Liposomes from jack beans phospholipid extract for delivering vitamin C

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Liposomes from Jack Bean's Phospholipid Extract for Delivering Vitamin C

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Abstract. Liposomes from jack bean's phospholipid extract were used to encapsulate vitamin C. The liposomes were made with cholesterol concentration between 0 and 40%. The encapsulation efficiency of liposomes was evaluated just after they were formed. Then the liposomes were stored under several temperatures namely 37, 25, and 5 °C. The vitamin C released from liposomes was monitored in 8 days. The highest value of vitamin C encapsulation efficiency (EE) in jack bean liposome was 86.61% once the cholesterol concentration was 40%. The cholesterol reduced the release of liposomes. The vitamin C optimum released from liposome was found at 20% cholesterol for the whole storing temperatures. The empty liposome zeta potential, particle size and polydispersity index were 71.36 mV, 59.27 nm and 0.39 while liposome encapsulated vitamin C were 71.65 mV, 78.40 nm, and 0.39, respectively.

INTRODUCTION

Jack bean (*Canavalia ensiformis* L.) is one of the local plants that have been cultivated in various regions in Indonesia. At the moment, jack bean utilization is still limited as snacks [1]. Research by Hudiyanti et al. found that in dried jack beans containing 0.1% phospholipids [2]. Nowadays phospholipids which are amphiphilic in nature are utilized as drug delivery system in the form of liposomal structures.

Hudiyanti et al. have used the natural phospholipids from sesame (*Sesamun indicum* L.) seeds, coconut (*Cocos nucifera* L.) and candlenut (*Aleurites moluccana*) endosperm for preparing liposomes with the size around 50 nm in diameter [3, 4]. These liposomes have been used to encapsulate vitamin C [5-8], beta-carotene [6, 7] and carboxyfluorecence [4]. Yang et al. [9] also have made liposomes from natural phospholipids, soybean phosphatidylcholine for encapsulation of vitamin C. Encapsulation of vitamin C is needed because vitamin C easily oxidized when dissolved in water [10]. Vitamin C is an essential vitamin that is important as an antioxidant protecting the body from the harmful effects of free radicals and pollution as well as important in the manufacture of collagen [10, 11]. As an antioxidant, vitamin C is easily oxidized when exposed to oxygen during storage. Therefore, it need protection before actually consumed.

Phospholipids from natural sources have been extensively investigated to maximize their potential. Soybean phospholipids have been widely utilized as liposomal materials in pharmaceutical products as well as foods [12]. The prospect of coconut and sesame phospholipids are currently studied and have given promising results [5-8, 13]. Meanwhile, the potential of jack bean phospholipids as active agent carriers have not been investigated.

This report describes a systematic approach to investigate liposomes from jack bean phospholipids as encapsulating agent of vitamin C. We aim to obtain the optimum ratio of jack bean phospholipid to cholesterol in liposome membrane in order to achieve the optimum encapsulation. We also determine their particle size and polydispesity index as well as their spontaneous release during 8 days storage at temperature 5, 25, and 37 °C.

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EXPERIMENTALS

Materials used were jack bean seeds obtained from local grower in Temanggung Indonesia, cholesterol p.a., various reagent grade organic solvents, nitrogen gas, vitamin C in the phosphate buffer solution at pH 7.4, and deionized water. Instruments used were GCMS-QP2010S Shimadzu to determine the acyl chains of jack bean phospholipids after subjected to esterification, Delsa™Nano Particle Analyzer, and UV-Vis spectrophotometer.

Liposome Preparation

A mixture of phospholipids with 0% cholesterol concentration of 100 ppm in chloroform was added to test tube and filled with nitrogen gas to form a thin layer. After that, 74 mL of 100 ppm vitamin C in the phosphate buffer solution pH 7.4 was added to the thin layer followed by freeze-thawing i.e. cooling at 4-6 °C, heating at 50 °C, and stirring with a vortex until thin layer at the bottom of a test tube disappeared. Liposome dispersion was then sonicated at room temperature for 60 minutes until the dispersion became clear. Liposomes with 0% cholesterol was formed and ready for analysis. The above procedure was repeated for a mixture of phospholipids with 10, 20, 30, and 40% cholesterol. Empty liposomes was prepared using phosphate buffer solution pH 7.4. Empty liposomes and liposomes containing vitamin C were subjected to encapsulation efficiency determination, release analysis, micromeritics and zeta potential analysis.

Micromeritics and Zeta Potential Analysis

Micromeritics and zeta potential analysis were performed by Delsa™ Nano Particle Analyzer with Diluent Properties as follows Diluent Name: WATER, Temperature : 25.0 °C, Refractive Index : 1.3332, Viscosity : 0.8878 cP, Dielectric Constant : 78.3 and Cell Condition in this manner Cell Type : Flow Cell, Avg. Electric Field : -9.82 V/cm and Avg. Current : -3.46 mA.

Determination of Vitamin C Encapsulation Efficiency and Release

The procedure of determining the encapsulation efficiency of vitamin C in liposomes was based on the procedures used by Yang et al. [9]. Immediately after formation the liposome dispersions were centrifuged for 30 min at 6000 rpm. One mL supernatant was mixed with 0.3 mL EDTA 0.25 M, 0.5 mL of acetic acid 0.5 M, and 1.25 mL of saline solution of fast blue B (2 g/L). The mixture was diluted with deionized water to 10 mL. The solution absorbance was analyzed using a UV-Vis spectrophotometer to determine the concentration of un-encapsulated vitamin C in the supernatant, C_{res}. The λ_{max} of vitamin C was determined beforehand and a standard curve was established. Encapsulation efficiency of vitamin C, EE_{vitC}, was calculated by equation (1).

$$EE_{vitC} = \left(1 - \frac{C_{res}}{C_i}\right) \times 100\% \qquad (1)$$

C_{res} was the concentration of unencapsulated vitamin C in the supernatant, and C_i was the initial concentration of vitamin C added in preparation. The spontaneous release of vitamin C from liposomes was determined by measuring the C_{res} after 8 days storage at 4, 25, and 37 °C for every liposome compositions.

RESULTS AND DISCUSSION

Acyl Chains Profile of Jack Bean Phospholipids

Acyl chains profile of phospholipids is an important factor since acyl chains are the non-polar part of phospholipids that contribute in self-assembly process during liposome formation. The acyl chains of jack bean phospholipids were determined from the composition and structure of the fatty acid obtained after their esterification and GC-MS analysis. Based on the results, there were four peaks that represent the fatty acyl residues of jack bean phospholipids on Table 1. However there were only two residues with abundance above 10% i.e. C16:0 (hexadecanoic acid, palmitic acid) and C18:1 (9(Z)-octadecenoic acid, oleic acid). These results were consistent

with previous studies by Hudiyanti et al. [2]. These long-chains fatty acid contributed to the liposome membrane thickness, fluidity and permeability.

TABLE 1. The fatty acids component of jack bean phospholipids

Peaks	t_R (min)	Abundance (%)	The fatty acid component	SI
1	37.251	0.43	9-octadecenoic acid	92
2	37.821	22.10	Hexadecanoic acid	97
3	41.404	74.03	9-octadecenoic acid	97
4	41.806	1.70	Octadecanoic acid	97

Micromeritics and Zeta Potential Analysis

Micromeritics analysis was carried out on empty liposomes and liposomes containing vitamin C. Results were presented in Fig. 1, 2 and 3. The zeta potential of both jack bean liposome with and without vitamin C were similar, around 71 mV, indicating that encapsulation of vitamin C did not affect the liposome surface charge. Particle size measurement showed that for empty liposomes, the particles size were around 59 nm while liposomes containing vitamin C were slightly bigger i.e. around 78 nm. These results were comparable to the coconut, sesame and candlenut liposomes [3]. The particle size were affected by encapsulation. The analysis also showed that the particle polydispersity index (PDI) was the same for both liposomes with the value 0.39. The PDI showed that the liposome dispersions were monodispers system. These particle size distributions and polydispersity index (PDI) were very significant physical features to be measured of liposome as drug delivery systems in pharmaceutical-grade products [14, 15]. Determination of the liposome size distribution were important quality control analyses for such products [16].

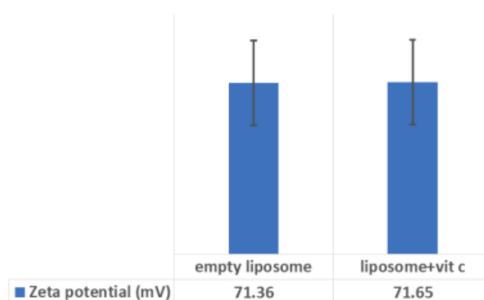

FIGURE 1. Zeta Potential of jack bean liposomes

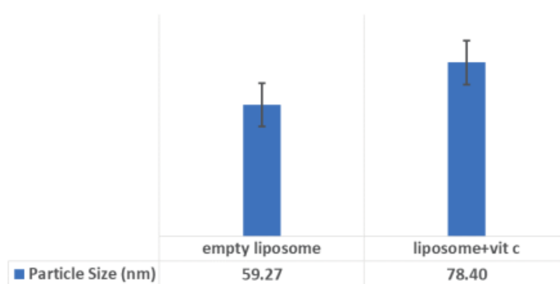

FIGURE 2. Particle size analysis jack bean liposome dispersions

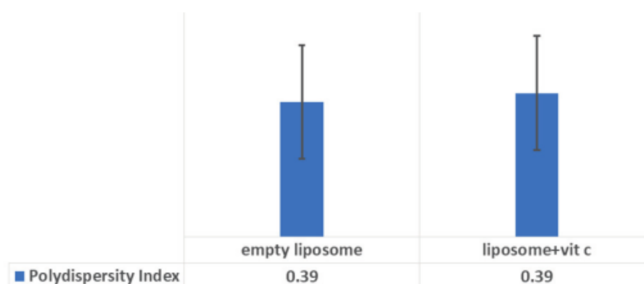

FIGURE 3. Polydispersity Index of jack bean liposome dispersions

Encapsulation Efficiency of Vitamin C

The purpose of vitamin C encapsulation efficiency (EE_{vitC}) analysis was to determine the ability of jack bean liposomes in encapsulating vitamin C. Besides, the cholesterol optimum concentration in the liposome membrane was also determined. Cholesterol modified liposome size, morphology, fluidity and permeability. The liposome spontaneous release also govern by the cholesterol content [17]. The optimum concentration of cholesterol would increase the fluidity and flexibility of the liposomal membrane that would optimize the liposome encapsulation efficiency as well. Based on the analysis, the highest EE_{vitC} value of jack bean liposomes was at cholesterol concentrations 40%. The encapsulation efficiency was upgraded about 13.23% compared to nil cholesterol. The vitamin C encapsulation efficiency of jack bean liposomes was presented in Fig. 4.

The EE_{vitC} value indicated that the presence of cholesterol would increase the liposomes ability to encapsulate vitamin C. The presence of cholesterol molecule in the phospholipid membrane was likely to fill the gap formed by phospholipid molecules. The hydroxyl head group of cholesterol would interact with the phosphate group while the bulky non polar chain of cholesterol would interact strongly to the acyl chains of phospholipid molecules. As a result more tightly bound liposome membrane and hence the increase of EE_{vitC} .

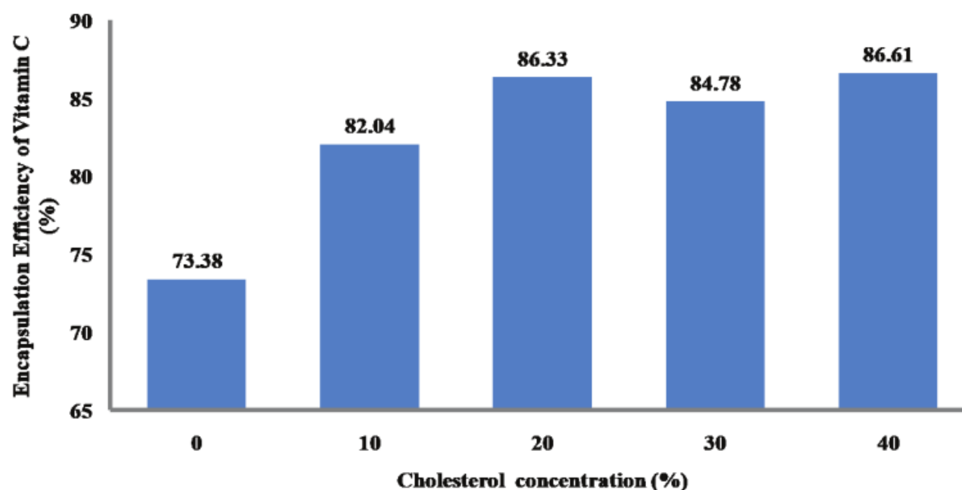

FIGURE 4. Vitamin C encapsulation efficiency of jack bean liposomes

Liposomes Release

Spontaneous release that occurred in jack bean liposomes caused by the membrane pores of liposome so that the vitamin C would diffuse because there was a concentration difference between the outer and core compartment of

liposomes. To determine the vitamin C release, the liposomes were stored for 8 days at a temperature of 5, 25, and 37 °C. Selection of temperature variations were based on the storage condition by consumers that ware at room temperature (25 °C) and refrigerator temperatures (5 °C). It was also adapted to the conditions in the body when consumed was at body temperature (37 °C).

Jack bean liposomes with cholesterol concentrations of 0, 20 and 40% were stored at 5 °C. After 8 days the concentration of free vitamin C (FVC) in the medium dispersion decreased by 1.91, 0.73, and 1.73 ppm, respectively (Fig. 5). The decline was due to the degradation of FVC faster than the release. According to Hudiyanti et al. [4], generally the liposomes at the beginning of the storage would release quickly after a while, and the release would continue at a slower pace. Jack bean liposomes with cholesterol concentrations of 10% and 30% increased concentrations of FVC by 1.91 and 7.30 ppm, respectively (Fig. 5). The increase was assumed due to a release of vitamin C that occurred from liposomes and greater than the degradation processes that occurred in FVC. Therefore, the concentration of FVC on day 8 was higher.

The concentration of FVC of jack bean liposomes with cholesterol concentration 0% and 20% after storage for 8 days at a temperature of 25 °C showed concentrations of FVC decreased by 9.76 ppm and 4.47 ppm, respectively. The concentration of FVC of the jack bean liposomes with the addition of cholesterol 10, 30, and 40% increased by 1.37, 3.92, and 6.67 ppm, respectively (Fig. 6). These results were assumed due to different self-assembly compact structure of cholesterol with phospholipids at different concentration.

At storage temperature of 37 °C, the concentration of FVC to the jack bean liposomes with cholesterol concentrations of 0, 10, 2, and 30% after storage for 8 days decreased by 19.61, 7.58, 1.28 and 1.46 ppm, respectively. The concentration of FVC to the jack bean liposomes with the addition of 40% cholesterol increased by 4.20 ppm (Fig. 7).

Jack bean liposomes that had the highest ability to storage of vitamin C at temperature 5, 25, and 37 °C was jack bean liposomes with 20% cholesterol. It was based on the release analysis. It showed the decline concentration of FVC measured from 0-8 day storage was not significant. This indicated that vitamin C release from the jack bean liposomes with the addition of cholesterol 20% were slower compared to the degradation process. Release data indicated that the addition of cholesterol in the liposome membrane reduced the release of vitamin C from liposomes and increasing the storage capacity of the liposomes for vitamin C. This was because cholesterol molecules were positioned in the gaps between the phospholipid molecules. Cholesterol would interact non-covalently with them, so that the membrane pores of jack bean liposomes were slightly blocked by the cholesterol molecules. Hence, they were still releasing a small amount of vitamin C.

The best storage temperature for encapsulating vitamin C of jack bean liposomes with the addition of cholesterol 20% was the temperature at 5 °C. At low temperatures, the movement of molecules in the liposome membrane and the degradation of vitamin C became slower, so that the liposomes membranes and vitamin C became more stable.

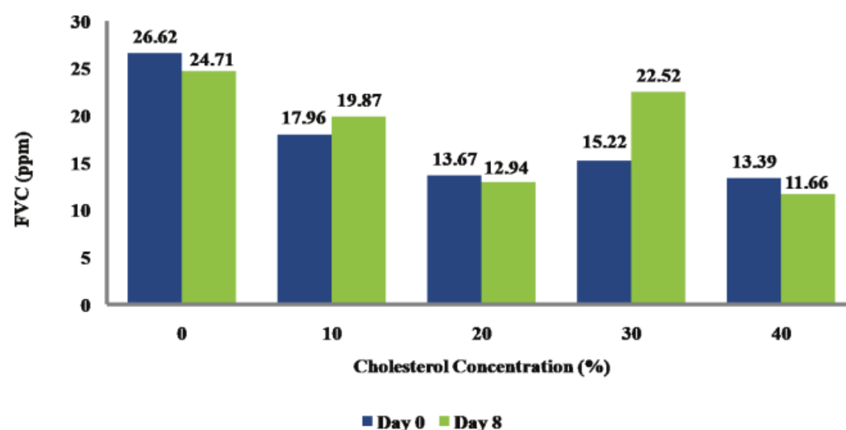

FIGURE 5. Liposomes release at a temperature of 5 °C

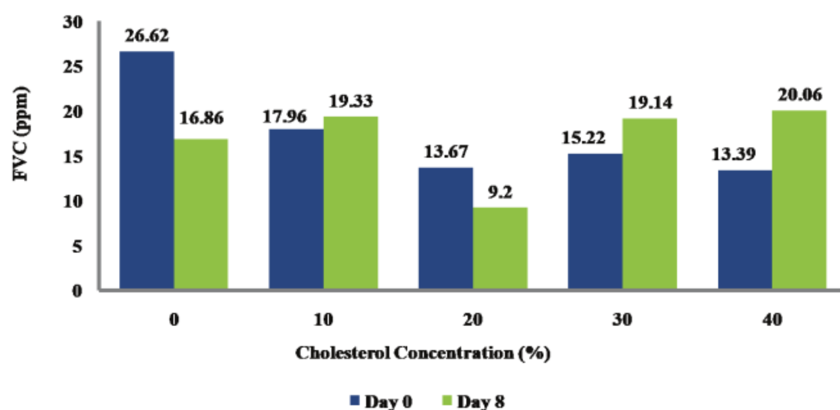

FIGURE 6. Liposomes release at a temperature of 25 °C

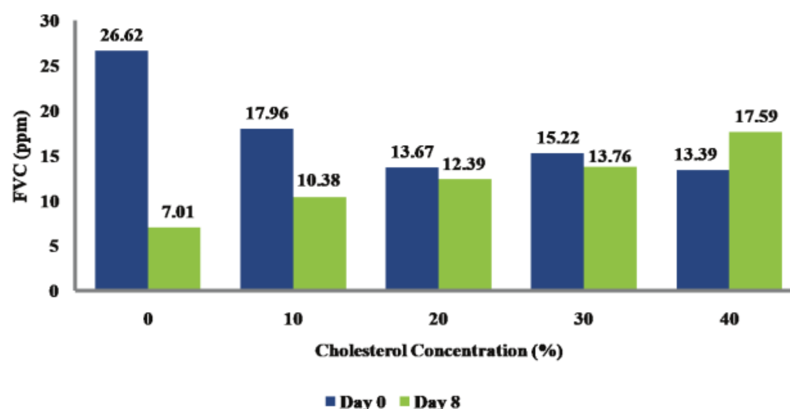

FIGURE 7. Liposomes release at a temperature of 37 °C

CONCLUSIONS

The results showed that the most abundant of acyl chains in the jack bean phospholipids was palmitic acid (C16:0) and oleic acid (C18:1). The addition of cholesterol in the jack bean liposome membrane increased encapsulation efficiency of vitamin C with the highest encapsulation efficiency value 86.61% on the addition of 40% cholesterol. Cholesterol also decreased spontaneous release of jack bean liposomes, in which the lowest release was found in the addition of 20% cholesterol at a temperature of 5 °C after storage for 8 days.

ACKNOWLEDGMENTS

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