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Research Article

Cyanidin, Malvidin and Pelargonidin Content of "Kolang-kaling" Jams Made with Juices from Asian Melastome (*Melastoma malabathricum*) Fruit, Java Plum (*Syzygium cumini*) Fruit Rind or Mangosteen (*Garcinia mangostana*) Fruit Rind

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Abstract

Background and Objective: A previous study showed that anthocyanins are present in Asian melastome fruit, java plum fruit rind and mangosteen fruit rind, as well as the juices from these fruits, but these juices impart different colors when added to "kolang-kaling" jam. This study was conducted to identify types of anthocyanin, Pelargonidin-3-glucoside chloride, malvidin-3-5-diglucoside chloride and cyanidin-3-0-glucoside chloride, in Asian melastome fruit, java plum fruit rind, mangosteen fruit rind and in "kolang-kaling" jam made with juice from these fruits and rinds. **Materials and Methdos:** Pelargonidin, malvidin and cyanidin content in the fruits and rinds was analyzed using a Thermo Ultimate 3000 HPLC with a CD-C18, 3 µm Cadenza column at a column temperature of 35°C. **Results:** The fruits and rinds did not contain pelargonidin-3-glucoside chloride, whereas, malvidin-3-5-diglucoside chloride was found only in java plum fruit rind and cyanidin-3-0-glucoside chloride was present only in Asian melastome fruit. **Conclusion:** The cyanidin-3-0-glucoside chloride and malvidin-3-5-diglucoside chloride contents increased linearly with addition of increasing amounts of juices from these fruits or rinds during "kolang-kaling" jam production.

Key words: Pelargonidin-3-glucoside chloride, malvidin-3-5-diglucoside chloride, cyanidin-3-0-glucoside chloride, *Malastoma melabathricum* fruit, *Syzygium cumini* fruit rind, *Garcinia mangostana* fruit rind, "kolang-kaling" jam

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Attractive color, in addition to flavor and aroma, is an important factor in consumer food choices. Currently, synthetic dyes continue to be widely used to enhance food colors. However, the presence of added synthetic dyes can contribute to unfavorable impressions by consumers and may have a negative impact on health. As such, natural dyes are needed to provide attractive food colorings that can be safely consumed over the long-term. Winarno[1] showed that several natural dyes can be derived from plant, animal and mineral sources. In plants, natural dyes and pigments are present in the form of chlorophyll, carotenoids, tannins and anthocyanins, which are all unstable following exposure to heat, light or variations in pH. Nonetheless, these pigments are generally safe for consumption and have not been shown to have negative health effects.

Fruits and fruit rinds from Asian melastome, java plum and mangosteen plants contain various natural pigments. These pigments arise during plant reproduction processes that occur after the flowers wilt. These fruits will split upon ripening and have pulp that is dark purple with orange seeds. The dark purple color of the fruit indicates the presence of anthocyanins[2]. Swami *et al.*[3] reported that java plum plants are rich in anthocyanin, glucoside and kaempferol. According to Siti-Azima *et al.*[4], java plum fruits contain anthocyanins such as cyanidin (approximately 8.71 mg g^{-1} extract) as well as flavonoids (17.02 mg QE g^{-1}) in total phenolic extracts (43.64 mg GAE g^{-1} extract). On the other hand, mangosteen fruit rind is red to purple in color and also contains anthocyanins[5]. According to Chaovanalikit *et al.*[6], the outer rind of mangosteen fruits have anthocyanin levels that are higher than the inner part of mangosteen fruit, which is pink in color.

Aishah *et al.*[7] showed that anthocyanins are water soluble plant pigments that are present in a wide variety of fruits and vegetables. Anthocyanin pigments have a spectrum of colors ranging from bright orange, pink, red, purple and blue that can be used as natural food colorants to replace synthetic dyes. So, far no negative side effects from the use of anthocyanins as food coloring agents have been found. In this study, it is analyzed the content of three anthocyanins in Asian melastome fruit, java plum fruit rind and mangosteen fruit rind that are used as natural dyes. The amount of anthocyanin in "kolang-kaling" jam produced using juices from these fruits was also assessed.

MATERIALS AND METHODS

Chemicals and standards: Three types of anthocyanin, cyanidin-3-0-glucoside chloride (Sigma-Aldrich, Fujicco, Japan, Cat. No. 1151935), pelargonidin-3-glucoside chloride (Cerilliant USA, Wako, Cat. No. PHY89753) and malvidin-3-5-diglucoside chloride (Cerilliant USA, Funakoshi, Japan, Cat. No. PHY89727) were used as standards for this study. Acetonitrile, acetic acid and methanol were all of HPLC grade.

Samples: Conventionally-grown Asian melastome fruit, java plum fruit and mangosteen fruit were obtained from gardens surrounding the city of Padang, West Sumatra, Indonesia. The fruits were sorted and selected based on maturity, uniform size and absence of visual defects. Only perfectly ripe fruits were used for sampling. Three forms of samples were used (1) Asian melastome fruit, java plum fruit rinds and mangosteen fruit, (2) Juice from the three fruit types and (3) "Kolang-kaling" jam made with natural dyes from the three fruits. In this study, it is referred to Asian melastome fruit as Asian melastome, java plum fruit rind as java plum and mangosteen fruit rind as mangosteen.

Research Procedures

Solvent preparation

Two solvents were prepared: Solvent 1: methanol:acetic acid 325:175 (v/v), pH 1-2; and Solvent 2: 2% acetate solution made from 20 mL acetate in 980 mL of distilled water and filtered with a 0.45 µm pore size filter.

Sample preparation: Approximately 500 mg of the different fruits and fruit juices as well as 5 g of jam were analyzed for anthocyanin content. Samples were placed in a test tube and 9 mL of solvent 1 was added before extraction using ultrasonication for 20 min. The extract solution was then centrifuged for 10 min at 3,500 rpm and 10 mL of the resulting supernatant that was first filtered through a 0.45 µm pore size filter was used as the sample solution.

HPLC analysis of anthocyanin content: Anthocyanin standards were weighed and dissolved in solvent 1 for use in generating a standard curve. The concentration of each standard solution was prepared from a range of 0.04-0.16 mg mL^{-1} for cyanidin-3-glucoside chloride and 0.03-0.12 mg mL^{-1} for pelargonidin-3-glucoside chloride and malvidin-3-5-diglucoside chloride.

A Thermo Ultimate 3000 HPLC equipped with a CD-C18, 3 µm, 250 mm Cadenza column and a column temperature of 35°C was used for analysis of anthocyanin contents. The HPLC was conditioned using the method described by Syukri *et al.*[8] with slight modifications. The mobile phase was (A) acetic acid 2% and (B) 100% Acetonitrile. Elution was achieved using a gradient of 0-10 min, mobile phase B increased

from 0-10%, 10-15 min, mobile phase B increased to 30% and was held at that percentage to 20 and 20-23 min, the mobile phase B returned to 0%. The anthocyanin spectrum was measured at 520 nm and a 10 mL injection volume was used.

RESULTS AND DISCUSSION

The color of Asian melastome, java plum and mangosteen, as well as that of juices and "kolang-kaling" jam, is derived from anthocyanins. Vargas *et al.*[9] showed that more than 540 types of anthocyanins have been described, but the most common anthocyanins in fruits and plants are cyanidin, peonidin, delphinidin, pelargonidin, malvidin and petunidin. Here, the content of three types of anthocyanins, pelargonidin-3-glucoside chloride, malvidin-3-5-diglucoside chloride and cyanidin-3-0-glucoside chloride anthocyanin, in Asian melastome, java plum and mangosteen, as well as juices from these fruits and "kolang-kaling" jam made using these fruit juices was analyzed. The three types of anthocyanins had slightly different retention times, with malvidin having the shortest retention time, followed by cyanidin and pelargonidin (Fig. 1). The analysis showed that pelargonidin-3-glucoside chloride was not present in the three natural colorants that were used, whereas malvidin-3-5-diglucoside chloride was only found in java plum and cyanidin-3-0-glucoside chloride was present only in Asian melastome. None of the three anthocyanins tested were present in mangosteen samples (data not shown). Thus, the skin of mangosteen fruit could contain anthocyanins other than pelargonidin, cyanin and 8,775 mg kg^{-1}. Notably, the cyanidin content of the juice was closer to 1/2 that of the fruit. Although the reason for this malvidin, as Chaovanalikit *et al.*[6] reported that mangosteen fruit does contain anthocyanins and has a higher anthocyanin content in the outer skin of the fruit.

Java plum and Asian melastome anthocyanin content

Malvidin-3-5-diglucoside chloride in java plums and juice: Analysis of anthocyanin content in java plum and its juice showed that malvidin-3-5-diglucoside chloride predominated (Fig. 2).

The amount of malvidine-3-5-diglucoside chloride was higher in java plum fruit (4,120 mg kg^{-1}) than in the juice (1,197 mg kg^{-1}). Java plum juice is produced by mixing java plum with water at a ratio of 1:1, such that the malvidine content of juice should be half that of the fruit. However, our results showed that the anthocyanin content juice was only 1/3 that of the fruit. This finding suggests that some malvidine may not be water soluble, which would be consistent with

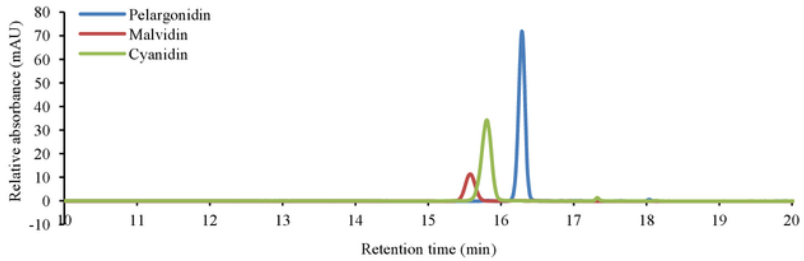

Fig. 1: Retention time (min) and relative absorbance unit at 520 nm of pelargonidin-3-glucoside chloride, malvidin-3-5-diglucoside chloride and cyanidin-3-0-glucoside chloride standards

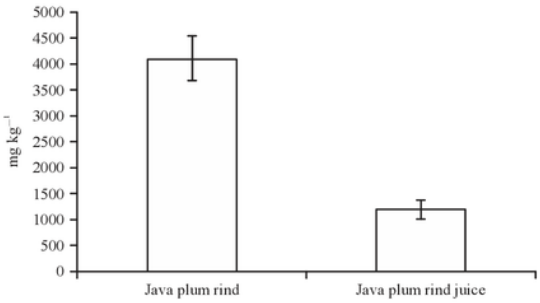

Fig. 2: Concentration of malvidin-3-5-diglucoside chloride in java plum fruit and juice

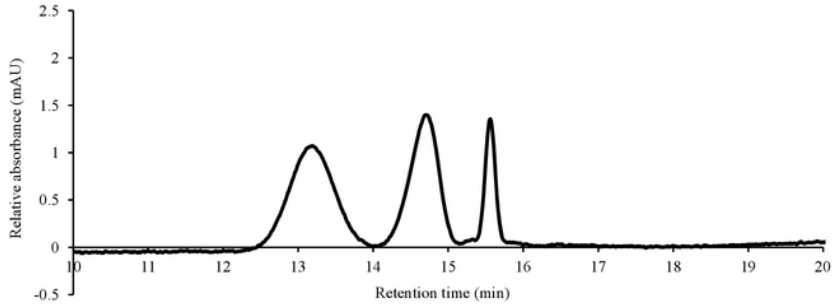

Fig. 3: Retention time (min) and relative absorbance unit at 520 nm of anthocyanins in java plum

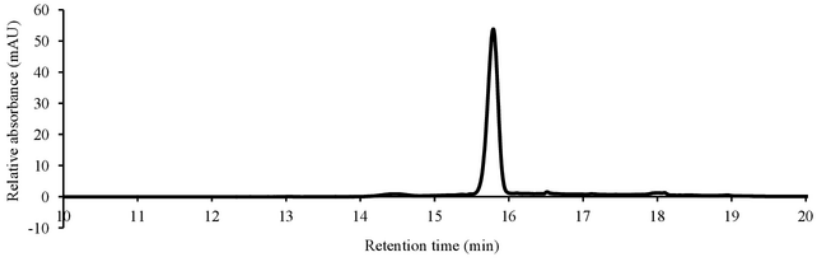

Fig. 4: Retention time (min) and relative absorbance unit at 520 nm of anthocyanin in Asian melastome

results by Sari *et al.*[10] showing that the highest anthocyanin concentration in plum leaf extract is in extracts made using a water-soluble solvent mixed with ethanol.

In analysis of anthocyanin content in java plum samples, the third of three peaks, having a retention time of between 15 and 16 min, was detected as malvidine-3-5-diglucoside chloride (Fig. 3). The two other peaks were likely the anthocyanin compounds petunidin and delphinidin that were reported in an earlier study by De Brito *et al.*[11].

Cyanidin-3-0-glucoside chloride in Asian melastome fruit and juice: Analysis of anthocyanins in Asian melastome fruit produced one peak, which was detected as cyanidin (Fig. 4). Meanwhile, cyanidine-3-0-glucoside chloride content in Asian melastome fruit (35,103 mg kg^{-1}) was significantly higher than that of the juice (16,474 mg kg^{-1}, Fig. 5). The juice was produced from a mixture of Asian melastome pulp with water at a ratio of 3:1. As such, the content of cyanidin in the juice should be 1/4 that of cyanidin in the fruit or about outcome awaits further study, it may be due to the water solubility of cyanidine. According to Ati *et al.*[12], anthocyanin polarity depends on the number of OH groups in the molecular structure, whereas.

DeMann[13] showed that most anthocyanins are soluble in water and other polar solvents. Thao *et al.*[14] showed that anthocyanins containing hydrocarbons and polyphenol components dissolve well in water and polar solvents. Generally, anthocyanin compound extraction is achieved using an organic solvent mixed with water. Moreover, a study by Salisbury and Ross[15] showed that anthocyanin pigments are soluble in water and impart red, purple and blue colors that are found in fruits and flowers. Anthocyanins thus are colorants that are polar and dissolve well in polar solvents.

"Kolang-kaling" jam made with Asian melastome, java plum or mangosteen juice: The addition of juices from java plum, Asian melastome and mangosteen produce different colors in "kolang-kaling" jam. Based on research conducted by Yenrina *et al.*[16], the °Hue value of "kolang-kaling" jam with added juice from Asian melastome fruit or java plum fruit rind ranged from 1.43-14.00, such that the resulting "kolang-kaling" jam had a purplish red color. The °Hue value of "kolang-kaling" jam made with mangosteen rind juice ranged from 20.97-32.33, consistent with its red color.

Although, the color of "kolang-kaling" jam was affected by the anthocyanin contained in the added juice, HPLC analysis showed that the three types of anthocyanin analyzed here were not present in jam made with mangosteen rind juice (data not shown). Thus, mangosteen rind juice likely contains other anthocyanin compounds, consistent with a study by Chaovanalikit *et al.*[6] reporting that anthocyanins are indeed present in mangosteen fruit rinds.

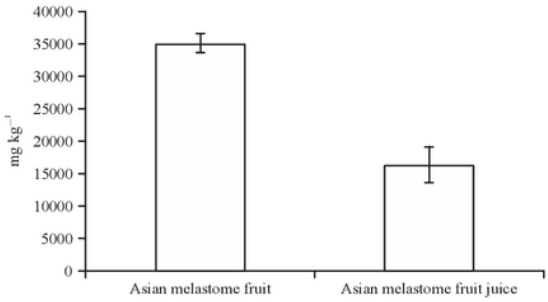

Fig. 5: Concentration of cyanidin-3-0-glucoside chloride in 500 mg and 10 mL Asian melastome fruit and juice, respectively

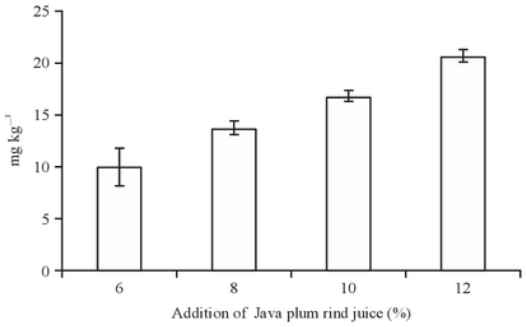

Fig. 6: Concentration of malvidin-3-5-diglucoside chloride in "kolang-kaling" jam with added java plum juice

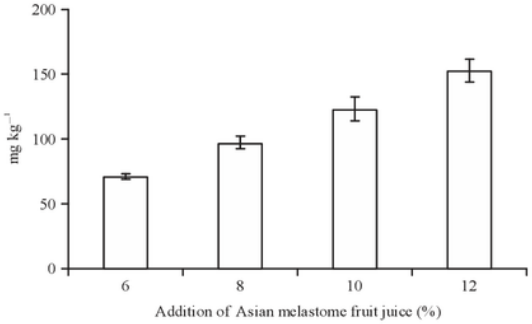

Fig. 7: Concentration of cyanidin-3-0-glucoside chloride in "kolang-kaling" jam with added Asian melastome juice

Malvidin-3-5-diglucoside chloride content of "kolang-kaling" jam with added java plum juice: The addition of 6, 8, 10 and 12% java plum juice increased malvidin-3-5-diglucoside chloride content in "kolang-kaling" jam by 10.1±1.8, 13.7±0.7, 16.8±0.6 and 20.7±0.6 mg kg^{-1}, respectively (Fig. 6). Addition of 2% java plum juice produced a consistent increase of about 3 mg kg^{-1} (data not shown).

According to Huang *et al.*[17], malvidin has antioxidant activity and thus may have a significant role in counter acting the effects of free radicals. Malvidin was also shown to have antihypertensive activity due to its ability to inhibit angiotensin converting enzyme. Furthermore, Passamonti *et al.*[18] found that malvidin had better bioavailability relative to other anthocyanidins due to its ability to bind organic anion membrane carriers.

Cyanidin-3-0-glucoside chloride in "kolang-kaling" jam with added Asian melastome juice: The cyanidin-3-0-glucoside chloride content in "kolang-kaling" jam increased with increasing amounts of Asian melastome juice (Fig. 7). Jam

made with 6, 8, 10 and 12% Asian melastome juice had increases in cyanidin content of 71.3±2.3, 97.4±4.9, 123.4±9.3 and 153.4 ± 8.9 mg kg^{-1}, respectively.

CONCLUSION

Pelargonidin-3-glucoside chloride was not found in any of the three fruits tested. Mangosteen rind samples lacked all of the three anthocyanins tested. Malvidin-3-5-diglucoside chloride was present only in java plum fruit rind, whereas, cyanidin-3-0-glucoside chloride was only found in Asian melastome fruit. The concentration of malvidin-3-5-diglucoside chloride was higher in java plum fruit than the juice. Similarly, cyanidin-3-0-glucoside chloride concentrations were higher in Asian melastome fruit compared to the juice. The concentration of cyanidin-3-0-glucoside chloride in Asian melastome was much higher than that of malvidin-3-5-diglucoside chloride in java plums.

The addition of juices from java plums and Asian melastome resulted in linear increases in the concentration of malvidin-3-5-diglucoside chloride and cyanidin-3-0-glucoside chloride in "kolang-kaling" jam.

SIGNIFICANCE STATEMENTS

This study shows that there were different types of anthocyanins in the tested fruits and juices. There were 3 types of anthocyanins in java plum and one type of anthocyanin in Asian melastome that was soluble in water. Mangosteen rind lacked these 3 anthocyanins. Asian melastome and java plum could thus be beneficial for use as natural dyes in foods. This study provides evidence that anthocyanins are safe to use as natural dyes in food due to the water solubility of these compounds.

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