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Application of RSM for optimizing 7-day fermentation conditions in rice wine production

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ABSTRACT: Rice wine is an ancient fermented beverage crafted primarily from rice starch. In this research, fermentation conditions of rice wine were optimized until the 7th day of fermentation. To optimize the fermentation conditions, the influence of three levels of independent variables including inoculum concentration (1-5 per cent), sugar content (0-40 per cent), and water content (30-70 per cent) was assessed using response surface methodology (RSM) through a central composite design. The interactive effects of the independent variables on response variables such as total score, alcohol content, pH, TSS, wine yield, and total sugar was studied. The results indicated that optimal fermentation conditions involved an inoculum concentration of 3.41 per cent, sugar content of 34.68 per cent, and water content of 60.93 per cent. Desirable outcomes included a total score of 14.20, alcohol content of 6.09 per cent, pH of 3.17, TSS of 15.26Brix, total sugar of 6.41 per cent, and wine yield of 52.50 per cent, with a desirability value of 0.98 for rice wine.

Key words: Central composite design, fermentation,response surface methodology, rice wine

Rice wine, a traditionally significant fermented beverage derived from the alcoholic fermentation of cereal carbohydrates, primarily rice, holds deep historical and cultural roots across the world. Its production begins with the saccharification of rice starch by microorganisms or enzymes, yielding a spectrum of products including rice wine, beer, and vinegar. For millennia, rice wine has been integral to societies globally, with traditional varieties like Thai rice wines, Japanese Sake, and Korean *yakju* and *takju* deeply embedded in local customs.
Beyond its cultural significance, the increasing recognition of rice wine's potential health benefits positions its commercialization as a promising pathway for economic growth and public health initiatives. Consequently, optimizing the production process is paramount to ensure consistent quality, enhance efficiency, and fully harness its multifaceted potential the using Indian rice varieties.

This research investigates the rice wine production process with 7 days of fermentation using selected underutilized local rice varieties. The study also optimized the inoculum concentration,sugar content, and water content of the rice wine,specifically tailored for Indian production specifications and consumer preferences.

MATERIALS AND METHODS

Collection of rice varieties

In this study, one of the underutilized white rice varieties of Kerala, Neeraja, was selected for the optimization of fermentation conditions of rice wine. It was collected from the Regional Agricultural Research Station,Pattambi, Kerala Agricultural University. The rice grains were cleaned, dehusked, and then used for fermentation. Baker's yeast and all other necessary ingredients were purchased from the local market.

Preparation of starter culture with baker's yeast

Sugar was dissolved in lukewarm water at 10 g per 100 ml of water. Baker's yeast was then added at 5 g per 100 ml of the sugar solution. The culture was kept as such for 30 minutes for vigorous frothing.

The population was enumerated by serial dilution plating on the Sabouraud's Dextrose Agar media contained in petri plates.

Preparation of substrate

A precisely weighed quantity of Neeraja rice was prepared for fermentation through a sequence of steeping and enzymatic saccharification steps. Initially, the rice grains were steeped in distilled water for 5 hours at ambient room temperature (28±2˚C) to facilitate grain softening and enhance subsequent gelatinization and enzyme penetration. Following the steeping period, the hydrated rice was steamcooked for 90 minutes. This thermal treatment facilitated the gelatinization of starch granules, rendering them highly amenable to subsequent enzymatic breakdown. Subsequently, 250 g of the gelatinized rice was transferred to a sterile glass container, to which 100 mL of distilled water was added to achieve optimal moisture content for the enzymatic reaction and diffusion. One per cent of a prepared á-amylase solution was then thoroughly mixed with the rice-water mixture to ensure homogeneous enzyme distribution (Karthikeyan*et al*., 2014). The resulting reaction mixture was incubated at 60±2°C for 5 hours in a thermostatically controlled incubator, a temperature range conducive to the optimal activity of diastase á-amylase for efficient hydrolysis of starch into fermentable sugars.

Preparation of wine

The cooled hydrolysate underwent initial amelioration and pH adjustment by the addition of cane sugar to enrich fermentable content, followed by dilution with sterile water according to the experimental design. The substrate pH was then precisely adjusted to 3.5, by using foodgrade lactic acid, measured with a calibrated pH meter. To inhibit the activity of indigenous microflora, sodium sulphite was incorporated at a concentration of 200 ppm, and the treated mixture was held undisturbed for 4–5 hours at room temperature (28±2˚C) to facilitate antimicrobial action. Di-ammonium hydrogen phosphate (DAP) was supplemented at 0.5 g/L to provide essential nitrogen and phosphorus for optimal yeast metabolism and ethanol production (Karthikeyan *et al.,* 2014).

The prepared yeast culture was subsequently introduced into the rice substrate at varying inoculum concentrations as per the experimental design, followed by thorough mixing. Anaerobic fermentation was then conducted at a controlled temperature of 28±2°C for 7 days. The resulting rice wine was filtered through a double-folded, sterile muslin cloth, then transferred into clean, sterilized glass bottles. These bottles were subjected to pasteurization at 65 °C for 30 minutes, followed by the addition of 100 ppm sodium benzoate as a preservative to ensure microbiological stability. Finally, the clear wine was transferred into clean bottles and matured under controlled low-temperature conditions (15–16 °C).

Sensory evaluation

Rice wines were rated into different categories based on the 20-point evaluation scale developed by the American Wine Society, where the total score determines the wine's rating according to the established criteria (Amerine,1959).

Physicochemical evaluation

Standard digital pH meter was used in determining the pH of rice wine. Total soluble solids were found out using a digital refractometer (range 0-30) and expressed as degrees Brix (^{0} Brix).

Estimation of Alcohol

The ethanol was estimated by the colorimetric method as described by Caputi *et al*. (1968). One ml of representative samples from each treatment was transferred to a 250 ml roundbottom distillation flask connected to the condenser and diluted with 30 ml distilled water. The sample was distilled at 74 -75°C. The distillated sample was collected in 25 ml of 0.23 N $K_2Cr_2O_7$ reagent, which was kept at the receiving end. The distillate containing alcohol was collected till a total volume of 45 ml was obtained. Similarly, standards (05-25 mg ethanol) were mixed with 25 ml of $K_2Cr_2O_7$ separately. The distillate of samples and standards was heated in water bath at 60 °C for

20 minutes and cooled. The volume was made up to 50 ml with distilled water, and the optical density was measured at 600 nm using a spectrophotometer. The standard curve was plotted considering the concentration against absorbance.

Estimation of Total sugars (per cent)

The filtrate of 50 ml used in the estimation of reducing sugars was taken into a 100ml volumetric flask and 5 ml of concentrated HCl was added for hydrolysing the sample. Then the hydrolysed solution was neutralized with 20 per cent NaOH by using one or two drops of phenolphthalein. Diluted HCl was added till it became colourless. Finally, the volume was made up to 100ml and it was titrated against standard Fehlings solution using methylene blue as an indicator (Ranganna, 1997). The total sugars were calculated as given below.

$$\text{Total sugars (per cent)} = \frac{\text{Fehling's factor x 250 x dilution}}{\text{Titre value x 50 x weight of sample}} \text{x } 100 \quad \ldots (1)$$

Estimation of Wine yield

The wine yield was expressed as a per centage of the weight of wine to the initial weight of fermenting substrate, including rice sugar and water.

$$\text{Wine yield} = \frac{\text{Weight of wine obtained}}{\text{Initial weight of fermenting substrate}} \text{X } 100 \quad \ldots (2)$$

Experimental design for process optimization of rice wine using response surface methodology

Independent variables (factors) such as inoculum concentration (1– 5 per cent), sugar content, (0–40 per cent), and water content (30–70 per cent), and the dependent variables (responses) were total score, alcohol content, pH, TSS, total sugar and wine yield of rice wine. Every factor was selected based on the preliminary trials and available literature. Excessively low or excessively high levels of inoculum, sugar, or water content can significantly impair both fermentation efficiency and the sensory quality of rice wine. For instance, moderate inoculum amounts are necessary to avoid sluggish fermentation or off-flavors due to overproduction of higher alcohols, while sugar levels outside optimal ranges can lead to excessive residual sweetness or undesirable acidity. Similarly, improper water ratios can hinder saccharification by decreasing enzyme efficacy, reducing nutrient availability, and impairing microbial metabolism all of which can compromise flavor, yield, and overall quality (Wang *et al.,* 2022). Experimental runs were obtained by using grapesAgri1 package in R (Gopinath *et al.,* 2020). Central Composite Design (CCD) was used to optimize the process conditions. The experimental design consisted of 20 runs with six centre points (Table 1).

The quadratic model was used to describe the response variables as per the following equation:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 \quad \ldots\ldots (3)$$

Where Y is the response, β_0 is the constant β_1 β_2, β_3 are linear coefficients β_{11} β_{22}, β_{33} are the squared coefficients, β_{12} β_{13}, β_{23} is the interaction coefficient. X_1 is the inoculum concentration, X_2 is the sugar content, X_3 is the water content.

Analysis of variance (ANOVA) was performed to check the significance model and process variables. The experiment data were run in triplicate for each run, and average values determined by fitting experimental data into a quadratic model of Equation 3.

Optimization technique

The numerical optimization technique was used for the simultaneous optimization of multiple responses. The desired goals set for each response were as follows: total score, alcohol content, and wine yield: Maximize. pH TSS, and total sugar: In range. The independent factors were kept within the experimental range. The desirability function was used to search for a solution for multiple responses, where all the goals were combined into an overall composite function as given below (Sharma and Khanna, 2013):

D (x) = (4) (d1 × d2 ×……. × dn) 11/n n . . . . (4)

where D (x) is the desirability function, d1, d2…dn are the responses, and n' is the total number of responses. The adequacies of the models were determined using model analysis, the lack-of-fit test, coefficient of determination (R^2), coefficient of variation (CV), and mean relative per cent deviation modulus (P).

Validation of models

Experimental validation of the optimized processing conditions was undertaken by preparing rice wine across a range of inoculum, sugar, and water combinations suggested by the Response Surface Methodology (RSM) software. The resulting experimental responses were then systematically compared to the predicted values derived from the RSM model. To ensure the model's validity, additional experiments were performed specifically under the established optimal conditions,and the relative per cent deviation modulus (P) was calculated according to Kaur *et al*. (2020) to evaluate the concordance between predicted and observed data.

$$\text{per cent} = \frac{\text{Predicted value} - \text{Actual value}}{\text{Predicted value}} \times 100. \quad (5)$$

RESULTS AND DISCUSSION

Experimental model fitting for process optimization of rice wine

Central Composite Design (CCD) with six replications at the centre point was used to optimize the process conditions during rice wine fermentation. The experimental design with various factors such as inoculum concentration (A), sugar content (B) and water content (C), along with responses such as organoleptic score, alcohol content, pH, TSS, total sugar and wine yield presented in Table 1. The experiment data were run in triplicate for each run. The total score varied from 4.81 to 14.06, alcohol content varied from 2.62 to 6.24 per cent, pH varied from 3.09 to 3.58, TSS varied from 11.07 to 17.56 Brix, total sugar varied from 4.24 to 7.9 per cent and wine yield varied from 43.47 to 52.5 per centduring fermentation process concerning variation in inoculum concentration, sugar content and water content. To choose the best model for fermentation process, the actual obtained data was fit into various regression models. The quadratic response surface model was fitted to each response variable (Eq. 3). Regression analysis and ANOVA were used to fit the model data and to examine the statistical significance of terms. The values of $R^2$and CV were determined to evaluate the adequacy of the selected model. The p-values (level of significance) were used as the tool to check the significance of every coefficient, which was necessary to understand the pattern of mutual interactions of test variable (Koocheki *et al*., 2014).

From ANOVA results (Table 2) show all the fermentation conditions of rice wine process were significantly dominated by higher F-values in positive quadratic terms, 31.65, 24.18,188.48,19.39,24.86 and 41.73for total score of organoleptic evaluation, alcohol content, pH, TSS, total sugar and wine yield respectively. The lack of fit test did not result in a significant F-value (Table 2) for all responses indicating that the models were adequate for predicting the responses. The standard deviationfor all responses were 0.7546, 0.2995, 0.0138, 0.4791, 0.2452, 0.5496 and the coefficient of determination (R_2) value was obtained as 0.9661, 0.9561, 0.9941, 0.9458, 0.9572, 0.9741for total score, alcohol content, pH, TSS, total sugar and wine yield, respectively. The R^2 value above 80 per cent indicates a good fit for the model. The CV values of 7.06, 6.18, 0.4103, 3.59, 4.47, and 1.16 for all the responses were found to be below 10 per cent. Higher R^2 value and low p-value (≤ 0.05) indicated the selected quadratic model for each response factor was highly significant and sufficient to represent the relationship between the process and response variables.

Total score

Total score of organoleptic evaluation observed in rice wines was found to be significantly influenced by inoculum concentration, sugar content, and water content. The highest total score (14.06) was obtained when the experiential combination included an inoculum concentration of 4.18 per cent, sugar content of 31.89 per cent, and water content of 61.89

per cent. In contrast, the lowest total score (4.81) was recorded at inoculum concentration of 3 per cent, sugar content 0 per cent, and water content was 50 per cent (as shown in Table. No.1). The analysis of variance was performed to understand the significance of linear, quadratic, and two-way interactions of different ingredient levels in the total score, The analysis of variance (ANOVA) table (Table.2), which indicates the overall significance of the model for total score (F= 31.65) Moreover, the linear terms of sugar content (F= 155.22) exhibited higher impact on total score followed by water content (F=55.98). The interaction terms of sugar content and water content (BC, F= 3.97)) have high impact on total sugar, followed by AB and AC. The model equation predicting the total score of rice wine influenced by inoculum concentration (A), sugar content (B) and water content (C) is presented as:

$$\text{Total score} = 12.73+0.5004\ A+4.28B+2.57C+0.3430AB - 0.3218AC-1.50BC - 3.01A^2 - 3.48\ B^2 - 1.98\ C^2 \quad \text{.........} (6)$$

Additionally, the 3D response surface plots illustrating the interactive effects of inoculum concentration, sugar content, and water content on the total score, consistently reveal a non-linear, quadratic relationship characterized by optimal points for each factor. This indicates that maximum or minimum concentration for any given parameter leads to suboptimal total scores while intermediate levels are crucial for maximizing the response.

Specifically, across all three plots (Fig.1a, 1b, 1c), the highest total score is achieved when inoculum concentration, sugar content, and water content are maintained within specific optimal ranges. For instance, when water content was fixed at 50 per cent (Fig.1a), an optimal Total score was observed at approximately 3-4 per cent inoculum concentration and 30-40 per cent sugar content. Similarly, fixing sugar content at 20 per cent (Fig.1b) yielded the highest scores with inoculum concentration around 3-4 per cent and water content between 50-60 per cent. Lastly, at a constant inoculum concentration of 3 per cent (Fig.1c), optimal total scores were attained with sugar content around 20-30 per cent and water content around 50-60 per cent.

The interactions between these three factors are significant and their effects on the total score are interconnected. Excessively low or high inoculum levels can hinder fermentation efficiency and sensory quality, as indicated by studies on rice wine sugar concentration and inoculum size work synergistically to influence fermentation kinetics and flavor development. Elevated sugar levels enhance yeast metabolism by providing ample substrate, while the inoculum level modulates the production of organic acids and esters, thereby shaping the sensory profile. Furthermore, both the quality and quantity of water play a crucial role in saccharification efficiency and flavor balance by affecting enzymatic activity and microbial dynamics, ultimately leading to variations in acidity, residual sugar, alcohol content, and overall sensory complexity (Peng *et al.,* 2025).

Alcohol content

Alcohol content observed in rice wines was significantly influenced by inoculum concentration, sugar content, and water content. The highest alcohol content (6.24 per cent) was obtained with an inoculum concentration of 4.18 per cent, sugar content of 31.89 per cent, and water content of 61.89 per cent. This was followed by an alcohol content of 6.04 per cent at an inoculum concentration of 3 per cent, sugar content of 40 per cent, and water content of 61.89 per cent. In contrast, the lowest total score (2.62) was recorded at an inoculum concentration of 1.81 per cent, sugar content of 8.10 per cent, and water content of 38.10 per cent (as shown in Table. No.1). Analysis of variance was performed to understand the significance of linear, quadratic, and two-way interactions of different ingredient levels on alcohol content. The ANOVA table (Table.2) indicates the overall significance of the model for alcohol content (F= 24.18). Moreover, the linear terms of sugar content (F= 130.01) had a higher impact on the total score, followed by water content (F=17.33). The interaction terms of inoculum concentration and water content (AC, F= 5.74) have high impact on alcohol content, followed by BC and AB. The model equation predicting the total score of rice wine influenced by inoculum concentration (A), sugar content (B), and water content (C) is presented as:

$$\text{Alcohol content} = 5.55 + 0.4274\,A + 1.55\,B + 0.5676\,C - 0.4562\,AB + 0.7179\,AC - 0.4774\,BC - 1.16\,A^2 - 1.01\,B^2 - 0.7493C^2 \quad \ldots\ldots\ldots (7)$$

These three 3D surface plots (Fig.2a, 2b, 2c) consistently demonstrate that the highest alcohol content is achieved when inoculum concentration, sugar content, and water content are maintained within specific optimal ranges. Across all figures, a clear dome-shaped response surface indicates that an optimal balance of these factors is crucial for maximizing alcohol yield. When water content was held constant at 50 per cent (Fig. 2a), the optimal alcohol content was observed with an inoculum concentration of approximately 3–4 per cent and a sugar content of 25-35 per cent. When the sugar Content was fixed at 20 per cent (Fig. 2 b), the highest alcohol yields were obtained with an inoculum concentration around 3-4 per cent and a water content between 50-60 per cent. This indicates the critical balance between microbial population and water availability. At a constant inoculum concentration of 3 per cent (Fig. 2c), optimal alcohol content was attained with a sugar content around 25-35 per cent a water content around 50-60 per cent. Alcohol yield exhibits a dome-shaped response to increasing sugar levels, at moderate sugar concentrations before declining as excessive osmotic stress impairs yeast viability and fermentation efficiency (Spinosa *et al.,* 2016) Wang *et al.* (2022) reported that controlling yeast species and inoculum concentration is essential for modulating higher alcohol production (isobutanol, isoamyl alcohol) since both yeast strain composition and population density affect alcohol profiles during fermentation.

pH

The effect of different levels of inoculum concentration, sugar content, and water content, and the respective pH obtained, was fitted to a second-order model, and the goodness of fit was examined. The highest pH (3.58) was obtained at an inoculum concentration of 1 per cent, sugar content 20 per cent, and water content was 50 per cent. In contrast, the lowest pH (3.09) was recorded at an inoculum concentration of 4.18 per cent, sugar content of 31.89 per cent, and water content was 61.89 per cent (Table No.1). The analysis of variance was performed to understand the significance of linear, quadratic, and two-way interactions of different ingredient levels in the pH. The analysis of variance (ANOVA) table (Table 2), which indicates the overall significance of the model for pH (F= 188.48). Moreover, the linear terms of inoculum concentration (F= 1236.05) exhibited higher impact on pH followed by sugar content (F= 382.23). The interaction terms of inoculum concentration and sugar content (AB, F= 29.15)) have high impact on pH, followed by BC and AC. The model equation predicting the pH of rice wine influenced by inoculum concentration (A), sugar content (B), and water content (C) is presented as:

$$\text{pH} = 3.36 - 0.2201\,A - 0.1224\,B - 0.0375\,C + 0.0177\,AC - 0.0389\,BC - 0.0032\,A^2 + 0.0018\,B^2 - 0.0182\,C^2 \quad \ldots\ldots\ldots (8)$$

These three 3D surface plots (Fig. 3a, 3b, 3c) consistently demonstrate that pH levels decrease as conditions for alcohol production become more optimal. Across all figures, a clear trend shows that an increase in factors promoting vigorous fermentation leads to a lower pH. This is a direct result of increased metabolic activity by microorganisms, which produce acidic by products.

When water content was held constant at 50 per cent (Fig. 3a), the pH generally decreased as both inoculum concentration and sugar content increased. The lowest pH values were observed at approximately 3–4 per cent inoculum and 30–40 per cent sugar content, suggesting that ample microbial activity combined with sufficient substrate leads to greater acid production. When sugar content was fixed at 20 per cent (Fig. 3b), pH consistently decreased with increasing inoculum concentration, particularly in the 4–5 per cent range. While the effect of water content was less pronounced, pH tended to be lower at 50–70 per cent water, especially when combined with higher inoculum levels. This indicates that a balanced microbial population and adequate water facilitate the production of acidic compounds. At a constant inoculum concentration of 3 per cent (Fig.3c), pH generally decreased as both sugar content and water content increased. The lowest pH values occurred with a sugar content of 25–35 per cent and water content of 50–60 per cent.

A higher inoculum concentration generally means more active yeast cells are present initially, leading to a faster and more vigorous fermentation onset. This accelerates the production of organic acids, resulting in a more rapid and pronounced pH drop. Likewise, sufficient sugar content (30–40 per cent) provides a rich substrate for sustained microbial metabolism. As sugars are consumed, by-products such as organic acids accumulate, further lowering the pH. During yeast fermentation, the primary organic acids produced include lactic, succinic, acetic, and pyruvic acids, arising from both yeast and bacterial activity. These acids directly influence the acidity profile of rice wine, impacting its taste, balance, and quality. Liu. *et al*. (2022) reported that, lactic acid levels increased from approximately 27.1 mg/L on day 1 to over 8,000 mg/L by the end of fermentation. This substantial lactic acid production imparts a soft sour tone and contributes to flavour balance via malolactic reactions. Thus, while achieving a lower pH is a key goal for optimal fermentation, a balanced approach involving appropriate inoculum concentration, sugar, and water content is essential for successful rice wine production.

TSS

The effect of different levels of inoculum concentration, sugar content, and water content, and the respective TSS obtained, was fitted to a second-order model, and the goodness of fit was examined. The highest TSS (17.56 Brix) was obtained at an inoculum concentration of 3 per cent, sugar content 40 per cent, and water content was 50 per cent. In contrast, the lowest TSS (11.07) was recorded at an inoculum concentration of 3 per cent, sugar content of 0 per cent, and water content was 50 per cent (Table 1). The analysis of variance was performed to understand the significance of linear, quadratic, and two-way interactions of different ingredient levels in the TSS, The analysis of variance (ANOVA) table (Table 2), which indicates the overall signifi-

Table 1: Experimental data of process optimisation of rice wine

Expt. Runs	Variable levels			Responses					
	Inoculum conc. (A)	Sugar content (B)	Water content (C)	Total score	Alcohol content (per cent)	pH	TSS (B^0)	Total sugar (per cent)	Wine yield (per cent)
1	1.811	8.109	38.109	5.22	2.62	3.54	13.06	4.75	43.98
2	4.189	8.109	38.109	5.34	3.24	3.32	12.56	4.51	44.85
3	1.811	31.891	38.109	11.15	5.49	3.47	15.53	6.65	47.01
4	4.189	31.891	38.109	12.44	4.85	3.15	14.25	6.32	47.12
5	1.811	8.109	61.891	9.42	3.67	3.51	12.23	4.89	49.43
6	4.189	8.109	61.891	9.77	4.69	3.32	11.56	4.75	49.44
7	1.811	31.891	61.891	13.91	5.25	3.39	15.06	6.22	52.02
8	4.189	31.891	61.891	14.06	6.24	3.09	14.39	5.89	51.76
9	1	20	50	8.48	3.96	3.58	14.25	5.72	47.54
10	5	20	50	9.76	4.84	3.13	13.3	5.15	48.98
11	3	0	50	4.81	3.06	3.48	11.07	4.24	45.55
12	3	40	50	12.5	6.04	3.24	17.56	7.9	50.78
13	3	20	30	7.82	4.53	3.38	13.06	5.05	43.47
14	3	20	70	12.49	5.1	3.3	12.05	4.51	52.5
15	3	20	50	12.53	5.9	3.34	13.25	5.42	47.88
16	3	20	50	12.96	5.54	3.35	13.19	5.44	48.78
17	3	20	50	12.8	5.04	3.39	12.5	5.432	48.01
18	3	20	50	13.24	5.54	3.36	12.7	5.45	46.98
19	3	20	50	13.05	5.54	3.34	12.5	5.43	48.28
20	3	20	50	12.02	5.74	3.36	12.9	5.94	48.78

cance of the model for TSS (F= 19.39) Moreover, the linear terms of sugar content (F= 137.16) exhibited higher impact on TSS followed by inoculum concentration (F= 7.10).). The interaction terms of sugar content and water content (BC, F= 1.23)) have high impact on TSS, followed by AB and AC. The model equation predicting the TSS of rice wine influenced by inoculum concentration (A), sugar content (B), and water content (C) is presented as:

$$TSS = 12.84 - 0.5810\,A + 2.55\,B - 0.4752\,C - 0.2758\,AB + 0.1556\,AC - 0.5304\,BC + 0.9284\,A^2 + 1.47\,B^2 - 0.2916\,C^2 \quad \text{....... (9)}$$

The three-dimensional response surface plots (Figures 4a, 4b, and 4c) provide comprehensive insights into the interactive effects of inoculum concentration (A), initial sugar content (B), and water content (C) on the final Total Soluble Solids (TSS) measured in Brix.

Figure (4a) showed that at 50 per cent water content, higher inoculum (4–5 per cent) and initial sugar (30–40 per cent) concentrations resulted in the lowest Brix values, indicating maximal substrate conversion. In Figure (4b), inoculum concentration was identified as the primary driver for Brix reduction at 20 per cent fixed sugar content, with moderate to high water content (50–70 per cent) also contributing to lower Brix. Figure (4. c) further revealed that, even with a constant 3 per cent inoculum, increasing both initial sugar (30–40 per cent) and water content (50–70 per cent) significantly reduced final Brix. These findings collectively emphasize that efficient sugar utilization, characterized by minimal residual Brix, is achieved through a synergistic interplay of optimal inoculum concentration, initial sugar content, and water activity. Rodrigues *et al*. (2015) reported that, reduction in Brix is also influenced by initial sugar concentrations where higher sugar levels correlate with lower residual Brix and sugar concentrations above 250 g/L can inhibit fermentation efficiency due to the Crabtree effect, emphasizing the need for balanced sugar levels.

Total sugar

The effect of different levels of inoculum concentration, sugar content, and water content, and the respective total sugar obtained, was fitted to a second-order model, and the goodness of fit was examined. The highest total sugar (7.9 per cent) was obtained at an inoculum concentration of 3 per cent, sugar content 40 per cent, and water content was 50 per cent. In contrast, the lowest total sugar (4.24) was recorded at an inoculum concentration of 3 per cent, sugar content of 0 per cent, and water content was 50 per cent (Table 1). The analysis of variance was performed to understand the significance of linear, quadratic, and two-way interactions of different ingredient levels in the total sugar, The analysis of variance (ANOVA) table (Table 2), which indicates

Table 2: The analysis of variance (ANOVA) table of process optimization of rice wine

Source	Total score		Alcohol content		pH		TSS		Total sugar		Wine yield	
	F -value	p-value	F -value	p-value	F -value	p-value	F -value	p-value	F -value	p-value	F -value	p-value
Model	31.65	< 0.0001	24.18	< 0.0001	188.48	< 0.0001	19.39	< 0.0001	24.86	< 0.0001	41.73	< 0.0001
A – Inoculum conc.	2.12	0.1758	9.83	0.0106	1236.05	< 0.0001	7.10	0.0237	4.87	0.0519	2.34	0.1570
B – Sugar content	155.22	< 0.0001	130.01	< 0.0001	382.23	< 0.0001	137.16	< 0.0001	185.34	< 0.0001	85.13	< 0.0001
C - Water content	55.98	< 0.0001	17.33	0.0019	35.91	0.0001	4.75	0.0543	2.35	0.1565	286. .67	< 0.0001
AB	0.2065	0.6592	2.32	0.1588	29.15	< 0.0003	0.3313	0.5776	0.1630	0.6949	0.4268	0.5283
AC	0.1818	0.6789	5.74	0.0376	1.65	0.2276	0.1054	0.7521	0.0208	0.8882	0.6087	0.4534
BC	3.97	0.0745	2.54	0.1422	8.00	0.0179	1.23	0.2942	3.20	0.1041	0.0612	0.8096
A^2	28.71	0.0003	27.21	0.0004	0.0992	0.7593	6.76	0.0265	0.0459	0.8347	0.1869	0.6747
B^2	38.25	< 0.0001	20.65	0.0011	0.0299	0.8661	16.92	0.0021	10.64	0.0085	0.0414	0.8428
C^2	12.37	0.0001	11.27	0.0073	3.16	0.1056	0.6675	0.4330	14.44	0.0035	0.0528	0.8229
Lack of fit	4.95	0.0519	1.14	0.441	0.0912	0.9900	3.21	0.1131	1.82	0.2644	0.3711	0.8497
R^2	0.9661		0.9561		0.9941		0.9458		0.9572		0.9741	
C. V (per cent)	7.06		6.18		0.4103		3.59		4.47		1.16	

Lack of fit is non-significant at p = 0.05, Significant at p = 0.05

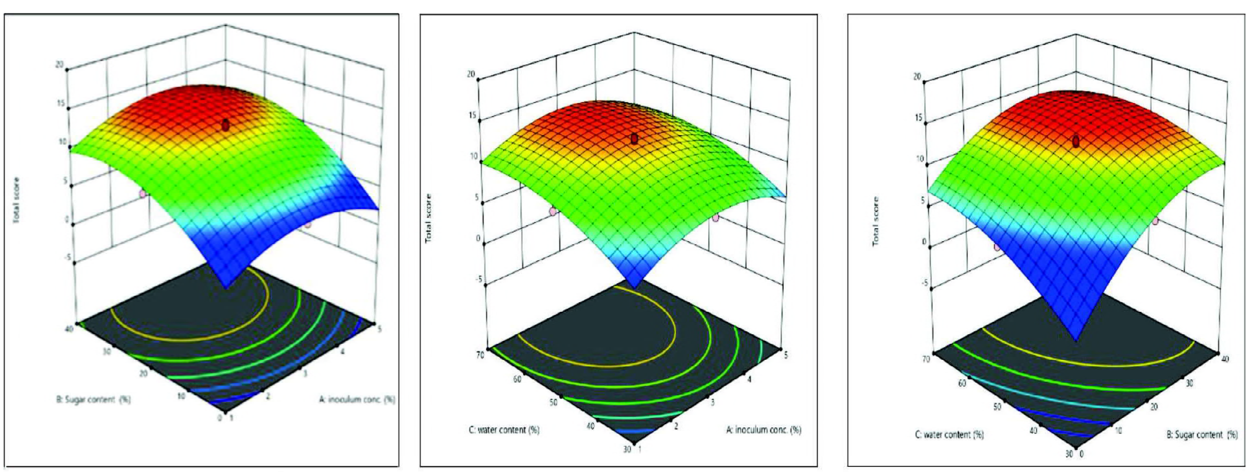
the overall significance of the model for total sugar (F= 24.86) Moreover, the linear terms of sugar content (F= 185.34) exhibited higher impact on total sugar followed by inoculum concentration (F= 4.87).). The interaction terms of sugar content and water content (BC, F= 3.20)) have high impact on total sugar, followed by AB and AC. The model equation predicting the total sugar of rice wine influenced by inoculum concentration (A), sugar content (B), and water content (C) is presented as:

$$\text{Total sugar} = 5.52 - 0.2462\,A + 1.52\,B - 0.1710\,C - 0.0990\,AB + 0.0354\,AC - 0.4385\,BC - 0.0391\,A^2 + 0.5959\,B^2 - 0.6941C^2 \quad \ldots\ldots (10)$$

The three-dimensional response surface plots (Figures 5a, 5b, and 5c) provide comprehensive insights into the interactive effects of inoculum concentration (A), initial sugar content (B), and water content (C) on the final total sugar. Figure (5a) revealed that at 50 per cent water content, higher inoculum concentrations (4-5 per cent) and initial sugar contents (30-40 per cent) led to the lowest residual sugar values. In Figure (5b), with fixed initial sugar at 20 per cent, residual sugar primarily decreased with increasing inoculum concentration, a trend augmented by moderate to higher water content (50-70 per cent). Furthermore, Figure (5c) illustrates that at a constant 3 per cent inoculum, increasing both initial sugar content (30-40 per cent) and water content (50-70 per cent) significantly reduced residual sugar. These response surface analyses consistently demonstrate that efficient sugar utilization, characterized by low residual total sugar per centages, is achieved through a synergistic optimization of inoculum concentration, initial sugar content, and water content. Higher initial sugar content leads to increased total sugar during fermentation. For instance, sweet glutinous rice wine fermentation shows a rapid increase in total sugar content during the first 48 hours, before declining and also water content is crucial for enzymatic activity during fermentation. Adequate moisture facilitates the breakdown of starch into sugars, enhancing the fermentation process (Li *et al.,* 2021).

Wine yield

The effect of different levels of inoculum concentration, sugar content, and water content, and the respective wine yield obtained, was fitted to a second-order model, and the goodness of fit was examined. The highest wine yield (52.5 per cent) was obtained at an inoculum concentration of 3 per cent, sugar content 20 per cent, and water content was 70 per cent. In contrast, the lowest wine yield (43.47) was recorded at an inoculum concentration of 3 per cent, sugar content of 20 per cent, and water content was 30 per cent (Table 1). The analysis of variance

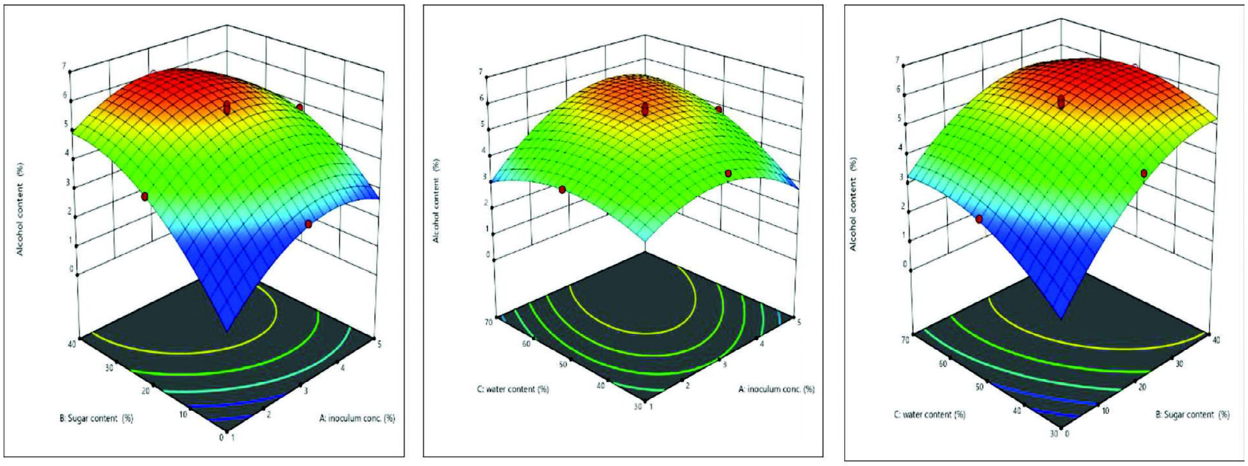


a. Inoculum concentration and sugar content (per cent)

b. Inoculum concentration and water content (per cent)

c. Sugar content and water content (per cent)

Fig.1: Effect of inoculum concentration, sugar content and water content on total score of rice wines

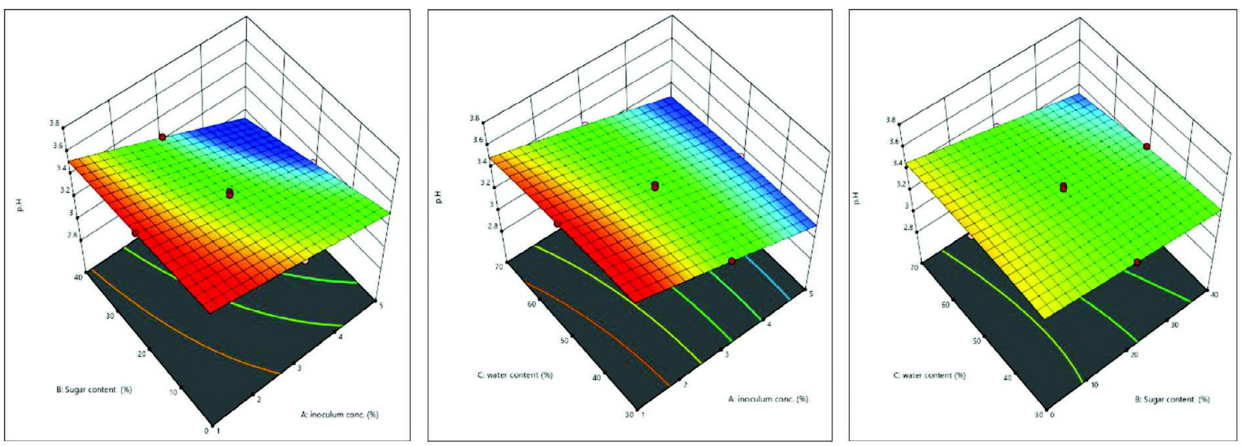


a. Inoculum concentration and sugar content (per cent)

b. Inoculum concentration and water content (per cent)

c. Sugar content and water content (per cent)

Fig.2: Effect of inoculum concentration, sugar content and water content on alcohol content of rice

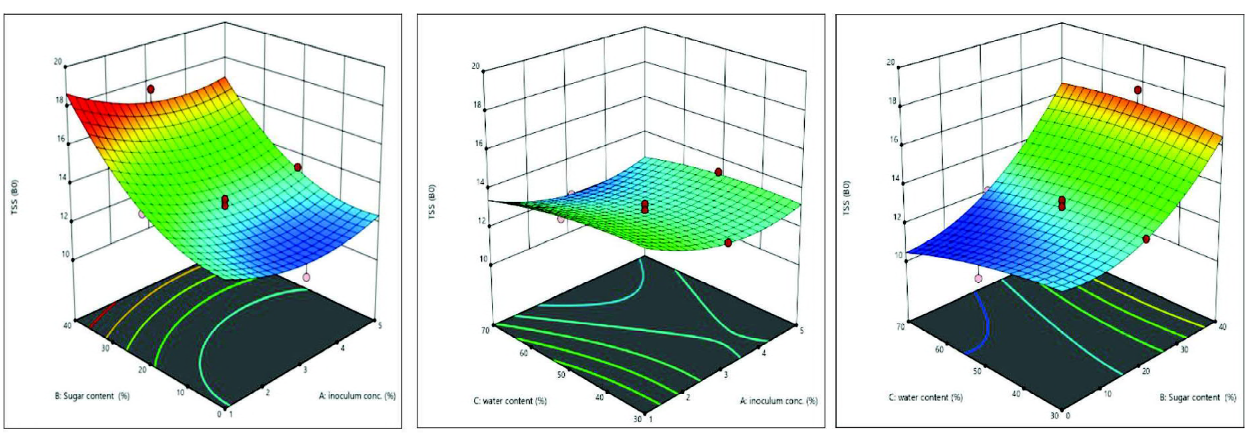


a. Inoculum concentration and sugar content (per cent)

b. Inoculum concentration and water content (per cent)

c. Sugar content and water content (per cent)

Fig.3: Effect of inoculum concentration, sugar content and water content on pH of rice wines

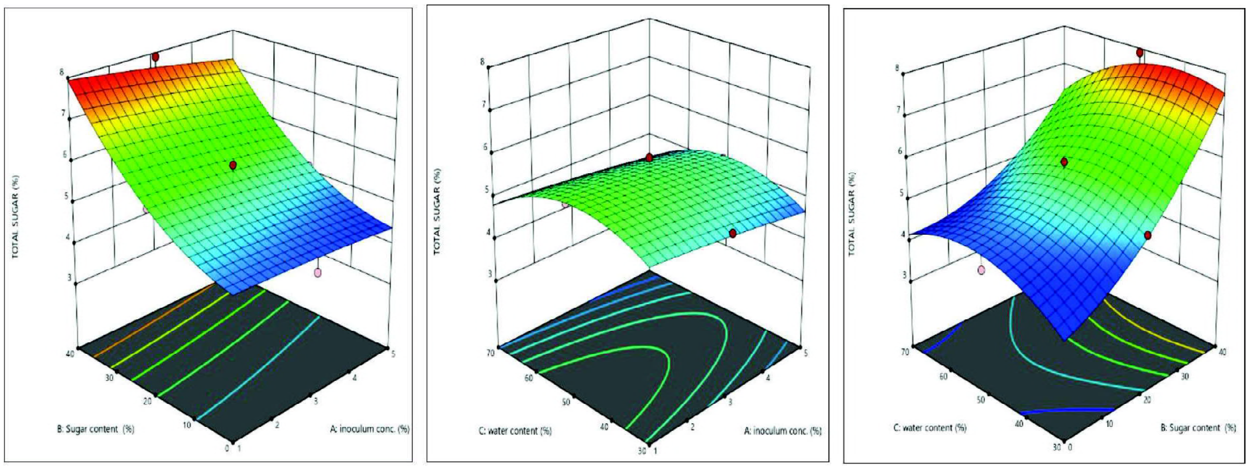


a. Inoculum concentration and sugar content (per cent)

b. Inoculum concentration and water content (per cent)

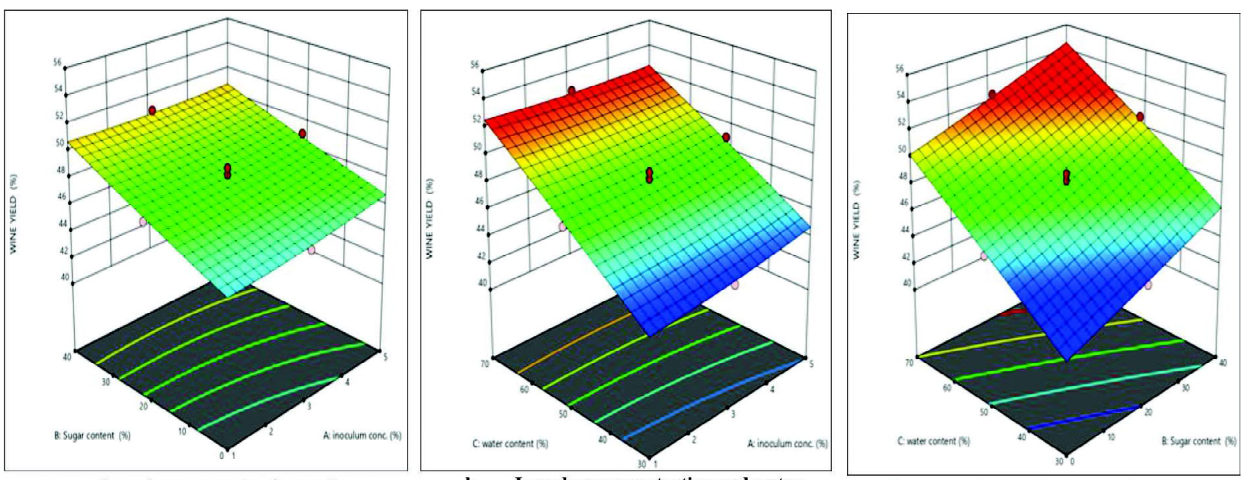
c. Sugar content and water content (per cent)

Fig.4: Effect of inoculum concentration, sugar content and water content on TSS of rice wines



a. Inoculum concentration and sugar content (per cent) **b. Inoculum concentration and water content (per cent)** **c. Sugar content and water content (per cent)**

Fig.5 Effect of inoculum concentration, sugar content and water content on total sugar of rice wines



a. Inoculum concentration and sugar content (per cent) **b. Inoculum concentration and water content (per cent)** **c. Sugar content and water content (per cent)**

Fig.6: Effect of inoculum concentration, sugar content and water content on wine yield of rice wines

was performed to understand the significance of linear, quadratic, and two-way interactions of different ingredient levels in the wine yield, The analysis of variance (ANOVA) table (Table2), which indicates the overall significance of the model for wine yield (F= 41.73) Moreover, the linear terms of water content (F= 286.67.) exhibited higher impact on wine yield followed by sugar content (F= 85.13).). The interaction terms of inoculum concentration and water content (AC, F= 3.97)) have high impact on wine yield, followed by AB and BC. The model equation predicting the wine yield of rice wine influenced by inoculum concentration (A), sugar content (B), and water content (C) is presented as:

$$\text{Wine yield} = 48.12 + 0.3882\,A + 2.34\,B + 4.30\,C - 0.3643\,AB - 0.4350\,AC - 0.1379\,BC + 0.1795\,A^2 + 0.0846B^2 - 0.0954\,C^2 \quad \text{………. (11)}$$

This study investigated the combined effects of inoculum concentration, initial sugar content, and water content on wine yield, revealing consistent positive correlations across the fermentation process. Figure 7a demonstrated that at 50 per cent water content, wine yield significantly increased with higher

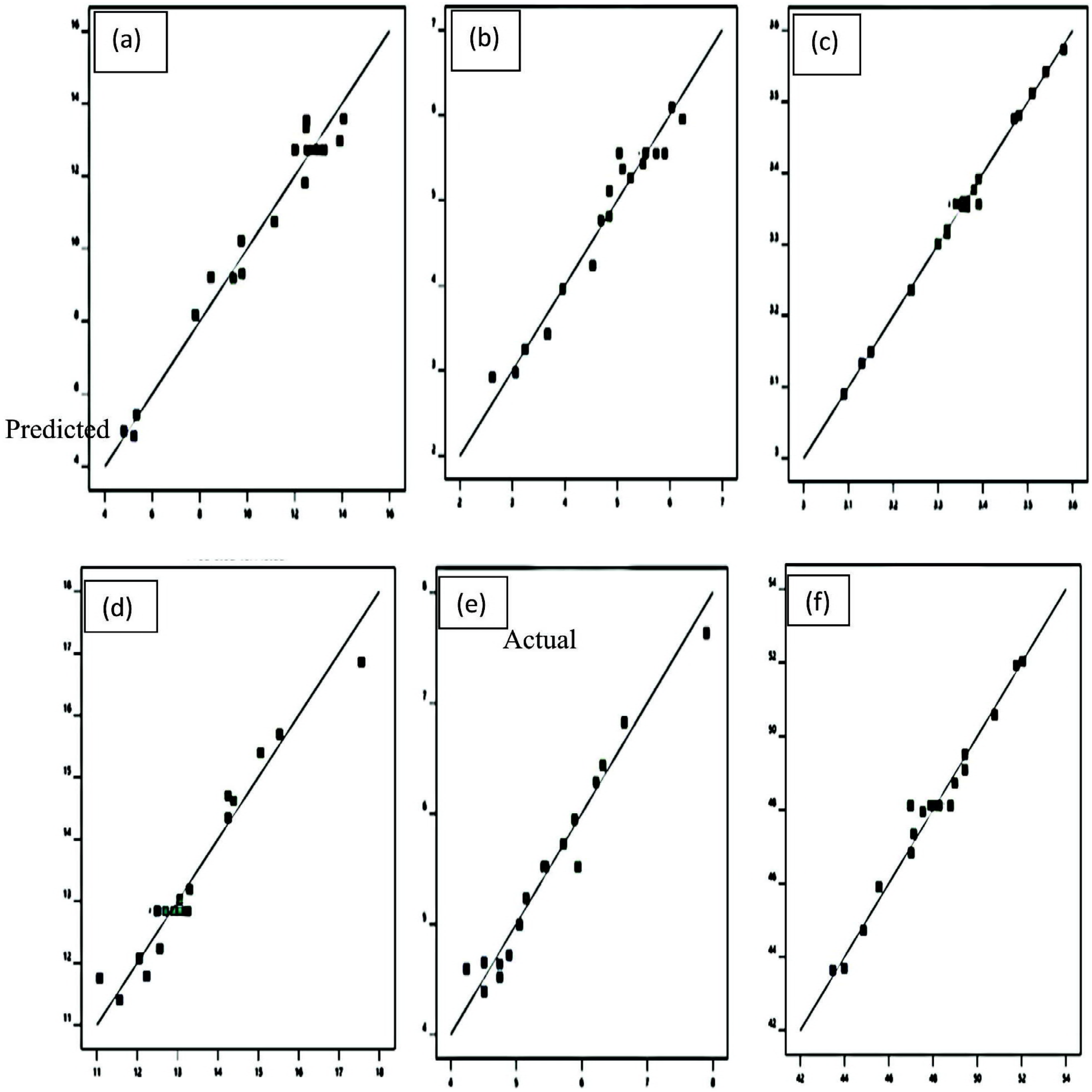


Fig.7: Comparison between predicted and actual values of Total score (a), Alcohol content (b), pH (c), TSS (d), Total sugar (e), and wine yield (f)

inoculum concentrations (4-5 per cent) and initial sugar contents (30-40 per cent). Similarly, Figure 7b showed that with a fixed initial sugar content of 20 per cent, higher inoculum concentrations (4-5 per cent) and water content (60-70 per cent) maximized wine yield, highlighting the crucial balance between microbial load and aqueous environment. Furthermore, Figure 7c illustrates that at a constant 3 per cent inoculum, wine yield consistently increased with higher initial sugar content (30-40 per cent) and water content (60-70 per cent). These findings collectively underscore that providing ample fermentable substrate, a robust microbial population, and optimal hydration are paramount for optimal fermentation. Mao *et al*. (2023) observes that variations in water content during saccharification and fermentation stages influence yield, acidity, and ester profile which contributing to the balance of sweet, sour, and bitter sensory notes.

Numerical Optimization

Optimal conditions were explored by numerical optimisation techniques. Here the aim is to optimize total score, alcohol content and wine yield by keeping TSS, total sugar and pH in intermediate range. Optimal fermentation conditions involved inoculum concentration of 3.41 per cent, sugar content of 34.68 per centand water content of 60.93 per cent. At the mentioned conditions, response variables of total score, alcohol content, pH, TSS, total sugar and wine yield were 14.20, 6.09 per cent, 3.17, 15.26 Brix, 6.41 per cent and 52.05 per cent,respectively, with desirability of 0.98.

Validation of optimized processing conditions

The model was validated by conducting experiments using the derived optimum processing conditions and their responses were also determined. The per centage error (per centP) of various responses such as total score, alcohol content, pH, TSS total sugar and wine yield were0.07 per cent,3.1 per cent,0.3 per cent, 0.5 per cent, 1.4 per centand0.86 per cent respectively, suggesting that the experimental data were in goodagreementwiththepredictedvalues and the response surface optimization model was adequate (Fig.7).

CONCLUSION

The present study successfully optimized fermentation conditions for 7-day rice wine production using a Central Composite Design (CCD) model under Response Surface Methodology (RSM). By analyzing the effects of inoculum concentration, sugar content, and water content on key quality parameters, including sensory attributes, alcohol content, pH, and yieldthe model provided optimum predictive equations. Validation experiments confirmed the model's accuracy, demonstrating strong agreement between predicted and observed values. These findings offer a valuable framework for standardizing traditional rice wine production, enhancingboth consistency and product quality. Furthermore, the approach can be extended in future research to explore the impact of different rice varieties and starter cultures, or prolonged fermentation periods beyond seven days, which potentially unlock novel flavour profiles and increase alcohol yield.

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