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Oxidative stability of encapsulated fish oil in electrospun zein fibres

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ABSTRACT

Zein fibres loaded with fish oil were generated by electrospinning of zein polymer solutions (20% w/w) prepared in 70% (w/w) aqueous ethanol and isopropanol solvents. Zein fibres with a diameter of about 300 nm with smooth surface morphology were produced from ethanol-based solutions. The fibre diameter increased to about 500 nm as a result of the addition of 30% (w/w) fish oil. Thinner fibres (190 ± 62 nm) with beads averaging up to 1 μm in size were produced from isopropanol zein solutions with decreasing bead formation due to the addition of fish oil by 30% (w/w). Transmission electron microscopy and florescence microscopy were utilized to examine the distribution of fish oil in the electrospun materials, revealing that the lipid phase tended to concentrate at the core of the fibres and beads, as a result of phase separation during the electrospinning process. This behaviour positively impacted the encapsulation efficiency, loading capacity, as well as the stability of the fish oil tested at 4, 25 and 60 °C. The encapsulation efficiency of the electrospun zein fibres reached as high as 91% for ethanol-based and 96% for isopropanol-based fibres, at 30% (w/w) loading level. The oxidative stability of the encapsulated and non-encapsulated fish oil was monitored over a period of 14 days by determining the formation of lipid peroxide using a modified ferrous oxidation–xylenol orange method. The secondary oxidative by-products were evaluated by p-anisidine value. The oxidative stability was further monitored via Fourier transform infrared spectroscopy. The results indicated that electrospun zein fibres provide a greater oxidative stability in comparison to non-encapsulated fish oil.

1. Introduction

Essential fatty acids facilitate absorption of fat soluble nutrients and of lipid metabolism by means of β-oxidation (Kim & Battaile, 2002; Wanders & Tager, 1998). Besides serving as an essential nutrient, fats and oils also play important roles in facilitating processing of foods, as well as defining the organoleptic and textural properties of many food products (Kinsella, 1988). Recent increased consumption of ω-3 fatty acids, including eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), as dietary supplements and fortified foods can be attributed to the increased consumers' awareness on the health benefits of these polyunsaturated fatty acids (PUFAs). Numerous studies have demonstrated that the consumption of ω-3 fatty acids provides protective roles against cardiovascular and cerebrovascular diseases in human (Hu et al., 2002; Leaf, Kang, Xiao, & Billman, 2003). In other diseases, such as rheumatoid arthritis, DHA and EPA consumption has been shown to diminish symptoms and lower the dosage of nonsteroidal anti-inflammatory drugs (NSAIDs) (Calder, 2008).

Despite the health promoting benefits of ω-3 fatty acids, high degrees of unsaturated carbon–carbon double bonds in these PUFAs render them highly susceptible to oxidative degradation, resulting in the production of unstable intermediary compounds such as free radicals and hydroperoxides. Further decomposition of these products results in the formation of the secondary oxidative compounds (e.g., aldehydes, ketones and alcohols) that not only result in loss in nutritional and sensory qualities, but also results in toxicity (Ladikos & Lougovois, 1990). Thus, these fatty acids have to be protected and their stability needs to be validated through quantification of primary and secondary products generated as a result of oxidation.

Encapsulation has been one of the strategies for protecting PUFAs against oxidative degradation and delivering them in food and supplements. The most common approach of encapsulation has been by dispersing the lipid in a polymer solution (often with the aid of a surfactant) to form an oil-in-water emulsion, followed by the removal of the water phase by a spray drying process to produce dry powder. The process is often carried out in nitrogen, instead of air, in conjunction with low spray drying temperature, to minimize oxidative degradation, albeit adding manufacturing cost (Baik et al., 2004; Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007). Various materials have been used for the microencapsulation of PUFAs, including maltodextrin, glucose syrup, proteins, sugars, gums, pectin, hydropropyl/methylcellulose, and modified starch (Drusch, Serfert, & Schwarz, 2006; Jónsdóttir et al., 2005; Kolanowski, Jaworska, Weibrodt, & Kunz, 2007). Other methods such as spray chilling, extrusion, and coacervation have also been employed for encapsulation with mixed success (Benczedi & Bouquerand, 2001; Blagdon & Morgan, 1993; Lamprecht, Schäfer, & Lehr, 2001). The abovementioned methods typically produce

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capsules of several microns to millimetres in size, which may be too large for certain applications.

Over the past few years, electrostatic spinning and spraying methods have captivated considerable interest for encapsulation of bioactive compounds (Jaworek, 2008; Peltonen, Valo, Kolakovic, Laaksonen, & Hirvonen, 2010). This process uses electrostatic force to draw polymer solutions from a spinneret towards a grounded collector, by exposing the solution in an electric field of 1–2 kV/cm. As the polymer solution takes flight in the air, repulsion of charges causes the polymer to stretch into ultrafine fibres, beaded fibres, or beads depending on the properties of the polymer solution and electrospinning process parameters (Alborzi, Lim, & Kakuda, 2013; Li, Lim, & Kakuda, 2009; Taylor, 1969; Vega-Lugo & Lim, 2012). Due to the simplicity of the process, its versatility for forming submicron polymeric fibres and beads, and the absence of heat, electrospinning is ideal for the encapsulation of bioactive compounds for food and nutraceutical applications. Fernandez, Torres-Giner, and Lagaron (2009) encapsulated β-carotene, a provitamin A and antioxidant, in ultrafine zein fibres via electrospinning. Confocal Raman imaging spectroscopy showed that the encapsulated β-carotene was stable and well dispersed inside the zein fibres. The electrospun β-carotene had significant higher light stability than the encapsulated control (Fernandez et al., 2009). Li et al. (2009) encapsulated (−)-epigallocatechin gallate (EGCG) in electrospun zein fibre to stabilize the polyphenol during simulated food processing conditions. The authors report that successful immobilization of EGCG occurred when the fibres were aged for at least 24 h under dry conditions at ambient temperature. Torres-Giner, Martinez-Abad, Ocio, and Lagaron (2010) encapsulated docosahexaenoic acid in zein capsules using an electrospraying process. They reported that the encapsulated fatty acid showed 2.5-fold reduction in degradation rate and longer induction time of oxidation as compared to the unencapsulated control. Reportedly, the fatty acid was more protected from oxidation when the capsules were exposed to elevated humidity than at dry condition. These studies showed that electrostatic encapsulation processes are very versatile for preparing a zein encapsulant polymer to protect bioactive compounds.

In view of its susceptibility to oxidative degradation and undesirable fishy odour, fish oil needs to be protected with a polymeric carrier for food fortification and enrichment purposes. The objectives of this study are: (1) to study the distribution of fish oil within electrospun zein fibres; (2) to determine fish oil encapsulation efficiency of the electrospun zein carrier; (3) to elucidate the oxidative stability of the fish oil encapsulated in electrospun zein fibres by a modified FOX method; and finally (4) to study the oxidative degradation of the encapsulated fish oil using attenuated total reflectance–Fourier transform infrared (FTIR) spectroscopy.

2. Materials and methods

2.1. Materials

Zein prolamine from corn (grade Z3625), xylenol orange sodium salt, hydrogen peroxide, anhydrous isopropanol, and hexane (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used as received, without further purification. Fish oil was kindly donated by Ocean Nutrition Canada Ltd. (Dartmouth, NS, Canada). Anhydrous ethanol was supplied by Commercial Alcohols (Brampton, ON, Canada). Barium chloride dihydrate, ferric chloride, and ferrous sulphate heptahydrate were procured from Fisher Scientific Company (Ottawa, ON, Canada).

2.2. Preparation of zein polymer solution for electrospinning

Polymer solutions were prepared by dissolving 20% (w/w, zein weight / (zein weight + weight of solvent)) zein powder in 70% (w/w, weight of solvent / (weight of solvent + weight of water)) aqueous ethanol or isopropanol solutions with the aid of a magnetic stirrer. Subsequently, 15 or 30% (w/w, weight of oil/weight of zein powder) of fish oil was added to the polymer solutions and stirred for 15 min in the dark at 21 ± 2 °C (Table 1).

Table 1
Treatment codes for zein samples electrospun using either ethanol or isopropanol solvent, with different levels of fish oil.

Treatment code	Solvent type	Fish oil loading
Z1	Ethanol	0%
Z2	Ethanol	15%
Z3	Ethanol	30%
Z4	Isopropanol	0%
Z5	Isopropanol	15%
Z6	Isopropanol	30%

2.3. Electrospinning of zein polymer solutions

The polymer solutions were electrospun with a feed rate of 1 mL/h controlled by an infusion pump (Model 780100; Kd Scientific Inc., Holliston, MA, USA) using a 16-gauge blunt end stainless steel needle (Fisher Scientific, Ottawa, ON, Canada) that acted as a spinneret. The spinneret was connected to the positive electrode of a direct current (DC) power supply (Model ES30R-5 W/DM; Gamma High Voltage Research, Ormond Beach, FL, USA). In order to minimize charge leakage, the disposable syringe, containing the polymer solution was covered with a rubber insulation to isolate it from the grounded infusion pump. Electrospun fibres were collected on a circular stainless steel collector plate covered with aluminium foil. The collector–spinneret tip distance was fixed at 20 cm. The voltage applied was 20 kV. The entire electrospinner setup was housed within an environmental test chamber (Model MLR-350; SANYO Electric Co., Ltd., Ora-Gun, GU, Japan), which was maintained at 21 ± 2 °C (Fig. 1).

2.4. Morphological characterization of encapsulated fish oil in zein fibres

A scanning electron microscope (Model S-570; Hitachi High Technologies Corp., Tokyo, Japan), operating at an accelerating voltage of 10 kV, was used to examine the morphology of the electrospun fibres. Fibre specimens were coated with gold/palladium (60:40) at a rate of 7 nm/min via a sputter coater (Model K550; Emitech, Ashford, Kent,

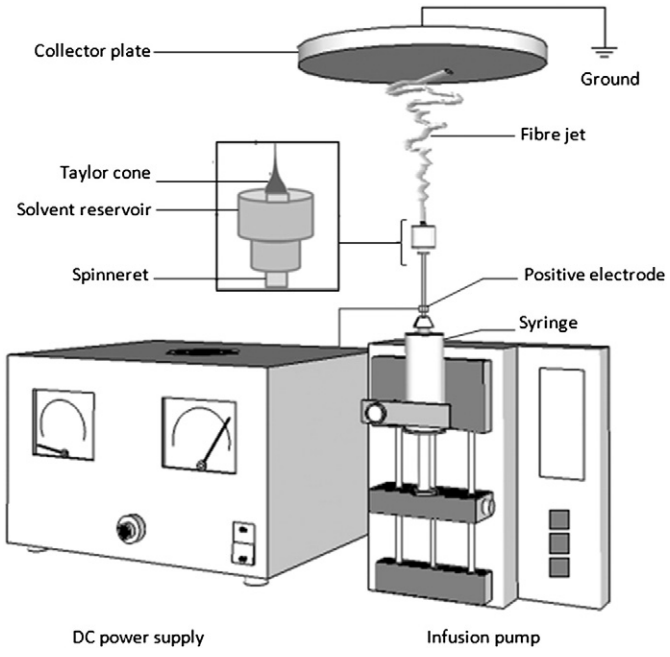

Fig. 1. Schematic diagram of the electrospinning setup used in this study.