

ORIGINAL RESEARCH

Valorising Defatted Soy Flour into High-Moisture Textured Vegetable Protein by Single-Screw Cooking Extrusion

Domas Galih Patria^{1,2}, Cindytia Selvina Bernas¹, Annur Ahadi Abdillah^{3,4}, Dodyk Pranowo², Jenshinn Lin^{1*}

¹Department of Food Science, College of Agriculture, National Pingtung University of Science and Technology, Pingtung, Taiwan

²Department of Agro-Industrial Technology, Faculty of Agro-Industrial and Biosystem Technology, Universitas Brawijaya, Malang, Indonesia

³Study Program of Fish Processing, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia

⁴Research Group of Post-harvest, Processing Technology and Bioproducts, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia

ARTICLE INFO

Article history:

Received: January 5, 2026

Accepted: March 10, 2026

Published: March 30, 2026

Keywords:

circular economy; defatted soy flour; extrusion; meat analogue; soy isoflavones

*Corresponding author:

Jenshinn Lin

E-mail address:

jlin@mail.npust.edu.tw

All authors have reviewed and approved the final version of the manuscript.

ORCID: <https://orcid.org/0000-0002-6292-3527>

ABSTRACT

Background: Conventional meat production is approaching ecological limits, motivating sustainable, circular-economy protein alternatives. Defatted soy flour (DSF), a low-value co-product of soybean oil extraction, is protein-dense, fibre-rich and abundant in Indonesia, yet its use in high-moisture textured vegetable protein (HM-TVP) is unexplored.

Objective: To valorise DSF into HM-TVP by single-screw cooking extrusion and determine how substitution ratio and screw speed govern its functional, proximate, textural, colour, microstructural, isoflavone and sensory quality.

Methods: Three DSF:pea-protein-isolate (PPI) ratios (0:70, 15:55, 30:40) and three screw speeds (30, 40, 50 rpm) were examined in a 3×3 factorial design (n=3) at the Food Pilot Plant, National Pingtung University of Science and Technology (NPUST), Pingtung, Taiwan. Water absorption/solubility indices, proximate composition, texture-profile analysis, HunterLab colour, SEM microstructure, RP-C18 HPLC isoflavones and hedonic scores were determined, and data were analysed by two-way ANOVA with Tukey HSD, effect sizes (η^2), Pearson correlation and principal component analysis.

Results: DSF substitution dominated most responses (partial $\eta^2=0.57-0.99$, $p<0.001$). Raising DSF from 0 to 30% increased water absorption index (1.92→3.43 g/g), crude fibre (2.79→6.40%), hardness (25.7→56.9 N) and chewiness, and shifted the isoflavone profile toward higher glycoside (genistin up to 1741 $\mu\text{g/g}$) and bioavailable aglycone concentrations, while reducing crude protein (77.1→63.3%), crude fat (1.86→0.93%), lightness (L^* 78.1→72.4) and springiness. Higher screw speed raised water-soluble index and soluble-fibre release but lowered hardness and browning. Crude protein of all extrudates (63–77%) far exceeded the $\geq 50\%$ benchmark for texturised soy products, and the 15% DSF blend at 40 rpm (D15S40) achieved the highest overall acceptability (4.10/5).

Conclusion: DSF can partially replace pea/soy protein isolates in sustainable, phytonutrient-enriched meat analogues at lower cost.

Introduction

Meat remains a primary source of high-quality protein, but conventional livestock production is approaching its ecological ceiling: it is a leading driver of greenhouse-gas emissions, land-use change and freshwater consumption, and rising affluence continues to inflate global meat demand.^{1,2} These pressures, together with animal-welfare and human-health concerns, have accelerated the shift toward plant-based meat analogues as a more sustainable route to dietary protein. Textured vegetable protein (TVP) is the structural foundation of such analogues, yet its cost, environmental footprint and nutritional balance still constrain wider adoption.³

TVP is produced by extruding vegetable proteins with water, converting flours and isolates into fibrous, meat-like matrices.³ Two classes are recognised: low-moisture TVP (<35% moisture, sponge-like) and high-moisture TVP (HM-TVP, >40% moisture, muscle-like).⁴ Commercial HM-TVP is dominated by soy protein isolate, wheat gluten and pea protein isolate (PPI), which nonetheless carry limitations — beany off-flavours linked to lipoxygenase, high production cost, and low fibre coupled with high caloric density.^{5,6} Incorporating polysaccharide- and fibre-bearing ingredients can act as dietary fibre within an otherwise protein-only matrix and diversify functionality.^{7,8}

Defatted soy flour (DSF), the residue remaining after soybean oil extraction, is an inexpensive, protein-dense (~60%) and low-fat material rich in dietary fibre and isoflavones.^{9,10} Redirecting DSF from animal feed or waste streams into human food embodies a circular-economy strategy that shortens the soybean value chain and improves protein-use efficiency. In Indonesia, where soybeans are processed at very large scale for tempeh, tofu and cooking oil, DSF is abundant and locally available, making its valorisation directly relevant to national food-security and sustainable-agriculture objectives. Functionally, DSF absorbs water and emulsifies fat, improving moisture retention and nutritional quality in baked and restructured foods.¹¹

The functional, textural and nutritional quality of extruded protein is governed by processing variables — principally screw speed, barrel temperature and feed moisture — and by feed composition. Higher screw speed shortens barrel residence time, mitigates browning, softens texture and enhances soluble-fibre release, whereas lower speed prolongs thermomechanical cooking and densifies the matrix.¹²⁻¹⁴ Standardised analytical methods — water absorption and water-soluble indices (WAI, WSI), AOAC proximate analysis, texture-profile analysis, HunterLab colorimetry, scanning electron microscopy and reversed-phase HPLC for isoflavones — allow rigorous quality benchmarking.¹⁵⁻¹⁸

For product safety and labelling, texturised and defatted soy-protein products are commonly benchmarked against national and international quality standards; Indonesian SNI 01-3020 and Codex CXS 175-1989 for soy-protein products indicate crude protein of at least ~50% for texturised grades, with low fat and, increasingly, elevated dietary fibre being desirable nutritional attributes (FAO/WHO guidance). Positioning DSF-based HM-TVP against these benchmarks is therefore essential for both regulatory alignment and consumer relevance.

To the best of our knowledge, no study has systematically produced HM-TVP from a DSF+PPI blend using an accessible single-screw cooking extruder — the configuration most relevant to small- and medium-scale processors — nor quantified how DSF substitution and screw speed jointly determine functional, proximate, textural, colour, microstructural, isoflavone and sensory quality with modern effect-size statistics. Figure 1 shows the single-screw cooking-extrusion process and the experimental design. This study aimed to valorise DSF into high-moisture TVP at the Food Pilot Plant of NPUST (Pingtung, Taiwan), evaluating three DSF:PPI ratios and three screw speeds, so as to identify sustainable formulations that meet quality standards while enhancing fibre and phytonutrient content.

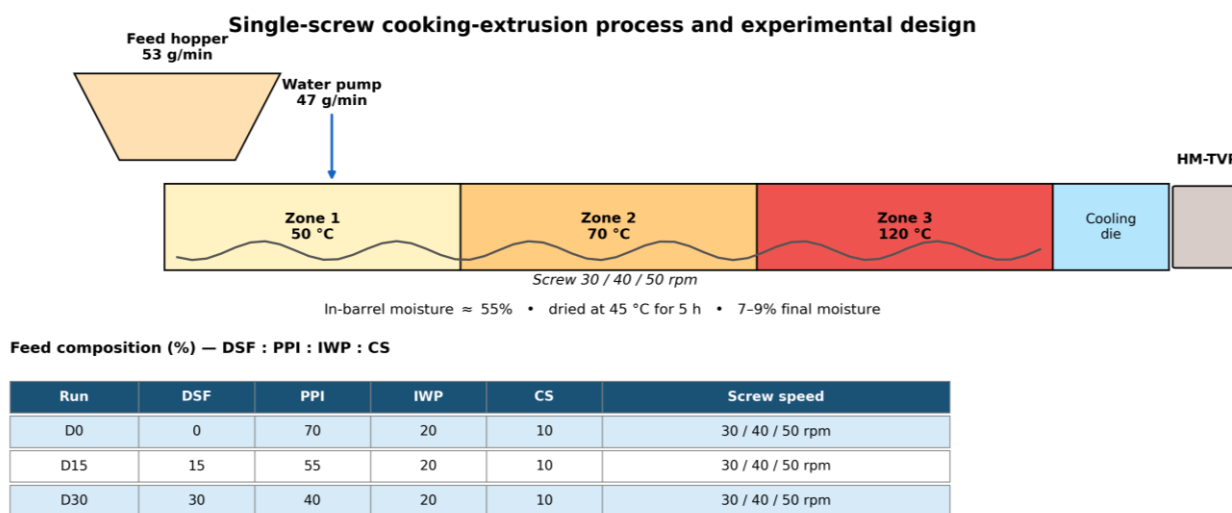


Figure 1. Single-screw cooking-extrusion process and experimental design, showing the feed hopper and water-pump rates, the three barrel temperature zones (50/70/120 °C), screw-speed levels (30/40/50 rpm), cooling die, and the DSF:PPI:IWP:CS feed-composition matrix for the nine runs.

Materials and Methods

Study design and location

A 3×3 full-factorial experiment crossed three DSF:PPI substitution ratios (0:70, 15:55, 30:40; coded D0, D15, D30) with three screw speeds (30, 40, 50 rpm), giving nine treatments each produced and analysed in triplicate (n=3). All extrusion, physicochemical, microstructural, chromatographic and sensory analyses were performed

at the Food Pilot Plant, Department of Food Science, National Pingtung University of Science and Technology (NPUST), Pingtung 91201, Taiwan (≈22.65°N, 120.60°E), in collaboration with Universitas Airlangga (Surabaya) and Universitas Brawijaya (Malang), Indonesia.

Materials

DSF (5% moisture, 60% protein, 31% carbohydrate, 4% ash) was supplied by Archer Daniels Midland (ADM) Co., USA. PPI (6% moisture, 82% protein, 2% fat, 6%

carbohydrate, 4% ash) was from Roquette Canada Ltd. Isolated wheat protein (IWP) was from the Manildra Group (USA) and corn starch (CS) from Ingredion SA (Pty) Ltd (South Africa). Analytical reagents — boric acid, hydrochloric, sulfuric acids, copper(II) sulfate pentahydrate, potassium sulfate, methyl red, bromocresol green, ethyl ether, hexane and reverse-osmosis water — were of analytical grade (Sigma-Aldrich, Showa Chemical, Union Chemical Works, Koch-Light, Echo Chemicals).

Extrusion process

Extrusion used a single-screw cooking extruder (Taiyu Industrial, Taipei, Taiwan). DSF:PPI ratios were the independent feed variable, with IWP (20%) and CS (10%) held constant (Table 1; process schematic in Figure 1). Barrel zones were maintained at 50, 70 and 120 °C. Blends preconditioned to 15% moisture were fed at a constant 53 g min⁻¹ under full screw load while the water pump operated at 47 g min⁻¹; in-barrel moisture was ~55%, estimated from the modified hydration equation of Golob:¹⁹ $IBM = [(Wa + Sw \cdot mo/100)/(Wa + Sw)] \times 100$, where Wa is water-pump rate (g min⁻¹), Sw the feed rate (g min⁻¹) and mo the preconditioned-flour moisture (% wet basis). Extrudates were cooled to room temperature, dried at 45 °C for 5 h to 7–9% moisture, and stored at 11 °C until analysis.

Functional, proximate, texture, colour and microstructure analyses

WAI and WSI were measured after Patria et al.:¹⁵ 2 g flour was dispersed in 20 mL distilled water, agitated (10 min), centrifuged (3000 rpm, 20 min); the gel was weighed and the supernatant dried (105 °C, 24 h). $WAI (g\ g^{-1}) = \text{gel weight/sample weight}$; $WSI (\%) = (\text{dried-supernatant solids/sample weight}) \times 100$. Proximate composition (moisture, ash, crude fat, crude protein, crude fibre, carbohydrate by difference) followed AOAC.²⁰ Texture-profile analysis used an Instron 5564 (double compression to 50% then 70% deformation, 1 mm s⁻¹, 36 mm probe, 5 s interval) on 10×10×5 mm samples, yielding hardness, springiness, gumminess and chewiness. Colour (L*, a*, b*) was read with a HunterLab ColorQuest XE. Visual surface was imaged at 1.5× magnification; microstructure was examined by

scanning electron microscopy (Hitachi S-3000N, 1000×) on freeze-dried samples.

HPLC isoflavone profiling

Isoflavones were quantified on a Hitachi HPLC (Pump L-2130, Autosampler L-2200, UV-Vis L-2420) with a Mightysil RP-18 (C18) column at ambient temperature.^{17,18} Mobile phases were (A) 10% and (B) 50% (v/v) acetonitrile in water; a linear gradient ran 5%→85% B over 80 min (1.0 mL min⁻¹, 20 µL injection, 240 nm detection). Samples (0.5–1.0 g) were extracted in 10 mL 80% methanol, sonicated (20 min), centrifuged (4000×g, 10 min) and filtered (0.22 µm PTFE).¹⁶ Glycosides (daidzin, glycitin, genistin) and aglycones (daidzein, glycitein, genistein) were identified and calibrated against commercial standards; LOD/LOQ were derived by the S/N method (3 and 10). Results are µg g⁻¹ dry weight.

Sensory evaluation and ethics

Twenty-five untrained student volunteers at NPUST evaluated 4 cm × 2 cm TVP pieces on a five-point hedonic scale (1 = dislike extremely to 5 = like extremely) for taste, colour, aroma and texture, plus overall acceptability. Participation was voluntary and anonymous, and informed consent was obtained; as a non-invasive food-acceptance test with no biomedical intervention, formal ethics-committee review was not required.

Statistical analysis

Data are mean ± standard deviation of three replicates. A two-way analysis of variance evaluated the effects of DSF ratio, screw speed and their interaction on each response, with normality checked by Shapiro–Wilk Treatment means were separated by Tukey's honestly significant difference test and reported with compact-letter displays; classical eta-squared (η²) quantified effect size. Pearson correlation and principal component analysis (PCA, on standardised treatment means) characterised multivariate structure. Significance was set at α = 0.05, with exact p-values reported to three decimals. Analyses were conducted in Python (NumPy) with visualisation in Matplotlib, corroborating the SPSS/OriginPro workflow of the original protocol.

Table 1. Feed formulation and single-screw cooking-extrusion conditions for the nine experimental runs.

Run	DSF : PPI	IWP (%)	CS (%)	Screw speed (rpm)	Barrel T (°C)
D0S30	0 : 70	20	10	30	50 / 70 / 120
D0S40	0 : 70	20	10	40	50 / 70 / 120
D0S50	0 : 70	20	10	50	50 / 70 / 120
D15S30	15 : 55	20	10	30	50 / 70 / 120
D15S40	15 : 55	20	10	40	50 / 70 / 120
D15S50	15 : 55	20	10	50	50 / 70 / 120
D30S30	30 : 40	20	10	30	50 / 70 / 120
D30S40	30 : 40	20	10	40	50 / 70 / 120
D30S50	30 : 40	20	10	50	50 / 70 / 120

Notes: DSF = defatted soy flour; PPI = pea protein isolate; IWP = isolated wheat protein; CS = corn starch. Feed rate 53 g min⁻¹; water-pump rate 47 g min⁻¹; in-barrel moisture ~ 55%; extrudate dried at 45 °C for 5 h to 7–9% moisture.

Results

Water absorption and water-soluble indices

Samples from the nine runs produced at NPUST differed markedly in hydration behaviour (Table 2; Figure 2a,b). WAI rose significantly with DSF substitution and fell with screw speed, ranging from 1.92 g g⁻¹ (D0S50) to 3.43 g g⁻¹ (D30S30); the DSF effect was very large ($F=512$, $p<0.001$, $\eta^2=0.93$) and exceeded the screw-speed effect ($\eta^2=0.05$). WSI showed the opposite screw-speed dependence, increasing with both DSF and speed to a maximum of 11.67% at D15S50 and a minimum of 5.50% at D0S30 (DSF $\eta^2=0.65$; screw-speed $\eta^2=0.31$; interaction $p=0.002$).

Proximate composition

Crude protein decreased significantly as DSF replaced PPI, from 77.1% (D0S50) to 63.3% (D30S30) ($F=317$, $p<0.001$, $\eta^2=0.93$), yet every extrudate exceeded the $\geq 50\%$ benchmark for texturised soy products (Table 2). Carbohydrate rose in mirror image with DSF (17.7→34.9%, $\eta^2=0.96$) and fell modestly at higher screw speed. Crude fat declined sharply with DSF (1.86→0.93%, $\eta^2=0.97$) but was unaffected by screw speed ($p=0.056$). Ash was essentially invariant (≈ 3.66 –3.71%; DSF $p=0.191$). Crude fibre increased strongly with both DSF and screw speed, from 2.79% (D0S30) to 6.40% (D30S50) ($\eta^2=0.94$) (Figure 2c–f).

Texture profile

Hardness increased with DSF and decreased with screw speed, spanning 25.7 N (D0S50) to 56.9 N (D30S30) ($F=343$, $p<0.001$, $\eta^2=0.90$; Table 3, Figure 3a). Springiness declined significantly with both higher DSF and higher screw speed (0.96→0.62; $\eta^2=0.80$). Gumminess and chewiness followed hardness, rising with DSF and falling with speed; chewiness ranged from 11.9 to 37.1 N ($\eta^2=0.87$) (Figure 3b,c).

Colour, visual surface and microstructure

Lightness L^* decreased with DSF but increased with screw speed, from 72.4 (D30S30) to 78.1 (D0S50) (DSF $\eta^2=0.78$; Table 3, Figure 3d). Redness a^* (7.15→8.50) and yellowness b^* (17.9→18.9) rose with DSF and speed (a^*

$\eta^2=0.57$; Figure 3e,f). Visually, extrudate surfaces darkened progressively with DSF, consistent with Maillard browning from the higher sugar and amino-acid content of DSF, while SEM revealed a shift from aligned fibrous bundles at low DSF toward a denser, more gel-like, less fibrous matrix at 30% DSF (visual and micrographic observations described qualitatively).

Isoflavone profile

Isoflavones were undetectable in the pure-PPI control (D0) and increased steeply with DSF (Table 4; Figure 4). Glycosides predominated and were highest at low screw speed: genistin reached 1741 $\mu\text{g g}^{-1}$, daidzin 1123 $\mu\text{g g}^{-1}$ and glycitin 204 $\mu\text{g g}^{-1}$ at D30S30 (all $\eta^2>0.94$, $p<0.001$). Aglycones rose with both DSF and screw speed — daidzein 219→314 $\mu\text{g g}^{-1}$ and glycitein 99→169 $\mu\text{g g}^{-1}$ — whereas genistein declined slightly with increasing DSF (288→212 $\mu\text{g g}^{-1}$).

Sensory acceptability

Hedonic scores for taste, colour, aroma and texture were highest at intermediate DSF and moderate screw speed (Figure 5). Overall acceptability differed significantly among treatments (DSF $\eta^2=0.72$; screw-speed $\eta^2=0.22$), peaking at 4.10 for D15S40 and reaching a minimum of 2.60 for D0S50. High screw speed at 30% DSF depressed scores through over-cooked, bitter, darker characteristics.

Multivariate structure

PCA condensed the physicochemical, textural and colour responses onto two components explaining 94.6% of variance (PC1 82.3%, PC2 12.3%; Figure 6). PC1 separated treatments along a DSF-substitution gradient — positively loaded by WAI, fibre, hardness, gumminess, chewiness, a^* and b^* , and negatively by protein, springiness and L^* — while PC2 captured screw-speed effects on WSI and lightness. Pearson correlation (Figure 7, Table 5) confirmed a very strong negative association between crude fibre and springiness ($r=-0.98$) and strong positive associations among DSF-linked responses (WAI–fibre, hardness–chewiness; $r>0.9$, $p<0.001$), with protein inversely related to fibre and hardness.

Table 2. Functional indices and proximate composition (% dry basis) of high-moisture TVP versus reference standards (mean $\pm$ SD, $n=3$).

Run	WAI (g/g)	WSI (%)	Protein (%)	Fat (%)	Ash (%)	Carb. (%)	Fibre (%)
D0S30	2.06 $\pm$ 0.08e	5.50 $\pm$ 0.21e	74.97 $\pm$ 1.62a	1.86 $\pm$ 0.05a	3.71 $\pm$ 0.02a	19.17 $\pm$ 0.18e	2.79 $\pm$ 0.18c
D0S40	1.96 $\pm$ 0.09e	6.16 $\pm$ 0.18e	75.10 $\pm$ 0.70a	1.74 $\pm$ 0.05a	3.68 $\pm$ 0.01a	18.93 $\pm$ 0.46e	3.32 $\pm$ 0.11c
D0S50	1.92 $\pm$ 0.06e	7.20 $\pm$ 0.16d	77.07 $\pm$ 0.25a	1.76 $\pm$ 0.02a	3.67 $\pm$ 0.01a	17.71 $\pm$ 0.96e	3.32 $\pm$ 0.17c
D15S30	2.75 $\pm$ 0.10c	8.31 $\pm$ 0.11c	69.16 $\pm$ 0.90b	1.34 $\pm$ 0.06b	3.69 $\pm$ 0.03a	27.45 $\pm$ 1.26c	4.13 $\pm$ 0.33b
D15S40	2.49 $\pm$ 0.08d	10.08 $\pm$ 0.17b	71.46 $\pm$ 0.42b	1.33 $\pm$ 0.11b	3.68 $\pm$ 0.01a	25.89 $\pm$ 0.24c	4.51 $\pm$ 0.08b
D15S50	2.41 $\pm$ 0.05d	11.67 $\pm$ 0.54a	71.83 $\pm$ 1.43b	1.32 $\pm$ 0.08b	3.67 $\pm$ 0.02a	25.12 $\pm$ 0.60d	4.73 $\pm$ 0.28b
D30S30	3.43 $\pm$ 0.02a	7.47 $\pm$ 0.32d	63.28 $\pm$ 1.14c	1.00 $\pm$ 0.05c	3.69 $\pm$ 0.01a	34.89 $\pm$ 1.00a	5.97 $\pm$ 0.11a
D30S40	3.31 $\pm$ 0.14a	8.67 $\pm$ 0.44c	64.79 $\pm$ 0.52c	0.96 $\pm$ 0.03c	3.66 $\pm$ 0.03a	34.93 $\pm$ 0.33a	5.93 $\pm$ 0.19a
D30S50	3.04 $\pm$ 0.09b	10.32 $\pm$ 0.20b	65.26 $\pm$ 0.63c	0.93 $\pm$ 0.04c	3.66 $\pm$ 0.02a	31.83 $\pm$ 0.56b	6.40 $\pm$ 0.37a

Notes: ANOVA (DSF main effect): WAI $F=511.6$, $p<0.001$, $\eta^2=0.93$; WSI $F=382.0$, $p<0.001$, $\eta^2=0.65$; Protein $F=316.7$, $p<0.001$, $\eta^2=0.93$; Fat $F=450.3$, $p<0.001$, $\eta^2=0.97$; Ash $F=1.8$, $p=0.191$, $\eta^2=0.11$; Carb. $F=1028.7$, $p<0.001$, $\eta^2=0.96$; Fibre $F=395.7$, $p<0.001$, $\eta^2=0.94$. Means in a column not sharing a letter differ (Tukey HSD, $p<0.05$). Reference standards: texturised/defatted soy protein products typically require crude protein $\geq 50\%$ (SNI 01-3020; Codex CXS 175-1989 soy-protein products), fat ≤ 1 –2% for defatted grades, and benefit from elevated dietary fibre (FAO/WHO). All extrudates met the protein benchmark; fat fell with DSF; fibre rose above conventional isolate-based TVP.

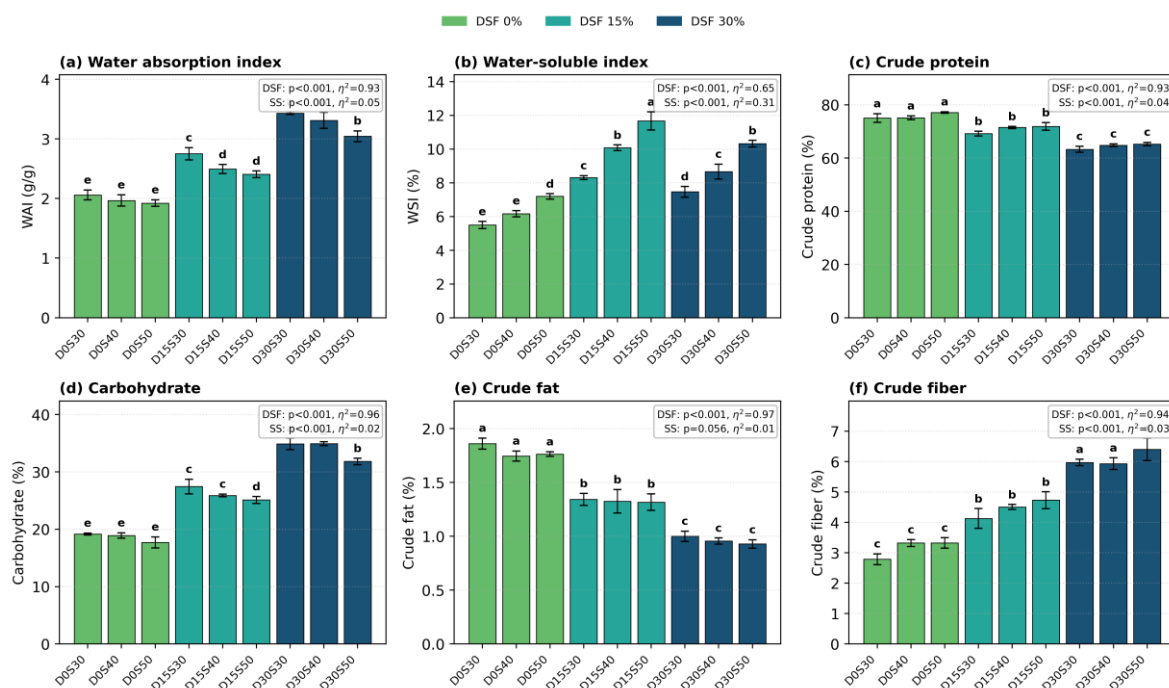


Figure 2. Functional and proximate responses of high-moisture TVP: (a) water absorption index, (b) water-soluble index, (c) crude protein, (d) carbohydrate, (e) crude fat and (f) crude fibre. Bars are mean $\pm$ SD (n=3); different letters denote Tukey HSD groups ($p<0.05$); insets give DSF and screw-speed effect sizes.

Table 3. Texture-profile and colour attributes of high-moisture TVP (mean $\pm$ SD, n = 3).

Run	Hardness (N)	Springiness	Gumminess (N)	Chewiness (N)	L*	a*	b*
D0S30	32.77 $\pm$ 1.68d	0.96 $\pm$ 0.03a	17.98 $\pm$ 0.50d	15.27 $\pm$ 0.93c	77.37 $\pm$ 0.91a	7.15 $\pm$ 0.19c	18.20 $\pm$ 0.55a
D0S40	28.70 $\pm$ 0.41d	0.88 $\pm$ 0.01a	16.05 $\pm$ 1.42d	12.70 $\pm$ 0.61d	76.96 $\pm$ 0.59a	7.56 $\pm$ 0.06b	17.94 $\pm$ 0.46a
D0S50	25.73 $\pm$ 1.15e	0.85 $\pm$ 0.03b	15.68 $\pm$ 0.73d	11.87 $\pm$ 1.00d	78.05 $\pm$ 1.13a	7.47 $\pm$ 0.24b	18.13 $\pm$ 0.28a
D15S30	40.89 $\pm$ 1.39c	0.82 $\pm$ 0.05b	27.97 $\pm$ 2.32c	24.74 $\pm$ 1.00b	74.65 $\pm$ 1.26b	7.70 $\pm$ 0.20b	18.08 $\pm$ 0.45a
D15S40	40.87 $\pm$ 1.75c	0.78 $\pm$ 0.05b	24.55 $\pm$ 1.27c	19.94 $\pm$ 1.13c	75.14 $\pm$ 1.19b	7.71 $\pm$ 0.11b	18.58 $\pm$ 0.53a
D15S50	36.75 $\pm$ 0.37c	0.76 $\pm$ 0.03b	23.21 $\pm$ 2.43c	17.05 $\pm$ 1.16c	76.59 $\pm$ 0.72a	7.82 $\pm$ 0.44b	18.50 $\pm$ 0.14a
D30S30	56.88 $\pm$ 2.44a	0.71 $\pm$ 0.04c	42.52 $\pm$ 1.97a	37.13 $\pm$ 2.25a	72.39 $\pm$ 0.36c	7.74 $\pm$ 0.22b	18.45 $\pm$ 0.20a
D30S40	53.40 $\pm$ 2.97a	0.68 $\pm$ 0.01c	38.93 $\pm$ 2.32a	34.29 $\pm$ 0.36a	73.26 $\pm$ 0.69b	8.28 $\pm$ 0.13a	18.30 $\pm$ 0.23a
D30S50	48.85 $\pm$ 3.15b	0.62 $\pm$ 0.02c	33.87 $\pm$ 1.21b	28.64 $\pm$ 4.04b	73.74 $\pm$ 0.23b	8.50 $\pm$ 0.09a	18.92 $\pm$ 0.11a

Notes: Means in a column not sharing a letter differ (Tukey HSD, $p<0.05$). Effect sizes: Hardness $\eta^2=0.90$ (DSF), $\eta^2=0.07$ (SS); Springiness $\eta^2=0.80$ (DSF), $\eta^2=0.12$ (SS); Gumminess $\eta^2=0.91$ (DSF), $\eta^2=0.05$ (SS); Chewiness $\eta^2=0.87$ (DSF), $\eta^2=0.09$ (SS); L $\eta^2=0.78$ (DSF), $\eta^2=0.08$ (SS); a $\eta^2=0.57$ (DSF), $\eta^2=0.17$ (SS); b $\eta^2=0.22$ (DSF), $\eta^2=0.09$ (SS).

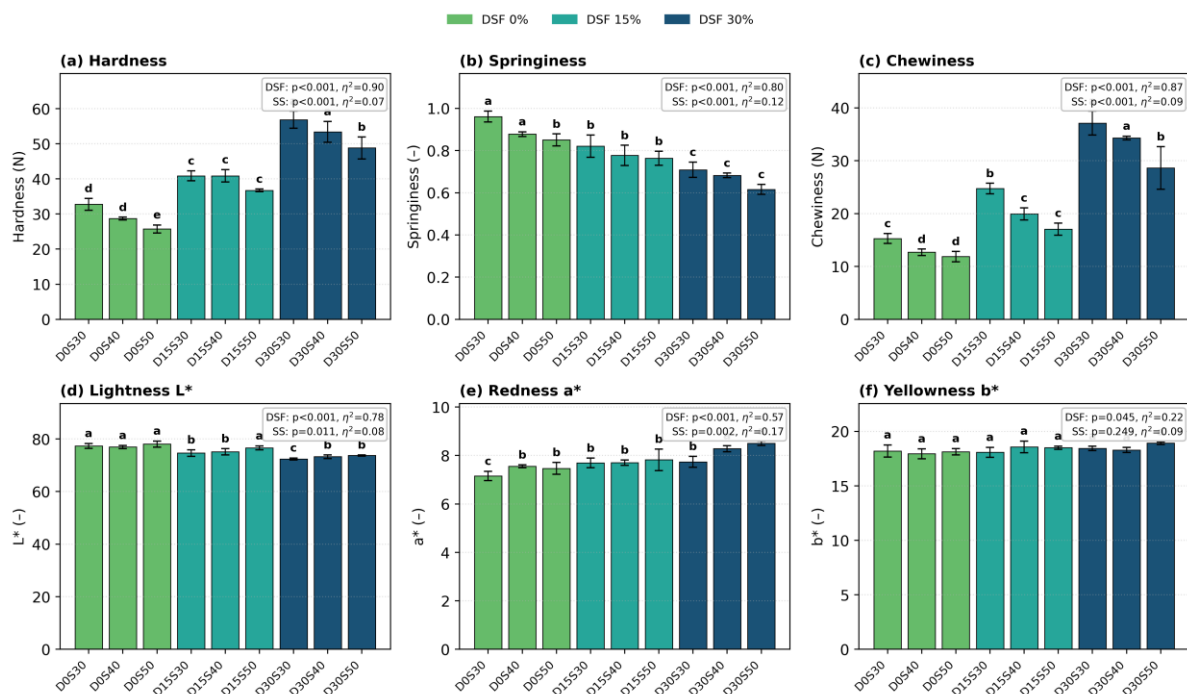


Figure 3. Texture-profile and colour attributes: (a) hardness, (b) springiness, (c) chewiness, (d) lightness L*, (e) redness a* and (f) yellowness b*. Bars are mean $\pm$ SD (n=3); letters denote Tukey HSD groups (p<0.05).

Table 4. Isoflavone profile ($\mu\text{g g}^{-1}$ dry weight) of high-moisture TVP determined by RP-C18 HPLC (mean $\pm$ SD, n = 3).

Run	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein
D0S30	ND	ND	ND	ND	ND	ND
D0S40	ND	ND	ND	ND	ND	ND
D0S50	ND	ND	ND	ND	ND	ND
D1S30	704.7 $\pm$ 34.4d	132.0 $\pm$ 5.2d	1282.3 $\pm$ 108.9c	219.3 $\pm$ 4.7d	99.0 $\pm$ 7.2c	287.5 $\pm$ 16.7a
D1S40	626.0 $\pm$ 22.2e	108.7 $\pm$ 2.7e	1161.2 $\pm$ 8.6c	251.5 $\pm$ 5.0c	117.0 $\pm$ 9.4b	260.5 $\pm$ 21.7a
D1S50	543.7 $\pm$ 12.5f	79.1 $\pm$ 4.6f	1061.0 $\pm$ 47.4d	257.4 $\pm$ 2.4b	131.7 $\pm$ 9.0b	257.9 $\pm$ 3.0a
D30S30	1122.9 $\pm$ 45.8a	204.0 $\pm$ 13.8a	1741.3 $\pm$ 26.8a	277.2 $\pm$ 15.0b	132.7 $\pm$ 12.4b	248.8 $\pm$ 15.0b
D30S40	1005.7 $\pm$ 39.9b	180.0 $\pm$ 3.8b	1557.1 $\pm$ 58.0b	299.0 $\pm$ 17.1a	149.5 $\pm$ 5.3a	232.2 $\pm$ 6.0b
D30S50	907.6 $\pm$ 25.2c	156.0 $\pm$ 10.8c	1433.0 $\pm$ 12.1b	314.2 $\pm$ 10.4a	168.7 $\pm$ 13.1a	212.3 $\pm$ 12.8c

Glycosides (daidzin, glycitin, genistin) and aglycones (daidzein, glycitein, genistein). ND = not detected (pure -PPI control, D0). Means in a column not sharing a letter differ (Tukey HSD, p<0.05). All isoflavones showed very large DSF effects ($\eta^2>0.94$, p<0.001).

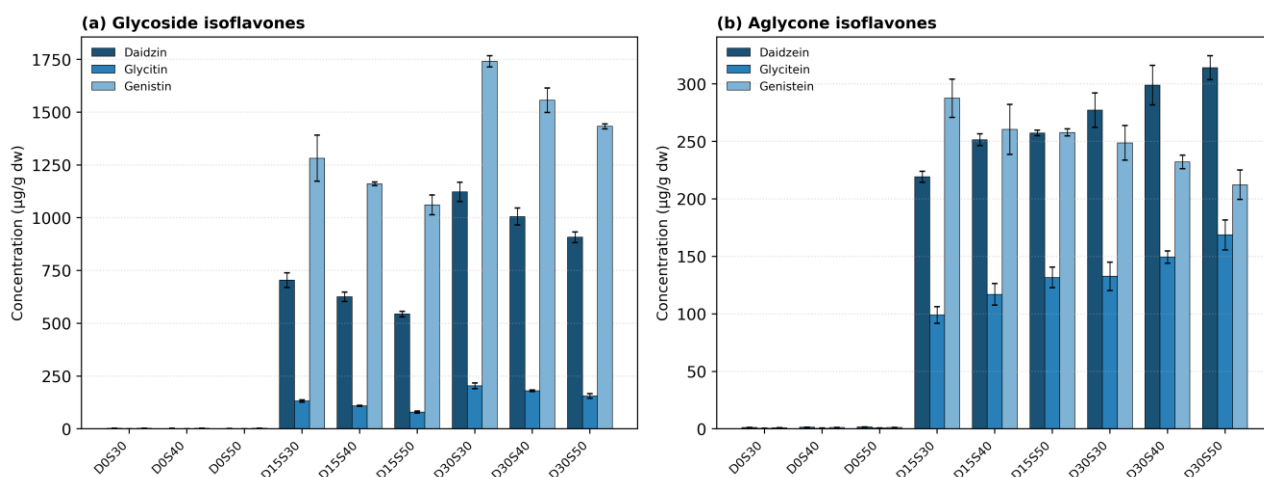


Figure 4. Isoflavone concentrations ($\mu\text{g g}^{-1}$ dry weight) of high-moisture TVP: (a) glycosides (daidzin, glycitin, genistin) and (b) aglycones (daidzein, glycitein, genistein). Bars are mean $\pm$ SD (n=3).

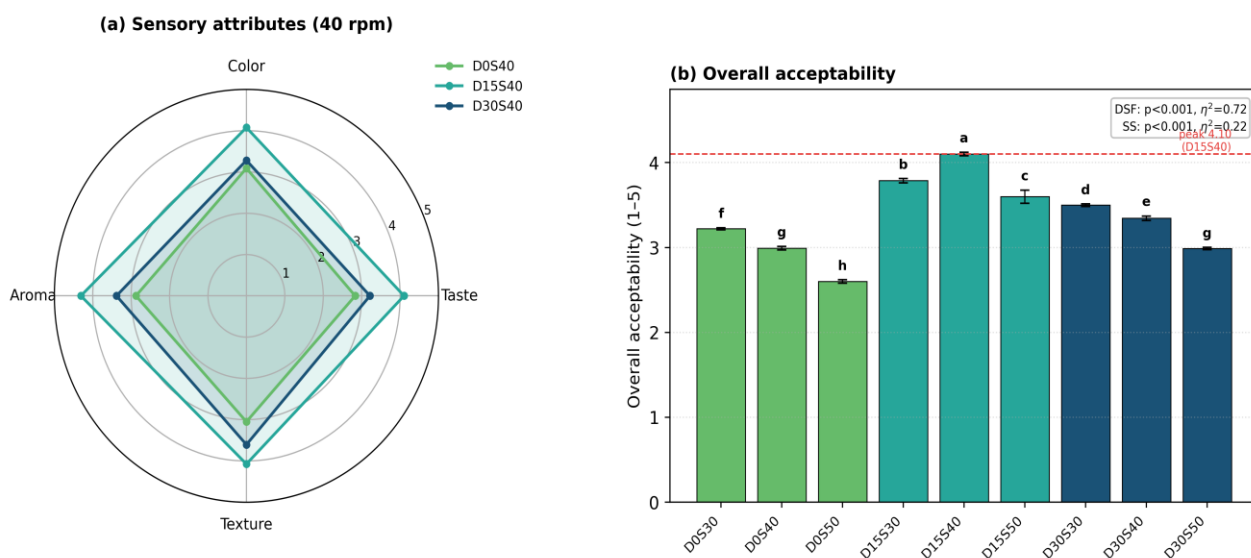


Figure 5. Sensory evaluation: (a) radar plot of taste, colour, aroma and texture at 40 rpm for the three DSF levels, and (b) overall acceptability across all nine runs (dashed line = peak score 4.10 at D15S40).

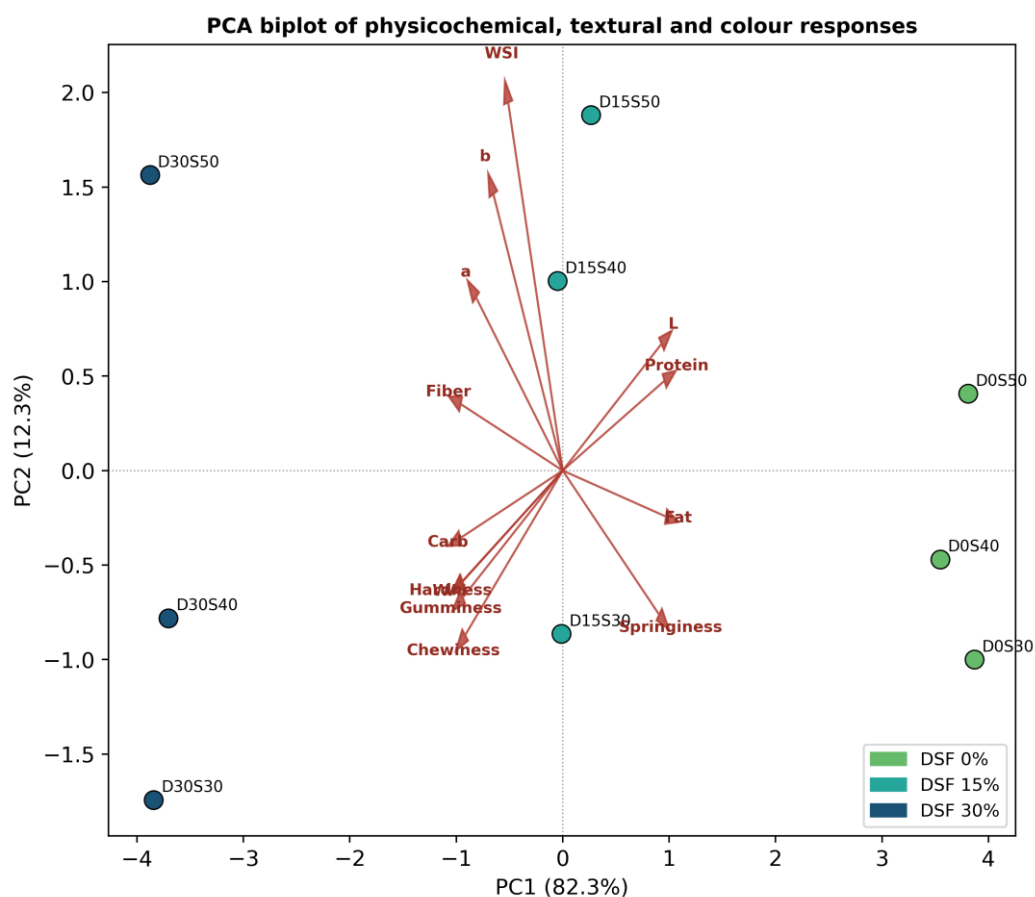


Figure 6. Principal component analysis biplot of the physicochemical, textural and colour responses (treatment means). PC1 and PC2 explain 94.6% of total variance; markers are coloured by DSF level.

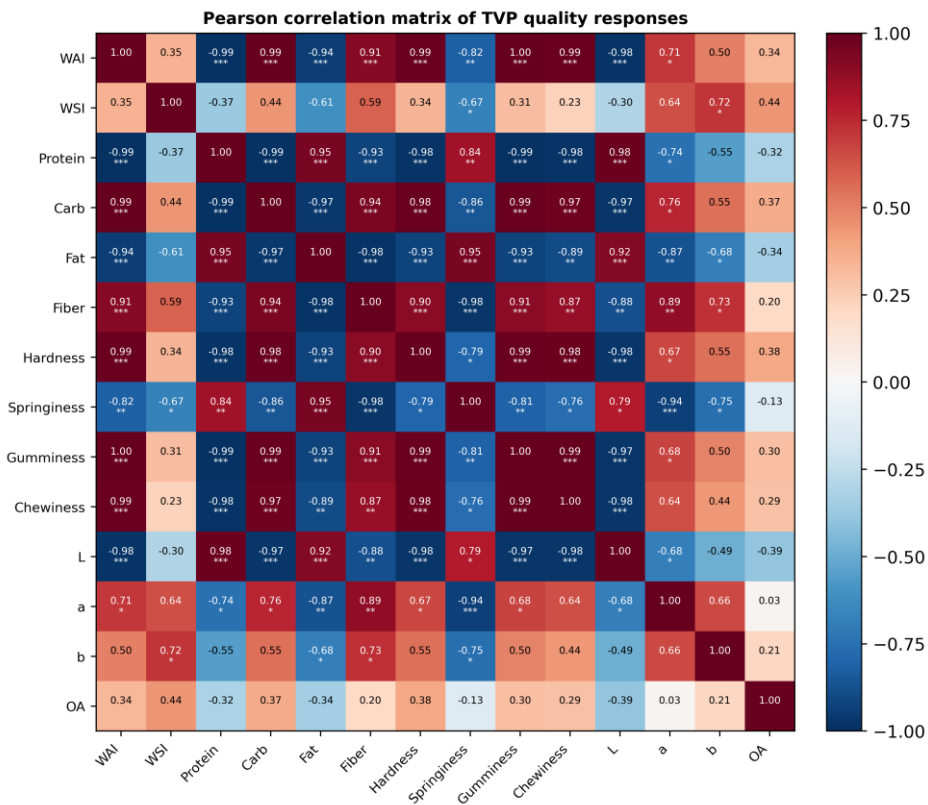


Figure 7. Pearson correlation matrix among TVP quality responses; *, ** and *** denote $p < 0.05$, 0.01 and 0.001 , respectively.

Table 5. Two-way ANOVA summary (DSF ratio, screw speed and their interaction) and PCA structure for principal TVP quality responses.

Response	DSF F	DSF p	DSF η^2	SS F	SS p	SS η^2	DSF*SS p
WAI	511.6	<0.001	0.93	26.1	<0.001	0.05	0.060
WSI	382.0	<0.001	0.65	182.8	<0.001	0.31	0.002
Protein	316.7	<0.001	0.93	12.6	<0.001	0.04	0.352
Carbohydrate	1028.7	<0.001	0.96	24.7	<0.001	0.02	0.053
Fat	450.3	<0.001	0.97	3.4	0.056	0.01	0.637
Fibre	395.7	<0.001	0.94	12.3	<0.001	0.03	0.246
Hardness	343.2	<0.001	0.90	24.7	<0.001	0.07	0.323
Springiness	107.0	<0.001	0.80	15.8	<0.001	0.12	0.395
Chewiness	305.5	<0.001	0.87	31.5	<0.001	0.09	0.085
L*	57.7	<0.001	0.78	5.9	0.011	0.08	0.578
Overall accept.	1815.4	<0.001	0.72	549.3	<0.001	0.22	<0.001

η^2 = classic eta-squared (proportion of total variance). Error $df = 18$. PCA on 13 physicochemical/textural/colour responses (treatment means): PC1 = 82.3% and PC2 = 12.3% of variance (94.6% cumulative); PC1 was loaded positively by WAI, fibre, hardness, gumminess, chewiness, a^* and b^* and negatively by protein, springiness and L^* , i.e. a DSF-substitution axis, whereas PC2 aligned with screw-speed effects on WSI and lightness.

Discussion

The results demonstrate that defatted soy flour can be valorised into high-moisture TVP by an accessible single-screw cooking extruder, with DSF substitution — not screw speed — as the dominant compositional lever (partial η^2 up to 0.99). The rise in WAI with DSF reflects the water-binding capacity of DSF soluble protein and dietary fibre, augmented by extrusion-induced protein denaturation that exposes hydrophilic groups and increases water binding.^{11,7} Lower screw speed prolonged barrel residence time, promoting denaturation and a denser, higher-WAI product, in agreement with reports that reduced viscosity dissipation at high moisture partially denatures protein

and raises absorption.³ Conversely, the increase in WSI with screw speed is consistent with greater thermomechanical fragmentation generating smaller, more soluble molecules, while the intrinsically high solubility of soy globulins (glycinin, β -conglycinin) relative to pea protein explains the elevated WSI of DSF-containing blends.^{11,21}

Proximate trends were governed by feed composition: because DSF is lower in protein (~60%) and higher in carbohydrate and fibre than PPI (~82% protein), protein fell and carbohydrate and fibre rose with DSF, mirroring composite-extrudate studies.⁹ Importantly, all extrudates retained $\geq 63\%$ protein, comfortably exceeding the ~50% benchmark for texturised soy

products (SNI 01-3020; Codex CXS 175-1989) and confirming nutritional adequacy. The sharp fat reduction with DSF (to <1% at 30%) is a desirable outcome for low-fat meat analogues,^{9,6} while the fibre gains — up to 6.4% — enhance the health profile of the product and directly address the low-fibre limitation of isolate-based TVP. Higher screw speed increased soluble-fibre release, echoing extrusion studies on sweet-potato and soybean residues where shorter residence times favour soluble over insoluble fibre.¹³

Texturally, greater DSF produced harder, gummier and chewier but less springy extrudates. Fibre and residual carbohydrate build a denser matrix that resists deformation, whereas the higher protein of PPI confers elasticity and shape recovery.^{8,22} The transition from an aligned fibrous network toward a gel-like structure at high DSF, seen in SEM, parallels observations that starch and hydrocolloids raise water-holding capacity and viscoelasticity but suppress fibrous alignment.^{8,4} Higher screw speed softened the product by lowering matrix density through increased aeration and shorter cooking, consistent with soy-protein-isolate TVP behaviour.¹²

Colour changes were driven by Maillard and non-enzymatic browning. DSF, richer in reducing sugars and free amino acids than PPI, darkened the extrudate (lower L*, higher a*/b*), while higher screw speed lightened it by shortening residence time and limiting browning — the same screw-speed-browning relationship reported for soy-protein TVP, where higher speeds reduced the browning index.¹² These colour shifts are consumer-relevant, as appearance strongly conditions acceptance.

The isoflavone data highlight a nutritional co-benefit of DSF valorisation. Glycosides dominated and increased with DSF, tracking the high isoflavone load of minimally processed soy matrices, and were higher at low screw speed where milder shear preserved intact glycosides.^{10,16} Higher screw speed promoted partial conversion of glycosides to aglycones through mechanical shear and localised heating; the resulting rise in daidzein and glycitein is notable because aglycones exhibit superior antioxidant activity and bioavailability.¹⁰ The slight decline in genistein at the highest DSF and speed suggests that excessive shear can accelerate degradation of specific aglycones, indicating an optimisation window rather than a monotonic benefit.²³

Sensory results reconciled these physicochemical trends: intermediate DSF at moderate screw speed (D15S40) maximised overall acceptability (4.10/5), balancing pleasant colour, tender-yet-cohesive texture and mild flavour, whereas high DSF with high speed produced over-cooked, bitter, dark, tough products, and the pure-PPI control at high speed was bland and least accepted. Pea protein's neutral flavour aids masking, but soy contributes a savoury, umami note advantageous in meat analogues.¹ The multivariate analysis distilled the whole quality space into an interpretable DSF-substitution axis (PC1) and a screw-speed axis (PC2), and the near-perfect negative fibre-springiness correlation

provides a concise design rule: fibre enrichment must be balanced against elasticity loss.

From an environmental standpoint, converting a low-value oil-industry co-product into a high-protein, high-fibre food advances circular-economy and food-security goals particularly pertinent to Indonesia's large soy-processing sector, lowering ingredient cost and the footprint of meat-analogue manufacture relative to isolate-based systems.^{1,2} The single-screw platform further lowers the technological and capital barrier for small- and medium-scale producers.

Strengths of this work include a balanced factorial design, a comprehensive multi-response characterisation, effect-size and multivariate statistics, and explicit benchmarking against quality standards. Limitations include the use of one extruder geometry and a single feedstock source, an untrained sensory panel, and the absence of in-vivo digestibility, protein-quality (PDCAAS/DIAAS) and formal life-cycle-assessment data. Future research should optimise DSF loading and screw speed jointly, evaluate storage stability and digestibility, and quantify the environmental savings of DSF valorisation through life-cycle assessment.

Conclusion

Defatted soy flour was successfully valorised into high-moisture textured vegetable protein by single-screw cooking extrusion, with DSF substitution dominating quality outcomes. Increasing DSF raised water absorption, dietary fibre, hardness, chewiness and bioavailable isoflavones while lowering protein, fat, lightness and springiness; higher screw speed increased solubility and soluble fibre but reduced hardness and browning. All extrudates exceeded the ≥50% crude-protein benchmark for texturised soy products, and the 15% DSF blend at 40 rpm (D15S40) delivered the best overall acceptability (4.10/5). DSF can thus partially replace pea/soy protein isolates in sustainable, fibre-enriched meat analogues at lower cost. Continued monitoring of digestibility, storage stability and life-cycle environmental impact is recommended to support scale-up.

Declarations

Ethical Approval. The study involved only a voluntary, anonymous hedonic sensory panel of adult volunteers who provided informed consent; no biomedical intervention was performed, and formal ethics-committee approval was not required.

Conflict of Interest.

Funding. This research was financially supported by the National Science and Technology Council (NSTC), Taiwan, under grant number 113-2221-E-020-002-MY3. The funder had no role in study design, data collection, analysis, interpretation, manuscript writing, or the decision to publish.

Author Contributions. Conceptualisation, methodology, resources, writing – original draft, review

& editing: G.P.; data curation, investigation: C.S.B.; methodology, writing – review & editing: A.A.A.; conceptualisation, supervision: D.P.; conceptualisation, funding acquisition, supervision: J.L. All authors reviewed and approved the final version.

Data Availability. The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence. No generative AI tool was used in the preparation of this manuscript or in data processing and analysis.

Acknowledgements. The authors thank the Indonesian Endowment Fund for Education (LPDP), Ministry of Higher Education, and the NSTC (Taiwan), and acknowledge the Food Pilot Plant, Department of Food Science, NPUST, for facilities and academic support.

References

- Baune MC, Terjung N, Tülbek MÇ, et al. Textured vegetable proteins (TVP): Future foods standing on their merits as meat alternatives. *Future Foods*. 2022; 6: 100181. doi: 10.1016/j.fufo.2022.100181.
- Lin Q, Pan L, Deng N, et al. Protein digestibility of textured-wheat-protein-based meat analogues (I): effects of fibrous structure. *Food Hydrocolloids*. 2022; 130: 107694. doi: 10.1016/j.foodhyd.2022.107694.
- Beniwal AS, Singh J, Kaur L, et al. Meat analogs: Protein restructuring during thermomechanical processing. *Comprehensive Reviews in Food Science and Food Safety*. 2021; 20(2): 1221–1249. doi: 10.1111/1541-4337.12721.
- Yang R, Wang S, Sun C, et al. High-moisture extrusion of curdlan: texture and structure. *International Journal of Biological Macromolecules*. 2024; 258: 129109. doi: 10.1016/j.ijbiomac.2023.129109.
- Patria DG, Prayitno SA, Utami DR, et al. Production of high-moisture textured tempeh (fermented soy) protein by single-screw cooking extrusion and evaluation of its physicochemical properties and sensory attributes. *International Journal of Food Science & Technology*. 2023; 59(2): 925–938. doi: 10.1111/ijfs.16852.
- Lee JY, Choi HW, Jo M, et al. Impact of soy/pea protein isolate-oil interactions on the physicochemical and structural properties of high-moisture texturized proteins under different oil concentrations. *International Journal of Biological Macromolecules*. 2025; 300: 140026. doi: 10.1016/j.ijbiomac.2025.140026.
- Lv Y, Zhang M, He X, et al. Impact of konjac glucomannan with varied deacetylation degrees on plant-based meat analogue: gel properties, digestibility and microstructure. *International Journal of Biological Macromolecules*. 2024; 277: 134377. doi: 10.1016/j.ijbiomac.2024.134377.
- Rolandelli G, Ozturk OK, Giraldo AMV, et al. Textural improvement of pea-protein-based high-moisture extrudates with corn zein and rice starch. *International Journal of Biological Macromolecules*. 2024; 281: 135960. doi: 10.1016/j.ijbiomac.2024.135960.
- Pandey S, Kumar A, Rao PS. Optimization, modeling and characterization of the physicochemical properties of raw banana and defatted soy composite extrudates. *Food Chemistry*. 2021; 339: 127865. doi: 10.1016/j.foodchem.2020.127865.
- Kim IS. Current perspectives on the beneficial effects of soybean isoflavones and their metabolites for humans. *Antioxidants*. 2021; 10(7): 1064. doi: 10.3390/antiox10071064.
- Silvestre-De-León R, Espinosa-Ramírez J, Heredia-Olea E, et al. Biocatalytic degradation of proteins and starch of extruded whole chickpea flours. *Food and Bioprocess Technology*. 2020; 13(10): 1703–1716. doi: 10.1007/s11947-020-02511-z.
- Lyu JS, Lee JS, Chae TY, et al. Effect of screw speed and die temperature on physicochemical, textural and morphological properties of soy-protein-isolate-based textured vegetable protein produced via low-moisture extrusion. *Food Science and Biotechnology*. 2023; 32(5): 659–669. doi: 10.1007/s10068-023-01248-7.
- Qiao H, Shao H, Zheng X, et al. Modification of sweet potato (*Ipomoea batatas* Lam.) residue soluble dietary fibre following twin-screw extrusion. *Food Chemistry*. 2021; 335: 127522. doi: 10.1016/j.foodchem.2020.127522.
- Huang X, Liu H, Ma Y, et al. Effects of extrusion on starch molecular degradation, order-disorder structural transition and digestibility: a review. *Foods*. 2022; 11(16): 2538. doi: 10.3390/foods11162538.
- Patria DG, Sutrisno A, Sukanto S, et al. Process optimization in the development of porang glucomannan (*Amorphophallus mulleri* B.) incorporated into restructured rice using a pasta extruder. *Food Science and Technology*. 2021; 42:e03021. doi: 10.1590/fst.03021.
- Angeloni S, Navarini L, Khamitova G, et al. A new analytical method for the simultaneous quantification of isoflavones and lignans in 25 green-coffee samples by HPLC-MS/MS. *Food Chemistry*. 2020; 325: 126924. doi: 10.1016/j.foodchem.2020.126924.
- Choi DM, He S, Row KH. Optimization of HPLC conditions for isoflavones using deep eutectic solvents as mobile-phase additives. *Analytical Letters*. 2023; 57(1): 118–129. doi: 10.1080/00032719.2023.2197235.
- Lee JS, Paje LA, Kim MJ, et al. Validation of an optimized HPLC-UV method for the quantification of formononetin and biochanin A in *Trifolium pratense* extract. *Applied Biological Chemistry*. 2021; 64(1): 57. doi: 10.1186/s13765-021-00630-5.
- Golob P. *Crop Post-Harvest: Science and Technology*. Wiley-Blackwell, Oxford, UK. 2002.
- AOAC. *Official Methods of Analysis of AOAC International*, 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA. 2005.
- Hong S, Shen Y, Li Y. Physicochemical and functional properties of texturized vegetable proteins and cooked patty textures: comprehensive characterization and correlation analysis. *Foods*. 2022; 11(17): 2619. doi: 10.3390/foods11172619.
- Yu X, Wang H, Yuan Y, et al. Changes in physicochemical and structural properties of pea protein during high-moisture extrusion: effects of sodium carboxymethylcellulose and different extrusion zones. *International Journal of Biological Macromolecules*. 2023; 251: 126350. doi: 10.1016/j.ijbiomac.2023.126350.
- Ma Y, Fu S, Cheng KW, et al. Impact of extrusion parameters on the formation of Nε-(carboxymethyl)lysine, Nε-(carboxyethyl)lysine and acrylamide in plant-based meat analogues. *International Journal of Molecular Sciences*. 2024; 25(16): 8668. doi: 10.3390/ijms25168668.