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Co-Encapsulation of Lycopene and Resveratrol in Polymeric Nanoparticles: Morphology and Lycopene Stability

Ho Thi Oanh[1, 2], Nhung Hac Thi[1, 2], Thanh Nhan Nguyen[1], Tuyet Anh Dang Thi[1, 2], Tuyen Van Nguyen[1, 2], and Mai Ha Hoang[1, 2, *]

[1] *Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[2] *Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

Lycopene and resveratrol are well-known for their high bioactivity, anti-inflammatory effects, and strong antioxidant properties. The combination of lycopene and resveratrol was synergistic in the potentializing immunity of the mammal body. In this study, the scalable co-encapsulation of lycopene and resveratrol into polymeric nanoparticles was performed using lycopene extracted from ripe gac fruit. These nanoparticles exhibited excellent water dispersion and spherical morphology with average particle diameters of 66–102 nm. The particle size was proportional to the lycopene/resveratrol ratio. The combinative use of lecithin and Tween® as surfactants and the use of a polylactide-polyethylene glycol copolymer as an encapsulation agent generated well-defined lycopene/resveratrol nanoparticles although the total content of these active compounds reached 12%. The stability of lycopene was enhanced when combined with resveratrol and antioxidants such as vitamin E and butylated hydroxytoluene. After 3 months of storage at −16 °C, the lycopene content in the lycopene/resveratrol nanopowder remained at ∼95%.

Keywords: Lycopene, Resveratrol, Nanoparticles, Stability, Co-Encapsulation.

1. INTRODUCTION

Lycopene is an unsaturated acyclic carotenoid found in gac fruit, tomatoes, watermelon, pink grapefruit, pink guava, papaya, and apricots [1, 2]. The content of lycopene in gac fruit is much higher than that in tomatoes, other fruits, and vegetables [3, 4]. Hence, gac fruit is well-known as the primary source of lycopene. However, there are limited studies on the extraction of lycopene from gac fruit. Commercial lycopene was extracted from tomatoes or obtained through synthetic pathways.

In recent years, lycopene received significant attention because of its excellent health benefits [5]. Previous reports suggested that the antioxidant properties of lycopene could reduce the risk of cervical and breast cancers, prostatic and pancreatic carcinoma, hepatic fibrogenesis, and cardiovascular diseases [6, 7]. Lycopene exhibited the highest singlet oxygen scavenging ability among all carotenoids and terpenoids [8]. Owing to the presence of 11 conjugated double bonds, lycopene is unstable when exposed to light, heat, and oxygen. Its autoxidation is irreversible, leading to color loss during storage and processing [2, 9, 10].

Resveratrol is a terpenoid known for its high antioxidant activity [11]. It appears in grape peel, peanut, pineapple, mulberry, and polygonum cuspidatum [12]. In nature, it acts as a phytoalexin that helps protect plants from UV irradiation, mechanical injury, and fungal attacks [13]. Resveratrol is able to prevent cancer and supports slow aging [14]. Its dual antioxidant property is higher than that of vitamin C and E, providing beneficial anti-aging efficiency in cosmetic products [15]. However, the poor water solubility of resveratrol is the major obstacle for utilizing it in pharmaceutical and food industries [16, 17].

After oral intake, adsorbed lycopene is mainly distributed to the prostate and other internal organs, while resveratrol is significantly dispersed in the heart,

*Author to whom correspondence should be addressed.

the circulation system, along the respiratory tract, and skin [18, 19]. Some studies demonstrated that the combination of lycopene and resveratrol possesses synergistic and complementary effects that lead to considerably higher activities than those of each of the compounds on its own. Therefore, supplement compositions comprising lycopene and resveratrol in different ratios were produced as pharmaceutical and beauty products [11, 20]. The composites attracted noticeable interest because of their potential effect in preventing chronic diseases such as prostate cancer, lung cancer, skin cancer, atherosclerosis, and cardiovascular disease [11]. However, the composites prepared by mixing microscopic crystalline lycopene and resveratrol exhibited poor water solubility and hence, their bioavailability was limited [20].

Thus, lycopene/resveratrol nanoparticles with a narrow polydispersity (PDI), good water dispersion, and high stability were prepared. Herein, lycopene/resveratrol nanoparticles were synthesized by the co-encapsulation method, using extracted lycopene from gac fruit, polylactide-polyethylene glycol (PLA-PEG) copolymer as an encapsulation agent, and Tween and lecithin as surfactants.

2. EXPERIMENTAL DETAILS

2.1. Materials

Reference lycopene (98%), resveratrol (purity >99%), 3,6-dimethyl-1,4-dioxane-2,5-dione (L-lactide), polyethylene glycol (PEG 6000), stannous octoate ($Sn(Oct)_2$), soybean lecithin, Tween 80, butylated hydroxytoluene (BHT), and vitamin E (VTE) were purchased from Sigma–Aldrich and Acros Organics and used without any further purification. All solvents used in this study were of analytical or high-performance liquid chromatography (HPLC) grade. Ripe gac fruits were randomly harvested in the Bacgiang province, Vietnam in December 2017.

2.2. Extraction of Lycopene from Dried Gac Aril by Soxhlet Method

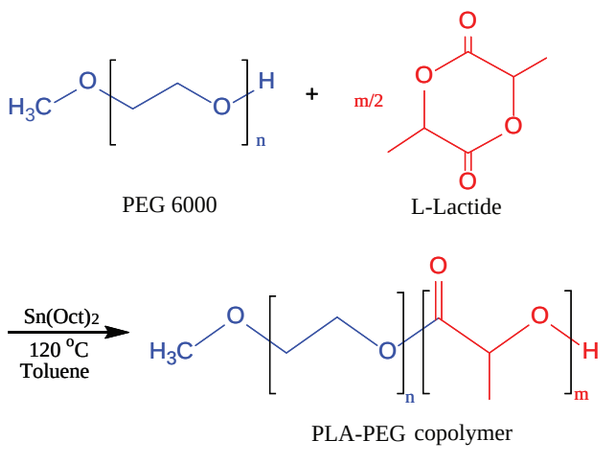
The red gac aril was separated from the gac seed and dried at 60 °C within 15 h in a convection oven to obtain a dried deep-red gac aril with a moisture content of ∼7% (Fig. 1(a)). The Soxhlet extraction method was used to extract lycopene from the dried gac aril. The dried gac aril sample was placed in a porous cellulose thimble and dichloromethane used as a solvent. The extraction process was continuously conducted for 3 h. The collected solution was concentrated by a vacuum distillation rotary evaporator. Then, ethanol was added slowly to precipitate the lycopene. The collected precipitate was dissolved in DCM and crystallized in ethanol. This step was repeated to obtain a purple powder (Fig. 1(b)).

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 6.66–6.17 (m, 14 H), 5.95 (d, $J = 11.0$ Hz, 2 H), 5.11 (bs, 2 H), 2.12 (bs, 8 H), 1.97 (s, 12 H), 1.82 (s, 6 H), 1.69 (s, 6 H), 1.61 (s, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 139.46, 137.37, 136.54, 136.16, 135.43, 132.65, 131.72, 131.56, 130.09, 125.76, 125.16, 124.81, 123.99, 40.24, 26.72, 25.68, 17.70, 16.96, 12.90, and 12.79.

2.3. Synthesis of PLA-PEG Copolymer

PLA-PEG copolymers were synthesized using a similar method reported in literature with some modifications [21]. Specifically, the synthesis of PLA-PEG copolymers was carried out with three different molar ratios of PEG-6000 and L-lactide (LA), 1:20, 1:50, and 1:100 (Table I). The mixture of LA, PEG, $Sn(Oct)_2$, and toluene was stirred at 110 °C for 6 h under nitrogen atmosphere. Then, the mixture was cooled to room temperature and concentrated by a rotary vacuum evaporator. The crude product was dissolved in dichloromethane and precipitated in hexane. The purification process was repeated twice to afford a white solid.



2.4. Preparation of Lycopene/ Resveratrol Nanoparticles

Using the emulsification/solvent evaporation method, lycopene/resveratrol nanoparticles with three different weight ratios (1:2; 1:1; 2:1 (w/w)) were prepared. Tetrahydrofuran (THF) was chosen as the organic solvent to prepare nanolycopene/resveratrol because it completely dissolves all ingredients in the system. The detailed synthesis is as follows: the mixture of lycopene, resveratrol, surfactants, encapsulation agent, and antioxidants (Table II) was dissolved in 400 mL THF, followed by thorough mixing and sonication for 10 min. This solution was added dropwise into 1 L of distilled water while stirring at 9000–10000 rpm for 30 min. Then, the nanosuspension was spray-dried to yield a deep plum-red nanolycopene/resveratrol powder.

VTE and BHT (4%) were employed as antioxidants to stabilize the nanolycopene/resveratrol combination (Table II). The same procedure was applied to prepare nanolycopene/resveratrol powders.

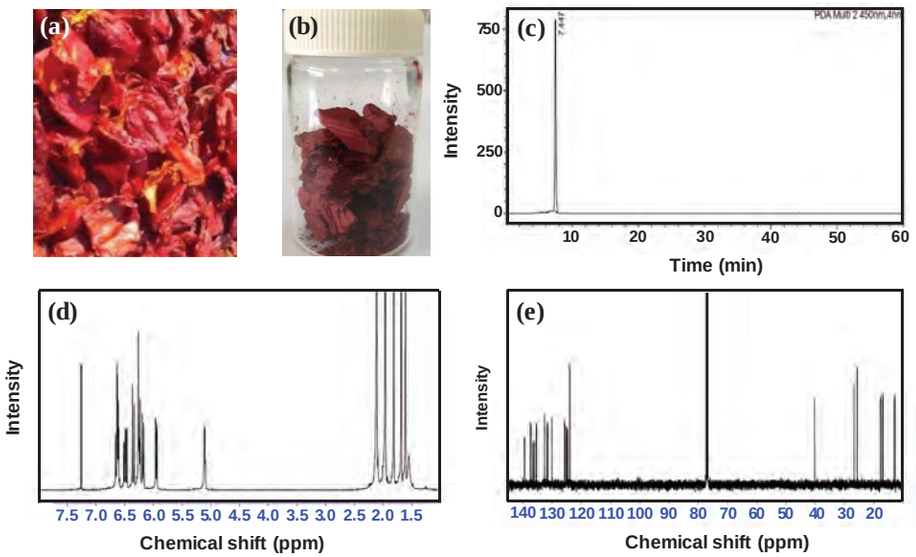


Figure 1. (a) Dried gac aril; (b) extracted lycopene; (c) chromatogram of lycopene developed with MeOH:ACN:DCM (10:50:40) system; (d) ^{1}H NMR and (e) ^{13}C NMR spectra of purified lycopene in $CDCl_3$.

Table I. Characteristics of PLA-PEG copolymers.

Samples	Molar ratio (PEG:LA)	LA (g)	PEG-6000 (g)	M_w (Da)	PDI
PLA-PEG.1	1:20	0.864	1.8	8400	1.2
PLA-PEG.2	1:50	2.16	1.8	11600	1.4
PLA-PEG.3	1:100	4.32	1.8	17200	1.7

2.5. Degradation Investigation of Lycopene in Nanosystems

The degradation of lycopene in the nanolycopene/resveratrol powders was evaluated using samples stored at room temperature and at −16 °C by using a UV-visible spectrophotometer (SP-3000 nano). Dry nanopowders (20 mg) were dissolved in 20 g THF. Then 300 mg of this solution was added to a conical flask containing 25 g of THF. The decomposition of lycopene was determined by comparing the absorbance intensity of the sample at 478 nm at the time of storage and after 15, 30, 60, and 90 days. The residual lycopene content was calculated based on the following formula:

$$\text{Residual lycopene content } (\%) = \frac{I_t}{I_o} \times 100$$

where I_0 is the initial absorbance intensity at 478 and I_t is the absorbance intensity at 478 nm after storage time t.

2.6. Instrumentation

NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer. The purity of extracted lycopene was determined by HPLC (Agilent series 1100, USA). The mobile phase consisted of methanol, dichloromethane, and acetonitrile. The molecular weight and PDI of the PLA-PEG copolymer were determined by gel permeation chromatography (GPC) using an Agilent add-on system with a refractive index signal detector recording at 212 nm. All transmission electron microscopy (TEM) images were taken by JEM 1400 (JEOL, Japan) with an acceleration voltage of ∼100 kV. The particle size

Table II. Feeding composition of nanolycopene/resveratrol samples.

Samples	Lycopene (g)	Resveratrol (g)	Lecithin (g)	Tween (g)	VTE (g)	BHT (g)	PLA-PEG.1 copolymer (g)	Lycopene content (%)	Resveratrol content (%)
S1	2	4	6	6	0	0	32	4	8
S2	2	4	6	6	2	0	30	4	8
S3	2	4	6	6	0	2	30	4	8
S4	3	3	6	6	0	0	32	6	6
S5	3	3	6	6	2	0	30	6	6
S6	3	3	6	6	0	2	30	6	6
S7	4	2	6	6	0	0	32	8	4
S8	4	2	6	6	2	0	30	8	4
S9	4	2	6	6	0	2	30	8	4

of nanolycopene/resveratrol was measured with an Anton Paar Litesizer 500 using a dynamic light scattering (DLS) technique.

3. RESULTS AND DISCUSSION

3.1. Extraction of Lycopene from Dried Gac Aril

The HPLC result (Fig. 1(c)) revealed 98% purity of the extracted lycopene. The extraction of 1 kg dried gac aril yielded ~3.6 g of pure lycopene. The obtained lycopene was characterized using NMR spectroscopy (Figs. 1(d, e)). Chemical shifts corresponding to 56 protons in the ^{1}H NMR and 40 carbon atoms in the ^{13}C NMR spectra matched well with previous reports [22]. Lycopene showed a relatively low solubility in organic solvents. The saturated concentrations of lycopene in tetrahydrofuran, chloroform, DCM, and hexane at 30 °C were 12.2, 6.9, 6.7,

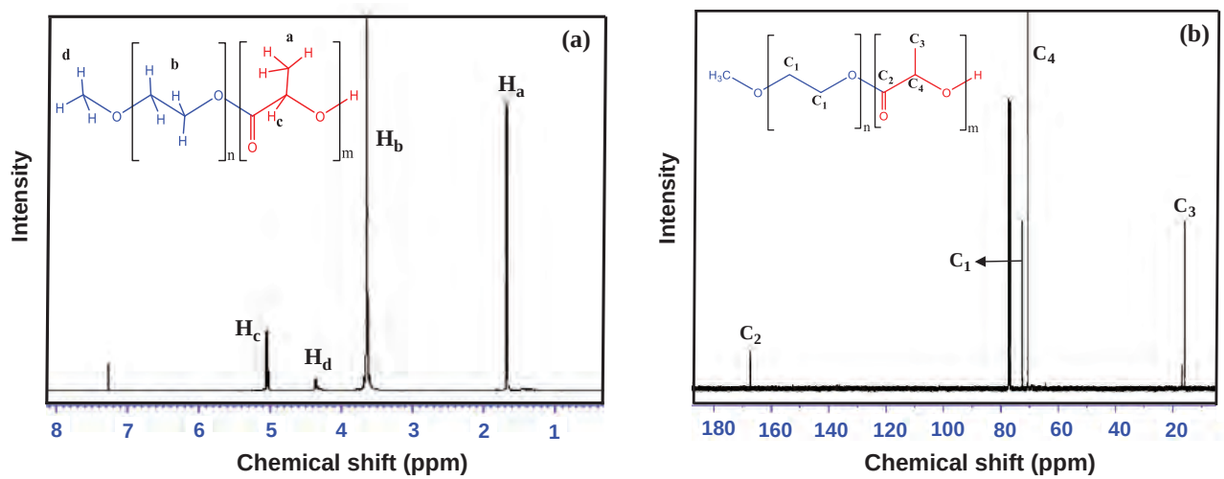


Figure 2. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of purified PLA-PEG.1 copolymer in $CDCl_3$.

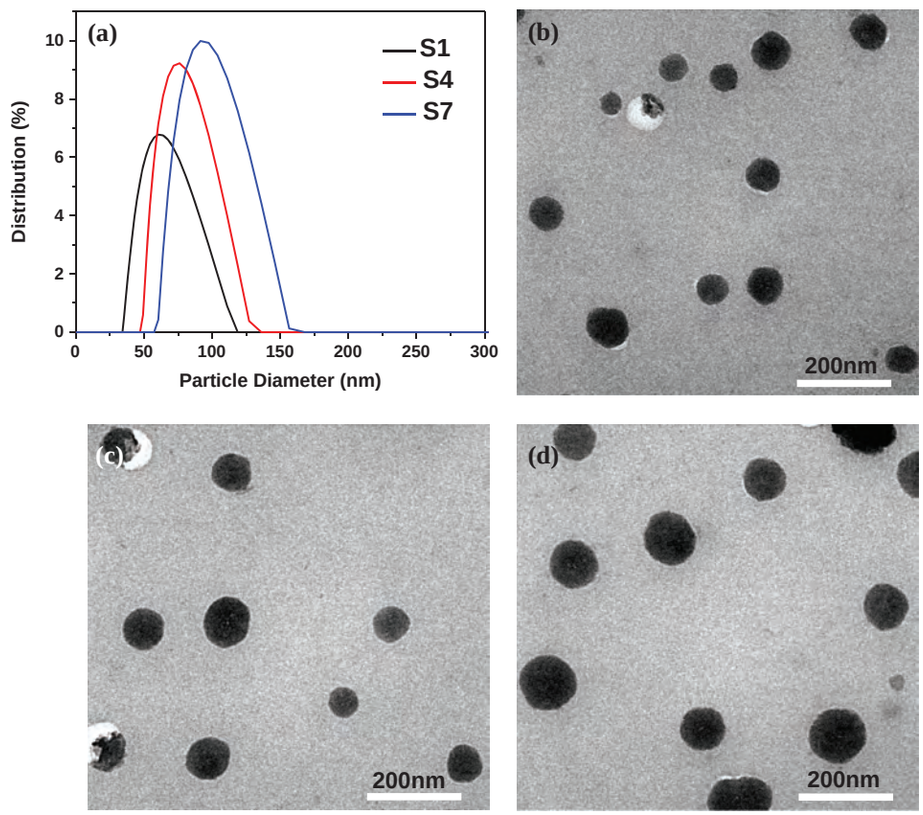


Figure 3. Size distribution of lycopene/resveratrol nanoparticles obtained by (a) DLS and TEM images of (b) S1, (c) S4, and (d) S7.

Table III. Stability evaluation of nanolycopene/resveratrol samples after storage at room temperature.

	Lycopene retention (%)				
Samples	0 day	15th day	30th day	60th day	90th day
S1	100	97.3	94.6	91.8	88.8
S4	100	96.5	92.9	89.0	85.0
S7	100	96.0	91.9	87.6	83.2

and 0.5 mg/g, respectively. The extraction of lycopene from dried gac aril by the Soxhlet method reduced the solvent quantity. Dichloromethane was used because of its lower toxicity compared to chloroform and tetrahydrofuran. In addition, dichloromethane has a low boiling point and a fast evaporation rate that makes Soxhlet extraction possible at low temperatures, resulting in a low lycopene decomposition. Moreover, purifying lycopene twice by recrystallization in DCM/ethanol afforded a high purity lycopene powder. After extraction, the concentrated mixture contained gac oils, VTE, and carotenoids such as lycopene, beta-carotene, lutein, and zeaxanthin. Lycopene, a highly crystalline carotenoid, is insoluble in ethanol while other carotenoids such as beta-carotene were sufficiently soluble in ethanol. Therefore, recrystallization in DCM/ethanol was a suitable method to purify lycopene.

3.2. Synthesis of PLA-PEG Copolymer

The identity of the three PLA-PEG copolymers was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy in $CDCl_3$. In the case of the PLA-PEG.1 copolymer (Fig. 2), the resonance and the proton positions were assigned as follows. The signal at 3.63 ppm was attributed to the methylene (CH_2) group of the PEG block in the copolymer chains. The position of the methine (CH) and methyl (CH_3) protons in the PLA block was detected at 5.03 ppm and 1.60 ppm, respectively. The ^{13}C NMR spectrum showed specific C atom signals. The position of the methylene (CH_2) carbon atom in the PEG block was related to 72.5 ppm. The position of the carbons in the carbonyl (C═O), methine (CH) and methyl (CH_3) group was observed at approximately 169.5, 70.5, and 16.7 ppm, respectively.

In addition, the molecular weight and PDI index data obtained from GPC analysis of these copolymers are listed in Table I. The molecular weights of PLA-PEG.1, PLA-PEG.2, and PLA-PEG.3 were 8400, 11600, and 17200, respectively, corresponding to a PDI of 1.2, 1.4, and 1.7. The glass transition temperature of these copolymers (35–40 °C) was much higher than that of PEG. Among them, the PLA-PEG.1 copolymer with the molar PEG: lactide ratio of 1:20 provided the best water solubility. Additionally, the good compatibility between lycopene/resveratrol with surfactants and the PLA-PEG.1 copolymer produced

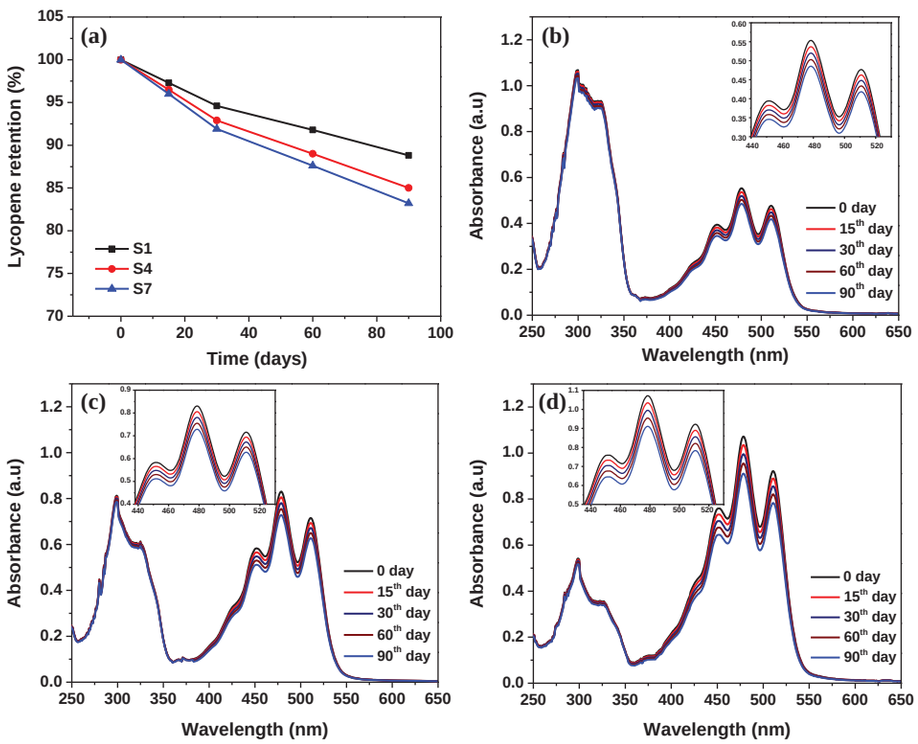


Figure 4. (a) Degradation of lycopene, UV-vis spectra in THF solution of nanolycopene/resveratrol samples (b) S1, (c) S4, and (d) S7 after a storage time of 15, 30, 60, and 90 days at room temperature.

Table IV. Stability evaluation of nanolycopene/resveratrol samples after storage at −16 °C.

Samples	Lycopene retention (%)				
	0 day	15th day	30th day	60th day	90th day
S1	100	99.2	98.2	96.5	94.8
S2	100	99.5	98.8	97.2	95.6
S3	100	99.4	98.4	96.9	95.3
S4	100	98.8	97.4	95.3	93.1
S5	100	99.1	98.0	96.1	94.2
S6	100	99.2	98.1	96.2	94.1
S7	100	98.0	96.0	93.8	91.5
S8	100	98.3	96.5	94.7	92.8
S9	100	98.4	96.7	94.9	93.0

nanopowders with considerable water dispersion and narrow size distribution. This copolymer not only had a positive impact on the co-encapsulation of lycopene and resveratrol, but also improved the smoothness of the nanopowder after the spray drying process. Hence, the PLA-PEG.1 copolymer was further used to synthesize lycopene/resveratrol nanoparticles.

3.3. Morphology of Lycopene/ Resveratrol Nanoparticles

The morphology of the S1, S4, and S7 samples with lycopene to resveratrol ratios of 1:2, 1:1, 2:1, respectively, is shown in Figure 3. The particle size distribution diagram of the S1, S4, and S7 samples in water revealed the presence of narrow polydispersed nanoparticles with an average particle diameter of 66, 79, and 102 nm, respectively. The TEM images (Figs. 3(b–d)) indicated that these nanoparticles had a well-defined spherical shape. The particle size was proportional to the lycopene/resveratrol ratio. In previous reports, nanolycopene was synthesized with average particle diameters of ∼200 nm [2, 5, 23]. The combination of lycopene and resveratrol reduced the particle sizes of the nanosystem to less than 100 nm although the total content of these compounds reached 12%. In addition, the narrow PDI of the spherical nanoparticles indirectly indicated the excellent miscibility of lycopene and resveratrol in the nanosystem.

Furthermore, the formation of lycopene/resveratrol nanoparticles was controlled by surfactants and the encapsulation agent. Lecithin is an amphiphilic surfactant and highly miscible in lycopene, thus forming a hydrophobic mixture. Tween 80 is a highly soluble aqueous emulsifier and solubilizes the lycopene/lecithin system in water. The combination of surfactants (Tween, lecithin) and the encapsulation agent (amphiphilic PLA-PEG copolymer) produced well-defined lycopene/resveratrol nanoparticles with high water dispersion. Moreover, all compounds used in this study were biocompatible with food and pharmaceutical products.

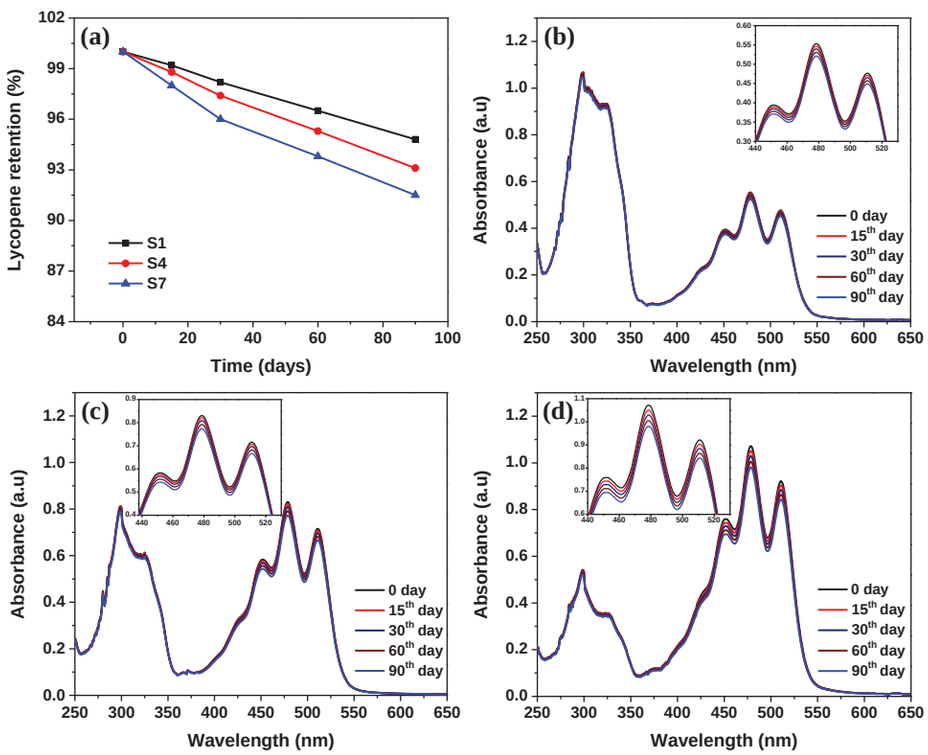


Figure 5. (a) Degradation of lycopene, the UV-vis spectra in THF solution of nanolycopene/resveratrol samples (b) S1, (c) S4, and (d) S7 after a storage time of 15, 30, 60, and 90 days at −16 °C.

To improve the lycopene stability, antioxidants such as VTE and BHT were incorporated into the nanosystems. With the same lycopene, resveratrol, lecithin, and Tween contents, the morphology of lycopene/resveratrol nanoparticles was not significantly affected by these antioxidants. All samples exhibited a good water dispersion, which improved their bioavailability.

3.4. Stability Investigation of Lycopene in Nanosystems

Owing to 11 conjugated carbon–carbon double bonds, lycopene is susceptible to heat, light, and oxygen. Some studies demonstrated that the lycopene stability improved by using antioxidants and nanoencapsulation techniques [1]. In this study, the role of resveratrol was not only a synergistic and complementary one, but it also served as antioxidant protecting lycopene. The stability of lycopene/resveratrol nanoparticles under different preservation conditions was investigated. Under the ambient condition, lycopene significantly degraded (Table III and Fig. 4). After 90 days of storage at room temperature, the remaining lycopene contents in the nanopowder of the S1, S4, and S7 samples were 88.8 85.0, and 83.2%, respectively. The most severe degradation was observed at the lycopene/resveratrol ratio of 2/1, followed by 1/1 and 1/2. The lycopene degradation was proportional to the lycopene/resveratrol ratio. The lycopene stability in these nanoparticles was much better than that reported in previous studies. For instance, Dos Santos et al. prepared lycopene-loaded lipid core nanocapsules and reported a

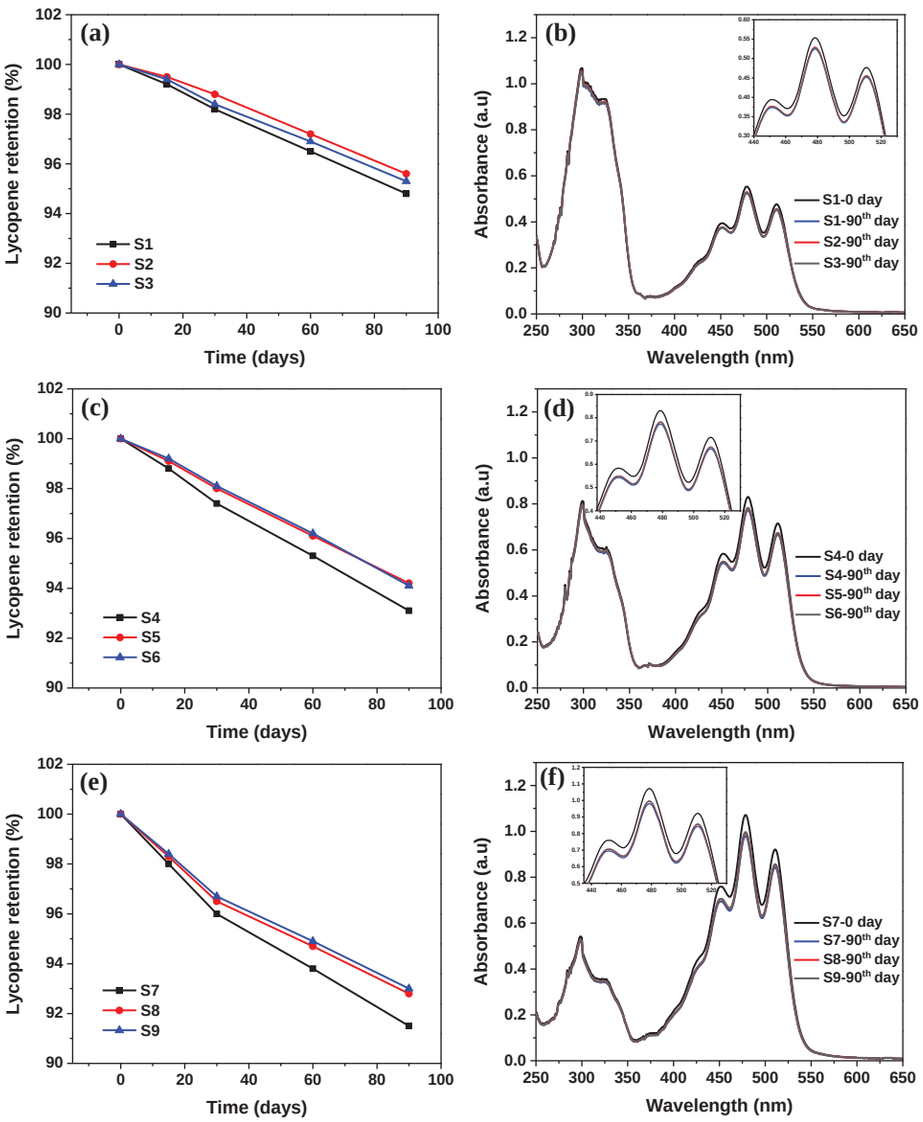


Figure 6. Degradation of lycopene in the nanolycopene/resveratrol samples: (a)-S1, S2, and S3; (c)-S4, S5, and S6; (e)-S7, S8, and S9; the UV-vis spectra in THF solution of (b) S1, S2, and S3; (d) S4, S5, and S6; (f) S7, S8, and S9 at the start of storage and after 90 days.

40% retention of lycopene after 14 days of storage under the same ambient condition [23]. Thus, the incorporation of lycopene with resveratrol could improve the stability of lycopene in the hybrid nanosystems.

In addition, the lycopene degradation was significantly affected by the storage temperature. The lycopene stability was improved by storing the samples at −16 °C (Table IV and Fig. 5). After 90 days of storage at this temperature, the remaining lycopene contents in the nanopowders of the S1, S4, and S7 samples were 94.8, 93.10, and 91.5%, respectively. The lycopene stability was also promoted by the resveratrol incorporation. The degradation rate at room temperature was approximately twice as high as that at −16 °C. Therefore, refrigeration increases the stability of nanolycopene.

Further, antioxidants such as VTE and BHT were incorporated to improve the lycopene stability in the nanosystems. Table IV and Figure 6 indicate that the presence of BHT or VTE in the lycopene/resveratrol nanopowder increases the lycopene stability. After 3 months of storage at −16 °C, the remaining lycopene content in the S2 sample was 95.6%. In the S2 and S3 samples, 4% lycopene was protected with 8% resveratrol and 4% of VTE or BHT. Thus, VTE or BHT did not play a significant role in protecting lycopene. However, in the S8 and S9 samples, 8% lycopene was protected by only 4% resveratrol and 4% of VTE or BHT. Hence, the lycopene stability in these samples was notably increased by these antioxidants. In comparison with BHT, VTE more efficiently protected lycopene because of its higher miscibility with lycopene. Owing to its natural origin and excellent benefits, VTE was a suitable antioxidant for the preparation of nanolycopene/resveratrol.

4. CONCLUSION

Lycopene was successfully extracted from dried gac aril with a high purity of 98%. The PLA-PEG copolymer was synthesized as an encapsulation agent for preparing lycopene/resveratrol nanoparticles. These nanoparticles have a well-defined spherical shape with average particle diameters ranging between 66–102 nm and good water dispersion. The particle size was proportional to the lycopene/resveratrol ratio. The stability of lycopene was significantly improved by combining lycopene with resveratrol and antioxidants such as VTE and BHT. After 90 days of storage at −16 °C, the lycopene–resveratrol system (ratio of 1:2) combined with VTE showed only a 4.4% loss of lycopene. These findings could potentially contribute to the further development of nanolycopene/resveratrol inclusion in pharmaceutical and food products.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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