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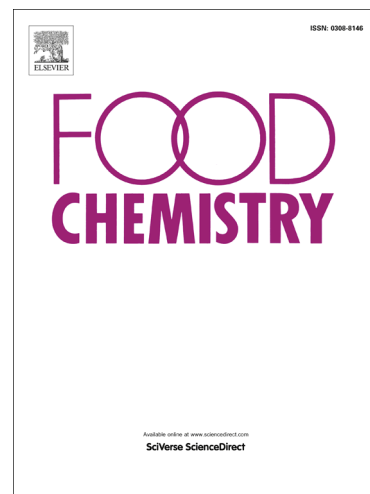
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Functional Properties of Navy Bean (*Phaseolus vulgaris*) Protein Concentrates Obtained by Pneumatic Tribo-Electrostatic Separation

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Abstract

A sustainable, chemical-free dry tribo-electrostatic separation approach was employed to fractionate navy bean flour. The resulting protein-enriched fractions had 36-38% protein on a moisture free basis, accounting for 43% of the total available protein. SDS-PAGE analysis of the dry-enriched protein fractions showed a similar protein profile to that of the original navy bean flour. The functional properties of these fractions were examined and compared with the commercial soybean protein concentrate as well as navy bean protein isolate obtained by a conventional wet fractionation process. These electrostatically separated protein fractions exhibited superior solubility at their intrinsic pH as well as superior emulsion stability (ES), foam expansion (FE) and foam volume stability (FVS) compared to the wet-fractionated navy bean protein isolate that was almost depleted of albumins, exhibiting poor solubility and foaming properties. These results suggest electrostatic separation as a promising route to deliver functional protein concentrates as novel food formulation ingredients.

Keywords Tribo-electrostatic separation; Protein purification; Protein solubility; Protein functional properties; Navy bean; *Phaseolus vulgaris*; Dry fractionation; Emulsifying and foaming properties.

1. Introduction

Plant-based proteins are the main sources of non-animal proteins that serve as part of the human diet as well as animal feed to supply energy and some of the essential amino acids for a nutritionally balanced diet. Besides their nutritional properties, plant proteins are also used to impart useful functional properties to food products including desirable solubility, emulsifying, foaming, and water/fat-binding properties, thus contributing to improved quality, sensory characteristics and shelf-life (Aiking, 2011; Boye, Zare, & Pletch, 2010; Day, 2013; Schutyser & van der Goot, 2011). Since the nutritional and functional benefits of plant proteins are highly dependent on the fractionation approach used, the development of a more sustainable fractionation that preserves native functionality is important.

Current plant protein separation technologies are based on wet fractionation approaches including conventional wet (Lusas & Rhee, 1995; Lusas & Riaz, 1995;; Xu & Diosady, 1994), aqueous and/or enzyme-assisted aqueous (Campbell *et al.*, 2011; Tabtabaei & Diosady, 2012, 2013; Tabtabaei, Hajar, Chen, & Diosady, 2017) extraction processes. These processes use large volumes of water along with solvents for de-oiling, and chemicals (alkali or acids) for protein solubilization and isoelectric precipitation. Relatively pure protein isolates (> 90% protein) can be obtained through wet fractionation routes at the expense of partially sacrificing native protein functionality as a result of protein denaturation caused by pH shifts and use of elevated temperatures during a final energy-intensive dehydration step (Pelgrom, Boom, & Schutyser, 2015a; Pelgrom, Vissers, Boom, & Schutyser, 2013; Schutyser & van der Goot, 2011).

More sustainable dry fractionation approaches do not require water and/or chemicals, thus eliminating the energy-intensive dehydration step as well as the costly treatment of proteinaceous effluent released as a by-product stream during wet fractionation (Mondor, Ippersiel, &

Lamarche, 2012). Dry fractionation technologies, on the other hand, result in the production of less refined protein concentrates (~30–80% protein) but with preserved native bio-functionality (Bergthaller, Dijkink, Langelaan, & Vereijken, 2001; Han & Khan, 1990; Jafari, Rajabzadeh, Tabatabaei, Marsolais, & Legge, 2016; Pelgrom, Boom, *et al.*, 2015a; Schutyser & van der Goot, 2011; Sosulski & McCurdy, 1987).

Many traditional foods have a complex mixture of protein, carbohydrate, oil and micronutrients; therefore, the use of high-purity protein isolates may not be essential for most food processing systems as they mainly require moderately enriched protein fractions with native bio-functionality to achieve desirable physical and structural formation (Boye *et al.*, 2010; Hüttner & Arendt, 2010; Pelgrom, Boom, *et al.*, 2015a; Schutyser, Pelgrom, van der Goot, & Boom, 2015; Schutyser & van der Goot, 2011). Dry-enriched protein concentrates in their native states can be potentially used in liquid food formulations such as protein shakes, for their high solubility, and also in solid and semi-solid formulations such as baked goods, soups, pasta, emulsified meat and texturized protein products, for their water/fat-binding capacity as well as foaming, gelation, and emulsification properties (Çelik, Yılmaz, Işık, & Üstün, 2007; Day, 2013; Pelgrom *et al.*, 2013; Sandoval & Orcutt, 2008; Schutyser *et al.*, 2015). Consequently, the production of dry-enriched protein fractions with enhanced bio-functionality and protein enrichment **is very attractive** for the development of novel food formulations and processing methods.

Dry milling followed by air classification is currently the most used dry fractionation technique through which legume or cereal seeds are first milled into very fine flours to physically disentangle small protein particles from large starch granules before being classified into starch-enriched (heavy coarse fraction) and protein-enriched (light fine fraction) particles in a spiral air classifier according to their particle size and/or density. Dry milling combined with air

classification has been effectively used to fractionate different legumes (Aguilera, Crisafulli, Lusas, Uebersax, & Zabik, 1984; Aguilera, Lusas, Uebersax, & Zabik, 1982; Bergthaller *et al.*, 2001; Han & Khan, 1990; Pelgrom, Berghout, van der Goot, Boom, & Schutyser, 2014; Pelgrom, Boom, *et al.*, 2015a; Pelgrom *et al.*, 2013; Sosulski & McCurdy, 1987; Tyler, Youngs, & Sosulski, 1981). Air-classified pea and faba bean protein concentrates showed high water solubility in their native states (Bergthaller *et al.*, 2001; Han & Khan, 1990; Pelgrom, Boom, *et al.*, 2015a; Pelgrom *et al.*, 2013; Sosulski & McCurdy, 1987). Protein-rich fractions obtained during dry milling and air classification of lupine seeds, faba beans, navy beans, pinto beans and peas exhibited superior emulsification, whip-ability and foam stability compared to wet-fractionated protein concentrates/isolates (Han & Khan, 1990; Pelgrom *et al.*, 2014; Sosulski & McCurdy, 1987). Unfortunately, dry milling and air classification processes require large and expensive equipment and cannot be effectively applied to a variety of agro-materials since the classification mechanism largely depends on particle size and density. Consequently, the protein enrichment level during air classification is sharply declined when disentangled particles vary little in size and/or density.

We have recently developed an approach where a tribo-charge transfer separation mechanism enabled classification of pin-milled navy bean (*Phaseolus vulgaris*) flour into protein- and carbohydrate-rich fractions (Tabtabaei, Jafari, Rajabzadeh, & Legge, 2016a, 2016b; Tabtabaei, Vitelli, Rajabzadeh, & Legge, 2017). Tribo-electrostatic separation has been long applied in the mining (Cangialosi, Notarnicola, Liberti, & Stencel, 2008; Dwari & Hanumantha Rao, 2007, 2009) and plastics (Park, Jeon, Yu, Han, & Park, 2007; Wu, Li, & Xu, 2013) industries, and is currently attracting attention by the food industry (Hemery *et al.*, 2009, 2011; Wang, Smits, Boom, & Schutyser, 2015).

Tribo-electrostatic separation of legume flours is performed by pneumatically conveying the milled particles through tubes or fluidized/vibrating beds, thus imparting a positive or negative charge to the surface of the constituent protein and carbohydrate particles depending on their tribo-charging behavior and the contact medium. Upon contact charging, the oppositely charged particles are separated in a strong electric field (Pelgrom, Wang, Boom, & Schutyser, 2015; Tabatabaei *et al.*, 2016b; Wang, de Wit, Boom, & Schutyser, 2015; Wang, de Wit, Schutyser, & Boom, 2014; Wang, Zhao, de Wit, Boom, & Schutyser, 2016). The dry fractionation of navy bean flour was accomplished using a triboelectric separator consisting of a fluidized bed vessel, a polytetrafluoroethylene (PTFE) tribo-charger tube, and a separation chamber supplied with a copper-plate electrode (Tabatabaei *et al.*, 2016b). Upon physical contact with PTFE, the protein particles acquired a net positive charge due to the presence of ionizable functional groups, whereas the carbohydrate particles acquired no charge and/or a weak negative charge as they are characterized by low ionizability (Mayr & Barringer, 2006; Tabatabaei *et al.*, 2016b). Optimized single-stage and two-stage tribo-electrostatic separation of navy bean flour resulted in protein-rich fractions containing ~38% protein accounting for 50 and 60% of the total protein, respectively. This approach is comparable to the navy bean protein fractions obtained after two-stage dry milling and air classification (Aguilera *et al.*, 1982). The resulting protein-rich fractions retained the native protein structure found in navy bean flour and had larger amounts of sulfur-containing amino acids compared to the navy bean protein isolate produced by a wet fractionation approach (Jafari *et al.*, 2016).

There is still limited information on the functional properties of electrostatically enriched protein fractions compared to the wet-fractionated plant protein products. The present study was carried out to characterize the biochemical, microstructural and functional properties of the protein-rich

fractions obtained using optimized single-stage tribo-electrostatic separation and to compare them to the functional properties of air-classified protein concentrates and other wet-fractionated protein isolates/concentrates as well as a commercially available soy protein concentrate.

2. Materials and methods

2.1. Materials

Pin-milled navy bean flour ($D_{3,2} = 35.1 \pm 1.9 \mu\text{m}$ and $D_{4,3} = 70.2 \pm 7.8 \mu\text{m}$) was supplied by the Canadian International Grains Institute (CIGI, Winnipeg, MB, Canada), and used for the pneumatic tribo-electrostatic separation experiments. Pin-milled navy bean flour was kept at -20°C , and dried at 70°C for 12 h before being used for tribo-electrostatic separation experiments. It contained 25.4 wt% protein (dry-basis), 2.5 wt% oil (dry-basis), 3.9 wt% ash (dry-basis), and 6.6 wt% moisture. The remaining 68.2 wt% (dry-basis) was allocated to the carbohydrates (fibers and polysaccharides). Sodium hydroxide pellets (reagent-grade, 98+%, anhydrous) and hydrochloric acid (ACS reagent, 37%) were purchased from Fisher Scientific Company (Ottawa, ON, Canada). Glycine (for electrophoresis, 99.0+%), glycerol (bioreagent, 99.0+%), Tris base (99.9+%), methanol (ACS reagent, 99.8+%), acetic acid (ACS reagent, 99.7+%), sodium dodecyl sulfate (SDS, anionic, electrophoresis grade), bromophenol blue sodium salt (for electrophoresis) and Coomassie brilliant blue R-250 were purchased from Sigma Aldrich (Oakville, ON, Canada) and used during SDS-PAGE analyses. Sulfuric acid (ACS reagent, 95.0-98.0%), selenium oxychloride (97%), and potassium sulfate (ACS reagent, 99.0+%) were also from Sigma Aldrich, and used during the digestion step of the Kjeldahl analysis method. Ricca Chemical Company (Arlington, TX, USA) provided the Nessler reagent used for the direct Nesslerization of the Kjeldahl digest solutions. Commercially available soy protein concentrate

powder (66.7% protein, 22.2% carbohydrate, and 2.8% oil) was purchased from Purely Bulk (Guelph, ON, Canada). Milli-Q® water (18.2 MΩ·cm at 25 °C) was used in all experiments.

2.2. Tribo-electrostatic separation

Pneumatic tribo-electrostatic separation experiments were carried out using an electrostatic gravity separator (Fig. 1a) designed by Advanced CERT Canada Inc. (Waterloo, ON, Canada) at laboratory-scale details of which have been previously described (Tabtabaei *et al.*, 2016a, 2016b). The separator consisted of a fluidized bed flour vessel (7.0 cm diameter × 12.0 cm height) where the particles were suspended in dry air before being pneumatically transferred into the main PTFE tribo-charger tube (4.76 mm inside diameter × 240.0 cm length) by which particle-wall contact resulted in imparting surface charge onto the flour particles. Earlier studies had shown that the protein particles are charged positively when contacted with PTFE while carbohydrate particles acquire no charge and/or a weak negative charge (Mayr & Barringer, 2006; Tabtabaei *et al.*, 2016b). Charged flour particles were then directed into a rectangular chamber containing a negatively charged copper-plate electrode to attract protein particles with a positive surface charge, thus separating the protein-rich particles from carbohydrate-rich particles that tended to accumulate by gravity in the bins at the bottom of the chamber (Fig. 1a).

Pin-milled navy bean flour (~223 g) was fractionated into protein- and carbohydrate-rich fractions (Fig. 1b) through seven single-stage tribo-electrostatic separation experiments. During each separation, ~30 g of the flour was located in the fluidized bed vessel and transferred pneumatically into the tribo-charger tube at a laminar air flow rate of 7 liters per minute (LPM), before fractionation in the separating chamber under a high-strength electric field of -5 kV. Following each electrostatic separation, six different fractions were obtained from the separation chamber. Three protein-rich fractions designated as “PF1”, “PF2” and “PF3” were collected

from the bottom, middle, and top sections of the electrode plate, respectively (Fig. 1a). Three carbohydrate-rich fractions designated as “CF1”, “CF2” and “CF3” were collected from the bins located at the bottom of the chamber (Fig. 1a). All similar fractions were combined and their weights recorded. The yields (%) were determined using Equation 1 based on the weight of each specific fraction.

$$\text{Yield (\%)} = (\text{mass (g) of each fraction} / \text{mass (g) of the original flour}) \times 100 \quad (1)$$

Protein, oil, ash and total carbohydrate composition was determined for each fraction. The percentages of total protein, oil, ash and carbohydrate were determined based on the composition and weight of each fraction and the starting navy bean flour using Equations 2, 3, 4 and 5, respectively.

$$\text{Percentage of total protein (\%)} = (\text{protein (g) in each fraction} / \text{protein (g) in original flour}) \times 100 \quad (2)$$

$$\text{Percentage of total oil (\%)} = (\text{oil (g) in each fraction} / \text{oil (g) in original flour}) \times 100 \quad (3)$$

$$\text{Percentage of total ash (\%)} = (\text{ash (g) in each fraction} / \text{ash (g) in original flour}) \times 100 \quad (4)$$

$$\text{Percentage of total carbohydrates (\%)} = (\text{carbohydrates (g) in each fraction} / \text{carbohydrates (g) in original flour}) \times 100 \quad (5)$$

All fractions were stored separately at 4 °C before functional property analysis.

2.3. Compositional analyses

Crude protein content of the original navy bean flour and all of the dry-enriched protein and carbohydrate fractions was analyzed in triplicate by micro-Kjeldahl determination of nitrogen,

based on the method of Lang (Lang, 1958) using a nitrogen to protein conversion factor of 6.25. The micro-Kjeldahl method consisted of three main steps of acid digestion, Nesslerization and absorbance determination at 420 nm using a microplate reader (BioTek Instruments, USA). The moisture content was analyzed gravimetrically by drying in a forced-air oven at 110 °C for 12 h. Oil content was measured in triplicate using the AOAC Mojonnier method 922.06 (AOAC International, 1995). Ash content was determined in triplicate based on AOCS Method Ba5a-49 (AOCS, 1998). The total carbohydrate content was estimated by the difference method, and therefore includes insoluble fiber.

2.4. Navy bean protein isolation by a wet fractionation approach

Navy bean protein isolate (NBI) was made based on the method of El-Adawy (2000) by first solubilizing the navy bean flour protein in water (1 g/20 ml) at alkaline pH of 9.0 ± 0.1 with 0.1 N NaOH followed by agitation at 50 °C for 2 h and centrifugation at 14,000 $\times g$ for 15 min. The supernatant from centrifugation was acidified to pH 4.0 ± 0.1 (isoelectric point of navy bean protein) (Han & Khan, 1990) by the addition of 1 N HCl, before being centrifuged at 14,000 $\times g$ for 15 min to precipitate the target proteins. The precipitated solids were washed two times (5 ml of water per 1 g of solids), followed by freeze-drying using a Labconco FreezeZone freeze dryer (Labconco Corp., Kansas City, MO) to produce the NBI. The protein content of NBI was determined by micro-Kjeldahl determination of nitrogen (Lang, 1958) as described in the compositional analyses section. The resulting navy bean protein isolate contained $97.0 \pm 1.3\%$ (dry-basis) protein.

2.5. Particle size distribution

Particle size distribution analysis of the original navy bean flour and the electrostatically separated protein- and carbohydrate-rich fractions were performed using an Aerodynamic

Particle Size Distribution Analyzer, TSI 3603 (TSI Incorporated, Shoreview, MN). Surface-weighted ($D_{3,2}$) and volume-weighted ($D_{4,3}$) mean diameters were measured in triplicate for all of the samples at room temperature.

2.6. Scanning electron microscopy (SEM)

The SEM images of the original pin-milled navy bean flour and all of the resulting protein- and carbohydrate-enriched fractions were obtained by field-emission scanning electron microscopy (Merlin FE-SEM, Carl Zeiss, Germany). All of the samples were initially placed onto carbon adhesive coated aluminum stubs, sputter coated (Denton Desk II sputter coater, Denton Vacuum, LLC, Moorestown, NJ) with pure gold before being imaged by SEM.

2.7. SDS-PAGE electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analyses were conducted to compare the type of proteins present in the navy bean flour and electrostatically separated protein- and carbohydrate-rich fractions with NBI. The samples were initially suspended in water (1 g/20 ml) followed by adjusting the pH of the solution to 9.0 ± 0.1 by the addition of 0.1 N NaOH. The samples were then centrifuged for 15 min at 14,000 $\times g$, and the supernatants analyzed for SDS-PAGE. The electrophoretic analysis was performed by mixing the resulting supernatants with SDS sample buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue) at a 1:1 volume ratio. The solutions were finally heated for 10 min at 95 °C before being loaded to a 4-15% polyacrylamide gel (30 μg protein/well). The protein ladder (PageRuler Plus, Thermo Scientific) was also loaded in the first well of the gel. The electrophoresis was carried out in 10X SDS-PAGE running buffer (250 mM Tris base, 1.92 M glycine, 1% SDS, pH 8.3) at 80 mA for 2 h using a Bio-Rad Mini-Protean 3 Gel Electrophoresis Unit (Bio-Rad Laboratories, Hercules, CA). After electrophoresis, the gels were

treated with a staining solution (40% methanol, 20% acetic acid and 0.1% Coomassie blue R-250) overnight and de-stained for 4 h using a solution of 7% acetic acid and 40% methanol.

2.8. Nitrogen solubility index (NSI)

The nitrogen solubility index (NSI) was determined in triplicate on dry-enriched protein fractions (PF1 and combined PF2-PF3 fractions), NBI and for comparison, on commercially available soybean protein concentrate based on AOCS method Ba 11-65 (AOCS, 1998) with some modifications. Protein dispersions (1 g/40 ml) were prepared with Milli-Q® water, mixed for 2 h at 30 °C and 120 rpm, centrifuged (4,000 xg, 20 min) and analysed for the nitrogen content in the supernatant by micro-Kjeldahl method (Lang, 1958) as depicted in the compositional analyses section. NSI was defined as a percentage of total nitrogen in the samples based on Equation 6. NSI measurements were initially performed at the intrinsic pH of the dispersed fractions: 6.5 ± 0.0 for dry-enriched protein fractions, 4.0 ± 0.3 for NBI and 6.7 ± 0.0 for commercial soy protein concentrate. The effect of pH on NSI was further evaluated for dry-enriched protein fractions (PF1 and combined PF2-PF3 fractions) as well as NBI. The pH was adjusted to the desired values (2.0 to 10.0) by addition of 1 N HCL or 1 N NaOH solutions.

$$\text{NSI \%} = (\text{nitrogen (g) in the supernatant} / \text{nitrogen (g) in the sample}) \times 100$$

(6)

2.9. Functional properties

Selected functional properties of dry-enriched protein fractions (PF1 and combined PF2-PF3 fractions), NBI and commercially available soy protein concentrate were investigated. The water absorption capacity (WAC) was characterized according to Naczki, Diosady, & Rubin (1985) by dispersing 0.5 g of each sample in 4.0 mL of Milli-Q® water in a 15 mL centrifuge tube. The

protein dispersions were vortex-mixed for 30 sec every 5 min during a 30-min interval, followed by 15 min centrifugation at 4000 xg. The supernatants were carefully removed and the tubes drained at a 45° angle for 15 min before being weighed. The WAC was reported as the percentage increase of sample weight.

The oil absorption capacity (OAC) was measured by the method of Sosulski, Humbert, Bui, & Jones (1976) where a half-gram sample was dispersed in 3 mL of canola oil in a 15 mL centrifuge tube. The contents were then vortex-mixed every 10 min for 30 sec during the 30-min time interval prior to centrifuging (4000 xg, 20 min). Free oil was finally removed, and the percentage of absorbed oil was calculated by weight difference.

The emulsifying activity index (EAI) was assayed according to Yasumatsu *et al.* (1972). Approximately a 3.5 g sample was suspended in 50 mL of Milli-Q® water. Canola oil (50 mL) was then added in two equal parts, and the mixture homogenized using a Silverson L2R laboratory homogenizer at ~10,000 rpm (Silverson Machines, Waterside, UK) for 30- and 90-sec periods after the first and second additions, respectively. The resulting emulsion was divided equally among four 50 mL tubes before being centrifuged for 5 min at 4000 xg. The calculation of the EAI was performed using Equation 7.

$$\text{EAI \%} = (\text{volume (ml) of the emulsified layer after centrifugation} / \text{volume (ml) of the whole layer in the centrifuge tube}) \times 100$$

(7)

The emulsion stability (ES) was determined based on the method of Naczka *et al.* (1985) using the emulsions previously prepared for the determination of EAI. All of the prepared emulsions were initially heated to 85 °C for 15 min followed by centrifugation. The ES value was defined as a percentage of the EAI remaining after heating.

The foam expansion (FE) and foam volume stability (FVS) values were determined by a whipping test (Naczka *et al.*, 1985) in which 3% dispersions of protein powder in water were initially homogenized for 6 min at ~10,000 rpm (Silverson Machines, Waterside, UK). The mixture was then decanted immediately into a graduated cylinder. The total volume of the foam (including any drained liquid) formed in the graduated cylinder was recorded and used for the calculation of FE as a percentage of the volume increase. In the next step, the foam was allowed to relax by quiescent periods of 20, 40, 60 and 120 min at room temperature. The FVS was finally calculated using Equation 8 by monitoring the volume of foam remaining over time.

$$\text{FVS \%} = (\text{foam volume (ml) at time } t / \text{initial volume (ml) of foam including liquid}) \times 100 \quad (8)$$

All of the functionality tests were carried out in triplicate at the intrinsic pH of the protein products.

2.10. Statistical analysis

The comparison of means was performed using the Least Significant Difference (LSD) analysis method (SAS University Edition Version, SAS Institute Inc., Cary, NC, USA) at a 5% significance level.

3. Results and discussion

3.1. Analysis of the optimized single-stage tribo-electrostatic separation

The single-stage tribo-electrostatic separation experiment was performed for pin-milled navy bean flour close to the optimal operating conditions of laminar air flow rate (7 LPM), plate voltage of -5 kV, plate angle of 0 degrees and tribo-charging tube length of 240 cm (Tabtabaei *et al.*, 2016a; Tabtabaei, Vitelli, *et al.*, 2017). To investigate the basis of this fractionation

approach, a total of six protein-rich and carbohydrate-rich fractions were collected from the electrode plate as well as the separator bins (Fig. 1a), and assessed for their composition, particle size, protein profile and microstructure as these properties could have influence the functional behavior of the navy bean fractions. Table 1 provides the composition of all fractions in terms of protein, carbohydrate, oil and ash content along with the distribution percentage of each component between the collected fractions.

All of the fractions collected from the bottom, middle, and top sections of the electrode plate (PF1, PF2, and PF3) had significantly higher protein contents and lower carbohydrate contents compared to the fractions collected from the bins at the bottom of the separator (CF1, CF2, and CF3). It should be noted that all of the plate fractions had considerably higher protein contents compared to the original navy bean flour, whereas the bin fractions were all depleted of protein, indicating the effective fractionation of navy bean flour into protein-rich and carbohydrate-rich fractions (Table 1). Combining CF1, CF2 and CF3 resulted in the production of the combined carbohydrate-rich fraction (CF) with 72.2% carbohydrate and 21.7% protein, accounting for 74.4 and 58.3% of the total carbohydrate and protein in the feed, respectively. Similarly, the combined protein-rich fraction (PF) contained 36.5% protein and 54.8% carbohydrate, accounting for 42.9 and 24.7% of total protein and carbohydrate in the feed, respectively. The protein enrichment level of the resulting PF fraction is slightly lower than the level obtained during our previous study using the same electrostatic separation factors (36.5 vs. 40.7% protein content). This might be associated with the increased loading of the original navy bean flour from 20 to 30 g per batch that results in accumulation of more protein-rich particles on the plate surface, thus slightly reducing the protein enrichment by shielding the electrode plate (Cangialosi

et al., 2008; Tabatabaei, Vitelli, *et al.*, 2017; Wang, de Wit, *et al.*, 2015). The increased loading was performed to produce sufficient material for functional analysis.

No statistical differences were observed between the protein contents of PF1, PF2 and PF3 fractions (Table 1) as they all have protein contents of 35.8-37.9%, substantially higher than the protein content of the navy bean flour (26.0%). In addition, as seen in Fig. 2a, the PF1, PF2, and PF3 fractions had statistically similar average particle sizes ($D_{3,2}$) of 20.3, 27.8, and 22.6 μm , considerably lower than the average particle size of navy bean flour ($D_{3,2}$ 35.1 \pm 1.9 μm). This observation is consistent with the air classification results, indicating that the air-classified fine fractions are enriched in protein while the air-classified coarse fractions are depleted in protein (Aguilera *et al.*, 1982; Pelgrom, Boom, & Schutyser, 2015b; Tyler *et al.*, 1981). Amongst the carbohydrate-rich fractions, the CF1 fraction collected from the bin closest to the copper-plate electrode had the highest protein content of 23.8%, not statistically different from the original navy bean flour. However, this was not the case with CF2 and CF3 fractions with around 22.9 and 20.6% protein. The particle size distributions and the average particle sizes of the CF1, CF2, and CF3 fractions are presented in Fig. 2b in comparison with the original flour. As can be seen, the CF1 fraction had the smallest average particle size ($D_{3,2}$ 40.4 \pm 4.1 μm) compared to the CF2 ($D_{3,2}$ 55.0 \pm 3.5 μm) and CF3 ($D_{3,2}$ 54.9 \pm 7.7 μm) fractions, and it was statistically similar to the average particle size of the pin-milled navy bean flour ($D_{3,2}$ 35.1 \pm 1.9 μm). The relationship between average particle size ($D_{3,2}$ and $D_{4,3}$) and protein content of all navy bean fractions (Fig. 2c and d; black lines) exhibited a moderate decrease in protein content as a function of particle size, comparable to the relationships observed by Pelgrom, Boom, *et al.* (2015b) for air-classified legume flours. However, a direct relationship was observed between the carbohydrate content and the average particle size (Fig. 2c and d; red lines). Consequently, the PF1, PF2 and

PF3 fractions with the highest protein-to-carbohydrate ratios had the smallest particle sizes (Fig. 2c), whereas the CF2 and CF3 fractions with the lowest protein-to-carbohydrate ratios possessed the largest particles (Fig. 2c and d). Navy bean flour and CF1 fraction shared similar characteristics in terms of protein-carbohydrate content and particle size. The relatively high protein content of the CF1 fraction might be associated with the presence of relatively large protein particles in that fraction. Larger protein particles have been shown to accumulate a relatively small charge-to-mass ratio due to their fewer particle-particle and particle-wall collisions (Wang *et al.*, 2014; Wang, Smits, *et al.*, 2015). It might be that some of the large and weakly charged protein particles deflected towards the electrode plate but cannot be attracted by the applied electric field due to their prevailing gravitational force, thus settling mostly in the bin close to the plate, resulting in the production of CF1 fraction with a greater protein content and fewer starch granules compared to the CF2 and CF3 fractions. Microstructure analysis of these electrostatically enriched fractions obtained by SEM (Fig. 3a-g) showed that the CF2 and CF3 particles (Fig. 3c and d) were large and rich in starch granules when compared to the protein-enriched PF1, PF2 and PF3 fractions (Fig. 3e-g) containing smaller particles. As can be seen, the CF1 fraction (Fig. 3b) contained starch granules as well as some large protein particles, resulting in an increase in its protein content to the level close to the protein content of original navy bean flour (Table 1).

Besides protein and carbohydrate, other components also distributed between the plate and bin fractions (Table 1). While the oil was originally 2.7% in navy bean flour, it increased to 3.5% in the combined protein-rich fraction (PF), and remained almost constant at 2.6% in the combined carbohydrate-rich fraction (CF). PF1, PF2, and PF3 fractions also contained higher oil contents than the original navy bean flour. Although CF2 had a lower oil content compared to the navy

bean flour, CF1 and CF3 fractions contained 2.6 and 2.7% oil, respectively, statistically similar to the oil content of the original flour. Similar distribution behavior was also observed for the ash content since the combined protein-rich fraction contained a higher ash content (6.9%) than the original flour (5.0%) and the combined carbohydrate-rich fraction (4.8%). No significant difference was observed between the ash content of the starting flour and the carbohydrate-rich fractions (CF1, CF2, CF3 and CF). As a consequence, all plate fractions were not only enriched in protein, but also slightly enriched in oil and ash. This observation is consistent with the air classification literature (Aguilera *et al.*, 1982; Pelgrom, Boom, *et al.*, 2015a; Schutyser *et al.*, 2015; Tyler *et al.*, 1981), reporting higher oil and ash contents in the resulting fine protein-rich fractions. However, the air-classified coarse starch-rich fractions produced in those studies were slightly depleted of oil and ash which was not the case for this study. This might be attributed to the chargeability of the oil and ash-producing components in the PTFE tribo-charge tube along with proteins that would result in the partial deflection of those components onto the plate. It is expected that the presence of oil, ash and carbohydrate in the protein-rich fractions may influence their functional properties.

3.2. Protein profile of navy bean flour and electrostatically separated fractions

SDS-PAGE profiles of the original navy bean flour and all of the electrostatically separated fractions are shown in Fig. 4a in comparison with the profile of navy bean protein isolate produced by a wet fractionation approach. The navy bean protein isolate exhibited a different protein profile when compared to the original navy bean flour. However, the protein profiles of all protein- and carbohydrate-enriched fractions were similar to that of navy bean flour (Fig. 4a), indicating that the triboelectrification-based approach preserves the protein content of the original source. All samples showed the presence of a thick band at ~ 45-52 kDa that could be

attributed to the 7S vicilin proteins that are dominant in beans and devoid of disulfide bonds between subunits (Gujska & Khan, 1991; Rui, Boye, Ribereau, Simpson, & Prasher, 2011; Sathe, 2002). The protein-rich fractions (PF1, PF2, and PF3) and navy bean protein isolate at ~ 45-52 kDa exhibited slightly thicker bands compared to the navy bean flour and carbohydrate-rich fractions (CF1, CF2, and CF3), indicating the presence of more vicilin in those protein fractions. The navy bean protein isolate gel pattern showed relatively weak bands in the 15- to 30-kDa range and almost no bands in the 10- to 15-kDa range. These bands mostly belong to the water-soluble albumin proteins (Gujska & Khan, 1991) which may have been lost during the acid precipitation stage because of their high solubility at the isoelectric point. The absence of albumin-type proteins in the protein isolate may negatively affect the NSI of this product. All of the samples exhibited some high MW bands (~ 100-250 kDa) which were considered to be 11S legumin, similar to the observations reported earlier for navy bean (Gujska & Khan, 1991; Kohnhorst, Smith, Uebersax, & Bennink, 1991) and other bean (*Phaseolus vulgaris*) proteins (Rui *et al.*, 2011). Moreover, navy bean protein isolate exhibited an additional weak band above 250 kDa when compared to the flour and electrostatically separated fractions. This could be caused by the protein aggregation due to heating (50 °C) as applied during the isolate preparation (section 2.4) (Pelgrom *et al.*, 2014).

3.3. Functional properties of electrostatically separated protein concentrates

The NSI was evaluated at different pH values (pH 2 to 10) for the protein-rich fractions (PF1 and combined PF2-PF3) and navy bean protein isolate as shown in Fig. 4b. The PF2 and PF3 fractions had almost similar composition (Table 1), microstructure (Fig. 2a and Fig. 3f and g) and protein profile (Fig. 4a); therefore, they were combined to provide sufficient material for the functionality tests. Navy bean protein isolate at its isoelectric pH (4.0 ± 0.3) had a very low NSI

value of 1.3%. This was expected as navy bean proteins were isoelectrically precipitated at pH 4.0, very close to the intrinsic pH of this product. The NSI values of the isolate increased sharply to 86.2 and 90.8% on the acidic and basic sides of the isoelectric point (pH 4). The NSI curve for the navy bean protein isolate is consistent with the profile reported earlier (Sai-Ut, Ketnawa, Chaiwut, & Rawdkuen, 2009). In the PF1 and combined PF2-PF3 fractions, 10.7-11.9% of the total nitrogen was soluble at pH 4.0, and solubility enhanced aggressively beyond this pH range, but not as aggressively as the curve observed for navy bean isolate. The nitrogen solubility curve of these electrostatically separated fractions was similar to the profiles reported earlier for air-classified protein fractions (Han & Khan, 1990). As can be seen from the solubility curves, the PF1 and combined PF2-PF3 fractions at their intrinsic pH of 6.5 had moderate NSI values of 40.5 and 34.1%, respectively, that are considerably higher compared to those for commercial soybean protein concentrate (9.0% NSI at intrinsic pH of 6.7) and navy bean protein isolate (1.3% NSI at intrinsic pH of 4.0). The NSI of navy bean protein isolate at pH 6.5 was improved to 44.2% that was statistically similar to that of PF1 fraction at its intrinsic pH (6.5) and slightly higher than that of PF2-PF3 fraction. The low nitrogen solubility of the commercial soybean protein concentrate at its intrinsic pH (6.7) and navy bean protein isolate between pH 4-6 might be associated to the alkaline solubilisation, acid precipitation and the effects of the final drying stage. An additional heating stage was probably applied to the soybean protein concentrate to remove the oil prior to alkaline solubilisation and acid precipitation, thereby reducing its NSI to the lowest level of 9.0%. This observation is consistent with the literature (Sosulski & McCurdy, 1987), exhibiting lower NSI values for the pea and faba protein isolates compared to their protein-rich fractions obtained by an air classification approach. It is expected that the proteins in the PF1 and PF2-PF3 fractions were still in their native state as no pH or temperature changes

were applied (Pelgrom *et al.* 2015a). Therefore, they exhibited relatively high solubility in water in their native states.

WAC can be used as an indicator of water-binding properties and represents the extent of protein-water interactions. The WAC values for PF1 and combined PF2-PF3 fractions (Table 2) were 131.8 and 134.5%, respectively. The WAC values obtained for the protein-rich fractions are statistically lower than those measured for navy bean protein isolate (189.3%) as well as commercial soybean protein concentrate (356.6%). The high WAC value for wet-fractionated protein isolates/concentrates (NBI and SPC) could be attributed to greater availability of the polar amino acids on the surface of the proteins. The WAC values found for PF1 and PF2-PF3 may functionally be desirable as high WAC values may attract a disproportionate amount of water, thereby dehydrating other components in the fractions (Xu & Diosady, 1994).

As seen in Table 2, PF1 and PF2-PF3 had OAC values of 82.4 and 91.3%, respectively, slightly lower than that measured for commercial soybean protein concentrate (119.6%). The navy bean protein isolate had an OAC value of 300.3%, which was higher than that of commercial soybean protein concentrate and other protein-rich fractions (PF1 and PF2-PF3). The results in Table 2 suggest that the navy bean protein isolate had high oil absorption capacity. This observation was also comparable to the OAC value reported earlier by Sai-Ut *et al.* (2009) for the navy bean protein isolate. High OAC values have also been reported for red kidney bean and azuki bean protein isolates (Sai-Ut *et al.*, 2009). The high OAC value for navy bean protein isolate could be attributed to the denaturation of the proteins that would expose the nonpolar side chains from the protein interior (Abbey & Ibeh, 1987).

Our results indicate that the WAC and OAC increased proportionally with the protein contents of the PF1 fraction (35.8% protein), combined PF2-PF3 fraction (37.9% protein), and navy bean

protein isolate (97% protein). This observation is also consistent with the WAC and OAC results reported earlier (Sosulski & McCurdy, 1987) for field pea and faba bean flours, air-classified protein fractions, and protein isolates. Samples with a higher protein content not only had more small protein particles, but also contained smaller starch fragments, contributing to both higher OAC and WAC values (Pelgrom *et al.*, 2013).

The emulsifying properties were characterized by the emulsifying activity index (EAI) and emulsion stability (ES) (Table 2). The EAI value for the PF1 and PF2-PF3 fractions were 59.0 and 58.6%, respectively, that were in the range of the commercial soy protein concentrate. The navy bean protein isolate had higher emulsifying activity of ~68.4% that was very close to the values reported earlier for the commercial soy protein isolate (Soybean Supro 500E) of 72.0% (Tabtabaei, Hijar, *et al.*, 2017; Xu & Diosady, 1994). Soybean flours and isolates are known as excellent binders and emulsifiers in different food products (Naczka *et al.*, 1985; Sosulski & McCurdy, 1987; Xu & Diosady, 1994). The present study demonstrated that navy bean protein fractions had similar emulsifying activity as soybean protein concentrate and might also serve as binders in different food products such as meat emulsion formation. As seen in Table 2, the emulsion stability (ES) of all products were high. Therefore, ES values for all the samples were not affected by heating the emulsions.

The electrostatically separated protein fractions showed excellent foaming properties. The PF1 and PF2-PF3 fractions had higher foam expansion (FE) values than those obtained for navy bean protein isolate and commercially available soy protein concentrate. Navy bean protein isolate obtained using wet fractionation had a lower foam expansion value than the dry-enriched fractions and it was even lower than the value measured for the commercial soybean protein concentrate. Very high foam expansion values have also been reported in the literature for air

classified protein fractions from navy bean, pinto bean and chick-pea (Han & Khan, 1990) as well as faba bean and field pea (Sosulski & McCurdy, 1987). The dry-enriched fractions (PF1 and PF2-PF3) had high foam stability values, retaining ~52% of their initial foam volume after 20 min and ~40% after 2 h (Table 2). The navy bean protein isolate, conversely, maintained up to 28.7% of its initial foam volume after 20 min and ~23.3% after 2 h. PF1 and PF2-PF3 fractions were relatively rich in albumins (Fig. 4a: bands in the 10- to 30-kDa range) compared to the navy bean protein isolate. The excellent whip-ability and foam stability for PF1 and PF2-PF3 can be explained by their high solubility as well as the availability of albumin-type proteins which have shown to possess superior foaming properties, similar to those of egg white (Day, 2013; Schutyser *et al.*, 2015). In contrast, the poor foaming properties of the navy bean protein isolate might be attributed to the low solubility of this product at its intrinsic pH (4.0 ± 0.3) as well as denaturation and aggregation of the proteins as exhibited in the SDS-PAGE (Fig. 4a).

4. Conclusion

Tribo-electrostatic separation of the pin-milled navy bean flour resulted in the production of protein- and carbohydrate-enriched fractions. The resulting protein-rich fractions possessed slightly more ash and oil, and had a smaller average particle size compared to the carbohydrate-rich fractions. The protein-rich fractions had functionality and protein content similar to those obtained by using an air classification approach. These electrostatically-enriched protein fractions contained more albumins compared to the navy bean protein isolate obtained using a wet fractionation approach. The resulting protein fractions had relatively high nitrogen solubility index **at their intrinsic pH**, excellent emulsifying and foaming properties. In contrast, navy bean protein isolate was almost depleted of albumins, exhibiting poor solubility and foaming properties, although high absorption of water and oil. **The proposed tribo-electrostatic separation**

approach is a viable alternative to the traditional wet protein separation, producing protein powders with improved food functionalities.. The application of this approach at a larger scale may effectively enable the sustainable production of functional plant protein concentrates.

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Figure Captions

Fig. 1 *a* Lab-scale experimental set-up for tribo-electrostatic separator; *b* Schematic diagram of the single-stage tribo-electrostatic separation approach. In Fig. 1 *a*, solid circles represent protein-rich particles and open circles represent starch-rich particles. “PF1”, “PF2”, and “PF3” are the protein-rich fractions from the bottom, middle and top sections of the plate electrode plate, respectively. “CF1”, “CF2”, and “CF3” are the carbohydrate-rich fractions collected from the bottom of the separation chamber.

Fig. 2 *a* Particle size distributions of the dry-enriched protein fractions (PF1, PF2, and PF3); *b* Particle size distributions of the dry-enriched carbohydrate fractions (CF1, CF2, and CF3); *c* Protein and carbohydrate content as function of volume weighted mean diameter ($D_{4,3}$) for all of the electrostatically separated fractions; *d* Protein and carbohydrate content as function of surface weighted mean diameter ($D_{3,2}$) for all of the electrostatically separated fractions. The particle size distribution curve, $D_{4,3}$ and $D_{3,2}$ of the navy bean flour (NBF) were also represented for comparison (Tabatabaei, Vitelli, *et al.*, 2017). Particle size distribution curves represent the average of three independent measurements. The vertical and horizontal error bars indicate the standard deviations (SD) of the means ($n = 3$).

^{ABC} Volume weighted means ($D_{4,3}$) sharing the same capital letters are not significantly different ($P < 0.05$).

^{abc} Surface weighted means ($D_{3,2}$) sharing the same lower case letters are not significantly different ($P < 0.05$).

Fig. 3 Scanning electron microscope (SEM) images of starting navy bean flour and electrostatically enriched fractions produced at the air flow rate of 7 LPM and plate voltage of -5

kV. (a) pin-milled navy bean flour-NBF; (b) CF1 fraction; (c) CF2 fraction; (d) CF3 fraction; (e) PF1 fraction; (f) PF2 fraction; (g) PF3 fraction. Blue and green arrows indicate starch granules (SG) and proteinaceous matrix (PM), respectively. Orange arrows indicate the presence of very large proteinaceous and/or seed-coat fibrous particles.

Fig. 4 a SDS gel electrophoresis of original navy bean flour (NBF), navy bean protein isolate (NBI) and all of the dry-enriched fractions produced during tribo-electrostatic separation process; **b** Influence of pH on the nitrogen solubility index (NSI) of the NBF, NBI and the protein-rich fractions (PF1 and combined PF2-PF3) produced during electrostatic separation approach. The intrinsic pH of the dry-enriched protein fractions (PF1 and combined PF2-PF3), NBI and commercially available soybean protein concentrate was 6.5 ± 0.0 , 4.0 ± 0.3 and 6.7 ± 0.0 , respectively.

^{ABC} Nitrogen solubility index means (NSI) sharing the same capital letters at each specific pH are not significantly different ($P < 0.05$).

Tables

Table 1: The proximate composition of the starting navy bean flour and various fractions obtained during electrostatic separation and the distribution of major components between them

Fractions ¹	Yield (%) ²	Protein (%) ³ dry-basis	Oil (%) ³ dry-basis	Ash (%) ³ dry-basis	Carbohydrate ⁴ (%) dry-basis	% of total protein ²	% of total oil ²	% of total ash ²	% of total carbohydrate ²
NBF	100	26.0 ± 1.3 ^B	2.7 ± 0.3 ^{BC}	5.0 ± 1.2 ^{BC}	67.5	101.2	103.7	108.5	99.1
PF1	18.0 ± 5.5 ^B	35.8 ± 0.6 ^A	3.1 ± 0.0 ^B	7.2 ± 0.2 ^A	55.6	24.9 ± 7.6 ^B	20.5 ± 6.3 ^B	25.9 ± 7.9 ^B	14.8
PF2	6.5 ± 2.4 ^D	37.3 ± 1.1 ^A	4.2 ± 0.1 ^A	6.9 ± 0.2 ^{AB}	53.2	9.4 ± 3.4 ^D	10.1 ± 3.7 ^{CD}	9.0 ± 3.3 ^{DE}	5.2
PF3	5.9 ± 2.0 ^D	37.9 ± 0.7 ^A	3.8 ± 0.3 ^A	5.9 ± 0.8 ^{ABC}	54.2	8.6 ± 3.0 ^D	8.1 ± 2.8 ^D	6.9 ± 2.4 ^E	4.7
CF1	12.6 ± 1.7 ^C	23.8 ± 0.7 ^{BC}	2.6 ± 0.0 ^{BC}	4.8 ± 0.0 ^C	70.3	11.5 ± 1.6 ^{CD}	12.1 ± 1.6 ^{CD}	11.9 ± 1.6 ^D	13.1
CF2	16.9 ± 1.7 ^B	22.9 ± 0.2 ^{CD}	2.2 ± 0.0 ^C	5.0 ± 0.0 ^{BC}	71.1	14.9 ± 1.5 ^C	13.6 ± 1.4 ^C	16.9 ± 1.7 ^C	17.8
CF3	40.1 ± 8.6 ^A	20.6 ± 0.6 ^D	2.7 ± 0.1 ^{BC}	4.7 ± 0.4 ^C	73.3	31.8 ± 6.8 ^A	39.3 ± 8.4 ^A	37.8 ± 8.1 ^A	43.5
PF⁵	30.5 ± 3.7	36.5 ± 0.3	3.5 ± 0.0	6.9 ± 0.3	54.8	42.9 ± 4.9	38.8 ± 4.5	41.9 ± 5.2	24.7
CF⁵	69.5 ± 5.2	21.7 ± 0.0	2.6 ± 0.0	4.8 ± 0.2	72.2	58.3 ± 4.1	65.0 ± 5.0	66.6 ± 4.9	74.4

¹ Single-stage tribo-electrification separation was performed close to optimized conditions: air flow rate of 7 LPM; plate voltage of - 5 kV; plate angle of 0 degree; tribo-charger tube diameter and length of 3/16" and 240 cm, respectively.

² Yield values were calculated using Equation 1. The percentages of total protein, oil, ash and carbohydrates were calculated using Equations 2, 3, 4 and 5, respectively. The yield results represent the means of seven determinations (n = 7) ± standard deviations.

³ The values of protein, oil and ash represent the means of three determinations (n = 3) ± standard deviations.

⁴ The carbohydrate content (dry-basis) was calculated by difference, and thus includes insoluble fiber.

⁵ PF is the combined protein-rich fraction obtained by uniformly mixing PF1, PF2, and PF3. CF is the combined carbohydrate-rich fraction obtained by the uniformly mixing CF1, CF2, and CF3 (Fig. 1b).

^{ABC} Means sharing the same capital letters in each column are not significantly different (P > 0.05).

Table 2: Functional properties of dry-enriched navy bean protein concentrates in comparison with the conventional wet navy bean protein isolate and commercially available soybean protein concentrate

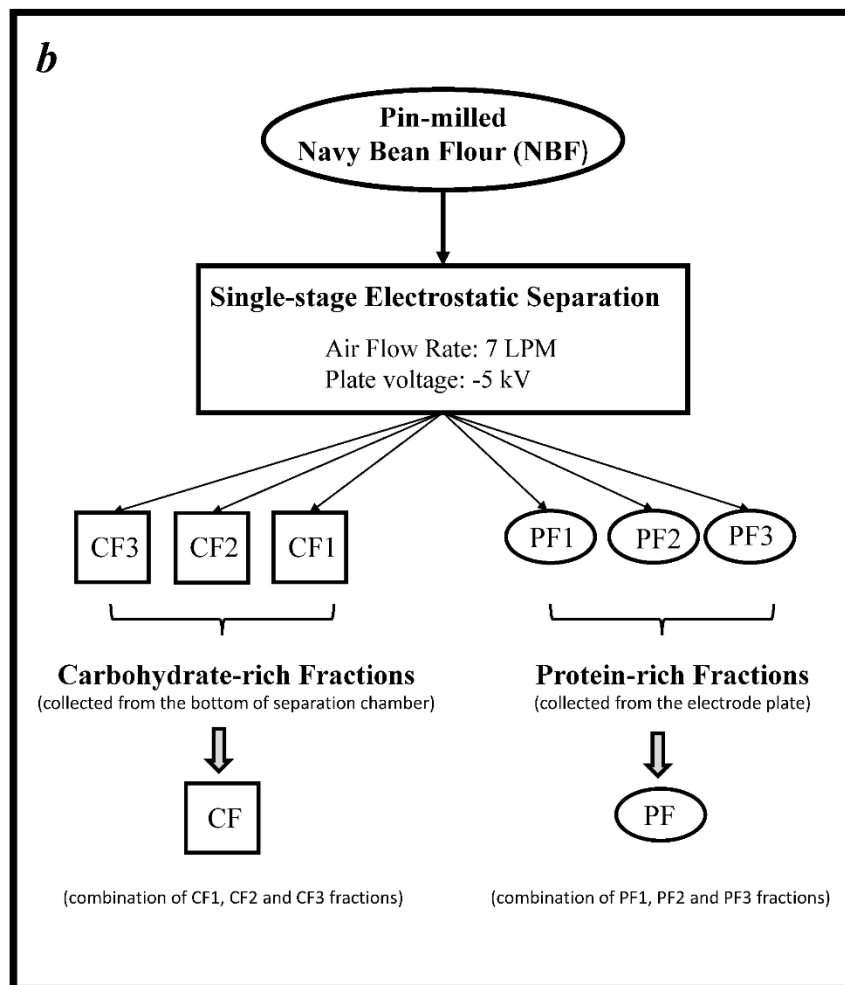
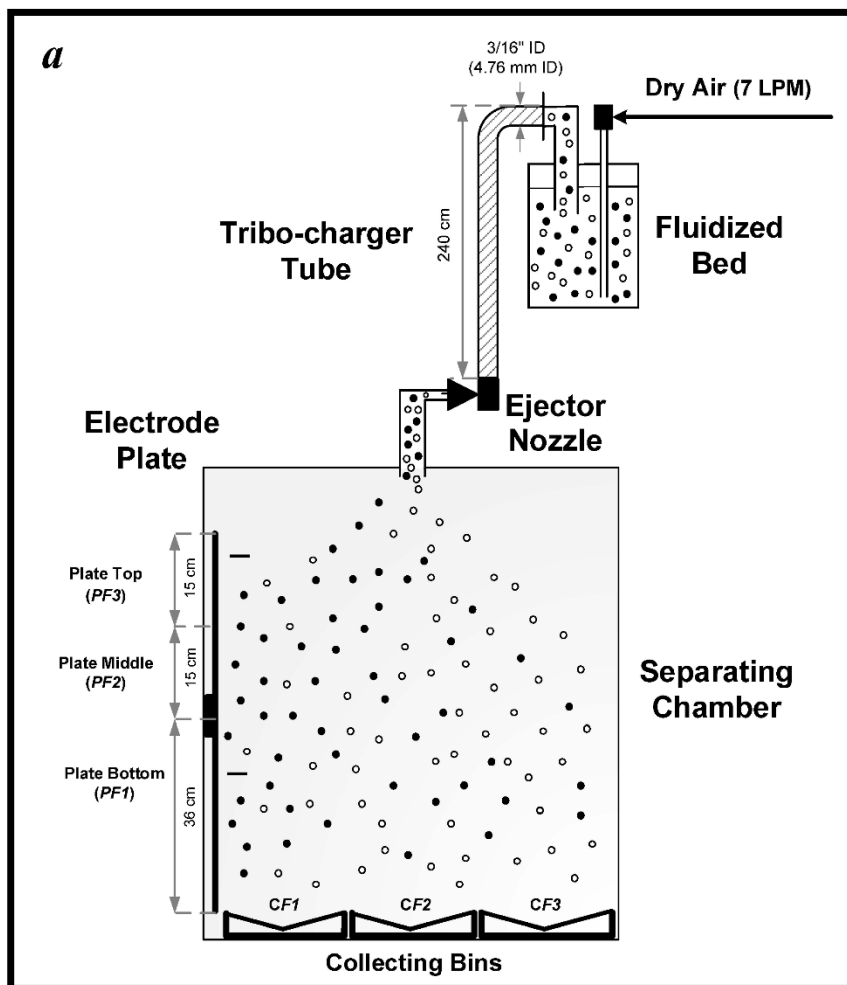
Fractions/Products ¹	WAC ² (%)	OAC ² (%)	EAI ² (%)	ES ² (%)	FE ² (%)	FVS ² (%)			
						20 min	40 min	60 min	120 min
PF1	131.8 ± 2.3 ^C	82.4 ± 1.4 ^D	59.0 ± 1.3 ^B	106.6 ± 3.9 ^A	88.0 ^A	51.1 ^A	47.3 ^A	44.1 ^A	41.5 ^A
PF2-PF3³	134.5 ± 1.5 ^C	91.3 ± 0.8 ^C	58.6 ± 0.4 ^B	112.3 ± 4.9 ^A	89.0 ^A	53.4 ^A	47.1 ^A	44.4 ^A	40.2 ^A
NBI³	189.3 ± 1.9 ^B	300.3 ± 1.5 ^A	68.4 ± 0.6 ^A	96.0 ± 0.4 ^B	50.0 ^C	28.7 ^C	28.0 ^C	28.0 ^C	23.3 ^C
SPC	356.6 ± 1.6 ^A	119.6 ± 2.0 ^B	57.7 ± 2.2 ^B	92.3 ± 3.8 ^B	63.0 ^B	38.0 ^B	36.8 ^B	36.8 ^B	30.7 ^B

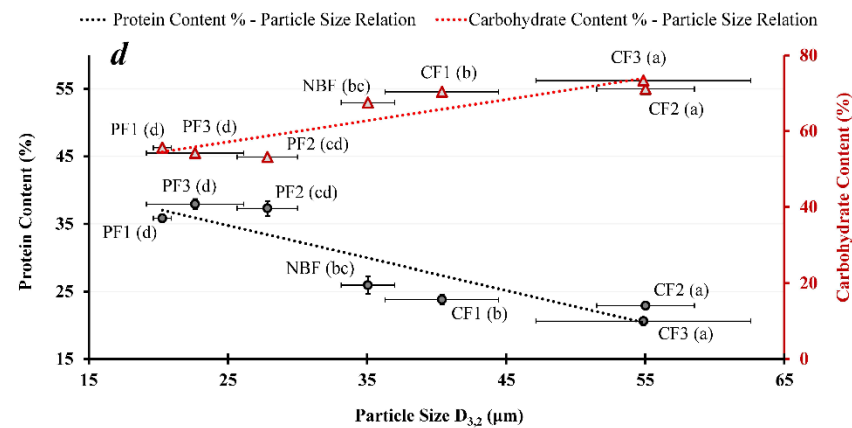
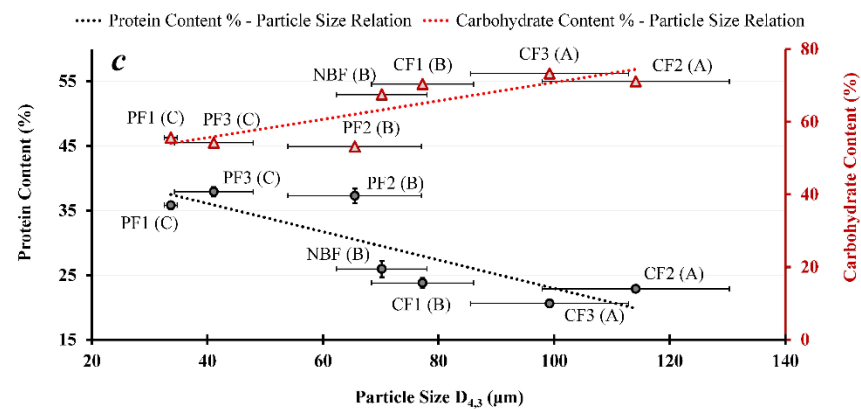
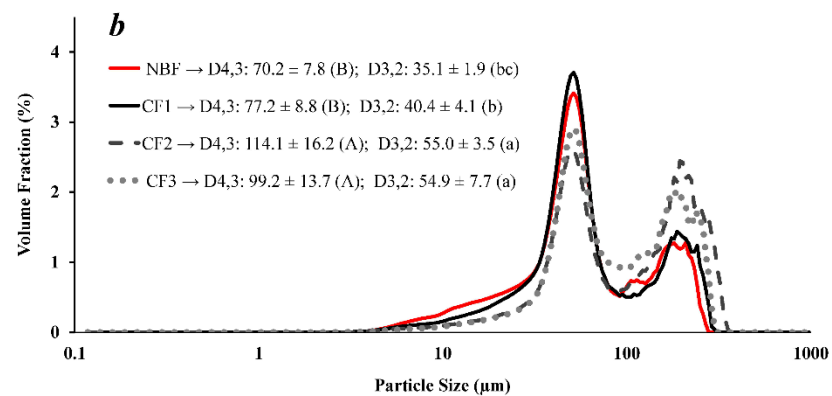
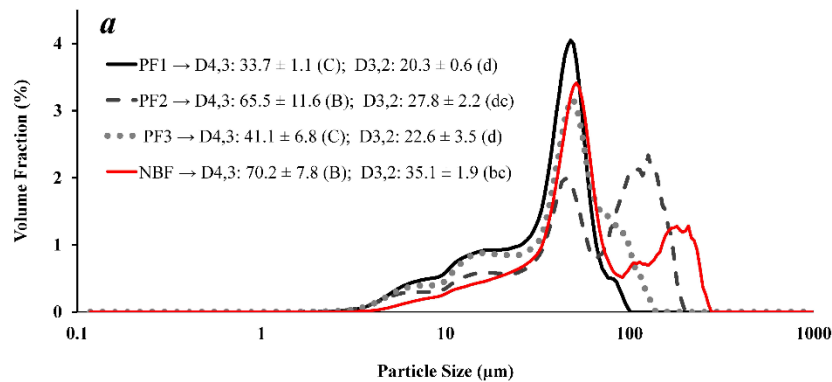
¹ PF1 is the protein-rich fraction collected from the bottom section of the copper-plate electrode (Fig. 1); PF2-PF3 is obtained by uniformly combining the PF2 and PF3 fractions from the middle and top sections of the plate, respectively. NBI is the navy bean protein isolate produced by a conventional wet fractionation process. SPC is the commercially available soybean protein concentrate.

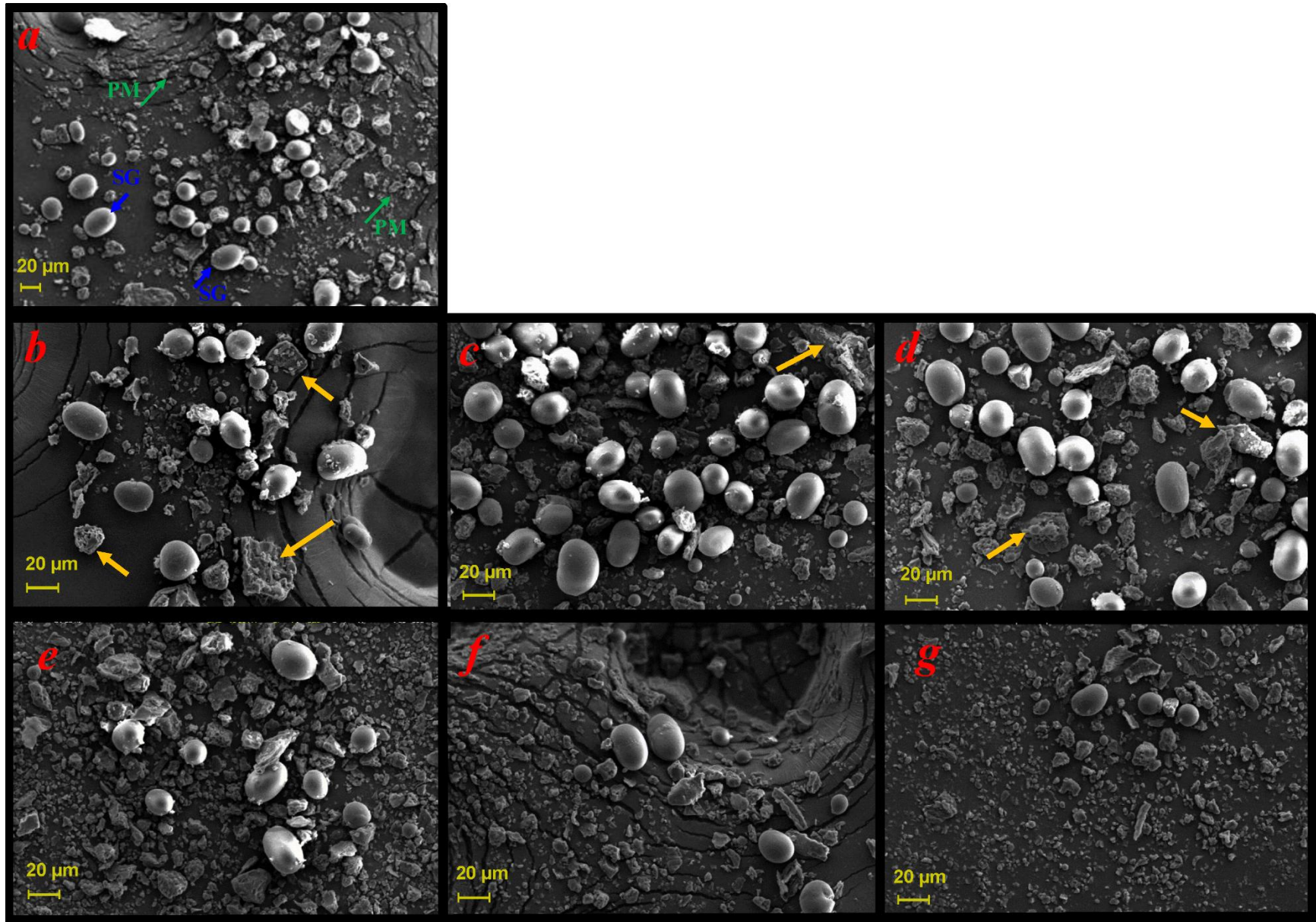
² All functional properties were determined in triplicate. Results are expressed as mean values ± standard deviations. WAC, water absorption capacity; OAC, oil absorption capacity; EAI, emulsifying activity index; ES, emulsion stability; FE, foam expansion; FVS, foam volume stability.

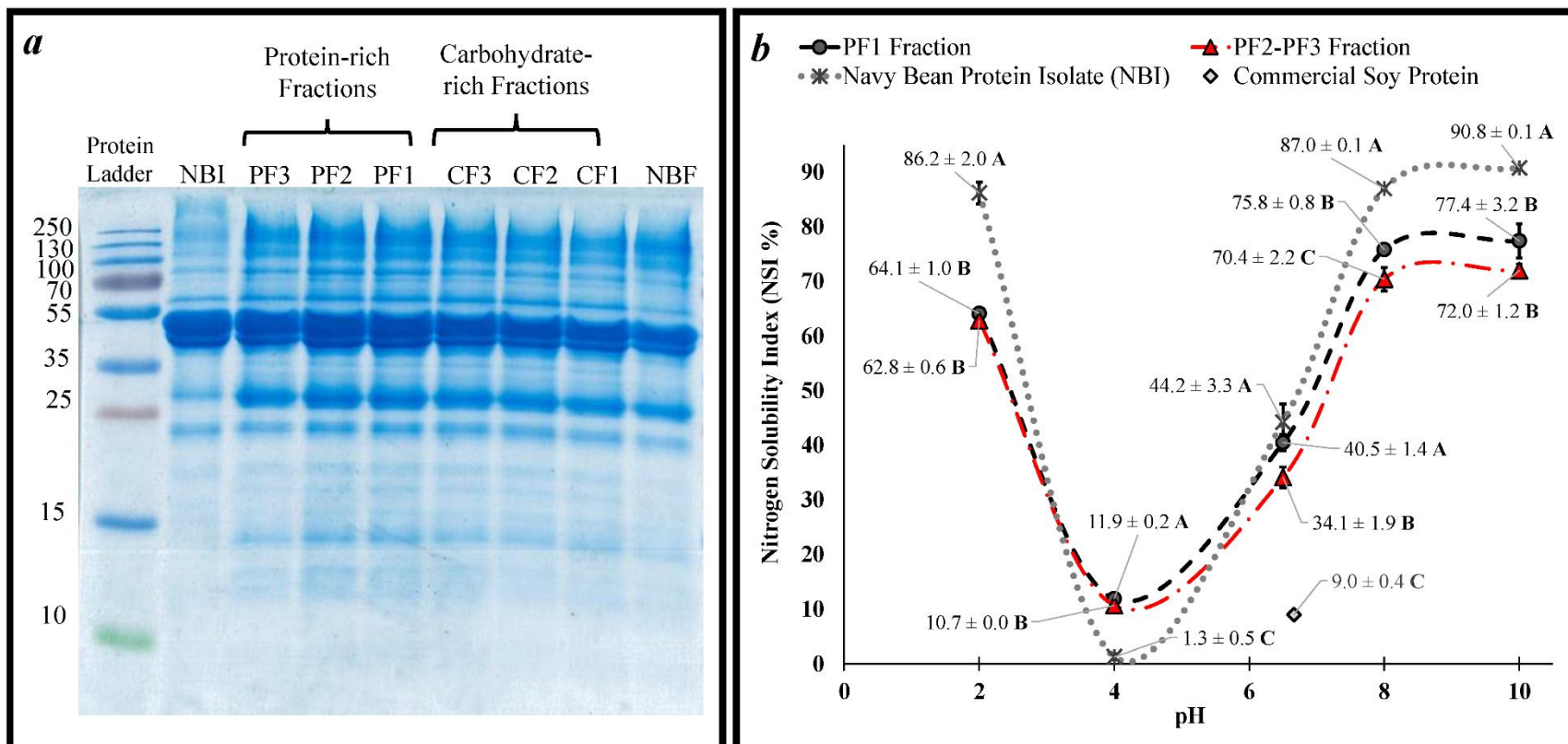
³ The protein content of the combined PF2-PF3 fraction was measured as 37.9 ± 0.8% (dry-basis). The protein content of the NBI was measured as 97.0 ± 1.3% (dry-basis).

^{ABC} Means sharing the same capital letters in each column are not significantly different ($P > 0.05$).









Highlights:

- Dry tribo-electrostatic separation can produce plant protein-rich fractions.
- Biochemical, microstructural and functional properties of fractions were analyzed.
- Protein-rich fractions showed superior solubility compared to wet process isolates.
- Protein-rich fractions also showed superior emulsifying and foaming properties.
- Dry tribo-electrostatic separation preserves native functionality of the proteins.

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