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Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt

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ABSTRACT

Fish oils have many dietary benefits, but due to their strong odors and rapid deterioration, their application in food formulations is limited. For these reasons, nano-liposome was used to nano-encapsulate fish oil in this study and encapsulated fish oil was utilized in fortifying yogurt. Physicochemical properties of produced yogurt including pH, acidity, syneresis, fatty acid composition, peroxide value as well as sensory tests were investigated during three weeks storage at 4 °C. Nano-liposome encapsulation resulted in a significant reduction in acidity, syneresis and peroxide value. The results of gas chromatography analyses revealed that after 21 days storage, yogurt fortified with nano-encapsulated fish oil had a higher DHA and EPA contents than yogurt containing free fish oil. Overall, the results of this study indicates that adding nano-encapsulated fish oil into yogurt gave closer characteristics to control sample in terms of sensory characteristics than yogurt fortified with free fish oil.

1. Introduction

There is evidence that poly unsaturated fatty acids (PUFAs) have beneficial effects on health, including prevention of cardiovascular diseases, decrease the risk of some types of cancer and autoimmune disorders, proper development and function of the brain and retina and prevention and treatment of many diseases. Thus, an adequate intake of omega PUFA is important. Fish and other sea animals are the richest sources of PUFA in human diet. Omega-3 fatty acids are long chain polyunsaturated fats containing methylene-separated double bonds starting from the third carbon atom counted from the methyl-terminus. The quantitatively most important long-chain n-3 PUFA in the diet are *cis*-5,8,11,15,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) (Kolanowski, Ziolkowski, Weißbrodt, Kunz, & Laufenberg, 2006; Ruxton, Reed, Simpson, & Millington, 2004; Siddiqui et al., 2004).

Recent evidence shows that the intake of EPA plus DHA is negatively related to cardiovascular risk in a dose-dependent way up to about 250 mg/d (1–2 servings of oily fish per week) in healthy populations (European Food Safety Authority, 2009). The proposed labeling reference intake value for long chain n-3 PUFA (200 mg) is lower than this value, as are observed average intakes of EPA plus DHA in adults in some European countries, which vary between 80 mg/d and 420 mg/d. The European Food Safety Authority proposes 250 mg/d as the labeling reference intake value for the long-chain n-3 PUFAs EPA plus DHA, which is in agreement with most recent evidence on the relationship between the intake of these fatty acids and cardiovascular health in healthy populations.

Due to low consumption of fish in many societies, supplementation of the diet with fish oil capsules seems to be the easiest way to elevate the level of omega-3 LC PUFA intake (Bender et al., 2014; Kolanowski, 2010). The increased interest of consumers in fortified foods, many containing micronutrients such as omega-3, has been significant (Siro, Kapolna, Kapolna, & Lugasi, 2008). Nevertheless, the challenge in producing fortified foods has been tremendous. The main challenge in producing these foods is related to the stability and undesirable flavors of fish oil. Using highly refined and odorless or microencapsulated fish oil may be an alternative way to mask undesirable sensory characteristics and thus protect the oil during processing (Iafelice et al., 2008).

Encapsulation is a unique way to package materials in the form of micro- and nano-particles and is defined as a process to entrap one substance (active agent) within another (wall material) (Jafari, Assadpoor, He, & Bhandari, 2008; Mahdavi, Jafari, Ghorbani, & Assadpoor, 2014). In the food industry, it involves the incorporation of ingredients such as polyphenols, volatile additives, colors, enzymes and bacteria in small capsules to stabilize, protect and preserve them against processing, nutritional and health losses (Zuidam & Shimoni, 2010). There are a lot of encapsulation techniques among them liposome encapsulation was used in this research.

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Nano-liposome technology is one of the most recent nano-encapsulation techniques (Munin & Edwards-Lévy, 2011). Liposomes (the term refers to artificially constructed capsules of phospholipid bilayers) are spherical particles with sizes in the nanometer to micrometer range. The vesicular particles may consist of one or more bilayer membranes. Liposomes have been widely used in food sectors both in research and industry; it has become feasible to use liposomes to deliver functional components such as nutraceuticals, antimicrobials, and flavors to foods because of having a number of benefits, e.g. possibility of large-scale production using natural ingredients and entrapment and release of water-soluble, lipid-soluble, and amphiphilic materials as well as targetability (Cui, Li, Li, Vittayapadung, & Lin, 2016; Lin et al., 2015; Mozafari et al., 2008). Different procedures have been developed to produce nano-sized liposomes; from thermal methods to non-thermal ones (Mozafari et al., 2008; Mozafari, Reed, Rostron, Kocum, & Piskin, 2002).

Current encapsulation technologies of fish oils are commonly based on spray-drying with the disadvantage that the high temperatures used during the drying process accelerates the oxidation of oils (Jafari, Assadpoor, Bhandari, & He, 2008; Kagami et al., 2003; Kolanowski et al., 2006; Pourashouri et al., 2014a,b). Moreover, there are various types of encapsulated fish oil structures which have been reviewed by Beindorff and Zuidam (2010), but still some challenges of fish oil encapsulation have not been solved; so more recent techniques can be applied to defeat these drawbacks. One of the solutions could be nano-encapsulation. Hence, the objective of this study was to evaluate the incorporation of fish oil nano-encapsulated by nano-liposomes into yogurt and evaluating their effect on the physicochemical and sensory quality of yogurt samples.

2. Materials and methods

Soy lecithin was purchased from Merck Company (Germany). Purified fish oil was obtained from Jiangyin Shuji International Trade Co., China, and sunflower oil (Nina, Iran) acquired at a local market. All other chemicals used in this study were of analytical grade and purchased from chemical suppliers.

2.1. Preparation of nano-liposomes

Nano-liposomes were prepared according to the modified method of Rasti et al. (Rasti, Jinap, Mozafari, & Yazid, 2012). Briefly, ingredients of the liposomal formulation (lecithin, sunflower oil) were mixed in a heating bath (IKA®HB4, USA) at 30 °C to ensure complete dissolving of lecithin in oil. Then, fish oil (preheated to 30 °C) was added drop wise into the lecithin-oil mixture while stirring at 1000 rpm (Hydolph, Germany) on a hotplate. Finally, this solution was hydrated by adding deionized water and glycerol (final concentration 2%, v/v and preheated to 30 °C) and homogenizing for 10 min by a rotor-stator homogenizer (Basic B50, IKA, Germany). The liposomal dispersions were subjected to sonication (7 min; 1 s on and 1 s off) at 25 °C using a probe sonicator (200 UPS, Dr. Heischler, Germany), and a nominal frequency of 20 kHz at 80% of full power before annealing. Final nano-liposomes were kept at 25 °C (ambient temperature) under nitrogen for at least 1 h after preparation to anneal and stabilize them.

2.2. Centrifugal stability measurement

Nano-liposome stability (NS), was determined by centrifugation 5 ml of nano-liposomes at 3500 rpm for 15 min. NS was calculated as:

$$NS = \frac{f_{eV}}{i_{eV}} \times 100 \quad (1)$$

where f_{ev} is the final volume and i_{ev} is the initial volume of liposomal dispersion (Sciarini, Maldonado, Ribotta, Pérez, & León, 2009).

2.3. Particle size and size distribution

Average particle size (PS), and size distribution (polydispersity index; PDI) of nano-liposome preparations were measured by dynamic light scattering (Nano ZS90, Malvern Instruments, Worcester, UK) technique at 25 °C, using a He-Ne laser of 633 nm and a detector angle of 173 °C. Three independent measurements were performed for each sample. The Malvern measures the time-dependent fluctuations of light scattered by the liposomes and uses it to calculate the average size and polydispersity of the liposomes. Samples were analyzed 24 h after preparation. Nano-liposomes were appropriately diluted with the aqueous phase of the formulations prior to the measurements. The particle size values given are averages of three measurements and are expressed as mean. An optical microscope (phase contrast) was used to confirm nano-liposome sizes.

2.4. Nano-encapsulation efficiency

In order to evaluate the effectiveness of nano-encapsulation, first the nano-liposome dispersions were centrifuged at 4200×*g* for 15 min (Hettich Lab Technology, Germany) to leave only the non-encapsulated active compound (fish oil). After phase separation, 1 ml of supernatant was collected. Then 5 mL chloroform was added and the samples were extracted for 5 min and filtered through 0.45-μm-sized Millipore membrane and left for 24 h after being well mixed to allow enough time for all entrapped active compound to be in the solution (Viriyaroj et al., 2009). The absorbance at 280 nm was measured by a spectrophotometer (PG-instrument-Ltd, UK). Similarly, the fish oil within the bottom layer of liposomal structures was extracted and its content was determined as "encapsulated fish oil". Encapsulation efficiency (%EE) was calculated according to Eq. (2) (Gomes, Moreira, & Castell-Perez, 2011; Hill, Gomes, & Taylor, 2013).

$$\%EE = \frac{(\text{Total fish oil within nanoliposomal disperions}) - (\text{non-encapsulated fish oil})}{\text{Total fish oil content}} \times 100 \quad (2)$$

2.5. Yogurt preparation

Yogurt formulations were made by adding 15 mL nano-liposomal emulsions into 100 g yogurt samples separately (8.2% fat, Pegah Dairy Company, Gorgan, Iran) and stored in glass containers in a refrigerator at 4 °C for further analysis. Physicochemical properties and sensory evaluations were studied over 21 days at 4 °C (weekly).

2.6. Physicochemical properties of yogurt samples

Titrable acidity and pH were measured according to the AOAC official method 942.15 (AOAC, 2000). Titrable acidity was