Optimization of substrate ratio for beer production from finger millet and barley

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Abstract: Seventeen designed experiments were conducted in three steps (malting, brewing and fermentation) to produce beer from barley, finger millet and the combination of both. Effects of independent variables with three levels for each i.e. blend ratios of grains (100:0, 50:50, 0:100), kilning temperature (50°C, 70°C, 90°C) and malted grain to water ratios (1:3, 1:5, 1:7) were investigated on product quality. The results of the study indicated that all the independent parameters i.e. blend ratio, kilning temperature and slurry ratio affected the responses (pH, colour, bitterness and alcohol content) significantly. Optimum values of parameters, from the simultaneous optimization done using Design Expert 8.0.6.1 software, for beer production, were found to be 68:32 blend ratio, 50°C kilning temperature and 1:7 slurry ratio. The model *F*-value was found to be highly significant at 1% level of significance for all the responses. All the responses could be predicted by fitting the second order mathematical model and adequacy checked by R^2.

Keywords: beer production, fermentation technology, blend ratio, alcohol content, bitterness, finger millet, barley

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1 Introduction

There are many crops including cereals and legumes which are locally cultivated in Uttarakhand, India in large quantity. Underutilized crops are the lesser known species in terms of trade and research, and often adapted to marginal and stress conditions. With ample nutritional benefits, these crops have the potential to be converted into the form of processed foods. Finger millet, one of the major underutilized crops of Uttarakhand, grows well in tropical countries and contains a good amount of reducing sugars. It can become a substitute for barley in beer production.

Malting characteristics of finger millet are superior to other millets and ranks next to barley malt[1,2]. Malting of finger millet improves its digestibility, sensory and nutritional quality. It also has pronounced effects in lowering anti-nutrients. Barley, rich in protein, carbohydrates, dietary fibers, minerals and vitamins, is the primary cereal used in the production of malt in the world and is the basic raw material for brewing. Its chemical composition, brewing and technological indices are highly determinative for beer quality and the economic efficiency of the brewing process[11].

The use of Indian finger millet in brewing has been investigated[3] but the detailed study related to

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optimization of fermenting[13] and process parameters is still scanty. Since finger millets have potentially useful characteristics with respect to brewing, studies on value addition of underutilized crops using fermentation technology need a radical approach as very little work has been done in India. Research and improvement efforts are needed to explore the potential of finger millet to increase agricultural production, crop diversification and improve nutritional environment. There is a clear indication that product development and value addition to these crops using established technologies can increase their utilization and improve incomes to small scale farmers/small entrepreneurs.

In the present study, an attempt to explore the utilization of underutilized crops using fermentation technology to produce beer has been made. Process parameters i.e. kilning temperature, blend ratio and malted grain to water ratio are responsible for beer quality and also important for beer yield. Variation in kilning temperature and dilution ratio plays an important role in colour, bitterness and alcohol content of beer hence a study needs to be done to optimize these parameters. Hence, the present study was undertaken keeping in view all the above factors with the following specific objectives:

• To develop and standardize a process for beer production using malted finger millet (Ragi) and barley;

• To optimize the process parameters responsible for beer production.

2 Methodology

2.1 Grains

Good quality raw grains i.e. finger millet (*Eleusinecoracana*), barley (*Hordeumvulgare*) of traditional (local) varieties were purchased from the local market of Uttrakhand, India. Hop (*Humuluslupulus*) species were procured from the beer industry. Yeast strain (*Saccharomyces cerevisiae)* was procured from the Department of Microbiology, College of Basic Science and Humanities, Pantnagar. All the chemical and reagents used for the analysis were of analytical grade. Grains were cleaned and washed thoroughly to remove immature grains, light materials and dirt. The production of fermented beverages were carried out in three steps i.e. malting, brewing and fermentation.

2.2 Experimental design

A total of seventeen sets of experiments using Box Behenken design having three factorial points, three levels of each were conducted[4]. The parameters that influence the product quality, acceptability and functionality were taken as responses. Blend ratio (barley: finger millet), kilning temperature, malted grain to water ratio (slurry ratio) were selected as independent variables and pH, colour (EBC), bitterness (IBU) and alcohol content (ABV) were selected as the responses. Each independent variable investigated in this experiment had three levels which were -1, 0, and +1 (Table 1). 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. Contour plots were drawn with the help of SURFER 9.0 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 (Barley: Finger millet)	X_1	100:0	50:50	0:100
Kilning temperature	X_2	50	70	90
Malted grain : water	X_3	1:3	1:5	1:7

Coded values and their levels

X_1	X_2	Runs
±1	±1	4
±1	0	4
0	±1	4
0	0	5

2.3 Experimental procedure

All the experiments were conducted in three steps.

2.3.1 Malting

Barley and finger millet were cleaned and washed thoroughly to remove immature grains, light materials, dirt and were steeped in surplus water at room temperature (28±2°C) for a period of 24 h. The water was changed every 6 to 8 h over a period of 24 h. After soaking, for a period of 24 h, the water was drained off and the grains were left on stainless steel sieves for germination process for a period of 30-36 h. Grains were gently disturbed in order to provide aeration and to prevent from matting. After germination, the germinated grains (green malt) were kilned in Integrated Malting Unit at different temperature (50, 70 and 90°C) for 14-20 h. Kilning was done till the desired moisture content was achieved (3%-5% for barley and 9%±1% for finger millet)[5]. The rootlets and broken fibers of kilned malt were removed by manual rubbing and winnowing. The cleaned malt was stored in air tight container for further experiments.

2.3.2 Brewing

The malt was crushed manually. This breaks down barriers in the grains, giving the enzymes full access to the carbohydrates, present in the grains, and facilitates the efficient extraction of the soluble material (extract) from the malt[6]. To prepare the wort, malted grain and water (1:3, 1:5 and 1:7 slurry ratios) was boiled. Firstly, only the water as per the ratio maintained (210, 350 and 490 mL) was heated at the temperature 68-70°C respectively, and after heating, the malted grain mixed as per the ratio (100:0, 50:50 and 0:100), soaked (70 g of barley/500 mL, 35 g barley +35 g finger millet/500 mL and 70 g of finger millet/ 500 mL). The total mixture was again boiled for 40 min at slow fire. Tap water 500 mL was heated at 68-70°C in another ware and sparging was done with hot water. As soon as the wort started boiling, 1 g of hops were added to enhance the flavor and colour of the final product and the whole mixture was boiled at 100°C for additional 1 h on slow fire. After 1 h of boiling, hops were separated by using strainer and muslin cloth. After hops separation, the wort was cooled to a temperature of 18-20°C which was best for yeast growth during fermentation [7].

2.3.3 Fermentation

When the wort was cooled at the temperature of 18-20°C, the level of wort was less than 500 mL, hence the volume was made up to 500 mL by adding simple tap water and then 30 mL of liquid yeast was transferred in 500 mL wort in Laminar flow chamber. After transferring the yeast, the flask was closed with cotton plug and placed in dark place. After three days, cotton plug was removed and flasks were again plugged using fermentation lock for a period of 14 days. This was done so that CO_2 evolved during fermentation process may pass to another flask within water. After 14 days of fermentation, fermented liquor was centrifuged at 4 000-5 000 r/min for 15 min in order to remove all yeast cells. Supernatant was stored in refrigerator at low temperature. The samples were analyzed immediately after the completion of the fermentation process[8].

2.4 Analytical evaluation

2.4.1 Colour of fermented liquor

Colour was estimated calorimetrically according to literature [9]. Fermented liquor was degased by gently stirring with a magnetic stirrer on low speed. Wavelength was kept 430 nm and absorbance was set 0.000 with distilled water. After setting the absorbance, sample was taken in 10 mm cuvette and absorbance was read. Colour was calculated by the formula given below.

$$\text{Colour} = A \times f \times 25.$$

where, A is absorbance at 430 nm in a 10 mm cuvette; f is dilution factor

2.4.2 pH of fermented liquor

The pH of sample was measured directly by digital pH meter (Triode India). The pH probe was calibrated using standard buffer solution (pH 4 and pH 7) prior to measurement of pH of sample at 30°C.

2.4.3 Bitterness of fermented liquor

Ten milliliters of fermented liquor was taken in a 35 mL centrifugal tube and degassed with the help of magnetic stirrer by stirring gently. Wavelength was kept 275 nm and absorbance was set 0.000 with 2, 2, 4-trimethyl pentane as a reference blank. Twenty milliliter 2, 2, 4-trimethyl pentane and 0.5 mL HCL (6 mol/L) was taken in centrifuge tube and rotated for 15 min in centrifuge. The centrifugation was done until maximum extraction had been achieved. The absorbance of the sample was recorded for centrifuged sample and this was repeatedly done till no change in absorbance was observed. Bitterness was calculated by the formula given below:

$$\text{BU} = A_{275} \times 50$$

where, A is absorbance at 275 nm in a 10 mm cuvette.

2.4.4 Ethanol estimation

Alcohol was estimated calorimetrically according to literature [10]. A standard curve was prepared by using 0.0 to 8.0 mg/mL of absolute alcohol to which 2 mL standardize (0.36 N) ceric ammonium nitrate reagent was added. After 5 min of mixing, the extinction was read at 486 nm on spectrophotometer. A blank was also prepared using 5 mL of distilled water. Calibration curve was plotted taking ethanol concentration on *x*-axis and absorbance on *y*-axis.

Samples (5 mL) collected after distillation in graduated tube were further diluted five times in order to get colour in the range of standard curve. Diluted distillate 5 mL was taken and 2 mL of ceric ammonium nitrate was added; after 5 min, extinction was determined by computing the absorbance against a standard curve of absolute alcohol. The regression analysis of the responses was conducted by fitting to the suitable model represented by the following equation:

$$Y = f(x) = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_{ii} X_i^2 + \sum_{i=1}^{k-1}\beta_{ii}\sum_{j=i+1}^{k}\beta_{ij} X_i X_j \quad (1)$$

where, Y is the response; k is the number of independent variables, X_i (i = 1, 2, 3, 4, 5) is the input predictor or controlling variable: β_0 is the constant coefficient, β_i, β_{ij}, and β_{ii} are the coefficients of linear, interaction and quadratic term, respectively. The coefficient parameters were estimated using a multiple linear regression analysis employing the software Design–Expert (version 8.0.1).

3 Results and discussion

3.1 Effect of independent variables on quality parameters of beer

3.1.1 pH

Maintaining the correct pH for enzyme during the mash ensures the proper conversion of starch and degradation of haze causing protein. The experimental data as tabulated in Table 2 shows the pH levels of end product (beer) for different combination of the experiments conducted. Data in the Table 2 shows that the lowest pH value (5.05) of beer was observed in Experiment No. 8 which had blend ratio 0:100 (X_1=1), kilning temperature 70°C (X_2=0), and 1:7 slurry ratio (X_3=1). The highest pH value (5.63) was observed for the Experiment No. 7 which had blend ratio 100:0 (X_1= -1), kilning temperature 70°C (X_2=0), and 1:7 slurry ratio (X_3=1). These data show that the pH was maintained throughout the entire range of experiments i.e. ranging from 5.05 to 5.63 which shows an acceptable range as brewers normally prefer a pH of 5.2 to 5.5.

Table 2 Experimental data for beer production from barley, finger millet and combination of both

Independent variables				Responses			
Exp. No.	Blend ratio	Kilning temperature/°C	Slurry ratio	pH	Colour (EBC)	Bitterness (IBU)	Alcohol content (ABV)
1	100:0	50	1:5	5.40	27.65	29.50	8.08
2	0:100	50	1:5	5.21	27.00	29.00	6.22
3	100:0	90	1:5	5.60	27.50	30.05	6.75
4	0:100	90	1:5	5.30	27.30	29.80	7.07
5	100:0	70	1:3	5.18	27.50	29.40	12.08**
6	0:100	70	1:3	5.45	28.50**	29.30	9.66
7	100:0	70	1:7	5.63**	27.70	30.50	4.17*
8	0:100	70	1:7	5.05*	27.60	29.35	8.60
9	50:50	50	1:3	5.24	25.60	28.50*	9.70
10	50:50	90	1:3	5.52	27.30	30.50	8.20
11	50:50	50	1:7	5.15	26.80	32.50**	7.50
12	50:50	90	1:7	5.29	25.85	30.50	5.80
13	50:50	70	1:5	5.50	26.40	29.50	4.60
14	50:50	70	1:5	5.45	25.60	29.50	4.80
15	50:50	70	1:5	5.51	25.50	29.60	5.50
16	50:50	70	1:5	5.55	25.40*	28.50*	4.20
17	50:50	70	1:5	5.42	25.50	28.60	4.40

Note: * Minimum value; ** Maximum value.

3.1.2 Colour

Differences in brewing conditions can lead to substantial colour change in final product (beer). The colour of end product (beer) was determined and is reported in Table 2. The least colour value (25.4) of beer was observed in experiment No. 16 which had blend ratio 50:50 (X_1=0), kilning temperature 70°C (X_2=0), and 1:5 slurry ratio (X_3=0). The maximum colour value (28.5) was observed for the experiment No. 6 which had blend ratio 0:100 (X_1= 1), kilning temperature 70°C (X_2=0), and 1:3 slurry ratio (X_3= -1).

The colour values ranged from 25.4 to 28.5 for entire range of experiment. As per the Beer Style SRM Colour Chart, ASBC, it has been reported that the colour for ale type (top fermented beer) comes in the range of 3 to 35. The data for colour in Table 2 indicate that the beer produced under different processing conditions had an acceptable range of colour i.e. 25.4-28.5. The variation in colour of beer may be due to the variation in kilning temperature and addition of hops.

3.1.3 Bitterness

Table 2 shows that the bitterness of the end product varied from 28.50 to 32.50. As per Beer Style International Bittering Unit, ASBC, the acceptable range for bitterness of ale type of beer is reported to be 12 to 40. The values of bitterness come in acceptable range for various set of experiments. The bitterness of end product (beer) is due to addition of hops. The least bitterness value (28.5) of beer was observed for experiment No. 9 and 16 with blend ratio 50:50 (X_1=0), kilning temperature 50°C (X_2=-1), and 1:3 slurry ratio (X_3 = -1) and blend ratio 50:50 (X_1 = 0), kilning temperature 70°C (X_2=0), and 1:5 slurry ratio (X_3=0), respectively. The maximum bitterness value (32.5) was observed for the experiment No. 11 which had blend ratio 50:50 (X_1=0), kilning temperature 50°C (X_2=-1), and 1:7 slurry ratio (X_3=-1).

It could be seen from Table 2 that highest value of bitterness was obtained for experiment No. 11 where the pH for the same experiment is quite low. The reason behind the increase in bitterness could perhaps be due to the fact that lower pH slightly decreases hop utilization and therefore, improves the quality of the bitterness, while higher pH slightly increases hop utilization and the harness of bittering compounds. The hop utilization is temperature dependent and could affect the bitterness of the beer.

3.1.4 Alcohol content

Major consideration for finished product (beer) is the level of alcohol content[12]. This parameter is very important and it helps the brewers to achieve consistence and produce flavor compounds. Beer is traditionally defined as an alcoholic beverage derived from fermented grains. The alcoholic strength of the beverage is determined by the choice and blend of grains, fermentation process, microorganism used and additives used. Table 2 summarizes the experimental data for the various processing conditions. Table 2 shows that the percentage of alcohol varies from 2.3% to 1.5% indicating the presence of alcohol.

The lowest alcohol content (1.5%) of beer was observed in experiment No. 13, 15 and16 which have blend ratio 50:50 (X_1=0), kilning temperature 70°C (X_2= 0), and 1:5 slurry ratio (X_3=0) in all three experiments. The highest alcohol content (2.3%) was observed for the experiment No. 1 which had blend ratio 100:0 (X_1=-1), kilning temperature 50°C (X_2=-1), and 1:3 slurry ratio (X_3 = -1). The highest alcohol content 2.3% was observed for blend ratio (100:0) which shows that barley alone could produce alcohol but the presence of alcohol as 1.5% also proves that if finger millet is mixed with barley (50:50), the alcohol could be produced to some extent. Hence, with full potential for being an important but underutilized source of starch, the finger millet could be used for beer production.

3.2 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.

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 3. The program provided the values of coefficients of model and related statistics in terms of lack of fit and *p*-value. The value 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.

Table 3 Results of regression analysis of quality parameters of beer

	pH		Colour		Bitterness		Alcohol	
	Coeff.	*P* value	Coeff.	*P* value	Coeff.	*P* value	Coeff.	*P* value
Cons	5.49	0.0031	25.68	0.0029	29.14	0.041	1.540	0.09
X_1	-0.100	0.0047***	0.00625	0.9660	-0.25	0.2706	-0.100	0.0198**
X_2	0.089	0.0086***	0.11	0.4527	0.17	0.4460	0.025	0.4769
X_3	-0.034	0.2119	-0.12	0.4290	0.64	0.0178**	0.075	0.0588
X_1X_2	-0.028	0.4546	0.11	0.5915	0.062	0.8386	0.050	0.3233
X_1X_3	-0.21	0.0005***	-0.27	0.2117	-0.26	0.4040	0.000	1.00
X_2X_3	-0.035	0.3473	-0.66	0.0129**	-1.0	0.0117**	-0.200	0.0038***
X_1^2	-0.041	0.2706	1.56	0.0001***	-0.21	0.4947	0.255	0.0009***
X_2^2	-0.068	0.0846	0.12	0.5499	0.65	0.0572	0.355	0.0001***
X_3^2	-0.12	0.0102**	0.59	0.0200**	0.71	0.0443	0.055	26.95
R^2 (%)	92.77%		92.91%		83.66%		95.01%	
F	9.98		10.20		3.98		14.81	
LOF	NS		NS		NS		NS	

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

The probability of significance of predictor's coefficient indicates the extent of effects of predictor on the response. The sign and magnitude of the coefficient explains the nature of the effects. Negative sign at linear level means decrease in response when the level of the predictor is increased while positive sign indicates increase in the response. Significant negative interaction suggests that the level of one of the predictors can be increased while that of other decreases for constant value of the response. Positive interaction means that the response is minimum at centre point and it increases with increase or decrease of both the variables from centre point. Positive coefficient of a quadratic term indicates the minimum response at centre value of the parameter and it increases with increase or decrease in parameter level. Negative coefficient of the quadratic term shows the maximum response at the centre value and it decreases with increase/decrease in parameter level.

3.3 Effects of Independent Variables on pH, colour, bitterness and alcohol content of beer

During the fermentation experiments, each response got affected by independent variables. It was determined by analysis using Design Expert 8.0.6.1. The analysis of variance (ANOVA) for each response is discussed in following sub-heading. Results of regression analysis for dependent parameters are reported in Table 3.

3.3.1 Effects on pH

Full second order mathematical model (Equation (1)) was fitted to the data of pH and experimental conditions using regression analysis to estimate the response of dependent variables and results are given in Table 3. Correlation coefficient *R* measures the goodness of fit of regression model. The coefficient of determination (R^2) of the regression model for pH was 92.77%, which implies that the model could account for 92.77% of data and 7.23% variation is not explained by the model. The R^2 value for the response variable was higher than 0.90 showing that the regression model explained the reaction well. Lack of fit was insignificant; therefore, second order model was adequate in describing pH. The predictive equation is given below.

$$\text{pH}=5.48600-0.100X_1+0.088750X_2-0.033750X_3-0.027500X_1X_2-0.21250X_1X_3-0.035X_2X_3-0.040500X_1^2-0.068X_2^2-0.11800X_3^2 \quad (2)$$

where, X_1, X_2 and X_3 are coded variables for blend ratios, kilning temperature, and slurry ratios respectively. The variance for each factor assessed was partitioned into linear, quadratic and interactive components. It can be seen from Table 3 that independent variables viz. X_1 (blend ratio) and X_2 (kilning temperature) affected pH at linear level. X_1 (blend ratio) and X_3 (slurry ratio) affected pH at interactive level. Slurry ratio (X_3) also affects pH at quadratic level. There were no significant effects of any other terms at any levels. Total effects of pH at linear, quadratic and interactive levels are reported in Table 4. It shows that the total effects were highly significant (P<0.05) at 5% level of significance at quadratic terms. The F-value (9.98) is greater than F-tab value (6.71) which indicates that the model is significant at 1% level of significance. Total effect of individual parameter pH was calculated using the sequential sum of squares, and it is given in the Table 5. It was observed that blending ratios (X_1), slurry ratios (X_3) affected the pH at 1% level of significance and kilning temperature (X_2) affected the pH at 5% level of significance. Lack of fit was insignificant; therefore, second order model was found to be adequate in describing pH value. The ANOVA conducted for pH value has an adequate precision; a measure of signal to noise ratio (11.728) indicates a better precision and reliability of the experiment carried out. A ratio greater than 4 is desirable. Hence, in the present study the ratio of 11.2728 indicates an adequate signal to use the model for prediction purposes.

Table 4 ANOVA for pH value

Source	*DF*	*SS*	*MS*	*F*-value	*F*-tab
Model	9	0.43	0.048	9.98***	6.71
Linear	3	0.1521	0.0507	1.4912	-
Quadratic	3	0.1781	0.0593	5.2389**	4.34
Interactive	3	0.0849	0.0283	2.4972	-
Error	7	0.034	0.0048		
Total	16	0.449143			

Note: ***, **, Significant at 1% and 5 % level of significance, respectively.

Table 5 Total effects of individual parameters on pH

Source	*DF*	*SS*	*MS*	*F*-value	*F*-tab
Model	9	0.43	0.048	9.98***	6.71
Blending ratios(X_1)	4	0.186	0.0465	9.68***	7.84
Kilning temperature (X_2)	4	0.08992	0.02248	4.68**	4.12
Slurry ratios (X_3)	4	0.2530	0.06325	13.17***	7.84
Error	7	0.034	0.0048		
Total	16	0.5629			

Note: ***, **, Significant at 1% and 5 % level of significance respectively.

3.3.2 Effects on colour

Full second order model Equation (1) was calculated with the help of statistical package Design expert 8.0.6.1 to assess the response of independent variables. Results are tabulated in Table 3. The coefficient of determination (R^2) of the regression model for colour was 92.91%, which implies that the model could account for 92.91% of data. Lack of fit was insignificant; therefore, second order model was adequate in describing colour. The predictive equation is given below:

$$\text{Colour}=25.6800+0.00625X_1+0.11250X_2-0.11875X_3+0.11250X_1X_2-0.27500X_1X_3-0.66250X_2X_3+1.56000X_1^2+0.12250X_2^2+0.58500X_3^2 \quad (3)$$

where, X_1, X_2 and X_3 are coded variables for blend ratios, kilning temperature, and slurry ratios respectively. It can be seen from Table 3 that independent variables viz. X_2 (kilning temperature) and X_3 (slurry ratio) affected colour at interactive level and X_3 (slurry ratio) affected colour at quadratic level. There were no significant effects of any other terms at any levels. Total effect of colour at linear, quadratic and interactive levels is reported in Table 6. It shows that the total effect was highly significant (P<0.01) at both quadratic and interactive terms. As the F-value (10.20) was observed to be greater than F-tab value (6.71). Model was found highly significant (P<0.01).

Table 6 ANOVA for colour

Source	*DF*	*SS*	*MS*	*F*-value	*F*-tab
Model	9	14.70	1.63	10.20***	6.71
Liner	3	0.2103	0.0701	0.4381	-
Quadratic	3	2.111	0.7036	4.3979***	8.45
Interactive	3	11.753	3.9176	24.4854***	8.45
Error	7	1.12	0.16		
Total	16	15.1943			

Note: *** Significant at 1% level of significance.

The total effect of individual parameter on colour was calculated using the sequential sum of squares, and it is given in Table 7.

Table 7 Total effects of individual parameters on colour

Source	*DF*	*SS*	*MS*	*F*-Value	*F*-tab
Model	9	14.70	1.63	10.20***	6.71
Blending ratios(X_1)	4	1.4062	0.351	2.1937	-
Kilning temperature (X_2)	4	-0.32	-0.08	-0.5	-
Slurry ratios(X_3)	4	-0.46	-0.115	-0.718	-
Error	7	1.12	0.16		
Total	16	0.316			

Note: *** Significant at 1% level of significance.

3.3.3 Effects on bitterness

Table 3 shows the regression analysis data of bitterness response. Full second order model, Equation (1) was fitted to the data of bitterness and experimental conditions. The coefficient of determination (R^2) of the regression model for bitterness was 83.66%, which implies that the model could account for 83.66% of data. Lack of fit was insignificant; therefore, second order model was adequate in describing bitterness. The predictive equation for estimating bitterness is given below

$$\text{Bitterness}=29.1400-0.2500X_1+0.16875X_2+0.64375X_3+0.062500X_1X_2-0.26250X_1X_3-1.0X_2X_3-0.20750X_1^2+0.6550X_2^2+0.70500X_3^2 \quad (4)$$

where, X_1, X_2 and X_3 are coded variables for blend ratios, kilning temperature and slurry ratios, respectively. It can be seen from Table 3 that only X_3 (slurry ratio) affects bitterness at linear level; X_2 (kilning temperature) and X_3 (slurry ratio) affect bitterness at interactive level; and X_3 (slurry ratio) affects bitterness at quadratic level. Total effects of bitterness at linear, quadratic and interactive levels are reported in Table 8. It shows that the total effects were significant (P<0.1) at 10% level of significance at all linear, quadratic and interactive terms. The F-value (3.98) is greater than F-tab value (3.67) indicating that the model was significant (P<0.05).

Table 8 ANOVA for bitterness

Source	DF	SS	MS	F-value	F-tab
Model	9	12.52	1.39	3.98**	3.67
Liner	3	4.050	1.35	3.857*	3.07
Quadratic	3	4.296	1.432	4.091*	3.07
Interactive	3	4.08	1.36	3.885*	3.07
Error	7	2.45	0.35		
Total	16	14.876			

Note: **,* Significant at 5% and 10 % level of significance, respectively.

3.3.4 Effects on alcohol content

Full second order model for the response alcohol content was fitted in Equation (1) using regression analysis and results are given in Table 3. The coefficient of determination (R^2) of the regression model for alcohol content was 95.01%, which implies that the model could account for 95.01% of data and 4.99% variation was not explained by the model. Lack of fit was insignificant; therefore, second order model was adequate in describing alcohol content. The predictive equation is given below

$$\text{Alcohol}=1.5400-0.100X_1+0.025000X_2+0.07500X_3+0.0500X_1X_2-2.7\times10^{-17}X_1X_3-0.2000X_2X_3+0.2550X_1^2+0.3550X_2^2+0.05500X_3^2 \quad (5)$$

where, X_1, X_2 and X_3 are coded variables for blend ratios, kilning temperature, and slurry ratios, respectively. It can be seen from Table 3 that independent variables viz. X_1 (blend ratio) affected alcohol content at linear and quadratic levels; X_2 (kilning temperature) affected alcohol content at quadratic level; and X_2 (kilning temperature) and X_3 (slurry ratio) affected alcohol content at interactive level. There were no significant effects of any other terms at any level. Total effects of alcohol content at linear, quadratic and interactive levels are reported in Table 9. It shows that the total effects were highly significant (P<0.01) at quadratic level. The F-value (14.81) is greater than F-table value (6.71) suggesting that the model was significant (P< 0.01).

Table 9 ANOVA for alcohol content

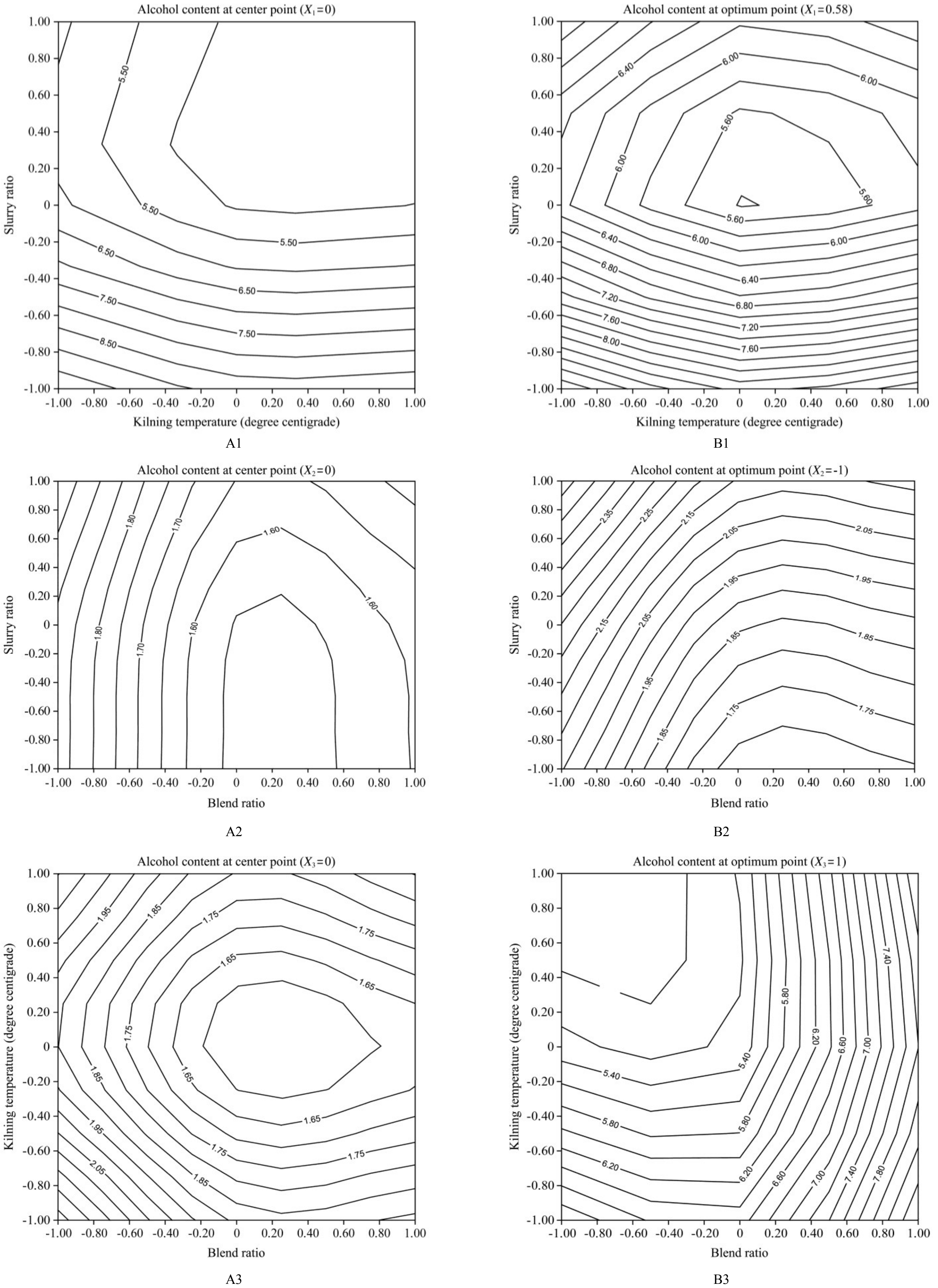
Source	DF	SS	MS	F-value	F-tab
Model	9	1.18	0.13	14.81***	6.71
Liner	3	0.85	0.2833	31.985***	8.45
Quadratic	3	0.17	0.0567	6.4017***	8.45
Interactive	3	0.813	0.271	30.59***	8.45
Error	7	0.062	0.0089		
Total	16	1.242			

Note: *** Significant at 1% level of significance.

The optimized values obtained for the selected independent variables for beer production are reported at blend ratio of finger millet: barley (68:32), kilning temperature at 50°C and slurry ratio at 1:7 (malt grain to water)

3.4 Graphical optimization of process parameters for beer production

Graphical optimization of processing conditions was carried out in order to show the effects of variables and to determine the operating range for best result. Contour plots were drawn and shown in Figure 1.



Note: A_1-A_3 -- At centre point; B_1-B_3 -- At optimum point.

Figure 1 Contour plots for alcohol

3.4.1 pH

The minimum change in pH at the optimum conditions was found to be 5.03. It was observed that pH was found to be highest at kilning temperature (70°C) and slurry ratio (1:7). As kilning temperature increases, pH was found to be increased; pH also increased with increasing in slurry ratio. As the kilning temperature increased, the pH also increased; with the increase in slurry ratio, pH decreased accordingly.

3.4.2 Colour

The minimum change in colour at the optimum conditions was found at 27.12. It was observed that colour value was highest at kilning temperature (70°C), and slurry ratio (1:3). The colour increased with increasing kilning temperature and slurry ratio, but decreased with increasing blend ratio.

Colour of the beer is important as it indicates the colouring potential in the end product. Differences in brewing conditions can lead to substantial colour change in final product (beer). As per the Beer Style SRM Colour Chart, ASBC, it has been reported that the colour for ale type (top fermented beer) comes in the range of 3 to 35. The data for colour in Table 2 indicate that the beer produced under different processing conditions has an acceptable range of colour i.e. 25.4-28.5. The variation in colour of beer may be due to the variation in kilning temperature and addition of hops.

3.4.3 Bitterness

The maximum change in bitterness at the optimum conditions was found to be 31.53. Contour plots were drawn in order to show the effect of variables and to determine the operating range for best results. Bitterness was found to be increased with increase in kilning temperature and slurry ratio. Contour plots also show that as the slurry ratio increased, the bitterness increased but not significant affected by blend ratio. Bitterness also decreased slightly with increasing kilning temperature and blend ratio.

3.4.4 Alcohol content

Figure 1 (A_1 and B_1) depict the effects of kilning temperature and slurry ratio on alcohol at centre and optimum point, respectively. It was observed that as kilning temperature and slurry ratio increased there was decrease in alcohol content. From Figure 1 (A_2 and B_2), it was observed that as the blend ratio and slurry ratio increased, the alcohol content was found to be decreased. Figure 1 (A_3 and B_3) show the effects of blend ratio and kilning temperature on alcohol content. From Figure 1 (A_3), it is predicted that alcohol content was decreased as blend ratio and kilning temperature increased. Figure 1 (B_3) shows as blend ratio increased alcohol content also increased but decreased as kilning temperature increased. Alcohol content was highly affected by blend ratio and kilning temperature as shown in Figure 1 ($A_3$and B_3).

4 Conclusions

It could be concluded that the beer could be produced using finger millet under natural fermenting conditions using *Saccharomyces cerevisiae* strains as the alcohol production at blend ratio 0:100 was found to be 9.66%. The process of beer production using underutilized crop, especially, finger millet is quite valuable as finger millet is a rich source of carbohydrate.

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