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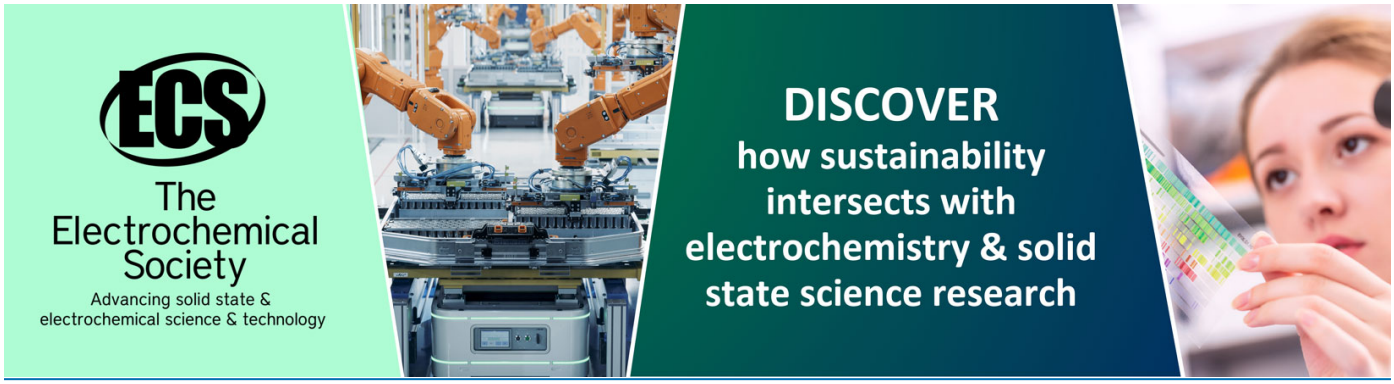

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The Effect of pH and Color Stability of Anthocyanin on Food Colorant

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Abstract. Anthocyanins are naturally occurring pigments of red and purple. Red anthocyanin pigments provide a strong and sharp and widely applied in various industries such as food coloring or drink. Anthocyanins isolated by maceration, extraction and thin layer chromatography (TLC). The extract has been obtained from the initial stages of maceration then separated into several fractions by chromatography to isolate fractions colored dark red. Identification of chemical compounds with TLC (Thin Layer Chromatography) is able to distinguish the fraction of anthocyanin produced. FTIR (Fourier Transform Infrared Spectroscopy) used to identification of the functional group of a compound. The UV-Vis absorption spectra have to produce maximum absorbance values that describe the intensity of anthocyanin spectra in different colors for different pH. Anthocyanins are more stable at low pH (acidic conditions) which gives a red pigment. Meanwhile, the higher the pH value of anthocyanin will provide color fading of the color blue. So as a food colorant, anthocyanin with a low pH or height pH has a significant effect on the food colorant.

1. Introduction

Anthocyanins are naturally occurring pigments of red and purple. Red anthocyanin pigments provide a strong and sharp and widely applied in various industries such as food coloring or drink. Anthocyanins are consumed as part of a normal diet and many times with higher levels than other flavonoid classes. The anthocyanin consumption in Europe are: 19.8 (Netherland) – 64.9 mg/d (Italy) (male), and 18.4 (Spain) – 44.1 mg/d (Italy) (female) [1].These compounds have been reported to exert health beneficial effects like cardiovascular protection and anti proliferative [2].

Anthocyanins are flavonoids of phenolic compounds. Whereas, the phenolic compounds are composed of a large group of organic substances, and flavonoids are an important subgroup [3].The flavonoid subgroup contains the anthocyanin's, one of the most broadly naturally source of colorants. On the anthocyanin structure (figure 1) there are 7 positions labeled R. R basically means that it can be occupied by almost any organic group like a methoxyl group, sugar, and the number of R that are occupied by specific substitutions would determine the color of the anthocyanin [4].

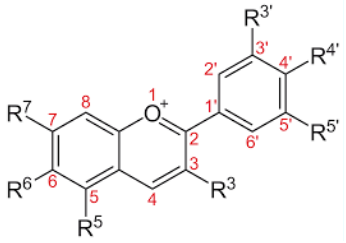


Figure 1. Structure of anthocyanin

Phenolic compounds are composed of a large group of organic substances, and flavonoids are an important subgroup. The flavonoid subgroup contains the anthocyanins, one of the most broadly naturally source of colorants including blue, purple, violet, red [5]. Anthocyanin comes from two words, "Antho" means flower and "cyanin: mean blue. Additionally, it is considered as a functional ingredient due to several anthocyanins has been shown to passes health-promoting properties [6]. The basic colors blue, purple, red and orange have a direct relation with the number of hydroxyl groups and indirect relation with the number of methoxyl groups. According to research the basic colors blue, purple, red and orange have a direct relation with the number of hydroxyl groups and indirect relation with the number of methoxyl groups [7]. Also, it has been showed that hydroxylation at position 4 changes the colors toward red tones. Anthocyanins are of great economic importance as pigments of fruit juices and wines. One of the main claims of the food industry nowadays is for natural colorants to replace synthetic red dyes, and anthocyanins are the principal candidates, however just anthocyanin and lees (sediment of the grape juice tanks) preparations are the only anthocyanin sources approved by FDA to be used for human food, while the main use is in the production of beverages and soft drinks [8]. However, anthocyanin instability limits their use, and different preparations have been evaluated to avoid anthocyanin degradation.

2. Experimental

2.1 Sample preparation

Rose flower was dried in an oven with temperature ± 50 °C. And then the dry sample was powdered using a blender.

2.2 Sample maceration and extraction

Extraction of rose flower powder used ethanol 96% and HCl 0.1 M as a solvent with ratio 4:1 in 24 hours. Residue and filtrate were separated by vacuum filtration. Then filtrate was evaporated to remove the solvent with a rotary evaporator, yielded concentrated extract.

2.3 Vacuum liquid chromatography (VLC)

Concentrated extract was fractionated by vacuum liquid chromatography to separate the polar and non-polar fraction. The eluent was used ethyl acetate and methanol. The eluate collected in a glass beaker and evaporated solvent with a rotary evaporator. Characterization eluate was conducted with Thin Layer Chromatography (TLC) and UV-vis spectrophotometry.

2.4 Gravity Chromatography

The chromatography used stationary phase Sephadex LH-20. 0.38 grams of sample eluted in the column of Sephadex. Samples eluted with solvent 0.1% HCl in methanol until the entire sample can be eluted out of the column. Each eluate evaporated solvent with a rotary evaporator. Characterization eluate was conducted with Thin Layer Chromatography (TLC), UV-vis spectrophotometry and Fourier Transform Infrared (FTIR).

2.5 Characterization of anthocyanin extracts in various pH

Measurement of the color intensity of anthocyanin extract was performed on six pH conditions (1, 3, 5, 7, 9, 11). Each solution was measured at the maximum wavelength with a spectrophotometer at range 400-800 nm.

3. Result and Discussion

3.1 Isolation, fractionation, and purification of compounds Roses Red

The maceration process of red roses carried out in acidic conditions with 0.1 M HCl (ratio 4:1). The addition of acid serves to denature cell membrane of plant, dissolve the pigment anthocyanin, flavonoid prevents oxidation and increases the polarity of the solvent that the dye is dissolved [9]. Figure 2 shows the results of maceration filtrate and concentrated extract of the red rose. Obtained concentrated extract as much as ± 422 g.

(a) (b)

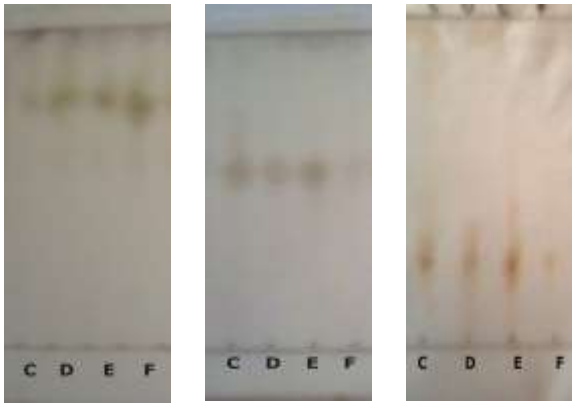
Figure 2. (a) Maceration filtrate (b) Concentrated extract of red rose

The next separation process was fractionated with vacuum liquid chromatography (VLC) (figure 3). In the process of VLC performed used eluent EtOAc: Methanol, because before it was did TLC to determine the appropriate eluent. The results of separation with VLC obtained 12 fractions and identified with Thin Layer Chromatography (TLC). Figure 3 shows the results of identification by TLC and the fraction C, D, E, F, G, H, I, J have 1 spot.

(a) (b) (c)

Figure 3. (a) Fractionation process (b) Filtrate of VLC (c) TLC of fraction (eluent butanol: ethanol: water (6: 3: 2))

From figure 3 was known fraction C, D, E, and F showed the color of the fraction is red. The fraction C, D, E, F were identification by TLC using different polarity eluent to know the pattern of the separation of compounds. The results of TLC analysis of the fraction of C-F shown in figure 4.



(a) (b) (c)

Figure 4. Identification of TLC with different eluent polarity (a) 2-propanol: acetic acid: water (7: 1: 2), (b) Butanol: Ethanol: water (5: 2: 3), (c) Butanol: acetic acid: water (4.1.5)

Figure 4 shown the fractions C, D, E and F have same separation profile, to determine the next phase separation did analysis with UV-Vis spectroscopy. The spectra of absorption UV-Vis is shown in figure 5 where the fraction C of the separation with VLC has a sharp peak in the 350-400 nm. The spectra indicated the fraction possibilities only one type, there are anthocyanin compounds. For further purification of fraction C was separated with chromatography gravity.

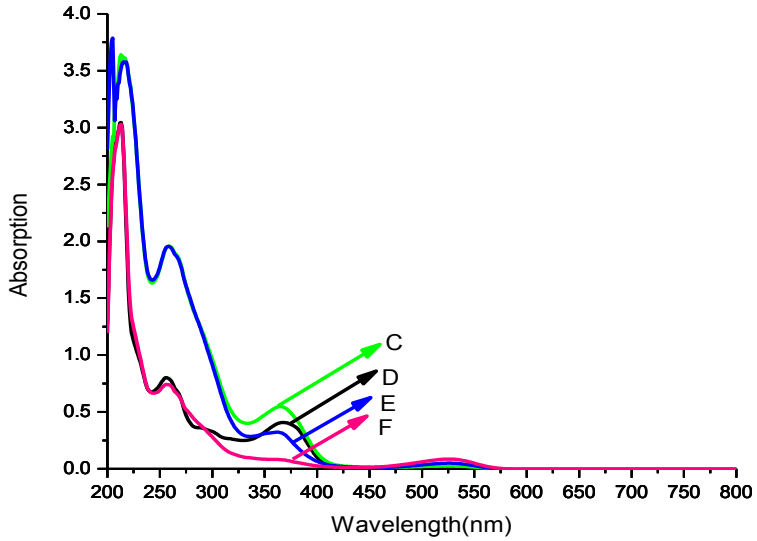
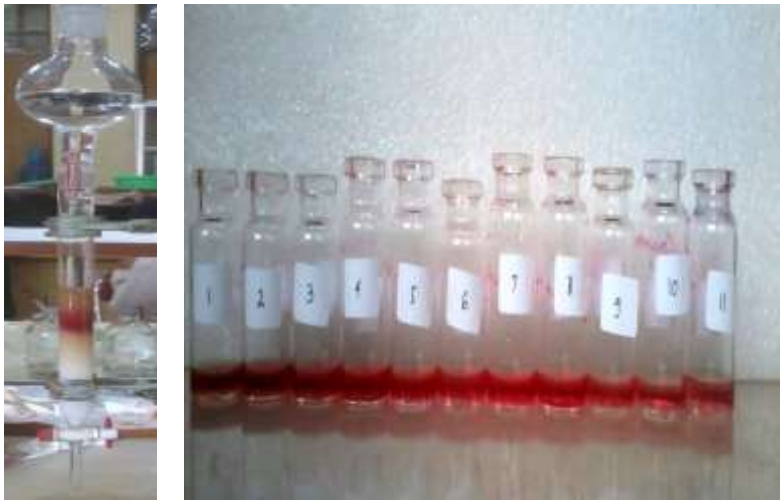


Figure 5. UV-Vis spectra of fractions C, D, E and F

The separation with gravity chromatography used stationary phase Sephadex LH-20 and eluent 0.1% HCl in methanol. Figure 6 shows the gravity separation process chromatography use Sephadex LH-20 column and the fraction result of separation. The figure show the color of the fraction is red, indicated anthocyanin compounds.



(a) (b)

Figure 6. (a) Process of separation by gravity column (b) Fraction of the result separation

The pattern of separation results gravity chromatographic can be determined by TLC identification with eluent butanol: ethanol: water (6: 2: 3) and shown in figure 7.

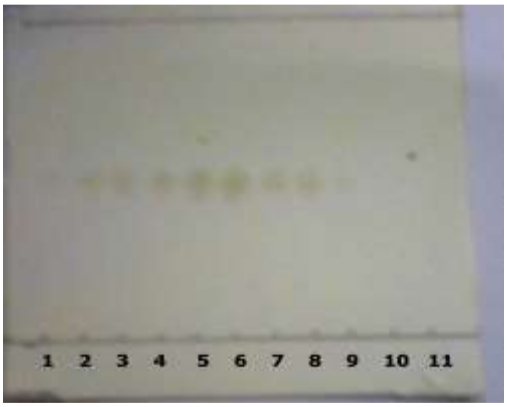
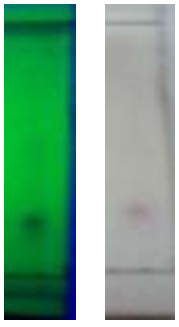


Figure 7. Results of TLC with eluent B: E: A = 6: 2: 3

From figure 7 it is known that the fraction of the number 7 is considered purer, that the purity test conducted using the eluent ethyl acetate: 2-propanol: water: formic acid (6: 2: 2: 1). Figure 8 shows the results of TLC provides one spot that it is possible to have been obtained anthocyanin pure compound. To ensure the purity of the compound then analysis of UV-Vis Spectroscopy and FTIR Spectroscopy.



(a) (b)

Figure 8. TLC with eluent ethyl acetate: 2-propanol: water: formic acid (6: 2: 2: 1) (a) In terms of a UV lamp 254 nm, (b) after sprayed with Ce $(SO_4)_2$

The results of UV-Vis spectra of the rose extract is shown in igure 9. Rose extracts contained 3 peaks in the area 268 nm, 364 nm and 526 nm [10]. Isolated compounds only appeared two peaks, namely 288 nm and 526 nm. The maximum wavelength can be seen in table 3.

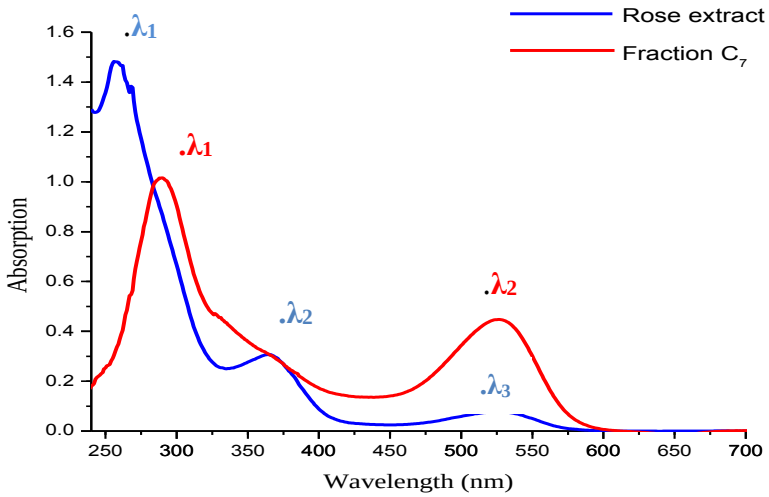


Figure 9. UV spectra extracts and isolates results (C7)

Table 1. The maximum wavelength of the compound isolates

λ	Wavelength (nm)			
	Extracts	Isolat	Anthocyanin	
			Cyanidin-3-sophoroside	Cyanidin-3,5-diglukoside
λ_1	256	288	283	270
λ_2	329	-	-	-
λ_3	526	526	526	526

Based on the results of spectra UV-Vis, C7 have aromatic groups in the area λ 288 nm. The aromatic group generally appears at λ 270-300 nm Beside it, in the area λ 526 nm originated from carbonyl groups conjugated (C=C). The wavelength of 526 nm is the wavelength anthocyanin cyanidin types [11].

From the IR analysis (table 2) can be seen that functional groups present in the compound C7. The existence of the aromatic group of spectra UV is amplified by the IR spectrum (figure 10) which shows the C = C aromatic absorption at wavenumber 1654 cm^{-1} and 677 cm^{-1} which indicate the presence of aromatic C-H bond. In addition, the aromatic group of IR also found in the C=C bonds alkenes appear in wavenumbers 1556 and 1516 cm^{-1}. C-O group detected at 1029 cm^{-1}, the hydroxyl group at wave number 3385 cm^{-1} and C-H absorption stretching appear at wave numbers 2943 and 2841 cm^{-1} which make up a group the sugar.

Table 2. Functional groups on the compound isolates

Functional group	Wavenumber (cm^{-1})	
	Isolate	Cyanidin
OH	3385	3370
C-H alifatic	2841	2840
C = C aromatic	1654	1660
C=C alkanes	1556 and 1516	1560
C-O	1029	1260
C-H aromatic	677	675

FTIR spectra C7 known that compounds have hydroxy groups, groups of alkanes and aromatic groups, which it can be concluded that the compound C7 isolate anthocyanin. In particular anthocyanins cyanidin compound has an absorption in the 3100-3400 cm^{-1} (OH);2900-2840 cm^{-1}(C-H aliphatic) 675-870 cm^{-1} (C-H aromatic) and 1660 cm^{-1} (C = C aromatic) [12].

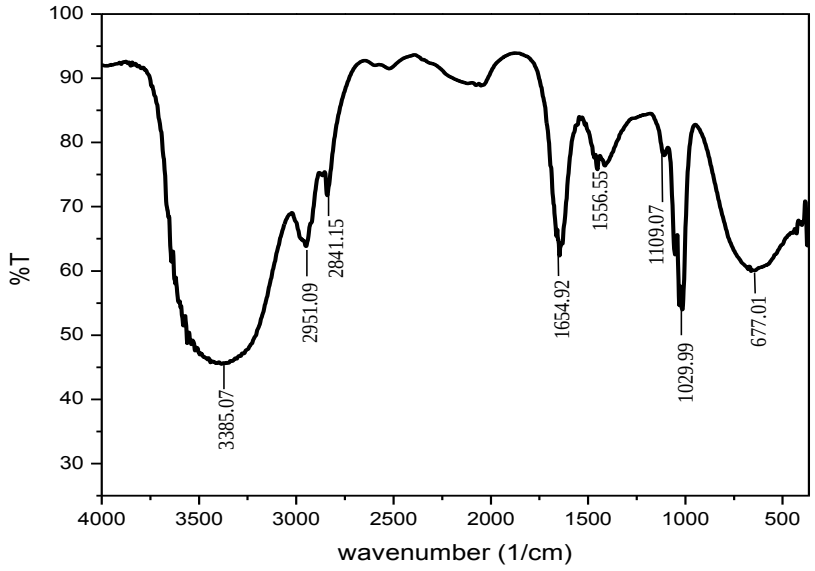


Figure 10. Infrared spectra of compound C7

3.2 Effect pH and stability color of anthocyanin

The stability of anthocyanin was influenced by several factors such as pH, temperature, light, and oxygen [13]. According to Clydesdale (1998) [14] and Markakis (1982) [15] Pigment anthocyanin (red, purple and blue) is an unstable molecule if there is a change in temperature, pH, oxygen, light, and sugar. Based on the analysis show that anthocyanins are red at low pH (acidic conditions), the higher the pH value of anthocyanins will provide color fading of colorless, yellow purple and blue.

Figure 11. Color of anthocyanin in variation pH.

Figure 12 shows the absorption spectrum of anthocyanin shift to a greater wavelength in base condition or pH increase. Overall the spectrum shows a peak of anthocyanin at range 400-600 nm. In the pH 2 give spectra with high absorption.

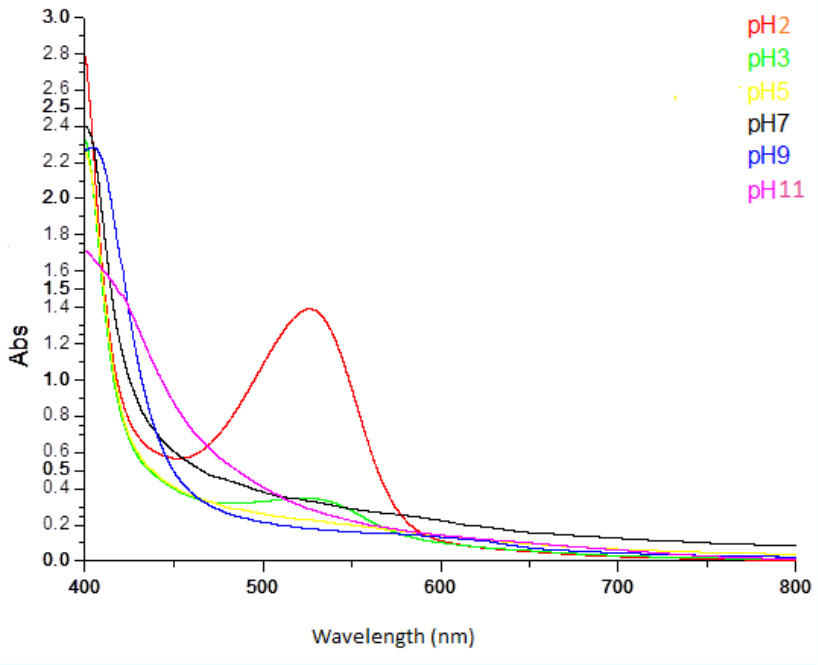


Figure 12. Effect of pH

Anthocyanins are stable at low pH. It becomes less stable when exposed to heat, causing a loss of color and browning. As a result, high temperature, increased sugar level, pH, and ascorbic acid can affect the rate of destruction. In solution, anthocyanins molecules are present in an equilibrium between the colored cationic form and the colorless pseudo base. This equilibrium is directly influenced by pH. pH is very important for the color of anthocyanins, some anthocyanins are red in acid solutions, violet or purple in neutral solutions, and blue in alkaline pH. The reason for this is right here this structure of the anthocyanins (red circle) is called flavylium cation the reason, at low pH the cyanidin molecule is protonated and forms a positive ion or cation, as the pH increases the molecules become deprotonated, at high pH the molecule forms a negative ion or anion [16, 17].This is the reason that most colorants containing anthocyanins can only be used at pH values below four. Additionally, anthocyanins can act as pH indicators.

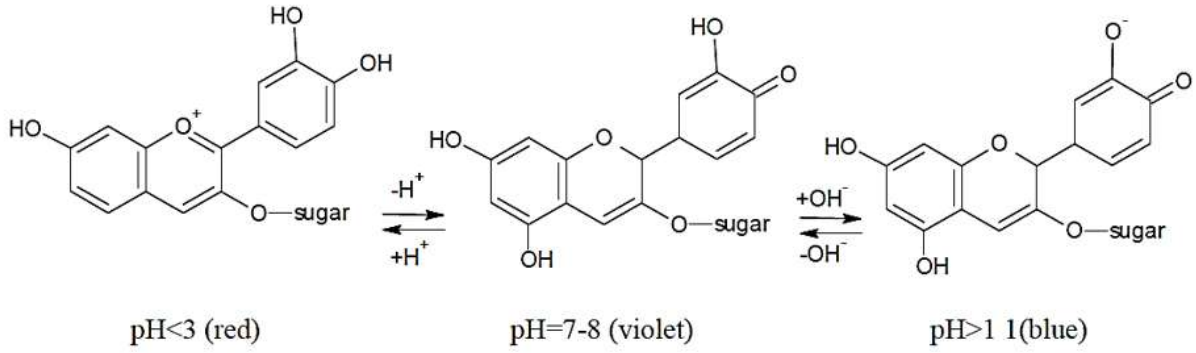


Figure 13. The equilibrium of anthocyanin

The anthocyanin belongs to the group of natural dyes responsible for several colors in the red–blue range, this spectrum depends on an abundance of anthocyanin in the natural source. As a result anthocyanin can be food colorant with variation pH.

4. Conclusion

The anthocyanin was successfully extracted from rose red flower by maceration method. The separation used vacuum liquid chromatography (VLC) and gravity chromatography. Characterization using TLC, UV-Vis, and FTIR shows the characteristic of anthocyanin compared by the standard of anthocyanin. Anthocyanin stable at pH low with red color. In the high pH, anthocyanin changed in to blue color. So, anthocyanin can be as food colorant.

References

[1] Zamora-Ros R, Knaze V, Lujan-Barroso L 2011 *Brit J Nut* **106** 1090–1099
[2] H Oliveira, N Wu, Q Zhang, J Wang, J Oliveira, V de Freitas, N Mateus and I Fernandes 2016 *Food & Function* **7** 2462-2468
[3] Yao LH, Jiang YM, Shi J F A, Tomas-Barber, Datta R N, Singanusong C, and Chen S S 2004 *Plant Foods for Human Nutrition* **59** 113–122
[4] Kong J M, Chia L S, Goh N K, Chia T.F, Brouillard R 2003 *Phytochem.* **64** 923
[5] Dai J and Mumper R J 2010 *Molecules* **15** 7313-7352
[6] A Mortensen 2006 *Pure Appl. Chem.* **78** (8) 1477–1491
[7] He F, Mu L, G-Liang Yan, Na-Na Liang, Qiu-Hong Pan, Wang J, Reeves M J and Chang-Qing Duan 2010 *Molecules* **15** 9057-9091
[8] Rymbai1 H, Sharma R R, Srivastav M 2011 *Int. J Pharm Tech Res CODEN (USA)* **3** (4) 2228-2244
[9] Arisasmita, Joek, Kuswardani I, dan Tjahjani L 1997 *Ekstraksi dan Karakterisasi Zat Warna dari Kulit Buah Manggis* (Surabaya: Universitas Katolik Widya Mandala)
[10] Saati E A, Simon B W, Yunianta and Aulanni'am 2011 *J Agr. Sci. Tech. A* **1** 1192-1195
[11] Harborne J B 1967 *Comparative Biochemistry of the Flavonoids* (London: Academic Press)
[12] Hurst W J 2002 *Functional Foods & Nutraceuticals Series (Book 4)* (New York: CRC Press)
[13] Basuki N, Harijono, Kuswanto, & Damanhuri 2005 *Agravita* **27** (1) 63-68
[14] Clydesdale F M 1998 *Color: origin, stability, measurement and quality in Storage Stability. Taub, I.A. & Singh, R. P (Ed)* (New York: CRC Press LCC)
[15] Markakis P 1982 *Anthocyanins as food additives: Anthocyanins as food colours* (New York: Academic Press)
[16] Brouillard R & Dangles O 1994 *Food Chem.* **51** 365–371
[17] Jackman R L & Smith J L 1992 *Anthocyanins and betalains In Hendry G A F & Houghton J D (Eds.), Natural food colorants* 182–241 (Glasgo: Blackie)