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Physicochemical properties and antioxidant potential of phosvitin–resveratrol complexes in emulsion system

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ABSTRACT

Egg yolk phosvitin is the most highly phosphorylated protein found in the nature. The physicochemical properties of phosvitin–resveratrol complexes and their synergistic antioxidant activities in microemulsions were investigated. The particle diameters of microemulsions containing 0.5%, 1.0% and 2.0% phosvitin were 2.660, 0.501 and 0.414 μm, respectively. The emulsifying activity index increased largely from 3.72 to 21.5 m^2/g with increasing phosvitin concentration from 0.5% to 2.0%. Fourier transform infrared spectroscopy and thermal analyses indicated that the microemulsions underwent a conformational change during homogenization. Antioxidant assays showed that phosvitin–resveratrol microemulsions exhibited a higher antioxidant activity than that of phosvitin–resveratrol primary emulsions. The MTT assay indicated that HepG2 cell viability remained higher than 80% at phosvitin concentration below 1.0 mg/ml. This suggested that phosvitin, when coupled with polyphenol, can effectively inhibit lipid oxidation in food emulsions, which provided valuable insights into deep processing and application of egg proteins in food industry.

1. Introduction

A wide range of modern processed foods, including milk, cream, mayonnaise and salad dressings, exist as emulsions (Zhang, Xiong, Chen, & Zhou, 2014). Due to the large contact surface between oxidizable fatty acids and oxidants, emulsified oil is much more susceptible to oxidation (McClements & Decker, 2000), initiating negative changes in food system that affect its nutritional quality, wholesomeness, safety, color, flavor and texture (Shahidi, Janitha, & Wanasundara, 1992). In recent years, food-derived proteins combined with phenolic compounds have been found to exhibit cooperative antioxidant effects in oil-in-water (O/W) emulsions (Cao, Jia, Shi, Xiao, & Chen, 2016; Di Mattia, Sacchetti, Mastrocola, Sarker, & Pittia, 2010). Due to their amphiphilic nature, many natural proteins, such as soybean protein isolates, egg white proteins, and sodium caseinates, could adsorb rapidly to the surface of oil droplets and thereby stabilize emulsions (Wang et al., 2016).

Improving the antioxidant potential of emulsions highly depends on the properties of the proteins used (Intarasirisawat, Benjakul, Visessanguan, & Wu, 2014). In O/W emulsions, the major prooxidant that decomposes lipid hydroperoxides into free radicals is ionic iron (Hu, McClements, & Decker, 2003). Phosvitin, a principal phosphoprotein in egg yolk, has shown strong antioxidant activity owing to its iron chelating activity (Chen et al., 2014). Phosvitin, in which approximately half of the amino acid residues are serine and nearly all of them occur in the phosphorylated form, is considered as the most highly phosphorylated protein found in the nature. This unique structure is accompanied by some excellent properties, such as emulsifying property, antibacterial activity and heat stability (Mine, 2008). These properties prompted us to wonder whether phosvitin could be an excellent choice providing simultaneous emulsification and antioxidant activities in food emulsion system.

Resveratrol, a non-flavonoid polyphenol, is mainly found in red grapes and peanuts and has exhibited health-beneficial properties including strong antioxidant activity (Sessa, Tsao, Liu, Ferrari, & Donsì, 2011; Zhang et al., 2015). However, the physicochemical properties of resveratrol, particularly its poor water solubility, limit its utilization as a nutraceutical ingredient within the food industry (Davidov-Pardo, Pérez-Ciordia, Marín-Arroyo, & McClements, 2015). Some stable protein-based systems are thought to be able to protect resveratrol against oxidation and improve its bioavailability (Cao et al., 2016; Wang et al., 2016). Recent research has indicated that soy protein isolate-resveratrol complexes were efficient emulsifiers and improved the oxidative stability of O/W emulsions (Wan, Wang, Wang, Yuan, & Yang,

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2014). Similarly, a cooperative inhibitory effect of licorice extract-pea protein hydrolysates on lipid oxidation in O/W emulsion was reported (Zhang et al., 2014). On the basis of this background, we hypothesized that phosvitin and resveratrol would exhibit cooperative antioxidant effects in emulsion system. To our knowledge, this is the first research to investigate the synergistic antioxidant activity of egg yolk proteins and polyphenol in food emulsion system.

Herein, the physicochemical properties and antioxidant potential of phosvitin–resveratrol complexes in emulsion system were investigated. The phosvitin–resveratrol microemulsions were prepared through high pressure homogenization. The physicochemical properties, including particle size, emulsifying activity index (EAI), morphology, as well as their thermal properties were characterized by laser light scattering instrument, invert fluorescence microscope, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA). The chemical antioxidant activities were determined by the ferric reducing power assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical assays and lipid peroxidation analysis.

2. Materials and methods

2.1. Materials

Egg yolk phosvitin was isolated according to the method of Jiang and Mine (2000). Resveratrol and the testing chemicals including DPPH, Nile Red, Nile Blue and MTT were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle medium (DMEM) and calf serum were purchased from Thermo Fisher Scientific Inc. (Shanghai, PR China). Salicylic acid, H_2O_2, thiobarbituric acid reactive substance (TBARS), dimethyl sulfoxide (DMSO), and other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and were of analytical grade. Ultrapure water (18 mΩ) was supplied by a Milli-Q apparatus (Millipore Corp.).

2.2. Preparation of microemulsion

Phosvitin was dispersed in pH 7.0 buffer (10 mM PBS) at room temperature and stirred for 3 h to form phosvitin solutions with concentrations of 0.5%, 1% and 2% (w/v), respectively. Because the oil phase alone did not increase sufficiently the solubility of resveratrol, a small amount of ethanol (ethanol/resveratrol weight ratio was 20:1) was used to dissolve resveratrol crystals prior to mixing with peanut oil (concentration of resveratrol was 0.2% in the eventual oil phase). The primary emulsions were prepared by mixing 5 ml of the eventual oil phase and 95 ml of the phosvitin solution, followed by high-speed homogenization at 10,000 rpm for 1 min using a high speed blender (IKA T25 Basic, Staufen, Germany). To prepare microemulsions, the primary emulsions were further homogenized twice with a high-pressure homogenizer at 100 MPa through the ATS homogenizer (ATS Engineer Inc., Shanghai, China). Samples were sealed and stored at 4 °C until analysis. A portion of each microemulsion was lyophilized for SEM, FTIR and thermal analysis. The primary emulsions without high-pressure homogenization were used as control groups for antioxidant assays.

2.3. Characterization of the microemulsions

2.3.1. Droplet size measurements

Droplet size distributions were determined using the granulometer Mastersizer 2000 laser light scattering instrument (Malvern Instruments Ltd., Worcestershire, UK). The samples were stirred continuously within the sample cell to ensure homogeneity at ambient temperature. Droplet size distributions were calculated by the instrument according to the Mie theory, which used the refractive index difference between the droplets and the dispersing medium to predict the intensity of scattered light. The refractive index values used for instrumental analysis of oil droplets and dispersant were 1.45 and 1.33, respectively. Droplet size measurements were reported as both

(a) volume-length mean diameter (d_{43})

$$d_{43} = \Sigma n_i \times d_i^4 / \Sigma n_i \times d_i^3$$

(b) surface-weighted mean diameter (d_{32})

$$d_{32} = \Sigma n_i \times d_i^3 / \Sigma n_i \times d_i^2$$

where n_i is the number of droplets of diameter d_i.

The span (the width of the distribution) was defined by the expression

$$\text{span} = (D_{90} - D_{10})/D_{50}$$

D_{90} was the drop diameter below which 90% of the distribution exists, D_{50} was the median diameter, and D_{10} was the drop diameter below which 10% of the distribution exists. The smaller the span value the narrower the particle size distribution.

2.3.2. Microstructure of emulsion droplets

Microscopic images of the microemulsions were taken using an inverted optical microscope (Olympus IX71, Olympus, Tokyo, Japan). A mix of Nile Blue (for protein) and Nile Red (for oil) dyes were added and the solution was thoroughly mixed. A small drop of microemulsions was placed onto a microscope slide and carefully covered with a coverslip. After having equilibrated for 2 min, the photomicrographs (40× magnification) were taken. Representative images were chosen from at least three similar images.

2.3.3. Scanning electron microscopy (SEM)

For scanning electron microscopy (SEM), the freeze-dried microparticles were sputter-coated with gold, and examined in a Hitachi S-3400N scanning electron microscope (Hitachi, Tokyo, Japan) at 5.0 kV.

2.3.4. FTIR

The FTIR spectra were recorded on a Bruker Fourier transform infrared spectrophotometer (Bruker, Germany). The freeze-dried microparticles were mixed with KBr in the ratio of 1:150 and ground in a mortar by hand with a pestle. The powders were pressed into pellets under a pressure of 4 t. The infrared radiation absorbency scans were analyzed in range of 4000–400 cm^{-1} for changes in the intensity of sample peaks, with air as the background. In addition, a physical mixture of phosvitin and resveratrol powders was used as control (phosvitin–resveratrol).

2.3.5. Thermal analysis of phosvitin

Thermal properties of phosvitin were analyzed by DSC and TGA. DSC was carried out using a DSC Q2000 (TA Instruments, New Castle, USA) machine. Freeze-dried microparticles were weighed in aluminum pans and heated in DSC from 20 to 180 °C at 10 °C/min using nitrogen gas at a flow rate of 50 ml/min. An empty pan served as the reference. The denaturation peak temperature (T_p) and enthalpy (ΔH) were obtained by DSC Q2000 V24.2 Build 107. Thermogravimetry and differential thermal analysis (TG/DTA) curves were measured using a Shimadzu DTG-60A