Food Hydrocolloids 51 (2015) 295–304

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Production of water soluble quercetin formulations by pressurized ethyl acetate-in-water emulsion technique using natural origin surfactants

V.S.S. Gonçalves [a, b, c], S. Rodríguez-Rojo [c, *], E. De Paz [c], C. Mato [c], Á. Martín [c], M.J. Cocero [c]

[a] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
[b] iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal
[c] Escuela de Ingenierías Industriales, Universidad de Valladolid, C/Dr. Mergelina s/n, 47011 Valladolid, Spain

ARTICLE INFO

Article history:
Received 23 October 2014
Received in revised form
4 May 2015
Accepted 6 May 2015
Available online 20 May 2015

Keywords:
Quercetin
OSA-starch
Lecithin
β-glucan
Encapsulation
High pressure emulsion technique
Nanosuspension

ABSTRACT

Quercetin is a strong antioxidant flavonoid with several bioactive properties such as anti-inflammatory and anticarcinogenic activities, becoming an interesting compound to be incorporated into pharmaceutical, cosmetic or food products. However, these applications are limited by the low bioavailability of this flavonoid. Quercetin is poorly soluble in aqueous media, such as gastrointestinal fluids, being also degraded by gut flora. Thus, it is necessary the development of quercetin's formulations capable of improving its water solubility resulting in increased bioavailability and thus higher biological activity of this compound.

The aim of the present work was the formulation of quercetin using three distinct natural origin surfactants, namely OSA-starch, Lecithin and β-glucan, by precipitation from a pressurized ethyl acetate-in-water emulsion. Formulations of quercetin with encapsulation efficiencies up to near 76% and a micellar particle size in the range of nanometers were obtained using lecithin. An improved antioxidant activity (3-fold higher per unit mass of quercetin) was also observed in these formulations, demonstrating that lecithin is a good emulsifier for the encapsulation of quercetin. Furthermore, the addition of glycerol as co-solvent increased the colloidal stability of the suspension and the encapsulation efficiency of the flavonoid.

1. Introduction

Quercetin (3,3′,4′,5,7-Pentahydroxyflavone), one of the most representative member of the flavonoid family with high antioxidant activity, is commonly found in several fruits and vegetables like onions, apples, grapes or strawberries, as well as in red wine or green/black tea (Priprem, Watanatorn, Sutthiparinyanont, Phachonpai, & Muchimapura, 2008; Souza et al., 2013). This compound has attracted the interest of the pharmaceutical and nutraceutical industries due to its bioactive properties, such as anti-inflammatory, anti-proliferative and neuroprotective effects (Boots, Haenen, & Bast, 2008; Priprem et al., 2008). In order to achieve quercetin plasma's concentration above 10 mM required for obtaining pharmacological activity, the ingestion of quercetin-enriched foods or supplements could not be enough due to the low bioavailability of this flavonoid (Russo, Spagnuolo, Tedesco, Bilotto, & Russo, 2012). The low water solubility (2 ppm at 25 °C to 60 ppm at 100 °C) allied with gastrointestinal degradation limits quercetin's biological effects *in vivo* (Souza et al., 2013; Srinivas, King, Howard, & Monrad, 2010). There are two main approaches to increase the bioavailability of this compound, whether by chemical modification or by the development of colloidal quercetin delivery systems (Barras et al., 2009).

Formulations of quercetin using different methods and distinct carrier materials have been developed by several authors. Regarding polymers, Kumari and co-authors achieved a controlled release of quercetin by its encapsulation into poly-D,L-lactide (PLA) nanoparticles through solvent evaporation technique (Kumari, Yadav, Pakade, Singh, & Yadav, 2010). Wu et al. have produced quercetin-loaded nanoparticles by a nanoprecipitation method using Eudragit® E and polyvinyl alcohol (PVA) as carriers, obtaining

* Corresponding author.
E-mail address: sorayarr@iq.uva.es (S. Rodríguez-Rojo).

http://dx.doi.org/10.1016/j.foodhyd.2015.05.006
0268-005X/

a quercetin's release 74-fold higher in comparison with the pure compound (Wu et al., 2008). Quercetin was also encapsulated in Pluronic F127 through supercritical antisolvent method by Fraile and co-authors, enabling an improved dissolution behavior of the compound in simulated physiological fluids (Fraile, Buratto, Gómez, Martín, & Cocero, 2014). Althans and co-authors have demonstrated that by using hyperbranched polymers and hydrogels as quercetin delivery systems it was possible to increase its water solubility and to enhance the chemical stabilization of the flavonoid, respectively (Althans, Schrader, & Enders, 2014). Cyclodextrins (CD), such as α-CD, β-CD or HP-β-CD, have also been used for the encapsulation of quercetin using the freeze-drying or co-evaporation method (Calabrò et al., 2004; Pralhad & Rajendrakumar, 2004). Barras and co-authors used lipids to encapsulate quercetin, being able to increase its apparent aqueous solubility by a factor of 100 (Barras et al., 2009). Besides solid lipid nanoparticles, nanostructured lipid carriers and lipid nanoemulsions were produced using a high pressure homogenizer for the encapsulation of quercetin by Aditya et al., achieving encapsulation efficiencies above 90% (Aditya et al., 2014). Quercetin-loaded liposomes have also been produced and are reported in the literature (Mignet, Seguin, & Chabot, 2013; Priprem et al., 2008). Inorganic materials can also be an option as carriers for the encapsulation of quercetin and, recently, quercetin-loaded silica microspheres were developed by Kim and co-authors using polyol-in-oil-in-water (P/O/W) emulsion and sol–gel methods to improve the flavonoid's stability as well as its properties (Kim et al., 2015).

In 2012, De Paz and co-authors (De Paz, Martín, Estrella, et al., 2012b) developed a novel method for the encapsulation of hydrophobic compounds, based on the production and processing of ethyl acetate-water emulsions at high pressure and temperature. The authors achieved stable aqueous suspensions of β-carotene with micellar particle sizes down to 400 nm and encapsulation efficiencies up to 80%. Moreover, the type of emulsifier used had been shown to affect the final properties of the suspension (De Paz, Martín, Bartolomé, Largo, & Cocero, 2014). This process is an attractive alternative to the conventional emulsion evaporation process, since it enables the acceleration of the mass transfer kinetics to the time scales of the precipitation processes. This intensification of the process allows an improved control over the precipitation, at the same time that the exposition of the product to degrading high-temperature conditions is decreased.

This work presents the development of water soluble formulations of quercetin through pressurized ethyl acetate-water emulsion technique. Ethyl acetate has been chosen as organic solvent because it is a Generally Recognized as Safe (GRAS) solvent with low toxicity (Lethal Dose LD50 in rats: 11.3 g/kg) and it can be safely used as a flavoring agent (Riemenschneider, & Bolt, 2000). Three natural origin surfactants, namely modified n-octenyl succinate anhydride (OSA) starch, soybean lecithin and barley β-glucan, were used in this work for the encapsulation of quercetin. Starch is the second most abundant biomass material present in nature being the most abundant storage polysaccharide in plants (Fathi, Martín, & McClements, 2014; Nitta & Numata, 2013). However, natural starch is mainly hydrophilic, which could limit its application in the encapsulation of hydrophobic compounds. Nevertheless, OSA (modified amphiphilic starch) is capable to overcome this drawback, and was already used for the encapsulation and delivery of compounds with distinct polarities (Fathi et al., 2014). Lecithin is a mixture of naturally occurring phospholipids, mainly phosphatidylcholine, which is usually available from sources such as soybeans or eggs. Phospholipids are amphiphilic molecules with antioxidant properties composed by hydrophobic tails and hydrophilic heads, being capable to rearrange themselves as liposomes, spherical and closed structures composed of lipid bilayers (Bouarab et al., 2014; Ramadan, 2008). Liposomes are interesting carrier materials for the delivery of hydrophobic/hydrophilic compounds, and since they have affinity to cellular membranes, they are capable to increase the absorption of several drugs (Hoeller, Sperger, & Valenta, 2009). β-glucans are soluble fibers present in cereal grains, especially in barley, constituted by linear polysaccharides of glucose units, connected by (1 → 3) or (1 → 4)-beta linkages. These carbohydrates are known for their therapeutic effects on coronary heart disease, diabetes and hypercholesterolemia, and have been used as encapsulating agents (Charalampopoulos, Wang, Pandiella, & Webb, 2002; Raemdonck, Martens, Braeckmans, Demeester, & De Smedt, 2013; Wang, Liu, Chen, & Zhao, 2013; Wood, 2007). The influence of the main process parameters has been studied, namely the effect of quercetin and emulsifier's concentration, the effect of the flows of organic solvent, suspension of quercetin and dissolution of emulsifier and also the organic to water ratio. By comparing the results obtained with the three different emulsifiers, their roles on the emulsion formation and quercetin's encapsulation can be established. Product analysis included particle size, encapsulation efficiency, antioxidant activity and structural characterization.

2. Materials and methods

2.1. Materials

Quercetin hydrate (Q) with a (purity ≥95%) was purchased from Acros Organicos. Ethyl Acetate with a purity of 99.5% and glycerol were purchased from Panreac Química (Barcelona, Spain). Modified OSA-starch refined from waxy maize was kindly provided by Ingredion Germany GmbH. Soybean lecithin (97% phospholipids) was obtained from Glama-Sot (SOTYA, Madrid, Spain). Glucagel™ (barley β-glucan) was kindly supplied by DKSH France (purity 78%, MW: 125–140 kDa).

2.2. Precipitation from pressurized ethyl acetate-on-water emulsions

The equipment used in this work, already described by De Paz et al. (De Paz, Martín, Estrella, et al., 2012b; De Paz et al., 2014), is represented in Fig. 1 with a schematic flow diagram.

Briefly, it consists of three small storages at ambient pressure, corresponding to the feed of pure ethyl acetate (V-1), quercetin suspension in the same organic solvent (V-2) and the aqueous solution of the emulsifier (V-3). The installation also counts with two piston pumps GILSON 305 (maximum flow rate: 25 mL/min; flow rate control with an a accuracy of 0.1 mL/min) used to feed the aqueous dissolution of the emulsifier and the quercetin suspension (pumps P-3 and P-2, respectively) and a piston pump JASCO PU-2080 plus (maximum flow rate: 10 mL/min; flow control with an a accuracy of 0.1 mL/min) used to feed the pure organic solvent (pump P-1). An oven (KNK-2000-C series GAS CHROMATOGRAPH) is used to preheat the organic solvent stream.

This process starts with the total dissolution of quercetin in hot and pressurized ethyl acetate, where by increasing temperature, it is possible to increase the solubility of quercetin in this solvent, which is around 1 g/L at ambient conditions. In this work, a temperature, typically, between 125 and 140 °C was used, keeping a constant pressure between 6.0 and 6.5 MPa in order to maintain the solvent in liquid state. The dissolution is achieved by mixing a flavonoid's suspension in pressurized ethyl acetate at ambient temperature, with a stream of preheated and pressurized ethyl acetate, using a T-mixer (M-1 in Fig. 1). In order to reduce the exposure of quercetin to high temperatures, this hot and pressurized solution is mixed with the ambient-temperature aqueous