Songklanakarin J. Sci. Technol.
46 (5), 406–413, Sep. – Oct. 2024

http://www.sjst.psu.ac.th

Original Article

Optimization of rice syrup production by solid-state fermentation using *Aspergillus tamarii*

Chanthana Chanchomsuk, Kamonlak Wicharaew, Kanok-orn Srimuang, Trakul Prommajak*, and Ratana Attabhanyo

School of Agriculture and Natural Resources, University of Phayao, Mueang, Phayao, 56000 Thailand

Received: 8 September 2023; Revised: 25 January 2024; Accepted: 4 July 2024

Abstract

This study aims to optimize the solid-state fermentation process for rice syrup production, focusing on the interplay between various fermentation parameters such as water-to-rice ratio, inoculum volume, and fermentation time. Quadratic models were employed to establish relationships between these independent variables and several response variables, including syrup volume, total soluble solids (TSS), and concentrations of reducing sugar, glucose, and maltose, as well as reducing sugar yield. The optimal conditions for maximizing these responses were determined to be a water-to-rice ratio of 3:1, an inoculum volume of 10 mL, and a fermentation time of 60 minutes. Validation of the models revealed a strong correlation between predicted and actual values for all response variables, confirming the models' robustness. Comparative analyses with traditional rice-based beverages, like amazake, showed that the reducing sugar and glucose concentrations achieved were competitive.

Keywords: fermentation, glucose, reducing sugar, response surface methodology

1. Introduction

Rice serves as a fundamental staple food in numerous countries around the world. Global rice production was more than 700 million tons since 2011, highlighting its significance in global agriculture and food security (Food and Agriculture Organization of the United Nations, 2023). However, many rice varieties are characterized by low market value, prompting a need for value-added processing to improve economic returns. Transforming rice into higher value products not only enhances the commercial worth of this raw material but also has the potential to elevate the income levels of both farmers and processors. This value addition aligns closely with the United Nations' Sustainable Development Goal 2 (SDG2), aimed at eradicating hunger through sustainable food production systems (United Nations, 2015).

One such value-added product derived from rice is rice syrup—a sweetening agent obtained through the hydrolysis of rice starch into low-molecular-weight sugars. This versatile sweetener finds applications in an array of foods and beverages. Rice syrup can be produced through various methods, including acid and enzymatic hydrolysis, as well as microbial fermentation (Akoh, Chang, Lee, & Shaw, 2008). The latter is a method steeped in tradition, used for centuries in the production of various alcoholic beverages like 'sato' in Thailand and 'sake' in Japan, as well as traditional desserts such as 'khao mark' (Okuda, 2019; Sivamaruthi *et al*., 2022).

Traditional fermentation methods often employ a starter culture, like 'Look-pang' or 'koji,' which contain a mixed microbial population consisting of bacteria, yeast, and molds. While these cultures facilitate the hydrolysis of starch to sugars, they often lead to the production of other compounds, including alcohols and flavors, that may not be desirable to all consumers. To tailor the process more precisely, researchers have been working on isolating amylolytic microorganisms from traditional starter cultures that produce a high concentration of glucose while minimizing ethanol content. This results in a rice syrup that is more universally palatable (Roongrojmongkhon, Rungjindamai, Vatanavicharn, & Ochaikul, 2020; Thancharoen & Malasri, 2023).

*Corresponding author
Email address: tpromjak@gmail.com

In our prior research, we successfully isolated the amylolytic fungus *Aspergillus tamarii* from the traditional 'Look-pang' starter culture (Chanchomsuk, Chailangka, Srimuang, Prommajak, & Attabhanyo, 2024). However, the optimal conditions for utilizing this fungal strain in rice syrup production remain unexplored. Therefore, the primary objective of this study is to employ response surface methodology to identify the optimal conditions for the production of rice syrup by *A. tamarii* during solid-state fermentation of cooked rice. By optimizing this process, we aim to not only enhance the commercial value of rice but also preserve cultural heritage related to traditional fermentation techniques.

2. Materials and Methods

2.1 Materials

The fungal strain *Aspergillus tamarii* NRRL 20818 isolated from the traditional starter culture Look-pang, was used in this study. The fungi were cultured on potato dextrose agar (PDA) and incubated at 30°C for 72 hours prior to use. Organic sticky rice of the RD6 variety was sourced from Ban Bua, Phayao. Other reagents included potato dextrose agar from Himedia (India), dinitrosalicylic acid (DNS) from Aldrich (India), and HPLC-grade acetonitrile from RCI Labscan (Thailand).

2.2 Experimental design

A three-level factorial design was implemented to investigate the influence of various parameters on the quality and yield of rice syrup obtained through solid-state fermentation using *Aspergillus tamarii*. The study focused on three independent variables: water-to-rice ratio, inoculum volume, and fermentation time. The dependent variables measured were syrup volume, total soluble solids (TSS), concentration of reducing sugar, glucose and maltose, and sugar yield. The levels for each factor are presented in Table 1.

2.3 Solid-state fermentation of rice syrup

The production process of rice syrup via solid-state fermentation is depicted in Figure 1. Organic sticky rice of the RD6 variety was prepared according to the ratios outlined in the experimental design and subsequently autoclaved at 120°C for 15 minutes. The sterilized rice was inoculated with a spore suspension containing 10^7 spore/mL and subjected to fermentation at 30°C. Both the inoculum volume and fermentation time were adjusted as per the experimental design. The liquid phase obtained as a result of the fermentation process was identified and utilized as rice syrup. Following fermentation, the solid matrix was separated from the liquid fraction by passing it through a double-layered cotton cloth. The filtrate was then centrifuged at 11,850 × g for 15 min. The collected supernatant was analyzed to determine rice syrup volume, total soluble solids, reducing sugar, glucose, and maltose concentrations. All fermentation experiments were conducted in triplicate.

Table 1. Factor levels for investigating solid-state fermentation of *A. tamarii* on cooked rice

Factor	Factor levels		
	-1	0	1
Water-to-rice ratio	3	4	5
Inoculum volume (mL)	10	20	30
Fermentation time (h)	36	48	60

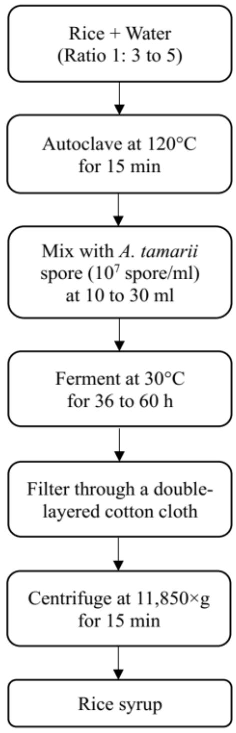

Figure 1. Rice syrup production by solid-state fermentation

2.4 Quality analysis of rice syrup

Total soluble solids (TSS) in the rice syrup were measured using handheld refractometer (Atago, Japan). The concentration of reducing sugar was quantified using the dinitrosalicylic acid (DNS) method using glucose as a standard (Miller, 1959). Reducing sugar yield, as an indicator for total sugar produced, was calculated by equation 1.

$$\text{Reducing sugar yield (g/100 g rice)} = \frac{\text{Reducing sugar concentration (mg/mL) x Syrup volume (mL)}}{1{,}000} \quad (1)$$

Glucose and maltose concentrations were determined using high-performance liquid chromatography (HPLC, 4000 series, Jasco, Japan) with a Phenomenex Lunar Omega Sugar column (250×4.6 mm). A 10 µL sample aliquot was eluted using a mixture of acetonitrile and water in a 75:25 ratio at a flow rate of 1.0 mL/min. The column temperature was maintained at 40°C. Sugar detection was conducted using a refractive index detector.

2.5 Statistical analysis

Statistical modeling was performed using EZR version 1.61 with 'rsm' and 'plot3D' package (Kanda, 2013; Lenth, 2009; Soetaert, 2021). Quadratic polynomial models were employed to characterize the relationship between the experimental factors and the measured responses. Non-significant equation terms were manually excluded from the model to maximize the adjusted R-squared (R^2). Optimal conditions for fermentation were determined based on those models with high R^2 values, specifically focusing on rice syrup volume, reducing sugar, glucose concentration and reducing sugar yield using desirability method (Amdoun *et al.*, 2018). The desirability value of individual model (d_i), targeting maximum value, was calculated by equation 2.

$$d_i = \begin{cases} 0 & Y_i \le L \\ \left(\frac{Y_i - L}{U - L}\right) & L < Y_i < U \\ 1 & Y_i \ge L \end{cases} \quad (2)$$

where Y_i is the ith response, and L and U are the lower and upper limits for the response Y_i, respectively. Then, overall desirability (D) for n responses was calculated by equation 3.

$$D = \left(\prod_{i=1}^{n} d_i\right)^{\frac{1}{n}} \quad (3)$$

The validity of these models was confirmed through experiments conducted under optimal conditions in triplicate. Analysis of variance (ANOVA) was utilized to evaluate the differences between actual and predicted values at the determined optimal conditions.

3. Results and Discussion

3.1 Regression models

Response values obtained from the experimental design are shown in Table 2. Quadratic regression models were employed to examine the relationships between three independent variables—water-to-rice ratio (W), inoculum volume (I), and fermentation time (T) —and multiple dependent variables, including syrup volume, total soluble solids (TSS), reducing sugar concentration and yield, as well as glucose and maltose concentrations, as shown in equations 4 to 9.

$$\text{Syrup volume (mL)} = -414.072 - 6.336W + 8.353I + 15.936T + 10.300W^2 - 0.104T^2 - 1.106WI - 0.773WT - 0.099IT \quad (4)$$

$$\text{TSS (\% w/w)} = 25.444 - 6.292W - 0.244I + 0.33T + 0.458W^2 + 0.004I^2 - 0.004T^2 \quad (5)$$

$$\text{Reducing sugar (mg/mL)} = 169.705 - 58.517W - 3.203I + 7.449T + 0.069I^2 - 0.091T^2 + 0.363WT \quad (6)$$

Table 2. Response values obtained from rice syrup production according to the experimental design.

No.	Water-to-rice ratio	Inoculum (mL)	Time (h)	Syrup volume (mL)	TSS (% w/w)	Reducing sugar (mg/mL)	Glucose (%)	Maltose (%)	Sugar yield (g/100 g rice)
1	3	10	36	33.5 ± 2.1	15 ± 1.4	155.0 ± 19.6	4.79 ± 0.01	0.24 ± 0.28	5.16 ± 0.33
2	3	10	48	76.0 ± 5.7	16.2 ± 0.4	166.0 ± 7.2	9.48 ± 0.39	0.58 ± 0.18	12.6 ± 1.48
3	3	10	60	96.5 ± 4.2	14.2 ± 2.5	141.0 ± 26.7	8.91 ± 1.34	0.43 ± 0.54	13.5 ± 1.97
4	4	10	36	69.0 ± 11.3	13.2 ± 1.1	145.0 ± 1.9	3.2 ± 0.05	0.27 ± 0.18	10 ± 1.77
5	4	10	48	83.8 ± 0.4	12.5 ± 0.7	132.0 ± 8.7	4.82 ± 0.54	0.24 ± 0.01	11 ± 0.68
6	4	10	60	89.5 ± 20.5	11.5 ± 2.1	111.0 ± 23.9	4.38 ± 0.33	0.68 ± 0.52	10.1 ± 4.41
7	5	10	36	83.8 ± 3.2	11 ± 0.0	52.6 ± 25.1	1.92 ± 0.67	0.49 ± 0.27	4.36 ± 1.94
8	5	10	48	153.0 ± 5.7	9.5 ± 2.1	77.4 ± 14.5	4.64 ± 0.9	0.29 ± 0.14	11.9 ± 2.66
9	5	10	60	154.0 ± 5.3	11 ± 0.0	97.3 ± 6.3	3.33 ± 0.37	0.06 ± 0.04	14.9 ± 0.45
10	3	20	36	46.5 ± 5.0	13.5 ± 0.7	137.0 ± 18.6	7.64 ± 1.31	0.18 ± 0.08	6.32 ± 0.19
11	3	20	48	65.8 ± 1.1	15.2 ± 1.8	149.0 ± 14.7	8.36 ± 0.74	0.07 ± 0.02	9.76 ± 0.81
12	3	20	60	94.2 ± 13.8	14.5 ± 2.1	163.0 ± 1.4	6.56 ± 1.27	0.21 ± 0.06	15.3 ± 2.37
13	4	20	36	71.5 ± 19.1	11.2 ± 0.4	119.0 ± 25.9	5.07 ± 0.18	0.17 ± 0.04	8.77 ± 4.13
14	4	20	48	104.0 ± 4.6	12.5 ± 0.7	121.0 ± 14.2	5.47 ± 1.9	0.23 ± 0.01	12.6 ± 2.02
15	4	20	60	66.8 ± 3.2	10.2 ± 0.4	105.0 ± 15.3	3.35 ± 0.3	0.21 ± 0.14	7.02 ± 1.35
16	5	20	36	91.8 ± 25.8	9.5 ± 0.7	45.8 ± 7.6	2.75 ± 0.34	0.06 ± 0.01	4.3 ± 1.88
17	5	20	48	101 ± 13.3	8.5 ± 0.7	76.8 ± 0.6	2.65 ± 0.51	0.1 ± 0.08	7.78 ± 0.96
18	5	20	60	80.5 ± 19.1	8.3 ± 0.4	59.6 ± 2.0	2.8 ± 1.1	0.19 ± 0.14	4.78 ± 0.98
19	3	30	36	44.0 ± 1.4	13.8 ± 1.8	141.0 ± 26.9	5.59 ± 0.59	0.17 ± 0.11	6.18 ± 0.98
20	3	30	48	84 ± 4.2	15 ± 1.4	169.0 ± 2.9	6.82 ± 0.98	0.2 ± 0.01	14.2 ± 0.97
21	3	30	60	88.8 ± 29.3	13.5 ± 2.1	158.0 ± 10.6	8.16 ± 2.01	0.2 ± 0.13	14.2 ± 5.56
22	4	30	36	68.0 ± 8.5	10.8 ± 0.4	100.0 ± 26.3	3.54 ± 0.54	0.39 ± 0.38	6.92 ± 2.64
23	4	30	48	87.5 ± 5.0	11.8 ± 1.1	125.0 ± 8.3	4.21 ± 0.99	0.1 ± 0.02	10.9 ± 1.34
24	4	30	60	48.5 ± 5.0	9.5 ± 0.7	77.5 ± 3.1	3.64 ± 2.43	0.09 ± 0.1	3.76 ± 0.53
25	5	30	36	99.2 ± 13.1	9.8 ± 1.1	62.4 ± 3.2	2.93 ± 0.52	0.23 ± 0.23	6.22 ± 1.13
26	5	30	48	84.5 ± 6.4	8.0 ± 0	80.2 ± 8.6	2.74 ± 0.18	0.13 ± 0.05	6.74 ± 0.22
27	5	30	60	84.8 ± 14.5	8.3 ± 1.8	85.0 ± 0.3	1.49 ± 0.78	0.17 ± 0.17	7.2 ± 1.2

$$\text{Glucose (\%)} = 0.51 - 7.608W + 0.148I + 0.935T + 0.900W^2 - 0.007T^2 - 0.039WT - 0.004IT \quad (7)$$

$$\text{Maltose (\%)} = -0.236 + 0.1736W - 0.039I + 0.024T + 0.001I^2 - 0.004WT - 0.0003IT \quad (8)$$

$$\text{Reducing sugar yield (g/100 g)} = -94.058 + 10.278W + 0.981I + 3.051T - 0.024T^2 - 0.218WI - 0.088WT - 0.011IT \quad (9)$$

All models were significant ($p < 0.05$), as shown in Table 3. Furthermore, each model contained at least one quadratic term, confirming that the relationships between the independent and dependent variables were non-linear. The adjusted R^2 values exceeded 0.72 for TSS, reducing sugar concentration, glucose and sugar yield, indicating a strong model fit. However, the adjusted R^2 values were lower for syrup volume (0.6428) and maltose concentration (0.1461). As for the lack-of-fit test, the values were generally insignificant, with the exceptions of syrup volume and reducing sugar concentration.

3.2 Syrup volume

The volume of rice syrup demonstrated a gradual increase during fermentation when a low water-to-rice ratio was employed, as depicted in Figure 2A. Interestingly, the graph exhibited a saddle point at higher water-to-rice ratios, indicating that a higher water-to-rice ratio could effectively reduce fermentation time while still achieving an increased syrup volume. The inoculum size appeared to have a minimal impact on syrup volume, as evidenced by its exclusion as a quadratic term in the model. The optimal syrup yield was 154.0 ± 5.3 mL, achieved with a water-to-rice ratio of 5 and an inoculum size of 10 mL over a 60-hour period. Importantly, this value was statistically indistinguishable from the 153 ± 5.7 mL yield obtained under the same conditions but with a 48-hour fermentation time.

3.3 Total soluble solids (TSS)

The TSS value measures all the solid components that are solubilized in water. During the cooking of rice, starch granules absorb water and create a "gel state," wherein water is entrapped between starch molecules. Subsequently, during fermentation, the mold releases enzymes that break down these starch molecules. This enzymatic action results in a viscous solution containing dextrins, sugars, and other starch hydrolysates (Das, Miyaji, & Deka, 2017). The water-to-rice ratio exhibited a negative impact on the TSS content of the rice syrup (Figure 2B). Since the amount of solids from the rice remained constant across all treatments, increasing the amount of water led to a dilution of these soluble solids in the syrup. Fermentation time had only a marginal impact on TSS values. This is likely because both starch and sugars count as soluble solids; thus, the enzymatic conversion of starch to sugar did not significantly alter the TSS content. Similarly, the inoculum size also had a minimal effect on TSS values. Importantly, no significant interaction was observed between the factor variables affecting TSS.

3.4 Reducing sugars, glucose and maltose concentrations

Reducing sugars serve as an indicator of the degree of starch hydrolysis. The breakdown of starch releases various compounds like dextrins, oligosaccharides, and sugars, culminating in glucose as the final hydrolysis product (Permanasari, Yulistiani, Purnama, Widjaja, & Gunawan, 2018). It's important to note that incomplete starch hydrolysis results in a lower concentration of reducing sugars compared to the TSS. This is because TSS includes all soluble components, not just reducing sugars, and the reducing power of polymers is less than that of monomers. Furthermore, as glucose and maltose are specific types of reducing sugars formed during starch breakdown, their combined concentration is inherently lower than the total reducing sugar concentration measured. The response surfaces for both reducing sugars and glucose exhibited similar trends and

Table 3. Model parameters for rice syrup production by *A. tamarii*

Model parameters	Response variables					
	Syrup volume	TSS	Reducing sugar	Glucose	Maltose	Sugar yield
Coefficients						
Intercept	-414.072*	25.444*	169.705	0.510	-0.236	-94.058
Water	-6.336	-6.292*	-58.517*	-7.608*	0.1736	10.278
Inoculum	8.353*	-0.244	-3.203	0.148	-0.039	0.981
Time	15.936*	0.330	7.449*	0.935*	0.024	3.051
Water^2	10.300*	0.458	-	0.900*		-
Inoculum^2	-	0.004	0.069	-	0.001*	-
Time^2	-0.104*	-0.004	-0.091*	-0.007*		-0.024
Water×Inoculum	-1.106*	-	-	-		-0.218
Water×Time	-0.773*	-	0.363	-0.039*	-0.004	-0.088
Inoculum×Time	-0.099*	-	-	-0.004	-0.0003	-0.011
P-value	0.0000	0.0000	0.0000	0.0000	0.0343	0.000
Adjusted R^2	0.6428	0.7782	0.7861	0.7668	0.1461	0.7202
Lack of fit	0.0021	0.7414	0.0466	0.1041	0.6851	0.0965

* Significant terms at $p < 0.05$

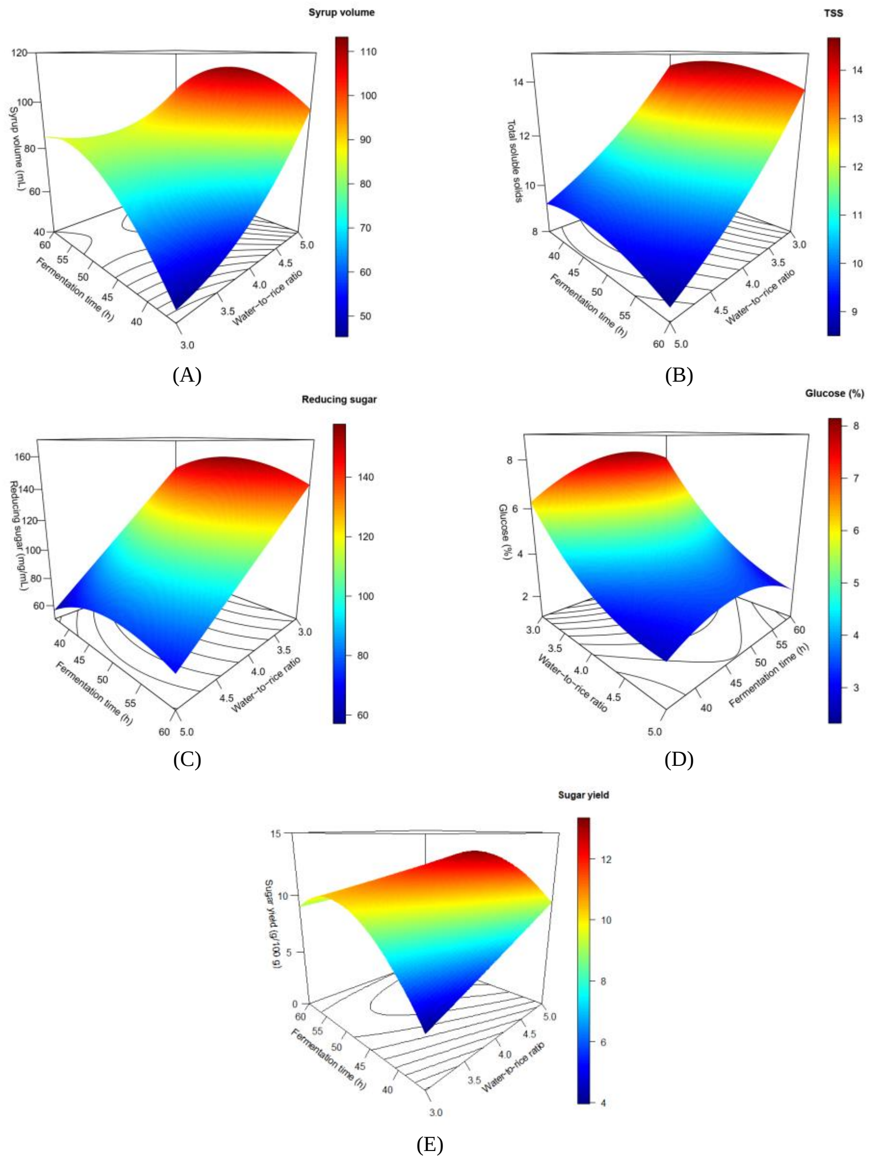

Figure 2. Response surface plot of (A) syrup volume, (B) total soluble solids, (C) reducing sugar concentration and (D) glucose concentration as functions of water-to-rice ratio and fermentation time. The inoculum volume was fixed at the center point.

shapes, as illustrated in Figure 2C and 2D. A correlation between these variables was also observed (Figure 3). The water-to-rice ratio had a negative impact on both response variables, likely due to a dilution effect. An increase in the water-to-rice ratio results in a larger syrup volume but a reduced sugar concentration. Interestingly, this leads to a net increase in the yield of reducing sugars, as depicted in Figure 2E. The role of water in solid-state fermentation is critical, especially in enhancing the solubility of substrate nutrients (Gervais & Molin, 2003). Notably, water has been found to boost the production of amylase in *Thermomyces lanuginosus* when used with solid agricultural wastes and generation of lignocellulose-degrading enzymes in solid-state fermentation of *Ganoderma lucidum* (Hu *et al.*, 2022; Kunamneni, Permaul, & Singh, 2005). In the early stages of fermentation, both reducing sugar and glucose concentrations increased. However, these values gradually declined during prolonged fermentation, which may be attributable to the microbial transformation of glucose into other products. This observed saddle point in the surface plot suggests that an optimal fermentation time could be determined for maximizing the concentrations of reducing sugars and glucose.

Maltose serves as an intermediate compound that is ultimately hydrolyzed into glucose. The concentration of maltose ranged from 0.06 ± 0.01% to 0.68 ± 0.52%, which is considerably lower compared to glucose, whose concentration ranged from 1.49 ± 0.78% to 9.48 ± 0.39%. This unstable behavior of maltose likely contributed to the low adjusted R^2 value for its corresponding model. Consequently, the surface plot for maltose is not presented, and this variable was excluded from the optimization process due to its low predictability.

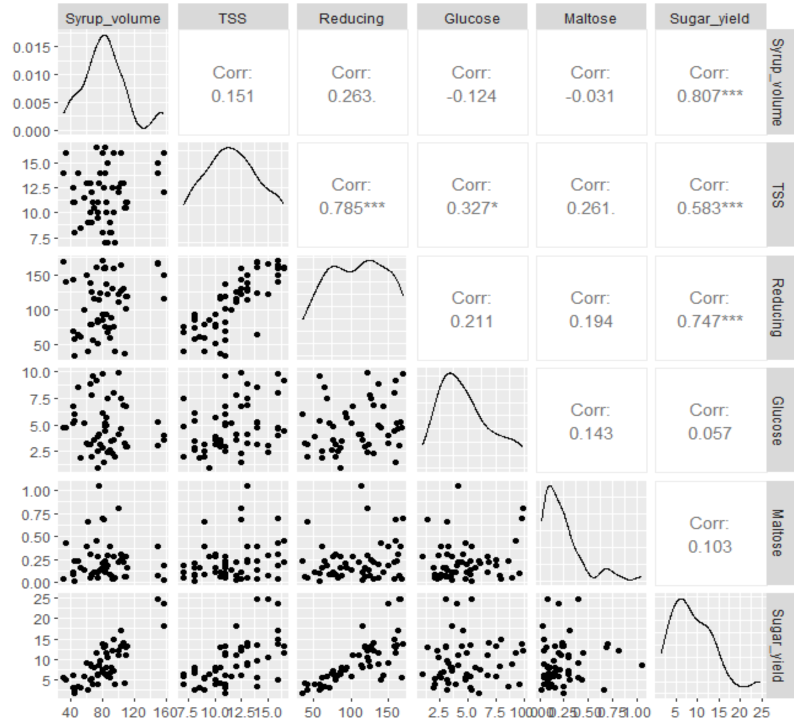

Figure 3. Correlation matrix between response variables during fermentation of rice syrup
Note: Values in the plot represent Pearson's correlation coefficient; *** $P < 0.001$

3.5 Optimal conditions

To calculate the optimal conditions for solid-state fermentation, the response values for all variables were targeted to reach their maximum levels. The optimal conditions identified were as follows: a water-to-rice ratio of 3:1, an inoculum volume of 10 mL, and a fermentation time of 60 min. The models were subsequently validated by conducting extractions under these optimal conditions. The actual values were not significantly different from the predicted response values for all variables, as shown in Table 4.

Although the correlation between syrup volume and sugar variables was negative, it was not statistically significant. This made it impossible to simultaneously achieve the highest syrup volume and the highest sugar concentrations. As a result, the values obtained under the optimal conditions were lower than the highest values achieved for each individual variable.

The maximum concentration of reducing sugar achieved under optimal conditions in this study was 152.47 ± 2.17 mg/mL. This is comparable to levels found in amazake, a Japanese sweet rice beverage, which has a reducing sugar content ranging from 137 to 169 mg/mL when using yellow koji for 10 hours and white koji for 6 hours, respectively (Saigusa & Ohba, 2007). On the other hand, our results surpassed those of rice beverages prepared using red koji or monascal rice, which reported a reducing sugar concentration of 44.2 mg/mL after 6 hours of fermentation (Kim, Kim, Kim, Kim, & Suh, 2008). This discrepancy may be attributed to variations in fermentation time and the specific fungal strains used.

Table 4. Actual and predicted responses obtained from extraction at optimal conditions.

Response variable	Predicted value	Actual value
Syrup volume (mL)	94.24	95.67 ± 3.17
TSS (˚Brix)	14.92	16.17 ± 0.24
Reducing sugar (mg/mL)	154.12	152.47 ± 2.17
Glucose (%)	8.59	8.58 ± 0.46
Sugar yield (g/100 g)	12.49	14.58 ± 0.55

Note: Actual and predicted values were not significantly different for all variables

In terms of glucose concentration, our study yielded a higher level (8.59%) compared to beverages made with monascal rice, which had a concentration of just 2.1%. Conversely, the maltose concentration in our study (0.68 ± 0.52%) was lower than that in beverages made with monascal rice (6.45%) (Kim *et al*., 2008). Another study on amazake fermented by rice koji at 50°C for 8 hours reported even higher glucose levels (13.5%), but lower maltose concentration (0.1%). This suggests that fermentation temperature can influence the saccharide composition in these beverages (Oguro, Nakamura, & Kurahashi, 2019).

Beyond sugar production, microbial fermentation of rice syrup also yields beneficial compounds such as prebiotic oligosaccharides, amino acids, and vitamin B, while enhancing antioxidant activity (Oguro *et al*., 2019; Saigusa & Ohba, 2007). These health benefits are not achievable through acid or enzymatic preparation methods for rice syrup. Future research should focus on optimizing these health benefits, as well as evaluating the flavor profile and consumer acceptance of the product.

4. Conclusions

This study successfully demonstrated the efficacy of using quadratic models to analyze the relationships between multiple independent variables—namely the water-to-rice ratio, inoculum volume, and fermentation time—and various response variables such as syrup volume, total soluble solids (TSS), and concentrations of reducing sugar, glucose, and maltose. The optimal conditions for achieving maximum response values were identified as a water-to-rice ratio of 3:1, inoculum volume of 10 mL, and a fermentation time of 60 minutes. Validation at these optimal conditions showed that the actual and predicted response values were not significantly different, thereby confirming the robustness of the models. However, the study also highlighted the limitations of aiming to optimize multiple variables simultaneously, particularly when these variables exhibit negative correlations. For example, it was challenging to maximize both syrup volume and sugar concentration.

Comparisons with existing literature revealed that the reducing sugar and glucose concentrations achieved in this study are competitive to traditional rice-based beverages like amazake. Future work should aim to refine the fermentation process to balance yield and nutritional value effectively.

Acknowledgements

This research was supported by the University of Phayao and the Thailand Science Research and Innovation Fund (Grant No. FF65-RIM017 and Fundamental Fund 2024).

References

Akoh, C. C., Chang, S. W., Lee, G. C., & Shaw, J. F. (2008). Biocatalysis for the production of industrial products and functional foods from rice and other agricultural produce. *Journal of Agricultural and Food Chemistry*, *56*(22), 10445–10451. doi:10.1021/jf801928e

Amdoun, R., Khelifi, L., Khelifi-Slaoui, M., Amroune, S., Asch, M., Assaf-Ducrocq, C., & Gontier, E. (2018). The desirability optimization methodology; a tool to predict two antagonist responses in biotechnological systems: case of biomass growth and hyoscyamine content in elicited datura starmonium hairy roots. *Iranian Journal of Biotechnology*, *16*(1), 11–19. doi:10.21859/ijb.1339

Chanchomsuk, C., Chailangka, K., Srimuang, K., Prommajak, T., & Attabhanyo, R. (2024). Isolation and screening of molds from traditional Look-pang starter cultures for rice syrup production. *Food and Applied Bioscience Journal*, *12*(1), 1.

Das, A. J., Miyaji, T., & Deka, S. C. (2017). Amylolytic fungi in starter cakes for rice beer production. *The Journal of General and Applied Microbiology*, *63*(4), 236–245. doi:10.2323/jgam.2016.11.004

Food and Agriculture Organization of the United Nations. (2023). *FAOSTAT: Food and agricultural data*. Retrieved from https://www.fao.org/faostat/en/#data/QCL

Gervais, P., & Molin, P. (2003). The role of water in solid-state fermentation. *Biochemical Engineering Journal*, *13*(2), 85–101. doi:10.1016/S1369-703X(02)00122-5

Hu, S., Zhu, Q., Ren, A., Ge, L., He, J., Zhao, M., & He, Q. (2022). Roles of water in improved production of mycelial biomass and lignocellulose-degrading enzymes by water-supply solid-state fermentation of *Ganoderma lucidum*. *Journal of Bioscience and Bioengineering*, *133*(2), 126–132. doi:10.1016/j.jbiosc.2021.10.006

Kanda, Y. (2013). Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplantation*, *48*(3), 452–458. doi:10.1038/bmt.2012.244

Kim, S. Y., Kim, Y. S., Kim, Y. S., Kim, J. M., & Suh, H. J. (2008). The application of monascal rice in rice beverage preparation. *LWT - Food Science and Technology*, *41*(7), 1204–1209. doi:10.1016/j.lwt.2007.06.011

Kunamneni, A., Permaul, K., & Singh, S. (2005). Amylase production in solid state fermentation by the thermophilic fungus *Thermomyces lanuginosus*. *Journal of Bioscience and Bioengineering*, *100*(2), 168–171. doi:10.1263/jbb.100.168

Lenth, R. V. (2009). Response-surface methods in R, using rsm. *Journal of Statistical Software*, *32*(7 SE-Articles), 1–17. doi:10.18637/jss.v032.i07

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428.

Oguro, Y., Nakamura, A., & Kurahashi, A. (2019). Effect of temperature on saccharification and oligosaccharide production efficiency in koji amazake. *Journal of Bioscience and Bioengineering*, *127*(5), 570–574. doi:10.1016/j.jbiosc.2018.10.007

Okuda, M. (2019). Rice used for Japanese sake making. *Bioscience, Biotechnology, and Biochemistry*, *83*(8), 1428–1441. doi:10.1080/09168451.2019.1574552

Permanasari, A. R., Yulistiani, F., Purnama, R. W., Widjaja, T., & Gunawan, S. (2018). The effect of substrate and enzyme concentration on the glucose syrup production from red sorghum starch by enzymatic hydrolysis. *IOP Conference Series: Earth and Environmental Science*, *160*(1), 12002. doi:10.1088/1755-1315/160/1/012002

Roongrojmongkhon, N., Rungjindamai, N., Vatanavicharn, T., & Ochaikul, D. (2020). Isolation and identification of fungi with glucoamylase activity from Loog-pang-khao-mak (A Thai traditional fermentation starter). *Journal of Pure and Applied Microbiology*, *14*(1), 233–246. doi:10.22207/JPAM.14.1.24

Saigusa, N., & Ohba, R. (2007). Effects of koji production and saccharification time on the antioxidant activity of amazake. *Food Science and Technology Research*, *13*(2), 162–165. doi:10.3136/fstr.13.162

Sivamaruthi, B. S., Alagarsamy, K., Suganthy, N., Thangaleela, S., Kesika, P., & Chaiyasut, C. (2022). The role and significance of *Bacillus* and

Lactobacillus species in Thai fermented foods. *Fermentation, 8*(11), 635. doi:10.3390/fermentation 8110635

Soetaert, K. (2021). *plot3D: plotting multi-dimensional data. R package version 1.4*. https://cran.r-project.org/ package=plot3D

Thancharoen, K., & Malasri, A. (2023). Diversity of yeasts and molds isolated from Look-pang. *RMUTP Research Journal*, *17*(1), 27–39. doi:10.14456/ jrmutp.2023.3

United Nations. (2015). *Transforming our world: The 2030 Agenda for Sustainable Development. A/RES/70/1*