

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

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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 investigation 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 (Tipco 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 redissolved 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 $^{\circ}H = \tan^{-1}(b^*/a^*)$.

Moisture and water activity

The moisture of mangosteen juice powder was determined by using AOAC method (925.10) while the water activity was measured by using Paw Kit (Dragon Devices Inc., WA, USA).

Anthocyanin

The monomeric anthocyanin content was determined using the pH differential method

(Giusti and Wrolstad, 2001). 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 100g fresh weight or 100 mL. The extinction coefficient of 26,900 Lcm-1mol⁻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 a 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 processess 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

Table 1. Total phenolic and anthocyanin content and EC₅₀ of each part of mangosteen

Part	Total phenolic content (mg GAE/100 g)	Anthocyanin content (mg Cyn-3-Glu /100 g)	EC ₅₀ (µg/mL)
Outer pericarp	2,930.49 ± 318.10 ^b	179.49 ± 10.80 ^a	4.73 ± 0.55
Inner pericarp	3,404.09 ± 321.92 ^a	19.71 ± 22.98 ^b	1.35 ± 0.13
Flesh	133.29 ± 20.44 ^c	Not detected	133.33 ± 25.17

Values are means ± SD

Means in column with different superscript letter differ significantly (p<0.05)

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

Properties	Mangosteen powder dried with vacuum dryer	Mangosteen powder dried with spray dryer
Moisture content	5.9%	3.5%
Water activity	0.34	0.37
Total phenolic content (mg GAE/100 g)	39.62 ± 0.13 ^b	42.31 ± 0.078 ^a
Anthocyanin content (mg Cyn-3-Glu/100 g)	3.59 ± 0.28 ^b	9.97 ± 0.21 ^a

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

Samples	Total phenolic content (mg GAE/100 mL)	Anthocyanin content (mg Cyn-3-Glu/100 mL)
Fresh mixed juice	39.26 ± 0.05 ^a	3.71 ± 0.20 ^a
Juice from powder dried with vacuum dryer	9.90 ± 0.32 ^c	0.90 ± 0.07 ^c
Juice from powder dried with spray dryer	10.58 ± 0.19 ^b	2.49 ± 0.01 ^b

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 challenged since mangosteen has the sweet and acid taste. Therefore, fruit juices, such as grape juice and 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.

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 5 presents the color values of all mangosteen juice. The L* value determine the lightness. The maximum for L* value is 100 representing white while the minimum for L* value is 0 representing black. Chroma is an index of the color intensity while the hue angle (°H) expresses the color tonality from 0/360° (magenta-red), 90° (yellow), 180° (bluish-green), to 270° (blue). The color values of mangosteen juice, CJV, and CJV+P were significantly different. Vacuum evaporator increased the lightness which is correlated with too dark in color score. Our result suggested that heating mangosteen concentrate in the presence of oxygen can produce darker color, more cloudiness and lower lightness (L value). This is possibly due to the role of oxygen in phenolic oxidation (Table 4 and 5). Moreover, the use of pectinase enzyme seemed to preserved the color intensity and increased the hue angle.

Mangosteen concentrate evaporated by vacuum evaporation contained higher total phenolic and anthocyanin content and lower polymeric color than that by direct heat evaporation (Table 6). When the thermal degradation occurred, the anthocyanin content was lost and the color appearance was fade because of the oxidation, cleavage of covalent bonds or enhanced oxidation reactions (Piffaut *et al.*, 1994; Patras *et al.*, 2010). Khanal *et al.* (2010) stated that heating grape and blueberry pomace at lower than 40 °C for up to 3 days may not be damaging their

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

Samples	Total phenolic content (mg GAE/100 mL)	Anthocyanin content (mg Cyn-3-Glu/100 mL)
Fresh mixed juice	39.26 ± 0.05 ^a	3.71 ± 0.20 ^a
Juice from powder dried with vacuum dryer	9.90 ± 0.32 ^a	0.90 ± 0.07 ^a
Juice from powder dried with spray dryer	10.58 ± 0.19 ^b	2.49 ± 0.01 ^b

Values are means ± SD
Means in column 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

Samples	L*	Chroma	°H
Fresh mangosteen juice	17.58±0.34 ^a	11.60±0.90 ^a	33.68±1.66 ^b
Mangosteen juice from concentrate under direct heat evaporator (CJH)	16.25±0.33 ^d	7.92±0.31 ^b	29.55±3.20 ^c
Mangosteen juice from concentrate under direct heat evaporator + pectinase (CJH+P)	17.68±0.81 ^a	11.80±2.64 ^a	39.78±6.22 ^a
Mangosteen juice from concentrate under vacuum evaporator (CJV)	18.54±0.38 ^b	8.94±0.27 ^b	30.24±3.86 ^c
Mangosteen juice from concentrate under vacuum evaporator + pectinase (CJV+P)	20.42±0.18 ^a	11.66±0.75 ^a	39.06±2.70 ^a

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

Samples	Total phenolic content (mg GAE/100 mL)	Anthocyanin content (mg Cyn-3-Glu/100 mL)
Fresh mixed juice	39.26 ± 0.05 ^a	3.71 ± 0.20 ^a
Juice from powder dried with vacuum dryer	9.90 ± 0.32 ^a	0.90 ± 0.07 ^a
Juice from powder dried with spray dryer	10.58 ± 0.19 ^b	2.49 ± 0.01 ^b

procyanidin and total anthocyanin while heating at higher temperature for more than 8 hours results in considerable loss. Hence, vacuum evaporation which the lower heating temperature was used and lower oxygen seemed to be appropriated for preserving total phenolic and anthocyanin. Adams (1973) reported that heating under aerobic condition (oxygen headspace) accelerate anthocyanin degradation. It also caused anthocyanin structure from cyanidin-3-glucoside to cyanidin which was very labile or cyanidin-3-rutinoside to cyanidin-3-glucoside.

The use of pectinase enzyme significantly increased total phenolic content (Table 6). Pectinase enzyme included pectin methyl esterase and depolymerizing enzyme. Since mangosteen is composed of oligomeric procyanidin bonded readily

to cell-wall polysaccharides through hydrogen-bonding and/or hydrophobic interactions (Renard, Baron, Guyot and Drilleau, 2001), pectinase enzyme can degrade the cell-wall and vacuolar membrane and increase the extraction of procyanidin (Oszmiański *et al.*, 2011). The result of Landbo and Meyer (2004) and Landbo *et al.* (2007) showed that the increased pectinase enzyme dosage and the maceration time and temperature had significantly positive effects on the juice yield and total phenol yield in the black currant juice and elderberry juice since the pectinase enzyme addition can breaking down the pectin in the plant cell walls and in the middle lamellae between the plant cells and releasing possible cell wall sited phenolics.

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.

Conclusion

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 phenolics of mangosteen juice concentrate. However, since mangosteen phenolics are composed of oligoprocyandin, the advance techniques, such as HPLC, are recommended for further analyzing changes during processing.

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