J. of Supercritical Fluids 104 (2015) 281–290

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Production of water-soluble quercetin formulations by antisolvent precipitation and supercritical drying

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ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 3 July 2015
Accepted 4 July 2015
Available online 13 July 2015

Keywords:
Quercetin
Antioxidant
Lecithin
Antisolvent precipitation
Turbulent mixing
Supercritical CO_2 drying

ABSTRACT

Quercetin is one of the most prominent dietary antioxidants. The application of quercetin in food, cosmetic and pharmaceutical industries due to its properties, requires an adequate formulation in order to provide protection against degradation, to increase the solubility in aqueous systems and to improve the bioavailability. This work presents a study of the encapsulation of quercetin in water-soluble carriers by antisolvent precipitation using three different techniques: shear mixing, continuous flow turbulent mixing at atmospheric pressure and ambient temperature and turbulent mixing at high pressure and high temperature. A preliminary study of quercetin formulation by shear mixing showed that modified OSA-starch and Pluronic L-64 were not good carrier materials due to the low encapsulation efficiencies and high particle sizes obtained. However, using lecithin as carrier material, it was possible to obtain stable and homogeneous aqueous dispersions of quercetin particles with encapsulation efficiencies up to 90%. By supercritical drying, spherical particles with particle sizes in the range of 10 μm were obtained that could be used to reconstitute the aqueous suspension, maintaining the encapsulation efficiency of quercetin.

1. Introduction

Quercetin (3,3′,4′,5′-7-pentahydroxy flavone), a member of the flavonoids family, is one of the most prominent dietary antioxidants. Quercetin can be found in fruits and vegetables such as apples, grapes, strawberries and onions, as well as in red wine and black tea [1,2]. Quercetin shows important biological activities, including anti-inflammatory, antibacterial and antiviral effects [3]. Moreover, some preliminary studies suggest that quercetin can provide protection against various diseases such as osteoporosis, certain forms of cancer, or pulmonary and cardiovascular diseases, and also against aging [4]. However, quercetin shows poor aqueous solubility (2 ppm at 25 °C [5]) and low bioavailability of less than 17% in rats [6] and 1% in humans [7]. In addition, quercetin is quickly degraded in alkaline conditions and it is also sensitive to heat exposure [8]. On the other hand, therapeutic antioxidant concentrations are between 10^{-6} and 10^{-5} M [9] while dietary blood concentrations are in range of 10^{-9}–10^{-7} M [10]. In order to avoid degradation, improve dispersibility in aqueous systems and increase bioavailability, an efficient quercetin formulation is required [11].

Formulations of quercetin with different carrier materials have been extensively studied due to the beneficial properties of quercetin for pharmaceutical, cosmetic and food applications. Li et al. [3] developed amorphous solid dispersions (ASD) of quercetin in cellulose derivative matrices by spray-drying technique. They demonstrated that these ASDs presented a superior stability against re-crystallization processes. Gao et al. [12] compared quercetin nanosuspensions prepared with Pluronic F68 and lecithin by evaporative precipitation into aqueous solution (EPAS) process and high pressure homogenization (HPH) process achieving better results by EPAS, with a drug solubility as high as 422 ppm. Dissolution enhancement of quercetin was studied by Kakran et al. [11] forming complexes with β-cyclodextrin and solid dispersions with polyvinylpyrrolidone and Pluronic F127 using evaporative precipitation of nanosuspensions, showing that the dissolution rate was much higher than that of the raw material. Pluronic F127 was also used by Fraile et al. [13] in order to encapsulate quercetin by supercritical antisolvent (SAS) process, obtaining an enhancement in solubility and dissolution rate. Li et al.[14] produced quercetin-loaded solid lipid nanoparticles prepared by emulsification and low temperature solidification method in order to study the gastrointestinal absorption of quercetin. They observed an improved

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http://dx.doi.org/10.1016/j.supflu.2015.07.006
0896-8446/

relative bioavailability (more than 5 fold) of the quercetin-loaded solid lipid nanoparticles to quercetin suspension in rats after oral administration. The same method was used by Chen-yu et al. [15] for the development of a quercetin loaded nanostructured lipid carriers as a topical delivery system. They achieved a high entrapment efficiency (90%) and results demonstrated that quercetin loaded nanostructured lipid carriers could promote the permeation of quercetin and increase the amount of quercetin retention in epidermis and dermis. Topical delivery of quercetin was also studied by Bose and Michniak-Kohn [16] using lipid based nanosystems (solid-lipid nanoparticle and nanostructured lipid carrier) prepared by probe ultrasonication method, obtaining the highest improvement in topical delivery of quercetin with the nanostructured lipid carrier system. Kumari et al. [1] studied the encapsulation of quercetin in poly-D-L-lactide (PLA) nanoparticles by solvent evaporation method, obtaining a quercetin formulation with an encapsulation efficiency of 96.7% and a particle size of 130 nm. Ha et al. [17] also investigated the encapsulation efficiency of quercetin using linoleic acid-modified chitosan oligosaccharide/β-lactoglobulin nanoparticles developed by subambient temperature treatment, achieving a maximum encapsulation efficiency of 55.6%. Quercetin nanoparticles were also developed by Wu et al. [18] They prepared the nanoparticles with a nanoprecipitation technique using Eudragit® E and polyvinyl alcohol (PVA) as carrier materials obtaining an encapsulation efficiency of 99% and a release of quercetin 74-fold higher compared to the pure quercetin. High pressure homogenization process was used by Aditya et al. [19] in order to encapsulate quercetin in lipid nanocarriers, achieving encapsulation efficiencies above 90%.

Several of these techniques produce aqueous suspension of quercetin particles. The removal of water from those suspensions is an important process step, as it is important to avoid a possible modification of the properties of particles or a chemical degradation of the compound during drying. Besides the conventional spray-drying o freeze drying methods, some innovative methods have been proposed which are based on the use of supercritical carbon dioxide, and achieve an efficient water removal while operating at moderate temperatures. Meterc et al. proposed a modification of the PGSS method denominated PGSS-drying for the processing of aqueous solutions, and applied it to the drying of green tea extracts [20]. Compared with a normal spray drying process, PGSS-drying enabled an improved atomization of the solution due to the effervescent boiling of CO_2 from the solution during the expansion [21] As a result of this improved atomization, it is possible to operate the process at lower temperatures than the conventional spray-drying method, thus making it possible to dry low-melting point carriers such as polyethylene glycol [22] or lecithin [23]. More recently, Nuchuchua et al. developed a supercritical fluid spray drying process [24]. This process can be operated at nearly isobaric conditions, which is an important aspect for an economically feasible scaling-up of the process.

The objective of this work is the development of formulations of quercetin with an increased solubility and stability in aqueous media. For this purpose, the encapsulation of quercetin in three different carrier materials has been studied: Pluronic L-64, modified OSA-starch and soybean lecithin. These materials have been chosen according to their successful use in previous studies, as described in the previous paragraphs, and as representative of important categories of hydrophilic carriers: Pluronic polaxamers are biocompatible synthetic surfactants with a high surfactant activity that are able to self-assemble into micelles in aqueous systems above the critical micelle concentration, allowing the encapsulation and the enhancement of the absorption of hydrophobic active compounds inside the micelles [25]. On the other hand, modified *n*-octenyl succinate anhydride (OSA) starch was chosen as a surfactant from natural origin because it has been demonstrated that it is suitable for encapsulating flavors, vitamins and spices, among others [26–29]. With respect to the lecithin, it is a mixture of naturally occurring phospholipids which are constituents of cell membranes and are present in food products from plant and animal sources. Liposomes are formed spontaneously by self-assembly of such phospholipids in water, forming vesicles consisting of an aqueous medium surrounded by a lipid membrane which can encapsulate hydrophilic substances in their inner cavity as well as hydrophobic compounds inside the lipid bi-layer [30,31].

In this work, a preliminary study of quercetin formulation by shear mixing has been developed using an Ultra-Turrax high-shear mixing device followed by solvent evaporation, as a simple, conventional method for the preparation of formulations. This method has been used to compare the use of the three different carrier materials, the influence of the organic/water ratio and the effect of using two different organic solvents. From these preliminary results, the formulation of quercetin by antisolvent precipitation under continuous flow turbulent mixing at ambient temperature and pressure has been developed. The influence of the main process parameters on product characteristics (encapsulation efficiency and particle size) has been studied, analyzing the concentrations of quercetin and carrier material, the organic/water ratio and the organic solvent used. Then, the same turbulent mixing process, but in this case operating at high temperatures and high pressures, has been investigated in order to observe the effects of those process conditions on the quercetin formulations. For further processing, quercetin solutions prepared by antisolvent precipitation have been dried with supercritical CO_2 in order to obtain a dry powder formulation, from which an aqueous dispersion can be reconstituted. The antioxidant activity of formulations has been characterized.

2. Materials and methods

2.1. Materials

Quercetin (QC) with a minimum purity of 95% was purchased from Sigma–Aldrich. Soybean lecithin (97% phospholipids) was obtained from De Tuinen B.V. (The Netherlands) and Glama-Sot (SOTYA, Madrid, Spain). Modified OSA-starch, an *n*-octenyl succinic anhydride (OSA)-dextrin derived from waxy maize, was supplied by National Starch Group (Hamburg, Germany). Poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) Pluronic L-64 (hydrophilic-lipophilic balance HLB in the range of 12–18) was provided by Sigma–Aldrich. Acetone, with a minimum purity of 99.7% (v/v) and 99.5% (v/v) was purchased from Carl Roth (The Netherlands) and Panreac Química (Spain). Ethanol with a purity of 100% (v/v), was supplied by Boom BV (The Netherlands). Carbon dioxide was provided by Linde (The Netherlands).

2.2. Ultra-Turrax high-shear mixing and organic solvent evaporation

An Ultra-Turrax®emulsifier (T25 basic IKA®-WERKE) was used to prepare solutions. A rotavapor (BÜCHI Switzerland Rotavapor R-210, BÜCHI Switzerland Vacuum Controller V-850, BÜCHI Switzerland Vacuum Pump V-700, BÜCHI Switzerland Heating Bath B-491) was used to eliminate the organic solvent from the prepared solutions. A dissolution of quercetin in acetone or in ethanol (quercetin concentration: 2 g/L) and a dissolution of the carrier material (using different concentrations) in distilled water were prepared. Three different carrier materials were used: modified OSA-starch, Pluronic L-64, and soybean lecithin. Both dissolutions were mixed and the mixture was stirred during 5 min in order to obtain a solution. The two dissolutions were mixed using different proportions of organic and aqueous solutions resulting in