The Potential of Malolactic Fermentation on Organic Acids Degradation in Mao (*Antidesma Thwaitesanum* Müell.) Wine Production

Wanphen Jitjaroen, Tunyaluk Bouphun, and Lachinee Panjai

***Abstract*—Commercial mao wines often show highly acidic level which results in a sourness taste of the products. Thus, the purpose of the study was to present the ability of malolactic fermentation in the reduction of acidity during mao wine production. The must was mixed with puree and adjusted to 3.7 g/L total acidity, 200 g/L sugar content, 0.6 mg/L thiamine hydrochloride, sulphited to a level of 50 mg/L, and fermented at 20 °C. The addition of three different industrial yeast strains, and ammonium phosphate levels (DAP): Rhone2323 with DAP 300, and 500 mg/L, and GHM with DAP 500 mg/L were prepared, and incubated at 20 °C until the end of alcoholic fermentation. Consequently, the commercial malolactic bacteria Elios1 was added until fermentation reached the end of the attenuation stage. The enological parameters were investigated to control a well-fermentation. Results showed that the malolactic fermentation affected the degradation of most organic acids in particular with malic acid from 1.34-1.76 g/L to nil, accompanying with the increase of lactic acid from 0.02-0.35 to 0.77-0.85 g/L, and slightly increase of a pH from 3.0 to 3.1-3.2. Overall acidity can be reduced in the range of 0.87 to 1.05 g/L.**

***Index Terms*—Mao wine, malolactic fermentation, yeast strain, ammonium phosphate, organic acid, malic acid, lactic acid.**

I. Introduction

Ma-mao or mao *(Antidesma* sp.) of the Stilaginaceae family is grown in the warm climate of Africa, Asia, Australia, Indonesia and the countries around the Pacific ocean. Its round or ovoid fruits with dark-red colour and fragrance are borne in clusters. The fruits are acid like cranberries, and less acidic and slightly sweet when fully ripe [1]. It is an indigenous fruit that could be used to produce fine wine, and is very well known in Thailand. Jitjaroen *et al*. (2011) [2] investigated the chemical composition of Thai commercial mao wines and found that most of them were identifiably sour, salty or bitter [3]. The main acids of mao fruit berry are 13.0 g/L citric, 1.1 g/L malic acids and 1.1 g/L tartaric which result in a main cause of much sourness [3]-[5]. The biological deacidification reaction is well recognized as one of the main metabolic capabilities of malolactic bacteria. It is known as the term malolactic fermentation (MLF) and its conduct is of major commercial importance to the winemaking process [6]. This technique was considered as secondary fermentation of the mao wine process in this study.

MLF describes the enzymatic conversion of L-malic acid to L-lactic acid and CO_2 by lactic acid bacteria [7]. In addition to the dependency of such effects on the initial concentration of malic acid, the actual changes in wine acidity and pH attributable to the MLF depend on other factors, including the buffering capacity of the wine as well as the initial pH [8]. In general, the overall decrease in wine acidity resulting from MLF can vary from 0.1%-0.3%, and pH may rise by 0.1-0.3 pH units [9]. Nevertheless, MLF can be desired in such wines to confer a degree of biological stability and/or to impart flavour complexity, necessitating the use of acidulants to adjust wine acidity and pH to acceptable levels after MLF. The increase in wine pH accompanying MLF can also influence wine colour [6].

Therefore, in this study the change of organic acid content before and after malolactic fermentation of mao wine was investigated. The objective of this study was to identify methods and techniques that would enable mao wine makers to decrease wine acidity.

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II. Material and Methods

A. Experimental Mao Wine Fermentation

Alcoholic fermentation: Three sets of mao juice with its puree (*Antidesma thwaitesanum* Müell.) variety "Fah-pra-tan" were fermented: yeast strain Rhône2323 in combination with diammonium phosphate (DAP) 300, and 500 mg/L, and yeast strain GHM in combination with DAP 500 mg/L (Lallemand, Australia). The must was adjusted to a sugar content up to 200 g/L initially by sucrose, the titratable acidity 3.7 g/L (as citric acid), thiamine hydrochloride 0.6 mg/L, and sulphited to a level of 50 mg/L. The must samples were made up to 1 L in 2.5 L glass bottles and mixed well with DAP and yeast strain, then fitted with a fermentation lock, and incubated at 20 °C until the end of yeast fermentation [3].

Secondary fermentation: The commercial malolactic bacteria Elios1was added and topped up the head space of bottle with carbon dioxide until fermentation reached the end of the attenuation stage. Consequently, sulphur dioxide was added to achieve a final concentration of 30 mg/L free sulphur dioxide in the finished wine. The pulp was separated from the wine into the bottles. They were stored for two weeks at 10-14 °C before analyzing fermentation parameter [3].

B. Analytical Methods

In order to control the well-fermentation parameters, mao

juice and wines were analyzed for pH value, titratable acidity [9], suphur dioxide by the Ripper titrametric method [10], total sugar in the form of D-glucose and D-fructose by high performance liquid chromatograph (HPLC) [11], acetaldehyde, pyruvic acid, α-ketoglutarate and sulphur binding capacity by enzymatic method [12], and alcohol content by ebulliometer [13]. Carbon dioxide production was examined by daily weighing during alcoholic fermentation.

Organic acids was examined by using a reversed-phase High performance liquid chromatography method. Separation was achieved using a column thermostat (35 °C on Zorbax SB-Aq, 4.6 mm x 150 mm, 5 µm) and diode array detector at 220 nm The mobile phase was 99% 20 mM NaH_2PO_4 at pH 2 and 1% acetonitrile. The flow-rate was 1 mL/min and injection volume was 10 µL [11].

All parameters were examined with three replications. The statistical analysis was analyzed by the method of Completely Randomized Design (CRD) at α=0.01. The significant different was interpreted by using Duncan Multiple Range Test (DMRT) [14], [15]. The report would be presented the changes of organic acid content before and after malolactic fermentations.

C. Culture Preparation

Yeast culture: The commercial dry yeast strains *Saccharomyces cerevisiae* Rhöne2323, and GHM were used for alcoholic fermentations obtained from Lallemand Co., Ltd., Australia, which was rehydrated for 25-30 min at 35 °C and was added to the must at 0.02% (v/v) [16].

Lactic acid bacteria culture: The commercial freeze-dried bacteria strain *Oenococcus oeni* Elios1 was used for secondary fermentation obtained from Lallemand Co., Ltd., Australia, which was rehydrated for 15 min at 20 °C and was added to the must at 0.001% (v/v) [16].

III. Result and Discussion

A. Mao Juice Components

Mao juice is highly acidic fruit with a deep red juice initially containing 157.55 g/L total sugar, 10.5 g/L total acidity (as citric acid) and a pH of 3.3. The main organic acids were 8.24 g/L citric acid, 3.08 g/L tartaric acid, and 0.87 g/L malic acid (result not shown). Therefore, the fermentation base was adjusted to a sugar content of 200 g/L initially by sucrose, whereas pH and acidity were appropriate for alcoholic fermentation [17], [18], and malolactic fermentation [3], respectively.

B. Fermentation Data

The informal sensory assessments of the wines after yeast fermentation were conducted, the wines were identifiably much acidic and sourness reflected from increased acidity from 3.7 g/L of must to 7.52-7.7 g/L of wine samples. Therefore, the MLF was conducted after alcoholic fermentation in order to reduce the acidic of the wine.

The wine components after MLF were obtained well-fermentation parameters. Different nutritive and yeast strains affected the max.CO_2 production at the range of 16-22.5 g/L/day on the second day of alcoholic fermentation. All wines completed their yeast fermentation after 17 days, following by 14 days of malolactic fermentation.

All treatments produced the expected ethanol level of 12.50-12.80 %vol. concomitant with residual sugar less than 5 g/L, 3.31-3.32 of a pH, 6.65 g/L total acidity, 18.27-18.88 mg/L acetaldehyde, 31.35-41.36 mg/L α-ketoglutarate, and 40.66-44.68 sulphur binding capacity (see Table I). The expected level of ethanol, the formation of appropriate organic acid concentrations and the carbonyl compounds that are present regularly in finished wine may be related to yeast capability of metabolizing glucose via glycolysis and to fermentation pathway forming pyruvic acid which is oxidized to acetaldehyde and subsequently reduced to ethanol [19]-[21].

C. Organic Acids Changed in Malalactic Mao Wine

The concentrations of most organic acids were within the range known from grape wines (2-8 g/L tartaric acid, up to 1 g/L malic acid, 0.1-1 g/L lactic acid and 0.6-0.9 g/L acetic acid), except for a large amount of 1.75-1.96 g/L citric acid, which was present in concentrations significantly beyond the upper limit as reported for good wine quality, e.g. in the range from 0.5 to 1 g/L [20], [22]-[24]. The occurrence of a large amount of citric acid is originated from the natural juice itself.

TABLE I: Chemical Component of Mao Wine Samples After the Malolactic Fermentation

Components[1]	Mao wine treatments		
	Rhöne2323 DAP 300 mg/L	Rhöne2323 DAP 500 mg/L	GHM DAP 500 mg/L
TSS (°Brix) [ns]	5.30±0.10	5.86±0.64	6.00±0.00
Glucose (g/L) [ns]	0.00±0.00	0.00±0.00	0.00±0.00
Fructose (g/L)	0.79±1.36 [b]	0.00±0.00 [c]	4.36±3.18 [a]
Sucrose (g/L) [ns]	0.00±0.00	0.00±0.00	0.00±0.00
Total sugar[1] (g/L)	0.79±1.36 [b]	0.00±0.00 [c]	4.36±3.18 [a]
Alcohol (% vol.) [ns]	12.56±0.11	12.80±0.26	12.50±0.00
Acetaldehyde (mg/L) [ns]	18.27±0.22	18.51±0.39	18.88±0.07
Pyruvate (mg/L)	nd[3]	nd	nd
α-Ketoglutarate (mg/L)	41.36±1.47[a]	31.41±1.17[b]	36.35±1.22[c]
Sulphur binding capacity[2] (mg/L)	44.68±0.86[a]	40.66±0.80[b]	43.37±0.49[a]

Means within the same column followed by different small letters are significantly different, p≤0.01.
[1] based on the calculation of totally glucose and fructose contents
[2] based on the calculation of acetaldehyde, pyruvate, and α-ketoglutarate with their factors
[3] not detected

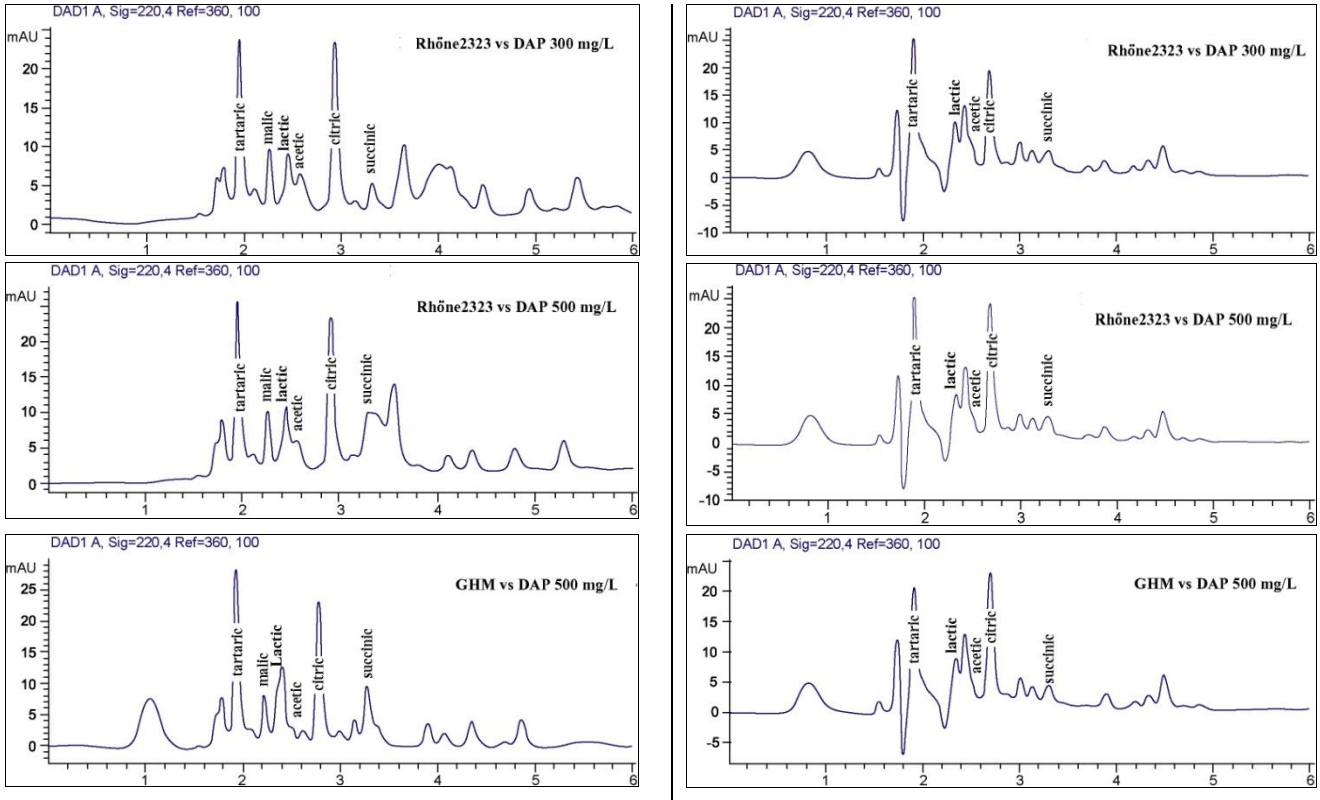
Some differences before and after MLF were noted between the treatments in terms of production and/or utilization of organic acids. These are reflected by the concentrations of malic acid in the wine. As shown in Table II and Fig. 1, the malolactic bacteria played a role by metabolizing the whole malic acid (from 1.34-1.76 g/L to nil) correspondent with the increase of lactic acid (from 0.02-0.35 to 0.77-0.85 g/L) and slightly increase of a pH (from 3.0 to 3.1-3.2). The decrease of some acids were observed such as citric acid (from 2.91-4.07 to 1.75-1.96 g/L), and succinic acid (from 0.53-0.78 to 0.21-0.35 g/L), whereas tartaric acid increased from 0.74-0.92 to 1.35-1.51 g/L. These resulted in decrease titratable acidity from 7.52-7.70 to 6.65 g/L. It was indicated that the MLF was able to

reduce acidity of mao wine ranging from 0.87 to 1.05 g/L (as citric acid), whereas acidity of malolactic grape wines range from 1.5-4.0 g/L (as tartaric acid) [25].

TABLE II: COMPARE ORGANIC ACIDS BEFORE AND AFTER THE MALOLACTIC FERMENTATIONS

Components[1]	Mao wine treatments								
	Yeast Rhône2323 DAP 300 mg/L			Yeast Rhône2323 DAP 500 mg/L			Yeast GHM DAP 500 mg/L		
	Before MLF[2]	After MLF	ΔT[3]	Before MLF	After MLF	ΔT	Before MLF	After MLF	ΔT
pH	3.30±0.00	3.32±0.01	0.02±0.01	3.31±0.01	3.31±0.00	0.00±0.01	3.31±0.00	3.31±0.00	0.00±0.01
Acidity[1] (g/L)	7.52±0.09	6.65±0.11	-0.87±0.02	7.70±0.02	6.65±0.05	-1.05±0.05	7.70±0.05	6.65±0.05	-1.05±0.10
Citric acid (g/L)	2.91±0.01	1.75±0.22	-1.16±0.23	3.85±0.04	1.82±0.36	-2.03±0.34	4.07±0.08	1.96±0.10	-2.11±0.19
Tartaric acid (g/L)	0.92±0.09	1.35±0.14	+0.43±0.04	0.91±0.16	1.49±0.17	+0.58±0.09	0.74±0.02	1.51±0.12	+0.77±0.15
Malic acid (g/L)	1.67±0.05	0±0.00	-1.67±0.05	1.76±0.16	0±0.00	-1.76±0.16	1.34±0.17	0±0.00	-1.34±0.17
Lactic acid (g/L)	0.02±0.01	0.85±0.06	+0.83±0.05	0.28±0.01	0.82±0.03	+0.54±0.04	0.35±0.03	0.77±0.16	+0.42±0.18
Acetic acid (g/L)	0.35±0.29	0.00±0.04	-0.35±0.27	0.00±0.00	0.00±0.02	0.00±0.02	0.00±0.00	0.00±0.01	0.00±0.01
Succinic acid (g/L)	0.78±0.17	0.35±0.07	-0.43±0.10	0.54±0.02	0.21±0.04	-0.33±0.02	0.53±0.05	0.31±0.04	-0.22±0.07

[1] as citric acid
[2] Malolactic fermentation
[3] Different between before and after the Malolactic fermentations



(a) (b)

Fig. 1. Malolactic acid fermentations influenced HPLC chromatographic peak of main organic acids as shown in (a) before the malolactic fermentation and (b) after the malolactic fermentation.

IV. CONCLUSIONS

The malolactic fermentation in mao wines were evaluated in terms of their impact on the reduction of wine acidity. In addition to the dependency of such effects on the initial concentration of malic acid, the actual changes in wine acidity and pH attributable to the MLF depend on other factors, including the mao fruit variety as well as malolactic bacteria strain. These results would be beneficial to wine makers to improve sourness in commercial mao wine. More research is needed to improve the potential of MLF, for examples, the metabolic activity of the MLB influences wine colour, aroma compounds of wine derived from fruit and the alcoholic fermentation, and biological stability on the final product.

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