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Stabilization of oil-in-water emulsions using mixtures of denatured soy whey proteins and soluble soybean polysaccharides

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ABSTRACT

Mixtures of denatured soy whey proteins (dSWP) and soluble soybean polysaccharides (SSPS) were used to stabilize 5 wt.% oil-in-water (O/W) emulsions against coalescence and phase separation. Emulsions prepared with either dSWP or SSPS at low concentrations demonstrated limited stability. At an optimal SSPS:dSWP ratio of 1.5:1.0 (corresponding to 2.5 wt.% biopolymer in the aqueous phase), emulsions did not phase separate for >60 days at pH 3 and 21 days at pH 8. Irrespective of the protein–polysaccharide ratio, emulsions prepared at lower pH (3–4) showed better long-term stability versus pH 5–8. The negligible surface charge (−2 mV) at low pH suggested the presence of dSWP–SSPS complexes that promoted emulsion stability via steric hindrance. The higher surface charge at pH 7–8 (near −20 mV) prevented mixed dSWP–SSPS layer formation around the dispersed oil droplets resulting in limited emulsion stability. A deleterious effect of 1 M NaCl on emulsion stability was noted, further confirming mixed dSWP–SSPS layer formation as the dominant mode of stabilization. Overall, this study showed that the presence of dSWP–SSPS interfacial layers promoted the capacity of O/W emulsions to resist oil droplet coalescence and phase separation.

1. Introduction

Mixed protein–polysaccharide systems are well-recognized macromolecular assemblies capable of stabilizing oil-in-water (O/W) food emulsions (Dickinson, 1992, 1993, 2003; Dickinson & Galazka, 1992; Tolstoguzov, 1997; Tolstoguzov, 2002, 2003a, 2003b). The presence of polysaccharides can significantly influence protein functionality (e.g., surface activity, solubility, emulsifying property, gel forming ability, foaming property, and conformational stability) (Lippi & Taranto, 1981; Mishra, Mann, & Joshi, 2001) though this depends on the extent of their interactions and mass ratio, local environmental conditions and external inputs such as temperature and shear (Benichou, Aserin, & Garti, 2002; Dickinson, 2003; Garti & Reichman, 1993; McClements, 1999; Tolstoguzov, 1997).

In the preparation and stabilization of O/W emulsions, oil–water interfacial adsorption of protein–polysaccharide complexes critically depends on whether complexation takes prior to, during or after emulsification, as the order of addition generates different structures and/or compositions at the interface (Braudo, Plaschina, & Schwenke, 2001; Dickinson & Galazka, 1991, 1992; Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008; Jourdain, Schmitt, Leser, Murray, & Dickinson, 2009; Kato, Tsutomo, & Kobayashi, 1989; Lippi & Taranto, 1981; Mishra et al., 2001). Complexation prior to or during emulsification may significantly alter the resulting emulsification efficacy of the protein–polysaccharide amphiphiles, particularly if the accompanying polysaccharide is surface-active (Ganzevles, Cohen Stuart, van Vliet, & de Jongh, 2006; Ganzevles, Zinoviadou, van Vliet, Cohen Stuart, & de Jongh, 2006). Such complexation may also modify protein conformational flexibility and de facto its capacity to appropriately unfold at an interface. Furthermore, as such complexation is typically electrostatic in nature, there is likely to be a reduction in the emulsion charge stabilization capacity of the biopolymer complex vis-à-vis the native protein (Braudo et al., 2001). As an example, a comparative study using a sodium caseinate–dextran sulfate complex to stabilize *n*-tetradecane–water emulsion suggested that improvements in interfacial film shear viscoelasticity with the pre-emulsified complex led to emulsions with an improved shelf life (Jourdain et al., 2009).

Both mixed film and layer-by-layer deposition of proteins and polysaccharides can create thick interfacial films that greatly minimize inter-droplet contact (Dickinson, 1995, 1996, 1998a, b). As well, compared to protein films, protein–polysaccharide films can provide superior resistance against environmental stresses such as large changes in pH or ionic strength and typical unit operations such as thermal processing or freezing (Aoki, Decker, & McClements, 2005; Gu, Decker, & McClements, 2005a, b; Guzey, Kim, & McClements, 2004; Moreau, Kim, Decker, & McClements, 2003). For example, O/W emulsions stabilized with β-lactoglobulin–pectin multilayers showed better resistance against salt-induced droplet flocculation compared to β-lactoglobulin-coated emulsions (Guzey & McClements, 2007).

Soy proteins, and in particular soy protein isolates, have been well-characterized as emulsifiers (Lippi & Taranto, 1981; Palazolo,

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Sorgentini, & Wagner, 2005). However, the soluble fractions of soy protein aqueous extracts, i.e., soy whey proteins, have seen comparatively little effort devoted to their structure–functionality relationship. Native soy whey proteins (nSWPs) are a waste by-product following the isoelectric precipitation of soy protein isolate. Their main components are lectin and the Kunitz trypsin inhibitor (KTI) with molecular weights of 120 kDa and 20–22 kDa, respectively (Koide & Ikenaka, 1973; Lotan, Siegelman, Lis, & Sharon, 1974; Sorgentini & Wagner, 1999). Soybean lectin is composed of four identical subunits of 30 kDa along with two carbohydrate binding sites (Liener, 1981). KTI is a monomeric non-glycosylated protein consisting of two disulfide bridges along with 12-criss-crossing antiparallel β-sheets (Roychaudhuri, Sarath, Zeece, & Markwell, 2003, 2004). The two disulfide bridges are stabilized by hydrophobic side chains, which show inhibitory action against trypsin and a high resistance towards thermal and chemical denaturation. Lectin and KTI are stable against pH-induced selective denaturation over wide pH ranges of 3–10 and 2.2–10.8, respectively (Koshiyama, Kikuchi, & Fukushima, 1981; Lotan et al., 1974). The limited literature available on SWPs has revealed that denatured soy whey proteins (dSWP) show somewhat better emulsifying activity compared to nSWP, but that they are incapable of preventing droplet coalescence over extended periods (Mitidieri & Wagner, 2002; Palazolo, Sorgentini, & Wagner, 2004). The isoelectric point of KTI and lectin have been reported as 4.5 and 5.8, respectively (Sorgentini & Wagner, 1999). Previous studies have demonstrated that raw soybean protein contains anti-nutritional components that may inhibit vertebrate pancreatic serine proteinases, resulting in a range of deleterious physiological effects (Tan-Wilson & Wilson, 1986). Though soybean trypsin inhibitor does not appear to be hazardous in humans, soymeal for use in animal feed must be heat-treated to minimize any potential health risks (Flavin, 1982).

Soy soluble polysaccharides (SSPS) are pectin-like acidic biopolymers also extracted from the residual carbohydrate by-product of soy protein isolate production. They consist of a main rhamnogalacturonan backbone branched with β-1,4-galactan and α-1,3 or α-1,5-arabinan chains, and homogalacturonan covalently bound to a ~50 kDa protein moiety (Nakamura, Yoshida, Maeda, & Corredig, 2006). SSPS is effective in stabilizing emulsions at pH 3–7 and ionic strengths up to 25 mM NaCl or $CaCl_2$ (Nakamura, Yoshida, Maeda, Furuta, & Corredig, 2004).

The characterization and utilization of SSPS and SWP from soymilk and tofu preparation may offer new avenues for the use of these undervalued agricultural resources. As use of nSWP or dSWP alone or in combination with native soy protein isolates for emulsification yields poorly-stable emulsions (Mitidieri & Wagner, 2002; Palazolo et al., 2004, 2005), the goal of this study was to develop O/W emulsions stabilized with mixed dSWP and SSPS to impart improved stability compared to protein-based systems. dSWP and SSPS dispersions were first analyzed and the resulting findings were extended to the stabilization of dilute O/W emulsions.

2. Materials and methods

2.1. Materials

Defatted solvent-free soy flour (7B Soy Flour: min. 53% protein, max. 9% moisture, 32% carbohydrates, max. 3% fat, 18% total dietary fiber) was obtained from Archer Daniels Midland Company (Decatur, IL, USA). Commercial-grade SSPS (SOYAFIBE-S-CA200: moisture: 5.8%, crude protein: 7.8%, crude ash, 7.8%. Saccharide composition (%): Rhamnose: 5.0, Fucose: 3.2, Arabinose: 22.6, Xylose: 3.7, Galactose: 46.1, Glucose: 1.2, Galacturonic acid: 18.2) was provided by Fuji Oil Ltd. (Osaka, Japan). Soybean oil (acid value < 0.2) (AOCS, 1997) was purchased from a local grocery store. All chemicals used were reagent grade. Deionized water was used throughout all experiments. Unless noted otherwise, all reagents were used without further purification.

2.2. Extraction of denatured soy whey proteins (dSWP)

The method of Sorgentini and Wagner (1999) was followed to extract the nSWP. After centrifugation of a 10 wt.% suspension of defatted solvent-free soy flour in water at $10^4 \times g$, the supernatant was brought to pH 4.5 using 0.1 M HCl and held 1 h at room temperature (Sorgentini & Wagner, 1999). The resulting suspension was again centrifuged at $10^4 \times g$. The collected supernatant was filtered through a defatted cotton sheet and brought to pH 8 with 0.1 M NaOH, then stirred for 1 h at room temperature and centrifuged (Sorvall 5C Plus Superspeed Centrifuge-Thermo-Scientific, Ottawa, ON, Canada) at $10^4 \times g$ for 30 min at 4 °C. The resulting clear supernatant was dialyzed for 24 h at 10 °C against a 0.02 wt.% sodium azide solution, resulting in an nSWP dispersion with a concentration of 0.1 wt.%. The nSWP dispersion was denatured at 100 °C for 45 min followed by freeze-drying for 4 days at −52 °C to yield a dSWP free-flowing powder (residual moisture content ~ 1.5 wt.%). Protein concentration was assayed spectroscopically (4284D reader, Thermo Labsystems Multiskan Ascent, Haverhill, MA, USA) (Sapan, Lundblad, & Price, 1999).

2.3. Preparation of dSWP and SSPS dispersions

Dispersions of dSWP (2 wt.%) and SSPS (3 wt.%) in deionized water were independently prepared, adjusted to pH 3 and stirred for 3 h. Sodium azide (0.02 wt.%) was added to prevent microbial growth. Based on preliminary emulsion droplet sizing and stability testing, sodium azide did not have any effect on protein–polysaccharide interactions in bulk or in emulsions at this low concentration.

2.4. Preparation of O/W emulsions

Emulsion consisted of 5 wt.% oil, 1 wt.% dSWP and 0.15–1.5 wt.% SSPS in deionized water. Volumes of stock dSWP and SSPS dispersions at pH 3 were mixed at appropriate ratios and stirred for 30 min. The emulsions were prepared by mixing 5 wt.% soybean oil with the dSWP–SSPS dispersion to produce 5% O/W emulsions. Coarse emulsions were prepared using a rotor/stator (Polytron PT-10-35 with PCU-2 control, Kinematica GmbH, Switzerland) at 27,000 RPM for 1 min followed by high-pressure valve homogenization (3 passes at 60 MPa) (APV, model 1000, Albertslund, Denmark) at room temperature (~25 °C). Emulsion pH was adjusted from pH 3 to 8 with 0.1 M NaOH or 0.1 M HCl. Emulsion samples were kept sealed at 4 °C until analysis.

2.5. Protein SDS–PAGE

Volumes (15 μl) of nSWP and molecular weight standards were resolved on a linear 8% acrylamide SDS–PAGE gel using a Bio-Rad mini electrophoresis system (Bio-Rad Laboratories, Mississauga, ON, Canada) in tricine electrophoresis buffer, following the method of Schägger and von Jagow (1987). The gel was run for 30 min at 30 V and then at 125 V until the tracking dyes reached the end of the gel. The gel was released from glass plates and the protein bands were stained with a Coomassie brilliant blue R-250 staining solution (Bio-Rad Laboratories, Mississauga, ON, Canada). Images of the gels were captured with a digital camera (Canon, Toronto, ON, Canada).

2.6. Circular dichroism (CD) spectroscopy

CD spectroscopy was performed on Jasco J-810 CD spectrophotometer (Jasco Inc., Easton, MD, USA). The secondary structures of nSWP and dSWP were determined by scanning 300 μl protein samples (1 wt.%) at pH 7 using a 0.1 cm path length cuvette. Samples were scanned from 250 nm to 190 nm with 1 s averaging time for each wavelength.