Anthocyanin and total phenolics content of mangosteen and effect of processing on the quality of mangosteen products

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Abstract: The study focuses on the anthocyanin and total phenolic content of mangosteen, the effect of drying on the quality of mangosteen mixed with fruit juice powder, and the effect of enzyme clarification and evaporation methods on the quality of mangosteen concentrate such as color value, anthocyanin and total phenolic content, and the percent of polymeric color. Mangosteen is composed of 17% of outer pericarp, 48% of inner pericarp, 31% of flesh and 4% of cap. Most of anthocyanin was found in outer pericarp (179.49 mg Cyanidin-3-glucoside (Cyn-3-glu) /100g) while most of total phenolic was found in inner pericarp (3,404 mg Gallic acid equivalent (GAE)/100 g). After juice process, the total phenolic content and anthocyanin of mangosteen juice is about 205.36 mg GAE/100 mL whereas the anthocyanin content is around 0.87 mg Cyn-3-Glu /100mL. Spray drying can preserve anthocyanin and total phenolic of mangosteen powder better than vacuum drying. After concentrated mangosteen juice by between two evaporation methods with and without pectinase enzyme, vacuum evaporation can prevent the degradation of anthocyanin better than the atmospheric evaporation. The enzymatic clarification can decrease the % polymeric color and increase the total phenolics of mangosteen juice concentrate.

Keywords: Mangosteen concentrate, mangosteen powder, anthocyanin, total phenolic, enzymatic clarification

Introduction

Mangosteen (Garcinia mangostana Linn) is a tropical fruit in Guttiferae family. Mangosteen is dark purple to red-purple fruits. The edible fruit aril is white, soft, and juicy with a sweet, slightly acid taste and a pleasant aroma (Martin, 1980). It is also known as “The queen of the fruit”. It is commonly cultivated in Thailand, Malaysia, and Indonesia. Mangosteen pericarp has been used in traditional Thai medicine for treating skin infections, wounds, and diarrhea for many years (Mahabusarakam et al., 1987; Moongkarndi et al., 2004). The major bioactive compounds found in mangosteens are phenolic acid, prenylated xanthone derivatives, anthocyanins, and procyanidins (Du and Francis, 1977; Fu et al., 2007; Zadernowski et al., 2009; Chaivisuthangkura et al., 2009). Ten phenolic acids were identified in mangosteen fruit. Of these, protocatechuic acid was the major phenolic acid in the peel and rind, while p-hydroxybenzoic acid was the predominant phenolic acid in the aril. (Zadernowski et al., 2007). The major anthocyanin in mangosteen was cyanidin-3-sophoroside (Du and Francis, 1977). Several researchers recognized phenolics and anthocyanin for their antioxidant properties (Robards et al., 1999; Karalaya et al., 2001; Rossi et al., 2003; Davalos et al., 2005; Balasundram, 2006).

Mangosteen season in Thailand is last from May to September. Most of mangosteen is consumed fresh or exported to foreign market. Extending shelf-life by using several food processing such as juice processing, concentrating, and drying could add value for mangosteen and create a new market. Recently, products such as mangosteen juices or dietary supplements have begun to be widespread around the world. Processing methods has an impact on phenolics and anthocyanins. Heating has a varied effect on several products depending on heating temperature. The retention of the total phenolic of bayberry juice after spray drying, a high temperature short time processes were 96%. Sterilization reduced total phenolic, procyanidin monomer, dimer, trimer, tetramer, pentamer, and hexamer of canned peach (Asami et al., 2003). Pasterurization could possibly increase procyanidin content in grape juice due to the depolymerization of flavan-3-ols especially oligomeric procyanidin (Fuleki and Ricardo-DA-Silva, 2003). Pectinolytic enzyme can be used in fruit processing due to increase the yield extraction or juice clarification. Landbo et al. (2007) reported
the use of pectinolytic enzyme increase juice yield and phenolic content in elderberry juice (Landbo et al., 2007). Landbo et al. (2006) reported that pectinase treatment may promote the formation of the immediate turbidity after treatment and decreased turbidity of blackcurrant juices during cold storage. Although there have been several investigations on the effect of processing on anthocyanins and phenolics in fruits, there is no study on the impact of processing on mangosteen products.

Therefore, our objective was to determine the total phenolic and anthocyanin content of mangosteen and measure their distribution in fruits. A second objective was to evaluate the impact of drying on the quality of mangosteen powder, and the effect of enzyme clarification and evaporation methods on the quality of mangosteen concentrate.

Material and Methods

Materials

Mangosteen and dried roselle were purchased from a local market in Bangkok, Thailand and stored at -18°C until further processed. Grape juice (Tipeco co.Ltd, Thailand) was purchased from a local store in Bangkok. Pectinase was purchased form D-I-Wine (Bangkok, Thailand).

Juice preparation

Mangosteen juice

Frozen mangosteens were thawed at 4°C and separated into pericarp, flesh, and juice. The flesh and juices were heat at 85°C for 2 min. Then, mangosteen juice was extracted using household extractor (Greenstar 1000, Tribest Corporation, CA, USA). The juices were centrifuged at 3000 x g (Falcon 6/300, UK) for 25 min. The clear juice was stored at -18 °C until further concentrated.

Roselle juice

Dried roselle was weighed for 50 grams and added 2 L of water. The mixture was boiled for 15 min. Then, the juice was filtered through thin white cloth. The juice was adjusted as 15 °Brix using the 33 °Brix syrup.

Mangosteen powder

Mangosteen juice mixed with roselle juice and grape juice (2:1:2 v/v) were mixed with maltodextrin DE 10 until the mixture has the total soluble solid as 30 °Brix. Then, the mixture was divided into 2 parts for drying at 2 different methods. The first was dried at 55°C for 2 hours by using the infrared vacuum oven (March Cool Industry Co., Ltd., Bangkok, Thailand). The other was dried by using spray dryer (Buchi, B-191, Switzerland). The flow rate was 15 mL per minute while the inlet and outlet temperature was 170 and 129°C. The sample powder was stored in polypropylene bag and stored in desiccators at room temperature for further analysis.

Mangosteen concentrate

Mangosteen clear juices were divided into 2 parts. The first one was directly concentrated into 47 °Brix while the other was depectinized with the 1000 ppm of pectinase at 40°C for 2 h. and heated at 85°C for 2 min to inactivate enzyme before concentrated. All samples were concentrated by 2 different methods: direct heat evaporator at 60°C and rotary vacuum evaporator at 40°C (Buchi R 114, Switzerland). All concentrates were pasteurized at 90°C for 2 min, bottled, and then stored at 4°C. To be compared with mangosteen juice, all concentrates were diluted as 15 ° Brix before analyzed.

Extraction of mangosteen anthocyanin and phenolics

Mangosteens were thawed and divided into flesh and pericarp. The pericarp were divided into two part: inner pericarp and outer pericarp. Each part was separately frozen in liquid nitrogen and cryogenically milled before extracted as follow. Each frozen powder (5 g) were mixed with 20 mL of acetone. The extract was sonicated for 10 min and centrifuged at 3000x g for 10 min. The supernatant was separated while the residue was reextracted twice with 10 mL of acetone. The supernatants were combined and evaporated in vacuo at 40°C until dried. The residue was redisolved with acidified water (0.1% HCl in water) and made up to the 25 mL. The extract was stored at -40°C for further analysis.

Physical and chemical analysis

Color measurements

The color of mangosteen juice and concentrate were determined as L*, a*, b* values using a colorimeter (BYK-Gardner, Germany). Chroma (C*) and the hue angle (H°) were calculated by the transformation of the a* and b* as the following equations: C*= (a*2 + b*2)1/2 and °H = tan-1(b*/a*).

Moisture and water activity

The moisture of mangosteen juice powder was determined as L*, a*, b* values using a colorimeter (BYK-Gardner, Germany). Chroma (C*) and the hue angle (H°) were calculated by the transformation of the a* and b* as the following equations: C*= (a*2 + b*2)1/2 and °H = tan-1(b*/a*).

Anthocyanin

The monomeric anthocyanin content was determined using the pH differential method
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(A) Shimadzu UV1601 spectrophotometer and 1 cm pathlength disposable cell were used for spectral measurement at 510 and 700 nm. Anthocyanin content was calculated as mg cyanidin-3-glucoside per 100 g fresh weight or 100 mL. The extinction coefficient of 26,900 L cm⁻¹ mol⁻¹ and the molecular weight of 449.2 gmol⁻¹ were used.

**Total phenolics**

Total phenolic content was determined using the modified Folin-Ciocalteu method described by Singleton and Rossi (1965). A 0.5 mL sample or a series of gallic acid standards (0, 40, 80, 120, 160, 200 ppm) were mixed with 0.5 mL of the Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO, USA) and 7.5 mL of deionized water. The mixture was held at room temperature for 10 minutes before adding 1.5 mL of 20% sodium carbonate (w/v). The mixture was heated in a 40°C water bath for 20 minutes and immediately cooled in an ice bath. The absorbance at 755 nm was determined. The total phenolic content was calculated as mg of gallic acid equivalent (GAE) per 100 g fresh weight or 100 mL.

**Antioxidant activity**

The effect of mangosteen and BHT on DPPH radical was estimated by the procedure described in Brand-Williams et al. (1995). A dilution series of sample and BHT was prepared. A 100 mL of sample or standard were mixed with 2.9 mL of DPPH solution (4.5 mg DPPH in 100 mL absolute methanol), followed by vortexing. The reaction was allowed to take place at room temperature for 30 min. The absorbances of DPPH remaining were determined at 517 nm. The reaction mixture without any sample was measured as control. The antioxidant activity was defined as the amount of sample to decrease the absorbance of DPPH by 50% (EC₅₀). BHT was used as a positive control.

**Sensory evaluation of mangosteen concentrate**

Thirty Srinakharinwirot University students who studied food science and nutrition were recruited to test mangosteen juice and juice dissolved from mangosteen concentrate. Samples were evaluated the color and cloudiness properties by using 5-point just right scales and the color property by 9-point hedonic scales.

**Statistical methods**

One-way ANOVA was used for determination of differences between mangosteen part and processes with SPSS V11.5. The Duncan Multiple Range Test was used to compare mean values. A probability level of p≤ 0.05 was considered to be significant. Pearson correlation was analyzed among total phenolic and anthocyanin content, and antioxidant capacity. All measurements and trials were done in duplicate.

**Results and Discussion**

**Distribution of total phenolic content, anthocyanin content, and antioxidant capacity of mangosteen**

Mangosteen is composed of 17% of outer pericarp, 48% of inner pericarp, 31% of flesh and 4% of cap. The total phenolic and anthocyanin content and EC₅₀ are showed in table 1. Both pericarps contained most of anthocyanin and phenolic. Inner pericarp, the soft pink pericarps, has the highest total phenolics content while outer pericarp, the hard purple pericarp, has the highest anthocyanin content. Anthocyanins in pericarp were reported as mostly cyanidin-3-soporoside with smaller amounts of cyanidin-3-glucoside (Du and Francis, 1977). Phenolics such as procyanidin, prodelphinidin, stereoisomers of afzekechin/epiafzelechin, catechin/epicatechin, and gallocatechin/epigallocatechin were also found in mangosteen pericarps (Fu et al, 2007). Mangosteen flesh contained a comparable amount of total phenolic as grape (158.0 mg GAE/100 g), black plum (143.5 mg GAE/100 g), and cherries (105.4 mg GAE/100 g) (Karakaya et al., 2001). Since mangosteen flesh is white, no anthocyanin was found.

According to Pearson correlation, both total phenolic and anthocyanin content was negative correlated with EC₅₀ values. The EC₅₀ values was more correlated total phenolic content (r=-0.965) than anthocyanin (r=-0.546). The negative correlation means when total phenolics or anthocyanin increase, EC₅₀ values, the amount that sample is required to decrease the 50% initial DPPH radical, decrease. Several studies also reported the high correlation among total phenolic content and antioxidant activity (Moyer et al., 2002; Proteggente et al., 2002). Moreover, outer pericarp and inner pericarp has EC₅₀ values comparable to BHT, the synthetic antioxidant (EC₅₀ = 7.5).

<table>
<thead>
<tr>
<th>Part</th>
<th>Total phenolic content (mg GAE/100 g)</th>
<th>Anthocyanin content (mg Cynidin-3-Glu/100 g)</th>
<th>EC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer pericarp</td>
<td>2.930 ± 0.106</td>
<td>179.49 ± 10.90</td>
<td>4.72 ± 0.55</td>
</tr>
<tr>
<td>Inner pericarp</td>
<td>3.464 ± 0.092</td>
<td>197.1 ± 22.90</td>
<td>1.35 ± 0.13</td>
</tr>
<tr>
<td>Flesh</td>
<td>132.29 ± 20.114</td>
<td>Not detected</td>
<td>133.35 ± 25.18</td>
</tr>
</tbody>
</table>

Values are means ± SD

Means in column with different superscript letter differ significantly (p<0.05)
The effect of drying method on mangosteen powder

A juice powder is a product that can be used as a drink or a flavor additive. Upon the preliminary study, the development of mangosteen powder is quite challenging since mangosteen has the sweet and acid taste. Therefore, fruit juices, such as grape juice and roselle juice were added to improve the flavor of mangosteen juice. After drying with two different methods, both mangosteen powders are a reddish pink. Powder dried with spray dryer contains higher total phenolic and anthocyanin content than that dried with vacuum dryer (Table 2). Comparing with fresh mixed juice, spray dryer can preserve total phenolic content and anthocyanin content about 26% and 67%, respectively, while vacuum dryer can preserve total phenolic content and anthocyanin content about 25% and 24%, respectively (Table 3). This may possibly due to the high temperature short time process in spray dryer. Although the increment of heating temperature decrease total phenolics, and antioxidant capacity (Chen and Lin (2007), Fang and Bhandari (2011) reported that the retention of total phenolic and anthocyanin content after spray drying bayberry juice were higher than 91%. They also recommended that the operating temperatures are very crucial for the spray drying of heat sensitive materials. The use of the inlet temperature of 150°C and outlet temperature of 80°C will appropriate for spray drying heat sensitive material since the temperature of the spray-droplets was about 40°C -55°C for only short periods (Fang and Bhandari, 2011). Therefore, to adjust the appropriate method for mangosteen powder in spray dry, the condition of inlet and outlet temperature should be considered.

The effect of evaporation and enzyme clarification method on mangosteen concentrate

Mangosteen concentrate was processed by using 2 different evaporation and pectinase and then sensory evaluated with mangosteen juice. According to 9-point hedonic scale (Table 4), mangosteen juice and mangosteen juice diluted from concentrate clarified by pectinase and evaporated by vacuum evaporator (CJV+P) had the highest color preference score followed by the juice diluted from concentrate evaporated by vacuum evaporator (CJV). No significant difference in color preference score was found between mangosteen juice and CJV+P. Most panelists suggested that the color and cloudiness of mangosteen juice, CJV+P and CJV were just right while those of juice diluted from concentrate evaporated by direct heat evaporator (CJH) and juice diluted from concentrate clarified by pectinase and evaporated by direct heat evaporator (CJH+P) were too dark and too cloud (Table 4).

Table 2. Moisture content, water activity, total phenolic and anthocyanin content of mangosteen powder dried with 2 different method

<table>
<thead>
<tr>
<th>Properties</th>
<th>Mangosteen powder dried with vacuum dryer</th>
<th>Mangosteen powder dried with spray dryer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>5.94%</td>
<td>5.94%</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.34</td>
<td>0.37</td>
</tr>
<tr>
<td>Total phenolic content (mg GAE/100 g)</td>
<td>39.6±0.13b</td>
<td>42.5±0.78b</td>
</tr>
<tr>
<td>Anthocyanin content (mg Cya-3-Glu/100 g)</td>
<td>3.59±0.28b</td>
<td>9.97±0.21b</td>
</tr>
</tbody>
</table>

Values are means ± SD
Means in row with different superscript letter differ significantly (p<0.05)

Table 3. Total phenolic and anthocyanin content of mangosteen juice mixed with grape and roselle juice and juice that dissolved from mangosteen powder dried with 2 different method

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolic content (mg GAE/100 ml)</th>
<th>Anthocyanin content (mg Cya-3-Glu/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh mixed juice</td>
<td>59.2±0.05b</td>
<td>3.77±0.29b</td>
</tr>
<tr>
<td>Juice from powder dried with vacuum dryer</td>
<td>982±2.32b</td>
<td>982±0.70b</td>
</tr>
<tr>
<td>Juice from powder dried with spray dryer</td>
<td>10.58±0.15b</td>
<td>2.49±0.01b</td>
</tr>
</tbody>
</table>

Values are means ± SD
Means in columns with different superscript letter differ significantly (p<0.05)

The effect of drying method on mangosteen powder

A juice powder is a product that can be used as a drink or a flavor additive. Upon the preliminary study, the development of mangosteen powder is quite challenging since mangosteen has the sweet and acid taste. Therefore, fruit juices, such as grape juice sand roselle juices were added to improve the flavor of mangosteen juice. After drying with two different methods, both mangosteen powders are a reddish pink. Powder dried with spray dryer contains higher total phenolic and anthocyanin content than that dried with vacuum dryer (Table 2). Comparing with fresh mixed juice, spray dryer can preserve total phenolic content and anthocyanin content about 26% and 67%, respectively, while vacuum dryer can preserve total phenolic content and anthocyanin content about 25% and 24%, respectively (Table 3). This may possibly due to the high temperature short time process in spray dryer. Although the increment of heating temperature decrease total phenolics, and antioxidant capacity (Chen and Lin (2007), Fang and Bhandari (2011) reported that the retention of total phenolic and anthocyanin content after spray drying bayberry juice were higher than 91%. They also recommended that the operating temperatures are very crucial for the spray drying of heat sensitive materials. The use of the inlet temperature of 150°C and outlet temperature of 80°C will appropriate for spray drying heat sensitive material since the temperature of the spray-droplets was about 40°C -55°C for only short periods (Fang and Bhandari, 2011). Therefore, to adjust the appropriate method for mangosteen powder in spray dry, the condition of inlet and outlet temperature should be considered.
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Table 4. Sensory characteristic of mangosteen juice and mangosteen concentrate diluted as 15° Brix by 9-point hedonic scale and 5-point just right scale

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolic content (mg GAE/100 mL)</th>
<th>Anthocyanin content (mg Cyn-3-Glu/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh mixed juice</td>
<td>39.36 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.71 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Juice from powdered dried with vacuum dryer</td>
<td>9.90 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.90 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Juice from powdered dried with spray dryer</td>
<td>10.38 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.49 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SD
Means in columns with different superscript letter differ significantly (p<0.05)
Level in just right scale was determined by the highest frequency panelist rated

Table 5. Color value of mangosteen juice and mangosteen concentrate diluted as 15° Brix

<table>
<thead>
<tr>
<th>Samples</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh mangosteen juice</td>
<td>17.38±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.60±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.68±1.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.76±0.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mangosteen juice from concentrate under direct heat evaporator (CR)</td>
<td>16.25±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.92±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.54±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.52±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mangosteen juice from concentrate under direct heat evaporator + pectinase (CR+P)</td>
<td>17.68±0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.80±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.76±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.76±0.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mangosteen juice from concentrate under vacuum evaporator (CV)</td>
<td>18.54±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.84±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.24±0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.52±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mangosteen juice from concentrate under vacuum evaporator + pectinase (CV+P)</td>
<td>20±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.66±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.66±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.66±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SD
Means in column with different superscript letter differ significantly (p<0.05)

Table 6. Total phenolic and anthocyanin content of mangosteen concentrate

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolic content (mg GAE/100 mL)</th>
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</tr>
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</tr>
</tbody>
</table>

Due to its flavors and its bioactive compounds, mangosteen products were introduced into markets. Most of anthocyanins and phenolics were in pericarp. The edible part of mangosteen contained comparable amount of phenolics as grape, plum, and cherries. Spray drying, a high temperature short time process, can prevent a loss of anthocyanin and total phenolic better than vacuum drying, a drying process under low oxygen condition. However, vacuum evaporation seemed to prevent a loss of anthocyanin and total phenolic better than atmospheric evaporation. The use of pectinase enzyme for juice clarification was tended to beneficially increase the total phenols of mangosteen juice concentrate. However, since mangosteen phenolics are composed of oligoprocyanidin, the advance techniques, such as HPLC, are recommended for further analyzing changes during processing.

**Conclusion**

The use of pectinase enzyme tentatively decreased anthocyanin content (Table 6). These result agreed with those of Versari et al. (1997) who suggested that some pectinase enzyme produced glucosidase activity which has a hydrolytic activity to degrade anthocyanin pigment. Jiang et al. (1990) also reported the 20% loss of raspberry anthocyanin after treated with pectinase enzyme was because some pectinase enzyme contained a glucosidase which can hydrolyzed the β1–2 glucosidic bonds of cyanidin-3-sophoroside and cyanidin-3-glucosylrutinoside into cyanidin-3-glucoside and cyanidin-3-rutinoside, which in turn resulted in decreasing the anthocyanin stability.

**Acknowledgement**

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References


