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# Optimization of microcapsules shell structure to preserve labile compounds: A comparison between microfluidics and conventional homogenization method

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ABSTRACT

A new technique is presented to optimize the formulation of microcapsules loaded with labile compounds. Fish oil was loaded into the microcapsule core and protected with a shell composed of whey protein microgel/beet pectin complexes. The microcapsules were formed using two different methods: microfluidics and homogenization. The microcapsules were further classified into three sub-groups. The first group was the microcapsules cross-linked with laccase (MCL), the second group was the microcapsules cross-linked with divalent cationic $CaCl_2$ salt (MCS), and the third group consisted of control microcapsules (CM), with no cross-linking. The microfluidics method enabled tracking of the effect of the shell cross-linking ability of laccase, or $CaCl_2$, on microcapsules. It was demonstrated that MCL obtained by microfluidics are more physicochemically stable than those produced via a homogenizer. The effect of cross-linking agents on the microcapsules were more significant when the microcapsules were produced by microfluidics.

## 1. Introduction

The ability of microcapsules to preserve labile nutrients and bioactive compounds is of considerable importance in the food industry (Evageliou & Saliari, 2017; Ravanfar, Tamaddon, Niakousari, & Moein, 2016; Rutz et al., 2017). The key element in fabricating suitable microcapsule structures is the design of a shell material that can protect its cargo from conditions such as thermal processing and oxidizing agents. Fish oil is a rich source of omega-3 long-chain polyunsaturated fatty acids—in particular, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Chen, McGillivray, Wen, Zhong, & Quek, 2013). Regular consumption of omega-3 fatty acids is associated with multiple health benefits, such as ameliorative effects on the brain and nervous system, and the prevention of diseases including diabetes, inflammatory and autoimmune disorders, cardiac arrhythmias and hypertension (Newton, 2001). However, omega-3 fatty acids are extremely susceptible to oxidative deterioration, and their oxidation products, such as aldehydes and ketones, have very low sensory thresholds (McClements, Decker, & Weiss, 2007), limiting the applications of omega-3 in functional foods. One approach to overcoming these issues is the encapsulation of fish oil to enhance its chemical stability, which masks unpleasant odors or tastes, improving palatability. Numerous groups have studied various formulations to encapsulate fish oil, improving its stability, thus increasing the health benefits of various functional foods (Choi, Decker, & McClements, 2009; Klinkesorn & McClements, 2009). In addition, a wide variety of methods including freeze drying (Heinzelmann, Franke, Jensen, & Haahr, 2000), spray drying (Chen et al., 2013), ultrasonic emulsification (Chatterjee & Judeh, 2015), as well as conventional homogenization methods (Frenzel & Steffen-Heins, 2015; Torres, Murray, & Sarkar, 2016, 2017) have been used to encapsulate fish oil. The main issue with such methods is that a change in one parameter can affect many other parameters at the same time in the emulsion, thus altering microcapsule properties. For instance, temperature and pressure are dependent parameters in high-pressure homogenization, thus alteration of temperature can affect the pressure. Therefore, it is challenging to track the effect of each parameter on resultant microcapsules to efficiently optimize formulations. There are also still challenges in the direct application of fish oil microcapsules to food systems, and their integration into the human diet. For example, the use of microencapsulated fish oil in the production of infant formula is still a major challenge due to the oxidative deterioration of fish oil during its shelf life, resulting in unpleasant odor and taste. Consequently, there is much room for optimizing the formulation of the microcapsule shell to extend shelf life of fish oil and improve its physicochemical stability against deterioration.

In this study, we utilized a drop-based microfluidic approach to

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optimize the formulation for preparation of the microcapsules, preserving labile compounds and improving their stability and potential for application in different food matrixes. Microfluidics is a promising technology for the production of microcapsules as it provides precise flow control, as well as highly monodisperse emulsions with controllable sizes and morphologies (Comunian, Abbaspourrad, Favaro-Trindade, & Weitz, 2014; Comunian et al., 2017). Moreover, the polymeric shell of microcapsules fabricated via microfluidics can be fine-tuned to optimize formulations (Choi et al., 2016). In this work, we also compared the physicochemical properties of microcapsules formed using microfluidics with that of microcapsules obtained by homogenization. As our model system, fish oil loaded microcapsules were prepared by whey protein microgel-beet pectin complexes, exploiting layer-by-layer electrostatic deposition. The microcapsule shell was further strengthened with laccase cross-linking or $CaCl_2$ salt cross-linking. Microfluidics enabled us to maintain precise control, holding all parameters constant in the microfluidic device and tracking the effects of shell cross-linking ability of laccase, or $CaCl_2$, on the physicochemical stability of fish oil loaded microcapsules. It was shown that microfluidics can optimize microcapsule formulations to preserve fish oil and enhance its physical and chemical stability. This approach may also prove useful in optimizing the formulation for microcapsules protecting other nutrients and labile bioactive compounds.

## 2. Materials and methods

### 2.1. Materials

Fish oil from menhaden and laccase from *Trametes versicolor* (activity ≥0.5 U/mg) were purchased from Sigma-Aldrich (St. Louis, MO, US). Calcium chloride was purchased from Fisher Scientific (Waltham, MA, USA). Beet pectin (GENU® BETA Pectin), extracted from sugar beet pulp, was kindly donated by CP Kelco (Atlanta, GA). *Bi*PRO whey protein isolate (moisture: 5%, protein (dry basis): 95%, fat: 1%, ash: 3%, lactose: 0.5%, pH = 6.7–7.5) was provided by Davisco Business Unit of Agropur Inc. (Eden Prairie, MN).

### 2.2. Methods

#### 2.2.1. Preparation of whey protein microgels

Whey protein microgel was prepared according to previous methods with slight modification (Donato, Schmitt, Bovetto, & Rouvet, 2009). Aqueous solution of whey protein (4%, W/V) was prepared and heated to 50 °C for 45 min under constant stirring. The solution was cooled to room temperature and the pH was adjusted to 5.9 with 0.1 M HCl. The solution was then transferred to glass vials and heated under constant stirring (300 rpm) in a water bath at 82 °C for 45 min. After heat treatment, the samples were rapidly cooled in ice to stop aggregation. The size of whey protein microgels was around 300 nm.

#### 2.2.2. Microencapsulation procedure

*2.2.2.1. Microfluidic devices*. Glass capillary-based microfluidic devices were used to prepare oil/water single emulsions. The round and square capillaries were purchased from World Precision Instruments, Inc., Sarasota, Florida and Harvard Borosilicate Square Tubing, Boston, MA respectively. A glass device designed for double emulsions was used to encapsulate fish oil, setting up the fish oil as the inner phase and a solution of 1% (w/w) whey protein microgels as the middle and continuous phases. The applied flow rates for these experiments were 2000 μL/h, 9500 μL/h and 30.50 mL/h for the inner, middle and continuous phases, respectively. Fluids were pumped through the microfluidic device by a syringe pump (New Era Pump Systems, Inc/Farmingdale, New York, USA). Whey protein microgels formed the inner layer of the microcapsule shell around fish oil. The single emulsions of fish oil/whey protein microgels (O/W) were collected in a beaker containing 0.5 or 1.0% (w/w) of beet pectin solution. Beet pectin formed an outer layer of the microcapsule shell, located on top of the whey protein microgel layer. Thus, the layer-by-layer structure of the microcapsule shell was formed around the fish oil. Then, different formulations were obtained by addition of either laccase (enzyme/polysaccharide ratio of 0.24/4 mg, respectively) or $CaCl_2$ (5 mM), or no addition (control) (Supplementary Table 1), followed by stirring for 12 h at room temperature. The samples were frozen at −80 °C and freeze-dried (Labconco/Kansas City, MO) for 48 h and stored at room temperature for 15 days.

*2.2.2.2. Conventional homogenization method*. Primary O/W emulsions were prepared using a high-shear homogenizer (VWR 200 Homogenizer Unit, Randor, PA, USA) at 15,000 rpm for 5 min, where fish oil was the oil phase and whey protein microgel was the aqueous phase. The whey protein microgels formed the inner layer around the fish oil. This O/W emulsion was dispersed in the beet pectin solution using a magnetic stirrer at 800 rpm. The beet pectin formed the outer layer located on top of the whey protein microgel layer to achieve layer-by-layer electrostatic deposition. Then, microcapsules were obtained by addition of laccase (enzyme/polysaccharide ratio of 0.24/4 mg, respectively), $CaCl_2$ (5 mM) or no addition (control) (Supplementary Table 1), followed by stirring for 12 h at room temperature. The samples were frozen at −80 °C and freeze-dried (Labconco/Kansas City, MO) for 48 h and stored at room temperature for 15 days.

#### 2.2.3. Characterization of microcapsules

Droplet morphology by optical microscopy.

Optical images were obtained using an inverted optical microscope (DMIL LED, Leica) connected to a fast camera (MicroLab 3a10, Vision Research) at different intervals after production (t = 0, 7 and 15 days).

*2.2.3.1. Cryo-scanning electron microscopy (Cryo-SEM)*. Using cryo-SEM, images of the structure of the microcapsules shell and the size of the pores in the interface of fish oil and pectin layer were assessed. Cryo-SEM experiments were performed using a Quorum P3010 system (Qurorum Technologies, Newhaven, UK). The samples were plunge-frozen in liquid nitrogen, transferred under vacuum to the P3010, and coated with gold-palladium (samples maintained at −165 °C in the preparation chamber). The samples were then transferred to the FIB to take images at −165 °C.

*2.2.3.2. Particle size*. The images of the samples obtained using the inverted optical microscope (DMIL LED, Leica) were analyzed using ImageJ program (Version 1.4.3.67) to measure the average particle size.

*2.2.3.3. Zeta potential*. The Zeta potential of the microcapsules was measured using a ZetaSizer Nano-ZS, Nano Series (Malvern, United Kingdom).

*2.2.3.4. Thermogravimetric analysis*. TGA curves were obtained using a Q500 Thermogravimetric Analyzer (TA Instruments/New Castle, DE). The freeze-dried microcapsules were positioned on platinum pans and nitrogen was used in the atmosphere of the furnace at a flow rate of 60 mL/min with a heating rate of 10 °C/min and within the range of 25–600 °C.

*2.2.3.5. Fourier transform infrared spectroscopy (FTIR)*. FTIR spectra of the microcapsules were investigated in the region from 4000 to 400 $cm^{-1}$ (50 scans, resolution of 4 $cm^{-1}$) using an IRAffinity-1S Fourier Transform Infrared Spectrophotometer (Shimadzu Scientific Instruments/Marlborough, Massachusetts-USA).

*2.2.3.6. Oxidative stability by thiobarbituric acid reactive substance (TBARS)*. The oxidative stability was measured with malonaldehyde (MDA) formation using the thiobarbituric acid reactive substance