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Effect of Ethanol Concentrations, Honey Adding and Maceration Times on Quality of Mao (*Antidesma thwaitesianum*) Liqueur

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Abstract

The Mao tree (*Antidesma thwaitesianum*) is a native and medicinal fruit tree found in Southeast Asia, thriving especially in dipterocarp forests, notably in Northeast Thailand's Phu Phan valley. Ripe Mao fruits are rich in various nutrients and antioxidants that have the potential to mitigate the risk of chronic diseases and cancer. A comprehensive study was conducted to assess the impact of ethanol at different concentrations (40 or 60 % v/v), with and without honey added. The effects of varying maceration times (0, 3, 6, 9, or 12 months) on Mao liqueur's properties were also investigated. The investigation explored various parameters, including color, total soluble solids, total acid content, alcohol concentration, and bioactive compounds. The results showed that the color lightness (L^*) values of all recipes decreased over the storage period. However, adding honey to the production of Mao liqueurs decreased the yellowness (b^*) value in the first 3 months but resulted in a higher total soluble solid content in Mao liqueur. Increasing the ethanol concentration from 40 to 60 % did not affect the total acid content (TTA 0.04 %). However, the addition of honey increased the total acid content from 0.04 to 0.08 % ($p \leq 0.05$). The maceration time did not affect the alcohol content of Mao liqueur ($p > 0.05$). The use of 60 % (v/v) ethanol with honey addition resulted in the highest alcohol content (42 % v/v). Increasing the ethanol solution concentration from 40 to 60 % with and without honey added increased the total phenolic content from 126 to 165 GAE mg/L and 136 to 160 GAE mg/L respectively. The addition of honey increased the anthocyanin content in the liqueurs in the 12th month.

Keywords: Bioactive compound, Ethanol, Honey, Liqueur, Maceration, Mao fruit

Introduction

Mao (*Antidesma thwaitesianum*) belongs to genus Antidesma. It is a medicinal plant in Southeast Asia and well known to the local people in Northeast Thailand. This plant is grown in dipterocarp forested areas in Southeast Asia; in Thailand it is found in dipterocarp areas in the Phu Phan Valley mountains in Sakon Nakhon province [1]. The Mao fruit is small, oval-shaped, and grouped in a long bunch. The unripe fruit is either light or dark green, whereas a nearly ripe specimen is red and transforms into a dark purple or blackish purple hue when fully mature. The raw and barely ripe Mao fruit has a sour taste, while completely ripened fruit has a sweet-and-sour and slightly astringent taste [2]. The ripening period of Mao starts in July-August; most harvested plants are grown and planted in residential areas. Both fresh fruit and processed Mao are consumed because of their unique color and flavor characteristics. *Antidesma bunius or* bignay fruit belongs to the same genus of *Antidesma thwaitesianum.* It was reported that this fruit is a good source of bioactive compounds; including flavonoid, phenolic compounds and anthocyanin. It also showed antioxidant scavenging activities [3]. These desirable properties of Mao, including its abundance of nutrients and active constituents, along with its distinctive color, aroma, taste, and high crop yield of ripe fruit, make it suitable for processing into various products. Mao fruit is processed into a variety of items, including fruit juice, wine, Mao jam, and preserved Mao. However, the specific production process for making liqueurs from Mao has not been reported.

Liqueur is an alcoholic beverage that is sweetened and flavored with, among other ingredients, fruits, herbs, spices, flowers, and nuts. It is often made by combining a distilled spirit with sugar and additional flavorings, and its alcohol content ranges from 15 to 55 % by volume [4]. Liqueurs can be consumed on their own or as a component in cocktails and other mixed drinks. In recent years, there has been an increase in public interest in fruit liqueurs and alcoholic beverages in Thailand, as customers increasingly seek traditional items of recognized quality and flavor. Alcohol treatment of fruit induces the release and extraction of the fruit's active constituents. Hanousek Čiča *et al.* (2020), studied physicochemical and aromatic characterization of carob macerates produced by different maceration conditions. Carob liqueur, a Mediterranean drink with a minimum 15 % alcohol and 100 g/L sugar, was studied for its aroma and color. Soaking carob pods in alcohol-water mix extracted bioactive and flavor compounds contributed to darkness-favoring phenolics. Sugar (mainly sucrose) was added at a concentration of around 96 - 107 g/L. The 26 aroma compounds were found, dominated by low-weight esters like ethyl hexanoate. Carob pod maceration in 50 % v/v hydroalcoholic base in darkness at room temperature for 8 weeks was recommended as optimal maceration conditions for the production of the aromatic carob macerate with functional properties [5]. The optimization of the coffee liqueur manufacturing process using caffeine content was also studied. Effect of different ethanol concentrations (20, 40, or 80 %) and soaking durations (14, 21, or 30 days) the physicochemical properties of liqueur were compared. Caffeine had an average content of 2,081.2 μg/mL. The highest concentration (2,793 μg/mL) of caffeine occurred in 80 % ethanol liquor. A 21-day soaking period in 40 % ethanol yielded the most consistent caffeine concentrations (RSD 7.8 %). This 40 % ethanol was suggested as the optimal coffee liqueur recipe for quality assurance. [6] Barros *et al.* [7], studied the production of grape stem liqueurs. The process of making liqueurs using grape stems. The stems are chopped and added to grape marc spirits, along with sugar. The mixture is then stored for 6 months and looks at the effect of different curing times (0, 90, or 180 days) on the physical characteristics, chemistry, and phytochemicals in liqueurs. They found that by curing the liqueurs for 90 days resulted in a high content of phenolic compounds, such as ortho-diphenol, flavanols, flavonols, and anthocyanins, which have interesting physical and chemical characteristics, as well as antioxidant activity [7].

Honey liqueur is an alcoholic beverage made by combining honey with a distilled spirit, such as brandy, rum, or whiskey. It is a sweet and flavorful drink with a rich history dating back centuries. The process of making honey liqueur typically involves blending honey with the base spirit and sometimes adding various spices, herbs, or other flavorings to enhance its taste profile. The sweetness of the honey adds a unique depth and complexity to the final product. In this study, Mao liqueur was combined with honey to achieve a new product. The impact of aging and different types of honey on the sensory properties of honey liqueur was studied. Aging increased honey, floral, and caramel aromas while reducing fruity and sour notes. Flavor-wise, aging heightened honey, floral, and sweet elements while lessening bitter and sour tastes. Color attributes like yellow and red were intensified with aging. Consumers favored honey liqueurs aged for at least 12 months and showed preferences for specific honey types based on sensory characteristics [8].

Therefore, the current research investigated the effect of maceration times, ethanol concentrations and the addition of honey on the physical and chemical properties of Mao liqueurs as an area-unique and highly nutritious product.

Materials and methods

Preparation of Mao liqueur

Mao fruits (Fapratan cultivar) in a color stage of red-purple grown in Phu Phan district, Sakon Nakhon Province, Thailand were harvested. Moa stems were separated from their fruits and then washed with tap and deionized water, respectively. Red-purple Mao fruits (each 50 g) were soaked in 250 g of 40 % (v/v) or 60 % (v/v) ethanol solution (L Pure, Liquor Distillery Organization, Thailand). A honey-added recipe (100 g of honey (Aro, Siammakro co. ltd, Thailand) symbolized as 40 % EtOH + Honey and 60 % EtOH + Honey and a recipe without honey were added to 100 g of distilled water (40 % EtOH and 60 % EtOH). After adding ethanol for liqueur maceration, the absolute alcohol contents based on the total weight of liqueur were 25.66, 32.51, 38.50 and 48.76 % (v/v), respectively. Samples were contained in an almost-filled amber bottle to minimize oxygen contact. In addition, the sample bottles were wrapped with aluminum foil, and stored at 15 °C in the dark; afterward, the liqueur samples were stored for 12 months and analyzed at months 0, 3, 6, 9, and 12, respectively. These are related to the period of aging time for native alcoholic beverages in the area of study. There was a study reported that consumers preferred honey liqueurs that had been aged for at least 12 months [8]. All experiments were repeated 3 times and then analyzed for the physical and chemical properties of the produced Mao liqueurs. The Mao fruits and

liqueurs were separated by using filter paper (No. 1) (Whatman, GE Healthcare Life Sciences, UK) before physical and chemical analysis.

Color measurements

The color of the Mao liqueurs was analyzed using a colorimeter (MiniScan EZ; HunterLab; USA). The colors of liqueurs were represented using CIE L^*, a^*, and b^* values [7]. In addition, the browning index (BI) Eq. (1) [9], was calculated to describe the alteration of Moa liqueur's color as the effect of formulas and maceration times.

$$BI = (100(x-0.31))/0.17) \quad (1)$$

where; $x = ((a^*+1.75L^*)/(5.645L^*+a^*-3.012b^*))$

Total soluble solid content measurement

The total soluble solids (TSS) of the Mao liqueurs were analyzed using a hand refractometer (N-1∞, ATAGO; Japan). The total soluble solid was expressed as ° Brix [7].

Total titratable acidity measurement

The total titratable acidity (TTA) of the Mao liqueurs was analyzed based on the AOAC method [10]. Mao liqueur (2 mL) was diluted with deionized water to reach a total solution of 10 mL, which was titrated with a 0.1 M sodium hydroxide (Merck, Merck KGaA, Germany) standard solution, using phenolphthalein (LABCHEM, Asia Pacific Specialty Chemicals Limited, Australia) solution as an indicator. The amount was reported as the total titratable acidity in grams per 100 mL of sample (% total titratable acidity content).

Alcohol content measurement

The alcohol content of the Mao liqueurs was evaluated using an ebulliometer and represented as the percentage of alcohol volume per 100 mL of liqueur (% vol.). Briefly, distilled water was added into the boiling chamber of the ebulliometer. The thermometer was inserted into the chamber to monitor temperature. After that, the chamber was heated until boiling water was noticed. The boiling temperature had to be stable for 30 s and then this temperature was recorded for scale calibration of 0 % alcohol. After that, the boiled water was drained from the chamber. The 50 mL of sample was added into the chamber, and heated for 15 min. The constant temperature for 30 s was recorded. The alcohol content was calculated by Ebulliometer Chart [11].

Total phenolic content measurement

A sample (0.1 mL) of Mao liqueur was pipetted into a 10 mL volumetric flask, 6 mL of distilled water were added, followed by 0.5 mL of Folin-Ciocalteu's solution (2N) (Sigma-Aldrich, Sigma-Aldrich, Co., USA), mixed thoroughly and left for 5 min. Then, 1.5 mL of 20 % (w/v) sodium carbonate (Ajax Finechem, Thermo Fisher Scientific, Australia) solution was added, followed by distilled water until the final volume was 10 mL. The solution was mixed thoroughly and incubated at room temperature for 2 h. Then, the incubated solution was measured for absorbance at 765 nm using a microplate reader (Synergy HT; BioTek Instruments; USA). The phenolic content in the Mao liqueurs was presented as gallic acid equivalent (GAE) (Sigma-Aldrich, Sigma-Aldrich, Co., USA) in milligrams per liter calculated using a linear equation for the GAE standard calibration curve of 0, 50, 100, 150, 250 and 500 mg/L respectively [12].

Determination of anthocyanin content using pH differential method

A 0.5 mL sample of Mao liqueur was added with 4.5 mL of 0.025 M potassium chloride (Ajax Finechem, Thermo Fisher Scientific, Australia) buffer solution at pH 1.0 and the absorbance was measured at 520 and 700 nm wavelengths, indicating absorbance values at A520 and A700 at pH 1.0, respectively ($A_{520,\ pH\ 1.0}$ and $A_{700,\ pH\ 1.0}$). In addition, the anthocyanin absorbance at pH 4.5 was measured by pipetting a 0.5 mL liqueur sample and adding 4.5 mL of 0.4M sodium acetate (Ajax Finechem, Thermo Fisher Scientific, Australia) buffer solution at pH 4.5. Absorbance was measured at wavelengths of 520 and 700 nm to obtain absorbance A_{520} and A_{700} values at pH 4.5 (A_{520}, pH 4.5 and A_{700}, pH 4.5). Then, the absorbance values were determined to calculate the anthocyanin content Eq. (2) according to the equations below [13].

Anthocyanin content (mg/L) = (A * MW * DF * 1,000) / (ε * 1) (2)

where A = $(A_{520nm}-A_{700nm})_{pH1.0}$ - $(A_{520nm}-A_{700nm})_{pH4.5}$; MW (molecular weight) = 449.2 g/mol for cyaniding-3-glucoside; DF = dilution factor established in D; 1 = pathlength in cm; ε = 26,900 molar extinction coefficient, in $L\times mol^{-1}\times cm^{-1}$, for cyd-3-glu; and 10^3 = factor for conversion from g to mg [13].

Statistical analysis

Sample variance and mean differences were analyzed using the SPSS program (IBM SPSS Statistics, USA) at the 95 % confidence level ($p \leq 0.05$). The variance was analyzed using a one-way ANOVA test and mean differences were analyzed using Duncan's new multiple range test methods.

Results and discussion

Color value (L^*, a^*,b^*) of Mao liqueurs

The L^*, a^* and b^* values of the Mao liqueurs are presented in **Table 1**. Considering the color values of different ethanol concentrations, the lightness (L^*) of the liqueur with 60 % EtOH was greater than that of one with 40 % at 0 months (not macerated). According to Ove *et al.* [14], this was a result of utilizing a higher ethanol concentration, which increased the solution's lightness. They reported that among other 30 and 40 % EtOH extracts, the 50 % EtOH extract had the highest level of lightness (L^*). When ethanol concentrations increased, the redness (a^*) of the Moa liqueur increased substantially, whereas the yellowness (b^*) decreased significantly ($p \leq 0.05$). As a consequence of honey's yellow natural color, the addition of honey significantly increased the b^* value of Mao liqueurs in all treatments. They seemed to become darker and more reddish brown.

Table 1 Color value (L^*, a^*, b^*) and browning Index (BI) of Mao liqueurs (*Antidesma thwaitesianum*) from different formulas and maceration times (0, 3, 6, 9, 12 months).

Treatments	Color value	Maceration time (months)				
		0	3	6	9	12
40 % EtOH	L^*	15.64 ± 1.74^{Ca}	7.26 ± 0.63^{Bb}	6.54 ± 1.07^{NSbc}	5.65 ± 0.49^{Ac}	6.16 ± 0.51^{Ac}
	a^*	19.13 ± 2.03^{Ba}	10.45 ± 0.77^{Ac}	13.27 ± 2.23^{Ac}	15.93 ± 1.62^{Ab}	15.85 ± 1.35^{Ab}
	b^*	-2.23 ± 0.60^{Cc}	3.33 ± 0.54^{Ab}	4.40 ± 1.06^{Ba}	3.22 ± 0.80^{Ab}	3.81 ± 0.34^{Aab}
	BI	57.98 ± 11.08^{Cc}	147.89 ± 17.69^{Ab}	213.19 ± 39.40^{Aa}	217.78 ± 36.45^{Aa}	219.09 ± 20.41^{Aa}
40 % EtOH + Honey	L^*	22.34 ± 1.13^{Aa}	11.41 ± 0.91^{Ab}	7.27 ± 0.62^{NSc}	3.81 ± 0.34^{Cd}	3.22 ± 0.80^{Cd}
	a^*	10.15 ± 0.23^{Ca}	$9.24 \pm 1.00^{B\ ab}$	10.00 ± 1.70^{Ba}	7.16 ± 1.52^{Bb}	6.87 ± 4.33^{BCb}
	b^*	19.31 ± 0.86^{Aa}	3.15 ± 0.60^{Ac}	5.58 ± 1.83^{Ab}	0.44 ± 0.50^{Bd}	0.44 ± 0.50^{Bd}
	BI	189.96 ± 21.20^{Aa}	86.34 ± 10.08^{Bb}	167.87 ± 46.35^{Ba}	116.10 ± 33.72^{Bb}	122.42 ± 44.47^{Bb}
60 % EtOH	L^*	17.24 ± 2.25^{BCa}	6.61 ± 0.68^{Bb}	6.73 ± 1.64^{NSb}	4.54 ± 0.32^{Bc}	4.54 ± 0.32^{Bc}
	a^*	21.96 ± 1.89^{Aa}	6.79 ± 1.26^{Cb}	8.66 ± 1.19^{Bb}	6.49 ± 4.17^{BCb}	7.27 ± 1.67^{Bb}
	b^*	-7.17 ± 1.17^{Dc}	1.46 ± 0.30^{Ba}	0.90 ± 0.22^{Cab}	0.13 ± 0.70^{Bb}	0.13 ± 0.70^{BCb}

Treatments	Color value	Maceration time (months)				
		0	3	6	9	12
60 % EtOH + Honey	BI	35.78 ± 5.00^{Db}	89.23 ± 10.52^{Ba}	94.67 ± 24.45^{Ca}	83.63 ± 60.27^{BCa}	94.25 ± 32.58^{Ba}
	L^*	18.16 ± 0.73^{Ba}	7.08 ± 0.6^{Bb}	7.70 ± 1.61^{NSb}	4.30 ± 0.17^{Bc}	4.30 ± 0.17^{Bc}
	a^*	10.93 ± 1.00^{Ca}	5.64 ± 0.64^{Db}	4.24 ± 1.02^{Cc}	4.70 ± 0.84^{Cbc}	4.70 ± 0.84^{Cbc}
	b^*	12.90 ± 0.90^{Ba}	1.43 ± 0.34^{Bb}	-0.05 ± 0.43^{Dc}	-0.16 ± 0.50^{Bc}	-0.16 ± 0.50^{Cc}
	BI	155.71 ± 18.40^{Ba}	74.72 ± 8.95^{Bb}	36.57 ± 12.42^{Dc}	62.41 ± 17.22^{Cb}	62.41 ± 17.22^{Cb}

Mean ± SD (n = 3). Different capital superscripts in column are significantly different due to Mao liqueur formula ($p \leq 0.05$). Different lowercase superscripts in row are significantly different due to maceration time ($p \leq 0.05$). NS is not significantly different ($p > 0.05$).

The results of the different maceration time on the color values showed that the L^* and a^* values of all liqueurs decreased at month 3 significantly ($p \leq 0.05$), except for a^* value of 40 % EtOH + Honey liqueur. However, the L^* and a^* values did not change significantly in month 6 and 12 ($p > 0.05$). This related to the darker brown appearance as well as the higher BI value of the Mao liqueur that had been macerated for a longer time. It may be due to both enzymatic and non-enzyme browning depending on the initial mechanism. Enzymatic browning occurs in an early stage of processing because of oxidoreductase (polyphenol oxidase: PPO and peroxidase: POD) [15]. The non-enzymatic browning for instance the Maillard reaction and/or ascorbic degradation could occur during storage [16]. An ascorbic acid content of 175.34 mg/100 mL was reported for Mao juice [17]. In addition, brown color might be influenced by the oxidation of phenols and the polymerization of the oxidized compounds [15]. The b^* values changed substantially during the first 3 months ($p \leq 0.05$). The b^* values of both 40 and 60 % EtOH tended to increase with increasing maceration time. However, the b^* values of the Mao liqueurs with honey added tended to decrease with increasing maceration time, especially in the first 3 months. This might be due to the honey could be act as anti-browning agent or antioxidant, thus it can prevent browning reactions in raisin processing [18], including grape juice preparations [19].

Table 2 Total soluble solid (° Brix) of Mao (*Antidesma thwaitesianum*) liqueur from different formulas and maceration times (0, 3, 6, 9, 12 months).

Treatment	Total soluble solid (° Brix)/Maceration time (months)				
	0	3	6	9	12
40 % EtOH	9.96 ± 0.08^{Dc}	10.96 ± 0.82^{D}	10.33 ± 0.10^{Dbc}	10.07 ± 0.10^{Dc}	10.51 ± 0.20^{Db}
40 % EtOH + Honey	30.62 ± 0.08^{Ba}	29.94 ± 0.59^{Bb}	29.80 ± 0.22^{Bb}	30.11 ± 0.33^{Bb}	30.07 ± 0.32^{Bb}
60 % EtOH	13.98 ± 0.08^{Cbc}	14.26 ± 0.24^{Cab}	13.84 ± 0.09^{Cc}	13.71 ± 0.48^{Cc}	14.44 ± 0.26^{Ca}
60 % EtOH + Honey	33.24 ± 0.08^{Aa}	33.11 ± 0.06^{Aa}	32.62 ± 0.19^{Ab}	33.02 ± 0.23^{Aa}	32.88 ± 0.79^{Aab}

Mean ± SD (n = 3). Different capital superscripts in column are significantly different due to Mao liqueur formula ($p \leq 0.05$). Different lowercase superscripts in row are significantly different due to maceration time ($p \leq 0.05$).

Total soluble solid content of Mao liqueurs

Total soluble solid content, as shown in **Table 2**, significantly increased with increasing ethanol concentration from 40 % EtOH (9.96 ° Brix) to 60 (13.98 ° Brix) at 0 months. These results demonstrated higher solubility of soluble solid in the water-ethanol solution with higher ethanol concentration [20]. With honey added, the total soluble solid-content of liqueurs increased by approximately 20 ° Brix. The initial soluble solid content of honey used in this study was 80 ° Brix.

The total soluble solid content of all Mao liqueurs was relatively similar during 0 - 12 months of maceration time. Only a slight increase was noticed. Thus, increasing the maceration time did not affect the total soluble solid content in Mao liqueurs. The total soluble solid content of all formulas seemed to be stable during the complete maceration period.

Total titratable acidity (TTA) of Mao liqueurs

Increasing the ethanol concentration from 40 to 60 % at 0 months maceration time had no significant affected on the % TTA in the Mao liqueurs, as shown in **Table 3**. However, the addition of honey significantly increased the % TTA ($p \leq 0.05$). The % TTA had increased of approximately 0.04 % in both 40 and 60 % EtOH Mao liqueurs. These showed an increase brought on by honey's free acids [19]. Approximately 120 organic acids have been reported in honey [20].

In addition, increasing the liqueur maceration times from 0 to 3 months increased the total titratable acidity of all liqueurs ($p \leq 0.05$). They continuously increased to 6 months and then were relatively stable during 6 - 12 months for all Mao liqueur formulas (**Table 3**). These results agreed with Barros *et al.* [7], who found that the total acid contents of grape stem liqueur increased when the maceration time increased from 0 to 90 days.

Table 3 Total titratable acidity (% TTA) of Mao (*Antidesma thwaitesianum*) liqueurs from different formulas and maceration times (0, 3, 6, 9, 12 months).

Treatment	Percentage total titratable acidity/Maceration times (months)				
	0	**3**	**6**	**9**	**12**
40 % EtOH	0.0433 ± 0.0050^{Bc}	0.0667 ± 0.0087^{Cb}	0.0944 ± 0.0053^{Ba}	0.0989 ± 0.0033^{Ca}	0.0967 ± 0.0050^{Ba}
40 % EtOH + Honey	0.0811 ± 0.0033^{Ac}	0.1378 ± 0.0067^{Aa}	0.1178 ± 0.0067^{Ab}	0.1411 ± 0.0093^{Aa}	0.1333 ± 0.0141^{Aa}
60 % EtOH	0.0456 ± 0.0073^{Bc}	0.0789 ± 0.0033^{Bab}	0.0900 ± 0.0071^{Ba}	0.0800 ± 0.0000^{Db}	0.0844 ± 0.0101^{Cb}
60 % EtOH + Honey	0.0767 ± 0.0071^{Ad}	0.1344 ± 0.0053^{Aa}	0.1156 ± 0.0053^{Ac}	0.1189 ± 0.0060^{Bc}	0.1256 ± 0.0053^{Ab}

Mean ± SD (n = 3). Different capital superscripts in column are significantly different due to Mao liqueur formula ($p \leq 0.05$). Different lowercase superscripts in row are significantly different due to maceration time ($p \leq 0.05$).

Alcohol content of Mao (*Antidesma thwaitesianum*) liqueurs

The 40 and 60 % EtOH were used as symbolics of the recipe using ethanol solution of 40 and 60 %. Khomdram *et al.* [21] found that the moisture content in Moa (*Antidesma bunius*) fruit was 79.28 %. As such, the ethanol concentrations in all of Moa liqueurs were calculated as 25.66, 32.51, 38.50 and 48.76 % (v/v) based on total water weight in formula of 40 % EtOH, 40 % EtOH + Honey, 60 % EtOH and 60 % EtOH + honey, respectively. The alcohol contents in filtrated Moa liqueurs at 0 - 3 months of 60 % EtOH were extremely lower than those of other maceration times (6, 9 and 12 months). Whereas other formulas had alcohol content relatively stable during maceration. It is due to the diffusion of alcohol into plant cells while water leaves to extraction phase (alcohol) resulting in a concentration of alcohol decreased during maceration [22].

Increasing the maceration time from 0 to 3 months slightly affected the alcohol content for the Mao liqueurs containing 40 % EtOH with and without honey. On the other hand, both Mao liqueurs of 60 % EtOH had large changes in their alcohol contents during the maceration time 3 - 6 months, and then it

remained stable during 6 - 12 months of maceration time. It might be due to the equilibrium mass transfer being reached [23]. However, the ethanol concentrations of Moa liqueur with the addition of honey seem to be much lower than the calculated ethanol concentration, which was mentioned earlier. It might be due to alcohol accelerating the loss of water because of the inflow of the strongly hygroscopic of alcohol into intercellular space [23]. According to Feng *et al.* [24], they found that the ethanol treatment enhanced pumpkin dehydration. In addition, sugar is often combined with alcohol for the osmotic dehydration of fruits because they were more effective than sucrose alone [25]. These results agreed with Barros *et al.* [7], who found similar alcohol contents in grape stem liqueur at maceration times of 0, 90, and 180 days.

Table 4 Alcohol content (% v/v) of Mao (*Antidesma thwaitesianum*) liqueur from different formulas and maceration times (0, 3, 6, 9, 12 months).

Treatment	Alcohol content (% v/v)/Maceration time (months)				
	0	**3**	**6**	**9**	**12**
40 % EtOH	25.25 ± 0.29^{Cb}	25.25 ± 0.29^{Db}	25.00 ± 0.87^{Db}	25.00 ± 0.00^{Db}	26.33 ± 0.50^{Ca}
40 % EtOH + Honey	26.00 ± 0.00^{Cc}	26.00 ± 0.00^{Cc}	26.22 ± 0.67^{Cc}	27.11 ± 0.60^{Cb}	28.22 ± 0.67^{Ba}
60 % EtOH	28.75 ± 0.87^{Bd}	27.13 ± 0.25^{Be}	36.78 ± 1.72^{Ac}	38.22 ± 0.97^{Bb}	40.33 ± 0.50^{Aa}
60 % EtOH + Honey	42.00 ± 0.82^{Aab}	42.75 ± 0.50^{Aa}	35.63 ± 0.52^{Bb}	40.89 ± 1.45^{Ab}	41.00 ± 1.22^{Ab}

Mean ± SD (n = 9). Different capital superscripts in column are significantly different due to Mao liqueur formula ($p \leq 0.05$). Different lowercase superscripts in row are significantly different due to maceration time ($p \leq 0.05$).

Total phenolic content (TPC) of Mao (*Antidesma thwaitesianum*) liqueur

At 0 months, the results showed that the TPC of the Mao liqueurs prepared from 60 % EtOH was higher than one with 40 % EtOH, with an increase from 126.19 mg GAE/L (40 % EtOH) to 165.24 mg GAE/L (60 % EtOH), as shown in **Table 5** ($p \leq 0.05$). These results corresponded to Thoo *et al.*, who reported that an ethanol concentration in the range 0 - 40 % (v/v) increased the total phenolic content of Noni extract; however, the TPC tended to decrease as the ethanol concentration increased from 60 to 100 % (v/v) because of the polar and non-polar components in the phenolic compounds. Therefore, polar and non-polar fractions in binary solvents are required for the extraction of phenolic compounds. However, plants are composed of various phenolic compounds with different structures that can result in different polarities for phenolic compounds. Therefore, the different polarities of solvents can be useful for phenolic compounds and for different plants [26]. At 0 month of maceration time, the TPC values in the liqueur with honey addition were similar to those for the liqueurs without honey addition. In addition, increasing the maceration time of the Mao (*Antidesma thwaitesianum*) liqueur, increased the TPC values for all formulas (**Table 5**). The TPC values of the Mao liqueur prepared using 40 % EtOH both with and without honey addition increased when the maceration time increased during 0 - 9 months. However, at 12 months, their TPC values had decreased ($p \leq 0.05$). For the Mao (*Antidesma thwaitesianum*) liqueurs prepared using 60 % EtOH both with and without honey, there was an increasing trend in TPC as the maceration time increased during 0 - 12 months, with the highest TPC at 12 months. The TPC substantially increased by adding honey to the Mao liqueur formulas 6 - 12 months, especially for the Mao liqueurs which were prepared using 60 % EtOH. The addition of honey to the Mao liqueur formulas could substantially increase the TPC during 6 - 12 months. This may have been due to the phenolic compounds that naturally occur in honey contributing to the increased TPC when honey was added [27]. Therefore, the TPC amounts in the Mao liqueur formulas with honey were higher than in those without added honey. In addition, the TPC of all Mao liqueurs seemed to increase with increasing maceration time, which was related to Barros *et al.*, who found that the total phenolic content of grape stem liqueur at 90 days (3,720 mg GAE/L) was higher than the total phenolic content of grape stem liqueur at 0 days (1,400 mg GAF/L). This may have been due

to the leaching of phenolic compounds from the grape stem during the maceration process [7]. However, Sokół-Łetowska *et al.* [4] found that the phenolic compound contents of 10 species of color fruit liqueurs are reduced during storage for 6 months at temperatures of 15 and 30 °C.

Table 5 Total phenolic content (mg GAE/L) of Mao (*Antidesma thwaitesianum*) liqueur from different formulas and maceration times (0, 3, 6, 9, 12 months).

Treatment	Total phenolic content (GAE mg/L)/Maceration time (months)				
	0	**3**	**6**	**9**	**12**
40 % EtOH	126.19 ± 4.57^{Be}	308.33 ± 4.46^{Dd}	653.20 ± 27.56^{Cb}	714.82 ± 59.87^{Ca}	578.45 ± 40.12^{Cc}
40 % EtOH + Honey	136.51 ± 4.11^{Be}	351.43 ± 15.41^{Cd}	824.63 ± 64.55^{Bb}	$1{,}078.28 \pm 72.16^{Aa}$	606.98 ± 26.93^{Cc}
60 % EtOH	165.24 ± 10.40^{Ad}	471.19 ± 3.66^{Ac}	686.53 ± 40.23^{Cb}	692.76 ± 104.13^{Cb}	876.59 ± 54.87^{Ba}
60 % EtOH + Honey	160 ± 26.19^{Ad}	405.71 ± 8.90^{Bc}	895.87 ± 63.68^{Ab}	916.25 ± 143.24^{Bb}	$1{,}109.45 \pm 67.05^{Aa}$

Mean ± SD (n = 3). Different capital superscripts in column are significantly different due to Mao liqueur formula ($p \leq 0.05$). Different lowercase superscripts in row are significantly different due to maceration time ($p \leq 0.05$).

Total anthocyanin content of Mao (*Antidesma thwaitesianum*) liqueur

At 0 months of maceration (**Table 6**), increasing the ethanol concentration from 40 to 60 % reduced the total anthocyanin content of Mao liqueur ($p \leq 0.05$). This result corresponded to Cortez *et al.*, who found that increasing the ethanol concentration affected a decrease in anthocyanin because its molecular structure is like water-soluble glycosides and AcylG that can be dissolved in water [28]. However, the anthocyanin contents of the Mao liqueurs prepared with 60 % EtOH and macerated during 3 - 12 months were higher than for 40 % EtOH. Considering the effect of maceration times, the results showed that 0 - 3 months for maceration resulted in a significantly increased total anthocyanin content. However, the total anthocyanin content decreased after 6 months, which corresponded with Barros *et al.* [7], who found that anthocyanin in the grape stem could be rapidly extracted in an ethanol solution on day 0. However, the anthocyanin content decreased when the maceration time was extended from 90 to 180.

In addition, increasing the liqueur maceration times from 0 to 3 months increased the total anthocyanin of all liqueurs ($p \leq 0.05$) and the total anthocyanin of Mao liqueurs containing 60 % EtOH with and without honey is higher than Mao liqueurs containing 60 % EtOH of maceration 3 months. This result corresponded to Tseng *et al.*, who found that increasing the ethanol concentration affected an increase in alvidin-3-glucoside. A higher concentration of ethanol corresponds to a higher extent of the bathochromic shift in the malvidin-3-glucoside ethanolic model solutions [29]. The major anthocyanins found in Mao fruit cultivars were cyanidin-3-O-glucoside, cyanidin 3-rutinoside, cyanindin, followed by malvidin 3,5-diglucoside, malvidin, pelargonidin, and delphinidin [30]. Ethanol concentration may affect the color phenomena of anthocyanin in the Mao liqueur (**Table 1**). However, the total anthocyanin of all liqueurs decreased after 6 months of maceration. This result corresponded to Sokół-Łetowska *et al.*, who found that the total anthocyanin content of 10 species of color fruit liqueurs decreased during storage for 6 months at temperatures of 15 and 30 °C [4].

Notably, the molecular structure instability of anthocyanin decreased the anthocyanin content [7]. However, adding honey and allowing Mao liqueur maceration for 3 - 12 months resulted in an increase in the anthocyanin content in the liqueurs compared to liqueurs without honey. This may have been due to sugar in the honey, which could have stabilized the anthocyanin structure during the maceration process. This was in accord with Nikkhah *et al.* [30], who found that a 20 % concentration of sucrose prevented the loss of anthocyanin in berry juice.

Table 6 Total anthocyanin content (mg/L) of Mao (*Antidesma thwaitesianum*) Liqueur from different formulas and maceration times (0, 3, 6, 9, 12 months).

Treatment	Total anthocyanin content (mg/L)/Maceration time (months)				
	0	**3**	**6**	**9**	**12**
40 % EtOH	2.11 ± 0.28^{Ad}	5.09 ± 0.03^{Da}	3.46 ± 0.38^{Cc}	4.16 ± 0.46^{Bb}	5.51 ± 1.13^{BCa}
40 % EtOH + Honey	1.78 ± 0.10^{Bc}	5.39 ± 0.12^{Cb}	0.69 ± 0.12^{Dd}	5.12 ± 0.52^{Ab}	6.58 ± 0.82^{Ba}
60 % EtOH	0.18 ± 0.04^{Cc}	7.96 ± 0.36^{Ba}	5.31 ± 0.30^{Ab}	5.60 ± 1.08^{Ab}	5.27 ± 1.22^{Cb}
60 % EtOH + Honey	0.32 ± 0.88^{Ce}	8.90 ± 0.05^{Aa}	3.83 ± 0.56^{Bd}	5.57 ± 1.07^{Ac}	7.93 ± 1.51^{Ab}

Mean ± SD (n = 3). Different capital superscripts in column are significantly different due to Mao liqueur formula ($p \leq 0.05$). Different lowercase superscripts in row are significantly different due to maceration time ($p \leq 0.05$).

Conclusions

In this study, alcohol concentration, the addition of honey and maceration time up to 12 months affected the quality of Mao liqueur. Increasing the ethanol concentration from 40 to 60 % (v/v) increased the values for L^*, a^*, TSS, alcohol content, and especially the TPC, while decreasing the b^* value and anthocyanin content substantially. While adding honey increased the values of L^*, b^*, TSS, TTA %, and TPC, it seemed to slightly alter the alcohol and anthocyanin contents. The Mao liqueur qualities seem to be significantly altered during the first 3 months of maceration. In conclusion, this research could lead to the development of Mao (*Antidesma thwaitesianum*) liqueur production as a new alternative food product for consumers. However, the sensory test of Mao liqueur should be further studied to gain more understanding of consumer perception.

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