

Physico-chemical and sensory properties of musts and wines from *Melodorum fruticosum* Lour.

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Abstract

In this study, the fully ripe fruits of *Melodorum fruticosum* Lour. were evaluated as material for red wine production. The effects of juice-to-water ratio (1:3 and 1:5v/v) and initial total soluble solids (18 and 20°Brix) on physico-chemical and sensory characteristics of musts and wines were examined. The results showed that during fermentation for 10 days the total soluble solids of all musts decreased to 7.5 - 9.3°Brix ($P \leq 0.05$) while the pH was decreased slightly from 3.7 to 3.5 - 3.6. The resultant wines had similar alcohol content (11.9 - 12.6%). The radical scavenging activities of most wines after ageing at 4°C for 4 weeks were similar to their corresponding musts, decreasing from 85 - 89% to 80 - 86%. Anthocyanin content was also found decreasing by 27 - 41% but total phenolics increased by 10 - 54%. The results of sensory evaluation showed a significant difference in terms of colour, clarity, sweetness, and bitterness, among the four aged wines. Samples made from juice-to-water ratio at 1:5 and initial soluble solid of 20°Brix showed the highest overall acceptance.

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Introduction

Wine is an alcoholic beverage prepared from juice of fruits or plants. Fermentation processes are done by the yeast *Saccharomyces*, whereby the sugars in the fruit juice are metabolised into alcohol and CO₂, that later react to form organic acid, aldehydes, esters and other chemical components (Boulton *et al.*, 1996). A number of commercial wines are mainly made from grapes. However, several other fruits e.g. mango, apple, blueberry, cherry, peach, pear, plum and pineapple have been used for forming musts and submitted to alcoholic fermentation (Czyzowska and Pogorzelski, 2002; Rupasinghe and Clegg, 2007).

Melodorum fruticosum Lour., locally known as Lumduan, Homnuan, Devil Tree and White Cheesewood belongs to the family Annonaceae. This fragrant plant is widely distributed throughout Indochina and Thailand (Rujjanawate *et al.*, 2008). A fruit of *M. fruticosum* has an oval shape, varying in sizes with average weight of 0.96 g. Similar to java plum (*Syzygium cumini* Linn), the edible pulp of *M. fruticosum* is sub-acid, juicy and aromatic, therefore this fruit would be used for beverage production. Importantly, the dark purple pulp and skin of *M. fruticosum* fruit would be a good source of biologically active compounds, mainly anthocyanin. According to Sonchai (2009), the ethanol extracts of *M. fruticosum* fruit had phenolic content of 30.40 mg/l gallic acid equivalents and exhibited IC₅₀ value of 11.92 mg/l. However, a limited amount of literature

exists on the utilization of *M. fruticosum* fruits in the food industries.

Therefore, the objective of this study was to use *M. fruticosum* fruits for the production of wine. An investigation was carried out to determine the effects of juice-to-water (J/W) ratio and initial total soluble solids (TSS) on the alcoholic fermentation of *M. fruticosum* juice and sensory properties of the final beverage. In addition, the influences of wine-making practices and ageing on the phenolic and anthocyanin contents, and the antioxidant activity of *M. fruticosum* musts and wines were accessed.

Material and Methods

Plant materials

The fresh fruits of *M. fruticosum* were purchased from a local market in Surin province, Thailand during June and July 2011. These fruits were transferred to storage at -18°C until further use. Prior to wine production, frozen fruits were allowed to reach room temperature (28 ± 2°C), cleaned with water and then air-dried.

Inoculum preparation

A 0.5 g of dry yeast (*Saccharomyces bayanus* Lavin EC-1118) was dissolved in 250 ml of sterilized pineapple juices (20°B). The cultures were grown at room temperature on a rotary shaker at 60 rpm for 24 h. This inoculum was used in the fermentation of *M. fruticosum* must to wine.

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Wine production

M. fruticosum fruits with average TSS content $15.98 \pm 0.27^{\circ}\text{B}$ and pH 3.83 ± 0.09 were submitted to four wine-making trials. Each of trials was performed two times. Fruits of *M. fruticosum* were weighed and squeezed coarsely through muslin cloth. The macerated pulp was collected whilst the juice was diluted with either three-fold or five-fold water. The juices obtained were analyzed for pH and TSS. The nutrients for yeast growth i.e. 0.05% diammoniumphosphate were supplemented to the juice. This was followed by the addition of sucrose to adjust the TSS to either 18 or 20°B . Five liters of *M. fruticosum* juice together with macerated pulp were filled in 6l plastic pail. After that, 250 ppm potassium metabisulphite was added to inhibit the growth of bacteria and wild yeasts and left for 24 h. After that a 24 h old yeast culture was taken to the juice and the fermentation was performed at room temperature until the gases terminated (10 days). When the fermentation was completed, a 200 ppm of potassium metabisulphite was added to stop yeast activities. Thereafter, wine was siphoned to another container and fined with bentonite (10 g/l) to remove the yeast lees from the must. Wine was aged at 4°C for 4 weeks before analyses.

Determination of pH, alcohol content and total soluble solids

The following parameters were analyzed on day 0, 1, 2, 3, 4, 6, 8 and 10 during the course of fermentation: pH, alcohol content and TSS. The pH of must was measured at 20°C using a bench-top pH-meter (Mettler Toledo, MP220, Germany). The TSS and alcohol content of the musts were analyzed in triplicate using a hand refractometer (Atago, Japan) and Ebuliometer, respectively.

Determination of total phenolic and anthocyanin contents

Samples were withdrawn and analyzed at the beginning of fermentation and at the end of ageing. The total phenolic content was measured spectrophotometrically at 765 nm according to the Folin-Ciocalteu method (Singleton *et al.*, 1999) and the results were expressed as g gallic acid equivalents per 100 ml. Total anthocyanin was analyzed using pH-differential spectrophotometry method (AOAC, 2000).

Determination of antioxidant activity

The antioxidant activities of musts and wines were determined through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method according to the method of Villano *et al.* (2006) with a slight modification. A diluted

sample (5 ml) was mixed with 5 ml of 0.06 mM DPPH in methanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the mixture was measured at 520 nm using a spectrophotometer. The control was conducted in the same manner but methanol was used instead of sample. DPPH radical scavenging activity (RSA) was calculated as follows: RSA (%) = $[1 - (A_{\text{sample}} - A_{\text{control}})] \times 100$, where A_{sample} is the absorbance of sample and A_{control} is the absorbance of the control.

Sensory evaluation

The sensory quality of the *M. fruticosum* wines in each trial was immediately assessed after 4-week ageing by 20 semi-trained taste panelists. Each panelist was served 30 ml of chilled wine sample (11°C) in transparent glasses and received a glass of water for rinsing and crackers for consumption between samples. All samples were coded with random three-digit numbers and served in a random presentation order (Jackson, 2002). The panelists were asked to rate each attribute on 9-point hedonic scale, where 1 correspond to "dislike extremely" and 9 correspond to "like extremely".

Statistical analysis

The results of physico-chemical and sensory evaluation were statistically analyzed using SPSS 14.0 software (SPSS Inc., Chicago, IL, USA). The data were expressed as means \pm SD. Duncan's Multiple Range test (DMRT) was applied for mean comparison when analysis of variance (ANOVA) showed significant differences at the 95% confidence level. In addition, data were subjected to Student's t tests when mean comparison of two groups was required.

Results and Discussion

Changes in pH, alcohol content and total soluble solids during fermentation

Tables 1-3 present changes in alcohol content, TSS and pH of four *M. fruticosum* musts during the course of fermentation for 10 days at room temperature. The results revealed that alcohol was produced rapidly within the first day of fermentation (2.43 - 2.73%), doubled after 3 days (5.29 - 6.69%) and reached 9.50 - 10.78% after 6 days. By the end of fermentation, there was no significant difference ($P > 0.05$) in final alcohol content of four wine samples, ranging between 11.89 and 12.61% (Table 1). The initial TSS of *M. fruticosum* musts were either 18 or 20°B . The addition of sugar at the beginning of fermentation is necessary to provide suitable

Table 1. Development of alcohol of musts during fermentation for 10 days

Fermentation days	Treatments			
	1 (1:3, 18° <i>B</i>)	2 (1:3, 20° <i>B</i>)	3 (1:5, 18° <i>B</i>)	4 (1:5, 20° <i>B</i>)
0	0.00±0.00 ^g	0.00±0.00 ^g	0.00±0.00 ^h	0.00±0.00 ^h
1	2.73±0.81 ^f	2.73±0.81 ^f	2.70±0.00 ^g	2.43±0.39 ^g
2	4.60±0.00 ^e	4.95±0.49 ^e	3.90±0.00 ^f	4.60±0.00 ^f
3	6.49±0.30 ^d	6.69±0.00 ^d	5.29±0.98 ^e	5.58±0.57 ^e
4	7.57±0.20 ^c	7.81±0.13 ^c	7.66±0.08 ^d	7.52±0.11 ^d
6	10.78±0.00 ^b	10.60±0.26 ^b	9.50±0.57 ^c	10.32±0.13 ^c
8	11.51±0.28 ^b	11.25±0.64 ^{ab}	10.83±0.06 ^b	11.45±0.15 ^a
10	12.61±0.27 ^a	12.18±0.46 ^a	11.89±0.05 ^a	12.45±0.15 ^a

1. Results shown are mean ± standard deviation of two experiments each with triplicate analyses (n = 6).

2. Different letters in the same column differ significantly at P ≤ 0.05 by DMRT.

Table 2. Changes in total soluble solids of musts during fermentation for 10 days

Fermentation days	Treatments			
	1 (1:3, 18° <i>B</i>)	2 (1:3, 20° <i>B</i>)	3 (1:5, 18° <i>B</i>)	4 (1:5, 20° <i>B</i>)
0	18.00±0.00 ^a	20.00±0.00 ^a	18.00±0.00 ^a	20.00±0.00 ^a
1	15.50±0.71 ^b	15.40±0.57 ^b	14.00±0.00 ^b	14.70±0.99 ^b
2	13.70±0.99 ^c	14.50±0.14 ^c	12.90±0.14 ^c	14.15±0.92 ^{bc}
3	11.90±1.27 ^d	13.65±0.21 ^d	11.70±0.14 ^d	13.60±0.85 ^{bc}
4	11.00±0.71 ^{de}	12.75±0.35 ^e	10.60±0.28 ^e	12.60±0.85 ^{cd}
6	9.60±0.57 ^{ef}	11.50±0.42 ^f	9.10±0.42 ^f	11.30±0.71 ^{de}
8	8.50±0.71 ^f	10.30±0.42 ^g	7.90±0.42 ^g	10.30±0.71 ^f
10	7.85±0.35 ^f	9.15±0.21 ^h	7.50±0.42 ^g	9.25±0.35 ^f

1. Results shown are mean ± standard deviation for two experiments each with triplicate analyses (n = 6).

2. Different letters in the same column differ significantly at P ≤ 0.05 by DMRT.

Table 3. Changes in pH of musts during fermentation for 10 days

Fermentation days	Treatments			
	1 (1:3, 18° <i>B</i>)	2 (1:3, 20° <i>B</i>)	3 (1:5, 18° <i>B</i>)	4 (1:5, 20° <i>B</i>)
0	3.71±0.00 ^a	3.72±0.01 ^a	3.71±0.00 ^a	3.71±0.01 ^a
1	3.67±0.02 ^{ab}	3.63±0.02 ^{bc}	3.52±0.02 ^b	3.54±0.05 ^b
2	3.65±0.02 ^{ab}	3.64±0.00 ^{bc}	3.51±0.03 ^b	3.50±0.03 ^b
3	3.62±0.01 ^c	3.61±0.01 ^c	3.47±0.04 ^b	3.48±0.04 ^b
4	3.63±0.01 ^{bc}	3.63±0.00 ^{bc}	3.48±0.03 ^b	3.47±0.04 ^b
6	3.61±0.03 ^c	3.65±0.02 ^{bc}	3.48±0.04 ^b	3.48±0.04 ^b
8	3.61±0.02 ^c	3.63±0.01 ^{bc}	3.48±0.02 ^b	3.49±0.03 ^b
10	3.60±0.03 ^c	3.62±0.01 ^{bc}	3.46±0.02 ^b	3.48±0.01 ^b

1. Results shown are mean ± standard deviation for two experiments each with triplicate analyses (n = 6).

2. Different letters in the same column differ significantly at P ≤ 0.05 by DMRT.

conditions for the growth of yeast and fermenting the sugar into ethanol. From the results in Table 2, a similar trend of reduction in TSS was observed in all samples in favour of ethanol formation. After 10 days of fermentation, the TSS of treatments 1 (1:3, 18°*B*) and 3 (1:5, 18°*B*) remained at 7.50-7.85°*B*, whereas those of treatments 2 (1:3, 20°*B*) and 4 (1:5, 20°*B*) remained at 9.15-9.25°*B*. Generally, about 95% of the total sugars in must is metabolized by the starter into ethanol and CO₂ whereas the remaining sugar (5%) is converted to cellular material and other products such as glycerol (Romano *et al.*, 1998).

All musts had similar initial pH values (3.71-3.72), which slightly reduced to pH 3.46-3.62 at the end of fermentation (Table 3). The observed decline of pH value could be due to increased microbial activities which led to the H⁺ ions and the formation of carbonic acid from the reaction of CO₂ and water (Jacques *et al.*, 2003).

These results are in agreement with Ifie *et al.* (2012) who reported the decline in TSS and pH, and increase in the yield of alcohol during the

fermentation of roselle wine. However, roselle wine had lower maximum ethanol production (9.6%), final TSS (4.8°*B*) and pH-value (3.09) than those of our study. Grape wine prepared in the study of Bindon *et al.* (2013) had alcohol content of 11.77 - 15.5% and pH of 3.46 - 3.62, comparable with *M. fruticosum* wine. However, in their study almost all sugar was consumed during fermentation. The different results among studies could be due to the fact that the acidity and ethanol content of wine depend on several factors, including type of fruit, type of yeast used, initial TSS in must and methods of wine production (Joshi and Sharma, 1994).

Total phenolic and anthocyanin contents

Total phenolic and anthocyanin contents of *M. fruticosum* must and wine as influenced by J/W ratio and initial TSS is shown in Table 4. *M. fruticosum* musts contained total phenolics 0.11 - 0.22 g/100 ml and anthocyanin 466.57 - 827.26 mg/l. The amount of water added to the juice significantly affected both parameters. It was found that the musts prepared with J/W ratio at 1:3 had higher total phenolics than those with J/W ratio at 1:5 (0.20 - 0.22 cf. 0.11 - 0.12 g/100 ml). Similar trend was also observed in anthocyanin content (726.90 - 827.26 cf. 466.57 - 491.11 mg/l).

Phenolic compounds are important to the organoleptic properties of wine by affecting the colour, astringency and aroma (Czyowska and Pogorzelski, 2002). The results of the study revealed that the addition of macerated pulps to the must during the period of fermentation had a positive effect on the phenolic content of the wine. All wine samples had the significant higher total phenolics (P ≤ 0.05) as compared to their corresponding musts, showing the values between 0.17 and 0.26 g/100 ml. Among these samples, treatment 3 (1:5 and 18°*B*) showed the highest increase from 0.11 to 0.17 g/100 ml, accounting for 54% of its initial concentration. It has been reported that the concentration and composition of the phenolics present in wines depends largely on the source of fruit, the maturity of fruit and the method of wine making (Rupasinghe and Clegg, 2007; Towantakavanit *et al.*, 2011). Fermentation and ageing could also modify the phenolic profile through process e.g. condensation and polymerization reactions, enzymatic activity, hydrolysis and oxidative processes (Martinez and Whitaker, 1995).

It was observed that the amounts of anthocyanin in all wine samples decreased significantly after fermentation and ageing (P ≤ 0.05), showing the values of 315.11 - 517.33 mg/l. Of the four samples, the loss of anthocyanins was highest in treatment 1 (1:3 and 18°*B*), equivalent to 41% of its initial

Table 4. Anthocyanin, total phenolics and antioxidant activity of musts and wines with different wine-making conditions

Contents	Treatments			
	1 (1:3, 18° <i>B</i>)	2 (1:3, 20° <i>B</i>)	3 (1:5, 18° <i>B</i>)	4 (1:5, 20° <i>B</i>)
Anthocyanin (mg/l)				
Must	827.26±70.38 ^{aA}	726.90±95.17 ^{aA}	491.11±42.27 ^{bA}	466.57±12.75 ^{bA}
Wine	485.60±45.34 ^{aB}	517.33±34.01 ^{aB}	357.36±12.75 ^{bB}	315.11±16.77 ^{bB}
Total phenolic (g/100ml)				
Must	0.20±0.02 ^{aB}	0.22±0.04 ^{aB}	0.11±0.10 ^{bB}	0.12±0.00 ^{bB}
Wine	0.22±0.01 ^{aA}	0.26±0.03 ^{aA}	0.17±0.00 ^{bA}	0.17±0.01 ^{bA}
Antioxidant activity (%)				
Must	84.76±0.41 ^{bA}	85.14±0.23 ^{bA}	89.06±2.04 ^{aA}	88.69±0.81 ^{aA}
Wine	80.26±1.75 ^{cB}	81.05±2.39 ^{bcA}	86.54±0.12 ^{aA}	84.55±0.58 ^{abA}

1. Results shown are mean ± standard deviation for two experiments each with triplicate analyses (n = 6).

2. Different small letters in the same row differ significantly at P ≤ 0.05 by DMRT.

3. Different capital letters in the same column differ significantly at P ≤ 0.05 by t-test.

Table 5. Sensory evaluation of wines using 9-point hedonic rating scale

Attributes	Treatments			
	1 (1:3, 18° <i>B</i>)	2 (1:3, 20° <i>B</i>)	3 (1:5, 18° <i>B</i>)	4 (1:5, 20° <i>B</i>)
Color	4.20±1.37 ^b	4.59±1.17 ^b	6.90±1.93 ^a	7.25±1.47 ^a
Clarity	5.10±1.67 ^c	6.05±1.30 ^b	5.90±1.73 ^{bc}	7.10±2.06 ^a
Aroma	6.80±1.73 ^{bc}	7.70±2.17 ^a	6.40±1.52 ^c	7.30±1.69 ^{ab}
Body ^{ns}	6.30±2.01	6.10±1.95	6.85±1.84	6.95±1.69
Sweetness	4.50±2.16 ^b	6.30±1.81 ^a	3.55±1.69 ^c	7.10±1.86 ^a
Bitterness	5.50±2.43 ^b	5.25±2.12 ^b	3.95±2.23 ^c	7.25±1.95 ^a
Overall	5.85±1.64 ^b	6.30±1.44 ^b	6.40±1.53 ^b	7.20±1.58 ^a

1. Results shown are mean ± standard deviation for two experiments each with 20 panelists.

2. Different letters in the same row of each sensory attribute differ significantly at P ≤ 0.05 by DMRT.

3. Ns = not significant (P > 0.05)

4. Scoring: 1 = Dislike extremely; Scoring 9 = Like extremely

concentration. The lowest decline in anthocyanins was equivalent to 27% in treatment 3 (1:5 and 18°*B*). The loss of anthocyanins could be ascribed by several reasons. It has been reported that the anthocyanins are relative unstable and they undergo structural transformations that affect colour and stability. Factors affecting anthocyanins in wine include pH, temperature, oxygen concentration, sugar, SO₂, polymerization and copigmentation (Somers and Evans, 1974). In addition, anthocyanin may possibly be adsorbed to the surface of bentonite and lost with other suspensions during the purification.

Antioxidant activity

Concerning the antioxidant activity, it was found that the RSA of all musts decreased from 85.14 - 89.06% to 80.26 - 86.54% after fermentation and ageing. This could possibly be due to the significant loss of anthocyanins during fining with bentonite as earlier described. However, three of four wine samples had statistically insignificant decrease in RSA (P > 0.05) as compared to their corresponding musts. Among these samples, wines with J/W ratio at 1:5 and initial TSS of 18°*B* exhibited the greatest ability to scavenge DPPH radical (86.54%). Similar results were observed by Towantakavanit *et al.* (2011).

Sensory properties

Table 5 summarizes the mean scores of sensory

evaluation of *M. fruticosum* wines based on a 9-point hedonic scale, where 1 was “dislike extremely” and 9 was “like extremely”. The findings indicated that the different initial TSS and J/W ratios yielded wines with different sensory characteristics. Overall, wines with J/W ratio at 1:5 were rated higher colour score than those with J/W ratio at 1:3. Samples with initial TSS of 20°*B* also had higher scores in terms of clarity, aroma and sweetness than those with TSS of 18°*B*.

As compared among the four wine samples, a significant difference (P ≤ 0.05) was found in terms of colour, clarity, sweetness and bitterness, ranging from “dislike moderately” (score = 3) to “like very much” (score = 8). However, there was no significance of the differences in body attribute ratings (P > 0.05), ranging from 6.10 - 6.95. This could be attributed to similar alcohol content of each wine. The highest scores of all attributes except aroma were seen in wine made with J/W ratio at 1:5 and initial TSS of 20oB. This could be due to the good appearance and high sugar content, which may have affected the taste of the sample.

Conclusions

Based on the level of health-promoting compounds present in its ripe fruits, the ability to support yeast growth and the high alcoholic content of the wine, *M. fruticosum* Lour. has been a promising raw material for production of red wine. The results indicated a decrease in anthocyanin content and a small increase in phenolic compounds of wines as compared to their corresponding musts. However, the antioxidant power of most wines was insignificantly affected. The sensory evaluation showed that all wines had an acceptable colour, clarity, aroma, sweetness and bitterness but further research is needed to improve the body of the final product. Wine prepared from J/W ratio at 1:5 (v/v) and initial TSS of 20°*B* showed the highest overall acceptance. The contents of alcohol and TSS of this wine sample were 12.45% and 9.25°*B*, respectively which was categorized as sweet wine.

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