Chemical and Chromatic Properties of Mao-Berry Fruit as a Key Parameter for Beverage Industry

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Abstract—The main physico-chemical composition of four mao cultivars (cv.) (Antidesma thwaitesanum Müell.: Fahpra-tann, Sann-home, Kham-ta, Heuv-bang) were investigated. The cv. Fah-pra-tann and Kham-ta had the highest sugar content (193.58, and 190.90 g/L), with the lowest acidity (6.55, and 6.76 g/L). The sugar acid ratio represent the balance taste ranging from 13.72 to 30.02. The cv. Fah-pra-tann and Kham-ta were in appropriate range of sugar x pH² (238, and 226). The main acid was citric (5.02-10.98 g/L), and tartaric (3.36-5.51 g/L) acids. They were rich in phenolic (349.18-845.97 µgGAE/ml), anthocyanin (583.07-2361.42 mg/l), and antioxidant activity (290.71-1213.80 µgQEAC/ml), being inversely proportional to colour intensity (6.16-8.39), and were highest in the cv. Kham-ta. They had similar contribution to the colouration between yellow and red pigments, and correlated with the results for the tint and anthocyanin of the juices. It indicates that mao had desirable characteristics to produce beverage products.

Index Terms—mao cultivars, acids, anthocyanin, antioxidant activity, colour intensity

I. INTRODUCTION

Ma-mao or mao (*Antidesma* sp.) of the Stilaginaceae family, is an indigenous fruit berry, 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].

Accordingly, mao-indigenous fruit berry has been booming in Thailand which are higher valuable in each year. It is produced for beverage, juice extract, dessert, jam, and wine. The consumers accept them as the healthy products which are addressed for high content of phenolic compounds depends upon the cultivars. The best mao quality can occur only through the achievement of optimal composition balance such as fruit sugar, acidity and secondary metabolites content, and anthocyanin composition, which effect from variety, ripening, and orchard management techniques. These compositions are considered as a key parameter in quality controlling and leading to develop the interest products. Mao is very well known in northern east of Thailand. The cultivar Fah-pratann, Kham-ta, Srang-kro, Heuy-bang, and Sann-home are suitable to produce beverage and wine.

With the trend interest in healthy drinks from red berries which are addressed for high content of phenolic

compounds [2]. Mao juice has stimulated the researchers to investigate the nutrients and chemical composition. Up to date there are only a few papers to report about these compositions. The objectives of this study were to determine the physico-chemical composition and antioxidant capacity of four different mao cultivars. The mao products industries would benefit from improving the product quality in terms of composition balance as well as colour and antioxidant characteristics. This would significantly contribute to the knowledge currently available.

II. MATERIALS AND METHODS

A. Mao Juice Preparation

Four dark-red colour and ripened of ma-mao or mao (*Antidesma thwaitesanum* Müell.) Fah-pra-tann, Sannhome, Kham-ta, and Heuy-bang cultivars (Fig. 1) were harvested from Phu-pann district, Sakolnakorn province, Thailand. The fruits were removed from the cluster, and extracted by using a hydraulic press. The juices were frozen and stored at -20 $^{\circ}$ C, and defrosted before chemical analyses.



Figure 1. The appearance of different mao cultivars.

B. Analytical Methods

Four cultivars of mao juice were analysed for pH value, titratable acidity by titration, and total soluble solids by hand refractometer [3].

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Organic acid and sugar were determined by using high performance liquid chromatograph (HPLC; Agilent 1200series). An isocratic HPLC system (1.0 ml/min flow rate) was set up with column thermostat. Ten μ l of sample was injected into the column. Sugar (D-glucose, D-fructose, and sucrose) were separated by Zorbax carbohydrate and Refractive index detector. The mobile phase ratio was 70:30 (ACN:H₂O), 30°C on 4.6 mm x 150 mm, 5 μ m. The sample was diluted by a factor of 10 with a ratio 1:1 (ACN:H₂O). Organic acids were separated by Zorbax SB-Aq column and diode array detector at 220 nm, 35 °C on 4.6 mm x 150 mm, 5 μ m. The mobile phase was 99% 20 mM NaH₂PO₄ at pH 2 and 1% acetonitrile [4].

Total anthocyanin was examined by pH-differential method and expressed as cyanidin, 3-glycoside [5]. Total phenolic content was examined by the Folin-Ciocalteu reagent, using gallic acid as standard. The results were expressed as μ g of gallic acid equivalents (GAE)⁻¹ [6]. The antioxidant activity was examined by the 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS⁺, radical cation) decolourisation method [7]. Quercetin solutions were used to obtain the calibration curve and calculate the antioxidant activity of juice as Quercetin-Equivalent Antioxidant Capacity (QEAC) [8], [9].

Colour Intensity (CI), Tint (T) were determined using the spectrophotometric absorbance (A) of the grape juices at 420 and 520 nm. CI was determined by using the equations of $CI = A_{520}+A_{420}$, and $T = A_{420}/A_{520}$ [3].

All parameters were examined with three replications. The statistical analysis was analysed by the analysis of variance [10], [11].

III. RESULT AND DISCUSSION

A. Physico-Chemical Components of Mao Cultivars



Figure 2. Peak chromatogram of sugar and organic acids of different Mao cultivars.

Sugar is a very good predictor of consumer acceptability. The more sugar (Brix level), the more people will like the fruit in question [12]. From the results presented in Fig. 2, Table I and Table II, it is obvious that the Fah-pra-tann and Kham-ta cultivars had the highest total soluble solids and sugar content in the level of 19.33 ^oBrix, and 193.58 and 190.90 g/L. respectively. The higher the total solids, the more convenient and desirable is the fruit for production of beverage, and sugar foods products. Generally, the highest share in total solids of fruit is contributed by carbohydrates, i.e. sugars [13]. Sugar of both varieties was predominated in the form of glucose 98.40 and 95.70 g/L, and fructose 95.18 and 95.20 g/L, respectively. In another study on strawberries, glucose was found to be the predominant sugar [14], [15]. Its concentration increased with ripeness.

Sugars, together with organic acids, play an important role in the sensory characteristics of fruit. The Brix-totitratable acidity ratio is an even better predictor to determine the balance between sweet and sour which relates to feel of a flavor [12]. In the study, mao juice is highly acidic, initially containing 6.55-9.48 g/L total acidity (as citric acid) and a pH of 2.81-3.48. The dominant acids were 5.02-10.98 g/L citric acid, and 3.36-5.51 g/L tartaric acid. The results showed that the cv. Fah-pra-tann, and Kham-ta had the less acidity and organic acid contents. These parameters were in the range of the previous study [16] reported the cv. Fah-pra-tann harvested in 2001 containing 157.55 g/L total sugar, 10.5 g/L total acidity and a pH of 3.3. The organic acids were 8.24 g/L citric acid, and 3.08 g/L tartaric acid. It is in the same range as strawberries and cherries, accumulate organic acids namely citric and malic acids [17]-[19]. In the study, the cv. Fah-pra-tann and Kham-ta had higher sugar of 190.90-193.58 g/L, concurrented with lower acidity of 6.55-6.76 g/L.

Referring juice industries, orange and grape juices are popular fruit drinks around the world. Thus the datas on orange and grape juice qualities were references in the fundamental juice industry. To ensure the consumer received an end product, fresh juice, that meets a continuous standard, taste and quality. The Brix/total acidity represents the balance between the acid and sweet taste of juice. The industry standards for fruits can vary in different states for example in some states of Australia are 9 to 1 for navel oranges, 8 to 1 for Mandarins, 5.5 to 1 for grapefruit and pummelos [20], [21], and 15 to 45 for grape juice [22]. The result showed that the ripeness of mao varieties were at level of 13.72-30.02. The cv. Fahpra-tann and Kham-ta with appropriate total sugar content may be considered the most appropriate for juice industry production. The high Brix/total acidity values obtained may be due to the low acidity of the juice.

In terms of mao wine production, the combination of sugar x pH^2 is a better indicator of optimum ripeness than sugar-to-acid ratio, due to the pH of the juice is probably the most important factor to determine microbial and chemical stability of wine. With this measurement, the best wines are made at index values ranging from 200 to

270 [23]. In the study, it was indicated that the cv. Fahpra-tann and Kham-ta had 238 and 226 within an optimum range of sugar x pH^2 for the alcoholic fermentation base production. This index would assist to control the optimum ripeness not overripe or green fruits, and gain the eventual quality of the wine.

Characteristics	Fah-pra-tann	Sann-home	Kham-ta	Heuy-bang
Glucose (g/l)	98.40±3.30 ^a	60.46±0.34°	95.70±0.71ª	85.52±2.3 ^b
Fructose (g/l)	95.18±2.90 ^a	61.73 ±0.47°	95.20±1.93 ^a	87.63 ±0.62 ^b
Total sugar (g/l)	193.58±6.19 ^a	122.19±1.18 ^c	190.90±4.24 ^a	173.15±0.95 ^b
Total soluble solids (°Brix)	19.33 ±0.58 ^a	13.0±0.00°	19.33 ±0.58 ^a	17.67 ±0.58 ^b
pH	3.48±0.01 ^a	2.81 ± 0.01^{d}	3.42±0.01 ^b	3.21±0.01 ^c
Acidity ¹ (g/l)	6.55 ± 0.07^{d}	9.48±0.04 ^a	$6.76 \pm 0.02^{\circ}$	8.44±0.01 ^b
Sugar acid ratio	30.02±0.63ª	28.66±0.81 ^b	20.92 ±0.68°	13.72 ±0.05 ^d
Sugar x pH ²	238.17±7.13ª	109.89 ± 0.42^{d}	226.56±6.41 ^b	181.68±6.58°

TABLE I. CHEMICAL COMPOSITION OF DIFFERENT MAO CULTIVARS

MEAN ±SD (N=3), (P<0.01).

DIFFERENT SUPERSCRIPT LETTERS WITH IN THE SAME ROW REPRESENT SIGNIFICANT DIFFERENCE AMONG CULTIVARS.

¹ COMPARE TO CITRIC ACID

TABLE II.	ORGANIC ACIDS OF DIFFERENT MAO CULTIVARS
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Characteristics (g/l)	Fah-pra-tann	Sann-home	Kham-ta	Heuy-bang
Citric acid	$6.44 \pm 0.05^{\circ}$	10.98±0.04 ^a	5.02 ± 0.03^{d}	8.23±0.09 ^b
Tartaric acid	5.29 ± 0.08^{b}	4.57±0.06°	5.51±0.02ª	3.36±0.01 ^d
Malic acid	1.29 ± 0.10^{a}	0.98 ± 0.02^{b}	0.62±0.04°	0.26 ± 0.04^d
Oxalic acid	0.34±0.00°	0.18 ± 0.00^{d}	0.43±0.01ª	0.37±0.01 ^b
Succinic acid	0.10±0.04 ^{ab}	0.11±0.01 ^a	0.06 ± 0.02^{b}	0.13±0.02 ^a

MEAN±SD (N=3), (P<0.01).

B. Phenolic Compounds, Anthocyanin, and Antioxidant Activity of Mao Cultivars

Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables [24], [25]. Phenolic compounds influence on astringency, bitterness and colour for food quality [26]. Among the fruit components, anthocyanins were the most effective in scavenging reactive oxygen species, inhibiting lipoprotein oxidation [27], and have attributed cancerpreventive properties to antioxidant effects [28], [29]. Anthocyanin as pigment, it is almost exclusively responsible for the red, blue and purple colours in fruits, and red berries which are addressed for high content of anthocyanin fruit source [30].

This statistical difference between the mao cultivars is shown in Table III. It was indicated that different cultivars different phenolic and anthocyanin contents, and antioxidant activity were remarked and wide range values. It contained high level of total phenolic compounds at 349.18-845.97 μ gGAE/ml (as gallic acid, R \geq 0.9984), anthocyanin at 583.07-2,361.42 mg/l, and antioxidant activities at 290.71-1,213.80 µgQEAC/gfw (fresh weight). The cv. Kham-ta had the highest content, followed by Fah-pra-taan, Saan-home, and Heuy-bang.

The total phenolic compounds in the fresh mao was less than in another fruits, for example, different source of grape juices (1117.10-3433.04 mgGAE/l) [22], different localities of wild bilberry (3.92-5.24 mgGAE/gfw [31], seven different locations of bilberries (1027-1629 mg/100gfw) [32], blackberries (486.53 mg/100gberry) [33], and 364.24 mgGAE/100gfw [34].

Whereas anthocyanin of mao juices was in the same range as bilberry (1210.3 mgCGE (cyanidin-3-O-glucoside equivalents)/100gfw), and blackberries (116.59 mg/100g) [33], and 121.82 mgCGE/100gfw [34], but was higher than grape juices (25.56-460 mg/L, and 28.7 mg/L) reported by Burin *et al.* [22], and Malacrida and Motta [35], respectively, sour cheery (600 mg/kg) [36], and purple wheat (491.3 mg/kg) [37]. Several factors could be responsible for differences in total phenolic content of foodstuffs of the same or similar origin. They include variation in fruit cultivars, harvest and post-harvest handling and storage conditions, and processing techniques [22].

Characteristics	Fah-pra-tann	Sann-home	Kham-ta	Heuy-bang
Total phenolic compounds (µgGAE ¹ /ml)	521.81±6.92°	349.18±2.94 ^d	845.97±3.12 ^a	663.45±8.22 ^b
Anthocyanin (mg/l)	983.13±0.77°	583.07±2.41 ^d	2361.42±2.93ª	1068.16±6.47 ^b
Antioxidant activity (µgQEAC ² /ml)	707.82 <u>+</u> 5.44°	290.71 ± 1.81^{d}	1213.80 <u>+</u> 10.88 ^a	849.28 <u>+</u> 1.81 ^b

TABLE III. TOTAL PHENOLIC, ANTHOCYANIN, AND ANTIOXIDANT CAPACITY OF DIFFERENT MAO CULTIVARS

MEAN±SD (N=3), (P<0.01).

TABLE IV. CHROMATIC PROPERTIES OF DIFFERENT MAO JUICE CULTIVA

Characteristics	Fah-pra-tann	Sann-home	Kham-ta	Heuy-bang
A ₄₂₀	3.08 ± 0.002^{d}	3.97±0.003 ^b	4.74±0.001 ^a	3.76±0.002°
A ₅₂₀	3.08±0.001°	3.10±0.002 ^b	3.6±0.006ª	3.10±0.001 ^b
Colour Intensity (CI)	6.16±0.00 °	6.85 ± 0.00 b	8.39±0.00 ^a	7.07 ±0.00 ^b
Tint ²	0.99 ± 0.00^{b}	1.22±0.00 ^a	1.30±0.00 ^a	1.28±0.00 ^a

MEAN±SD (N=3), (P<0.01).

DIFFERENT SUPERSCRIPT LETTERS WITH IN THE SAME COLUMN REPRESENT SIGNIFICANT DIFFERENCE AMONG CULTIVARS.

C. Chromatic Properties of Mao Cultivars

Colour of red berry is the most important attribute used, along with other variables, as an indicator of the quality of juice. This characteristic is directly dependent on the phenolic composition of the juice and the anthocyanins present in the fruit skin; for example, reported on the grape [38].



Figure 3. Representation of yellow and red colour pigments in different mao cultivars.

The results for the colour characterization of the mao juices are represented in Fig. 3 and Table IV. The mao juice samples showed a significant difference regarding CI. The CI represents the amount of colour present in the mao juices with a range from 6.16 to 8.39. Most mao juice samples showed slightly higher percentages of yellow pigment than red pigment, with contribution to the colouration ranged from 50% to 56.83%, and 43% to 50%, respectively. These datas are correlated with the results for the Tint ranging from 0.99 to 1.30 of the juice samples. The cv. Fah-pra-tann was red shade (less than 1.0), and the other was brownish shade (more than 1). These were in the same range of different localities of grape juices of B ôrdo grapes (*Vitis labrusca*) ranged from 5.37 to 21.12 for CI, and 0.57 to 1.04 for Tint (Burin *et al.*, 2010). A strong correlation was observed between the antioxidant activity, phenol and anthocyanin contents, and colour intensity in all mao cultivars.

Most samples had the brownish shade because mao juices were fresh extracted with no preservative treatment. Phenolic compounds exposed to oxidative reactions, the colour modification from red-violet to the red-brick was observed. As well as the changing of red wine to browning wine involved the oxidative reactions of phenolic compounds with the elaboration of some xantiliu derivatives [39]. The yellow pigment participates with approximate in the same measure as red pigment at total colour of red pigment; in these cases the mao juice colour was perceptible as intensive red.

IV. CONCLUSION

This study is likely to represent the first data on the sugar and organic acid of different cultivars mao juices. Generally, the mao juice was identified as citrus and acidic fruits. The cv. Fah-pra-tann and Kham-ta were observed that they had higher sweetness, but lower acidity other than the other cultivars, with a reasonable range of sugar acid balance, and sugar x pH². The content of phenol, anthocyanin, and antioxidant activity differed significantly among the cultivars, values similar to those reported for berry fruits. A strong correlation was observed between the phenol and anthocyanin contents, antioxidant activity, and colour intensity among cultivars, and higher in the cv. Fah-pra-tann and Kham-ta. It indicates that mao juice is a source of healthy products and desirable composition to produce some drinks, wine and related products.

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