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ANTHOCYANIN AS NATURAL COLORANT: A REVIEW

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ABSTRACT

On this era, people very concern to their food. The first sensory quality which is seen to choose the food are the color. However, during food processing is often occurred the color degradation, so the colorant is added to the food. Today, natural colorant is consumer’s selection because it has functional function. One of natural colorant is anthocyanin. Anthocyanin gives red, blue, and purple color. Anthocyanin has different types, it is depended to sugar and hydroxyl which bounded into structure. The types are pelargonidin, malvidin, cyanidin, delphinidin, petunidin, and peonidin. This pigment is stable on acid condition, away from light and oxygen, cold temperature, and away from polyphenol oxidase enzyme. Beside as colorant, anthocyanin also act as antioxidant because the structure is very reactive. The consequences of having antioxidant activity, anthocyanin can prevent cardiovascular disease, cholesterol, atherosclerosis, or colon cancer by blocking fat oxidation and DNA mutation. Anthocyanin source is very broad like from flowers, fruits, tubers, or fruit peels.

Keywords: Antioxidant, anthocyanin, colorant

INTRODUCTION

On this era, people are very concerning with their food that they eat. They are not only interested in its taste, but also have noticed to sensory quality of the food that they eat, like color. Color is often used as first assessment of food quality determining. The example, if the color of apple peeled is brown, people will not accept it because of enzymatic browning, or the color of whole bread is greyish green, also people will not accept it because the mold is grow. On the other hand, also color can make food more attractive and increase food acceptability. One example, people will be more interested to bright color in food like rainbow cake than brown cake (Nurhadi & Nurhasanah, 2008).

Food processing often make the decreasing of sensory quality, including the color. Therefore, manufacturers often add colorant to their products. Food synthetic colorant are often used because of their high stability, but nowadays people are more attention to the functional properties of their food that they eat, including natural food colorant. One of the natural food colorant is anthocyanin.

Anthocyanin is a pigment found in flowers, fruits, vegetables, and tubers. This pigment gives red, blue, and purple colors and there is a lot of availability in nature. This pigment can replace amaranth which are banned in the United States and Europe. Another advantage of anthocyanin is that it has a high antioxidant content that is good for health (Cahyadi, 2006; Sampebarra, 2018).

STRUCTURE AND CHARACTERISTIC OF ANTHOCYANIN

Anthocyanin word comes from the Greek word *anthos* which means flower and *kyanos* which means dark blue. It is categorized as flavonoid pigment which is soluble in water and one of the main pigment groups in higher-order plants (Fennema, 1996). Anthocyanin pigment is found in plant cell vacuoles and it is flavonoid which is naturally in the form of glycosides from flavylium or 2-phenyl benzopyrilium. This pigment belongs to the benzopiran derivative. The main structure of benzopiran is characterized by the presence of two benzene aromatic rings which are linked to three carbon atoms forming a ring. Anthocyanin is a glycoside group formed by aglycone and glycon (Hendry & Houghton, 1996). The basic structure of anthocyanin (Figure 1) consists of 2-phenyl-benzopirylium or flavylium with a number of hydroxy and methoxy.

Most anthocyanin come from 3,5,7-trihydroxyflavylium chloride and the sugar part is usually bound to the hydroxyl group on number 3 carbon (Hendry & Houghton, 1996). If the sugar part of the anthocyanin is removed through the hydrolysis process, it remain aglycone and sugar-free pigment called anthocyanidin (Hendry & Houghton, 1996). The following structure of the anthocyanidin molecule can be seen in Figure 2.

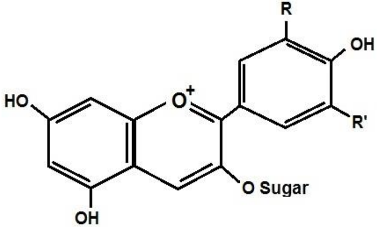

Figure 1. Anthocyanin structure (Winarno, 1998)

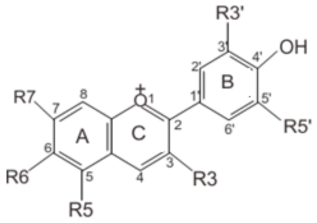

Figure 2. Anthocyanidin structure (Hendry and Houghton, 1996)

A,B : Substituted Benzene (C6)
C : Three carbon atom aliphatic chain
R3’ : H,OH/OCH$_3$
R5’ : H,OH/OCH$_3$
R3 : O-Glycosyl
R5 : O-H/Glycosyl
R6 : H
R7 : OH

In Figure 2, it show that the position of R3 is always filled with sugar. The sugar which can be bound is glucose, rhamnose, arabinose, xylose, and sometimes di- or trisaccharide. The position of R5 consists of glucose or rhamnose, while the position of R7, R3', R5', and sometimes R4 'can be substituted by glucose. Each anthocyanidin is distinguished by each R substitute group whereas most anthocyanidin is a derivative of cation 3,5,7-trihydroxyflavylium (Hendry & Houghton, 1996).

The type of anthocyanidin are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin. The differences of all the type are on R3’ and R5’. The R3’ and R5’ of pelargonidin consists of atom H and the color expression are orange and reddish orange to dark red. R3’ and R5’ in cyanidin are OH and H, the expression are reddish orange, dark orange, and purplish red to bluish red. OH is on R3’ and R5’ of delphinidin and red, purplish red, and purple to blue are the color expression. The R3’ and R5’ of peonidin consists of OCH3 and H, the color expression are purple, red, to reddish orange. R3’ and R5’ in petunidin are OH and OCH3, the expression are blue and purple to red. OCH3 is on R3’ and R5’ of malvidin and blue and light purple to red are the color expression (Hendry and Houghton, 1996).

The concentration of the pigment is very important on determining of the colour expressed by anthocyanin. In lesser concentration, the colour is blue. Meanwhile in higher concentration, the colour is red. In medium concentration, the colour is purple. The increase of the hydroxyl group tends to strengthen the color to be more bluish (Fennema, 1996).

The double bond conjugated to the chromophore group in the anthocyanin structure makes it to be able to absorb the light with maximum absorbance in the visible light region. The more and the longer conjugated the double bond to anthocyanin, the stronger the colour. It will cause the light absorption to the longer wavelength. It is because the energy needed to the transition to the conjugated double bond is getting smaller, so that the light absorption will shift more to the larger wavelength (Mahmudatussaadah et al., 2014).

Anthocyanin can absorb light radiation in the ultraviolet area (UV) absorption until the visible light, but it absorbs stronger in the visible light area. It can absorb light at the wavelength of 250 - 700 nm, with two peaks as sugar groups (glycon) at the wavelength of 278 nm, and the main peaks as anthocyanin (aglycones) at the wavelength 490-535 nm (Mahmudatasaadah et al., 2014). The following is the maximum wavelength of each aglycone which can be seen in Table 1.

Table 1. Maximum Absorption Visible Light Wavelength of Aglycons

Aglycon	**λmax (nm)**	**Color**
Pelargonidin	494 nm	Orange
Cyanidin	506 nm	Reddish Orange
Peonidin	506 nm	Reddish Orange
Delphinidin	508 nm	Red
Petunidin	508 nm	Red
Malvidin	510 nm	Bluish Red

Sumber: Farahmandazad (2015)

ANTHOCYANIN STABILITY

Anthocyanin is a reactive compound. This reactivity is because the flavylium cation nucleus experiences a lack of electrons, so that its colour easily degraded. It occurs due to changes in the red flavylium cation to an alkali colourless carbinol and eventually it becomes a colorless chalcone (Markakis, 1982). The changes in anthocyanin structure can be seen in Figure 3.

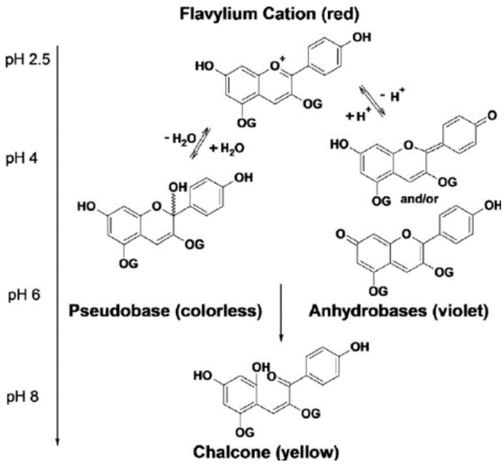

Figure 3. The changes of anthocyanin structure (Barnes, *et al*., 2009)

Anthocyanin is hydrophilic which is soluble in water easily (Husna et al., 2013). However, it can also be dissolved in polar organic solvents such as ethanol, methanol, acetone, and chloroform (Kristiana et al., 2012). Anthocyanin is very stable in acidic conditions, so that when extraction can be added organic acids such as acetic acid, citric acid, or hydrochloric acid (Sipahli et al., 2017). A mixture of polar solvents and organic acids that will produce very acidic conditions can stabilize the anthocyanin which gives a red color. However, if the solvent is mixed with a weak acid, the anthocyanin will change into faded red at pH 3; purplish red at pH 4; purple at pH 5-6; and purple blue at pH 7. The form of anthocyanin at pH 1 is a flavylium cation, at pH 2-4 in the form of a mixture of flavylium and quinoidal cations, and at pH 5-6 form two colorless compounds namely pseudo-alkali carbinol and chalcone (Pedro et al., 2016; Sitepu et al., 2016).

Beside the acid, many other factors can influence the stability of anthocyanin, which is the presence of the polyphenol oxidation enzyme, temperature, light, and oxygen (Fennema, 1996; Hendry and Houghton, 1996). The enzyme reacts in the form of o-diphenol and oxygen which will oxidize anthocyanin. Polyphenol will initially oxidize o-diphenol to benzoquinone which will later react with anthocyanin in non-enzymatic reactions to form oxidized anthocyanin and other degradation products. High temperature can change the structure of anthocyanin in equilibrium reactions from flavyllium cations to chalcones. The forming of chalcone because of high temperature occur in two stages, there are hydrolysis of glycosidic bonds and producing labile aglycone, and the ring of aglycone is opened and forms a colorless carbinol and chalcone group (Markakis, 1982; Hendry & Houghton, 1996; Fennema, 1996).

Chalcone compounds are able to degrade to form simpler colorless compounds, carboxylic acids such as substituted benzoic acid and carboxyl aldehyde compounds namely 2,4,6- trihydroxy benzaldehyde (Hendry and Houghton, 1996). Oxygen can affect the stability of anthocyanin directly and indirectly. The direct effect of oxygen is that it can oxidize anthocyanin into a colorless compound, whereas for indirectly effect, some hydroxyradical compounds can oxidize anthocyanins to form colorless compounds such as chalcone (Rein, 2005).

Light can degrade anthocyanin because it has energy that stimulates photochemical reactions (photooxidation). This reaction can cause the opening of the aglycone ring which begins with the opening of the carbon ring number two so as to form colorless compounds such as chalcone (Hendry and Houghton, 1996). The anthocyanin stability can be maintained by a copigmentation reaction. It is the interaction between the structure of anthocyanin with other molecules such as metals (Al^{3+}, Fe^{3+}, Sn^{2+}, Cu^{2+}) and organic molecules such as flavonoids (flavones, flavonoons, and flavonols), alkaloid compounds (caffeine), and so on. The presence of copigmentation with metals and other organic molecules tends to increase the color stability of anthocyanins (Hendry and Houghton, 1996).

THE ROLE OF ANTHOCYANIN AS ANTIOXIDANT

The existence of the conjugated double bond in the anthocyanin structure can make it very reactive and can function as an antidote to radical compounds or antioxidants (Barrowclough et al., 2015). The more phenolic hydroxyl groups that are bound to anthocyanin, the stronger the antioxidant activity (Han et al., 2017). Anthocyanin can react with various types of free radicals derived from reactive oxygen, such as peroxyl ($ROO^{\cdot}$), hydroxyl ($^{\cdot}OH$), and singlet oxygen ($O2^{\cdot}$). The free radicals are compounds that can be formed in the strucrure itself by prooxidative enzymes as well as those from the environment such as cigarette smoke, pollution, fat oxidation, exhaust fumes, and exposure to other chemicals (Muttalib et al., 2014).

Because anthocyanin has antioxidant activity, it can prevent various degenerative diseases such as cardiovascular disease, cholesterol, colon cancer, and atherosclerosis. The mechanism of anthocyanin in reducing cholesterol levels in the blood is to oxidize LDL (low density lipid). Forbes-Hernandez et al., (2017) who tested strawberry methanol extract on HepG2 cells showed a decrease in cholesterol levels by 13.6% compared to before being given strawberry methanol extract. The mechanism that occurs is the termination of the chain of propagation of free radicals and all hydroxyl groups on ring B can contribute electrons so that free radical inhibition occurs. (Forbes-Hernandez et al., 2017).

The mechanism of antioxidants in preventing colon cancer is by blocking the cancer initiation stage by inhibiting DNA damage caused by carcinogens (Manson, 2003), in addition antioxidants can prevent the mutation of DNA by stabilizing free radicals and inhibit the occurrence of chain reactions from the formation of free radicals that can cause stress oxidative. Antioxidants can also act as free radical scavenger, decomposer peroxide, and reduce singlet oxygen (Glasauer et al., 2014).

SOURCES AND EXTRACTION OF ANTHOCYANIN

Anthocyanin sources are very abundant in nature such as flowers, leaves, fruits, tubers, and fruit peels. The following anthocyanin sources and levels can be seen in Table 2.

Table 2. Anthocyanin Contents in Various Sources

Sources	Content
Rose	0,0925%/g
Hibiscus	0,0739%/g
Rosella	0,0795%/g
Four o'clock flower	0,0977%/g
Red spinach	6350 ppm
Purple sweet potato	0,24-0,44 mg/g
Red cabbage	11,11-17,80 mg/g
Strawberry	20,8 mg/g
Wine	0,267 – 1,9 mg/g
Mulberry	19,93 mg/g
Red dragon fruit	0,088 mg/g
Jamblang	1,61 mg/g
Red dragon fruit skin	22,593 ppm
Rambutan skin	4,1.10-3 mg/ml
Mangosteen skin	593 ppm
Jamblang skin	0,19 mg/g
Eggplant skin	750 mg/g
Jenitri's skin	0,2387 mg/g

Source: Djaeni *et al.* (2017); Sangadji *et al.* (2017); Ahmadiani *et al.* (2014); Pebrianti *et al.* (2015); Winata *et al.* (2015); Anggraini *et al.* (2018); Winarti *et al.* (2018); Zulfajri *et al.* (2018); Lestario *et al.* (2011); Handayani *et al.* (2012); Farida *et al.* (2015).

The table shows that anthocyanin sources are found in nature abundantly. Vegetable sources such as radish, purple sweet potato or red cabbage have been shown to provide higher percentage of acylated anthocyanins than fruits. Beside that, radish and red potato have the potential to be used as an alternative for Federal Food Drug and Cosmetic Red No. 40 (Allura red). Acylated pelargonidin extracted from red radish given a red color close to Allura red at pH 3.5 (Shipp and Abdel-Aal, 2010).

Black carrot is a good anthocaynin source not only for its color, but also it has high ratio of monoacylated structures increasing color retention at low pH like small amounts of non-anthocyanic phenolics which is oxidize easily. Red wine is very good anthocyanin source because it can be reduced to form stable complexes during fermentation and generated intermediate products further react to yield color stable anthocyanin derivatives.

Red rice is commonly used as a food colorant in China and was approved by the Chinese Ministry of Health to improve the color of meat, fish, and soybean products. Red cabbage and radish extracts not only improve the color of food but also reduce unnecessary aroma and flavour compounds. Another applications of anthocyanin is to acid fruit preparations, jams and preserves. The viability of acylated anthocyanins from red radish, red cabbage, black carrot and grape skin extract to color dairy products such as yogurt and sour cream having pH levels around 4.2-4.5 (Shipp and Abdel-Aal, 2010; Giusti and Wrolstad, 2003).

Anthocyanin can be obtained by extraction process with acidic condition. Because cation flavylium form is very stable and give strong color on acid environment. The best organic solvent to extract is aquadest and tartrate acid as acidulant. This acidation is done to decrease pH so flavylium cation still stable and increase the extraction efficiency. Anthocyanin extraction process consist of destruction of anthocyanin source, maceration extraction about 6-10 hours, decantation, centrifugation to remove slurry, vacuum filtering, and vaporization to have concentrate of anthocyanin (Tensiska, 2007).

Beside water, anthocyanin can be extracted by methanol or ethanol with small amount of acid. Ethanol is more preferable than methanol because of less toxic in food. If the extract contains lipid part, organic solvent like hexane must be added to the extract for eliminate lipid-containing substances. The pH level has a significant influence on the color of the anthocyanin extracts. At lower pH ($pH < 2$), the color extract is a red to dark and at a higher pH ($pH > 4$) extracts exhibited a yellow color, and anthocyanin is decreased 94% in pH 5 than lower pH (pH 1) (Shipp and Abdel-Aal, 2010).

CONCLUSION

Anthocyanin is one of pigment commonly applied as natural colorant in food. This pigment gives red, blue, and purple colors. Anthocyanin is a reactive compound. This reactivity is because the flavillium cation nucleus experiences a lack of electron, so that its color easily degraded. Anthocyanin is hydrophilic and very stable in acidic conditions. In addition to acidic conditions, it is stable when it is stored in cold temperatures, no oxygen, and no light exposed, and no polyphenol oxidase enzyme.

Besides acting as a coloring agent, anthocyanin can play as an antioxidant role. It is because the conjugated double bond in the anthocyanin structure makes it very reactive and can act as an antidote to radical compounds. Anthocyanin sources in nature are very abundant which come from fruits, flowers, and tubers.

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