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Influence of the protein type on the stability of fish oil in water emulsion obtained by glass microfluidic device

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ABSTRACT

The objective of this study was the encapsulation of fish oil (source of omega-3 fatty acids) by glass microfluidic devices using different types, concentrations and combinations of proteins in oil-in-water emulsions to protect the fish oil against oxidation. Formulations of fish oil in gelatin, casein and hydrolyzed whey protein were analyzed via optical and cryo-transmission electron microscopy, and assessed in regards to particle size, zeta potential, structural stability under different stress conditions, interfacial tension, and oxidative stability by thiobarbituric acid reactive substance over 15 days. Following sample preparation and after 15 days storage, zeta potentials ranged from −24 to +11 mV (0 days) and from −16 to −0.54 mV (15 days); particle sizes ranged from 77 to 97 μm and from 79 to 107 μm, respectively. The formulations were stable under different stress conditions; however, the combination of 0.5% (w/w) gelatin and casein (ratio 1:1) promoted the best protection to the fish oil against oxidation. The microfluidic methods presented herein offer a new alternative to encapsulate fish oil and promote its protection and future application in food products.

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1. Introduction

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) in the food industry due to their beneficial anti-inflammatory and anti-arrhythmic properties (Binsi et al., 2017; Endo & Arita, 2016). These two ω-3 fatty acids are part of the composition of fish oil, which makes the inclusion of this oil in human diet essential. However, polyunsaturated fatty acids are extremely susceptible to oxidation when in contact to high temperature, oxygen or light, which makes the handling and application of fish oil in food products more difficult. Additionally, the strong odor associated with fish oil and its degradation often results in the rejection by the consumer.

Encapsulation offers an alternative to minimize the problems related to PUFA instability and the unpleasant odor presented by fish oil. According to Comunian and Favaro-Trindade (2016), the process of microencapsulation consists of the formation of small reservoirs, which are structures that have one or more bioactive materials protected by a polymer or a lipid. Several common microencapsulation techniques include complex coacervation (Comunian et al., 2016a, 2016b), spray drying (Mohammed, Tan, Manap, Alhelli, & Hussin, 2017), liposomes (Ghorbanzade, Jafari, Akhavan, & Hadavi, 2017), ionic gelation (Martins, Poncelet, & Renard, 2017), solid lipid nanoparticles (Ravanfar, Tamaddon, Niakousari, & Moein, 2016), and spray chilling (Matos-Jr, Comunian, Thomazini, & Favaro-Trindade, 2017). These techniques present interesting advantages; complex coacervation and ionic gelation allow the possibility of working with different biopolymers, there is no use of organic solvent and process conditions do not compromise the compounds. With complex coacervation, a real microcapsule (with a core surrounded by one or two polymers) is formed. Spray drying and spray chilling techniques are considered the most versatile and flexible due to their low cost of operation, excellent yield, and production of material with great solubility in water (spray drying) or in oil (spray chilling). In addition, they may be used with solutions, suspensions, and emulsions, among others. Encapsulation within liposomes is advantageous due to their high biocompatibility and versatility. Overall, all of these encapsulation techniques result in the formation of microcapsules/microparticles within a wide range of particle size distribution ranging from μm to mm; this can be problematic since particle size can often have significant influence on texture and behavior when

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these microcapsules/microparticles are applied in food products.

The method by glass microfluidic devices consists of the elaboration of single, double or multi-compartment emulsions that enables highly-controlled production of monodisperse droplets, factors that are difficult to control in other microencapsulation techniques. Moreover, these devices are fabricated with rigid and chemically resistant glass capillaries (Duncanson et al., 2012) that allow the particle size to be controlled according to the capillary diameter and flow rates. This technique additionally offers advantages from an economic and technological point of view, allowing the integration of multiple steps in the same device, and then the increase of the scale. It is a method that has rarely been explored for the encapsulation of food ingredients (Comunian, Abbaspourrad, Favaro-Trindade, & Weitz, 2014, Ravanfar, Comunian, Dando, & Abbaspourrad, 2018).

Additionally, proteins -macromolecules consisting of amino acids formed by carbon, a carboxylic acid moiety, an amine moiety and a radical-have different and important functions, such as acting as essential nutrients for humans, as well as antibodies and enzymes which are crucial for growth and maintenance of the human body, besides its function as surfactants (Damodaran, Parkin, & Fennema, 2010). However, the emulsifying ability may vary from one protein to another, influencing the stability of the system. Some examples of important proteins are gelatin, casein, soy protein and whey protein. Gelatin is the most utilized hydrocolloids in the food industry, having low cost and surfactant properties. Casein, a protein derived from milk, has been of interest to food researchers due to its composition rich in common and essential amino acids. Soy protein isolates offer a low cost protein source with wide availability, and whey proteins are widely used by the food industry and researchers due to their nutritional and functional properties, most notably their surfactant properties (Comunian & Favaro-Trindade, 2016).

In this respect, the objective of this study was to investigate different types of proteins and their combination as surfactants in preparation of fish oil in water emulsions by glass microfluidic devices. Since proteins can be supplemented into food products, this sort of work should allow the development of better carriers with novel functionality and promote both the delivery and protection of fish oil and other nutrients.

2. Material and methods

2.1. Material

Fish oil from menhaden (Sigma Chemical Co., St. Louis, MO, USA) was used as a core in oil-in water emulsion. Gelatin from bovine skin (Sigma Chemical Co., St. Louis, MO, USA), casein sodium salt from bovine milk (Sigma Chemical Co., St. Louis, MO, USA), enzymatically hydrolyzed soy protein (Proyield Soy SE50MK-NK, Friesland Campina Ingredients, Paramus, NJ, USA) and enzymatically hydrolyzed whey protein isolate (BiPro; Agropur Inc, Davisco, Eden Prairie, MN, USA) were used as surfactant.

2.2. Protein preparation

The solutions of gelatin, casein and soy protein were prepared by dissolving 0.5, 1.0 and 1.5% (w/w) proteins in water and keeping them on magnet stirring to obtain completely homogeneous solutions (concentrations determined according to viscosity of solutions). In the case of gelatin, the solution was stirred at 40 °C; for the other proteins, the solutions were prepared at room temperature. The hydrolyzed whey protein was obtained by magnetic stirring of a mixture of whey protein in distilled water for 2 h, following by addition of fungal protease (Mak Wood, Inc; Grafton, WI) at 1 g enzyme per 100 g protein at pH 8.0. The mixture was kept at 40 °C for 24 h. The solution was then spray dried (Spray Dryer Armfield, Ringwood, England) with inlet and outlet air temperatures of 170 and 70 °C and feed flow rate of 3.5 L/h. The powder obtained after drying was used for the preparation of all hydrolyzed whey protein solutions.

2.3. Encapsulation method

In order to obtain the fish oil-in water (O/W) emulsions, the technique of glass microfluidic device was used according to Comunian et al. (2014). The round and square capillaries were purchased from World Precision Instruments, Inc., Sarasota, Florida, United States and Harvard Borosilicate Square Tubing, respectively. A glass device designed for double emulsion was used (Fig. 1) so that the middle and continuous phase were the same solutions, consisting of different types and concentrations of proteins (Table 1). The ratio of 1:1 was used for the formulations with two proteins. The inner phase consisted of fish oil. The flow rates used for the elaboration of each fish oil-in water emulsion were presented in Table 1. All fluids were pumped through microfluidic device by using the syringe pump (New Era Pump Systems, Inc/Farmingdale, New York, USA). The samples were collected in a falcon tube containing the same aqueous solution which was used as continuous phase.

2.4. Characterization of the emulsions

2.4.1. Morphology of the droplets by optical microscopy

The optical images were obtained using an inverted optical microscope (DMIL LED, Leica/Buffalo Grove, United States), with a magnification of 100×, connected with a fast camera (MicroLab 3a10, Vision Research) with the samples stored at room temperature during 15 days.

2.4.2. Particle size

The average particle size was analyzed using the ImageJ program (Version 1.4.3.67), where 50 droplets of each treatment were measured individually. The images were obtained by inverted optical microscope (DMIL LED, Leica/Buffalo Grove, United States), with a magnification of 100× with the samples stored at room temperature for 15 days.

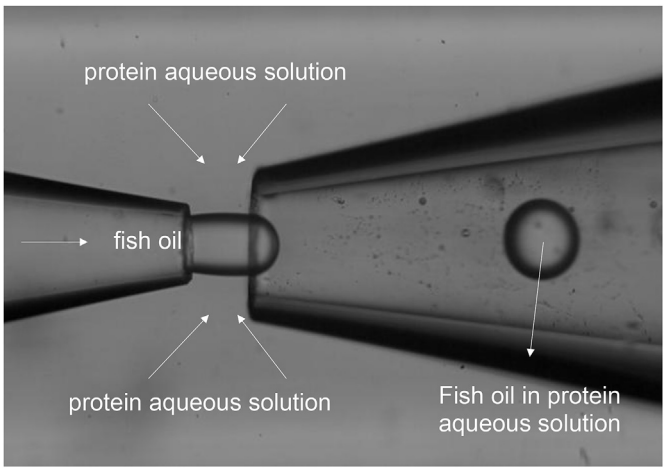

Fig. 1. Scheme of the single emulsion formation inside the glass microfluidic device.

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