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Fermentation Kinetic and Alpha-Amylase Inhibition Studies of Mao Wine Fermented by Three Commercial *Saccharomyces cerevisiae* Yeasts

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Abstract

Mao wine is produced from mao (*Antidesma thwaitesianum*) and is a local beverage promoting Sakon Nakhon province, Thailand. Understanding the fermentation process is necessary in order to enhance the quality of mao wine products. This study aims to examine the kinetics of mao wine fermentation using 3 commercial types of *Saccharomyces cerevisiae* yeasts, namely Lalvin 71B, Lalvin RC212 and Lalvin K1-V1116, with an initial pH of 3.4, a sweetness of 22 % Brix and a yeast concentration of 0.4 g L^{-1} in controlled conditions at 25 °C for 10 days. The results revealed that the kinetic models, including logistic, Luedeking-Piret and modified Luedeking-Piret equations, are appropriate for analyzing the kinetics of yeast growth, alcohol production and reducing sugar, respectively. Lalvin K1-V1116 yeast yielded the highest capacity for alcohol production (~113 g L^{-1}), sugar-to-alcohol conversion (45 % conversion), and product efficiency (88 %), indicating its suitability for mao wine production. Furthermore, the investigation of the alpha-amylase inhibitory activity revealed that the IC_{50} values of mao wine samples were 2.88 - 4.78 mg mL^{-1}, which were roughly 410 - 680 times greater than those of acarbose. These findings revealed the low efficacy of mao wine in inhibiting alpha-amylase activity. Nevertheless, a decrease in the level of inhibition may result in a decreased likelihood of side effects or undesirable reactions. This treatment is especially beneficial for people who have medication sensitivities. Additional research is required in order to substantiate its applicability in the management of type 2 diabetes.

Keywords: Mao, Wine, *S. cerevisiae*, Fermentation kinetics, Yeast growth, Alcohol production, Reducing sugar consumption, Alpha-amylase inhibition, Diabetes

Introduction

Mao (*Antidesma thwaitesianum*) is a wild fruit found in Sakon Nakhon province in the northeast of Thailand. Mao is known to possess a variety of phytochemicals that exhibit antioxidant features, including polyphenols and anthocyanins [1,2]. Ripe fruits are frequently processed into a variety of products such as wine, jam, juice and others. Mao products are a delicious reddish-purple color and have a wide range of therapeutic benefits [3,4]. Drinks containing anthocyanin-rich fruit extract decrease postprandial blood glucose [5]. Mao is a promising agent for reducing monosaccharide-induced protein glycation, inhibiting the activity of carbohydrate-digesting enzymes, including alpha-amylase [6,7]. Alpha-amylase is an enzyme that plays a role in the digestion of starches into simpler sugars. Although it is not a direct cause or treatment for diabetes, it could be relevant in the context of diabetes management and monitoring. Generally, wine is an alcoholic beverage with an alcohol concentration that does not exceed 14 %. It is produced through the process of fermenting juice and yeast under specific conditions, such as controlled air exposure. The process described is commonly referred to as alcoholic fermentation, wherein the reaction progresses in the following manner: $C_6H_{12}O_6$ + yeast → $2C_2H_5OH + 2CO_2$ + energy. Mao wine is a local beverage that promotes Sakon Nakhon province because of its delicious flavor and stunning rich crimson hue. The investigation of the fermentation process of mao wine and the various aspects that influence its

production are essential for improving the quality of mao wine products within the region. Winemakers in the region face various challenges related to environmental factors and traditional conservation methods. Producing mao wine is a complex and delicate process. This requires careful attention to these variables for successful production.

Kinetic studies of wine play a crucial role in understanding and controlling the chemical and physical transformations that occur during winemaking, aging and storage [8]. These data offer winemakers to enhance procedures, maintain quality and guarantee the creation of wines with desired taste characteristics and stability [9]. By monitoring and analyzing key kinetic parameters, such as sugar consumption, acid formation, volatile compound evolution and tannin polymerization, winemakers can make informed decisions to ensure the production of high-quality wines [10]. The goal of a kinetic model created for wine fermentation is to predict how yeast will behave kinetically during the fermentation process based on the juice's initial properties [11,12]. In the study of yeast behavior and metabolic regulation, it is crucial to find a reliable mathematical model of the fermentation kinetics [13-15]. A suitable model of fermentation that takes into account its technical, economic and physiological implications would be an effective tool for forecasting and managing problematic fermentations as well as for better understanding the fermentation process [16]. Several physical and mathematical models for wine fermentation have been examined, and kinetic methods have provided extensive documentation of many factors that can cause problematic fermentations, including nitrogen and oxygen limitation, temperature extremes and others [11,14,17,18].

To the best of our current knowledge, the specific variables that influence the kinetics of fermentation in mao wine remain unidentified as well as the acclaimed type 2 diabetes mellitus management with mao wine is yet reported scientifically. Type 2 diabetes mellitus is a chronic metabolic disorder characterized by elevated blood sugar levels (hyperglycemia) due to insulin resistance and impaired insulin secretion. Thus, controlling blood sugar levels is another option to reduce the severity of the disease. The inhibitory effect of mao wine on alpha-amylase is of particular relevance due to its potential benefits for patients diagnosed with type 2 diabetes.

Therefore, the present study was undertaken to investigate the kinetics of mao wine fermentation process using 3 different types of *Saccharomyces cerevisiae* yeasts, including Lalvin 71B, Lalvin RC212 and Lalvin K1-V1116. These 3 commercial yeast strains are used to ferment red wine or berry wine, which are popular products in Sakon Nakhon province. Thus, the primary factor that led to their selection mostly relied on the prevalence of utilization among winemakers in Sakon Nakhon province. Conducting study on the kinetics of mao wine fermentation utilizing different yeast strains could be advantageous for winemakers. The utilization of this study facilitates the improvement of wine quality, the creation of unique wine varieties and the optimization of fermentation procedures, ultimately advancing the art of winemaking. Furthermore, the efficacy of mao wine was assessed in terms of its potential to suppress the enzymatic activity of alpha-amylase during starch hydrolysis, thereby leading to a reduction in starch digestibility. While not directly causing or treating type 2 diabetes, it can play a critical role in managing and monitoring the disease.

Materials and methods

Materials

The following were the materials used in this work: Mao fruit (*Antidesma thwaitesianum* Müll. Arg.) of the Fa Prathan variety (brought from Phu Phan district, Sakon Nakhon province, Thailand); potassium metabisulfite ($K_2S_2O_5$) campden tablets (LD Carlson company, USA); commercial yeast strains of *S. cerevisiae*, including Lalvin 71B, Lalvin RC212 and Lalvin K1-V1116 (Lallemand Inc., Canada); 3,5-dinitrosalicylate ($C_7H_4N_2O_7$) 98 %, AR grade (Loba, India); alpha-amylase from *Bacillus* sp., type II-A, lyophilized powder, ≥ 1,500 units mg^{-1} protein (biuret) (Sigma-Aldrich, Germany); D(+)-glucose monohydrate, AR grade (Qrec, New Zealand); acarbose ≥ 95 % (Sigma-Aldrich, Germany), sodium hydroxide (NaOH) pellet 99 % AR grade (Qrec, New Zealand); UV-visible spectrophotometer (UV-1800, Shimadzu, Japan); microplate reader (CYATION B Imaging reader, Bio Tek, USA); shaking incubator (Floor Model, SHEL LAB, USA); Colorimeter, ColorFlex EZ (HunterLab, USA).

Study of fermentation kinetics

Ripe maos were washed with 0.116 g L^{-1} $K_2S_2O_5$ before being crushed in a 1:2 ratio with water (w/v). The juice was heated to 80 °C and sugar was added until the desired sweetness level of 22 % Brix was achieved. The acidity was evaluated based on 0.46 g L^{-1} of tartaric acid at pH 3.4. For each batch sample, 0.04 g of dry *S. cerevisiae* yeast (Lalvin 71B, Lalvin RC212, or Lalvin K1-V1116) was mixed with 5 mL of mao juice and left to activate at 42 °C for 30 min. It was then mixed with a volume of 95 mL of mao

juice in a 150 mL bottle. Three replicates were conducted for each sample. For fermentation kinetic study, the samples were incubated in an incubator for 10 days at a temperature of 25 °C [15]. Daily measurements were taken for the yeast concentration, remaining sugar amount, alcohol production, pH and acidity percentage. The yeast concentration was determined using the dry weight method [19]. The sample of 5 mL was centrifuged at 1500× g for 5 min. Following the removal of the transparent portion, the sediment was washed 3 times with distilled water. Subsequently, it was subjected to drying at a temperature of 105 °C until a consistent weight was achieved. The weight of the resulting sludge was then determined. The gravimetric method was employed to estimate the amount of alcohol produced [20]. The sample was filtered before measuring. Meanwhile, the remaining sugar content was determined using the dinitrosalicylate (DNS) method [21]. The sample was 25-fold diluted. The volume of 0.5 mL of 3,5-dinitrosalicylate was added to 0.5 mL of the diluted sample, then the mixture was heated for 10 min, followed by cooling and being diluted with 5 mL of distilled water. The absorbance of the sample was then measured at a wavelength of 540 nm, and glucose was employed as a reducing sugar standard for evaluating the sample concentration. The simulated kinetic models and statistical analyses were conducted using the OriginPro 2018 software [22].

Yeast growth kinetic study

The yeast growth kinetics was investigated using the logistic equation which is expressed as:

$$X(t) = \frac{X_0 e^{\mu_m t}}{1-\left(X_0/X_m\right)\left(1-e^{\mu_m t}\right)} \tag{1}$$

where μ_m is the specific growth rate (day^{-1}), X_m is the maximum yeast concentration (g L^{-1}), X_t is the number of yeasts (g L^{-1}) at time t (day) and X_0 is the starting number of yeasts (g L^{-1}).

Alcohol kinetic study using the Luedeking-Piret equation

The investigation of alcohol production kinetics utilized the Luedeking-Piret equation, which was first developed by Luedeking and Piret [15]. The observed correlation between rates of product formation and cell growth demonstrates that the process of alcohol formation exhibits a temporal delay in relation to cell growth, rather than occurring simultaneously. Consequently, the process of alcohol formation exhibits a partial coupling with cell growth, which can be mathematically represented by the following equation:

$$P(t) = P_0 + \alpha X_0\left(\frac{e^{\mu_m t}}{1-\left(X_0/X_m\right)\left(1-e^{\mu_m t}\right)}-1\right) + \beta\frac{X_m}{\mu_m}\cdot\ln\left(1-\frac{X_0}{X_m}\left(1-e^{\mu_m t}\right)\right) \tag{2}$$

where $P(t)$ is the alcohol concentration (g L^{-1}) at time t (day), P_0 is the initial alcohol concentration (P_0 = 0 g L^{-1}), α is the stoichiometry of yeast growth over time (g day^{-1}), and β is the coefficient of specificity of yeast (g day^{-1}).

Sugar consumption kinetic study

The process of alcohol fermentation involves the conversion of a sugar into products. An appropriate kinetic equation for this process can be derived by modifying the Luedeking-Piret equation, which is written as:

$$S(t) = S_0 - \gamma X_0\left(\frac{e^{\mu_m t}}{1-\left(X_0/X_m\right)\left(1-e^{\mu_m t}\right)}-1\right) - \eta\frac{X_m}{\mu_m}\cdot\ln\left(1-\frac{X_0}{X_m}\left(1-e^{\mu_m t}\right)\right) \tag{3}$$

where $S(t)$ is the sugar concentration (g L^{-1}) at time t (day), S_0 is the initial sugar concentration (g L^{-1}), $\eta = m_s + \beta/Y_{P/S}$, and $\gamma = (1/Y_{x/S}) + \alpha(\beta/Y_{P/S})$. Here, m_s is the yeast metabolic constant (gram of sugar per gram of yeast), $Y_{P/S}$ and $Y_{x/S}$ are the stoichiometry coefficient of ethanol to sugar formation (gram of ethanol per gram of sugar) and the stoichiometry of yeast growth to sugar (gram of yeast per gram of sugar), respectively.

Wine characteristic

In order to investigate the characteristics of mao wines, the samples underwent a fermentation process for 28 days. The production process was followed by the measurement of many parameters, including pH, total acidity percentage, production efficiency and color assessment.

Total acidity

A wine sample of 1 mL was diluted with 100 mL of distilled water and the solution was then titrated with 0.1 N NaOH using bromothymol blue as an indicator. The overall acidity percentage was determined in terms of the tartaric acid equivalent, which is expressed as:

$$\%\text{Total acidity} = \frac{V_1 \times N \times 75}{10 \times V_2} \tag{4}$$

where V_1 and N are the volume and concentration of NaOH used in the titration, respectively. V_2 is the sample volume, and the value 75 corresponds to the molecular weight of tartaric acid.

Production efficiency

The investigation focused on evaluating the efficiency of wine production by yeast through the conversion of total reducing sugars into ethanol. The calculation is represented as:

$$\text{Production efficiency}(\%) = \frac{A}{0.51} \times 100 \tag{5}$$

where A is the conversion of sugar to alcohol which is determined based on the linear relationship between alcohol produced and sugar intake [23]. A number 0.51 refers to the value of theoretical alcohol produced according to alcoholic fermentation.

Color assessment

The color of the wine was assessed using the CIELAB color system. The color described in this method is defined by 3 parameters: L^* representing perceptual lightness, and a^* and b^* representing hue directions. The numbers $+a^*$, $-a^*$, $+b^*$ and $-b^*$ refer to the color characteristics of red, green, blue and yellow, respectively [24-26]. The ΔE^* value, used for evaluating the color difference, is also incorporated within the CIELAB system. The color measurement in transparency mode was conducted using a colorimeter. In the context of color analysis, the wine samples were filtered before being analyzed without dilution. This analytical procedure was repeated 3 times.

Alpha-amylase inhibition

Mao wine has been mentioned among locals for its potential as a diabetes treatment. Therefore, the investigation of its potential as a therapeutic option for managing type 2 diabetes mellitus was carried out, focusing on the assessment of its inhibitory activity of the alpha-amylase. To assess and compare the inhibitory activities of mao wine, samples including crude extracts from fresh mao, mao wines fermented by *S. cerevisiae* yeasts and the medication acarbose were prepared. A 0.01 u L^{-1} of alpha-amylase was incubated with a 25 mL of sample at 37 °C for 30 min. Then, it was taken out of incubation and left for 10 min and a 2 % starch solution was added. The analysis of the remaining reducing sugars was performed using the DNS method [27,28]. The alpha-amylase inhibitory activity was expressed as percentage inhibition and was calculated using the equation given below. The inhibitory ability was determined by calculating the IC_{50} value, which was obtained from the plot of % inhibition *versus* sample concentration, and finding the concentration of the sample required to inhibit 50 % of alpha-amylase from the graph.

$$\%\text{Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \tag{6}$$

where A_{control} is the absorbance of the inhibitor's absence, where it was set up to achieve 100 % enzyme activity, and A_{sample} is the absorbance of the inhibitor's presence.

Results and discussion

Kinetics of mao wine fermentation process

The wine fermentation process was monitored for 10 days, with the objective of enhancing comprehension regarding the kinetics of wine fermentation when using different yeast strains. The kinetic investigation was divided into 3 sections, including yeast growth, alcohol production and reducing sugar consumption.

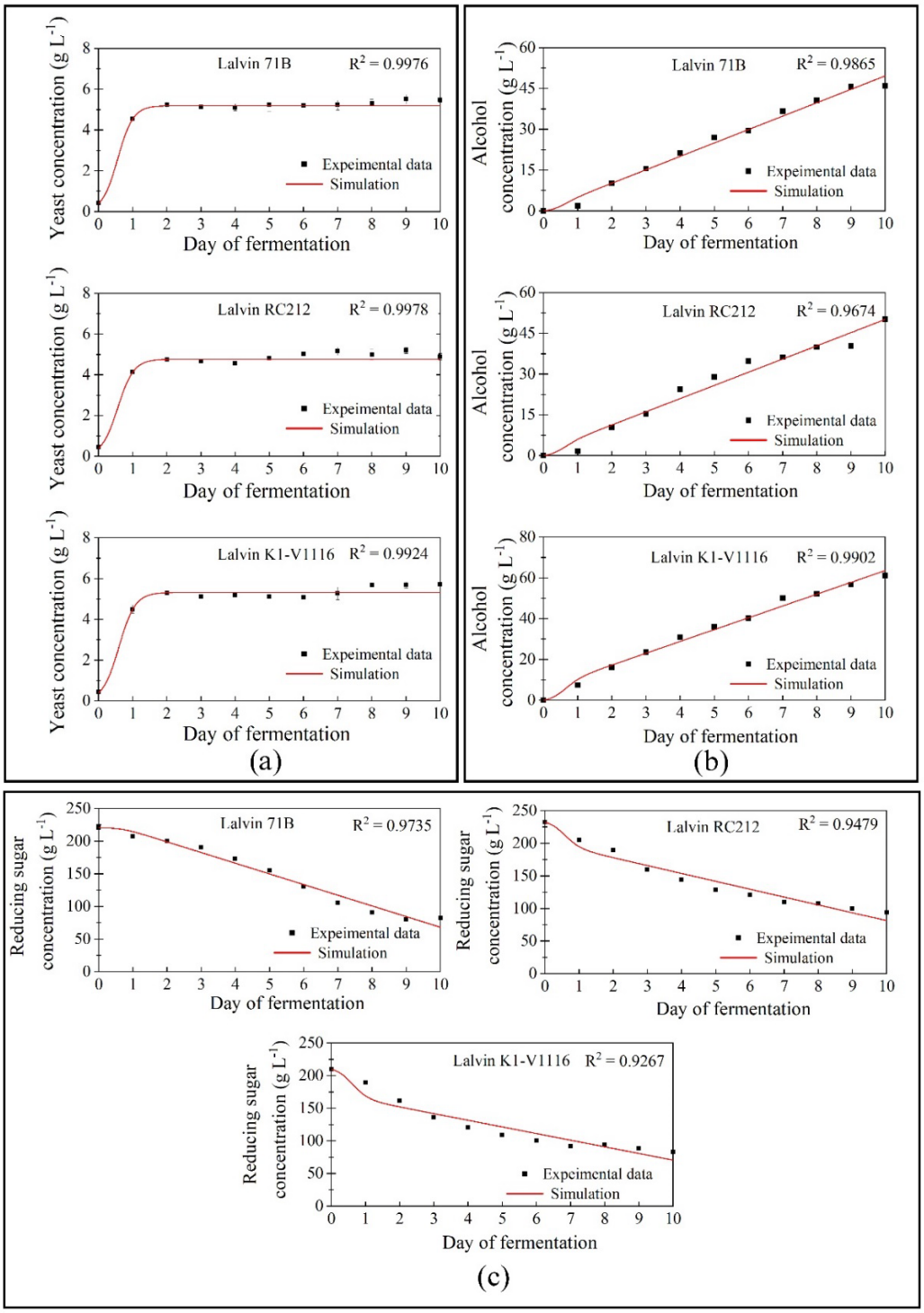

Figure 1 Simulated kinetic models of a) yeast growth, b) alcohol production and c) reducing sugar concentration of mao wines fermented by Lalvin 71B, Lalvin RC212 and Lalvin K1-V1116.

Table 1 Kinetic parameters for mao wines fermented by 3 *S. cerevisiae* yeasts.

Parameter		*S. cerevisiae* yeast		
		Lalvin 71B	**Lalvin RC212**	**Lalvin K1-V1116**
Yeast growth	X_0 (g L^{-1})	0.40	0.40	0.40
	X_m (g L^{-1})	5.19 ± 0.11^b	4.77 ± 0.06^a	5.32 ± 0.13^b
	μ_m (day^{-1}),	4.43 ± 0.21^a	4.27 ± 0.57^a	4.21 ± 1.68^a
	R^2	0.9976	0.9978	0.9924
Alcohol production	α (g day^{-1})	0.67 ± 0.27^a	1.01 ± 0.46^a	1.89 ± 0.27^b
	β (g day^{-1})	0.95 ± 0.04^a	1.02 ± 0.07^{ab}	1.09 ± 0.04^b
	R^2	0.9865	0.9674	0.9902
Reducing sugar consumption	γ	-0.36 ± 0.19^a	8.52 ± 1.64^b	8.92 ± 1.59^b
	η	3.15 ± 0.19^c	2.53 ± 0.26^b	1.91 ± 0.26^a
	R^2	0.9735	0.9479	0.9267

Note: $^{a-d}$ represent the significant differences of the kinetic parameters ($p \leq 0.05$). Statistic calculation was calculated using the Tukey test.

Yeast growth kinetics

The logistic equation was employed to investigate the kinetics of yeast growth. The growth rate of yeast throughout the fermentation process was shown to exhibit an "S-pattern" consistent with the logarithmic equation. This pattern revealed evidence of the inhibitory effect of cell concentrations on cell growth. The fermentation conditions employed are intended to encourage the growth of *S. cerevisiae*. The initial day of the fermentation process exhibited the most dramatic growth of yeast, consistent with previously reported observations [15,29]. The yeast population showed rapid multiplication during the initial stages, followed by a stabilization at a particular quantity, as depicted in **Figure 1(a)**. The parameters derived from the simulated equations are presented in **Table 1**. The specific growth rate observed in each yeast fermentation exhibited minimal variation, ranging from 4.21 to 4.33 day^{-1}. Nevertheless, it was observed that the Lalvin K1-V1116 yeast strain demonstrated the highest yeast concentration, with a maximum value (X_m) of 5.32 g L^{-1}, although exhibiting a slightly lower growth rate in comparison to the other strains. Based on the results obtained, it was observed that the yeast exhibited a rapid rate of proliferation during the fermentation process of mao wine.

Alcohol production kinetics

Wine is an alcoholic beverage that is produced by the fermentation of fruit juice, wherein yeast is employed to convert sugars into alcohol. Consequently, the quantity of alcohol produced is directly related to the quantity of yeast utilized during the fermentation process. The relationship can be predicted using the Luedeking-Piret equation, as demonstrated by the curve fitting depicted in **Figure 1(b)**. The equation demonstrates a high consistency with the experimental findings, as evidenced by a coefficient of determination (R^2) exceeding 0.9, as indicated in **Table 1**. The stoichiometry of yeast growth over time (α) and the coefficient of specificity of yeast (β) obtained from the Lalvin K1-V1116 strain (α = 1.89 and β = 1.09) exhibit notable distinctions when compared to those of the Lalvin 71B and Lalvin RC212 strains. The observed higher value indicates that the Lalvin K1-V1116 system exhibits greater activity and possesses a larger capacity for alcohol production compared to other systems under identical fermentation conditions.

Reducing sugar consumption kinetics

The process of fermentation involves the conversion of sugars into alcohol, other products and energy. The kinetic model that the sugar is used throughout the fermentation process of mao wine was found to be compatible with the modified Luedeking-Piret equation, with a coefficient of determination (R^2) exceeding 0.9 (**Table 1**). The inquiry findings indicated that the γ parameter of Lalvin 71B exhibited a negative value, whereas the remaining systems showed positive values, reaching up to ~9. The observed negative value could possibly be attributed to the lower relevance of the stoichiometry coefficient of yeast growth to sugar ($Y_{x/s}$), which is evident from the graph that there exists a slightly positive peak in the 1st simulation curve of sugar consumption kinetic (**Figure 1(c)**). The yeast metabolic constant (m_s), which reflects the η parameter, reveals how much yeast contributes to its metabolic processes. Therefore, variations in the yeast's ability to convert sugars into other metabolites can be responsible for differences in η value. Yeast

strain Lalvin 71B provided the highest η value, which was then followed by Lalvin RC212 and Lalvin K1-V1116, respectively.

It is noteworthy that the simulated equations exhibit suitability for the study of kinetics, particularly the logistic equation used for the investigation of yeast growth kinetics. This equation yielded R^2 values more than 0.99 for all strains of yeast.

Wine characteristic

Total acidity and production efficiency

The original mao juice had a pH range of 3.3 - 3.5, which has been found to be suitable for optimal yeast growth [30]. During the fermentation process, it was observed that the total acid content ranged from 0.52 to 0.69 %. **Figure 2** illustrates that the concentration of acid increases after the fermentation process. Nevertheless, it was discovered that all strains of yeast yielded similar pH values. The pH values observed before and after fermentation ranged from 3.4 to 3.6, suggesting that the fermentation process does not exert significant effects on the pH level. It should be noted that fruit wines commonly exhibit standard values of approximately 3.4 - 3.5 for pH and 0.8 - 0.9 % for total acidity [9].

The investigation of the efficiency of wine production by analyzing the process of converting reducing sugars into alcohol over a period of 28 days during the fermentation phase. The production efficiency can be used to assess the survival of *S. cerevisiae* strains throughout the fermentation process. According to the data presented in **Table 2**, The results indicated that the alcohol content ranged from around 92 to 113 g L^{-1}, with an alcohol by volume percentage (%ABV) of approximately 12 to 14 %. These findings correspond with the average alcohol concentration observed in wines. The amount of alcohol produced correlates with the amount of reducing sugar intake, indicating a high positive correlation coefficient ($R \approx 0.99$). The results obtained from the mao wine fermentation process show that Lalvin 71B, RC212 and K1-V1116 strains of *S. cerevisiae* yielded the production efficiencies of around 73, 78 and 88 %, respectively. According to the given information, Lalvin K1-V1116 exhibited the most notable degree of fermentation efficiency. Conversely, Lalvin 71B and Lalvin RC212 resulted in comparable levels of efficiency.

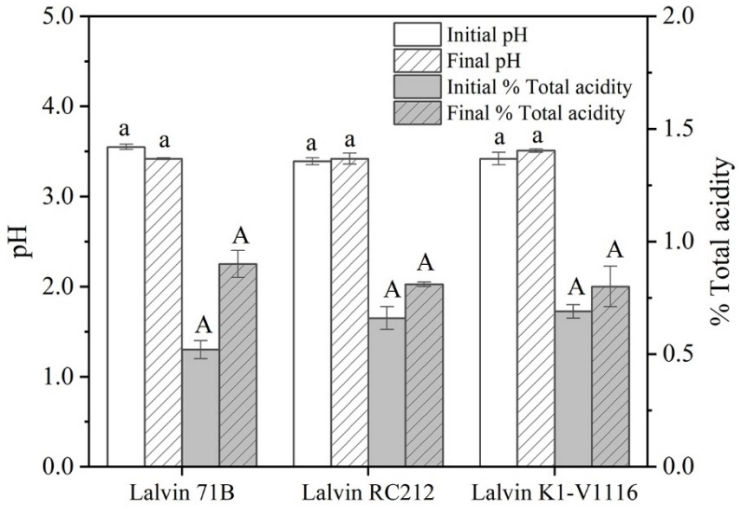

Figure 2 pH and %Total acidity of mao wines fermented by Lalvin 71B, Lalvin RC212 and Lalvin K1-V1116. The letters "a" and "A" represent the significant differences in pH value and %Total acidity, respectively ($p \leq 0.05$).

Table 2 Production efficiency of mao wines fermented by 3 yeast strains.

Yeast type	%ABV	Produced alcohol (g L^{-1})	Reducing sugar intake (g L^{-1})	Sugar-to-alcohol conversion	Correlation coefficient (R)	Production efficiency (%)
Lalvin 71B	11.68	92.15	246.42	0.37	0.9934	72.55
Lalvin RC212	12.96	102.57	252.83	0.40	0.9911	78.43
Lalvin K1-V1116	14.27	112.90	248.53	0.45	0.9887	88.24

Color assessment

The color of wine products was determined using the ColorFlexEZ instrument and reported in the CIELAB system. The measurements were compared to the values of wine from Chateau de Phu Phan (Sakon Nakhon winery), which is a well-known wine used as a benchmark. This benchmark provided L^* (brightness), a^* (red-green color) and b^* (yellow-blue color) values of 3.26, 1.74 and –0.23, respectively (**Table 3**). The L^* values of wine samples obtained from 3 different yeast strains ranged from 1.00 - 1.43, which were observed to be lower than those of the benchmark wine. This suggests that Chateau de Phu Phan wine demonstrates a higher level of brightness compared to the wine samples. This phenomenon may be attributed to the characteristic of the reference wine as a long-aging wine, resulting in enhanced clarity and more vibrant. The fermented mao wines exhibited a higher a^* value, suggesting a darker red color in the wine samples. In similar fashion, the wine samples showed higher b^* values, indicating a greater presence of the blue color in comparison to the benchmark wine. Furthermore, the color difference of mao wine fermented by Lalvin K1-V1116 strain showed a higher data (ΔE^* of 5.09) than other wines when compared to the Chateau de Phuphan wine. When comparing mao juice to mao wine, it was observed that all mao wine samples were found to be brighter and darker hues, possibly due to the formation of anthocyanin copigments and other substances within the mao, which may cause changes in coloration [31].

Table 3 Colors of mao wine products reported in CIELAB parameters.

Mao wine	L^*	a^*	b^*	ΔE^*
Chateau de Phuphan	3.26 ± 0.01^{e}	1.74 ± 0.07^{b}	$–0.23 \pm 0.07^{a}$	-
Mao juice	0.76 ± 0.03^{a}	1.38 ± 0.05^{a}	0.31 ± 0.01^{b}	2.74 ± 0.04^{a}
Mao wine (Lalvin 71B)	1.00 ± 0.03^{b}	4.02 ± 0.01^{c}	1.05 ± 0.05^{c}	3.41 ± 0.05^{b}
Mao wine (Lalvin RC212)	1.14 ± 0.03^{c}	4.99 ± 0.06^{d}	1.18 ± 0.07^{c}	4.07 ± 0.03^{c}
Mao wine (Lalvin K1-V1116)	1.43 ± 0.05^{d}	6.25 ± 0.24^{e}	1.49 ± 0.06^{d}	5.09 ± 0.21^{d}

Note: $^{a\text{ - }e}$ represent the significant differences of L^*, a^*, b^* and ΔE^* ($p \leq 0.05$).

Alpha-amylase inhibition

The investigation focused on determining the inhibitory activities of alpha-amylase in mao wines fermented by 3 distinct strains of *S. cerevisiae* yeasts. Additionally, the inhibitory activities of fresh mao and acarbose were examined in the study for comparison. The results, in terms of IC_{50} values, are presented in **Figure 3**. The experimental findings revealed that the IC_{50} values of the mao wine samples ranged from approximately 2.9 to 4.8 mg mL^{-1}, which were approximately 410 - 680 times higher than that of acarbose (0.007 mg mL^{-1}). The results are consistent with the data reported by Aksornchu *et al*. [6], which demonstrated that the mao extract acquired after a separation procedure had inhibitory effects on alpha-amylase, with an IC_{50} value exceeding 4 mg mL^{-1}. This finding suggests that mao wines have a relatively low capacity to inhibit the activity of alpha-amylase in comparison to acarbose. Hence, it may not help control blood sugar levels in people with type 2 diabetes. Furthermore, there exists a discrepancy between the prevailing belief held by the local people and the potential of mao wine in the context of diabetes treatment, unless it may have potential for controlling alternative kinds of diabetes mellitus. When comparing the inhibitory activity of mao wines with that of fresh mao, it was shown that the IC_{50} values of mao wines were higher than those of fresh mao, approximately 26 to 43 times. This result demonstrates that the process of fermentation can decrease the fruit's ability to inhibit the alpha-amylase enzyme. The possible reason for this phenomenon may be attributed to the degradation of the bioactive compound present in fresh mao during the fermentation process. The amount of phenolic compound families may exhibit a decline over the course of fermentation. Furthermore, it is worth noting that the production of alcohol during the fermentation process can cause the loss of anthocyanins. The involvement of anthocyanin derivatives in mao encompasses various processes, including pH control, copigmentation reactions, polymerization, degradation, proanthocyanin synthesis and reactions between anthocyanins and tannins [31,32].

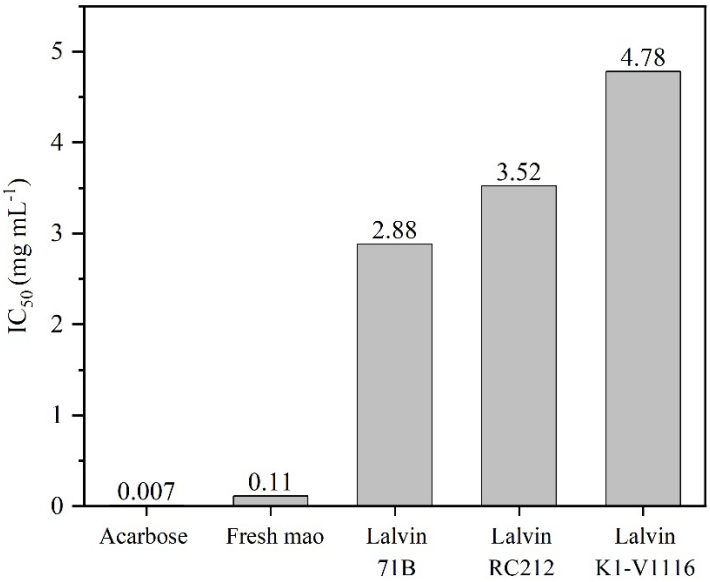

Figure 3 The IC_{50} values of acarbose, fresh mao and mao wine fermented by 3 *S. cerevisiae* yeasts.

Conclusions

Three commercial *S. cerevisiae* yeast strains, including Lalvin 71B, Lalvin RC212 and Lalvin K1-V1116, were used for mao wine fermentation. After that, kinetic experiments on yeast growth, alcohol production and reducing sugar consumption were performed on the mao wines. As a result, the chosen kinetic models are suitable for these kinetic studies. Lalvin 71B yeast was shown to grow well under the given conditions, but it still has a lower ability than Lalvin RC212 and Lalvin K1-V1116 to convert sugar into products. In particular, Lalvin K1-V1116 strain has the maximum capacity for converting sugar to alcohol and yields 88 % of product efficiency. Therefore, it can be concluded that Lalvin K1-V1116 is the most suitable yeast for making mao wine, followed by Lalvin RC212 and Lalvin 71B.

The investigation of the alpha-amylase inhibitory activity of mao wines demonstrated that their capacity to inhibit alpha-amylase was notably inferior to that of acarbose. This discovery revealed the poor therapeutic efficacy of mao wine in lowering blood sugar levels. However, this lower level of inhibition could potentially lead to a reduction in the occurrence of side effects or adverse reactions. This is especially beneficial for patients who are allergic to certain medications. Further study is necessary to validate its relevance to the treatment of type 2 diabetes.

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