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Characterization of Red Dragon Fruit Wine Fermented with a Newly Identified Yeast Strain *Saccharomyces cerevisiae* M7

Running head: Characterization of Red Dragon Fruit Wine Fermentation

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SUMMARY

Research background. Dragon fruit (*Hylocereus* spp.) has been known to be a rich source of bioactive compounds, such as anthocyanins, betacyanin, betaxanthin and other phenolic substances, and possesses a nutritional profile suitable to produce wine with functional properties. The aim of this study was to characterize the wine fermentation from red dragon fruit juice by a newly identified yeast strain.

Experimental approach. Yeast strains from *banh men*, a Vietnamese traditional alcoholic fermentation starter, were screened for ethanol production using thermally pretreated red

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dragon fruit juice. The most potent candidate was identified by the DNA sequencing method and subjected to an optimization study using a one-factor-at-a-time approach to optimize the conditions for red dragon fruit wine fermentation.

Results and conclusions. Results showed that thermal pretreatment of the red dragon fruit juice at 70 °C for 10 mins resulted in a higher level of phenolic and antioxidants compared with other pretreatment temperatures. Among the 4 isolates, M7 was the most potent alcohol fermenter, which was then identified to be *Saccharomyces cerevisiae* using a DNA sequencing method. The optimal conditions for wine fermentation from red dragon fruit juice by *S. cerevisiae* M7 included a pitching rate of 10^8 CFU/mL, an initial sucrose content of 18 % *m*/*V*, an initial pH of 4.5, fermentation temperature of 30 °C and a fermentation time of 6 days. In such conditions, the wine fermented by *S. cerevisiae* M7 had an ethanol concentration of (12.12±0.15) % *V*/*V* total phenolic content of (37.78±0.38) mg GAE/mL, anthocyanin content of (11.22±0.31) mg CGE/L, betacyanin content of (65.18±0.82) mg/L, betaxantin content of (60.47±1.29) mg/L and antioxidant activity measured by DPPH scavenging capacity of (65.41±0.44) %.

Novelty and scientific contribution. This study used a novel yeast strain *Saccharomyces cerevisiae* M7 for fermentation. In addition, the results of the study present novel data such as the optimal parameters and the accumulation of bioactive compounds (phenolics, anthocyanins, betalains) related to fermentation of red dragon fruit wine.

Keywords: *Saccharomyces cerevisiae*; yeast; wine; red dragon fruit; anthocyanin

INTRODUCTION

Wine is traditionally produced from the fermentation of the grape must. However, the development of wine from fruits other than grapes has gained much interest due to its multiple properties, such as color, flavor, and nutritional values (*1*). Many fruits from temperate regions (*e.g*. apples and berries) and tropical regions (*e.g*. banana, mango, pineapple, and sweet potatoes) can be used to produce wines. As such, wines made from cherries, raspberries, and blueberries contain a significant amount of polyphenols, flavonoids, and anthocyanins that give the wines remarkable antioxidant, anti-proliferative, anti-inflammatory and anti-aging capacities (*2*–*4*). Among the fruits from tropical and subtropical regions, dragon fruit (*Hylocereus* spp.) possesses suitable properties to produce wine, evidenced by the pH of the juice ranging from 4.3 to 5 depending on cultivars, while glucose and fructose of the juice being in the ranges of 49.1 to 104 g/L and 19.2 to 29 g/L, respectively (*5*). The red dragon fruit is also a rich source of flavonoids, betacyanin and betaxanthin, and carotene (*6*,*7*), making wine produced from these fruits a potential functional food.

During the production of fermented beverages, the yeast *Saccharomyces cerevisiae* plays an essential role in converting carbohydrates to ethanol and participating in secondary fermentation, which influences flavor and aroma development (*8*). Therefore, *S. cerevisiae* has been used as a starter culture for various fermented beverages, such as wine, whisky, cognac, sake, and beer (*9*). In Vietnam, however, *banh men* has been used as a starter to produce traditional alcoholic beverages from rice for centuries. *Banh men* is produced from uncooked rice dough and oriental herbs inoculated with a starter from the previous batch (*10*). According to Lee and Fujio (*11*), *banh men* is similar to fermentation starters traditionally used in other Asian countries in terms of microbial composition. Among the microflora present in *banh men*, *S. cerevisiae* strains were reported as the main ethanol producer (*10*). In this study, we aimed to screen the *S. cerevisiae* strains from *banh men* capable of fermenting red dragon fruit juice into wine and investigate the effect of fermentation conditions on the quality of wine produced by the most potent strain.

MATERIALS AND METHODS

Chemicals

All of the reagents used in experiments such as glucose, sucrose, peptone, yeast extract, Hansen broth, Hansen agar, buffer, gallic acid, cyanidin 3-glucoside, Folin-Ciocalteu's phenol, cyanidin-3-glucoside, phenol, chloroform, isoamyl alcohol, ethanol, agarose, Na_2CO_3, KCl, CH_3COONa, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Isolation of yeasts

One gram of '*banh men*' was finely ground and suspended in 9 mL of saline. The suspension was further serially diluted with saline and spread onto Hansen agar plates. The plates were then incubated at 28 to 32 °C for 24 to 48 h until obtaining isolated colonies. The colonies were subsequently transferred to the new Hansen agar including 2 % *m*/*V* of glucose, 1 % *m*/*V* of peptone, 0,1 % *m*/*V* of yeast extract and 1.5 % *m*/*V* of agar. The colonies that are round, smooth, and white to whitish cream and the cells that have an oval shape and budding characteristics were suspected to be yeast and used for further studies.

Red dragon fruit juice preparation

The red dragon fruits were washed under running water for about 1 min and drained for 30 min. The fruits were then peeled, and the juice was extracted from the fruit pulp using an electric slow juicer (Hurom H200, Korea). The juice had a total soluble solid of 12.5±0.30 % and a pH of (4.53±0.12). The total phenolic, anthocyanin, betacyanin and betaxanthin contents of the juice were (23.11±0.69) mg GAE/mL, (5.09±0.42) mg CGE/L, (31.21±0.56) mg/L and (23.12±0.39) mg/L, respectively.

Thermal treatment of dragon fruit juice and screening of yeast strains

The dragon fruit juice was thermally treated at temperatures 60, 70 and 80 °C for 10 min, followed by cooling to ambient temperature. Sucrose was added to the juice samples to obtain a total soluble solid of 18 % *m*/*V*. The yeast isolates were then inoculated into the juice at 10^7 CFU/mL density and kept at 25 °C for fermentation. When the formation of bubbles ceased, as an indication of primary fermentation completion, the fermented juice samples were analyzed for various quality attributes. The thermal treatment regimen and yeast isolate that yield the highest levels of ethanol, anthocyanins, total phenolic compounds, betacyanin and betaxanthin and antioxidant activity will be selected for further studies.

Identification of selected yeast strain

The yeast isolate that produces the wine with higher levels of ethanol and antioxidant substances was identified by Internal Transcribed Spacer Regions (ITS) sequencing method. The ITS sequence was compared against accession numbers available in the GenBank database using the Basic Local Alignment Search Tool (BLAST)(*12*).

Total genomic DNA extraction

The selected yeast isolate was grown in Hansen broth for 24 h, followed by centrifugation at 8000×*g* (Sigma 1-16; Sigma, Osterode am Harz, Germany) for 2 min to obtain biomass. The biomass was washed with 700 µL of sterile distilled water. The genomic DNA of the yeast was extracted using the TopPURE® Genomic DNA extraction kit (ABT Biomedical Solutions, HCM city, Vietnam) following the manufacturer's instructions. Briefly, an aliquot of 800 µL lysis buffer was added to the tube containing the biomass, mixed, and added with 40 µL of 20 % *m*/*V* SDS. The mixture was vortexed for 2 min and incubated at 65 °C for 30 mins, followed by centrifugation at 10 000×*g* for 15 min at 4 °C (Sigma 1-16, Sigma). The collected supernatant was added with an equivalent volume of phenol:chloroform:isoamyl alcohol

(25:24:1), mixed and centrifuged at 10 000×*g* for 15 min at 4 °C (Sigma 1-16, Sigma, Germany). The top layer was collected, mixed with an equivalent volume of isopropanol, and incubated at – 40 °C for 2 h. The mixture was subsequently centrifuged at 10 000×*g* for 15 min at 4 °C (Sigma 1-16, Sigma, Germany) to collect genomic DNA. The genomic DNA was washed with 500 μL of 70 % *m*/*V* ethanol twice and resuspended in 30 μL of sterile water. The quality of genomic DNA was verified by electrophoresis on 1 % *m*/*V* agarose gel. An aliquot of 1 μL of RNase (100 μg/μL) was added to the DNA solution to eliminate RNA.

DNA amplification and sequencing analysis

The genomic DNA was amplified by using yeast universal primers ITS1 (5'TCCGTAGGTGAACCTGCGG 3') and ITS4 (5'TCCTCCGCTTATTGATATGC 3'). The PCR reaction volume was 60 μL consisting of 30 μL of 2X Go Tag Green Master Mix, 3 μL of each primer (10 pmol/μL), 6 μL of genomic DNA, and 18 μL of H_2O. The PCR was carried out at the following conditions: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 1 min, annealing at 53 °C for 1 min, and extension at 72 °C for 1 min; and final extension at 72 °C for 10 min. The PCR product was checked by electrophoresis at 70 V for 30 min on 1 % agarose gel, stained by SafeView Classic Nucleic Acid Stains (ABM, Inc. USA). The DNA bands on the gel were visualized by using Ultra Slim LED Illuminator and the size of DNA was estimated using GeneRuler 1kb DNA Ladder (Thermal Scientific Inc. USA). The PCR product was sent to 1st BASE (Apical Scientific Sdn. Bhd., Malaysia) for DNA sequencing. The obtained sequences were then aligned and compared with sequences of species available in the NCBI database using BLAST (*13*).

Dragon fruit wine fermentation by selected strain

Effects of fermentation conditions on the quality of fermented dragon juice were evaluated by varying several parameters one by one. The parameters of interest investigated in this study were in the order of pitching rate (10^5–10^9 CFU/mL), initial total soluble solid (12–21 °Bx), initial pH (3.5–5.5), fermentation temperature (20–35 °C) and fermentation time (1–7 d). The quality of the wine and the efficacy of the yeast strain will be assessed via ethanol concentration, anthocyanins, phenolic content, and antioxidant activity.

Wine quality analysis

Ethanol concentration

The ethanol concentration of the fermented juice was determined using the AOAC method 920.57 (*14*). Briefly, ethanol from 200 mL of the fermented juice was separated by distillation. A hydrometer was used to measure the gravity of the distillates, which were then used to calculate the ethanol concentration.

Total phenolic content

An aliquot of wine was centrifuged at 6000×*g* for 15 min and used to determine the total phenolic content and antioxidant activity. The total phenolic content was determined according to the method of Singleton and Rossi (*15*). Briefly, 200 μL of the extract was mixed with 1 mL of 10 % *m/V* Folin-Ciocalteu's phenol reagent and 1.2 mL of 10 % *m/V* Na_2CO_3 solution. The mixture was then allowed to react for 2 h at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a standard and the total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per milliliter of the juice (mg/mL).

Total anthocyanin content

The total anthocyanin content was determined by the pH differential method described by Lee *et al.* (*16*). Briefly, the absorbances of samples diluted in 0.025 M potassium chloride buffer (pH 1) or 0.4 M sodium acetate buffer (pH 4.5) were measured concurrently at 520 and 700 nm, respectively, after 20 mins incubation at ambient temperature. The total anthocyanin content was calculated as milligrams of cyanidin 3-glucoside equivalents (CGE) per litter of the juice (mg/L) using the following equation:

$$\text{Total anthocyanin content} = \frac{A\cdot \mathrm{M}\cdot \mathrm{DF}\cdot 10^3}{\varepsilon\cdot \mathrm{L}} \qquad /1/$$

where $A = (A_{520\ \mathrm{nm}} - A_{700\ \mathrm{nm}})_{\mathrm{pH}=1} - (A_{520\ \mathrm{nm}} - A_{700\ \mathrm{nm}})_{\mathrm{pH}=4.5}$ /2/

$A_{520\ \mathrm{nm}}$ is the absorbance of the samples at 520 nm; $A_{700\ \mathrm{nm}}$ is the absorbance of the samples at 700 nm; M is the molecular mass of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, 10^3 is the coefficient for conversion from gram to milligram; ε is the molar extinction coefficient of cyanidin-3-glucoside (26 900 L/(mol·cm)) and L is the path length (1 cm).

Antioxidant activity

Antioxidant activity was determined by the DPPH radical scavenging method (*17*). Briefly, 0.4 mL of the sample or blank (ethanol) was mixed with 3.6 mL of 0.1 mM DPPH. The reaction was allowed to occur in the dark for 1 h at room temperature. The absorbance of the resulting solution was measured at 517 nm and used to calculate the antioxidant activity of the juice as the DPPH scavenging capacity (%) using the following equation:

$$\text{DPPH scavenging capacity} = \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \cdot 100 \qquad /3/$$

where A_{Blank} is the absorbance of blank and A_{Sample} is the absorbance of the juice extract sample.

Betalain content

The betalains content was quantified as described previously (*18*). Briefly, the optical density of the samples was measured at 480 nm and 540 nm for determining betacyanin and betaxantin contents, respectively. The contents of each betalain compound (mg/mL) were calculated using the following equations:

$$\gamma_{\text{bc}} = \frac{A_{540\ \text{nm}} \cdot D_{\text{f}} \cdot M_1 . 1000}{e_1 \cdot L} \qquad /4/$$

$$\gamma_{\text{bx}} = \frac{A_{480\ \text{nm}} \cdot D_{\text{f}} \cdot M_2 \cdot 1000}{e_2 \cdot L} \qquad /5/$$

where γ_{bc} and γ_{bx} are the betacyanin and betaxantin mass content (mg/L), respectively, $A_{540\ \text{nm}}$ and $A_{480\ \text{nm}}$ are the absorbance of the samples at 540 and 480 nm, respectively, M_1 and M_2 are the betaxanthin (308 g/mol) and betacyanin (550 g/mol) molecular mass, respectively, e_1 and e_2 are the molar extinction coefficient of betaxanthin (48000 L/mol) and betacyanin (6000 L/mol), respectively, D_{f} is the dilution factor, 1000 is the coefficient for conversion from gram to milligram, and L is the path length (1 cm).

Statistical analysis

Data were reported as mean value±SD of triplicate experiments. One-way ANOVA, followed by Duncan's test, was used to determine the difference between the means at a significant level of 0.05. Statistical analyses were performed using SPSS V17.0 software (*19*).

RESULTS AND DISCUSSION

Isolation and screening of yeast strains

Among 32 isolated colonies obtained from Hansen agar plates, 18 isolates (designated as strains M1 to M18) were considered as yeasts based on their cell morphology observed using electron microscopy. These isolates were screened for ethanol production by fermenting red dragon juice for 24 h at 28 °C. The results showed that four isolates (M2, M7, M11 and M17) produced ethanol (Table S1). These four strains were selected for further experiments.

Effect of thermal pretreatment and yeast isolates on the quality of wine

Temperature is one of the most important factors affecting the extraction of bioactive ingredients from biomass (*20*). Thus, the thermal pretreatment of dragon fruit juice in this study was expected to affect the quality of the wine in terms of bioactive components, such as phenolics, anthocyanins, betacyanin, betaxantin and their antioxidant activity.

On the other hand, ethanol concentration is another key element determining the yield and activity of bioactive compounds (*21*). During the fermentation, the yeasts continuously produce ethanol, which consequently alters the ethanol concentration of the fermented juice, hence the extraction of bioactive substances. In this experiment, we study how yeast isolates from *banh men* influence the quality attributes of dragon fruit wine made from juice that was thermally pretreated with different temperatures. The two-way ANOVA showed that thermal pretreatment and yeast isolates and the interaction between these two factors affect the concentrations of ethanol, total phenolic content, anthocyanin content, betacyanin content, betaxantin content and antioxidant activity of the dragon fruit wine ($p<0.05$). As such, yeast isolates produced higher levels of ethanol at a pretreatment temperature of 70 °C than at 60 or 80 °C (Table 1). Also, the concentrations of phenolic compounds, bioactive compounds (anthocyanins, betacyanin and betaxantin) as well as antioxidant activity of the wine produced from the juice pretreated at 70 °C were significantly higher than at other temperatures. This result suggested that phenolic compounds were most effectively extracted from dragon fruit pulp particles to the juice at 70 °C. The results from this experiment were in line with a study reported by El Darra *et al*. (*22*) that thermovinification pretreatment at 70 °C promoted the release of phenolic compounds from grape skin cells. Among the isolates capable of ethanol fermentation, the M7 yielded higher levels of ethanol ((6.29±0.33) % (v/v)) than M2, M11, and M17 at a pretreatment temperature of 70 °C. In addition, the total phenolic ((27.22±5.41) mg GAE/mL), anthocyanins ((10.02±0.25) mg CGE/L), Betacyanin ((50.37±0.99) mg/L), Betaxanthin (43.60±0.72) mg/L) contents, and DPPH scavenging capacity (57.65±0.35) % of the juice pretreated at 70 °C and fermented by M7 were markedly greater than by other isolates. In short, results from this study showed that the pretreatment temperature of 70 °C and isolate M7 appeared to be most suitable for producing wine from red dragon fruit juice and were therefore selected for further experiments.

Identification of yeast strain

The BLAST search of the ITS sequence of isolate M7 against reference sequences of *Saccharomyces cerevisiae* species from GenBank (accession numbers of the ITS sequences are KY109257.1 and MZ452353.1) showed a high similarity (>99 %), indicating that isolate M7 belongs to this species (Table 2). Thus, the strain was named *S. cerevisiae* M7.

Optimization of red dragon juice fermentation by the yeast strain

Effect of pitching rate

Studies showed that pitching rate is a crucial factor affecting the wine fermentation from different fruit juices by yeasts (*23*,*24*). In this study, the effect of pitching rate on ethanol production, total phenolic, anthocyanin contents, and antioxidant activities of the dragon fruit wine was investigated. Results show that ethanol and phenolic concentrations, anthocyanin content, betacyanin and betaxanthin contents, and the DPPH scavenging capacity of the fermented juice increased with the increase of pitching rate and reached the maximum levels of (12.5±0.3) % *V*/*V*, (35.19±0.90) mg GAE/mL, (9.9±0.20) mg CGE/L, (62.61±0.70) mg/L, (60.51±0.68) mg/L and (63.2±0.3) %, respectively, at the pitching rate of 10^8 CFU/mL, then leveled off with the further increment of pitching rate (Fig. 1). The increase in ethanol production in response to the increment of inoculation size to a certain level was also observed in a study by Huan *et al*. (*25*) who reported that the maximum level of ethanol of around 3.5 % *V*/*V* was obtained at a yeast rate of 2 % *V*/*V* after 40–48 h of fermentation, while the lower or higher amount of yeast added to the juice gave lower ethanol productivity. Similarly, Samson *et al.* (*26*) also documented that ethanol production in pomegranate fruit juice fermentation increases with the increase in the pitching rate from 2 to 8 % *V*/*V*, then decreases at higher inoculation sizes. In another study of wine produced from cactus pear and lantana camara fruit juice, a mild level of 10 % *V*/*V* of yeast inoculation, compared with 8 or 12 % *V*/*V*, was found to be most favorable for wine fermentation (*27*). In the current study, the improvement of anthocyanin and phenolic compound concentrations and antioxidant activity of the red dragon wine at a higher pitching rate (*e.g.* 10^8 CFU/mL) was likely to be yielded by the higher ethanol concentration obtained at this pitching rate, which enhanced the extraction of these substances from the red dragon fruit pulp particles.

Effect of initial sucrose content

As one of the important carbon sources for wine fermentation by yeasts (*9*), sucrose has been documented as the most influential factor affecting the quality of wine produced from fruit juice compared with other factors, such as SO_2 treatment, yeast inoculation, and fermentation time (*24*). In this study, we investigated the fermentation of red dragon fruit wine using *S. cerevisiae* M7 by varying the initial sucrose content from 12 to 21 % *m/V* while the pitching rate and the juice pH were kept constant at 10^8 CFU/mL and 4.5, respectively. Results showed that higher initial sucrose content led to higher ethanol production and the maximum ethanol yield (12.3±0.33) % *V*/*V* was obtained at the initial sucrose concentration of 18 % *m/V* (Fig. 2a). Further elevation of initial sugar content did not result in higher ethanol accumulation. Quality attributes, including total phenolic content, anthocyanin content, betacyanin content, betaxantin content, and antioxidant activity of the wine, were improved with the increase in initial sugar content up to 18 % *m*/*V* and leveled off beyond this concentration (Fig. 2b, Fig. 2c, Fig. 2d, Fig. 2e and Fig. 2f). A maximum level of (36.15±0.43) mg GAE/mL, (10.55±0.37) mg CGE/L, (66.53±0.84) mg/L, (64.31±0.64) mg/L and (64.13±0.88) % was obtained for phenolic, anthocyanin, betacyanin and betaxantin contents, and antioxidant activity measured by DPPH scavenging capacity, respectively. However, these results were discordant with those reported by Yuan *et al.* (*24*) that a higher concentration of initial sugar content (*e.g.* 24 % *m/V*) is more favorable for ethanol accumulation. In contrast, Arroyo-Lopez *et al.* (*28*) documented that a sugar concentration higher than 20 % *m/V* decreased the yeast cell growth rate, which may retard the ethanol production rate. In addition, wine fermentation with high sugar concentration negatively affected the production of volatile compounds, which might be detrimental to the quality of the wine (*29*). The high sucrose content might impose a high osmosis pressure on the yeast cells, reducing their fermentation performance. It was concluded from this study that an initial sucrose concentration of 18 % *m/V* was the most favorable to obtain red dragon fruit wine with a high antioxidant profile.

Effect of initial pH

Environmental pH is another critical factor for the growth of yeast and wine fermentation (*28*), as it has been known to alter the conformation, hence the function of cellular membrane-embedded proteins and eventually affect the fermentation rate and constitutions of fermentation products (*30*). To investigate the effect of initial pH on the fermentation of red dragon fruit wine by *S. cerevisiae* M7, the pH of the juice was adjusted to 3.5, 4.5, and 5.5 before yeast inoculation. Results showed that ethanol productivity was ~ 12 % *V*/*V* at the initial pH levels of 3.5 and 4.5 (Fig. 3a). The total phenolic compounds were ~ 37 mg GAE/mL at initial pH 3.5 (Fig. 3b). Anthocyanin content was slightly higher at initial pH of 3.5 than at 4.5 (Fig. 3c) while there was no significant difference in betacyanin and betaxantin contents at these initial pH (Fig. 3d and Fig. 3e). The antioxidant activity was ~65 % at the initial pH 3.5 (Fig. 3f). Increasing the initial pH of the juice to 5.5 substantially decreased ethanol production as well as the phenolic, anthocyanin, betalanins contents and antioxidant activity of the wine (Fig. 3). The results from our study agreed with those from Liu *et at*. (*30*) reporting that an initial pH of 4.5 is most favorable for the growth and alcoholic fermentation of several *S. cerevisiae* strains, including Freddo, BH8 and N˚.7303. It must be noted, however, optimal pH for wine fermentation might be strain dependent as *S. cerevisiae* NCIM 3045 give the highest yield of ethanol at a pH of 5.5 in the palm wine fermentation (*31*). Nonetheless, results from the current study indicated that the initial pH of 4.5 was optimal for wine fermentation from red dragon fruit juice by *S. cerevisiae* M7 regarding the ethanol productivity, phenolic, betacyanin, betaxantin contents and antioxidant activity of the wine.

Effect of temperature

Culture temperature is an important factor affecting the physiology of microoganisms during fermentation. Consequently, the accumulation of fermented products in the culture is impacted (*26*). In this work, the strain *S. cerevisiae* M7 was used for the fermentation of red dragon fruit juice at various temperatures, ranging from 20 to 35 °C, with pitching rate of 10^8 CFU/mL, 18 % *m*/*V* of sucrose concentration supplement in red dragon fruit juice, and an initial pH of 4.5. The results (Fig. 4) showed the highest content of alcohol (13.1±0.24) % *V*/*V*, total phenolic compounds ((37.58±0.87) GAE/mL), anthocyanin ((11.41±0.38) CGE/L), betacyanin ((65.63±0.49) mg/L) and betaxantin ((61.15±0.95) mg/L) at a fermentation temperature of 30 °C (Fig. 4a, Fig. 4b, Fig. 4c, Fig. 4d and Fig. 4e). There was no significant difference in antioxidant activities at 25 °C and 30 °C (Fig. 4f). The concentration of all these compounds in culture was significantly lower at fermentation temperatures of 20 and 35 °C. These results are similar with those of Patil *et al.* (*32*) who reported an optimal temperature of 27.5 °C for fermentation of sugarcane–papaya juice by *S. cerevisiae* (EC1118), while the optimal temperatures in fermentation of palm juice (*31*) and pomegranate juice (*26*) were reported to be higher, at 32 °C and 37 °C, respectively.

Effect of fermentation time

Fermentation time is critical to determine the quality of wine as well as to obtain a wine with a high level of bioactive compounds. In this study, the changes in ethanol production, phenolic compounds, and antioxidant of red dragon wine were investigated during the primary fermentation period. As shown in Fig. 5a, the ethanol concentration of wine gradually increased to a maximum level of ((12.12±0.15) % *V*/*V*) from the first to the sixth day of fermentation, while further fermentation beyond this time did not yield any additional amount of ethanol. In parallel with the increase in ethanol concentration, the levels of phenolic content, anthocyanins, betacyanin, betaxantin contents and antioxidant activity of the wine also steadily increased during the first six days of fermentation and reached a maximum level of (37.78±0.38) mg GAE/mL, (11.22±0.31) mg CGE/L, (65.18±0.82) mg/L, (60.47±1.29) mg/L and (65.41±0.44) %, respectively (Fig. 5b, Fig. 5c, Fig. 5d, Fig. 5e and Fig. 5f). These results indicated that the primary fermentation period of six days was optimal for *S. cerevisiae* M7 to produce red dragon fruit wine. In other studies, Samson *et al.* (*26*) and Yuan *et al*. (*24*) reported that the fermentation time of 7 or 8 days is optimal for wine fermentation from pomegranate or green jujube juices, respectively. The variation in the optimal time for wine fermentation between studies might be attributed to the differences in yeast trains used, inoculation size, fermentation temperature, and the nature of juices as these parameters greatly affect the growth and fermentation ability of yeast strains.

CONCLUSION

Our result demontrate that the phenolic, anthocyanin, betacyanin, betaxantin content and antioxidant ability of the wine product produced from the red dragon fruit juice pretreated at 70 °C significantly increased. Among the yeast isolates tested, *Saccharomyces cerevisiae* M7 exhibited the highest levels of these compounds in wine production. The optimal fermentation parameters for this strain was established. They are a pitching rate of 10^8 CFU/mL, an initial sugar content of 18 % *m*/*V*, an initial pH of 4.5, a temperature of 30 °C, and a fermentation time of 6 days. Under these conditions, the levels of ethanol, total phenolics, anthocyanins, betacyanins, betaxantins, and antioxidant activity in this wine product were remarkably high. These findings highlight the potential of red dragon fruit juice as a substrate for producing high-quality wine with enhanced bioactive properties using the novel strain *Saccharomyces cerevisiae* M7 as a starter.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

T. T. Q. Anh contributed to the design of the study, data collection, and drafting of the article. N.T. An contributed to data analysis, interpretation, and drafting of the article. D. T. B. Thuy contributed to overall project management, study design, and drafting of the article. All authors contributed to the final approval of the version to be published.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at: www.ftb.com.hr.

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TABLES AND FIGURES

Table S1. Screening of yeast strains for ethanol production

Yeast strain No.	Ethanol concentration/%	Yeast strain No.	Ethanol concentration/%
M1	-	M10	-
M2	3.97	M11	5.12
M3	-	M12	-
M4	-	M13	-
M5	-	M14	-
M6	-	M15	-
M7	5.16	M16	-
M8	-	M17	4.35
M9	-	M18	-

"-" represents no ethanol production

Table 1. Effect of thermal pretreatment and yeast isolates on the quality of dragon fruit wine

Pretreatment temperature/°C	Yeast isolate	Ethanol concentration/%	Total phenolic content as *w*(GAE)/(mg/mL)	Anthocyanin content as *w*(CGE)/(mg/L)	Antioxidant activity (% DPPH scavenged)	*γ*(betacyanin)/(mg/L)	*γ*(betaxanthin)/(mg/L)
60	M2	(3.97±0.13)^{ef}	(14.80±2.10)^{cde}	(3.60±0.45)^{g}	(38.12±0.46)^{ef}	(26.87±1.14)^{f}	(26.41±0.60)^{g}
	M7	(5.16±0.38)^{bcd}	(13.39±1.28)^{def}	(6.71±0.38)^{cde}	(42.43±0.60)^{c}	(46.63±0.92)^{b}	(38.47±0.65)^{c}
	M11	(5.12±0.39)^{bcd}	(18.95±2.16)^{bcd}	(5.64±0.46)^{ef}	(39.37±0.65)^{de}	(44.67±0.93)^{b}	(35.53±0.84)^{de}
	M17	(4.35±0.24)^{def}	(12.10±1.80)^{ef}	(5.92±0.39)^{def}	(28.12±0.11)^{i}	(35.40±0.75)^{de}	(30.62±1.00)^{f}
70	M2	(4.70±0.29)^{cde}	(22.71±1.14)^{ab}	(4.93±0.54)^{f}	(46.11±0.42)^{b}	(26.64±0.97)^{f}	(28.37±0.73)^{fg}
	M7	(6.29±0.33)^{a}	(27.22±5.41)^{a}	(10.02±0.25)^{a}	(57.65±0.35)^{a}	(50.37±0.99)^{a}	(43.60±0.72)^{a}
	M11	(5.55±0.20)^{abc}	(20.28±1.63)^{bc}	(7.457±0.40)^{bc}	(40.42±0.67)^{d}	(46.15±0.48)^{b}	(39.13±0.88)^{b}
	M17	(5.00±0.19)^{bcd}	(20.57±1.16)^{bc}	(6.86±0.35)^{cde}	(35.58±0.59)^{fg}	(44.49±0.77)^{b}	(36.50±1.23)^{cde}
80	M2	(3.97±0.23)^{ef}	(16.50±1.41)^{bcde}	(5.01±0.40)^{f}	(30.66±1.04)^{h}	(28.29± 0.94)^{f}	(28.29±0.94)^{fg}
	M7	(5.72±0.41)^{ab}	(15.63±1.20)^{cde}	(8.62±0.55)^{b}	(42.76±0.70)^{c}	(41.41±0.49)^{c}	(41.41±0.49)^{ab}
	M11	(4.69±0.43)^{cde}	(11.85±1.24)^{ef}	(6.93±0.32)^{cd}	(35.28±0.33)^{g}	(37.70±0.58)^{d}	(37.70±0.58)^{cd}
	M17	(3.65±0.16)^{f}	(8.91±1.21)^{f}	(6.51±0.53)^{cde}	(24.25±0.58)^{j}	(34.97±0.69)^{e}	(34.97±0.69)^{e}

CGE=cyanidin 3-glucoside equivalent, GAE=gallic acid equivalent. Results are mean values of triplicate analyses±standard deviation. Mean values in the same column without a common letter differ significantly, $p \leq 0.05$

Table 2. Identification result of M7 strain

Yeast strain No.	Accession numbers of the ITS sequences	Species	Similarity (%)
M7	KY109257.1	*Saccharomyces cerevisiae*	100
	MZ452353.1	*Saccharomyces cerevisiae*	99

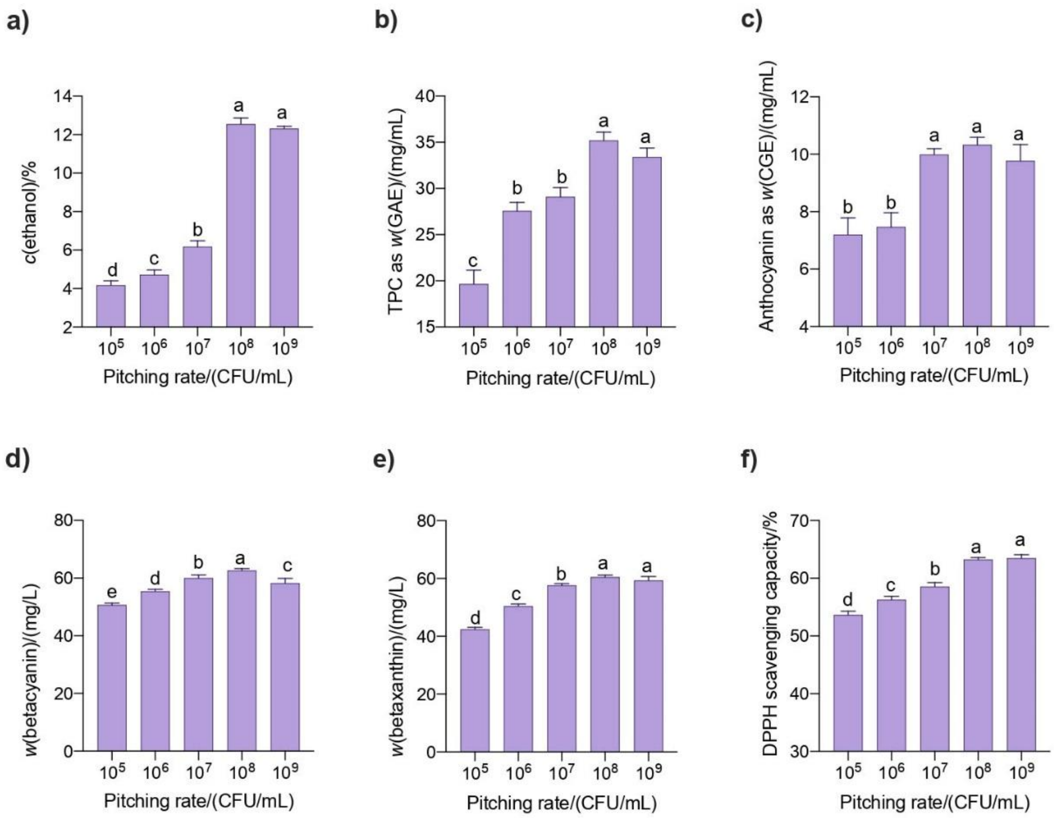

Fig. 1. Effect of pitching rate on: a) ethanol concentration, b) total phenolic content, c) total anthocyanin content, d) betacyanin content, e) betaxanthin content, and f) antioxidant activity of the red dragon fruit wine. The juice, with a total soluble solid content of 18% w/v and a pH of 4.5, was fermented by S. *cerevisiae* M7 at different pitching rates for 7 days at 25 °C. Data are means of triplicate analyses±standard deviation. Mean values in each graph without a common letter differ significantly ($p \leq 0.05$)

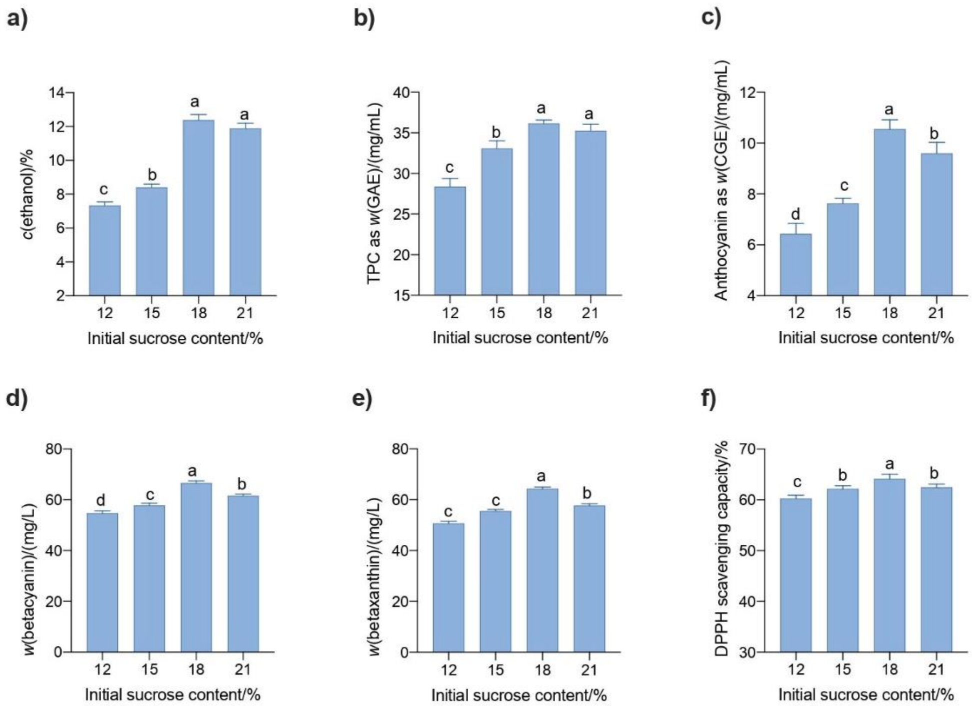

Fig. 2. Effect of initial sugar content on: a) ethanol concentration, b) total phenolic content, c) total anthocyanin content, d) betacyanin content, e) betaxanthin content, and f) antioxidant activity of the red dragon fruit wine. The juice with various initial sugar content and a pH of 4.5 was fermented by *S. cerevisiae* M7 at a pitching rate of 10^8 CFU/mL at 25 °C for 7 days. Data are means of triplicate analyses ± standard deviations. Means in each graph without a common letter differ significantly, $p \leq 0.05$

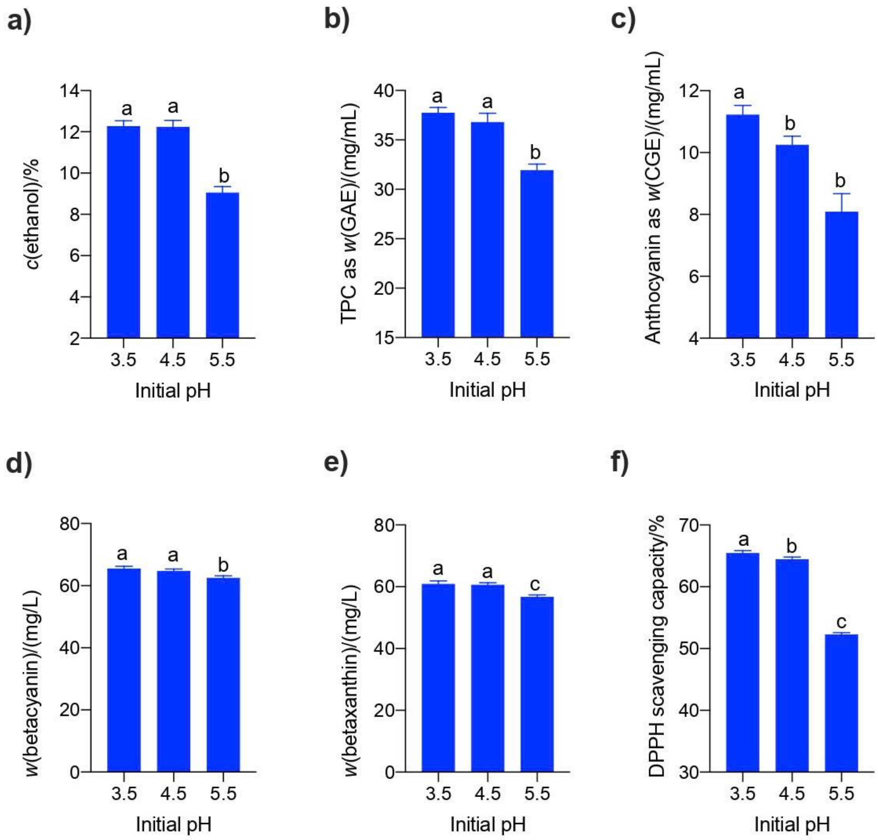

Fig. 3. Effect of pH on: a) ethanol concentration, b) total phenolic, c) total anthocyanin, d) betacyanin, e) betaxanthin contents, and f) antioxidant activity of the red dragon fruit wine. The juice with various initial pH values and a sugar content of 18 % w/v was fermented by *S. cerevisiae* M7 at a pitching rate of 10^8 CFU/mL at 25 °C for 7 days. Data are means of triplicate analyses±standard deviations. Mean values in each graph without a common letter differ significantly, $p\leq0.05$

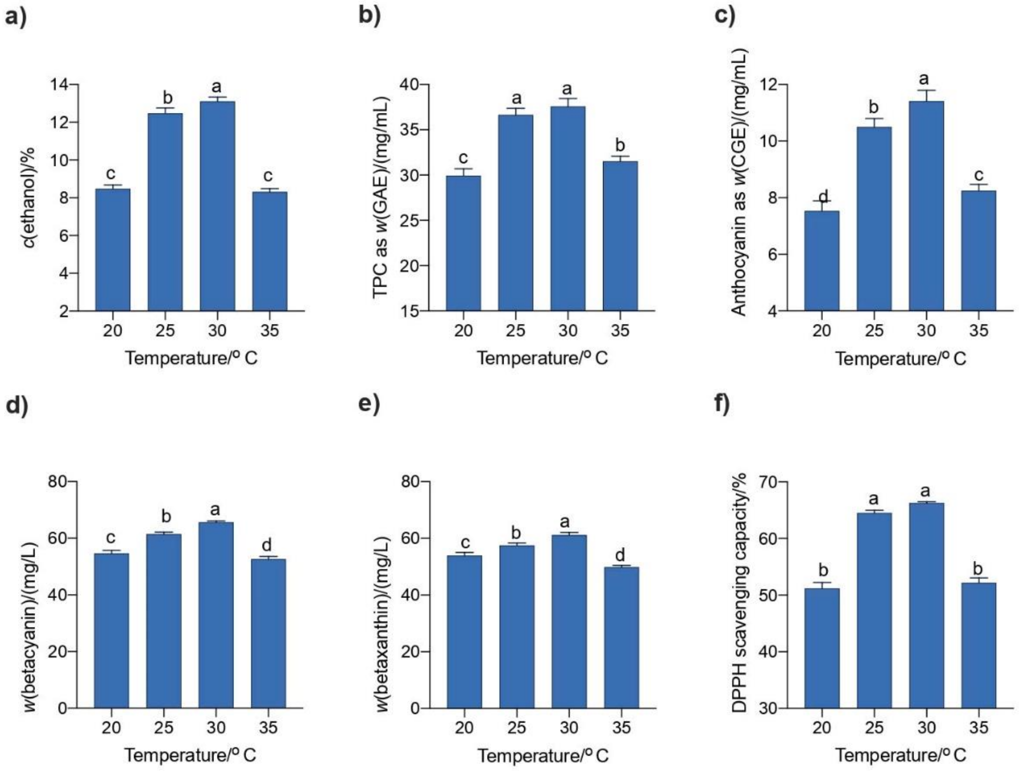

Fig. 4. Effect of fermentation temperature on: a) ethanol concentration, b) total phenolic content, c) total anthocyanin content, d) betacyanin content, e) betaxanthin content, and f) antioxidant activity of the red dragon fruit wine. The fermentation temperature was varied from 20 to 35 °C. The initial pH was 4.5, sugar content was 18 % w/v, and *S. cerevisiae* M7 was inoculated at a pitching rate of 10^8 CFU/mL with a fermentation time of 7 days. Data are means of triplicate analyses±standard deviations. Means values in each graph without a common letter differ significantly ($p\leq0.05$)

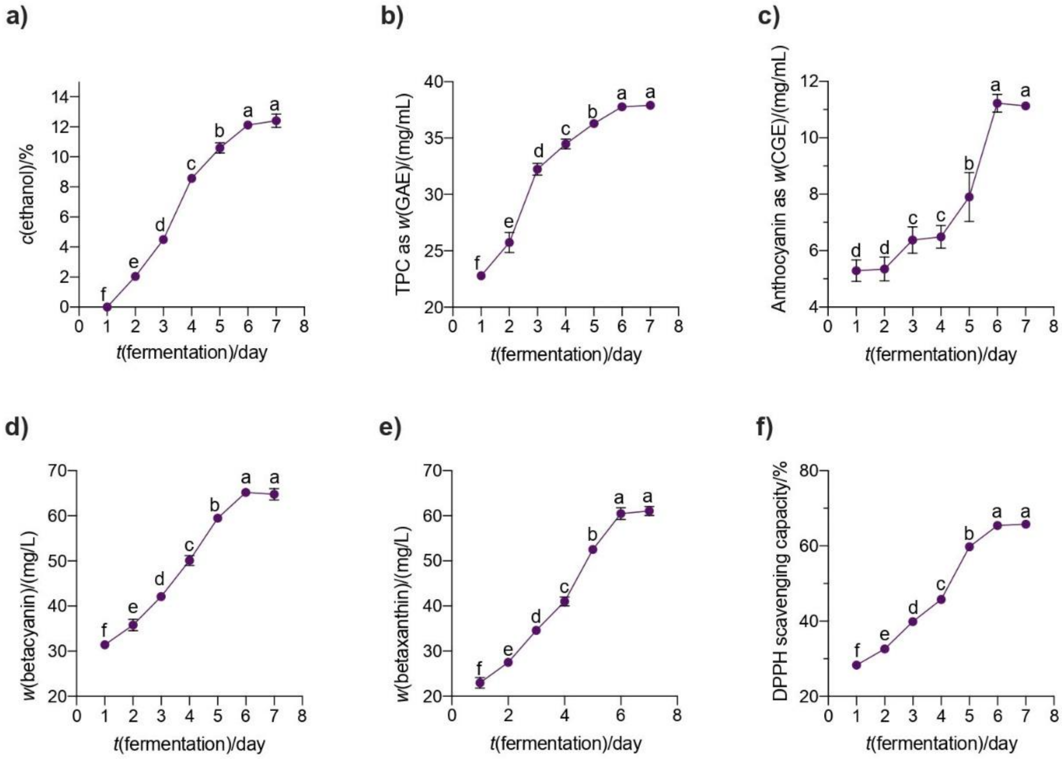

Fig. 5. Changes in: a) ethanol concentration, b) total phenolic content, c) total anthocyanin content, d) betacyanin content, e) betaxanthin content, and f) antioxidant activity of the red dragon fruit wine during fermentation. The juice with an initial pH of 4.5 and a sugar content of 18% w/v was fermented by *S. cerevisiae* M7 at 30 °C and a pitching rate of 10^8 CFU/mL for different times (day). Data are means of triplicate analyses±standard deviations. Means values in each graph without a common letter differ significantly, $p \leq 0.05$