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In-vitro digestion of probiotic bacteria and omega-3 oil co-microencapsulated in whey protein isolate-gum Arabic complex coacervates

Divya Eratte [a,*], Kim Dowling [a,*], Colin J. Barrow [b], Benu P. Adhikari [c,d]

[a] Faculty of Science and Technology, Federation University Australia, Ballarat, VIC 3353, Australia
[b] Centre for Chemistry and Biotechnology, Deakin University, Geelong, VIC 3217, Australia
[c] School of Science, RMIT, Melbourne, VIC 3083, Australia
[d] CSIRO Manufacturing Flagship, Clayton South, VIC 3169, Australia

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ABSTRACT

Solid co-microcapsules of omega-3 rich tuna oil and probiotic bacteria *L. casei* were produced using whey protein isolate-gum Arabic complex coacervate as wall material. The *in-vitro* digestibility of the co-microcapsules and microcapsules was studied in terms of survival of *L. casei* and release of oil in sequential exposure to simulated salivary, gastric and intestinal fluids. Co-microencapsulation significantly increased the survival and surface hydrophobicity and the ability of *L. casei* to adhere to the intestinal wall. No significant difference in the assimilative reduction of cholesterol was observed between the microencapsulated and co-microencapsulated *L. casei*. The pattern of release of oil from the microcapsules and co-microcapsules was similar. However, the content of total chemically intact omega-3 fatty acids was higher in the oil released from co-microcapsules than the oil released from microcapsules. The co-microencapsulation can deliver bacterial cells and omega-3 oil to human intestinal system with less impact on functional properties.

1. Introduction

There is a significant commercial interest for functional foods containing long chain omega-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The estimated global demand for omega-3 ingredients was 1.6 billion USD in 2010 and is expected to be greater than 4.0 billion USD in 2018 (Transparency Market Research, 2013). Long chain PUFAs play an important role in human health (Shahidi, 2015). The dietary intake of these fatty acids has been associated with improving cardiovascular health (Bonafini, Antoniazzi, Maffeis, Minuz, & Fava, 2015), combating neural disorders (Blondeau, 2016), helping in infant brain and vision development (Wu, Zhou, Ma, Yuan, & Peng, 2015), and combating some forms of cancer (Rose & Connolly, 1999). However, omega-3 fatty acids are highly susceptible to oxidative degradation due to high degree of unsaturation (Jacobsen, 2010). Lipid oxidation impairs flavour and nutritional value, and shortens the shelf-life of products containing omega-3 fatty acids (Kaushik, Dowling, Barrow, & Adhikari, 2015). Microencapsulation is effective in stabilizing omega-3 fatty acids and for masking the inherent fishy odour of fish oils (Nickerson, Yan, Cloutier, & Zhang, 2014). Microencapsulated omega-3 fatty acids have been used to fortify a range of food products such as breads, milk, fruit juices, tortillas, chocolate, and yogurt drinks (Kaushik et al., 2015; Kadam & Prabhasankar, 2010).

Alternatively, probiotic bacteria are live microorganism which provide numerous health benefits in human (Salminen, Kneifel, & Ouwehand, 2016). These benefits include control of serum cholesterol levels and intestinal infection, positively influencing the immune system (de Roos & Katan, 2000), improving lactose utilization (Post, 2013) and providing anticarcinogenic activities (Maleki, Homayouni, Khalili, & Golkhalkhali, 2016). Many studies report on various aspects of incorporation of probiotic bacteria into a range of food products (Farnworth & Champagne, 2016; Shori, 2016). One of the major challenges regarding the application of probiotic bacterial cultures in functional foods is the maintenance of their viability during processing and passage through the host's gastrointestinal system (De Prisco & Mauriello, 2016). Microencapsulation is a promising technology for introducing viable probiotic

* Corresponding authors.
E-mail addresses: d.eratte@federation.edu.au (D. Eratte), k.dowling@federation.edu.au (K. Dowling).

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bacteria in various foods as the encapsulating shell material provides protection to the encapsulated bacterial cells against environmental stressors such as temperature, pH and mechanical shear (Mattila-Sandholm et al., 2002). The microcapsules containing bacterial cells, if properly designed, are able to maintain their integrity during passage through the gastrointestinal tract until they reach their target destination (colon), where they should break down and release the probiotic bacteria (Del Piano et al., 2011).

In our previous work, we reported the co-microencapsulation of omega-3 rich tuna oil and probiotic bacteria *L. casei* in whey protein isolate-gum Arabic complex coacervate matrix and detailed the synergistic effect of this co-encapsulation to bacterial survival and stability against oxidation during storage (Eratte, Wang, Dowling, Barrow, & Adhikari, 2016; Eratte et al., 2015). In this study, address the *in-vitro* digestion of co-microcapsules containing omega-3 oil and probiotic bacteria when they are sequentially exposed to simulated salivary (SSF), gastric (SGF) and intestinal (SIF) fluids. *In-vitro* digestion behaviour of individual microcapsules containing probiotic bacteria (WPI-P-GA) or tuna oil (WPI-O-GA) distinctly were also studied as controls. To date, there is no study which reports the digestion behaviour of co-microcapsules containing two distinct bioactive ingredients such as omega-3 oil and probiotic bacteria.

The site-specific delivery of bioactive ingredients to a targeted site in the gastrointestinal tract is of great interest (Hirsch, Binder, Schehlmann, Kolter, & Bauer, 1999; Kosaraju, Weerakkody, & Augustin, 2009). Hence, in order to ensure the delivery of co-microencapsulated omega-3 oil and probiotic bacteria to targeted sites of the human digestive system, it is important to test the stability and release of both omega-3 and probiotic bacterial cells either *in-vivo* or *in-vitro* using simulated digestive fluids. In the human digestive system, food is first exposed to salivary enzymes at pH ~ 7.0 and then digested by gastric pepsin at low pH of ~3.0, and eventually reaches the intestine having pH ~ 7.0 (Minekus et al., 2014). To understand the possible release of omega-3 oil and survival of probiotic bacteria during their passage through the human gastrointestinal digestion system, the co-microcapsules were subjected to SSF, SGF and SIF and the survival of *L. casei* and release of omega-3 oil were evaluated. The novelty of this study lies in that it advances the concept of co-microencapsulation of probiotic bacteria and omega-3 fatty acids by quantifying viability of the former and release of the latter *in-vitro* using SSF, SGF and SIF.

In this context, this study addresses two major research objectives. Firstly, to quantify the survival of *L. casei* in co-microcapsules (WPI-P-O-GA) when sequentially exposed to SSF, SGF and SIF, in comparison to microcapsules containing only *L. casei* (WPI-P-GA). Secondly, to quantify and compare the release properties of major omega-3 fatty acids from co-encapsulated tuna oil (WPI-P-O-GA) when sequentially exposed to SSF, SGF and SIF with that of microcapsules containing only tuna oil (WPI-O-GA).

2. Materials and methods

2.1. Materials

L. casei 431 was used as a model probiotic bacteria and was kindly donated by Chr.Hansen (Horsholm, Denmark). Tuna oil (HiDHA) was donated by NuMega Ingredients Ltd. (Melbourne, Australia). Tuna oil is rich in omega-3 fatty acids particularly DHA. The oil samples were stored at 4 ± 0.5 °C until use. Whey protein isolate (WPI 895™) was donated by Fonterra Cooperative (Melbourne, Australia). Gum Arabic was purchased from Sigma-Aldrich Ltd. (Sydney, Australia). Enzymes including α-amylase from human saliva (Type 1X-A, 1000–3000 units/mg protein), pepsin from porcine gastric mucosa (3200–4500 units/mg protein), pancreatin from porcine pancreas, bile salt (B8756) were obtained from Sigma–Aldrich Ltd. (Sydney, Australia). All other chemicals were purchased from Sigma–Aldrich and were of analytical grade. All of the above ingredients and chemicals were used as received without further purification or alteration.

2.2. Preparation of L. casei culture

L. casei was cultured for 18 h in sterile MRS broth (1%, w/v) at 37 ± 0.5 °C. This culture was further sub-cultured (37 ± 0.5 °C, 18 h) twice in the same broth to activate bacterial cells and to allow them to adapt. All inoculation works were carried out under a sterile biological hoods with laminar air flow (Auramini, Laftech, Australia). Finally, 1000 ml of media was used for bulk culturing and cells were harvested at the stationary growth phase by centrifuging at 2200*g* for 15 min at 5 °C. The supernatant was discarded and the precipitated cell mass was washed twice with sterile peptone water (0.2%, w/v). The final wet cell mass was weighed and divided into two equal portions. One portion (wet mass ~5 g) was used to prepare WPI-P-GA microcapsules and the second equal half was used to prepare WPI-P-O-GA co-microcapsules (Eratte et al., 2015).

2.3. Microencapsulation process

The complex coacervation and microencapsulation procedures were carried out according to the following procedures described in our previous works (Eratte, Wang, Dowling, Barrow, & Adhikari, 2014; Eratte et al., 2015) with slight modification. These processes are briefly presented in Sections 2.3.1,2.3.2 and 2.3.3.

2.3.1. Microencapsulation of tuna oil

A solution of 15 g tuna oil and 250 ml WPI solution (12%, w/v) were stirred (IKA® RW 20 digital overhead stirrer, Staufen, Germany) at 800 rpm for 10 min and then homogenized using a microfluidizer at 45 MPa for 3 passes (M110L, Microfluidics, Newton, MA, USA) to produce an O/W emulsion. Then a 250 ml GA solution (4%, w/v) was added drop wise into this O/W emulsion with continuous stirring at 400 rpm and the pH adjusted to 3.75 by adding 1% citric acid drop wise in order to induce complexation between WPI and GA.

2.3.2. Microencapsulation of L. casei

Similar to above, the previously prepared probiotic cell mass (Section 2.2) was added slowly into a WPI solution, and a GA solution was then added drop wise with continuous stirring, after which the pH was adjusted to 3.75.

2.3.3. Co-microencapsulation of omega-3 oil and L. casei

As above, tuna oil (15 g) was dispersed into a WPI solution and stirred for 10 min, followed by homogenizing using a microfluidizer to produce an O/W emulsion. Previously prepared probiotic cell mass was then added into this O/W emulsion and GA solution was added with continuous stirring at 400 rpm, and the pH adjusted to 3.75 as above.

The microencapsulation procedure in all three cases were carried out at 25 °C, followed by keeping the liquid microcapsules at 5 °C for 24 h to ensure the complete formation of complex coacervates. After maintaining the sample at 5 °C for 24 h, 100 ml of 4% (w/w) transglutaminase dispersion was added to induce the crosslinking. The slurry was heated to 25 °C to activate the enzyme and was kept at 25 °C for 2 h to allow complete crosslinking. Finally, the microcapsules/co-microcapsules were dried to produce