The Influences of Antioxidants on the Stability of Coix Seed Oil Liposomes Under Ultraviolet Irradiation

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Abstract: In this research, coix seed oil (CSO) liposomes containing antioxidants were prepared by ethanol injection method. The influence of types and concentrations of antioxidants on the physicochemical stability of CSO liposomes irradiated by ultraviolet (UV) light was investigated in terms of particle size distribution and malondialdehyde (MDA) production. The results showed that lipid peroxidation and liposomal particle size change were induced by UV irradiation. The tert-butylhydroquinone (TBHQ) and dibutyl-hydroxytoluene (BHT) exhibited better resistance on size change and peroxidation induced. However, β- carotene exerted good anti oxidative activity at low concentration; the antioxidant effect was weaken and even promoted oxidation at higher concentration. Although the antioxidant effect of α-tocopherol was enhanced as the concentration increased, its influence on liposomal size varied and dependent largely on exposed time. In addition, the lower MDA value of CSO liposomes than that of control indicates the oil could supply anti oxidative activity against the peroxidation of liposomal membrane. This research would supply good foundation for prolonging the shelf of CSO liposomes.

1 INTRODUCTION

Coix seed is a traditional herbal planted in many Asian countries, such as Indian, China, and so on, where the seed could be consumed as medicine and food (Bai, 2019). According to literatures, the oily abstracts named as coix seed oil was the main active ingredients in the seed. Numerous experiments have proved that the oil exhibited excellent antitumor, anti-inflammation and analgesia activities (Zhu, 2017). However, the drawbacks of water water-insolubility, low accessibility, combined with poor oxidative stability significantly confined the wide utilization of CSO. Thus, strategies that could solve the above problems are needed to be thought out.

To deal with these drawbacks of CSO, various delivery systems (microcapsule, microsphere, microemulsion, and liposomes) have been developed by many researchers (Nakhaei, 2021; Chen, 2022). During the past several years, we also carried out relative researches, and liposomes were used to encapsulate CSO. The delivery system was proved to be an efficient carrier that could supply good protection for CSO from adverse environments and promote controlled release of CSO in gastrointestinal tract (Bai, 2019). However, phospholipids, as the main membrane materials for liposomes, contain certain amount of unsaturated fatty acids. That means the bilayer is sensitive to external environment (oxygen, lights, and so on) and easily to be oxidized and decomposed, producing harmful chemicals, including lysophospholipids etc. Introducing antioxidant into liposomes was a useful way to delay the degradation (Rafaela, 2021; Walker, 2017; Palmina, 2021). Usually, the efficiency of the antioxidant activity was largely dependent on the propertied of liposomes themselves as well as the types and concentrations of antioxidant used. However, nothing was known about how to effectively protect liposomes containing CSO.

In this sense, four common used antioxidants (TBHQ, BHT, β-carotene, and α-tocopherol) were chosen and encapsulated together with CSO to delay the oxidant of CSO liposomes. The physical and chemical stability of the liposomes during UV-light exposure was monitored and determined in details. This research would supply certain foundation for the development of stable CSO liposomes.

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2 MATERIALS AND METHODS

2.1 Materials

Egg yolk phospholipids and cholesterol were bought from Shanghai Lanji Technology Development Co., Ltd (Shanghai, China). Coix seed oil was purchased from Hecheng Sanxian Biotechnologies Co., Ltd. (Guangzhou, China). TBHQ, BHT, α-tocopherol, β-carotene, and 2, 2 -azobis(2-methypropionamidin) dihydrochloride (AAPH) were kindly supplied by Sigma-Aldrich (Shanghai, China). All other chemicals used were of analytical grade.

2.2 Preparation of Liposomes

Liposomes were prepared by ethanol injection method as described previously by us with slight modification (Bai, 2019). Briefly, weighted amounts of antioxidants were dissolved in CSO, and diluted with the oil to obtain a serial concentration of antioxidants/CSO solution. The mixture, egg yolk lecithin and cholesterol were all added into ethanol and mixed thoroughly until all the agents were solute. The mixture was then dropped into to a phosphate buffer solution maintained at 45℃ under stirring. After 20 mins, the sample was subjected to rotary evaporation and then sonication treatment. The obtained samples were stored in refrigerator at 4 ℃ until further use. The nothing loaded liposomes (control) and CSO loaded liposomes were also prepared by the same procedure for comparison.

2.3 Particle Size Distribution

The particle size of all liposomal formations were analyzed on a zeta sizer instrument (Nano ZS90, Malvern Instruments, Malvern, UK)

2.4 Measurement of Lipid Oxidation

The extent of lipid oxidation in liposomes was determined by an assay (thiobarbituric acid reactive substances, TBARS) as described by Walker et al., 2017 (Walker, 2017). Briefly, 1 mL of liposomes was added into 5 ml of TBA working solution, mixed thoroughly and then heated in a water bath at 75℃ for 15 min. This mixture was cooled to room temperature, and then centrifuged at 2500 rpm for 5min. The absorbance of supernatant at 532 nm was recorded on a UV-visible spectrophotometer. The TBARS content was calculated and expressed as ng MDA equivalent per mg phospholipids.

2.5 UV-light Exposure Stability

In order to accelerate the instability induced by UV-light irradiation, APPH was added into all liposomes systems (Pires, 2019). Freshly prepared liposomes added with APPH (0.05 mol/L) were placed in closed quartz cells and irradiated with a 254 nm UVC germicide lamp (Philips TUV PL-S 5W/2P 1CT) at a radiance of 1.9W/m2. After exposure for 15, 30, 45, 60, 90, 120 mins, samples were withdrawn. The instability caused by UV radiation was recorded by the determining size distribution and MDA values.

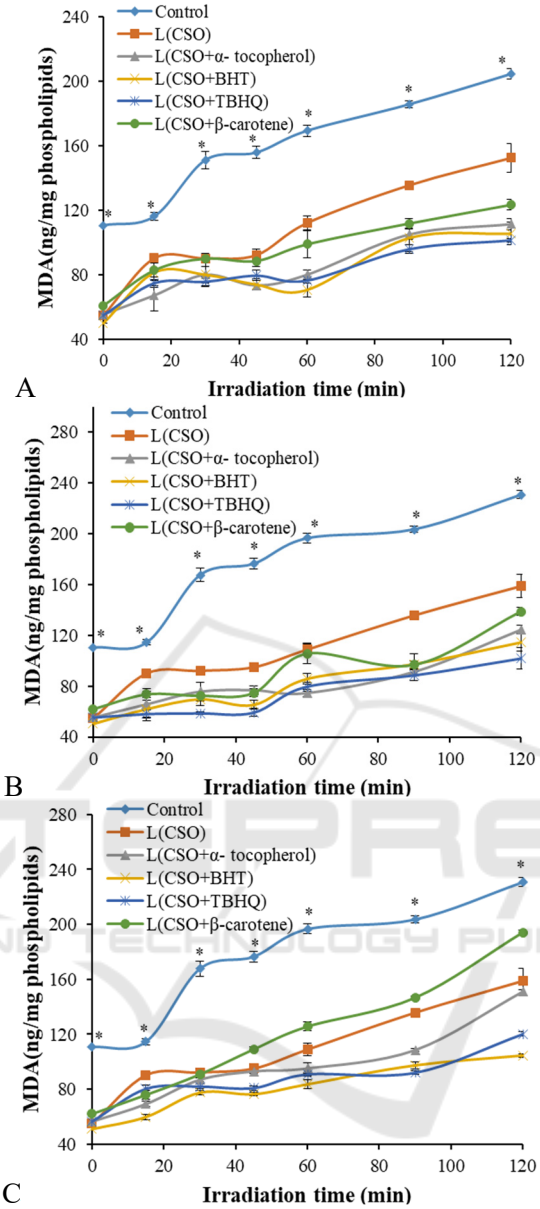
2.6 Statistical Analysis

All determinations were repeated triplicate, and presented as means ± standard deviations (SD). The results were analyzed statistically for significance ($p \leq 0.05$) using SPSS 18.0 software.

3 RESULTS & DISCUSSION

3.1 The Irradiation Stability of Liposomes at 0.002% Antioxidant

Fig. 1A shows changes of the peroxidation product in liposomes during irradiation. Obviously, the MDA values of the control (nothing was loaded) increased as the exposure time increased, indicating oxidation of the liposomal bilayer occurred. Meanwhile, the values of CSO loaded liposomes were generally lower than that of the control ($p<0.05$), suggesting the embedding CSO could somewhat delay the oxidative degradation of the membrane. This may be accounted to the antioxidant activity of un-entrapped CSO. According to literatures, the content of unsaturated fatty acid in CSO was higher than 70% (Xiao, 2019). The protective role of CSO may be originated from the oxidization itself. In addition, the peroxidation produced in liposomes containing antioxidants was lower than that in CSO liposomes. What's more, the MDA values of them was in the order of THBQ>BHT>α-Tocopherol>β-carotene. The higher antioxidant effect of β-carotene may be explained by its conjugated polyenes structure, which could help to remove free radicals and quench singlet oxygen, as a result, inhibiting the decomposition of primary oxidation products to secondary oxidation products (Walker, 2017). The result was consistent with our previous report that the co-loaded CSO and β-carotene exhibited synergistic antioxidant (Bai, 2019).



*indicates the value of the Control is significantly different from that of all the other liposomes at each time point (p<0.05).

Figure 1: The effects of antioxidant on oxidation stability of CSO liposomes during UV irradiation. A (0.002%), B (0.01%), C (0.02%).

Figure 2 shows that particle size distribution of all samples generally shifted to higher values after UV irradiation. What's more, the longer the exposure time the larger the particle was. The results were in consistence with previous reports (Palmina, 2021; Pires, 2019). Pires et al. (Pires, 2019) found that UV irradiation affects the phosphate and carbonyl groups of 1, 2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol) in liposome. The enlarged particle size may be due to the oxidation of phospholipids induced by UV irradiation, which might change the emulsifying property of phospholipids, and disturbing the integrity and stability of liposomes in the end. Meanwhile, more encapsulated CSO may have leaked out from liposomes and absorbed on the surface of bilayers as the result of the changed integrity, that the increased viscosity may promote particle aggregation (Sabet, 2021). In addition, the particle size changes for liposomes containing CSO+BHT was the smallest, indicating incorporating BHT into liposomes could supply better shield against UV radiation.

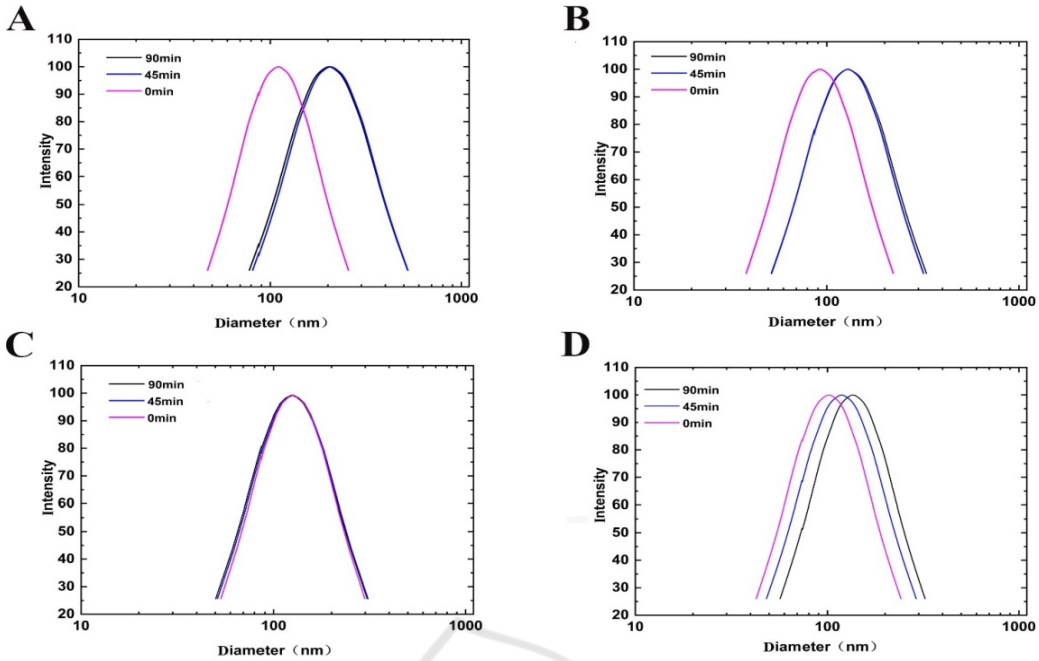


Figure 2: The effects of 0.002% antioxidant on the particle size distribution of liposomes during irradiation. A: L(CSO+β-carotene), B: L(CSO+TBHQ), C: L(CSO+BHT), D: L (CSO+α- tocopherol).

3.2 The Irradiation Stability of Liposomes at 0.01% Antioxidant

Similarly, the MDA value were all increased as the function of time, and the values were in the order of control>CSO liposomes>CSO + antioxidant liposomes at fixed irradiation time when 0.01% antioxidant was embedded (Fig. 1B). It indicates that all types of antioxidant exhibited certain inhibition effect on the degradation of liposomes (Temprana, 2011). In addition, comparing with Fig. 1A, the MDA values increased more slowly, indicating fewer lipids were decomposed and the anti-oxidative activity of antioxidant were increased when the concentration of antioxidant increased (Feng, 2018). However, nearly no significant difference was detected among the liposomes loaded with antioxidant.

Figure 3 shows that the particle size distribution of the liposomes added with BHT or TBHQ exhibited slightly shifts during irradiation, while that of ones enclosed with α-tocopherol or β-carotene was more complex. This suggests that BHT and TBHQ at higher concentration could supply good protection against the size change induced by UV-light. It could be found that, the size of CSO+α-tocopherol co-loaded liposomes became larger after exposure for 45 mins, whereas, the size changed to much smaller after 90 mins' irradiation. According to literatures, there are continuous movement and collision among liposomal particles (Brilliantov, 2007). What's more, the leakage of embedded materials and state transition of bilayer membranes co-existed during irradiation and affected the collision (Pires, 2019). When the irradiated time was short, α-tocopherol could supply efficient protection for phospholipids, resulting in low extent of oxidation. At this stage, the increase in particle size caused by the collision may be dominantly influenced by the leakage of embedded materials, which enhanced the surface viscosity of liposomes and increased the chances adhering with each other. However, a large part of unsaturated fatty acids might have undergone peroxidation during long-term irradiation since the limited ant oxidative activity of α-tocopherol. As a result, the melting point of phospholipid bilayer membrane was increased and transferred to gel phase, leading to increased rigidity with decreased deformability for liposomal membrane, which in turn inhibited the leakage of CSO.

3.3 The Irradiation Stability of Lipidosomes at 0.02% Antioxidants

Fig. 1C showed that the general trends of all samples at 0.02% antioxidants were increased as increasing the irradiation time. What's more, the

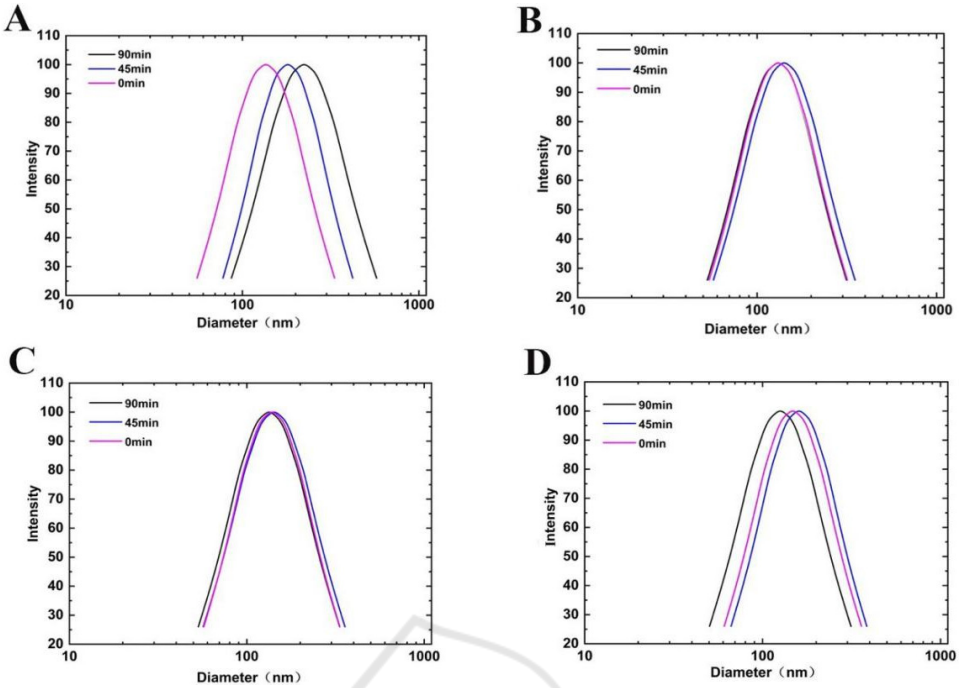


Figure 3: The effects of 0.01% antioxidant on the particle size distribution of liposomes during irradiation. A: L(CSO+β-carotene), B: L(CSO+TBHQ), C: L(CSO+BHT), D: L (CSO+α- tocopherol).

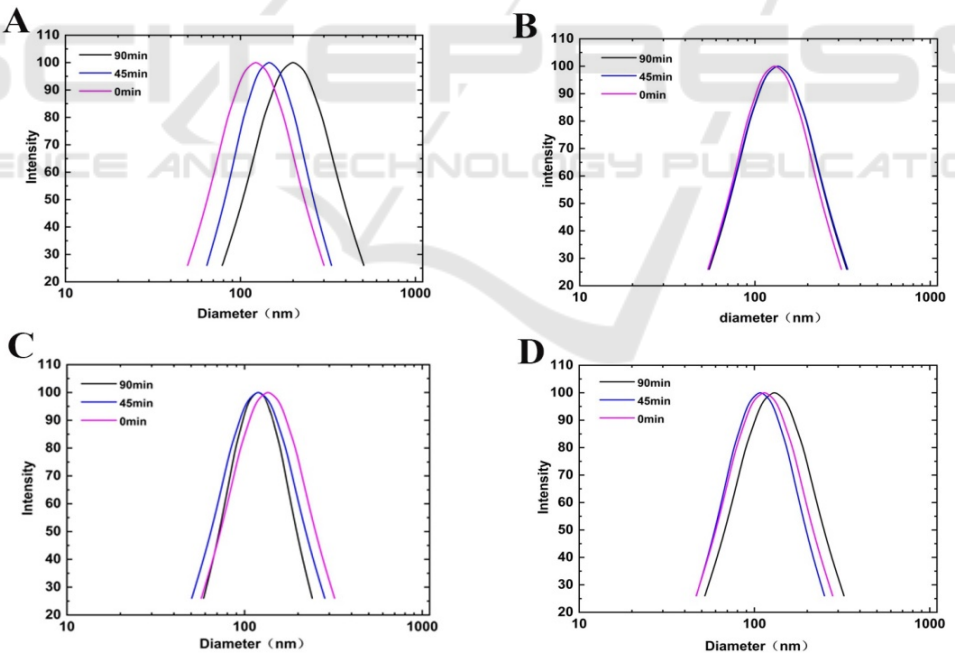


Figure 4: The effects of 0.02% antioxidant on the particle size distribution of liposomes during irradiation. A: L(CSO+β-carotene), B: L(CSO+TBHQ), C: L(CSO+BHT), D: L (CSO+α- tocopherol).

MDA value was lower than that with less antioxidant, except for the one with β-carotene. Obviously, the MDA value of liposomes containing CSO+ β-carotene was higher than that containing less β-carotene after irradiation for the same time. What's more, some values were even higher than that of CSO liposomes. This suggests that β-carotene might exhibit good antioxidative at

limited concentration, whereas would promote oxidation when excess certain concentration. Scoccianti et al. reported tocopherols could help against lipid peroxidation induced by 1 mM Cr(III), but generated oxidative stress at the highest concentration (Scoccianti, 2016).

Figure 4A shows that there are significant changes in particle size distribution of L (CSO+β-carotene) during irradiation. And the mean particle size was in the order of 90 mins treated > 45 mins treated > freshly prepared, indicating irradiation induced polymerization of the liposome. As to L (CSO+α- tocopherol), the mean particle size of 90 mins treated was smallest, while 45 mins treated samples were the largest. On the contrary, the changes in L (CSO+TBHQ), L (CSO+BHT) were not so significant, indicating that TBHQ and BHT had a strong irradiation stabilization effect at this concentration.

4 CONCLUSIONS

In this research, the influence of TBHQ, BHT, α-Tocopherol and β-carotene on the physiochemical stability of CSO liposomes during UV irradiation were investigated. The results showed that CSO could exert certain protection for liposomal bilayer from oxidation. The ant oxidative efficiency of antioxidant was largely dependent on the type, concentrations, and exposure time. β -carotene could supply good shield at low concentration, whereas promote oxidation when the concentration increased to 0.2%. On the contrary, TBHQ and BHT exhibited good irradiation stabilization effect, and nearly no particle size changes were detected for all concentrations. Although the oxidation of CSO liposomes could be inhibited by α- tocopherol; its particle size stabilization function was limited. However, whether these antioxidants influence the leakage of CSO and the structural integrity of liposomal bilayer need further investigation.

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