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Preparation and characterization of novel nanocarriers containing krill oil for food application

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ABSTRACT

The encapsulation of krill oil high in docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and astaxanthin as a multifunctional dietary supplement for the purpose of enhancing its physical and chemical stability and to expand its application in aqueous-based food was attempted. Nanostructured lipid carriers (NLCs) containing high content of krill oil were successfully prepared using palm stearin as a solid lipid and lecithin as a surfactant. The results demonstrated that the developed NLC had spherical or ovoid structure with small size (<150 nm), narrow polydispersity index (<0.2) and high entrapment efficiency (>96%). DSC (differential scanning calorimetry) result showed a less-ordered crystalline structure leading to high loading capacity. NLC was found to offer bioactives in krill oil significant protection against photooxidation upon exposure to UV light. Good physical and chemical stabilities were revealed by long-term storage at different temperatures. Feasibilities of pasteurization and lyophilization were also demonstrated, showing promise for application in functional drinks and milk powder fortification.

1. Introduction

Krill oil has recently emerged as a new dietary supplement abundant in long chain omega-3 polyunsaturated fatty acids. Essential omega-3 fatty acids, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) account for over 30% of the total fatty acids in Antarctic krill oil (Kolakowska, Kolakowski, & Szczygielski, 1994). Unlike fish oil, approximately 30–65% of the fatty acids in krill oil are in the phospholipid form, providing krill oil with a proposed better bioavailability (Schuchardt et al., 2011; Ulven et al., 2011). Krill oil also possesses 48 times higher antioxidant potency than fish oil on the basis of ORAC (oxygen radical absorption capacity) values as a result of containing various kinds of powerful antioxidants, such as vitamins E, A and D, astaxanthin, and canthaxanthin (Farooqui, 2009). A number of animal and human studies have suggested that krill oil has a variety of biological functions, including positive effects on cardiovascular disease, non-alcoholic fatty liver disease, metabolic syndrome, premenstrual syndrome, endocannabinoids, inflammation, colon cancer and attention deficit hyperactivity disorder (Batetta et al., 2009; Bunea, El Farrah, & Deutsch, 2004; Deutsch, 2007; Ierna, Kerr, Scales, Berge, & Griinari, 2010;

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Sampalis et al., 2003; Vigerust et al., 2013; Zhu, Shi, Qian, Cai, & Li, 2008). It is possible that the proposed benefits of krill oil might arise from the synergism between EPA, DHA, phospholipids, astaxanthin and other bioactive constituents like vitamins and flavonoids. However, the poor solubility of krill oil has limited its application in food, especially in aqueous-based foods. On the market, krill oil is sold to consumers in the capsule form. Furthermore, due to the high unsaturation of EPA, DHA and astaxanthin, they are prone to degradation and exert an undesirable effect on sensory acceptance of enriched foodstuffs when exposed to oxygen, heat, light and metal ions. Seafood-derived products fortified with krill oil are more susceptible to oxidation than those enriched with other n-3 rich oils (Pietrowski, Tahergorabi, Matak, Tou, & Jaczynski, 2011). Consequently, development of a delivery system which enhances its solubility in water and minimizes oxidation is considered desirable for future use of krill oil in foods.

Nanostructured lipid carriers (NLCs) derived from oil/water (O/W) nanoemulsions have great potential to serve as a carrier system for bioactive compounds of foods (Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013). Possessing small size, high entrapment efficiency and the potential of mass production, has made it very promising to the food industry (Fathi, Mozafari, & Mohebbi, 2012). NLC consists of solid lipid, liquid lipid, surfactant and water as major ingredients, thus both solid and liquid lipids exist in the lipid phase of NLC at room temperature. Müller, Radtke, and Wissing (2002a, 2002b) developed partly crystallized lipid droplets which constituted a less-ordered crystalline structure for NLC, in order to get over the defects of solid lipid nanoparticles(SLN) in the late 1990s. Since the liquid oil is incorporated into the centre of the solid lipid in NLC, the bioactives dissolved in the liquid oil are simultaneously entrapped in the solid lipid, which results in a higher drug loading and controlled drug release (Varshosaz, Eskandari, & Tabakhian, 2010). NLC were initially intended for pharmaceutical and cosmetic applications. Nonetheless, they may improve bioavailability and nutritional value of bioactives, and increase their functionality (consumer acceptability, shelflife, stability and safety of foods), and offer controlled release of the entrapped nutrients. In recent years, NLC loaded with omega-3 polyunsaturated fatty acids of fish oil and algal oil have been successfully prepared (Averina, Müller, Popov, & Radnaeva, 2011; Lacatusu et al., 2013; Wang et al., 2014) while little attention has been drawn to krill oil.

When dispersed in an aqueous medium, hydrophobic bioactive compounds require stabilization and protection against adverse factors (Zimet, Rosenberg, & Livney, 2011). It has been shown that entrapment of n-3 unsaturated fatty acids for food fortification dramatically reduced its oxidation (Garg, Wood, Singh, & Moughan, 2006; Tamjidi, Nasirpour, & Shahedi, 2012). The challenges to use of carotenoids as nutrition enhancers are their poor solubility in water, low bioavailability, high melting point and chemical instability (Qian, Decker, Xiao, & McClements, 2012). For these reasons, we focussed on the astaxanthin in krill oil.

The objective of this research was to firstly study the suitability and the effectiveness of NLC as a delivery system to encapsulate krill oil and secondly to investigate the chemical and physical stability of the prepared NLC.

2. Materials and methods

2.1. Materials

Antarctic krill oil (about 14.8% DHA, 22.5% EPA and 250 mg/kg astaxanthin) was purchased from Shandong Keruier Biotechnology Co., Ltd (Shandong, China). This krill oil was obtained by solvent extraction from fresh Antarctic krill (Euphausia superba) and then underwent multistage purification. The lipid classes of this krill oil are predominantly phospholipids (50%) carrying more than 95% of total omega-3 fatty acids, polar non-phospholipids (29%) and triacylglycerols (21%) and the composition of astaxanthin was free astaxanthin (15%), astaxanthin monoesters (28%) and astaxanthin diesters (57%). Palm stearin (melting point 58.6 °C, iodine value <17) was purchased from Guangdong Jinzhi Co., Ltd (Guangdong, China). DHA methyl ester (≥98%, capillary GC), EPA methyl ester (≥97%, capillary GC), astaxanthin (HPLC), lecithin (L-α-phosphatidylcholine, analytical grade) were supplied by Sigma (St. Louis, MO, USA). Isopropyl alcohol (HPLC) and hexane (GC) were purchased from Tianjin Shield Chemicals Co., Ltd (Tianjin China). All other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of analytical grade.

2.2. Methods

2.2.1. NLC production

Nanostructured lipid carriers composed of different ratios of krill oil, solid lipid (palm stearin) and emulsifiers were produced by the optimized hot homogenization method with ultrasonication technique. Palm stearin acid and krill oil were mixed together and melted at 70 °C until the total lipid phase was entirely melted. Meanwhile, the aqueous surfactant solution consisting of dispersing surfactant lecithin in double distilled water at the same temperature was agitated at 600 rpm by a hot plate stirrer. Then, the melted lipid phase was added to the aqueous phase drop by drop using an agitation at 600 rpm for 60 s and then further dispersed at 10,000 rpm for 5 min with the aid of a high-speed stirrer (FA25, Fluko, Shanghai, China). Finally, the preemulsion was treated by an ultrasonic cell disruption system (JY-92-II, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) for 5 min (active for 2 s at intervals of 2 s, 200 W). After recrystallization upon cooling to room temperature, NLC dispersions were prepared.

2.2.2. Formulation optimization

The optimum formulation for the NLC was ascertained using response surface methodology (RSM) and a two-factor inscribed central composite design CCD (two factors at five levels). The krill oil content in total lipid phase (w/w) (X_1) and level of the surfactant lecithin (w/w) (X_2) were the input variables. The parameter of probe-ultrasonic disruptor (200 W, 5 min) and the total lipid concentration (10%) was applied according to many references and our preliminary single-factor experiments (Averina et al., 2011; Lacatusu et al., 2013; Luo, Zhao, Zhang, & Pan, 2011; Wang et al., 2014). Altogether 13 experiments were conducted to evaluate the effects of the two variables on the particle size and size distribution (polydispersity index) of NLC as they are the most important features