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Encapsulation of resveratrol using food-grade concentrated double emulsions: Emulsion characterization and rheological behaviour

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ABSTRACT

The aim of this work was to prepare concentrated water-in-oil-in-water ($W_1/O/W_2$) double emulsions to entrap resveratrol (RSV) with high encapsulation efficiency, good stability, and appropriate rheological behaviour.

W_1/O was formulated with an ethanol/water RSV solution (W_1) dispersed in Miglyol 812 (O) (20 vol%) with polyglycerol polyricinoleate as stabiliser. W_2 was a 2% w/v Tween 20 solution with and without sodium carboximethylcellulose as thickening agent. Different volumetric ratios (20/80 to 80/20) of W_1/O dispersed into W_2 were used.

$W_1/O/W_2$ emulsions were characterized in terms of visual inspection, droplet size distribution, and stability. The rheology of these double emulsions was fully studied.

Actual encapsulation efficiency was determined considering the non-encapsulated RSV recovery yield.

The concentrated $W_1/O/W_2$ double emulsions with the optimum formulation showed high encapsulation efficiency (up to 58%), good storage stability, shear-thinning behaviour, and dominant elastic character, with 6.2 mg/L of encapsulated RSV. These double emulsions may be suitable for food applications.

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1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene, RSV) is a natural polyphenol present in many plants. Therefore, RSV can be got from different sources, such as grapes, nuts, berries, dark chocolate, and especially red wine, where the highest RSV concentration is found (Guerrero et al., 2009; Mullin, 2011).

Many in vitro and in vivo studies have suggested that RSV has a great number of potential health benefits in combating diseases, such as cancer, diabetes, neurodegeneration, cardiovascular disorders, inflammation, and other age-related pathologies (Diaz-Gerevini et al., 2016; Murtaza et al., 2013; Novelle et al., 2015; Saiko et al., 2008; Yang et al., 2014).

However, this great RSV potential is hindered by its poor pharmacokinetic properties, such as low aqueous solubility, low photostability, short biological half-life, and rapid metabolism and elimination (Amri et al., 2012; Francioso et al., 2014; Gomes-Silva et al., 2013; Neves et al., 2012; Yang et al., 2015). To overcome these limitations, encapsulation of RSV seems to be a good alternative for using this valuable ingredient in food, pharmaceutical and cosmetic industries.

Several encapsulation studies have been conducted to protect RSV from degradation, increasing its solubility in water and improving its chemical stability and bioavailability via multiparticulate forms and colloidal carriers (Amri et al., 2012; Davidov-Pardo and McClements, 2014; Ganesan and Choi, 2016; Pangeni et al., 2014; Sessa et al., 2014; Soo et al., 2016; Summerlin et al., 2015).

Double emulsions, such as water-in-oil-in-water ($W_1/O/W_2$) emulsions, are one of these colloidal delivery systems available to encapsulate and protect RSV (Hemar et al., 2010; Matos et al., 2014; Wang et al., 2017).

$W_1/O/W_2$ double emulsions consist of small water droplets

Abbreviations: BS, backscattering profile; CMC, sodium carboximethylcellulose; EE, encapsulation efficiency; HIPE, high internal phase emulsion; HLB, hydrophilic-lipophilic balance; O, oil phase; PGPR, polyglycerol polyricinoleate; RP-HPLC, reversed-phase high-performance liquid chromatography; RSV, resveratrol; TS, transmission profile; UV–vis, ultraviolet–visible; W_1, inner aqueous phase; W_2, external aqueous phase; W_1/O, primary emulsion; $W_1/O/W_2$, water-in-oil-in-water double emulsion.

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trapped within larger oil droplets that are themselves dispersed in a continuous water phase (Aserin, 2008; Kim et al., 2003; McClements and Li, 2010; Muschiolik, 2007).

One of the main drawbacks of $W_1/O/W_2$ double emulsions is their low stability, due to the excess free energy associated with droplets surface of the primary and secondary emulsions. The stability of the W_1/O primary emulsion must be ensured to obtain a stable $W_1/O/W_2$ double emulsion, which depends on droplet sizes, amounts of dispersed and continuous phase, and emulsifier affinity for both phases (Garti, 1997; Muschiolik and Dickinson, 2017; Schuch et al., 2013; Yildrim et al., 2017).

Although $W_1/O/W_2$ double emulsions have shown their suitability to encapsulate RSV, encapsulation efficiencies (EE) were not as high as those for other types of colloidal systems (Matos et al., 2014), even when it was enhanced by membrane emulsification techniques (Matos et al., 2015a).

Also, the concentration of encapsulated RSV in the final double emulsion should be clearly improved regarding previous works. Wang et al. (2017) reported high EE values but with very low RSV concentration, and Hemar et al. (2010) used an initial RSV concentration larger than the RSV solubility in the W_1 phase, what means that RSV was not completely dissolved under those conditions. Moreover, it is well known that the determination of EE in double emulsions requires the emulsion destabilization (frequently by centrifugation) and subsequent extraction of a sample of W_2 previously filtered to analyse the non-encapsulated RSV (Hemar et al., 2010; Wang et al., 2017). However, with this experimental procedure some amount of non-encapsulated RSV could remain retained either by interactions with filter materials or with other formulation components, which would be considered as encapsulated RSV providing an overestimated EE value.

On the other hand, high internal phase emulsions (HIPEs) are concentrated systems with a large volume of internal (or dispersed) phase. HIPEs contain more than 74 vol% of internal phase, which corresponds to the Ostwald critical volume. But it is possible to reach higher packing values by the concentration of polydisperse emulsions, since small droplets may fill the voids between the large ones even losing their spherical shape becoming polyhedral (Babak and Stebe, 2002; Cameron and Sherrington, 1996). These systems have wide application in cosmetics, foodstuffs, emulsion explosives, drug delivery systems, reaction media, and especially in the production of porous polymers (emulsion-templated porous polymers, PolyHIPEs) (Llinàs et al., 2013; Park et al., 2003; Silverstein, 2014; Solans et al., 2003; Zhang and Guo, 2017).

Moreover, stability studies revealed that HIPEs are very stable against destabilization phenomena and lead to high EE values (Gutiérrez et al., 2014; Matos et al., 2015b).

In addition to that, a few studies with new approaches to produce concentrated double emulsions have been recently published (Leal-Calderon et al., 2012; Lei et al., 2016; Li et al., 2014).

In this paper, the preparation of concentrated $W_1/O/W_2$ double emulsions containing RSV through a two-step mechanical emulsification process is reported. Also, the RSV amount lost during centrifugation, filtration, and W_2 sample extraction processes was measured and taken into consideration for determining the actual EE value. Furthermore, the rheology of these double emulsions was fully studied since it is largely influenced by the emulsion concentration and the emulsion rheological behaviour is also a key parameter in the final application of the product (Pal, 2011).

The main purpose of this work was to prepare a product with high RSV content and to enhance the EE through the use of concentrated $W_1/O/W_2$ double emulsions with large stability and adequate rheological behaviour, which could be suitable for food applications.

2. Materials and methods

2.1. Materials

RSV ($C_{14}H_{12}O_3$), absolute ethanol, Tween® 20 (polyoxyethylenesorbitan monolaurate, $C_{58}H_{114}O_{26}$), and sodium carboximethylcellulose (CMC, $C_8H_{16}NaO_8$) with polymerization degree 1100 (molar mass = 982 kg/kmol) were purchased from Sigma Aldrich (USA). Miglyol® 812 (density 945 kg/m^3 at 20 °C), which is a neutral oil formed by esters of caprylic and capric fatty acids and glycerol, was supplied by Sasol GmbH (Germany). Polyglycerol polyricinoleate (PGPR, $C_{21}H_{42}O_6$) was obtained from Brenntag AG (Germany). Sodium chloride was supplied by Panreac (Spain).

The hydrophilic-lipophilic balance (HLB) values of the emulsifiers tested in this study are: Tween 20 = 16.7; PGPR = 3.0.

HPLC-grade methanol, acetonitrile, 2-propanol, and acetic acid were obtained from Sigma Aldrich (USA).

Paraffin oil supplied by VWR International (USA) was used as dispersant for droplet size measurements of W_1/O emulsions.

2.2. W_1/O emulsion preparation

The primary W_1/O emulsion was formulated with 20 vol% of inner aqueous phase (W_1) and 80 vol% of oil phase (O).

Miglyol 812 containing 5 wt% of the hydrophobic emulsifier (PGPR) previously dissolved by magnetic stirring at 50 °C for 30 min was used as the oil phase. PGPR is highly effective for stabilizing W/O emulsions (Márquez et al., 2010; Wolf et al., 2013).

In order to increase RSV solubility, a 20 vol% ethanol/water solution with 50 mg/L of RSV was selected as the inner aqueous phase.

0.1M NaCl was added to the inner aqueous phase in all double emulsions to ensure W_1 droplets stability because it had been previously reported that electrolytes increase W_1/O emulsion stability (Jiang et al., 2013; Márquez et al., 2010).

Both phases were emulsified in glass vessels by high shear mixing (Silentcruser M Homogenizer, Heidolph, Germany) using a 6 mm dispersing tool at 15,000 rpm for 10 min.

2.3. $W_1/O/W_2$ double emulsions preparation

The $W_1/O/W_2$ double emulsions were prepared dispersing the W_1/O primary emulsion into the external aqueous phase (W_2) at several volumetric ratios of W_1/O in W_2: 20/80, 40/60, 50/50, 60/40, 70/30, and 80/20. W_2 was a 2% (w/v) Tween 20 solution. Emulsification was carried out by mixing the continuous and dispersed phases with the aforementioned Silentcruser M Homogenizer at 5000 rpm for 2 min.

These double emulsions were also formulated with 0.5% (w/v) CMC in the W_2 phase. Overnight magnetic stirring was needed to completely dissolve CMC. Then, 2% (w/v) Tween 20 was added and dissolved proceeding with the stirring for 30 min.

0.1M NaCl was also added to the W_2 phase to maintain an appropriate osmotic balance between W_1 and W_2, the inner and outer aqueous phases, in all emulsions.

2.4. Emulsion characterization

2.4.1. Droplet size and visual inspection

Emulsion droplet size distributions were analysed using laser light scattering technique in a Mastersizer S long bench apparatus (Malvern Instruments, Ltd. UK).

For single W_1/O emulsion measurements, the samples were dispersed in paraffin oil, whereas $W_1/O/W_2$ double emulsion samples were diluted with deionized water.

Micrographs of the emulsions were obtained with a light

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