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In situ examination of starch granule-soy protein and wheat protein interactions

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Abstract

The objective of this study was to evaluate the association between heat and pressure-treated soy proteins and wheat prime starch relative to the level of granule surface proteins present. Soy isolates were manufactured from defatted soy flour and from two types of textured soy flour. Conditions for binding between wheat starch and soy fractions were established by altering pH, protein and sucrose concentrations. Conformations of exogenous proteins bound to the wheat starch granule surface were evaluated using an amyloglucosidase assay. Proteins present on the granule surface were removed and soy protein binding was reevaluated. Thermal and pressure processing of soy protein significantly influenced binding kinetics. Textured soy proteins exhibited increased wheat starch granule adsorption characteristics compared to untreated soy protein. Removal of native wheat starch granule surface proteins decreased the binding of added proteins. This suggests that native granule proteins may mediate the binding of exogenous protein.
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1. Introduction

Association between starch and protein in wheat flour dough and subsequent quality changes have been investigated by numerous researcher groups (Eliasson & Tjerneld, 1990; Kulp & Lorenz, 1981; Ryan & Brewer, 2005a, 2005b). Using light microscopy, Baker (1941) reported a relationship between the continuity of a developing gluten film and the location of starch granules within the developed dough. This relationship appears to be dependent on the water content of the dough, with lower water content favoring a stronger starch-protein bond (Huang & Moss, 1991). From electron microscopy studies, Evans, Pearson, and Hooper (1981) concluded that the starch granule surface serves as an important anchor for (other) proteins during bread dough formation. Protein addition increases this relationship (Moss, Gore, & Murray, 1987), with gluten protein strengthening dough protein–starch interactions (Gan, Ellis, Vaughan, & Galliard, 1989). Soy, sunflower, faba bean and field bean have the opposite effect (Fleming & Sosulski, 1978). Soy protein's detrimental effect has been hypothesized to be due to its hydrophilicity; it competes with wheat proteins and starch for water decreasing their hydration (Tsen, 1976; Yamazaki, Donelson, & Kwolik, 1977).

Minimizing the negative effects of soy protein on baked product quality has been achieved using sucrose esters and surfactants (Tsen, Peters, Schaffer, & Hoover, 1973), however, the mechanism by which this improvement occurs is not fully understood. Hyder, Hoseney, Finney, and Shogren (1974) suggested that soy protein-supplemented doughs lacking emulsifiers have weak protein–starch interactions. Emulsifiers, such as sodium stearoyl lactylate, may serve to strengthen the bond between starch, protein and lipid in the developing dough matrix. However, the mechanism of soy protein–wheat starch interaction in baked products has not been fully elucidated.

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Small, B-type (~5 μm) wheat starch granules have a surface area of 0.25–0.90 m^2/g of dry starch (Morrison & Scott, 1986) suggesting a large potential area for starch-protein interaction. Eliasson and Tjerneld (1990) determined protein binding by measuring protein concentration in the supernatant before and after adsorption. They found that wheat starch granules preferentially adsorbed higher molecular weight proteins with long, fibrillar rather than globular structures. Heat denaturing these proteins decreased adsorption.

Barlow, Buttrose, Simmonds, and Vesk (1973) and Seguchi (2001) reported the presence of low molecular weight proteins and lipids on the surface of the wheat starch granule which may influence protein–starch interaction. Eliasson, Carlson, Larsson, and Meizis (1981) suggested that these proteins and lipids may greatly affect the proclivity of starch–protein interactions. Of the protein fraction, the most studied group is collectively referred to as friabilins or puroindolines. These proteins impart hydrophobic characteristics to the granule surface. Greenwell and Schofield (1986) reported that soft wheat starch contained approximately 10 times the amount of friabilin as hard wheat starch. Chemical alteration of this protein component of soft wheat starch has been shown to affect the texture and consistency of pancakes (Seguchi, 2001). Addition of 0.1% puroindoline to bread dough formulated with puroindoline-free hard wheat flour alters the rheological character of the dough and improves bread crumb structure (Dubreil et al., 1998).

The objective of this study was to investigate the binding of heat and pressure-treated soy proteins to wheat prime starch in relation to the level of proteins present on the granule surface. To this end, soy isolates were manufactured from defatted soy flour and textured soy flour. Binding conditions between wheat starch and soy fractions were established. The conformation of protein bound to the wheat starch granule surface was assessed, proteins present on the granule surface were removed and the binding of soy protein was reevaluated.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Fisher Scientific (Hanover Park, IL 60103) and were of reactant grade, unless otherwise noted.

2.2. Wheat flour fractionation

Commercially milled hard and soft wheat flours were donated by Archer-Daniels Midland Milling (8000 West 110th St. Overland Park, Kansas 66210). Prime starch was isolated using the dough-ball method of Wolf (1964). Flour (500 g) was hand mixed with distilled water (315 mL) to form a cohesive mass. Starch was washed away from the gluten with distilled water (22 °C) until water ran clear. The gluten fraction was then lyophilized. The starch suspension was allowed to stand overnight (~8 h, 4 °C) then centrifuged (5000*g* for 10 min; Sigma 2-5 Benchtop Centrifuge, Sigma International Osterode and Harz, Germany). Supernatant containing the water-soluble fraction was decanted, shell frozen and lyophilized. The top layer of precipitate (tailings) was removed and discarded. The bottom white layer (prime starch) was removed and air-dried (~8 h). All fractions were ground in a burr mill (Mr. Coffee coffee grinder IDS55, Sunbeam Products, Hattisburg, MS, 39401) to pass a #40 mesh sieve (#40 sieve, Endecotts Limited, Lombard Road, London SW193TZ, England) then stored in polyethylene bags at 22 °C until used (<30 d).

Glutenins were extracted as described by Melas, Morel, Autran, and Feillet (1994) and Uthayakumaran, Gras, Stoddard, and Bekes (2000). Soft wheat flour (16 g) was extracted three times for 30 min (stirring with magnetic stirrer) with 190 mL of 50% propan-2-ol at 22 °C. Suspension was centrifuged (20,600*g*, 15 min) discarding the supernatant after each extraction. The resulting pellet was resuspended in 100 mL of dithiothreitol (DTT; 1 g DTT, 50 mL propan-2-ol, 50 mL 160 mM Tris–HCl, pH 8.0), shaken at 65 °C in a shaking water bath for 3 min, then centrifuged (29,600*g*, 20 min). Supernatant was decanted, acetone was added (40% v/v), solution was allowed to precipitate overnight (4 °C) then centrifuged (29,600*g*, 20 min). The pellet was resuspended in 100 mL distilled water and centrifuged (20,600*g*, 10 min). This procedure was repeated and the final pellet (glutenin) was suspended in 0.1% acetic acid (v/v), lyophilized, ground using a mortar and pestle to pass a # 40 Taylor sieve then stored in polyethylene bags at 22 °C until use (<30 d).

Gliadins were extracted as described by Wieser, Antes, and Seilmeier (1998). Soft wheat flour (10 g) was extracted twice with a salt solution (67 mM $HKNaPO_4$, pH 7.6, + 0.4 M NaCl, 100 mL) at 22°C. After centrifugation (20,000*g*, 10 min, 20°C), the combined supernatants were dialyzed against distilled water (25 °C) using a semi-permeable membrane (Sigma–Aldrich, St. Louis, MO), lyophilized, then ground to pass a #40 Taylor sieve using a mortar and pestle.

Flour fractions were loaded onto a 4–12% gradient Tris–Bis SDS–PAGE (Invitrogen Corp., Carlsbad, CA) to determine approximate molecular weight distributions.

2.3. Soy isolate production

Defatted, toasted soy flour (SF) and solvent-extracted, textured protein (textured vegetable protein [TVP]) were donated by Archer Daniels Midland (Decatur, IL 62526). Extruded-expelled (non-solvent extracted) soy flour (TSP) was donated by Insta-Pro International (Des Moines, IA) Soy protein isolates of textured (ground to pass #40 sieve) and nontextured soy flour were prepared by extracting the flours with alkaline water (pH 8.0 adjusted with 2 M NaOH) for 2 h at 22 °C (water: flour, 10:1 v/v). Suspension

was centrifuged (1000*g*, 30 min) and the resulting supernatant adjusted to pH 4.5 with 2 N HCl. Precipitate was centrifuged (5000*g* for 15 min), suspended in water (5% protein mass/v) and adjusted to pH 8.0 with 1 M NaOH. Isolates were lyophilized and stored in polyethylene bags at 22 °C until use (<30 d).

Soy fractions were loaded onto a 4–12% Bis–Tris gradient SDS–PAGE (NOVEX Xcell SureLock Mini-Cell System, NOVEX USA, San Diego, CA) to determine approximate molecular weight distributions using standards (Novex Mark12 Wide Range Protein Standard, NOVEX USA, San Diego, CA).

2.4. Proximate analysis of flours

Moisture content of soft and hard wheat flours and soy flours was determined using Approved Method 44-15A and Method 44-31, respectively (AACC, 2000). Starch content of flours was determined enzymatically (AACC Method 76-13, Megazyme International Wicklow, Ireland). Protein and ash content are as reported by the supplier. All analyses were triplicated.

2.5. Starch–protein binding affinity

Protein binding to wheat starch granules was assessed under various conditions including changes in protein concentration, solution pH and sucrose concentration. The method of Eliasson and Tjerneld (1990) was used to assess binding affinity of soluble protein to ungelatinized starch. To avoid error due to starch swelling, prior to binding affinity assessment, starch (0.5 g) was hydrated with 5 mL distilled water for 30 min (22 °C). The mixture was centrifuged (15 min, 1100*g*) and the supernatant discarded. Prior to adsorption experiments, hydrated starch was suspended in distilled water (2 mL, 22 °C).

Protein suspensions were prepared by mixing protein texturized soy protein (TSP), texturized vegetable protein (TVP), SF (soy flour), BSA (bovine serum albumen), gliadin, or glutenin in distilled water which had been adjusted to the desired pH (3.5, 4.5, 5.5, 6.5, 7.5) using NaOH or HCl for 2.5 h. Solutions were centrifuged (15 min, 1100*g*), then supernatant was diluted with the same solvent used to dissolve the protein. Final solutions consisted of five protein concentrations (0.2, 1.0, 2.5, 3.5 and 5.0 mg/mL) at each pH.

Protein solution (5 mL) was added to a test tube containing starch (0.5 g) suspended in 2 mL distilled water or aqueous sucrose solution (0 mg/mL, 2.5 mg/mL, 3.5 mg/mL, and 5 mg/mL) at the various pH levels. Adsorption was allowed to occur for 30 min at 22 °C. Samples were centrifuged (15 min, 1100*g*) and protein content of the supernatant was determined using the Biuret method (Ohnishi & Barr, 1978). Adsorbed protein was calculated based on decrease in protein content in the supernatant and is expressed as mg protein/g starch. Protein solutions with and without starch in water served as controls.

After protein-containing supernatant was removed, the same volume of water or sucrose solution was added back. Starch granules were resuspended, then desorption was allowed to proceed for 30 min at 22 °C. The solution was centrifuged and protein content was determined as previously described. Experiments were triplicated. Adsorption/desorption data were subjected to the Student's *t*-test to determine differences. Differences were considered significant at $p < 0.05$ (SAS, 2002).

2.6. Amyloglucosidase activity assessment of protein–starch interaction

Gelatinized samples were prepared as described by Guerrieri, Eynard, Lavelli, and Cerletti (1997). Hard or soft wheat prime starch (500 mg) was combined with 50 mg protein (BSA, gliadin, glutenin, TSP, TVP, or SF). Distilled water (330 mL) was added, then mixture was heated in a waterbath (Precision Reciprocal Shaking Bath, Jouan, Inc., Winchester, VA) at 100 °C for 10 min to allow gelatinization to occur, followed by rapid cooling in an ice bath (5 min). Preparations were freeze-dried and ground to pass a #40 mesh sieve. Ungelatinized samples were prepared without heating. Gelatinized and ungelatinized starches without protein were also prepared to serve as controls.

Amyloglucosidase assay was conducted as described by Guerrieri et al. (1997). Native or gelatinized sample (100 mg, dwb) was dispersed at 22 °C in 25 mL of distilled water and stirred for 10 min; 1 mL of this mixture plus 2 mL of 0.1 M sodium acetate buffer was heated to 60 °C. Amyloglucosidase (0.25 mg in 50 μL buffer) was added. Reaction was allowed to proceed at 60 °C for 10 min then stopped with 0.5 mL of 2.5 M NaOH. Liberated glucose was determined enzymatically (Megazyme International, Wicklow, Ireland). Assays were triplicated. Blanks (50 μL of buffer replacing amyloglucosidase) were also prepared.

2.7. Wheat prime starch oil binding

Wheat prime starch oil binding capacity was determined as described by Seguchi (2001). Soft wheat prime starch (0.5 g) and corn oil (1.0 mL) were shaken vigorously by hand for 2 min, centrifuged (8000*g*, 10 min) then volume of oil bound to starch granules was determined by difference. Resulting mixture was held at 22 °C for 24 h then recentrifuged (8000*g*, 10 min) to assess binding stability.

2.8. Determination of starch granule protein density

Amido black (1 g) was mixed with 100 mL of *n*-propanol, acetic acid, and water (3:1:6,v/v) then membrane filtered (Whatman GF/A, Clifton, NJ) with suction prior to use. Starch samples (0.5 g) from hard and soft wheat flours in water (1 mL) were mixed with amido black solution (1 mL) then allowed to stand for 20 min (22 °C). After

centrifugation (600g, 5 min), supernatant was discarded. Pellet was suspended in 100 mL of 90% methanol containing 2% acetic acid and centrifuged (600g, 5 min). This washing process was repeated three times. The washing process was repeated 10 times using distilled water (100 mL). The washed starch pellet was suspended in 100 mL of 50 mM NaOH solution containing 0.1% SDS, shaken for 2 h (22 °C), then centrifuged (500g, 5 min). Supernatant volume was determined then diluted with the same volume of absolute ethyl alcohol. Absorbance was determined at 630 nm (Beckman Spectrophotometer, DU 640, Fullerton, CA).

2.9. Quantification of starch granule surface protein

Protein was extracted from hard and soft wheat prime starch as described by Seguchi and Yamada (1989). Wheat prime starch (10 g) was combined with 200 mL of 1% 2-mercaptoethanol. Solution was stirred (300 rpm) for 24 h in a 300 mL conical flask then centrifuged (600g, 10 min). Supernatant was decanted and 40 mL was concentrated to 4 mL by dialyzing against distilled water. Concentrated protein (5 μg) was subjected to SDS–PAGE using 4–12% Bis–Tris gel following manufacturer's instructions (NOVEX Xcell SureLock Mini-Cell System, NOVEX USA, San Diego, CA). Samples were electrophoresed against a protein marker (range = 77,000–6000 kDa) for comparison (Novex Mark12 Wide Range Protein Standard, NOVEX USA, San Diego, CA). Gels were stained using colloidal silver and digitally scanned to jpeg format (HP ScanJet 6200, Hewlett Packard Company, Palo Alto, CA).

2.10. Starch granule surface protein stripping on protein affinity

Protein remnants were removed from starch as described by Delcour et al. (2000). Prime starch (100 g) was shaken for 120 min at 22 °C with a solution of 0.1% dithiothreitol and 0.1% acetic acid (w/v = 1/2). Resulting solution was Buchner-filtered, washed 10 times with distilled water and air-dried. Starch was suspended in distilled water until testing (<1 h).

Suspensions were prepared by mixing protein (TSP isolate, TVP isolate, SF isolate, BSA, gliadin, or glutenin) at five concentrations (0.2 mg/mL, 1.0 mg/mL, 2.5 mg/mL, 3.5 mg/ mL, or 5.0 mg/ mL) in 0.1 M Tris/HCl solutions at five pHs (3.5, 4.5, 5.5, 6.5, 7.5). Solutions were centrifuged (15 min, 1100g) then supernatant was diluted with the same solvent to produce five protein concentrations at each of 5 pHs/protein source.

Protein solution (5 mL) was added to a 15 mL test tube containing control or de-proteinated starch (0.5 g) suspended in 2 mL 0.1 Tris/HCl buffer. Adsorption was allowed to proceed for 30 min at 22 °C. Samples were centrifuged (15 min, 1100g) and protein content of the supernatant was determined using the Biuret method (Ohnishi & Barr, 1978). Protein adsorbed (mg/g of starch) was calculated by difference. Control solutions contained protein without starch or starch without protein.

After the protein-containing supernatant was removed, the same volume of water was added back. Starch granules were suspended and desorption was allowed to occur for 30 min at 22 °C. Solution was centrifuged as previously described and protein content of supernatant was determined.

2.11. Oil binding of stripped starch granules

The oil binding assay was repeated to compare binding affinity between stripped and unstripped starch using the method of Seguchi (2001) as previously described.

2.12. Statistical analyses

Data were analyzed as a 4 (levels of sucrose) by 5 (pH values) factorial design within starch and soy fraction type. Means were considered significant at $p < 0.05$ and were separated using least significant difference. Adsorption/desorption data were subjected to the Student's t-test to determine differences. Differences were considered significant at $p < 0.05$ (SAS Systems 2002).

3. Results and discussion

Proximate composition of soy and wheat flours are shown in Table 1. Soy flour, TVP, and TSP were similar in moisture content (7.8–8.3%), starch content (0.9–1.6%) and protein content (50–53%). Soft and hard wheat flours were similar in moisture content (10–13%), starch content (~79%), and protein content (8–12%).

3.1. Protein binding to wheat starch granules

Maximum adsorption of protein fractions on wheat starch granules is shown in Fig. 1. In general, as protein concentration (mg protein/mg starch) increased adsorption of protein to the starch granule increased, however it did not occur equally for all proteins. At the two lowest protein concentrations, granules adsorbed similar amounts of all five proteins. With the exception of SF which adsorbed

Table 1
Proximate analysis of soft wheat and soy flours

Component	Component, % on a dry weight basis			
	Moisture	Starch	Protein	Ash
Soft wheat cookie flour	12.87[a] (0.14)	78.54 (1.24)	8.4	1.56 (0.09)
Hard wheat	10.04 (0.19)	79.32 (1.54)	12.2	1.13 (0.23)
Soy flour	7.8 (0.76)	1.2 (0.03)	53	NA
TVP[b]	8.3 (2.12)	0.9 (0.20)	50	NA
TSP[c]	7.9 (1.33)	1.6 (0.25)	50	NA

[a] Means ± SD of three determinations.
[b] TVP = solvent extracted textured soy flour.
[c] TSP = non-solvent extracted textured soy flour.

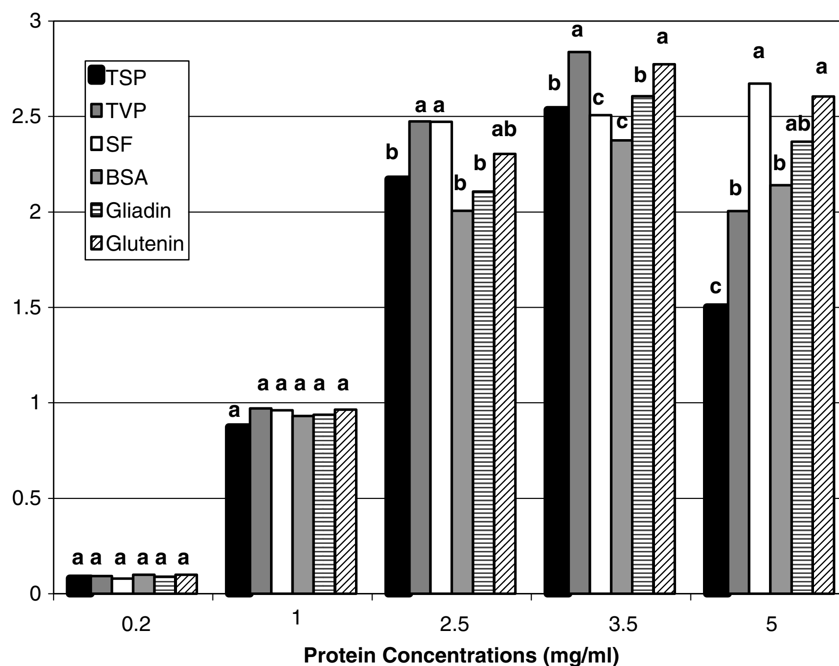

Fig. 1. Maximum adsorption of protein (mg/g) on starch granules at various protein concentrations (mg/ml).

maximally at 5 mg/mL, all proteins appeared to reach maximum adsorption at protein concentrations of 2.5–3.5 mg/mL beyond which additional protein did not increase binding to the granule surface. These data are similar to those of Eliasson and Tjerneld (1990) and Eliasson (1993) who discussed the plateau effect of wheat proteins in terms of protein precipitation at high concentrations.

Fig. 2 shows the adsorption of proteins at 0.2, 3.5 and 5.0 mg/mL. It is apparent from these data that the pH of the system influences protein adsorption to some degree. Using both pH and protein concentrations, our research shows a trend analogous to that of Eliasson and Tjerneld (1990) and Eliasson (1993). Adsorbance of proteins onto solid surfaces is generally highest near the protein's isoelectric points (MacRitchie, 1978). Earlier findings suggest that soy, BSA and gliadin exhibit maximal adsorption at their isoelectric points (pI = 4.4–4.5; pI = 4.7; pI = 8.1, respectively) proteins, however that was not evident here. In addition, glutenin adsorbed at a pH value which was not near its isoelectric point (pI = 7.1). Based on data in Tables 2 and 3 at 0% sucrose, it is apparent that pH affected adsorption of some of the proteins but not others. At the lower protein concentration (2.5 mg/mL) pH had little effect on TVP or TSP; increasing pH increased binding of BSA and gliadin and decreased binding of soy flour and glutenin. At the higher protein concentration (5.0 mg/mL), increasing pH increased protein binding of TVP, decreased that of glutenin, and had inconsistent effects on binding of the other protein sources. Isolation procedures used in the purification of these proteins may have altered their ter-

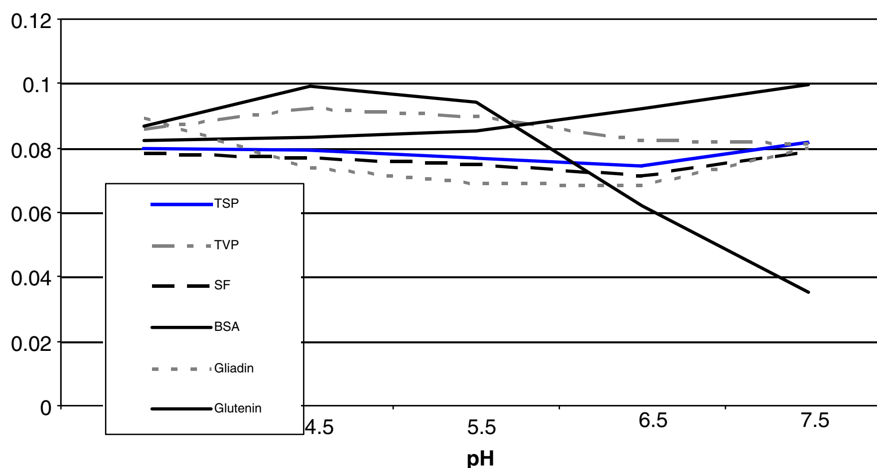

Fig. 2. Average absorbance of protein on starch using 0.2 (top), 3.5 (middle), and 5.0 (bottom) mg/ml protein solution of varying pH.

Table 2
Effect of pH and sucrose concentration on TVP, TSP and soy flour binding affinity

	pH value				
	3.5	4.5	5.5	6.5	7.5
Texturized vegetable protein		2.5 mg/ml protein solution			
0% Sucrose	2.34^{d}	2.4^{c}	2.38^{c}	2.44^{c}	2.47^{c}
2.5% Sucrose	1.40^{e}	1.62^{e}	1.60^{e}	1.59^{e}	1.61^{e}
3.5% Sucrose	2.13^{c}	2.27^{d}	2.24^{d}	2.22^{d}	2.40^{c}
5.0% Sucrose	2.23^{cd}	2.30^{d}	2.27^{d}	2.25^{d}	2.38^{c}
		3.5 mg/ml protein solution			
0% Sucrose	2.67^{c}	2.80^{c}	2.80^{c}	2.84^{c}	2.77^{c}
2.5% Sucrose	1.98^{d}	2.35^{c}	1.96^{d}	1.90^{d}	1.93^{d}
3.5% Sucrose	3.32^{b}	3.72^{a}	3.09^{b}	3.37^{b}	3.25^{b}
5.0% Sucrose	3.50^{a}	3.79^{a}	3.46^{ab}	3.51^{a}	3.42^{ab}
		5.0 mg/ml protein solution			
0% Sucrose	1.44^{e}	1.47^{e}	1.70^{e}	1.90^{d}	2.00^{d}
2.5% Sucrose	0.89^{fg}	0.91^{fg}	1.20^{f}	1.34^{f}	1.26^{f}
3.5% Sucrose	1.26^{f}	1.29^{f}	1.72^{e}	1.92^{d}	1.82^{e}
5.0% Sucrose	1.34^{ef}	1.38^{e}	1.81^{e}	2.02^{d}	2.03^{d}
Texturized soy protein		2.5 mg/ml protein solution			
0% Sucrose	1.47^{d}	1.61^{d}	1.77^{c}	1.97^{c}	2.17^{b}
2.5% Sucrose	0.99^{e}	1.24^{e}	1.15^{e}	1.28^{d}	1.41^{d}
3.5% Sucrose	1.39^{d}	1.84^{c}	1.72^{cd}	1.91^{c}	2.15^{c}
5.0% Sucrose	1.60^{d}	1.83^{c}	1.70^{cd}	1.90^{c}	2.09^{c}
		3.5 mg/ml protein solution			
0% Sucrose	1.60^{d}	1.74^{cd}	1.97^{c}	2.20^{b}	2.54^{b}
2.5% Sucrose	1.35^{d}	1.29^{d}	1.46^{d}	1.63^{d}	1.88^{c}
3.5% Sucrose	1.94^{c}	2.03^{c}	2.30^{b}	2.77^{a}	3.15^{a}
5.0% Sucrose	2.42^{b}	2.07^{c}	2.35^{b}	2.63^{b}	3.02^{a}
		5.0 mg/ml protein solution			
0% Sucrose	1.30^{d}	1.30^{d}	1.40^{d}	1.44^{d}	1.50^{d}
2.5% Sucrose	0.81^{e}	0.80^{e}	0.86^{e}	0.89^{e}	0.95^{e}
3.5% Sucrose	1.11^{e}	1.16^{e}	1.24^{e}	1.28^{d}	1.36^{d}
5.0% Sucrose	1.25^{d}	1.29^{d}	1.31^{e}	1.34^{d}	1.52^{d}
Soy flour		2.5 mg/ml protein solution			
0% Sucrose	2.47^{bc}	2.27^{c}	2.24^{c}	2.04^{c}	2.01^{d}
2.5% Sucrose	1.48^{de}	1.53^{de}	1.51^{de}	1.33^{de}	1.31^{de}
3.5% Sucrose	2.21^{c}	2.31^{c}	2.27^{c}	2.00^{c}	1.98^{d}
5.0% Sucrose	2.19^{c}	2.47^{bc}	2.44^{bc}	2.15^{c}	1.93^{d}
		3.5 mg/ml protein solution			
0% Sucrose	2.51^{bc}	2.34^{c}	2.27^{c}	2.21^{c}	2.10^{c}
2.5% Sucrose	1.75^{d}	1.96^{d}	1.59^{de}	1.54^{de}	1.47^{de}
3.5% Sucrose	2.97^{ab}	2.83^{b}	2.28^{c}	2.62^{b}	2.47^{bc}
5.0% Sucrose	3.10^{ab}	3.54^{a}	2.86^{b}	2.77^{b}	2.36^{bc}
		5.0 mg/ml protein solution			
0% Sucrose	2.14^{c}	2.37^{bc}	2.67^{b}	2.47^{bc}	2.27^{c}
2.5% Sucrose	1.32^{de}	1.47^{de}	1.88^{d}	1.74^{d}	1.43^{de}
3.5% Sucrose	1.88^{d}	2.09^{b}	2.66^{b}	2.47^{bc}	2.03^{d}
5.0% Sucrose	2.00^{d}	2.28^{c}	2.91^{b}	2.69^{bg}	2.31^{c}

^{abcdefg} Means with like superscripts do not differ ($p > 0.05$).

Table 3
Effect of pH and sucrose concentration on BSA, gliadin and glutenin binding affinity

	pH value				
	3.5	4.5	5.5	6.5	7.5
Bovine serum albumin^{g}		2.5 mg/ml protein solution			
0% Sucrose	1.84^{e}	1.87^{e}	1.91^{e}	2.01^{d}	2.01^{d}
2.5% Sucrose	1.10^{h}	1.26^{h}	1.28^{h}	1.30^{h}	1.30^{h}
3.5% Sucrose	1.66^{fg}	1.90^{e}	1.93^{d}	1.97^{d}	1.98^{d}
5.0% Sucrose	1.56^{g}	2.04^{cd}	2.08^{cd}	2.11^{c}	1.93^{d}
		3.5 mg/ml protein solution			
0% Sucrose	2.07^{cd}	2.14^{c}	2.17^{bc}	2.27^{b}	2.37^{b}
2.5% Sucrose	1.48^{gh}	1.79^{efg}	1.52^{g}	1.59^{g}	1.66^{fg}
3.5% Sucrose	2.34^{b}	2.58^{ab}	2.18^{bc}	2.70^{ka}	2.78^{a}
5.0% Sucrose	2.62^{ab}	2.89^{a}	2.44^{ab}	2.55^{ab}	2.67^{ab}
		5.0 mg/ml protein solution			
0% Sucrose	2.07^{cd}	2.14^{c}	2.07^{cd}	2.10^{c}	2.14^{c}
2.5% Sucrose	1.29^{h}	1.33^{h}	1.45^{gh}	1.48^{gh}	1.35^{h}
3.5% Sucrose	1.83^{e}	1.82^{e}	1.99^{cd}	2.10^{c}	1.91^{e}
5.0% Sucrose	1.94^{d}	2.06^{cd}	2.25^{b}	2.29^{b}	2.17^{bc}
Gliadin		2.5 mg/ml protein solution			
0% Sucrose	1.91^{c}	1.84^{c}	1.81^{c}	2.01^{c}	2.11^{bc}
2.5% Sucrose	1.14^{de}	1.42^{d}	1.39^{d}	1.55^{d}	1.37^{d}
3.5% Sucrose	1.72^{cd}	2.11^{bc}	2.07^{bc}	2.30^{b}	2.08^{bc}
5.0% Sucrose	1.69^{cd}	2.10^{dc}	2.06^{bc}	2.29^{b}	2.03^{c}
		3.5 mg/ml protein solution			
0% Sucrose	2.20^{b}	2.24^{b}	2.30^{b}	2.37^{b}	2.33^{b}
2.5% Sucrose	1.37^{d}	1.39^{d}	1.62^{cd}	1.66^{cd}	1.50^{cd}
3.5% Sucrose	1.94^{c}	1.90^{c}	2.21^{b}	2.36^{b}	2.13^{bc}
5.0% Sucrose	2.06^{bc}	2.15^{bc}	2.51^{ab}	2.58^{ab}	2.41^{b}
		5.0 mg/ml protein solution			
0% Sucrose	2.20^{b}	2.24^{b}	2.30^{b}	2.37^{b}	2.33^{b}
2.5% Sucrose	1.37^{d}	1.39^{d}	1.62^{cd}	1.66^{cd}	1.50^{cd}
3.5% Sucrose	1.94^{c}	1.90^{c}	2.21^{b}	2.36^{b}	2.13^{bc}
5.0% Sucrose	2.06^{bc}	2.15^{bc}	2.51^{ab}	2.58^{ab}	2.41^{b}
Glutenin		2.5 mg/ml protein solution			
0% Sucrose	2.24^{bc}	2.27^{bc}	2.30^{bc}	1.64^{cd}	0.91^{e}
2.5% Sucrose	1.34^{d}	1.75^{cd}	1.77^{cd}	1.26^{d}	0.59^{ef}
3.5% Sucrose	2.02^{c}	2.64^{b}	2.68^{ab}	1.90^{c}	0.89^{e}
5.0% Sucrose	1.90^{c}	2.48^{b}	2.52^{b}	1.79^{cd}	0.84^{e}
		3.5 mg/ml protein solution			
0% Sucrose	2.64^{b}	2.77^{ab}	2.74^{ab}	1.95^{c}	1.13^{de}
2.5% Sucrose	1.89^{c}	2.33^{bc}	1.91^{c}	1.36^{d}	0.79^{e}
3.5% Sucrose	2.98^{ab}	3.68^{a}	3.02^{ab}	2.15^{c}	1.33^{d}
5.0% Sucrose	3.34^{ab}	3.75^{a}	3.44^{a}	2.45^{b}	1.42^{d}
		5.0 mg/ml protein solution			
0% Sucrose	2.44^{b}	2.50^{b}	2.61^{b}	1.99^{c}	1.45^{d}
2.5% Sucrose	1.50^{d}	1.76^{cd}	1.83^{cd}	1.40^{d}	0.93^{e}
3.5% Sucrose	2.13^{c}	2.41^{b}	2.51^{b}	1.99^{c}	1.32^{d}
5.0% Sucrose	2.42^{b}	2.83^{ab}	2.94^{ab}	2.25^{bc}	1.50^{d}

^{abcdefgh} Means with like superscripts do not differ ($p > 0.05$).

tiary structure resulting in distorted binding (Damodaran, 1996). Protein binding onto wheat starch granules in the present study differed from that reported in other systems (MacRitchie, 1978).

Adsorption and desorption can be considered in terms of protein molecular weight. The wheat starch granule surface contains 10 major protein groups ranging from 5 to 149 kDa, as well as several lipid components (Baldwin, 2001). BSA (MW = 66 kDa) and gliadin (MW = 67 kDa; Burk, 1939) were the smallest proteins evaluated while the major soy proteins, β-conglycinin MW = 105–193, glycinin (MW = 309–394; Monaghan, 2003) and glutenin (low MW < 90 kDa, high MW > 90 kDa; Damodaran, 1996; Liu, 1999) were the largest proteins evaluated in this study. Although glutenin consistently adsorbed to the highest degree, soy isolates ranged in adsorption depending on pre-isolation processing

procedures. At 2.5 mg protein/g starch, TVP and soy flour exhibited significantly higher adsorption and desorption than gliadin and glutenin. At 3.5 mg protein/mg starch, TVP, gliadin and glutenin adhered the most and BSA adsorbed the least. However, at 5 mg protein/mg starch, adsorption began to drop off for TSP and TVP. Soy flour, TVP and TSP isolate represent nearly identical protein subunits, but their adsorbance patterns indicate the possibility of quite different mechanisms of attraction and adhesion. The larger protein molecules adsorbed to a greater degree than smaller proteins. Larger proteins generally have larger surface areas, and thus a greater propensity to contain active areas for adhesion. Processing may denature the proteins opening up the polypeptide chain increasing the surface area, however, factors that reduce the surface area may cause aggregation of the protein (Damodaran, 1996). Either of these effects may increase adsorption of processed soy flour depending on the nature of the granule surface. Polypeptide chain unfolding during processing may expose hydrophobic groups normally buried in the protein interior. If the granule surface components have hydrophobic properties, this would likely increase protein binding. However, if the protein is denatured and folded into a tighter configuration, it may be more attracted to hydrogen bond-forming granule surface components.

The lower protein concentrations (0.2% and 1.0%) in the solution had equivalent ($p > 0.05$) effects on protein adsorption at all sucrose concentrations and at all pH values (data not shown). At 2.5% sucrose, additional protein tended to decrease protein adsorption, although not necessarily significantly at all pH values, adsorption by 20–50% (Tables 2 and 3). Sucrose significantly altered protein adsorption to wheat starch granules regardless of protein type or pH. For some proteins, the highest sucrose concentration (5.0%) increased protein adsorption, sometimes to levels equal to or greater than those occurring in solutions with no added sucrose. Alteration of pH appeared to have little, if any, effect on the adsorption pattern of proteins in a sucrose system. Although protein adsorption is altered by sucrose, maximum adsorption ranges were identical to those found in the systems without sucrose. The effect of sucrose on protein binding appeared to be independent of pH and protein concentration.

These data support several thermodynamic mechanisms hypothesized regarding the effect of sucrose on protein stability and interactivity. Kulmyrzaev, Cancelliere, and McClements (2000) reported that low sucrose concentrations (0–100 g/kg) decrease protein gelation by increasing the viscosity of the aqueous phase. Higher sucrose concentrations (100–300 g/kg) increase gelation by competing with protein for water, increasing protein–protein interactions. Lee and Timasheff (1981) found that sucrose is preferentially excluded from the protein surface increasing chemical potential in direct proportion to the surface area exposed to the sucrose solution. Our data can be explained based on three stages of binding (Lee & Timasheff, 1981). In stage one, low sucrose concentrations (0.2 and 1.0 mg/mL) increased aqueous phase viscosity sufficiently to slow binding of proteins to the granule surface, but not sufficiently to structurally affect the exposed amino acid chains. Stage two occurred when sucrose concentrations reached 2.5–3.5 mg/mL wherein proteins are surrounded by a hydrophilic sucrose solution that decreases protein–water interaction and increases protein–protein and protein–starch granule interaction. Using ribonuclease, Wang, Robertson, and Bolen (1995) noted that sucrose pulled water from the protein surface causing transient fluctuations in the protein surface area. Using hydrogen–deuterium exchange, protein in this environment was shown to have a larger surface area and enhanced interactive capability. In stage three (5.0 mg/mL), sucrose behaved as an osmolyte and was preferentially excluded from the protein surface. The system minimizes the thermodynamically unfavorable effect of sucrose by favoring the protein state with the smallest exposed surface area. Hence, denatured proteins fold into forms thermodynamically similar to the stable native state, surface area is reduced and protein binding is increased.

3.2. *Amyloglucosidase evaluation of the interaction between protein and starch granules*

The interaction between native hard wheat starch, soft wheat starch and gelatinized soft wheat starch and various proteins using amyloglucosidase hydrolysis of glucose is shown in Fig. 3. In the presence of gliadin, glucose production from either native or gelatinized starches was significantly ($p < 0.05$) lower than when starches alone or in the presence of BSA were subjected to amyloglucosidase hydrolysis for 30 min. TSP and TVP provided a small degree blocking from enzymatic attack while glutenin and soy flour isolate provided intermediate degrees of blocking. Proteins that affected hydrolysis rate also decreased the degree of hydrolysis, indicating that these proteins blocked amyloglucosidase active sites on the granule. Native hard and soft wheat starches and gelatinized soft wheat starch interacted with these proteins similarly.

Guerrieri et al. (1997) reported similar effects using high molecular weight (HMW) gluten fractions and gliadin. These results suggest that the molecular configuration of proteins is primarily responsible for the efficiency of the protein–starch granule interaction. Because gliadins are flexible, single-chained monomers, they may conform more easily to the starch granule surface interacting primarily through hydrophobic and hydrogen bonding. In contrast, glutenins, extensively branched, multi-chained polypeptides that are compact due to extensive interior disulfide bonding are less prone to monolayer formation on a solid surface (MacRitchie, 1978). Texturization can severely alter the three-dimensional structure of soy proteins. Evidence from scanning electron microscopy and chemical studies suggest that soy proteins denature and aggregate during the early stages of the texturization process. Subse-

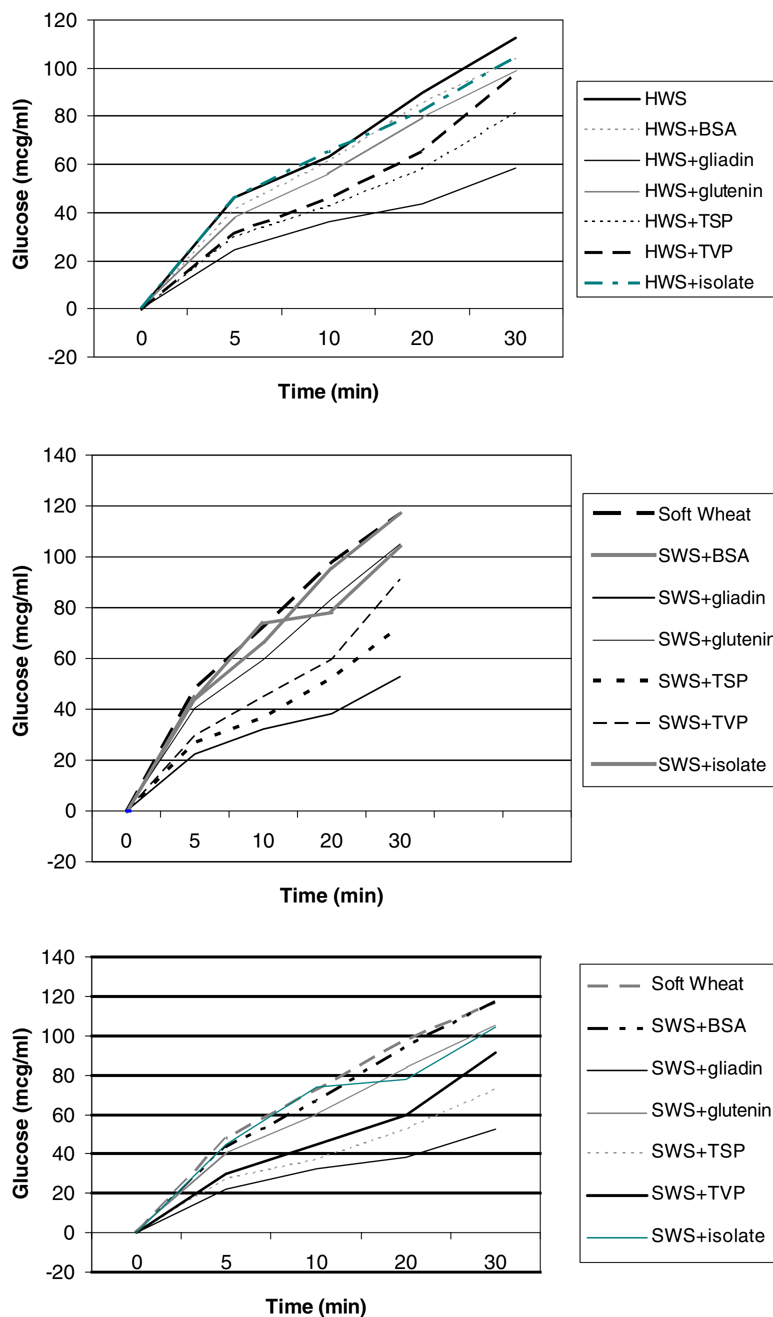

Fig. 3. Effect of amyloglucosidase on native hard (top), native soft (center) and gelatinized soft (bottom) wheat starch with added protein. SWS + BSA: soft wheat starch + bovine serum albumen; SWS + gliadin: soft wheat starch + gliadin; SWS + glutenin: soft wheat flour + gliatin; SWS + TSP: soft wheat starch + texturized soy protein; SWS + TVP: soft wheat starch + texturized vegetable protein; SWS + isolate: soft wheat starch + soy protein solate.

quent steps in extrusion result in protein rearrangement into long, sheet-like structures (Harper, 1981; Kokini, Ho, & Karwe, 1992). Processing may elongate the protein, increasing its availability for granule attachment, and thereby improve soy protein–starch granule association. In addition, heat and pressure processing may expose previously buried amino acid groups, improving potential binding potential. Guerrieri et al. (1997) reported that protein molecular specificity was the most important factor in granule binding assays, however structure alone may not be responsible for protein binding to the starch granule surface.

The lack of difference between hard and soft wheat hydrolysis rates suggests that protein binding to the granule surface is either not mediated through granule surface components (i.e. surface proteins or lipids) or that very low amounts of these components are required for this event to occur. Based on hydrolysis of hard and soft wheat flours, protein attachment and binding geometry appears to be similar in the two starches.

3.3. Oil binding of wheat starch granules

Soft wheat prime starch oil binding was 0.33 ml oil/g starch initially and 0.30 ml oil/starch after 24 h indicating that, overall, the starch granule surface is relatively hydrophilic (data not shown). These results are consistent with those reported by Seguchi (2001) for non-heat treated wheat starch. Oil binding stability measurements suggest that strongly hydrophobic pockets are present on the granule surface which may attract proteins and lipids with strong hydrophobic regions. Blochet et al. (1993) found that puroindoline contains a tryptophan-rich region in its sequence. These hydrophobic areas may be accessible to hydrophobic proteins during granule binding.

3.4. Starch granule protein density and quantification

Adsorption studies indicated that approximately 0.26% of the starch surface of soft wheat prime starch granules was protein (~127 μm of protein on granule surface) which is slightly higher than that reported by other groups (Seguchi, 2001). SDS–PAGE of the extracted surface proteins indicated that no gluten proteins were present on the starch used in the present study (data not shown), therefore, all

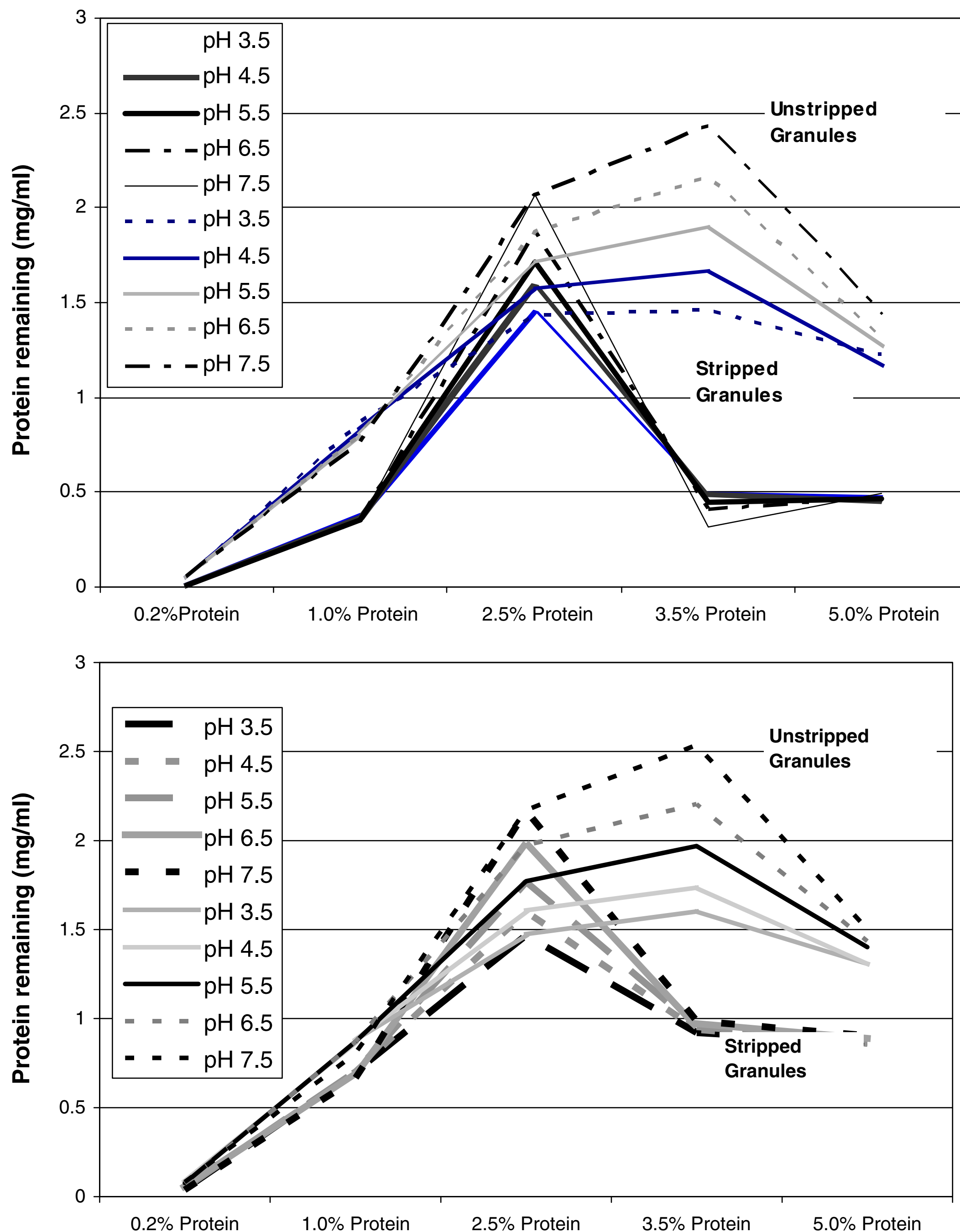

Fig. 4. Adsorption (top) and desorption (bottom) of TSP on stripped and unstripped wheat starch granule.

protein was considered to be granule surface protein, without gluten contamination. All known surface protein groups were evident from the electrophoresed sample, including puroindoline (≈15 kDa). Amido black adsorption of hard wheat granules indicated a protein level of 0.07% (∼33 μ protein on granule surface) with no evidence of residual gluten.

3.5. Effects of granule stripping and adsorption

The binding affinity of stripped and unstripped starch granules for TSP is shown in Fig. 4 (top); patterns for TVP and soy flour isolate were similar (data not shown). Stripping endogenous protein from the wheat starch granule did not inhibit protein binding below a protein concentration of 2.5%. At higher protein concentrations, bound protein level either dropped and remained fixed, or continuously decreased, a pattern that was consistent for all the proteins used in this study.

Desorption patterns in the stripped granules indicate an alteration similar to that seen in binding. Desorption profiles for TSP are shown in Fig. 4 (bottom); profiles of TVP and soy flour isolates were similar (data not shown). Again, at a concentration of 2.5% protein, the granule lost the ability to bind and retain the attached protein. This change in binding ability appears to indicate that the starch granule surface proteins must participate in the mechanism by which non-starch proteins associate with the granule surface. It is hypothesized that surface proteins serve to anchor binding proteins to the granule, increasing the amount of protein able to bind, and maintaining this interaction against desorption.

In comparison to the unstripped granule, the affinity of any particular protein for the stripped granule surface did not change upon alteration of the pH of the surrounding environment at the higher protein concentrations. Therefore, trends in protein binding must be associated only with the pH alteration of the binding protein and not with the native starch granule surface protein.

Oil binding of stripped granules was 0.09 ml oil/g starch (data not shown) which indicated a significant decrease (decreasing from 0.33 ml oil/g starch) in hydrophobic areas present on the granule surface. These areas were assumed to be due to granule surface proteins. The hydrophobic nature of these starch granule surface proteins may anchor binding proteins, especially at higher levels of protein addition. The increased binding observed with processed soy proteins is evidence of this type of mechanism.

4. Conclusions

Soy protein texturization significantly influenced starch granule surface binding. Oil binding and protein stripping studies implicate hydrophobic interactions in the protein–starch granule association. However, collective analysis of the data indicates that several factors may be involved in this interaction, including protein shape, size and charge. Overall, the native starch granule protein appears to affect the binding of added proteins to the granule surface. Stripping data support a mechanism of association wherein the native protein attracts and holds added protein to the granule surface. From these data, it appears that surface proteins serve to anchor binding proteins to the starch granule surface, increase the amount of protein able to bind, and maintain this interaction against desorption.

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