Journal of Functional Foods 47 (2018) 491–502

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Enhancing the lipase-mediated bioaccessibility of omega-3 fatty acids by microencapsulation of fish oil droplets within porous silica particles

Paul Joyce, Hanna Gustafsson, Clive A. Prestidge*

School of Pharmacy and Medical Sciences, University of South Australia, City East Campus, Adelaide, South Australia 5000, Australia
ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, University of South Australia, Mawson Lakes Campus, Mawson Lakes 5095, Australia

ARTICLE INFO

Keywords:
Fish oil
PUFA
In vitro lipolysis
Lipid digestion
Porous silica
^{1}H NMR

ABSTRACT

Solid-state fish oil formulations composed of omega-3 (ω-3)-rich lipid droplets internalised within a nanostructured porous silica matrix have been engineered for the first time to optimise the bioaccessibility of ω-3 fatty acids. Silica-fish oil hybrid (SFOH) particles were fabricated by spray drying a submicron fish oil emulsion stabilised by hydrophilic porous silica particles. The integrity of the bioactive polyunsaturated acyl chain was preserved during synthesis and mid-term storage, highlighting the ability for silica to impart oxidative stability towards PUFA. ^{1}H NMR analysis elucidated that the nanostructured silica network acted as a substrate-enzyme immobilisation template that facilitated enhanced fish oil hydrolysis kinetics through the interfacial activation of lipolytic enzymes, resulting in a 1.4-fold improvement in bioaccessibility compared to crude fish oil. Subsequently, this novel solid-state fish oil formulation has the potential to overcome stability drawbacks associated with PUFA and optimise the degree of ω-3 absorption into the systematic bloodstream.

1. Introduction

Long-chain omega-3 (ω-3) polyunsaturated fatty acids (PUFA), specifically eicosapentaenoic acid (EPA; C20:5 ω3) and docosahexaenoic acid (DHA; C22:6 ω3), are bioactive compounds considered essential for human nutritional health (Ruxton, Reed, Simpson, & Millington, 2007) due to their reported ability to reduce the prevalence of cardiovascular disease (Bowen, Harris, & Kris-Etherton, 2016), neurodegenerative disorders (Thomas, Thomas, Radcliffe, & Itsiopoulos, 2015), and several forms of cancer (Fabian, Kimler, & Hursting, 2015; Larsson, Kumlin, Ingelman-Sundberg, & Wolk, 2004). Furthermore, ω-3 fatty acids play an important role in infant brain, visual and neural development (Innis, 2008, 2009). Despite the plethora of health benefits associated with PUFA, the average human consumption is considerably lower than that recommended by the World Health Organization, which corresponds to 300–500 mg DHA equivalent per day (Arab-Tehrany et al., 2012; Kris-Etherton, Harris, & Appel, 2002). Studies have indicated that low consumption of ω-3 fatty acids does not deliver the associated health benefits, and therefore, higher consumption of PUFA is required for an extended period of time to be considered beneficial (Swanson, Block, & Mousa, 2012).

Marine and fish oils are widely considered to contain the richest and most bioaccessible natural sources of DHA and EPA (Cardoso, Afonso, & Bandarra, 2016). However, global sustainability issues due to overfishing and the lack of accessibility to fresh seafood make it difficult for many regions of the world to consume the recommended intake of DHA and EPA (Greene, Ashburn, Razzouk, & Smith, 2013; Jenkins et al., 2009). As a result, supplementing healthy diets with ω-3-rich functional foods and nutraceuticals has emerged as the most promising approach to increase PUFA intake. Yet, the incorporation of PUFAs and fish oils within foods and health supplements has limited success due to its low solubility in most food systems and its high susceptibility to oxidation (Arab-Tehrany et al., 2012; Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert, 2016). Fish oils are highly sensitive triglycerides that rapidly undergo oxidation when exposed to light, heat, oxygen or water moisture due to their high degree of unsaturation (D'Andrea, 1994). This has led to many consumers avoiding the commercially available, soft gel fish oil capsules due to their large size, which is required for the daily recommended consumption of ω-3 fatty acids, and their poor shelf life that results in the rapid formation of a fishy odour

Abbreviations: %H, percent fish oil hydrolysis; H NMR, proton nuclear magnetic resonance; 4-BBA, 4-bromophenylboronic acid; CLSM, confocal laser scanning microscopy; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acids; *k*, pseudo-first-order rate constant; H_{max}, maximum fish oil hydrolysis; MCT, medium-chain length triglycerides; N_{FA}, molar concentration of fatty acids; N_{MG}, molar concentration of monoglycerides; N_T, total molar concentration of lipids; PDI, polydispersity index; PUFA, polyunsaturated fatty acids; SEM, scanning electron microscopy; SFOH, silica-fish oil hybrid; SFOLH, silica-fish oil-lecithin hybrid; SLH, silica-lipid hybrid; *t*, time; TBU, tributyrin units; TGA, thermogravimetric analysis; ω-3, omega-3; ω-3_{BA}, bioaccessible omega-3

* Corresponding author at: School of Pharmacy and Medical Sciences, University of South Australia, City East Campus, Adelaide, South Australia 5000, Australia.
E-mail address: clive.prestidge@unisa.edu.au (C.A. Prestidge).

https://doi.org/10.1016/j.jff.2018.06.015
Received 26 February 2018; Received in revised form 30 May 2018; Accepted 13 June 2018

due to rancidification. Consequently, the development of solid-state fish oil formulations, which impart high chemical stability on the hosted PUFA, has received considerable interest in recent years, by utilising common microencapsulation techniques to maximise fish oils' full therapeutic potential (Rodríguez, Martín, Ruiz, & Clares, 2016). Furthermore, microencapsulation of PUFA not only improves oxidative stability, but also transforms the formulation state from liquid to solid, which has considerable consumer and commercial benefits (easier transportation, storage and processing) for functional food and pharmaceutical applications (Christophersen, Yang, & Mu, 2016).

The majority of microencapsulation excipients used to chemically stabilise PUFA consist of food-grade matrices formed from carbohydrates (Shen, Augustin, Sanguansri, & Cheng, 2010), gums (Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007) and proteins (Legako & Dunford, 2010). Various methodologies may be used to achieve this, including spray drying (Encina et al., 2016), electrospraying (García-Moreno et al., 2018), freeze drying (Heinzelmann, Franke, Jensen, & Haahr, 2000), phase coarcervation (Wu & Xiao, 2005) and ultrasonic atomisation (Klaypradit & Huang, 2008). However, spray drying has emerged as the most commonly used fish oil microencapsulation technique due to its economic feasibility, high encapsulation efficiency and low residual water content (Bakry et al., 2016). While these studies successfully exerted oxidative stability on the encapsulated ω-3 fatty acids, the potential effect of restructuring the oils on the enzyme-mediated bioaccessibility has commonly been ignored. The oral bioavailability of triglycerides (*i.e.* the extent absorbed into the systematic bloodstream or target site) is controlled by enzyme-mediated digestion and absorption in the gastrointestinal tract (GIT). As fish oil passes through the gut into the small intestine, gastric and pancreatic lipases hydrolyse the triglyceride molecules into ω-3 fatty acids and monoglycerides, which can then be absorbed across the epithelium into the blood and surrounding tissues (Porter, Trevaskis, & Charman, 2007). Lipase activity is modulated by the interfacial composition of oil droplets (Klinkesorn & McClements, 2010), and therefore the bioaccessibility of the fish oil-in-water interface can be optimised through colloidal engineering techniques. While several microencapsulation techniques have demonstrated equivalent oral bioavailibities to crude fish oil (Christophersen et al., 2016), it is desirable to optimise and increase the extent of ω-3 fatty acid absorption through intelligent formulation design. In doing so, this will reduce the required dosing of fish oil and increase the feasibility of consuming the required intake for the associated health benefits.

One microencapsulation technique, which enhances the *in vitro* bioaccessibility of medium- and long-chain length triglycerides is that of silica-lipid hybrid (SLH) microparticles (Joyce, Whitby, & Prestidge, 2016b). SLH particles are engineered by spray drying a silica nanoparticle-stabilised lipid emulsion, which results in the formation of a highly organised three-dimensional hybrid matrix nanostructure (Simovic et al., 2009; Tan, Simovic, Davey, Rades, & Prestidge, 2009). SLH particles enhance the lipase-mediated hydrolysis of encapsulated triglyceride molecules by acting as an immobilisation template for the interfacially active digestive enzymes (Tan, Colliat-Dangus, Whitby, and Prestidge, 2014). The enhanced release of fatty acids and monoglycerides from SLH particles has considerably improved the bioavailability of a wide range of poorly water-soluble drugs that were incorporated within the lipid phase of the formulation (Rao et al., 2015; Simovic et al., 2010; Tan, Davey, & Prestidge, 2011; Tan, Eskandar, Rao, & Prestidge, 2014). Furthermore, nanostructuring lipid within porous silica particles has shown to impart long-term (up to 12 months) chemical and thermodynamic stability on both the lipid phase and the encapsulated drug molecules (Tan et al., 2009). Therefore, this has led to the hypothesis that nanostructuring fish oil droplets within a porous silica matrix, in an equivalent manner to SLH particles, will induce oxidative stability to the PUFA, while facilitating enhanced lipase-mediated bioaccessibility and release of ω-3 fatty acids.

In this study, hydrophilic porous silica nanoparticles were used as a novel excipient for the microencapsulation of fish oil droplets to systematically investigate their potential in (i) imparting oxidative stability to ω-3 triglycerides, and (ii) enhancing the digestive enzyme-provoked bioaccessibility of ω-3 fatty acids. Porous silica, specifically Aerosil 300 fumed silica, was selected as the stabilising colloid due to its FDA approval to be used as a food and pharmaceutical grade excipient, biocompatibility, ease of handling, cheap manufacturing costs, and high porosity (AG., 2013). By developing this unique solid-state ω-3 fatty acid formulation, silica-fish oil hybrid (SFOH) particles have demonstrated the promising potential for combining porous colloids with fish oil droplets to enhance the bioavailability of ω-3 fatty acids.

2. Experimental section

2.1. Materials

Food-grade fumed hydrophilic silica (Aerosil 300) existing of primary particles with an average diameter of 7 ± 1 nm that randomly cluster to form aggregates with an estimated particle size of 0.5–5.0 μm and a pore size of 2–7 nm in powder form (Joyce, Whitby, & Prestidge, 2016a), were supplied by Evonik Degussa (Germany). The porous silica particles used within this study were negatively charged and had a zeta potential of −24.1 mV when dispersed in water. SEM images and contact angle measurements of initial porous silica particles are provided within Supplementary Information Figs S1 and S2, respectively. Commercial grade liquid fish oil (Nature's Own) composed of 77% natural fish oil, 14% equivalent EPA and 9% equivalent DHA was purchased from Woolworths Limited (Australia). Soybean lecithin (containing > 94% phosphatidycholine and < 2% triglycerides) was obtained from BDH Merck (Sydney, Australia). Fluorescent dyes, coumarin 6 and rhodamine B, were obtained from Sigma-Aldrich (Australia). FaSSIF/FeSSIF/FaSSGF biorelevant powder was purchased from Biorelevant.com Ltd (London, United Kingdom). Other materials used for *in vitro* lipolysis studies, including *Candida* lipase (6000 TBU/mL), sodium taurodeoxycholate (NaTDC), trizma maleate, type X-E L-α-lecithin (consisting of approximately 60% pure phosphatidylcholine), 4-bromophenylboronic acid (4-BBA), calcium chloride dihydrate and sodium hydroxide pellets were also purchased from Sigma-Aldrich (Australia). Porcine pancreatin extract (activity equivalent to 8× USP specification) was supplied by MP Biomedicals (Australia). Deuterated chloroform ($CDCl_3$) was supplied by Cambridge Isotope Laboratories (USA).

2.2. Synthesis of silica-fish oil hybrid (SFOH) and silica-fish oil-lecithin hybrid (SFOLH) microparticles

Fish oil was added to water (10% w/v) to form a coarse fish oil-in-water emulsion. For SFOLH particles, the anionic stabiliser, soybean lecithin (6% w/w) was dissolved within the lipid phase prior to addition to water. The coarse emulsion was homogenized at a pressure of 1000 bar for 5 cycles (Avestin EmulsiFlex-C5 Homogenizer, Ottawa, Canada). An aqueous suspension of silica particles (2% w/v) was prepared by sonication (Bransonic Ultrasonic Bath; 40 Hz, 100%) for 2 h to allow for effective dispersion. The fish oil emulsions were added to the silica dispersion, immediately after homogenisation to prevent phase separation, with stirring (750 rpm, room temperature) for 1 h. The initial silica:fish oil ratio was varied for SFOH particles, but only a ratio of 50:50 was selected for SFOLH particles (Table 1). The fish oil-in-water emulsions partially stabilised by silica particles (Tan et al., 2010) were subsequently spray dried (Büchi Mini Spray Dryer B-290 apparatus, Flawil, Switzerland) to form SFOH and SFOLH particles under the following conditions: product flow rate, 420 mL/h; inlet temperature, 140 °C; outlet temperature, ~70 °C; aspirator setting, 10. The product yield of SFOH and SFOLH particles was determined as the ratio between the mass of product captured after spray drying and the initial mass of the combined silica and fish oil phases, given as a percentage.