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Optimisation of the microencapsulation of tuna oil in gelatin–sodium hexametaphosphate using complex coacervation

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ABSTRACT

The microencapsulation of tuna oil in gelatin–sodium hexametaphosphate (SHMP) using complex coacervation was optimised for the stabilisation of omega-3 oils, for use as a functional food ingredient. Firstly, oil stability was optimised by comparing the accelerated stability of tuna oil in the presence of various commercial antioxidants, using a Rancimat™. Then zeta-potential (mV), turbidity and coacervate yield (%) were measured and optimised for complex coacervation. The highest yield of complex coacervate was obtained at pH 4.7 and at a gelatin to SHMP ratio of 15:1. Multi-core microcapsules were formed when the mixed microencapsulation system was cooled to 5 °C at a rate of 12 °C/h. Crosslinking with transglutaminase followed by freeze drying resulted in a dried powder with an encapsulation efficiency of 99.82% and a payload of 52.56%. Some 98.56% of the oil was successfully microencapsulated and accelerated stability using a Rancimat™ showed stability more than double that of non-encapsulated oil.

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1. Introduction

The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have proven health benefits in humans and they play functional roles in cell membrane fluidity, cellular signalling, gene expression and eicosanoid metabolism (Ruxton, Reed, Simpson, & Milington, 2004). A substantial body of knowledge generated through clinical studies indicates that EPA and DHA can help prevent a number of chronic diseases including cancer (De Deckere, 1999), cardiovascular disease (Yokoyama et al., 2007), depression (Sontrop & Campbell, 2006), Alzheimer's disease (Cole, Ma, & Frautschy, 2009) and other diseases associated with excess inflammation (Wall, Ross, Fitzgerald, & Stanton, 2010). Tuna oil is an excellent source of omega-3 oils, particularly DHA, and is widely used as an additive to infant formula and some food products (Jacobsen, Nielsen, Horn, & Sørensen, 2013). However, the incorporation of omega-3 oils into food products is limited by the high susceptibility of these highly unsaturated oils to oxidation, primarily on exposure to air, light and elevated temperatures (Lytle, Lytle, Newmark, & Deschner, 1992). Even low levels of oxidation of EPA and DHA from fish or microbial oil leads to objectionable off-flavour, and therefore caused sensory problems in food and beverage products (Kampa, Nifli, Notas, & Castanas, 2007). Hence, microencapsulation needs to be employed in order to stabilize these omega-3 oils and minimise their oxidative degradation for their incorporation in foods and beverages where direct oil incorporation leads to sensory problems (Barrow, Wang, Adhikari, & Liu, 2013).

Coacervation refers to the phase separation of a liquid phase into a polymer-rich phase (coacervate) and a polymer-poor phase. The coacervation process is widely used in the food and pharmaceutical industries to encapsulate bioactive ingredients. Complex coacervation, in which two or more oppositely charged polymers are involved, has also been used to encapsulate fish oils (Barrow et al., 2013). The complex coacervation process occurring between proteins and polyanions has been extensively investigated (Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2004). In the modern food industry, gelatin is the primary protein used in microencapsulation using complex coacervation, and studies have been performed with a variety of anionic polymers such as gum Arabic (Nakagawa & Nagao, 2012), sodium dodecyl sulphate (SDS) (Li, Wu, Chen, & Wang, 2009), pectin (Saravanan & Rao, 2010) or chitosan (Kang, Dai, & Kim, 2012).

Complex coacervation was not used successfully for the delivery of fish oil into food products at an industrially scale until the development of "multicore" complex coacervation (Barrow, Noleen, & Holub, 2009). In this process rather than single oil zdroplets covered by an outer shell, the resultant product is an

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agglomeration of micron sized single cores surrounded by a secondary outer shell (Barrow et al., 2009). In this process there are four key steps: The first step is an emulsion preparing step in which oil-in-water emulsions are prepared, defining the size of the inner single core structures. The second step is the coacervation step in which the oil droplets in the emulsion are coated by the coacervates through adjusting the pH of the emulsion. During this step, coated oil droplets tend to agglomerate to form particles of defined size, normally 30–50 microns. The third step involves modification of the temperature so that an outer shell forms around the agglomeration. The final step involves the hardening of the microcapsule shell by enzymatic or chemical crosslinking, so that the particles cannot redissolve without breakage of amide bonds. Compared to the conventional microencapsulation techniques such as spray drying of oil-in-water emulsion, this "multicore" complex coacervation process achieves greater than 50% payload and provides a thicker outer shell, improving oxidative stability, together with decreasing the surface oil content (Barrow, Nolan, & Jin, 2007). A limitation of this process is that gelatin is the only protein that will form a thick outer shell and so systematic optimisation of this process may enable the design of modified processes that enable the use of other proteins as shell materials and alternative core materials to be used without extensive modification by trial and error approaches. In the current study, gelatin and sodium hexametaphosphate (SHMP) were used as wall materials to microencapsulate tuna oil through multicore complex coacervation. Firstly, the optimum coacervation pH and ratio between gelatin and SHMP were determined. Secondly, formation of the outer shell and transglutaminase crosslinking were investigated. Finally, the microcapsules were freeze dried and oil payload, encapsulation efficiency and oxidative stability index values of the freeze dried powder particles were determined. Based on the systematic optimisation of processing for the formation of "multicore" microcapsules undertaking in this study, the impact of key parameters on coacervate formation has been evaluated.

2. Materials and methods

2.1. Materials and solution preparation

Tuna oil (HiDHA) provided by NuMega Ingredients Ltd (Altona North, Victoria, Australia) and stored at 4 °C before use. The tuna oil had the following major fatty acid content: DHA 29.4%, decosapentaenoic acid (DPA) 1.2%, EPA 6.0%, arachidonic acid 2.0%, stearidonic acid 0.6%, linolenic acid 0.4%, linoleic acid 1.2%, oleic acid 12.8%, stearic acid 5.3%, palmitic acid 19.2%, myristic acid 2.4%. Type A gelatin (from porcine skin type A, 300 bloom) and sodium hexametaphosphate (SHMP) were purchased from Sigma–Aldrich Corporation (Sydney, NSW, Australia). Nine fat-soluble antioxidants were provided by Kemin Nutrisurance Incorporation (Des Moines, IA, USA) and Kalsec Incorporation (Kalamazoo, MI, USA). Transglutaminase (Activa® KS–LS) was purchased from Ajinomoto (Tokyo, Japan). All other chemicals used in this study were purchased from Sigma–Aldrich Australia (Sydney, NSW, Australia).

Gelatin solution was prepared by wetting in Milli-Q water (18.2 MΩ cm, Advantage A10, Millipore Corporation, Billerica, MA, USA) followed by heating at 50 °C for 20 min until a clear solution was obtained. SHMP powders were dissolved in Milli-Q water in a separate beaker also at 50 °C. Tuna oil was allowed to melt at the same temperature for 10 min before the microencapsulation. A continuous flow (flush) of nitrogen was maintained to prevent the oxidation of oil when it was being heated. Transglutaminase powder was fully dispersed into Milli-Q water at room temperature and stored at 4 °C before use.

2.2. Accelerated oxidation by Rancimat

Accelerated oxidation tests for the liquid oil and final microcapsule products were performed using a Rancimat (model 743, Metrohm Corporation, Herisau, Appenzell Ausserrhoden, Switzerland) as a fast and reliable analytical method (Farhoosh, 2007). Specifically, 4 mL tuna oil or 1.5 g freeze dried microcapsules was heated under a purified air flow rate of 20 L/h at 90 °C. The induction time of test samples was recorded and was used as the oxidative stability index (OSI).

2.3. Optimisation of incorporated antioxidants

The nine commercial antioxidants were incorporated into tuna oil individually at 50 °C, from half to double the recommend dosage (RD) from the manufacture, as Table 1 shows. Then stability of the tuna oil with each antioxidant dose was determined using a Rancimat™ at 90 °C with air flow rate of 20 L/h (OSI values).

2.4. Coacervation behaviour study

An understanding of the extent and nature of phase separation of aqueous gelatin–SHMP solution is required in order to optimise the coacervation process. The phase separation of gelatin–SHMP aqueous solutions depends on several factors such as pH and gelatin-SHMP ratios.

Table 1
The concentration of antioxidants used in tuna oil for stability tests (provided by supplier).

Antioxidant	Code	Incorporated dosage (%, w/w)					Description of antioxidant
		0.5 RD	0.75 RD	RD	1.5 RD	2 RD	
Herbalox XT	A	0.05	0.1	0.2	0.3	0.4	Rosemary leaves extracts
Duralox OMB MAN-1	B	0.1	0.2	0.4	0.6	0.8	Mixed tocopherols, natural flavour, ascorbic acid, citric acid
Duralox blend AN 110 XT	C	0.1	0.2	0.4	0.6	0.8	Vegetable oils, natural flavours, ascorbic acid
Verdilox RP liquid	D	0.1	0.2	0.3	0.45	0.6	Mixed tocopherols and botanical extracts
Verdilox GT liquid	E	0.1	0.2	0.3	0.45	0.6	Mixed tocopherols and botanical extracts
Naturox R30 liquid	F	0.1	0.2	0.3	0.45	0.6	Mono- and diglycerides, blended tocopherols, citric acid, rosemary extracts
Naturox premium liquid	G	0.1	0.2	0.3	0.45	0.6	Mono- and diglycerides, blended tocopherols, citric acid, rosemary extracts
Pet-ox premium liquid	H	0.05	0.1	0.15	0.2	0.3	Vegetable oils, butylated hydroxygoluence, mono- and diglycerides, butylated hydroxuanisole, citric acid
Rendox AET liquid	I	0.05	0.075	0.1	0.15	0.2	Vegetable oils, butylated hydroxytoluence, lecithin, ethoxyquin, butylated hydroxyanisole, mono- and diglycerides

Where RD stands for recommended dosage from manufacture (%, w/w).

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