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Facile Synthesis of Lycopene Reduced Graphene Oxide in Different Solvent Polarity

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Abstract. Graphene is a two-dimensional carbon which has many applications. Due to high utilities of graphene, the synthetic procedures should be developed to increase its production. The synthesis of reduced graphene oxide (rGO) using lycopene were carried out in the presence of polar and non-polar solvent. The lycopene was extracted from watermelon by solid-liquid extraction using n-hexane/acetone/ethanol in 2:1:1 (v/v/v) and gave 5 mg/g of watermelon. The reduction of GO was performed by dissolved 100 mg of GO in 50 mL deionized water followed by sonication for 120 min, then added with 15 mL of 37 mM lycopene, reflux for 3-24 h in hexane/water and acetone/water in 3:10 (v/v) ratio, respectively. The result showed that the reaction using acetone/water in 3 h produce rGO. Replacing the solvent into hexane/water and reacted for 24 h produce rGO with similar characteristic. FT-IR analyses showed typical absorption of C=C alkenes at 1651 cm^{-1} . The XRD showed 2-theta at 21.5° that indicate a typical peak of rGO. The UV-Vis analysis revealed a maximum wavelength that shifted from 231-271 nm. All characteristic of the synthesized rGO showed that lycopene can be used as natural reductor for rGO synthesis.

1. Introduction

Graphene is a carbon allotrope that has an arrangement of hexagonal skeletal atoms and forms one sheet as thin as one atom. Experimentally, this carbon allotrope was discovered in 2004 in the most outer layer of carbon [1]. The graphene lattice is composed of two layers of carbon atoms which have sigma bond. Each carbon atom in this layer has phi-orbital. Graphene is commonly used as an adsorbent and material in supercapacitors [2].

Various methods have been carried out to synthesize graphene, that is by the growth of silicon carbide (SiC) and Chemical Vapor Deposition (CVD) in Ni and Cu metal. However, this method is less efficient and requires expensive costs. Recently, Hummer's developed an efficient method to obtain graphene by sequence reaction started from oxidation of graphite into graphite oxide followed by reduction into graphene [3]. Then, the development of reduction graphite oxide become widely explored.

Stankovich and co-workers (2007) was reduced graphite oxide using hydrazine hydrate (N_2H_4) [4]. Shin *et al.* (2008) reported the used of 150 mM NaBH_4 solution for reducing agent [5]. However, the use of N_2H_4 and NaBH_4 is dangerous due to its toxicity. Further research is the used of metal as reducing agent, such as Zn which is harmless, unpoison and react in acid or basic solution [3]. Nevertheless, metal reductor is environmentally not friendly reagent just like as organic compound. To overcome this problems, the use of natural reagent is developed as eco-friendly reducing agent, such



as ascorbic acid [6], sugar [7], hydrolyzed tannins [8], hydroxylamine [9], yeast [10], phenolic compounds such as polyphenols in tea [11] and beta-carotene [12]. These compound is reported as deoxygenation agent which have similar reactivity with hydrazine.

The reduction of graphene oxide into graphene also influenced by reaction time and solvent type. Sungjin, *et al* (2009) reported the reduction of graphene oxide using hydrazine with various of organic solvent (DMF, NMP, ethanol, DMSO, and acetonitrile). They reported after addition some of hydrazine monohydrate in suspension of graphene oxide, DMF/water solvent had a stable one than the other solvent. The results indicating the best combination of solvent that used is DMF/water.

Lycopene is one of carotenoid groups which consist of 8 isoprene units, containing 11 conjugate bonds and 2 non-conjugated double bonds between carbon atoms. Lycopene can be isolated from many fruits, such as watermelon (45.32 mg/kg), tomatoes (25.73 mg/kg), apricots (3.2mg/kg), red wine (14,19mg/kg), and carrots (0.02 mg/kg) [13]. Similar with beta-carotene, lycopene is reported as reductor in oxygen singlet formation and inhibit lipid oxidation [14]. Sukarnawan (2008) reported that antioxidant compoundis able to be reductor because of easy to oxidaze, free radical capture, metal chelating, and reducing formation of oxygen singlet [15]. Lycopene is not degraded when heated and the bioavailability is increasingby heating process. Based on the properties of lycopene, we proposed the used of lycopene as prospective reducing agent for graphene synthesis in various organic solvent.

2. Experimental procedures

2.1. Chemical and instrumentation

The lycopene is isolated from watermelon (*Citrullus lanatus*), graphite (Merck), H₂SO₄ 98% (Sigma Aldrich), KMnO₄ (Merck), H₂O₂ 30% (Merck), demineralized water (HYDROBAT), HCl 37% (Smart Lab Indonesia), n-hexane (Smart Lab Indonesia), ethanol (Merck), acetone (Smart Lab Indonesia), methanol (Merck), dichloromethane (Merck), acetonitrile (Merck).

The instrumentation used in this research is spectrophotometer UV-Vis Shimadzu 1601 type, spectrophotometer FT-IR 8400S, Sonicator-210 BRANSON, HPLC Shimadzu LC-20AD Prominence, X-Ray Diffraction and Ultima IV Diffractometer, Oven-MEMMERT, Hot Plate stirrer-PMC.

2.2. Isolation of lycopene

Isolation of lycopene was carried out according to Syafaatur method [16], with modifications in the amount of watermelon and solvent volume. Isolation of lycopene was carried out using solid-liquid extraction method with n-hexane:ethanol:acetonein the ratio of 2:1:1(v/v/v). Watermelon juice (10 g) was macerated using 40-100 mL solvent, stirred for 30 minutes. After stirring, the residue was separated using separating funnel. 10 mL of distilled water was added into the filtrate and the mixture was shaken 15 minutes and separated the organic layer. The solvent was evaporated using rotary evaporator without heating. The lycopene was then obtained and analyzed by FTIR, UV-Vis, and HPLC.

2.3. Synthesis of graphene oxide

Synthesis of graphene oxide was performed using the Hummer method, referred to Zaid, *et al.* [12] with modification. Graphite powder (4 g) was added with H₂SO₄ 98% solution (92 mL) and stirred. 12 g KMnO₄ powder was slowly added in the mixture and the temperature was maintained at 20°C. The temperature of the mixture was then increased to 35°C and continued to stir for 2 hours. Water (200 mL) was added into the mixture and stirred for 15 minutes. Additional 600mL of distilled water was added into the solution, and continued stirring for additional 5 minutes. After that, 10 mL of H₂O₂ 30% was added slowly while the solution still being stirred. The precipitate was separated and washed with 20 mL HCl 37%: distilled water (0.2: 2 (v/v)), followed by washing with distilled water until neutral. Graphite oxide were obtained after separation and drying at 60°C until constant weight. Graphene oxide was obtained by dissolving 0.1 g of graphite oxide in 50 mL of distilled water, then sonicated (200 W) for 2 hours. Graphene oxide is dispersed in the supernatant as it was centrifuged

3000 rpm to separate from unreacted graphite oxide. Dispersed graphene oxide produced as yellowish brown powder after additional centrifugation at 9000 rpm.

2.4. Reducing of graphene oxide by lycopene


The reducing of graphene oxide is according to Zaid, *et al.* (2015) [12], with the modifications on the initial volume. During the reaction, O₂ gas was flowed to initiate an oxidation reaction on lycopene. The polarity of solvent and reduction time for the synthesis is modify to increase the obtain product. For each reactions, 0.3 g lycopene and 0.1 g graphene oxide dissolved in 65 mL of n-hexane/water (3:10 (v/v) with reduction time 4, 8, and 24 hours, respectively. Similar reaction is carried out by modify the solvent into polar organic solvent, that is, acetone/water in 3:10 (v/v) in 3, 7, and 16 hour, respectively. The mixture is then refluxed at 95 °C. After reflux the mixture is filtered using a vacuum pump and washed with distilled water. The solids obtained is dried for a night at 60°C until constant weight.

3. Result and discussion

3.1. Isolation of lycopene from watermelon

The isolation of lycopene was carried out by maceration using mix solvent. The macerate consists of three phases, that is water, solidify materials and organic phase. Lycopene is dissolved in organic phase and separated by separating funnel. The isolated lycopene is obtained in different mass based on the volume of the solvent (**Table 1**).

Table 1. The isolated lycopene from watermelon in various solvent.

No	Entry sample	Sample mass (g)	Solvent (mL)	Isolated lycopene (g)	Extracted lycopene
1	Watermelon juice	10	40	0.04	
2	Watermelon juice	10	50	0.05	
3	Watermelon juice	10	60	0.05	
4	Watermelon juice	10	70	0.05	
5	Watermelon juice	10	100	0.05	
6	Watermelon pulp	10	50	0.085	
7	Watermelon filtrate	10	50	0.028	

In Table 1 (entry 1-5), the isolated lycopene from watermelon juice did not increase significantly by the increasing of solvent volume. For the next experiment, the ratio of sample and solvent used is 1:5 (w/v) (entry 2). This result showed the more effective sample/solvent ratio for lycopene extraction compared with Syafaatur *et al.*, (2015) [16]. In Table 1 (entry 6), the isolated lycopene is 0.085 g by modifying the sample into watermelon pulp. This result suggested that lycopene is mainly obtained in the watermelon pulp compared to watermelon juice. In our knowledge, this is the first report for the isolation of lycopene from watermelon pulp.

The isolated lycopene is obtained as reddish orange solids which were soluble in n-hexane and partially in acetone (**Table 1**) [17]. The isolated lycopene is stored at room temperature in a dark vial and tightly closed to avoid oxidation. Analysis of lycopene by FTIR showed the stretching of C=C conjugated alkenes and -CH sp³. The weak stretching of *trans*-C=C character is identified at 1651 cm⁻¹. Pavia *et al.* (2009) reported that very weak stretching at 1600 and 1650 cm⁻¹ indicate the structure of unsaturated C=C *trans*- isomerism [18]. However, the peak for -CH sp² are not identified. Additional peak at 1742 cm⁻¹ indicated as astaxanthin which is one of carotenoid compound (**Figure 1**) [19].

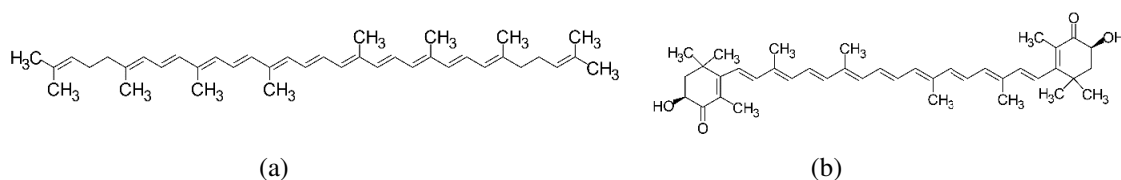


Figure 1. (a) Structure of lycopene and (b) astaxanthin

The UV-Vis analysis showed the maximum wavelength of isolated lycopene is 450, 477, and 508 nm using n-hexane as a solvent. Lycopene has pi-conjugated systems which gives color to its compound and detected in detected visible area. The electron transition that occurs is from $\pi \rightarrow \pi^*$ from the C=C alkene. The results of the UV-Vis analysis is in accordance with Xu *et al.* (2006) [20]. The HPLC analysis revealed that the retention time of lycopene was shorter than beta-carotene [20]. In this analysis, lycopene is eluted at 8.9 minutes and beta-carotene in 11.2 minutes.

3.2. Synthesis of graphene oxide

Graphene oxide synthesis consists of two steps, that is, oxidation of graphite into graphite oxide followed by exfoliation of graphite oxide into graphene oxide. Oxidation of 4 g graphite gave 5.65 g graphite oxide. The exfoliation of 0.1 g graphite oxide by sonication gave 0.01 g graphene oxide which is dispersed in supernatant [12]. Analysis by FTIR are shown in **Figure 2**.

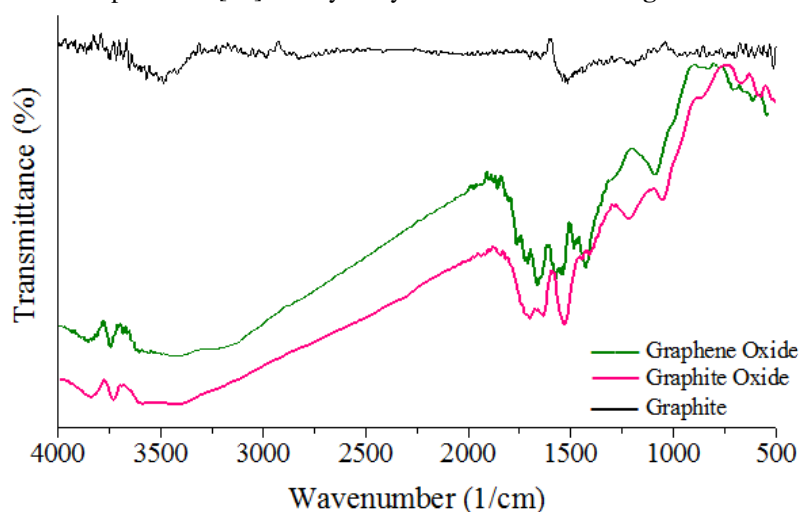


Figure 2. FTIR spectra of graphite, graphite oxide, and graphene oxide

Based on FTIR spectra (**Figure 2**), graphite has C=C stretching at 1501 cm^{-1} ; 1609 cm^{-1} ; 1497 cm^{-1} and bending C=C at 1327.6 cm^{-1} . After oxidation into graphite oxide, a new peak observed, such as, ketones, carboxyl (epoxy) at wave numbers 1701 cm^{-1} and hydroxyl at 3437 cm^{-1} . The C–O ether function group appeared at 1217 cm^{-1} and C=C cyclic at 1535 cm^{-1} . Kartick *et al.* (2013) were reported that graphite oxide has –OH strain, C=O (carboxyl) strain, C–O, C–OH, and C=C [21]. The presence of new peaks in graphite oxide compounds indicates that the oxidation process was successfully carried out. Graphene oxide (**Fig. 2**, red line) have similar profile with graphite oxide, except the shift of C=O carbonyl to the larger wavenumber. This is explained that there are structural changes in the structure [12].

UV-Vis analysis of graphene oxide showed the maximum wavelength in 231 nm occurs due to the transition of $\pi \rightarrow \pi^*$ from the C=C bonds. Another specific wavelength appear at ~ 300 nm for electronic transition of $n \rightarrow \pi^*$. This UV-Vis analysis is accordance with Khanra *et al.* (2012) which states that pure graphene oxide in water absorbed at ~ 230 nm and ~ 300 nm [21].

3.3. Reduction of graphene oxide by lycopene in n-hexane/water.

The reduction graphene oxide into reduced graphene oxide (rGO) was carried out for 4, 8, and 24 h by refluxed under O_2 atmosphere (**Table 2**). Reaction at 8 h gave the highest product. Analysis by FTIR (**Figure 3**) showed that rGO which reacted for 4 h gave high intensity of C=C, C=O carbonyl and -OH stretching but the character of epoxy group does not appear. The strong intensity of $-CH_{sp^3}$ may indicated that the residue of lycopene on the rGO surface due to uncomplete washing. Prolonged the reaction for 8 and 24 h resulted the reducing intensity of C=O, C-O and -OH functional group followed by the increasing intensity of C=C stretching. Based on the Zaid *et al* report, the increasing intensity of C=C indicated that the reduction is occurred [12].

Table 2. Reduction of lycopene using n-hexane/water solvent.

Volume of GO (mL)	Lycopene Mass (g)	Reflux time (h)	rGO (g)
50	0.3	4	0.07
		8	0.11
		24	0.09

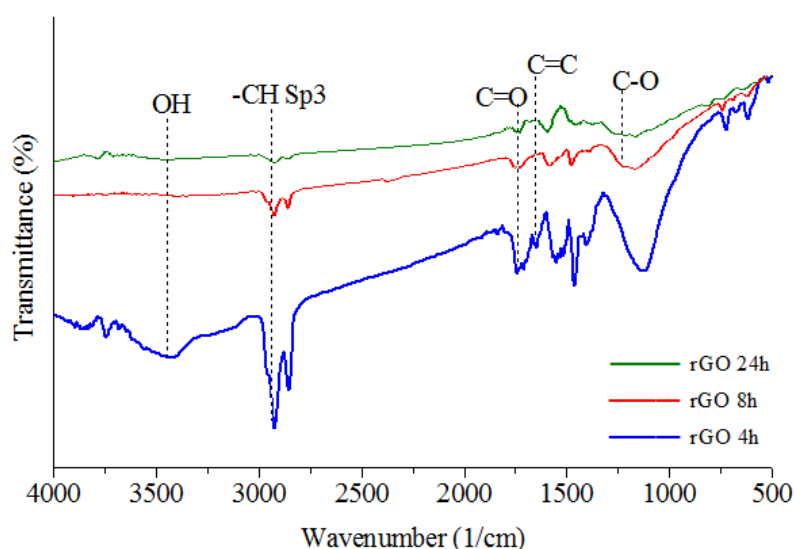


Figure 3. FTIR spectra of rGO-4, rGO-8, and rGO-24

The crystallinity of rGO was confirmed by XRD analysis. **Fig. 4** shows the XRD patterns of rGO-4, rGO-8, and rGO-24. The rGO-4 and rGO-8 exhibits an intense diffraction at 21.5° , while in rGO-24 showed at 18° and 21.5° . This data is in accordance with research of Chang *et al.* (2013) which states that rGO has a peak at 21.4 - 21.5° [9].

3.4. Reduction of graphene oxide by lycopene in acetone/water.

The reduction was performed at 3, 7, and 16 h in acetone/water as a solvent. The result is tabulated in **Table 3**. FTIR analysis of rGO-3, rGO-7 and rGO-16 are shown in **Fig. 5**. The presence of stretching at 1632 cm^{-1} (C=C, weak intensity) on rGO-3 indicate formation of C=C double bond which is the

characteristic of rGO [8][9]. The broad spectrum of -OH vibration at 3458 cm^{-1} is still detected but almost all the vibration of oxygen-functional group, such as, C=O carbonyl and C-O epoxy is diminished. In addition, the shifted wavenumber is detected due to the hydrophobic solvent (contains merely carbon-carbon bonds) [12].

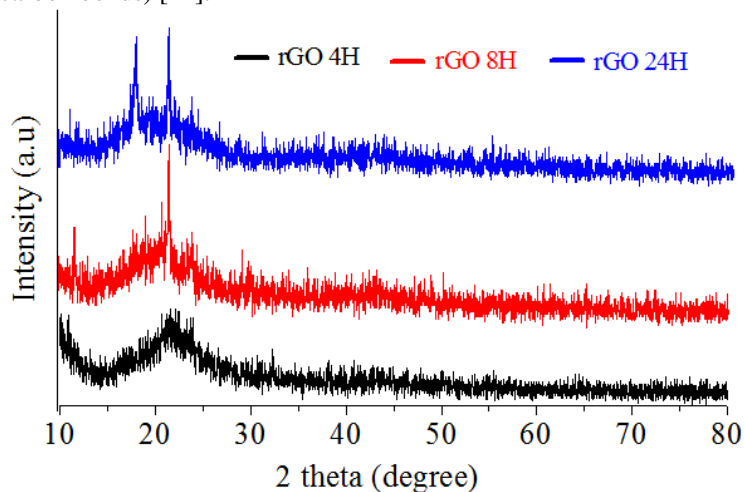


Figure 4. XRD of rGO-4, rGO-8, and rGO-24

Table 3. The results of reduction process using acetone /water solvent.

Volume of GO (mL)	Lycopene mass (g)	Reflux time (H)	rGO (g)
50	0.3	3	0.02
		7	0.03
		16	0.07

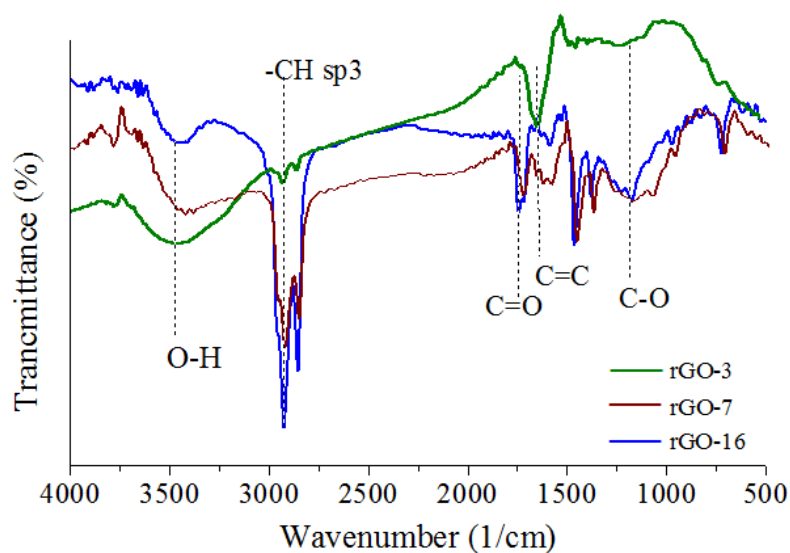


Figure 5. FTIR spectra of rGO-3, rGO-7, and rGO-16

The rGO-7 and rGO-16 showed different characteristic compared to rGO-3 by the appearance of vibration of C-O at $1269\text{--}1273\text{ cm}^{-1}$; 1730 , 1742 , and 1717 cm^{-1} for C=O stretching vibrations. In addition, rGO-7 and rGO-16 also showed C=C stretching at 1576 and 1586 cm^{-1} which are specific to aromatic compounds and cyclohexene. The presence sharp peak at $2925\text{--}2800\text{ cm}^{-1}$ for C-H sp^3 stretching may indicate the residue of lycopene. UV absorption shifted to the bathochromic shift when the graphene oxide reduced into rGO. The initial position of absorption peak is at 231 nm and shifting to 273 nm due to restoration of electronic conjugation during reduction process [12].

The color changes for the lycopene, before and after the reaction was also observed (**Fig. 6**). By prolonged the reaction time, the color of lycopene after reaction become faded. It may be explained that the conjugated double bond in the lycopene is oxidized into aldehyde/alcohol by the graphene. The plausible mechanism is explained by Zaid *et al.* (2015) [12].

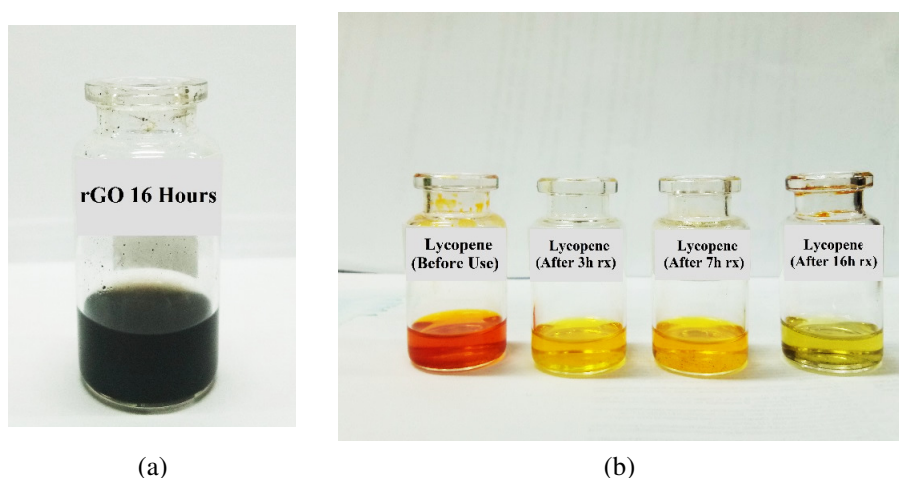


Figure 6. (a) Dissolved rGO in hexane, (b) color degradation of lycopene

4. Conclusion

Isolation of lycopene from watermelon fruit was conducted by solid-liquid extraction with watermelon mass ratio and solvent is 1:5 (m/v). The highest yield is 0.5%. The reduction of graphene oxide at 24 h using n-hexane/water gave the best character of rGO. However, the shorter reaction time, that is 4 h is needed for the reduction in acetone/water solvent. FTIR characterization of rGO showed the increasing intensity of C=C group which is the main character of rGO.

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