



Original Research Article

Development of Hom Nil and Hom Ubon Rice Wine

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ARTICLE INFO

Article history:

Received 7 September 2022

Received in revised form 9 November 2022

Accepted 10 November 2022

Published 13 December 2022

Keywords:

Thai rice

Hom Nil rice

Hom Ubon rice

Rice wine

Wine fermentation

ABSTRACT

Thai aromatic rice is one of the well-renowned rice varieties which is an essential crop in Thailand. Hom Nil rice (HNR) and Hom Ubon rice (HUR), aromatic black and brown rice, that are generally rich in nutrients are potential to be used as the raw material for wine fermentation in order to add value to the product. This study, thus, aimed to develop the rice wine produced by using HNR and HUR. Results indicated that, of both rice wine samples, as the fermentation time increased, the total soluble solids (TSS) content decreased, while their pH and alcohol content increased. HNR and HUR wine fermented for seven days had the lowest TSS (7.6% and 7.8%, respectively) ($p < 0.05$). The highest alcohol content of HNR wine (13.7%) and HUR wine (13.2%) was found on the seventh day of fermentation. HNR wine was higher in phenolic compound and antioxidant activity than HUR wine ($p < 0.05$). Phenolic compound and antioxidant activity of the samples increased with a longer fermentation period. The highest phenolic compound and antioxidant activity were determined when fermenting HNR (340.52 mg GAE/l; 158.47 mg Trolox/l) and HUR (299.78 mg GAE/l; 57.97 mg Trolox/l) for eight days. However, HUR wines fermented for seven and eight days were not statistically different in the antioxidant activity. The sensory evaluation revealed that HNR and HUR wine with eight days of fermentation received the highest sensory acceptability scores (ranked between like slightly to like moderately), rated by 30 untrained panelists on a 9-point hedonic scale, among other samples with a shorter fermentation period, but not statistically different from those fermented for seven days. Therefore, such fermentation period was selected to evaluate the sensory acceptability when a black mint flavor (BMF) was added to the samples. Accordingly, a BMF increased the score of flavor and aroma of both samples. HNR wine with a BMF received the highest overall sensory acceptability scores (like slightly) ($p \geq 0.05$). Thus, HNR and HUR could be used as an alternative source of raw material for wine production, which would be beneficial for adding value to the Thai aromatic rice crop and improving the health advantages of the rice wine.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops that is commonly consumed worldwide. Rice with different varieties has a unique aroma, flavor, and texture (Champagne *et al.*, 2010), as well as a difference in nutritional quality (Verma *et al.*, 2019). Thai aromatic rice is one of the well-renowned rice varieties which is an economic crop in Thailand. Hom Nil rice (HNR) and Hom Ubon rice (HUR) are two of those rice that are generally rich in nutrients. HNR is a black fragrant rice that contains a higher amount of γ -oryzanol, anthocyanins, phenolic, flavonoid compounds, and protein than white rice (Daiponmak *et al.*, 2014; Peanparkdee *et al.*, 2019; Sivamaruthi *et al.*, 2018; Tangsrianugul *et al.*, 2019). Accordingly, it could be used as a health-promoting food for reducing the risk of diabetes, cancer, and coronary heart diseases (Guo *et al.*, 2007; Sivamaruthi *et al.*, 2018). HUR is one of the aromatic brown or unpolished rice that generally consists of higher protein, minerals, dietary fiber, and phenolic compounds contents than polished rice, indicating a greater potential in reducing the risk of several chronic diseases (Butsat & Siriamornpun, 2010; Peanparkdee *et al.*, 2019; Tangsrianugul *et al.*, 2019; Tian *et al.*, 2004). Thus, the application of HNR and HUR in food and beverage products would potentially provide health benefits for consumers.

Wine is fermented juice or beverage that is generally made from grapes. However, wines have been produced from several kinds of fruits, for example, apple, plum, peach, pear, berries, strawberries, cherries, currants, and apricots (Joshi *et al.*, 2017). A moderate wine consumption has been reported to be beneficial for human health in reducing the incidence of gastrointestinal infections and the risks of cardiovascular disease, diabetes, osteoporosis, neurological diseases and mortality (Artero *et al.*, 2015; Jackson, 2016; Maksimović & Dragišić Maksimović, 2017). Antioxidants, anthocyanin, flavonoids and other polyphenolic compounds extracted from fruits during wine fermentation play a significant role in these positive health effects. Rice wine is an alcoholic beverage fermented from cereals that is widely consumed, especially in Asian countries (Koay *et al.*, 2022). It has also been reported in containing high amounts of antioxidant and polyphenolic compounds (Amatayakul *et al.*, 2017; Cai *et al.*, 2019; Koay *et al.*, 2022; Rhee *et al.*, 2004). Mainly, these phenolic contents were derived from raw materials or fermentation substrates that could be glutinous rice, non-glutinous rice, maize, millet or wheat. Due to the chemical composition, the variety of rice substrates affects flavor, alcoholic contents and quality of rice wine (Koay *et al.*, 2022). For example, rice wine produced from pigmented rice was higher in antioxidant activity than the non-pigmented one (Koay *et al.*, 2022). Another study indicated that rice wine made of black rice and purple rice showed higher total phenolic contents and antioxidant activity than white rice (Cai *et al.*, 2019). Since HNR and HUR are generally high in anthocyanins and polyphenolic compounds, they are a potential source for rice wine production to produce a product with more health advantages. Therefore, this study aimed to develop rice wine produced by using HNR and HUR as a raw material, in order to enhance the health benefits of rice wine and add value to HNR and HUR.

MATERIALS AND METHODS

Materials

HNR, HUR, and black mint flavor (BMF) were obtained from Rai Ruen Rom Organic Farm (Chiang Rai, Thailand). Chemical reagents such as Folin-Ciocalteu, and sodium carbonate (analytical grade) were purchased from Carlo Erba (Chaussée du Vexin, France). Gallic acid was purchased from TCL (Tokyo chemical industry Co., LTD. Tokyo, Japan).

Rice wine preparation

HUR and HNR wine processing was conducted by following the method from Rittiplang (2006) with some modifications. Initially, HUR and HNR were cooked in a rice cooker at a ratio of 1:2 (rice:water). The cooked rice was cooled down to room temperature (27-30°C). Four grams of yeast starter (Loogpang Kaomark from a local market, Chiang Rai, Thailand) were mixed with 1 kg of cooked rice and fermented undisturbed in a tray covered with a filter cloth at room temperature for four days. Then, the fermented cooked rice was added with syrup (360 g of sugar boiled with 1200 ml of water). The mixture was allowed to further ferment for one to four days at room temperature. After every 24 hours of further fermentation, the fermented rice wine sample was filtered with a filter cloth and kept in a refrigerator for further analysis. HUR and HNR wines with the highest alcohol content, total phenolic content and antioxidant activity, and the highest sensory score were selected for sensory evaluation when adding with the 0.15% (v/v) of BMF.

Determination of pH, total soluble solid (TSS) and alcohol content of rice wine

The pH, TSS and alcohol content of all rice wine samples were determined by following the method of Boonsupa *et al.* (2016) with some modifications. The pH was measured by a pH-meter (Mettler Toledo Co., Ltd., Ohio, United States). TSS was measured by a hand-held refractometer (Atago Co., Ltd., Tokyo, Japan). The alcohol content was measured by an alcohol refractometer (Jedto, Fujian, China).

Determination of total phenolic content

The total phenolic content of rice wine was determined using the method of Pengkumsri *et al.* (2015) with some modification. One milliliter of rice wine was mixed with 5 ml of Folin-Ciocalteu reagent and kept at room temperature for 8 min. Then, 10 ml of sodium carbonate was mixed, and the total volume was adjusted to 100 ml by distilled water. The solution was kept in the dark for 60 min at room temperature (27-30°C). The absorbance was measured at 765 nm, using a UV-Visible Spectrophotometer (Shimadzu, Kyoto, Japan). The total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE/l rice wine).

Antioxidant activity determination

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of rice wine was determined using the method of Hong *et al.* (2009) and Büyüktuncel *et al.* (2014) with some modification. A 200 μ l of the rice wine was added with 600 μ l of 0.8 mM DPPH solution and 5.20 ml of ethanol. The solution was kept in the dark for 30 min. The absorbance of the solution was measured at 517 nm, using a UV-Visible Spectrophotometer. Trolox was used as a standard for the measurement, accordingly, the results were reported as mg Trolox/1 rice wine.

Sensory evaluation

All rice wine samples were evaluated for sensory characteristics including color, aroma, flavor and overall acceptability by 30 untrained panelists. The rice wines were served in a half of 60-ml glass container, and the serving temperature was approximately 6-8°C. The liking scores were rated using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely).

Statistical analysis

All experiments and measurements were performed in triplicate. The determination of physicochemical properties, total phenolic content and antioxidant activity was arranged in a Completely Randomized Design (CRD). The Randomized Complete Block Design (RCBD) was applied in the sensory evaluation experiment, as a sensory panelist was used as a block. All data were analyzed by the analysis of variance (ANOVA) and Duncan's new multiple range test. The different data were reported as significant at $p < 0.05$, using SPSS version 12.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Physicochemical properties of HNR and HUR wines

The physicochemical properties of HNR and HUR wines are reported in Figure 1. The pH of HNR and HUR wines fermented for five to eight days increased from 3.55 to 3.70, and 3.45 to 3.59, respectively. This trend was also found in milled glutinous rice wine (Mamucod *et al.*, 2004). These pH values were lower than those reported in previous studies on Chinese rice wine (pH 4.1) (Chen & Xu, 2012; Liu *et al.*, 2007), and rice wines produced by using waxy pigmented (pH 3.7 – 4.3) and non-pigmented rice (pH 4.4 – 4.6) (Chay *et al.*, 2017). However, Núñez *et al.* (2021) discussed that pH values ranging from 3.10 to 3.86 were the optimal values to maintain wine stability and prevent the possibility of wine oxidation and browning. Also, the pH of wines generally depends on the chemical characteristics, specifically the acid and sugar contents of the wine materials (Chay *et al.*, 2017; Woldemariam *et al.*, 2014).

Results also indicated that the TSS content of both HNR and HUR wines decreased with a longer fermentation period (Figure 1). Similar findings were reported in previous studies (Mamucod *et al.*, 2004; Woldemariam *et al.*, 2014). The decrease in TSS contents might be the result of the decrease in the reducing sugar used for alcohol fermentation and production process (Koay *et al.*, 2022;

Woldemariam *et al.*, 2014). These results linked with the increase in their alcohol content of HNR and HUR wines, which is the important parameter to measure wine quality. The highest alcohol content was found in HNR and HUR wines fermented for seven and eight days (13.7% and 13.2%, respectively). These amounts were less than those of rice wine fermented for six to seven days (14.10%-15.47%) reported in previous study (Woldemariam *et al.*, 2014), but similar and higher than those from rice wines reported in some studies (Koay *et al.*, 2022). Following the same manner of TSS content, alcohol is generated due to alcoholic fermentation, the process which yeast transforms sugars to ethyl alcohol and other by-product (Ciani *et al.*, 2013). Thus, variation in fermentable sugars, starter cultures, capacity of sugar uptake and alcohol tolerance limit (Koay *et al.*, 2022; Woldemariam *et al.*, 2014).

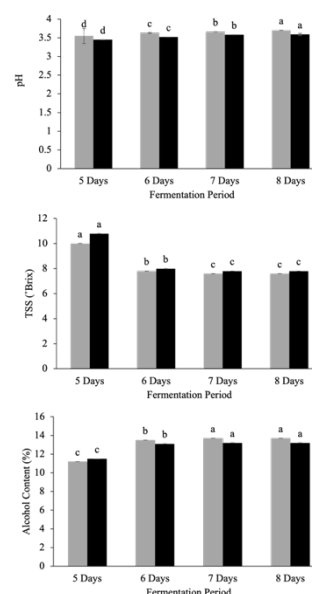


Figure 1. Physicochemical properties of HNR and HUR wines with 5-8 days of fermentation.

^{a-d} Different letters in the same type of rice wine sample indicate significant difference at $p < 0.05$.

Phenolic compound and antioxidant activity of HNR and HUR wines

The total phenolic compound and antioxidant activity of HNR and HUR wines with different fermentation periods are presented in Figure 2. Results indicated that the phenolic compound and antioxidant activity of the samples increased with a longer fermentation period, and the highest values were determined when fermenting HNR and HUR for eight days.

HNR wine was higher in the phenolic compound than HUR wine ($p < 0.05$). Specifically, the phenolic contents of HNR rice wine were between 228.67 to 340.52 mg GAE/l, while those of HUR samples were between 196.81 to 299.78 mg GAE/l. This variation is due to the composition and concentration of the phenolic compound naturally contained in the source of raw material of winemaking process (Koay *et al.*, 2022; Núñez *et al.*, 2021; Vasantha Rupasinghe *et al.*, 2017). Since black rice bran was found to contain the higher phenolic contents than brown rice bran (Ghasemzadeh *et al.*, 2018) and it is rich in anthocyanin contents (Daiponmak *et al.*, 2014; Sivamaruthi *et al.*, 2018), it is possibly lead to the higher phenolic contents of HNR wine.

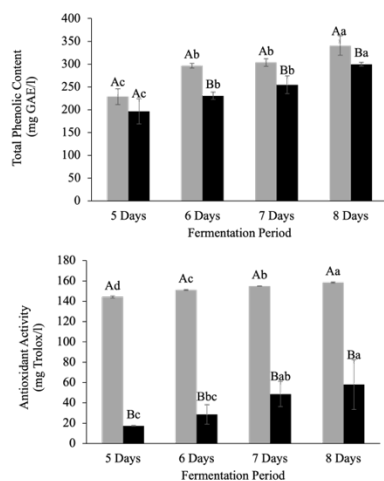


Figure 2. Total phenolic content and antioxidant activity of HNR and HUR wines with 5-8 days of fermentation.

^{a-d} Different letters in the same type of rice wine sample indicate significant difference at $p < 0.05$.

^{A-B} Different letters in the same fermentation period indicate significant difference at $p < 0.05$.

Meanwhile, a similar trend was also found in the results of their antioxidant activity, but with a more pronounced effect. The antioxidant activity measured by DPPH assays of the HNR and HUR wines ranged from 144.16 to 158.47 mg Trolox/l, and 17.31 to 57.97 mg Trolox/l, respectively. The highest concentration values were obtained in HNR wine with eight fermentation days. However, HUR wines fermented for seven and eight days were not statistically different in antioxidant activity. A large gap in antioxidant activity between HNR and HUR samples is possibly because HNR is a black fragrant rice that is rich in phytochemicals which act as free-radical scavengers when measuring the ability of antioxidants to capture free radicals by DPPH radical-scavenging assay (Ghasemzadeh *et al.*, 2018; Núñez *et al.*, 2021). Moreover, the higher levels of secondary metabolites in black rice, as compared to brown rice, could be responsible for the high antioxidant activity (Ghasemzadeh *et al.*, 2018). Additionally, the activity of the enzymes, such as β -glucosidase and α -amylase, produced by the starter culture during fermentation, could enhance the mobility of free hydroxyl groups on the phenolic structure that, accordingly, heightening the content of free phenolic compounds and

antioxidant activity of the substrate. Therefore, the total phenolic compound and antioxidant activity of the samples increased with a longer fermentation (Abd Razak *et al.*, 2015).

Sensory evaluation of HNR and HUR wines

The results of the sensory evaluation are shown in Table 1. Color of both rice wines were not significantly different among the samples ($p \geq 0.05$). However, their odor, taste and overall acceptability scores were significantly different ($p < 0.05$). HNR wine fermented for eight days was rated highest in the liking score in overall acceptability and taste that ranked between like slightly to like moderately; however, they were not statistically different from those of the sample fermented for seven days in the scores of all sensory attributes. Similarly, the liking scores of HUR wine sample with eight-day fermentation period were not significantly different from that with the seven-day fermentation period, and the liking score in a taste of HUR wine fermented for seven days (like moderately) was the highest among other samples. Therefore, the seven-day fermentation period was selected to evaluate the sensory acceptability when a black mint flavor was added to both samples of rice wine produced from HNR and HUR.

Sensory evaluation of HNR and HUR wines with black mint flavor (BMF)

HNR and HUR wines with seven fermentation days were selected to study the consumer sensory acceptability when adding a BMF for enhancing the flavor and aroma of the samples. Results of the sensory evaluation with 30 untrained panelists indicated that a BMF increased the liking score of both rice wine samples in all sensory attributes (Table 2). Among the two conditions, HNR and HUR wine with a BMF received the higher overall sensory acceptability scores (like slightly) ($p < 0.05$). Thus, the addition of BMF could enhance consumer preferences in HNR and HUR wines. However, due to the limitation in different groups of panelists for two sensory evaluation experiments, the liking scores of sensory acceptability among these evaluations were different. Thereby, further studies should be carried out regarding their physical properties to link with consumer sensory preferences, as well as their nutritional composition and more antioxidant activity to confirm their nutritive values and health benefits.

Table 1. Sensory evaluation of HNR and HUR wines with 5-8 days of fermentation

Sample	Fermentation Period	Sensory Acceptability Scores ^{1,2}			
		Color	Odor	Taste	Overall Acceptability
HNR Wine	5 Days	7.03±0.96 ^a	5.83±0.70 ^b	5.97±0.89 ^b	5.87±0.78 ^b
	6 Days	6.83±0.65 ^a	5.70±0.84 ^b	6.20±0.76 ^b	6.10±0.76 ^b
	7 Days	7.10±0.71 ^a	6.60±0.77 ^a	6.63±0.72 ^a	6.80±0.76 ^a
	8 Days	7.00±0.70 ^a	6.40±0.77 ^a	6.93±0.64 ^a	6.93±0.74 ^a
HUR Wine	5 Days	5.77±1.10 ^a	6.10±0.92 ^b	5.77±0.77 ^b	5.50±0.57 ^c
	6 Days	6.10±0.89 ^a	6.43±1.01 ^a	6.23±0.81 ^b	6.30±0.70 ^b
	7 Days	6.23±0.97 ^a	6.57±0.93 ^{ab}	7.17±0.65 ^a	6.93±0.64 ^a
	8 Days	5.90±0.99 ^a	6.70±1.02 ^a	6.93±0.74 ^a	7.03±0.72 ^a

¹ Means \pm standard deviations from 30 untrained panelists.

² Rated on a 9-point hedonic scale: 1=dislike extremely, 5=neither like nor dislike, 9=like extremely.

^{a-c} Means with different superscripts within the same column of each rice wine sample are significantly different ($p < 0.05$).

Table 2. Sensory evaluation of HNR and HUR wines with black mint flavor (BMF)

Formula	Sensory Acceptability Scores ^{1,2}			
	Color	Odor	Taste	Overall Acceptability
HNR Wine				
Without BMF	5.60±1.43 ^b	5.05±1.73 ^b	4.25±1.83 ^b	4.70±1.72 ^b
With BMF	6.60±1.47 ^a	6.40±1.23 ^a	5.25±1.45 ^a	5.95±1.32 ^a
HUR Wine				
Without BMF	5.70±1.59 ^b	3.65±1.95 ^c	4.35±1.95 ^b	4.65±1.60 ^b
With BMF	5.75±1.62 ^b	6.15±1.18 ^a	5.10±1.77 ^a	5.55±1.47 ^{ab}

¹ Means ± standard deviations from 30 untrained panelists.

² Rated on a 9-point hedonic scale: 1=dislike extremely, 5=neither like nor dislike, 9=like extremely.

^{a-c} Means with different superscripts within the same column are significantly different ($p < 0.05$).

CONCLUSIONS

The findings of this study reveal that rice wines fermented using HNR and HUR as a source for the alcoholic fermentation process were successfully developed. According to the physicochemical properties, the highest alcohol content, which is the critical parameter for assessing rice wine quality, was found in HNR and HUR wine samples with seven days of fermentation. Regarding their phenolic compound and antioxidant activity, all HNR wine samples were higher in these contents than those of HUR samples. The longer fermentation period, the higher contents of phenolic compound and antioxidant activity. However, in terms of sensory preferences, these two samples with seven days of fermentation received high liking scores in all sensory attributes, rated by 30 untrained panelists on a 9-point hedonic scale. When added BMF to HNR and HUR wines fermented for seven days, it increased the liking score in flavor, aroma and overall acceptability of both samples, when compared to those without the addition of BMF. Therefore, HNR and HUR could be used as alternative sources of raw material for wine production, which would be beneficial for adding value to the Thai aromatic rice crop and improving the health advantages of the rice wine.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge to the School of Food Industry, King Mongkut's Institute of Technology Ladkrabang for the chemicals and instruments support, and Rai Ruen Rom Organic Farm for the supply of Hom Nil rice and Hom Ubon rice used as the raw materials for this study.

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