Original Article

Liposomal Green Tea Extract: Optimization and Physicochemical Characterization

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Abstract
Introduction: Although green tea is a rich source of antioxidant, using this herb in food industries is limited due to its oxygen and light instabilities.
Materials and Methods: Green tea polyphenols were encapsulated into liposomes using the Mozafari method (with no solvents and detergents) to improve the bioavailability of tea polyphenols. Screening design was used to find the major variables within all possible process variables. Then, optimal conditions were studied using response surface.
Results: The most appropriate condition was achieved using phosphatidylcholine of 4.5% (w/w), extract concentration of 0.7%, mixing time of 30 minutes and temperature of 50°C. Encapsulation efficiency (EE) depended on concentrations of the green tea extract and phosphatidylcholine. The EE in optimal formulation reached 53.58%. The particle size and Z-potential of liposomal green tea extract were assessed at 419 nm and -59.7 mV, respectively. The total polyphenol content (TPC) of green tea included 164.2 mg gallic acid/g extract. Free radical scavenging activities of free and liposomal extracts were calculated as 90.6% and 93.37%, respectively, using the 2-2-diphenyl-1-pycrylhydrazyl (DPPH) method.
Conclusions: Results revealed that liposomal green tea extract can be used extensively in food industries due to its high antioxidant activity. No size decreasing methods (e.g. sonication and homogenization) were needed to produce nano size liposomal extracts to avoid structure instability.
Keywords: Green Tea Extract, Liposome, Polyphenols, Process Variables, Plackett-Burman Design, Response Surface Method
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Introduction

One of the most important reasons for deterioration of oils and lipids is oxidation. Formed products from oxidation decrease sensory assessment and nutritive quality. These products of oxidation can negatively affect human health and lead to a number of diseases such as cardiovascular diseases (CVDs) and cancers.[1] The use of antioxidants is a major technique against oil oxidation. Despite their effectiveness, cheap cost and good stability the use of synthetic antioxidants in common processes is limited because of possible toxic effects. Therefore, extensive studies on natural antioxidants have been carried out. One of the plants rich in antioxidative components is green tea derived from *Camellia sinensis L.*[2] Green tea includes high quantities of antioxidant components, known as polyphenols. Antioxidants neutralize free radicals under physiological stress conditions and hence protect the body against various diseases, including cancers, CVDs and neurodegenerative diseases. One of the most

important polyphenol components of green tea includes catechins, which can be used in clinical fields. Nevertheless, the consumption of green tea polyphenols is limited because of its poor solubility and low bioavailability. To overcome this problem and in order to improve the herbal bioavailability properties, green tea can be incorporated in appropriate delivery systems. Green tea polyphenols include special characteristics and liposome is considered as an appropriate carrier system for their encapsulation.[3] Liposomes and nanoliposomes are lipid-based carriers capable of providing protection and controlled release of various active agents, including food and drug compounds at an appropriate time and location.[4] Liposomes are formed of a central aqueous part surrounded by one or more concentric phospholipid layers. Therefore, the liposome structure possess hydrophilic and hydrophobic components and is able to be incorporate in an extensive spectrum of bioactive components. There are several methods to prepare liposomes such as spray

drying, hydration of a thin lipid film and the solvent injection technique.[5] One of the regular methods to produce liposomes is the heating method developed by Mozafari. This method is based on the hydration of the phospholipid components in an aqueous solution containing 3% (vol) glycerol. Temperatures, depending on presence or absence of cholesterol, shift between 60 and 120°C, respectively. Glycerol in liposome structures is a water soluble compound, which prevents coagulation and sedimentation of the lipid vesicles and hence increases lipid vesicle stability.[6] This method can be used at large-scales in one step with no use of toxic solvents and detergents.[7] Noudoost et al prepared liposomes containing green tea extract by thin film layer method. The results determined that liposome containing green tea polyphenols had significantly more antioxidant activity than free extract.[8] Naghavi et al used of green tea liposomes for developing shelf life of fresh orange and pomegranate juices. The results showed, the destruction of ascorbic acid during storage significantly reduced. The main reason this effect was antioxidant green tea extract incorporated into liposome.[9] Lu et al reported liposomes containing green tea extract had more antioxidant properties than free green tea extract.[10] A report showed microencapsulated green tea extract had more stability and the antioxidant capacity of the mango drink than the non-encapsulated powder at the end of the storage period.[11] In all previous reports, common methods applied organic solvents or detergents during their manufacture causes toxicity and instability of liposome structure.[6] Removal of residual solvent as an additional time and cost consuming process can not be done completely.[12] According to above mentioned gap of research, the purpose of this research was application of liposome to encapsulate natural antioxidant of green tea extract via Mozafari method to increase the oxidation resistance and improve the storage time of tea polyphenol. In this method, has not use any organic solvents or detergent. Lasic et al. explained phospholipid bilayers normally trend to be as a flat surface and to curve them, energy is required.[13] In Mozafari method, the needed energy provides by heating energy during production liposome and stirring process that cause to homogeneous distribution of the ingredients.[12] It should be noted, in this research, did not use of any method for particle size reduction (e.g. sonication, homogenization or extrusion). This matter caused to reduce executive stages, as well as to be economic. Using of high stress and pressure, can destroy the membrane of liposome. In this study, operational process conditions were optimized, and the physicochemical properties of tea polyphenol liposome were measured. Rasti et al investigated the oxidative and physical stability of liposomes and nanoliposomes containing docosahexaenoic and eicosapentaenoic acid, prepared by the thin film hydration technique and Mozafari method. They found, prepared liposomes by Mozafari method, duo to the smaller size of the particles and increasing surface charge had more physical and oxidative stability.[14]

Materials and Methods
Materials
Green tea plant (*Camellia sinensis*) of the *Theaceae* family was purchased from Isfahan province, Iran. Lecithin (99%, granular phosphatidylcholine) was purchased from Across, USA. Ethanol, glycerol, 2-2-diphenyl-1-pycrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were provided by Merck, Germany.

Preparation of the Green Tea Extract
Fresh green tea leaves were collected from tea gardens of Mazandaran province, Iran. These were sorted, washed and dried under sunlight. Dried tea leaves were crushed using a blender and sieved using 70/100 mesh sieve-set.[15] Extraction was carried out by mixing 100 g of the green tea powder in 1000 mL of 70% ethanol in distilled water. The mixture was agitated for 24 hours at 40°C and the extract was separated by filtration. To achieve a concentrated extract, the extract was evaporated at 40°C using an oven. Then, the extract was transformed to powder at -50°C using freeze dryer and stored at -18°C in air-tight dark bottles until use.[16]

Liposome Preparation
In this study, the Mozafari method was used to prepare liposomes containing green tea extracts. One of the most important advantages of this method includes the production of liposomes without using solvents or detergents.[12] Furthermore, this method does not use homogenization or sonication methods for decreasing liposomal particle sizes, which may destruct liposome structure. In Table 1, various independent variables are shown. At first, the green tea extract (0.7–2%) was dissolved in distilled water. Then, phosphatidylcholine (2.5–4.5%) and glycerol (3% v/v) were added to the solution and agitated 1000 rpm for various time intervals of 30–60 minutes at 50–70°C. This mixture was set at room temperature for 1 hour to increase stability of the liposomes. The formula was stored at 4°C in dark bottles under nitrogen gas.

Central Composite Design (CCD) and the Expert Design software v.10 were used to analyze data. Levels of variables and experiment design are listed in Table 1. In this study,

Table 1. Independent Variables in Liposome Preparation for Encapsulation of Green Tea Extract

Variable	Variable Levels			References
	+α	0	-α	
Phosphatidylcholine concentration (%)	5.5	3.5	1.5	9,10
Extract concentration (%)	2.65	1.35	0.05	10
Mixing temperature (°C)	80	60	40	5
Mixing time (min)	75	45	15	14

three factors were used to design the experiments, including: (1) Factorial fraction design; (2) Determination of α as the distance of central point; and (3) Determination of central point.

Encapsulation Efficiency

Encapsulation efficiency (EE) was reported as the ratio of encapsulated polyphenols to the total polyphenol content (TPC) expressed in percentage. To calculate EE, the liposomes with green tea extract were separated from an unencapsulated extract at 36 000 g for 30 min at 4°C using ultracentrifuge (Herolab, Hicen, Germany). This was washed with deionized water and recentrifuged. Separated liposomes were suspended in deionized water and the final volume was adjusted to 2 mL. Then, liposomal membrane was disrupted using 0.02% Triton X-100 solution. Briefly, 1 mL of Triton X-100 solution was added to 1 mL of the suspended liposomes and the encapsulated extract was released. To create uniformity of the process conditions, 1 mL of the supernatant was mixed with 1 mL of 2% Triton X-100 solution. Then, EE was calculated using the following formula[17]:

$$\%EE = \frac{P}{S+P} \times 100$$

Where, P was the concentration of phenolic compounds inside liposomes that was assessed after liposome disruption by Triton X-100, and S was the quantity of unentrapped phenolic compounds in supernatants.

Assessment of Permeability

To assess the permeability rate, EE was calculated before and after one-month storage of liposomes containing green tea extract at 4°C and permeability was calculated as follows[10]:

$$Permeability = \frac{EE_o - EE_{3o}}{EE_o}$$

Where, EE_o and EE_{3o} were efficiencies of encapsulation after preparation of liposomes (day 0) and one month after storage, respectively.

Particle Size Assessment

Particle size and the polydispersity index (PDI) were assessed for optimal liposomes with the most EE after and before encapsulation of the green tea extract. These parameters were assessed using the light scattering method and Zeta-Sizer Analyzer (Horiba Scientific Instruments, SZ-100 Series, Germany). Assessments were carried out at 25°C and the medium viscosity and the angle of assessment included 0.896 mPa.s and 90°, respectively. The instrument was able to assess the particle size in a range of 0.3 nm to 8 μm. Samples were diluted 100-folds using distilled water to prevent light scattering due to interactions between the particles.[10]

Assessment of Zeta Potential

Zeta potential is a physical property which shows the interaction magnitude between colloidal particles. This index is commonly used to assess colloidal system stability.[10] In this study, zeta potential was assessed at 25°C using Zeta-Sizer Analyzer (Horiba Scientific Instruments, SZ-100 Series, Germany) with an electrode voltage of 3.3 V.

Assessment of Total Phenolic Content

To assess TPC in green tea extracts, Folin-Ciocalteu assay was used. Polyphenols react with Folin-Ciocalteu reagent, forming a blue complex. Absorbance of the complex is measured at 720 nm.[18] Briefly, 1 ml of the extract (5 mg/mL) was mixed with 1 ml of Folin-Ciocalteu reagent. After 1 min, 1 ml of 7.5% saturated sodium carbonate solution was added to the mixture and mixture was diluted to 10 ml using distilled water. Mixture was stored at dark for 90 minutes and then its absorbance was recorded at 720 nm using SpectroFlex spectrophotometer Model UV-6600, Germany. The TPC was assessed using calibration curve from specified concentration of gallic acid absorbance and results were reported as mg/L of gallic acid equivalents (GAE).

Free Radical Scavenging Method

To assess antioxidant activity of the green tea extract, the DPPH method was used. Mechanism of this reaction involves discoloration of a methanolic solution of DPPH. Solution was prepared by mixing 0.0025 g of DPPH with 100 mL of methanol. Then, 3.9 mL of DPPH methanolic solution were separately added to 100 μL of encapsulated and free green tea extracts. This solution was set at dark for 60 minutes and its absorbance was recorded at 517 nm using UV/VIS spectrophotometer. The proportion of DPPH radical inhibition by the extract was calculated using the following equation:

$$Free\ radical\ scavenging\ rate\ (\%) = \frac{Ac - As}{Ac} \times 100$$

Statistical Analysis

Analysis of results was carried out using Design Expert software v.10. Based on previous studies, the range of the independent variables was calculated. Using the software, process conditions, including four independent variables of phosphatidylcholine concentration, green tea extract concentration, time and temperature of mixing process were optimized.

Results and Discussion
Encapsulation Efficiency

As shown in Table 2, EE of the encapsulated green tea was 43.51%–53.71%. Results were analyzed using the Design Expert software v.10. The regression equation was as follows:

$$EE = +50.89 + 1.00A - 0.44B + 0.66B^2$$

Analysis of EE variance is shown in Table 3. The P values demonstrated significant linear relationships between the independent and dependent variables. As seen in Table 3, effects of phosphatidyl choline concentration (A), extract concentration (B) and B^2 were significant at $P < 0.05$. Various factors such as phosphatidylcholine concentration, extract concentration, temperature and process time and their effects on EE were investigated Lu et al reported EE of the encapsulated green tea extract in liposomes using thin film

Table 2. The Experimental Design and Response Result

St. Order	A: Phosphstydil Choline (%)	B: Extract (%)	C: Temperature (°C)	D: Time (min)	Response 1: Encapsulation Efficiency (%)	Response 2: Permeability (%)
1	2.5	0.7	50	30	51.12	4.15
2	4.5	0.7	50	30	53.58	2.40
3	2.5	2.0	50	30	49.76	4.06
4	4.5	2.0	50	30	43.51	3.76
5	2.5	0.7	70	30	51.50	2.26
6	4.5	0.7	70	30	52.23	2.90
7	2.5	2.0	70	30	50.04	2.00
8	4.5	2.0	70	30	52.43	2.10
9	2.5	0.7	50	60	51.03	10.60
10	4.5	0.7	50	60	53.71	3.20
11	2.5	2.0	50	60	50.28	2.28
12	4.5	2.0	50	60	54.46	6.20
13	2.5	0.7	70	60	51.56	1.20
14	4.5	0.7	70	60	52.50	2.80
15	2.5	2.0	70	60	49.72	0.92
16	4.5	2.0	70	60	52.06	3.01
17	1.5	1.35	60	45	48.34	1.60
18	5.5	1.35	60	45	52.48	3.25
19	3.5	0.05	60	45	48.94	2.30
20	3.5	2.65	60	45	50.01	1.40
21	3.5	1.35	40	45	50.42	6.59
22	3.5	1.35	80	45	51.87	1.90
23	3.5	1.35	60	15	51.48	2.60
24	3.5	1.35	60	75	50.67	2.40
25	3.5	1.35	60	45	52.22	2.60
26	3.5	1.35	60	45	49.42	2.60
27	3.5	1.35	60	45	50.86	1.40
28	3.5	1.35	60	45	50.78	1.00
29	3.5	1.35	60	45	51.41	2.30
30	3.5	1.35	60	45	46.50	2.38

Table 3. Analysis of Variance for Encapsulation Efficiency

Source	Df	Mean Square	F value	P value (Probe > F)
Model	3	13.75	37.26	<0.0001**
Phosphatidylcholine	1	24.1	65.29	0.0001**
B-Extract	1	4.71	12.75	0.0014*
B²	1	12.45	33.73	0.0001**
Residual	26	0.37		
Lack of Fit	21	0.25	0.3	0.9784
Pure Error	5	0.86		
Core Total	29			

R-adjusted=0.79%, R-square=0.81%, R-prediction=0.75%.
**$P<0.01$; *$P<0.05$.

ultrasonic dispersion technology at various ratios of extract to lecithin as 45–61.52%.[10]

As shown in Figure 1, EE increased with increases in the phosphatidylcholine concentration. Moreover, EE decreased when the concentration of the green tea extract increased.

By increasing the proportion of phosphatidylcholine, more volume of the green tea extract could be incorporated into liposomes. These results were similar to the results of other studies.[20] Takahashi et al prepared food material loaded liposomes using lecithin and a mechanical method. They reported that EE clearly increased with increases in lecithin concentration up to 10 weight %.[21] Lu et al showed that EE increased when the ratio of tea polyphenols to lecithin shifted from 0.333:1 to 0.111:1. They investigated that whether the concentration of phosphatidylcholine decreased, the liposome vesicles available for the entrapment of tea polyphenols were limited.[10] Khosravi-Darani and Khoosfi produced liposomes loaded with *Zataria multiflora* Boiss essential oil. They demonstrated that EE increased at higher phosphatidylcholine proportions.[22] Naghavi et al reported that EE decreased with increases in green tea extract. By increasing the extract concentration, negative charges and repulsive forces in the system increased and hence increased the particles size and unstable liposomes.[9] Gülseren and Corredig showed
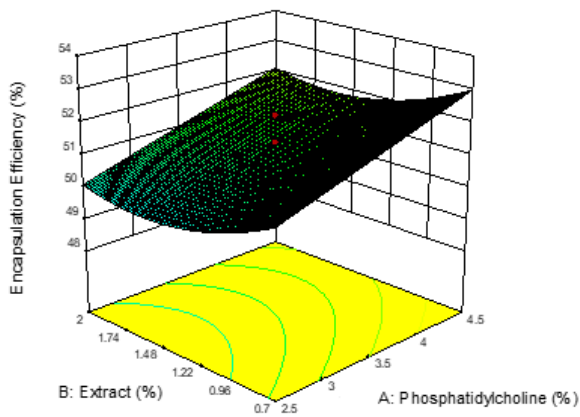
Figure 1. Response Level Charts on the Encapsulation Efficiency; Effects of Phosphatidylcholine and Green Tea Extract Concentrations.

that EE decreased when the concentration of the extract in liposomes increased from 60 to 40%.[23] Nakayama et al reported that liposomes mildly leaked at low concentrations of epigallocatechin gallate and epicatechin gallate. The major reason of this effect was linked to the content of gallic acid esters, which were responsible for their bilayer affinity while polyphenols could interrupt the membranes at high concentrations.[24] Jaafar-Maalej et al demonstrated that concentrations of phospholipids positively affected EE. By increasing phospholipid concentration from 40 to 60 mg/mL, the proportion of EE was nearly tripled (from 5.8 to 15.6%).[25] Results showed that the time and temperature of liposome production period did not significantly affect EE. This could occur because the most abundant component of polyphenols in green tea is catechin family. Applied ranges of time and temperature in mixing process were not enough to play destructive roles for green tea polyphenols. Su et al reported that use of 100°C for 3 h degraded nearly 25% of the green tea catechins, while theaflavins was completely degraded. Heating at 70°C for 3 hours degraded 56% of the theaflavins, while only 29% of catechins were destroyed.[26]

Permeability
For the assessment of the permeability rate, EE of liposomes containing green tea extract was calculated before and after 30 days of storage. Permeability rates of various formulations are shown in Table S1 (Supplementary file 1). As seen in Table S1, the effects of phosphatidyl choline concentration (A), extract concentration (B), temperature (C), time (D), AD, BC and C² were significant ($P < 0.05$). The permeability rate is a major index to show liposome stability. In this study, the permeability rate included 0.92–10.60% after 30 days of storage at 4°C. Results were analyzed using Design Expert software v.10 and the regression equation was as follows:

$$Premeability = +2.36 + 0.74A + 0.084B - 0.83C - 0.12D + 0.66AD - 0.38BC + 0.46C^2$$

Effects of phosphatidylcholine and time are shown in Figure 2a. By increasing the concentration of phosphatidylcholine, the proportion of permeability gradually increased. This effect could be clearly seen as when the concentration of

phosphatidylcholine increased, the liposomal particle size increased as well, which as a result increased the permeability rate. Lu et al reported a permeability range of 0.43–0.28 after 15 and 30 days of storage at 3–5°C, respectively.

Low permeability demonstrated conditions of polyphenols in liposomes during storage, which slowly changed at low temperatures. In fact, high temperatures of storage increased the mobility phase of liposome membranes and facilitated leakage.[10] Results demonstrated that the permeability rate decreased by increasing process time. When mixing time increased in liposome preparation, it possibly provided an appropriate opportunity to import phosphatidylcholine molecules to the liposome structure and hence improved stability. Figure 2b shows effects of the extract concentration and process time on permeability rate. Results showed that permeability decreased when the extract concentration in liposomes decreased. At high concentrations of the green tea extract, negative charges and repulsive forces increased and hence increased the permeability rate. This resulted instability of the liposome structure.[9]

Mixing temperature effects are illustrated in Figure 2b. Data revealed that the permeability rate decreased by increasing temperature to 60°C and was then constant. The permeability
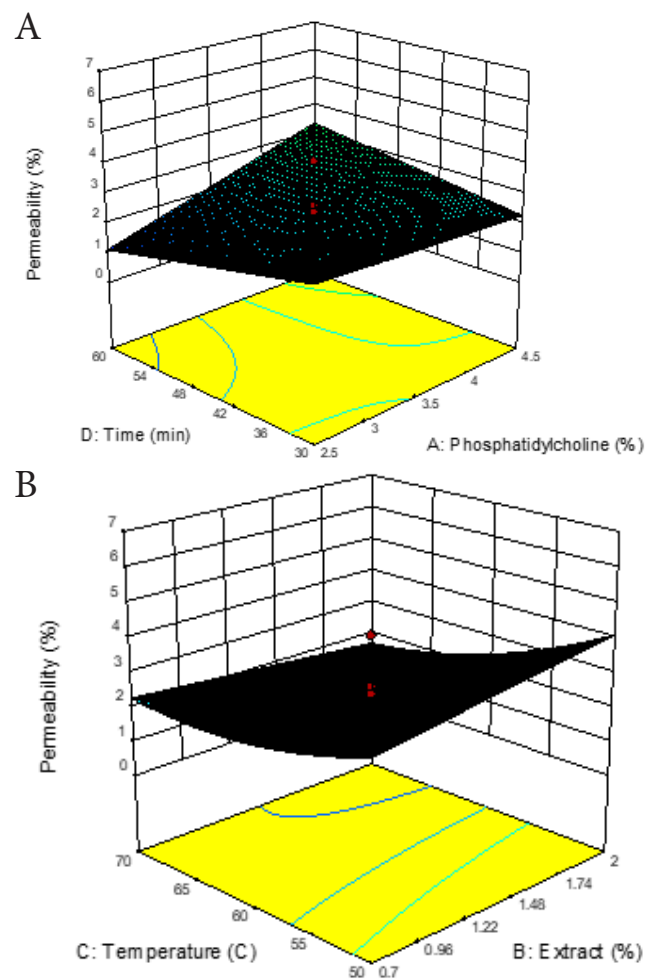
A

B

Figure 2. Response Level Charts on the Permeability of Liposomes; (a) Effects of Phosphatidylcholine Concentration and Time; (b) Effects of Extract Concentration and Process Temperature.

rate decreased with increased process temperature in liposome production. The phospholipids constituent liposome includes a phase transition temperature. At temperatures greater than the phase transition temperature, phospholipids form a liquid crystal phase. In temperatures lower than the phase transition temperature, molecules of phospholipids form a gel phase. Soy lecithin includes a transition temperature of 50–60°C.[27] In general, the Mozafari method can be carried out at a range of 60–70°C.[5] Jahadi et al used a mixing temperature of 50–60°C to prepare liposomes.[28] Putri et al explained that the use of mixing temperatures greater than the phase transition temperature of phospholipids decreased the particle size and thus the permeability rate decreased.[29]

Particle Size

The average particle sizes of empty and green tea loaded liposomes are shown in Figure 3a and 3b, respectively. The average size of the empty liposomes was 265.4 nm while the average size of the green tea extract loaded liposomes was 419 nm. Results revealed that empty liposomes were smaller than liposomes with green tea extract on an average particle size. This can be justified by entering polyphenols of the green tea within the liposome bilayers.

Putri et al reported that the Mozafari method was able to produce liposomes with a mean particle size of 600 nm with no use of particle size reduction procedures.[29] In fact, PDI is an indicator for particle size distribution. In this study, The PDI was 0.28 and PDI < 0.3 showed a narrow distribution.[30] Dag and Oztop reported that the average particle size of liposomes containing green tea extract was higher than that of the empty liposomes.[31] The results of the present study were generally similar to the results of Gibis et al. They reported that the size of the liposome particles depended on the encapsulated bioactive components. This could be

Figure 3. Particle Size in (a) Empty Liposomes; (b) Liposomes Encapsulating Green Tea Extract.

explained by hydrogen interactions between the polar head group phospholipids and phenolic compounds of the green tea extract as well as hydrophobic interactions between the fatty acid tails of lipids and further hydrophobic sections of the phenolic compounds.[32] Fang et al prepared tea catechin loaded liposomes using the reverse phase evaporation method. They showed that the size of the prepared liposomes varied between 100 and 700.[3] Lu et al reported the mean size of green tea polyphenol liposomes included 160.4 nm using ultrasound method to decrease particle sizes.[10] The necessary energy to produce small liposomal particles was supplied by magnet stirrer with an agitation speed of 1000 rpm and thermal treatments.

Total Polyphenolic Content

Various methods such as boiling, heating and refluxing have been described to extract sufficient quantities of polyphenols. Several studies have demonstrated that extraction of polyphenols using high temperatures degrade polyphenols and volatile compounds. In addition, the use of high temperatures increase protein and pectin extraction of the green tea leaves and degrade the quality of the green tea extracts.[33] In the present study, a temperature of 40°C was used to prevent degradation of thermally unstable components. Setyopratomo used temperatures of 40, 50 and 60°C to extract polyphenols from green tea and protect its susceptible components to high temperatures.[15] In this study, quantities of the polyphenols in green tea samples included 164.2 mg gallic acid/g extract. However, this was different in other studies. For example, Tsai et al. reported TPC of green tea as 237 mg gallic acid/g extract.[34] Dag and Oztop reported polyphenols within liposomes produced by ultrasonication and microfluidization as 78.795 and 62.340 mg GAE/L sample.[31] Noudoost et al calculated polyphenols of green tea in nanoliposomes as 88.86 mg ±3.84 gallic acid/g extract.[8] Quantities of the polyphenols of green tea leaves could be affected by several parameters, including climate, harvest time, leaf maturity, processing method and storage temperature.[35] As several investigations demonstrated, quantities of the extracted polyphenols from green tea leaves ranged between 280 and 580 g/kg dry extract using various solvents.[36]

Antioxidant Activity of the Green Tea Extract using DPPH Method

Results showed that free radical scavenging activities of the green tea extracts in free and liposomal forms included 90.6 and 93.37%, respectively, using the DPPH method. These findings were similar to findings by Zokti et al, who reported that encapsulated green tea extract included a higher antioxidant activity than that of the free green tea extract of mango drinks.[11] Noudoost et al described that the green tea incorporated in liposomes included more antioxidant activity, compared to that of the free green tea extract.[8] Khalaf et al. reported that the inhibition rate of 10 μg/mL green tea (*Camellia sinensis* Linn.) extract was 69.4%.[37] Bastos et al showed the free radical scavenging activity of ethanolic green tea extract as 88.93 ±0.22 using the DPPH method.[38] Dehkharghanian et al showed that the maximum antioxidant
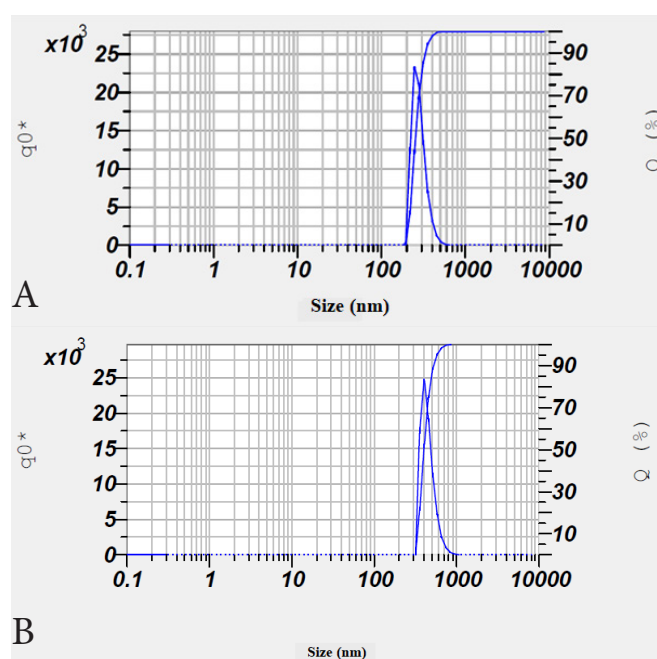
activity of the green tea extract in 2 mg/mL concentration reached 90%.[39]

Z-Potential

The Zeta potential (Z-potential) is an important index to assess surface charges of colloidal systems. Therefore, it is a useful indicator to display associations between encapsulated bioactive materials and the liposome bilayers. In this study, the Z-potential of liposomes containing green tea extract was -59.7 mV. The high value of Z-potential revealed a stability of the suspension as the charged particles repelled each other and avoided natural tendency of their aggregation.[4] Manosroi et al reported that liposomes containing negative charges were more stable and their leakage rate was lower than the positive liposomes.[40] To achieve good stabilities, it is necessary to set the Z-potential above ±30.[10] In the current study, values of the Z-potential demonstrated appropriate electrostatic stabilizations for liposomes loaded with green tea extracts. In extract loaded liposomes, wide associations can be created between polyphenols in extracts and liposome bilayers. The most important bond includes hydrogen interactions between the polar moieties in extract polyphenolic components and liposome bilayers or hydrophobic bonds between the hydrophobic polyphenols and hydrophobic parts of the fatty acid tails in phospholipid molecules.[32] Lu et al reported Z-potentials of nanoliposomes containing green tea polyphenol as -67.2 mV. Indeed, higher values of Z-potential demonstrate higher stabilities of nanoliposome particles due to higher repulsive forces that prevent aggregation of the particles.[10] Dag and Oztop reported Z-potential values of green tea extract loaded liposomes as -35 mV.[31]

Conclusions

In this study, encapsulation of green tea extract was carried out using the Mozafari method, resulting in protective effects on antioxidant activity of the green tea polyphenols. The size of the liposomes containing green tea extract was 419 nm using no particle size reduction methods. The Z-potential was calculated as -59.7 mV, demonstrating a good stability for the production of liposomes. Furthermore, results showed that optimal conditions to achieve the most value of EE and the lowest value of permeability included 4.5% of phosphatidylcholine, 0.7% of extract concentration and 30 min and 50°C of processing time and temperature, respectively. Ranges of EE and permeability included 42–54 and 0.92–6.59, respectively. Moreover, results showed that antioxidant activity of the encapsulated green tea polyphenols was more than that of free polyphenols. These results are promising to use of green tea extracts instead of the synthetic antioxidants for example BHT in food sciences.

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