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Effects of rutin incorporation on the physical and oxidative stability of soy protein-stabilized emulsions

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ABSTRACT

We investigated the effects of rutin additive on the physical (droplet size, zeta potential, and interfacial adsorption) and oxidative stability of emulsions stabilized by native soybean protein isolate (SPI) and heat-denatured SPI. The fluorescence spectra and interfacial tension of SPI–rutin mixture were also measured. The formation of SPI–rutin complex mainly driven by hydrophobic interactions was verified. The addition of rutin reduced interfacial tension of native SPI at the oil–water interface, while that of HSP was less influenced. In the case of native SPI emulsion, rutin particles could competitively adsorb into the oil–droplet interface with high interfacial accumulation. The formation of native SPI–rutin mixed interfacial layers resulted in the reductions in oil droplet size of emulsions and evident improvement of physical and oxidative stability. However, the stability of heated SPI emulsions showed no remarkable improvement due to lower interfacial accumulation and physical location of rutin. Rutin particles were believed to mainly adsorb outside protein layer. These findings showed that rutin can be used as a native stabilizer for protein emulsions to enhance its physical and oxidative stability.

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1. Introduction

Soybean protein isolate (SPI) is widely used as a proteinaceous emulsifier in food emulsions such as soups, sausages, and coffee whitener, due to its excellent functional properties and low cost. However, protein-stabilized emulsions are more prone to destabilization, including coalescence, flocculation, and eventually oxidation, than droplets covered by surfactants (Berton, Ropers, Bertrand, Viau, & Genot, 2012; Guzey & McClements, 2006; Hu, McClements, & Decke, 2003). Thus, antioxidants, such as butylatedhydroxy toluene and tert-butylhydroquinone, are usually added to emulsions to prolong their storage stability. In terms of food safety, however, the requirement that synthetic emulsifiers should be replaced with natural "label-friendly" alternatives has intensified in the food industries (Qian & McClements, 2011).

Many native stabilizers have been proposed by researchers as surface-active substances, which are capable of enhancing the stability of protein emulsions. These include polysaccharides (Dickinson, 2011; McClements, 2006; Murray, 2011) and polyphonic compounds (Brewer, 2011; Wan, Wang, Wang, Yang, & Yuan, 2013), among others. Rutin, a kind of secondary plant metabolite and phenolic compound is usually described as a glycoside comprised of quercetin (3, 5, 7, 30, 40-pentahydroxyflavone) and disaccharide rutinose (rhamnose and glucose) (Yang, Huang, & Chen, 2009; Yu et al., 2011). Rutin exhibits excellent antibacterial as well as antioxidant properties (Watt & Pretorius, 2001) and was beneficial to human health when consumed via food or drug systems (Enkhmaa et al., 2005; Jung, Lee, Cho, & Kim, 2007; Lucci & Mazzafera, 2009). In addition, rutin can act as a stabilizer in Pickering emulsions (Luo et al., 2011, 2012). It is inclined to adsorb at the oil–water interface owing to its amphiphilicity and surface activity although it exists in the form of insoluble particle in the aqueous phase.

Recently, resveratrol (a natural polyphenol compound) was reported to enhance the emulsions' stability by forming a mixed resveratrol–protein interfacial layers due to competitive adsorption (Wan et al., 2013). Thus, the accumulation of rutin at the interface is also expected to increase through the competitive adsorption between SPI and rutin, which may be beneficial for improving the stability of emulsions stabilized by SPI. Atarés, Marshall, Akhtar, and Murray (2012) found that rutin could be incorporated into the interface of an emulsion stabilized by whey protein, leading to partially replacing proteins at the interface and further improvement of the microstructure and oxidative stability of emulsion. Soy protein isolate (SPI), mainly composed of glycinin and β-conglycinin, is the most commercially available soy protein product, which is widely used as a proteinaceous emulsifier in food

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emulsions. Little research has been conducted on the effects of rutin on the stability of emulsions stabilized by soy protein. Heat treatment during food processing may cause protein denaturation, leading to the improvement of adsorption efficiency (Cui, Chen, Kong, Zhang, & Hua, 2014; Tcholakova, Denkov, Sidzhakova, & Campbell, 2006) and the formation of aggregates (Li et al., 2007, 2009). Therefore, the effects of rutin incorporation on the properties of the emulsions stabilized by native SPI and heat-denatured SPI, need to be explored.

In the present work, emulsions stabilized by rutin-incorporated soy protein were successfully prepared using high-pressure homogenization. Particular attention was paid to the emulsions' physical properties (particle-size distribution, physical stability, and the extent of interfacial adsorption) and their oxidative stability. In addition, the underlying mechanism of emulsion stability was investigated by determining the fluorescence quenching and interfacial tension of protein–rutin mixture.

2. Materials and methods

2.1. Materials

Low-denatured, defatted soy flakes were provided by Shandong Gushen Industrial and Commercial Co., Ltd (Dongying, Shandong, China). Soybean oil was purchased from a local supermarket and purified with Florisil (60–100 mesh, Sigma Aldrich) to remove surface-active impurities. All of the other reagents and chemicals were of analytical grade.

2.2. Isolation and heat treatment of SPI

The SPI was prepared as described by Li, Kong, Zhang, and Hua (2011). Protein content of the prepared soy protein was 90.6% (*w*/*w*) as determined by the micro-Kjeldahl method (AACC, 2000) with a nitrogen conversion factor of 6.25. Protein dispersions (0.5%, *w*/*v*) were prepared by adding soy protein to a 10 mM sodium phosphate buffer (pH 7.0), and stored overnight at 4 °C to ensure full hydration. The pH was adjusted to 7.0 by adding 1 M NaOH or 1 M HCl, as appropriate. The protein dispersions were filtered (Φ50 mm*0.45 μm, low protein binding, Sinopharm Chemical Reagent Co., Ltd.) before heating. Specific volumes of the dispersions were heated to 95 °C in water for 30 min. After treatment, the dispersions were rapidly cooled to room temperature in an ice bath. The protein content of final solution was determined by the Lowry, Rosebrough, Farr, and Randall (1951) method. In our experiment, high purity native soy protein was used as a protein standard, which was prepared in this lab and had a protein content of 99.0% as determined by the micro-Kjeldahl method.

The molecular weight distribution was determined by high performance size-exclusion chromatography (HPSEC) according to the method of Wu et al. (2010). Native and heat-treated protein solutions were filtered through a cellulose acetate membrane with a pore size of 0.45 μm (Sartorius Co., Ltd., Gottingen, Germany). About 20 μL of protein solutions was injected into the column and the fluent was monitored at 280 nm.

2.3. Preparation of rutin dispersion

The rutin dispersion was prepared using the method described by Medina et al. (2010), with some modifications. Rutin was added to a screw-capped 50 mL Erlenmeyer flask containing methanol solution, and the mixture was stirred for 1 h. Subsequently, the methanol in the mixture was removed under a stream of nitrogen. The rutin was dispersed again using a 10 mM phosphate buffer (pH 7.0), then sonicated for 5 min.

2.4. Fluorescence-spectroscopy measurement

The fluorescence spectra were recorded using a F7000 fluorescence spectrophotometer (Hitachi Co., Japan). The intrinsic fluorescence of the protein was measured at a constant SPI concentration (0.3 mg/mL) and different rutin concentrations in a 10 mM phosphate buffer (pH 7.0). The emission spectra were recorded from 300 to 500 nm at an excitation wavelength of 280 nm. Both the excitation and the emission slit widths were set to 5 nm. The fluorescence spectra of the phosphate buffer were subtracted from the respective spectra of the samples. The fluorescence quenching was carried out according to the Stern–Volmer equation (Equation (1))

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (1)$$

In this equation, F_0 and F are the fluorescence intensities in the absence and presence of a quencher, respectively. [Q] is the quencher concentration, K_{SV} is the Stern–Volmer quenching constant, k_q is the bimolecular quenching-rate constant, and τ_0 is the lifetime of the fluorescence in the absence of a quencher, the value of τ_0 for the biopolymer is 10^{-8} s^{-1} (Lakowicz & Weber, 1973). The value of K_{SV} was obtained by performing a linear regression of a plot of F_0/F versus [Q].

2.5. Interfacial tension

The interfacial tensions between soybean oil and 10 mM phosphate buffer or the protein–rutin mixture were measured by the Wilhelmy plate method (Sakuno et al., 2008), using a dynamic contact angle meter and tensiometer (DCAT21, Dataphysics, Germany). The aqueous phase was water or SPI solution (0.3%, *w*/*v*) in water, pH adjusted to 7.0.

2.6. Emulsion preparation

The oil–water emulsion was prepared by dispersing 10% (*w*/*v*) soybean oil in a 10 mM phosphate buffer (pH 7.0) containing 0.3% (*w*/*v*) native SPI (NSP) or heat-denatured SPI (HSP). The emulsions containing rutin (0.05–0.02 mg/mL) were prepared by adding rutin to the SPI/phosphate buffer aqueous phase prior to preparation by homogenization. Emulsions without the rutin additive were used as control samples. Coarse emulsions were prepared using a high-shear homogenizer (FA25, Fluko Equipment Co., Ltd., Shanghai, China) at 10,000 rpm for 1 min. Fine emulsions were prepared by homogenization at 40 MPa of pressure with two passes through the homogenizer (APV1000, APV Co., Crawley, U.K.). Each emulsion was prepared at least three times to confirm the repeatability of the process. The pH of each emulsion was measured and adjusted to 7.0 by adding 0.1 M or 1 M HCl or NaOH. Sodium azide (0.02%, *w*/*v*) was added to the emulsions to prevent microbial growth.

2.7. Physical characterization of emulsions

The particle-size distribution of the emulsions was determined using a Mastersizer 2000 laser particle-size analyzer (Malvern Instruments, Malvern, U.K.). The particle sizes were obtained in the form of area-weighted mean diameter, d_{32}. The emulsions were diluted 1000 times using 10 mM sodium phosphate buffer (pH 7.0), and zeta (ζ) potential was measured at 25 °C using a Zetasizer Nano ZS (Malvern Instruments, Malvern, U.K.).

The microstructure of the emulsions was examined using a confocal laser scanning microscope (LSM710, Zeiss Microsystems Inc., Germany) with a 40 mm objective lens. Aliquots (1 mL) of emulsion were mixed with 20 μL of Nile-red solution (0.02%, *w*/*v*)

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