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Encapsulation of resveratrol in biopolymer particles produced using liquid antisolvent precipitation. Part 2: Stability and functionality

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ABSTRACT

Resveratrol is a polyphenol that is believed to have beneficial effects on human health. However, its low water-solubility and poor chemical stability hamper its use as a functional food ingredient. The goal of this study was to assess the effect of resveratrol encapsulation within biopolymer particles on its UV-induced isomerization and the effect of particle structure on particle stability to environmental stresses and redispersion of powdered particles. Biopolymer particles were formed by antisolvent precipitation of hydrophobic proteins (zein or gliadin) and coated with hydrophilic copolymers (pectin or sodium caseinate). The stability and functionality of bare zein and gliadin particles (0.5% (w/v) in suspension) were compared to zein and gliadin particles coated with sodium caseinate (1.0% (w/v)) or pectin (0.1% (w/v)), respectively. Resveratrol was encapsulated at a concentration of 250 mg/l and the effect of particle structure on resveratrol UV-stability was monitored. The suspensions of coated particles were physically stable after being held at temperatures up to 90 °C (30 min). Both bare protein particles and pectin coated gliadin nanoparticles aggregated when increasing the ionic strength, while sodium caseinate coated zein particle suspensions remained stable. The pH stability of the particle suspensions was related to the isoelectric point of the nanoparticles. After freeze-drying, only the sodium caseinate coated zein nanoparticles could be fully redispersed in water. All particle formulations protected resveratrol against UV-light to some extent. However, resveratrol was best protected when encapsulated in sodium caseinate-coated zein particles. In conclusion, these particles seem promising encapsulation systems to stabilize resveratrol upon supplementation to functional foods.

1. Introduction

Several health benefits have been linked to the consumption of resveratrol (Alves, 2012; Catalgol, Batirel, Taga, & Ozer, 2012; Neves, Lucio, Martins, Lima, & Reis, 2013), a polyphenol that can be isolated from by-products of the wine industry (Walle, Hsieh, DeLegge, Oatis, & Walle, 2004). Nevertheless, its use as a nutraceutical ingredient within the food industry is currently limited because of its poor water-solubility, low oral bioavailability, and chemical instability (Hung, Chen, Liao, Lo, & Fang, 2006; Patel et al., 2011; Trela & Waterhouse, 1996). Different strategies have therefore been developed in an attempt to overcome the challenges of incorporating resveratrol as a nutraceutical component (Davidov-Pardo & McClements, 2014). For example, resveratrol has been encapsulated in alginate microspheres hardened with calcium chloride (Cho, Chun, Kim, & Park, 2014), in liposomes (Isailovic et al., 2013), in emulsions where resveratrol was dissolved in the oil phase (Davidov-Pardo & McClements, 2015) or in emulsions prepared using a solvent removal method (Lee et al., 2012). In all cases, at least one of the issues of using resveratrol as a nutraceutical component was solved. Protein-based delivery systems have proven to be a particularly promising way to incorporate polyphenols and hydrophobic compounds into food products (Li, Percival, Bonard, & Gu, 2011; Shutava et al., 2009). Using a biomimetic approach, casein has been used to create micelles in order to encapsulate vitamin D (Haham et al., 2012). Gelation of proteins has also been investigated to create micro- or nano-structured delivery systems for nutraceuticals (hydrogel particles). This

Abbreviations: EE, encapsulation efficiency; GSE, grape skin extract; SEM, scanning electron microscopy.

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procedure usually involves protein gelation through thermal denaturation (Jones & McClements, 2010) or addition of cross-linking agents such as divalent cations (Liang & Subirade, 2012). The gelled nanoparticles can subsequently be coated with another biopolymer to reduce hydrophobic attraction and/or increase steric repulsion (Jones & McClements, 2010). Protein-based delivery systems can also be created based on the complexation of hydrophobic bioactive molecules to non-polar functional groups/patches on proteins. Complexes with dairy proteins such as casein, β-lactoglobulin or whole buttermilk have been successfully used to improve the stability and solubility of a variety of compounds including resveratrol (Acharya, Sanguansri, & Augustin, 2013; Shpigelman, Shoham, Israeli-Lev, & Livney, 2014; Ye, Thomas, Sanguansri, Liang, & Augustin, 2013).

In this study, protein particles produced by liquid antisolvent precipitation (LAS) were used as potential delivery systems for resveratrol. LAS is a simple and versatile method for fabricating biopolymer nanoparticles that has already been used to produce particles encapsulating several types of bioactive molecules (Duclairoir, Orecchioni, Depraetere, Osterstock, & Nakache, 2003; Ezpeleta et al., 1996; Gan, Ju, Zhang, & Wu, 2011; Li, Yin, Yang, Tang, & Wei, 2012; Subia & Kundu, 2013; Yoo, Choi, & Park, 2001). Biopolymeric nanoparticles or microparticles are spontaneously formed when an antisolvent is added to a solvent containing dissolved biopolymer molecules (Joye & McClements, 2013). The addition of the antisolvent reduces the solvent quality, which favors biopolymer–biopolymer interactions over biopolymer–solvent interactions, thereby promoting self-association of the biopolymer molecules (Li, Jiang, Xu, & Gu, 2015). After formation, the physicochemical properties, surface characteristics, and stability of the biopolymer particles can be controlled by coating them with polymers and/or surfactants (Joye, Nelis, & McClements, 2015; Kim, Ng, Dong, Das, & Tan, 2012; Zhang, Le, Wang, Zhao, & Chen, 2013).

In a previous paper, we already described the encapsulation of resveratrol in biopolymeric particles fabricated from hydrophobic proteins (zein and gliadin) using LAS (Davidov-Pardo, Joye, & McClements, 2015). Zein particles had a substantially higher encapsulation efficiency (EE) than gliadin particles (67.5 and 44.9%, respectively), which may have been because zein contains more hydrophobic amino acids than gliadin. The EE of resveratrol in these particles could be further increased by coating them with hydrophilic copolymers (86.3 and 52.9% for the sodium caseinate coated zein particles and pectin coated gliadin particles, respectively) (Davidov-Pardo, Joye, et al., 2015).

In the current paper, the stability of these biopolymeric nanoparticles is assessed under conditions that mimic conditions found in real-world food systems and during food processing, *i.e.*, thermal processing, exposure to UV light, and changes in ionic composition. Furthermore, special attention was given to particle redispersability as commercial delivery systems are often added to food products in a powdered form in order to reduce transport and storage costs. The results of our study are useful to evaluate the potential of biopolymeric particles to supplement food products and beverages with resveratrol without compromising product quality.

2. Materials and methods

2.1. Materials

All chemicals, reagents, and solvents were purchased from Fisher Scientific (Waltham, MA, USA) and were of analytical grade unless stated otherwise. Resveratrol standard (purity ≥ 99%) was purchased from Sigma–Aldrich Co. (St. Louis MO, USA). Resveratrol (99%) from grape skin extract (GSE) was purchased from Changsha Organic Herb Inc. (Changsha, China). Gluten was kindly provided by Cargill (Minneapolis, MN, USA). High methoxyl pectin [Genu® pectin (citrus), degree of esterification 61.4%, HMP] was obtained from CP Kelco (Atlanta, GA, USA). Food grade zein (F4000) was purchased from Flo Chemicals (Ashburnham, MA, USA). Sodium caseinate was purchased from American Caseinate Company (Burlington, NJ, USA).

2.2. Fabrication of nanoparticles

The nanoparticles were fabricated following the procedure explained in a previous paper (Davidov-Pardo, Joye, et al., 2015). Briefly, the protein solution [containing 3.0% (w/v) protein and 0.15% (w/v) GSE] was added to the antisolvent (aqueous phase) at a 1:5 ratio under continuous stirring (440 rpm) at room temperature. After addition, the particle suspension was stirred for an additional 2 min. Ethanol was removed from the mixture using a rotary evaporator at 30 °C (Buchi RE 111, Flawil, Switzerland). Sodium caseinate and pectin were dissolved in the antisolvent phase at 0.1 and 1.0% (w/v), respectively. The pH of the antisolvent phase containing pectin was adjusted to 4.5, while the pH of the sodium caseinate solution was brought to 6.5.

2.3. Resveratrol quantification

The resveratrol level contained in the nanoparticles was measured using a UV–visible spectrophotometer (Ultrospec 3000 pro, Biochrom Ltd., Cambridge, England) set at 307 nm. Resveratrol was dissolved in dimethyl sulfoxide at a concentration of 100 μg/ml and then diluted to create a calibration curve ranging from 0.2 to 6.0 μg/ml. Suspensions without resveratrol were used as blanks and their absorbance was used to correct for any minor absorbance from the proteins at 307 nm.

2.4. Particle size and ζ-potential

Mean particle diameter, polydispersity index (PDI) and particle size distributions were measured using a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). The particle size distribution of the samples was based on intensity measurements. The ζ-potential was determined by particle electrophoresis using the same instrument. Samples were equilibrated at 25 °C prior to analysis. All data reported are the average values of at least three different samples.

2.5. Particle stability to environmental stresses

2.5.1. Ionic strength

After production, the particle dispersions were diluted two-fold using NaCl solutions (0.0–1000 mM) to obtain systems with final NaCl concentrations ranging from 0.0 to 500 mM. The samples were then stored for 24 h at room temperature and the particle size distribution was analyzed as described above (Section 2.4).

2.5.2. Temperature stability

Freshly prepared particle dispersions were diluted two-fold in double distilled water and incubated in water-baths set at different temperatures (30–90 °C) for 30 min. The samples were then allowed to cool down to 23 °C, stored for 24 h, and the particle size distribution was analyzed as described above (Section 2.4).

2.5.3. pH stability

Freshly prepared particle dispersions were diluted ten-fold in double distilled water and their pH was adjusted at room