# Co-encapsulation of echium oil and sinapic acid or quercetin by combined methods: microfluidic devices and ionic gelation

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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 co-encapsulation of echium oil and quercetin or sinapic acid by microfluidic and ionic gelation techniques. The treatments were analyzed according to morphology, encapsulation yield, particle size, thermogravimetry, stability of phenolic compounds and oxidative stability by thiobarbituric acid reactive substance. 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 one ($0.34x10^{-6}$ and $2.42x10^{-6}$ 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.*

**Key-Words**: *ômega-3 fatty acids; phenolic compounds; microencapsulation, double emulsion, sodium alginate*

**Introduction:** Omega-3 fatty acids (ω-3) are used in food products due to their benefits to human health. A source of ω-3 is echium seed oil (*Echium plantagineum* L.), which has a ratio of ω-3 to ω-6 ideal for health [1]. However, they are unstable, what makes their application into food industry difficult. Two strategies that could minimize these limitations are: (1) adding a compound with antioxidant activity—phenolic compound—and (2) microencapsulation. Phenolic compounds have been of great interest because they can act as antioxidant agents [2]. Two examples of phenolic compounds are sinapic acid and quercetin. Moreover, microencapsulation is a technique in which one or more materials are contained by one or more polymer, that would protect the encapsulated material against environmental conditions [3]. Ionic gelation is one technique used to form microparticles whose wall material is composed of a gel-forming polymer and does not require the use of solvents or high temperature; however, it does not afford good control of particle size, which negatively affects food texture. By coupling the ionic gelation technique with the microencapsulation technique by microfluidics, this limitation could be overcome. Microfluidics is a promising method for the production of monodisperse droplets, which allows greater control and optimization of the encapsulation efficiency [4]. The objective of this work was the encapsulation of echium oil by a combination of ionic gelation and microfluidic techniques using quercetin or sinapic acid at different concentrations.

**Experimental:** The encapsulation process was performed on a glass microfluidic device [4]. An oil-in-water-in-oil double emulsion was produced, 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, obtaining five treatments (Table 1). A calcium-EDTA complex was prepared by mixing a solution of calcium chloride and a solution of EDTA disodium salt. This complex was added to a solution of sodium alginate. The pH of the solution was adjusted to 10. The double emulsion droplets were collected in corn oil, soy lecithin and glacial acetic acid, with constant stirring. After sedimentation of the capsules, they were transferred into an aqueous solution and centrifuged at 4000 g/ 10 min and the microcapsules remained in the aqueous phase. The microcapsules were analyzed regarding to encapsulation yield (EY) of sinapic acid, quercetin and oil, morphology by optical and scanning electron microscopy, particle size, thermogravimetry (TGA), stability of quercetin and sinapic acid and oxidative stability by thiobarbituric acid reactive substance (TBARS).

**Results and discussion:** For the production of microcapsules, a Ca-EDTA complex was added into the sodium alginate solution. The gelation of alginate occurred when the droplets were collected in corn oil containing acetic acid. The acid diffuses into the droplets, causing the pH reduction and the dissociation of calcium ions from the Ca-EDTA complex. The released calcium ions react with the alginate chains, promoting the gelation of the polymer and the formation of the microcapsules [5].

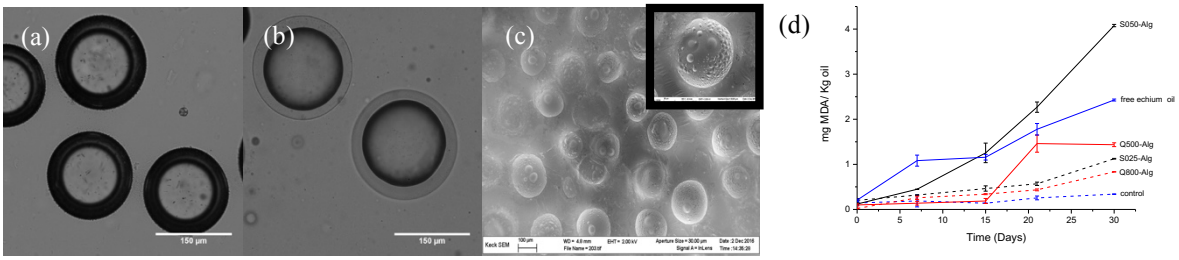
In relation to morphology, images before and after gelation and a SEM are shown in Figures 1a, 1b and 1c. The treatments did not show morphological differences and presented particles adhering to the surface of the microcapsule (due to the presence of soy lecithin). There were no significant differences in relation to the EY among treatments (Table 1), so the addition of phenolic compound does not influence EY. Values for particle size were in the range of 117-213 and 115-227 µm before and after gelation. The control produced smaller particle size due to presence of phenolics, which caused an increase in solution viscosity and influenced the size. Regarding to TGA, all treatments displayed mass loss in three steps: 25-120, 200-350 and 350-450 °C, which correspond to the loss of water, decomposition of alginate and oil; and Q800-Alg was the most thermally stable. Regarding to stability of phenolic compounds, retention of the encapsulated ones was 25–30% after a 30-day storage, while for the non-encapsulated compounds, it was 16 and 24% for sinapic acid and quercetin. Oxidative stability by TBARS (Figure 1d) showed that non-encapsulated oil presented a higher formation of malonaldehyde compared to the other treatments, with the exception of S050-Alg. It was expected since the presence of a higher concentration of sinapic acid in the capsule wall destabilized its structure.

Table 1: Composition of each treatment and values of encapsulation yield for phenolic compounds and oil.

| Treatments | Inner phase | Middle phase | EY for phenolic compounds (%) | EY for the oil (%) |
|---|---|---|---|---|
| Control | Echium oil | Alginate | -- | 85.39 ± 10.74[a] |
| S025-Alg | Echium oil | Alginate + 0.025g sinapic acid/ g alginate | 91.33 ± 18.74[a] | 83.29 ± 10.56[a] |
| S050-Alg | Echium oil | Alginate + 0.05g sinapic acid/ g alginate | 94.04 ± 23.19[a] | 77.81 ± 1.80[a] |
| Q500-Alg | Echium oil + 500 ppm of quercetin | Alginate | 97.90 ± 4.60[a] | 90.61 ± 3.99[a] |
| Q800-Alg | Echium oil + 800 ppm of quercetin | Alginate | 93.19 ± 13.98[a] | 82.47 ± 1.41[a] |

Equal letters in the same column do not differ statistically at level of 5% by the Tukey test.

Figure 1: (a) Optical microscopy before and (b) after gelation; (c) scanning electron microscopy and (d) oxidative stability of encapsulated and non-encapsulated echium oil by thiobarbituric acid reactive substance (TBARS).




**Conclusion:** Microencapsulation of echium oil by a combination of microfluidic and ionic gelation techniques is feasible, since high values of encapsulation yield and oxidative stability of echium oil during storage at 40 °C were obtained. The Q800-Alg treatment showed the best thermal stability, followed by the control, suggesting that this type of microcapsule can be applied to different products.

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