

Extraction of Lycopene from Gac Fruit (*Momordica cochinchinensis* Spreng) and Preparation of Nanolycopene

Ho Thi Oanh^{1,2}, Hac Thi Nhung¹, Nguyen Duc Tuyen¹, Le Thi Kim Van³,
Trinh Hien Trung⁴, Hoang Mai Ha^{1,2*}

¹*Institute of Chemistry, Vietnam Academy of Science and Technology (VAST)*

²*Graduate University of Science and Technology, VAST*

³*National Institute of Medicinal Materials*

⁴*School of Pharmacy, Haiphong University of Medicine and Pharmacy*

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Abstract

In this work, we extracted high purity lycopene from dried Gac (*Momordica Cochinchinensis*) aril using organic solvents. We also succeeded in the preparation of nanolycopene. The effect of temperature on the drying of Gac aril and suitable solvents on the extraction of lycopene has been investigated. The results showed that the suitable drying temperature for Gac aril was 60-70 °C. The suitable solvents for lycopene extraction were dichloromethane or chloroform. The lycopene content in dried Gac aril is about 0.28-0.46 %. Nanolycopene was prepared successfully by freeze-dried method, with relatively small particles size of 40-60 nm. Nanolycopene is relatively stable in the inert environment in the presence of antioxidants such as butylated hydroxytoluene (BHT).

Keywords. *Momordica cochinchinensis* Spreng, Gac Fruit, lycopene, nanolycopene, extraction.

1. INTRODUCTION

Gac fruit (*Momordica Cochinchinensis* Spreng) is a special fruit of Southeast Asia, especially familiar to Vietnamese people due to its high nutritional value [1-3]. Gac aril is a thin aril surrounding a gac fruit seed, containing some carotenoids (lutein, beta-cryptoxanthin, zeaxanthin, alpha-carotene, beta-carotene, cis-lycopene, trans-lycopene, vitamin C, vitamin E, and some fatty acids (omega-3, omega-6)) [4-5]. Among these carotenoids, lycopene has attracted much interest because of its antioxidant activity in vitro [6]. It is commonly extracted with organic solvents such as chloroform, toluene, petroleum ether, acetone, hexane and ethanol. Amount of lycopene in Gac aril ranged from: 0.380-0.408mg/g, respectively (Aoki et al., 2002; Vuong et al., 2006) [7-8]. While, Ishida et al. (2004) reported that lycopene concentration in gac aril was ranged from 1.546-3.053 mg/g [9]. Nhung et al. (2010) reported higher concentration of lycopene at 2.378-3.728mg/g [10]. According to research from the University of California, the lycopene content in Gac fruit was 70 times higher

than lycopene content in tomatoes and much higher than that in other vegetables such as watermelon, papaya, red guava, red grapefruit or strawberries [8].

Lycopene is a nonpolar and acyclic carotenoid completely insoluble in water and only slightly soluble in vegetable oil. Thus, only a minor part of lycopene is absorbed by humans during consumption of raw vegetables and fruits. [5] Regardless of the promising results, the extensive use of lycopene has met only limited success, largely due to its instability, poor solubility, inefficient systemic delivery and low bioavailability. To overcome these physicochemical and pharmacokinetic limitations, the encapsulation of lycopene into nanostructure is a major challenge, and nanotechnology represents a powerful strategy [11].

In this study we selected the solvents and appropriate conditions to extract lycopene from gac fruit. Moreover, in order to improve the absorption of lycopene into the body, this active element was made in the form of nanoparticles by emulsification/solvent evaporation follow by freeze-dried method.

2. EXPERIMENTAL

2.1. Materials

Materials used in this study are ripe Gac fruits collected in Bac Ninh, Bac Giang, Hai Duong and Nghe An province in the January of 2017.

Chemical standards including Lycopene (> 90 %) were purchased from Sigma Aldrich.

All solvents used in this study were freshly dried under the standard distillation method. All deuterated solvents were purchased from Cambridge Isotope Laboratory.

2.2. Extraction of Lycopene from Gac fruit

The extraction process of lycopene from Gac fruit was shown in figure 1. The whole Gac fruit was scooped out and the red aril surrounding the seeds was completely separated. Gac aril was dried in the convection oven under drying temperature of 60-80 °C. Lycopene was extracted at least three times from the dried Gac aril using organic solvents such as dichloromethane (DCM), chloroform, tetrahydrofuran (THF), toluene, ethylacetate, petroleum ether, hexane and ethanol. The collected solution was concentrated under reduced pressure; then, ethanol was added slowly to precipitate out the lycopene. The solid was filtered, recrystallized in DCM/ethanol to afford a purple powder.

2.3. Preparation of nanolycopene

Nanolycopene was prepared by emulsification/solvent evaporation method. Firstly, lycopene (10 mg), tween (10 mg), span (10 mg), BHT (1 mg) were dissolved in 3 mL DCM. The organic phase was added dropwise to 10 mL polyvinyl alcohol (PVA) solution (0.7% w/v) with an Ultra-Turrax (T18 IKA, Germany) homogenizer at 8400 rpm for 10 min. The nanosuspension was subjected to a Buchi freeze-dryer (Inlet temperature: 150 °C, outlet temperature: 60 °C, aspirator 100 %, pumb 46 %) to afford a deep red nanolycopene powder.

2.4. Instrumentations

NMR spectra were recorded on a Varian AS400 (399.937 MHz for ¹H and 100.573 MHz for ¹³C) spectrometer. The HPLC analysis was performed with LC-20A System (Shimadzu Co. Ltd, Japan) equipped with a high pressure pump and UV-Vis detector. All scanning electron microscopy (SEM) images were obtained using a Jeol JSM-6500F scanning electron microscope.

3. RESULTS AND DISCUSSION

3.1. Drying of Gac aril

Suitable temperature for the drying of Gac aril was investigated. Figure 2 showed a general tendency of the aril mass loss within 15 hours. At the drying temperatures from 60 to 80 °C, the moisture content was rapidly decreased within first 5 hours and slowly down in the following hours. After 15 hours of drying at 60-70 °C, the weight of dried Gac aril was fixed at 31.5%. This weight was remained event after drying up to 20 hours because the moisture was not significantly reduced further.

By drying Gac aril at 80 °C, the moisture rapidly decreased in the first 5 hours. However, after 15 hours, the weight of Gac films was still remained at 33 %. It is because the high drying temperature led to the rapidly evaporation of water on the surface, resulted on the shrink of outer capillaries and cells that prevent the transfer of moisture from the inside to the surface. By drying Gac aril at high temperature (> 80 °C) on a long time, Gac aril lost their characteristic color and odor. In order to avoid the oxidation of the carotenoids by thermal air, the drying of Gac aril at 60-70 °C within 15 hours in the convection oven was suitable condition to obtain high quality dried aril for the extraction of lycopene.

3.2. Extraction of lycopene from dried Gac aril using organic solvents

Lycopene was extracted from dried Gac aril using various organic solvents including DCM, chloroform, THF, toluene, ethylacetate, petroleum ether, hexane and ethanol. Because of the nonpolar and strong intermolecular interaction structure, lycopene showed a poor solubility in polar solvent such as alcohols. This molecule exhibited a highly solubility in DCM, chloroform, THF. Because of the low toxicity, DCM is a suitable solvent for the extraction of lycopene.

The average content of lycopene extracted from dried Gac aril in different provinces was shown in table 1. The average lycopene content in dried Gac aril from Bac Giang and Bac Ninh province was surround 0.44-0.46 % while dried Gac aril from Nghe An showed lower content of 0.28 %.

Table 1: The average lycopene content extracted from dried gac aril in different provinces.

Sample	Bac Giang	Bac Ninh	Nghe An	Hai Duong
Lycopene content (%)	0.46	0.44	0.28	0.37

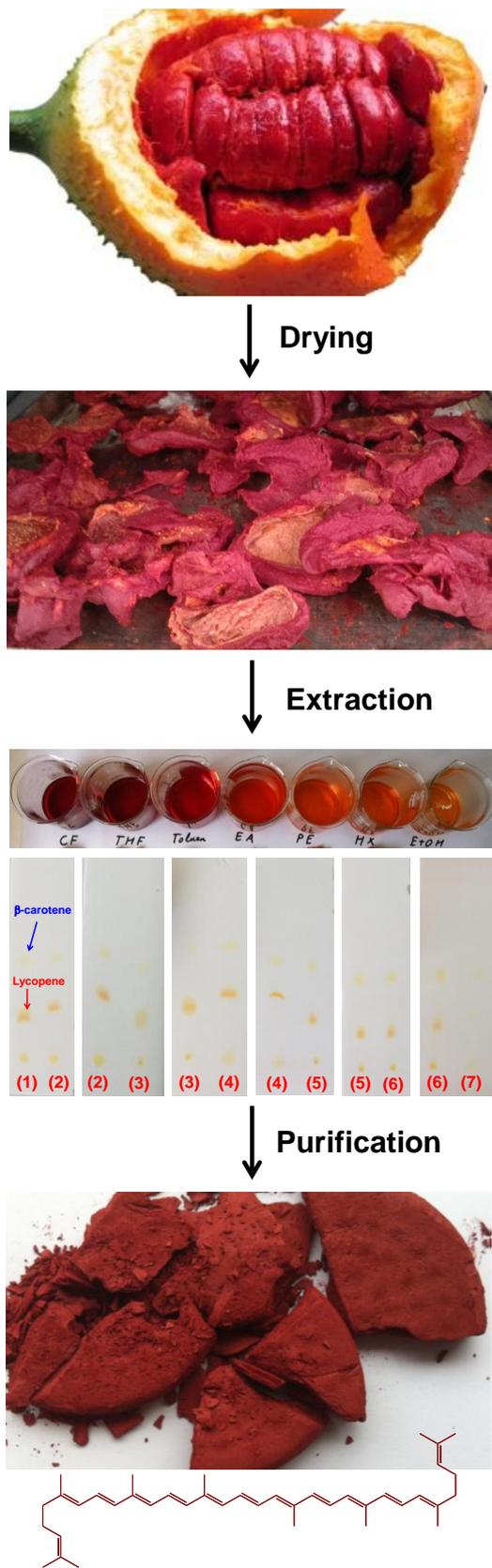


Figure 1: The extraction process of lycopene from dried Gac aril: (1)- Chloroform or DCM; (2)- Tetrahydrofuran (THF); (3)- Toluene; (4)- Ethylacetate; (5)- Petroleum ether; (6)- Hexane; (7)- Ethanol

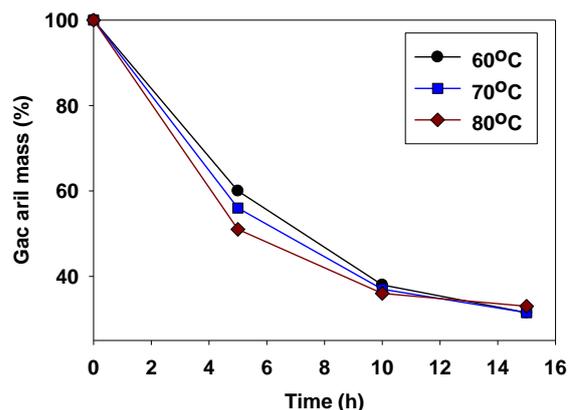


Figure 2: The tendency of the Gac aril mass loss

The HPLC analysis was performed with LC-20A System. Separation was conducted on Supelco C₁₈ (250 mm, 4.6 mm ID, 5 μm) column. The solvent system was composed isocratic of MeOH:ACN: DCM (10:50:40). This eluent contained 0.05 % BHT as antioxidant agent. The flow rate was 1.3 ml/min. Injection volume was 10 μl. The quantification of lycopene and beta carotene was performed at 472 nm. Identification of lycopene and beta carotene was carried out by comparisons of the HPLC retention time and absorption spectra in 472 nm of unknown peaks and chromatography with standards (figure 3). The HPLC result showed the purity of extracted lycopene was higher than 90 %.

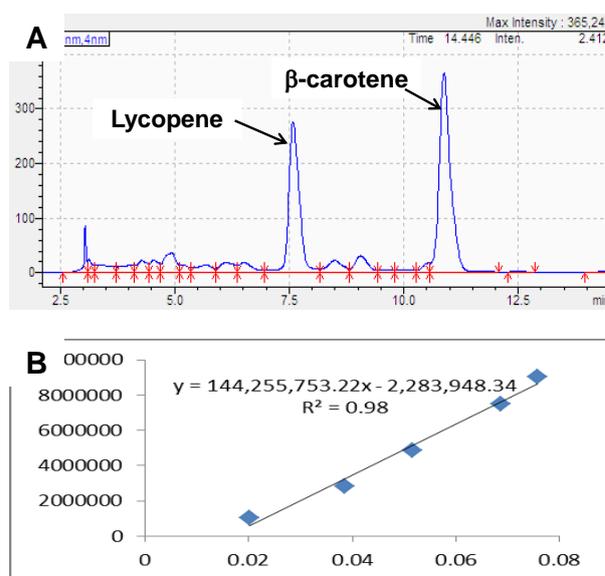


Figure 3: Chromatogram of Gac aril extract developed with MeOH:ACN:DCM (10:50:40) system (A) and the standard line of lycopene quantification (B)

The structure and purity of extracted lycopene

were analysed by $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ using CDCl_3 (Fig. 4). Chemical shifts (in ppm) corresponding to 56 protons in the $^1\text{H-NMR}$ spectrum of Fig. 4A and to 40 carbons in the $^{13}\text{C-NMR}$ spectrum of Fig. 4B were assigned. The chemical shifts were reported in ppm; the CHCl_3 (7.28 ppm for ^1H) and CDCl_3 (77.02 ppm for ^{13}C) signals were used as the internal standard reference. $^1\text{H-NMR}$ (CHCl_3 at 7.28 ppm): 6.68–6.20 (14H, m); 5.98 (2H, d, $J = 10.0$ Hz); 5.135 (2H, bs); 2.15 (8H,

bs); 1.99 (12H, s); 1.84 (6H, s); 1.71 (6H, s); 1.64 (6H, s). The value of the coupling constant of the signals centred at 5.98 ppm is assigned for a *trans* configuration around a partial double bond. $^{13}\text{C-NMR}$ (CDCl_3): 139.50 (2C); 137.38 (2CH); 136.56 (2C); 136.18 (2C); 135.43 (2CH); 132.66 (2CH); 131.75 (2C); 130.10 (2CH); 125.76 (2CH); 124.82 (2CH); 123.97 (2CH); 40.25 (2CH₂); 26.71 (2CH₂); 25.71 (2CH₃); 17.71 (2CH₃); 16.97 (2CH₃); 12.92 (2CH₃); 12.81 (2CH₃).

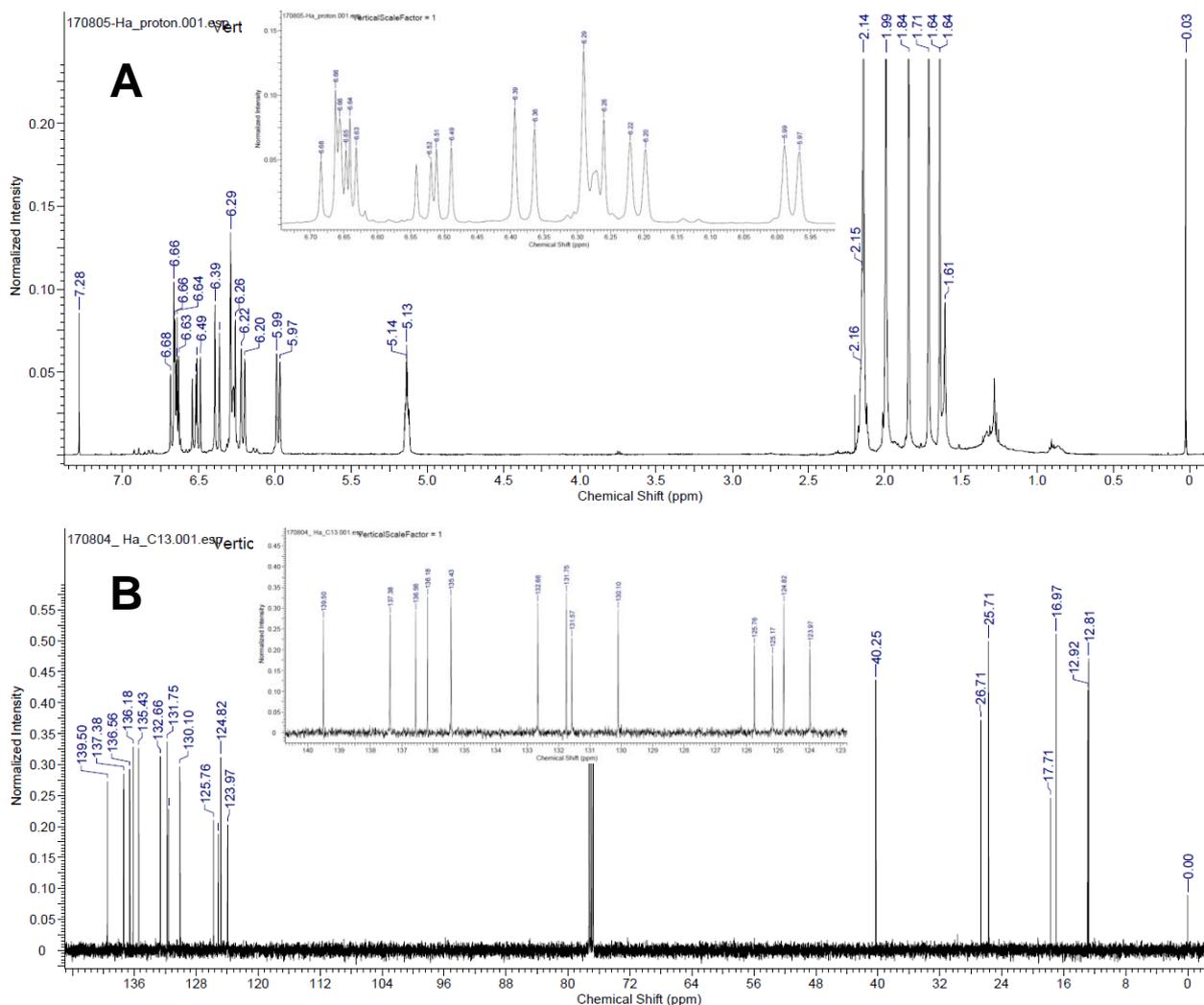


Figure 4: $^1\text{H-NMR}$ (A) and $^{13}\text{C-NMR}$ (B) spectrum of purified lycopene in CDCl_3

Table 2: Feeding composition of nanolycopene samples

Sample	Lycopene (mg)	DCM (g)	BHT (mg)	Tween 80 (mg)	Span 80 (mg)	PVA (mg)	H ₂ O (mL)	Dispersion in water	Lycopene content (%)*
S1	10	5	-	10	10	70	20	Well	9.9
S2	10	5	1	10	10	70	20	Well	9.8

* Lycopene content in nanolycopene powder analysed by HPLC.

3.3. Preparation of Nano lycopene

Nanolycopene was prepared by emulsification/solvent evaporation method using an Ultra-Turrax homogenizer. The feeding composition of sample S2 included 1 mg of BHT as anti-oxidation agent (table 2) while sample S1 was prepared without any anti-oxidation agent. The HPLC analysis showed that the lycopene content of S1 nanoparticles was 9.9 % while lycopene content of S2 nanoparticles was 9.8 %.

Figure 5 showed SEM images of S1 and S2 samples. Similar morphological aspects of nanoparticles with distinct spherical shape were observed. The results indicated that the average particles size of both samples were about 40-60 nm, independently of the initial BHT amount loaded.

Figure 6 showed the stability of sample S1 and S2 on the ambient and inert environment. By the presence of BHT, sample S2 was much more stable than sample S1. Otherwise, the degradation of lycopene was inhibited if these samples were stored in inert environment. For example, in the case of sample S2, with the presence of 1% BHT, 92 % of lycopene was remained if the sample was stored in nitrogen.

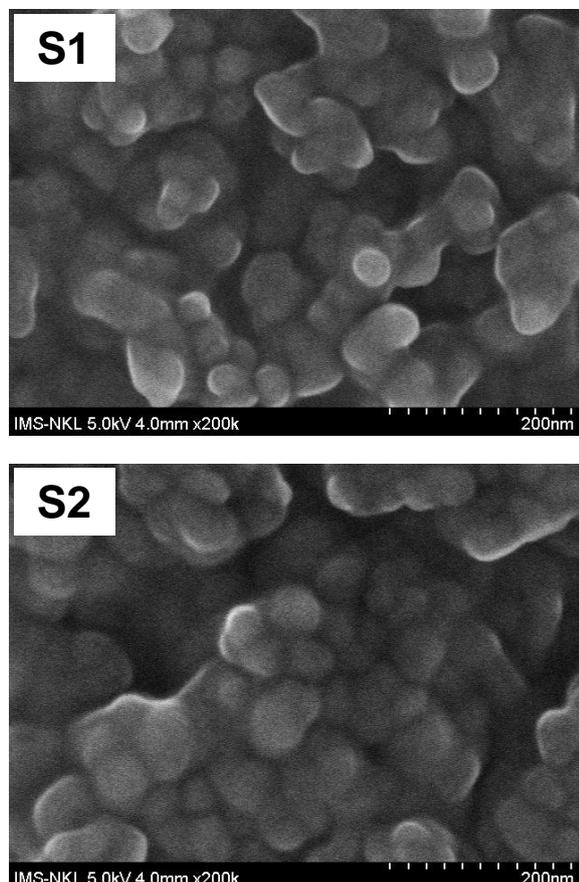


Figure 5: SEM images of S1 and S2 samples

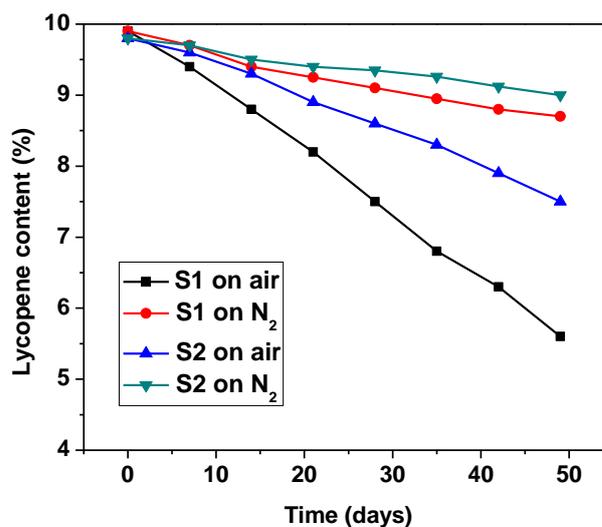


Figure 6: The stability of lycopene nanoparticles

4. CONCLUSION

We have successfully extracted lycopene (the purity > 90 %) from Gac fruit with the average lycopene content in dried Gac aril was surround 0.28-0.46 %. Nanolycopene was prepared by emulsification/solvent evaporation method. These particles showed spherical shape with average particles size of 40-60 nm. BHT was used as an antioxidant agent to improve the stability of nanolycopene.

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Corresponding author: **Hoang Mai Ha**

Institute of Chemistry, Vietnam Academy of Science and Technology
18, Hoang Quoc Viet road, Cau Giay district, Hanoi, Viet Nam
E-mail: hoangmaiha@ich.vast.vn
Tel: 84-4-38361282, Fax: 84-4-38361283.