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Functional Properties of Select Edible Oilseed Proteins

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Borate saline buffer (0.1 M, pH 8.45) solubilized proteins from almond, Brazil nut, cashew nut, hazelnut, macadamia, pine nut, pistachio, Spanish peanut, Virginia peanut, and soybean seeds were prepared from the corresponding defatted flour. The yield was in the range from 10.6% (macadamia) to 27.4% (almond). The protein content, on a dry weight basis, of the lyophilized preparations ranged from 69.23% (pine nut) to 94.80% (soybean). Isolated proteins from Brazil nut had the lightest and hazelnut the darkest color. Isolated proteins exhibited good solubility in aqueous media. Foaming capacity (<40% overrun) and stability (<1 h) of the isolated proteins were poor to fair. Almond proteins had the highest viscosity among the tested proteins. Oil-holding capacity of the isolated proteins ranged from 2.8 (macadamia) to 7 (soybean) g of oil/g of protein. Least gelation concentrations (% w/v) for almond, Brazil nut, cashew, hazelnut, macadamia, pine nut, pistachio, Spanish peanut, Virginia peanut, and soybean were, respectively, 6, 8, 8, 12, 20, 12, 10, 14, 14, and 16.

KEYWORDS: Seed; proteins; functional properties; foaming; viscosity; gelation; solubility; oil holding capacity; electrophoresis; Western blotting

INTRODUCTION

Edible nut seeds are globally valued for their sensory and nutritional attributes. Often used as snack foods in variously processed forms, nut seeds are also incorporated in a variety of foods to impart the desirable quality attributes that include flavor and texture. The type of nut seed used in a food partly depends on the nut seed type and the final product quality attribute. For example, almond, cashew nut, macadamia, pecan, pistachio, and peanut are widely used in dry or oil roasted, salted, or unsalted forms as snack food. Chocolate-coated almonds, macadamias, peanuts, and others are popular confectionery items. Pine nuts typically find use in salad toppings or in the preparation of nut pastes. Almonds and cashew nuts are used in the production of pastes and butters.

Lipids (notably triglycerides) and proteins in edible nut seeds account for the major portion, typically 50−90% (as-is basis) (*6*), of seed weight and are therefore thought to significantly influence seed properties. In recent years nut seed lipids have received significant attention due to not only their importance in sensory properties (mild flavors and smooth texture) but also their possible role in human health (*1*−*3*) and weight management (see refs *4* and *5* and references cited therein).

Edible nut seeds contain 7−25% protein on an as-is basis (see ref *6* and references cited therein). The number and type of proteins present are dependent on the seed type, and typically these proteins are rich in acidic amino acids Glx and Asx and the basic amino acid Arg (*6*). With the increased awareness of tree nut-induced allergies, tree nut proteins have received increased scrutiny in recent years (*7*−*13*).

Proteins perform a variety of functions in food systems, and therefore protein functional properties are of interest (see ref *14* and several references cited therein). Although tree nut seeds contain significant amounts of proteins, investigations focusing on their functional properties are limited. Whereas premium quality whole nut kernels may not be economically practical for the purpose of preparing protein concentrates/isolates, cull seeds may provide suitable raw materials for such products. Extraction of nut seed lipids (e.g., gourmet oils) provides defatted high-protein flours that may also be used as ingredients or in the preparation of protein concentrates/isolates. With the exception of a few limited studies on almond (*15*−*17*) and cashew (*18*, *19*) nut proteins, tree nut protein functional properties remain largely unexplored. The purpose of the current investigation was to assess certain functional properties of isolated proteins from commercially important tree nut seeds. Soybean and peanut seed proteins were included in the study for comparative purposes.

MATERIALS AND METHODS

Materials. Sources of seeds, chemicals, and supplies have been reported earlier (*20*). Brazil nuts, cashews, hazelnuts, macadamia nuts, pine nuts, and Spanish peanuts were purchased from local grocery stores. Almonds (Nonpareil marketing variety; Almond Board of California, Modesto, CA), pecans (cultivar Desirable, Dr. T. Thompson, USDA-ARS, Pecan Breeding and Genetics, Somerville, TX), pistachio (Paramount Farms, Inc., Los Angeles, CA), walnuts (Blue Diamond Growers, Sacramento, CA), Virginia peanuts (VA 98R, Dr. Sean F. O'Keefe, VPI&SU, Blacksburg, VA), and soybean Williams 82 (Dr. W. J. Wolf, USDA, NRRL, Peoria, IL) were gifts.

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Methods. *Preparation of Seed Proteins*. Whole seeds were ground in an Osterizer blender (Galaxie model 869-18R, Jaden Consumer Solutions, Boca Raton, FL) at speed setting "grind" until a uniform flour was obtained. The flour was defatted for 6−8 h in a Soxhlet apparatus using petroleum ether (boiling point range of 38.2−54.3 °C) as the extraction solvent. The defatted flour was thinly spread on aluminum foil and dried overnight in a fume hood. Dried defatted samples were ground to a fine flour (Osterizer blender) to pass through 40 mesh sieve before storage in tightly capped plastic containers at −20 °C. Soluble proteins from defatted seed flours were extracted (flour to solvent ratio of 1:10 w/v) using borate saline buffer (BSB; 0.1 M H_3BO_3, 0.025 M $Na_2B_4O_7$, 0.075 M NaCl, pH 8.45) solvent for 2 h with constant magnetic stirring at room temperature (25 °C, RT). Samples were centrifuged at 15000*g* for 30 min at 4 °C. The supernatant was vacuum filtered through 185 mm diameter Whatman filter paper no. 4, dialyzed against 5 L of distilled deionized (DI) water for 48 h at 4 °C with six water changes, lyophilized, and stored at −20 °C in airtight plastic bottles until further use. Yield (%) was calculated as follows: yield (%) = [lyophilized protein powder (g)/defatted flour used for protein powder preparation (g)] × 100.

Proximate Composition

Moisture AOAC Official Method 925.40 (21). Accurately weighed samples (~0.1 g) were placed in an aluminum pan and dried in a previously heated vacuum oven (Barnstead Lab-line, Melrose Park, IL; model 3608-5; 95−100 °C, 25 in. of Hg) until constant weight.

Ash (AOAC Official Method 923.03). Accurately weighed samples (~0.1 g) were placed in a ceramic crucible and subjected to ashing in a muffle furnace (Thermolyne type 10500 furnace, a subsidiary of Sybron Corp., Dubuque, IA) maintained at 550 °C until a constant final weight for ash was achieved.

Protein AACC Official Method 46-12 (22). Total protein was determined using the micro-Kjeldahl method. The conversion factors used were 6.25 for soybean (*23*), 5.18 for almond, 5.46 for peanut, and 5.3 for the rest (*21*).

Soluble Protein. Soluble protein content of samples was determined according to the method of Lowry et al. (*24*) using bovine serum albumin (fraction V, purity 98%, Sigma Chemical Co., St. Louis, MO) as the standard protein (0−100 μg/mL).

Tannins. A known weight of isolate (~0.1 g) was extracted for 1 h in 1 mL of acidified (1% HCl, v/v) methanol with continuous vortexing followed by centrifugation (16000*g*, 10 min, room temperature). Aliquots of the supernatant were immediately analyzed for tannin using a 4% (w/v) vanillin assay (*6*). A catechin (98% purity, lot 58H1174, Sigma Chemical Co.) standard curve (0−1 mg/mL) was prepared simultaneously, and the tannin content was expressed as catechin equivalents (mg/100 g).

Color. Samples were placed in the glass sample cup (2.5 in. diameter, part 04-7209-00) of the LabScan XE spectrocolorimeter (Hunter Associates Laboratory, Reston, VA) using 1 in. (diameter) view with 0°/45° geometry and 10° observer. Care was taken to ensure the sample cup bottom was completely covered with the sample. The L^*, a^*, and b^* values were measured with EasyMatch QC software (version 3.90) using the 1 in. sample view port A (*25*), and the average values of four measurements, two readings for each of the duplicate preparations, were reported.

Apparent Viscosity. Stock protein solutions (10% w/v) were prepared by suspending the freeze-dried protein powders in DI water for 30 min at RT with constant magnetic stirring provided. Working protein solutions of the desired protein concentrations (1, 2, 3, 4, 5, 7, and 10% w/v) were prepared from the appropriate stock solution. The transit time between the fixed markings on an Ostwald type viscometer (Cannon Instrument Co., State College, PA; size 150) was determined using a stop watch (0.01 s accuracy). Apparent viscosity was determined using the equation

$$\text{apparent viscosity (cP)} = \text{kinematic viscosity (cS)} \times \text{sample density (g/mL)}$$

where kinematic viscosity = transit time in seconds × 0.035.

Foaming Capacity. Foaming capacity and stability were determined according to the method of Sathe and Salunkhe (*26*). Briefly, 50 mL of 1% (w/v) protein solution prepared in DI water was whipped for 3 min in an Osterizer blender at "stir" setting and then immediately poured into a 100 mL graduated cylinder. The total sample volume was monitored at 0 min for foam capacity and for up to 120 min for foam stability.

Oil Absorption Capacity. Oil absorption was determined by vortex mixing 0.1 g of protein and 1 mL of vegetable oil, density = 0.9239 g/mL, for 30 s and allowed to stand for 30 min. The mixture was centrifuged (13600*g*, 10 min, RT), and the weight of the supernatant was determined. The weight (g) of oil absorbed per gram of protein on a dry weight basis (dwb) was reported.

Least Gelation Concentration (LGC). LGC was determined according to the method of Sathe and Salunkhe (*26*) with slight modifications. Duplicate protein suspensions in DI water at 2, 4, 6, 8, 10, 12, 14, 16, and 20% (w/v) in a final volume of 200 μL were prepared in a 1.5 mL microcentrifuge tubes. Samples were briefly vortexed and heat denatured in a boiling water bath (100 °C) for 0.5 h, cooled quickly with running tap water, and kept at 4 °C (in a cold room) for 2 h before checking for gelation. LGC was the lowest concentration at which the sample did not fall after inversion of the tube and 10 slow taps with an index finger.

Rabbit Polyclonal Antibodies (pAbs). Production and characterization of the rabbit pAbs used were described earlier (*20*).

Electrophoresis and Western blotting. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) was done as described by Fling and Gregerson (*27*). Protein samples were boiled in SDS-PAGE sample buffer (50 mM Tris-HCl, pH 6.8, 1% SDS, 30% glycerol, 0.01% bromophenol blue) in either the absence or presence of 2% v/v β mercaptoethanol (β-ME) for 10 min, and suitable protein amount was loaded on the gels. Electrophoresis was carried out using an 8−25% linear monomer acrylamide gradient (separating gel) and 4% monomer acrylamide (stacking gel) with continuous cooling using running tap water. The gel was run at a constant current, typically 10 mA per gel overnight followed by 20 mA per gel until the dye migrated to the gel edge. The gels were either stained overnight with 0.25% w/v Coomassie Brilliant Blue R (CBBR) containing 50% v/v methanol and 10% v/v acetic acid or used for transfer onto 0.22 μm nitrocellulose membrane as described by Towbin et al. (*28*). The CBBR stained gels were destained with 50% v/v methanol containing 10% v/v acetic acid until the blue background was clear. Following the protein transfer, the unbound sites on the nitrocellulose membrane were blocked by incubation in Tris-buffered saline (TBS-T; 10 mM Tris, 0.9% w/v NaCl, 0.05% v/v Tween 20, pH 7.6) containing 5% w/v nonfat dried milk (NFDM) for 1 h at RT. The membrane was washed three times each with TBS-T for 5 min and then incubated with anti-nut protein rabbit antiserum in TBS-T at appropriate dilution (almond, 10000×; Brazil nut, 8000×; cashew nut, 3000×; hazelnut, 2000×; macadamia, 2000×; pine nut, 2000×; pistachio, 2000×; Spanish peanut, 5000×; Virginia peanut, 5000×; soybean, 2000×; all v/v) overnight at 4 °C. The membrane was rinsed once with TBS-T and thoroughly washed three times with TBS-T for 15 min each, followed by incubation with horseradish peroxidase-labeled goat anti-rabbit IgG antibody in TBS-T at 1:40000 v/v dilution for 1 h at RT. The membrane was then rinsed with TBS-T and washed three times with TBS-T for 15 min each. The reactive bands were developed by incubating the membrane with a luminol/*p*-coumaric acid system for 5 min and exposure to X-ray film for autoradiographic visualization. The exposure time was 5−60 s, depending on the signal intensity.

Statistics. All analyses were done at least in duplicate, and data are reported as mean ± standard deviation. When appropriate, data were analyzed for statistical significance ($p = 0.05$) using one-way ANOVA by SPSS statistical software (SPSS 15.0 for Windows, Microsoft Corp., Chicago, IL) and Fisher's least significant difference (LSD) as described by Ott (*29*).

RESULTS AND DISCUSSION

Composition. Isolated, freeze-dried proteins (**Table 1**) contained <4% moisture, an ash content of 1−4%, and 69−95% protein, on a dry weight basis. Protein preparations with ≥90% protein are termed protein isolates, whereas those with protein content of ≥65% but <90% are referred to as protein concentrates (*30*,*31*). By this definition, almond, Brazil nut, and soybean preparations in the current study may be classified as protein isolates with the rest as protein concentrates. Tannin content was highest in pistachio, whereas the pine nut sample was devoid of detectable tannins.

Table 1. Yield and Chemical Composition of Protein Preparations on a Dry Weight Basis[a]

seed	yield (%)	moisture (%)	ash (%)	protein (%)	tannin (mg/100 g)
almond	27.4 ± 5.9	3.56 ± 0.29	1.35 ± 0.39	92.72 ± 1.52	70 ± 20
Brazil nut	18.6 ± 0.3	2.73 ± 0.79	1.03 ± 0.44	92.29 ± 2.35	30 ± 10
cashew	13.6 ± 5.1	3.71 ± 0.55	2.08 ± 0.39	88.20 ± 3.27	30 ± 10
hazelnut	12.6 ± 2.0	3.43 ± 0.28	1.31 ± 0.48	89.95 ± 0.72	90 ± 10
macadamia	10.6 ± 0.8	3.42 ± 0.60	2.01 ± 0.15	78.40 ± 1.88	30 ± 0
pine nut	21.3 ± 2.5	2.51 ± 0.38	2.20 ± 0.28	69.23 ± 0.41	0 ± 0
pistachio	21.4 ± 6.5	3.89 ± 0.39	4.16 ± 0.47	80.34 ± 1.22	130 ± 30
Virginia peanut	21.0 ± 0.8	3.22 ± 0.82	1.69 ± 0.45	82.45 ± 1.87	90 ± 10
Spanish peanut	17.1 ± 2.7	3.26 ± 0.79	1.60 ± 0.35	79.98 ± 4.02	150 ± 20
soybean W82	12.4 ± 0.0	2.97 ± 0.74	1.64 ± 0.47	94.80 ± 0.60	20 ± 10
LSD (p = 0.05)[b]	3.08	0.46	0.37	2.16	10

[a] Data expressed as mean ± standard deviation (n = 4) except for yield values (n = 2). [b] Differences between means within the same column exceeding the LSD value are significant.

Figure 1. Color of isolated proteins from nut seeds.

Table 2. Hunter Color L^*, a^*, b^* for Seed Proteins[a]

protein source	L^*	a^*	b^*
almond	78.51 ± 1.08	3.97 ± 0.15	15.74 ± 0.22
Brazil nut	88.57 ± 0.29	0.15 ± 0.06	10.54 ± 0.32
cashew	82.98 ± 0.89	1.25 ± 0.15	10.74 ± 0.86
hazelnut	62.29 ± 0.66	6.30 ± 0.04	14.01 ± 0.14
macadamia	79.95 ± 0.40	1.42 ± 0.08	18.02 ± 0.27
pine nut	77.23 ± 2.03	1.60 ± 0.24	16.05 ± 0.89
pistachio	77.71 ± 1.89	2.48 ± 0.40	21.53 ± 0.68
Virginia peanut	72.61 ± 0.08	4.24 ± 0.00	14.29 ± 0.07
Spanish peanut	69.76 ± 0.45	4.74 ± 0.21	17.02 ± 0.49
soybean W82	77.09 ± 0.29	0.22 ± 0.11	10.08 ± 0.53
white[b]	93.08 ± 0.00	0.00 ± 0.00	−0.17 ± 0.00
LSD (p = 0.05)[c]	3.77	0.82	2.87

[a] Data are mean ± standard deviation (n = 4). [b] White refers to the standard white tile used as the reference. [c] Differences between the means within the same column exceeding the LSD value are significant.

Color. The protein preparations were typically pale white to light beige to brown colored (**Figure 1** and **Table 2**). The tannin content and the L value did not always correlate. For example, L^* values for Brazil nut and cashew were significantly different, although both had the same tannin content. Similarly, L^* values for Brazil nut and cashew were statistically different from the L^* value for macadamia despite their same average tannin content. The a^* and b^* values, respectively indicative of redness and yellowness, were generally consistent with the sample visual color appearance. The sample color is a result of the combination of several components including quantity of total phenolics, presence of nonprotein components such as coloring pigments and minerals, and type of phenolic compounds.

Protein Solubility. Protein solubility results (**Figure 2**) revealed the seed proteins to be quite soluble in tested solvents, a finding consistent with our earlier observation on defatted tree nut flour protein solubility (*20*). For a fixed nut seed, protein solubility was comparable in the tested solvents, suggesting several aqueous buffers may be suitable solvents for solubilization of the isolated proteins. The tree nut seed proteins are known to contain high amounts of acidic (Asx and Glx) and basic (particularly Arg) amino acids (*6*), and therefore good solubility of the isolated proteins in aqueous buffers was expected.

Apparent Viscosity. Apparent viscosity of the protein solutions increased with increased protein concentration (**Figure 3**). At a fixed protein concentration (≤ 5% w/v), the viscosities of protein preparations were comparable. Cashew nut proteins appeared to be the most viscous among the tested samples. The difference in viscosity was especially significant at protein concentrations of 7 and 10%. A recent study (*32*) reported that replacement of wheat flour with 5, 10, and 15% (by weight) nut pastes (almond, hazelnut, peanut, and walnut) produced acceptable, on the basis of sensory evaluations, breads that contained the tree nut and not the peanut pastes. Of particular note was the improvement of adhesive properties of the bread crumb and delayed staling of the bread samples containing the tree nut pastes over the corresponding controls.

Foaming Properties. Foaming capacity, percent increase in the volume also known as percent overrun, of the tested protein preparations was ≤40%, and the foam stability was typically < 1 h (**Figure 4**). Hazelnut proteins had the least foaming capacity

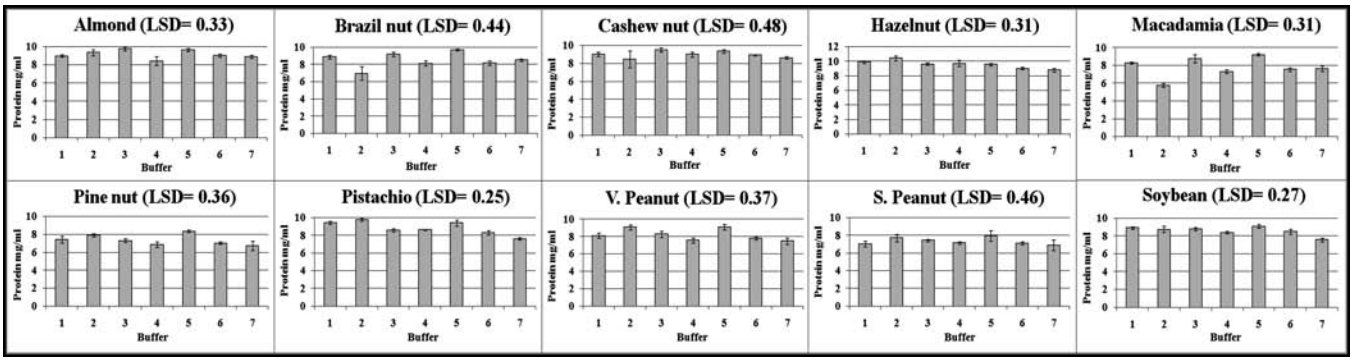

Figure 2. Protein solubility in different buffers as measured by the Lowry et al. method. Data are expressed as mean ± standard deviation ($n = 4$). 1, BSB, pH 8.45; 2, DI water; 3, 0.1 M $NaHCO_3$; 4, 20 mM Tris-HCl buffer, pH 8.5; 5, 0.1 N NaOH; 6, 50 mM phosphate buffer, pH 7.5; 7, 4 M aqueous NaCl.

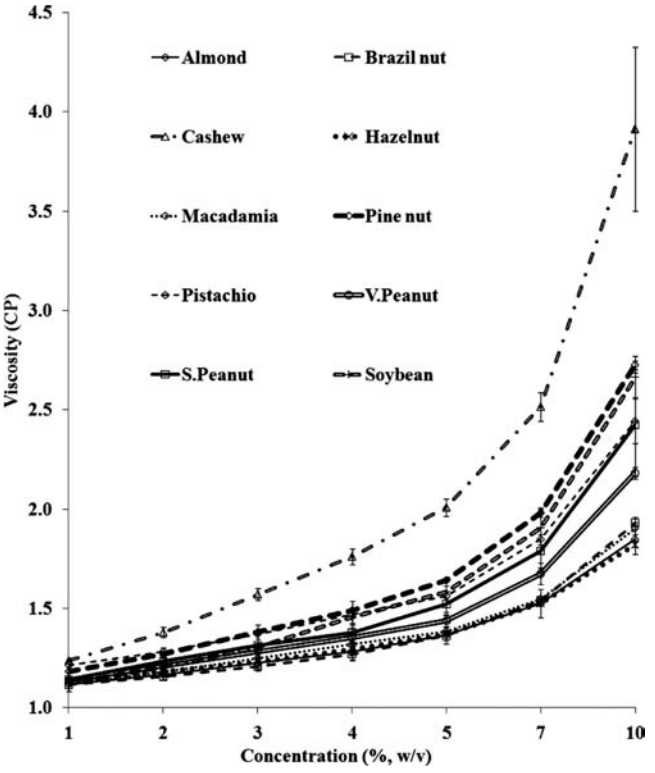

Figure 3. Apparent viscosity of proteins as a function of concentration. Data are expressed as mean ± standard deviation ($n = 6$).

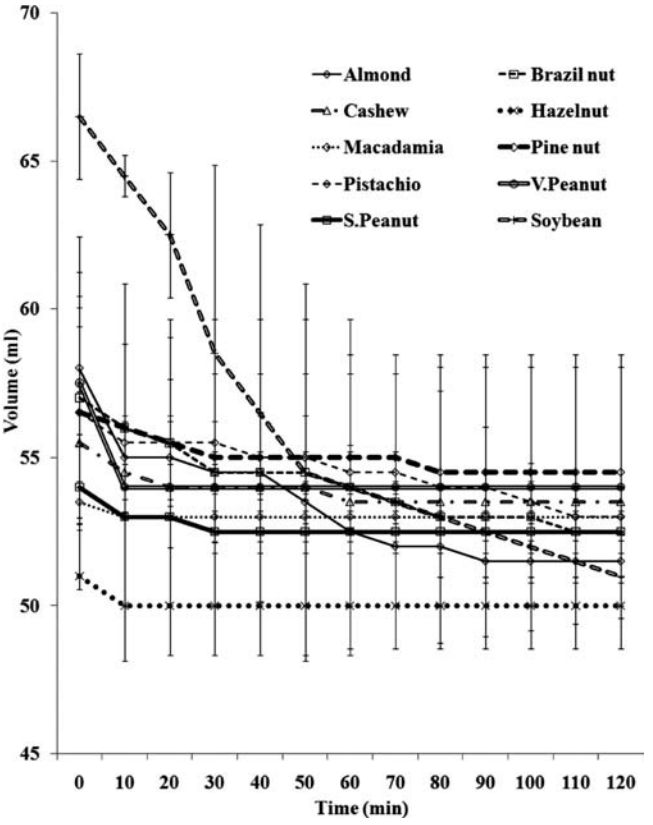

Figure 4. Foaming capacity and stability of seed proteins. Data are expressed as mean ± standard deviation ($n = 2$).

and stability among the tested samples. The differences in foaming capacity of the tested samples cannot be simply attributed to the amount of protein. For example, almond, cashew nut, and soybean protein isolates had comparable protein contents, 92.72, 92.29, and 94.80%, respectively. However, foaming capacity and stability of almond and cashew nut protein isolates were significantly lower than the corresponding values for soybean protein isolate. The results therefore suggest that in addition to protein amount, the type of protein may also be an important factor in determining foaming properties.

Neto et al. (*18*) reported poor foaming properties (low foam volume) of cashew nut protein isolate. In a subsequent study Bora and Neto (*19*) reported an improvement in foaming properties of cashew nut protein isolate subjected to heat denaturation or when exposed to NaCl (up to 0.5 M). These investigators found that at 0.75 M NaCl the foaming capacity of undenatured protein isolate improved from 4 to 5.5 mL/mg of protein as compared to 0−8.9 mL/mg of protein for the heat denatured one. Ogunwolu et al. (*33*) reported improvement in foaming properties of cashew nut protein concentrate and isolate when the pH of protein solutions was in the range of 6−8 as compared to an acidic pH range of 2−4 and attributed this improvement mainly to improved protein solubility in the alkali pH. Jitngarmkusol et al. (*34*) investigated foaming properties of macadamia nut defatted flour and found foaming capacity to greatly vary depending on the cultivar. Foaming capacity of defatted flour for cultivar PDF PY 741 was 22.67% as opposed to 126% for the cultivar TDF PY 741. Such a large, 5.56 times, difference in foaming capacity of the two cultivars may be partly attributed to the significant difference in the protein content of the two cultivars, 30.96% for PDF PY 741 and 36.45% for TDF PY 741, on a dry weight basis, respectively. Similarly, Yusuf (*35*) reported poor foaming capacity for almond flour (38% overrun) and protein concentrate (69% overrun). Foaming capacity of almond protein isolate in the current study (< 15% overrun) was comparable to the one reported earlier (*17*) and lower compared

to the one reported by Yusuf (*35*). The higher foaming capacity observed by Yusuf (*35*) is likely due to some protein denaturation/unfolding as a consequence of exposure to alkali pH during the protein solubilizaton step.

Peanut protein concentrates (78−86% protein) are reported (*36*) to have limited foaming capacity (0.03−0.8 mL/g). The foaming capacity of < 40% observed in the current investigation for peanut proteins (82.45 and 79.98% protein, respectively, for Virginia and Spanish peanuts, on a dry weight basis) was therefore not unexpected and was also comparable to the 50% foaming capacity recently reported for peanut protein isolate (96.65% protein on a dry weight basis) by Wu et al. (*37*). Interestingly, although Wu et al. used alkali solubilization followed by isoelectric precipitation (sample PPI in their study) as opposed to buffer solubilization (borate buffer, pH 8.45) and dialysis in the current investigation, both the foaming capacity and stability of the peanut protein preparations was similar in the two studies. Chovea et al. (*38*) found the foaming capacity of soy protein isolate to be 45−70% (overrun) and that 7S globulin (β-conglycinin) subunits (particularly β- and α-) were important for good functional properties of soy protein isolates. Hojilla-Evangelista et al. (*39*) reported the foaming capacity of soybean and lupin seed proteins to be low to moderate (4−44% increase in volume).

For good foaming properties, the protein should be soluble in the water phase and be able to lower interfacial tension to enable formation of strong elastic films around the dispersed air bubbles (*40*, *41*). From the foregoing discussion, tested seed proteins do not appear to be good foaming agents, perhaps due to their compact globular structures as they are unable to form strong elastic films around air bubbles. Partial protein denaturation (facilitates protein unfolding) and limited protein hydrolysis (generates water-soluble polypeptides from large complex proteins) have been suggested to partially overcome this limitation (*42*−*44*). Protein properties including surface charge, surface hydrophobicity, and molecular flexibility are also important determinants of foaming properties. Earlier we have reported that almond, Brazil nut, hazelnut, pine nut, pistachio, and Virginia peanut proteins possess comparable solubilities in water and several aqueous buffers (*20*), so these seed proteins would appear to be good candidates for further studies. Published reports and the results of current investigation suggest studies focused on protein structure−foam function are required.

LGC. Gelation studies indicated LGC (%, w/v) for almond, Brazil nut, cashew, hazelnut, macadamia, pine nut, pistachio, Spanish peanut, Virginia peanut, and soybean to be 6, 8, 8, 12, 20, 12, 10, 14, 14, and 16, respectively. The LGCs observed in the current investigation are comparable to the reported LGCs for several seed proteins including Great Northern bean protein concentrate (8%) and isolate (12%) (*25*), lupin protein concentrate (8%) (*45*), winged bean protein concentrate (14%) (*46*), pumpkin (8%) (*47*), quinoa (16%) (*48*), chickpea flour (8−12%) (*49*), cowpea protein isolate (6%) (*50*), African locust bean (4−18%) (*51*), jack bean proteins (4−12%) (*52*), sunflower proteins (10%) (*43*), and canola (14.9−15.7%), soybean (9.7−11%), and flaxseed (8.5−9.7%) meals (*53*). Yusuf (*35*) found LGC for almond protein concentrate (89.95% protein, 3.15% carbohydrates) to be 25% compared to LGC of 14% for almond flour (30.13% protein, 47.70% fat, and 9.63% carbohydrates) indicating the importance of nonprotein components in the gel formation.

Gelation is a multistep process that is partly dependent on the protein source and type as well as experimental conditions. In the current investigation, proteins were suspended in water without pH adjustment. On the basis of the results, almond proteins, with cashew nut, and Brazil nut proteins as close seconds, appear to be suitable for developing heat-set gels as they formed gels at lower protein concentrations than the rest and therefore should be further investigated for protein gel based product formulations. We have previously reported that almond protein isolates form gels at 4% (w/v) protein concentration (*16*). The lower protein concentration required in that study is likely due to the pH adjustment (final pH was adjusted to 8.2 with dilute alkali) prior to the protein suspension being subjected to heating. Legumin (or 11S storage protein) is reported to be the dominant storage protein in almond (*54*), cashew nut (*55*), and Brazil nut (*56*) seeds, accounting for approximately 60−65% of total seed proteins. In soybeans, both 11S and 7S are known to have important roles in gel formation as well as gel firmness, and the soy protein gel quality that is important in products such as soybean tofu (see ref *57* and references cited therein). Dependence of soybean protein gel formation on the 11S quantity, 11S subunit polypeptide composition (*58*), and amino acid composition of 11S and 7S is known (*59*).

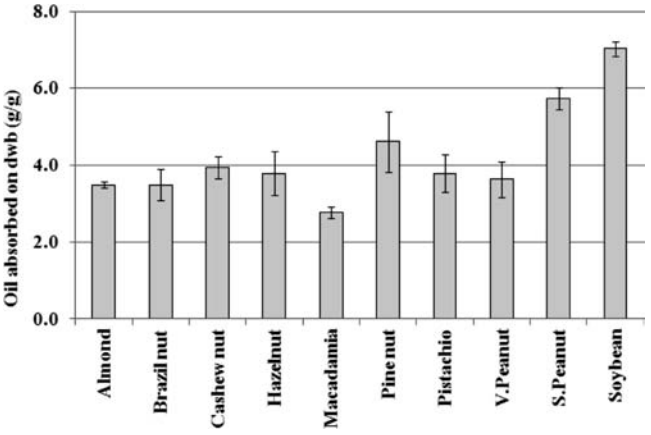

Figure 5. Oil absorption capacity (g of oil/g of protein) of seed proteins. Data are expressed as mean ± standard deviation (n = 4). LSD (p = 0.05) = 0.329.

Ogunwolu et al. (*33*) reported the LGC for cashew nut protein isolate to be 13.5%, which is significantly higher than the 8% in the current study. Alkali pH was used for protein extraction in the current study as well as the one conducted by Ogunwolu et al. (*33*). Following the procedure developed in our laboratory (*60*) Ogunwolu and co-workers used 0.1 N NaOH for protein extraction and therefore likely exposed the seed proteins to higher pH, pH ∼10, compared to the pH 8.45 BSB buffer used in the current study. It is therefore possible that the cashew seed proteins extracted in our investigation may be less denatured. A difference in the degree of denaturation of the extracted proteins is thus one possible reason for the observed differences in LGC in the two studies.

Additional sources of variation in protein gelation properties include protein type (*25*) and the presence/absence of nonprotein components such as fiber (*61*), salts (*52*, *62*), and other agents (*43*).

The results of the current study suggest that gelation properties of tree nut proteins should be further investigated to understand the critical parameters for defining optimum conditions for gel formation. Investigations aimed at defining mechanical properties of the heat-set gels may provide a basis for the development of targeted gels and gel-based products for the purpose of generating diversified uses of the tree nut proteins.

Oil Absorption. Soybean proteins had the highest oil absorption followed by Spanish peanut and pine nut proteins (**Figure 5**). Among the remaining, macadamia proteins exhibited the lowest oil

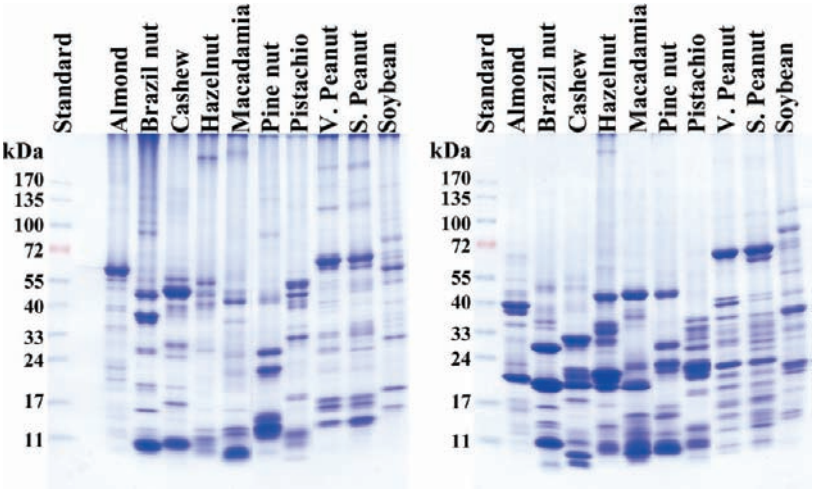

Figure 6. SDS-PAGE profiles in the absence (left) and presence (right) of 2% (v/v) β-ME. Except for the standard marker proteins, the protein load in each lane was 50 μg.

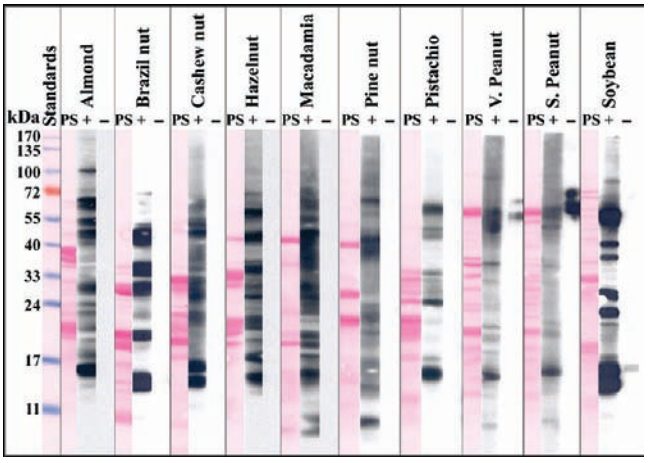

Figure 7. Western blot of proteins probed with corresponding rabbit polyclonal antibodies. PS, Ponceau S stained protein profile for the proteins transferred onto the nitrocellulose membrane and used for Western blotting; +, rabbit antisera; −, rabbit preimmune sera. Except for the standard marker proteins, the protein load in each lane was 30 μg.

absorption. All protein preparations absorbed oil more than twice their own weight, ranging from 2.8 (macadamia) to 7 (soybean) g of oil/g of protein. Earlier (*17*) we reported an oil absorption capacity of 3.6 for almond protein isolate compared to 2.93 g of oil/g of protein for soy protein isolate. The difference in the previously reported oil absorption for soy protein isolate and the current study is mainly due to the method of protein preparation. In the current study the use of reducing agent (2% v/v β-ME) was avoided to prevent disulfide bond reduction during protein solubilization. As a consequence, disruption in soy protein structure as a result of disulfide bond cleavage, and the resulting protein unfolding, may have been minimal in the current study. Oil-holding capacity of proteins is known to be dependent on protein surface properties (e.g., area, hydrophobicity, and electrical charge) (*63*). Many plant proteins typically exhibit oil-holding capacities of ~5 g/g of protein (*31*), and the results of current study are in agreement with this observation. High oil absorption by the protein preparations indicates their suitability as emulsifying agents in food applications where final product moistness and oil holding are desirable traits (e.g., ice creams, cakes, and shortbread cookies). In addition, these proteins may be used as ingredients in developing high protein, value-added, novel food products. Several legume proteins have been reported to be excellent emulsifying agents (*25*, *63*). Understanding oil-holding and emulsification properties of nut seed proteins would be beneficial when nut seed proteins are used in developing innovative and value-added products and therefore should be explored.

Electrophoresis and Western Blotting. SDS-PAGE patterns in the absence (**Figure 6**, left) and presence (**Figure 6**, right) of the reducing agent (2% v/v β-ME) indicate the presence of disulfide bond-linked protein polypeptides in all of the samples. These SDS-PAGE protein polypeptide profiles indicate that the molecular mass range for the tree nut seed proteins is ~3−200 kDa. This molecular mass range of the polypeptides is within the reported range for several seed protein polypeptides (*20*, *64*). Type I food allergies are generally attributed to food proteins (*65*). Therefore, allergenic tree nut proteins are of interest to consumers, food processors, and regulators. Food allergen labeling laws in the United States and European Union have been in use since 2006 to protect sensitive consumers from unintended exposure to offending allergens. A lack of unambiguous labeling, incorrectly following the label information, and the inability to follow the label information (or ignoring the information on the label), among others, still pose challenges to ensuring the safety of sensitive individuals (*13*). Therefore, sensitive, robust, and specific methods to detect trace quantities of offending agents are needed. To this end, we have developed rabbit polyclonal antibody-based immunoassays for sensitive detection of almond (*66*, *67*), cashew (*68*), Brazil nut (*69*), and pecan (*70*) seed proteins. Additionally, we have developed rabbit pAbs against several edible seed proteins and have demonstrated that these pAbs recognize substantially the same polypeptides as those recognized by the corresponding pooled allergenic patients' sera (*20*). As can be seen from **Figure 7**, rabbit pAbs recognized numerous polypeptides in the corresponding protein extracts, indicating the possible utility of rabbit pAbs for the purpose of detecting the presence of the targeted tree nut seed proteins.

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