Microbial Strains as a Key Role Played on Aroma Profiles of Mao-Berry Fruit Wine

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Abstract-Using appropriate yeast strain and ammonium phosphate (DAP) was to evaluate aroma profiles in mao wine fermentation. The three different industrial veast strains, and DAP levels: Rh öne2323 with DAP 0.3, and 0.5 gL⁻¹, and GHM with DAP 0.5 gL⁻¹ were added, and incubated at 20°C, consequently the malolactic fermentation by the commercial malolactic bacteria Ellios1. The modeling of mao wine aroma profiles by gas chromatography and descriptor intensity by quantitative descriptive analysis were determined. The matrix of fermentations affected the aroma constituents and flavors complexity. Fifty-five aroma components were identified, mainly acetate, fatty acid and acid esters, higher alcohol, and terpenes. The twenty nine descriptors were intensified by judgments and discriminated into six groups (fruity, vegetable, floral, spice, sugar and trait) in a spider graph. The attributes were intensified ranking from mao fruit, ripe tamarind, dried rosella, prune, orange, bell pepper, and honey, respectively. The yeast strains with different ammonium phosphate levels enhanced aroma compounds in the mao wine. Esters, terpenes, and higher alcohols were synthesized by yeast strain Rhöne2323 in combination with DAP 0.5 gL⁻¹. While yeast strain GHM with DAP 0.5 gL⁻¹ received the highest intensity scores in most attribute.

Index Terms—mao wine, yeast, aroma profiles, aroma descriptor, ammonium phosphate

I. INTRODUCTION

An indigenous fruit berry, namely 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. It is a round or ovoid fruit with a dark-red colour, and fragrance is borne in clusters. The fruit is acidic like cranberries, and less acidic and slightly sweet when fully ripe [1]. It can be used to produce fine wine. Jitjaroen et al. [2], [3] investigated the chemical composition of Thai commercial mao wines and found that most of them were identifiably sour, salty or bitter. The main acids of mao fruit berry were 13.0 gL⁻¹ citric acid, 1.1 gL⁻¹ malic acid and 1.1 gL⁻¹ tartaric acid. The addition of three different industrial yeast strains, and ammonium phosphate levels consequently, the commercial malolactic bacteria caused to the degradation of most organic acids in particular with malic acid from 1.34-1.76 gL⁻¹ to nil, with the increase of lactic acid from 0.02-0.35 to 0.77-0.85 gL⁻¹, 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 gL⁻¹.

However, the key factors of wine aroma and flavour influence the sensory experience of wine and consumer perception [4]. Compounds contributing to wine aroma and flavour are classified according to the different sources from which they originate. These include fermentative flavour (produced by yeast and bacteria during alcoholic and malolactic fermentation [5]. While wine yeast and lactic acid bacteria function and mechanism are still speculative. There are only a few papers that report data about the aroma compounds in mao wine. The objectives of this study were to determine the aroma constituents of mao wine systematically using GC-chromatogram, linked to the aroma descriptors by judgments, continuing from the previous study of Jitjaroen [3]. The mao wine and fruit wine industry would benefit from improving the wine perception. This would significantly contribute to the knowledge currently available.

II. MATERIAL AND METHODS

A. Experimental Mao Wine Fermentation

Three sets of mao juice with their puree (Antidesma thwaitesanum Müell) were fermented: yeast strain Rhöne2323 in combination with DAP 0.3, and 0.5 gL^{-1} , and yeast strain GHM in combination with DAP 0.5 gL¹ (Lallemand, Australia). The must was adjusted to a sugar content up to 200 gL^{-1} initially by sucrose, the titratable acidity 3.7 gL^{-1} (as citric acid), thiamine hydrochloride 0.6 mgL⁻¹, and sulphited to a level of 50 mgL⁻¹. 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]. The commercial malolactic bacteria Elios1 was added and topped up the headspace 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 mgL⁻¹ free sulphur dioxide in the finished wine. In order to control the well-fermentation parameters, mao juice and wines were analyzed for pH values, titratable acidity, sulphur dioxide, D-glucose and D-fructose, sulphur binding capacity, and alcohol content [6]. The pulp was separated from the wine in the bottles, and stored for six months at 10-14°C before analyzing the aroma profiles.

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B. Analytical Methods

Aroma volatile compounds were examined by using solid phase micro-extraction (SPME) and GCMS method. Adsorption was achieved using 100 µm PDMS fiber to adsorb volatile components in headspace oven at 85 °C for 30 min. GC-MS analysis was performed on an Agilent gas chromatograph model 7890C coupled to an Agilent 5975C mass selective detector. Analytes were separated on an HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm) increased to 250 °C at 5 °C min maintained for 15 min. increased to $250 \,^{\circ}\text{C}$ at $5 \,^{\circ}\text{C}$ min⁻¹, maintained for 15 min. Transfer line temperature was 280°C. Mass detector conditions were electronic impact (EI) mode at 70 eV, source temperature at 280 °C, scanning rate was 2.88 scan s⁻¹; mass scanning range at 29-540 mz gL⁻¹. The tentative identification of volatile components were measured by comparing the mass spectra with the Wiley and NIST data system library [7].

All parameters were examined with three replications. The statistical analysis was determined by Analysis of variance (ANOVA, α =0.01). The significant difference was interpreted by using Duncan Multiple Range Test (DMRT) [8]. The report would present the wine compositions after fermentations.

C. Intensive Aroma Assessment

Systematic descriptive sensory analysis was performed using Meilgaard *et al.* [9]. In brief, ten tasters from the staff of Rajamangala University of Technology Lanna Lampang, participated. They had experience from previous wine sensory tasting experiments. A total of five training and vocabulary development sessions were conducted following a modified QDA consensus method, comprising a 90 minute-long discussion in each session. In a series of subsequent formal rating sessions the tasters rated 29 aroma attributes for the wines in duplicate. The perceived intensity of each aroma attribute was rated using an unstructured 15 cm line scale with indented anchor points of 'low' to 'high'.

The data for each attribute were determined by ANOVA (α =0.05). The significant difference was interpreted by using DMRT [8]. The report will present the comparative intensity of wine aroma attributes in the spider graph.

III. RESULT AND DISCUSSION

A. Qualification of Volatile Aroma Compounds by Gas Chromatography

A number of studies have indicated that the volatiles in wine are responsible for the characteristic bouquet of wines. There is a relationship between specific volatile compounds and aroma in the wine, and therefore some sensory descriptors can be predicted by gas chromatographic data [10]. Fifty-four aroma component peaks of mao wine samples were consistently determined in the form of peak area contribution (%), including 27 esters, 4 terpenoids, 5 alcohols, 3 acids and 15 miscellaneous (Fig. 1 and Table I). The yeast strains with different ammonium phosphate levels enhanced aroma compounds in the samples.

Ester compounds are qualitatively one of the most important groups of volatile compounds in determining wine flavour. They are mainly produced as secondary products of yeast sugar metabolism during alcoholic fermentation [11]. From the wine maker's point of view, the most important esters found in the wine samples were acetic acid 2 and 3-methylbutyl ester, acetic acid 2phenylethyl ester, octanoic acid ethyl ester, butanoic acid ethyl ester, decanoic acid ethyl ester. They play a major role in the fruity and floral aromas of young wines and also positively influence the general quality of wine [12]. These compounds were prominent in the addition of yeast stain Rhöne2323 and DAP 0.5 gL⁻¹, which was higher than of DAP 0.3 gL⁻¹. The quantity and quality of ester related to nitrogen level presenting in the must like isoamyl and ethyl acetate, but not for phenylethyl acetate [13]. The addition of DAP up to 2 gL^{-1} increased esters and acids, but decreased alcohols [14].

In addition, the contribution of MLF to the ester profile of wine has been shown by a number of wine volatile profiling studies. Strain specific changes in ester concentration during MLF, in particular with the increase of ethyl-2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-hydroxypropanoate, ethyl 3-hydroxypropanoate, ethyl hexanoate, 3methylbutyl acetate, ethyl 2-phenyl-acetate, 2phenylethyl acetate and hexyl acetate [15]. With the same bacterial strain in the MLF of mao wine, ethyl-2methylpropanoate (fruity, strawberry, lemon), 2phenylethyl acetate (flowery, rose), and ethyl hexa-noate (fruity, strawberry, green apple, anise) were mostly prominent in the sample with the addition of yeast strain Rhöne2323 and DAP 0.5 gL⁻¹, and found 3-methylbutyl acetate (banana, fruity) in both yeast strains with the addition of DAP 0.5 gL⁻¹

Terpenes are typical components of the essential oils of flowers and fruits. They are present in wine, originate from grape and not fermentation. The biosynthesis of monoterpenes by S. cerevisiae in the absence of grape derived precursors in the condition of higher concentration of assimilable nitrogen increased accumulation of linalool and citronellol [16]. The samples showed slight peak area distribution of terpene. The odoriferous limonene responsibles for a citrus aroma [17], received mostly in fermenting with yeast strain Rhöne2323 and DAP 0.5 gL⁻¹, which was twofold of DAP 0.3 gL⁻¹, and found β -linalool (flowery, sweet and rose), and β -myrcene (peppery and spicy) [18] only in fermenting with yeast strain GHM. Higher alcohols are synthesized as a consequence of amino acid metabolism and considered to contribute to the complexity and fruity aroma of wine when present at concentrations lower than 300 mgL^{-1} . However, at concentrations above 400 mgL^{-1} , these compounds could impart harsh, solvent, chemicallike aromas detrimental to wine aroma [19]. The influence of MLF on concentrations of higher alcohols appears to be inconclusive. A number of studies reported no change [20], or an insignificant increase [21] in the concentrations of 1-propanol, isobutanol, isoamyl alcohol and 2-phenylethanol. Maicas et al. [22] observed the

production of isobutanol, 1-propanol, 1-butanol and isoamyl alcohol to be dependent on the strain used to perform MLF. It found significant increases in the concentrations of isoamyl alcohol [23], isobutanol and 2phenylethanol [24]. The highest peak contribution samples contributed 2, and 3-methyl-l butanol (fruit and onion), and phenethyl alcohol (rose) in particular with yeast strain Rhöne2323 and DAP 0.5 gL⁻¹. This probably was synthesized by different yeast strains and amino acid concentration during the alcoholic fermentation [25].

The metabolism of organic acids during fermentation can have a significant impact on the flavour of wine. The samples received acetic acid (pungent, vinegar) fermenting in particular with yeast strain Rhöne2323, and found significant in addition with Rhöne2323 and DAP 0.5 gL⁻¹. Whilst octanoic acid is a volatile fatty acid, it has the ability to add complexity when present in lower quantities and be detrimental to wine quality when present at higher concentrations, as they impart unpleasant odours of rancid, pungent, cheese, sweaty and fat-like aromas [4]. A positive contribution to the wine aroma profile can develop when volatile compounds such as esters, ketones and aldehydes are derived from these fatty acids [26]. It found only slightly in the fermenting with Rh \ddot{o} ne2323 and DAP 0.5 gL⁻¹.

Moreover, the other compound significant such as 2,3butanediol (fruity and buttery notes), eicosane (waxy), pal-mitaldehyde (cardboard), hexahydro-farnesyl acetone (herbal, jasmine, cerely, woody), butylated hydroxyanisole (BHA), and butylated-hydroxytoluene (BHT). The two latters are defined as natural antioxidant substances. Currently, there is a growing interest in using natural antibacterial compounds, as these possess a characteristic flavour, the preservation of foods, antioxidant activity as well as antimicrobial activity [27].



Figure 1. Example of GC-MS-chromatograms of volatile aroma compounds from different mao wines fermentation.

TABLE I. % PEAK AREA CONTRIBUTION1 RELATED TO AROMA DESCRIPTORS OF DIFFERENT MAO WINES FERMENTA	ATION
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			RhÖr	ne2323	GHM	
Compounds	CAS no.	Molecu lar	DAP 0.3 gL ⁻	DAP 0.5 gL ⁻	DAP 0.5 gL ⁻¹	Aroma descriptors [17]
esters			45.77	41.93	24.88	
2-methylbutylethanoate	000624-	$C_7H_{14}O_2$	5.29	8.5	nd	sweet, fruity, banana [19]
2-phenylethyl acetate	000103-	$C_{10}H_{12}O$	0.16	0.39	nd	floral, rose sweet, honey, fruity, tropical
2-propenyl acetate	000591-	C11	nd	0.04	nd	green, galbanum, fruity, pineapple
butyl butyrate	000109-	$C_9H_{18}O_2$	nd	nd	0.17	mild amber, balsam, fruity
dimethyl heptyladipate	001330-	$C_{22}H_{42}O$	nd	0.45	2.03	amber liquid, mild
diethyl phthalate	000084-	$C_{12}H_{14}O$	3.8	3.67	0.12	odorless
dibutyl phthalate	000084-	$C_{16}H_{22}O$	1.05	1.65	nd	-
diisooctyl phthalate	027554-	$C_{24}H_{38}O$	nd	nd	0.7	oily
diisobutyl phthalate	000084-	$C_{16}H_{22}O$	0.67	0.34	0.89	faint odor
diethyl butanedioate	000123-	$C_6H_{10}O_4\\$	nd	0.06	0.42	apple, apricot, chocolate, grape, floral, fruity, peach,
	25-1					pear, waxy, wine, earthy
ethyl hexanoate	000123-	$C_8H_{16}O_2$	1.4	2.63	1.31	sweet, fruity, pineapple, waxy green, banana
ethyl octanoate	000106-	$C_{10}H_{20}O$	5.91	11.88	7.74	waxy, sweet, musty, pineapple and fruity with a
	32-1	2				creamy, dairy nuance
ethlylbutanoate	000105-	C6 H12	0.38	0.26	0.31	fruity, pineapple [19]
ethyl decanoate	000110-	$C_{12}H_{24}O$	5.87	7.17	5.95	floral, soap [19]
ethyl 9-decanoate	067233-	$C_{10}H_{18}O$	nd	0.15	nd	waxy, green, fatty, soapy, cheese type nuance

			RhÖne2323		GHM		
Compounds	CAS no.	Molecu lar	DAP 0.3 gL ⁻	DAP 0.5 gL ⁻	DAP 0.5 gL ⁻¹	Aroma descriptors [17]	
ethyl tetradecanoate	000628-	C18H36O	18.32	nd	nd	waxy, fruity, creamy and milky with a balsamic	
ethyl nonanoate	000123-	$C_{11}H_{22}O$	nd	0.5	nd	fat, waxy, cheese, coconut, fruity, rose, rum, wine	
ethyl isobutyrate	000097-	C_6H_{12}	nd	0.47	nd	strawberry [28]	
ethyl cyclohexanepropionate	10094-36-	$C_{11}H_{20}O$	nd	0.13	nd	powerful fruity, sweet, pineapple peach, pear	
ethyl 2-phenylethanoate	000101-	$C_{10}H_{12}O$	nd	nd	0.11	sweet, floral honey rose balsam cocoa	
ethyl isobutyrate	000097-	$C_4H_8O_2$	nd	nd	0.14	sweet, etherial and fruity with pungent, alcoholic, fusel,	
ethyl hexadecanoate	000628-	$C_{18}H_{36}$	0.14	nd	nd	green apple [29]	
hexyl formate	000629-	C_7H_{14}	nd	2.2	nd	apple, unripe plum, banana	
isopentyl decanoate	002306-	$C_{15}H_{30}O$	nd	0.19	nd	waxy, banana, fruity, sweet cognac, green	
isoamyl acetate	000123-	$C_7H_{14}O_2$	1.29	nd	1.34	sweet, fruity, banana solvent	
Isoamyl octanoate	002035-	$C_{13}H_{26}O$	0.21	0.78	0.25	sweet, fruity, waxy, pineapple, fruity, greeny,	
phenyl formate	000103-	C ₉	nd	nd	3.4	rose, green, hyacinth, herbal, watercress	
terpenes			3.35	6.2	4.74		
β -myrcene	000123-	$C_{10}H_{16}$	nd	nd	0.33	peppery, terpene, spicy, balsam	
β -linalool	000078-	$C_{10}H_{18}O$	0.16	0.2	0.2	citrus, floral, sweet, rosewood, woody, green, blueberry	
farnesyl alcohol	004602-	$C_{15}H_{26}O$	0.09	nd	nd	mild, fresh, sweet, linden floral	
limonene	000138-	$C_{10}H_{16}$	3.10	6.0	4.21	fresh, sweet, orange, citrus	
alcohols			14.08	23.93	16.98		
amyl alcohol	999064-	$C_5H_{12}O$	nd	3.29	nd	roasted wine, onion, fruity	
isoamyl alcohol	000123-	$C_5H_{12}O$	11.87	17.08	13.02	fusel, alcoholic, pungent, etherial, cognac, fruity,	
hexyl alcohol	000111-	$C_6H_{14}O$	0.27	nd	0.21	pungent, etherial, fusel, fruity, alcoholic, sweet and	
phenethyl alcohol	000060-	$C_8H_{10}O$	nd	3.56	3.75	floral, rose, dried rose, flower rose, water	
palmityl alcohol	036653- 82-4	$C_{16}H_{34}O$	1.94	nd	nd	waxy, floral	
acids			1.40	0.90	12.86		
5-t-butyloxycarbonylamino-	003789-	-	nd	nd	12.77	-	
salicyclic acid	85-3						
acetic acid	000064-	$C_3H_6O_2$	1.4	0.54	0.09	pungent, sour, vinegar	
octanoic acid	000124-	$C_8H_{16}O_2$	nd	0.36	nd	acidic, fruity, soapy, sour	
miscellaneous			35.4	27.04	40.54		
1-eonadecane	018435-	$C_{19}H_{38}$	0.98	0.14	-	-	
1-dodecane	000112-	$C_{12}H_{24}$	nd	nd	5.81	-	
2,3-butanediol	000513-	$C_{4}H_{10}$	nd	1.18	0.16	fruity, creamy, buttery	
2,6-di(t-butyl)-4-hydroxy-4-	000000-	$C_{15}H_{24}O$	nd	0.30	nd	rancid, buttery	
methyl-2,5-cyclohexadiene-	00-0	2					
butylated hydroxylanisol	025013-	C ₁₁ H ₁₆ O	0.28	nd	nd	mild rubbery	
butylated hydroxytoluene	000128-	C ₁₅ H ₂₄ O	13.64	3.47	4.24	mild phenolic, camphor	
cyclotetradecane	000295-	C ₁₄ H ₂₈	0.11	0.08	nd	-	
eicosane	000112-	C ₂₀ H ₄₂	16.19	19.25	27.53	waxy	
nonadecane	000629-	C ₁₉ H ₄₀	0.24	nd	nd	bland	
octadecane	000593-	C ₁₈ H ₃₈	0.44	0.96	nd	-	
tetradecane	000638-	$C_{14}H_{30}$	0.33	nd	nd	mild waxy	
hexahydrofarnesyl acetone	000502-	C ₁₈ H ₃₆	1.86	0.87	0.64	oily, herbal, jasmine, celery, woody	
heneicosane	000629-	C ₂₁ H ₄₄	nd	0.09	nd	waxy	
palmitaldehyde	000629-	C ₁₆ H ₃₂ O	1.33	0.70	1.03	cardboard	
tetradecanal	000124-	$C_{14} H_{28}$	nd	nd	1.13	fatty, waxy, dairy, creamy and fishy with a fruity, pear	

 $^{1}(100 \text{ x curve area of each compound of the sample})/total curve area of the sample nd = not detected$



B. Intensive of Aroma Attributes by Judges and Relevant for Chromatograms

Figure 2. Spider graphical presentation of 29 descriptors selected for the quantitative descriptive sensory profiling analysis of the three different mao wines, with addition of the yeast strain Rh \ddot{c} and DAP 0.3 gL⁻¹(---), Rh \ddot{c} and DAP 0.5 gL⁻¹(---), GHM and DAP 0.5 gL⁻¹(----), (average of 10 tasters).

It has been demonstrated that the profile of aroma characters of wine have been used to identify different wine products [19]. Recent work of Zhang *et al.* [29] investigated volatile composition and sensory properties of Merlot wines from different districts in China and found fifty-seven aroma compounds with thirty active compounds. Its main flavour is that of some tropical and temperate fruits, along with a lactic flavour from the malolactic fermentation. As highlighted in the work of Aznar *et al.* [30], overall pleasant descriptions are positively correlated with the chemicals with a pleasant aroma, but unpleasant compounds have a much more destructive effect on global aroma quality.

The odoriferous samples were intensified as shown in Fig. 2. The winemaking techniques enhanced aroma characteristics of mao wines. They received twenty-nine attributes representing a broad range of aroma characteristics. The descriptors were discriminated into six main groups; fruit, floral, vegetable, spice, sugar, and trait.

Fruit notes with raisin, pineapple, strawberry, orange peel, mao fruit, prune, tamarind, passion fruit, banana, blueberry and apple, linked to mostly acetate esters, fatty acid and acid esters, higher alcohol, and terpenes as shown in Table I.

Vegetative notes with green grass, bell peper, mint, and dried grass, related to isoamyl octanoate, ethyl hexoate, ethyl isobutyrate, ethyl 9-decanoate, and β -linalool.

Spicy notes with pepper, clove, cinnamon, vanilla, oak and coffee, associated with 2-phenethyl acetate, β -myrcene, butylated hydroxytoluene (BHT), and hexahydrofarnesyl acetone.

Caramel notes with caramel, and honey, linked to ethyl 2-phenyl ethanoate, 2 and 3-methyl-l butanol.

Floral notes with rose, and dried rosella, enhanced ethyl nonanoate, diisobutylphthalate, ethyl 2-phenylethanoate, butyl butyrate, 2-phenethyl acetate as well as β -myrcene, β -linalool, phenethyl alcohol, farnesyl alcohol, 1-hexadecanol, and hexahydrofarnesyl acetone.

The trait notes with pickled, musty, vinegar and sulphur, linked to ethyl nonanoate, ethyl tetradecanoate, diisobutylphthalate, ethyl 9-decanoate, ethyl isobutyl-rate, 1-hexanol, octanoic acid, 2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexa-diene-1-one,butylated

hydroxylanisol (BHA), 2,3-butanediol, and palmitaldehyde.

The notable descriptors represent high intensive aroma ranking from mao fruit (6.66-7.06) ripe tamarind (6.10-6.97), rosella (6.41-6.96), prune (6.10-6.76), orange (5.19-5.41), bell pepper (5.11-5.78) and honey (5.0-5.78), respectively. The wine sample with addition of yeast strain GHM received the highest intensity scores in most attributes, while yeast strain Rhöne2323 and DAP 0.3 gL⁻¹ was in last position.

IV. CONCLUSION

The different mao wine making techniques were determined in terms of their impact on the aroma profile and its intensity. The differences in gas chromatogram and QDA intensity were investigated systematically. Fifty-four aroma components were identified in mao wine, twenty-nine descriptors were detectable, and high intensive score of mao fruit, ripe tamarind, dried rosella, prune, orange, bell pepper and honey. Furthermore, some aroma compounds such as BHA and BHT would be beneficial in terms of the antioxidant effect. These results would support wine makers in improving the pleasant aroma in mao wine production. Further study would find out the accurate concentration of these aroma compounds affecting each descriptor.

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