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Finger Millet: A Potential Source For Production of Gluten Free Beer

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ABSTRACT
Studies related to value addition of underutilized crops using fermentation technology need a radical approach. Present study has been made to explore the underutilized crops utilization for production of beer.
Designed experiments were conducted to find the effect of three independent variables having three levels of each i.e. (blend ratio-80:20:0,80:10:10, 80:0:20 , α-amylase enzyme concentration - 0%, 0.4%, and 0.8%) and slurry ratio -1:5, 1:7 and 1:9) on pH, colour and alcohol content of beer prepared from finger millet, barnyard millet and paddy. The data from all experiments were analyzed statistically using Design Expert 8.0.7.1 and the response functions were developed using the regression analysis.
Result of fermented studies reveals that blend ratio had maximum effect on alcohol content,pH and colour but enzyme concentration had maximum effect on alcohol content while slurry ratio affected the colour of beer. Statistical analysis resulted in the optimum conditions of the independent variables as blend ratio (80):(9.6):(10.4), enzyme concentration 0.45% and slurry ratio 1:6.82 for maximum beer production.
The second order model was found to be fitted to predict all the responses i.e., pH, colour and alcohol content.
Key words: Underutilized crops, millets, Fermentation technology, beer, alcohol content, pH , colour.

I. INTRODUCTION

Barley is the most suitable cereal for production of beer owing to its chemical, physical characteristics and its optimal content of amylolytic enzymes. Due to the presence of gluten proteins, celiacs can not drink most beers **[1].** Finger millet and Barnyard millet, grown in Uttarakhand state, contain good amount of reducing sugars and free from gluten, can become a substitute for barley in beer production.
Production of these crops is underutilized crops is around 821076 MT from approximately 522486 ha area (Directorate of Agriculture, UK 2010-11) **[3].** Out of total production approximate 10-12% goes waste because of inappropriate processing technologies. The use of Indian finger millet in brewing has been investigated by **[9].** It has potential useful characteristics with respect to brewing. Using the fermentation technology, a gluten free beer from fingermillet, barnyard millet could be prepared.

The present study focuses on production of fermented beverage (beer) using fingermillet and barnyardmillet incorporated with paddy. The combination of ragi, barnyard millet and rice malt is found to be ideal and hence could be used for preparation of beverages. It was found that beer could be prepared using finger millet and barley in combinations using the yeast isolates (*Saccharomyces cerevisiae*) **[5].** Commercial amylolytic enzyme is used for increasing the fermentable carbohydrates and increasing the viscosity, leading to higher filtration rates and yield **[4].** The main purpose of enzyme (α-amylase) addition is to increase the free α- amino nitrogen contents into the wort and to enhance the efficiency of beer production. Parameters like pH, colour and alcohol content are need to be considered to enhance the efficiency of beer production.

Keeping this in view, present investigation has been planned to optimize the blend ratio (fingermillet:barnyard millet :paddy) slurry ratio and enzyme concentration and to see their effect on pH, colour and alcohol content of beer.

II. MATERIALS AND METHODS

The raw grains i.e. Finger millet (*Eleusine coracana*), Barnyard millet (*Echinochloa frumentacea*) and Paddy (*Oryza sativa*) Sarjoo 52 of traditional varieties, , were purchased from the local market of Haldwani and Pantnagar, Uttarakhand. To impart a bitter and tangy flavour to beer, hops were procured from the Beer industry located in Ghaziabad. Raw grains were cleaned manually and kept in moisture proof plastic containers prior to use.

Selection of variables: The final **e**xperiments were designed by considering the constant, parameters as reported in Table 1.

The independent variables were selected as blend ratio (80:20:0, 80:10:10, 80:0:20 for fingermillet,

barnyardmillet and paddy respectively), α-amylase.concentration (0%, 0.4%, 0.8%) and slurry ratio (1:5, 1:7, 1:9). The responses considered during the study were as pH, colour and alcohol content.

Table 1 Constant variables taken for experiment

Parameters	Range
For steeping process	Soaking time, hrs.
Finger millet	24
Barnyard millet	24
Paddy	48
For germination process	Time, hrs.
Finger millet	36-48
Barnyard millet	48
Paddy	72-96
For kilning process	Temp, °C, Time, hrs.
Finger millet	50, 12
Barnyard millet	65, 7
Paddy	70, 7
For brewing process	
Wort boiling and hopping	
Time, hrs.	1-1.30
Temperature, °C	100
Hops separation and wort cooling	
Time, min.	80
Temperature, °C	18-20
For fermentation	
Time, days	8-14
Fermentation Temperature, °C	18-24
Inoculum Size, %	5
Yeast	*Saccharomyces cerevisiae*
Carbonation	
Pressure,	10psi
Temperature, °C	25
Pasteurisation	
Temperature, °C	65-70
Time, minute	15

To find out all possible combinations of parameters, experiments were designed using Box Benkhen methodology. List of independent variables along with range/levels is reported in Table 2 as given below-

Table 2 Independent variables (coded and actual values)

S.N	Variables	Code	Coded Levels		
			-1	0	1
			Actual Levels		
1.	Blend ratios (FM:BM:PADDY)	X_1	80:20:0	80:10:10	80:0:20
2.	Enzyme concentration (α-amylase)	X_2	0%	0.4%	0.8%
3.	Malted grains : water	X_3	1:5	1:7	1:9

pH of wort was measured directly by digital pH meter (Triode India). Color was determined calorimetrically according to method given by **[2]** and alcohol content was calculated directly by the difference of specific gravity of wort and beer **[7].** Total experiments were carried out in three phases –Malting, brewing and fermentation. During malting, malt of grains was obtained which was kept for further studies. During brewing, wort was prepared by boiling the slurry of malted grain and water. After preparation of wort , it was kept for fermentation for 14 days by adding 30 ml of liquid yeast. After 14 days of fermentation, fermented liquor was centrifuged at 4000-5000 rpm for 15 minutes in order to remove all yeast cells, Supernatants was stored in refrigerator at low temperature for further analysis. Maturation of samples was done at 7-8°C for 2-3 weeks followed by filtration and carbonation. Carbonated beer was filled into the precleaned sterilized bottles and then pasteurization was done at 70°C for 15 minutes. (Plate 1).

Plate 1 Pant Beer

III. RESULTS AND DISCUSSION

The responses (pH, colour, alcohol content) were determined for different combinations of the experiments. Results of experiments have been reported in Table 3. Following form of full second order mathematical model was fitted into each response.

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

Effect of Independent Variables on Various Responses

1) pH of wort

The least pH (3.98) of beer was observed in experiment No. 16 which had blend ratio 80:10:10, enzyme concentration 0.4% and slurry ratio 1:7. The maximum pH (4.73) was observed in the Experiment No. 8 which had the blend ratio 80:0:20, enzyme concentration 0.4% and slurry ratio 1:9 (Table 3).

These data shows that the pH was maintained throughout the entire range of experiments i.e. ranging from 3.98 to 4.73 which shows the acceptable range of pH for beer. The lower pH of beer in the range (3.8-4.7) increases the shelf life of beer because it provides the unfavorable medium for the growth of beer spoilage microorganisms **[6].**

Full second order equation was fitted to responses observed for levels of pH and various experimental conditions using multiple regression analysis .Results obtained are given in Table 4. The coefficient of determination (R^2) for the regression model for this parameter was 0.99 implying that the model could account for 99% data. Model was highly significant ($p < 0.01$) with F-value of 63.72. Therefore, second order model was found to be adequate in describing change in pH .

2) Colour of beer

The least color value (23) of beer was observed in Experiment No.16 which had the bled ratio 80:10:10, enzyme concentration 0.4% and slurry ratio 1:7. The maximum color value (26) was observed in the Experiment No. 8 which had the blend ratio 80:0:20, enzyme concentration 0.4% and slurry ratio 1:9.

The color values ranged from 23 to 26 for entire range of experiment. As per the Beer Style SRM color chart, ASBC, **[8].** It has been reported that the color for ale type (top fermented beer) comes in the range of 3 to 35. The data for color in Table 3 indicates that the beer produced under different processing conditions had an acceptable range of color i.e. 23-26. The variation in color of beer might be due to the variation in blend ratio, slurry ratio and addition of hops. The similar result of beer color was found in the range of (25.4-28.5) by **[5]** when he worked on in vitro synthesis of beer by optimizing fermenting parameters and blend ratios of barley and finger millet.

Full second order equation was fitted to responses observed for levels of colour content and various experimental conditions using multiple regression analysis . (Table 4).The coefficient of determination (R^2) for the regression model for this parameter was 0.96 implying that the model could account for 96% data. Model was highly significant ($p<0.03$) with F-value of 20.15. Therefore, second order model was found to be adequate in describing change in colour.

Table 3 Experimental data for beer production from finger millet, barnyard millet and paddy

Variables				Responses		
Exp. No.	**Blend ratio (X_1)**	**Enzyme conce.(%) (X_2)**	**Slurry ratio (X_3)**	**pH**	**Colour**	**Alcohol content (%ABV)**
1	80:20:0	0	1:7	4.69	25.7	4.82
2	80:0:20	0	1:7	4.71	25.9	4.6
3	80:20:0	0.8	1:7	4.59	25.5	5.5
4	80:0:20	0.8	1:7	4.62	25.6	5.38
5	80:20:0	0.4	1:5	4.5	25.2	6.18
6	80:0:20	0.4	1:5	4.65	25.6	5.1
7	80:20:0	0.4	1:9	4.53	25.5	6
8	80:0:20	0.4	1:9	4.73**	26**	4.45*
9	80:10:10	0	1:5	4.35	24.7	7.69
10	80:10:10	0.8	1:5	4.21	24.5	8.05
11	80:10:10	0	1:9	4.4	24.8	7.36
12	80:10:10	0.8	1:9	4.33	24	7.82
13	80:10:10	0.4	1:7	4.03	23.4	10.3
14	80:10:10	0.4	1:7	4	23.1	10.47
15	80:10:10	0.4	1:7	4.03	23.5	10.2
16	80:10:10	0.4	1:7	3.98*	23*	11.55**
17	80:10:10	0.4	1:7	4.05	23.8	9.86

Table 4 Results of regression analysis of quality parameters of beer

Source	pH		Color		Alcohol content	
	Coeff.	**P (%)**	**Coeff.**	**P (%)**	**Coeff.**	**P (%)**
Model	4.02	0.01***	23.36	0.03***	10.48	0.01***
X_1	0.050	1.80**	0.15	20.32	-0.37	11.32
X_2	-0.050	1.80**	-0.19	12.28	0.29	20.73
X_3	0.035	6.85*	0.038	73.60	-0.17	42.49
X_1X_2	0.0025	91.65	-0.025	87.33	0.025	93.37
X_1X_3	0.012	60.39	0.025	87.33	-0.12	69.75
X_2X_3	0.017	47.18	-0.15	35.40	0.025	93.37
X_1^2	0.46	0.01***	1.69	0.01***	-3.85	0.01***
X_2^2	0.18	0.01***	0.62	0.4***	-1.55	0.09***
X_3^2	0.13	0.08***	0.52	0.96***	-1.19	0.39***
R^2	**0.99**		**0.96**		**0.97**	
Adj R^2	**0.97**		**0.92**		**0.94**	
F-value	**63.72*** **		**20.15 *** **		**28.69*** **	
LOF	**NS**		**NS**		**NS**	

3) Alcohol content of beer

Alcohol % varied from 4.45 to 11.55 indicating the presence of alcohol. These findings are in agreement with (Kumar, 2013) who also reported 4.2 to 12.8 % alcohol under various processing conditions when finger miller and barley were used as substrate. The least alcohol content (4.45) of beer was observed in Experiment No. 8 which had the blend ratio 80:0:20, enzyme concentration 0.4% and slurry ratio 1:9. (Table 3) The maximum alcohol content (11.55) was observed for the Experiment No. 16 which had the blend ratio 80:10:10, enzyme concentration 0.4% and slurry ratio 1:7. (Table 3) The maximum alcohol content 11.55% observed for blend ratio (80:10:10) Full second order equation was fitted to responses observed for levels of alcohol content and various experimental conditions using multiple regression analysis . (Table 4).

The coefficient of determination (R^2) for the regression model for this parameter was 0.97 implying that the model could account for 97% data. Model was highly significant ($p <0.01$) with F-value of 28.69. Therefore, second order model was found to be adequate in describing change in alcohol.

Multiple regression equation generated to predict the pH, colour and alcohol content affected by different factors in terms of actual and quadratic factors is depicted in Table 5.

Table 5 Predictive equations of second order model

S.No.	Parameters	Actual equations	Quadratic
1.	pH	$pH = 4.02 + 0.050X_1 - 0.050X_2 + 0.035X_3 + 0.0025X_1X_2 + 0.012X_1X_3 + 0.017X_2X_3 + 0.46X_1^2 + 0.18X_2^2 + 0.13X_3^3$	$pH = 4.02 + 0.050X_1 - 0.050X_2 + 0.035X_3 + 0.46X_1^2 + 0.18X_2^2 + 0.13X_3^3$
2.	Colour	$Colour = 23.36 + 0.15X_1 - 0.19X_2 + 0.038X_3 - 0.025X_1X_2 + 0.025X_1X_3 - 0.15X_2X_3 + 1.69X_1^2 + 0.62X_2^2 + 0.52X_3^2$	$Colour = 23.36 + 1.69X_1^2 + 0.62X_2^2 + 0.52X_3^2$
3.	Alcohol content	$Alcohol\ content = 10.48 - 0.37X_1 + 0.29X_2 - 0.17X_3 + 0.025X_1X_2 - 0.12X_1X_3 + 0.025X_2X_3 - 3.85X_1^2 - 1.55X_2^2 - 1.19X_3^2$	$Alcohol\ content = 10.48 - 3.85X_1^2 - 1.55X_2^2 - 1.19X_3^2$

X_1, X_2 and X_3 are coded variables for blend ratios α-amylase enzyme concentration and slurry ratios respectively.

Effect of independent variables on pH , colour and alcohol content at linear, quadratic and interactive level was revealed using ANOVA. The results showed that the effect was highly significant ($p < 0.01$) at linear level. The model F-value was also found to be highly significant at 1% level of significance.

Optimization of Process Parameters

The optimum levels of variables for blend ratio, enzyme conc. and slurry ratio in coded and actual form are -0.04, 0.12, -0.09 and 80:9.6:10.4 (56+6.72+7.28= 70 gm), 0.45% (0.7x0.45= 0.315 gm), 1:6.82 (70x6.82= 477.4 ml) respectively.

IV. CONCLUSION

Experimental studies followed by statistical analysis concluded that the effect of blend ratio was found to be most significant on all responses considered during the study. The enzyme concentration and slurry ratio also affected the pH, colour and alcohol content of beer. Optimum value of parameters for beer production were found to be 80:9.6:10.4 (56+ 6.72+7.28 =70 gm) blend ratio, 0.45% (0.7× 0.45=0.315 gm) enzyme concentration and 1:6.82 (70×6.82 = 477.4ml) slurry ratio. The values for pH, colour and alcohol content at optimum condition were found to 4.0109, 23.346 and 10.501% respectively. Beer having 10.5% alcohol could be produced using 56 gm finger millet, 6.72 gm barnyard millet and 7.28 gm paddy of 70 gm malt using 0.45% concentration (0.315gm/70gm of malt) of α-amylase enzyme and 1:6.28 (477.4 ml/70gm of malt) slurry ratio.

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