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The Effect of Acetic Acid in Anthocyanins Extraction from Mangosteen (*Garcinia mangostana* L.) Pericarp

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ABSTRACT

Anthocyanins are pigments in mangosteen (*Garcinia mangostana* L.) pericarp. This pigments extracted with acidified solvent easily, but strong acids can degrade anthocyanins. The aim of this study is to investigate the effect of acetic acid in anthocyanins extraction from mangosteen pericarp. The steps of this study consist of anthocyanins extraction, quantify and analysis of its stability with visible spectrophotometric and chromameter. The results showed that 2% acetic acid in 95% ethanol gave the highest anthocyanins content. The anthocyanins stability is affected by sunlight exposure, hydrogen peroxide, and storage condition.

Keywords: Acetic acid, Anthocyanin, Mangosteen, Pericarp

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INTRODUCTION

The mangosteen (Garcinia mangostana L.) tree is presumed to have originated in Southeast Asia. The mangosteen rind, leaves, and bark have been used as folk medicine for thousand years. The thick mangosteen rind is used for treating catarrh, cystitis, diarrhea, dysentery, eczema, fever, intestinal ailments, pruritis and other skin ailments. The positive physiological effect of mangosteen could be related to the presence of anthocyanins with potent antioxidant activity [1].

The purple color of the mangosteen pericarp is mainly due to anthocyanins [2]. Anthocyanins are the most important group of water soluble pigments, after chlorophyll, are visible to the human eyes. They are responsible for many of the attractive colours, from scarlet to blue, of flowers, fruits, leaves and storage organs [3]. The major anthocyanins found in the mangosteen pericarp were cyanidin-3-sophoroside and cyanidin-3-glucoside [2, 4]. The extraction of bioactive compounds from natural sources become very important for the utilization of phytochemicals in the preparation of dietary supplements or neutraceuticals, functional food ingredients and additives to food, pharmaceutical and cosmetic products. Low stability of anthocyans is the limiting factor in their application. The main goal of this study was to establish the effect of acetic acid in anthocyanins extraction from mangosteen (Garcinia mangostana L.) pericarp.

MATERIALS AND METHODS

Mangosteen were grown in the field of Ghaliafarmindo, Bogor, West Java. The fruits harvested on 20 weeks after flowering. After harvest, the outer and inner pericarps were seperated from the fruits and homogenized. Analytical grade ethanol, acetic acid, hydrochloride acid, potassium chloride, sodium acetate, and hydrogen peroxide were purchased from Merck (Germany).

Anthocyanin Extraction

The homogenized pericarp was extracted by CH_3COOH/EtOH (0, 1, 2, 3, 4, 6, 8, and 10 mL concentrated acetic acid in 95% ethanol) solutions with solid/solvent ratio of 10 g/100 mL. After 1 h, each sample was filtrated in 100 mL volumetric flask and filled to 100 mL with the mixing solution [5].

Buffer Solution Recipe

Potassium chloride buffer, 0.025 M, pH 1.0. Mix 1.86 g KCl and 980 mL of distilled water in a beaker. Measure the pH and adjust to 1.0 with concentrated HCl. Transfer to a 1 L volumetric flask and fill to 1 L with distilled water [5].

Sodium acetate buffer, 0.4 M, pH 4.5. Mix 54.43 g CH_3CO_2Na. 3H2O and 960 mL distilled water in a beaker. Measure the pH and adjust to 4.5 with concentrated HCl. Transfer to a 1 L volumetric flask and fill to 1 L with distilled water [5].

Anthocyanin Analysis

Prepare two dilutions of the sample, one with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer, pH 4.5, diluting each sample by the previously until the absorbance of the sample at the λvis-max is within the linear range of the spectrophotometer. Divide the final volume of the sample by the initial volume to obtain the dilution factor. Let these dilutions equilibrate for 15 min. Measure the absorbance of each dilution at the λvis-max and at 700 nm, against a blank cell filled with distilled water**.** The anthocyanin contents were expressed as cyanidin-3-glucoside. Calculate the absorbance of the diluted sample (A) using formula (1) and calculate the anthocyanin concentration in the original sample using formula (2) [5].

$$A = (A_{\lambda vis\text{-}max} - A_{700})_{pH\ 1.0} - (A_{\lambda vis\text{-}max} - A_{700})_{pH\ 4.5} \quad (1)$$

$$\text{Anthocyanin (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon/l) \quad (2)$$

where MW is the molecular weight of cyanidine-3-glucoside, DF is the dilution factor, and ε is the molar absorptivity.

Extract Color Analysis

Extract color was measured using Minolta CR-300 chromameter (Minolta, Osaka, Japan) as L*, a*, b* values (CIELab) and converted to hue angle (color wheel, with red–purple at an angle of 0◦, yellow at 90◦, bluish-green at 180◦). The ΔE* was calculated.

Anthocyanin and Color Stability Test

Serial extract were stored in three different storage condition, i.e. (a) Sunlight influence: Each extract were stored in the transparent vial and permitted to sunlight exposure. Anthocyanin was measured every hour for 8 h. (b) Oxidator influence by hydrogen peroxide: Each extract were stored in amber vial and added with hydrogen peroxide 5 mmol/L. The mixture solution is stored at room temperature. Anthocyanin was measured every 30 minutes for 6 h [6, 7]. (c) Storage condition influence: Each extract was stored at three different of storage condition, i.e. in transparent vial at 27˚C and sunlight exposure (P1), n amber vial at 27˚C without sunlight exposure (P2), and in transparent vial at 4˚C without sunlight exposure (P3). Anthocyanin content and color measured every three days for 28 days.

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RESULTS AND DISCUSSION

The mangosteen pericarp were homogenized to mix the outer and the inner pericarp, anthocyanins content in the outer pericarp higher than the inner pericarp [4]. Maseration was choosen to extracted the anthocyanins from mangosteen pericarp, because anthocyanins extraction is commonly carried out under cold conditions with methanol or ethanol containing a small amount of acid with the objective of obtaining the flavylium cation form, which is red and stable in a highly acid medium [8]. Therefore, maseration also choosen because anthocyanins are relatively unstable and often undergo degradative reactions during processing and storage. Anthocyanins are unstable in neutral or alkaline solution, even in an acidic solution, the color slowly fading due to light. After one hour, extract was filtered to separated the small particles. Extract should be clear with no particles dispersed, because the dispersed particles will interference measurement [5].

The anthocyanin content in extracts containing other phenolic materials has been determined by measuring absorptivity of the solution at a single wavelength. This is possible because anthocyanins have a typical absorption band in the 490 to 550 nm region of the visible spectra. This band is far from the absorption bands of other phenolics, which have spectral maxima in the UV range [9]. Anthocyanin undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra. The red colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and rapid measurement of anthocyanin, even in the presence of polymerized degraded pigments and other interfering compounds [5]. The maximum wavelength was 527 nm (Fig 1). Haze or sedimentation that formed in diluting process corrected by measuring the absorbance at 700 nm, i.e the wavelength for the measurement of light scattered by solid particles suspended in solution. The more haze or sediment, so the higher the absorbance at 700 nm [5].

The solvent for extraction was 95% ethanol which acidified with concentrated acetic acid at concentration 0, 2, 4, 6, 8, and 10% from total solvent volume. Acid helps extracting anthocyanins from plant tissues, because it lysis of the plant cells, so anthocyanins retrieved more easily [5]. Acid made dehydrated sugar, because of water is lost upon forming a glycosidic bond. Acetic acid is chosen, because it is a weak acid, so it not damage the anthocyanins, especially at evaporation [10]. The optimum concentration of concetrated acetic acid to acidified 95% ethanol was 2% (Fig 2). Ethanol which acidified with 2% concetrated acetic acid produced the highest anthocyanin content from mangosteen pericarp. Higher concentration than 2% made hydrogen ion not dissociated prefectly, so the ability to protonated the anthocyanin is not optimal [10]. Anthocyanins content decreased due to sunlight exposure (Table 1), because sunlight increase the temperature of the extract, while anthocyanins are thermostable [5]. Hydrogen peroxide as oxidizing agent will react with anthocyanins that have antioxidant activity, resulting in lower levels of anthocyanins (Fig 3). The degradation effect of hydrogen peroxide on anthocyanins through direct oxidative mechanism increases brown products concentration. As a result the oxidized components of the media further react with anthocyanins giving rise to colourless or brown products [11]. The curve of decreasing of

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anthocyanins content versus time was not linear, so the reaction not follow zero-order. Then, we made the logarithmic curve of decreasing of anthocyanins content versus time, the result was linear, so the reaction follow the first-order (Fig 4). The half time of anthocyanin degradation was 97 min 27 sec, mean anthocyanin is unstable.

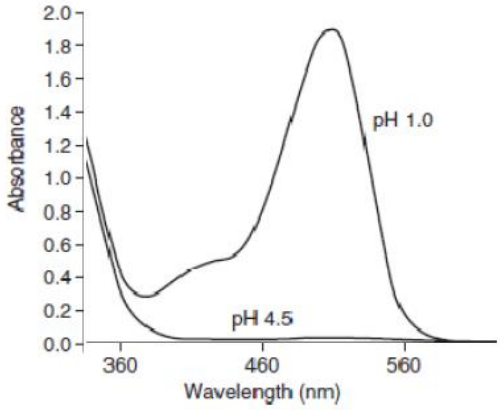

Fig 1: The spectra of mangosteen anthocyanins in pH 1.0 and pH 4.5 buffer

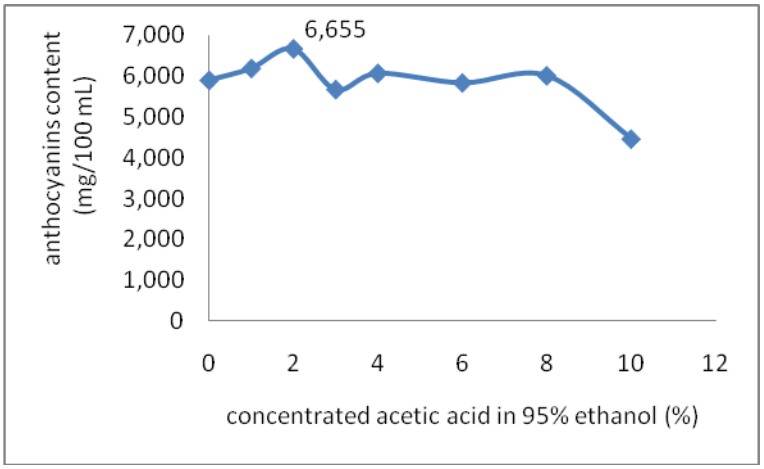

Fig 2: Anthocyanins content in extract which acidified with concentrated acetic acid

Table 1 Anthocyanin Stability to Sunlight Exposure

Time	Anthocyanin (mg/100 mL)		Temperature (°C)
	Control	Treatment	
08.00	2.61	2.61	29
09.00	2.62	2.75	35
10.00	2.77	2.74	41
11.00	2.82	2.36	43
12.00	2.74	2.16	44
13.00	2.77	1.85	38
14.00	2.76	1.62	36
15.00	0.87	1.41	36

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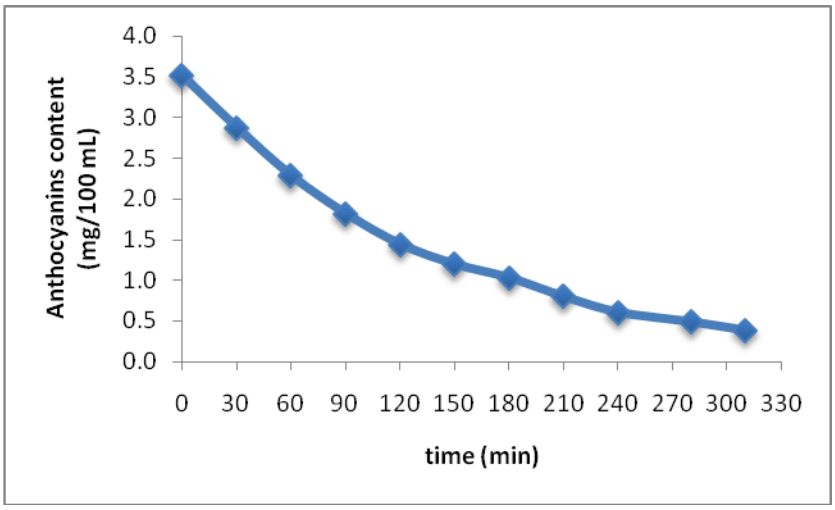

Fig 3: The influence of hydrogen peroxide on anthocyanins content (mg/100 mL extract)

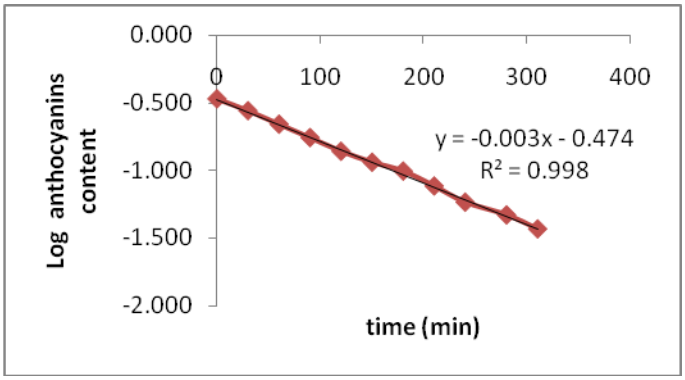

Fig 4: The influence of hydrogen peroxide on logarithmic of anthocyanins content (mg/100 mL extract)

Anthocyanins degradation in transparent vial at 27˚C and sunlight exposure, in amber vial at 27˚C without sunlight exposure, and in transparent vial at 4˚C without sunlight exposure were 98.0% (from 12.01 to 0.22 mg/mL), 92.7% (from 12.01 to 0.88 mg/100 mL), and 42.3% (from 12.01 to 6.93 mg/100 mL), respectively (Table 2). This degradation occurs because of unstable anthocyanins were exposure to the sunlight and temperature.

Value of L*, a*, and b* used to determine the identity of discoloration. When the absorbance of anthocyanins reduced, so value of L* increasing which means the color become more lighter; when the value of a* and b* reduced, so the red and yellow diminishing (Fig 5). Value of ΔL*, Δa*, and Δb* showed the color identity. Negative value of ΔL* showed that in the end study, the color more lighter than the initial color. Positive value of Δa* and Δb* showed that the red and yellow diminishing (Table 3). The value of ΔE* express the difference distance between the color of an object with another object. The normal human eye began to difficult in distinguishing colors when value of ΔE* below 4.

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Table 2: The Anthocyanin Stability Test Against Variation in Storage Condition

Storage condition	Time (day)	Anthocyanin (mg/100mL)	L*	a*	b*
in transparent vial at 27˚C and sunlight exposure	0	12.01	31.35	25.17	21.37
	3	8.28	33.18	23.57	19.95
	7	5.93	36.79	20.41	16.14
	10	4.80	36.80	20.40	16.58
	14	2.94	39.78	15.82	11.36
	16	2.50	43.63	10.59	5.34
	21	1.20	47.09	5.48	1.68
	24	0.66	47.88	5.28	-0.95
	28	0.22	47.94	5.20	-0.94
in amber vial at 27˚C without sunlight exposure	0	12.01	31.35	25.17	21.37
	3	8.73	33.00	23.77	19.86
	7	6.37	35.65	20.79	16.40
	10	6.01	36.78	20.46	16.31
	14	3.95	37.93	18.57	13.91
	16	3.29	39.88	15.93	11.38
	21	2.03	46.40	6.73	3.11
	24	1.45	46.79	5.48	2.18
	28	0.88	47.74	5.25	-0.96
in transparent vial at 4˚C without sunlight exposure	0	12.01	31.35	25.17	21.37
	3	8.06	33.09	22.32	18.58
	7	9.72	32.22	24.68	20.88
	10	9.49	32.26	23.48	19.73
	14	8.75	33.01	23.56	20.21
	16	8.57	33.33	23.73	20.01
	21	7.36	34.95	21.94	18.62
	24	7.08	35.15	22.22	18.33
	28	6.93	35.00	21.86	18.10

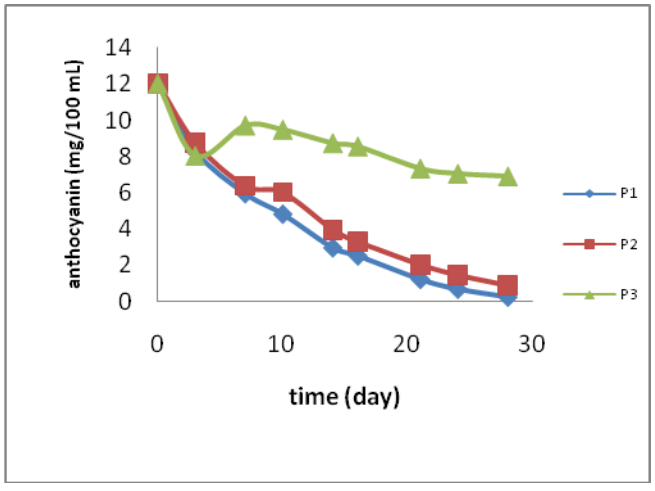

Fig 5: The anthocyanin stability against variation in storage condition

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Table 3: Color Identity and Total Color Difference

Storage condition	ΔL*	Δa*	Δb*	ΔE*
P1	-16,59	19,97	22,31	34,23
P2	-16,39	19,92	22,33	34,12
P3	-3,65	3,31	3,27	5,91

CONCLUSION

The 95% ethanol acidified with 2% acetic acid is the most optimal solvent to extracting anthocyanins. Anthocyanin stability is affected by sunlight exposure, hydrogen peroxide, and storage condition.

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