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THE EFFECT OF pH AND TEMPERATURE ON THE STABILITY OF ANTHOCYANINS FROM BLACK SOYBEAN SKIN EXTRACTS

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Abstract

Anthocyanins are natural dyes of purple and red. Anthocyanin dyes are applied in various industries as a drink or food coloring. As natural dyes, anthocyanins have an important role in human health. This research aimed to analyze the stability of anthocyanins from black soybean skin extract at varying pH and temperature. Anthocyanins from black soybean skins are macerated using two kinds of solvents. The solvents are distilled water + 30% citric acid and 70% ethanol + 1% HCl. Maceration was carried out at a temperature of 4℃. Then the anthocyanin was treated at a temperature range of 40-90℃ and pH of 5-9 and its absorbance was measured using UV-Vis's spectrometry. The concentration of anthocyanin from black soybean skins is from the solvent distilled water + 30% citric acid of 1.992 mg/L, while from the solvent 70% ethanol + 1% HCl it was 1.936 mg/L. The color stability temperature t of black soybean skin extract was carried out at temperatures of 40°C, 50°C, 60°C, 70°C, 80°C, and 90°C. with respective absorbance of 0.274; 0.247; 0.237; 0.227; and 0.214. The higher the temperature, the anthocyanin content decreases. pH test was carried out at pH 5, 6, 7, 8, and 9 with respective absorbance values of 1.831; 1,759; 1,229; 1,118; and 1,066. The pH test showed that the higher the pH, the anthocyanin content was reduced, while the application of additional anthocyanin to white agar as food coloring showed that the anthocyanin content was only 0.125. It can be concluded that anthocyanins have good stability at low temperatures and acidic pH.

INTRODUCTION

Dyes consist of natural dyes and synthetic dyes. Natural dyes are pigments obtained from plants, animals, or from mineral sources. These dyes have been used for a long time and are generally considered safer than synthetic dyes [1,2,3]. Since the discovery of synthetic dyes, the use of pigments has decreased, although it has not disappeared completely. In the last few decades, efforts have emerged to explore the ins and outs of pigments, especially to determine changes in the color of food ingredients due to the influence of various processing and cooking treatments [2,4,5].

Excessive use of synthetic dyes can have a negative impact on the health of the human body, because they contain toxins and are carcinogenic [6]. Recently, after learning about the negative impacts of using artificial dyes, humans have been doing a lot of research on natural dyes from plants and animals [2,4,6].

One of the natural coloring substances in plants is anthocyanin; a group of reddish-blue natural dyes that are visible to our eyes [7,8]. Anthocyanins are less stable in various environmental conditions. They have many varieties of colors from orange, red, maroon to blue [7,9,10]. Anthocyanins do not cause damage to food or food packaging and are not a substance that is toxic to the body, so internationally it has been permitted as an additional food coloring agent. Anthocyanins are also believed to play a role in biological systems, including their ability as prebiotics, because they contain bioactive compounds [2,11,12]. Apart from acting as a coloring agent in food, anthocyanins are also believed to play a role in biological systems, including capabilities as a scavenger of free radicals scavenging), cardioprotective capacity, and the ability to delay the initiation stage of the chemical reaction that causes carcinogenesis [6].

There are around 700 types of anthocyanins that have been isolated from plants and have been identified. Some of them have an important role in food ingredients, including pelargonidin, cyanidin,

peonidin, delphinidin, petunidin, malvidin, and anthocyanidin glucosides [13,14,15].

The structures of anthocyanins are dependent on different conditions such as temperature, pH, and also solvent [16,12,17,18,19].

Black soybeans are abundant and abundant in nature, black soybeans contain anti-oxidants, isoflavones, saponins, anthocyanins, and vitamin E, while the anthocyanin content is in the skin of the black soybeans [20,21]. Black soybeans have long been consumed in Indonesia, soybeans have been grown by Javanese and Balinese farmers and produced as soy sauce, even in the process of making soy sauce, black soybean skins become waste [22]. Black soybean skins are currently only used for animal feed, and their use is less than optimal, even though black soybean skins contain anthocyanins, the anthocyanin content in black soybean skins is not very-well-known [20].

However, anthocyanin instability limits their use. Black soybean is an Eeast Asian legume rich in anthocyanins with antioxidant, anti-obesity, antihyperglycemic, and anti-hyperlipidemic effects, making it a valuable addition as functional food [23,24]. So, the objective of the research is to investigate the stability of anthocyanins from black soybean skin at different pH and temperature.

EXPERIMENT

Material

The materials used are black soybean shells, 1% citric acid (Merck, p.a), HCl (Merck), 70% ethanol (technical grade), pH buffer, white agar, and sugar.

Instrument

The instrumentation used was UV-Vis Spectrophotometer Thermo Scientific Evolution 200.

Procedure

Making Black Soybean Extracts

Black soybean skins taken from soy sauce production waste are dried in Cabinet Dyer C for ± 6 hours. This drying method is intended to avoid damage to the anthocyanin substances in the black soybean skin. The dried black soybean skins are ground until they become powder. The resulting powder is then sieved using a 40-mesh sieve to produce maximum extract. The 60 g of black soybean skins were macerated using distilled water + 3% citric acid and 70% ethanol + 1% HCL, after adding the solvent in a magnetic stirrer first for 1 hour at a speed of 125 pm. After using a magnetic stirrer, this maceration is carried out once for 24 hours in the refrigerator in cold conditions. This step aims to get more anthocyanin. The maceration results were centrifuged for 10 minutes at a speed of 200 rpm, and the supernatant was filtered.

Determination of Color Stability

After filtering the two solvents, color stability was carried out using a UV-vis spectrophotometer for the two solvents. Optimum color stability is taken based on the best color stability seen from the results of the UV-vis spectrophotometer.

Making Extracts Using Appropriate Solvents (Distilled Water + 3% Citric Acid)

Making 200 ml of extracts using appropriate solvents is carried out based on the best color stability from preliminary research. This main research includes making extracts with solvents that have seen the best color stability in preliminary tests. In this main research, distilled water + 3% citric acid was used. Black soybean skins were added with distilled water + 3% citric acid solvent, after adding the solvent, a magnetic stirrer was first carried out for 1 hour at a speed of 125 pm. After being magnetic.

Stirrer maceration is carried out, this maceration lasts for 24 hours in the refrigerator at a temperature of 4℃, this aims to get more anthocyanins. The maceration results were centrifuged for 10 minutes at a speed of 200 rpm, and the supernatant filtered was centrifuged to obtain the supernatant from black soybean skin extract after which it was freeze-dyed.

pH Test and Temperature Test

Several tests were carried out for black soybean skin extract, namely temperature test, pH test, and white gelatin test. This test looks at the stability of the color using a UV-vis spectrophotometer.

Application of anthocyanins as coloring food

Black soybean shell extract is added to the white agar extract, adding only 25% black soybean skin extract to the white agar, making white agar 10.88 grams of agar powder, put into 1050 ml of water, heated until boiling while stirred, then added

186.66 grams of granulated sugar, stirred again until dissolved, and added 350 ml of black soybean shell extract.

RESULT AND DISCUSSION

The first stage of this research is to determine the optimum concentration of anthocyanin from two solvents. The solvents are distilled water + 3% citric acid and 70% ethanol + 1% HCl. The concentration of anthocyanins from the solvent of 70% ethanol + 1% HCl 1% is 1.936 mg/mL, while distilled water + 3% citric acid is 1.992 mg/mL. The color of anthocyanins from both solvents is different can be seen in **Figure 1**.

Figure 1. Black soybean skin extract (left) with 70% ethanol + 1% HCl and (right) with distilled water + 3% citric acid.

Anthocyanin is a phenolic compound so a test was carried out by adding a few drops of 1% $FeCl_3$ to get positive results, the black soybean skin extract changed color from pink to dark green. The free radical scavenging activity and reducing the power of anthocyanins is highest at the lowest pH because the dominant anthocyanin structure is in the form of flavylium cations so that it can easily donate hydrogen cations and reduce Fe^{3+} to Fe^{2+} from $FeCl_3$ solution and change the color of the solution [25].

In **Figure 2** can be seen, there are nine principal peaks of anthocyanins. Peak 3,5,6 are major peaks had been identified as delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, and petunidine-3-O-glucoside, respectively [26].

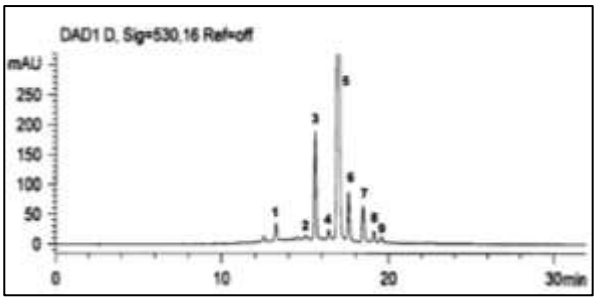

Figure 2. Chromatogram of HPLC from methanolic crude extract of black soybean seed [26].

Figure 3 shows three major structures of anthocyanins of black soybean seed [26].

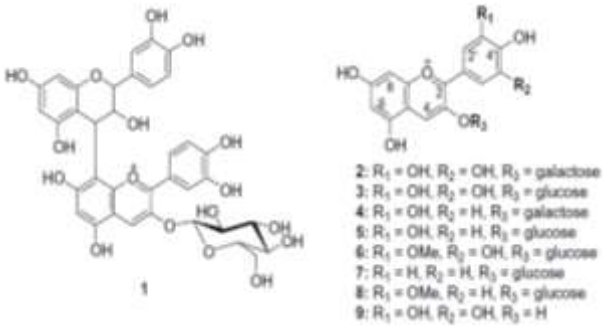

Figure 3. Structure of anthocyanins from black soybean seed [26].

pH analysis of the black soybean skin extract, a pH buffer of 5, 6, 7, 8, and 9 was added to the anthocyanins extract. It had an absorbance between 1,066 and 1,831 at a wavelength of 510 nm. The stability of anthocyanin is influenced by several factors such as pH, light, oxygen, and temperature [4]. Dye anthocyanin is an unstable molecule if there is a change in temperature, oxygen, pH, and light [4,7]. Anthocyanin is red at acidic conditions, at the higher pH value anthocyanin will change to color fading of colorless, yellow purple, and blue [4]. Under neutral or slightly acidic conditions, the anthocyanins exist predominantly in their colorless forms, due to the instability of the anhydrous base. Rate of the anthocyanin degradation has long been known to be pH-dependent [18].

Based on our results, pH has a great influence on the stability of anthocyanin dye. In **Figure 4**, pH 5 shows the biggest absorbance of 1.831, while the lowest absorbance at pH 9 is 1.066.

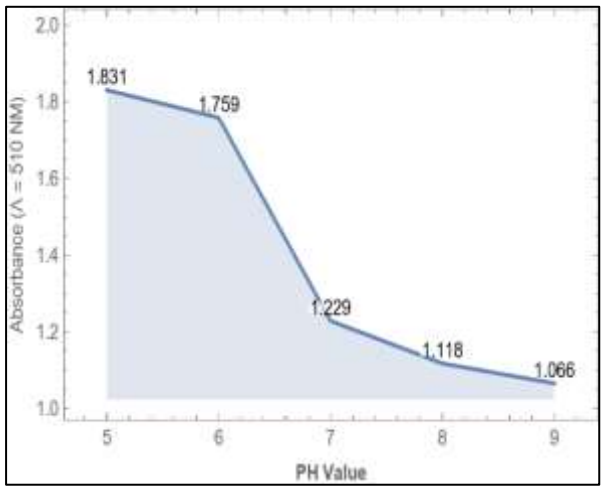

Figure 4. The absorbance of anthocyanins in various pH.

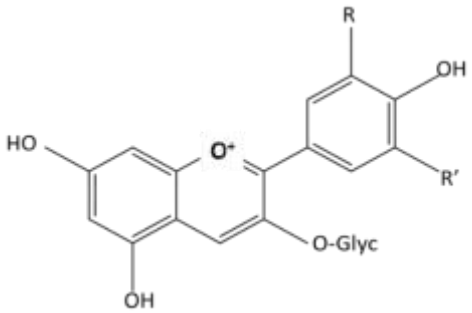

Figure 5. Structure of anthocyanins in the form of flavylium ion.

It shows that the lower the pH value, the higher the absorbance value. The higher the pH value, the lower the stability of the color extract, this indicates damage to anthocyanins. A significant decrease in color stability occurs at pH 7. Anthocyanins are generally more stable in acidic conditions than in neutral and alkaline atmospheres [6]. The anthocyanin structure in the form of flavylium ion is shown in **Figure 5**. The acid nature structure is due to the conjugation of double bonds in the rings of the main structure and hydroxyl groups at C4', C5, and C7 respectively. The hydroxyl group at C7 is the strongest acid. The deprotonation can be produced at acid pH 4 yielding a neutral quinonoid base stabilized by tautomerization with the hydroxyl group at C5. The hydroxyl group at C4 is also susceptible to be deprotonated at higher pH 7 yielding the anionic base. If the pH level is still rising to the basic pH, higher than 8, the deprotonation is produced in C5 yielding the dianionic base which can lead to the chalcone anion. So, pH must be controlled during the extraction process [16,15].

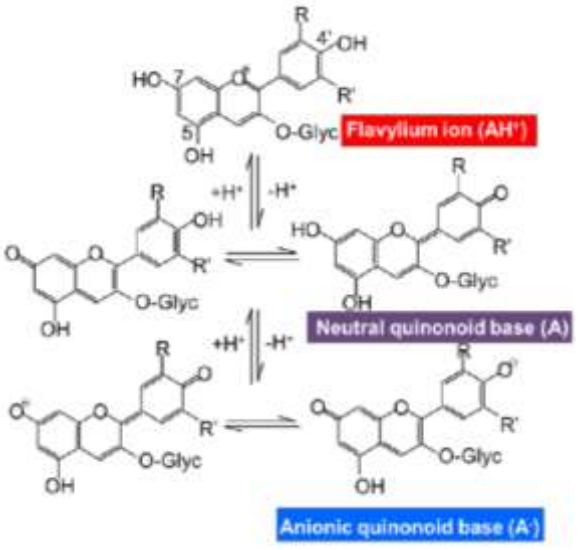

Figure 6. Structure of anthocyanins at different pH values [14].

Anthocyanin is soluble in water because of hydrophilic. Anthocyanins also can be dissolved in polar organic solvents such as acetone, ethanol, methanol, and chloroform. Anthocyanin is very stable in acidic conditions. So that extraction can be added organic acids, citric acid, or hydrochloric acid [15,14]. Anthocyanin structure can be changed due to pH conditions shown in **Figure 6**. Anthocyanins at pH >7 will be degraded according to the substituent group [14].

The color stability temperature test of black soybean skin extract was carried out at temperatures of 40, 50, 60, 70, 80, and 90℃. In this temperature test, anthocyanin from black soybean skin extract was heated in a water bath for 30 minutes. Its absorbance was measured at a wavelength of 510 nm and had an absorbance of 0.214-0.274 shown in **Figure 7.**

Anthocyanin is stable in acidic pH. It becomes less stable when exposed to high temperatures, causing loss of color and browning. At high temperatures, the sugar level increased, rate of destruction also increased [4]. In solution, anthocyanin is present in an equilibrium between a colorless pseudo base and colored cationic form. This equilibrium is controlled by pH. Temperature also has a great influence on the stability of anthocyanin dye, in the presence or absence of light. The color loss with an increase in temperature. Increasing the temperature of heating results in changes in anthocyanin and co-pigmentation complex which increase in absorbance or hyperchromic effects and bathochromic shift in solution [18].

Based on **Figure 7**, the highest absorbance temperature treatment was at 40℃ is 0.274, while the lowest absorbance was at 80℃ is 0.214.

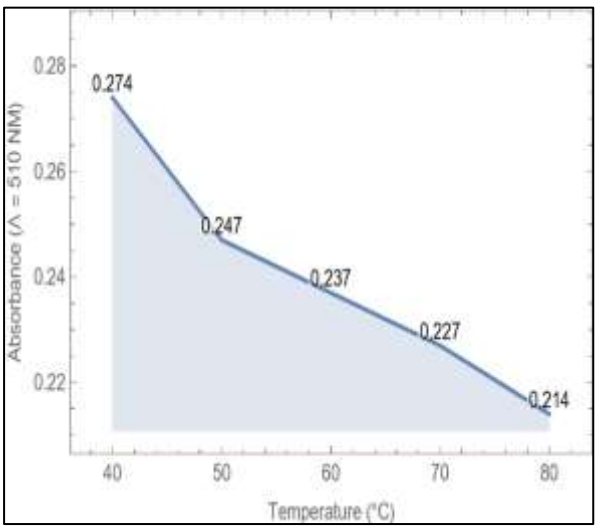

Figure 7. The absorbance of anthocyanins in various temperature.

Acylated anthocyanin is significantly more stable than non-acylated anthocyanin at all temperature [18]. Shown in **Figure 8** possible mechanism of thermal degradation for non-acylated anthocyanin [10].

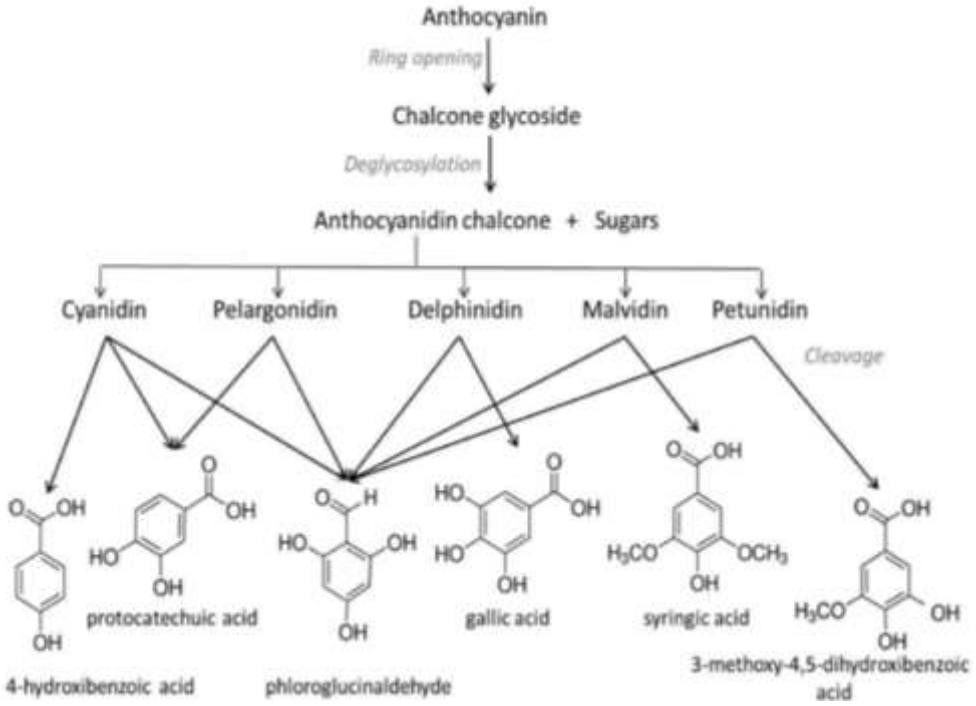

Figure 8. Possible mechanism of anthocyanins of thermal degradation for most common non-acylated anthocyanins [10].

High temperatures can change the structure of anthocyanin in equilibrium reactions from flavylium cations to chalcones. The forming chalcone because of high temperature occurs in two stages, there are hydrolysis of glycosidic bonds and producing labile aglycone, and the ring of the aglycone is opened so it forms a colorless carbinol and chalcone group. Chalcone compounds can degrade to form simpler colorless compounds, carboxylic acids such as substituted benzoic acid and carboxyl aldehyde compounds namely 2,4,6-trihydroxy benzaldehyde [10,15].

The results of agar with natural coloring dye using anthocyanins from black soybean skin are in **Figure 9**.

Figure 9. Agar with anthocyanin from black soybean skin as coloring food.

The absorbance of extracted agar using a UV-vis spectrophotometer with a wavelength of 510 nm is 0.125. The absorbance is smaller than the absorbance obtained at pH 8 and temperature 80℃. Anthocyanins from black soybean skin have degraded in colorless form due to the temperature effect of cooking [27,4].

CONCLUSION

Anthocyanins of black soybean skin have concentration from the solvent of 70% ethanol + 1% HCl 1% is 1.936 mg/mL, while from the solvent of distilled water, + 3% citric acid is 1.992 mg/mL. Anthocyanin's stability depends on pH and temperature conditions. In an acidic condition, anthocyanin is in the form of a flavylium cation that has a good stability structure, while in base condition anthocyanin has a colorless form and instability structure. In high temperatures, anthocyanin is in the form of a co-pigmentation complex.

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