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RESPONSE SURFACE OPTIMIZATION OF FERMENTING PARAMETERS FOR THE PRODUCTION OF BEER FROM FINGER MILLET AND APPLE JUICE BY USING BOX-BEHNKEN DESIGN

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ABSTRACT

The experiments were planned using Response Surface Methodology, Box Behnken design was used and total seventeen designed experiments were conducted to produce beer from finger millet and apple juice. Effects of independent variables with three levels for each i.e. blend ratios ((Finger millet: Apple Juice) (90:10, 85:15, 80:20)), yeast concentration (6%, 8%, 10%) and malted grain to water ratios (1:8, 1:9, 1:10) were investigated on beer quality. During study it was observed that all the independent parameters i.e. blend ratio, yeast concentration and malted grain to water ratios affected the responses (pH, Titrable acidity, colour, bitterness and alcohol content) significantly. Optimization was done using Design Expert 10.0.1 software, for free beer production. The optimum values were found to be 80.24:19.76 blend ratio, 10% enzyme concentration and 1:8 slurry ratio. The model F-value was found to be highly significant at 1% level of significance for all the responses. The values for pH, titrable acidity, colour, bitterness and alcohol content at optimum conditions were found to be 5.12, 0.12, 17.312, 18.95 and 9.25 respectively all the responses could be predicted by fitting the second order mathematical model and adequacy checked by R2.

1.Introduction

India leads the world in production of millets. In Uttarakhand traditional crops like, finger millet, barnyard millet, black soyabean, horse gram etc. are cultivated in a wide range of soils and under diverse climate conditions. In India, finger millet (*Eleusinecoracana*) (locally called by various name including ragi and nachani) is mostly grown and consumed in Karnataka, Rajasthan, Andhra Pradesh, Tamil Nadu, Orissa, Maharashtra, Kumaon (Uttarakhand) and Goa. Finger millet, one of the major underutilized crops of Uttarakhand, grows well in tropical countries and contains a good amount of reducing sugars (Kumar*et al.*, 2015).It is mainly grown for food grain for human consumption (Upadhyaya *et al.,* 2006).It is rich in calcium and protein and also has good amount of iron and other minerals (Khandelwal *et al.,*2012).Because of its good nutritional value finger millets can be used for brewing process. Malting of finger millet improves its digestibility, sensory and nutritional quality as well as pronounced effect in lowering the antinutrients (Desai *et al.*, 2010).In the past, these cereals were used locally both in malted and unmalted forms for the production of some types of alcoholic beverages in the tropics (Odunfa 1985).The grain is made into a fermented drink (or beer) in Nepal and in many parts of Africa. The use of cereals offers great advantages in brewing (Okafor and Aniche, 1980; Glennie *et al.,* 1983; Ugboaja*et al.,* 1991).So it is estimated that finger millets can be used for the preparation of Beer single handly because of its rich carbohydrates content.

Fruits wines are prepared from fruits namely apples, peaches, oranges, bananas, blackberries, mangoes pumpkin etc.Apple (*Pyrus malus*) belongs to the family Rosaceae. Apple according to scientists is a miracle fruit because of the several health benefits it offers (Kaur *et al.,* 2004). Apple fruits are consumed directly as whole or in form of juice, jams and jellies etc. Apple juice is one of the most widely consumed juices in temperate regions. Apple contains high levels of antioxidants, vitamins, minerals and phenolic compounds (Yazdanshenas*et al.,* 2010). It is rich source of phytochemicals; these phytochemicals are unaffected or affected by some extent during storage (Boyer and Liu, 2004).

Alcoholic beverages like beers are legally consumed in most countries. Beer is an alcoholic beverage made from cereals like barley, corn, rice, oat, sorghum, etc. and tuber crops like cassavaand most widely consumed. Use of finger millets with apple Juice for making beer to increase versatility and add to novelty is not reported.The aspect of blending desired and nutritive fruit juices in such alcoholic beverages could add more acceptability with nutrition. With these considerations, the aim of present studies related to value addition of underutilized crops using fermentation technology and was focused on utilization of underutilized millets namely finger millet (ragi) and apple fruit juiceto develop a new variety of alcoholic beverages like beer.An attempt has been made to explore the underutilized crops utilization (finger millet) using fermentation technology.

2. Materials and methods

2.1.Materials

Raw finger millet (*Eleusinecoracana*) and Apple fruit of good quality was purchased from the local market of Dehradun, Uttrakhand, India. Hop (*Humuluslupulus*) species were procured from the DVKSP Impex Pvt. Ltd. Yeast strain (*Saccharomyces cerevisiae*) was taken from food microbiology lab, UCALS, Uttaranchal University.

2.2. Experimental design

A total of seventeen sets of experiments by using Box Behenken design a total 17 experiments having three factorial points, three levels of each were conducted. Blend ratio (finger millet : apple juice), yeast concentration, malted grain to water ratio (slurry ratio) were selected as independent variables with three levels which were -1, 0, and +1 (Table 1). pH, Titrable acidity, colour, bitterness and alcohol content were selected as the responses. 3D curves were drawn with the help of Design Expert 10.0.1 to get the range of independent variables for product development.

Table 1. Independent variables levels and experimental design

Independent variables		Coded Levels		
Name	Code	-1	0	1
		Actual Levels		
Blend ratio (Finger millet: Apple Juice)	X_1	90:10	85:15	80:20
Yeast Concentration (%)	X_2	6	8	10
Malted grain : water	X_3	1:8	1:9	1:10

2.3. Procedure

All the experiments were conducted in three steps.

2.3.1. Malting

After cleaning , finger millet were soaked in water at room temperature (28±2°C) for 24 h. for proper aeration of grain the water was changed after every 6 to 8 h over a period of 24 h. After soaking the water was drained off and the grains were left on stainless steel sieves for germination process for a period of 24-36 h. After germination, the germinated grains were dried at 90°C for 12 h in Integrated Malting Unit developed by (Sanjay *et al.*, 2016).
Afterdryingrootlets were removed manually and cleaned malt was stored air tightly for further experiments.

2.3.2. Brewing

Before the preparation of wort, the malt was crushed coarsely in mechanical grinder, after that malted grain and water (1:8, 1:9 and 1:10 slurry ratios) for 100 ml of beer was boiled for 40 min at slow fire. In another ware 100 mL tap water was heated at 68-70°C for and sparging repeat the sparging process 2-3 time for maximum extraction of carbohydrates from finger millet malt. Again boil the wort at 70-80°C for 1 h, as soon as the wort started boiling, 1 g of hops were added to enhance the flavor and colour of the final product. Hops were separated by using strainer and muslin cloth and the wort was cooled to a temperature of 18-20°C for yeast growth during fermentation (Logan *et al.,* 1999).Apple juice at different concentration was added as per the experimental designshown in table 2.

Table 2. Experimental Design

Expt. No.	Coded Levels			Real Levels		
	X_1	X_2	X_3	Blend ratio finger millet: apple juice	Yeast concentration (%)	Slurry ratio (malted grain : water)
1	-1	-1	0	90:10	6	1:9
2	1	-1	0	80:20	6	1:9
3	-1	1	0	90:10	10	1:9
4	1	1	0	80:20	10	1:9
5	-1	0	-1	90:10	8	1:8
6	1	0	-1	80:20	8	1:8
7	-1	0	1	90:10	8	1:10
8	1	0	1	80:20	8	1:10
9	0	-1	-1	85:15	6	1:8
10	0	1	-1	85:15	10	1:8
11	0	-1	1	85:15	6	1:10
12	0	1	1	85:15	10	1:10
13	0	0	0	85:15	8	1:9
14	0	0	0	85:15	8	1:9
15	0	0	0	85:15	8	1:9
16	0	0	0	85:15	8	1:9
17	0	0	0	85:15	8	1:9

2.3.3. Fermentation

After cooling,liquid yeast was transferred (6%, 8% and 10%) in Laminar flow chamber and placed in dark place for fermentation for a period of 14 days. After fermentation, fermented liquor was centrifuged at 5000 rpm for 15-20 min in order to remove all yeast cells.

Supernatant was collected and stored in refrigerator at 4°C for further analysis.

2.4. Analytical Procedure

2.4.1. pH

The pH values of all samples were measured by digital pH meter of TOSCHON.

2.4.2.Titratable Acidity

Titratable acidity of fermented beverages was determined by the method of (Rangana, 2010), by using N/10 NaOH and expressed in term of malic acid.

2.4.3.Colour

Colour was estimated calorimetrically according to (Daniels, 1995).Degased sample was taken in 10 mm cuvette and absorbance was taken at 430 nm. Colour was calculated by the formula given below.

Calculations:

Colour = A × f ×25

Where:

A is absorbance at 430 nm in a 10 mm cuvette

f is dilution factor

2.4.4. Bitterness

Bitterness was estimated by the international method using iso octane extraction and bitterness was given in Bitterness Units (BU). http://dx.doi.org/10.1094/ASBCMOA-Beer-23.Briefly, in 10.0 ml Transfer 10.0 ml chilled sample a minute amount of octyl alcohol , 1 ml 3*N* HCl (reagent b) and 20 ml 2,2,4-trimethylpentane was added and centrifuge for 15 min. As soon as possible, transfer sufficient clear, upper (isooctane) layer to cuvet of spectrophotometer and absorbance was taken at 275 nm with 2, 2, 4-trimethylpentane-octyl alcohol as blank

Calculations:

Calculate bitterness units of beer by the formula,

BU = absorbance$_{275}$ × 50.

2.4.5 Ethanol content

Ethanol content in fermented liquor was estimated by the spectrophotometric method of (Caputi*et al.,* 1968).In brief, 1 ml of alcoholic sample was added directly to 30 ml with distilled water and then distilled at 70±2˚C. 20 ml of distillate was collected in a 50 ml volumetric flask containing 25 ml of potassium dichromate solution. The contents in the volumetric flask were heated at 60˚C in a water bath for 20 minutes and final volume was made to 50 ml with distilled water. After mixing and cooling the contents of the flask, the absorbance was recorded at 600 nm. The amount of ethanol in each sample was determined by using the standard curve of ethanol [0 – 20 % ethanol (v/v)].

2.5 Development of second order model

A complete second order mathematical model (Equation (1)) was fitted to the data and adequacy of the model was tested considering R^2 (the coefficient of multiple determination) and fisher's F-test. The models were then used to interpret the effect of various parameters on the response. Optimization of process parameters was carried out to predict the optimized values of selected independent variables.

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j + \sum_{i=1}^{3}\beta_{ii}X_i^2 \tag{1}$$

Where,

β_0, β_{ii}, β_{ij} are constants

X_i, X_j are coded variables

The experimental data were then analyzed by multiple regression techniques to develop response functions and variable parameters optimized for best outputs. The regression coefficients of complete second order model and their significance are reported in Table 4. Thevalue of p represented the probability of significance.A high p-value indicated that the model had a significant lack of fit and therefore, considered to be inadequate. The lower the values of p, the better the model would be. The models having p-value lower than 0.1 (indicating the lack of fit is insignificant at 90% confidence level) were accepted.

3.Results and discussions

3.1. Process Optimization

3.1.1. Numerical optimization of process parameters for beer production

Numerical optimization of independent variables (blend ratio, yeast concentration and slurry ratio) was carried out by using software Design expert 10.0.1. The goal was fixed for all independent variables as per the objectives of the study. The responses namely pH, titrable acidity, colour, bitterness and alcohol content were considered for optimization. The goal setup for optimization is given in the Table 5

The optimization was carried out as per the criteria mentioned in table 5. During optimization, total 40 solutions were obtained out of which the one that suited the criteria most (as per the desirability/ objectives) was selected. Choice of the solutions was automatically retrieved by the software. The optimized values are given in table 6

3.2. Response Surface Analysis of process parameters for beer production

The effect of differentindependent variables levels treatment on pH, titrable acidity, color, bitterness and alcohol content is given in Table 3. A series of three-dimensional response surfaces were drawn using Design Expert Software 10.0.1 for the visualization of variation in responses (pH, titrable acidity, color, bitterness and alcohol content) with respect to processing variables (finger millet to apple juice ratio (X_1), yeast concentration(X_2) and malt to water ratio(X_3)**)**. Since the present study involved three variables, it was necessary to fix the value of one variable in order to see the effect of two variables on the response.

Table 3. Experimental Data for beer production from combination of finger millet and apple juice

Variables				Responses				
Exp. No.	**Blend ratio of Finger millet: Apple Juice**	**Yeast concentration**	**Slurry ratio of Malted Grain: Water**	**pH**	**Titrable Acidity**	**Colour**	**Bitterness**	**Alcohol content**
1	90:10	6	1:9	5.5	0.35	34.7	17.9	10.5
2	80:20	6	1:9	5.8	0.21	36.7	17	9.4
3	90:10	10	1:9	5.2	0.12*	34.7	18.01	11.2**
4	80:20	10	1:9	5.5	0.13	37.8**	17.7	9.9
5	90:10	8	1:8	5.6	0.26	21.2	17.4	8.9
6	80:20	8	1:8	5.4	0.54	22.8	17.3	8.1
7	90:10	8	1:10	5.1	0.61	19.8	18.4	6.9
8	80:20	8	1:10	4.9	0.45	15.2*	17.5	5.5*
9	85:15	6	1:8	5.4	0.56	22.6	16.5*	8.9
10	85:15	10	1:8	5.1	0.65	15.7	18.5	8.6
11	85:15	6	1:10	5	0.63	16.2	19.8**	7.4
12	85:15	10	1:10	4.6*	0.7	17.2	16.5*	7.1
13	85:15	8	1:9	5.9**	0.78**	33.4	17.5	11.1
14	85:15	8	1:9	5.7	0.69	31.5	17.6	10.2
15	85:15	8	1:9	5.5	0.65	32.5	16.5*	10.1
16	85:15	8	1:9	5.6	0.73	33.6	16.5*	9.6
17	85:15	8	1:9	5.7	0.76	33.6	16.6	9.5
***Minimum value**				****Maximum value**				

Table 4. Results of Regression Analysis of Quality Parameters of Beer

	pH		Titrable Acidity		Colour		Bitterness		Alcohol	
	Coeff.	**P value**	**Coeff.**	**P value**	**Coeff.**	**P value**	**Coeff.**	**P value**	**Coeff.**	**P value**
Cons	5.680	0.0119**	0.7260	0.0020***	32.920	< 0.0001***	16.940	0.0275**	10.10	0.0016***
X_1	0.025	0.6990	-1.250×10^{-3}	0.9681	0.26250	0.6720	- 0.27625	0.1688	- 0.5750	0.0263**
X_2	-0.1625	0.0344**	- 0.018750	0.5533	- 0.60	0.3463	-0.061250	0.7437	0.0750	0.7251
X_3	-0.23750	0.0065***	0.04750	0.1588	- 1.73750	0.0222**	0.31250	0.1262	- 0.950	0.0024***
X_1X_2	-1.279×10^{-16}	1.0	0.03750	0.4078	0.2750	0.7531	0.14750	0.5805	- 0.050	0.8679
X_1X_3	1.4229×10^{-18}	1.0	- 0.110	0.0363**	-1.550	0.1077	- 0.20	0.4579	- 0.150	0.6206
X_2X_3	-0.0250	0.7839	-5.0×10^{-3}	0.9098	1.9750	0.0511*	- 1.3250	0.0012***	-1.96262×10^{-16}	1.0
X_1^2	0.02250	0.80	- 0.34675	< 0.0001***	2.440	0.0205**	0.26875	0.3147	- 0.250	0.4053
X_2^2	-0.20250	0.0497**	- 0.17675	0.0038***	0.6150	0.4772	0.44375	0.1169	0.40	0.1995
X_3^2	-0.45250	0.0011***	0.085750	0.0777*	- 15.610	<0.0001***	0.44125	0.1186	-2.50	<0.0001***
R^2 (%)	**89.03**		**93.70**		**98.22**		**85.68**		**94.06**	
F LOF	**Not Significant**		**Not Significant**		**Not Significant**		**Not Significant**		**Not Significant**	

***, **, * Significant at 1, 5 and 10 % level of significant respectively
Cons = Constant and Coeff.= Coefficient

Table 5. Goals for optimization for independent variables/ dependent variables

Name of Independent/ Dependent variables	Goal	Lower limit	Upper limit
Blend Ratio	In range	-1	+1
Yeast Concentration	In range	-1	+1
Slurry Ratio	In range	-1	+1
pH	In range	4.6	5.9
Titrable Acidity	In range	0.12	0.78
Color	Minimize	15.2	37.8
Bitterness	Maximize	16.5	19.8
Alcohol Content	Maximize	5.5	11.2

Table 6. Optimum values of variables

Value	Blend ratio (X_1)	Yeast concentration (X_2)	Slurry ratio (X_3)
Coded	-0.952	1	-1
Actual	80.24	10	8

3.2.1. Effect of yeast concentration and malt to water ratio on pH of beer

The effect of malt to water ratio and yeast concentration on pH is depicted in figure 1.Response surface indicate that pH decreases as the yeast concentration increases. This may be because yeast concentration significantly affects the rate of fermentation. From table 3 it was also observed thatyeast concentration (X_2) and malt to water ratio (X_3) affects the pH at 1% ($P \leq 0.01$) and 5 % ($P \leq 0.05$) level of significance at both linear and quadratic term. The similar finding was observed by (Khandelwal *et al.,* 2012) who observed that pH decreases as yeast concentration increases during the preparation of blended low alcoholic beverages from under-utilized millets with zero waste processing methods.It was also observed that the pH was significantly affected by slurry ratio. The maximum pH was observed at the center value (1:9) of slurry ratio.

3.2.2. Effect of finger millet to apple juice and yeast concentration on titrable acidity of beer

The effect of finger millet to apple juice ratio and yeast concentrations on titrable acidity as shown in figure 2 it was observed that titrable acidity was significantly affected by the blend ratio. As the volume of apple juice increases and finger millet decreases the titrable acidity was found to be increased significantly.From table 3 it was also observed thatfinger millet to apple juice ratio (X_1) and yeast concentration (X_2) affects the titrable acidity at 1% ($P \leq 0.01$) level of significance at quadratic term. Our finding favor the findings of (Khandelwal *et al.,* 2012) who observed that the titrable acidity increases as the volume of apple juice increases during the preparation of blended low alcoholic beverages from under-utilized millets with zero waste processing methods.

3.2.3. Effect of malt to water ratioand finger millet to apple juice ratio on colorof beer

Figure 3 shows theeffect of malt to water ratio and finger millet to apple juice on color of beer. From figure 3 it was observed that the color was maximum at the center value (1:9) and decreased as the malt to water ratio increased.From table 3 it was also observed thatfinger millet to apple juice ratio (X_1) and malt to water ratio (X_3) affects the color of beer at 5% ($P \leq 0.05$) and 1% ($P \leq 0.01$) level of significance at quadratic term.

3.2.4. Effect offinger millet to apple juice ratio and malt to water ratioonbitterness of beer

Effect of finger millet to apple juice and malt to water ratio on bitterness of beer depicted in figure 4.Response surface shows that bitterness decreases with increase in malt to water ratio and is not significantly affected by the finger millet to apple juice ratio. The increase bitterness is due to the addition of hops. From table 3 it was also observed thatfinger millet to apple juice ratio (X_1), yeast concentration (X_2) and malt to water ratio (X_3) not affects the bitterness of beer at any level of significance but finger millet to apple juice ratio (X_1) and malt to water ratio (X_3) affects bitterness of beer at 1% ($P \leq 0.01$) level of significance at interactive term. The similar finding was observed by (Kumar *et al.*, 2015) who observed that bitterness increases with the increase in slurry ratio and kilning temperature.

3.2.5. Effect ofyeast concentration and finger millet to apple juice ratio on alcohol content of beer

Figure 5 shows the Effect of yeast concentration and finger millet to apple juice ratio on alcohol content of beer. From figure 5it was found that alcohol content increases as yeast concentration increase while finger millet to apple juice ratio not affected significantly. From table 3 it was also observed thatfinger millet to apple juice ratio (X_1) affects the alcohol content at 5% ($P \leq 0.05$) level of significance at liner term, while malt to water ratio (X_3) affects the alcohol content of beer at 1% ($P \leq 0.01$) level of significance at linear and quadratic term. The similar finding was observed by (Amadi and Ifeanacho, 2016)who obsereved that fermentation is affected by subrate volume, mass of yeast and fermentation time.

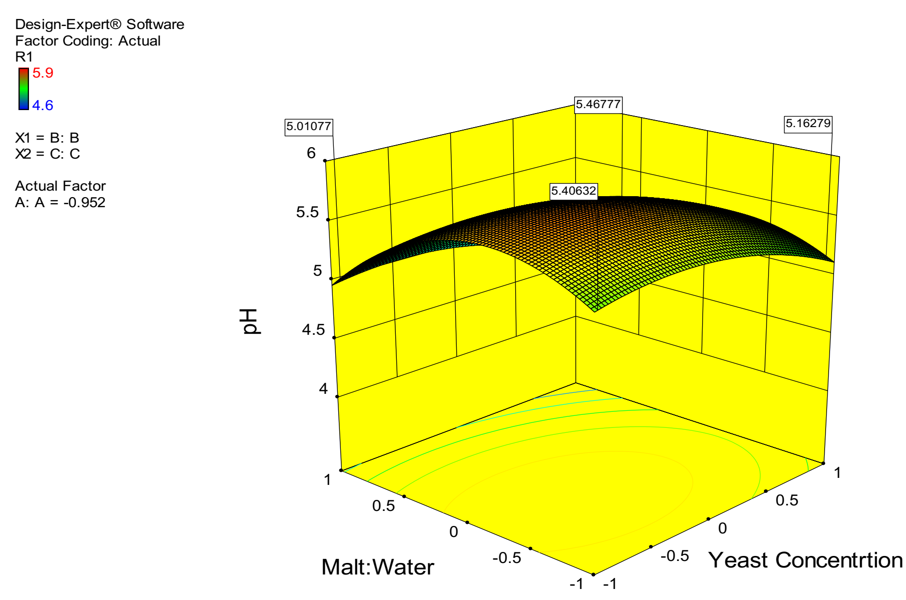

Figure1. 3D Response Surface showing the effect of malt to water ratio and yeast concentration on pH at the optimum value of finger millet to apple juice ratio

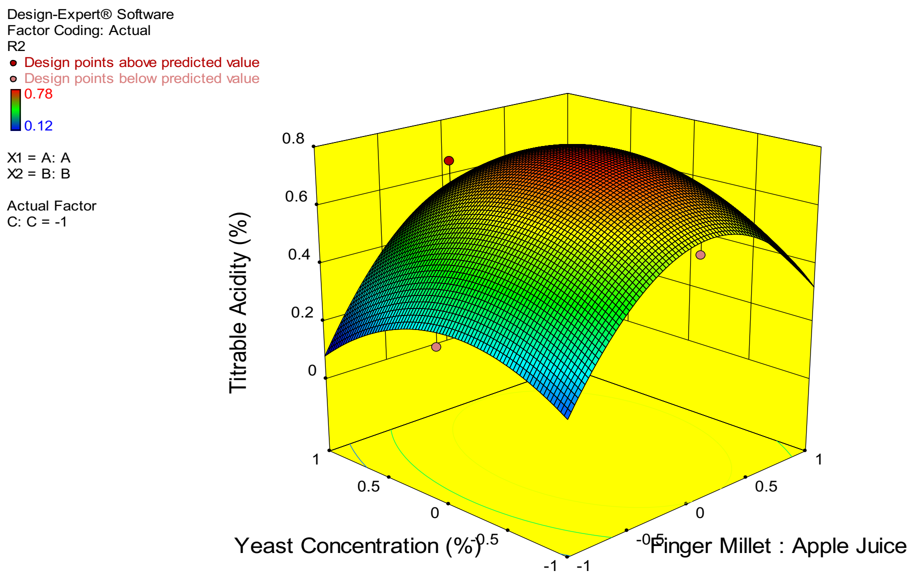

Figure 2. 3D Response Surface showing the effect of finger millet to apple juice ratio and yeast concentrations on titrable acidity at the optimum value of malt to water ratio

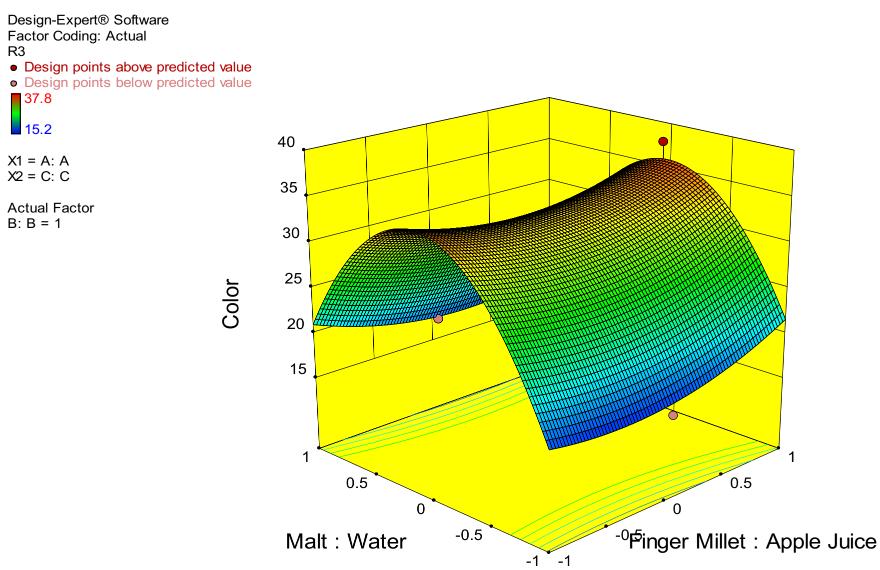

Figure 3.3DResponse Surface showing the malt to water ratio and finger millet to apple juice ratio on color of beer at the optimum value of yeast concentration

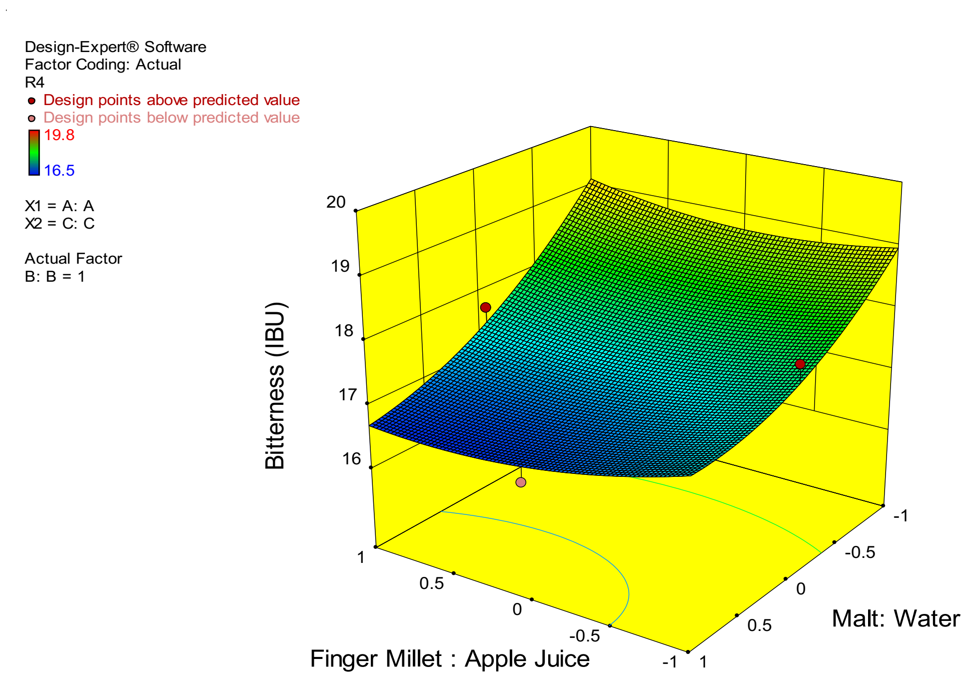

Figure 4. 3D Response Surface showing Effect of finger millet to apple juice and malt to water ratio on bitterness of beer at the optimum value of yeast concentration

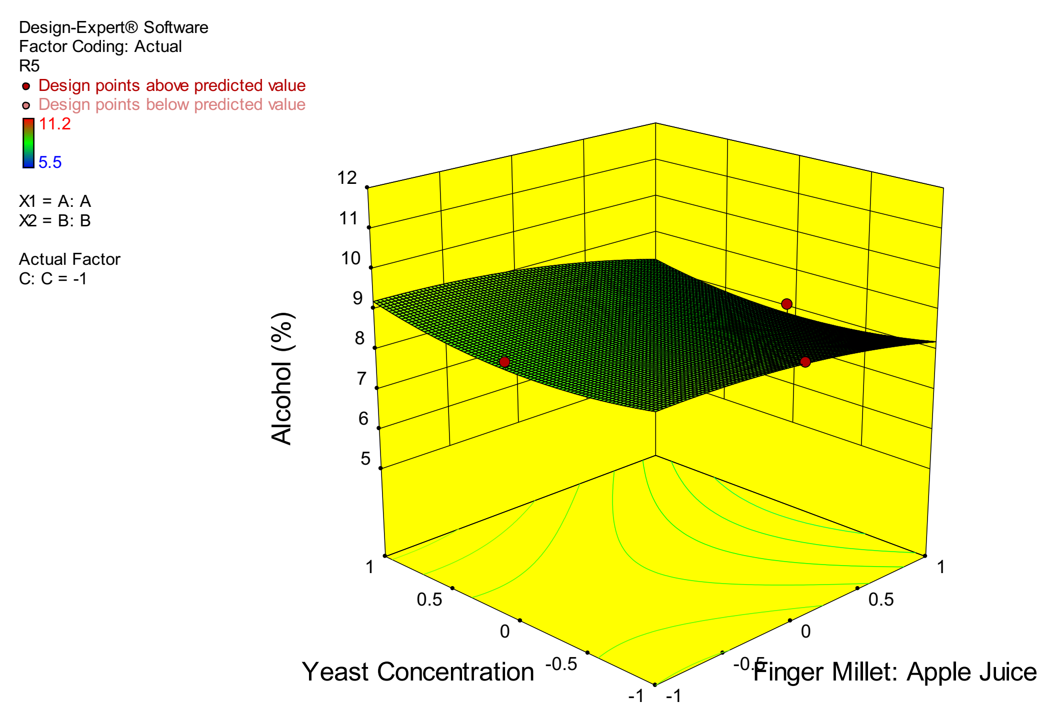

Figure 5.3D Response Surface showing effects of yeast concentration and finger millet to apple juice ratio on alcohol content of beer at the optimum value of malt to water ratio.

4. Conclusions

It could be concluded that the beer could be produced using finger millet and apple juice combination under natural fermenting conditions using 10 % yeast concentration strains as the alcohol % for finger millet to apple juice ratio 90:10 was found to be 11.2%. Optimized values of parameters for beer production were found to be 80.24:19.76 finger millet to apple juice ratio, 10% yeast concentration and 1:8 slurry ratios. The values for pH, titrable acidity, colour, bitterness and alcohol content at optimum conditions were found to be 5.12, 0.12, 17.312, 18.95 and 9.25 respectively.

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