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Innovation of Lycopene Isolation Procedure from Tomatoes (*Lycopersicum esculentum*)

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Abstract. Tomatoes (*Lycopersicum esculentum*) are horticulture plants frequently cultivated in Indonesia. Lycopene is one of tomatoes largest chemical content in tomatoes. This research had been able to isolate lycopene with extraction method on 65 °C temperature using hexane and methanol antisolvents. Isolation procedure in this research was modification from previous research. Sample in this research were dry tomatoes powder. Research result showed that hexane solvent yielded lycopene level of 3.10 mg/100 g tomatoes powder. Functional group analysis using Fourier Transform InfraRed (FT-IR) spectroscopy detected C = C group at wavelength 1674,91 cm⁻¹ and 1639,65 cm⁻¹, aliphatic CH (stretching) was indicated by the appearance of a sharp absorption peak at number wave 2853,12 cm⁻¹, CH (CH₃). Indicates absorption at wave number 1378,71 cm⁻¹. The sharp peak at the wave number 2924,16 cm⁻¹ shows the presence of C-H alkene (stretching), and C-H alkene (bending) at the wave number 1498,86 cm⁻¹.

Keywords: Isolation, Lycopene, *Lycopersicum esculentum*

INTRODUCTION

Tomatoes (*Lycopersicum esculentum*) are horticulture plants with abundant availability in Indonesia. Tomatoes are also food material with high folic acid, vitamin C, and potassium content. Potassium content within one hundred grams of tomatoes are 245 mg. Potassium could reduce blood pressure by lowering sodium in urine and water with diuretic way [11].

Compound content within tomatoes (*Lycopersicum esculentum* Mil) amongst them are lycopene, solanine, saponine, folic acid, malic acid, citric acid, bioflavonoid (including lycopne, α and β -carotene), protein, fat, vitamin, mineral and histamine [5]. The most dominant compound is lycopene as of 63.6% [17].

The maturity level of tomatoes affects the lycopene content produced, the young green tomato contains lycopene about 25 ug / 100 g fresh weight, yellowish tomatoes 370 ug / 100 g (fresh weight, red tomato 4600 ug / 100 g fresh weight, and tomato pass mature contains lycopene about 7050 ug / 100 g fresh weight [6]. The tomato skin is dried in the sun, containing crude protein of 13.8 g / 100 g of dry matter, lycopene 112 mg / 100 g dry matter [3].

Lycopene frequently referred to as α -carotene, bright red pigment carotenoid that mostly encountered within tomatoes and another red fruits. Lycopene in nature are in form of trans which is stable form thermodynamically, dissolve in non polar solvent and found in 446-50nm wave length range. Light and heating existence potentially turn trans isomer into cis [15].

Based on previous research, [2] had been able to isolate lycopene from tomato sauce with solid liquid extraction method and crystallization using methanol and carbon tetrachloride mixture solvent yielding extraction yield of 2.313 mg/100 g of tomato sauce. [13] were able to perform extraction

with soxhlet and antisolvent (methanol) method with hexane, ethyl acetate and ethanol solvents with Ethyl Acetate extract yield of 4.39 ± 0.27 mg/g, Hexane: 3.38 ± 0.38 mg/g, Ethanol: 1.25 ± 0.29 mg/g. [18] successfully isolated lycopene from tomato juice using liquid-liquid method, using methanol and ethanol as antisolvent with the results of 3.2 mg / 150 ml and 2.8 mg / 150 ml. Therefore isolation procedure innovation are necessary to obtain higher lycopene compound content.

Table 1. Tomatoes Nutrition Content within 100 gram of Tomatoes [14]

Nutrition	Unit	Content per 100 grams tomatoes
Vitamin C	mg	22.8
Vitamin B-6	g	0.078
Folic	µg	13
Vitamin A	IU	489
Lycopene	µg	3,041
B-carotene	µg	293
Lutein	µg	94
Vitamin E	mg	0.56
Vitamin K	µg	2.8

Lycopene is carotenoid with $C_{40}H_{56}$ and has 536.85 g / mol molecule weight with the following structure formula:

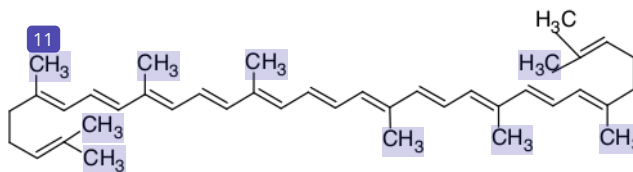


Figure 1. Lycopene Chemical Structure

Lycopene is non polar compound easily dissolved in chloroform, hexane, benzene, ethyl acetate, petroleum ether and others [9]. Method used in this research was liquid-liquid extraction. Liquid-liquid extraction is separation carried out should the separated mixture is homogenous solution (liquid – liquid) in which one component's boiling point with other component consisted within mixture are nearly similar or adjacent.

Antisolvent crystallization stage carried out with methanol addition as antisolvent. Antisolvent crystallization is an effective separation and purification method. Antisolvent usage in this crystallization reducing certain dissolved substance solvability and forming crystal in timely manner. Crystallization experiment parameter highly affected particle formation mechanism and regulated crystal size form and its distribution. Generally, antisolvent include hydrophilic stabilizer such as surfactant absorbed on crystal surface to block crystal growth [1].

This antisolvent crystallization benefit is that the process could be carried out on temperature close to room temperature. This is highly comfortable for heat-sensitive substance. In addition, this process require low energy compared to solvent evaporation process [12]. Methods in this research were extraction using reflux and tomatoes powder. In reflux there is warming of mixture of simplicia and solvent in a round bottom flask. The solvent condenses with the coolant in the condenser through which the solvent vapor passes [8].

MATERIALS AND METHODS

The materials used are n-hexana, methanol, aquadest, and fresh tomatoes which are washed using water, tomato seeds are discarded. Tomato is cut and dried using an oven for 3 days at a temperature of 60°C until a moisture content of around 0.8% is obtained. Then the dried tomatoes are ground. The tools used are: Blender, 1 set of reflux tools, scales, beaker glass, erlenmeyer, measuring cup, drop pipette, separating funnel, FT-IR spectroscopy.

100 grams of tomatoes powder inserted into 500 ml round flask bottom, added with 300 ml of hexane, that subsequently extracted on 65 °C temperature for 3 hours. Extract then separated from its raffinate. The obtained extract then added with 100 ml aquadest to eliminate impurities included with extract then separated using separating funnel. Subsequently, 100 methanol were added as antisolvent that it produced lycopene crystal. Obtained lycopene crystal in optimum condition then analyzed using Fourier Transform Infrared Spectroscopy (FTIR).

RESULT AND DISCUSSION

FTIR spectrophotometry is a method that observes the interaction of molecules with electromagnetic radiation in the wavelength region of 0.75-1000 μm or at wave numbers of 13,000-10 cm^{-1} [7]

FTIR (*Fourier Transform Infra Red*) characterization result of lycopene extract from tomatoes using hexane solvent with methanol addition as antisolvent, aimed to identify lycopene compound functional groups. FTIR (*Fourier Transform Infra Red*) result is presented in the following figure 2.

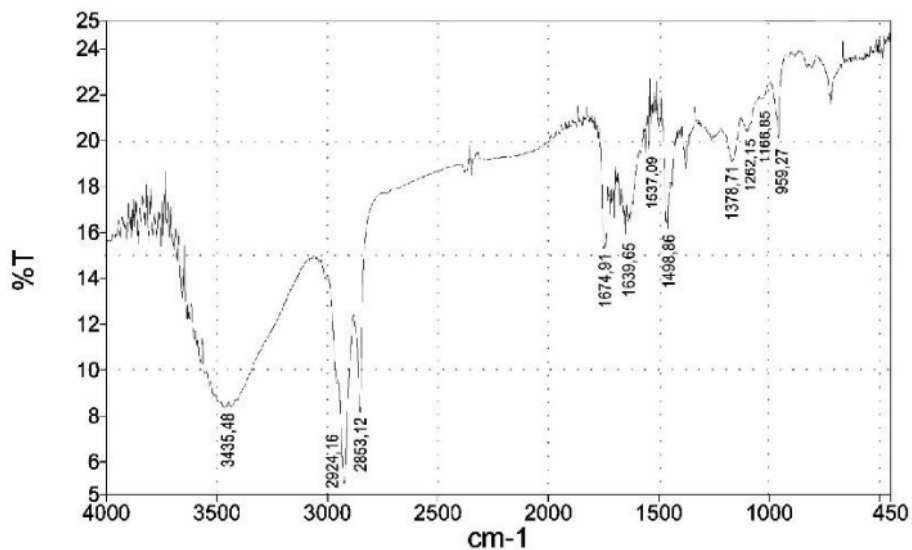


Figure 2: Characteristics of FTIR (*Fourier Transform Infra Red*) Lycopene in Comparison of 1: 3 Feed and Solvent (F / S) with Antisolvent volume: 100 ml

Table 2. Results of Characteristic Analysis (Fourier Transform Infra Red) of Lycopene Based on Its Function Group.

Functional groups	Wavelength (cm ⁻¹)		
	Kamil et al (2011) [10]	Bunghez et al (2011) [4]	Lycopene results from Analysis
Aromatic ring stretch (C=C)	1510		1537,09
Symmetrical of CH ₂ lycopene	1444		1498,86
Stretching OH	3450		3435,48
CH ₂ asymmetrical	2856		2853,12
R-CH=CH-R lycopene	960		959,27
C-H bending		1477-1400	1498,86
C-C and C-C-H Stretching		1400-1100	1262,15
C-O stretching		1170-1115	1166,85
V(C-O-C)		900-1200	959,27

Table 2 shows the results of the functional group analysis on lycopene crystals from tomato extract through FTIR spectroscopy performance which showed that lycopene has a double bond C = C at a wavelength of 1537.09 cm⁻¹. There is a symmetric functional group of CH₂ lycopene at a wavelength of 1498.86 cm⁻¹. O-H range groups occur at a wavelength of 3435.48 cm⁻¹ which allows water vapor to be included in lycopene. The R-CH group = CH-R in lycopene has a wavelength absorption of 959.27cm⁻¹. Whereas the bent C-H group in lycopene is located at a wavelength of 1498.86 cm⁻¹. In the C-C and C-C-H strain groups, each occurs at a wavelength of 1262.15 cm⁻¹. For strain groups C-O has a wavelength of 1166.85 cm⁻¹ and for stretch vibration C-O-C has a region with a wavelength of 959.27 cm⁻¹ which indicates that there are residues of solvents and other compounds that are joined with lycopene.

Based on these comparisons it can be concluded that lycopene has been analyzed according to standards based on previous research.

In general, maximum carotenoid absorption at three wavelengths that appear in the form of three spectrum peak. Compounds with a greater number of conjugated double bonds have a higher wavelength value. Lycopene with 11 conjugated double bonds absorbs at the highest wavelength compared to other carotenoids.

The solvent used is very influential on the amount of lycopene obtained, this is because the interaction of the lycopene compound with the solvent used is the dispersion of solvent molecules. Lycopene compounds tend to be perfect if the solvents used are non-polar. This happens because the intermolecular forces between similar compounds tend to have the same strength. This tendency causes the rules "like dissolves like".

Antisolvent crystallization is a method of separation and purification which is an effective way to produce nano-sized particles [19]. The use of antisolvent in the crystallization process reduces the solubility of solutes in the solution and causes rapid crystallization. The crystallization parameter greatly influences the mechanism of particle formation, crystal size, and distribution [16]. So from the research that has been done, it can be concluded that the results of the research are in accordance with the theory.

CONCLUSION

Based on the results of research and analysis of FTIR (*Fourier Transform Infra Red*) spectroscopy it could be concluded that lycopene level obtained using hexane and methanol as an antisolvent were as of 3,10 mg/100 g (tomatoes powder).

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