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Research Article
Effect of Natural Colorants on Color and Antioxidant Activity of "Kolang Kaling" (Sugar Palm Fruit) Jam

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Abstract
Background: The colors of Asian melastome fruit, Java plum rind and Mangosteen rind range from red to dark purple. These fruits are also rich in anthocyanins, making them useful as natural colorants. **Materials and Methods:** In this study, it was evaluated that the color and antioxidant activity of jams produced with different concentrations of juice extracted from these fruits. The juice extracts were added at concentrations of 6, 8, 10 and 12% during the production of sugar palm fruit jam. **Results:** Analysis of sugar palm fruit jam with added juice from Asian melastome fruits, Java plum rinds and Mangosteen rinds produced colors with ^{0}hue values of 6.90-14.00, 1.43-12.87 and 20.97-32.33, respectively and anthocyanin levels of 3.50-8.57, 3.28-11.19 and 1.61-3.73 mg L^{-1}, respectively, the total phenol levels of 1.20-1.60, 1.32-1.94 and 0.83-3.51%, respectively. The antioxidant activity for each treatment exceeded 5,000 ppm, indicating a lack of activity. **Conclusion:** These results show that the addition of different amounts of natural colorants significantly affected the color of sugar palm fruit jam, as well as the total phenol and anthocyanin levels but did not improve antioxidant activity.

Key words: Natural colorant, color, total phenol, anthocyanin, antioxidant activity

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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

Sugar palm fruit contains the polysaccharide galactomannan[1]. The main advantage of galactomannan over other polysaccharides is its ability to form highly viscous solutions at low concentration. Galactomannans are not significantly affected by pH, ionic strength or heating[2]. Galactomannan solutions maintain constant viscosities over a pH range of 1-10.5, which is likely due to the neutral charge of this molecule. Galactomannan is degraded only under very acidic or alkaline conditions or at high temperatures[3].

Galactomannan can also act as an antioxidant. Boual *et al.*[4] showed that a galactomannan-rich water-soluble polysaccharide mixture had antioxidant activity with an IC_{50} of 330 μg mL^{-1}, which exceeds the <500 pg mL^{-1} IC_{50} value that is commonly thought to define antioxidant potential. Because of its contribution to viscosity and antioxidant properties, galactomannan from sugar palm fruit is often used as a raw material for jam production. Jam from sugar palm fruit is colorless, so natural colorants, such as those contained in Asian melastome fruit or rinds from Java plums or mangosteens are often added during jam production.

The purple color of Asian melastome (*Melastoma malabathricum*, L.) fruit is due to its flavonoid content. Anthocyanins are stable across a temperature range[5] of 30-100°C. Between pH 1-3 and 5-9, anthocyanins are red and light blue-purple, respectively.

Anthocyanins extracted from *Musa acuminata* bracts are quite resistant to changes in pH (5.1 and 6.0) and temperature (20 and 30°C) both in the presence and absence of light[6]. Meanwhile, sorghum rich in 3-deoxyanthocyanins has a yellow to orange color in acidic media. Sorghum bran also has a high content of anthocyanins that have characteristics that are similar to those from other plants[7].

However, increased temperature can decrease the total anthocyanin content in foods. For instance, during Java plum powder production, increased drying temperature and periods decrease the total anthocyanin content and the colors brighten[8]. Anthocyanin content in foodstuffs can be preserved by using a processing temperature below 60°C[9]. In addition to anthocyanins, fruits contain other bioactive compounds, including xanthones, prenylated derivatives and procyanidin[10,11].

In this study, we examined how natural colorants derived from Asian melastome, Java plums and Mangosteens can affect the color and antioxidant activity in sugar palm fruit jam.

MATERIALS AND METHODS

Materials: The main materials in this study are sugar palm fruit and natural colorants from Asian melastome fruit, Java plum rind and Mangosteen rind.

Design: The experimental design of this study involved 3 factors (3 natural colorants) and 4 different concentrations. Statistical analysis of data was performed using analysis of variance (ANOVA) and continued by Duncan's New Multiple Range Test (DNMRT) at 5% significant level. The colorant concentrations were 6, 8, 10 and 12% (w/w).

Sugar palm fruit jam production: Sugar palm fruit jam was made by mixing sugar palm fruit pulp and sugar at a ratio of 45:55 (w:w) and adding fruit extracts at increasing concentrations. Natural colorants were prepared as slurries by adding water at a ratio of 1:3 (fruit:water) for Asian melastome fruit and Mangosteen rind and 1:1 for Java plum rind.

Sugar palm fruit jam characterization: To assess the resulting sugar palm fruit jam, color tests were performed according to the Hunter lab system[12]. Total anthocyanin content was determined using the pH differential method[13]. Total phenol content was assessed using the Folin-Ciocalteu method[14] and antioxidant activity was determined by measuring absorbance of 2,2-diphenyl-1-picryhydrazyl (DPPH) at 517 nm[15].

RESULTS AND DISCUSSION

Raw materials: The total phenol and anthocyanin contents, as well as antioxidant activity of Asian melastome fruit juice and juice extracted from Java plum and Mangosteen rinds were assessed before use in jam production (Table 1).

The total phenol content in Asian melastome fruit juice was higher relative to juice extracted from Java plum and Mangosteen rinds (Table 1). Meanwhile, Java plum rind extracts had the highest total anthocyanin content (Table 1), which was expected given that Java plum rind juice

Table 1: Chemical contents and antioxidant activity of Asian melastome fruit juice and juice extracted from Java plum and Mangosteen rinds

	Fruit juice		
Variables	Asian melastome	Java plum rind	Mangosteen rind
Total phenol (%)	1.93±0.66	1.07±0.08	1.67±0.02
Anthocyanin (mg L^{-1})	38.91±21.9	71.30±3.19	20.48±1.99
Antioxidant activity (ppm)	1726,83	178,92	>5000

Numbers are the Mean±SD

Table 2: Color of sugar palm fruit jam with additives

Juice	Percentage added	L*	a*	b*	Chroma	^{0}hue
Asian melastome	6	24.46 ± 0.04^c	18.62 ± 1.12^c	4.50 ± 0.01^c	18.50 ± 0.03^d	14.00 ± 0.1^d
	8	24.21 ± 0.07^b	12.08 ± 0.05^b	2.24 ± 0.00^b	12.31 ± 0.07^c	10.47 ± 0.05^c
	10	23.06 ± 0.04^a	8.35 ± 0.05^a	1.06 ± 0.03^a	8.41 ± 0.05^a	7.20 ± 0.26^a
	12	24.17 ± 0.06^b	8.75 ± 0.03^a	1.07 ± 0.00^a	8.81 ± 0.03^b	6.90 ± 0.00^b
p-value		0.00	0.00	0.00	0.00	0.00
Java plum rind	6	24.94 ± 0.05^d	15.84 ± 0.04^d	3.65 ± 0.02^d	16.25 ± 0.05^d	12.87 ± 0.05^d
	8	23.58 ± 0.07^c	9.49 ± 0.01^c	1.46 ± 0.01^c	9.60 ± 0.01^c	8.70 ± 0.10^c
	10	23.44 ± 0.03^b	8.00 ± 0.00^b	0.87 ± 0.01^b	8.04 ± 0.00^b	6.20 ± 0.10^b
	12	15.81 ± 0.05^a	4.63 ± 0.04^a	0.12 ± 0.01^a	4.63 ± 0.04^a	1.43 ± 0.11^a
p-value		0.00	0.00	0.00	0.00	0.00
Mangosteen rind	6	27.56 ± 1.14^a	26.68 ± 0.04^c	16.92 ± 0.03^c	31.58 ± 0.05^c	32.33 ± 0.05^d
	8	32.73 ± 0.18^c	25.45 ± 0.15^b	13.15 ± 0.12^b	28.64 ± 0.19^b	27.30 ± 0.10^b
	10	32.62 ± 0.10^b	28.22 ± 0.10^d	14.93 ± 0.09^d	31.92 ± 0.13^d	27.83 ± 0.05^c
	12	28.06 ± 0.02^c	20.63 ± 0.02^a	7.93 ± 0.01^a	22.10 ± 0.02^a	20.97 ± 0.05^a
p-value		0.00	0.00	0.00	0.00	0.00

Numbers are the Mean±SD, means within the same column having different superscripted letters showed a significant difference ($p<0.05$) by Duncan's New Multiple Range Test (DNMRT), L*: Value of dark and light colors. Low L* values (0-50) indicate darker color and L* >50 indicates bright colors, a*: Represents red and green. Positive and negative values indicate red and green, respectively, b*: Represents yellow and blue. Positive and negative values indicate yellow and blue, respectively

was obtained from a 1:1 juice:water mixture, whereas Asian melastome fruit and Mangosteen rind juice was a 1:3 juice:water mixture.

The phenol content of Asian melastome fruit was previously determined as 1.92 g gallic acid equivalent[16] (GAE)/100 g and the anthocyanin level in this fruit[17] is 49.90 mg L^{-1}. Java plum rind juice had 43-64 mg GAE g^{-1} and an anthocyanin level of approximately 8.71 mg cyanidin g^{-1} extract[11]. Mangosteen rind juice contained 50.51-54.60 mg g^{-1} total phenol and had an anthocyanin level[17] of 5.70-6.20 mg g^{-1}. Antioxidant activity can be analyzed by determining IC_{50} values that are defined as the concentration of antioxidant compounds that cause the loss of 50% DPPH activity[18]. Compounds having lower IC_{50} values have higher antioxidant activity. Here, the IC_{50} value of Java plum rind juice was lower (101-250 ppm) than that of Asian melastome fruit juice and Mangosteen rind juice (>500 ppm).

Color test: Color is an essential element of food products and is important for consumer preference. Mixing thoroughness during food production is characterized by prevalent and evenly distributed color[19]. Here, the Hunter lab system was used to test whether the addition of natural colorants improved the color of sugar palm fruit jam (Table 2).

Sugar palm fruit jam with added Asian Melastome fruit juice or rinds from Java plum or Mangosteen rind as natural colorants had varying values for L*, a* and b*, chroma and ^{0}hue (Table 2). The Hunter system is widely used to describe food colors[20]. In this system, color is divided into 3 dimensions. The symbol a* refers to the red/green component, whereas b* describes the yellow/blue component. The 3rd dimension of color L* indicates lightness or brightness.

According to Hunter system notation, a* values between 0 and +100 indicate red color and those between 0 and -80 indicate green. Meanwhile, b* color intensity expresses a chromatic mixture of blue-yellow wherein a*+b* values from 0 to +70 are yellow and values from 0 to -70 are blue. Values for ^{0}hue represent the dominant wavelength that determines whether colors are red, green and yellow, whereas the chroma value expresses color intensity[19,21]. The ^{0}hue values can be grouped as follows:

^{0}hue 342-18:	Red purple	^{0}hue 162-198: Green
^{0}hue 18-54:	Red	^{0}hue 306-342: Purple
^{0}hue 54-90:	Yellow red	^{0}hue 270-306: Blue purple
^{0}hue 90-126:	Yellow	^{0}hue 198-234: Blue green
^{0}hue 234-270:	Blue	^{0}hue 126-162: Yellow green

The addition of Asian melastome fruit juice or juice extracted from Java plum or Mangosteen rinds to sugar palm fruit jam decreased values for L*, a* and b*, chroma and ^{0}hue relative to jam without additives (Table 2). The decline in L* was associated with a darker color, whereas positive values for a* and b* indicated that the jam had a more yellow and red color, respectively.

The ^{0}hue value for sugar palm fruit jam with Asian melastome juice ranged from 1.43-14.00, which translates to a purplish-red color (Table 2). Addition of juice extracted from rinds produced hue values between 20.97 and 32.33, which are associated with a red color (Table 2). The changes in color of sugar palm fruit jam were likely due to the anthocyanin content of the additives[13].

Total anthocyanins: Given the change in color produced by the jam additives, we next assessed the anthocyanin content

Table 3: Total anthocyanin content of sugar palm fruit jam with additives

Percentage added	Asian melastome fruit (mg L^{-1})	Java plum rind (mg L^{-1})	Mangosteen rind (mg L^{-1})
6	3.50 ± 1.46^{a}	3.28 ± 2.27^{a}	1.61 ± 0.254^{a}
8	5.51 ± 0.60^{ab}	4.17 ± 0.60^{a}	2.22 ± 0.253^{b}
10	8.12 ± 1.84^{b}	4.90 ± 1.18^{a}	2.61 ± 0.255^{b}
12	8.57 ± 2.61^{b}	11.19 ± 1.04^{b}	3.73 ± 0.258^{c}
p-value	0.026	0.00	0.00

Values are Mean±SD, means within the same column with different superscripted letters showed a significant difference

Table 4: Total phenol of sugar palm fruit jam with additives

Percentage added	Asian melastome fruit	Java plum rind	Mangosteen rind
6	1.22 ± 0.08^{a}	1.32 ± 0.09^{a}	0.83 ± 0.03^{a}
8	1.60 ± 0.27^{b}	1.48 ± 0.07^{ab}	1.03 ± 0.09^{a}
10	1.27 ± 0.01^{a}	1.62 ± 0.17^{b}	3.04 ± 0.20^{b}
12	1.20 ± 0.11^{a}	1.94 ± 0.13^{c}	3.51 ± 0.30^{c}
p-value	0.05	0.02	0.00

Values are Mean±SD, means within the same column with different superscripted letters showed a significant difference

of the jam. The palm fruit jam with added Asian melastome fruit juice had anthocyanin contents that ranged between 3.50 and 8.57 mg L^{-1}, whereas the addition of Java plum or Mangosteen rind juice produced lower anthocyanin contents that ranged between 3.28 and 11.19 mg L^{-1} and 1.61 and 3.73 mg L^{-1}, respectively (Table 3). The highest values were seen for jams that had the highest amount (12%) of additive (Table 3).

These results are in contrast to previous analyses which showed that the anthocyanin content in Java plum rind juice was 71.30 mg L^{-1} juice, whereas Asian melastome fruit juice and Mangosteen rind juice had lower values of 38.91 and 20.48 mg L^{-1} juice, respectively. The differences between this study and these values may be due to the influence of several factors, including pH, temperature, enzymes, metals and co-pigments on anthocyanin stability. Indeed, Nugraheni[22] found that anthocyanin degradation could be influenced by temperature. Moreover, hydroxylated anthocyanin is less heat stable than methylated or glycosylated anthocyanin.

Total phenol: Next measured the effect on phenol content of adding natural colorants during sugar palm fruit jam production. Java plum rind juice added across the 6-12% range produced increases of about 0.6% by weight. Jam with added Asian melastome juice extract had the lowest phenol content (between 1.20 and 1.60%) and Mangosteen fruit rind juice increased the phenol content to 3.51% when added at 12% (Table 4). Jam with added 8% Asian melastome juice had the higher phenol level and then decreased with added 10 and 12% of the fruit juice (Table 4), the more Asian melastome juice added, the higher phenol in the jam, it was assumed that it does not enough solvent to extract al phenols in jam, so not all phenols present in the jam is detected.

Phenolic compounds (polyphenols) are thought to act as antioxidants in Java plums. The astringent and sour taste of Java plum fruits is due to its content of tannin polyphenols, anthocyanins, glycosylated cyanidin, petunidin, malvidin, gallic acid, delphinidin-3-gentiobioside and eyanidindicli glycoside[23].

Polyphenols are also naturally present in vegetables (broccoli, cabbage, celery), fruits (apple, pomegranate, melon, cherry, pear and strawberries), bean nuts (walnut, soybean, peanut), olive oil and beverages (tea, coffee, chocolate and wine)[24]. Polyphenols are generally plentiful in fruit rinds. Polyphenol compounds consist of several subclasses namely, flavonol, tannin, isoflavone (soy), flavanone, anthocyanidin, catechin and biflavan. In general, the antioxidant activity of phenolic compounds depends on several factors such as the position and bonding of hydroxyl groups on the aromatic ring, the ability to act as a hydrogen or electron donor and its ability to degrade free radicals.

Phenolic compounds can react with fatty radicals due to the substitution of alkyl groups at positions 2, 4 or 6 that increases the electron density on the hydroxyl group. Radical phenols that form after phenol reacts with fatty radicals are stabilized by delocalization of unpaired electrons to the aromatic ring[25]. Ukieyanna *et al.*[25] asserted that the total phenolic content accounts for 77% of antioxidant activity in plants.

Antioxidant activity: An antioxidant activity analysis was conducted to determine the IC_{50} values of the different jams prepared with Asian melastome fruit juice or juice extracts from Java plum or Mangosteen rinds (Table 5). As determined by a DPPH test, all sugar palm fruit jam with additive concentrations ranging from 6-12% had IC_{50} values >5000 ppm (Table 5). The IC_{50} values can be defined as the concentration needed to inhibit free radical activity of DPPH by 50%. The IC_{50} value is inversely proportional to the ability of a substance or substances to act as an antioxidant, wherein smaller IC_{50} values are associated with greater antioxidant activity[19]. A compound has very high antioxidant activity if its IC_{50} value is less than 50 ppm and is inactive if the IC_{50} value is >500 ppm[26]. Thus, the additives in this study had no antioxidant activity.

Antioxidants are low molecular weight electron donors or reductant compounds that can inactivate oxidation reactions by preventing the formation of radicals. Antioxidants study by donating an electron to compounds that are oxidized that in

Table 5: Antioxidant activity (IC_{50}) of sugar palm fruit jam with additives

Percentage added	Asian melastome fruit (ppm)	Java plum rind (ppm)	Mangosteen rind (ppm)
6	>5000	>5000	>5000
8	>5000	>5000	>5000
10	>5000	>5000	>5000
12	>5000	>5000	>5000

turn inhibits the oxidant activity of the recipient molecules. Antioxidant compounds also can inhibit oxidation reactions and mitigate cell damage by binding free radicals and highly reactive molecules[27].

Based on the mechanism of action, antioxidants can be classified into three groups: (1) Primary antioxidants (antioxidants endogenous), which stop the chain reaction of radical formation by providing hydrogen atoms to stabilize radicals, (2) Secondary antioxidants (Exogenous antioxidants), such as metal chelate compounds containing Cu or Fe that act as a proxidant and (3) Tertiary antioxidant, which are compounds that can repair damage caused by radicals and include DNA-repair enzymes and methionine sulfoxide reductase[14].

According to Swami *et al.*[28], Java plum fruit is rich in anthocyanin compounds, glucoside and kaempferol, as well as alkaloids, jambosine and jamboline glycosides that can act as antioxidants. Asian melastome fruit has a total antioxidant activity of 5.878 µM Acid Equivalent Antioxidant Capacity (AEAC) g^{-1} dry weight and 157.15 mg/100 g dry weight as determined with DPPH[16]. Meanwhile, Mangosteen rind is rich in pectin and bioactive compounds, such as phenolic compounds, anthocyanins and xanthone, which is a powerful antioxidant compound[29]. Xanthone compounds are organic compounds derived from diphenyl-γ-pyron that can be grouped according to the phenol or polyphenolic type[25].

The very low antioxidant activity we found in sugar palm fruit jam is likely not due to processing factors but instead may be because the additives had a very low phenolic content. Antioxidant activity can be influenced by physical factors, such as high oxygen pressure, heating or irradiation and moisture content of materials[30]. Thus, the temperature used in jam processing should be kept below 50°C to avoid damage to compounds with antioxidant activity.

CONCLUSION

Here we found that the addition of Asian melastome fruit juice or juice extracts from Java plum rind or Mangosteen rind added during production of sugar palm fruit jam significantly affected the color, total anthocyanin content and total phenol content of the jam that was produced. However, the antioxidant capacity of the jam was not affected (>5,000 ppm). Color test results indicated that the addition of Asian melastome fruit juice and Java plum rind juice gave a red purple color to the jam (^{0}hue: 1:43-14:00), whereas jam made with Mangosteen rind juice was red (^{0}hue: 20.97-32.33).

Anthocyanin levels in sugar palm fruit jam with added Asian melastome fruit juice, Java plum rind juice and Mangosteen rind juice increased with increasing amounts of additive.

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