Identification of chemical structure of anthocyanin and other active substances of red color melinjo peels by FTIR and LC-MC analysis

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Abstract. Research on red color melinjo peels containing anthocyanin active substance has been studied since 2011, starting from anthocyanin extraction stage (maceration) by using both polar and non-polar solvents, a variation of citric acid addition and stability of anthocyanin extract storage and application of anthocyanin for food and beverage. However, research on the identification of anthocyanin chemical structures and other active substances of red color melinjo peels is also necessary. The purpose of this study was to identify the structure of anthocyanin and other active substances from red color melinjo peels with FTIR and LC-MS analysis. The results obtained: from FTIR analysis shown wavelength, among others: 3425 cm⁻¹, 1725 cm⁻¹, 1632 cm⁻¹ and 1219 cm⁻¹, it shows that inside the red color melinjo peels contained OH function group, C=O, C=C, and CO. From the LC-MS analysis shown at a minute of 1.62 observed BM = 302.236, which is the molecular weight of the Quercetin compound. The mass spectrum with the base peak height of m/z = 165, at min 3.83 h as 100% abundance, whereas the 50% abundance at peak M+m/z = 242 has BM = 271 with the anthocyanin molecule of red color melinjo peels C₁₅O₅H₁₁. The anthocyanin type of red color melinjo peels is a cyanine compound.

1. Introduction
Anthocyanin is a natural coloring which belongs to the class of flavonoids which are widely used as dyes, especially drinks. These anthocyanin pigments can replace commercial synthetic dyes that are known to be toxic and carcinogenic [1]. In addition to acting as food and beverage coloring agents, anthocyanins are also believed in biological systems with the ability to bind free radicals and the ability to inhibit chemical reactions at the initiation stage which causes carcinogenesis [2]. According to Jackman and Smith [3], the source of anthocyanins is found in some plants, among others; strawberry, cherry, plum, cabbage, grapes, and red peppers. The anthocyanin concentration of the plant was calculated from the total anthocyanin content after being separated from the solvent. Mateus and Freitas [4] explained that there were 6 types of anthocyanins in foodstuffs such as pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. The type and chemical structure of anthocyanin are influenced by the raw materials used in obtaining the anthocyanin extract. Preliminary research has been carried out by Ermiziar et al [5] where the results of red color melinjo extract with ethanol solvent containing anthocyanin which has antioxidant activity (IC₅₀) of 28.43 mg. The function of these antioxidants is as anti-free radicals. Therefore, it is necessary to know other active substances besides anthocyanins from red color melinjo which can also function as antioxidants. Ermiziar et al [6] explained that red color melinjo waste containing anthocyanin can be used as a coloring agent for food and beverages. The red melinjo skin waste is obtained from the production of chips which is usually only used for vegetables and chips but the rest is wasted.

The concentration of anthocyanins contained in the skin melinjo red is influenced by the purity,
chemical structure, and type of anthocyanin. Where in obtaining anthocyanin extract from melinjo red skin can be done by maceration process using polar solvents [6]. While the stages in determining the active substances contained in anthocyanins were carried out by characterization using FTIR and LC-MS analysis. The FTIR (Fourier Transform Infrared) analysis function is to determine the functional group based on the number of melinjo red skin anthocyanin waves. While the analysis of LC-MS (Liquid Chromatography - Mass Spectrometry) is an analytical technique used by combining the ability to separate sample components and detect sample components using mass spectrometry. LC-MS data can be used to provide information about molecular weight, chemical structure, identity and quantity of certain sample components [7]. In this study, the identification of chemical structures and other active substances from the red-skinned melinjo anthocyanin was carried out using FTIR and LC-MS from the anthocyanin extract samples obtained in previous studies. An example of anthocyanin produced in previous studies [5] is shown in Figure 1.

![Figure 1. Anthocyanin Sample [5]](image1)

2. Experimental
The material used in the study was red color melinjo peels, 96% alcohol (Food Grade) and citric acid with the analytical grade. While the method used in anthocyanin isolation is maceration process as follows: 200 grams of red melinjo peels was cut into small pieces and put into 1000 mL of 96% alcohol solvent with a ratio (w/v) of red color melinjo peels / alcohol solvent of 1:5 for maceration limes for 3 days. In the maceration process citric acid was added with a concentration of 3%. The next step was filtering to separate red melinjo peels and filtrates containing mixtures of anthocyanin extract and solvent. The last step was to separate the solvent and anthocyanin extract using a rotary evaporator. The anthocyanin extract produced was then characterized using FTIR and LC-MS analysis.

3. Results and Discussion
3.1 Characterization of anthocyanin samples by FTIR analysis
The FTIR analysis is used to identify functional groups and compounds contained in anthocyanin samples produced from red color melinjo peels as shown in Figure 2.

![Figure 2. The FTIR spectrum of anthocyanin samples from red color melinjo peels](image2)
Figure 2 showed the FTIR spectrum results of red color melinjo peels which had a wave absorption band of 3425 cm⁻¹. According to Ghassempour (2008) [10], the vibration characteristics of hydroxyl groups were seen in the wave number area of 3700 cm⁻¹-3100 cm⁻¹. So that the absorption shown at a wavelength of 3425 cm⁻¹ is characteristic for absorption for OH groups. The spectrum in the area of 1725 cm⁻¹ was stretching vibration C=O, absorption at 1632 cm⁻¹ is the vibration of C=C, and the last absorption at 1219 cm⁻¹ is the C-O stretch vibration. The results of FTIR analysis looked that the wave number range was the same as the wave number range of quercetin compounds. The obtained function groups can be seen in Table 1.

Table 1. The functional groups of FTIR analysis from anthocyanin samples

| No. | Wave number | Literature | band | Function group |
|-----|-------------|------------|------|----------------|
| 1   | 3425 cm⁻¹   | 3700-3100 cm⁻¹ | widen | O-H            |
| 2   | 1725 cm⁻¹   | 1705-1725 cm⁻¹ | sharp | C=O (ketone)   |
| 3   | 1632 cm⁻¹   | 1500-1600 cm⁻¹ | medium | C=C (Aromatic) |
| 4   | 1219 cm⁻¹   | 1080-1300 cm⁻¹ | sharp | C-O           |

3.2 Characterization of anthocyanin samples by LC-MS analysis

Liquid Chromatography Mass - Spectrometry (LC-MS) is an analytical technique that combines the physical separation capability of liquid chromatography with the specificity of mass spectrometry detection. Liquid chromatography separates the sample components and then these ions are detected by a mass spectrometer. LC-MS data can be used to provide information about the molecular weight, chemical structure, identity and quantity of certain sample components [7]. The result of LC-MS analysis for anthocyanin samples from red color melinjo peels could be looked in Figure 3.

Figure 3. The LC-MS spectrum of anthocyanin spectrum from red color melinjo peels

Figure 3 showed the results of compound identification using the LC-MS method. The chromatogram results showed that anthocyanin extract from red color melinjo peels at 1.62 min produced a molecular weight of 302.236 m/z. This molecular weight was Quercetin compound. The following is a picture of the Quercetin structure.
Figure 4. The chemical structure of Quercetin[8]

From the LC-MS spectrum in Figure 3 looked that the base peak height of m/z = 165, at 3.83 min has 100% abundance, whereas the 50% abundance at peak M+m/z = 242 has BM = 271 with the anthocyanin molecule of red color melinjo peels was C_{15}O_{5}H_{11}. This anthocyanin type of red color melinjo peels was a cyanine compound. The following is a picture of the cyanine structure

Figure 5. The chemical structure of cyanine[9]

4. Conclusion
The conclusion from the results of the characterization of anthocyanin samples using FTIR and LC-MS analysis was as follows: The FTIR analysis looked wavelength, among others: 3425 cm\(^{-1}\), 1725 cm\(^{-1}\), 1632 cm\(^{-1}\), and 1219 cm\(^{-1}\). It showed that anthocyanin from red color melinjo peels contained OH function group, C=O, C=C, and CO. From the LC-MS analysis shown at a minute of 1.62 has observed BM = 302.236, which is the molecular weight of the Quercetin compound. The mass spectrum with the base peak height of m/z = 165, at 3.83 min has 100% abundance, whereas the 50% abundance at peak M+m/z = 242 has BM = 271 with the anthocyanin molecule of red color melinjo peels was C_{15}O_{5}H_{11}. The anthocyanin type of red color melinjo peels was a cyanine compound.

Acknowledgments
This research was carried out at organic chemistry laboratory at Department of Chemical Engineering, Institut Teknologi Indonesia. The authors are grateful for the main financial supports from RISTEK_DIKTI budget 2018.

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