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Physicochemical properties and antioxidant activity of α-tocopherol loaded nanoliposome's containing DHA and EPA

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Dipalmitoyl phosphatidylcholine (PubChem CID: 160339)
Propyl paraben (PubChem CID: 7175)
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ABSTRACT

The aim of this study was to prepare α-tocopherol loaded nanoliposomes as carriers of DHA and EPA and to investigate their physicochemical properties, such as peroxide value (PV), volatile compounds (VOCs), particle size, size distribution, zeta potential and morphology of the liposomes. The particle size of liposomes was in the range of 82.4–107.2 nm. The highest extent of lipid oxidation was observed at 40 °C for 90 days, with the lowest PV and propanal levels for a nanoliposome formulation in comparison with the control sample. The zeta potential of the nanoliposomes was decreased during storage. No significant change in the PV and zeta potential of the liposome formulations with α-tocopherol was observed at 4 °C after 90 days (0.14 meq/kg and −43.5 mV, respectively). This study demonstrated that incorporation of α-tocopherol into liposomes contributes a significant antioxidant effect on DHA and EPA.

1. Introduction

The beneficial role of bioactive lipids in health has been investigated since the early 1950s (Moghadasian & Eskin, 2012). Epidemiological evidence of associations between omega-3 fatty acids and reduced cardiovascular risk has provided the rationale for many experimental studies conducted in this area. The relationships between docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and α-linolenic acid and improved blood lipids and endothelial function is gaining increased attention (Grienke, Silke, & Tasdemir, 2014; Moghadasian & Eskin, 2012). EPA and DHA are highly susceptible to lipid oxidation. The autoxidation of unsaturated fatty acids is a chain process that occurs auto-catalytically

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through free radical intermediates that are related to the number of bis-allylic positions in the fatty acids (Arab-Tehrany et al., 2012). Autoxidation implies that this reaction occurs spontaneously under mild conditions, and it generally is initiated by promoter-induced oxidants in food and biological lipid systems. The oxidation of lipids proceeds like that of many other organic compounds, i.e., by a free radical chain mechanism, which can be described in terms of initiation, propagation, and termination processes. These processes often consist of a complex series of sequential and overlapping reactions (Frankel, 2014). The autoxidation of DHA and EPA generates eight and 10 hydroperoxide isomers. The secondary oxidation products obtained via the subsequent oxidation of the initial products of polyunsaturated fatty acids, are potential sources of VOCs, such as hydrocarbons, acids, alcohols, aldehydes, and ketones, via further degradation (Frankel, 2014).

Studies have indicated that the physicochemical instability of omega-3 fatty acids limits their applications (Kaushik, Dowling, Barrow, & Adhikari, 2014). DHA and EPA are more prone to oxidation, several strategies can be used to protect them from oxidative degradation.

Oxidation of omega-3 fatty acids can be overcome by antioxidant compounds and using different encapsulation techniques (Jacobsen, Nielsen, Horn, & Sørensen, 2013). Thus, α-tocopherol may be incorporated into formulations in order to control their oxidative degradation, Nacka, Cansell, Méléard, & Combe, 2001).

Extensive research has indicated that membrane phospholipids have positive effects on marine lipids (Lyberg, Fasoli, & Adlercreutz, 2005). Other findings have indicated that the encapsulation of lipophilic substances into a liposome would affect its bilayer nature (Nara, Miyashita, Ota, & Nadachi, 1998). Encapsulation of omega-3 polyunsaturated fatty acids (PUFAs) might be used to prolong the shelf life of products (Kaushik et al., 2014).

The primary purpose of encapsulation is to stabilize the internally-trapped omega-3 PUFAs oil against oxidative degradation. The physical barrier is important, because it can prevent contact with oxygen, transition metal ions, and light. Several studies have indicated that liposomes can act as useful carriers for incorporating lipophilic and hydrophilic functional molecules (Torchilin & Weissig, 2003). Furthermore, the bioavailability of encapsulated bioactive components in nanoliposomes is higher than that in conventional liposomes (Tiwari & Takhistov, 2012). Liposomes have been studied extensively as delivery systems for the improvement of the physical and chemical stabilization of omega-3 fatty acids during processing, storage, and transit in the gastrointestinal tract (Nacka et al., 2001). Previous studies have confirmed that antioxidants and phospholipids have a synergistic effect on the stability of omega-3 fatty acids (Onuki, Morishita, Chiba, Tokiwa, & Takayama, 2006). Different components were used for the fabrication of stable liposome formulations. α-Tocopherol and its derivatives are important due to their physicochemical properties, including biocompatibility, improved solubility of hydrophobic compounds, increased encapsulation efficiency, improved properties of bio-pharmaceuticals, and existing FDA approval (Duhem, Danhier, & Préat, 2014). Liposomes formulation also can comprise other components that may be chosen depending on the carrier and/or the intended use of the formulation. Additional components are not limited to antioxidants; chelating agents and preservatives. Various preservatives are used in a wide variety of cosmetics, as well as foods and drugs, to extend shelf life inexpensively. Methyl and propyl paraben are recognized as safe preservatives under FDA regulations and are used in drugs, foods and cosmetics for their anti-microbial effect and their relatively low toxicity in humans (Soni, Carabin, & Burdock, 2005).

Because of the potential benefits of liposome technology for improving the nutritional value of active ingredients in foods, it is important to continue to study liposome behavior during processing and storage (Garti & McClements, 2012). Following our previous research, nanoliposomes had a positive effect on the encapsulation of DHA and EPA; the aims of this study were to measure both PV and volatile aldehyde compounds and to monitor lipid oxidation, particle size, size distribution (PDI), zeta potential and morphology of vesicular bilayers. Hence, we studied and compared these characteristics of four liposome formulations in addition to control samples during storage.

2. Experimental

2.1. Materials

The D-Lα-tocopherol, DHA oil (⩾96% purity) and EPA oil (⩾96% purity), phosphate buffer solution, and Sephadex G-50 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dipalmitoylpho sphatidylcholine (DPPC) was acquired from Lipoid (⩾95% purity, Ludwigshafen, Germany). Polycarbonate filter paper was supplied by Millipore Company (Maidstone, UK). All other chemicals, including methanol, chloroform, methyl/propyl paraben, ethanol, 12% boron trifluoride, propanal, pentanal, hexanal, heptanal, n-dodecane (99% purity), pentadecanoic acid, 2,5-dimethyl furan, and all solvents were of analytical grade (Merck, Darmstadt, Germany). Palmito-olein oil was obtained from Behshahr industrial group in Iran.

2.2. Preparation of nanoliposomes

Liposomes were prepared in two steps according to the film hydration method as previously described by Kudsiova, Arafiena, and Lawrence (2008). Table 1 shows the list of four nanoliposome formulations (LOE, LOEP, LO, and LOP). Briefly, 257 mg of DPPC was dissolved in 10 mL of chloroform/methanol as the solvent (2:1 v/v). Then, α-tocopherol at a 5 mol% was added to the mixture in a round flask (pH = 7.4). The solvents were evaporated in a rotary evaporator at 50 °C and dry lipid sediment remained on the wall of the flask. Hydration of the lipids was carried out by adding 10 mL of distilled water; a rotary evaporator was used to form multi lamellar vesicles (MLVs). The MLVs were subjected to probe-sonication (200 W, 20 kHz, Hielscher UP200H, Germany) at 30% sonication strength for 10 min in an ice bath. Titanium particles in the lipid suspension were removed by centrifugation at 13,000 rpm (Heraeus PIC6 Biofuge) for 10 min. Next, DHA and EPA (3.02 mg mL^{-1} and 1.64 mg mL^{-1}, respectively) were added to the liposomes suspension and incubated at 42 °C for 2 h. Then, 1 mL of sample was taken and free DPPC was separated from liposome associated DHA-EPA-lipids by size exclusion chromatography using a Sephadex G-50 column. The fraction containing liposomes was collected in the first 10 mL by washing the column with phosphate buffer (50 mM). Thus, SUV liposomes loaded with DHA and EPA with a molar ratio of 70:20:10 (DPPC: DHA: EPA) were prepared. Total lipid concentration after adding free omega-3 fatty acids into liposomes was determined in a range of

Table 1
Nanoliposome composition.

Component	Formulation code			
	LOE	LOEP	LO	LOP
Ratios (DPPC:DHA:EPA)[a]	70:10:20	70:10:20	70:10:20	70:10:20
α-Tocopherol[b]	5	5	0	0
Methyl paraben[c]	0	0.2	0	0.2
Ethyl paraben[c]	0	0.02	0	0.02

[a] wt/wt.
[b] Mol% lipid weight.
[c] % w/v.