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Production of stabilized quercetin aqueous suspensions by supercritical fluid extraction of emulsions

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ABSTRACT

Quercetin is a flavonoid with highly promising bioactivity against a variety of diseases, due to its strong antioxidant, antiviral and antihistaminic effect, but these applications are limited by the low solubility of quercetin in gastrointestinal fluids and the correspondingly low bioavailability. The objective of this work is to produce encapsulated quercetin particles in sub-micrometric scale, in order to increase their low bioavailability. These particles were produced by extraction of organic solvent from oil in water emulsions by supercritical fluid extraction of emulsions (SFEE). Due to the rapid extraction of organic solvent by this method, the disperse organic phase becomes rapidly supersaturated, causing the precipitation of quercetin particles in sub-micrometric scale, encapsulated by the surfactant material. Two different biopolymers (Pluronic L64® poloxamers and soy bean lecithin) were used as carriers and surfactant materials. In experiments with Pluronic, needle quercetin particles were obtained after SFEE treatment, with particle sizes around 1 μm and poor encapsulation efficiency. In case of soy lecithin, quercetin-loaded multivesicular liposomes were obtained, with a mean particle size around 100 nm and around 70% encapsulation efficiency of quercetin, without presence of segregated quercetin crystals.

1. Introduction

Quercetin (3,3′,4,4′,5,7-pentahydroxyflavone, chemical structure presented in Fig. 1) is a bioflavonoid, available in various fruits, vegetables and oils. It can scavenge reactive oxygen species, and down-regulate lipid peroxidation due to its ion chelating and iron stabilizing effect [1]. Furthermore it can promote the oxidation of Fe^{2+} to Fe^{3+}, which is less effective in generating free radicals. These effects of quercetin may be explained by its o-diphenol B-ring structure [2] and the ability of donating π electrons from the benzene ring, while it is remaining relatively stable [3]. It has also anti-proliferative effects in a wide range of human cancer cell lines [4]. Due to these properties, quercetin is a highly promising active compound against a wide variety of diseases.

A major limitation for the clinical application of quercetin is its low bioavailability, that makes it necessary to administrate high doses (50 mg/kg) [5]. Due to the low water solubility of quercetin, it has a minimal absorption in the gastrointestinal tract, and its oral bioavailability is lower than 17% in rats [6] and lower than 1% in humans [7]. Different approaches have been proposed in literature to increase the bioavailability of quercetin. Mulholland et al. [8] synthesized a water-soluble derivative of quercetin, but its bioavailability was only 20%. Also, to increase the bioavailability of this poorly water soluble compound, drug loaded solid lipid nanoparticles could be a promising alternative, and the complexation of quercetin with lecithin and cyclodextrin in aqueous solution has been tested [9,10]. Li et al. [11] produced lecithin encapsulated quercetin by emulsification and low-temperature solidification, with over 90% drug entrapment efficiency in spherical particles of an average diameter of 155 nm was observed. Heterogeneous morphologies were obtained with a co-existence of additional colloidal structures, like micelles, liposomes, supercooled melts, drug nanoparticles, which caused a certain scatter in the particle size distribution, with particle sizes spanning the range from 20 nm to 500 nm. The absorption rate of quercetin loaded solid lipid nanoparticles was studied by in situ perfusion method in rats, obtaining a 6-fold relative increase in bioavailability, compared to unprocessed quercetin.

Supercritical fluids are another promising alternative in the processing of natural bioactive compounds, such as quercetin, because they allow carrying out the encapsulation process at near ambient temperatures, and in an inert atmosphere, thus avoiding the thermal degradation or oxidation of the product and reducing its contamination with organic solvents. Several authors have

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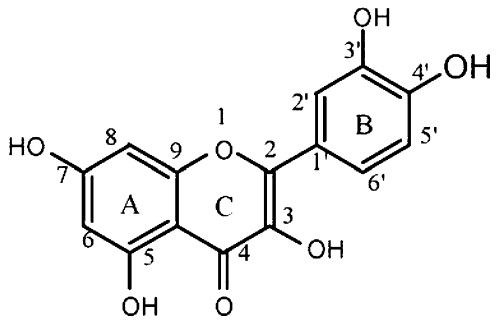

Fig. 1. Chemical structure of quercetin [5].

studied the processing of quercetin by supercritical fluid technologies. Due to the low solubility of quercetin in supercritical carbon dioxide [12], supercritical antisolvent (SAS) experiments have been particularly successful. By SAS processing of pure quercetin, crystalline particles with particle sizes in the micrometer range (1–6 μm) have been obtained [13–15]. Fraile et al. [16] produced quercetin particles encapsulated with Pluronic F127 by SAS technology. As in previous works, SAS-processed pure quercetin crystallized as needle like particles, meanwhile quercetin co-precipitated with Pluronic had a totally different spherical morphology, indicating that Pluronic F127 was able to successfully encapsulate quercetin. Higher particle sizes were obtained when the quercetin/Pluronic mass ratio was increased, due to the possible aggregation of the polymer shells. Obtained morphologies indicate, that quercetin particles acted as nucleation sites for the formation of a polymer film, and this film of polymer restrained the growth of quercetin particles above the mass ratio of 1/1 = quercetin/Pluronic. With this encapsulation method, the solubility of quercetin in simulated intestinal fluid was increased by a factor of 8.

Supercritical fluid extraction of emulsions (SFEE) technology can be considered as an evolution of SAS technology, which is especially suitable to encapsulate poorly water soluble drugs in an aqueous suspension. The process consists of forming an oil-in-water emulsion, containing the water-insoluble drug in the dispersed organic phase. By SFEE, the organic solvent is extracted from this emulsion by the supercritical solvent, which should have high affinity to the organic solvent and a low affinity to the active compound of interest. Due to the solubility differences, the supercritical solvent quickly extracts the organic solvent from the emulsion, leading to the rapid super-saturation of active compound, and hence a fast precipitation. Meanwhile in the SAS antisolvent precipitation method particle nucleation and growth occur across the whole solution volume, in the case of SFEE the formation of particles is confined within the emulsion droplets. This restrains the size of the particles obtained that can be one order of magnitude smaller than particles produced by solution precipitation [17].

Mattea et al. studied the precipitation of β-carotene by continuous SFEE in order to model the process [18]. In this study submicro- and nano-particles were obtained with a residual organic content as low as 1 ppm. The obtained particle size distribution was directly related with the droplet size distribution of the initial emulsion, while residual organic content depended on the process parameters, such as the pressure and the temperature. Model results showed that the saturation of organic phase droplets with CO_2 caused a rapid antisolvent effect, which in the continuous implementation of the process can take place during the drop fly time, while the elimination of the residual organic solvent was much slower. Based on experimental and model results, a two-step process strategy can be proposed. The first step would involve contact between emulsion and CO_2, to ensure the saturation of the disperse phase, in order to achieve precipitation by antisolvent effect. A second step would involve an extended contact between CO_2 and emulsion, in order to eliminate the remaining organic solvent. This step might be slower than the first, because once the particles are formed, emulsion destabilization is no longer a problem. In a subsequent work, Santos et al. extended this approach to the precipitation of lycopene [19].

The aim of this study is to apply the supercritical fluid extraction of emulsion process to the encapsulation of quercetin. Based on the available information, two different carrier materials that have been found to increase the water solubility and the bioavailability of quercetin have been tested: Pluronic block copolymers, and soybean lecithin. The influence of the main process parameters has been studied, including properties of the initial emulsion, extraction time and extraction conditions. The performance of the process has been evaluated analysing the encapsulation efficiency and particle size and morphology of the final aqueous suspensions.

2. Experimental

2.1. Materials

Quercetin hydrate ($C_{15}H_{10}O7{\cdot}H_2O$, 95% purity, CAS: 849061-97-8) was obtained from Acros Organics (New Jersey, USA). The surfactant material poly-(ethylene glycol)-block–poly-(propylene glycol)-block-poly-(ethylene glycol) (Pluronic L64, CAS: 9003-11-6) was obtained from Sigma–Aldrich (St. Louis, USA). Soy lecithin was obtained from Glama-Sot (SOTYA, Madrid, Spain). Ethyl acetate (EtAc, CAS: 141-78-6) and methanol (MeOH, CAS: 67-56-1), with a purity of 99% and 99.9%, respectively, were obtained from Panreac Química (Barcelona, Spain). Acetonitrile (CAS: 75-05-8); acetic acid (reference number: 211008.1211) with a purity of 99.9% and 99.5%, respectively, were obtained from Panreac Química (Barcelona, Spain). Carbon dioxide was provided by Carburos Metálicos (Barcelona, Spain).

2.2. Emulsion preparation and supercritical extraction of the emulsion

The initial emulsion was prepared using an Ultraturrax IKA LABOR-PILOT 2000/4 (IKA-WERKE GMBH&CO.KG) high frequency mixing device with a cooling jacket. The required amount of quercetin was dissolved in an organic solvent (ethyl acetate), and a required amount of surfactant material (Pluronic L64 or lecithin) was dissolved in water, purified by Millipore Elix. Then these two solutions were mixed together by a magnetic stirring for 5 min, in order to obtain a homogeneous dispersion. Afterwards, the dispersion was mixed by the Ultraturrax emulsifier at 70 Hz frequency for a predefined time.

To extract the organic solvent from the initially prepared emulsion, a batch SFEE equipment – presented in Fig. 2 – was used. The equipment consists of two vessels: an extractor vessel with a volume of 85 mL, and a buffer vessel with a volume of 100 mL. The vessels are located in a thermostated oven, and are separable from each other by two valves.

Firstly, the equipment was pressurized with $scCO_2$ and thermostated (typically, at 110 bar and 40 °C). Afterwards 25 mL of the initially prepared emulsion was injected by an HPLC into the extraction vessel. The emulsion was loaded after the pressurization of the system, as otherwise the disturbances caused by the addition of CO_2 can spill the emulsion out from the extraction vessel into the recirculation circuit, making it difficult to recollect the treated emulsion after the experiment. Then the HPLC pump was isolated from the circuit by closing the valve in its impulsion, and the CO_2 recirculation pump was switched on, starting the circulation of the $scCO_2$ between the CO_2 buffer vessel and the extraction vessel. During