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Synergistic effects of ovalbumin/gum arabic complexes on the stability of emulsions exposed to environmental stress

Fuge Niu [a, b], Jianzhong Zhou [a, c], Debao Niu [d], Chenying Wang [a, b], Yuntao Liu [a, b], Yujie Su [a, b], Yanjun Yang [a, b, *]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, PR China
[b] School of Food Science, Jiangnan University, Wuxi, Jiangsu, 214122, PR China
[c] School of Food Science and Pharmacy, Xinjiang Agricultural University, Urumchi 830052, PR China
[d] Guangxi Subtropical Crops Research Institute, Nanning, Guangxi, 530001, PR China

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ABSTRACT

This study investigated the complexes formed between ovalbumin (OVA) and gum arabic (GA) and their ability to stabilize oil-water emulsions. The stability of the emulsions stabilized by the OVA/GA complexes was evaluated by measuring storage time (1–7 days), salt concentration (0–100 mM NaCl), and heat (40–90 °C, 30 min) stability at pH 3.8–7.0. The results showed that the stability of the OVA-stabilized emulsion was greatly improved by an OVA/GA ratio of 1:2 and acidic pH. Above or below 1:2, the emulsion was unstable due to depletion or bridging flocculation. Emulsions saturated with OVA/GA complexes were stable with changing pH, storage time, and heat treatment (up to 90 °C). However, the OVA/GA complexes did not improve the stability of the emulsions when salt concentrations were varied. The experimental results demonstrated that OVA/GA complexes could be used to prepare stable emulsion structures, which may be useful in the beverage industry.

1. Introduction

Proteins are natural biopolymers that are extensively used as surface activity ingredients in the food industry, to stabilize emulsions and foams, and to protect and deliver bioactive ingredients to target sites (Dickinson, 2009; Jeong et al., 2004). Their surface activity can be attributed to the molecular flexibility, amino acid composition, and amphiphilicity of their polypeptide chains (Damodaran, 1997). Once adsorbed, rapid conformational change at the interface is required for the protein to reorient its hydrophobic amino acid residues in the oil phase. At the same time, the hydrophilic groups rearrange and protrude away from the surface into the aqueous phase (Husband, Garrood, Mackie, Burnett, & Wilde, 2001; Walstra, 2002). The adsorbed protein molecules can form a strong viscoelastic membrane, which stabilizes the droplet against aggregation and coalescence through electrostatic and/or steric repulsive forces (Dickinson, 2009; Tcholakova, Denkov, Ivanov, & Campbell, 2006). However, the stability of emulsions prepared with proteins is affected by solvent conditions (e.g., pH and salts). Changes in the pH and/or ionic strength of the aqueous phase can cause flocculation/aggregation of oil droplets, thereby decreasing the stability of the emulsion (Tcholakova et al., 2006). Furthermore, the stability decreases in the presence of competing biopolymers and/or surfactants or changes in protein conformation during aging (Djordjevic, McClements, & Decker, 2004; Tcholakova et al., 2006).

Polysaccharide is widely used in the food industry for its gelling, thickening, and stabilizing properties (Stephen & Phillips, 2010). Emulsions containing droplets coated with protein-polysaccharide complexes are more resistant to environmental stress than those coated with protein alone. This may be attributable to the formation of a thick and compact interfacial layer around the oil droplet surface, providing strong steric stabilization against aggregation/flocculation and coalescence (Dickinson, 2008). The level of protein-polysaccharide interaction is affected by physicochemical parameters such as the solvent conditions (pH, salts, and temperature) and biopolymer characteristics (e.g., mixing ratio,

* Corresponding author. State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, PR China. Tel./fax: +86 0510 85329080.
E-mail addresses: niufg123@hotmail.com (F. Niu), yangyj@jiangnan.edu.cn (Y. Yang).

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concentration, charge density, type, etc.). Previous studies have shown that the ability of deamidated ovalbumin to stabilize oil-in-water emulsions can be improved by forming conjugate (Maillard) complexes with polysaccharides (dextran, galactomannan) (Kato, Sasaki, Furuta, & Kobayashi, 1990; Nakamura, Kato, & Kobayashi, 1992). Galazka, Dickinson, and Ledward (2000) investigated emulsions made with pressurized OVA in the presence of polysaccharide (carrageenan, dextran sulphate) and showed that protein-polysaccharide interactions protected the protein against loss of functionality from aggregate formation after high-pressure treatment. The pressure-processed emulsion had better emulsifying efficiency and stability. Padala, Williams, and Phillips (2009) reported that the droplet sizes of emulsions prepared using gum arabic (GA)/egg white protein mixtures (1:0.05% w/w) were significantly larger at pH 7.5 than that of 3.5. However, the physicochemical properties of emulsions stabilized by OVA/GA complexes against environmental stress are still unclear and the mechanisms need to be clarified.

The natural biopolymers used in this work are ovalbumin and gum arabic. Gum arabic is a complex polysaccharide exuded from Acacia Senegal (L.) Willd. It is an anionic arabinogalactan polysaccharide-protein complex, composed of three fractions (Randall, Phillips, & Williams, 1989; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). These are commonly referred to as the arabinogalactan-protein, arabinogalactan, and glycoprotein fractions, comprising 10.4%, 88.4%, 1.24% of the total volume, respectively, and 50%, 0, 25%, of the protein content, respectively. Chicken egg ovalbumin is the main constituent of egg white protein (~65%). The molecular mass of OVA is 42.7 kDa and it is an important food ingredient with structural functionality, including emulsifying and foam stabilizing properties. Egg white proteins are extensively used in processed foods (Choi, Kim, Park, & Moon, 2005). In a recent study, we formulated complexes of OVA and GA and found that the addition of GA could induce the protein solutions to go through four different successive phase diagrams, in terms of pH and OVA/GA ratios (Niu, Dong, et al., 2014; Niu, Su, et al., 2014).

In this study, the relationship between the emulsifying functionality and the phase diagram was established using the OVA/GA system. The purpose of the study was to investigate the possibility of blending OVA and GA to prepare a stable emulsion and to further extend the application field of eggs. The microstructure of the emulsion was observed to better understand the relationship between the microstructure characteristics and emulsion stability. Moreover, the underlying mechanism of emulsion stability was investigated against changes induced by storage time, pH, salt, and thermal treatment. The results could be useful for designing food and beverage emulsions with improved properties and consumer acceptance.

2. Materials and methods

2.1. Materials

Ovalbumin (from egg white, molecular weight 42.7 kDa) was purchased from Amresco Chemical Co. (Boise, USA). According to the accompanying report, the composition of the powder was 90.3% total protein (% N × 6.25), 5.92% moisture, 0.2% fat, and 2.82% ash.

Gum Arabic was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The powder contained 9.2% moisture, 89.24% dry solid, and 4.91% ash (w/w). Commercial sunflower oil was purchased from a local supermarket, and contained approximately 13.0, 26.0, and 61.0 wt % of saturated, monounsaturated, and polyunsaturated fats, respectively. All other chemicals used were of analytical grade and were purchased from either Sigma Chemical Co. (St. Louis, MO, USA) or Sinopharm Chemical Reagent Co., Ltd. (Shanghai, P.R. China).

2.2. Emulsion preparation

Mixtures of OVA and GA at various ratios (0.6% w/v) were obtained by dissolving each powder in deionized water containing 0.02% (w/v) sodium azide as an antimicrobial agent, stirring gently (500 rpm) at room temperature for 4 h, and then overnight at 4 °C to ensure biopolymer dissolution (Niu, Dong, et al., 2014; Niu, Su, et al., 2014). The OVA/GA mixtures were adjusted to pH 7.0 using 0.1 M NaOH, before homogenization. The oil-in-water (O/W) emulsions were prepared by dispersing 5% (v/v) sunflower oil in an OVA/GA mixture solution. Primary emulsions were prepared using an Ultra-Turrax blender (IKA T25 Basic, Staufen, Germany) at 11,000 rpm for 2 min. Fine emulsions were prepared by homogenization twice with a high-pressure homogenizer at 40 MPa through the homogenizer (APV1000, APV Co., Crawley, U.K.). The pH of the emulsion was adjusted with 0.1 M HCl or 0.1 M NaOH to the desired pH. Samples were sealed and stored at 4 °C until analysis.

2.3. Particle size measurements

The droplet size distribution and mean particle diameter of the emulsions were determined using the laser diffraction apparatus Zetasizer nano ZS (Malvern Instruments Ltd., Malvern, U.K.). Emulsion samples were diluted 1:100 using deionized water of the appropriate pH. Samples were equilibrated for 60 s inside the instrument before data was collected at least 12 sequential readings. Reported z-averages were the average of three independent replicates.

2.4. ζ-potential measurements

The ζ-potential of the emulsion samples was calculated by measuring the electrophoretic mobility of the droplets using a capillary electrophoresis cell (Zetasizer nano ZS, Malvern Instruments Ltd., Malvern, U.K.). The samples were diluted to a droplet concentration of approximately 1:100 using deionized water of the appropriate pH. Samples were equilibrated for 60 s inside the instrument before data were collected over at least 10 sequential readings and the Smoluchowsky mathematical model was used to convert the electrophoretic mobility measurements into the ζ-potential values (Salminen & Weiss, 2014). All measurements were made in triplicate.

2.5. Microstructure of emulsion droplets

Microscopic images of the emulsions covered with biopolymer complexes were taken using a light microscope (Leica DM2000, Leica Microsystems Wetzlar GmbH, Germany). A small drop of emulsion was placed onto a microscope slide and carefully covered with a coverslip. After having equilibrated for 2 min, the photomicrographs (20 × magnification) were taken. Representative images of microscopic imaging were chosen from at least four similar images.

2.6. Physical stability of emulsions

2.6.1. Storage stability

Emulsions were prepared with 5% oil and an OVA/GA ratio of 1:2, placed in transparent screw-capped vials, and stored in a dark oven at 25 °C for 7 days. The particle size, ζ-potential, and