PRODUCTION AND EVALUATION OF NANOLIPOSOMES LOADED WITH *CAPPARIS SPINOSA* EXTRACT

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Abstract

In this study, after extracting capparis spinosa extract and determining the amount of its phenolic compounds, nanoliposomes containing capparis spinosa extract were prepared in different ratios of lecithin and cholesterol. Then, the effect of cholesterol-lecithin concentrations on particle size, particle size distribution, encapsulation efficiency (EE), physical stability of nano- liposomes and stability of phenolic compounds loaded in nano-liposomes during the storage time were evaluated. The amount of phenolic compounds were obtained 6.328 mg of GAE/g dry sample. The average particle size as Z-Average was in the range of 95.05-164.25 nm. By increasing the cholesterol concentration, particle size increased. The particle size distribution was in the acceptable range of 0.3-0.5 (PI≤0.5). Addition of cholesterol increased zeta potential from -60.40 to -68.55. Higher zeta potential values, indicate a higher and longer- term stability of the particles. Also, cholesterol led to increase encapsulation efficiency (EE) and even the stability of phenolic compounds loaded in nano-liposomes during storage time. The results showed that the problem of instability of phenolic compounds, which leads to their limited commercial application, can be solved by encapsulation.

Keywords: *Capparis spinosa, phenolic compounds, nanoliposome*

1. INTRODUCTION

Capparis spinosa extract is a rich source of phenolic compounds (Anwar et al., 2016; Adias et al., 2012). The instability of phenolic compounds in environmental conditions as well as their bitter or astringent taste has created challenges for the use of these compounds in food; To overcome these challenges, these compounds was tried to encapsulate using nanoliposomes. Nanoliposomes are the nanometric version of liposomes. Liposomes are spherical particles composed of lipid molecules (mainly phospholipids) that tend to accumulate in polar solvents such as water in the form of bilayer membranes (Mozafari et al., 2008; Mozafari et al., 2010). Encapsulation with liposomes is an effective way to preserve the intrinsic properties of bioactive compounds during storage and production of foods fortified with them, as well as a physicochemical barrier against peroxidan agents such as free radicals, oxygen and UV (Ghanbarzadeh et al., 2015). In this study, after extracting *capparis spinosa* extract and determining the amount of its phenolic compounds, nanoliposomes containing *capparis spinosa* extract were prepared in different ratios of lecithin and cholesterol. Then, the effect of lecithin-cholesterol concentrations on particle size, particle size distribution, encapsulation efficiency (EE), physical stability of nano- liposomes and stability of phenolic compounds loaded in nano-liposomes during the storage time were evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Capparis spinosa fruits, were collected from subtropical regions of Ilam province (Iran). Folin ciocalteu, gallic acid and tween 80 were obtained from Merck (Germany) and cholesterol and phosphatidylcholine were obtained from Sigma- Aldrich (Germany).

2.2. Methods

2.2.1. Extraction

The extract obtained from *capparis spinosa* fruit powder (50 g) in 1000 mL of ethanol %96 (1:20) using ultrasonic bath (Backer, vCLEAN 1- L6, Iran) with 40 kHz at 30 ºC for 30 and 60 min. The extracted solution was filtered and lyophilized.

2.2.2. Phenolic content measurement

The phenolic content was measured by folin ciocalteu method (Kossah et al., 2011). Gallic acid was used to produce a standard curve (0-200 mg/L). Measurements of the phenolic compounds were expressed as mg of gallic acid equivalents (GAE)/ g of the dry weight of plant material.

2.2.3. Preparation of nanoliposomes containing Capparis spinosa extract

Nanoliposomes containing *capparis spinosa* extract were prepared in ratios of 60- 0, 50- 10, 40- 20 and 30- 30 lecithin- cholesterol. For this purpose, after preparing sodium acetate buffer 250 mM and adjusting its pH to 3.5, different amounts of lecithin in buffer was mixed by magnetic stirrer with heater (Alpha Model D500, Iran) for 2 hours and put in the refrigerator overnight to be well hydrated. Then, 25 mg of extract, 20 mg Tween 80 and the mentioned ratios of cholesterol were added to the hydrated lecithin for the ratio of 10 ml of buffer. The resulting mixture was stirred with a heater magnetic stirrer and after complete mixing the particle size was reduced to nanoscale with a homogenizer (Ika model digital T25, Germany) for 5 minutes at 20,000 rpm at a temperature higher than the phase transition temperature of lecitin. The final step was ultrasonic process for 5 min at a power of 40 kHz frequency in the cycles of 15 s on and 5 s off, whereafter nanoliposomes were obtained (Chi et al., 2019).

2.2.4. Evaluation of nanoliposomes containing Capparis spinosa extract

2.2.4.1. Particle size and zeta potential

Particle size and zeta potential were measured by DLS (Horiba, Japan) at 25 ºC.

2.2.4.2. Encapsulation efficiency of polyphenlos

Encapsulation efficiency (EE) of polyphenlos were obtained using method described by Tavakoli et al. (2018). At first, the free (unencapsulated) phenolic compounds were separated from the entrapped parts via centrifugation at 5000 rpm for 15 min. Afterward, chloroform was added into the encapsulated phase so that the liposomal membrane could be solved and the phenolic compounds are liberated. Ultimately, the concentration of phenolic compounds in each sample was determined based on the gallic acid standard curve as mentioned in Section *2.2.2*. The encapsulation efficiency (EE) was measured using the following equation:

$$EE\% = ((C_{total} - C_{free})/C_{total}) \times 100$$

Where C_{total} is the concentration of the total phenol content in the liposomal suspension and C_{free} is the concentration of the free phenolic content.

2.2.4.3. The stability of polyphenols during storage

The stability of the samples was evaluated by visual observation of phase separation and the release rate of phenolic compounds encapsulated in nano- liposomes at ambient temperature over a period of 60 days. In addition, the phenolic compounds stability was also monitored within 60 days (days 0, 15, 30, 45 and 60) of storage at 25 ºC.

2.3. Statistical analysis

All the data were subjected to one way analysis of variance using a completely randomized factorial design and means were compared with Donkan test ($p<0.05$) using version 9.1.3 of the SAS (Statistical Analysis System) statistical program.

3. RESULTS

The amount of phenolic compounds were obtained 6.328 mg of GAE/g dry sample. The average particle size as Z- Average was in the range of 95.05- 164.25 nm. The particle size distribution was in the acceptable range of 0.3- 0.5 (PDI≤0.5). The Zeta potential was in the range of -60.4 to -68.55 mV. Addition of cholesterol increased zeta potential from -60.40 to -68.55. Also, cholesterol led to increase encapsulation efficiency (EE). The encapsulation efficiency of containing *Capparis spinosa* extract with 0-60 and 10-50 ratio of cholesterol-lecithin was 89.35% and 95.75%, respectively. Solutions containing *Capparis spinosa* extract loaded nanoliposomes with ratios of 0-60 and 10-50 cholesterol-lecithin showed good physical stability, so that partial biphasic was observed on day 60 of storage. Cholesterol effected on stability of phenolic compounds loaded in nano-liposomes during storage time.

Nanoliposomes containing *Capparis spinosa* extract with different ratio of cholesterol-lecithin	**Average size (nm)**	**Polydispersity index (PDI)**	**Zeta potential (mV)**
0-60 cholesterol-lecithin	95.05±5.30^{c}	0.517±0.06^{a}	-60.4±0.42^{b}
10-50 cholesterol-lecithin	118.5±0.28^{b}	0.329±0.02^{b}	-63.05±2.75^{b}
20-40 cholesterol-lecithin	130.05±5.58^{b}	0.382±0.02^{b}	-68.55±0.35^{a}
30-30 cholesterol -lecithin	164.25±14.49^{a}	0.364±0.05^{b}	-68.45±1.20^{a}

Table 1. Average particle size and zeta potential of the nanoliposomes containing *Capparis spinosa* extract with different ratio of cholesterol-lecithin.

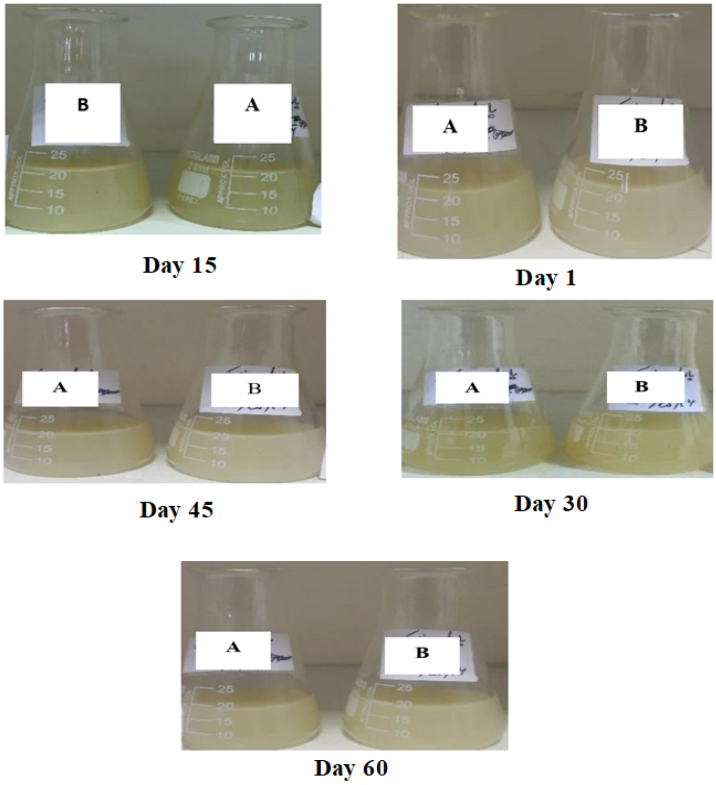


Fig. 1. Physical stability of solutions containing *Capparis spinosa* extract loaded nanoliposomes in 60 days storage at room temperature; A: nanoliposomes with ratios of 0-60 cholesterol-lecithin, B: nanoliposomes with ratios of 10-50 cholesterol-lecithin.

4. DISCUSSION

The total phenol content of *Capparis spinosa* can vary depending on geographical conditions, plant growth stage and different parts of the plant. Total phenols and carbohydrates increase during fruit ripening (Sharifi et al., 2017). In a study, the total phenol content of *Capparis spinosa* was reported 37 mg of GAE/100 g dry sample (Aliyazicioglu et al., 2013). According to the results of this study, *Capparis spinosa* fruit has appropriate amounts of phenolic compounds and more research is needed in food models to be able to introduce it as a suitable source of antioxidants for the production of food products to the food industry.

Particle diameter and particle size distribution (PI) are two important factors in determining the characteristics of colloidal systems. The values of these parameters show the stability of colloidal systems as well as the encapsulation efficiency of polyphenlos (Gharehbeglou et al., 2019). By increasing the cholesterol concentration, particle size increased. It can be stated that cholesterol can reduce the binding of hydrophobic molecules of dual membrane through one of these mechanisms: 1- Cholesterol competes with phospholipid molecules to capture the lipophilic space in the lipid membrane (Fang al et., 2001). 2- Cholesterol causes lipophilic molecules to attach to lipid membranes by reducing the flexibility of the bilayer membrane (Mohammed et al., 2004). These results are in line with the results reported by Malheiros et al. (2012) on liposome-encapsulated nisin.

Zeta potential is one of the factors that play an important role in electrostatic interactions and stability of nanodelivery systems (Gharehbeglou et al., 2019). In general, particles are electrostatically stable if the zeta potential of the colloidal system is higher than ±30 mV (Lu et al., 2011). Addition of cholesterol increased zeta potential from -60.40 to -68.55 mV. Higher zeta potential values, indicate a higher and longer- term stability of the particles. In fact, cholesterol stabilizes liposomes by tightening the membrane structure and increasing the zeta potential and electrostatic repulsion between particles (Liu, 2000). These results are in line with the results reported by the other researchers (Mohammadi et al., 2013).

With the addition of cholesterol to the nanoliposome membrane, although the particle diameter increased, it had a positive effect on PI, reducing the size difference and smoothing the size of the nanoliposomes.

In the present study, the ratio of 0-60 cholesterol-lecithin with the smallest particle size and the ratio of 10-50 with the smallest particle size among cholesterol nanoliposomes were selected for further tests.

The encapsulation efficiency indicates the potential of wall materials in trapping of polyphenlos as well as the shelf life of the material enclosed in nanostructures (Soleimanifard et al., 2017). Since the hydrophilic space of liposomes is more than their hydrophobic space, the high percentage of encapsulation in both ratios can be due to the higher hydrophilic part of the extract and its connection to the inner and outer surface of nanoliposomes as well as being in the inner aqueous phase of nanoliposomes. Higher encapsulation efficiency of cholesterol samples may be due to the effect of cholesterol on the reduction of membrane fluidity. Fan et al. (2008) expressed the similar reasons for the effect of cholesterol on encapsulation efficiency of nanoparticles.

Solutions containing *Capparis spinosa* extract loaded nanoliposomes with ratios of 0-60 and 10-50 cholesterol-lecithin showed good physical stability, so that partial biphasic was observed on day 60 of storage. The physical stability of liposomes depends on the particle size of the liposome, the number of layers, the structure of phospholipids and the production method of liposomes (Laridi et al., 2003). In a research, formulations with the highest and lowest rates the phenolic compounds of olive leaf as the core material showed the lowest and highest stability, respectively. The phenolic compounds stability results within 60 days (days 0, 15, 30, 45 and 60) of storage at 25 ^{o}C showed that until day 45 of storage, the stability of phenolic compounds in nanoliposomes with cholesterol (10-50) was significantly higher than nanoliposomes without cholesterol (0-60) ($p<0.05$), but then there was not a significant difference in the percentage of phenolic compounds stability. Cholesterol effected on stability of phenolic compounds loaded in nano- liposomes during storage time by reducing fluidity and permeability of liposome membrane. Cholesterol inhibits of rupture and changes in membrane, too.

5. CONCLUSIONS

In the present study, nanoliposomes containing *Capparis spinosa* extract were successfully produced in the particle size range of 95-165 nm. The addition of cholesterol to the liposomal structure, although increasing the particle size, increased the repulsion and electrostatic stability of nanoliposomes by increasing the zeta potential, as well as the encapsulation efficiency. *Capparis spinosa* extract loaded nanoliposomes showed good physical and chemical stability within two months of storage at room temperature. The results showed that the problem of instability of phenolic compounds, which leads to their limited commercial application, can be solved by encapsulation.

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REFERENCES

1. Adias, T. C., Moore-Igwe, B., & Jeremiah, Z. A., 2012, Storage related haematological and biochemical changes of CPDA-1 whole blood in a resource limited setting. *Journal of Blood Disorders & Transfusion*, 3(3), 124.
2. Aliyazicioglu, R., Eyupoglu, O. E., Sahin, H., Yildiz, O., & Baltas, N., 2013, Phenolic components, antioxidant activity, and mineral analysis of *Capparis spinosa* L. *African Journal of Biotechnology*, 12(47), 6643-6649.
3. Anwar, F., Muhammad, G., Hussain, M. A., Zengin, G., Alkharfy, K. M., Ashraf, M., & Gilani, A. H., 2016, *Capparis spinosa* L.: A plant with high potential for development of functional foods and nutraceuticals/pharmaceuticals. *International Journal of Pharmacology*, 12(3), 201-219.
4. Chi, J., Ge, J., Yue, X., Liang, J., Sun, Y., Gao, X., & Yue, P., 2019, Preparation of nanoliposomal carriers to improve the stability of anthocyanins. *LWT*, 109, 101-107.
5. Fan, M., Xu, S., Xia, S., & Zhang, X., 2008, Preparation of salidroside nano-liposomes by ethanol injection method and in vitro release study. *European Food Research and Technology*, 227(1), 167-174.
6. Fang, J. Y., Hong, C. T., Chiu, W. T., & Wang, Y. Y., 2001. Effect of liposomes and niosomes on skin permeation of enoxacin. *International Journal of Pharmaceutics*, 219(1-2), 61-72.
7. Ghanbarzadeh, B., Pezeshky, A., Hamishekar, H., & Moghadam, M., 2015, Vitamin A palmitate-loaded nanoliposomes: study of particle size, zeta potential, efficiency and stability of encapsulation. Iranian Food Science and Technology Research Journal, 12(2), 261-275.
8. Gharehbeglou, P., Jafari, S. M., Hamishekar, H., Homayouni, A., & Mirzaei, H., 2019, Pectin-whey protein complexes vs. small molecule surfactants for stabilization of double nano-emulsions as novel bioactive delivery systems. *Journal of Food Engineering*, 245, 139-148.
9. Kossah, R., Zhang, H., & Chen, W., 2011, Antimicrobial and antioxidant activities of Chinese sumac (*Rhus typhina* L.) fruit extract. *Food Control*, 22(1), 128-132.
10. Laridi, R., Kheadr, E. E., Benech, R. O., Vuillemard, J. C., Lacroix, C., & Fliss, I., 2003, Liposome encapsulated nisin Z: optimization, stability and release during milk fermentation. *International Dairy Journal*, 13(4), 325-336.
11. Liu, D. Z., Chen, W. Y., Tasi, L. M., & Yang, S. P., 2000, Microcalorimetric and shear studies on the effects of cholesterol on the physical stability of lipid vesicles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 172(1-3), 57-67.
12. Lu, Q., Li, D.-C., Jiang, J.-G., 2011, Preparation of a tea polyphenol nanoliposome system and its physicochemical properties. *Food Chemistry*, 59 (24), 13004−13011.

13. Malheiros, P.D.S., Sant Anna, V., Barbosa, M.D.S., Brandelli, A., Franco, B.D.G.D.M., 2012, Effect of liposome-encapsulated nisin and bacteriocin-like substance P34 on Listeria monocytogenes growth in Minas frescal cheese. *International Journal of Food Microbiology*, 156(3), 272-277.
14. Mohammadi, M., Jahadi, M., Ehsani, M. R., & Khosravi-Darani, K., 2013, Application of liposome nano carrier in cheese production and ripening. *Iranian Journal of Nutrition Sciences and Food Technology*, 7(4, Suppl.).
15. Mohammed, A. R., Weston, N., Coombes, A. G. A., Fitzgerald, M., & Perrie, Y., 2004, Liposome formulation of poorly water soluble drugs: optimisation of drug loading and ESEM analysis of stability. *International Journal of Pharmaceutics*, 285(1-2), 23-34.
16. Mozafari, M. R., 2010, Nanoliposomes: preparation and analysis. In Liposomes (pp. 29-50). Humana press.
17. Mozafari, M. R., Khosravi-Darani, K., Borazan, G. G., Cui, J., Pardakhty, A., & Yurdugul, S., 2008, Encapsulation of food ingredients using nanoliposome technology. *International Journal of Food Properties*, 11(4), 833-844.
18. Sharafi Z, Ghani A, Salehi S, Mohtashami S., 2017, Antioxidant activity, phenolics content and total flavonoids of caper fruit (*Caparis spinosa*) at different maturity stages. *Research Journal of Pharmacognosy*, 4(Supplement), 83.
19. Soleimanifard, M., Sadeghi mahoonak, A., Ghorbani, M., Azizi, KH., & Sepahvand, A., 2017, Optimization of Khorramabad Olive Leaf Extract Extraction and Investigating the physical properties of nano-structured lipid carriers containing it. Journal of Nutrition Sciences & Food Technology, 13(3), 81-92.
20. Tavakoli, H., Hosseini, O., Jafari, S. M., & Katouzian, I., 2018, Evaluation of physicochemical and antioxidant properties of yogurt enriched by olive leaf phenolics within nanoliposomes. *Journal of Agricultural and Food Chemistry*, 66(35), 9231-9240.