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Model system analysis of wheat starch-soy protein interaction kinetics using polystyrene microspheres

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Abstract

The objective of this study was to identify the interaction of puroindoline and starch lipids with soy proteins when coupled to polystyrene microspheres. Manipulation and removal of wheat starch granule surface proteins and lipids may damage granule integrity and hinder studies of ungelatinized starch systems. Therefore, 5 μm polystyrene microspheres were used to evaluate the role of starch granule surface components on exogenous protein binding. Puroindoline, phospholipid, glycolipid and triglyceride were passively adsorbed to the surface of polystyrene microspheres. Binding assays using soy protein indicated that puroindoline-lipid coated microspheres adsorbed soy protein to a greater extent than did those coated with puroindoline alone. Phospholipids increased binding more than glycolipids and triglycerides.

Keywords: Starch; Soy; Puroindoline; Friabilin; Microspheres

1. Introduction

Starch–protein interactions are important in cereal processing and in the production of cereal-based products because of their impact on the texture of wheat kernels and the rheological properties of wheat doughs. In baked bread dough systems, protein and starch are tightly associated. Scanning electron microscopy has consistently demonstrated this association in bread at various stages of processing (Pomeranz, El-Baya, Seibel, & Stephan, 1984a; Pomeranz, Meyer, & Seibel, 1984b).

By using glass beads in place of starch in a model bread dough system, researchers have reported that starch interacts with protein in some manner beyond that of the beads providing a simple hydrophobic support (Hibberd, 1970; Rasper & deMan, 1980). Edwards, Dexter, and Scanlon (2002) confirmed the importance of starch granule surface interactions by incorporating glass powder coated with bovine serum albumen (BSA) into a wheat flour dough. BSA masked the hydrophobic character of the glass surface and decreased gluten protein interaction. This loss of interaction was directly correlated to a change in the moduli and compliance functions of the viscoelastic dough. These studies suggest that starch granule surface components dictate the manner in which starch interacts with its surrounding protein environment.

Little research has investigated the adhesion between wheat starch and non-wheat protein in composite doughs. Dahle (1971) and Larsson and Eliasson (1997), in non-wheat proteins to wheat starch granule binding studies, found that wheat starch granules adsorb proteins at a different rate and in a different configuration depending on their origin and environmental conditions. In a wheat dough system containing added soy protein, the manner in which soy proteins and wheat

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starch surface components interact may dictate the resulting texture of the baked product.

The wheat starch granule surface contains 10 major protein groups ranging from 5 to 149 kDa, as well as several lipid components (Baldwin, 2001). Researchers have hypothesized that the protein portion of the granule surface partially mediates starch–protein functionality in doughs and dough products (Seguchi, 1990; Seguchi, 1993; Seguchi, Hayashi, Kenegaga, Ishihara, & Noguchi, 1998). Of the proteins, puroindoline (≈15 kDa) has been studied the most due to its possible involvement in wheat kernel hardness and dough foam stabilization. Addition of puroindoline to flours genetically free of this protein produces breads with improved crumb structure compared to breads from puroindoline-free flours (Igrejas et al., 2002). It has been hypothesized that lipids present on the starch granule surface, often identified as non-starch lipids to distinguish them from lipids bound to the starch granule interior, also play a role in starch–protein interaction. The removal of this lipid has been reported to result in a detrimental dough rheology in bread (Marston & Macritchie, 1985) and poor internal structure in cookies (Clements & Donelson, 1981). Specifically, Pomeranz et al. (1984a) found that polar lipids significantly improve dough protein–starch interactions in toast bread. Identification of the mechanism by which starch granule surface proteins and lipids function in a dough matrix would aid in the incorporation of non-wheat proteins into bakery products.

The difficulty in measuring the contribution of starch granule surface components to dough functionality is in the separation of the fractions in vivo. Removal of each protein fraction requires different methods, several of which disrupt the integrity of the granule (Baldwin, 2001). Removal of all protein and lipid from the starch granule surface is difficult thereby making model studies involving starch interaction impractical. The objective of this study was to identify the interaction of puroindoline and starch lipids coupled to polystyrene microspheres with added soy proteins. Differing levels of puroindoline and/or starch lipids were serially adsorbed to the surface of 5 μm polystyrene microspheres. These modified microspheres, representing starch in the experimental model, were then exposed to soy protein (extracted from textured and untextured soy flours) and the level of binding was determined.

2. Materials and methods

2.1. Reagents and equipment

Rabbit antibodies to whole soybean protein and goat anti-rabbit IgG peroxidase were purchased from Accurate Chemical and Scientific Corp. (Westbury, NY). Other reagents were purchased from Fisher Scientific (Hanover Park, IL) and were of reactant grade, unless otherwise noted.

2.2. Soy isolate and fraction production

Defatted, toasted soy flour (SF) (53% protein, 9.7% moisture) and solvent-extracted 13 textured soy flour (TVP; 50% protein, 10.2% moisture) were obtained from Archer Daniels Midland (Decatur, IL). Extruded-expelled soy flour (TSP; 49% protein, 10.8% moisture) was provided by Insta-Pro International (Des Moines, IA). The manufacturing processes by which these flours were generated is proprietary.

Soy protein isolates of textured, ground (#40 sieve) and non-textured soy flour were produced by extracting the flour with alkaline water (pH 8.0, adjusted with 2 N NaOH) for 2 h at room temperature (~22 °C; water:flour 10:1 mass/v). Suspension was centrifuged (1000*g*, 30 min) and the resulting supernatant adjusted to pH 4.5 with 2 N HCl. Precipitate was separated by centrifugation (5000*g*, 15 min), suspended in water (5% protein mass/v) and adjusted to pH 8.0 with 1 N NaOH (Ortiz & Anon, 2001). Isolates were lyophilized and stored in polyethylene bags at room temperature until use (<30 days). Major soy protein fractions (11S and 7S) of non-texturized soy flour and textured soy flour were separated via isoelectric precipitation (Than & Shibasaki, 1979). Flour (100 g) was extracted with 0.03 M Tris buffer (flour:buffer 1:20, pH 8.0) containing 0.01 M mercaptoethanol at room temperature for 1 h. Solution was centrifuged (20,000*g*, 20 min), adjusted to pH 6.4 with 2 N HCl then re-centrifuged (20*g*, 20 min, 4 °C). Precipitate (11S globulin) was suspended in 50 mM Tris–HCl (pH 7.8) containing 20 mM NaCl and dialyzed against 20 mM NH_4HCO_3 (6 h). Aggregated globulins were removed by gel filtration (Sepharose CL-6B; flow rate = 0.5 ml/min). Resulting protein was lyophilized. Supernatant was adjusted to pH 4.8 with 2 N HCl then centrifuged (20,000*g*, 20 min, 4 °C). Resulting pellet was suspended in 0.03 M Tris–HCl with addition of 2 N NaOH until dissolution (pH 7.6). Supernatant (7S globulin) was dialyzed against 20 mM NH_4HCO_3 (6 h), and lyophilized. To check for purity, an 18 ml gel filtration column (Kontes Flex Column, Kontes-Kimble, Vineland, NJ) was loaded with Sepharose CL-6B and gravity flow equilibrated with 100 mM Tris/HCl buffer (pH 7.8, flow rate of 0.5 ml/min). Samples of each of the soy fractions (20 μM) were injected into the column to establish normal retention times, which were checked against molecular weight standards. Fractions were checked for purity using 4–12% gradient Bis-Tris SDS–PAGE (NOVEX Xcell SureLock Mini-Cell System, NOVEX USA, San Diego, CA).

2.3. Protein dispersibility

Protein dispersibility was determined as an indirect measure of solubility. Protein solubilities in water were determined using a modification of the method of Mohammed, Hill, and Mitchell (2000). Samples (0.1 g) of lyophilized protein (SFI, soy flour S, TVP isolate, TVP 7S, TVP 11S, TSP isolate, TSP 7S, TSP 11 S, puroindoline, gliadin, glutenin) were mixed individually with 10 ml of 10 mM phosphate buffer (pH 7.5), 10 mM acetate buffer at pH 7.5 for 8 h. Solutions were held in a water bath (25 °C) for 5 min, centrifuged (1500*g*, 10 min) and filtered (Whatman No. 4). An aliquot (0.4 ml) of the solution was diluted to 10 ml with the appropriate buffer and the protein content determined using the Lowry method (Lowry, Rosenbrough, Farr, & Randall, 1951). Results are expressed as a percentage of the total protein content in the original sample.

2.4. Puroindoline purification

Puroindolines were separated from soft wheat starch granules using the detergent method described by Bloch, Darlington, Shewry, Darlington, and Shewry (2001). Soft wheat flour (100 g) was extracted for 8 h at 4 °C with 500 ml of 100 mM Tris/HCl buffer (5 mM EDTA, 100 mM potassium chloride and 9 4%(w/v) Triton X-114 pH 7.8). Solution was centrifuged (5000*g*, 15 min), incubated at 37 °C until phase separation occurred then re-centrifuged (5000*g*, 15 min). Upper phase of supernatant was removed, replaced with an equal volume of Tris/HCl buffer minus Triton X-114 and phase separation procedure was repeated. Lower phase was precipitated for 8 h at −18 °C, followed by addition of 200 ml of ice-cold diethylether and ethanol (1:3). Solution was centrifuged (2000*g*, 15 min) and the resulting pellet was washed with solvent and dried under vacuum for 8 h. Pellet was resuspended in 5 ml of 50 mM acetic acid, centrifuged (20,000*g*, 5 min; Sanyo/Harrier 18–80 Refrigerated Centrifuge, Belton Park, Loughborough, Leics, LEI 15XG, UK), and the supernatant loaded onto a Sephadex G50 column equilibrated with the Tris/HCl buffer. Column fractions were analyzed using 4–12% Bis-Tris gels (NOVEX Xcell SureLock Mini-Cell System, NOVEX USA, San Diego, CA). Eluted fractions containing M_r 15,000 proteins were pooled and lypholized.

2.5. Preparation of microspheres

Polystyrene microspheres (d = 5 μm, supplied at 10% solids) were purchased from Bangs Laboratories, Inc. (Fishers, IN). Calculation of microsphere/puroindoline ratio necessary for surface saturation (“region of independence”) was achieved based on information provided by the manufacturer (Bangs Laboratories, 1999; Cantarero, Butler, & Osborn, 1980):

$$S = (6/\rho D)(C),$$

where S = puroindoline necessary for monolayer adsorption (mg puroindoline/g microspheres); C = capacity of the microsphere for puroindoline, based on size and MW (~0.8 mg/m^2); $6/\rho D$ = surface area/mass (m^2/g) of microsphere of given diameter (polystyrene density = 1.05 g/cm^3); D = diameter of microsphere (5.0 μm).

Therefore, $S = (6/1.05\ \text{g/cm}^3 \times 5.0\ \mu\text{m})(0.8\ \text{mg/m}^2)$, ~1.0 mg of puroindoline was necessary to saturate 1 g of 5 μm polystyrene microsphere.

2.6. Passive adsorption of puroindoline onto microspheres

The scheme for all microsphere adsorption studies is shown in Fig. 1. Adsorption buffer (pH 9.6) was prepared by combining 16 ml sodium carbonate with 34 ml sodium bicarbonate and adjusting the final volume to 200 ml with ultrapure water (Millipore Corporation, Billerica, MA). Storage buffer was prepared by combining adsorption buffer with 0.5% polyethylene glycol as a blocking agent. Control procedures were conducted to assess the amount of protein adsorbed to the walls of untreated centrifuge tubes and those treated with a blocking solution. Puroindoline (200 μg) was dissolved in 10 ml adsorption buffer. Aliquots (2 ml) of this solution were added to untreated tubes and tubes that had been incubated with storage buffer for 2 h then rinsed with excess adsorption buffer. Suspension was centrifuged (1500*g*, 15 min), supernatant removed and protein content assessed using the Lowry method (Lowry et al., 1951). Data revealed that significant protein adsorption on microcentrifuge tubes was blocked by polyethylene glycol. Subsequent adsorption studies were conducted using tubes treated with storage buffer (i.e. blocked).

For adsorption assays, microspheres were diluted to 1% solids (10 mg/ml) with adsorption buffer. Interaction between puroindoline and microspheres was initiated by adding 10 ml microsphere suspension to 10 ml puroindoline solution. Suspension was incubated (25 °C) for 2 h vortexing every 10 min. Suspension was pipetted into blocked microcentrifuge tubes and centrifuged (1500*g*, 15 min). Supernatant was removed and the binding capacity of the microspheres was assessed by quantifying the protein concentration of the supernatant. Concentrations were expressed as ►g puroindoline/g microspheres. All assays were performed in triplicate.

2.7. Interaction of soyprotein with puroindoline-coated microspheres

To ease difficulty of immunological measurement of soy protein fractions adsorbed to puroindoline-coated

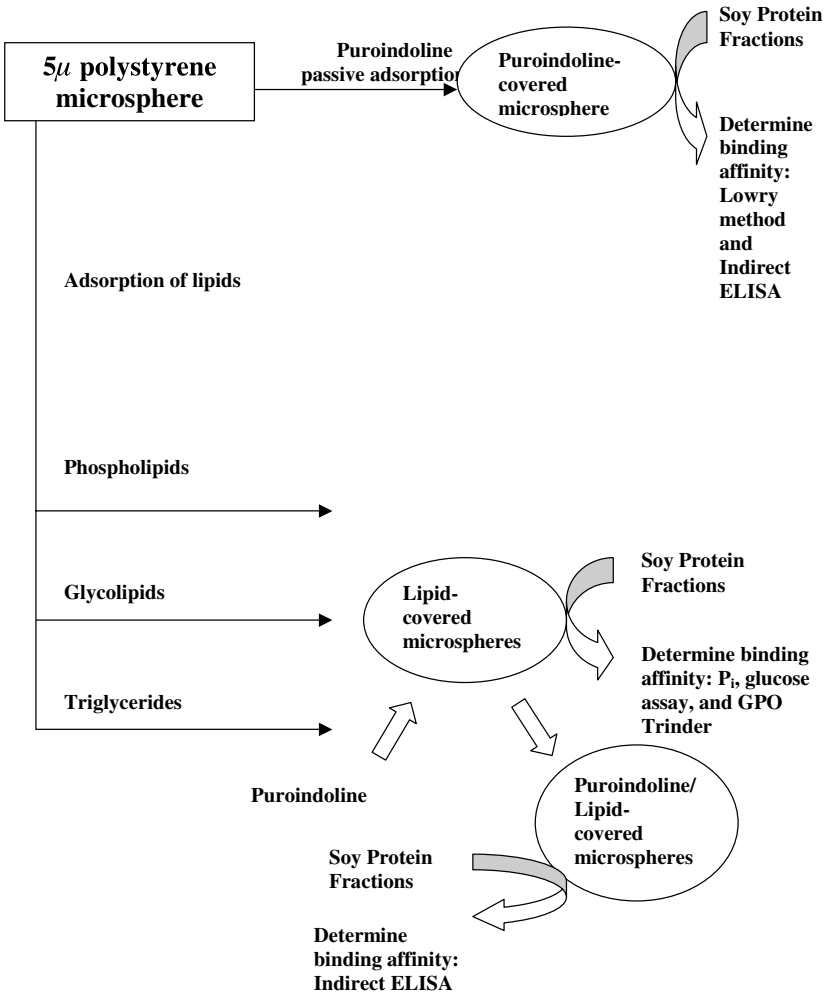

Fig. 1. Soy fraction adsorption to puroindoline- or lipid-coated microspheres.

microspheres, the adsorption efficiency of puroindoline and propensity of displacement via soy protein was assessed (Bale, Danielson, Daiss, Goppert, & Sutton, 1989). Adsorption efficiency of puroindoline was determined using the desorption characteristics of this protein. Puroindoline was adsorbed onto polystyrene microspheres by adding 10 ml of microsphere suspension to 10 ml puroindoline solution, incubating (25 °C) for 2 h, vortexing every 10 min, followed by centrifugation (1500*g*, 15 min). Supernatant was removed, replaced with an equal amount of adsorption buffer then incubated at room temperature (~22°C) for 30 min to allow desorption to occur. Suspension was centrifuged (1500*g*, 15 min), then supernatant was removed and protein content was determined. Concentrations were expressed as mg puroindoline remaining/g microspheres.

Displacement characteristics were assessed by dissolving soybean 11S protein fraction (150 μg in 10 ml adsorption buffer). Puroindoline-coated microspheres in storage buffer solution (10 ml) were added to 11S solution (10 ml) and incubated (25 °C) for 2 h vortexing every 10 min. Resulting solutions were pipetted into blocked microcentrifuge, centrifuged (1500*g*, 15 min), then supernatant was removed and protein content was determined. Soy protein content was determined directly using a modified indirect ELISA assay (Hitchcock, Bailey, Crimes, Dean, & Davies, 1981; Fig. 2).

Supernatant aliquots (1 ml) from each sample were used for the ELISA assay. Standards 9 (0.05 μg/μL soybean 11S protein) were prepared to contain 0, 0.05, 0.10, 0.40, 0.80, and 1.5 μg of protein. Standards and samples were applied to the interior walls of polystyrene microcentrifuge tubes. Adsorption buffer was added making the volume up to 2 ml. Blanks containing only adsorption buffer and only antibody enzyme-conjugate-antiglobulin were prepared to determine non-specific binding to the polystyrene surface. Tubes were incubated 16 h (4 °C) to allow antigen binding. Tubes were then washed four times with 4.0 ml of PBS-T (0.05% Tween 20 in phosphate buffer solution [PBS]: 0.041 M $NaH_2PO_4 \cdot H_2O$, 0.061 M Na_2HPO_4, 0.01% thimersol, 0.9% NaCl, pH 7.0). Tubes were blocked with 2.0 ml

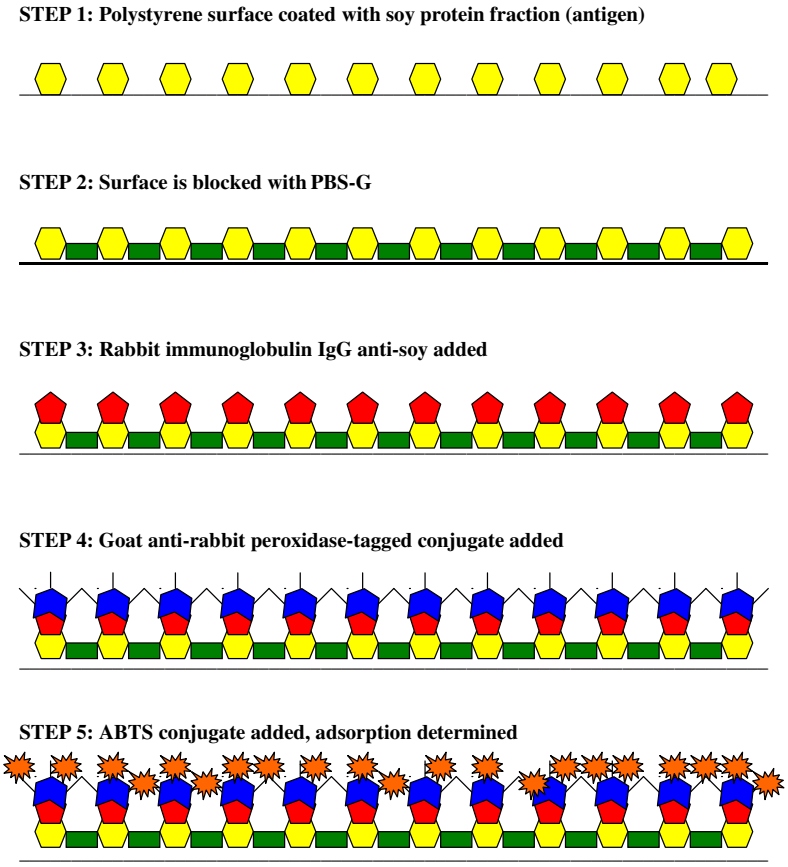

Fig. 2. Indirect ELISA procedures for soy protein determination on polystyrene microspheres.

of PBS-G (1% gelatin in PBS), incubated (37 °C, 30 min) and washed as previously described. Bound soy fractions were incubated (37 °C, 30 min) with 2.0 ml rabbit immunoglobulin IgG raised against soy protein (1:5000). Excess reagent was removed by washing, and 2.0 ml of goat anti-rabbit peroxidase-tagged conjugate (1:750) was added and allowed to incubate (37 °C, 90 min). Tubes were washed four times, and 2.0 ml of substrate containing 0.22% w/v ABTS (2,2′-azinobis[3-ethylbenzthiazolinesulfonic acid]) and 30% v/v H_2O_2 in citrate buffer (0.23 M sodium citrate monohydrate, 0.36 M citric acid, pH 4.0) were added. After developing for 15 min at 25 °C, the reaction was stopped with the addition of 1.0 ml of 1.25% aqueous KF. Absorbance was determined at 410 nm.

Results of adsorption efficiency and displacement assays indicated that, under the conditions of the procedure, puroindoline was permanently fixed to the polystyrene microspheres and did not exist in significant quantities in the supernatant. Therefore, subsequent assays measuring binding of soy protein fractions were conducted assuming that the protein in the supernatant was entirely soy protein.

The remaining soy protein fractions (SF isolate, SF 7S, TVP isolate, TVP 11S, TVP 7S, TSP isolate, TSP 11S, TSP 7S) were evaluated for binding to puroindoline-coated microspheres by dissolving 150μg of each protein in 10 ml adsorption buffer. Puroindoline-coated microspheres in storage buffer solution (10 ml) were added to soy protein solution (10 ml) and incubated (25 °C, 2 h) vortexing every 10 min. Resulting solutions were pipetted into blocked microcentrifuged tubes, centrifuged (1500*g*, 15 min) and supernatant removed. Protein content was assessed. Soy protein adsorbed to the microspheres was expressed as mg soy protein/g microspheres.

2.8. Preparation of lipid-coated microspheres

Lipid-coating was initiated by individually adsorbing one of three lipids (phospholipid, purified triglyceride, or glycolipid) to the surface of polystyrene microspheres. Adsorption of phospholipid to the surface of polystyrene microspheres was accomplished using modifications of the methods of Carmona-Ribeiro and Herrington (1993). Egg phosphatidylcholine (PC) was purchased from Supelco Inc. (Bellefonte, PA). Aqueous phospholipid suspensions were prepared by ethanol injection (Kremer, Esker, Pathmamanoharan, & Wiersema, 1977). Aliquots of the PC-ethanol suspension

(25 μM/ per ml) were injected using a glass syringe into 10 ml of a magnetically stirred adsorption buffer solution (200 rpm, 35 ° C). The solution was filtered (Millipore, 450 nm) and dialyzed against adsorption buffer for 4 h to eliminate the ethanol from the solution. Microsphere-PC interaction was initiated by combining 5.0 ml microsphere suspension (1% solids) with 5.0 ml of PC suspension. Mixtures were incubated (24 h, 25 °C), centrifuged (20,000*g*, 60 min, 15 °C) and the supernatant removed. PC remaining on microsphere surface was determined by measuring inorganic phosphorus (P_i) in supernatant. Briefly, an aliquot of supernatant (1.0 ml) was heated in a test tube until solvent was completely evaporated. Perchloric acid (0.65 ml) was added and solution was heated until yellow color disappeared. Tubes were cooled and water (3.30 ml), 2.5% ammonium molybdate (0.50 ml) and 10% ascorbic acid solution (0.50 ml) were added, in order, vortexing after each addition. Color was developed by heating the solutions for 5 min in a boiling water bath (Precision Reciprocal Shaking Bath, Jouan, Inc., Winchester, VA). Solutions were transferred to cuvettes and absorbance was determined at 800 nm. Blanks were prepared containing the same combination of water, ammonium molybdate and ascorbic acid solution. Adsorption was expressed as μg PC adsorbed/g microspheres.

Adsorption of glycolipid onto microsphere surfaces was accomplished using a modification of the methods of Yang, Zeller, and Schnaar (1996). Glycolipid (digalactosyldiglyceride) was obtained from Supelco Inc. (Bellefonte, PA). Glycolipid–ethanol solution (30 μM/ml) was prepared and vortexed for 30 s. Microsphere suspension (5.0 ml) and glycolipid solution (5.0 ml) were combined in a 10 ml glass test tube, vortexed 30 s and air dried (25 °C). Glycolipid-adsorbed microspheres were washed three times with ultra-pure water, resuspended in 5 ml adsorption buffer, centrifuged (20,000*g*, 60 min, 15 °C) and the supernatant removed. Glycolipid content in the supernatant was determined by transferring 1.0 ml supernatant to a 5 ml glass test tubes and allowing solvent to evaporate (25 °C). Orcinol 2 solution (2.0 ml, 2 mg/ml 5-methylresorcinol/70% sulfuric acid, v/v) was added to the dry glycolipid and heated for 20 min at 80 °C. Absorbance of cooled solutions was determined at 505 nm. Blanks containing orcinol only were also evaluated. Glycolipid concentration was determined based on a standard curve using glucose standards (10, 20, 30 and 40 μg). Glycolipid adsorption to microsphere surface was expressed as μg glycolipid/g microspheres.

Triglycerides (TG) were adsorbed to microsphere surface as described by Yang et al. (1996). A triglyceride mixture (50 μl; ~20%, triacetin, ~20% tributyrin, ~20% tricaproin, ~20% tricaprylin, and ~20% tricaprin by weight; Supelco Inc., Bellefonte, PA) was combined with ethanol (5.0 ml) in a 10 ml test tube, vortexed for 30 s and combined with 10 ml of the microsphere suspension. Microsphere-triglyceride mixture was vortexed for 30 s and air dried (25 °C). Triglyceride adsorbed microspheres were resuspended in 10 ml of adsorption buffer, vortexed 30 s, and mixture was centrifuged (20,000*g*, 60 min, 15 °C). Supernatant was removed and triglyceride concentration was determined spectrophotometrically (490 nm) using a Sigma Diagnostic kit according to manufacturer's directions (Triglyceride GPO-Trinder #339, Sigma–Aldrich, St. Louis, MO). Adsorption was expressed as μg glycolipid/g microspheres.

2.9. Adsorption of soy fractions to lipid-coated microspheres

Microspheres containing each of the three lipids were prepared as previously described and suspended in 10 ml adsorption buffer. Soy protein fractions (SF isolate, SF 11S, SF 7S, TVP isolate, TVP 11S, TVP 7S, TSP isolate, TSP 11S, TSP 7S) were evaluated for binding to lipid-coated microspheres by dissolving 150 μg of each protein in 10 ml adsorption buffer. Lipid-coated microspheres in adsorption buffer solution (10 ml) were added to soy protein solution (10 ml) and incubated (25 °C, 2 h) vortexing every 10 min. Resulting solutions were centrifuged (1500*g*, 15 min), supernatant was removed and protein content was determined. Soy protein adsorbed to the lipid-covered microspheres was expressed as mg soy protein/g microspheres.

2.10. Adsorption of puroindoline to lipid-coated microspheres

Each of the three lipids was adsorbed to microspheres using procedures as described above. Concentrations were expressed as mg puroindoline remaining/g microspheres.

2.11. Adsorption of soy fractions to microspheres containing puroindoline and lipids

Soy protein fractions (SF isolate, SF 11S, SF 7S, TVP isolate, TVP 11S, TVP 7S, TSP isolate, TSP 11S, TSP 7S) were evaluated for binding to lipid-coated microspheres by dissolving 150 μg of each protein in 10 ml adsorption buffer. Lipid-coated microspheres in adsorption buffer solution (10 ml) were added to soy protein solution (10 ml) and incubated (25 °C, 2 h) vortexing every 10 min. Resulting solutions were pipetted into blocked microcentrifuge tubes, centrifuged (1500*g*, 15 min), and supernatant was removed. Soy protein content was determined by indirect ELISA assay as previously described. Concentration of soy fractions remaining on microspheres was expressed as mg soy fraction remaining/g microspheres.

2.12. Statistical analyses

Each adsorption assay was triplicated. Data were treated as a 9 (soy fractions) by 7 (microsphere treatment) factorial design and subjected to two-way Analysis of Variance. Means for significant effects ($P < 0.05$) were separated using least significant difference (LSD; SAS, 2002).

3. Results and discussion

Based on the traces of glycinin (11S; Fig. 3) and conglycinin (7S; Fig. 4) in the native state, and heated to 100 or 150 °C, these fractions appeared to be relatively pure.

3.1. Protein dispersibility

Dispersibility of TSP isolate was highest (>90%) while those of soy flour 11S and soy flour protein isolate were lowest (53–56%; Table 1). Dispersibilities of soy flour 7S and TSP 7S fractions were >87% while those of TVP 11S, puroindoline, gliadin and glutenin were between 80% and 87%. Ryan and Brewer (in press) noted that, as temperature increased from 25 °C, to 50 °C, to 100 °C, solubility of the 7S fraction derived from soy flour (not texturized) was 84–88% depending on pH. However, the 11S fraction was about 85% soluble at these temperatures at pH 5.5, it decreased to <70% when heated at pH 7.5. The solubility of the 7S and 11S fractions derived from textured soy was about 88% at pH 5.5 and 90–97% at pH 7.5. However, 100 °C was the highest temperature they used for solubility evaluation.

3.2. Puroindoline and lipid adsorption

Monolayer coverage results for puroindoline and lipids are shown in Table 2. Data is consistent with that reported in previous studies in regard to protein and lipid adsorption to polystyrene surfaces (Carmona-Ribeiro &

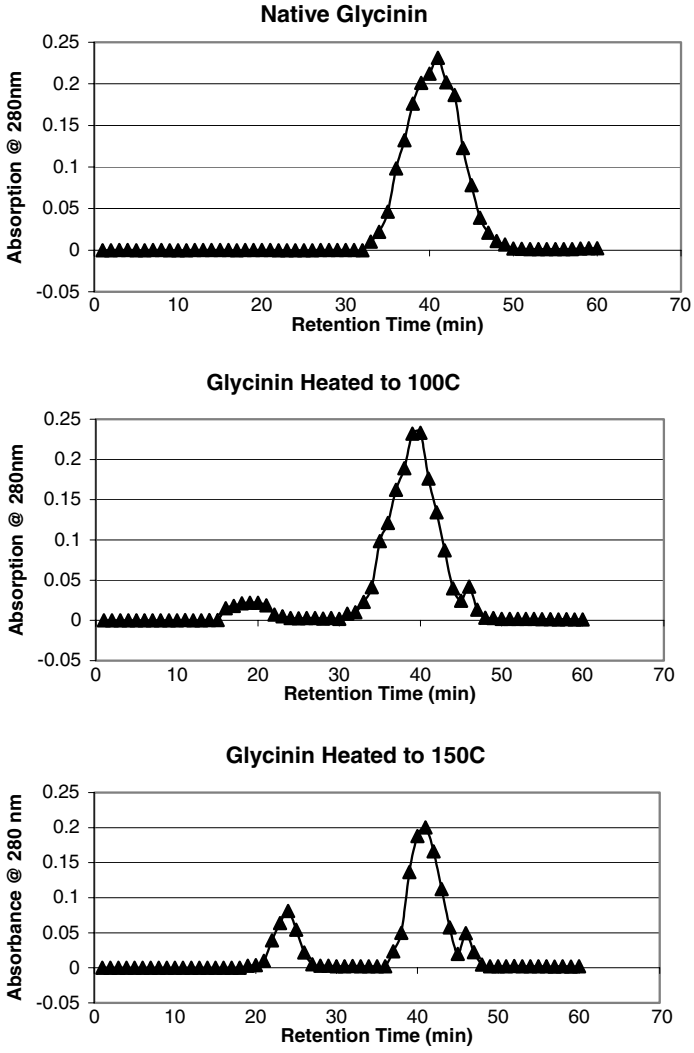

Fig. 3. Gel filtration trace: Glycinin (11S).

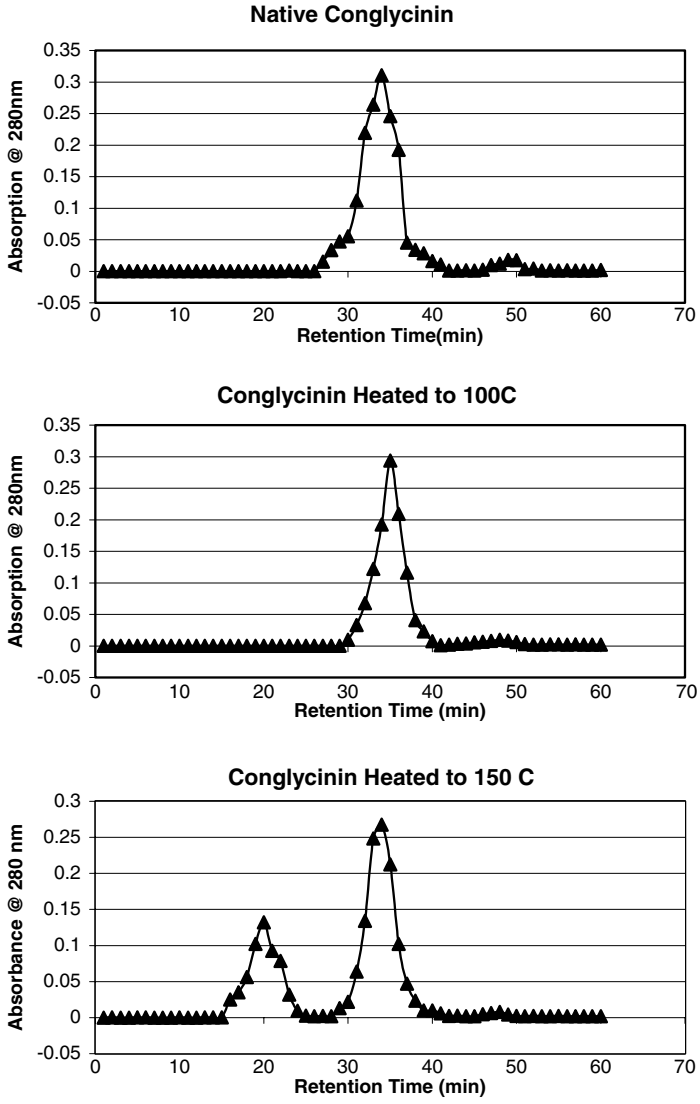

Fig. 4. Gel filtration trace: Conglycinin (7S).

Table 1
Protein dispersibility as a percent of total protein

Fraction	Percent
Soy flour protein isolate	56.4(0.02)
Soy flour 7S	87.4(0.01)
Soy flour 11s	53.1(0.02)
TSP isolate	91.2(0.14)
TSP 7S	87.4(0.05)
TSP llS	70.9(0.01)
TVP llS	79.6(0.09)
Puroindoline	83.9(0.04)
Gliadin	86.8(0.03)
Glutenin	87.0(0.09)

Herrington, 1993; Yang et al., 1996; Michalski & Saramago, 2000). Adsorption efficiency of puroindoline and propensity of displacement via soy protein fraction interaction were similar to results reported by Baty, Suci, Tyler, and Geesey (1996). That is, concentration of puroindoline adsorbed to microsphere exterior did not change significantly after exposure to the soy protein fractions. Desorption profiles of puroindoline and lipids after the 30 min period indicated an equilibrium system (data 20 not shown).

3.3. Soy fraction adsorption

Results of soy fraction adsorption onto treated microspheres is shown in Table 3. Maximum adsorption, across all microsphere treatments, was observed with TVP and TSP isolates. TVP isolate, TSP isolate, soy flour 11S and TSP 11S fractions were adsorbed to a significantly greater degree by puroindoline- or puroindoline/phosphatidylcholine-coated microspheres than were all other fractions This affinity was mirrored in the behavior of TVP and TSP 11S fractions. Yagasaki, Takagi, Sakai, and Kitamira (1997) and Peng, Quass,

Table 2
Monolayer coverage for puroindoline and lipids adsorbed onto polystyrene microsphere surface

Adsorbing material	Monolayer (μg/g microsphere)
Puroindoline	623 ± 23
Phosphatidylcholine[a]	487 ± 32
Digalactosyldiglyceride	323 ± 16
Triglyceride mixture	207 ± 24
Puroindoline on PC[b]	201 ± 13
Puroindoline on GL[c]	176 ± 28
Puroindoline on TG[d]	187 ± 22

[a] Forms mono- and bi-layers under preparation conditions.
[b] PC, phosphatidycholine.
[c] GL, digalactosydiglyceride.
[d] TG, triglyceride mixture.

Dayton, and Allen (1984) hypothesized that the 11S fraction is largely retained during soy protein extrusion, compared to the 7S fraction, and is responsible for the majority of isolate functionality. A combination of increased hydrophobicity and altered shape may result in this heightened adsorption. During processing, soy protein configuration is radically altered to expose a greater proportion of hydrophobic residues (Hermansson, 1978). Hydrophobic portions of the processed fractions may increase adsorption affinity to the starch granule. The shape of the extruded proteins may also have some effect. Ornebro, Wahlgren, Eliasson, Fido, and Tatham (1999), in a study on gliadin fraction adsorption to hydrophobic surfaces, hypothesized that the filamentous nature of particular proteins increases their adsorption profile. Zhang, Mungara, Jane, Mungara, and Jane (2001) found that extrusion of soy protein can elongate the polymer, resulting in a fibrous end product. Soy protein exhibiting a long, strand-like profile may have increased microsphere coverage due to increased polymer flexibility and surface area.

The combination of puroindoline and lipid increased the binding efficiency for all soy fractions except the 7S, in comparison to either puroindoline or lipid alone. The soy protein 7S fraction has been shown to break down into an insoluble aggregate at extrusion temperatures and pressures, severely decreasing adsorption propensity (Peng et al., 1984). The increased adsorption of the processed isolate and 11S moiety may indicate a duel binding mechanism. These data show that soy fraction affinity in puroindoline-lipid microspheres increases in the following order: phospholipid > glycolipid > triglyceride. Isolated soy fractions contain almost three times as many polar side groups compared to their non-isolate counterparts (Chiang, Shih, & Chu, 1999). These polar side groups have been shown to participate in binding of substances containing a polar charge within their environment (Deeslie & Cheryan, 1988). The interaction of soy protein with the puroindoline-lipid microsphere may involve both the hydrophobic portion of the puroindoline and the polar fraction of the adsorbed phospholipid. These data indicate that this interaction trend did not occur in the untextured soy fractions. Lack of substantial hydrophobic regions may inhibit binding.

Polar lipids (e.g. phosphatidylcholine) have been used extensively in the baking industry to improve baked wheat products. Kissell and Yamazaki (1975) found that phospholipids improve the top surface and spread ratio characteristics of sugar-snap cookies compared to other extracted flour lipids. Researchers have hypothesized that polar lipids enhance the stability of developing air cell in cookies and breads (Daftary, Pomeranz, Shogren, & Finney, 1968; Hoseney, Finney, & Pomeranz, 1970). However, Wehrli and Pomeranz (1970) documented the hydrophobic and polar interaction of glycolipids with the glutenin and gliadin fractions. They conjectured that polar lipids form a bridge between gluten proteins

Table 3
Maximum adsorption (μg/g microsphere) of soy protein fractions onto puroindoline and lipid-coated microspheres

Adsorbing soy protein fraction	Microsphere treatment						
	Puroindoline	PC[A]	GL[B]	TG[C]	Puro/PC	Puro/GL	Puro/TG[C]
Soy flour isolate[D]	640.1^{d}	331.3^{g}	303.9^{g}	489.2^{e}	855.4^{b}	722.1^{e}	704.9^{c}
Soy flour 11S[D]	809.3^{c}	417.2^{f}	388.8^{fg}	492.1^{e}	966.4^{b}	633.9^{d}	573.1^{de}
Soy flour 7S[D]	443.4^{f}	492.1^{e}	488.3^{e}	378^{g}	509.3^{e}	633.5^{d}	504.2^{e}
TVP isolate[E]	853.1^{bc}	452.8^{f}	412.2^{f}	567de	1334.7^{a}	873.3^{bc}	730.1^{c}
TVP 11S	653.2^{d}	509.3^{e}	400.5^{f}	204.2^{h}	799.3^{c}	563.4^{de}	509.9^{e}
TVP 7S	209.1^{h}	332.1^{g}	204.7^{h}	388.1^{fg}	409.2^{f}	319.1^{g}	417.3^{f}
TSP isolate[F]	877.5^{bc}	503.2^{e}	477.2^{ef}	601.3^{d}	1203.4^{a}	789.9^{c}	844.1^{bc}
TSP 11S	708.4^{c}	600.4^{d}	514.3^{e}	301.4^{g}	643.2^{de}	588.1^{de}	466.3^{f}
TSP 7S	177.3^{i}	307.7^{g}	211.2 ^{h}	308.4^{g}	300.7^{g}	230.1^{h}	319.3^{g}

[abcdefghi] Means with like superscripts do not differ ($P < 0.05$).
[A] PC, phosphatidylcholine.
[B] GL, digalactosydiglyceride.
[C] Puro, puroindoline; TG, triglyceride mixture.
[D] Soy flour, toasted soy flour.
[E] TVP, hexane extracted, extruded ground soy flour.
[F] TSP, expelled, extruded, ground soy flour.

and the starch fraction, improving dough structure. Soy flour texturization may alter the protein fraction so as to mimic the bonding sites present in the gluten proteins, both hydrophobic and hydrophilic.

4. Conclusions

These results indicate that polar lipids and puroindoline may be necessary for maximum adhesion of protein fractions to the exterior of starch granules. Data from this simplified model system may partially explain why polar lipids improve the qualities of wheat-based dough systems. Polar lipids, in comparison to neutral triglycerides, increased adsorption of added protein to the surface of model starch granules. In a dough system, this enhanced adsorption may improve dough functionality and negate the adverse effects of soy flour addition.

These data show that soy fraction affinity in the puroindoline-lipid microsphere system increases in the following order: phospholipid > glycolipid > triglyceride. Isolated soy fractions contain almost three times as many polar side groups in comparison to their non-isolate counterparts. These polar side groups have been found to participate in binding of substances containing a polar charge within their environment.

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