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A Semi-Pilot-Scale Procedure for Isolating and Purifying Soybean (*Glycine max*) Lectin

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Availability of gram quantities of purified soybean lectin (SBL) to scientists will foster discovery of novel biomedical applications of the lectin and provide the opportunity to investigate the antinutritional effects of SBL in soybean-consuming food animals and poultry. Therefore, a semi-pilot-scale procedure for isolating and purifying SBL was designed. Defatted soyflour was extracted overnight with 0.9% NaCl at 4 °C. The extract obtained was filtered (0.45 μm membrane) and subjected to affinity chromatography using a column containing *N*-acetyl-D-galactosamine resin that is specific for SBL. Bound SBL was eluted off the column with 0.14 M galactose solution. The eluent was ultrafiltered (30 kDa), and the resulting solution (SBL and water) was freeze-dried. Electrophoretic analysis and hemagglutination assay revealed that the freeze-dried SBL was similar to Sigma-grade SBL in purity and activity (35 and 33 HU/mg protein, respectively). The procedure yielded 141 mg of SBL/100 g of soyflour.

KEYWORDS: Soybean lectin (SBL); *Glycine max*; lectin purification; scale-up; affinity chromatography; SDS- and native-PAGE

INTRODUCTION

Lectins are proteins (or glycoproteins) of non-immunoglobin nature capable of specific recognition of and reversible binding to carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognized glycosyl ligands (*1*, *2*). Soybean lectin binds specifically to *N*-acetyl-D-galactosamine (including its glycosides and oligosaccharides containing a terminal *N*-acetyl-D-galactosamine residue) with greatest affinity and also binds to galactose and its derivatives with lesser affinity (*3*). The lectin is localized in the protein bodies of cotyledon cells in soybean seeds (*4*). It is a tetrameric glycoprotein with a molecular weight (MW) of 120 KDa and consists of four identical subunits (*5*). Each subunit carries an oligosaccharide chain $Man_9(GlcNAc)_2$ (*6*) and has a MW of 30 kDa (*7*).

Purified soybean lectin (SBL) is highly valued for its use in a variety of biomedical applications including analytical, diagnostic, and clinical uses (*8*−*13*). Access of researchers to gram quantities of the lectin will facilitate the exploration and conduction of more medical research geared toward the discovery of new or improved beneficial application(s).

Despite the beneficial biomedical applications of lectins, they may be detrimental to food animals and poultry since they are known to induce antinutritional effects in laboratory rodents (*2*). Most ingested dietary lectins are able to resist gut proteolysis to varying degrees and bind to their target glycoproteins on the intestinal surface (*14*, *15*). In particular, SBL is known to survive enzyme degradation in the gut to a considerable extent (*16*, *17*). Lectins that survive intestinal enzyme degradation bind to their target glycoprotein receptors in the small intestine, thereby causing disruption of the brush border membrane. This membrane disruption usually results in antinutritional effects such as loss of brush border enzymes, reduced nutrient digestibility, reduced growth, and occasionally death (*2*, *18*−*22*). Studies investigating the antinutritional effects of SBL have been done mostly with laboratory rats. A number of researchers have observed a significant reduction in feed consumption and growth of rats and mice fed diets containing purified SBL or raw soybeans (*2*, *23*−*26*).

Among farm animals, the poultry industry is the largest consumer of soybean products as animal feed. Some experiments investigating the antinutritional effect of SBL have also been done with chickens (*27*−*33*). These investigators observed that feeding raw conventional soybean meal (SBM) or raw trypsin inhibitor-free SBM to chickens reduced weight gain. In addition, they found that SBL contributed to the growth-depressing effects observed. In fact, some researchers reported that SBL accounted

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for approximately 15% of the growth depression observed for raw soybeans in chicks (*34*). Lectins (SBL) and trypsin inhibitors (TI) are the major antinutrients present in soybeans due their high levels and to the severity of the antinutritional effects they cause (*20*).

Typically, soybeans are included at high levels (approximately 25−50%) in poultry diets as defatted toasted meal or as expanded or extruded soybeans (*35, 36*). Lectin levels in these soybean products can vary considerably. For example, variable residual lectin activity (0.2−0.6 mg of SBL/g of meal) have been observed among samples of commercially processed meals (*37*). Also, it has been speculated that the residual lectin levels present in processed soybean products may still affect growth and nutrient utilization in animals (*38*). As such, it is important to determine safe lectin levels for broiler chicken and turkey diets. Completion of such research requires isolation of gram quantities of purified SBL to add in graded levels to semi-purified diets intended for feeding chicks or poults in a nutritional study. The use of purified SBL and a semi-purified diet will eliminate the presence of any other antinutrient in the diet. As such, all effects observed in these experiments will be attributed only to SBL.

Considering the valuable applications of SBL in biomedical research and the requirement of gram quantities of SBL for nutritional studies in animals and poultry, an on-site process for producing the amount of SBL needed at any point in time would be of great value to researchers. Studies describing small-scale procedures for isolating and purifying SBL exist (*37, 39, 40*), but these procedures cannot be directly used as published to purify gram quantities of the lectin. Therefore, this report describes a semi-pilot-scale procedure that is designed for isolating and purifying gram quantities of SBL.

MATERIALS AND METHODS

Extraction and Centrifugation. Defatted soyflour was purchased from Archer Daniels Midland Company (Protein Specialties Division, Dacatur, IL). Soyflour was extracted overnight at 4 °C with 0.9% saline as follows: 320 g of soyflour was weighed into a container, and 4 L of saline was added. A wooden paddle was used to manually suspend the soyflour into the saline for about 15 min. The mixture was then refrigerated overnight for extraction to occur. Three 4 L batches of extract were prepared each time to make 12 L of extract. The next morning, two distinct layers were observed in each container: (1) a top layer which was a clear yellow fluid that contained the soluble components of soyflour, and (2) the cloudy bottom layer that consisted of insoluble components with some fluid trapped within. Soybean lectin is a soluble protein (*41*) and thus would be in the fluid top layer. Hence, the top layer was decanted, and the bottom layer was centrifuged (Sorvall RC-5B superspeed centrifuge, Du Pont Company, Wilmington, DE; Sorvall GS-3 rotor; Sorvall polypropylene bottles, #03943) at 4225 × g and 4 °C for 20 min to recover trapped fluid. Supernatants (or extracts) were pooled and subjected to microfiltration to remove insoluble particles.

The efficiency of the extraction process was compared to a small-scale extraction procedure (*37*) by measuring protein content in the extract from each procedure. In the small-scale procedure, 2 g of a soybean sample was extracted with 25 mL of 0.9% saline by constantly stirring with magnetic stir-bars. After 2 h, the mixture was centrifuged, supernatant decanted, and the pellet resuspended in 15 mL of 0.9% saline. Residual soluble protein was then extracted and the mixture centrifuged again as previously described. The resulting supernatants were pooled for analyses. For the purpose of our evaluation, supernatants obtained from each procedure were prepared from similar quantities of soyflour. As such, the major difference between the two procedures was in the degree of laboriousness of the extraction process. Extracts from the semi-pilot and small-scale procedures were subjected to the Bradford dye-binding assay (*42*) for the determination of protein content, using bovine serum albumin as the standard.

Microfiltration. Microfiltration (MF) is a membrane separation system used for clarification or the separation of two or more components from a fluid stream on the basis of size differences (*43, 44*). Membranes used for microfiltration typically have a pore-size of 0.1−2 μm and can selectively separate particles with molecular weights greater than 200 kDa (*43*). In this study, the pooled extract (12 L) was passed through a 20 L filtration system (model DC10L, Amicon Co., Lexington, MA) that had a 0.45 μm polysulfone membrane (CFP-4-E−6A, A/G Technology Co., Needham, MA). This membrane size was used to remove insoluble particles, thereby clarifying the extract. The 12 L clarified extract was kept refrigerated until time for application unto an affinity column.

Affinity Chromatography. Affinity chromatography makes use of biological-like interactions for the separation and specific analysis of sample components (*45*). Since SBL binds specifically to *N*-acetyl-D-galactosamine with high affinity (*3*), an aqueous column (D = 10 cm and H = 30 cm; Spectrum Chromatography, Houston, TX) containing 225 mL of *N*-acetyl-D-galactosamine resin (Sigma St. Louis, MO) was prepared. The binding capacity of the resin was 7 mg of lectin/mL of the resin. The size of column used in this process was carefully selected to maintain good hydrodynamic characteristics in the column. The column was stored in a cold room (4 °C), and a peristaltic pump (model LP-1; Amicon Co., Lexington, MA) was connected to allow the movement of fluids through the column at optimum flow rates. The resin was equilibrated with 0.9% saline until the absorbance of the eluent (at 280 nm) remained constant at zero for 10 min. Subsequently, 4 L of the clarified extract was pumped onto the column (30 mL per min) to allow for the SBL to bind to the *N*-acetyl-D-galactosamine resin. Afterward, saline was pumped through the column to remove unbound proteins. The absence of unbound proteins in the column was indicated when the absorbance (at 280 nm) of the eluent from the column was zero. Elution of SBL from the resin was accomplished by pumping 2 L of 0.14 M galactose in saline solution through the column. The galactose displaces the lectin from the immobilized ligand as the affinity eluent that is collected and frozen until time for ultrafiltration.

A total of 12 L of clarified extract was subjected to affinity chromatography over a period of 3 days at a rate of 4 L per day. Approximately 1.2 L of eluent was obtained from each 4 L run. After 3 days, the column was cleaned and regenerated with 1 N NaCl in readiness for the next batch of 12 L extract. Thus, the same resin can be used indefinitely. A total of 48 L of extract was processed with the affinity column over a period of 3 weeks, yielding approximately 15 L of affinity eluent. For long-term storage, the resin was stored in 1 M NaCl with 1% sodium azide.

Ultrafiltration. Ultrafiltration (UF) is a rapid and scaleable separation process that uses membranes with a molecular weight cutoff in the range of 1−200 kDa (*43*). The 15 L of affinity eluent (containing essentially SBL, galactose, NaCl, and deionized water) was thawed and pumped into 20 L filtration equipment containing a membrane with a 30 kDa molecular weight cutoff (UFP-30-E−6A, A/G Technology Co., Needham, MA). This membrane was used to remove galactose (180 Da), NaCl (58 Da), and deionized water from the affinity eluent, thereby leaving a concentrated solution of SBL (120 kDa) (*5*). Efficiency of the UF process was assessed by measuring the amount of galactose left in the concentrated lectin solution. Galactose concentration was determined by liquid chromatography (*46*). Approximately 2 L of concentrated lectin solution was obtained from the UF process.

Freeze-Drying and Storage. The 2 L of concentrated lectin solution was poured into stainless steel freeze-drying trays and frozen overnight in preparation for freeze-drying. The trays were then placed in the freeze-dryer (model 79480, Labconco Corporation, Kansas City, MO) for freeze-drying to occur over 24−36 h. The dried lectin (a white fluffy powder) was then collected into preweighed sterile plastic bags, weighed, and placed in a refrigerated desiccator until needed. A total of 5.4 g of purified lectin was obtained from 3840 g of soyflakes within 3 weeks to constitute one round of operation.

Repeatability. This semi-pilot-scale procedure was repeated 2 times over a period of 7 weeks in order to assess the consistency and repeatability of the process. In the end, 3 rounds were completed. The freeze-dried lectin sample obtained from each round was analyzed for purity by electrophoresis and Western blotting. Similar electrophoretic

Table 1. Properties of Molecular Weight Markers Selected for Native-PAGE Analysis (*54–57*)

protein	molecular weight (kDa)	isoelectric point (pI)	Stokes radius (nm)
myoglobin	17	6.8	2.02
hemoglobin	64	7.0	3.32
Sigma-grade SBL	120	5.8–6.2	unknown
human IgG	153	5.8–7.3	5.50
catalase	230	5.4	5.20

profiles were obtained for each isolate, and thus, samples from the 3 rounds were pooled for determination of lectin activity.

Lectin Yield and Recovery. Lectin yield and recovery was estimated for each of the 3 rounds of purification. Lectin yield was calculated as the amount of purified lectin obtained from 100 g of soyflour. Lectin recovery was calculated as "the lectin yield as a percentage of the original lectin content in the extracted soyflour".

Evaluation of Lectin Purity and Biochemical Characteristics. Purity of the SBL was assessed using SDS-polyacrylamide gel electrophoresis (PAGE) and Western blot analyses. A native-PAGE analysis was also done to examine the quality of the purified lectin and to determine the Stokes radius of the lectin. Samples used for these electrophoretic analyses were the pooled purified SBL sample (denoted as L_1), Sigma-grade SBL (denoted as L_2), and the clarified extract/supernatant (denoted as E) obtained after extraction and filtration.

SDS−PAGE. A 100 μL amount of each sample (L_1 and L_2) was mixed with 100 μL of reducing sample buffer (Novex, Inc.) and heated at 100 °C for 10 min. Samples were then loaded onto two 4−20% Tris-Glycine gradient gels (Novex, Inc. San Diego, CA.), such that each sample was present in both gels. A multicolored standard (Novex, Inc.) containing glutamic dehydrogenase (60 kDa), carbonic anhydrase (42 kDa), myoglobin-blue (30 kDa), myoglobin-red (22 kDa), lysozyme (17 kDa), aprotinin (6 kDa), and insulin (4 kDa) was used for molecular weight calibration. Electrophoresis was carried out using the Xcell II Mini Cell apparatus (Novex, Inc.). After electrophoresis, the first gel was stained for protein using a colloidal Coomassie blue staining reagent (Novex, Inc.). Proteins in the second gel were transferred onto a polyvinyldinitrofluoride (PVDF) membrane (Novex, Inc.), using an electroblotting module (Novex Inc.) fitted to the Mini Cell apparatus.

Native-PAGE. For native-PAGE, 50 μL of each sample (L1, L2, and E) was mixed with 50 μL of native sample buffer (Novex, Inc.). Each sample along with selected molecular weight markers was electrophoresed in a pair of 6%, 10%, 12%, and 14% acrylamide Tris-Glycine gels (Novex, Inc.). Since separation of proteins on a native gel is based on differences in molecular charge, size, and shape, proteins that were selected as molecular weight markers had known Stokes radii and isoelectric points (pI) close to that of soybean lectin. All molecular weight markers (Table 1) were obtained from Sigma, St. Louis, MO. Electrophoresis was carried out using the Xcell II Mini Cell apparatus (Novex, Inc.). After electrophoresis, one gel was stained with a colloidal Coomassie blue (Novex, Inc.) to visualize protein bands, while the proteins on the second gel were transferred onto a PVDF membrane for Western blotting.

Western Blotting. After transfer of proteins, the PVDF membranes obtained from both SDS- and native-PAGE were similarly processed/treated for immunodetection of SBL bands. The membranes were blocked overnight at room temperature using a 3% solution of nonfat dry milk and then washed 3× with phosphate-buffered saline containing 0.02% Tween (PBS/Tween). Next, the membranes were incubated overnight with polyclonal rabbit anti-SBL antibody (1:2000 dilution; obtained from Sigma Inc.), after which membranes were washed 3× to remove unbound antibody. The bound antibody was detected by incubating the membrane with goat anti-rabbit IgG-horseradish peroxidase (HRP) conjugate (1:2000 dilution; obtained from Sigma Inc.) for 3−4 h. Membranes were washed again (3×), and peroxidase activity was detected by the addition of HRP substrate solution (1 mL of diaminobenzidine added to 9 mL of hydrogen peroxide; obtained from Pierce Inc., Rockford, IL). Soybean lectin-positive bands were then visualized on the PVDF membranes.

Table 2. Relative Mobility (R_f) and Retardation Coefficient (KR) of Molecular Weight Markers and Samples in Gels Differing in Acrylamide Concentration

% acrylamide in gels	myoglobin	hemoglobin	human IgG	catalase	L1	L2	E
6	0.467	0.467	0.217	0.367	0.317	0.333	0.283
10	0.456	0.421	0.193	0.281	0.263	0.281	0.211
12	0.443	0.415	0.170	0.264	0.245	0.264	0.189
14	0.438	0.396	0.167	0.250	0.229	0.250	0.177
KR value	0.0036	0.0087	0.0152	0.0211	0.0158	0.0177	0.0261

[a] L_1 = purified SBL extracted using the semi-pilot scale procedure in this study. L_2 = purified SBL purchased from Sigma Chemicals, St. Louis, MO. E = the clarified extract/supernatant obtained after extraction and filtration.

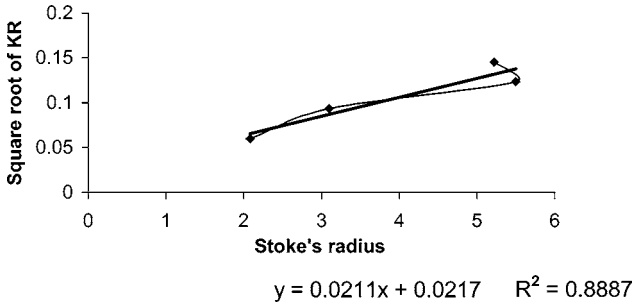

Figure 1. Standard curve for Stokes radius estimation. The equation ($y = 0.0211x + 0.0217$; where y = square root of KR of the protein in question and x = SR of the protein in question) obtained from a linear fit to the curve was used to estimate the SR of L_1, L_2, and E.

The SDS−PAGE gel and membrane were assessed for lectin purity, while the native-PAGE gel and membrane were used for assessing lectin quality and for estimating the Stokes radius of the native lectin.

Determination of the Stokes radius of native SBL. Stokes radius (SR) is the hydrodynamic radius of a protein. In this study, the SR of native SBL was determined in samples L_1, L_2, and E. The relative mobility (Rf) of each molecular weight marker and sample was measured on each gel concentration (i.e., 6%, 10%, 12%, and 14% acrylamide gels stained for protein). Data obtained (Table 2) were used to construct the Ferguson plot (log of Rf versus gel concentration) for each protein. Retardation coefficient (KR) for each protein (Table 2) was calculated from the slope of each plot. Then, the square roots of KR of the molecular weight markers were plotted against their known Stokes radius values (Figure 1). A linear fit of this plot yielded an equation from which the Stokes radii of the native soybean lectin present in L_1, L_2, and E were estimated.

Determination of Lectin Activity. Lectin activity was determined by measuring the hemagglutination activity (HA) of the lectin samples (L_1 and L_2). Hemagglutination activity (*47*) was determined for L_1 and L_2 by incubating each lectin sample with erythrocyte suspension from rabbit red blood cells (RBC). The lectin agglutinates the RBC, thereby causing a reduction in the amount of erythrocytes present in suspension. Hemagglutination activity was expressed as hemagglutinating unit (HU)/mg of protein. Typically, one HU is defined as the amount of material that is required to cause a 50% decrease in the absorbance of an erythrocyte suspension within 2.5 h at room temperature (*48*).

RESULTS AND DISCUSSION

The supernatant obtained after extraction and centrifugation was subjected to protein assay in order to compare the extraction efficiency of this semi-pilot procedure to that of the small-scale extraction procedure (*37*). Results of this analysis are presented in Table 3. The amount of protein extracted by the semi-pilot process in this study (93.4%) was comparable to that obtained (89.6%) for the small-scale procedure.

The membrane used for microfiltration had a low protein-binding capacity (A/G Technology Co., Needham, MA). Thus,

Table 3. Comparison of Extraction Efficiency

extraction procedure	% protein extracted
semi-pilot-scale extraction procedure[a]	
decanted supernatant containing soluble protein (first layer)	49.77
recovered trapped supernatant (second layer)	43.61
pooled supernatants	93.38
small-scale extraction procedure[b]	
supernatant from first extraction (2 h)	75.58
supernatant from second extraction (2 h)	14.01
pooled supernatants	89.59

[a] This procedure involved 15 min of manual stirring followed by overnight extraction, decanting of soluble supernatant, and centrifugation to retrieve trapped supernatant within the layer of insoluble particles. Lectin yield for this procedure was 2.75 mg/g meal. [b] In this procedure, sample is first extracted with 0.9% saline over 2 h. The mixture is then centrifuged and supernatant decanted. The pellet obtained is resuspended in 0.9% saline, extracted again for 2 h, centrifuged, and the second supernatant is collected. The first and second supernatants can be analyzed separately or pooled (*37*). Lectin yield for this procedure was 2.71 mg/g meal.

it is reasonable to assume that protein loss during microfiltration was minimal. Clarification of the extract during MF enhanced the operation of the affinity column by preventing decreasing flow rates due to clogging. This ultrafiltration processing of each batch of 15 L of affinity eluent took only 4 h compared to alternative procedures such as dialysis and gel filtration that would have taken much longer (at least overnight). The effectiveness of the UF process in removing galactose from the affinity eluent was assessed by measuring galactose concentration in the eluent (before UF) and in the concentrated lectin solution (after UF). Analysis was performed on samples from each of the three rounds of purification. Results obtained indicated that the UF process removed 97.1% ± 3.5% of the original galactose concentration in the affinity eluents.

The semi-pilot-scale procedure described in this study produced 5.4 ± 0.15 g of purified lectin over a period of 3 weeks. Lectin yield was 141.5 ± 4.0 mg/100 g of soyflour. This yield was comparable to values reported in the literature (113−150 mg/100 g of defatted SBM) for small-scale affinity chromatography procedures (*39, 40, 49*). Lectin recovery was 60% and similar to the value (59%) reported earlier by other authors (*50*).

The subunit size homogeneity of the extracted lectin (L_1) was compared to that of the Sigma product (L_2) using SDS−PAGE. We define a pure lectin sample as one that contains no other protein. Thus, all the protein bands in SBL samples on the SDS−PAGE gel should stain positive for lectin on the Western blot. The gel stained for protein (Figure 2, lanes 2 and 3) showed that both L_1 and L_2 had similar profiles of protein bands, with each sample showing one major band and two smaller bands. The major band is the lectin subunit band (MW = 30 kDa), while the two smaller bands are possibly lectin fragments that exist as a result of the lectin protein being nicked by inherent proteolytic activity in the soybean seed. The corresponding Western blot analysis (Figure 2, lanes 4 and 5) revealed that all the bands in the lanes for L_1 and L_2 stained positive for SBL, thereby indicating that both L_1 and L_2 are homogeneous with respect to lectin protein. These results imply that the semi-pilot-scale procedure produced lectin (L_1) of similar purity to the Sigma-grade SBL (L_2).

Native-PAGE analysis was performed to compare the charge homogeneity of L_1 to L_2 and to determine the Stokes radius of native SBL in samples L_1, L_2, and E. Lectin quality describes the relative proportion of native and denatured or nicked lectin

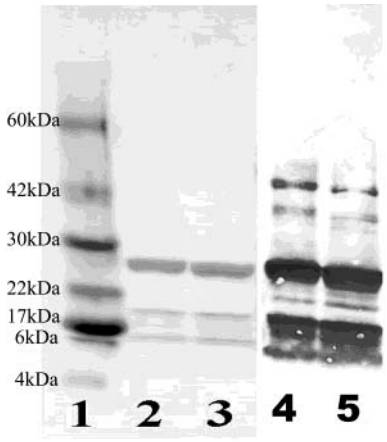

Figure 2. SDS–PAGE and Western blot analysis of L_1 and L_2. Lane 1 = MW markers; Lanes 2 and 3 presents protein bands detected in L_1 and L_2 after staining the SDS–Tris–glycine gel with colloidal blue. Lane 2 = profile of protein bands in L_2; Lane 3 = profile of protein bands in L_1; Lanes 4 and 5 present protein bands that stained positive for soybean lectin on the PVDF membrane after probing with polyclonal rabbit anti-soybean lectin antibody. Lane 4 = profile of lectin-positive bands in L_2; Lane 5 = profile of lectin-positive bands in L_1.

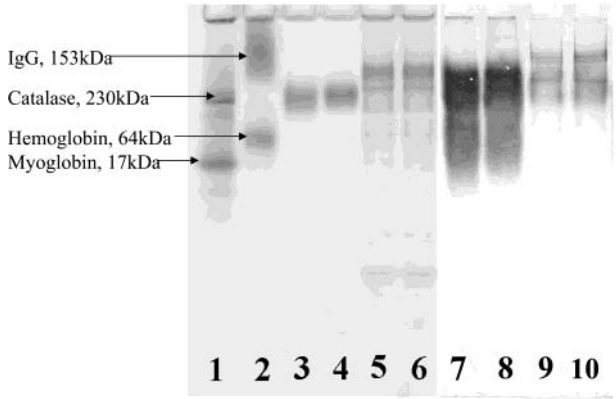

Figure 3. Native-PAGE and Western blot analysis of L_1, L_2, and E on a 12% acrylamide gel. Lanes 1 and 2 = MW markers; Lanes 3–6 present protein bands detected in samples after staining the gel with colloidal blue. Lane 3 = L_2; Lane 4 = L_1; Lanes 5 and 6 = E. Lanes 7–10 present protein bands that stained positive for soybean lectin on the PVDF membrane after probing with polyclonal rabbit anti-soybean lectin antibody. Lane 7 = L_2; Lane 8 = L_1; Lanes 9 and 10 = E.

present in the sample. Typically, the higher the proportion of native form of a protein, the better the protein quality. The native-PAGE gel stained for protein and the corresponding Western blot results are both presented in Figure 3. Only one broad protein band was observed in the lanes 3 and 4 for L_2 and L_1 respectively (Figure 3). However, on the more sensitive Western blot (Figure 3, lanes 7 and 8), the broadening of the lectin bands was more pronounced for both L_1 and L_2, suggesting the possible presence of lectin fragments as observed for the denaturing SDS−PAGE analysis in Figure 2. Under native conditions, a nicked protein that still maintains its tertiary and quaternary structure would exhibit only a subtle change in mobility, resulting in broadening of the protein band. Nonetheless, both L_1 and L_2 contained a higher proportion of the native lectin (which is the upper thick portion of each band) compared to the fragments (which are possibly the smeared portion of the L_1 and L_2 bands). Hence, we believe that the amount of denatured forms detected by the sensitive Western blot analysis

in L_1 and L_2 was very small and therefore undetected by native-PAGE. These results indicate that L_1 and L_2 are of similar quality.

The "denatured" lectin forms of L_1 and L_2 are probably a result of imprecise proteolytic processing of C-terminal ends of the lectin within the plant seed (*51*). Isolectins resulting from "ragged C-terminal ends" are a major source of heterogeneity in lectins (*51*). Hence, the smeared portion of the lectin bands in L_1 and L_2 (Figure 3, lanes 7 and 8) probably represents soybean isolectins that resulted from proteolytic processing of C-terminal ends of the native lectin. In addition, some degree of proteolysis usually occur during protein isolation and purification. Alternatively, the smeared portion of the lectin bands may represent genetic isoforms or glycan variants of the lectin (*51*).

Sample E (Figure 3, lanes 9 and 10) also contained both the native and denatured lectin forms that had slower mobilities compared to their counterparts in L_1 and L_2. The lower mobility of the lectin forms in E was probably because the lectins in E associated with other compounds in the raw extract giving rise to complexes that moved slower in the electrophoresis gel.

The Stokes radius of native SBL was estimated to be 5.3 nm for L_1, 4.9 nm for L_2, and 6.7 nm for E. The results indicate that L_1 and L_2 had similar Stokes radii. Native SBL in E had a higher SR probably because it was associated with other components present in the crude extract. To the best of our knowledge, this is the first time the SR of native soybean lectin has been determined. Stokes radius values are very useful in the study of proteins that have changes in the quaternary structure or different conformations (*52*). For instance, knowing the SR of native SBL should enable the separation of the native lectin from its denatured forms, using size-exclusion chromatography. Furthermore, knowledge of SR can be applied in the study of equilibria in reactions accompanied by a change in dimensions (*53*).

Lectin activity was assessed by measuring the hemagglutination activity (HA) of the lectin samples (L_1 and L_2). Hemagglutination activity values of 35 HU/mg of protein and 33 HU/mg of protein were obtained for L_1 and L_2, respectively. Both lectins had similar activity. Some authors have reported a comparable but lower HA value of 20 HU/mg of protein when they used untreated rabbit RBC for the assay (*47*) as we did in this study. Their lower value may be due to differences in the age of the RBC at the time of assay and differences in laboratory experimental techniques.

A recently published small-scale procedure for isolating and purifying soybean lectin (*37*) was compared to the semi-pilot scale described in this study. The small-scale procedure involved extracting raw soyflour with saline, microfiltration of extract using 1.6 μm Whatman GF/A filter (Sigma Chemicals, St. Louis, MO), processing of filtered extract in an affinity column containing *N*-acetylgalactosamine resin, desalting affinity eluent by gel filtration technique, and freeze-drying of the resulting pure lectin solution to obtain a dry lectin powder. The semi-pilot-scale procedure designed in this study utilized similar principles of extraction, microfiltration, affinity chromatography, and freeze-drying, except in the desalting stage where a faster and scaleable ultrafiltration process was employed.

Although the efficiency of protein extraction was similar for both the small-scale and the semi-pilot-scale procedures (Table 3), the former procedure is more laborious and is only well suited for extracting small quantities (2 g) of soyflour at a time, compared to the latter procedure that can be scaled to process large quantities of sample. The obviously larger scale and faster speed of the semi-pilot-scale procedure makes it a superior technique for isolating and purifying gram quantities of SBL. In addition, the lectin extracted in this study was further characterized by ascertaining its purity and activity by electrophoretic techniques, Western blotting, and hemagglutination assays.

In conclusion, a semi-pilot-scale procedure has been designed and implemented for the isolation and purification of gram quantities of SBL. Furthermore, the modified procedure should be scaleable to a large process. Moreover, the cost of the purified SBL obtained in this study was nearly 15-fold less than that of commercially available SBL of similar quality. It is anticipated that scientists engaged in medical and animal research involving the use of SBL will adopt this procedure. This will facilitate the discovery of new and/or improved medical application(s) of SBL and the determination of safe inclusion levels of the lectin in poultry and other animal diets.

ABBREVIATIONS USED

SBL, soybean lectin; TI, trypsin inhibitor; SBM, soybean meal; MF, microfiltration; UF, ultrafiltration; L1, soybean lectin isolated in this study; L2, Sigma-grade soybean lectin; E, filtrate obtained after extraction and microfiltration; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; PVDF, polyvinyldinitrofluoride membrane; PBS/Tween, phosphate-buffered saline containing 0.02% Tween; HRP, horseradish peroxidase; SR, Stokes radius; Rf, relative mobility; KR, retardation coefficient; MAT, minimum agglutination titer; HA, hemagglutination activity; RBC, red blood cell(s).

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