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Research Article
Effect of 'Jamblang' (*Syzygium cumini*) Peel and Citric Acid Addition on Antioxidant Activity of 'Kolang-kaling' Jam

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Abstract

Background and Objective: The addition of 'jamblang' peel could increase the beneficial properties of 'Kolang-kaling' jam, including antioxidant activity. This study was conducted to evaluate the effect of 'jamblang' peel and citric acid addition on the antioxidant activity of 'Kolang-kaling' jam. **Materials and Methods:** A factorial design with a completely randomized design that considered 2 factors was used. Factor A was addition of 'jamblang' peel, at four concentrations: A1 (10%), A2 (15%), A3 (20%) and A4 (25%) and factor B was the addition of citric acid at three concentrations: B1 (0.2 g), B2 (0.3 g) and B3 (0.4 g). 'Kolang-kaling' jam with the various additions was analyzed for total phenol and total anthocyanin content, as well as antioxidant activity (IC_{50}). **Results:** The addition of 'jamblang' peel and citric acid significantly ($p<0.05$) and synergistically ($p<0.05$) increased the total phenol and anthocyanin content of 'Kolang-kaling' jam in a dose-dependent manner. Meanwhile, the IC_{50} of the jam dose-dependently decreased with 'jamblang' peel and citric acid addition and was negatively correlated with total phenol content ($r = -0.881$) and anthocyanin levels ($r = -0.683$). **Conclusion:** The highest antioxidant activity, (1,376 ppm) was seen in jam production with 25% 'jamblang' peel and 0.4 g citric acid. Anthocyanin had a stronger effect on antioxidant activity than did citric acid. Although the antioxidant activity in 'Kolang-kaling' jam was relatively weak even with 'jamblang' peel and citric acid supplementation, this food still could be used as an antioxidant substance.

Key words: Kolang-kaling, 'jamblang' (*Syzygium cumini*) peel, citric acid, anthocyanin, total phenol and 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

The endosperm of palm tree seeds (Kolang-kaling) has various nutrients that are beneficial for human health. 'Kolang-kaling' has a very high water content (~97.00%) and also contains 0.01% ash, 0.10% fat, 1.21% protein and 2.37% carbohydrate. This fruit contains fiber that can aid digestion and serve as a source of galactomannan polysaccharide that carries mannose and galactose[1]. 'Kolang-kaling' has a crude fiber and galactomannan content of 0.97 and 2.16%, respectively[2]. Galactomannan confers a high viscosity when mixed in liquid and thus is widely used as a thickener, stabilizer, emulsifier and additive in various foods and medicines[3].

The rate of development of products derived from 'Kolang-kaling' is increasing. 'Kolang-kaling' jam is one such product. However, 'Kolang-kaling' produces jam with a pale color, which is an important consideration for consumer appeal. To increase the consumer value of 'Kolang-kaling' jam, shredded 'jamblang' peel containing anthocyanin pigments can be added as a natural colorant[4]. Anthocyanin also has antioxidant, anti-carcinogenic, anti-diabetic, neuroprotective and anti-inflammatory activity[5].

Despite the addition of 'jamblang' peel during juice production, the anthocyanin content of 'Kolang-kaling' jam often remains low due to several processing factors[4]. Moreover, addition of low concentrations of 'jamblang' peel juice decreases the antioxidant activity of the jam. Therefore, to increase the appeal and antioxidant activity of 'Kolang-kaling' jam, the optimal amount of added 'jamblang' peel should be determined.

Another characteristic of jam is texture, which can be enhanced through the formation of pectin-sugar-acid gels[6]. Citric acid is frequently used to acidify, neutralize and maintain the degree of acidity of foodstuffs as well as to alter taste and color. Citric acid is also used as a preservative. In jam production, the addition of citric acid can enhance gel formation[7]. The level of acidity also inversely affects the stability of anthocyanin, which is more stable at acidic pH than at alkaline pH[8]. This study was designed to examine how the addition of varying amounts of both 'jamblang' peel and citric acid affected the anthocyanin content and antioxidant activity of 'Kolang-kaling' jam.

MATERIALS AND METHODS

Materials: 'Kolang kaling' and sugar were obtained from a traditional market in Padang City and 'jamblang' fruit was obtained from a region near Padang City, West Sumatra. Citric acid were obtained from Smart Lab, Indonesia.

Design: A completely randomized design with 2 factors was used. Factor A represented the 'jamblang' peel concentration and factor B was the citric acid concentration. Three replications were performed for each concentration.

Factor A: Concentration of 'jamblang' peel added to the following amounts:

- A1 = 10% 'jamblang' peel
- A2 = 15% 'jamblang' peel
- A3 = 20% 'jamblang' peel
- A4 = 25% 'jamblang' peel

Factor B: Amount of citric acid added:

- B1 = 0.2% citric acid
- B2 = 0.3% citric acid
- B3 = 0.4% citric acid

'Kolang-kaling' pulp production: 'Kolang-kaling' pulp was produced according to the method described by Sayuti *et al.*[4].

Shredded 'Jamblang' peel preparation: 'Jamblang' fruit was washed and the peel was removed. The peel was then shredded using a blender.

Determination of jam composition

Total phenol: The total phenol content was determined according to the method described by Arnous *et al.*[9]. Briefly, 1 g of sample was added to 10 mL of methanol (Smart Lab, Indonesia) and 1 mL of this mixture was added to 2 mL distilled water containing 1 mL of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany). The solution was mixed for 1 min before 1 mL Na_2CO_3 (7.5%, Merck, Darmstadt, Germany) was added. After mixing, the solution was allowed to stand at room temperature for 60 min in the dark. The absorbance of the solution at 725 nm was measured with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) and the total phenol content was expressed as gallic acid equivalents (GAE).

Total anthocyanin levels: The pH-differential method described by Lee *et al.*[10] was used to determine the total anthocyanin levels of the jams. In brief, 1 g of sample was mixed with 9 mL ethanol (Smart Lab, Indonesia) and 1 mL HCl

(Smart Lab, Indonesia). Then, buffer containing 4 mL KCl (pH 1.0, Merck, Darmstadt, Germany) and 4 mL sodium acetate (pH 4.5, Merck, Darmstadt, Germany) was added. Mixed absorbance was measured at 510 and 700 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The absorbance was calculated as A = [(A510-A700) pH 1.0]-[(A510-A700) pH 4.5] with a molar extinction coefficient of 26.900 for anthocyanin. Total anthocyanin was calculated as cyanidin-3-glucoside using the following equation:

$$\text{Anthocyanin content (mg)} = \frac{(A \times MW \times DF \times V \times 10^3)}{\varepsilon \times L \times m}$$

Where:

A = Absorbance
MW = Molecular weight of cyanidine-3-glucoside (449.2 Da)
DF = Dilution factor
V = Final volume (mL)
10^3 = Conversion factor from g to mg
ε = Absorbance of cyanidine-3-glucoside molar (26.900)
L = Cuvette width (1 cm)
m = Sample weight (g)

Antioxidant activity: The antioxidant activity of the jam was described as DPPH IC_{50} determined as described by Mosquera *et al.*[11]. Briefly, 1 g of sample was dissolved in 10 mL methanol and homogenized. The indicated volume of solution (0.5, 1.0, 1.5, 2.0 mL) was diluted with methanol and distilled water was added 1:1 in a 10 mL volume. Then, 1 mL of this solution was added to 1 mL DPPH (5 mg/100 mL methanol) (Sigma, USA) and the mixture was incubated at room temperature in the dark for 15 min. The absorbance of the solution at 517 nm was measured using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The percentage of each inhibition was calculated as:

$$\text{Free radical activity (\%)} = \frac{A - B}{A} \times 100$$

Where:

A = Absorbance of blank
B = Absorbance of sample

The percentage of free radical amounts was plotted relative to the concentration of the tested solution. The IC_{50} can then be determined using a near regression equation. The IC_{50} value describes the concentration of sample needed to counteract half (50%) of the DPPH free radicals.

Statistical analysis: The data obtained were first statistically analyzed by F-test. For factors showing significant differences, a Duncan's new multiple range test (DNMRT) test at 5% significance level was then performed.

RESULTS AND DISCUSSION

Total phenol analysis of variance (ANOVA) showed an interaction between factor A ('jamblang' peel) and factor B (citric acid) on the total phenol content of 'Kolang-kaling' jam (Table 1).

Total phenol can be measured using the Folin-Ciocalteau method, which involves an oxidation-reduction reaction that forms blue phosphotungstat-phosphomolibdat[10] that can be used to estimate all phenolic compounds present, including flavonoids, anthocyanin and non-flavonoid phenolic compounds. The total content of phenol in each extract was expressed in gallic acid equivalents (GAEs). Gallic acids are phenol compounds that have antioxidant activity[7].

The total phenol content in the 'Kolang-kaling' jam increased as the concentration of 'jamblang' peel increased. Total phenol also increased with increasing citric acid

Table 1: Total phenol content of 'Kolang-kaling' jam made with different amounts of 'Jamblang' peel and citric acid

Total phenol (mg GAE g^{-1}) ± Standard deviation

Factor A ('Jamblang' peel) (%)	Factor B (Citric acid)			
	B1 (0.2 g)	B2 (0.3 g)	B3 (0.4 g)	Average ± SD
A1 (10)	11.90±0.282	14.50±0.282	15.65±0.070	14.01±1.727^{a}
A2 (15)	14.15±0.070	15.20±0.141	17.60±0.282	15.65±1.588^{b}
A3 (20)	16.50±0.282	17.05±0.070	16.20±0.144	16.58±0.411^{c}
A4 (25)	17.75±0.212	17.90±0.141	20.45±0.212	18.70±1.365^{d}
Average ± SD	15.07±2.404^{A}	16.16±1.469^{B}	17.47±1.992^{C}	

Coefficient of variation (%) = 0.052

Columns and values followed by different letters (upper and lowercase, respectively) were significantly different according to DNMRT at 5% significance level. p-values for Factor A: 0.000, Factor B: 0.000 and Factor A*B: 0.000 were <0.05, <0.5 and >0.05, respectively

Table 2: Total anthocyanin content of 'Kolang-kaling' jam containing different amounts of 'Jamblang' peel and citric acid

Anthocyanin (mg L^{-1}) ± Standard deviation				
Factor A ('Jamblang' peel) (%)	Factor B (Citric acid)			
	B1 (0.2 g)	B2 (0.3 g)	B3 (0.4 g)	Average ± SD
A1 (10)	2.8508 ± 0.8078	4.9551 ± 1.0855	9.8870 ± 1.0873	5.8976 ± 3.3202[a]
A2 (15)	11.9777 ± 0.2713	12.7382 ± 0.8092	15.8142 ± 0.8387	13.5101 ± 1.8941[b]
A3 (20)	22.0330 ± 0.5458	25.0661 ± 1.0724	28.7203 ± 0.8291	25.2731 ± 3.0654[c]
A4 (25)	29.9926 ± 0.5114	32.3161 ± 1.0753	34.9808 ± 1.0704	32.4298 ± 2.3445[d]
Average ± SD	16.7135 ± 10.952[A]	18.7689 ± 11.369[B]	22.3506 ± 10.690[C]	
Coefficient of variation (%) = 0.189				

Columns and values followed by different letters (upper and lowercase, respectively) were significantly different according to DNMRT at 5% significance level. p- values for Factor A: 0.000, Factor B: 0.000 and Factor A*B: 0.182 were <0.05, <0.5 and >0.05, respectively

concentration. The phenol content of 'Kolang-kaling' jam ranged from 11.90-20.45 mg GAE g^{-1} sample tested at a concentration of 100,000 ppm. 'Kolang-kaling' jam produced with the A_4B_3 treatment (25% 'jamblang' peel and 0.4 g citric acid) had the highest phenol content at 20.45 mg GAE g^{-1}. Meanwhile, the lowest total phenol content (11.90 mg GAE g^{-1}) was obtained with treatment A_1B_2 (10% 'jamblang' peel and 0.2 g citric acid) . This effect was likely due to the phenol content of the 'jamblang' peel, which contained 1.07 total phenol in the extract, whereas, 'Kolang-kaling' jam with added 'jamblang' fruit juice contained 1.32-1.94 mg GAE g^{-1} total phenol tested at a concentration of 100,000 ppm[4]. The total content of phenol in 'jamblang' peel ranges between 18.2 and 49.1 mg GAE g^{-1} [12].

The addition of citric acid with 'jamblang' peel had a synergistic effect on polyphenol content. Anthocyanin is a polyphenol that is stable at low pH and contributes to the total polyphenol content. Citric acid is a secondary antioxidant that is often added to fats and oils in combination with primary antioxidants and thus yields a synergistic effect to increase the activity of primary antioxidants. Citric acid is also a chelating agent that synergizes with polyphenols to bind oxidizing metals[13]. Some antioxidants such as citric acid, ascorbic acid and phospholipids are synthetic antioxidants that in themselves have no antioxidant activity but can enhance the activity of actual antioxidants[6].

Total anthocyanin: The peel of 'jamblang' fruit contains anthocyanin, which imparts color to jam products. Due to its polarity, anthocyanin is soluble in polar solvents and thus ethanol was used a solvent for analysis of total anthocyanin content[14]. This study showed no interaction between factor A and factor B for the total anthocyanin content of 'Kolang-kaling' jam (Table 2).

The results of the analysis showed that total anthocyanin in 'Kolang-kaling' jam made with different concentrations of 'jamblang' peel ranged from 5.89-32.42 mg L^{-1} tested at 100,000 ppm. The anthocyanin content increased concurrently with increasing amounts of added 'jamblang' peel. Levels of anthocyanin in 'jamblang' fruit juice had a value of 73.14 mg L^{-1}, whereas the resulting jam products with added juice had anthocyanin values ranging from 4.34-10.60 mg L^{-1}. Anthocyanins generally are present on the surface (peel) of the fruit. Mature jamblang peel contains anthocyanin levels of 731 mg/100 g fruit peel (wb), whereas, ripe fruit has an anthocyanin content of 161 mg/100 g fresh fruit (wb)[15].

Citric acid also affects anthocyanin stability in 'Kolang-kaling' jam. Here, the anthocyanin content of 'Kolang-kaling' jam made with different amounts of citric acid ranged between 16.71 and 22.35 mg L^{-1}. Citric acid is an organic solvent that releases protons in acidic solutions. Here, the presence of citric acid released protons and denatured cell membranes in the 'jamblang' peel and in turn dissolved cellular anthocyanin pigments[16]. Anthocyanins are more stable at acidic pH[8] and stability is also affected by solvent, temperature, anthocyanin structure and concentration, oxygen, light and enzymes. For example, the presence of vanillyacetone enhances the color of anthocyanin in berries[17] and thus the addition of other compounds could enhance anthocyanin-induced color of 'Kolang-kaling' jam.

IC_{50} antioxidant activity: Antioxidants can counteract or reduce the negative effects of oxidants in the body[18]. Most antioxidants in fruits and vegetables originate from vitamin C, vitamin E or β-carotene[19]. Here, analysis of antioxidants in 'Kolang-kaling' jam was expressed quantitatively in terms of IC_{50}. Jam samples dissolved in methanol to 100,000 ppm were then diluted to 100, 150, 200 and 250 ppm to generate an IC_{50} curve from which antioxidant activity could be determined (Table 3). The inhibitory concentration (IC_{50}) is a parameter used to denote the amount of substance that inhibits 50% of DPPH free radicals. Substances having lower IC_{50} values will have greater antioxidant activity[20].

Table 3: Antioxidant activity of 'Kolang-kaling' jam with different amounts of 'Jamblang' peel and citric acid

Antioxidant activity IC_{50} (ppm)			
Factor A ('Jamblang' peel) (%)	Factor B (Citric acid)		
	B1 (0.2 g)	B2 (0.3 g)	B3 (0.4 g)
A1 (10)	5,773	4,003	2,921
A2 (15)	5,537	3,036	2,762
A3 (20)	3,585	2,643	2,592
A4 (25)	3,270	1,654	1,376

Here the antioxidant value of 'Kolang-kaling' jam ranged between 1,376 and 5,773 ppm. Although relatively weak, 'Kolang-kaling' jam could still provide antioxidant benefits. A previous study showed that antioxidant activity was obtained in jam with the addition juice from 'jamblang' peels to >5,000 ppm[4]. The antioxidant content of jamblang peel extract was 178.92 ppm. IC_{50} values indicated that 'Kolang-kaling' jam made with shredded 'jamblang' peel had a greater antioxidant value than 'Kolang-kaling' jam made with 'jamblang' juice. 'Jamblang' peel contained 165 μg mL^{-1} antioxidant as determined using the IC_{50} method[21], whereas 'Kolang-kaling' itself had an IC_{50} antioxidant activity of 24,456.82 mg L^{-1} [2]. These results showed that with higher concentration of 'jamblang' peel, the IC_{50} antioxidant value of the'Kolang-kaling' jam decreased, indicating that 'jamblang' peel had a higher capcity to inhibit free radicals. The addition of acid also affected the antioxidant content of 'kolang kaling' jam as the IC_{50} antioxidant content decreased as the citric acid content increased and in turn the pH decreased.

The antioxidant compounds in 'kolang kaling' jam were phenol and anthocyanin derived from the peel of the 'jamblang'[12], although galactomannan in 'kolang kaling' also has antioxidant activity[22]. A statistical analysis using a correlation test between phenol and anthocyanin levels and antioxidant activity showed a strong negative correlation between antioxidant activity and both total phenol (r = -0.881, R^2 = 0.776) and anthocyanin levels (r = - 683). This finding is consistent with studies by Esmaeili *et al.*[23] (R^2 = 0.745) and Perwiratami *et al.*[24] (r = 0.974).

CONCLUSION

The addition of 'jamblang' peel and citric acid significantly and dose-dependently affected ($p<0.05$) the total phenol and anthocyanin of 'Kolang-kaling' jam. Jam produced with higher amount of 'jamblang' and citric acid had higher total phenol and anthocyanin content, as well as higher antioxidant activity, as evidenced by lower IC_{50} values. The highest antioxidant activity (1,376 ppm), was seen for jam made with 25% 'jamblang' peel and 0.4 g citric acid. The value of antioxidant activity in 'Kolang-kaling' jam obtained was relatively weak, but this food could still have potential use as an antioxidant substance.

SIGNIFICANCE STATEMENT

This study discovered that the addition of 'jamblang' fruit peel and citric acid to the pulp of 'kolang kaling' can produce 'kolang kaling' jam containing antioxidant compounds, which have beneficial health effects.

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