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MODELING AND OPTIMIZATION OF SACCHARIFICATION AND FERMENTATION OF BROKEN RICE

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ABSTRACT

Rice wine is a traditional alcoholic beverage derived from fermented glutinous rice or broken rice. The method is separated into two steps: first, the rice is cooked and liquefied/saccharified by molds and enzymes, followed by fermentation. The study examined how *Aspergillus oryzae* (0.1 - 0.2%) and α-amylase (0.01 - 0.04%) affect starch liquefaction and saccharification, as well as how *Saccharomyces bayanus* concentration (0.02 - 0.05%) and total soluble solids content (22 - 26%) impact rice wine fermentation. To improve process prediction and optimization, an artificial neural network integrated with a genetic algorithm (ANN-GA) was applied to model the nonlinear relationships between process variables and fermentation performance. The optimization approach utilizing a machine learning-based model demonstrated better prediction ability. Compared with conventional regression approaches, the ANN-GA model provided improved predictive accuracy and enabled the identification of optimal processing conditions for both saccharification and fermentation stages. The optimum content of *Aspergillus oryzae* and α-amylase was 0.181% and 0.036%, respectively, resulting in high starch saccharification efficiency with a total soluble solids content of 27.2°Brix. The volume of sugar solution achieved was 34.01 mL (from 50 g rice, yield 68.02%). In addition, using the optimal content of *Saccharomyces bayanus* of 0.043% and fermenting in an environment with high soluble solids content of 24.88°Brix produced wine with high ethanol and ester content, 12.19% by volume and 0.93 g/L, respectively. The methanol content of the fermented product under these optimal conditions was lower (49.8 mg/L). These findings demonstrate that the integration of machine-learning-based optimization can effectively enhance fermentation efficiency while maintaining product safety. Overall, the optimized saccharification and fermentation parameters provide a viable approach for producing rice wine with higher quality and safety assurances for this traditional product.

Keywords: broken rice, rice wine, optimization, saccharification, fermentation, modeling

INTRODUCTION

Vietnam's economy is primarily agricultural, with rice (*Oryza sativa*) being the main product. Rice kernels are composed of ~20% rice husk, 11% rice bran, and 69% starchy endosperm (milled rice) **(Dhankhar & Hissar, 2014)**. Rice milling generates a number of byproducts, including husks, bran, milled bran, and broken rice; around 20% of the rice is broken. Broken rice is a key raw material in the production of beer, wine, noodles, vermicelli, rice paper **(Abdel-Haleem, 2016; Ahmed *et al.*, 2015; Li *et al.*, 2013)**, animal feed, and even cosmetics. IR504 is a rice variety commonly grown in Vietnam's Mekong Delta region, generating excellent output because to its better pest and disease resistance, as well as its adaptation to varied soil and climate conditions, thereby considerably contributing to the local agricultural economy. Finding new and more practical applications for IR504 broken rice is critical, and fermented drinks are one of the most promising approaches for producing a high-value product. Rice wine is a traditional alcoholic beverage created by saccharifying cooked rice and fermenting it. Fermentation is a viable technique for developing new products with appealing sensory properties, and the wine business is actively looking to diversify (Thuy *et al.*, 2023). As the worldwide rice wine industry expands, quality and safety become critical issues.

Saccharification is one of the two key processes in rice wine production; it is a chemical reaction that turns starch into fermentable sugars through the hydrolysis activity of amylase enzymes. The process of making koji involves saccharification. Koji (*Aspergillus oryzae*) is a cultured fungus that is a significant ingredient in traditional East Asian fermented cuisine. This filamentous fungus is known for its exceptional enzymatic abilities, since it produces a diverse variety of hydrolytic enzymes, including proteases and amylases (Straka *et al.*, 2025). Koji fermentation has a direct impact on product quality. Wang *et al.* (2023) reported that the koji-making method has a significant impact on the volatile flavor compounds in the finished wine, with microorganisms converting starch into sugars and producing compounds such as alcohols, organic acids, and compounds with distinctive odors, all of which contribute to improved product quality. In addition, commercial enzymes such as α-amylase and glucoamylase are also commonly used to hydrolyze starch into sugars. Adding starch hydrolyzing enzymes to the koji fermentation process shortens the time and improves the efficiency of starch saccharification, thereby improving the yield and economic value of wine products. In addition to its excellent amylase and protease production, it also has the ability to convert protein and starch into sugars and amino acids (Watarai *et al.*, 2019).

Rice wine's quality is determined by appropriate fermenting conditions. **Thuy *et al.* (2023)** discovered that *Saccharomyces bayanus* outperformed the yeast strain *Saccharomyces cerevisiae* in terms of growth under vitamin-deficient conditions. *Saccharomyces bayanus* is currently accepted to be the result of numerous hybridization events between three purebred species, *Saccharomyces uvarum*, *Saccharomyces cerevisiae*, and *Saccharomyces eubayanus* **(Libkind *et al.*, 2011; Nguyen *et al.*, 2011; Thuy *et al.*, 2023)**. Furthermore, *Saccharomyces bayanus* imparts a distinct flavor to wine by extensively utilizing carbon sources to make vast amounts of volatile chemicals such as 2-phenylethanol, ethyl lactate, 2-phenylethyl acetate, and a few other esters **(Januszek *et al.*, 2020)**.

Many studies on koji fermentation and rice wine quality have been conducted **(Kim & Seo, 2021; Wang *et al.*, 2023; Yang *et al.*, 2013)**; however, the interaction between microorganisms and other enzymes influencing the quality of koji fermented rice wine remains unknown. As a result, it is necessary and worthwhile to consider the impact of microorganisms combined with enzymes on the quality of koji fermented white rice wine in order to close the rice utilization cycle, contributing to increasing the value of broken rice/low-cost rice and opening up new opportunities for using agricultural by-products in the food technology industry. Utilizing byproducts from manufacturing is critical to improving the value chain of the region's rice economy. Thus, with sustainable and environmentally friendly development, the circular economy will assist in optimizing resource utilization, reducing waste, lowering greenhouse gas emissions, and providing value to the rice business. Especially, the increasing number of studies on utilizing the machine-learning based model as artificial neural network coupled with genetic algorithm (ANN-GA) for optimizing several processes were found **(Van Tai *et al.*, 2024; Zeng *et al.*, 2025)**. This integrated method is especially important since ANN can capture complex nonlinear interactions between processing variables, whilst GA offers an effective global optimization strategy that avoids local optima. GA is used to determine optimal weights, biases, and network topology, enhancing overall ANN performance.

Hybrid optimization models using GA reduce training error and achieve high learning rates compared to standard methods. GA helps select or optimize input factors, improving ANN model accuracy and reducing experimental requirements **(Kundu *et al.*, 2015; Luong & Poeaim, 2025; Zeng *et al.*, 2025)**. The application of ANN-GA in this study is novel since it is used to optimize both saccharification and fermentation conditions in rice wine production from IR50404 broken rice, a raw material that has not previously been fully modeled using advanced computational methodologies. Therefore, the goal of this research is to produce a high-quality rice wine using IR504 broken rice. Prediction of dependent variables in the saccharification and fermentation according to multiple regression analysis and ANN-GA were also performed..

MATERIAL AND METHODS

Raw materials used

Broken rice IR504 (rice milling company in Vietnam); commercial *Aspergillus oryzae* (Vietnam), α-amylase (132.5 Unit/gram - Novozymes, Vietnam); glucose-amylase (Amyloglucosidase 296.5 Unit/gram, Vietnam); pure *Saccharomyces bayanus* (France). .

Preparation of rice *koji* and saccharification

50 g of cooked rice was placed in a 250 mL glass jar with gauze (steamed at 121°C for 20 minutes) and varying quantities of *Aspergillus oryzae* (X_1, 0.1%-0.2%) and α-amylase (X_2, 0.02 to 0.04%). The prepared raw materials were cultivated for three days in an incubator (Constant Temperature And Humidity Incubator LB-HSP-80BEII, China) at 30°C and 70-75% relative humidity. The fixed ratio of gluco-amylase was 0.077% **(Thủy & Tuyền, 2015)**. After incubation, the mixture was transferred to sealed 45 mL cylinders and centrifuged at 4000 rpm for 20 minutes to separate the residue. The volume of sugar solution (Y_1) and the total soluble solids content (Y_2) were determined.

Koji mass fermentation

The koji mass was fermented at room temperature (28±2°C) with *Saccharomyces bayanus* (X_3) dry yeast at a concentration of 0.02-0.05%. Before fermentation, the yeast was pre-activated in a 5% glucose solution at 35-38°C per the manufacturer's instructions. The pH of the fermentation broth was adjusted to 4.0-4.5 using citric acid. Water was boiled and cooled before being added to the koji mass at a 2:1 ratio. The mixture is adjusted to a TSS concentration (X_4) of 22 to 26°Brix (using sucrose) using **Equation 1 (Thuy *et al.*, 2022)**.

$$\frac{A}{100} = \frac{a + x}{100 + x} \qquad (1)$$

Where A is the required °Brix, a is the °Brix of the measured sugar solution, x is the amount of sugar to be added (g/100 g).
The fermentation vessel is then sealed, enabling the anaerobic fermentation to continue for around 18-20 days. When the fermentation process is complete (the alcohol content reaches around 12% by volume), a layer of wine lees accumulates at the bottom. The wine is then decanted, and the lees are removed and used for another purpose. The finished rice wine is allowed to settle naturally for 20-30 days before bottling. The product is stored in a cold, dry location. The assessed quality parameters were ethanol concentration (Y_3) (% volume), ethyl acetate ester (Y_4) (g/L), methanol (mg/L), and total acidity (g/L).

Physicochemical properties analysis

Total soluble solids (TSS) content was determined using a refractometer having a range of 0-32°Brix (Atago, Japan). Determination of ethanol and acid content was followed by **Liu *et al.* (2020)**. Methanol content was determined according to the **Li *et al.* (2018)**. Ester content was measured using titration, as described by **Ouyang *et al.* (2018)**.

Microstructure and morphology of molds

Lactophenol cotton blue was used as a staining solution to visualize the microstructure and morphology of *Aspergillus oryzae* after koji incubation was observed under a microscope (Olympus CX23, China).

Statistical analysis

Multiple regression analysis

Statgraphics Centurion software version XV.I (USA) was used for statistical analysis. Analysis of Variance (ANOVA) and Least Significant Difference (LSD) tests were performed to determine whether the difference between the treatment means was statistically significant at the 95% confidence level (5% significance level). A quadratic polynomial equation (**Equation 2**) was created to describe the relationship between the dependent and independent variables.

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

In which, Y is the acquisition index; β_o is a constant; β_i is a linear coefficient; β_{ii} is a square coefficient; β_{ij} is an interaction coefficient; X_i, X_j are the survey variables; k is the number of optimized factors. The suitability of the predictive model is assessed through the coefficient of determination R^2 and the P value of Lack-of-fit.

Artificial neural network coupled with genetic algorithm (ANN-GA)

The data analyzed from previous analysis is utilized in the network input via the deep learning toolbox of MATLAB R2023b (Mathworks, Inc., MA, USA). The phases in neural network design include data collection, network creation, network configuration, weight and bias initialization, network training, network validation, and network use. The multilayer perceptron network architecture comprises three levels of nodes: input, hidden, and output layers. The datasets were divided into three subsets: training (70%), validation (15%), and testing (15%). The training dataset was used to develop the model, while the validation dataset was applied to monitor model performance and prevent overfitting during the training process. The testing dataset was used to evaluate the predictive ability of the developed model. The process modeling was configured with 10 hidden neurons, and the model performance was evaluated based on the mean squared error (MSE) function. During the training phase, a feed-forward network utilizing the Levenberg-Marquardt back-propagation algorithm (trainlm) was employed to reduce the MSE between the target outputs and the actual outputs produced by the network. The data produced by the ANN network were utilized as the initial population in the GA, employing the optimization toolbox of MATLAB R2023b (Mathworks, Inc. MA, USA). GA is an iterative, population-based, parallel global search algorithm that has been extensively utilized for optimizing complex problems **(Kundu *et al.*, 2015)**. The GA parameters were set up with 500 individuals in the initial population and 1000 generations. After each generation, 80% of the best individuals were selected and continued to mate with their parents in the next generation (0.8 of crossover fraction) **(Luong & Poeaim, 2025)**.

RESULTS AND DISCUSSION

Effect of *Aspergillus oryzae* and α-amylase on koji preparation (saccharification and liquefaction)

Analysis of variance for total soluble solid content and volume of sugar solution

TSS content

The ANOVA findings (Table 1) for TSS content revealed that the regression model was highly statistically significant and well characterized the data fluctuation. *Aspergillus oryzae* (X_1) and α-amylase (X_2) significantly affected TSS, with F values of 185.36 and 135.84, respectively ($p < 0.0001$). This indicated that variations in the concentrations of these components resulted in significant changes in TSS. Furthermore, the quadratic effects of X_1 (X_1X_1) and X_2 (X_2X_2) were statistically significant ($p < 0.05$), showing that the TSS response did not fluctuate linearly but had a curvilinear tendency depending on the level of each factor. The interaction between the two components (X_1X_2) was not significant ($p = 0.6195$), indicating that combining *Aspergillus oryzae* and α-amylase did not significantly affect TSS. The P-value of Lack-of-fit was non-significant ($p = 0.4935$), indicating that the model suited the experimental data well. The model's R^2 = 94.67% and adjusted R^2 = 93.40% indicate that it explains the majority of the variation in TSS. The minimal standard error of estimate (SEE = 0.23) confirms its accuracy and reliability.

Table 1 Analysis of variance (ANOVA) for TSS content and volume of sugar solution

Source	TSS content		Volume of sugar solution	
	F-ratio	*P-value*	*F-ratio*	*P-value*
X_1: *Aspergillus oryzae*	185.36	0.0000	216.97	0.0000
X_2: α-amylase	135.84	0.0000	121.15	0.0000
X_1X_1	30.01	0.0000	70.74	0.0000
X_1X_2	0.26	0.6195	4.76	0.0427
X_2X_2	12.34	0.0025	49.13	0.0000
Lack-of-fit	0.83	0.4935	2.01	0.1482
R^2	94.67%		95.06%	
R^2 (adjusted for d.f.)	93.40%		93.89%	
Standard Error of Estimate (SEE)	0.23		0.29	

The X_1X_2 interaction had no significant effect, so it was removed from the correlation model, and a new model (**Equation 3**) was developed. With this new model, the high values of R^2 (94.6%) and R^2_{adj} (93.62%), together with the P value of Lack-of-fit > 0.05 (P = 0.6) showed that the selected model was accurate enough to predict the soluble solids content from the independent variables.

$$Y_1 = 14.8 + 76.0X_1 + 259.4X_2 - 204.4X_1^2 - 3277.8X_2^2 \qquad (3)$$

In which: Y_1 is TSS (ºBrix), X_1 is *Aspergillus oryzae* (%), X_2 is α-amylase (%).

The volume of sugar solution

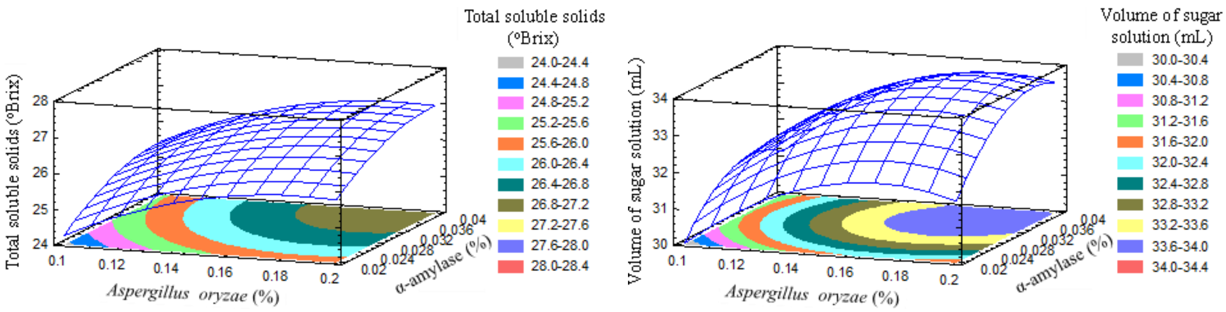
Similarly, optimization of the volume value of the glucose solution according to the influencing factors was also performed (**Table 2**). The multivariate regression model is an appropriate statistical model for forecasting the volume of a sugar solution. *Aspergillus oryzae* (X_1), α-amylase (X_2), square interaction (X_1X_1, X_2X_2), and pair interaction (X_1X_2) all significantly impact sugar solution volume (P < 0.05). The model is based on a strong connection between experimental and anticipated data (**Equation 4**). The R^2 statistic indicates that the appropriate model explains 95.06% of the variation in the volume of sugar solution. The R^2_{adj} statistic, which is useful for comparing models with varying numbers of independent variables, is 93.89%. The estimate's standard error indicates that the residual has a standard deviation of 0.29.

$$Y_2 = 13.3 + 129.2X_1 + 520.6X_2 - 400.0X_1^2 + 366.7X_1X_2 - 8333.3X_2^2 \qquad (4)$$

In which: Y_2 is the volume of sugar solution (mL), X_1 is *Aspergillus oryzae* (%), X_2 is α-amylase (%).
A Multiple Regression Analysis model with high R^2 and R^2_{adj} values, low standard error, and P value of Lack-of fit > 0.05 (P = 0.15), indicates a high goodness of fit.

Contour and surface plots of total soluble solids (TSS) content and volume of sugar solution - Optimization

The response surface and contour plots show the simultaneous influence of *Aspergillus oryzae* and α-amylase on the total soluble solids (TSS) content and volume of sugar solution are presented in Figure 1.



a. **b.**

Figure 1 The 3D response surface and contour plots show the simultaneous influence of Aspergillus oryzae and α-amylase on the total soluble solids content (a) and volume of sugar solution (b)

TSS content

The mesh surface of Figure 1a illustrates that when both elements' concentrations grow, the TSS value rises dramatically in the low to medium concentration zone before stabilizing above the optimal level. This is consistent with the regression equation, which contains negative quadratic components, showing a nonlinear relationship between the factors and the response. The contour plots on the bottom plane aid in identifying the optimum region. High color bands (green, yellow, and red) indicate greater TSS and are primarily concentrated in the region with *Aspergillus oryzae* at 0.15-0.19% and α-amylase at 0.03-0.04%. This is the region where TSS reaches its peak, approximately 26.5-28.2°Brix. Both variables contribute to the increase in TSS, but α-amylase has a stronger influence, as evidenced by the larger slope of the surface along the X_2 axis. Furthermore, the contour lines are not significantly distorted in the figure, showing that the interaction between the two factors (X_1X_2) is unimportant. This is consistent with earlier ANOVA results. Thus, each component influences TSS independently in a curvilinear fashion, with each element having its own optimal level. Response optimization was performed to maximize TSS with the optimal value 27.03ºBrix at optimum saccharification conditions of *Aspergillus oryzae* and α-amylase at 0.186% and 0.04%, respectively.
Aspergillus oryzae affects the resulting wort by breaking down complex carbohydrates into simpler sugars, producing hydrolytic enzymes (such as amylase and protease) and influencing the fermentation process, as well as the products of fermentation. In addition, this fungus is capable of producing flavor and aroma compounds, changing the color of the wort. The α-amylase hydrolyzes starch into shorter chain dextrins, creating more intermediate sugar molecules and helping the gluco-amylase enzyme to further convert into glucose, thereby also increasing the TSS of the sugar solution. At the same time, the addition of enzymes contributes to shortening the saccharification time as well as improving the efficiency of saccharification of starch. It was observed that *Aspergillus oryzae* and α-amylase synergize in the liquefaction and saccharification. Perhaps as the mold ratio increased, so did the enzyme activity. The endogenous enzyme produced by the mold enhanced the efficiency of starch hydrolysis, resulting in an increase in the Brix of the sugar solution. **Coulibaly and Agathos (2007)** investigated the starch hydrolysis process and discovered that the ability to degrade starch improved as the density of *Aspergillus niger* increased. Mold endogenous enzymes (such as cellulase, protease, and amylase) contributed to the hydrolysis of amylose and amylopectin chains during the liquefaction process, resulting in shorter molecules such as maltose, maltotriose, and dextrin, lowering the viscosity of starch paste. However, the more endogenous enzymes rapidly increase the quantity of glucose, resulting in suppression of carbon catabolism, which partially limits the formation of starch-degrading enzymes **(Gomi, 2019)**. However, it is necessary to balance the ratio of mold and additional enzymes for effective saccharification.

The volume of sugar solution

Figure 1b depicts the response surface and the contour plots illustrate the simultaneous influence of *Aspergillus oryzae* (%) and α-amylase (%) on the volume of sugar solution (mL). The response surface shows that with increasing concentrations of both factors, the wort volume tends to increase significantly in the low to medium concentration range, then decreases the rate of increase as it approaches the optimum. This demonstrates a nonlinear response, consistent with the quadratic coefficients in the regression model. The contour plots at the bottom plane show the distribution of different volumes. The dark colored region (from dark blue to yellow to purple) corresponds to the highest volume of sugar solution, concentrated when the *Aspergillus oryzae* concentration is 0.15–0.18% and α-amylase is in the range of 0.03–0.04%. In this region, the volume of the wort reaches 33.6–34.4 mL, which is considered the optimum region. The figure also shows that α-amylase has a stronger effect than *Aspergillus oryzae*, as shown by the larger slope of the surface along the X_2 axis. In addition, the contour lines are quite smooth and not distorted. Overall, the figure confirms that both *Aspergillus oryzae* and α-amylase contribute to the increase of wort volume, with a specific optimum for each factor; excessive increase may slow down the increase efficiency. Optimal volume of sugar solution of 33.92 mL/50 g broken rice was achieved through response optimization using *Aspergillus oryzae* and α-amylase for saccharification settings of 0.178% and 0.035%, respectively. **Rami Tzafriri and Edelman (2007)** stated that the reaction rate is affected by the difference in enzyme and substrate concentrations. When the enzyme concentration is high, the reaction rate is fast, the substrate is quickly exhausted, leading to enzyme waste. On the contrary, low enzyme concentration causes enzyme saturation and reduces the liquefaction rate. Adding α-amylase early in the process helps accelerate starch liquefaction and saccharification. The glucoamylase converts dextrin and maltose into glucose, improving saccharification efficiency.

Simultaneous multivariate regression for total soluble solids and volume of sugar solution

Simultaneous optimization of dependent variables is a statistical or modeling approach in which many dependent variables are optimized concurrently rather than one at a time. It also seeks to identify optimal settings that increase many response variables at the same time, while accounting for interdependence. As a result, in this investigation, RSM was used to choose the parameters that produced the highest or desired response. To improve the TSS concentration and volume of the sugar solution, a simultaneous optimization procedure was used. After analyzing the effects of the factors (*Aspergillus oryzae* and α-amylase addition), the optimal parameters (TSS content and volume of sugar solution obtained) were chosen. Using Statgraphic software, the overlay plot displaying the influence of *Aspergillus oryzae* (%) and α-amylase (%) was optimized in general, as shown with (*) in Figure 2. At a given gluco-amylase enzyme ratio of 0.077%, the optimal values for *Aspergillus oryzae* and α-amylase were found to be 0.184% and 0.037%, respectively. With these ideal liquefaction and saccharification settings, the volume of sugar solution reached 33.87 mL (from 50 g of raw material), with a TSS content of 27ºBrix. The research data series yielded optimal values for all input variables.

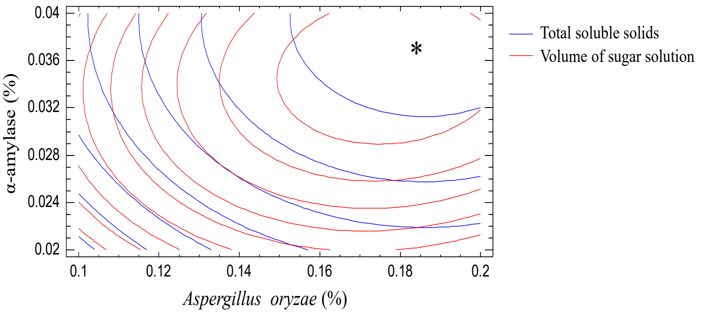


Figure 2 Overlapping plot of TSS and volume of syrup and optimal values* (with different levels of independent variables)

To verify the adequacy of the model and the optimal conditions developed, a confirmation run experiment was performed. The *Aspergillus oryzae* and α-amylase contents were arranged in the optimal ratio, and 0.077% glucoamylase was fixed. The actual experimental results determined the TSS content and the volume of the sugar syrup to be 27.1±0.20ºBrix and 33.43±0.21 mL (with 50 g of raw materials used), respectively. The predicted values and the actual experimental values were compared. It was observed that most of the values determined experimentally were similar to those determined by the developed models, which definitely proved the validity of the experiments. This result also showed good applicability to actual production.

ANN-GA optimization of saccharification and liquefaction process

ANN model was develop with the structure of 2-10-2, with two input variables as *Aspergillus oryzae* and α-amylase content, 10 nodes in the hidden layer and two ouput variables (TSS content and the volume of the sugar syrup). As seen in Figure 3, the model has the high regression coeficient with the value over 0.9. GA optimization was done based the ANN model created. The GA-ANN approach was chosen to maximize saccharification and liquefaction process after the previously mentioned analysis. Under ideal circumstances, which included *Aspergillus oryzae* of 0.181% and α-amylase of 0.036%, the ANN model projected an optimal sugar solution of 34.01 mL (from 50 g of raw material) and TSS content of 27.2ºBrix. The experimental sugar solution and TSS measured under this combination of parameters were 34.11±0.17 mL and 27.18±0.05ºBrix, there was no statistically significant difference between the predicted and experimental results.

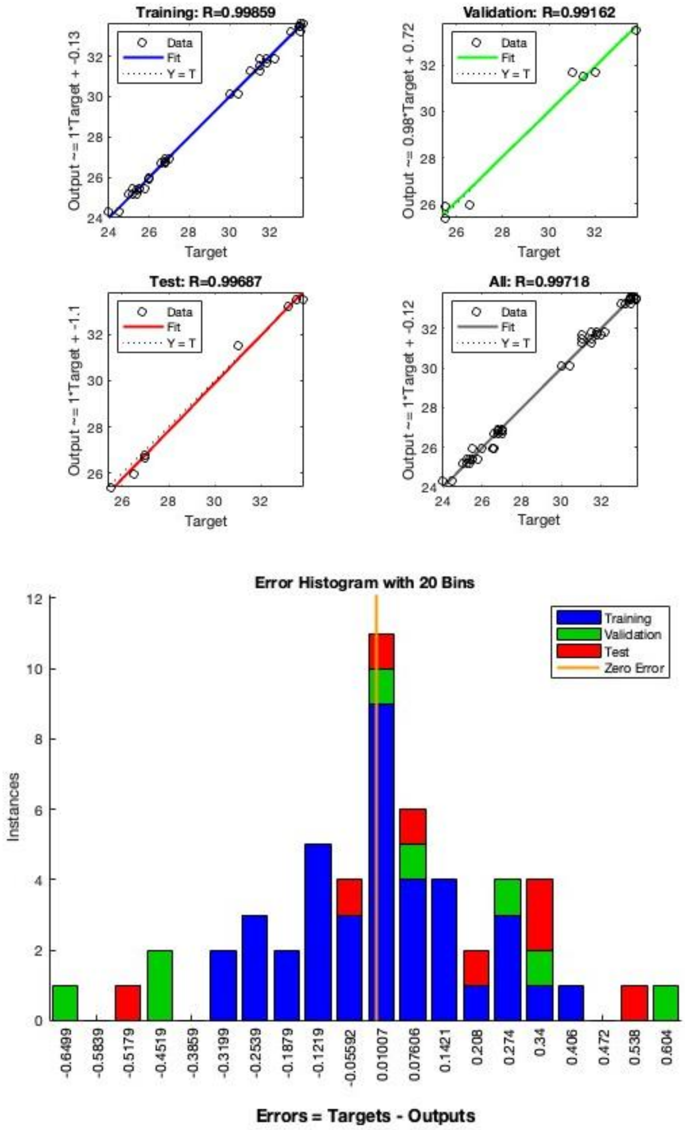
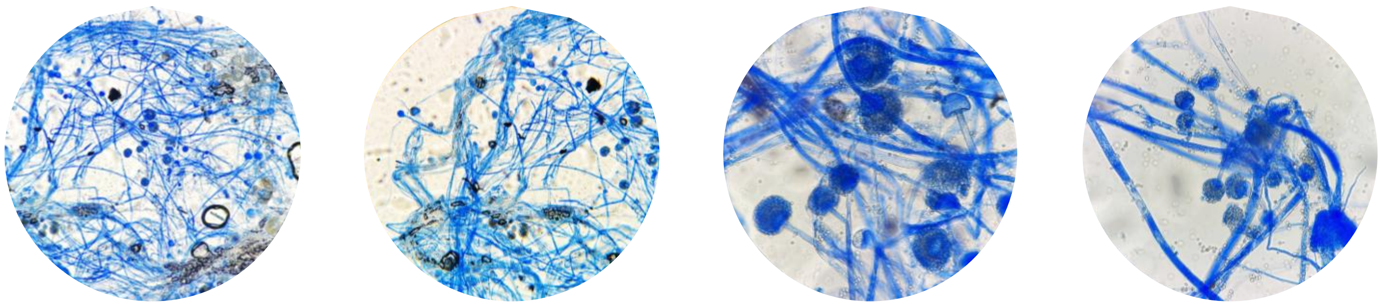


Figure 3 Regression analysis and error histogram of ANN model

Microstructure

Applying the optimal conditions to the experiment, observing the growth of koji mold on the substrate surface showed that the filamentous fungi developed favorably after 3 days of culture. Figure 4 shows the microstructure of *Aspergillus oryzae* after 3 days of koji incubation, observed under an optical microscope at 10× and 40× magnification. At 10× magnification (Figure 4a), a dense, intertwined, and diffusely branched mycelium network is observed. The strong growth of mycelium indicates that the culture medium ensures sufficient humidity, temperature, and oxygen, which are essential factors for the growth of this aerobic mold **(Kitamoto, 2015)**.



a. 10× **b. 40×**

Figure 4 *Aspergillus oryzae* at 10× (a) and 40× (b) magnification under the light microscope

At 40× magnification (Figure 4b), the image clearly shows the conidiophore and conidial head radiate with globose to subglobose vesicles, uniseriate. The sporophore wall was thin and smooth, conidiophore stipes were hyaline and coarsely roughened. The spore structure was tightly arranged on the vesicle, reflecting the asexual reproductive stage of the fungus. The observed morphology is consistent with the typical characteristics of the genus *Aspergillus*, in which *Aspergillus oryzae* has smooth, spherical, light-colored spores, and unbranched conidiophore **(Gomi, 2014)**. After three days, *Aspergillus oryzae* reached its maximum physiological condition with high enzyme activity, particularly α-amylase, glucoamylase, protease, lipase, and others **(García-Conde *et al.*, 2024; Gómez *et al.*, 2016)**. These enzymes play crucial roles in hydrolyzing starch, proteins, and lipids in the substrate into simpler molecules such as glucose, amino acids, and free fatty acids, providing the foundation for subsequent fermentation processes **(Al-Maqtari *et al.*, 2019)**. This process contributes to the distinctive flavor and aroma of the finished koji fermented product in addition to facilitating the growth of the yeast (*Saccharomyces cerevisiae/Saccharomyces bayanus*) for the subsequent fermentation. The stable development and robust metabolic activity of *Aspergillus oryzae* during koji fermentation are also demonstrated by the clear spore morphology observed under a microscope (after three days).

Koji mass fermentation - Effect of *Saccharomyces bayanus* and total soluble solid content on rice wine quality

Analysis of variance for ethanol and ester content

ANOVA (Table 2) revealed that *Saccharomyces bayanus* (X_3) and TSS soluble solids content (X_4) had substantial effects on ethanol and ester content, with high F-ratio values and p-values < 0.0001. The lack-of-fit test for both models was non-significant (p > 0.05), indicating that the model fit the experimental data accurately. The R^2 and adjusted R^2 values varied from 80.52% to 86.92%, demonstrating excellent explanatory power. The model's low SEE estimate errors (0.27 for ethanol and 0.03 for ester) demonstrated good predictive accuracy.

Ethanol content

For ethanol content, both X_3 and X_4 were the most important factors, and the quadratic effects (X_3X_3 and X_4X_4) were also statistically significant, demonstrating a nonlinear link between the factors and the response. However, the X_3X_4 interaction was not significant (p = 0.9207), implying that the combination of yeast strain and TSS had no meaningful effect on ethanol production.
The optimal conditions of the fermentation process with the influence of yeast and TSS were obtained from the solution of regression Equation 3 based on the experimental results arranged in two factors and three levels. The relatively high R^2 and R^2_{adj} values, low SEE and P-value of Lack-of- fit > 0.05, all imply that the quadratic model utilized here is a good fit.

$$Y_3 = -44.03 + 146.67X_3 + 4.11X_4 - 1555.56X_3^2 - 0.08X_4^2 \quad (3)$$

In which: Y_3 is the ethanol (%), X_3 is the *Saccharomyces bayanus* (%), X_4 is the TSS (ºBrix).

R^2 = 86.92%, R^2 (adjusted for d.f.) = 85.23%, SEE = 0.272, P (Lack of fit) is 0.586 (> 0.05)

Table 2 Analysis of variance (ANOVA) for ethanol contet and ester content

Source	Ethanol content		Ester content	
	F-ratio	*P-value*	*F-ratio*	*P-value*
X_3: *Saccharomyces bayanus*	86.59	0.0000	124.06	0.0000
X_4: TSS content	87.78	0.0000	33.55	0.0000
X_3X_3	11.75	0.0022	20.05	0.0002
X_3X_4	0.01	0.9207	6.43	0.0182
X_4X_4	11.10	0.0028	8.10	0.0089
Lack-of-fit	0.95	0.4814	2.42	0.0567
R^2	86.92%		83.30%	
R^2 (adjusted for d.f.)	84.74%		80.52%	
Standard Error of Estimate (SEE)	0.27		0.03	

Ester content

In contrast, for ester content, both the quadratic effect and the X_3X_4 interaction were statistically significant (p < 0.05), demonstrating a high contribution of *S. bayanus* and TSS to ester formation and a synergistic effect between them. Equation 4 expresses Y_4 (ester content) as a function of *Saccharomyces bayanus* concentration (X_3) and TSS (X_4), as follows.

$$Y_4 = -5.52 + 41.21X_3 + 0.45X_4 - 247.22X_3^2 - 0.77X_3X_4 - 0.01X_4^2 \quad (4)$$

In which: Y_4 is the ester content (g/L), X_3 is the *Saccharomyces bayanus* (%), X_4 is the TSS (ºBrix).
R^2 = 83.30%, R^2 (adjusted for d.f.) = 80.52%, SEE = 0.033 and P-value (Lack-of-fit) = 0.06 (>0.05)
The regression coefficients provided by Statgraphic XV.I can clarify the relevance and impact of variables on yield. Positive coefficients suggest a linear effect on the reactions, whereas negative coefficients indicate the opposite tendency.

Contour and surface plots of ethanol and ester – Optimization

Figure 5 illustrate how the yeast ratio and fermentation broth concentration affect the ethanol and ester content of the rice wine product. The ethanol and ester concentrations grew from 9.93 to 12.17% vol and 0.68 to 0.91 g/L when the yeast ratio increased from 0.02 to 0.05% and the TSS of the fermentation broth ranged from 22 to 26ºBrix, respectively. However, they did not rise in a linear fashion with the concentration of the fermentation broth.

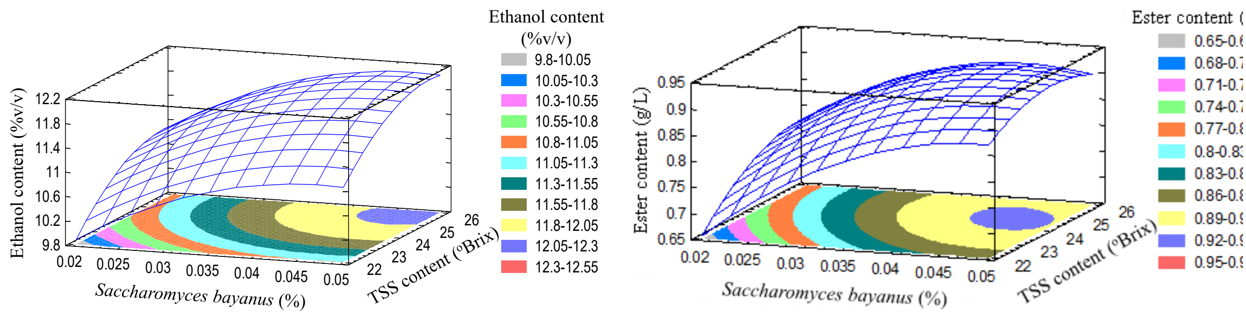


Figure 5 The 3D response surface and contour plots show the simultaneous influence of *S. bayanus* and TSS content on the ethanol content (a) and ester content (b)

Ethanol content

Figure 5a depicts a 3D response surface and contour plot showing the effect of *Saccharomyces bayanus* (X_3) and TSS concentration (X_4) on ethanol content (% v/v). The upward curve of the surface along both axes indicates that the ethanol content increases dramatically as yeast and TSS concentrations increase. This reveals that X_3 and X_4 have a beneficial and significant impact on ethanol output. The curvature of the surface represents the nonlinear effect of the two elements, which is consistent with the ANOVA results, which show statistical significance for the quadratic components X_3X_3 and X_4X_4. However, the surface shows no twist or change in form, which is typical of a strong interaction between the two elements, indicating that X_3X_4 has a minor interaction with ethanol. The contour lines at the bottom of the figure demonstrate that the maximum ethanol production zone (12.0-12.55% v/v) occurred when *S. bayanus* was about 0.045-0.05% and TSS was 25-26 °Brix. In contrast, the lowest ethanol (about 9.8-10.5% v/v) was found at low yeast and TSS concentrations. Overall, the graph demonstrates that simultaneous adjustment of *S. bayanus* and TSS concentrations is required to produce high ethanol yields during fermentation. The predicted ethanol value (% vol) under the optimal conditions was 12.09% vol% when *S. bayanus* yeast was used at the optimal concentration of 0.047% and the TSS of the fermentation broth was 25.62ºBrix. **Matsushika and Sawayama (2010)** found that a high initial yeast cell concentration considerably boosted both substrate utilization and ethanol production rates. When the yeast content is low, the low yeast cell biomass will lead to a delay in fermentation, creating conditions for competing bacteria to grow. However, if the yeast density is too high, the yeast may die due to lack of nutrients to grow. **AbdElhafez *et al.* (2022)** stated that in order for the fermentation process to be as efficient as possible, the yeast ratio must be optimized; utilizing a yeast ratio that is greater or lower than the optimal level will result in a drop in fermentation productivity. A decrease in yeast growth and viability occurred when the sugar concentration in the medium increased, possibly due to osmotic stress of the yeast **(Yamaoka *et al.*, 2014)**. High initial glucose concentrations during fermentation can impede glucose consumption and lower ethanol fermentation efficiency **(Chang *et al.*, 2018)**. **Thuy *et al.* (2023)** found that the optimal conditions for fermenting mulberry juice with the *Saccharomyces bayanus* strain were pH 3.93, TSS content of 26ºBrix, and yeast ratio of 0.22 g/L to achieve the highest ethanol content (12.97% vol). Previous studies tested *Aspergillus oryzae* and *Rhizopus oryzae* derived from Korean fermentation starter strains **(Seo *et al.*, 2012; Yang *et al.*, 2013)**, as well as *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 newly isolated from Korean fermentation starter strains **(Kim & Seo, 2021)**, for koji and rice wine production, with finished wine alcohol concentrations ranging from 10 to 14%.

Ester content

Figure 5b depicts a 3D response surface that shows how the *Saccharomyces bayanus* (%) and initial TSS (°Brix) affect the ester content generated during fermentation. As these parameters grow, so does the measured ester content, but in a nonlinear fashion, as evidenced by the surface's severe curvature. TSS has a more critical function because high solids concentrations supply an abundance of sugars for metabolism to create ethanol and intermediate acids, which are crucial precursors for ester production. At the same time, a minor increase in yeast ratio adds to an increase in ester content through higher cell density and enzyme activity in esterification activities. The contours below reveal a considerable interaction between the two factors, with the highest ester-enhancing impact observed at *S. bayanus* levels of 0.04-0.05% and TSS of 24.5-26°Brix. The yellow and red sections on the contour represent the highest ester levels (0.90-0.98 g/L), which corresponds to the model's estimated optimal range. Overall, the figure shows that both factors positively influence ester formation; however, TSS is the stronger factor, and the combination of the two variables clearly defines the optimum conditions for enhancing the aroma characteristics of the fermented product. The interaction between *Saccharomyces bayanus* and TSS showed that the maximum ester content (0.925 g/L) was detected at the optimum ratio of *Saccharomyces bayanus* used as 0.045% and TSS of the fermentation broth as 24.71ºBrix.
The total ester content, which directly reveals the level of esterification throughout the brewing process, is regarded as a significant brewing metric. Higher ester concentrations correlate with better flavor and product quality. Several parameters are known to influence ester formation during alcoholic fermentation, including the yeast strain used, the composition of the fermentation medium, and the fermentation conditions **(Saerens *et al.*, 2008)**. **Du *et al.* (2025)** reported a relationship between the yeast ratio and total ester concentration, showing that ester content increased with increasing yeast density. **Scott *et al.* (2023)** discovered that the yeast ratio influences the metabolism of acetyl-CoA and CoA, consequently changing ester content.

Simultaneous multiple regression of ethanol and ester content

The overlay plot shows the effect of *Saccharomyces bayanus* (%) and TSS (ºBrix) which were optimized simultaneously using Statgraphic Centurion XV.I software. From the response surface methodology analysis, with two responses (outputs) optimized simultaneously, the desired optimization at the optimum value of 0.945035, *i.e.* the best possible combined desirability, was selected for implementation. The optimal values for *Saccharomyces bayanus* and TSS were found to be 0.045% and 25.18ºBrix, respectively, resulting in maximum ethanol and ester concentrations of 12.07% vol and 0.92 g/L, respectively, as shown with asterisks (*) in Figure **6**. The research data set had all of the optimal values for the independent variables. The experimental or model circumstances attained 94.5% of the maximum combined desirability, indicating an outstanding overall optimization result.

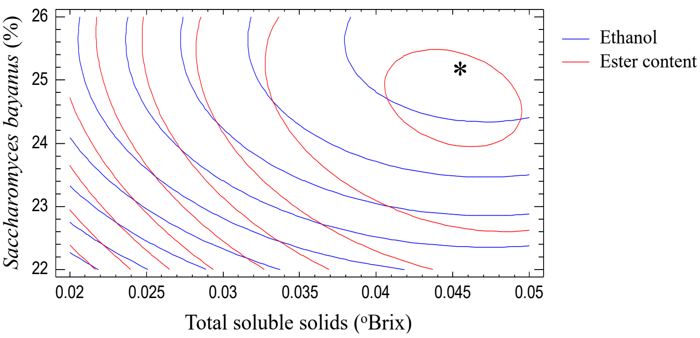


Figure 6 Overlapping plot of ethanol and ester content and optimal values* (with different levels of independent variables)

A validation experiment was run to ensure that the developed model and anticipated optimal values were correct. The *Saccharomyces bayanus* and TSS content were prepared at the appropriate ratio. The finished rice wine contained ethanol and ester levels of 12.4% vol and 0.90 g/L, respectively. The experimental data was consistent with the predicted values (ethanol and ester contents were 12.07% vol and 0.92 g/L, respectively), indicating the accuracy of the model. Thus, the model validation results contributed to the model's scientific value, reliability, and practical application.

ANN-GA optimization of fermentation process

The ANN moldel also developed for predicting the change of ethanol and ester level under different fermentation conditions. It was similar to the previous process, ANN model with structure of 2-10-2 showed the best prediction capacity with regression coeficient value over 0.9 as seen in Figure 7. For optimization process, the ANN model hybirded with GA for finding the best conditions for maximizing the ethanol and ester content in the wine. It was found that optimal values for *Saccharomyces bayanus* and TSS were found to be 0.043% and 24.88ºBrix, respectively, resulting in maximum ethanol and ester concentrations of 12.19% vol and 0.93 g/L, respectively. The model validation was conduted at the optmial conditions and found that there was no significant difference between actual and predicted value. However, it could be seen that in the both processes, the ANN-GA optimization showed the better prediction than the multiple regression analysis. It also was consitent with other processes as drying **(Yang *et al.*, 2023)**, foaming **(Van Tai *et al.*, 2024)**, extraction **(Zeng *et al.*, 2025)**, etc… It show the potential application of ANN-GA in various process.

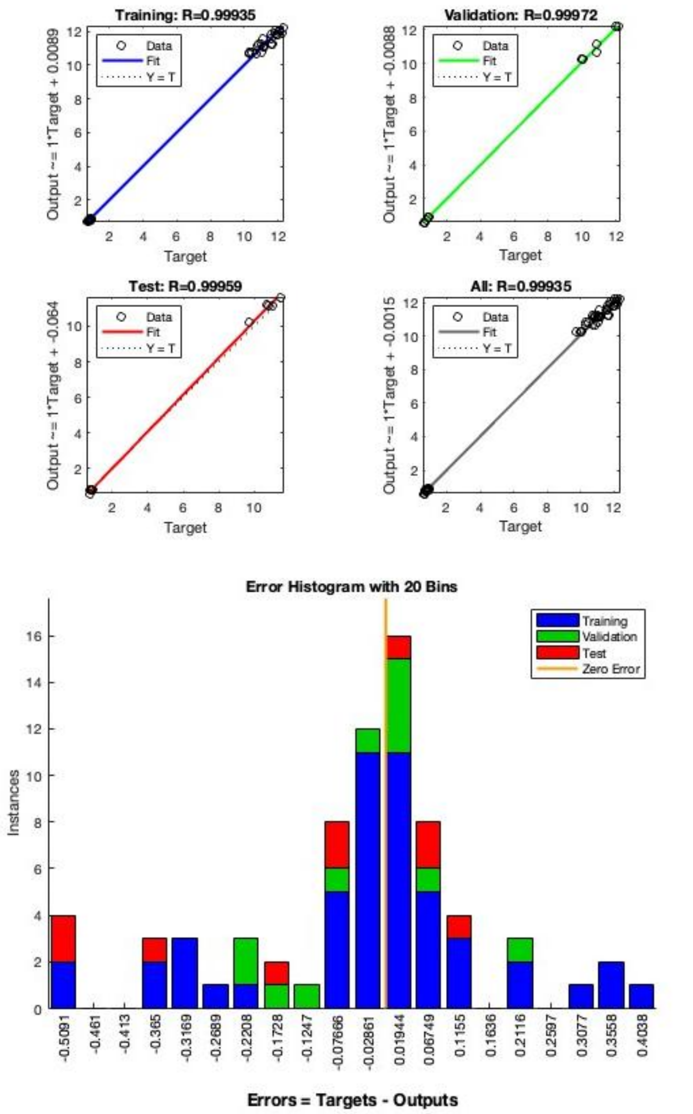


Figure 7 Regression analysis and error histogram of the ANN model for the fermentation process

The quality of rice wine is produced according to optimal parameters from two processes of saccharification and fermentation

In addition to the ethanol and ester content determined during fermentation, other key parameters of the wine made from broken rice (using the optimal fermentation and alcoholization techniques) were examined. The results showed that the acid content was less than 1.2 g/L, within the published standard for wine produced in the country (TCVN 7045:2013). The acid level also influences the flavor of the product. **Lee *et al.* (2013)** assessed the quality of rice spirits using various distillation and filtration procedures, and concluded the wine's acid level (as acetic acid) to be 4.67 g/L. According to **Molina *et al.* (2007)**, fermentation temperature plays a substantial role in the final fragrance profile of the wine, making it an important control parameter for fine-tuning wine quality throughout winemaking. The optimization technology for rice wine production not only improves the fermentation speed but also gives the rice wine a unique aroma, while providing a more perfect route for the fermentation process, thereby promoting the fermentation of rice wine to the most optimal level **(Liu *et al.*, 2020)**. The optimized parameters from the two saccharification and fermentation produced a finished product with high alcohol and ester content but low acid level. The Vietnamese Standard (TCVN 7045:2013) specifies a methanol content limit of < 400 mg/L for red wine and < 250 mg/L for white and rose wines. The test findings at the competent authority (CATECH Center, Vietnam) show that the methanol content in the rice wine product is 49.8 mg/L, indicating that the product fits the regulations for rice wine.

CONCLUSIONS

This study effectively used cheap domestic broken rice sources, well controlled the input factors of saccharification (*Aspergillus oryzae* and α-amylase) and fermentation (*Saccharomyces bayanus* and TSS of fermentation broth), and optimized these two processes (by mathematical modeling and ANN) to improve the finished product's quality. The application of machine-learning-based modeling, particularly the artificial neural network (ANN) combined with optimization techniques, demonstrated strong capability in predicting the nonlinear relationships between process variables and fermentation performance. This approach provided higher prediction accuracy and enabled efficient determination of optimal operating conditions compared with conventional regression models. The utilization of broken rice has numerous economic and environmental benefits because it is a byproduct of milling that retains high nutritional content. Using this source to manufacture value-added products (such as wine in this study) reduces resource waste, increases farmer income, and contributes to the rice industry's circular economic model. Furthermore, the integration of machine learning with fermentation process optimization provides a promising data-driven strategy for improving traditional fermentation technologies and supporting industrial scale-up. The developed modeling approach can also be applied to other cereal-based fermentation processes to enhance productivity and product quality. In the future, new research and technology will focus on increasing the quality of traditional rice wine products in Vietnam, including an in-depth investigation of mold strains used for koji production and various yeast strains used for rice wine fermentation. Aroma compounds and antioxidant compounds will be identified to improve the manufacturing process, boost the efficiency of broken rice utilization, and generate green, sustainable products that are appropriate for current consumption trends.

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REFERENCES

Abdel-Haleem, A. M. (2016). Production Of Gluten-Free Rolled Papers From Broken Rice By Using Different Hydrothermal Treatments. *Egyptian Journal of Agricultural Sciences*, *67*(2), 167-179.

AbdElhafez, S. E., Taha, T., Mansy, A. E., El-Desouky, E., Abu-Saied, M. A., Eltaher, K., Hamdy, A., El Fawal, G., Gamal, A., Hashim, A. M., Elgharbawy, A. S., El-Latif, M. M. A., Hamad, H., & Ali, R. M. (2022). Experimental Optimization with the Emphasis on Techno-Economic Analysis of Production and Purification of High Value-Added Bioethanol from Sustainable Corn Stover. *Energies*, *15*(17), 6131. https://doi.org/10.3390/en15176131

Ahmed, I., Qazi, I. M., & Jamal, S. (2015). Quality evaluation of noodles prepared from blending of broken rice and wheat flour. *Starch - Stärke*, *67*(11-12), 905-912. https://doi.org/10.1002/star.201500037

Al-Maqtari, Q. A., Waleed, A., & Mahdi, A. A. (2019). Microbial enzymes produced by fermentation and their applications in the food industry-A review. *International Journal of Agriculture Innovations and Research*, *8*(1), 2319-1473.

Chang, Y.-H., Chang, K.-S., Chen, C.-Y., Hsu, C.-L., Chang, T.-C., & Jang, H.-D. (2018). Enhancement of the Efficiency of Bioethanol Production by *Saccharomyces cerevisiae* via Gradually Batch-Wise and Fed-Batch Increasing the Glucose Concentration. *Fermentation*, *4*(2), 45. https://doi.org/10.3390/fermentation4020045

Coulibaly, L., & Agathos, S. N. (2007). Effects of *Aspergillus niger* inoculum concentration upon the kinetics of starchy wastewater pretreatment in a tanks-in-series bioreactor under transitory conditions. *Brazilian Journal of Chemical Engineering*, *24*, 499-507. https://doi.org/10.1590/S0104-66322007000400004

Dhankhar, P., & Hissar, T. (2014). Rice milling. *IOSR Journal of Engineering*, *4*(5), 34-42. https://doi.org/10.9790/3021-04543442

Du, Y., Sun, Y., Chen, R., Xu, R., Li, Q., Liu, X., Li, L., & Zhao, T. (2025). Staged Fermentation with a *Saccharomyces cerevisiae* Consortium and Monascus purpureus for Sustainable Valorization of Persimmon into High-Quality Wine. *Fermentation*, *11*(5), 278. https://doi.org/10.3390/fermentation11050278

García-Conde, K. B., Cerna-Chávez, E., Ochoa-Fuentes, Y. M., & Velázquez-Guerrero, J. J. (2024). *Aspergillus oryzae*: Una oportunidad para la agricultura. *Revista mexicana de fitopatología*, *42*(1). https://doi.org/10.18781/r.mex.fit.2302-2

Gómez, S., Fernández, F. J., & Vega, M. C. (2016). Chapter 4 - Heterologous Expression of Proteins in Aspergillus. In V. K. Gupta (Ed.), *New and Future Developments in Microbial Biotechnology and Bioengineering* (pp. 55-68). Elsevier. https://doi.org/10.1016/B978-0-444-63505-1.00004-X

Gomi, K. (2014). *Aspergillus: Aspergillus oryzae*. In *In Encyclopedia of Food Microbiology: Second Edition* (Second ed., Vol. 1). Academic Press. Oxford, UK. https://doi.org/10.1016/B978-0-12-384730-0.00011-2

Gomi, K. (2019). Regulatory mechanisms for amylolytic gene expression in the koji mold *Aspergillus oryzae*. *Bioscience, Biotechnology, and Biochemistry*, *83*(8), 1385-1401. https://doi.org/10.1080/09168451.2019.1625265

Januszek, M., Satora, P., Wajda, Ł., & Tarko, T. (2020). *Saccharomyces bayanus* Enhances Volatile Profile of Apple Brandies. *Molecules*, *25*(14), 3127. https://doi.org/10.3390/molecules25143127

Kim, M., & Seo, J.-A. (2021). Fermentation profiling of rice wine produced by *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 newly isolated from Korean fermentation starter. *Applied Biological Chemistry*, *64*(1), 25. https://doi.org/10.1186/s13765-020-00582-2

Kitamoto, K. (2015). Cell biology of the Koji mold Aspergillus oryzae. *Bioscience, Biotechnology, and Biochemistry*, *79*(6), 863-869. https://doi.org/10.1080/09168451.2015.1023249

Kundu, P., Paul, V., Kumar, V., & Mishra, I. M. (2015). Formulation development, modeling and optimization of emulsification process using evolving RSM coupled hybrid ANN-GA framework. *Chemical Engineering Research and Design*, *104*, 773-790. https://doi.org/10.1016/j.cherd.2015.10.025

Lee, Y., Eom, T., Cheong, C., Cho, H., Kim, I., Lee, Y., Kim, M., Yoo, S., & Jeong, Y. (2013). Quality characteristics of spirits by different distillation and filtrations. *Journal of the Korean society of food science and nutrition*, *42*.

Li, H., Jiao, A., Xu, X., Wu, C., Wei, B., Hu, X., Jin, Z., & Tian, Y. (2013). Simultaneous saccharification and fermentation of broken rice: an enzymatic extrusion liquefaction pretreatment for Chinese rice wine production. *Bioprocess and Biosystems Engineering*, *36*(8), 1141-1148. https://doi.org/10.1007/s00449-012-0868-0

Li, Y.-S., Mo, L.-M., & Gao, X.-F. (2018). Direct automatic determination of the methanol content in red wines based on the temperature effect of the $KMnO_4/K_2S_2O_5$/fuchsin sodium sulfite reaction system. *RSC Advances*, *8*(15), 8426-8434. https://doi.org/10.1039/C8RA00307F

Libkind, D., Hittinger, C. T., Valério, E., Gonçalves, C., Dover, J., Johnston, M., Gonçalves, P., & Sampaio, J. P. (2011). Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proceedings of the National Academy of Sciences*, *108*(35), 14539-14544. https://doi.org/10.1073/pnas.1105430108

Liu, L., Nan, L., Zhang, X., Wang, T., Zhong, Y., Yang, R., Tao, F., & Huang, J. (2020). The optimum technology of the mixed rice wine. *IOP Conference Series: Earth and Environmental Science*, *559*(1), 012018. https://doi.org/10.1088/1755-1315/559/1/012018

Luong, T. T. H., & Poeaim, S. (2025). Multi-objective optimization of lignocellulolytic enzyme cocktail production from Pseudolagarobasidium acaciicola TDW-48 by artificial neural network-genetic algorithm (ANN-GA) strategy and its application in lignocellulose waste bioconversion. *Biomass Conversion and Biorefinery*, *15*(6), 9283-9298. https://doi.org/10.1007/s13399-024-05802-z

Matsushika, A., & Sawayama, S. (2010). Effect of Initial Cell Concentration on Ethanol Production by Flocculent *Saccharomyces cerevisiae* with Xylose-Fermenting Ability. *Applied Biochemistry and Biotechnology*, *162*(7), 1952-1960. https://doi.org/10.1007/s12010-010-8972-6

Molina, A. M., Swiegers, J. H., Varela, C., Pretorius, I. S., & Agosin, E. (2007). Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Applied microbiology and biotechnology*, *77*(3), 675-687. https://doi.org/10.1007/s00253-007-1194-3

Nguyen, H.-V., Legras, J.-L., Neuvéglise, C., & Gaillardin, C. (2011). Deciphering the hybridisation history leading to the lager lineage based on the mosaic genomes of *Saccharomyces bayanus* strains NBRC1948 and CBS380T. *PloS one*, *6*(10), e25821. https://doi.org/10.1371/journal.pone.0025821

Ouyang, X., Zhu, B., Liu, R., Gao, Q., Lin, G., Wu, J., Hu, Z., & Zhang, B. (2018). Comparison of volatile composition and color attributes of mulberry wine fermented by different commercial yeasts. *Journal of Food Processing and Preservation*, *42*(2), e13432. https://doi.org/10.1111/jfpp.13432

Rami Tzafriri, A., & Edelman, E. R. (2007). Quasi-steady-state kinetics at enzyme and substrate concentrations in excess of the Michaelis–Menten constant. *Journal of Theoretical Biology*, *245*(4), 737-748. https://doi.org/10.1016/j.jtbi.2006.12.005

Saerens, S. M. G., Delvaux, F., Verstrepen, K. J., Van Dijck, P., Thevelein, J. M., & Delvaux, F. R. (2008). Parameters Affecting Ethyl Ester Production by *Saccharomyces cerevisiae* during Fermentation. *Applied and Environmental Microbiology*, *74*(2), 454-461. https://doi.org/10.1128/AEM.01616-07

Scott, W. T., Henriques, D., Smid, E. J., Notebaart, R. A., & Balsa-Canto, E. (2023). Dynamic genome-scale modeling of *Saccharomyces cerevisiae* unravels mechanisms for ester formation during alcoholic fermentation. *Biotechnology and Bioengineering*, *120*(7), 1998-2012. https://doi.org/10.1002/bit.28421

Seo, W. T., Cho, H. K., Lee, J. Y., Kim, B., & Cho, K. M. (2012). Quality characteristics of wheat-rice makgeolli by making of rice nuruk prepared by

Rhizopus oryzae CCS01. *Korean Journal of Microbiology*, *48*(2), 147-155. https://doi.org/10.7845/kjm.2012.48.2.147

Thuy, N. M., Tan, H. M., & Van Tai, N. (2022). Optimization of ingredient levels of reduced-calorie blackberry jam using response surface methodology. *Journal of Applied Biology and Biotechnology*, *10*(1), 68-75. https://doi.org/10.7324/JABB.2021.100109

Thuy, N. M., Tien, V. Q., Giau, T. N., Van Hao, H., Van Thanh, N., Thanh, N. N., Van Tai, N., Dang, P. C., & Minh, V. Q. (2023). Box–Behnken design to determine optimal fermentation conditions for apple-fortified mulberry wine using *Saccharomyces bayanus*. *Food Science and Technology*, *43*. https://doi.org/https://doi.org/10.5327/fst.00036

Thủy, N. M., & Tuyền, N. T. M. (2015). Tối ưu hóa quá trình thủy phân tinh bột bằng enzyme amylase trong chế biến sữa gạo sử dụng mô hình phức hợp trung tâm và bề mặt đáp ứng. *Tạp chí Khoa học Đại học cần Thơ*(37), 30-38. https://ctujsvn.ctu.edu.vn/index.php/ctujsvn/article/view/1457

Van Tai, N., Van Hao, H., Han, T. T. N., Giau, T. N., Thuy, N. M., & Van Thanh, N. (2024). Effect of foaming conditions and drying temperatures on total polyphenol content and drying rate of foam-mat dried banana powder: Modeling and optimization study [Article]. *Journal of Agriculture and Food Research*, *18*, 101352, Article 101352. https://doi.org/10.1016/j.jafr.2024.101352

Wang, Y., Cai, W., Gai, J., Liu, H., Hou, Q., Zhao, H., Shan, C., & Guo, Z. (2023). Analysis of the effect of rice wine koji on the fermentation quality of rice wine based on high-depth sequencing. *Food Bioscience*, *56*, 103109. https://doi.org/10.1016/j.fbio.2023.103109

Yamaoka, C., Kurita, O., & Kubo, T. (2014). Improved ethanol tolerance of *Saccharomyces cerevisiae* in mixed cultures with Kluyveromyces lactis on high-sugar fermentation. *Microbiological Research*, *169*(12), 907-914. https://doi.org/10.1016/j.micres.2014.04.007

Yang, S., Choi, S. J., Kwak, J., Kim, K., Seo, M., Moon, T. W., & Lee, Y.-W. (2013). Aspergillus oryzae strains isolated from traditional Korean Nuruk: Fermentation properties and influence on rice wine quality. *Food Science and Biotechnology*, *22*(2), 425-432. https://doi.org/10.1007/s10068-013-0097-6

Yang, T., Zheng, X., Vidyarthi, S. K., Xiao, H., Yao, X., Li, Y., Zang, Y., & Zhang, J. (2023). Artificial Neural Network Modeling and Genetic Algorithm Multiobjective Optimization of Process of Drying-Assisted Walnut Breaking. *Foods*, *12*(9), 1897. https://doi.org/10.3390/foods12091897

Zeng, Y., Zhong, Y., Li, S., Deng, Y., Ma, Y., Huang, J., Li, H., & He, Q. (2025). Integration of COSMO-RS and ANN-GA for extraction of echinacoside and acteoside from Cistanche deserticola using natural deep eutectic solvents. *Food Chemistry*, *492*, 145440. https://doi.org/10.1016/j.foodchem.2025.145440