

ISOLASI, ANALISIS, DAN MIKROENKAPSULASI ANTOSIANIN DARI SEMANGGI UNGU (*Oxalis triangularis*)

*Isolation, Analysis, and Microencapsulation of Anthocyanin from False Shamrock (*Oxalis triangularis*)*

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ABSTRAK

Oxalis triangularis atau semanggi ungu adalah tanaman hias yang memiliki warna ungu. Warna ungu yang terdapat pada daun adalah indikator keberadaan pigmen antosianin. Antosianin dapat dimanfaatkan sebagai pigmen alami untuk makanan dan minuman. Makanan dan minuman yang ditambahkan antosianin akan mendapat nilai tambah berupa warna yang menarik dan senyawa antioksidan. Tujuan dari penelitian ini adalah untuk mengisolasi, mengidentifikasi, dan mikroenkapsulasi pigmen antosianin dari *O. triangularis*. Metode yang digunakan adalah ekstraksi pigmen dengan aquades, identifikasi pigmen dengan KLT dan Spektrofotometer UV-Vis, mikroenkapsulasi pigmen dengan maltodekstrin, dan pengujian termostabilitas dengan pemanasan selama 0 – 50 menit pada suhu 100°C. Hasil yang diperoleh terdapat 2 fraksi warna dari hasil KLT, dan 2 puncak serapan berdasar pemindaian dengan spektrofotometer UV-Vis. Total antosianin sebesar 1,073569 mg/g untuk ekstrak pigmen dan 0,147799 mg/g untuk mikroenkapsulasi pigmen. Hasil uji termostabilitas, menunjukkan pigmen antosianin termikroenkapsulasi stabil di suhu panas. Dengan kandungan antosianin yang cukup tinggi, bisa dimikroenkapsulasi, dan memiliki termostabilitas yang baik maka bisa dijadikan sumber pigmen alami untuk makanan dan minuman.

Kata kunci : antosianin, mikroenkapsulasi, *Oxalis triangularis*, pigmen, termostabilitas.

ABSTRACT

Oxalis triangularis or false shamrock is an ornamental plant that has a purple color. The purple color found on the leaves is an indicator of the presence of anthocyanin pigments. Anthocyanin can be utilized as a natural pigment for foods and beverages. Foods and beverages that added up with anthocyanin would gain the additional worth of enchanting colors and antioxidant compounds. This study aims to isolate, identify, and microencapsulated anthocyanin pigments of *O. triangularis*. The method began with pigment extraction using aquadest, pigment identification with TLC and Spectrophotometer UV-Vis, the microencapsulation pigment with maltodextrin, and thermostability testing by heating for 0 – 50 min at 100°C. The results obtained were 2 color fractions from TLC and 2 peaks based on the UV-Vis spectrophotometer. Total anthocyanin was 1.073569 mg/g for pigment extract and 0.147799 mg/g for microencapsulated pigment. The results of the thermostability test showed that the microencapsulated anthocyanine pigment was stable at high temperatures. From this result, we can conclude that anthocyanin, which contained high at *oxalis triangularis*, can be microencapsulated thus had a good thermostability, which can be a natural pigment source for food and beverages industry.

Keywords : anthocyanin, microencapsulation, *oxalis triangularis*, pigment.

INTRODUCTION

False shamrock (*Oxalis triangularis*) is a perennial plant. *O. triangularis*, which is part of the *Oxalidaceae* family, is a plant that comes from South America (Argentina, Bolivia, Brasil, and Paraguay) (Nesom, 2009). The plant belongs to a decorative and edible plant.

O. triangularis contains oxalate in its leaves and trunk. Oxalate is a natural organic acid compound that is relatively stronger than other organic acids, such as acetic. This substance functions as a self-defense mechanism to a predator (herbivore). Oxalate acid will give strong taste and in lower dosage, it will not give any effect to health, yet in higher dosage, oxalate acid can irritate and cause discomfort in the alimentary canal since oxalate acid can bind to calcium. The heating technique by boiling the plant can neutralize the oxalate acid so that it can reduce its effectiveness. Behind its negative effect, *O. triangularis* has purple leaves that can be natural pigment sources. Purple color signs anthocyanin in the plant. Anthocyanin is a natural pigment that is responsible for purple pigment.

Anthocyanin is a single aromatic structure derivative of cyanidin with addition and reduction of hydroxyl, methylation, and glycosylation clusters (Harborne, 2005). The compound is amphoteric, that it can react well to both acid and alkali. When it is acidic, anthocyanins will be in dark red and when it is alkaline, it turns to be purple and blue.

The anthocyanin color is formed of long conjugated double-bond so that it can absorb light waves longer. The bond can function as an antioxidant to catch free radicals. Antociacine can delay the atherogenesis process by oxidizing the bad cholesterol (LDL) in the body. Besides, anthocyanin can protect endothelial cell's integrity that layer blood vessels walls for blocking destruction (Ginting, 2011). Anthocyanin in plants can give dark red, purple, and blue color to fruits, leaves, and flowers. In the food industry, it can be used as a safe natural food coloring.

Microencapsulation is one of the efficient ways to put in a compound to a product. Microencapsulation is defined as a process to trap the active substance in the coating material. Maltodextrin is dextrose solid that can be used as the coating material with its high solubility in water, low viscosity, low sugar level, and uncolored solubility. Those characters are the pre-requisites of a general coating material used in microencapsulation (Mahdavi, et.al., 2006). To keep the endurance of saving period, simplify storage and use, it needs to microencapsulate the antociacine. Microencapsulated antociacine is hoped to give the additional value specifically in the utilization technique.

Color is usually seen as a psychological factor in food product acceptance and the criteria used by consumers to choose food products. In the food industry, raw materials are modified by technology to get natural coloring. To get the most

appropriate color, each of the food materials is given synthetic coloring in its processing (Alcade-Eon, 2004). The wide use of synthetic coloring as an additive to food and beverages can give a negative impact on the consumer. Consuming synthetic coloring might cause poisoning and its accumulation potentially causes cancer. As an alternative solution to the problem is using *O.triangularis* as a natural pigment. The purpose of the research is to isolate, analyze, and produce the pigment of *O.triangularis* as a natural pigment.

METHOD

The research is a laboratory experimental research conducted at the CARC (*Carotoneid Antioxidant Research Center*) laboratory, Universitas Kristen Satya Wacana. The research phases included pigment extraction, pigment crystallization, thermostability test, and pigment identification.

Pigment Extraction

10 grams of *O. triangularis* leaves were pulverized to become soft by using mortar in a porcelain bowl. Slowly, the researcher added 25ml of aquades and filtered it. Filtering remain was mashed and added with 25 ml of Aquades. The treatment must have been done four times so that the total solution was 100 ml. Then, the 100 ml liquid was centrifuged by applying 4,000 RPM speed for 10 minutes. The supernatant was taken for the next process.

Pigment Microencapsulation

90ml of supernatants were added with 30 gr (33.533%) of maltodextrin and they were mixed to become soluble. The mixed result of the two materials was then moved in a baking pan and put into the oven at 80°C for 90 minutes. After 90 minutes, formed rough crystals. The crystals were then softened by mashing them using mortar and porcelain bowl. They were then sifted and there would be pigment powders.

Thermostability Test

1 gram of pigment powder was dissolved in a 99ml of aquades in a beaker glass. The solution was then heated in a hot plate until it reached the boiling point. After reaching the boiling point, 100°C, the sampling was taken in the 10th, 20th, 30th, 40th, and 50th minute.

Pigment Identification

The pigment identification was conducted by using UV-Vis spectrophotometer in the 400 – 800 nm wavelength. The tested pigment was supernatant, dissolved pigment powders, and sampling test in thermostability test. Pigment identification was also conducted by using Thin-Layer Chromatography from concentrated supernatant which was steamed in hot air. The solvents in Thin-Layer Chromatography were hexane, acetone, and aquades that were separated. The dyeing was done for 15 minutes in each solvent and the Thin-Layer Chromatography plate was dried before.

The total calculation of anthocyanin was done through the following formulation:

Anthocyanin total : $((\text{abs } 530 - 0,25 \times \text{abs } 657) \times \text{total volume extract(ml)}) / \text{weight sample (gr)}$

RESULT

The result of pigment scanning by using a UV-Vis spectrophotometer with 400 – 800 nm wavelength is shown in Figure 1. In Figure 1, there are two maximum absorption peaks in the pigment (Figure 1A). In Figure 1B, there is a pattern of pigment spectra that had been microencapsulated. The wave peak had shifted and the shift was

caused by maltodextrin addition. Figure 1C. shows the result of microencapsulation by using 25% of maltodextrin.

There were two color fractions which were separated by hexane, ethanol, and aquades eluent. Two fractioned colors were green with RF 1.5 cm and purple with RF 4.5 cm. Table 2 shows the total anthocyanin was gr/100ml from sampling test; supernatant, microencapsulation (0-minute heating), microencapsulation (10-, 20-, 30-, 40-, and 50-minute heating).

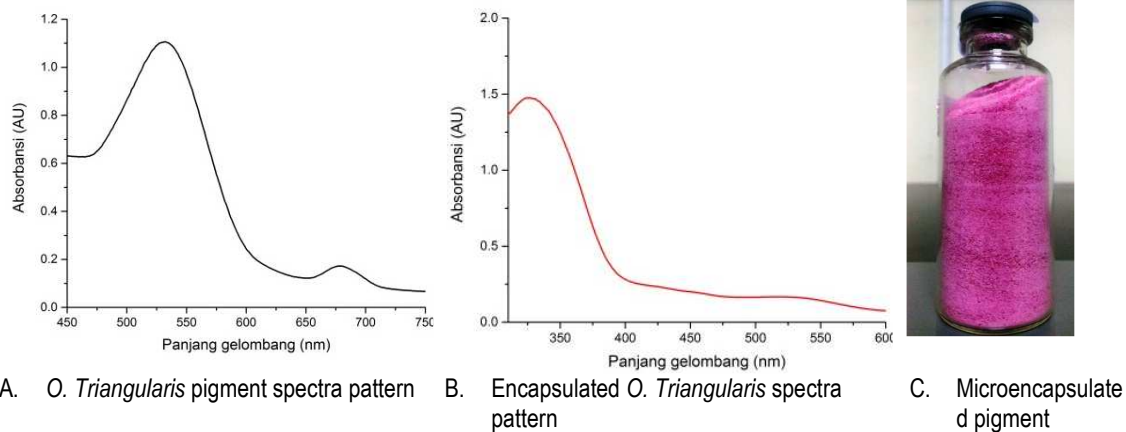


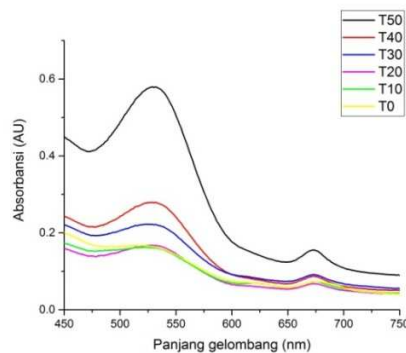
Figure 1. Spectra pattern (A,B) and microencapsulated pigment (C)

Table 1. Thin-Layer Chromatography Test Result

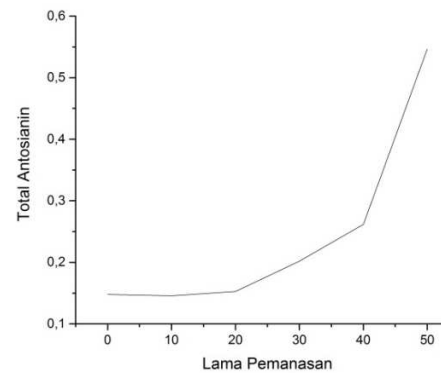
TLC	Eluent	Color	RF
	1. Hexane	Green	1.5 cm
	2. Ethanol		
	3. Aquades	Purple	4.5 cm

Table 2. Anthocyanin total from several sampling tests

Sample	λ 530 (nm)	λ 657 (nm)	Anthocyanin (mg/g)
Supernatant	1.105326	0.12703	1.073569
Micro	0.163175	0.061505	0.147799
10-minute heating	0.160896	0.061119	0.145616
20-minute heating	0.167167	0.057531	0.152785
30-minute heating	0.221563	0.078657	0.201899
40-minute heating	0.279671	0.072307	0.261595
50-minute heating	0.578971	0.130839	0.546261



A. Spectra pattern of thermostability test



B. Chart of the increasing total of anthocyanin because of heating (minutes)

Figure 2. The pattern of heated anthocyanin spectra (A) and chart of total anthocyanin during the heating (B)

The thermostability test is shown in Figure 2. It shows that the pattern was increasing from the 10th to the 50th minute (Figure 2A). It was suspected that the increase happened because of pigment coating by maltodextrin that was able to protect pigment from the heating exposure. The increasing total of microencapsulated anthocyanin is shown in Figure 2B.

DISCUSSION

Oxalis triangularis Pigment

The attractive color of anthocyanin extract from *O. triangularis* leaf and the edible character

show that the pigment can be used as a natural coloring source. *O. triangularis* is proved to be rich and can be the source of anthocyanin. It contains 195 mg/100 g monomeric anthocyanin in leaf or 2.42 g/100 gr in dried leaf (Duran, et.al., 2001). Based on this research result, the total anthocyanin was 107.3569 mg/100ml leaf. The result is different from the research of Duran, et.al. (2001) that has a bigger total anthocyanin. The difference is maybe caused by different extraction methods. Duran, et.al. (2001) used 0.15% HCL to extract anthocyanin. On the other hand, this research only used aquades. The extraction using acid addition

will optimize the extraction process and make anthocyanin stable in its acidic. For food products, extraction should use safe solvent, such as aquades.

Anthocyanin Isolation

Anthocyanin pigment can be extracted by using several kinds of polar solvents such as methanol, ethanol, acetone, water, and/or the mixture of those kinds of solvents. Methanol is the most used solvent in industry. However, since it is toxic, it is not allowed to be used in the food industry. Ethanol is used among the others because of its friendly and non-toxic character. But, based on the economic calculation, water is much cheaper than ethanol. Water, as anthocyanin solvent, is much cheaper and safer, so that it is good to be used in the food industry.

The purple color in the pigment extract happened because it was extracted by using aquades containing pH 7. Anthocyanin is vulnerable to pH changes that the color will change as well. In low pH (1-2), anthocyanin will be in dark red, and in neutral pH, it will become purple and it will become green in alkaline (Gross, 1987). The purple color, as is shown in Figure 1, was the extraction result with aquades that contained Ph 7.

Anthocyanin Analysis

There are about 17 anthocyanins found in nature, but there are only six (cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin) that

are mostly found (Miguel, 2011). Anthocyanin has a peak absorption point of 520 nm (pelargonidin), 535 nm (cyanidin), dan 546 nm (delphinidin) (Gross, 1987). Based on the spectrophotometer analysis result, it was assumed that the absorption peak was in the 546 nm wavelength and the absorbed color was delphinidin. According to Jing (2006), the delphinidin color is reddish-blue as shown in Figure 3.

From the TLC result (Table 1), it is shown that two-color fragments green and purple. The green color could be predicted as chlorophyll. The pigment extract came from *O. triangularis* leaf where chlorophyll is mostly found in the leaf. The chlorophyll number should have been more, but based on the spectra analysis result, it was found that there was a peak between 650 – 700 nm wavelength. In that wavelength, according to Gross (1987), it is an optimal absorption point for Chlorophyll A and it is exactly in the 662 nm wavelength.

Pigment Microencapsulation

Encapsulation is a technique where active solids, liquids, or gases are put into a matrix or polymer wall system to protect its active material from the environmental factor effects. The polymer that is used in microencapsulation is called encapsulation agent (AE). The microparticle, which is produced, is vesicle or small particle that has various sizes, from sub-micron to some millimeters (Robert & Fredes, 2015). Several kinds of

encapsulation agents are gummi arabicum, gum acacia, whey, and polysaccharides such as maltodextrin from solid dextrose, inulin, corn starch, and modified polysaccharides (Ozkan & Bilek, 2014).

Choosing an encapsulation agent is very important for appropriate efficient encapsulation, because of the stability of active compounds in microparticles during the storage and the release character in food products and alimentary canal. In anthocyanin microencapsulation, maltodextrin has been proved significant to maintain anthocyanin integrity for encapsulation (Ozkan & Bilek, 2014). Several techniques can be used for natural coloring microencapsulation, which are spray drying, freeze-drying, coacervation, and emulsion. The emulsion was chosen because the technique is very simple, that is by mixing pigment supernatant with maltodextrin and then drying with heating technique.

The microencapsulation result is shown in Figure 2. The purple color dominated, although the color was not homogenous yet. It happened because the mixture and drying were uneven so that the pigment was not coated perfectly. The most used technique in microencapsulation is spray drying that will produce more homogenous colors. The microencapsulation technique with emulsion is more accurate for the products that have different polarity levels, such as water and oil (Ozkan & Bilek, 2014). Mahdavi, et.al. (2016) mentioned that the optimal value of coating by using maltodextrin is 25%, this researched used 33.33% coating.

Anthocyanin Thermostability Test

The test by using heat exposure aims at finding the durability and level of anthocyanin degradation. Some natural pigments cannot stand for high heat exposure, and if the exposure happens, the colors will be fading. As a natural coloring product that will be used in various food products, it needs to have some information on the durability of color exposures, since many food products are processed by heating. The availability of thermostability information will become such a recommendation for suggested heat exposure so that the colors will not be fading and the color destruction will be minimized.

The maltodextrin that is used as coating materials can protect pigment from environmental effects, and one of them is heat. Principally, microencapsulation can protect pigment and improve its storage period, but heat exposure can cause anthocyanin degradation. Based on the heat exposure test for 0 – 50 minutes, there was an increasing spectrum pattern (Figure 2). However, the increase could be caused by several factors. The first factor was the addition of pigment concentration since the evaporation solvent was water. The continuous heating made water evaporate so that the anthocyanin concentration increased. The second factor was the protection of maltodextrin. Maltodextrine, as a coating material, was able to protect from heat exposure. It took some time so that maltodextrin could release its tied anthocyanin by heating. The longer the heating, the more released anthocyanin was so that the

concentration level became higher. The third factor was the legibility of the spectra pattern that still reads anthocyanin compound, but not in the color change. Based on the result of color compare and 0 – 50-minute heating, it shows the color change that became red, dark red, and brown. The color change happened because of the temperature change.

Hongmei and Meng (2015) said that encapsulated anthocyanin and non-encapsulated anthocyanin have a significant difference towards half of the pigment degradation storage period due to temperature exposure. In the exposure of 40°C, the anthocyanin degradation that is not encapsulated is $6 \times 10^{-3}/d$ within half of the storage period for 115 days and the anthocyanin degradation that is encapsulated is $3,5 \times 10^{-3}/d$ within half of the storage for 198 days.

Temperature change, mainly heat can shift the anthocyanin equilibrium. The heat exposure can cause anthocyanin equilibrium to tend to be non-colored forms, which are carbinol and calcon alkali. The destruction due to the heat can happen through two phases. First, hydrolysis happens in glycosidic anthocyanin bond so that it produces unstable aglycons. Second, aglycon rings are open to forming carbional and calcon clusters. The degradation can happen further if there is an oxidant so that it forms dark or brown compound. Therefore, in food processing, the application of anthocyanin coloring must be done in the last phase where the heating process has become minimal.

Anthocyanin Pigment Utilization

Anthocyanin gives interesting colors such as orange, red, and blue. This pigment is dissolved in water that can facilitate pigment mixture in the watery food system. Anthocyanin is widely spread in nature and not dangerous so that this quality makes anthocyanin interesting to be natural coloring (Duran, et.al., 2001).

Anthocyanin pigment can be used as a food coloring. Its character that is dissolved in water makes it easy for the mixture of food and beverage materials that are dissolved in water. Microencapsulated anthocyanin can be directly used by dissolving it in the water. The anthocyanin microparticle is dissolved in water and food matrix, so that anthocyanin can be released fast (Robert & Fredes, 2015). Ozkan dan Bilek (2014) mentioned microencapsulation coloring can be used commercially as a food coloring. The food materials that might be added with encapsulated pigments are yoghurt, ice cream, yoghurt candy, soft drink, and syrup.

CONCLUSION

O. triangularis can be used as a natural pigment source that is anthocyanin. Anthocyanin pigment can be microencapsulated. The total content of anthocyanin in the pigment extract was 1.073569 mg/g and 0.147799 mg/g for microencapsulated pigment. In the thermostability test, there was an increasing pattern of anthocyanin content which was caused by the released

anthocyanin concentration from maltodextrin coating.

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