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Nutraceutical delivery systems: Resveratrol encapsulation in grape seed oil nanoemulsions formed by spontaneous emulsification

Gabriel Davidov-Pardo [a,*], David Julian McClements [a,b]

[a] Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA
[b] Department of Biochemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Saudi Arabia

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ABSTRACT

The aim of this work was to fabricate nanoemulsions-based delivery systems to encapsulate resveratrol. Nanoemulsions were formed using spontaneous emulsification method: 10% oil phase (grape seed oil plus orange oil) and 10% surfactant (Tween 80) were titrated into 80% aqueous phase. An optimum orange oil-to-grape seed oil ratio of 1:1 (w/w) formed small droplets ($d \approx 100$ nm) with good stability to droplet growth. The maximum amount of resveratrol that could be dissolved in the oil phase was 120 ± 10 μg/ml. The effect of droplet size on the chemical stability of encapsulated resveratrol was examined by preparing systems with different mean droplet diameters of 220 ± 2; 99 ± 3; and 45 ± 0.4 nm. Encapsulation of resveratrol improved its chemical stability after exposure to UV-light: 88% retention in nanoemulsions compared to 50% in dimethylsulphoxide (DMSO). This study showed that resveratrol could be encapsulated within low-energy nanoemulsion-based delivery systems and protected against degradation.

1. Introduction

Marcs are by-products of the wine making process that consist of mixtures of grape seeds and peels (Lafka, Sinanoglou, & Lazos, 2007). The conversion of these by-products into value added ingredients is of considerable interest to the wine industry since around 13–14 million ton are generated per year. Grape seeds contain health promoting unsaturated oils and polyphenols (such as flavan-3-ols or condensed tannins), while grape skins contain anthocyanins and resveratrol (Chira, Suh, Saucier, & Teissèdre, 2008).

Grape seed oil (GSO) is rich in unsaturated fatty acids, which represent more than 89% of the total oil composition with many of them being essential fatty acids (Table S1 in Supplementary materials). GSO is also rich in antioxidants such as tocopherols and phytosterols that may exert anti-artheriosclerotic activity (Yu & Ahmedna, 2013). Resveratrol (trans-resveratrol; trans-3,5,4′-trihydroxystilbene) is a polyphenol from the stilbens family that is found at relatively high levels in grape skins (Walle, Hsieh, DeLegge, Oatis Jr., & Walle, 2004). It is of interest to the food and pharmaceutical fields due to its potential beneficial effects on human health, including cardio-protective, neuro-protective, antioxidant, anti-inflammatory, anti-carcinogenic, and anti-obesity effects (Alves, 2012; Catalgol, Batirel, Taga, & Ozer, 2012; Neves, Lucio, Martins, Lima, & Reis, 2013). Despite the potential health benefits of resveratrol, its utilisation as a nutraceutical ingredient within the food industry is currently limited due to its poor water-solubility, chemical instability, and low bioavailability (Hung, Chen, Liao, Lo, & Fang, 2006; Patel et al., 2011; Trela & Waterhouse, 1996).

Various encapsulation technologies are available that may be able to overcome the challenges associated with utilising resveratrol as a bioactive agent in foods. In particular, emulsion-based delivery systems are a promising encapsulation technique due to the fact that lipophilic bioactive components can be encapsulated within the hydrophobic core of the lipid droplets where they may be protected from degradation during storage and then released after ingestion (McClements, Decker, & Weiss, 2007; McClements & Rao, 2011). If the bioactive compound to be encapsulated is crystalline (such as resveratrol), it is necessary to ensure that it is used at a level below its saturation concentration in the carrier oil to avoid precipitation and sedimentation during storage (McClements, 2012). The two most common forms of emulsion-based delivery systems are nanoemulsions (radius $\leqslant$ 100 nm) and emulsions (radius > 100 nm), which are both thermodynamically unstable systems but that can be designed to have sufficient kinetic stability for many food applications. Microemulsions may also be

* Corresponding author. Address: Department of Food Science, Room 232, University of Massachusetts, Amherst, MA 01003, USA. Tel.: +1 (413) 545 7157; fax: +1 (413) 545 1260.
E-mail address: gdavidov@foodsci.umass.edu (G. Davidov-Pardo).

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used as delivery systems for lipophilic agents. This type of colloidal dispersion has some characteristics similar to nanoemulsions (e.g., radius $\leqslant$ 100 nm), but they are thermodynamically stable systems, whereas nanoemulsions are not (Rao & McClements, 2012a).

Previous studies have shown that resveratrol can be encapsulated in either emulsions or nanoemulsions using various carrier oils, emulsifiers, and particle dimensions. Emulsions formed using both natural and synthetic emulsifiers have been shown to improve the stability of resveratrol (Donsi, Sessa, Mediouni, Mgaidi, & Ferrari, 2011; Sessa, Tsao, Liu, Ferrari, & Donsi, 2011). The influence of oil phase composition on the formation and stability of emulsion-based delivery systems for resveratrol has also been studied (Donsi et al., 2011; Nemen & Lemos-Senna, 2011; Spigno et al., 2013). The development of novel emulsion-based delivery systems to encapsulate resveratrol fabricated using structural design principles has also been examined, such as multiple emulsions (Hemar, Cheng, Oliver, Sanguansri, & Augustin, 2010; Lee, Ahn, & Kwak, 2013; Matos, Gutiérrez, Coca, & Pazos, 2013) and solid lipid nanoparticles (Neves et al., 2013). In all cases at least one of the drawbacks of using resveratrol as a functional compound was overcome using emulsion-based delivery systems.

In the current study, we focus on the utilisation of nanoemulsions to encapsulate resveratrol. Two approaches can be used to fabricate nanoemulsions: high-energy and low-energy approaches (Rao & McClements, 2011). High-energy methods use mechanical devices that generate intense disruptive forces to break the oil phase into tiny droplets that are then dispersed within the aqueous phase. On the other hand, low-energy methods are based on the spontaneous formation of nano-sized oil droplets in surfactant–oil–water mixtures when the composition or temperature of the system is changed in a particular manner. Two approaches can be distinguished for preparing low-energy emulsions: phase inversion and spontaneous emulsification methods. The phase inversion methods rely on the modification of the optimum curvature or solubility of the surfactant by changing the temperature (phase inversion temperature) or composition (phase inversion composition) of the system. On the other hand the spontaneous emulsification methods rely on the movement of surfactants from their original location in one phase into the other phase due to solubility preferences. This movement causes an increase in oil–water interfacial area, interfacial turbulence, and spontaneous formation of droplets (McClements & Rao, 2011). The main advantage of using low-energy methods over high-energy methods is the reduction in equipment and operating costs and the ease of implementation, whereas the main disadvantage is that they require considerably higher amounts of surfactant (Saberi, Fang, & McClements, 2013).

Citrus oils are a complex mixture of various types of molecules, with some of the major components being cyclic hydrocarbons with double bonds (Santana, Perrechil, & Cunha, 2013). Low energy nanoemulsions have previously been shown to have smaller droplet sizes when fabricated from citrus oils than from long chain triacylglycerol oils (Ostertag, Weiss, & McClements, 2012). In addition, citrus oils have been shown to have a higher loading capacity than long chain triacylglycerol oils for hydrophobic bioactive compounds (Li, Zheng, Xiao, & McClements, 2012), which may be an advantage for increasing the amount of bioactive agent that can be incorporated into a functional food or beverage product. On the other hand, studies have shown that the bioavailability of lipophilic nutraceuticals may be decreased when they are encapsulated within flavour oils (indigestible) rather than triacylglycerol oils (digestible) (Qian, Decker, Xiao, & McClements, 2012; Rao, Decker, Xiao, & McClements, 2013).

Our hypothesis is that it is feasible to combine two by-products from the wine industry, GSO and grape skin extract (GSE), to create a stable colloidal delivery system for resveratrol. The aim of this work was therefore to design stable nanoemulsion-based delivery systems to encapsulate GSE rich in resveratrol using a mixture of grape seed oil (digestible) and orange oil (indigestible) as carrier oils, and spontaneous emulsification as the fabrication technique. In addition, we aimed to determine whether nanoemulsion encapsulation would have an impact on the stability of resveratrol to exposure to UV-light stability.

2. Materials and methods

2.1. Materials

Resveratrol standard (purity $\geqslant$ 99%), polysorbate 80 (Tween 80), sodium phosphate monobasic (NaH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4) were purchased from Sigma–Aldrich Co. (St. Louis MO, USA). Dimethylsulphoxide (DMSO) and chloroform were purchased from Thermo Fisher Scientific Inc (Waltham MA, USA). Organic virgin grape seed oil was purchased from Jedwards International, Inc. (Braintree MA, USA). Orange oil (10 $\times$ fold) was provided by International Flavors and Fragrances Inc. (New York, NY, USA). Resveratrol (99%) from grape seed extract was purchased from Changsha Organic Herb Inc. (Changsha, China).

2.2. Resveratrol quantification

The concentration of resveratrol was determined from absorbance measurements (307 nm) made using a UV–visible spectrophotometer (Ultrospec 3000 pro, Biochrom Ltd., Cambridge, England). A stock solution was prepared by dissolving resveratrol in DMSO at a level of 100 μg/ml, and then this solution was diluted using either DMSO or chloroform to create a calibration curve in the range from 0.2 to 6 μg/ml. The curves were linear for both solvents (DMSO r^2 = 0.9999 and chloroform r^2 = 0.9995).

2.3. Resveratrol solubility

The solubility of resveratrol was assessed by quantifying the amount of resveratrol dissolved in the oil phase after 96 h storage at 5, 20 and 37 °C (Pool, Mendoza, Xiao, & McClements, 2013). Different amounts of grape seed extract (30–1000 μg/ml GSE) were dispersed in the oil phase using an incubator shaker (Innova 4080, New Brunswick Scientific, Eppendorf, Enfield CT, USA) at 37 °C and 170 rpm for 24 h (Hung et al., 2006). After this storage period the samples were centrifuged for 30 min at 4000 rpm using a centrifuge (Sorvall ST 8, Thermo Fisher Scientific Inc., Waltham, MA, USA). An aliquot of the supernatant was collected, diluted in chloroform (1:100 v/v), and the absorbance at 307 nm was measured using a UV–visible spectrophotometer (Ultrospec 3000 pro, Biochrom Ltd., Cambridge, England). The diluted oil phase without GSE was used as a blank.

2.4. Low-energy emulsion preparation

Emulsions fabricated by spontaneous emulsification were prepared based on the method described by Saberi et al. (2013) with slight modifications. Briefly, the two oils (OO and GSO) were mixed together for 1 h and then surfactant (Tween 80) was added and the system was mixed for another hour. The resulting organic phase was added to an aqueous phase (5 mM phosphate buffer pH 7 solution) over a 10 min period under constant magnetic stirring (700 rpm) at ambient temperature. After adding the organic phase, the resulting emulsion was left for another 5 min of stirring. For the emulsions containing resveratrol, 100 μg of GSE per ml of oil were dissolved as explained in Section 2.3. The oil containing the GSE was centrifuged prior to its utilisation to remove any non-dissolved components.