Chapter 5: Laboratory scale optimization of rice beer making process and sensory evaluation of the products

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5.1 Introduction

Optimization of fermentation conditions has been used to substantially enhance yield and productivity of many bioprocesses. Statistical optimization methods can take into account the interaction of variables in generating the process response. Factorial design of optimization is suitable and efficient to account for the interactions among the various factors [1]. Response surface methodology (RSM) is a multivariate equation solving technique which uses a collection of mathematical and statistical methods to evaluate relationships between a group of quantitative independent variables and one or more responses. The RSM enables to evaluate operation variables that may or may not have significant effect in the main response and helps in optimizing a set of operational variables of the process [2-4]. One of the most popular RSM designs is the central composite design (CCD) which is applied to estimate the coefficients of a particular model equation. In this design, a minimum number of experiments are suggested and at the same time provides sufficient information about the effects of variables and overall experimental error [5,6].

Instead of using the conventional ways to quantify and analyze sensory responses, an alternative could be introduced which applies fuzzy sets instead of average scores to compare the various sensory attributes of foods [7]. Since human expressions on sensory feelings for foods are fuzzy rather than deterministic, the fuzzy sets have a merit in sensory evaluation as they are not confined to a deterministic value [8]. The uncertainty of humans' expression is resolved by the mathematical methods provided by the fuzzy sets. The fuzzy linguistic approach assesses the variables in the problem by using of linguistic terms instead of numerical values [9] and a subject can be represented by fuzzy sets with a series of elements and their membership degrees compared to crisp sets without membership [8]. The concept of the membership given to each element makes it possible to represent fuzzy states with linguistic variables like ''very sweet'' rather than a using a particular preference score. The main advantage of fuzzy logic over other statistical technique is that the fuzzy systems separate the space into several rule areas whose partial shapes are determined by membership functions and rule output [10]. Fuzzy logic approach is an important tool for dealing with the uncertain and vague data obtained in linguistic form. Certain conclusions regarding the acceptance, rejection, ranking and the strong and weak quality attributes of the tested food

could be obtained using this approach [11,12]. Thus, fuzzy logic enables us to quantify linguistic term of expert's opinion. The different kinds of linguistic variables or taste indices used to evaluate beer taste could be synthesized wholly and systematically with the help of mathematical statistics technology [13]. The study of Liu et al. [14] indicated that fuzzy mathematics comprehensive evaluation (FCE) method could be used for beer sensory evaluation as an objective method for breweries to produce beers compatible with consumer preference.

Aspergillus oryzae is a filamentous fungus, which has an ability to secrete large amounts of hydrolytic enzymes such as neutral and alkaline protease, amylase, glutaminase, and metallopeptidase [15]. It is widely used in preparing traditional fermented products in Asia. Yeasts are the most commonly used microorganisms for ethanol fermentation and *Saccharomyces cerevisiae* is one of the well-known ethanol producers [16]. *Lactobacillus plantarum* is a lactic acid bacteria (LAB) frequently occurring in fermented foods of plant origin. There is a high prevalence of *L. plantarum* in the human intestinal mucosa and it possesses probiotic properties [17]. In this chapter, these three microorganisms have been used as the mixed consortium to carry fermentation, and an experiment was designed using RSM in order to optimize conditions for fermentation of rice. In addition, these optimized conditions were further applied to prepare beer from plantain and cassava and some important biochemical properties of the beers were also studied. The sensory characteristics of the five different types of beer prepared using the three substrates were also studied by application of fuzzy logic.

5.2 Materials and methods

5.2.1 Raw materials

Rice (*Oryza sativa* var. *Mahsuri*) and cassava (*Manihot esculenta* var. *Sri Bijoya*) were procured form Assam Agricultural University, Assam (26°43'N; 94°11' E). Plantains (*Musa* ABB) were collected from the experimental plot of Tezpur University, Assam (26°42'N; 92°49'E). The plantains and cassava tubers were washed on running tap water to remove dirt etc. Both the substrates were peeled manually with the aid of stainless kitchen knife and kept in a bowl containing water, and allowed until the peeling process was completed to prevent browning of the resultant chips. Slicing was done longitudinally to

about 5 mm thickness using a mechanical slicer and dried in a tray dryer (IK-112, IKON Instruments, India) at 50 °C for about 6 h. All the three types of raw materials are shown in Fig. 1.1.

Fig. 5.1 The three basic substrates used for preparation of beer

5.2.2 Microbial inoculums

The microbial strains used for fermentation were *Aspergillus oryzae* (ATCC 10124), *Saccharomyces cerevisiae* (ATCC 9763) and *Lactobacillus plantarum* (ATCC 8014). They were obtained from the Department of Food Engineering and Technology, Tezpur University, Assam. The bacterium and yeast were grown on MRS broth and yeast mould broth for 48 h and the mould was grown on potato dextrose broth for 4 days, followed by centrifugation (8000 rpm, 10 min) and the pelleted cells were suspended in 0.89 % NaCl solution, such that the count of *L. plantarum* in the suspension was 6.83 log CFU ml^{-1}, that of *S. cerevisae* was 7.90 log CFU ml^{-1} and that of *A. niger* was 7.61 log CFU ml^{-1}. The amylolytic mould *A. oryzae* was used in order to carry out saccharification of the starches present in the substrates and the yeast *S. cerevisae* was responsible for alcoholic fermentation of the sugars produced during the saccharification process. *L. plantarum* was used in order to incorporate probiotic properties in the beers.

5.2.3 Preparation of plantain and cassava chips

The plantain and cassava tubers were cleaned in running tap water to remove dirt and possible chemical residue. They were peeled manually with the aid of steel knife and kept in a bowl containing water, and allowed to remain in water until the peeling process is

completed to prevent browning of the resultant chips. Then sliced longitudinally to about 5 mm thickness using a mechanical slicer and dried in a tray dryer (IK-112, IKON Instruments, India) at 50 ^{o}C for about 6 h. The rice grains were also washed with water after weighing the amount needed for fermentation.

5.2.4 Substrates used for preparation of beer

The various combinations of substrates used are shown in Table 5.1.

Table 5.1 The combinations of substrates used for preparation of beer

Substrate	Beer Code	Composition
Rice	RB	100 %
Kachkal chips	KB	100 %
Kachkal chips + Rice	KRB	1:1 ratio
Cassava chips	CB	100 %
Cassava chips + Rice	CRB	1:1 ratio

5.2.5 Biochemical analysis

The moisture, ash, crude fibre, crude fat, protein, total soluble sugars, reducing sugars and starch were estimated according to the official methods of AOAC [18].

5.2.6 Estimation of alcohol content

The volumetric alcohol content was estimated according to the colorimetric method of Magri et al. [19] by using potassium dichromate solution and concentrated perchloric acid.

5.2.7 Total polyphenols content (TPC) analysis

The concentration of total phenolic compounds was determined according to Bray and Thorpe [20]. The sample extracted was treated with Folin-Ciocalteu reagent and the absorbance was read at 650 nm in a UV-Vis spectrophotometer (Spectrascan UV- 2600, Thermo Scientific).

5.2.8 pH and acidity

The pH and acidity were determined according to AOAC Official Method 945.10 and 950.07 respectively [18]. The undiluted test portions were tested in a digital pH meter (pH510, Eutech Instruments) and the indicator titration method was used to obtain the total acidity of the samples. The results were reported as % of lactic acid (1 ml of 0.1M alkali = 0.0090 g lactic acid).

5.2.9 Total soluble solids (TSS) measurement

The TSS values were measured in a digital Abbe refractometer (DR-A1, Atago, Japan) at room temperature and the results were expressed as ^{o}Brix.

5.2.10 Colour measurement

The colour measurement of the beer samples was done by analyzing in a Hunter Lab Color Quest (Ultrascan Vis- Model, USA) by placing the beers in 20mm glass cuvettes. The measurement was done without altering the original shape and size of the cakes. The results were expressed in Commission Internationale de I'Eclairage L, a and b (CIELAB) systems. L indicates the degree of lightness or darkness (L=0 indicates perfect black and L=100 indicates most perfect white); “a” indicates degree of redness (+) and greenness (-) and “b” indicates degree of yellowness (+) and blueness (-).

5.2.11 Microbial count

All the samples were serial diluted with 0.86 % NaCl solution and plated on specific growth media by pour plate or spread plate methods. MRS agar supplemented with $CaCO_3$ and bromocresol purple indicator was used for enumeration of *L. plantarum* in an anaerobic gas pack system (LE012, HiMedia, India) at 37 °C. Yeast malt agar was used for *S. cerevisae* at 27 °C and PDA was used for *A. niger* at 27 °C.

5.2.12 Optimization of fermentation parameters

5.2.12.1 Experimental design

The central composite rotatable design (CCRD) was used in this experiment which is represented by Eq. 5.1.

$$y = f(x_1, x_2, x_3, \dots\dots, x_k) \quad \text{Eq. (5.1)}$$

Where, y represents the answer of the system, and x_i represents the variables of action called factors. It is assumed that the independent variables are continuous and controllable by experiments with negligible errors. The goal here is to optimize the response variable (y) [21].

CCRD requires fewer tests than the full factorial which saves time and cost in research. It has been shown to be sufficient to describe the majority of steady-state process responses. Moreover, CCRD checks the effect of extreme points on product response. The factorial design component of CCRD is of the class $2k$ factorial where k represents the number of relevant factors or variables. If k is the number of variables, then the number of tests required for the CCRD includes the standard 2^k factorial with its origin at the center, $2k$ points fixed axially at a distance, sayγ, from the center to generate the quadratic terms, and replicate tests at the centre [21]. The axial component of CCRD refers to the points that are equidistant from the center of the cube formed for the factorial design. A spherical design is obtained for the reason and there is an equal variance from the center to all the points in the sphere. In consequence, there is a positive axial value (+α) and a negative axial value (-α). The axial points add two more levels in each variable. The α value is calculated from the equation $\alpha = (n_i)^{1/4}$. Where, n_i represents the number of interactions obtained from the factorial design [3].

For two variables, the recommended number of tests at the center is 5 and hence the total number of tests required for the two independent variables is given by the Eq. 5.2.

$$N = 2^k + 2 \cdot k + 5 \quad \text{Eq. (5.2)}$$

Each of the variables is taken at two levels meaning that each variable has a low and high numeric value. A coded numeric value of -1 and +1 is assigned to represent the

variable's low and high values, 0 for the center points and $\pm\gamma$ for the axial points. The central point or zero point may be defined as the region where the optimal conditions are supposedly met [3]. When the response data are obtained from the test work, a regression analysis is carried out to determine the coefficients of the response model ($\beta_1, \beta_2, \ldots\ldots\ldots\ldots, \beta_n$), their standard errors and significance. In addition to the constant (β_0) and error (ε) terms, the response model incorporates linear terms in each of the variables ($x_1, x_2, \ldots\ldots\ldots\ldots\ldots, x_n$), squared terms in each of the variables ($x_1^2, x_2^2, \ldots\ldots\ldots\ldots\ldots, x_n^2$) and first-order interaction terms for each paired combination ($x_1x_2, x_1x_{3,\ldots\ldots\ldots\ldots}, x_{n-i}x_n$). Thus, for the two variables under consideration, the response model will be given by the Eq. 5.3.

$$y = (\beta_0 + \varepsilon) + \sum_{i=1}^{2} \beta_i x_i + \sum_{i=1}^{2} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{2} \beta_{ij} x_i x_j \qquad \text{Eq. (5.3)}$$

The β coefficients, which should be determined in thesecond-order model, are obtained by the least squares method. In general Eq. 5.3 can be written in matrix form as given in Eq. 5.4.

$$Y = \beta X + \varepsilon \qquad \text{Eq. (5.4)}$$

Where, Y is defined to be a matrix of measured values and X to be a matrix of independent variables. The matrices b and e consist of coefficients and errors, respectively. The solution of Eq. 5.4 can be obtained by the matrix approach.

$$\beta = (X' \cdot X)^{-1} X' \cdot Y \qquad \text{Eq. (5.5)}$$

Where, X' is the transpose of the matrix X and $(X' \cdot X)^{-1}$ is the inverse of the matrix $X' \cdot X$ [21].

The experimental runs which were carried out according to the CCRD design were for the two identified design independent variables, namely, fermentation time in hours (β_1) and incubation temperature in °C (β_1), with low (−1) and high (+1) levels. The levels were selected based on the results of preliminary study. The design factors (variables) with low (−1) and high (+1) levels, were, namely, β_1 (24 and 216 h) and β_2 (25 and 40°C). The central values; middle level chosen for experimental design were, 120 h and 32.5 °C for β_1 and β_2 respectively (Table 5.2). The responses which were considered in this particular design were

protein content, alcohol content, *L. plantarum* count, total polyphenols content (TPC), reducing sugars content (RSC) and titratable acidity.

Table 5.2 Variables and their levels used in the experimental design

Variables	Symbol coded	Range and levels		
		Low	Center	High
Fermentation time (h)	β_1	24	120	216
Fermentation temperature (°C)	β_2	25	32.50	40

5.2.12.2 Fermentation procedure

Rice was first used as a substrate for carrying out the optimization process, only later on all the other substrates were used to make beer. Substrates (30 g each) were taken in 250 ml Erlenmeyer flasks and the mouths of it were sealed with cotton plugs. Three number of flasks for each type of beer was used. These flasks were then sterilized by autoclaving at 121 °C at 15 psi for 15 min. After this, 160 ml of separately sterilized distilled water was added to the flasks and boiled for 10 min on a hot plate with constant stirring and then allowed to cool to room temperature. Suspensions (1 ml each) of *S. cerevisae*, *A. oryzae* and *L. plantarum* were inoculated into the flasks under a laminar air flow hood and fermentation was allowed to take place under conditions given by the experimental design in an incubator shaker (Excella E24R, NBS, USA) at 150 rpm. At the end of fermentation, each of the products were strained using a cheese cloth of grade 50 (28x24 threads per inch) and the filtrates were diluted in 1:1 ratio with water and stored in clean and sterile containers. The flow chart for the methodology followed is depicted in Fig 5.2. The beers prepared were immediately kept under refrigerated condition (4 ^{o}C) and brought to room temperature before analysis. All the five different beers prepared by this methodology are shown in Fig. 5.3.

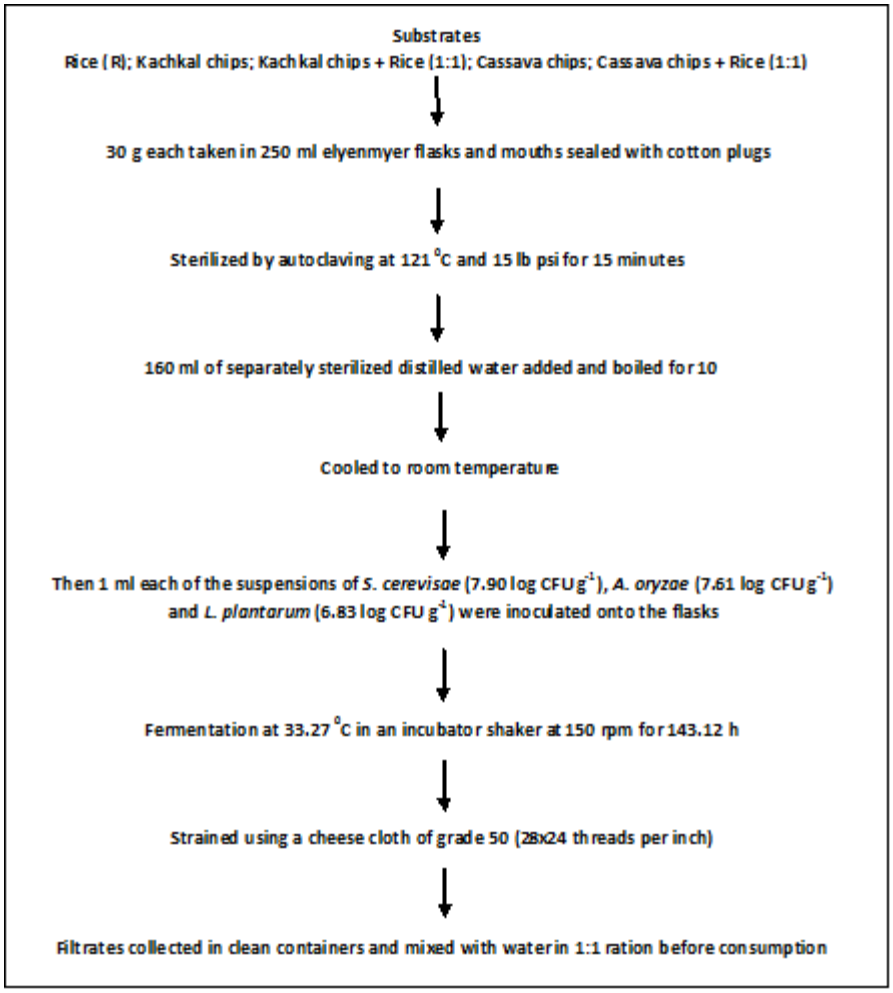

Fig 5.2 Flow chart of the methodology followed for preparation of beer for one flask. Same methodology was followed for all the sets of beers (10 flasks per beer and 50 flasks in total)

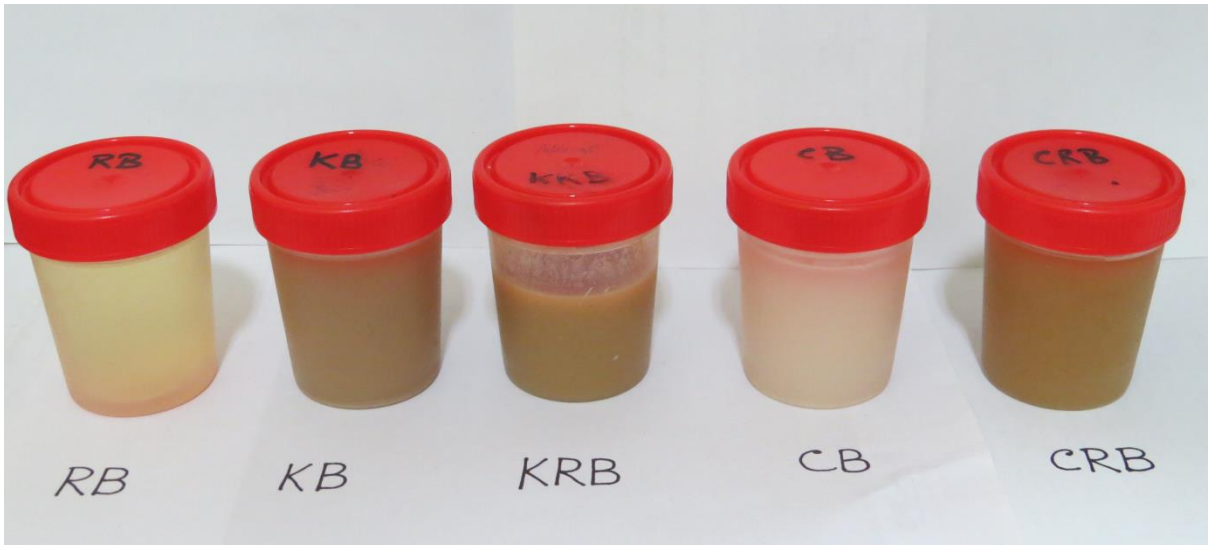

Fig 5.3 The five different varieties of beer prepared in the laboratory

5.2.13 Sensory evaluation of the products

5.2.13.1 Obtaining sensory evaluation response score from panellists

The panel for sensory evaluation consisted of 25 members comprising of research scholars, staffs and faculties of the Department of Food Engineering and Technology, Tezpur University, Assam, India. All the judges were in the age group between 24 to 50 years (8 females and 17 males) and all the panelists were beer consumers. All the members were healthy, non-smokers and non-beetle leaf chewers. All the members of panelists volunteered owing to their interest in sensory evaluation and familiarity with beer in general. Following the guidelines of Meilgaard et al. [22], a screening test was done to determine difference among the candidates, discriminate character difference among products and difference in the intensity or strength of the characteristic. The candidates scoring less than 75 % correct matches and less than 60 % in choosing the correct descriptor for attribute were rejected. The ability to detect and describe difference, the ability to apply abstract concepts, and the degree of positive attitude and predilection for the tasks of descriptive analysis were determined through a series of tests which included a set of prescreening questionnaires, a set of acuity, ranking/rating tests and a personal interview. The panelists were given certain guidelines like arrival time, scheduling of practice and product orientation, scheduling of study and vacations and downtime.

The panelists were suitably trained and familiarized for over a week about the characteristics of good and poor quality beer before the sensory evaluation. They were also educated about the meaning of different terminologies used in the sensory evaluation, definition of quality attributes selected for sensory evaluation, explaining the score sheet and method of scoring. The training was conducted in a controlled sensory facility. They were apprised on the precondition and sensory modality and on any day subject suffering from cold, headache, lack of sleep etc were exempted from training. The subjects were taught the correct method for handling the sample and ways to eliminate or reduce sensory adaptation. They were taught the importance of disregarding personal preference and concentrating on the detection of difference. They were initially presented with beer samples that represent large, easily perceived sample difference and gradually smaller but easily perceived difference was presented. Validation was carried out to document the panel member's mean and standard deviations in relationship to the panel as a whole and within the individual. This

was done by measuring the reliability of sample repetition by panelist for the entire sensory factor [22].

The quality attributes of “colour”, “aroma”, “taste” and “alcoholic strength” were selected for sensory evaluation. The members were asked to judge the samples quickly but not hurry and to take two short sniffs of the samples before ‘tasting’ the samples and give the score for the ‘smell’ first in the scorecard. After evaluation of each sample, a 5 min rest was given during which they were instructed to rinse their mouth with deionized water and crackers to prevent carryover effects. The panellist members were asked to give a tick (√) mark against respective fuzzy scale factor for each of the quality attributes of the samples. The response for quality attributes of the sample was taken on a five-point linguistic scale viz. “poor”, “fair”, “good”, “very good” and “excellent”. The individual preferences of the members to the weightage or importance of the quality attributes were also obtained as “not at all important”, “some-what important”, “important”, “highly important” and “extremely important” [23,24].

All the samples were presented in separate 100 ml glassware (of same shape, size and colour) at room temperature to each of the panelist. The room was away from other noise and odour sources. The walls were off-white and shadow free illumination of 70-80 Foot Candles was used. The area was air conditioned at 72-75 ^{o}F and 45-55 % R.H. The samples were presented in a randomized order to account for presentation and carryover effects. Each of the beers was presented in quadruplicate to each member of panelist and data were collected on paper ballots.

5.2.13.2 Fuzzy analysis

The major steps which were involved in the fuzzy modeling of linguistic evaluation are given below

(1) Triplets associated with linguistic scale,

(2) Triplets for linguistic score of beer samples,

(3) Triplets for linguistic score quality attributes,

(4) Triplets for relative weightage of quality attributes,

(5) Triplets for overall linguistic score of beer samples,

(6) Calculation of membership function on standard fuzzy scale,

(7) Calculation of overall membership function of linguistic scores on standard fuzzy scale,

(8) Estimation of similarity values for beer samples,

5.2.13.2.1 Triplets associated with linguistic scale

Triangular membership function distribution pattern of sensory scales were represented by a set of three numbers, called “triplet”. The distribution pattern of five-point linguistic scales, viz. poor/not at all important, fair/somewhat important, good/important, very good/highly important, and excellent/extremely important is shown in Fig. 5.4 [25]. For instance, triangle *a b c* represents membership function for poor/not at all important category, triangle *g i j* represents distribution function for excellent/extremely important category, etc. Symbols F1, F2, F3, F4, F5, and F6 represent sensory scales. Membership function of each of the sensory scales follows triangular distribution pattern where maximum value of membership function is 1.

The various triplets associated with five-point linguistic scale are shown in Table 5.3. First number of the triplet denotes the coordinate of the abscissa at which the value of the membership function is 1. Second and third numbers of the triplet designate the distance to the left and right, respectively, of the first number where the membership function is 0.

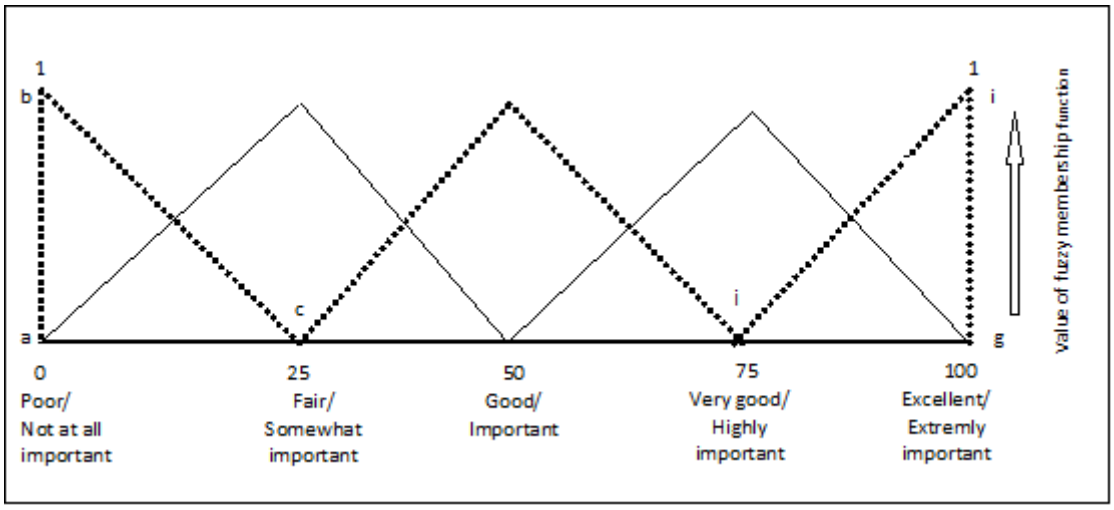

Fig 5.4 Triangular membership function for fuzzy analysis

Table 5.3 Triplet associated with the five-point linguistic scale

Linguistic Attributes	Triplet Score
Poor/ Not at all important	(0 0 25)
Fair/ Somewhat important	(25 25 25)
Good / Important	(50 25 25)
Very good / Highly important	(75 25 25)
Excellent / Extremely important	(100 25 0)

5.2.13.2.2 Triplets for linguistic score of beer samples

For a five point sensory scales the distribution pattern, viz. poor/not at all important, fair/somewhat important, good/important, very good/highly important, and excellent/extremely important based on triplets is shown in Fig. 5. Each triangle on the scale represents membership functions for a particular sensory scale factors and these are represented by the triplets whereby the first number denotes the value of abscissa at which the value of membership function is 1. Second and third numbers of the triplet designate the distance to the left and right, respectively, of the first number where the membership function is 0. As such the values of the triplets for the five point sensory scale are mentioned under the individual scale factors in Fig. 5 will be: not at all important (0,0,25); somewhat important (25,25,25); important (50,25,25); highly important (75,25,25) and extremely important (100,25,0).

For a particular sample, the triplet corresponding to a particular quality attribute can be obtained from the sum of scores obtained for each of the sensory scale factors, the values of triplets associated with the sensory scales and the number of judges. Similarly, for a particular quality attribute of the sample, the aggregated fuzzy value for the judge's opinion on that particular attribute can be denoted by an equation with fuzzy arithmetic for scalar multiplication. The equation for the calculation of triplets for linguistic score is shown as Eq. 5.6.

$$T = \frac{n_1(0\ 0\ 25)+n_2(25\ 25\ 25)+n_3(50\ 25\ 25)+n_4(75\ 25\ 25)+n_5\ (100\ 25\ 0)}{n_1 + n_2 + n_3 + n_4 + n_5} \qquad \text{Eq. (5.6)}$$

In this case, the triplets for the sensory scores of the "taste" attribute of a sample is being given by T, where the total number of judges are $n_1 + n_2 + n_3 + n_4 + n_5$ and n_1 judges

gave the score as "not satisfactory", n_2 judges gave "fair", n_3 judges gave "medium", n_4 judges gave "good" and n_5 judges gave "excellent".

5.2.13.2.3 Triplet for linguistic score of the quality attributes

From the weightage given by judges to the quality attributes of samples in general, the triplet for sensory score of the quality attributes was also calculated.

5.2.13.2.4 Triplets for relative weightage of quality attribute

Relative weightage of quality attributes were calculated as follows.

$Q_{summation}$ = First digit of triplets Q_C, Q_A, Q_T and Q_S = $Q_C+Q_A+Q_T+Q_S$ Eq. (5.7)

$$Q_{avg} = \text{Average of } Q_{summation} = \frac{Q_C+Q_A+Q_T+Q_S}{\text{Number of capabilities (4)}} \quad \text{Eq. (5.8)}$$

Relative weightage of Q_C termed as $Q_{C\,rel}$ will be as follows.

$$Q_{Crel} = \left(Q_{C1}/Q_{avg}\ Q_{C2}/Q_{avg}\ Q_{C3}/Q_{avg}\right), \text{ similarly,} \quad \text{Eq. (5.9)}$$

$$Q_{Arel} = \left(Q_{A1}/Q_{avg}\ Q_{A2}/Q_{avg}\ Q_{A3}/Q_{avg}\right), \quad \text{Eq. (5.10)}$$

$$Q_{Trel} = \left(Q_{T1}/Q_{avg}\ Q_{T2}/Q_{avg}\ Q_{T3}/Q_{avg}\right) \text{ and} \quad \text{Eq. (5.11)}$$

$$Q_{Srel} = \left(Q_{S1}/Q_{avg}\ Q_{S2}/Q_{avg}\ Q_{S3}/Q_{avg}\right) \quad \text{Eq. (5.12)}$$

5.2.13.2.5 Triplets for overall linguistic score of different beer samples

To find out the triplets for overall linguistic scores of different beer samples, triplet for linguistic score for each quality attributes were multiplied with the triplet for relative weightage of that particular quality attribute, and the sum of resultant triplet values for all quality attributes was taken. The overall linguistic score for rice beer is given by the Eq. 5.13.

$$LRB = RB_C \times Q_{Crel} + RB_A \times Q_{Arel} + RB_T \times Q_{Trel} + RB_S \times Q_{Srel} \quad \text{Eq. (5.13)}$$

Where, RB_C, RB_A, RB_T and RB_S represent the triplets corresponding to the quality attributes of rice beer and, Q_{Crel}, Q_{Arel}, Q_{Trel} and Q_{Srel} denote the triplets corresponding to the relative weightage of quality attributes. Using similar equations, the overall scores for all the beer samples were calculated, such as LKB, LKRB, LCB and LCRB. The rule was applied here for multiplication of triplet (a b c) with triplet (x y z) is given by the Eq. 5.14.

$$(a\ b\ c) \times (x\ y\ z) = (a \times x \quad a \times y + x \times b \quad a \times z + x \times c) \qquad \text{Eq. (5.14)}$$

5.2.13.2.6 Calculation of membership function on standard fuzzy scale

This is calculated from the standard fuzzy scale (Fig. 5.5) [25]. Values of membership function of F1 through F6 are defined by a set of ten numbers as shown in Table 5.4.

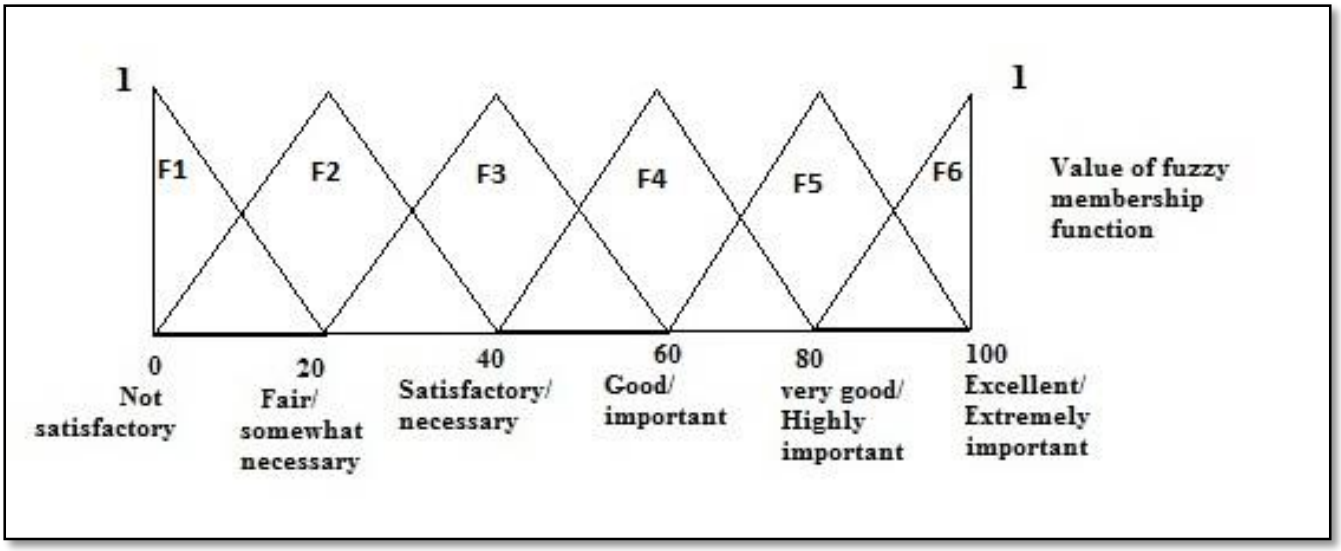

Fig 5.5 Standard fuzzy scale

Table 5.4 Membership function of six point linguistic scale

Scale Factors	Symbols	Attribute values in Fuzzy Scale
Not satisfactory	F_1	(1,0.5,0,0,0,0,0,0,0,0)
Fair	F_2	(0.5, 1, 1, 0.5, 0, 0, 0, 0, 0, 0)
Satisfactory	F_3	(0, 0, 0.5, 1, 1, 0.5, 0, 0, 0, 0)
Good	F_4	(0, 0, 0, 0, 0.5, 1, 1, 0.5, 0, 0)
Very Good	F_5	(0, 0, 0, 0, 0, 0, 0.5, 1, 1, 0.5)
Excellent	F_6	(0, 0, 0, 0, 0, 0, 0, 0, 0.5, 1)

5.2.13.2.7 Calculation of overall membership function of linguistic scores on standard fuzzy scale

Graphical representation of membership function of a triplet (a b c) is shown in Fig. 5.6 [25]. The figure showed that for a triplet (a b c), when the value of abscissa is a, value of membership function is 1, and when it is less than a−b or greater than a+c, the value is 0. For a given value of x on abscissa, value of membership function B_x can be expressed as given in Eq. 5.15.

$$\left.\begin{aligned} B_x &= \frac{x-(a-b)}{b} \text{ for } (a-b) < x < a \\ B_x &= \frac{(a+c)-x}{c} \text{ for } a < x < (a+c) \\ B_x &= 0 \text{ for } x < (a-b) \text{ and } x > (a+c) \end{aligned}\right\} \quad \text{Eq. (5.15)}$$

For each of the samples and its triplets, the value of membership function B_x at x=0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 can be found out from Eq. 15. This membership function value of samples on standard fuzzy scale will be given a set of ten numbers which are "(maximum value of B_x at 0<x<10), (maximum value of B_x at 10<x<20), (maximum value of B_x at 20<x<30), (maximum value of B_x at 30<x<40), (maximum value of B_x at 40<x<50), (maximum value of B_x at 50<x<60), (maximum value of B_x at 60<x<70), (maximum value of B_x at 70<x<80), (maximum value of B_x at 80<x<90) and (maximum value of B_x at 90<x<100)."

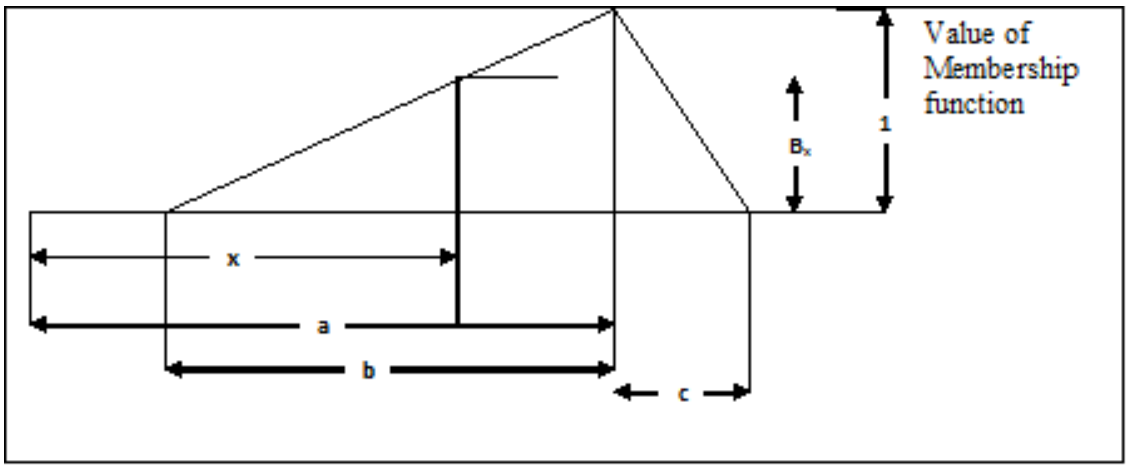

Fig 5.6 Graphical representation of triplet (a,b,c) and its membership function

5.2.13.2.8 Estimation of similarity values for beer samples

The similarity value representing the overall quality of any beer sample as a crisp number can be found out by finding the defuzzified numeric form of fuzzy triangular distribution function (a, b, c), which is represented by Eq. 5.16.

$$y_a = \frac{1}{3}(3a - b + c) \qquad \text{Eq. (5.16)}$$

Similarity values under the six categories of linguistic scales were compared to find out the highest similarity value. The category corresponding to the highest similarity value was considered responsible for its capability. For example, if the similarity value under "good" category is the highest, the overall capability of that beer was regarded as "good". Using similar procedure, the overall capability of each of the beer samples was defined. By combining the defined overall capabilities of the beer samples as calculated by the above procedure, all the five beers were ranked.

5.2.14 Statistical analysis

The statistical software Design Expert Ver. 6.0.11 (Stat-Ease Inc., Minneapolis, USA) was used for design of experiments, regression and graphical analyses of the data obtained, and statistical analysis of the model to evaluate the analysis of variance.The software MATLAB Version 7.1 (developed by MathWorks, USA) was used for carrying out the statistical analyses for fuzzy analysis.

5.3 Results and Discussions

5.3.1 Biochemical composition of the substrates

The initial biochemical compositions of the various substrates are shown in Table 5.5 and all the calculations have been done on wet basis. Moisture content was low in all the three kinds of substrates and ranged from 8.02 % (cassava chips) to 13.23 % (rice). Higher content of crude protein (8.18 %) and crude fiber (6.29 %) was found in rice as compared to the other two substrates. The highest content of crude fats (3.67 %) was found in cassava chips, while *kachkal* chips had the lowest content (0.58 %). The ash content was in the range of 1.5 % in both rice and cassava chips, while *kachkal* chips had the lowest content of 0.83 %. Starch was the major constituent in all the substrates and the highest content of 73.80 % was seen in cassava. This was followed by rice (65.62 %) and plantain (46.31 %). The soluble sugars were in close range in all the three types of substrates.

Table 5.5 Initial composition of the various substrates

Composition	Substrates		
	Rice	Cassava Chips	*Kachkal* Chips
Moisture	13.23±0.15	8.02±1.25	10.16±0.65
Crude protein	8.18±1.44	5.07±0.88	4.99±0.57
Crude fibre	6.29±0.69	2.42±0.88	2.66±0.05
Crude fats	2.47±0.27	3.67±0.58	0.58±0.06
Ash	1.58±0.32	1.50±0.85	0.83±0.20
Starch	65.62±1.65	73.80±3.24	46.31±0.99
Soluble sugars	2.64±0.22	3.37±0.24	2.64±0.03

Note: Results are mean of three replicates ± SD

5.3.2 Experimental runs and the responses

The total numbers of experimental runs generated according to the Eq. 5.2 under the variables ranges (Table 2) were 13. The existing traditional methodologies followed for the preparation of rice beer in Northeast India does notfollow anydefinite conditions of time and temperature and fermentation is usually carried out at room temperature. Since, it is a

temperate region with seasonal and geographical temperature fluctuations, the temperature range of 25 to 40 °C was selected. Also the fermentation is carried out for indefinite period and varies with region. As such the time period chosen for fermentation was wide (24 and 216 h). Moreover, some preliminary runs and experimental data were considered in fixing the levels for the independent variables. The conditions for fermentation and the responses of protein content, alcohol content, *L. plantarum* count, TPC, RSC and titratable acidity obtained in case of rice beer are shown in Table 5.6.

5.3.3 Statistical analysis and model fitting

RSM was applied to see the effect of time and temperature on the response values, namely protein content, alcohol content, *L. plantarum* count, total polyphenols, reducing sugars and titratable acidity for the fermentation of rice beer. The statistical data representing the analysis of variance (ANOVA) of the test are shown in Table 5.7. The sequential sum of squares, *F*-value and the corresponding coefficient of determination (R^2) and adjusted coefficient of determination (Adj. R^2) are also presented. Variance and regression analysis was carried out to fit the suggested quadratic models and investigate the statistical significance of model factors. The adequacy of the model was investigated by the *F*-values and corresponding *p* values of the regression models. It was observed that, the predicted models for all the response variables were adequately fitted to the observed experimental data ($p \leq 0.001$). The effect of linear, square and interaction effect of each response variables are also presented in the Table 5.8. The accuracy of the fitness of the models was also judged by the lack of fit values for each response and it was observed that there were no lack of fits ($p > 0.05$) in any response model. Non significant lack of fit tests also suggested that quadratic models were best fitted for the fermentation of rice beer. Fittness of quadratic models was ascertained by computing the R^2 and adj. R^2 values. The R^2 values for protein content, alcohol content, *L. plantarum* count, total polyphenols, reducing sugars and titratable acidity of rice beer were 0.93 %, 0.82 %, 0.83 %, 0.79 %, 0.74 % and 0.85 % respectively. The difference between the R^2 and R^2_{adj} values were less than 0.2 implying there are no insignificant terms added to the models [26]. Thus these results showed that the models can establish optimum condition for preparation of beer from rice.

Table 5.6 Response sheet for CCRD experimental design with process variables and experimental results of rice beer fermentation

Run	Factors		Responses					
	Time (h)	Temperature (°C)	Protein content (%)	Alcohol content (%)	*L. plantarum* count (Log CFU ml^{-1})	Total polyphenols content (mg/100g)	Reducing sugars content (%)	Acidity (%)
1	52.12	37.80	0.17	0.00	4.02	1.22	1.50	0.05
2	120	40.00	0.32	2.15	6.00	7.90	8.12	0.22
3	120	32.50	0.40	3.19	6.04	16.87	8.11	0.24
4	24	32.50	0.14	0.00	4.28	1.18	0.26	0.03
5	187.88	37.80	0.88	7.00	5.0	31.98	1.08	0.42
6	120	25.00	0.27	0.86	5.24	3.21	4.20	0.10
7	187.88	27.20	0.82	6.99	5.12	32.68	1.58	0.46
8	120	32.50	0.40	3.16	5.70	17.92	8.09	0.24
9	120	32.50	0.41	3.91	6.08	18.11	8.00	0.25
10	120	32.50	0.42	6.80	6.38	37.12	7.84	0.38
11	52.12	27.20	0.16	0.00	4.08	1.02	0.28	0.03
12	120	32.50	0.60	6.81	6.00	34.28	3.12	0.39
13	216	32.50	0.90	6.98	5.33	32.13	1.89	0.40

Table 5.7 Analysis of variance (ANOVA) showing the linear, quadratic interaction and lack of fit of the response variables

Source of variation	DF	Response variables											
		Protein content		Alcohol content		*L. plantarum* count		TPC		RSC		Acidity	
		Sequential sum of square	F	Sequential sum of square	F	Sequential sum of square	F	Sequential sum of square	F	Sequential sum of square	F	Sequential sum of square	F
Regression	5	0.80	18.38^{***}	85.24	6.40^{**}	99.82	4.00^{*}	1906.24	5.24^{*}	6.41	6.74^{**}	0.25	7.95^{**}
Linear	2	0.75	33.34^{***}	71.59	11.08^{**}	6.17	0.24^{*}	1414.24	7.06^{**}	1.64	1.34^{*}	0.22	15.70^{***}
Square	1	0.005	0.05^{ns}	0.0002	0.00006^{ns}	0.74	0.05^{ns}	0.20	0.01^{ns}	0.009	0.01^{ns}	0.009	0.12^{ns}
Interaction	2	0.05	2.92^{ns}	13.65	2.56^{ns}	92.91	9.31^{**}	491.80	3.38^{ns}	4.78	12.56^{**}	0.03	2.07^{ns}
Residual error	7	0.06	-	18.64	-	34.91	-	509.62	-	1.33	-	0.04	-
Lack of fit	3	0.03	1.28^{ns}	4.53	0.43^{ns}	15.74	1.09^{ns}	113.01	0.38^{ns}	1.10	6.24^{ns}	0.02	1.08^{ns}
Pure error	4	0.03	-	14.11	-	19.81	-	396.61	-	0.23	-	0.02	-
Corr Total	12	0.86	-	103.88	-	134.74	-	2415.86	-	7.75	-	0.29	-
R^2	-	92.92%	-	82.05%	-	74.09%	-	78.91%	-	82.81%	-	85.02%	-
Adjusted R^2	-	87.87%	-	69.24%	-	55.58%	-	63.84%	-	70.53%	-	74.32%	-

Note: *significant at P≤0.05; **significant at P≤0.01; ***significant at P≤0.001; ^{ns}not significant

Table 5.8 Estimated regression coefficients of the fitted second order polynomial for response variables (coded)

Coefficients	Estimated coefficients					
	Protein content	Alcohol content	*L. plantarum* count	TPC	RSC	Acidity
β_0	0.45^{***}	4.77^{**}	6.04^{**}	24.86^{*}	7.03^{*}	0.30^{**}
β_1	0.31^{***}	2.98^{***}	0.44^{*}	13.27^{***}	0.40^{ns}	0.17^{***}
β_2	0.017^{ns}	0.23^{ns}	0.11^{ns}	0.77^{ns}	0.78^{ns}	0.019^{ns}
β_1^2	0.062^{ns}	-0.39^{ns}	-0.78^{**}	-2.70^{ns}	-3.61^{**}	-0.029^{ns}
β_2^2	-0.050^{ns}	-1.38^{ns}	-0.37^{*}	-8.25^{*}	-1.06^{ns}	-0.057^{ns}
$\beta_1 \beta_2$	0.012^{ns}	0.0025^{ns}	-0.015^{ns}	-0.22^{ns}	-0.43^{ns}	-0.015^{ns}
Probability of F value	0.0007	0.0152	0.0132	0.0256	0.0491	0.0084
Probability of lack of fit	0.3961	0.7441	0.0546	0.7737	0.4480	0.4528

*significant at P≤0.05, **significant at P≤0.01, ***significant at P≤0.001, ^{ns}not significant

5.3.4 Effect of the process variables on various responses of rice beer

5.3.4.1 Protein content

The values of the coefficients for protein content were used for a final predictive equation neglecting the non-significant cross-terms as given in Eq. 5.17.

$$Y = 0.45 + 0.31X_1 + 0.017X_2 + 0.12X_1X_2 + 0.062{X_1}^2 - 0.050{X_2}^2 \quad \text{Eq. (5.17)}$$

To determine the optimal levels of variables (time and temperature) for obtaining the maximum protein content, a three-dimensional surface plot was constructed according to Eq. 5.17 which is shown in Fig 5.7. A significant linear effect (P<0.001) of fermentation time with protein content (Table 5.8) was observed and it might be attributed to increase in microbial mass during fermentation. It was supported by the favourable pH for the growth of lactic acid bacteria with the progress of fermentation [27] and in turn caused extensive hydrolysis of the protein molecules to amino acid and other simple peptides [28]. However,

the increase in protein content with temperature of fermentation did not reveal significant effect.

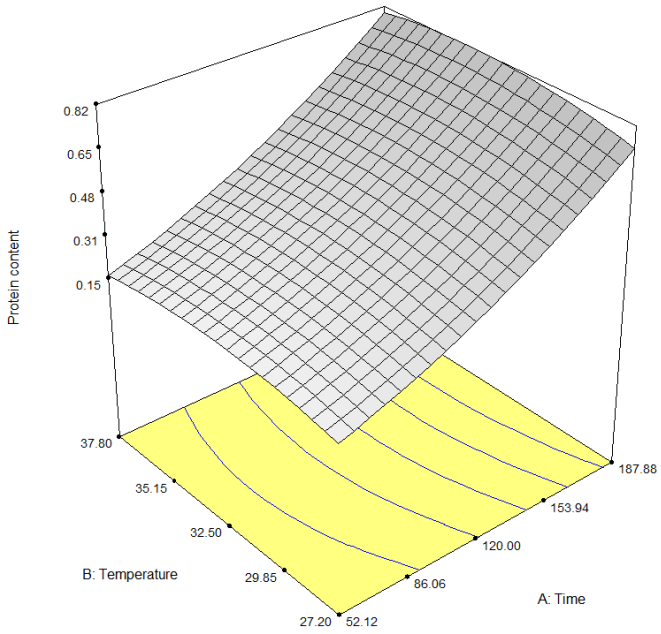

Fig 5.7 The effect of time and temperature on protein content (%)

Protein content is an important criterion for the quality of beer. Its content along with alcohol is responsible for the characteristic properties of beer. The stability and organoleptic characteristics of beer depends on the interaction among proteins, amino acids and polyphenols like proanthocyanidins. The amino acid residues also take part in the production of aromatic compounds [29].

5.3.4.2 Alcohol content

The values of the coefficients for alcohol content were used for a final predictive equation neglecting the non-significant cross-terms as given in Eq. 5.18.

$$Y = 4.77 + 2.98X_1 + 0.23X_2 + 0.0025X_1X_2 - 0.392{X_1}^2 - 1.38{X_2}^2 \qquad \text{Eq. (5.18)}$$

The three-dimensional surface plot (Fig 5.8) was constructed according to Eq. 5.18 in order to determine the optimal levels of variables (time and temperature) for obtaining the maximum alcohol content. It was observed that among the temperature and time of fermentation, time exhibited a strong positive effect on alcohol content. This may be

attributed to the reason of accumulation of alcohol as a by product of yeast metabolism in the fermenting mass with the progress of time.

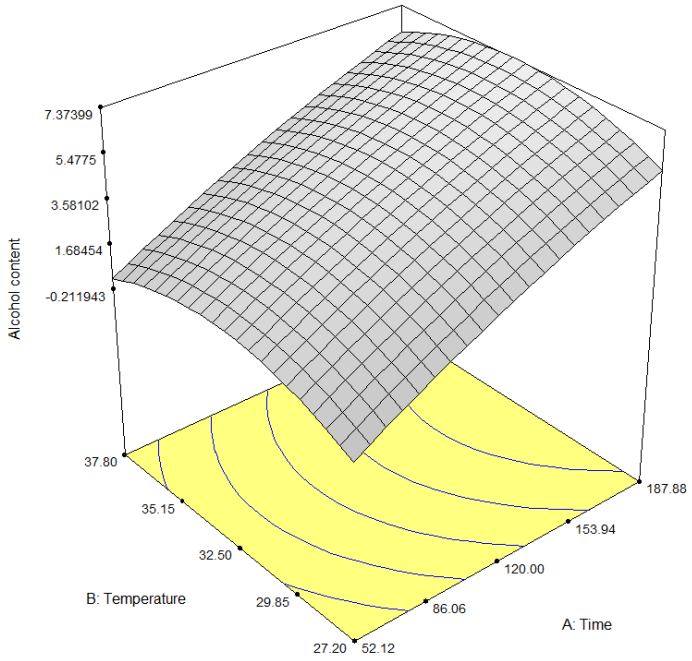

Fig 5.8 The effect of time and temperature on alcohol content (%)

Alcohol and water are the main components of beers. The amount of alcohols in different beers varies based on the quantity of fermentable sugars in the wort, the variety of yeast used and the fermentation conditions. Alcohol is a by-product of yeast metabolism and typical brewing yeast can survive up to alcohol concentrations of 15 % by volume [30]. The lager beers usually contain around 5 % alcohol and the effect of alcohol on the sweet and bitter tastes of beer has also been reported [31].

5.3.4.3 *Lactobacillus plantarum* count

The values of the coefficients for *L. plantarum* count were used for a final predictive equation neglecting the non-significant cross-terms as given in Eq. 5.19.

$$Y = 6.04 + 0.44X_1 + 0.11X_2 - 0.015X_1X_2 - 0.78{X_1}^2 - 0.37{X_2}^2 \qquad \text{Eq. (5.19)}$$

To determine the optimal levels of variables (time and temperature) for obtaining the maximum *L. plantarum* count, a three-dimensional surface plot was constructed according to

Eq. 5.19. Time and temperature of fermentation had a negative quadratic effect on *L. plantarum* survivability (P<0.01). It was observed that *L plantarum* population increased up to 150 h of fermentation, followed by decrease in survivability as illustrated in Fig 5.9.

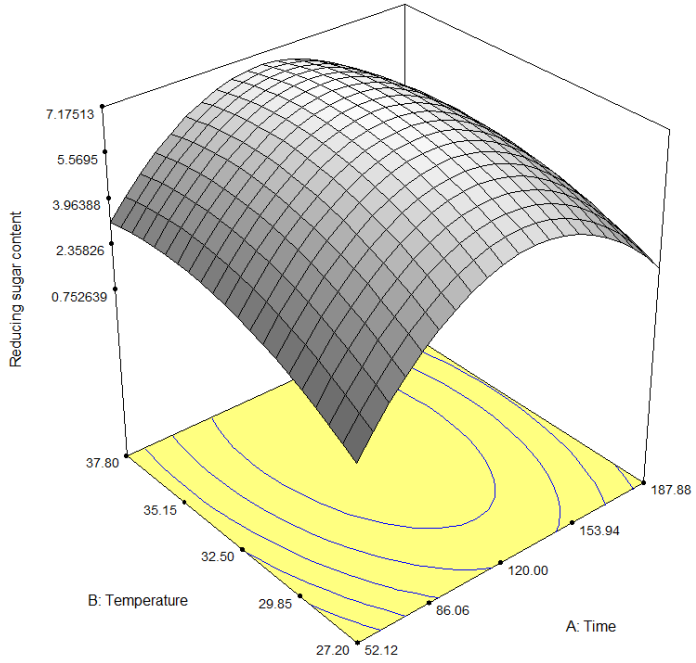

Fig 5.9 The effect of time and temperature on *L. plantarum* count (log CFU ml^{-1})

L. plantarum is a facultatively hetero fermentative lactobacilli and is considered as a safe probiotic organism which can survive gastric transit and colonize the intestinal tract of humans and other mammals. *L. plantarum* has been associated with various special therapeutic or prophylactic properties and has been found to limit the amount of pathogenic bacteria [32].

5.3.4.4 Total polyphenols content (TPC)

The values of the coefficients for total polyphenol content were used for a final predictive equation neglecting the non-significant cross-terms as given in Eq. 5.20.

$$Y = 24.86 + 13.27X_1 + 0.77X_2 - 0.22X_1X_2 - 2.70{X_1}^2 - 8.25{X_2}^2 \qquad \text{Eq. (5.20)}$$

The Eq. 5.20 has been used for the construction of a three-dimensional surface plot (Fig 5.10) which determines the optimal levels of time and temperature for obtaining the

maximum TPC. It exhibited a linear positive effect with time of fermentation (P<0.001) whereas temperature had a negative quadratic effect on TPC. The increase in polyphenols content with fermentation time may be attributed to an increase in the level of free soluble phenolics due to hydrolysis of the glycosidic bonds of bound phenolics by microbial-secreted hydrolytic enzyme [33]. Further the reduction in polyphenols content with increase in temperature may be due to thermal degradation of the phenolic compounds [34].

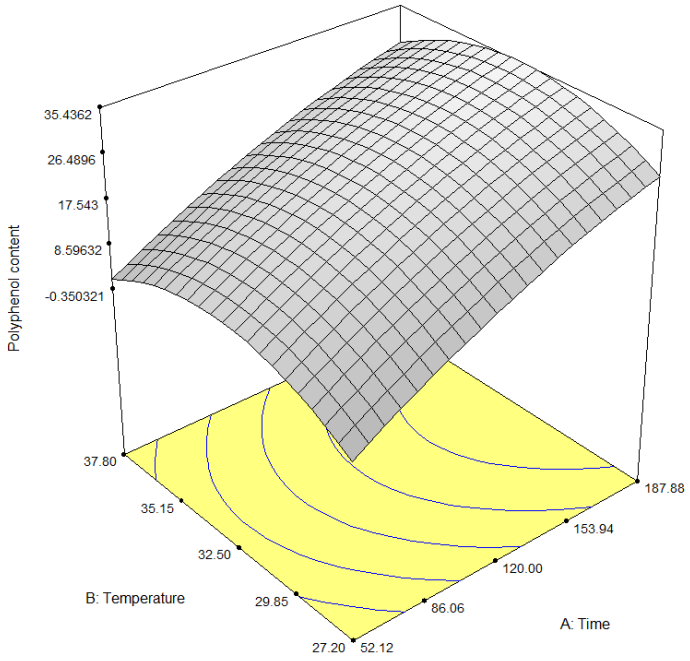

Fig 5.10 The effect of time and temperature on TPC (mg/100g)

Polyphenols are the most important antioxidant compounds in beer. These phenolic compounds have several functional properties in the beer influence on its colloidal stability, savour, aging and colour [35]. During beer storage, phenolic compounds react with proteins andform high molecular weight species and hazes. The polyphenolic compounds are also important antioxidants, and due to its antioxidant capacity and low alcoholic content, consumption of beer helps to improve the plasma antioxidant activity and reduce the risk of cardiovascular diseases [36].

5.3.4.5 Reducing sugars content (RSC)

The values of the coefficients for reducing sugars content were used for a final predictive equation neglecting the non-significant cross-terms as given in Eq. 5.21.

$$Y = 7.03 + 0.40X_1 + 0.78X_2 - 0.43X_1X_2 - 3.61{X_1}^2 - 1.06{X_2}^2 \quad \text{Eq. (5.21)}$$

The optimal levels of time and temperature for obtaining the maximum reducing sugars contenthas been by the three-dimensional surface plot (Fig 5.11) and it was constructed according to Eq. 5.21. As the fermentation progressed, the reducing sugars content decreased. A quadratic relationship was observed between the RSC and time of fermentation (P<0.01).

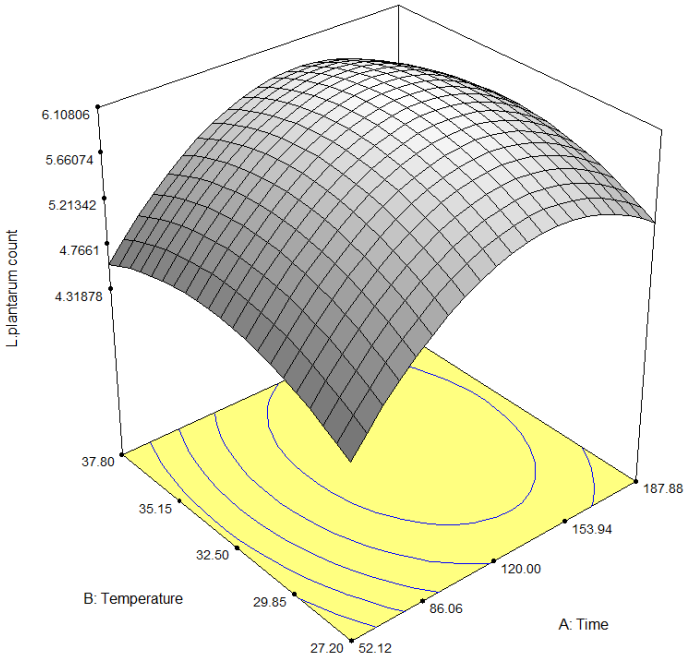

Fig 5.11 The effect of time and temperature on RSC (%)

The reducing sugars are responsible for imparting sweetness to the beer and some amount of sweetness in beer is desirable from sensory point of view [37].

5.3.4.6 Titratable acidity

The values of the coefficients for titratable acidity content were used for a final predictive equation neglecting the non-significant cross-terms as given in Eq. 5.22.

$$Y = 0.30 + 0.17X_1 + 0.019X_2 - 0.015X_1X_2 - 0.029{X_1}^2 - 0.057{X_2}^2 \quad \text{Eq. (5. 22)}$$

To determine the optimal levels of variables (time and temperature) for obtaining the minimum titratable acidity, the three-dimensional surface plot (Fig 5.12) was constructed according to Eq. 5.22. Fermentation temperature had little effect on titratable acidity. However, the time of fermentation exhibited a strong relationship with acidity and it increased with the progress of fermentation (Table 5.8).

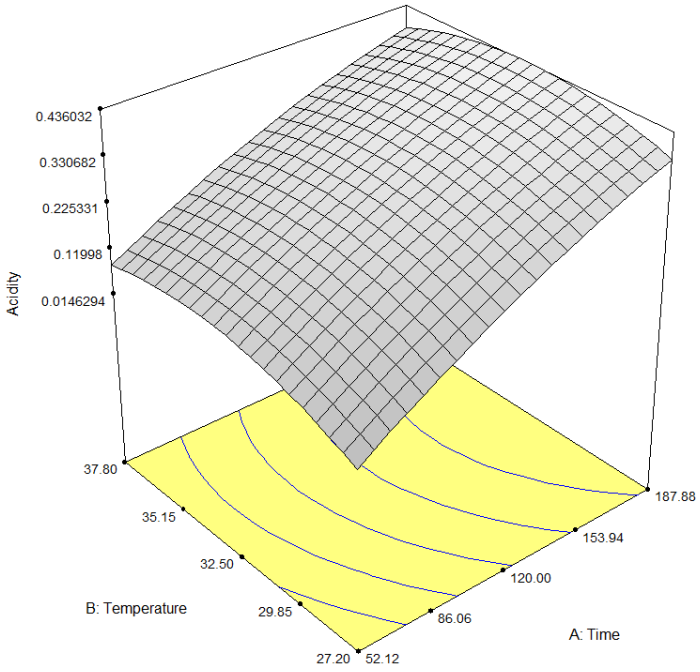

Fig 5.12 The effect of time and temperature on titratable acidity (%)

Acidity has high impacts on the sensory profiles of beer and consumers dislike beers with high acidity [14]. Also high acidic beverages are thought to increase the potential for dental erosion [38]. Hence, acidity was considered as a limiting factor in the current experiment.

5.3.5 Optimization of fermentation parameters for rice beer

The independent variables were optimized numerically using statisticalsoftware Design Expert, Ver. 6.0.11. For this purpose the goals for the variables i.e. fermentation time and fermentation temperature were kept in range. In case of the response parameters, the protein content, alcohol content, *L. plantarum* count, total polyphenols content (TPC) andreducing sugars content (RSC) and were set at maximum, whereas the titratable acidity was set at minimum. The optimal conditions, predicted values and experimental values for various responses are shown in Table 5.9. Numerical analysis revealed that a fermentation

period of 143.12 h at a temperature of 33.28 °C gave an optimized product with maximum content of protein (0.56 %), alcohol (5.75 %), *L. plantarum* count (6.11 log CFU ml^{-1}), TPC (28.99 mg/100g), RSC (6.82 %) and a minimum of acidity (0.35 %). When the experiment was actually performed under the optimized conditions the protein content (0.60 %), alcohol content (6.29 %), *L. plantarum* count (7.08 log CFU ml^{-1}) and TPC (30.46mg/100g) were found to be higher than the predicted values, while the RSC (5.76 %) and acidity (0.34 %) were lower than the respective predicted values.The residual values in between the predicted values and the experimental data were calculated for all the responses. The residuals were found to vary from 2.85 to 15.87 %, which validates the accuracy of the optimization process.

Table 5.9 Optimal conditions, predicted values and experimental values for various responses

Responses	Predicted value	Experimental value	Residual values
Protein content (%)	0.56	0.60±0.06	7.14%
Alcohol content (%)	5.75	6.29±0.10	9.39%
L. plantarum count (log CFU ml^{-1})	6.11	7.08±0.79	15.87%
TPC(mg/100g)	28.99	30.46±0.01	5.07%
RSC (%)	6.82	5.76±0.76	15.54%
Acidity (%)	0.35	0.34±0.08	2.85%

5.3.6 Attributes of the final products

Biochemical composition and microbial load of the five different beers prepared using optimized conditions are shown in Table 5.10 and Table 5.11 respectively. It was seen that the properties of all the types of beer prepared from cassava and plantain were at par with rice beer. The pH of all the beers remained in the range of 4.94 (KRB) to 5.98 (RB) and in case of acidity, except for RB (0.34 %) the values remained in the range of 51 % (KB) to 0.58 % (CRB). The alcohol and reducing sugars content were also highest in RB with values of 6.99 % and 2.39 %, which was followed by KRB with values of 6.15 % and 2.37 % respectively. Thapa and Tamang [39] in their study on *kodo ko jaanr*, a similar kind of product found the pH, acidity and alcohol content to range from 3.7–4.5, 0.23–0.5% and 1.8–8.7% respectively. The alcohol contents were found to be similar to that of *zutho* (5.0 %) [40], *bhaati jaanr*

(5.9%) [41] and *kodo ko jaanr* (4.8 %) [39]. The concentration of reducing sugars in the beers was higher than other products like *tapuy* (0.07 % - 0.21 %) [42] and *zutho* (6.3 mg/ml) [40]. The protein content was found to be lowest in RB (0.77%), while in the remaining beers the content was above 1 %. The results can be corroborated to the protein content of *Ou* samples which vary from 0.45 to 0.99 % [43]. Phenolic compounds were found to be present in all the beers and the highest content was seen in PRB (45.86 mg/100g), while the lowest content was seen in CB (16.67 %).

The highest count of the probiotic bacteria *L. plantarum* was found in CB (8.41 log CFU ml^{-1}), while in all the other beers its count remained in the range of 7–8 log CFU ml^{-1}. This count was higher than other products like *kodo ko jaanr* [39], where the count of LAB was found to range from 4.1 to 6.5 log CFU g^{-1}. The count of *S. cerevisiae* also remained in the range of 7–8 log CFU ml^{-1} in all the beers expect for RB where the count was 6.69 log CFU ml^{-1}. The count of yeasts in *bhaati jaanr* [41] was also found to increase from 5 log CFU g^{-1}on day 1 of fermentation to 8 log CFU g^{-1} on day 2, and then gradually decreased to level of 10^5 CFU g^{-1} on day. The mould *A. oryzae* was however absent in all the final products. The moulds are responsible for amylolytic or proteolytic enzyme activities during the initial phase of fermentation. Their absence from the final product may be attributed to the production of antifungal metabolites by lactic acid bacteria i.e. *L. plantarum* such as organic acids, reuterin, hydrogen peroxide, hydroxylated fatty acids and phenolic compounds [41]. The disappearance of moulds from the final product of alcoholic fermentation has also been reported by others such as Blagojev et al. [44].

Table 5.10 Some biochemical attributes of the beers prepared in laboratory

Attribute	Beer type				
	RB	KB	KRB	CB	CRB
pH ±SD	5.98±0.21	5.74±0.67	4.94±0.72	5.29±0.63	5.69±0.45
Acidity (%)±SD	0.34±0.08	0.51±0.12	0.62±0.06	0.54±0.04	0.58±0.04
Alcohol (%)±SD	6.99±0.14	5.27±0.07	6.15±0.07	3.81±0.19	4.99±0.15
TSS (^{o}Bx)±SD	18.40±0.16	12.80±0.50	16.27±0.18	7.07±0.12	10.29±0.12
Reducing sugar (%)±SD	2.39±2.57	2.30±0.17	2.37±0.57	0.63±0.05	1.55±0.31
Protein (%)±SD	0.77±0.63	1.60±0.06	1.56±0.12	1.26±0.134	1.15±0.01
TPC (mg/100g)±SD	34.46±0.01	45.86±0.63	42.60±1.18	16.67±1.53	22.67±1.79

Table 5.11 Microbial load in the beers

Beer Code	Microbial load (Log CFU ml^{-1})		
	L. plantarum	*S. cerevisiae*	*A. oryzae*
RB	7.08±0.79	6.69±0.56	0
KB	7.46±0.18	7.58±0.48	0
KRB	7.29±0.16	7.35±0.66	0
CB	8.41±0.20	7.90±0.84	0
CRB	7.89±0.19	7.72±.052	0

5.3.7 Colour of the different beers

The colour of all the five types of beer is CIELAB expression is given in Table 5.12. The L parameter is an indication of the lightness or darkness and the capacity of the samples to reflect or transmit light and hence the samples with the higher L values are clearer than the others. The L values of all the beers were to range from 23.42 in the beer RB to 37.40 in the beer KRB. The yellow component (b) was higher in the beers RB, KB, CRB and CB than the red component "a" and thus whitish yellow contributed the most to the colour characteristics of these beers. However, in the sample CRB the component "a" was higher than the "b" component.

Table 5.12 Colour of the beers in CIELAB expression

Beer type	Colour		
	L±SD	a	b
RB	23.42±0.38	0.21±0.03	0.77±0.03
KB	32.92±0.06	0.24±0.03	3.52±0.05
KRB	37.40±0.73	1.81±0.16	7.57±0.42
CB	32.32±0.23	0.28±0.04	0.72±0.06
CRB	31.52±0.62	0.80±0.13	0.26±0.28

Note: Values are means of three replicated followed by the standard deviation

5.3.8 Sensory evaluation

5.3.8.1 Triplets for linguistic score of beer samples

This was obtained from the sum of linguistic scores (Table 5.13), triplets associated with linguistic scale (Table 5.3) and the number of judges (Table 5.13).

For example, for the colour parameter of rice beer (RB_C), the total number of experts was 25 and out of the total experts, no expert gave "poor" score, one gave "fair" score, five gave the score as "good", ten gave "very good" and nine gave "excellent", the evaluation process for triplets for the linguistic scores for color is given as follows.

$$LRB_C = \frac{0(0\ 0\ 25) + 1(25\ 25\ 25) + 5((50\ 25\ 25) + 10(75\ 25\ 25) + 9(100\ 25\ 0)}{0 + 1 + 5 + 10 + 9}$$

LRB_C represents linguistic scores of colour parameter of rice beer. Similar values were obtained for each type of beer for all the sensory quality parameters, such as LRB_A, LRB_T, LRB_S, LKB_C, LKB_A, LKB_T, LKB_S, $LKRB_C$, $LKRB_A$, $LKRB_T$, $LKRB_S$, LCB_C, LCB_A, LCB_T, LCB_S, $LCRB_C$, $LCRB_A$, $LCRB_T$ and $LCRB_S$, which are shown in Table 5.14.

Table 5.13 Sum of the number of judges with varied preferences against the quality attributes for the four different kinds of beer

Sensory quality attribute	Sample	Number of judges					Total
		P	F	G	VG	E	
Colour	RB	0	1	5	10	9	25
	KB	7	9	8	1	0	25
	KRB	4	12	8	1	0	25
	CB	10	12	3	0	0	25
	CRB	6	10	7	2	0	25
Aroma	RB	1	2	2	12	8	25
	KB	4	10	8	3	0	25
	KRB	4	9	12	0	0	25
	CB	9	11	4	1	0	25
	CRB	6	8	5	5	1	25
Taste	RB	0	2	3	14	6	25
	KB	5	15	4	1	0	25
	KRB	5	11	8	1	0	25
	CB	7	8	10	0	0	25
	CRB	7	11	4	3	0	25
Alcoholic strength	RB	0	2	4	11	8	25
	KB	5	7	7	6	0	25
	KRB	2	10	12	1	0	25
	CB	8	10	6	1	0	25
	CRB	4	12	5	3	1	25

Note: P – Poor; F – Fair; G – Good; VG – Very Good; E – Excellent

Table 5.14 Triplets for linguistic score of the beer samples

Sample codes	Quality attributes	Triplets for linguistic scores
RB	Colour	28 18 25
	Aroma	35 21 25
	Taste	26 20 25
	Alcoholic strength	39 20 25
KB	Colour	18 15 25
	Aroma	22 16 25
	Taste	28 18 25
	Alcoholic strength	25 17 25
KRB	Colour	77 25 16
	Aroma	74 24 17
	Taste	74 25 19
	Alcoholic strength	75 25 17
CB	Colour	31 21 25
	Aroma	33 21 25
	Taste	30 20 25
	Alcoholic strength	37 23 25
CRB	Colour	30 19 25
	Aroma	37 19 24
	Taste	28 18 25
	Alcoholic strength	35 21 24

5.3.8.2 Triplets for linguistic score of quality attribute

Similarly, from the general weightage given by experts for the quality attributes of beer in general (Table 5.15), the triplet for linguistic score of colour, aroma, taste and alcoholic strength were also calculated.

For example, capability of colour (Q_C) in general calculated as follows

$$Q_C = \frac{1(0\ 0\ 25) + 2(25\ 25\ 25) + 7((50\ 25\ 25) + 10(75\ 25\ 25) + 5(100\ 25\ 0)}{1+2+7+10+5}$$

Similarly capability of aroma (Q_A), taste (Q_T) and alcoholic strength (Q_S) were calculated and shown in Table 5.16.

Table 5.15 Sensory score for quality attributes

Quality attribute	Number of judges					Total
	NI	SI	I	HI	EI	
Colour	1	2	7	10	5	25
Aroma	0	1	2	18	4	25
Taste	0	0	1	21	3	25
Alcoholic strength	1	3	4	15	2	25

Note: NI- Not at all important; SI- Somewhat important;
I- Important; HI- Highly important; EI- Extremely important

Table 5.16 Triplet for quality attributes of beer samples

Quality attributes	Triplets for linguistic scores
Colour	66 24 20
Aroma	75 25 21
Taste	77 25 22
Alcoholic strength	64 24 23

5.3.8.3 Triplets for relative weightage of quality attribute and overall linguistic score of different beer samples

The triplets associated with the relative weightage of the beer samples are shown in Table 5.17 and the triplets for overall linguistic score of different beer samples are given in Table 5.18.

Table 5.17 Triplets for relative weightage of quality attribute

Quality attributes	Triplets for relative weightage
Colour	0.2340 0.0851 0.0709
Aroma	0.2660 0.0887 0.0745
Taste	0.2730 0.0887 0.0780
Alcoholic strength	0.2270 0.0851 0.0816

Table 5.18 Triplets for overall linguistic score of different beer samples

Beer samples	Triplets for overall linguistic score
RB	31.8121 30.9078 34.8014
KB	23.3830 24.6312 32.1383
KRB	74.9291 50.7908 40.1738
CB	32.6206 32.5532 35.0142
CRB	32.4504 30.4752 34.4291

Table 5.19 Similarity values for beer samples

Scale factors	RB	KB	KRB	CB	CRB
Not satisfactory	0.0388	0.1166	0.1174	0.1830	0.1091
Fair	0.1467	0.4265	0.4195	0.5128	0.4182
Satisfactory	0.3135	0.4783	0.4803	0.3840	0.4851
Good	0.6013	0.2902	0.3011	0.2174	0.2990
Very Good	0.6710	0.4223	0.4222	0.4502	0.4272
Excellent	0.2701	0.2053	0.2039	0.2189	0.2072

5.3.8.3 Similarity value of beer samples

Similarity values of beer samples are given in Table 19. The highest score i.e. 0.6710 was obtained for the factor of “very good” by the RB variety of beer. The subsequent highest score of 0.6013 was also obtained by the same beer for the “good” factor. This suggests that the beer prepared from rice i.e. RB had better sensory characteristics as compared to the other beers. The CRB variety of beer had the highest score of 0.4851 for the factor “satisfactory” followed by KRB (0.4803) and KB (0.4783). This signifies a similar pattern of sensory quality amongst these three types of beer. Only the beer CB had the highest score of 0.5128

for the factor “fair”, suggesting that this beer is less preferable over the remaining beers. Among all the varieties overall quality of rice beer is under the “very good” category. Beer obtained from *kachkal* starch is under “satisfactory” category. But when rice is integrated with *kachkal* the obtained beer is under “satisfactory” category but the similarity value is little bit higher which implies the positive effect of rice starch on sensory value. Beer obtained from cassava starch is under “fair” category from sensory analysis. Similarly when rice is integrated with cassava starch the similarity value of cassava-rice beer is increased and it rises to “satisfactory” category from “fair” category which also signifies the positive effect of rice on sensory value. The decreasing order of ranking of the beer samples are RB, CRB, KRB, KB, CB respectively.

5.4 Conclusions

The aim of the experimental design was to select a representative set of data from the entire lot. Statistical analysis was done to fit this data set in a mathematical model and to see its adequacy of fitting by correlation coefficient. The relationship between the dependent and independent variables were established and the significance of changes was seen through F-test. Time and temperature were found to have profound effect on the final biochemical and microbial properties of beer. The optimal conditions for the production of probiotic beer using starchy substrates and particular fermenting microbes were established, and these conditions were successfully applied in the production of beer from cassava and plantain. These optimal conditions obtained can thus be successfully applied in the production technology for a wide variety of beers from starchy substrates. This study also broadens the prospects of the particular bacterial and yeast strains to be utilized in the preparation of fermented alcoholic beverages. Implementation of fuzzy logic and the linguistic variables helped to rank the five beer varieties and beer made from rice clearly shown better sensory characteristics and acceptability as compared to the beer made from other substrates. Beer prepared from infusion of rice with cassava and *kachkal* evinced better sensory characteristics compared to substrates cassava and *kachkal* alone. The ranking of the beers shown rice beer under “very good” category, *kachkal* beer, *kachkal* rice beer and cassava rice beer under “satisfactory” category and cassava beer under “fair” category. In this study, a procedure for sensory evaluation, capable of describing the sensory profile of different varieties of beer is being elucidated and will help in precise documentation of the sensory properties and perception of the products prepared from various substrates. The brewing industry has

immense interest for effective and quicker sensory evaluation techniques for its products. Therefore, the fuzziness which is associated with the panellists' perception in the characterization of sensory profile of beers could be well represented using fuzzy logic and will make important contribution towards product development.

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