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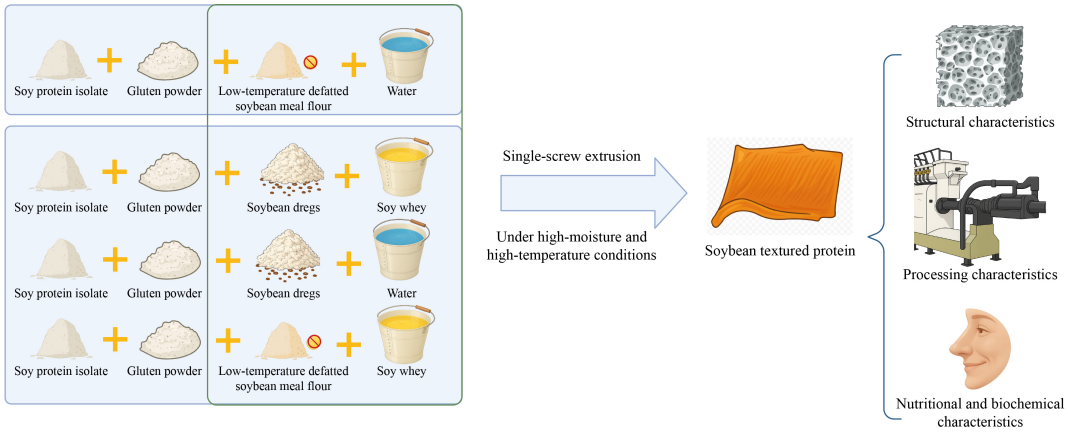
Direct Incorporation of Wet Soybean Dregs and Soy Whey into Textured Protein Products by Single-Screw Extrusion

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GRAPHICAL ABSTRACT



HIGHLIGHTS

- Effective high-value utilization of wet soybean dregs and soy whey.
- Formulation affected the nutritional, structural, and functional properties of textured proteins.
- Single-screw extrusion technology is used to realize the immediate utilization of by-products and achieve low-cost processing.
- The process offers a pathway for soybean by-product valorization.

ABSTRACT Soybean dregs and soy whey are by-products generated in large quantities during soybean processing. Their collection, transportation, and stabilization present challenges because of their large volume, high moisture content, and limited storage stability, and their high-value utilization remains limited. Therefore, this study evaluated fresh wet soybean dregs and soy whey as replacements for low-temperature defatted soybean meal flour and process water in textured protein prepared by single-screw extrusion. We compared four formulations for structure, processing behavior, and nutritional composition. Formulations containing fresh wet soybean dregs had water absorption indices 3.57%–10.00% higher than that of the control (SF + W). Formulations containing soy whey had elongation at break values 8.49%–39.47% higher than that of the control. The formulation combining both by-products (SD + Y) had 43.42% more total dietary fiber and 26.79% less trypsin inhibitor than the control (SF + W). *In vitro* protein digestibility ranged from 77.91% to 80.68%, with SD + Y showing the highest mean value.

KEYWORDS Fresh wet soybean dregs; Soy whey; Textured protein; Single-screw extrusion technology; Protein properties; Sustainable development

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1 Introduction

Soybean processing by-products include soybean dregs and soy whey. Soybean dregs are the insoluble residue generated during raw soymilk production, whereas soy whey is the liquid by-product produced during pressing. For every kilogram of soybeans processed, approximately 1.1 kg of soybean dregs and 9 kg of soy whey are generated [1,2]. The simultaneous generation of these two wet streams creates a practical challenge for soybean processors because their high moisture content increases handling volume and limits storage stability. Although these quantities are substantial, most of these by-products are currently used only as low-value feed or subjected to end-of-pipe treatment [3,4]. Fresh wet soybean dregs are particularly susceptible to microbial deterioration, while soy whey contains dissolved organic matter that contributes to high biochemical and chemical oxygen demand. At a given wastewater flow rate, discharging soy whey increases the organic load entering the treatment system. In aerobic biological treatment, degrading this organic matter increases oxygen demand and associated aeration energy requirements. It also generates additional biological sludge, increasing the requirements for sludge handling and disposal. These demands can increase wastewater treatment costs [5]. Delayed utilization can therefore cause spoilage, odor formation, and additional wastewater-treatment costs [6,7]. This disposal-oriented approach also loses food-grade components that remain in the two by-products.

Reported compositions of soybean dregs include (38.9 ± 0.8) g of protein and (46 ± 2) g of total dietary fiber per 100 g dry matter, with isoflavone concentrations ranging from 20 mg/100 g to 120 mg/100 g dry matter across previous studies [8,9]. Soy whey obtained during tofu production also retains these components: protein and dietary fiber contents of (15.1 ± 0.7) g/100 g and (14.2 ± 2.0) g/100 g dry matter, respectively, have been reported, together with an isoflavone content of approximately 120 mg/100 g lyophilized dry matter in a separate study [10,11]. Their composition makes them potential food ingredients, but conventional stabilization, drying, extraction, or fermentation can extend the processing chain and increase energy or water use. A food-manufacturing route that accepts both materials soon after production would therefore meet two linked needs. It could reduce the deterioration and treatment burden of wet by-products while retaining their nutritional components in a value-added product [12].

Although these by-products contain valuable nutrients, using them effectively in food requires a protein matrix that can be structured and remain processable. Soy protein is widely used in textured protein because of its favorable amino acid profile and Protein Digestibility Corrected Amino Acid Score (PDCAAS), as well as its gelation, emulsifying, and water-holding properties [13]. Wheat gluten can complement soy protein nutritionally and functionally, supporting fibrous structure formation during extrusion [14]. Extrusion is relevant to this application because heat, moisture, and shear can transform mixed plant proteins into a coherent structure within a continuous process [15,16]. It may also reduce selected anti-nutritional factors and modify hydration, digestibility, and volatile formation. However, the direction and magnitude of these changes depend on formulation and processing conditions [17,18]. Fresh wet soybean dregs and soy whey, however, cannot be treated simply as dry flour and process water. The insoluble fiber and residual protein in soybean dregs can alter water distribution, melt continuity, and protein-network development. Meanwhile, soluble solids in soy whey can affect composition and thermal reactions. Direct incorporation may improve fiber retention and by-product utilization but may also create trade-offs in texture, protein accessibility, anti-nutritional factors, and flavor. Previous studies have examined dried soybean residue, isolated residue components, or extruded soybean dregs-containing foods [19–22]. However, limited attention has focused on using fresh wet soybean dregs together with soy whey as direct formulation inputs for textured protein. It therefore remains necessary to determine whether single-screw extrusion can accommodate both streams without separate drying. The effects of replacing low-temperature defatted soybean meal flour and process water on product structure, processing-related functionality, and nutritional properties also require evaluation.

This study aimed to evaluate the feasibility of directly incorporating fresh wet soybean dregs and soy whey into textured protein produced by single-screw extrusion without separate drying of the by-products. It also sought to identify trade-offs among product structure, processing behavior, and nutritional properties to inform the use of these wet streams as food ingredients.

2 Materials and Methods

2.1 Materials

To ensure experimental control, we selected the HH43 soybean variety, which has the largest planting area in China. Each tofu preparation batch used 130 g of soybeans. After cleaning, the soybeans were soaked in deionized water at a dry bean-to-water ratio of 1:5 (g/mL) for 12 h at room temperature. The soaked soybeans were

then ground with water at a dry bean-to-water ratio of 1:9 (g/mL). The raw soymilk was filtered through a 120-mesh sieve to separate it from fresh wet soybean dregs, which were collected for later use. The raw soymilk was heated in a 90 °C water bath for 10 min and then brought to a boil on an electric stove for 2 min. Subsequently, it was stirred at 150 r/min using a high-speed mixer and cooled to approximately 85 °C, after which 2.8 g of $MgCl_2$ was added for coagulation. After thorough mixing, the coagulated curd was quickly transferred to an insulated container and left to stand for 12 min. The curd was then poured into a mold and pressed with a 24 lb (10.89 kg) steel plate for 15 min, and the pressed-out soy whey was collected for later use. Each tofu preparation batch yielded (123 ± 5.00) g of fresh wet soybean dregs and (1000 ± 90.00) mL of soy whey. We repeated tofu preparation to obtain sufficient by-products for the extrusion batches. The other three protein materials were commercial products: soy protein isolate and low-temperature defatted soybean meal flour were purchased from Wandefu Bio-Technology Co., Ltd. (Shandong, China), and wheat gluten was purchased from Kangdi Powder Industry Co., Ltd. (Henan, China).

For each independent extrusion batch, the feed masses prepared and processed were as follows: SF + Y, 666 g soy protein isolate, 334 g wheat gluten, 500 g low-temperature defatted soybean meal flour, and 1500 g soy whey (3000 g total); SD + Y, 356 g soy protein isolate, 177 g wheat gluten, 356 g fresh wet soybean dregs, and 311 g soy whey (1200 g total); SD + W, 356 g soy protein isolate, 177 g wheat gluten, 356 g fresh wet soybean dregs, and 311 g water (1200 g total); and SF + W, 666 g soy protein isolate, 334 g wheat gluten, 500 g low-temperature defatted soybean meal flour, and 1500 g water (3000 g total). Feed was added in successive portions of (300 ± 10) g. Once a smooth, continuous extrudate was obtained, extrusion was continued for a further 3 min before sampling. The four formulations were produced during a single extrusion campaign, with one extrusion batch prepared for each formulation.

All chemicals and reagents used for sample preparation, structural characterization, nutritional analysis, and exploratory volatile screening were of analytical grade unless otherwise specified. Deionized water was used throughout the experiments, including sample preparation, extraction, dilution, and preparation of analytical solutions (hereinafter referred to as water).

2.2 Extrusion conditions and experimental design

Based on the laboratory formulation for textured protein (soy protein isolate:wheat gluten:low-temperature defatted soybean meal flour:water = 4:2:3:9), fresh wet soybean dregs and soy whey were incorporated into the raw materials. Fresh wet soybean dregs typically contain 80%–85% moisture [7]. In this study, we measured the moisture content of fresh wet soybean dregs for each preparation batch according to GB 5009.3–2016 and expressed it on a wet basis [23]. The measured moisture contents of fresh wet soybean dregs and total solids contents of soy whey for each preparation batch are presented in Table S3. Fresh wet soybean dregs replaced part of the soybean meal and process water, whereas soy whey replaced deionized water as the liquid ingredient. The four treatments were defined as SD + Y (soy protein isolate + wheat gluten + fresh wet soybean dregs + soy whey, 8:4:8:7), SD + W (soy protein isolate + wheat gluten + fresh wet soybean dregs + water, 8:4:8:7), SF + Y (soy protein isolate + wheat gluten + low-temperature defatted soybean meal flour + soy whey, 4:2:3:9), and SF + W (soy protein isolate + wheat gluten + low-temperature defatted soybean meal flour + water, 4:2:3:9; control). The two solid formulations (including 8:4:8:7 and 4:2:3:9) were selected through preliminary trials based on their ability to achieve stable, continuous extrudate formation when incorporating the wet by-products. All formulations targeted a feed moisture content of 50% (*w*/*w*, wet basis) and were processed using the same extrusion equipment and operating settings. Fresh wet soybean dregs accounted for approximately 30% of the total feed. We selected this moderate inclusion level because increasing soybean dregs incorporation has been associated with reduced hardness in high-moisture extruded meat analogs [24]. We compared the four formulations to evaluate the direct incorporation of wet soybean-processing by-products and the resulting product properties. The SF and SD formulations differed in the proportions of soy protein isolate and wheat gluten and were not compositionally matched. Accordingly, comparisons between these formulations represent their combined compositional effects, whereas comparisons between soy whey and water were made within each solid formulation.

Textured protein was prepared using a YYSR-42 single-screw extruder from Yuya Soybean Machinery Manufacturing Co., Ltd. (Shandong, China). The die consisted of two cylindrical sections, one larger and one smaller in diameter. The screw speed was maintained at 15–17 r/min; the temperatures of zones Ⅰ–Ⅲ were set at 160, 180, and 220 °C, respectively; and the cooling zone was water-cooled at 15 °C. Before extrusion, all feed materials were equilibrated to an actual feed temperature of 25 °C. We defined a stable extrusion state as the production of a smooth, continuous extrudate at the die exit. After reaching this state, extrusion continued for 3 min before sampling, and material was collected

directly at the die exit. The initial transient extrudate was discarded. We applied this fixed stabilization criterion and sampling position to all formulations to improve comparability of processing conditions and product structure. One portion of the samples was stored at −20 °C. Before analysis, frozen samples were thawed at room temperature for 30 min. For analyses requiring powdered samples, the samples were first frozen at −20 °C for 5 h, then oven-dried at 100 °C for 5 h, ground for 5 min, and passed through an 80-mesh sieve.

The four formulations were compared to assess the effects associated with the different ingredient combinations. Characterization included circular dichroism spectral characteristics, Fourier transform infrared spectra, extrudate appearance, and scanning electron microscopy (SEM) observations of prepared particle surfaces. Processing-related functionality and mechanical properties were assessed using the water absorption index, water solubility index, tensile measurements, and texture profile analysis. Nutritional characterization included trypsin inhibitor, phytic acid, lectin, *in vitro* protein digestibility, basic nutritional composition, isoflavones, and dietary fiber. Free sulfhydryl groups were measured to assess protein-group accessibility across the four formulations. The analytical procedures are described in Sections 2.3–2.5. The extrusion experiments were conducted in a pilot-scale workshop. After extrusion, the samples were temporarily packaged in individually labeled sealed plastic bags. Once all experimental groups had been prepared, the samples were transported to the laboratory for subsequent analyses. TPA and tensile testing of the undried extrudates were initiated approximately 1 h after completion of all extrusion runs. During this interval, the samples were kept in sealed plastic bags and underwent natural cooling. Analyses requiring dried powder samples were performed after the corresponding sample preparation procedures described above.

2.3 Structural characteristics

2.3.1 Circular dichroism spectroscopy

The processed sample (80-mesh) was prepared as a 1 mg/mL suspension in 1 mL of phosphate-buffered saline (PBS). The sample to be tested was placed in an optical cell (quartz) and passed through a BioLogic-500 spectrometer (BioLogic, France). The optical path length was 1 mm, and the spectral resolution was 0.1 nm. CD spectra were recorded over the wavelength range of 185–260 nm at 20 °C. The CD spectra were normalized, and the wavelength range of 190–260 nm is presented for qualitative comparison (Fig. S1). Considering the potential effects of PBS absorption and light scattering from insoluble particles, the CD spectra were used only for qualitative comparisons of spectral characteristics among the samples.

2.3.2 Fourier transform infrared spectroscopy

One milligram of the processed sample (80-mesh) was mixed with KBr powder at a ratio of 1:100, and spectra were collected using a Nicolet iS5 spectrometer (Thermo Fisher, Waltham, MA, USA). At room temperature, spectra were recorded at a resolution of 4 cm^{-1} over the wavenumber range of 4000–400 cm^{-1}, with a wavenumber accuracy of 0.01 cm^{-1}, and were saved for analysis.

2.3.3 Appearance images and scanning electron microscopy images

Appearance images were obtained by placing the sample in a dark imaging box and photographing it with an electronic camera at the same angle. For SEM, 1 mg of the processed sample (80-mesh) was immersed in 2.5% glutaraldehyde solution (pH 7.2–7.4) for 10 h, washed three times with PBS, dehydrated stepwise in ethanol (30%, 50%, 70%, 85%, 95%, and 100%), dried, sputter-coated, and observed using a JSM-IT800 field-emission scanning electron microscope (JEOL, Japan) at 5000× magnification (scale bar: 1 μm). SEM was used to compare the surface morphology of dried and ground particles, rather than the internal structure of intact extrudates.

2.4 Processing characteristics

2.4.1 Water absorption index and water solubility index

Two grams of the processed sample (80-mesh) (M_0) was transferred into a centrifuge tube with a known mass (M_1). Subsequently, 25 mL of deionized water was added, and the mixture was shaken until a homogeneous suspension was formed. The system was then incubated in a 30 °C water bath for 30 min, with shaking every 10 min. After incubation, the suspension was centrifuged at 4000 r/min for 15 min. The resulting supernatant was collected, transferred to a pre-weighed petri dish (M_2), and dried at 110 °C to a constant mass (M_3). Concurrently, the total mass of the centrifuge tube and the remaining sediment was recorded (M_4). The calculation formulas are as follows:

$$\mathrm{WSI}\,(\%) = \frac{M_3 - M_2}{M_0} \times 100. \quad (1)$$

$$\text{WAI}\,(\%) = \frac{M_4 - M_1}{M_0} \times 100. \quad (2)$$

2.4.2 Tensile strength and elongation at break

The extruded products were elongated strips with a C-shaped cross-sectional profile. Rectangular strip specimens measuring 100 mm in length and 6 mm in width were prepared parallel to the extrusion direction. Tensile testing was performed parallel to the extrusion direction, with an initial grip distance of 40 mm and a tensile speed of 5 mm/s. The sample was placed in a texture analyzer, fixed at both ends, and measured. The following values were recorded: maximum tensile force (P), sample cross-sectional area (S), extension at break (ΔL), and original length (L). The calculation formulas are as follows:

$$\text{Tensile strength (MPa)} = P/S. \quad (3)$$

$$\text{Elongationat break}\,(\%) = (\Delta L/L) \times 100, \quad (4)$$

where ΔL is the extension at rupture (mm) and L is the initial grip distance (40 mm).

2.4.3 Texture profile analysis

Undried and unfrozen extruded samples were used for texture profile analysis (TPA), which was initiated approximately 1 h after completion of all extrusion runs using a TA.new plus Texture Analyzer. Samples were cut into discs with a radius of 1 cm using a circular cutter. Texture profile analysis was performed according to Lyu et al. [25], with modifications. The analysis was performed in TPA mode using a P/36R probe, with pre-test, test, and post-test speeds of 2, 1, and 2 mm/s, respectively, and a compression ratio of 50%.

2.5 Nutritional and biochemical characteristics

2.5.1 Anti-nutritional factor content

Phytic acid content was determined using a phytic acid assay kit (ADS-W-QT017; Addison Biotechnology, Jiangsu, China). Trypsin inhibitor and lectin contents were determined using ELISA kits (SMK30075A and SMK0278PLA, respectively; Meikebio, Jiangsu, China).

2.5.2 *In vitro* protein digestibility

In vitro protein digestibility was determined using the three-enzyme pH-drop method described by Tinus et al. [26], with modifications. The specific procedure is as follows: For each formulation, the mass of the processed sample (80-mesh) was calculated from its measured protein content to provide 62.5 mg of protein. The weighed sample was dispersed in 10 mL of deionized water, and the pH was adjusted to 8.0. The solution was hydrated in a 37 °C water bath for 1 h. Subsequently, 1 mL of multi-enzyme solution (pH = 8) was added to the mixture. The multi-enzyme solution was prepared by dissolving 16 mg of trypsin (T6325, biotechnology grade), 31 mg of α-chymotrypsin (C804761a, 800 USP U/mg), and 13 mg of pronase (P861439, activity > 7000 U/g) in 10 mL of deionized water, and it was kept in an ice bath before addition. The pH values at the start of digestion (pH_0) and after 10 min of digestion (pH_{10min}) were recorded. The signed pH change was defined as $\Delta pH_{10min} = pH_{10min} - pH_0$. IVPD was calculated as follows:

$$\textit{In vitro}\ \text{protein digestibility(IVPD)} = 65.66 - 18.1\Delta pH_{10min}. \quad (5)$$

Note: ΔpH_{10min} is negative when the pH decreases during digestion.

2.5.3 Basic nutritional composition and isoflavone content

The isoflavone content was determined according to Lyu et al. [25] using high-performance liquid chromatography (HPLC) for four sample types. The processed sample (80-mesh) was accurately weighed (0.1 g) and extracted with an acetonitrile-water solution by shaking for 2 h. After centrifugation, the supernatant was concentrated, made up to volume with 80% methanol, and filtered. Chromatographic analysis was performed on a C18 column (5 μm, 4.6 mm × 250 mm) with gradient elution using mobile phase A (0.1% acetic acid in water) and mobile phase B (0.1% acetic acid in acetonitrile), at a flow rate of 1.2 mL/min, column temperature of 35 °C, detection wavelength of 260 nm, and injection volume of 10 μL. Quantification was carried out using the external standard method. Dietary fiber content was determined according to AOAC Official Method 2017.16 [27].

Protein, fat, and ash contents were determined according to the Chinese National Food Safety Standards GB 5009.5–2025 [28], GB 5009.6–2025 [29], and GB 5009.4–2016 [30], respectively. Protein, fat, ash, and total dietary fiber contents were expressed as g/100 g dry matter.

2.5.4 Exploratory volatile screening

Exploratory screening of the four formulations was performed using headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME–GC–MS). Samples were oven-dried at 100 °C for 5 h before grinding and sieving. Ground samples (80 mesh,

1 g) were placed in headspace vials with 2 g NaCl, sealed, and equilibrated in an 80 °C water bath for 20 min. A DVB/C-WR/PDMS solid-phase microextraction fiber was exposed to the vial headspace at 80 °C for 20 min, followed by thermal desorption in the GC inlet for 5 min in splitless mode. Separation was performed on a TG-5 column (30 m × 0.25 mm × 0.25 μm), with helium at 1.2 mL/min. The oven temperature was held at 60 °C for 5 min, increased to 100 °C at 3.5 °C/min, and then raised to 200 °C at 4.5 °C/min. Mass spectral library searches were performed using Thermo NIST MS Search 2.3. No *n*-alkane reference mixture was analyzed. Experimental retention indices, documented reverse-match scores, and authentic-standard confirmation were unavailable. The analysis was therefore treated as exploratory screening.

2.5.5 Free sulfhydryl group content

Free sulfhydryl group content in textured protein was determined using the Solarbio Free Sulfhydryl Group Assay Kit (Micro Method) (Cat. No. BC5895, Beijing). The specific procedure was as follows: Approximately 0.1 g of the processed sample (80-mesh) was precisely weighed and mixed with the extraction solution at a mass (g) to volume (mL) ratio of 1:10. The mixture was homogenized in an ice bath and then centrifuged at 4 °C and 3000 r/min for 10 min; the supernatant was discarded. Add 2 mL of Reagent One to the pellet, dissolve it by stirring, and centrifuge again at 4 °C and 3000 r/min for 3 min. The resulting supernatant served as the test sample. We measured absorbance at 412 nm using a microplate reader. The absorbance of the test sample was recorded as ATest, and that of the control tube as AControl. Simultaneously, a standard tube containing a 0.125 μmol/mL standard was measured to obtain AStandard, and a blank tube was measured to obtain ABlank.

$$X = \Delta A_{\text{Test}} / (\Delta A_{\text{Standard}} / C_{\text{Standard}}) \times V_{\text{Reagentone}} / W \times F \quad (6)$$

X: content of free sulfhydryl groups in the sample (μmol/g);

ΔA_{Test}: $A_{\text{Test}} - A_{\text{Control}}$;

$\Delta A_{\text{Standard}}$: $A_{\text{Standard}} - A_{\text{Blank}}$;

C_{Standard}: standard tube concentration, 0.125 μmol/mL;

W: sample mass;

$V_{\text{reagentone}}$: volume of Reagent One added during extraction (mL);

F: dilution factor.

2.6 Statistical analysis

Measurements were performed in triplicate for each formulation. Results are presented as mean ± standard deviation (SD), rounded to two decimal places. The number of extrusion batches and the type of replication are specified for each endpoint in the table notes and supplementary data. Repeated measurements within a batch were averaged before comparisons among independent batches. One-way ANOVA in SPSS 20.0 was applied to endpoints with appropriate independent replication. Endpoints represented by a single extrusion batch were summarized descriptively, with SD reflecting variability among specimens or repeated measurements.

3 Results and Analysis

3.1 Structural characteristics

3.1.1 Circular dichroism spectral characteristics

The CD spectra of the four formulations are shown in Fig. S1. All samples exhibited a pronounced negative band at approximately 205–208 nm, followed by a gradual return toward the baseline at around 225 nm. Although the overall spectral profiles were similar, differences in signal intensity were observed among the formulations, with SD + Y exhibiting a more pronounced negative band than SF + W. Previous studies have demonstrated the applicability of CD spectroscopy for examining protein conformational characteristics, including those of soy proteins [31,32].

However, the presence of insoluble particles and the use of PBS may affect the reliability of far-UV CD measurements, particularly at shorter wavelengths, due to light scattering and buffer absorption [32,33]. Therefore, the CD spectra were interpreted qualitatively rather than used to calculate the proportions of individual protein secondary structure components. The observed spectral differences were considered supplementary information and were not used to establish quantitative changes in protein secondary structure.

3.1.2 Fourier transform infrared spectral characteristics

All formulations showed similar absorption bands at approximately 3430, 2921, 2852, 1652, 1538, 1227, and 1049 cm^{-1} (Fig. 1). These bands correspond to O–H stretching, C–H stretching, amide Ⅰ, amide Ⅱ, and carbohydrate vibrations. Their similar positions suggest that fresh wet soybean dregs and soy whey did not substantially change the main functional groups in the extruded protein system.

Protein aggregation and matrix organization may contribute to the contrasting hydration and mechanical properties despite the broadly similar FTIR profiles [34]. The carbohydrate-related bands may contain overlapping

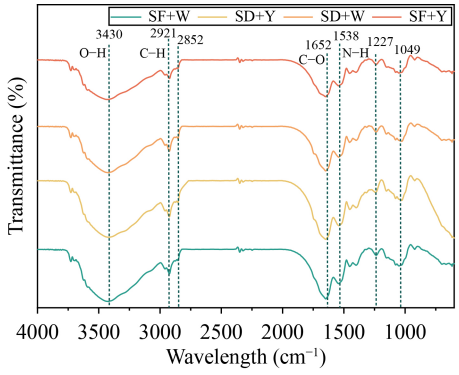


Fig. 1 Fourier transform infrared (FTIR) spectra of four textured protein samples. SF + W: low-temperature defatted soybean meal flour + Water; SD + Y: fresh wet soybean dregs + Soy Whey; SD + W: fresh wet soybean dregs + Water; SF + Y: low-temperature defatted soybean meal flour + Soy Whey.

contributions from soybean dregs, soy whey solids, and the other ingredients.

3.1.3 Appearance and particle surface morphology

All samples had similar yellowish-brown surfaces and C-shaped cross sections after extrusion. SEM nevertheless showed differences in particle surface morphology. SF + W had a relatively compact surface, whereas SD + Y and SD + W showed surface openings and flaky features. SF + Y remained comparatively dense but showed irregular surface openings. Thus, the formulations produced similar external shapes but differed in particle surface morphology (Fig. 2).

The similar surface features of SD + Y and SD + W may reflect their shared solid formulation. Differences in ingredient composition and fracture behavior may also influence the surfaces exposed during drying and grinding. These SEM observations describe the morphology of the prepared particles and should be interpreted in the context of the sample preparation procedure.

3.2 Processing characteristics

3.2.1 Water absorption index and water solubility index (WAI and WSI)

Formulations containing fresh wet soybean dregs showed higher water absorption indices and lower water solubility indices than SF + W. WAI increased from 523.57% in SF + W to 542.26% in SD + Y and 575.95% in SD + W. In contrast, WSI decreased from 13.53% in SF + W to 9.69% in SD + Y and 7.35% in SD + W. SD + W had the highest WAI and the lowest WSI among the four formulations (Table 1).

Hydroxyl-rich polysaccharides and insoluble fiber in soybean dregs may promote water retention, while protein composition and aggregation can affect hydration and soluble-material release. Physical modification and extrusion have likewise been reported to alter the hydration properties of soybean dregs [21,22]. The contrast between SD + Y and SD + W suggests an additional contribution from the liquid-phase ingredients. Together, these responses reflect the combined composition of each formulation.

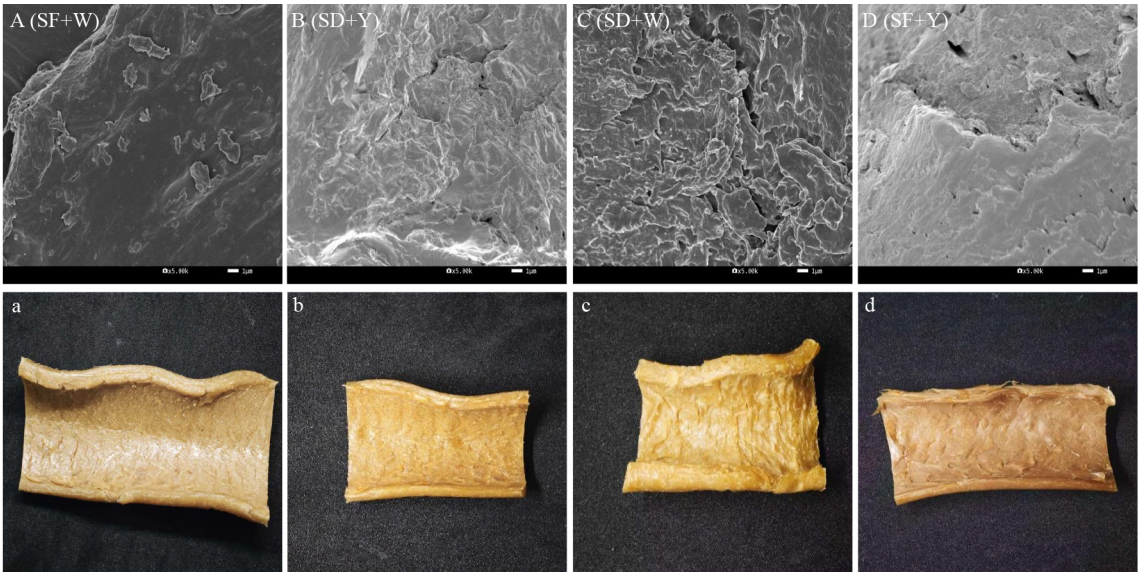


Fig. 2 Appearance of extrudates and surface morphology of dried and ground particles from four textured protein formulations (Aa, Bb, Cc, and Dd).

Table 1 WAI, WSI, tensile strength, and elongation at break of four textured protein samples.

Samples	WAI (%)	WSI (%)	Tensile strength (MPa)	Elongation at break (%)
SF + W	523.57 ± 5.71^{c}	13.53 ± 1.03^{a}	0.36 ± 0.01^{b}	89.40 ± 1.97^{c}
SD + Y	542.26 ± 7.63^{b}	9.69 ± 0.21^{b}	0.33 ± 0.01^{c}	97.00 ± 2.64^{b}
SD + W	575.95 ± 8.09^{a}	7.35 ± 0.03^{c}	0.43 ± 0.01^{a}	92.83 ± 1.50^{bc}
SF + Y	505.91 ± 8.96^{d}	12.30 ± 0.60^{a}	0.35 ± 0.01^{bc}	124.69 ± 2.41^{a}

Note: results are expressed as mean ± SD (n = 3); different letters indicate significant differences in the same column ($P < 0.05$).

3.2.2 Tensile strength and elongation at break

Soy whey was associated mainly with a change in the balance between tensile strength and extension. Tensile strength was 0.33 MPa in SD + Y, 0.43 MPa in SD + W, 0.35 MPa in SF + Y, and 0.36 MPa in SF + W. Elongation at break was highest in SF + Y (125%), followed by SD + Y (97%), SD + W (93%), and SF + W (89%). The effect was most evident when soy whey was combined with low-temperature defatted soybean meal flour (Table 1).

The tensile results show that soy whey changed the balance between resistance to rupture and deformation capacity, rather than uniformly strengthening or weakening the textured protein. Tensile strength represents the maximum stress sustained by the matrix, whereas elongation at break describes deformation before failure. The hydration results suggest that differences in water distribution may contribute to the observed tensile behavior. Changes in matrix plasticization and molecular mobility may also influence extensibility, although the present measurements do not establish a specific secondary-structure mechanism. Soluble carbohydrates, minerals, and other small constituents in soy whey may change water distribution and protein-chain mobility during extrusion, thereby affecting melt flow and network stabilization. Similar trade-offs between strength and extensibility have been reported for structured plant protein blends, although the direction and magnitude of the response depend on protein compatibility, moisture, and processing history [35]. The small difference in tensile strength between SF + Y and SF + W, together with the clearer difference in elongation, suggests that soy whey had a stronger effect on deformability in the low-temperature defatted soybean meal flour system.

3.2.3 Texture profile analysis

The TPA measurements also differed among formulations. SF + W had the highest hardness [8379.40 gf (gram-force)], chewiness (7140.44 gf), and gumminess (7537.94 gf), whereas SD + Y had the lowest values. SD + W and SF + Y were intermediate. Relative to SF + W, the other formulations showed lower compression-related values, whereas springiness and cohesiveness did not differ significantly among formulations (Table 2).

Table 2 Texture data of four textured protein samples.

Sample	Hardness (gf)	Chewiness (gf)	Springiness	Gumminess (gf)	Cohesiveness
SF + W	8379.40 ± 558.19^{a}	7140.44 ± 977.44^{a}	0.95 ± 0.02^{a}	7537.94 ± 857.03^{a}	0.90 ± 0.04^{a}
SD + Y	4065.03 ± 420.05^{d}	3736.95 ± 471.84^{c}	0.98 ± 0.02^{a}	3807.40 ± 411.07^{c}	0.94 ± 0.01^{a}
SD + W	5147.65 ± 90.63^{c}	4523.79 ± 296.98^{bc}	0.97 ± 0.01^{a}	4641.71 ± 272.82^{bc}	0.90 ± 0.04^{a}
SF + Y	6192.29 ± 246.82^{b}	5477.72 ± 562.55^{b}	0.98 ± 0.02^{a}	5610.07 ± 501.40^{b}	0.90 ± 0.05^{a}

Note: results are expressed as mean ± SD (n = 3); different letters indicate significant differences in the same column ($P < 0.05$).

The lower hardness may reflect the combined effects of soy protein isolate and wheat gluten proportions, fiber content, and water retention. Insoluble fiber particles from fresh wet soybean dregs may disrupt compact protein domains, and greater water retention may reduce the force needed to compress the matrix. Soluble constituents in soy whey may further increase matrix plasticization, particularly in the presence of the fiber-rich phase. Soybean-dregs-enriched extrudates have also shown lower instrumental hardness when insoluble particles disrupted matrix continuity or increased water retention [19]. This result is consistent with the tensile data, although the two methods measure different deformation modes. Tensile testing measures failure during extension, whereas TPA measures repeated compression and recovery. The lower tensile resistance and compression-related parameters in SD + Y therefore indicate a more deformable matrix, rather than a change confined to one measurement. Because springiness and cohesiveness did not differ significantly, the softer texture was mainly related to lower resistance to applied force.

3.3 Nutritional and biochemical characteristics

3.3.1 Anti-nutritional factor content

The contents of the three anti-nutritional factors varied across the four textured protein formulations, with different patterns observed for each compound. Lectin content was lowest in SF + Y (178.44 pg/mg) and highest in SD + W (240.75 pg/mg). Phytic acid content ranged from 10.45 μg/mg in SD + Y to 11.91 μg/mg in SF + W. Similarly, trypsin inhibitor content was lowest in SD + Y (0.41 μg/mg) and highest in SD + W (0.64 μg/mg). SD + W had the highest lectin and trypsin inhibitor contents, whereas SF + W had the highest phytic acid content. SF + Y had the lowest lectin content, while SD + Y had the lowest phytic acid and trypsin inhibitor contents (Table 3).

Table 3 Antinutritional factors content and IVPD of four textured protein samples.

Samples	Lectin content (pg/mg)	Phytic acid content (μg/mg)	Trypsin inhibitor content (μg/mg)	IVPD (%)
SF + W	201.60 ± 8.10^{b}	11.91 ± 0.06^{a}	0.56 ± 0.03^{b}	77.91 ± 0.46^{c}
SD + Y	220.68 ± 8.96^{a}	10.45 ± 0.24^{c}	0.41 ± 0.01^{c}	80.68 ± 0.65^{a}
SD + W	240.75 ± 11.63^{a}	11.49 ± 0.25^{ab}	0.64 ± 0.03^{a}	79.60 ± 0.54^{ab}
SF + Y	178.44 ± 18.22^{b}	11.17 ± 0.19^{b}	0.51 ± 0.01^{b}	78.45 ± 0.28^{bc}

Note: results are expressed as mean ± SD ($n = 3$); different letters indicate significant differences in the same column ($P < 0.05$).

The measured contents of lectin, phytic acid, and trypsin inhibitor showed distinct patterns across the four formulations. These differences may reflect both ingredient composition and the recovery of the compounds during sample extraction. SD + Y had a lower measured trypsin inhibitor content than SD + W, suggesting that replacing water with soy whey affected the amount detected after processing. Soluble constituents in soy whey may influence protein aggregation and associations with other matrix components, potentially affecting extraction efficiency and analytical recovery. Previous reviews have described compound-dependent responses of anti-nutritional factors to thermal processing and extrusion conditions [17,36]. In the present study, ELISA provided estimates of trypsin inhibitor and lectin contents in the prepared extracts. The lower trypsin inhibitor content in SD + Y therefore supports a difference in measured content between the two formulations. Future work combining extraction-recovery checks with trypsin inhibition and lectin activity assays would clarify the contributions of analytical recovery and changes in biological activity.

3.3.2 *In vitro* protein digestibility

IVPD ranged from 77.91% to 80.68% across the four formulations. SD + Y showed the highest mean IVPD (80.68% ± 0.65%), followed by SD + W (79.60% ± 0.54%), SF + Y (78.45% ± 0.28%), and SF + W (77.91% ± 0.46%). SD + Y had significantly higher IVPD than both SF formulations, while SD + W had significantly higher IVPD than SF + W ($P < 0.05$) (Table 3).

The SD formulations showed higher mean IVPD alongside their higher dietary fiber contents. Differences in protein ingredient proportions and matrix organization may have contributed to this pattern. The digestibility of plant-based meat analogues has also been linked to protein source, processing history, aggregation state, and food-matrix organization [37]. As the SF and SD formulations differed in ingredient proportions, their IVPD values reflect the combined effects of the complete formulations.

3.3.3 Basic nutritional composition and isoflavone content

The protein, fat, ash, and total dietary fiber contents of the four textured protein formulations are presented in Table S2 and Table 4. Protein contents ranged from 67.94 to 76.61 g/100 g dry matter, with higher values in the SD formulations than in the SF formulations. Fat and ash contents ranged from 1.92 to 3.16 and from 3.58 to 4.70 g/100 g dry matter, respectively.

The two by-products affected different parts of the nutritional composition. SF + Y contained the highest glycoside content (21.96 μg/g), aglycone content (8.34 μg/g), and total quantified isoflavones (30.30 μg/g). SD + Y contained the most total dietary fiber (16.35 g/100 g) and insoluble dietary fiber (9.27 g/100 g). SD + W had the highest aglycone proportion but the lowest total isoflavone content (Table 4 and Table S1).

Fresh wet soybean dregs and soy whey made different nutritional contributions to the textured protein. Fresh wet soybean dregs mainly supplied insoluble dietary fiber, whereas soy whey provided soluble constituents, including isoflavones. Their effects were complementary at the compositional level, although they varied with the accompanying ingredients. The SD formulations combined higher dietary fiber contents with higher estimated IVPD than the SF formulations. Nutritional evaluation should therefore consider both enrichment and

Table 4 Glycoside content, aglycone content, total and insoluble dietary fiber and free sulfhydryl content of four textured protein samples.

Samples	Glycoside content (μg/g)	Aglycone content (μg/g)	Total dietary fiber content (g/100g)	Insoluble dietary fiber content (g/100g)	Free sulfhydryl content (μmol/g)
SF + W	19.76 ± 0.76^{b}	6.76 ± 0.12^{b}	11.40 ± 1.48^{bc}	5.15 ± 0.11^{b}	0.76 ± 0.12^{ab}
SD + Y	13.97 ± 0.35^{c}	5.96 ± 0.07^{c}	16.35 ± 1.14^{a}	9.27 ± 0.10^{a}	0.63 ± 0.06^{bc}
SD + W	11.72 ± 0.32^{d}	5.33 ± 0.09^{d}	13.44 ± 0.74^{b}	4.28 ± 0.09^{c}	0.44 ± 0.07^{c}
SF + Y	21.96 ± 0.30^{a}	8.34 ± 0.15^{a}	9.29 ± 0.77^{c}	4.05 ± 0.10^{d}	0.93 ± 0.09^{a}

Note: results are expressed as mean ± SD ($n = 3$); different letters indicate significant differences in the same column ($P < 0.05$).

digestibility. Differences in total isoflavones and aglycone proportions may reflect dilution by solid ingredients, binding to proteins or fiber, thermal stability, and conversion within the matrix during extrusion. Soybean dregs are reported to be a concentrated source of dietary fiber, whereas soy whey contains soluble isoflavones and oligosaccharides [38]. In this system, SF + Y had higher quantified isoflavone content than SF + W, while the SD formulations had higher dietary fiber contents than the SF formulations. The two by-products therefore affected different nutritional targets, and their combined value depended on the desired balance among fiber, isoflavones, and protein digestibility.

3.3.4 Targets for future volatile analysis

The available library-matching results were insufficient to establish compound identities or compare volatile composition among formulations. Hexanal and 1-octen-3-ol are proposed as targets for future confirmation using authentic standards and experimental retention indices (Table S4). These targets do not represent confirmed findings. Future measurements should examine the fresh product because the present screening used powder oven-dried at 100 °C for 5 h.

3.3.5 Free sulfhydryl groups content

Free sulfhydryl group content ranged from 0.44 μmol/g in SD + W to 0.93 μmol/g in SF + Y. The two formulations therefore represented the lowest and highest measured accessibility of sulfhydryl groups, respectively. This difference indicates that the protein chains responded differently to the four ingredient combinations during extrusion (Table 4).

Differences in free sulfhydryl group content show that the formulations varied in thiol exposure, protein aggregation, or sulfhydryl participation in cross-linking during extrusion. Because the assay measures accessible free groups, a lower value may reflect disulfide-bond formation or the burial of thiol groups within protein aggregates. The free sulfhydryl measurements can be interpreted alongside the tensile results. Greater sulfhydryl accessibility was observed in the formulation with the highest elongation at break, whereas lower sulfhydryl accessibility was associated with greater tensile resistance. These observations suggest possible differences in protein aggregation and thiol-group accessibility among the formulations, although they do not directly establish changes in secondary structure or the extent of disulfide-bond formation. Extrusion texturization has been shown to alter protein aggregation and intermolecular interactions, including disulfide exchange, with consequences for the structure and functionality of plant protein extrudates [34]. The free sulfhydryl results thus support the mechanical differences reported earlier. Although all formulations were designed to have a feed moisture content of 50% (*w*/*w*), differences in ingredient composition may have influenced water distribution and protein organization, contributing to the observed differences in tensile behavior. SD + W was associated with a more resistant protein network, whereas SF + Y showed a more deformable structure.

4 Conclusions

This work demonstrated the technical feasibility of directly incorporating fresh wet soybean dregs and soy whey into a single-screw extrusion process for textured protein production. All four formulations produced structured extrudates, while the incorporation of the two soybean processing by-products generated distinct and measurable product responses. Compared with the formulations containing soybean meal flour, the soybean-dregs-containing formulations exhibited higher water absorption indices, lower water solubility indices, and lower hardness. SD + Y achieved the highest total dietary fiber and insoluble dietary fiber contents, reaching 16.35 and 9.27 g/100 g, respectively. Within the dregs-containing system, replacing process water with soy whey reduced tensile strength from 0.43 to 0.33 MPa, increased elongation at break from 93% to 97%, and lowered trypsin inhibitor content by 35.9%.

SD + Y combined the highest total dietary fiber content and mean IVPD with lower hardness, whereas SD + W exhibited greater tensile resistance. The mean IVPD of SD + Y was significantly higher than those of SF + W

and SF + Y. The choice between these formulations depends on the intended balance among fiber content, protein digestibility, and mechanical properties. Because the solid formulations differed in ingredient proportions, these results describe the performance of the complete formulations. Compositionally controlled comparisons are needed to determine the independent contribution of soybean dregs.

The feasibility demonstrated here concerns direct incorporation under the tested extrusion conditions. Future studies should compare freshly generated and stored by-products and evaluate microbiological quality, shelf life, sensory acceptance, energy demand, and economic feasibility. Measurements of water distribution, protein aggregation, and disulfide bonding would help explain the formulation-dependent responses. Volatile analysis of fresh products should include compound confirmation and quantitative measurements. Further validation should assess prolonged continuous operation, performance at higher throughput, and consistency across independent soybean batches.

Supporting Information Supporting Information is available in the online version of this article at https://doi.org/10.2738/ENGF.2026.0026 and is accessible for authorized users.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Competing interests The authors declare no conflicts of interest.

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