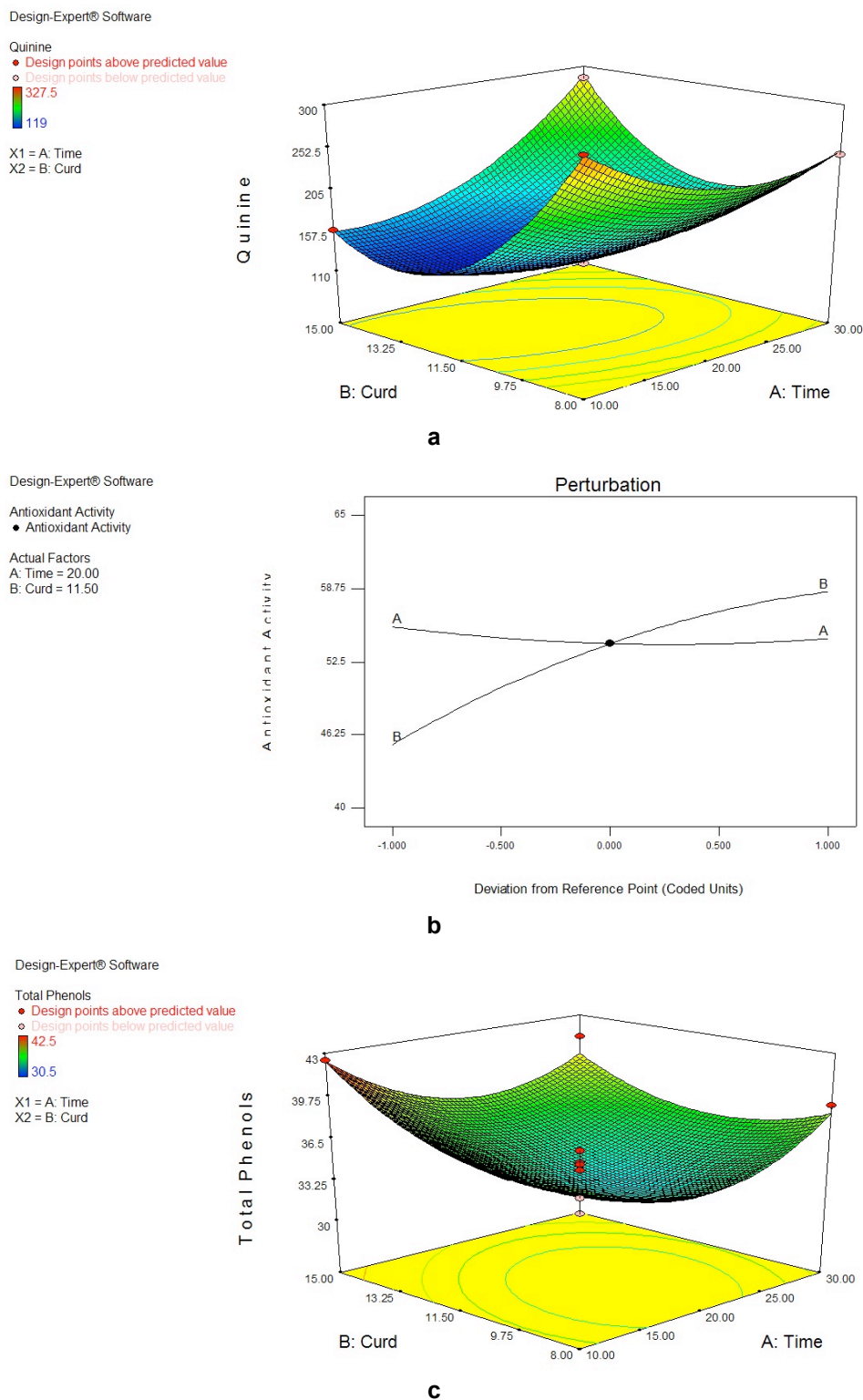
content where as it had a significant (p<0.0001 & p<0.05) positive effect on antioxidant activity and total phenol content respectively at linear levels. At quadratic level both fermentation time and curd concentration had a positive highly significant level at p<0.0001 for quinine and p<0.001 on total phenols, while antioxidant activity was negatively affected by curd at p<0.001. Fermentation time and curd had positive significant (p<0.0001) effect (p<0.01) on quinine level and variables showed negative significant (p<0.0001) effect on antioxidant activity at interactive level. The multiple coded equations in terms of coded factors generated for their responses are shown below:

$$\begin{aligned} \text{Quinine} = & 128.38 + 20.519 X_1 - 25.656 X_2 \\ & + 38.16 X_1^2 + 81.01 X_2^2 \\ & + 45.5 X_1 X_2 \quad R^2 = 0.99 \end{aligned}$$

$$\begin{aligned} \text{Antioxidants} = & 54.08 - 0.502 X_1 + 6.514 X_2 \\ & + 0.903 X_1^2 - 2.096 X_2^2 \\ & - 4.725 X_1 X_2 \quad R^2 = 0.99 \end{aligned}$$

$$\begin{aligned} \text{Total Phenols} = & 33.85 - 0.358 X_1 + 1.609 X_2 \\ & + 3.247 X_1^2 + 2.322 X_2^2 \\ & - 0.95 X_1 X_2 \quad R^2 = 0.85 \end{aligned}$$

The response surface for quinine, total phenols and antioxidants in relation to fermentation time and curd is



**Figure 1:** Perturbation graphs depicting effect of independent variables on functional parameters of the fermented beverage.

shown in Figure (1a, 1b and 1c) respectively. As curd concentration increased total phenols and antioxidants increased and as fermentation decreased there was increase in total phenol level of beverage. Kubola *et al.* [10] reported that bittergourd fractions are rich in phenolics and have a strong antioxidant activity and a radical-scavenging action.

### 3.2. Effect of Variables on Nutritional Parameters

Since the time fermented products have had an image of possessing almost magical health-giving properties. However, the knowledge of how lactic acid fermentation influences the nutritional value of products is poor and many of the nutritional aspects of

fermented products still have to be subjected to in depth scientific investigations. Thus the effect of fermentation time and starter culture concentration on water soluble vitamins formation was studied in the current study. The model obtained from the regression can be written as follows for nutritional parameters.

$$\begin{aligned} \text{Thiamine} = & 0.38 - 0.069 X_1 - 0.165 X_2 \\ & + 0.060 X_1^2 - 0.0478 X_2^2 \\ & + 0.173 X_1 X_2 \quad R^2 = 0.99 \end{aligned}$$

$$\begin{aligned} \text{Riboflavin} = & 0.222 + 0.0455 X_1 + 8.6925 X_2 \\ & - 0.035 X_1^2 - 0.040 X_2^2 \\ & + 0.00025 X_1 X_2 \quad R^2 = 0.95 \end{aligned}$$

$$\begin{aligned} \text{Niacin} = & 0.87 - 0.019 X_1 + 0.016 X_2 \\ & + 0.0956 X_1^2 - 0.229 X_2^2 \\ & - 0.0625 X_1 X_2 \quad R^2 = 0.98 \end{aligned}$$

$$\begin{aligned} \text{Pyridoxine} = & 101.141 + 10.6268 X_1 + 0.3855 X_2 \\ & + 33.7317 X_1^2 - 6.3412 X_2^2 \\ & - 22.53 X_1 X_2 \quad R^2 = 0.99 \end{aligned}$$

$$\begin{aligned} \text{Vitamin C} = & 25.36 + 5.6969 X_1 + 2.471 X_2 \\ & + 0.445 X_1^2 - 0.78 X_2^2 \\ & + 0.325 X_1 X_2 \quad R^2 = 0.99 \end{aligned}$$

From the above equation, it can be concluded that thiamine had significant ( $p < 0.0001$ ) negative effect on linear model. Curd concentration had a highly significant ( $p < 0.0001$ ) positive effect on riboflavin and vitamin-C where as it affected pyridoxine levels negatively at  $p < 0.001$  and niacin was insignificant at linear level. At linear level fermentation time had a highly significant ( $p < 0.0001$ ) positive effect on pyridoxine and vitamin C, while riboflavin had significant ( $p < 0.01$ ) positive effect and niacin was insignificant. Fermentation time had negative effect on riboflavin at quadratic level with the significance level of  $p < 0.01$  and curd concentration had negative effect on thiamine, riboflavin, vitamin C with significance level of  $p < 0.01$ , niacin with significance level of  $p < 0.0001$  while it was positively significant ( $p < 0.0001$ ) for pyridoxine at quadratic level. Fermentation time affected pyridoxine at significance level of  $p < 0.0001$ , thiamine and niacin at  $p < 0.001$  and vitamin C at  $p < 0.01$  at quadratic level

while it affected negatively on riboflavin ( $p < 0.001$ ). At interactive level, fermentation time and curd concentration affected the thiamine significantly at  $p < 0.0001$ , niacin at  $p < 0.01$  while variables had no significant effect on riboflavin, pyridoxine and Vitamin C retention. It is clear from Figure (2a to 2e), as there was increase in fermentation time there was increase in riboflavin, pyridoxine and vitamin C content and with increase in curd concentration, there was increase in riboflavin and vitamin C content. Thus the graph explains that fermentation time and curd concentration has great influence on vitamin profile.

### 3.3. Optimization of Independent Variables

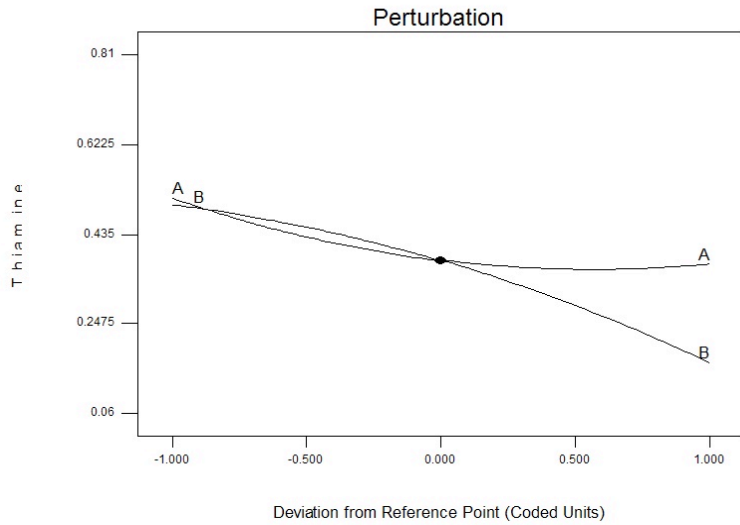
Numerical optimization of independent variables, fermentation time and curd concentration were optimized using Design Expert Software. The criteria used along with the predicted and actual values of the response are given in Table 4. The aim of the experiment was to maximize the formation of water soluble vitamins and increase the antioxidant activity. The solution which was obtained was 30 hours of fermentation time and 15% of curd with best fit desirability of 0.80. The optimized results were quinine 287.9mg%, antioxidants 54.2%, total phenols 39.7 mg%, vitamin B<sub>1</sub> 0.33mg%, B<sub>2</sub> 0.27mg%, B<sub>3</sub> 0.67mg%, B<sub>6</sub> 0.27mg% and vitamin-C 33.5mg%. The predicted response value as against actual value for responses as shown in Table 4 were in concurrence with each other, hence the similar fitted models are suitable for predicting the responses.

When compared with the bittergourd juice, optimized fermented bittergourd beverage was higher in antioxidant levels, total phenols content, thiamine, riboflavin and vitamin C content (Table 5). Sensory scores of bittergourd juice, bittergourd diluted beverage, bittergourd fermented beverage and bittergourd diluted fermented beverage is presented in Table 6. The bittergourd juice and fermented bittergourd beverage were analysed for microbiological status and the results revealed that the products were free from TPC, coli and yeast and moulds and were fit to drink. The safe product was subjected to sensory analysis and product bittergourd fermented beverage with the good taste and flavour was acceptable with 6.7 score on 9-point hedonic scale in diabetic panelists and 6.2 score in non diabetic panelists. The sensory quality of the fermented bittergourd beverage was scored above acceptable range when compared with fresh bittergourd juice and the preferred quantity of fermented beverage consumption was higher than the fresh bittergourd juice.

Design-Expert® Software

Thiamine  
 • Thiamine

Actual Factors  
 A: Time = 20.00  
 B: Curd = 11.50

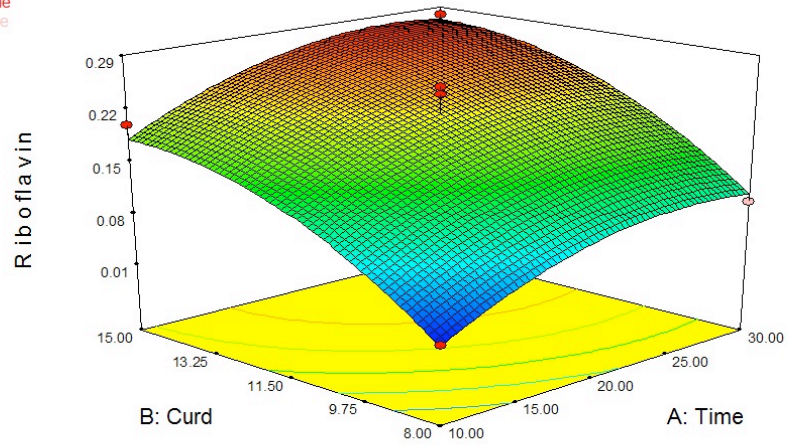


a

Design-Expert® Software

Riboflavin  
 • Design points above predicted value  
 ◊ Design points below predicted value  
 0.28  
 0.019

X1 = A: Time  
 X2 = B: Curd

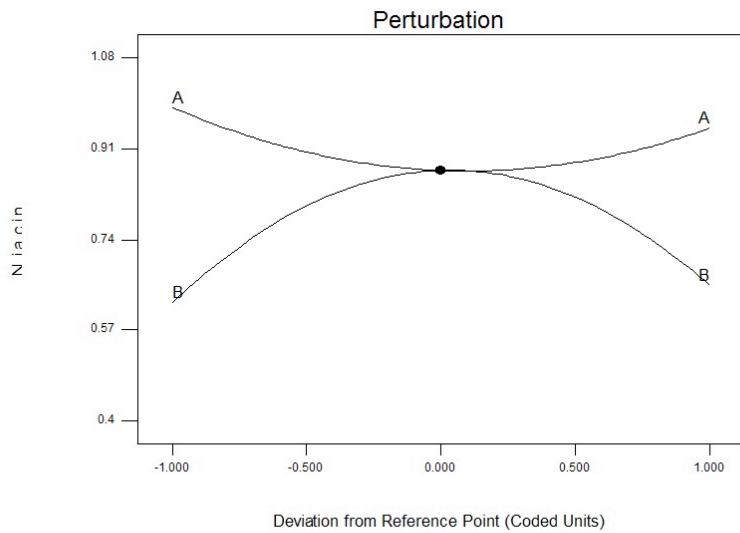


b

Design-Expert® Software

Niacin  
 • Niacin

Actual Factors  
 A: Time = 20.00  
 B: Curd = 11.50



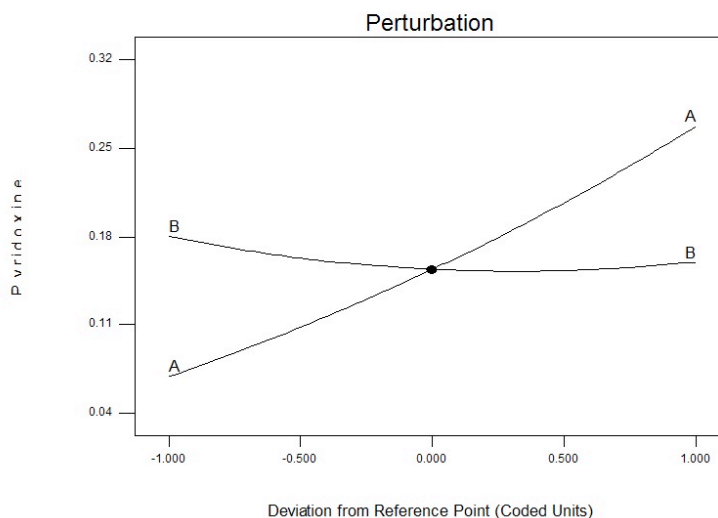
c

Figure 2: continued

Design-Expert® Software

Pyridoxine  
 ● Pyridoxine

Actual Factors  
 A: Time = 20.00  
 B: Curd = 11.50

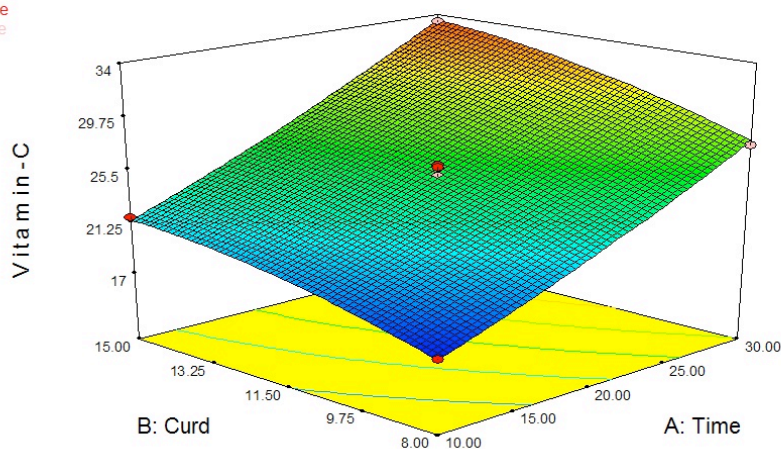


d

Design-Expert® Software

Vitamin-C  
 ● Design points above predicted value  
 ○ Design points below predicted value  
 34.6  
 17.2

X1 = A: Time  
 X2 = B: Curd



e

Figure 2: Perturbation graph and 3D plots depicting effect of independent variables on Nutritional Parameters of the fermented beverage.

Table 4: Predicted and Actual Response Values

Responses	Actual	Prediction
Quinine (mg%)	287.9	297.4
Antioxidants (%)	54.2	55.6
Total Phenols (mg%)	39.7	43.0
Thiamine (mg%)	0.33	0.38
Riboflavin (mg%)	0.27	0.32
Niacin (mg%)	0.67	0.74
Pyridoxine (mg%)	0.27	0.28
Vitamin C (mg%)	33.5	34.3



**Table 5: Functional and Nutritional Parameter of Bittergourd Juice and Bittergourd fermented beverage**

Parameters	Fresh Juice	Fermented Juice
Quinine (mg %)	426	287.9
Antioxidants (%)	44	54.2
Total Phenols (mg %)	20	39.7
Thiamine (mg %)	0.16	0.33
Riboflavin (mg %)	0.15	0.27
Niacin (mg %)	0.7	0.67
Pyridoxine (mg %)	0.3	0.27
Vitamin C (mg %)	28	33.5

**Table 6: Sensory Score of Bittergourd Juice and Fermented Beverage, n=10**

Types	Subjects	Bitterness	Color	Aroma	Taste	OAA*	Preferred quantity to drink
Bittergourd Juice	Non - Diabetic	6.4 ± 1.4	6.8 ± 0.8	5.1 ± 2.6	5.4 ± 1.8	5.2 ± 1.4	34 ± 32
	Diabetic	6.5 ± 1.6	6.5 ± 0.8	5.5 ± 0.8	5.5 ± 0.8	4.5 ± 0.8	45 ± 12
Bittergourd Beverage (Diluted)	Non - Diabetic	6.2 ± 1.5	6.6 ± 1.5	5.8 ± 2.4	6.2 ± 1.2	6.0 ± 1.4	36 ± 31
	Diabetic	4.8 ± 1.2	6.5 ± 0.8	5.7 ± 0.5	6 ± 1.3	5.7 ± 1.5	58 ± 20
Bittergourd Fermented Beverage	Non - Diabetic	5.8 ± 1.5	6.8 ± 0.9	5.9 ± 2.5	6.1 ± 1.6	6.2 ± 1.5	34 ± 32
	Diabetic	5.3 ± 0.8	6.5 ± 0.8	5.8 ± 0.8	6.3 ± 1.6	6.7 ± 2.1	75 ± 27
Bittergourd Fermented Beverage (Diluted)	Non - Diabetic	5.2 ± 2.2	6.6 ± 0.8	6.1 ± 1.6	6.3 ± 1.8	5.9 ± 2.1	38 ± 31
	Diabetic	4.3 ± 0.5	6.5 ± 0.8	6 ± 1.3	6.1 ± 1.3	5.8 ± 1.6	58 ± 20

Note: \* Overall acceptability.

#### 4. CONCLUSION

The application of RSM for optimizing the ingredients and process showed that quadratic response surface models were fitted. F values were significant in all selected responses and a high R<sup>2</sup> value of >90% showed fitness of the polynomial regression models for describing the effect of variables. The results of the study indicated that the effect of fermentation time and curd concentration were significant to all the selected responses and RSM could be useful in optimizing the curd concentration and fermentation time with maximum retention of vitamins. Using the optimized condition the prepared beverage from fermentation process contained more total phenolics, antioxidants, Vitamin B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub> and Vitamin C when compared with fresh bittergourd juice. Overall the fermentation time and curd concentration is optimized with maximum retention of vitamins, antioxidants and total phenols with 30 hours of fermentation time and 15% curd concentration. The product with the good taste and flavour was acceptable with 6.7 score on 9-point hedonic scale in diabetic panelists and 6.2 score in non diabetic panelists. The

sensory quality of the fermented bittergourd beverage was scored above acceptable range when compared with fresh bittergourd juice and the preferred quantity of fermented beverage consumption was higher than the fresh bittergourd juice. From the study, it can be concluded that the fermented bittergourd beverage was nutritionally and functionally strengthful and highly acceptable.

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