



Improving oxidative stability of echium oil emulsions fabricated by Microfluidics: Effect of ionic gelation and phenolic compounds



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ABSTRACT

Echium oil is rich in omega-3 fatty acids, which are important because of their benefits to human health; it is, however, unstable. The objective of this work was the coencapsulation of echium oil and quercetin or sinapic acid by microfluidic and ionic gelation techniques. The treatments were analyzed utilizing optical and scanning electron microscopy, encapsulation yield, particle size, thermogravimetry, Fourier transform infrared spectroscopy, stability under stress conditions, and oil oxidative/phenolic compound stability for 30 days at 40 °C. High encapsulation yield values were obtained (91–97% and 77–90% for the phenolic compounds and oil) and the encapsulated oil was almost seven times more stable than the non-encapsulated oil (0.34 vs 2.42 mg MDA/kg oil for encapsulated and non-encapsulated oil, respectively). Encapsulation was shown to promote oxidative stability, allowing new vehicles for the application of these compounds in food without the use of solvents and high temperature.

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1. Introduction

Omega-3 fatty acids (ω -3) are widely used in food and pharmaceutical products due to their benefits to human health (Ghorbanzade, Jafari, Akhavan, & Hadavi, 2017). A source of ω -3 is the echium seed oil (*Echium plantagineum* L.), which contains from 9 to 16% stearidonic fatty acid, an intermediate in the biosynthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), also important for human nutrition (Berti et al., 2007; Clough, 1993; Payne, Lad, Foster, Khola, & Gray, 2014). Echium oil also has a ratio of omega-3 to omega-6 ideal for health, which is not found in any another type of oil (Berti et al., 2007). The use of this oil in the food industry is hampered due to the instability of its unsaturated fatty acids when they come into contact with light, oxygen and heat. Two strategies that could minimize these limitations, singly or together, are: (1) adding compounds with antioxidant activity—such as phenolic compounds—or (2) microencapsulation.

Phenolic compounds have been of great interest in the food industry due to their activity as antioxidant agents (Abdallah, Salama, Abd-Elrahman, & El-Maraghy, 2011; Lee et al., 2013), in

addition to their other health benefits, such as the prevention of cancer, inflammation and neurodegenerative diseases (Esfanjan & Jafari, 2016). Sinapic acid is the major phenolic acid of canola (Rawel & Rohn, 2010) and has been studied for its neuro-protective effects against Alzheimer's disease (Lee et al., 2012), cardiac hypertrophy and dyslipidemia (Pari & Jalaludeen, 2011; Roy & Prince, 2013). Quercetin, another phenolic compound, is one of the most common flavonoids present in nature and has exhibited anti-stress, anti-inflammatory and anti-cancer properties (Waterhouse, Wang, & Sun-Waterhouse, 2014).

Microencapsulation consists of a broad class of techniques in which one or more bioactive materials are contained or immobilized by one or more polymers or lipids, protecting the encapsulated material against environmental conditions to facilitate handling, application and storage of these materials (Comunian & Favaro-Trindade, 2016). Ionic gelation is one such technique, often used to form microparticles whose wall material is composed of a gel-forming polymer, not requiring the use of solvents or high temperatures. Ionic gelation, however, does not afford good control of particle size (which can subsequently range from μ m to mm), which can negatively affect resultant food texture. By coupling ionic gelation with microencapsulation using microfluidics, this limitation could be overcome.

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Microencapsulation by microfluidics is a promising method for the production of monodisperse droplets—not to mention capsules with multiple compartments—which allows greater control and optimization of the encapsulation efficiency and release of active agents (Zhao et al., 2011; Comunian, Abbaspourrad, Favaro-Trindade, & Weitz, 2014). A microfluidic device consists of coaxial assemblies of a series of rigid glass capillaries resistant to chemicals; their three-dimensional geometry allows for the controlled production of multiple emulsions. This technique has been little explored for the encapsulation of food ingredients.

The objective of this work was the encapsulation of echium oil by using a combination of ionic gelation and microfluidic techniques. In addition, a phenolic compound (quercetin or sinapic acid) was also tested at various concentrations, offering benefits to the consumer's health, protection to the encapsulated compounds and the possibility of controlled release. No research related to the encapsulation of these compounds by the combination of these technologies was found in the literature, which suggests the innovative nature of this study.

2. Material and methods

2.1. Materials

Echium oil (NEWMega™ Echium Oil, Ref.15200, De Wit Specialty Oils, De Waal, Tescel, The Netherlands) was used as core. Sinapic acid and quercetin (Sigma Chemical Co., St. Louis, MO, USA), sodium alginate (Manugel GHB, FMC/Philadelphia, Pennsylvania), corn oil (Mazola, ACH Food Companies), soy lecithin Ultralect P (ADM/Decatur, IL, USA) and Tween 80 (Pepsico/Delaware, USA) were used as antioxidants, wall material, continuous phase, and hydrophobic and hydrophilic surfactant, respectively. Calcium chloride hexahydrate (Fisher Scientific, Waltham, MA, USA) and EDTA disodium salt (Fisher Scientific/Waltham, MA, USA) were used for the formation of a complex that would promote the gelation of the polymer.

2.2. Methods

2.2.1. Microencapsulation process

The encapsulation process was performed using a glass microfluidic device (Fig. 1a and b) as described by Comunian et al. (2014). The cylindrical capillaries used (World Precision Instruments, Inc., Sarasota, Florida, United States) had a diameter and length of 1 mm and 6 in, respectively. These capillaries were inserted into a square capillary (Harvard Borosilicate Square Tubing), with outer and inner diameters of 1.5 and 1.05 mm, respectively. An oil-in-water-in-oil (O/W/O) double emulsion was produced using this device, composed of echium oil in the internal oil phase, sodium alginate aqueous solution in the middle phase, and corn oil and soy lecithin in the continuous phase.

Five encapsulation treatments were performed, which either had quercetin added to the inner phase or sinapic acid added to the middle phase (at two different concentrations each), or no phenolic compound added at all (see Table 1). The flow rates used were 1300, 870, and 5500 $\mu\text{L/h}$ for the inner, middle and continuous phases for both the control treatment and the S050-Alg treatment; 1300, 870, and 5700 $\mu\text{L/h}$ for the Q500-Alg treatment; 1300, 970, and 5300 $\mu\text{L/h}$ for the S025-Alg treatment; and 1500, 870, and 5000 $\mu\text{L/h}$ for the Q800-Alg treatment. The flow rates were determined in preliminary testing, pumped into the microfluidic device using a syringe pump (New Era Pump Systems, Inc/Farmingdale, New York, USA).

In combination with the microfluidic technique, the internal ionic gelation technique was used according to Utech et al.

(2015). In order to prevent the coalescence of the microcapsules and to obtain a faster gelation process for sodium alginate, a calcium-EDTA complex was prepared by mixing a solution of calcium chloride and a solution of EDTA disodium salt, both at 0.1 M. This complex was added to a 2% (w/w) solution of sodium alginate, in equal ratios, resulting in a final alginate concentration of 1% (w/w). The pH of the solution was adjusted to 10. The double emulsion droplets were collected in a plastic beaker containing corn oil, 2% (w/w) soy lecithin and 5% (v/v) glacial acetic acid, with constant stirring, which promoted the gelation of sodium alginate and the formation of microcapsules.

During production of the O/W/O double emulsion (Fig. 1a and b), after the sodium alginate gelation, the microcapsules were transferred from an oil medium to a water medium to facilitate handling and control of the material. After sedimentation of the capsules, they were transferred with a pipette into an aqueous solution with 1% (w/w) Tween 80 and centrifuged at 4000g for 10 min to separate the oil and aqueous phases. Due to the difference in density, the microcapsules remained in the aqueous phase. The aqueous solution was washed several times to remove Tween 80 from the media. For specific characterization analysis, the aqueous phase with the microcapsules was frozen in an ultra-freezer (-80°C) and lyophilized (LabConco freeze-dryer, LabConco Corporation/Kansas city, MO) for 24 h to produce a powder.

2.2.2. Encapsulation yield of sinapic acid, quercetin and oil

The yield was determined as a function of the mass difference between the total amount used in the encapsulation process and the amount of compound actually retained in the microcapsules after the process. The capsules were ruptured by adding a 0.1 M EDTA disodium salt solution and then it was mixed using a vortex mixer for 1 min. For the encapsulation yield of the phenolic compounds, methanol was added to the mixture in order to extract them. The samples were stirred with vortex again for 1 min and centrifuged (Heraeus Multifuge X1R Centrifuge, Thermo Scientific/Waltham, Massachusetts – USA) at 5000g for 3 min at room temperature. The concentrations of sinapic acid and quercetin were determined in the supernatant using UV–Vis spectrophotometry (UV–Vis Spectrophotometer UV-2600, Shimadzu Scientific Instruments/Marlborough, Massachusetts – USA) at wavelengths of 321 and 371 nm for sinapic acid and quercetin, respectively.

For the encapsulation yield of echium oil, after the capsule rupture, hexane was added to the solution in order to extract the oil, then the solution was mixed with a vortex for 1 min. The sample was centrifuged (Heraeus Multifuge X1R Centrifuge, Thermo Scientific/Waltham, Massachusetts – USA) at 8000g for 3 min at room temperature, and the supernatant containing hexane and oil was transferred to an eppendorf and maintained at 60°C until complete evaporation of the solvent. The same extraction process was performed twice to ensure that all the oil was quantified.

2.2.3. Morphology of the microcapsules by optical and scanning electron microscopy

The optical images were obtained using an inverted optical microscope (DMIL LED, Leica) connected to a fast camera (Micro-Lab 3a10, Vision Research). The scanning electron microscopy images of the microcapsules were obtained using a scanning electron microscope (LEO 1550 FESEM (Keck SEM) (Carl Zeiss, New York/USA)). Before taking the SEM images, the wet emulsions were placed on the SEM stub and allowed to dry for 24 h at room temperature.

2.2.4. Particle size and size distribution

The particle size and particle size distribution analyses were performed using ImageJ (NIH, Bethesda, MD), where 100 microcapsules for each treatment were measured individually.

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