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Simultant encapsulation of vitamin C and beta-carotene in sesame (*Sesamum indicum l.)* liposomes

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Abstract. In this study sesame liposomes were used to encapsulate both vitamin C and beta-carotene simultaneously. Liposomes were prepared with addition of cholesterol. The encapsulation efficiency (EE) of sesame liposomes for vitamin C in the present of beta-carotene was 77%. The addition of cholesterol increased the encapsulation efficiency. The highest encapsulation efficiency was 89% obtained in liposomes with 10% and 20% cholesterol. Contrary to that, the highest beta-carotene encapsulation efficiency of 78%, was found in the sesame liposomes prepared without the added cholesterol. Results showed that sesame liposomes can be used to encapsulate beta-carotene and vitamin C simultaneously. When beta-carotene and vitamin C were encapsulated concurrently, cholesterol intensified the efficiency of vitamin C encapsulation on the contrary it diminished the efficiency of beta-carotene encapsulation.

1. Introduction

Ascorbic acid or vitamin C is an antioxidant needed in human bodies [1-5]. Vitamin C serves to reduce the risk of cancer (such as prostate and gastric cancer) and cardiovascular diseases [6-8]. Therefore, vitamin C is an important nutrient that is widely consumed [9]. Not only vitamin C, beta-carotene also acts as an antioxidant which is needed by human bodies [4, 5]. Beta-carotene is a pro vitamin A that prevents vitamin A deficiency [10-12]. Beta-carotene serves to reduce the risk of lung cancer and cardiovascular diseases, and eye deseases [5, 13, 14]. However, vitamin C and beta-carotene have disadvantages which may reduce their benefits. The role of vitamin C may be disrupted due to its high reactivity and its poor stability in solutions. Vitamin C may also easily be oxidized by the presence of oxygen, which is catalyzed by transition metal ions such as iron and copper [15]. Beta-carotene is a highly hydrophobic compound with high melting point, low chemical stability, and low bioavailability [16, 17]. These disadvantages can be overcome by the encapsulating vitamin C and beta-carotene in liposomes. Liposome is able to protect vitamin C from being oxidized and increases its stability [18, 19]. Liposome may increase the bioavailability and the stability of beta-carotene [20].

Liposome is a lipid vesicle which contains hydrophilic core and hydrophobic bilayer membrane [21, 22]. Its biocompatible structure and characteristics make it possible to be utilized as a drug delivery system [23-25]. Liposomes can encapsulate hydrophobic compounds inside its bilayer membrane and hydrophilic compound inside its core [21]. Some evidences have demonstrated that liposome is able to encapsulate beta-carotene and vitamin C singly [18, 19, 26]. Liposome is mostly built up by amphiphilic molecules such as phospholipids [27]. Phospholipid can be isolated from natural materials, like egg, soybean, coconut, and sesame seeds [28-30]. Phospholipid will create double layers similar to biologic membrane [31, 32]. The stability of liposomes can be optimized by adding cholesterol [33, 34]. Cholesterol is able to reduce the permeability of the membrane and rigidify the fluidity of the intravesical interaction [35, 36].

This research was conducted to investigate the capability of the sesame liposomes in encapsulating both beta-carotene and vitamin C simultaneously. The liposomes analysis was done by calculating the value of encapsulation efficiency (EE) and analyzing the influence of cholesterol towards the encapsulation efficiency value. Our new finding was that sesame liposomes can be used to encapsulate both beta-carotene and vitamin C simultaneously. When beta-carotene and vitamin C were encapsulated concurrently, cholesterol amplified the efficiency of vitamin C encapsulation but cut down the efficiency of beta-carotene encapsulation.

2. Materials and method

2.1. Materials

The materials used in this research were isolated sesame phospholid (Sesamum Indieum L.), chloroform p.a (Merck No.Batch 1.02445.2500), ethanol 96% (Brataco), cholesterol (Sigma Aldrich No. 11145-50G), vitamin C uncoated (Brataco No. Batch 1140870083), beta-carotene (Sigma No. C4582-25MG), soy phospholipid (Sigma Aldrich No. P3644-100G), $Na_2HPO_4.2H_2O$ (Merck No. Batch 2121439), $NaH_2PO_4.2H_2O$ (Merck No.Batch 1.06342.0250), demineralized water (Brataco).

2.2. Encapsulation beta-caroten and vitamin C in liposomes

The preparation of liposomes was done according to the method by Hudiyanti et al. (2015) with the addition of cholesterol 0%, 10%, 20%, 30%, 40% (w/w). Before creating the liposomes, the partition coefficient of vitamin C and beta-carotene was determined using MarvinSketch program. It was done by making thin layer, hydration, and ultrasonication. Beta-carotene was added to phospholipid with ratio 20% (w/w). Cholesterol was added to the mixture with ratio as above. The mixture was dissolved in chloroform to prepare thin layer. Vitamin C in phosphate buffer solution was added in the hydration process. Liposome's capability in encapsulating beta-carotene and vitamin C, and the influence of cholesterol towards its efficiency can be calculated by encapsulation efficiency value. The solution was centrifuged in order to separate the encapsulated molecules from those which was not encapsulated inside the liposomes. The supernatant which contained unencapsulated molecules (C_t) was analyzed by using spectrometer UV-Vis at the wavelength of vitamin C (265 nm) and beta-carotene (453 nm). The encapsulation efficiency of the vitamin C and beta-carotene was determined by equation 1.

$$EE = [1 - (C_t / C_0)] \times 100\% \quad (1)$$

3. Result and discussion

3.1. The encapsulation efficiency of vitamin c

The encapsulation efficiency value of vitamin C in the sesame liposomes was 88%. Cholesterol reduced the encapsulation efficiency of vitamin C as shown in the Figure 1. This reduction maybe caused by the sludge resulted from centrifugation. During the centrifugation process, the particles inside the tube were separated and spread according to the specific weight of each particle by centrifugal force [37]. This sludge was unstable and could not be dispersed back to the supernatant. It affected the absorbance of vitamin C at 265 nm wavelength. The measured absorbance becomes higher hence the unencapsulated vitamin C concentration increased. As a result the encapsulation efficiency values were reduced.

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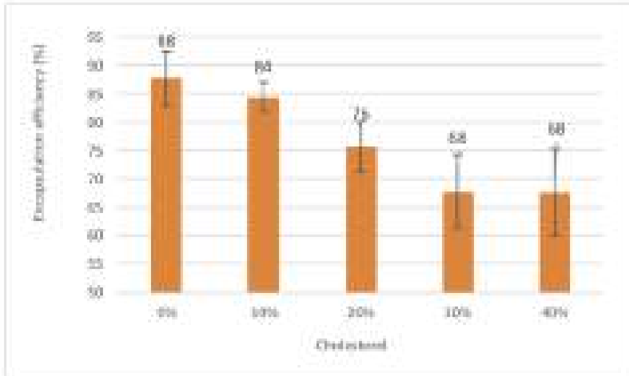

Figure 1. The encapsulation efficiency value of vitamin C in sesame liposomes.

The addition of 20% beta-carotene lowered the encapsulation efficiency value of the vitamin C inside the sesame liposomes to 77%. It was due to the big structure of beta-carotene with several double bonds. Beta-carotene interacted with sesame phospholipid which also contains many double bonds and created liposomes in the big shapes. As a result, there were less liposomes particles and encapsulated vitamin C. Cholesterol also unexpectedly increased the encapsulation efficiency of the vitamin C inside the sesame liposomes which contains beta-carotene as shown in Figure 2. Phospholipid which has been mixed by cholesterol has more well-ordered membrane. This regularity reduced the permeability of the membrane and rigidified the fluidity of the intravesical interaction, made it more effective in encapsulating a molecule [33].

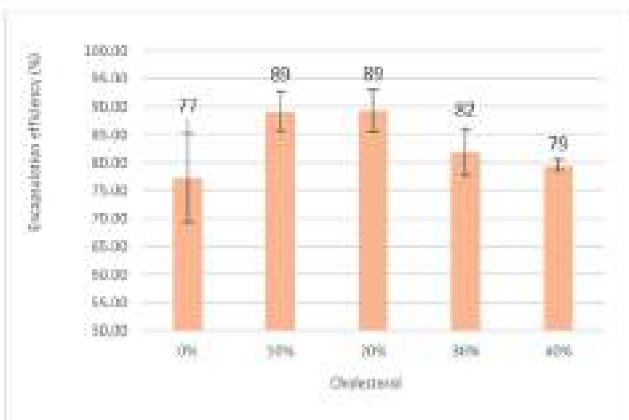

Figure 2. The encapsulation efficiency of vitamin C in sesame liposomes which contains beta-carotene

Figure 3. Sesame liposomes solution after centrifugation. The yellow solution represents sesame liposomes containing beta-carotene and the white one was sesame liposomes solution which do not containing beta-carotene.

The effect of cholesterol towards the encapsulation efficiency of vitamin C in the sesame liposomes which contains beta-carotene was contradictory with the sesame liposomes with out beta-carotene. It could happen because beta-carotene could increase the specific weight of the sediment after centrifugation process, so the sediment became more stable (Figure 3).

The encapsulation efficiency value of vitamin C inside non cholesterol soy liposomes without and with beta-carotene was 78% and 84% respectively. Cholesterol increased the encapsulation efficiency value of the soy liposomes with or without beta-carotene as seen in Figure 4.

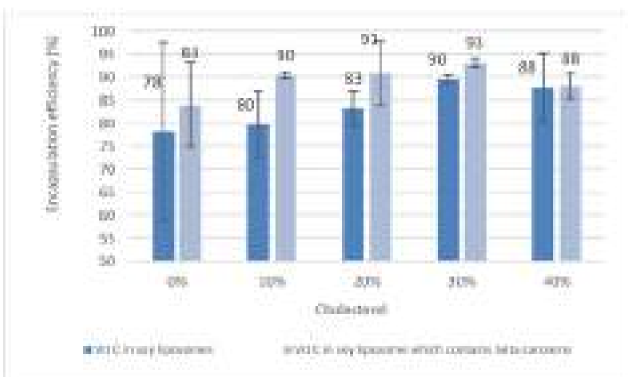

Figure 4. The encapsulation efficiency of vitamin C in soy liposomes.

The encapsulation efficiency of vitamin C inside sesame and soy liposomes with beta-carotene has increased compared to those without beta-carotene. The partition coefficient of beta-carotene which was 11,12 suggested that this compound would resided inside liposomes membrane. Meanwhile vitamin C with partition coefficient -1,91 would be in the hydrophilic part or liposomes core. Beta-carotene can

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alter membrane microviscosity, hydrophobicity, membrane permeability, and also protect phospholipid from being oxidized [38].

3.2. The encapsulation efficiency of beta-carotene

The encapsulation efficiency of beta-carotene inside sesame liposomes was 79%. The addition of cholesterol reduced the encapsulation efficiency of beta-carotene inside sesame liposomes as shown in Figure 5. This reduction was caused by the high competition between cholesterol and beta-carotene to be in the liposomes membrane [38]. It would decreased the concentration of beta-carotene inside the sesame liposomes membrane. The position of beta-carotene and cholesterol in the sesame liposomes membrane was predicted as shown as in Figure 6. Although both cholesterol and beta-carotene competed to be in the bilayer membrane, cholesterol could easily adapt compared to beta-carotene, due to its tiny shape and amphiphilic characteristic like phospholipid [38, 39].

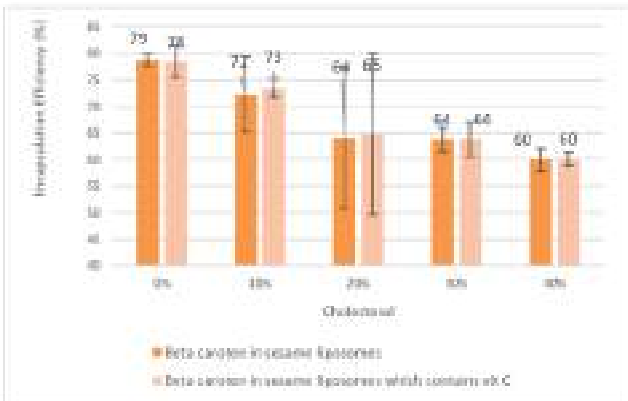

Figure 5. The encapsulation efficiency of beta-carotene in sesame liposomes.

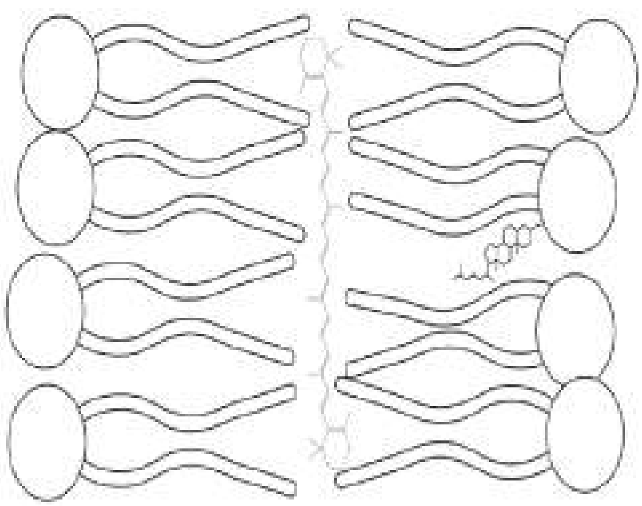

Figure 6. Prediction of position of beta-carotene and cholesterol in sesame liposomes membrane.

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The results also showed that the encapsulation efficiency value of beta-carotene in sesame liposomes with or without loaded vitamin C was similar (Figure 5). It can be concluded that vitamin C did not affect the encapsulation efficiency value of beta-carotene.

Cholesterol showed the same effect on soy liposomes and sesame liposomes encapsulation efficiency of beta-carotene. Cholesterol reduced the encapsulation efficiency value of beta-carotene as depicted in Figure 7. These results can be explained by the similar lipophilic part of the phospholipids [30]. The decrease in beta-carotene encapsulation efficiency is possibly due to competition between cholesterol and beta-carotene to reside in the liposomal bilayer membrane.

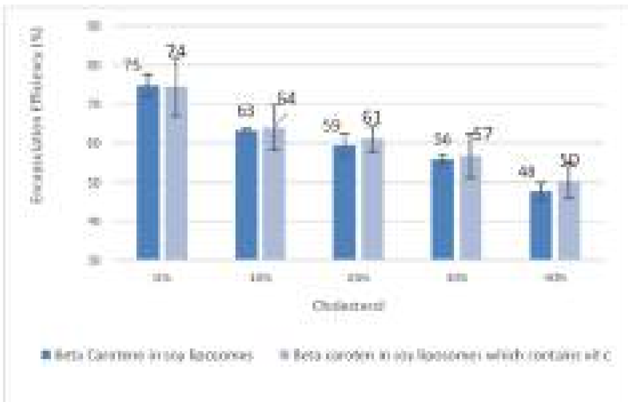

Figure 7. The encapsulation efficiency of beta-carotene in soy liposomes

4. Conclusion

The encapsulation efficiency of sesame liposomes for vitamin C in the present of beta-carotene was 77%. Addition of cholesterol increased the encapsulation efficiency. The highest was 89% obtained in liposomes with 10% and 20% cholesterol. On the contrary, the highest beta-carotene encapsulation efficiency, i.e. 78%, was found in the sesame liposomes prepared without cholesterol. Sesame liposomes can be used to encapsulate both beta-carotene and vitamin C simultaneously. When beta-carotene and vitamin C were encapsulated concurrently, cholesterol increased the efficiency of vitamin C encapsulation but lowered the efficiency of beta-carotene encapsulation.

Acknowledgments

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