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The Observation of Interactions Between Yeast Strain and Nitrogen Reducing Succinic Acid in Mao (*Antidesma thwaitesanum Müell.*) Wine Fermentation

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Abstract. Commercial mao wines often show a succinic acid content as high as 13.26 g/L which results in a salty taste in the products. Thus, the purpose of the study was to select suitable industrial yeast strains, and ammonium phosphate levels, to decrease the succinic acid production in mao wine. The must was added to 3 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 must was fermented by three yeast strains (Rhône, GHM, V1116) and two levels of ammonium phosphate (300, 500 mg/L). Results showed that the yeast strain and ammonium phosphate affected the potential of succinic acid production after fermentation. The addition of ammonium phosphate at the level of 500 mg/L produced higher succinic acid than 300 mg/L, and was significant in combination with yeast strains GHM and V1116. They were evaluated at the lowest rank sum score of saltiest and accepted in the same level as the commercial grape wine.

Keywords: Mao wine, Succinic acid, Yeast strain, Ammonium phosphate

1. Introduction

Mamao 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.

Succinic acid has been reported to have an 'unusual salty and bitter taste.[3] It is a main non-volatile carboxylic acid produced by yeasts during wine fermentations, and generally varies between 1 to 4 g/L. It accounts for 90% of the observed increase in acidity,[4, 5] as 1 g/L of succinic acid will contribute approximately 1.3 g/L to the titratable acidity value expressed as tartaric acid.[6] It might derive from either sugar or amino acid catabolism by yeast, depending on growth conditions and available nitrogen sources, and its direct formation is dependent on the reactions of the tricarboxylic acid cycle.[7]

Therefore, in this study the relationship between yeast strains and ammonium phosphate levels was investigated for the succinic acid content, concurrent with the saltiness evaluation after yeast fermentation. The objective of this study was to identify methods and techniques that would enable mao wine makers to improve wine quality.

2. Material and Methods

2.1. Succinic Acid in Commercial Mao Wines

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The commercial mao wines were collected from markets in Thailand. The five saltiest out of thirteen samples were selected to determine succinic acid content.

2.2. Mao Wine Fermentation with Different Yeast Strains and Ammonium Phosphate levels

Mao juice and puree (*Antidesma thwaitesanum* Müell.) were fermented with three yeast strains: Rhône, GHM and V1116 (Lallemand, Australia). Fermentations were conducted using two ammonium phosphate levels: 300 and 500 mg/L. Thus they gave a matrix of six different fermentations. The must was adjusted to a sugar content up to 200 g/L initially by sucrose, the titratable acidity 3 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 different ammonium phosphate levels and yeast strains, then fitted with a fermentation lock, and incubated at 20 ^{o}C 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. They were stored for two weeks at 10-14 ^{o}C before analyzing succinic acid and saltiness assessment.[8]

In order to control the well-fermentation parameters, mao juice and wine were analyzed for pH value, titratable acidity,[9] glucose and fructose, and organic acids including succinic acid content.[10] The saltiness was determined by sensory evaluation. All parameters were examined with three replications. The report would be present only the values of succinic acid content and ranking sum score of saltiness.

Succinic acid was examined by using a reversed-phase High performance liquid chromatography method. Separation was achieved using a column thermostat (35 ^{o}C on Zorbax SB-Aq, 4.6 mm x 150 mm, 5 µm) and diode array detector at 220 nm (Agilent Technologies, Germany). 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.[10]

The saltiness was determined by 24 trained panelists at the department of Agro industry, Rajamangala University of Technology Lanna Lampang, Thailand. The ranking of saltiness intensity was evaluated by the method of the multiple ranking no ties allowed-with Balanced Incomplete Block Design (BIB). The statistical analysis was analyzed at α=0.05 by Friedman's statistic (T) for BIB 11.7 (t=7, k=3, r=3, b=7, λ=1, p=1). The significant different was interpreted by using Turkey's HSD test.[11, 12]

3. Results and Discussion

3.1. Succinic Acid Production

The concentration of succinic acid of the products depends upon various factors including the vintage and wine type. It is normally found 0.5-1.5 g/L succinic acid in the wines[13, 14] but higher concentrations up to 3.0 g/L have been detected within certain red wine.[6] The Australian wines during the period 1991 to 2003 were found with succinic acid at the level of 0.1 to 2.6 g/L in red wines, and 0.1 to 1.6 g/L in white wines.[6] The commercial mao wine samples in Fig. 1 shows succinic acid in a wide range 0.93 to 13.26 g/L. The informal sensory assessments that were conducted, the wines were identifiably less to much saltiness and accounted for low quality wine. The saltiness might deal with the succinic acid content which was produced during wine fermentation.[4, 5]

3.2. Yeast Strains and Ammonium Phosphate Levels Influenced Succinic Acid Production

Some of the main factors influencing the production of succinic acid during fermentation are yeast strain, and nitrogen. The mesophilic strains AWRI 796 and Enoferm M2 appear to be relatively high producers of succinic acid. Yeast strain WE372 produced the largest amount of succinic acid (1.436 g/L) in synthetic grape juice at 28 ^{o}C when compared with EC1118, DV10, U43 and WE14 (0.626-0.813 g/L).[15] In addition, increased succinic acid production might occur with increased nitrogen up to 500 mg total N/L.[11] Jean-Louise (2011)[15] indicated that succinic acid production by fermenting yeasts will be flavored by moderate amounts of metabolically available nitrogen (approx. 300 mg/L).

The interaction of yeast strains and ammonium phosphate levels as a nitrogen source influenced to the succinic acid production in mao wines as shown in Fig. 2 and 3. The wines with yeast strains GHM and V1116 produced more succinic acid at the level of 0.67-1.39 g/L than yeast strain Rhône at the level of 0.52-

0.61 g/L. The addition of ammonium phosphate at 500 mg/L enhanced succinic acid production when compared with 300 mg/L, but not influenced in yeast strain Rhône.

These results were correlated with the saltiness assessment of mao wine as present by ranking sum score in Fig. 2. The saltiest was detected in wine samples with yeast strain V1116 (score 22 and 24) and less in commercial grape wine as a control (score 10). The wine samples with yeast strain Rhône2323 (score 21), GHM (score 14), as well as the control, were within the same rank sum perception and were acceptable. Previous research reported that the solutions of succinic acid in water at level of 0.5, 1.0 and 2.0 g/L to be unpleasant when compared to solutions of tartaric acid, and the unusual taste lingered after expectorating.[6] Whilst the saltiness of mao wine might interweave between sourness, saltiness and bitterness appear to be relatively the saltiness acceptable which could be initially concluded up to 1.38 g/L.

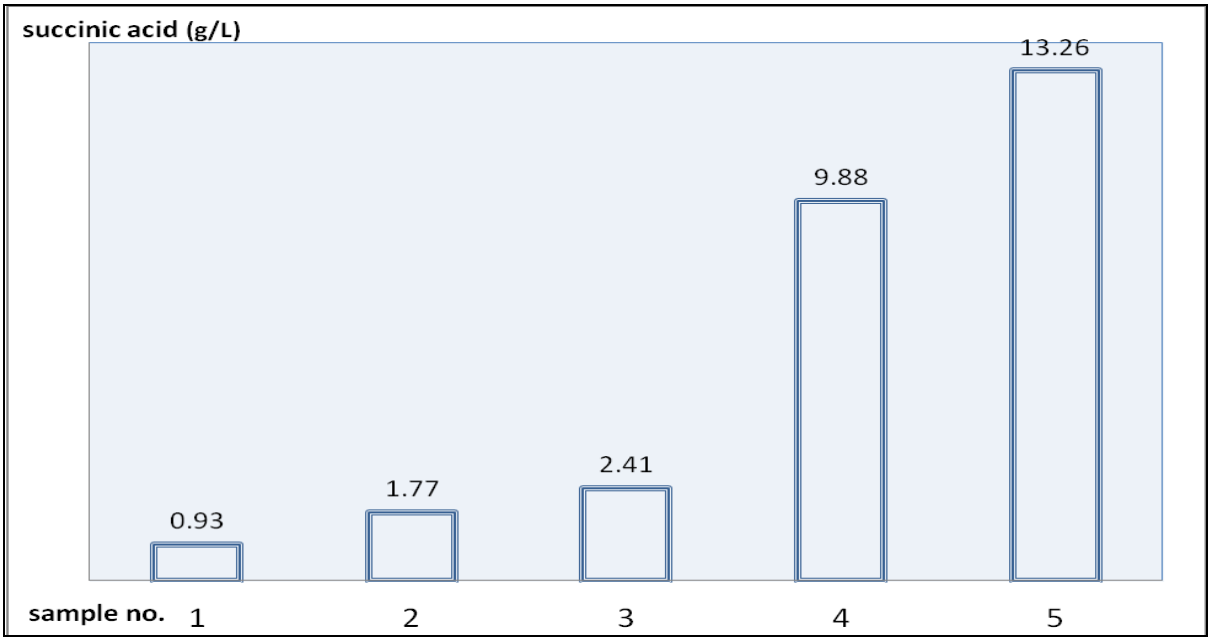

Fig. 1:Succinic acid content in commercial mao wines collected from markets in Thailand.

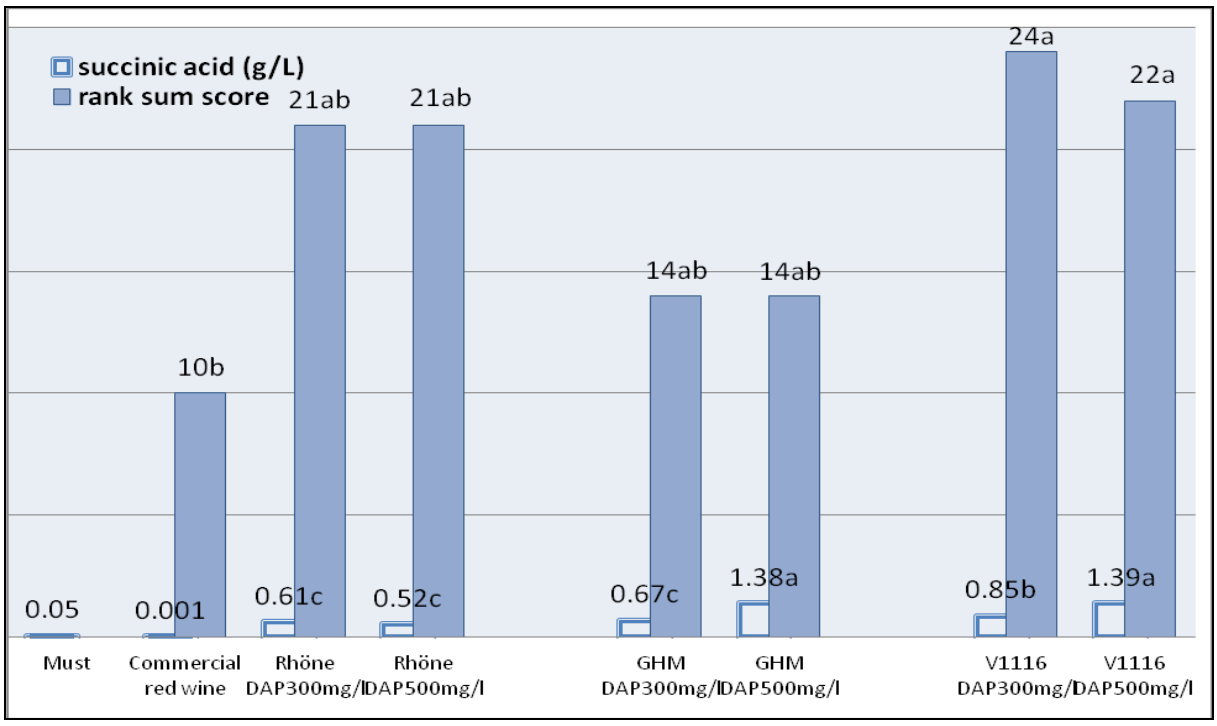

Fig. 2: Yeast strains and ammonium phosphate (DAP) levels influencing succinic acid production of mao wine fermentation, and sensory rank sum score of saltiness by multiple ranking no ties allowed with BIB design (score line 7= most salty, 1=least salty).

4. Conclusions

Selected yeast strains and nitrogen sources in mao wine fermenting were evaluated in terms of their impact on the succinic acid production. Some yeast strains like Rhône and GHM influenced to reduce succinic acid production. The increased nitrogen source like ammonium phosphate up to 500 mg/L tended to enhance succinic acid production, but insignificant in the combination with yeast strain Rhône. These results appear to lower the succinic acid content in commercial mao wine in which wine makers can control succinic acid production during mao wine fermentation. More research is needed to minimize the succinic acid production of wine as fermentation temperature, pH, oxygen, vitamin, sulfur dioxide, sugar concentration, clarification, variety, and vintage.[6]

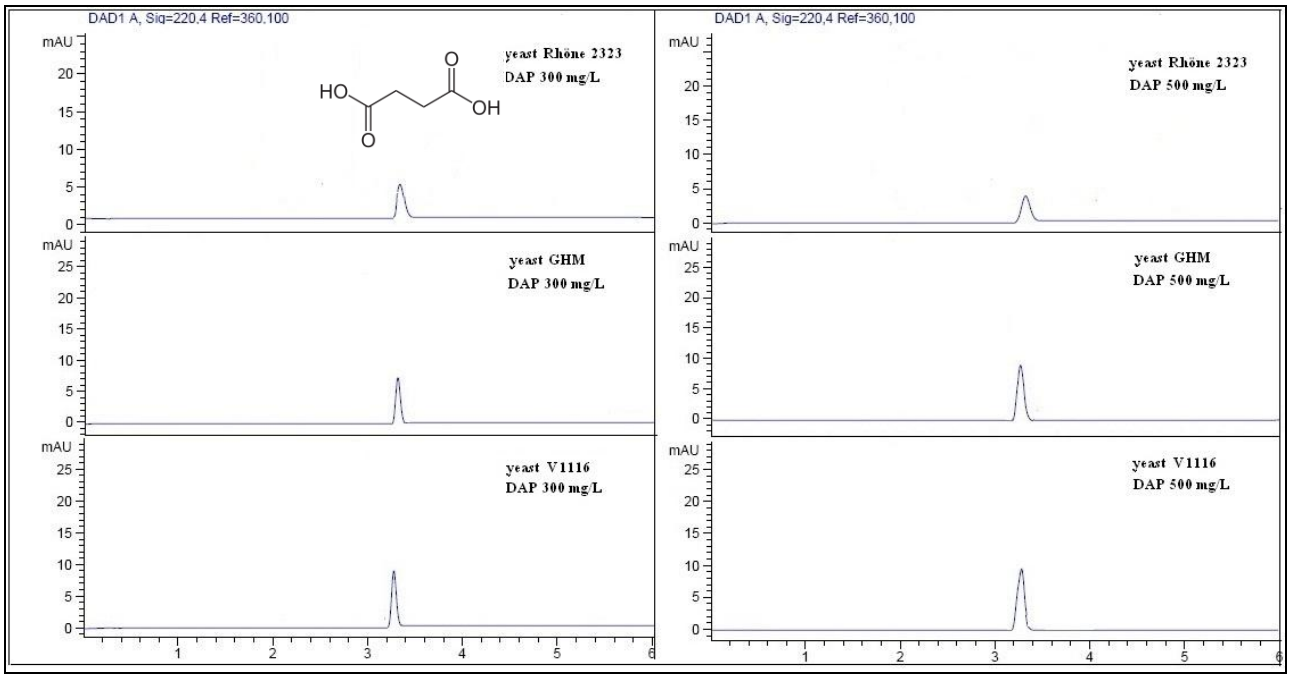

Fig. 3: Yeast strains and ammonium phosphate (DAP) levels influenced HPLC chromatographic peak of succinic acid in mao wine fermentation.

5. Acknowledgements

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