

Comparative impact of steaming and dry heat on functional components of soy hulls

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Article history

Received:

26 June 2024

Received in revised form:

6 June 2025

Accepted:

9 June 2025

Keywords

soy hull,
thermal processing,
trypsin inhibitor reduction,
protein solubility,
polyphenol extractability

Abstract

Soy hulls, a major by-product of the soy processing industry, are composed of varying amounts of cellulose, hemicellulose, and lignin. They also contain significant levels of proteins (up to 15%) and valuable phytochemicals, including polyphenols, trypsin inhibitors, phytic acid, and phytates. Thermal treatment is required prior to their utilisation. In the present work, the effects of two types of thermal treatment steaming and dry heating on the protein contents and compositions, trypsin inhibitor (TI) activity, polyphenol contents, and phytate levels of soy hulls were investigated. Protein fractions were analysed by measuring total and water-soluble protein contents, and performing SDS-PAGE under reducing conditions. The heating method significantly affected protein solubility and TI activity. Moist heat improved protein solubility by 34.5%, while dry heating reduced it by 28.9%. Residual TI activity was 18.5 and 84.8% for steamed and dry-heated samples, respectively. Additionally, steaming enhanced the water extractability of polyphenols and phytates. The heating method did not significantly influence the mineral profile of soy hulls.

DOI

<https://doi.org/10.47836/ifrj.32.3.20>

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Introduction

Soy hulls are a major by-product of soybean processing, produced in large volumes during dehulling operations. Globally, countries such as the United States, Brazil, and China generate significant quantities of soy hulls, yet they remain underexploited in human nutrition (Sari *et al.*, 2020; Chen *et al.*, 2021). Despite their low commercial value, soy hulls possess favourable nutritional and functional attributes, including high dietary fibre, protein, and phenolic contents (Porrás *et al.*, 2022). Their valorisation aligns with sustainability goals and the development of functional food ingredients derived from agro-industrial residues.

Thermal treatments are commonly employed to reduce anti-nutritional factors such as trypsin inhibitors, enhance protein functionality, and improve the release of bioactive compounds (Lee *et al.*, 2023). Moist and dry heat treatments, such as steaming and oven-drying, have distinct impacts on structural and compositional properties. Steaming facilitates cellular softening and solubilisation of matrix components, while dry heat favours dehydration, and promotes Maillard reactions, thus

influencing protein-polyphenol interactions (Wang *et al.*, 2021).

While several studies have evaluated thermal effects on soybean meals and flours, the functional modulation of soy hulls remains poorly characterised. Most notably, comparative studies focusing on moist versus dry heat treatments on soy hulls are scarce, and existing data often overlook mechanistic interpretations regarding protein denaturation, antioxidant capacity shifts, or mineral chelating changes (Martínez-Villaluenga *et al.*, 2020; Li *et al.*, 2022). Furthermore, the influence of these treatments on the interaction between phenolics, proteins, and chelators like phytic acid remains to be elucidated.

Therefore, the present work aimed to fill this knowledge gap by systematically comparing the effects of steaming and dry heat on key functional characteristics of soy hulls. Emphasis was placed on evaluating protein solubility, trypsin inhibitor activity, phenolic extractability, antioxidant capacity, and mineral chelation. The results would contribute to a better understanding on how different thermal modalities affect the functional potential of soy hulls, and support their application in value-added food formulations.

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Materials and methods

Commercial soy hulls obtained after the production of extruded-expelled soy at Agrolek d.o.o. (Novi Sad, Serbia) were used in the present work. The hulls were washed with distilled water, drained, and subjected to thermal treatment. A portion of the washed hulls (100 g) was steamed, while an equal quantity was treated in a hot air oven based on their experimental design presented in Table 1. Following treatment, the samples were dried in convection dryer at 60°C to approximately 10% moisture content, ground using a mortar, and defatted with *n*-hexane for 3 h. After filtration, the defatted material was dried overnight at room temperature, finely powdered, and stored at -20°C until further analysis. These conditions were selected based on preliminary

experiments and literature reports, aiming to induce moderate structural changes in the soy hull matrix while preserving thermolabile bioactive compounds (Kim *et al.*, 2017). The pressure of 1.3 bar corresponds to a saturated steam temperature of approximately 120°C, simulating mild industrial steaming conditions. The oven treatment at 120°C was chosen to allow direct comparison between moist and dry thermal effects. Untreated soy hulls (*i.e.*, without thermal processing) were used as a control group for all analytical comparisons. This allowed the evaluation of the specific effects of steaming and dry heat on the measured parameters. All soy hull samples originated from a single homogenised batch. Each thermal treatment was performed in triplicate to minimise batch-to-batch variation.

Table 1. Experimental design for thermal processing of soy hulls.

Sample ID	Treatment type	Temperature (°C)	Pressure (bar)	Time (min)	Airflow / Humidity control
C	Control (untreated)	—	—	—	—
S	Steaming (dynamic)	~120	1.3	10	Continuous flow of pressurized saturated steam
D	Dry heat (oven)	120	Atmospheric	10	Static air, ambient humidity

Determination of total phenolic content

The total phenolic contents of soy hulls' aqueous and methanolic extracts were determined using Folin-Ciocalteu method according to Kostić *et al.* (2021). Briefly, 0.250 g of powdered soy hull was extracted in 10 mL of deionised water or in 80% methanol on a vortex mixer for 1 h at room temperature. After that, the extracts were centrifuged at 2,400 g (DMO412, DLAB, China) for 15 min. Next, 182 µL of diluted methanolic extract or the same amount of undiluted aqueous extract was mixed with 909 µL of Folin working solution (1:9). After 5 min, 909 µL of 7.5% sodium carbonate solution was added. The reaction mixtures were left in the dark for 90 min, and the absorbance was measured at 765 nm using UV-1800 Shimadzu spectrophotometer (Shimadzu, Nipon). The content of total phenolics was expressed as mg equivalents of ferulic acid (FAE) per gram of dry sample (mg·g⁻¹ FAE) based on a standard curve of ferulic acid previously prepared

following the same procedure.

Determination of total flavonoid content

The total flavonoid contents of soy hulls' aqueous and methanolic extracts were determined according to Kostić *et al.* (2021). Briefly, 682 µL of water and 50 µL of 5% sodium nitrite solution were added to 150 µL of undiluted extracts prepared as in the case of total phenolic determination. After 6 min, 90 µL of 10% aluminium chloride solution was added. After an additional 5 min, 300 µL of 1 mol·L⁻¹ sodium hydroxide and an additional 165.5 µL of distilled were added. After centrifugation, the absorbance was measured at 510 nm. A blank was prepared using 150 µL of water. The content of flavonoids was expressed as mg of rutin equivalents (RE) per gram of dry sample based on a standard curve made using rutin solutions of the known concentrations.

Determination of total dihydroxycinnamic acid derivative

The total HCA contents of soy hulls' aqueous and methanolic extracts were determined by the spectrophotometric method using standard Arnov's reagent following a slightly modified procedure suggested by Kilibarda *et al.* (2022). Briefly, 200 μL of soy hull extract (aqueous or methanolic extract) prepared as previously described, was mixed with 0.4 mL of 0.5 $\text{mol}\cdot\text{L}^{-1}$ HCl, 0.4 mL of Arnov's reagent, 0.4 mL of 2.215 $\text{mol}\cdot\text{L}^{-1}$ NaOH, and 0.6 mL of H_2O . Blank was prepared by replacing 0.2 mL of hull extract with distilled water or 80% methanol. After that, the reaction mixtures were intensively vortexed and left in the dark at room temperature for 20 min. The absorbance was measured at 525 nm. Chlorogenic acid (CGA) was used as standard for the calibration curve preparation. Total HCA content was expressed as mg of CGA equivalents (CGAE) per g of dry matter.

Determination of total tannin content

The total tannin contents of soy hulls' aqueous and methanolic extracts were determined according to Vijayalaxmi *et al.* (2015). Briefly, to 100 μL of aqueous or methanol extract, 1 mL of 1% potassium ferricyanide and 8% of aqueous solution of ferric chloride was added. The mixture was kept for 5 min at room temperature. The absorbance was measured at 720 nm. The standard curve was prepared using tanninic acid (5 - 50 $\text{mmol}\cdot\text{L}^{-1}$). The results were expressed as mg of tanninic acid equivalents per g.

Determination of phytate content

The phytate contents of soy hulls' aqueous and methanolic extracts were determined according to Vaintraub and Lapteva (1988). Briefly, 1 mL of Wade's reagent (containing 0.03% solution of $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ and 0.3% sulfosalicylic acid in water) was added to 3 mL of the extract, and the mixture was vortexed for 5 min. The absorbance of mixture was measured at 500 nm. The standard curve was prepared using phytic acid sodium salt solution, and expressed as mg of Na-phytate equivalents per g of dry matter.

Protein characterisation

The protein fractions of defatted soy hulls were characterised by total and water-soluble protein contents, as well as with the SDS-PAGE under reducing conditions. The total protein content of

soybean hulls was determined by the standard Kjeldahl method (AOAC, 1998), and expressed as total nitrogen multiplied with 6.25. The dry matter was determined by the standard drying method at $102 \pm 2^\circ\text{C}$. The water-soluble protein content was determined according to Bradford method (1976). Briefly, soy hull samples (0.350 mg) were extracted in 10 mL of deionised water for 1 h. As a standard, BSA (bovine serum albumin) was used.

The SDS-PAGE under reducing conditions was performed according to Fling and Gregerson (1986). For the SDS-PAGE, 5% (w/v) stacking and 12.5% (w/v) resolving gels were used (Gel Electrophoresis Apparatus, LKB-2001-100, LKB, Uppsala, Sweden). Defatted soy hull sample was extracted in 0.055 $\text{mol}\cdot\text{L}^{-1}$ Tris-HCl sample buffer (pH 6.8) for 1 h. The sample buffer contained 2% SDS, 5% β -mercaptoethanol (v/v), 7% glycerol, 6 $\text{mol}\cdot\text{L}^{-1}$ urea, and 0.0025% bromophenol blue. Next, 25 μL of protein extracts were loaded per well. Molecular weights of the identified polypeptides were determined using the Low Molecular Weight Calibration Kit (Pharmacia, Uppsala, Sweden), as well as previously reported data (Barac *et al.*, 2014a).

Determination of trypsin inhibitor activity

The trypsin inhibitor activity of soy hull samples was assayed according to Liu and Markakis (1989) using α -N-benzoyl-DL-arginine-p-nitroanilide (BAPA) as substrate and bovine trypsin (Sigma, USA). The samples were extracted in distilled water (1:100, defatted hull/water, w/v). The inhibitor activity was expressed in trypsin units inhibited (TUI) per mg of dry sample.

Determination of ferric-reducing antioxidant power

The ferric-reducing antioxidant power (FRAP) of aqueous and methanolic extracts of soy hull were determined according to Thaipong *et al.* (2006). Briefly, 150 μL of extracts was mixed with 2,850 μL of FRAP solution (50 mL of sodium acetate buffer (pH 3.6), 5 mL of a 10 $\text{mol}\cdot\text{L}^{-1}$ TPTZ (2,4,6-tripyridyl-s-triazine) solution prepared in 40 $\text{mol}\cdot\text{L}^{-1}$ HCl, and 5 mL of 20 $\text{mol}\cdot\text{L}^{-1}$ $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$). Prior to addition, the FRAP reagent was tempered in a water bath at 37°C . The mixture was then incubated for 30 min at room temperature, after which the absorbance at 593 nm was measured. The calibration curve was prepared using a standard solution of Trolox, and the results were expressed as $\text{mmol}\cdot\text{L}^{-1}$ equivalents of Trolox per g of dry sample.

Iron (II) chelating ability

The ability of soy hull extracts to chelate Fe(II)- ions was determined according to Meira *et al.* (2012). Briefly, 250 mg of defatted and powdered hull samples was extracted in 10 mL of deionised water or methanol on a vortex mixer for 30 min, then centrifuged at 2,500 g for 15 min. Next, 1 mL of the clear extract was mixed with 3.7 mL of deionised water, 0.1 mL of 2 mmol·L⁻¹ FeSO₄, and 0.2 mL of 5 mmol·L⁻¹ ferrozine; and after 10 min, the absorbance of the reaction mixture was measured at 562 nm. The control sample was prepared by same procedure using 1 mL of deionised water instead of the extracts. The Fe(II) ion chelating ability was then determined using Eq. 1:

$$\text{Chelating ability (\%)} = (1 - \text{sample absorbance} / \text{control absorbance}) \times 100 \quad (\text{Eq. 1})$$

ABTS radical scavenging activity

The free-radical scavenging activity was determined according to Arnao *et al.* (2001). The stock solution (7 mmol·L⁻¹ aqueous solution of ABTS (2, 2-azino-bis/ 3-ethyl-benothiazoline-6-sulphonic acid) with 2.45 mmol·L⁻¹ potassium persulfate) was allowed to stand in the dark for 16 h. The working solution of ABTS was prepared by diluting the stock solution with methanol to obtain an absorbance between 0.7 and 0.8 at 734 nm. Thereafter, 1 mL of working solution of ABTS was mixed with 50 µL of aqueous extract or with 100 µL of methanolic extract, and stirred vigorously. At the same time, a control was prepared, only instead of the sample, 50 µL of deionised water or 100 µL methanol was mixed with 1 mL of ABTS. After 7 min, the absorbance was measured at 734 nm. The radical scavenging activity for standard and samples were calculated using Eq. 2:

$$\text{ABTS scavenging activity (\%)} = (\text{Ac} - \text{As} / \text{Ac}) \times 100 \quad (\text{Eq. 2})$$

where, Ac = absorbance of ABTS working solution, and As = absorbance of sample or standard solution mixed with ABTS working solution. Solutions of Trolox with concentration ranging from 10 to 100 µg·mL⁻¹ were used to construct a calibration curve. The free-radical scavenging activity was expressed as the percent of inhibition (%) as well as the Trolox equivalent in micrograms of Trolox per g of dry sample (µg Trolox·g⁻¹).

Statistical analysis

All measurements were performed in triplicate. Data were expressed as mean ± standard deviation. Normality and homogeneity of variances were verified prior to statistical analysis. One-way analysis of variance (ANOVA) was conducted using IBM-SPSS v20 (IBM Corp., Armonk, NY, USA), followed by Tukey's HSD *post hoc* test at a significance level of $p < 0.05$.

Results and discussion

Effect of thermal treatments on soy hull proteins

The possible influence of thermal treatment on protein fraction of commercial soy hull was characterised by the change of total protein and water-soluble contents (Table 2), as well as by the SDS-PAGE under denaturing and reducing conditions (Figure 1). The effect of thermal treatments on the activity of trypsin inhibitors (TI) was also determined. As expected, heat treatments did not significantly affect ($p > 0.05$) the total content of soy hull proteins. The total protein content of the examined samples was in the range of 22.4 - 24.5%. Based on SDS-PAGE analysis (Figure 1), the extracts

Table 2. Moisture, total, and water-soluble protein contents, and TI activities of defatted commercial soybean hulls.

Soy hull	Moisture (%)	Total protein (% on D.M.)	Water-soluble protein (mg·g ⁻¹)	Trypsin inhibitor activity (TUI·mg ⁻¹)*	Inhibitors (%)**	
					BBI	KTI
Non-treated	8.1 ± 0.11 ^b	22.4 ± 1.24 ^a	11.20 ± 0.11	65.20 ± 0.45	4.3 ^b	5.7 ^a
Steamed	9.0 ± 0.05 ^a	24.5 ± 0.64 ^a	15.07 ± 0.21 ^a	12.10 ± 0.21	6.0 ^a	3.8 ^b
Dry-heated	7.0 ± 0.07 ^c	23.2 ± 0.42 ^a	7.96 ± 0.09	55.31 ± 0.07	4.3 ^b	5.5 ^a

*Means ± S.D. Means followed by different lowercase superscripts within similar column are significantly different at $p < 0.05$; **means of three densitometric analyses of total protein extracts by SDS-PAGE.

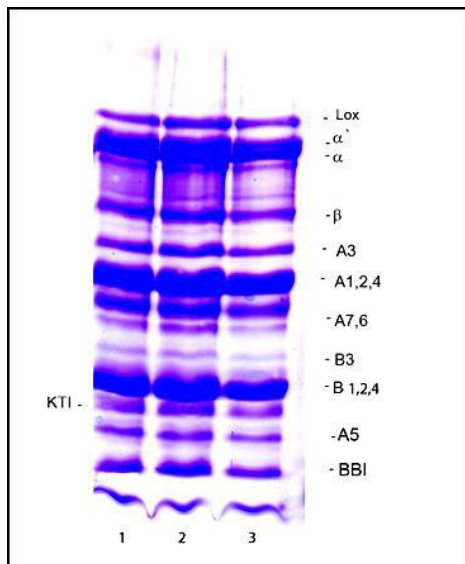


Figure 1. SDS-PAGE patterns of soy hull proteins, 1: non-treated; 2: steamed; and 3: dry-heated. A: acidic subunits of glycinin; B: basic subunits of glycinin; KTI: Kunitz trypsin inhibitor; and BBI: Bowman-Birk inhibitor.

of soy hull contained subunits and polypeptides of almost all reserved soybean proteins. With this method, regardless of the used thermal treatment method in the area of molecular masses 94.000 - 5.000 Da, 17 polypeptide fractions were detected, most of which belonged to the dominant proteins, glycinin, and β -conglycinin (Figure 1 and Table 2).

Acidic- (A3-, A1,2,4-, A5-, A7,6-) and basic (B3-, B1,2,4) subunits of glycinin and α -, α' -, and β -subunits of β -conglycinin with molecular weights similar to those reported by Barac *et al.* (2006) were observed. Besides them, both types of trypsin inhibitors, Bowman-Birk's and Kunitz inhibitors, as well as polypeptides of lipoxygenase were detected. As shown in Figure 1 and Table 3, the SDS-patterns of soy hull proteins and the relative contents of the major protein subunits were similar. However, steamed hulls contained lower level of acidic subunits of glycinin compared to non- and dry-treated samples (Table 2). A summary has been added to clarify the relevance of these fractions in relation to protein structure changes following thermal treatment.

The choice of the method of thermal treatment affected soy hull protein solubility as well as the activity of trypsin inhibitors (Table 2). Namely, aqueous extracts of the examined samples were characterised by significantly different ($p < 0.05$) soluble protein contents, which ranged from 7.96 to 15.07 mg·g⁻¹. While the steaming improved protein solubility by 34.5%, the solubility of dry heat-treated hull proteins was reduced by 28.9%. It is known that thermal treatment significantly alters protein conformation. Dry heat, in particular, is known to promote irreversible protein unfolding, aggregation, and cross-linking through Maillard-type reactions

Table 3. Relative content (%) of major protein subunits in commercial soy hull.

Protein / subunit	Soy hull (%)		
	Non-treated	Steamed	Dry-heated
β-conglycinin			
α - and α' -	17.9 ^a	17.1 ^b	17.7 ^a
β -	6.3 ^b	6.8 ^a	7.1 ^a
SUM	24.3 ^a	23.9 ^a	24.8 ^a
Glycinin			
A ₃ -	5.2 ^b	5.9 ^a	5.5 ^a
A _{1,2,4} -	17.6 ^a	15.4 ^c	16.9 ^b
A _{7,6} -	8.2 ^a	7.7 ^b	7.38 ^c
A ₅ -	3.1 ^b	3.4 ^b	3.4 ^a
Sum of Acidic	34.1 ^a	32.5 ^b	33.7 ^a
B ₃ -	1.0 ^b	1.5 ^a	1.7 ^a
B _{1,2,4} -	15.6 ^a	15.5 ^a	15.8 ^a
Sum of Basic	16.6 ^c	17.0 ^b	17.6 ^a
Sum A+Sum B	50.8 ^a	49.5 ^a	51.3 ^a

Values are means of three densitometric analyses of total protein extracts by SDS-PAGE; means followed by different lowercase superscripts within similar row are significantly different at $p < 0.05$.

(Wang *et al.*, 2021). These structural modifications can increase surface hydrophobicity, and decrease trypsin inhibitor activity, as observed in the present work. Steaming, by contrast, appeared to favour limited denaturation with increased protein solubility, possibly due to softer matrix disruption without extensive browning reactions. These changes aligned with previous findings on heat-modified soy protein isolates (Lee *et al.*, 2023).

Moist heat treatment proved to be more effective way of trypsin inhibitor inactivation compared to dry heat treatment. Steaming of soy hulls for 10 min reduced inhibitor activity (TI) to 12.10 ± 0.21 TUI·mg⁻¹, whereas the TI-activity of dry heated samples was 55.31 ± 0.07 TUI·mg⁻¹ (Table 1). In another words, residual TI activity of steamed and dry heated samples was 18.6 and 84.8%, respectively. The residual TI activity of the steamed soy hulls was in the range of values (< 20%) that have no adverse effects in nutrition. Based on the results of SDS-PAGE both types of inhibitors, KTI and BBI were responsible for the TI activity of raw as well as treated soy hulls. Their relative content in raw soy hull proteins was 4.3% (KTI) and 5.7% (BBI). After steaming, due to the higher thermal stability of BBI compared to KTI (Barac *et al.*, 2014b), the relative content of BBI increased whereas KTI's content decreased to 3.8% (Table 2). However, relatively high content of both inhibitors and low residual activity of steamed hulls suggested that the most of these inhibitors existed in partially disrupted and inactive forms. These forms of inhibitors are a rich source of amino acids with sulphur, which are generally deficient in most proteins of plant origin. Furthermore, BBI is a source of bioactive polypeptide named Lunasin, which is associated with anti-inflammatory, antioxidant, and anticancer properties (Clemente and Arques Mdel, 2014; Wan *et al.*, 2017; Carbonaro, 2021). Therefore, the presence of inactivated forms of inhibitors, especially those of BBI, is desirable from a nutritive and health-promoting aspect of the potential use of soy hulls as food ingredient.

Effect of thermal treatments on phytochemical extractability

The impact of thermal treatments on the extractability of selected phytochemicals from soy hulls is presented in Table 4. Two types of extracts were analysed: aqueous and 80% methanol.

The results indicated that both the thermal treatment and the choice of extractant significantly influenced the content of total phenolics, flavonoids, and hydroxycinnamic acid derivatives. In general, aqueous extracts contained higher levels of total phenolics and hydroxycinnamic acid derivatives, while methanolic extracts were richer in flavonoids. Among the thermally-treated samples, steamed soy hulls exhibited the highest concentrations of total phenolics and dihydroxycinnamic acid derivatives. An exception was the methanolic extract of steamed hulls, in which dihydroxycinnamic acid derivatives were below the detection limit.

The observed differences between dry-heated and steamed samples may be partially explained by protein-phenol interactions. Both steaming and dry heat can either enhance or hinder phenolic availability, depending on the nature of protein-phenol binding. Phenolics may interact with protein residues through hydrogen bonding, hydrophobic interactions, or covalent cross-linking under thermal stress (Jakobek, 2016). These interactions can reduce the extractable free phenolic content and modulate antioxidant activity. In steamed samples, increased solubilisation and reduced protein aggregation likely contributed to higher phenolic release.

Aqueous extracts contained significantly more total phenolics than methanolic extracts. Specifically, their content ranged from 10.47 to 13.06 mg·g⁻¹ FAE in aqueous extracts, compared to 3.09 - 4.02 mg·g⁻¹ FAE in methanolic extracts (Table 4), representing approximately 3- to 4-fold higher levels. Phenolics encompass a broad group of structurally diverse compounds (*e.g.*, phenolic acids, acetophenones, hydroxycinnamic acids, flavonoids, coumarins, and stilbenes) with varied polarity and solubility characteristics (Jakobek, 2016). In this case, water appears to be a more suitable solvent for extracting phenolics from soy hulls, which agreed with the findings of Cabezudo *et al.* (2021), who reported over six-fold higher phenolic content in aqueous versus methanolic extracts after two hours of extraction.

As previously noted, the highest levels of total phenolics and dihydroxycinnamic acid derivatives were detected in the aqueous extract of steamed hulls (13.06 mg·g⁻¹ FAE and 3.68 mg·g⁻¹ CGAE, respectively). In plant tissues, phenolic compounds occur both in free form and as complexes with macromolecules such as proteins, polysaccharides, and lipids (Jakobek, 2016). The elevated phenolic

Table 4. Phytochemical contents and antioxidant properties of soy hull extracts.

Treatment	Total phenolic content (mg·g ⁻¹) FAE	Total flavonoid content (mg·g ⁻¹) RE	Total dihydroxy cinnamic acid derivatives (mg·g ⁻¹) CGAE		Total tannins (mg·g ⁻¹) TAE	Total phytates (mg·g ⁻¹) Na-phytate E	ABTS scavenging		Chelating ability (%)	FRAP (mmolE Trolox·g ⁻¹)
							(% of inhibition)	(mgE Trolox g ⁻¹)		
Aqueous extract										
Non-treated	12.07 ± 0.09 ^b	1.21 ± 0.17 ^c	3.23 ± 0.08 ^b		1.05 ± 0.04 ^d	2.54 ± 0.02 ^b	62.1 ^c	4.57 ^b	23.9 ± 0.67 ^c	37.90 ^b
Steamed	13.06 ± 0.13 ^a	0.75 ± 0.09 ^d	3.68 ± 0.25 ^a		1.29 ± 0.07 ^c	2.66 ± 0.01 ^a	69.7 ^a	4.91 ^a	14.8 ± 0.27 ^c	45.72 ^a
Dry-treated	10.49 ± 0.15 ^c	1.16 ± 0.14 ^c	3.0 ± 0.04 ^c		0.92 ± 0.06 ^d	2.53 ± 0.02 ^b	66.9 ^b	4.61 ^b	18.7 ± 0.41 ^d	35.26 ^c
80% methanolic extract										
Non-treated	3.09 ± 0.16 ^c	1.66 ± 0.21 ^δ	2.25 ± 0.01 ^d		1.39 ± 0.04 ^b	2.52 ± 0.03 ^b	58.9 ^d	2.14 ^c	14.1 ± 0.51 ^e	9.48 ^f
Steamed	3.86 ± 0.18 ^d	2.73 ± 0.11 ^a	n.d. ^e		1.58 ± 0.07 ^a	2.69 ± 0.01 ^a	57.6 ^e	2.03 ^c	27.9 ± 0.22 ^a	17.06 ^d
Dry-treated	4.02 ± 0.05 ^d	1.68 ± 0.04 ^δ	n.d. ^e		1.17 ± 0.05 ^c	25.70 ± 0.01 ^b	62.8 ^c	2.16 ^c	15.1 ± 0.47 ^e	16.01 ^e

Means followed by different lowercase superscripts within similar column are significantly different at $p < 0.05$. n.d.: not detected.

levels observed in steamed samples suggested that moist heat may disrupt these complexes, enhancing the release of bound phenolics. This is further supported by the highest content of extractable tannins observed in steamed hulls (Table 4). Conversely, aqueous extracts contained significantly lower flavonoid levels ($0.75 - 1.21 \text{ mg} \cdot \text{g}^{-1} \text{ RE}$) than methanolic extracts ($1.66 - 2.73 \text{ mg} \cdot \text{g}^{-1} \text{ RE}$). This difference likely reflected the poor water solubility of isoflavones, which represent the dominant class of soybean flavonoids.

Phytic acid, traditionally considered an antinutritional factor due to its mineral-binding capacity, has also been recognised for its antioxidant, anti-inflammatory, and anticancer potentials (Abdulwaliyu *et al.*, 2019; Li *et al.*, 2020; Vucenik *et al.*, 2020). In the present work, soy hulls were found to be a good source of phytates, with both extract types yielding comparable amounts, ranging from 2.53 to $2.69 \text{ mg} \cdot \text{g}^{-1}$.

Antioxidant properties of soy hulls

Antioxidant properties of aqueous and methanolic extracts of investigated hulls were determined using three parameters, the ability to scavenge free radicals, the reducing power (FRAP), and the ability to chelate Fe(II)-ions. The obtained results are shown in Table 4. The ability to scavenge free radicals is expressed as % of inhibition, and as mgE Trolox per gram of dry matter.

From the results listed in Table 4, it is evident that regardless of the parameter by which it is expressed, aqueous extracts had significantly greater scavenging ability of free radicals compared to methanolic extracts. The only exception was the absence of a statistically significant ($p > 0.05$) difference in the % of inhibition between the aqueous extract of the non-treated hulls and the methanolic extract of the dry-treated samples. The percentage of inhibition of these extracts was 62.6 and 62.8%, respectively. However, when the ability to scavenge free radicals was expressed in mgE Trolox per g of dry matter, these samples differed significantly.

The ability of aqueous extracts of soy hull to scavenge free radicals expressed as mgE Trolox per g of dry matter ranged was from $4.57 - 4.91$, whereas those of methanolic extract were between $2.03 - 2.1$. In other words, ABTS scavenging activity of aqueous extracts was two times higher than those of methanolic extracts. This could partly be attributed to the higher content of total phenolics detected in the

aqueous extracts (Table 3), but also to a large extent to the composition of the extracts themselves, *i.e.* the presence of soluble proteins in the aqueous extracts, and their absence in the methanolic extracts. This is supported by the fact that no significant ($p > 0.05$) differences between radical scavenging activities of methanolic extracts were found.

Aqueous extracts of soy hulls also had better reducing power than methanolic extracts; FRAP values of aqueous extracts were in the range of $35.26 - 45.72 \text{ mmol Trolox} \cdot \text{g}^{-1}$, while the reducing power of methanolic extracts was $9.48 - 17.06 \text{ mM Trolox} \cdot \text{g}^{-1}$. Moreover, both types of the extracts of steamed hulls had the highest ability to reduce ferric ions, which was in agreement with the highest content of soluble proteins and total polyphenols determined in these extracts (Tables 2 and 4).

Notably, in contrast to the reducing power and the free radical scavenging ability, the aqueous extract of steamed hulls showed the lowest Fe^{2+} -chelating ability, which was unexpected. One possible explanation lies in the complex interactions that occur between proteins, phenolic compounds, and phytic acid during extraction and/or assay conditions. These interactions may interfere with reactive groups on amino acid residues (*e.g.*, histidine, aromatic, or polar acidic residues) that are typically involved in metal binding. This assumption is supported by the fact that the methanolic extract of the same sample lacking proteins exhibited the highest Fe^{2+} -chelating activity. Interestingly, despite having a slightly higher phytic acid content, the steamed aqueous extract still exhibited poor chelation activity. This indicated that steaming may have altered the structural integrity or availability of phytic acid and other intrinsic chelators, potentially through partial hydrolysis or complex formation. It is also possible that thermal treatment promoted the formation of insoluble aggregates or co-precipitates involving phytic acid, proteins, and metals, thereby reducing the pool of free chelators. These observations were consistent with earlier reports that phytic acid can undergo structural modifications such as hydrolysis, and form insoluble complexes with proteins and minerals under thermal conditions (Liu *et al.*, 2021; Roy and Kumari, 2023). Further analyses, such as detailed phytic acid profiling and FTIR characterisation of chelating functional groups, would be necessary to elucidate this mechanism. Furthermore, soy hull extracts represent chemically complex systems, and their antioxidant properties

result not only from the sum of individual compounds, but also from their synergistic or antagonistic interactions (Cömert and Gökmen, 2017).

Principal component analysis (PCA; Figure 2) was used to visualise differences among aqueous and methanolic extracts based on their phenolic content, antioxidant activity, and chelating ability. The first two components explained the major portion of variance. Water-steamed samples showed distinct clustering due to high total phenolics and ABTS activity, while MeOH-steamed samples stood out due to elevated FRAP capacity. Dry-treated samples

occupied intermediate positions. These trends supported the conclusion that thermal treatment modality influenced the distribution and activity of functional components in soy hulls.

Mineral content of soy hulls

Table 5 presents the mean contents of macro-, micro-, and trace elements found in whole soybean and soy hulls. Soy hulls are a good source of minerals, especially Ca, P, K, and Mg. The average content of these minerals found in non-treated hulls was 442, 252, 653, and 180 mg·100g⁻¹, respectively. The Ca:P ratio of non-treated hull was 1.75. The

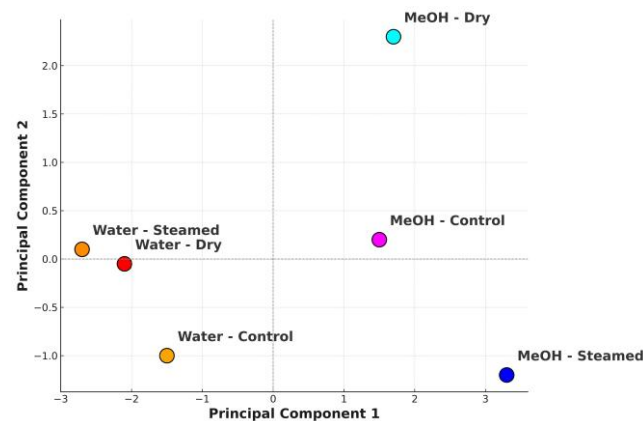


Figure 2. Principal component analysis (PCA) of functional components in soy hull extracts.

Table 5. Mineral contents of soybean seed and hulls.

	Whole seed	Hull		
		Non-treated	Steamed	Dry-heated
		(mg·100 g ⁻¹)		
Ca	825 ± 15.7 ^a	442 ± 28.1 ^b	424 ± 16.2 ^b	431 ± 12.5 ^b
Mg	410 ± 10.20 ^a	180 ± 10.4 ^b	165 ± 3.5 ^b	145 ± 7.60 ^c
Na	3.38 ± 0.24 ^a	2.25 ± 0.158 ^b	1.86 ± 0.08 ^c	1.37 ± 0.05 ^d
K	1690 ± 31.4 ^a	653 ± 43.1 ^c	643 ± 11.4 ^c	710 ± 7.4 ^b
P	710 ± 4.5 ^a	252 ± 5.30 ^b	237 ± 8.2 ^c	260 ± 6.8 ^b
S	189 ± 7.6 ^a	65.9 ± 1.68 ^b	72.4 ± 3.41 ^b	80.4 ± 4.2 ^b
Fe	60.50 ± 4.1 ^a	39.60 ± 3.56 ^c	42.30 ± 1.70 ^c	39.40 ± 2.45 ^c
B	9.10 ± 0.31 ^a	3.43 ± 0.29 ^b	2.67 ± 0.11 ^c	3.10 ± 0.44 ^b
Zn	9.50 ± 0.17 ^a	3.12 ± 0.32 ^c	4.11 ± 0.27 ^b	3.87 ± 0.21 ^b
Cu	3.10 ± 0.22 ^a	0.978 ± 0.094 ^b	0.892 ± 0.06 ^b	0.884 ± 0.11 ^b
Mn	6.70 ± 0.34 ^a	2.77 ± 0.24 ^d	3.20 ± 0.32 ^c	4.10 ± 0.23 ^b
		(μg·100 g ⁻¹)		
Co	61.0 ± 1.72 ^a	33.4 ± 2.90 ^b	37.40 ± 1.50 ^b	36.20 ± 2.10 ^b
Cr	111.3 ± 2.01 ^a	97.5 ± 7.47 ^b	91.10 ± 3.20 ^b	97.60 ± 4.11 ^b
Mo	50.8 ± 4.57 ^a	n.d. ^b	n.d. ^b	n.d. ^b
Ni	31.40 ± 2.80 ^a	28.10 ± 1.10 ^a	29.20 ± 1.60 ^a	28.80 ± 2.20 ^a

Means followed by different lowercase superscripts within similar row are significantly different at $p < 0.05$. n.d.: not detected.

observed values, except that of P content, agreed well with the data reported by Peripolli *et al.* (2012). Namely, these authors reported much higher content of P ($1220 \text{ mg} \cdot 100 \text{ g}^{-1}$) and consequently much lower Ca:P ratio (0.43). The content of Mg and K in non-treated hull represents 43.9 and 38.6% of the amount of these minerals present in whole seed.

Mineral profiles of heat-treated hulls were similar to non-treated sample. It seemed that heat treatments had no significant effect on mineral composition of soy hulls. In steamed samples, slightly lower contents of Na, K, P, and B were detected compared to non-treated hull, whereas significant ($p < 0.05$) differences between heat-treated samples were observed for Mg, Na, K, S, and Mn.

Iron deficiency anaemia is the most common and widespread nutritional disorder in the world, which is presented in both industrialised countries as well as in developing ones. Based on the results presented in Table 4, soy hull can be a good source of iron. The whole soybean seed contained $60.50 \text{ mg} \cdot 100 \text{ g}^{-1}$ of Fe. After seed dehulling, more than 60% remained in the hull. In contrast to soybean meal that has very poor iron bioavailability, the iron in hulls is readily assimilated by humans (O'Bryan *et al.*, 2014). The higher iron bioavailability attributed the lower phytic acid content. Therefore, soybean hulls have the potential as source of iron fortification, for example, in baked products.

Conclusion

The present work clearly demonstrated that the type of thermal treatment significantly influenced the functional potential of commercial soy hulls. Steaming was shown to enhance protein solubility, and markedly reduce trypsin inhibitor activity, resulting in soy hulls becoming a rich source of inactivated forms of Kunitz and Bowman-Birk inhibitors, which are recognised for their health-promoting properties. In addition, steaming improved the water extractability of phenolic compounds, phytates, and other antioxidant constituents. Furthermore, the present work revealed that the choice of extraction solvent was critical, as it strongly influenced both the free radical scavenging activity and reducing power of soy hull extracts. On the other hand, Fe(II) chelating ability appeared to be largely governed by complex interactions among extract components, particularly between proteins and

phenolics. Future research should explore the application of thermally processed soy hulls in functional food formulations, with a focus on enhancing their bioactivity and techno-functional properties. Additionally, investigation into bioaccessibility and bioavailability of key compounds would provide valuable insight into their nutritional potential. These findings suggested that thermal treatment, especially steaming, could significantly improve the functional and nutritional properties of soy hulls, supporting their use as a valuable ingredient in health-promoting food products.

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