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Complexation of resveratrol with soy protein and its improvement on oxidative stability of corn oil/water emulsions

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ABSTRACT

This work was to evaluate the potential of soy protein isolate (SPI)–resveratrol (RES) complex as an emulsifier to improve the effectiveness of RES as a natural antioxidant in corn oil-in-water emulsions. The physical properties and oxidative stability of emulsions stabilized by the native SPI–RES and heated SPI–RES complexes were evaluated. The water solubility of RES was enhanced by complexation with SPI, which was mainly driven by hydrophobic interactions. Heat treatment favoured the formation of the SPI–RES complex and endowed it with a higher antioxidant activity. Furthermore, the emulsions stabilized by the SPI–RES complexes showed an increased oxidative stability with reduced lipid hydroperoxides and volatile hexanal. This improving effect could be attributed to the targeted accumulation of RES at the oil–water interface accompanied by the adsorption of SPI, as evidenced by the high interfacial RES concentration. These findings show that the soy protein–polyphenol complex exhibited a good potential to act as an efficient emulsifier to improve the oxidative stability of emulsions.

1. Introduction

Oil-in-water (O/W) emulsions form the basis for many food products, such as milk, beverages, sauces and dressings. Lipid oxidation in O/W emulsions is believed to be more likely to occur due to the higher interfacial area of emulsions, where the oxidative reaction has been suggested to be initiated (Berton, Ropers, Viau, & Genot, 2011; Frankel, Huang, Kanner, & German, 1994; McClements & Decker, 2000). The oxidative degradation of lipids causes unpleasant quality changes of food emulsions, such as the development of unpalatable flavours and odours, nutrient degradation and colour changes (McClements & Decker, 2000; Shahidi & Zhong, 2010). Therefore, various antioxidants are usually incorporated into O/W emulsions to improve their oxidative stability. Recently, there has been growing interest in the use of natural polyphenols to retard lipid oxidation due to their putative health-promoting properties and remarkable antioxidant activity (Brewer, 2011).

Resveratrol (trans-3,5,4′-trihydroxystilbene, RES) is a natural polyphenol found in the skin of grapes, apples, peanuts and in some traditional herbs. It has been associated with many benefits for human health, including antioxidant, anti-inflammatory, anticancer, antiplatelet aggregation, cardioprotective and antiobesity effects (Vang et al., 2011). The antioxidant capacity of RES has been verified by the inhibition of lipid peroxidation induced by many *in vivo* and *in vitro* systems (Gülçin, 2010; Kimura et al., 1983; Soares, Andreazza, & Salvador, 2003). However, scarce reports of the use of RES as a food antioxidant have been found so far (Filip et al., 2003; Medina et al., 2010). In fact, the extremely low solubility of RES in both water and oil has limited its application in functional foods, especially in emulsified systems, as an antioxidant due partly to the unpredictable physical distribution of RES (Laguerre et al., 2009). Therefore, in a previous study, to strengthen the effectiveness of RES as an antioxidant in O/W emulsions, a water-soluble RES was prepared by the encapsulation of RES in stevioside self-assembled micelles, and enhanced the antioxidant efficiency of RES by purposefully accumulating RES at the oil–water interface (Wan, Wang, Wang, Yang, & Yuan, 2013). Moreover, many other approaches, such as complexation with cyclodextrin and its derivatives, or using bile acid micelles, nanoemulsions and nanoparticle delivery systems, have also been made to increase the water solubility and bioavailability of RES (Amri, Chaumeil, Sfar, & Charrueau, 2012).

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Food proteins, such as soy protein, hold the attractive advantages of being natural, nontoxic, and widely available, are ideal binders of polyphenol by formation of protein–polyphenol complex and have been widely used to improve the water solubility, stability, and bioavailability of RES. Liang, Tajmir-Riahi, and Muriel (2008) reported that complexing with β-lactoglobulin provides a significant increase in the solubility of RES and a slight increase in its photostability. The work from Hemar, Gerbeaud, Oliver, and Augustin (2011) showed that a whey protein isolate and its β-lactoglobulin- and α-lactalbumin-rich fractions could form 1:1 complexes with RES, thus providing an excellent carrier for RES. In addition, many investigators also observed the stabilizing effects on RES of various proteins, such as collagen, sodium caseinate and bovine serum albumin (BSA) (Acharya, Sanguansri, & Augustin, 2013; Bourassa, Kanakis, Tarantilis, Pollissiou, & Tajmir-Riahi, 2010; Zhang, Mi, & Shen, 2012). Moreover, previous studies have shown that *in vitro* bioaccessibility and antioxidant activities of polyphenols were not influenced by the interaction with proteins (Tapal & Tiku, 2012; van der Burg-Koorevaar, Miret, & Duchateau, 2011).

In searching for food proteins to form complexes with RES, we focused on soy protein isolate (SPI). SPIs are used extensively in the food industry, due to their functional properties, low cost, availability and high nutritional values. Recently, Tapal and Tiku (2012) demonstrated that SPI could be used as a carrier for water-insoluble curcumin, and the water solubility and stability of curcumin were thus enhanced. Grace et al. (2013) and Roopchand et al. (2013) found that soy proteins could stably bind and concentrate cranberry polyphenols to form a cranberry polyphenol–SPI complex, thus creating protein/polyphenol-enriched matrices. On the other hand, soy proteins are also widely used as emulsifiers in foods because of their excellent ability to form and stabilize O/W emulsions.

Considering the fact that the effectiveness of antioxidants in O/W emulsions would be promoted by their accumulation at the oil–water interface (Laguerre et al., 2009; Lucas et al., 2010), we thus postulated that the use of protein–RES complex as emulsifiers in an O/W emulsion system may result in the targeted accumulation of RES at the oil–water interface, due to the adsorption of surface-active proteins at oil droplet surface, and thus could improve the efficiency of RES in the interfacial resistance to lipid oxidation (Frankel et al., 1994; McClements & Decker, 2000). Therefore, the objective of this work was to improve the water solubility of RES by complexation with SPI, and then to purposefully accumulate RES at the oil–water interface by using the SPI–RES complex as an emulsifier, thus enhancing the effectiveness of RES as a natural antioxidant in O/W emulsions. The potential of SPI as a carrier for RES was first studied through interactions between SPI and RES using fluorescence spectroscopy. In general, heat treatment could induce structural changes of proteins, affecting the binding of RES, and thus the interaction between SPI and RES during heating was examined. The antioxidant activities of the native SPI–RES and heated SPI–RES complexes prepared were also evaluated. Subsequently, the physical properties and oxidative stability of corn oil/water emulsions stabilized by the native SPI–RES and heated SPI–RES complexes were investigated using lipid hydroperoxides and headspace hexanal analysis.

2. Materials and methods

2.1. Materials

RES (purity >98%) was purchased from Shanxi Tianrun Phytochemical Co., Ltd., China. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azo-bis(2-amidinopropane) dihydrochloride (AAPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma–Aldrich (St. Louis, MO). Defatted soy flour was provided by Shandong Yuwang Industrial and Commercial Co., Ltd., China. SPI was prepared from flour by alkaline extraction (pH 8.0) followed by precipitation at pH 4.5, as described by Wang et al. (2012). The precipitate collected was washed twice with distilled water, re-dispersed in distilled water, and then neutralised to pH 7.5 with 2 M NaOH. Subsequently, the protein solution was dialyzed against distilled water at 4 °C for 48 h before freeze-drying. The protein content of SPI was 88.8%, determined by the Dumas method (N × 5.71, wet basis) in a Rapid N Cube (Elementar France, Villeurbanne, France). Corn oil was purchased from a local supermarket and used without further purification. The selection of corn oil was made due to its common use as cooking media and also relatively high reaction rate of oxidative deterioration (Naz, Sheikh, Siddiqi, & Sayeed, 2004). All other chemicals used were of analytical grade.

2.2. Preparation of SPI–RES complex

The SPI dispersions (2.0%, w/v) were prepared by dissolving lyophilized SPI in distilled water and stirring at room temperature (22 °C) for 2 h. The pH of the prepared SPI dispersions was adjusted to pH 7.0 with either HCl or NaOH. RES (0.1005 g) was mixed with 50 ml of the SPI dispersions by homogenising at 8000 rpm for 5 min using an Ultra-Turrax T25 (IKA-Werke GMBH & CO., Germany). In a typical heat treatment, the resulting mixture was heated at 90 °C for 30 min in a water bath (TW12; Julabo, Seelbach, Germany), then immediately cooled in an ice bath. This heat treatment ensured extensive denaturation of SPI (Renkema & Van Vliet, 2002), which might affect the binding of RES. The free RES was removed by centrifugation (10,000*g*, 30 min) and the supernatant was freeze-dried in a Christ DELTA 1-24 LSC freeze-dryer (Christ, Germany) to get the dry SPI–RES complex powder. The freeze-dryer was set at a shelf temperature of −30 °C and a condenser temperature of −55 °C before the main drying was initiated by lowering the vacuum to 0.340 mbar. The shelf temperature was gradually increased to 30 °C, which was maintained for 24 h.

The SPI–RES complex powder was suspended at 2.0% w/v in distilled water and was stirred at room temperature (22 °C) for 2 h. Then, the SPI–RES dispersions (1 ml) were added to 9 ml of acetonitrile and stirred for 5 min. After centrifugation at 15,000*g* for 20 min, the supernatant was used for RES quantification. Each sample was filtered through a 0.22 μm filter (Millipore, Billerica, MA, USA) prior to HPLC determination. Quantitative analysis of RES was performed according to the method described in the previous study (Wan et al., 2013)

2.3. Fluorescence spectroscopy

The fluorescence spectra were recorded using an F7000 fluorescence spectrophotometer (Hitachi Co., Japan). RES was dissolved in ethanol at a concentration of 1 mg/ml as a stock solution, which was diluted with 10 mM phosphate buffer (pH 7.0) before use. The fluorescence of RES was measured by fixing its concentration at 5 μg/ml and by varying the concentration of SPI from 0 to 2.5 mg/ml. The emission spectra were recorded from 350 to 550 nm with an excitation wavelength of 320 nm. Protein intrinsic fluorescence was measured at a constant SPI concentration (0.5 mg/ml) in the presence of 0–20 μg/ml RES. Emission spectra were recorded from 300 to 500 nm at an excitation wavelength of 280 nm. Both the excitation and emission slit widths were set at 5 nm.

Fluorescence quenching is described according to the Stern–Volmer equation (Eq. 1) (Lakowicz, 2006): $F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q]$. In this equation F_0 and F are the fluorescence