

Development of fruit tea from Mao (*Antidesma* sp.) seeds; by-products from Mao juice and wine processing

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ABSTRACT: This study aimed to value added of by-products from Mao juice and wine production. Mao seeds after pulp separation by machines were dried at 5 different temperatures (50, 60, 70, 80 and 90°C) in hot air oven for 8 hours. After being dried, the 5 grams of Mao seeds were packed in a tea sachet paper. Tea solution from Mao seeds was obtained by soaking the tea bag in 170 ml of hot water at 90°C (as common ratio for one cup of tea) for 2 minutes. The results showed that the highest of DPPH^o inhibition (20.17%) was obtained when drying the Mao seeds at the temperature of 50°C as well as those of anthocyanin ($0.32 \pm 0.008\text{mg}/100\text{ml}$) with significantly difference ($p \leq 0.05$) from those of other temperature samples. However, Mao seeds dried at the temperature of 90°C resulted in with the highest red value (a^*) ($p \leq 0.05$) and the highest acceptability score (appearance, color, odor, taste and overall acceptability). To develop fruit tea from Mao seeds considering both consumer's perception (dried at 90°C) and antioxidant properties (dried at 50°C), the ratio of Mao seeds of those drying temperatures was investigated. It was found that the best ratio of dried Mao seeds (90°C to 50°C) was 75%:25% (w/w) with 14.25% DPPH^o inhibition. The antioxidant activity (DPPH^o inhibition) of this developed tea was similar to antioxidant activity of commercial green tea and Oolong tea available in the markets.

Keywords: *Antidesma* sp., Mao seeds, By-products, Fruit tea

Introduction

Mao (*Antidesma* sp.) is one of local fruits in the Northern and the Northeast of Thailand, especially in Phupan Mountain, Sakon Nakhon. Mao is a perennial plant with approximately 6-12 meters in height. The leaves have lanceolate shape. Its infructescence is look like that of pepper. The harvesting period of Mao is usually between Augusts to October. It becomes red and black when it is ripe. It has sweet and sour taste, and astringent taste. Local people are usually cooked as sour and spicy salad calls "Tum Mak Mao". Recently, Mao is processed to various products such as Mao juice, Mao jam, and Mao wine because of its unique taste and full of phytochemicals and antioxidant capacities. (Jorjong et al., 2015).

Mao has properties in preventing diseases. Steenkamp et al. (2009) found that Mao extract inhibited Hela cancer cell which the inhibition of proliferation (IC_{50}) was 25.4 $\mu\text{g}/\text{ml}$. Puangpronpitag et al. (2011) reported that Mao seeds and marcs had a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay with IC_{50} of 0.85-1.21 $\mu\text{g}/\text{ml}$ and DPPH and ABTS assays were 3.74 and 3.80 $\mu\text{g}/\text{ml}$ trolox equivalent/g, respectively. In addition, Mao extract contained 97.32-130.00 mg GAE/g of polyphenols and proanthocyanidins which can prevent lipid peroxidation as grape seed extract had. Kukongviriyapan et al., (2105) reported that hypertension and oxidative stress in nitric oxide deficient rats could be reduced by Mao pomace

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extract. El-Tantawy et al. (2015) reported that rats supplemented with daily dose Moa extract (250 mg/kg body weight) could significantly reduce in blood glucose level along with an increase in serum insulin. It was suggested that Mao could be used as a dietary supplement against hypertension.

At presently, Mao is processed to various types of products such as Mao juice, Mao jam, and Mao wine. Those products are highly accepted from the consumers. In addition, Mao is approved as a geographical indication (GI) plant as well as a 5-star OTOP products of Sakon Nakhon. There are more than 10 processing plants of Mao products in Sakon Nakhon. Normally, Mao processing can produce about 70% of product from raw material that mean by-products or waste are left for about 30% (primarily data from in-house pilot factory). Although Mao had been developed into many products but by-products such as seeds and marcs are lack of utilization. It was found that there are a lot of waste (Mao seeds) after processing process which can be calculated as 30 percent (primarily data from in-house pilot factory). Therefore, this study aimed to development of fruit tea products from Mao seeds to increase the value of by-products from Mao processing plant.

Material and methods

Materials

The Mao seeds in this study was collected from Mao processing plant at pilot of Food science and technology department, Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon campus (RMUTI), on September-November, 2013. The

Mao seeds were the waste of Mao juice producing after juice press and pomace separator machine (OFM, Bangkok, Thailand) and were kept at -20°C before being processed to the fruit tea (not more than 2 months).

Tea preparation

Firstly, frozen Mao seed sample (flat ovals shape with 0.4-0.7 cm length) was thawed in room temperature for 2 hours. Then, seeds were dried at five different drying temperatures (50, 60, 70, 80 and 90°C) in hot air oven (Model 800, Memmert, USA) for 8 hours. After cooling down (about 1 hour), Mao seeds were measured activity value (a_w) by using Water Activity Meter (Aqua Lab, Model 4TE, Decagon Devices, Inc. Pullman, USA). Consequently, 5 grams of processed Mao seeds was packed in a tea sachet (50×50 cm. pulp paper, Cheevarom, Thailand). Secondly, the fruit tea from Mao seeds was prepared for later investigation by soaking a tea sachet in 170 ml of hot water (90°C) for 2 minutes.

Development of Mao tea for commercial purposes

To develop the commercial Mao tea, the two drying temperatures with the highest DPPH° free radical scavenging assay (A) and the high sensory evaluation score (B) were mixed together in percentage ratio of A and B (100:0, 75: 25, 50:50, 25:75 and 0:100, respectively). DPPH° inhibition, colors and sensory evaluation of each ratio were investigated for the optimum formula.

Colors and pH

The fruit tea samples were evaluated in CIELAB color system (L^* , a^* and b^*) using Hunter Lab (Color Flex, USA) and pH value was evaluated using pH meter (Horiba F-12, Japan).

DPPH° free radical scavenging assay

DPPH° free radical scavenging assay was measured according to the method of Butsat and Siriamorpun (2010) with some modification. Briefly, 0.1 ml tea solution from Mao seeds dried at different temperatures was reacted with 1.9 ml of

2-diphenyl-2-picrylhydrazyl (DPPH) (0.1 mg/ml) and kept in a dark room for 30 minutes for completed interaction. After that, the obtained solution was measured absorbance at 517 nm. The percentage of DPPH° inhibition activity was calculated by using the following formula.

$$\% \text{ DPPH}^\circ \text{ inhibition activity} = [(A_o - A_e)/A_o] \times 100 \text{ (1)}$$

A_o = Absorbance of blank without the sample tea, A_e = Absorbance of the sample tea

Anthocyanin Measurement

Anthocyanin was measured by pH differential method (Shafazila, et al., 2010). Briefly, the obtained tea solution from Mao seeds dried at different temperatures (0.2 ml) were put into 3 ml of different buffer solutions as follows: 1) Buffer solutions with pH 1.0 which anthocyanin structure was in Oxonium form with colored objects. 2)

Buffer solutions with pH 4.5 which anthocyanin structure was in Hemiketal form and each pH buffer was kept in stirring for 15 minutes. The obtained solution was measured absorbance at 521 and 700 nm. Total anthocyanin (TA) in form of cyanidin-glucoside was calculated by the following equation.

$$A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}} \text{ (2)}$$

$$\text{TA content (mg/l)} = (A \times \text{Mw} \times \text{dilution factor} \times 1000) / (\epsilon)$$

$$(\text{Mw} = 449.2 \text{ g mol}, \epsilon = 26900 \text{ M cm}, \text{dilution factor} = 15)$$

Sensory evaluation

Tea samples were evaluated the acceptability using 9-point hedonic scale by 40 un-train panelists. The evaluation was conducted at sensory evaluation room at Food science and technology department, RMUIT.

Data Analysis

The experimental designs were done by using completely randomized design (CRD). Analysis of variance (ANOVA) and mean comparison with Duncan's new multiple range test (DMRT) was analyzed by using SPSS software version 12 (SPSS, Chicago, IL). The criterion for statistical significance was $p \leq 0.05$. The results

were reported as mean $\pm$ SD of 3 replications.

Results

Effect of different drying temperatures on color, pH, DPPH° and anthocyanin values

Dried Mao seeds at the different temperature had a low a_w at the range of 0.52-0.55 which was lower level to inhibit the growth of fungi and bacteria. Therefore, the obtained Mao tea can be kept in tea bag for long time and suitable for commercial aspects. The color of Mao tea solution after soaked in 170 ml of hot water was shown in

Table 1

Table 1 Effect of drying temperatures on Mao tea colors

Drying temperature (°C)	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> ^{* ns}
50	74.84 ± 2.47 ^b	1.99 ± 1.42 ^b	5.54 ± 0.37
60	76.21 ± 0.23 ^a	0.54 ± 0.29 ^b	5.37 ± 0.28
70	76.31 ± 0.24 ^a	0.69 ± 0.56 ^b	5.55 ± 0.42
80	75.55 ± 1.12 ^a	1.31 ± 0.76 ^b	5.01 ± 0.32
90	74.40 ± 0.96 ^b	5.20 ± 2.11 ^a	5.71 ± 0.94

Note: mean±SD (n=3) values with different lowercase letters in the column are significantly different ($p \leq 0.05$) and ^{ns} means no significantly different ($p > 0.05$)

Lightness value (*L*^{*}) has a range of 0-100 which means the lowest lightness to the highest lightness respectively. The results showed that *L*^{*} of Mao tea solution from Mao seed dried at 60, 70 and 80°C were not significantly different ($p > 0.05$) but significantly higher than *L*^{*} value of Mao tea from Mao seed dried at 50 and 90°C.

a^{*} shows redness value. The positive value is red color and negative value is green color. It was found that *a*^{*} of Mao tea at the temperature

of 50, 60, 70 and 80°C were not significantly different ($p > 0.05$). However, the *a*^{*} value of Mao tea prepared from Mao seed dried at 90°C was significantly higher ($p < 0.05$) at 5.20±2.11. For *b*^{*} value, the positive value is yellow and negative value is blue. All sample of Mao tea in this study were not significantly different ($p > 0.05$). This means drying temperature of Mao seeds did not affect the *b*^{*} value tea, prepared from Mao seeds with different drying temperatures.

Table 2 pH, DPPH^o scavenging activity and anthocyanin content of fruit tea from Mao seeds dried at different temperatures

Drying temperature (°C)	pH ^{ns}	DPPH ^o (% Inhibition)	Anthocyanin (mg/100ml)
50	3.31 ± 0.34	20.07±0.008 ^a	0.32±0.008 ^a
60	3.49 ± 0.33	20.05±0.005 ^a	0.28±0.008 ^a
70	3.27 ± 0.33	18.70±0.019 ^{ab}	0.25±0.03 ^{ab}
80	3.38 ± 0.33	16.39±0.007 ^{ab}	0.14±0.032 ^b
90	3.33 ± 0.34	13.29±0.012 ^b	0.10±0.041 ^c

Note: mean±SD (n=3) values with different lowercase letters in the column are significantly different ($p \leq 0.05$) and ^{ns} means no significant differences ($p > 0.05$)

pH of the dried Mao tea solution dried at the temperature of 50, 60, 70, 80 and 90°C was between the range of 3.31-3.49 which were not significantly different ($p > 0.05$). While the DPPH^o scavenging activity of dried Mao tea at the

temperature of 50 and 60°C significantly higher different from Mao tea dried at 70, 80 and 90°C. DPPH^o was decreased when the drying temperatures of Mao seeds was increased. The highest DPPH^o was 20.07% inhibition when the

temperature was at 50°C. This result was related to the amount of anthocyanin that was decreased with increasing drying temperature. The highest amount of anthocyanin was 0.32 mg/100 ml at 50°C. It could be explained that high temperature on food processing had an effect on degradation of anthocyanin (Odriozola-Serrano et al., 2010). Anthocyanins are water-soluble vacuolar pigments (a group of flavonoids) that could be found in tissues of plants, including leaves, roots, stems, fruits and flowers with high antioxidant activities. With these properties, anthocyanin could be used for medical purposes, uses for reducing oxidative stress for cardiovascular diseases (Puangprongpitag et al., 2008; Ferroni et al., 2006).

DPPH^o and anthocyanin observed in Mao tea solution was not from activities of polyphenolic

compounds inside Mao seeds because Mao seeds had hard endocarp which it had to be crushed and extraction process to obtain the polyphenolic compounds. Therefore, DPPH^o inhibition activity and anthocyanin in this study was activity of the remained Mao marcs around the seeds. Mao marcs had polyphenolic compounds and proanthocyanidins which had high antioxidant activities (Puangprongpitag et al., 2008) which remained around 20.69% in our preliminary study (data not shown).

Sensory evaluation

The results of sensory evaluation of Mao tea from different drying temperature by 40 untrained panels were shown in **Table 3**.

Table 3 Hedonic scores of Mao tea from different drying temperatures

Temperature (°C)	Appearance	Color	Odor	Taste ^{ns}	Overall liking
50	5.67 ± 1.71 ^b	5.03 ± 1.56 ^{bc}	6.10 ± 1.06 ^a	5.27 ± 1.72	5.80 ± 1.45 ^b
60	5.60 ± 1.59 ^b	5.50 ± 1.36 ^b	5.97 ± 1.54 ^a	5.47 ± 1.53	5.77 ± 1.25 ^b
70	5.23 ± 1.55 ^b	4.77 ± 1.70 ^c	5.23 ± 1.22 ^b	5.07 ± 1.68	5.50 ± 1.50 ^b
80	6.90 ± 0.71 ^a	7.43 ± 0.82 ^a	6.17 ± 1.18 ^a	5.93 ± 1.41	6.53 ± 1.14 ^a
90	7.23 ± 0.77 ^a	7.97 ± 0.76 ^a	6.47 ± 1.66 ^a	5.93 ± 2.15	6.63 ± 1.56 ^a

Note: mean±SD (n=3) values with different lowercase letters in the column are significantly different ($p \leq 0.05$) and ^{ns} means no significantly different ($p > 0.05$)

Drying temperature of Mao seeds affected the color liking of Mao tea. Mao tea from Mao seeds dried at 80 and 90°C showed higher significant differences in overall liking score than those of Mao tea with drying seeds at 50, 60 and 70°C. Mao tea of dried seeds at 90°C showed the highest score for appearance (7.23 ± 0.77), odor (6.47±1.66), and overall liking (6.63±1.56),

respectively. Moreover, Mao tea with red color were highly recommended based on sensory score paper by panelists (data not shown).

Taste score was not significantly different. This might be due to all samples had the similar sour taste or similar pH value. On the other hand, it might be due to the panelists were not familiar with these Mao tea taste that was not sweet as

other commercial tea that were sold in the markets.

The regression model of overall liking and other attributes (appearance, color, odor and

taste) was investigated. The color and taste score significantly affected to overall liking score as shown in **Table 4**.

Table 4 Regression model and ANOVA of appearance, color, odor and taste to overall liking

Model	Coefficients	Standardized Error	t	Sig.
Constant	3.364	0.373	9.012	0.000
Appearance	0.46	0.840	0.544	0.584
Color	0.287	0.076	0.544	0.000
Odor	0.092	0.071	-1.296	0.196
Taste	0.246	0.054	4.534	0.000

Appearance and odor had not affected to overall liking while color and taste showed significant ($p < 0.05$). The colors of Mao tea solution became red (a^*) if the drying temperature was high. The increasing of a^* value in this study that occurred by heating might due to Maillard reaction (Birlouez-Aragon et al., 2010; Van Lancker et al., 2011). Maillard reaction was non-enzymatic browning between amino acid and sugar that occurred when process foods with heating. Mao contained various kinds of amino acids (Lokaewmanee and Sansupha, 2015) and amino acid left on Mao pulp might be reaction

with fruit sugar when the temperature was increased, therefore a^* of Mao tea with high temperature became increasing.

Development of Mao tea for commercial purposes

Interestingly, it was found the temperature inversely affected on DPPH^o value and the acceptance of the consumers. Therefore, to develop Mao tea to market, it is necessary to blend formula for customer's satisfaction. **Table 5** showed proportion of different drying temperature of Mao seed and the sensory analysis and its anti-oxidant properties.

Table 5 Ratio of Mao seed dried at 50 and 90°C and results of DPPH^o, colors and sensory analysis of Mao tea

Ratio (%)		DPPH ^o	Colors				Sensory Analysis			
		(%)	L^*	a^*	b^{*ns}	Appearance	Color	Odor	Taste	Overall
50°C	90°C	Inhibition)								liking
100	0	20.85±0.01 ^a	69.94±3.6 ^a	-0.51±0.3 ^c	4.23±0.8	4.80±1.83 ^c	4.29±1.9 ^d	5.66±1.53 ^c	4.32±1.8 ^d	5.20±1.45 ^c
75	25	18.03±0.01 ^a	67.38±0.5 ^{ab}	0.35±0.6 ^{ac}	4.46±0.5	5.39±1.53 ^c	5.10±1.56 ^c	5.44±1.40 ^d	4.59±1.96 ^c	5.44±1.47 ^c
50	50	16.10±0.02 ^b	66.56±0.9 ^{ab}	1.25±0.9 ^b	4.50±0.5	6.10±1.20 ^b	5.90±1.37 ^b	5.88±1.47 ^c	5.07±1.94 ^b	6.17±1.18 ^b
25	75	14.25±0.01 ^{bc}	66.56±2.1 ^{ab}	2.21±1.0 ^{ab}	4.92±0.8	6.39±1.32 ^b	6.96±1.40 ^{ab}	6.17±1.48 ^b	5.49±1.9 ^{ab}	6.34±1.32 ^{ab}
0	100	13.58±0.01 ^c	65.23±0.3 ^b	3.12±1.3 ^a	4.52±0.6	7.07±1.40 ^a	7.34±1.11 ^a	6.32±1.54 ^a	5.76±2.11 ^a	6.88±1.3 ^a

Note: mean±SD (n=3) values with different lowercase letters in the column are significantly different ($p \leq 0.05$) and ^{ns} means no significant differences ($p > 0.05$)

It was found that the Mao seeds dried at the temperature 50°C had the highest DPPH^o inhibition, while Mao seeds dried at the temperature 90°C had the lowest DPPH^o inhibition. It was found that the best mixture ratio of the Mao seeds tea was 25% of Mao seeds dried at the temperature of 50°C and 75% of Mao seeds dried at the temperature of 90°C. For this proportion, sensory analysis such as color, taste and overall liking and color measurement (L^* and a^*) had high scores. Although, DPPH^o inhibition was quite low (14.25±0.01% inhibition at this ratio, but when comparing with other kinds of tea available in the market in the same condition, it showed almost the same DPPH^o inhibition with green tea (15.25±0.008%) and Oolong tea (13.68±0.072%).

Conclusion

Mao seeds were dried at temperature 50°C in hot air oven for 8 hours showed the highest DPPH^o inhibition while Mao seeds dried at the temperature of 90°C showed the highest red value (a^*) and customer acceptance. To develop Mao tea for commercial purposes, it was found that the best mixture proportion of the Mao seeds which were dried at the temperature of 90 and 50°C was 75%:25%. The antioxidant properties (DPPH^o inhibition) of obtained Mao tea was similar to green tea and Oolong tea available in the markets.

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