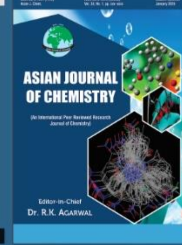
Asian Journal of Chemistry; Vol. 38, No. 4 (2026), 871-880

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2026.35348



Impact of Phenolic Removal on the Molecular and Functional Properties of Sunflower Seed Cake Proteins

POOJA VARTIYA and PIYUSH KASHYAP*

Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Jalandhar-Delhi GT Road, Phagwara-144411, India

*Corresponding author: E-mail: p18.kashyap@gmail.com

Received: 28 November 2025 *Accepted*: 21 January 2026 *Published online*: 8 April 2026 AJC-22307

Sunflower seed cake (SSC), the major by-product of oil extraction, is considered an under utilised protein resource due to its abundance. The nutritional quality and functionality of protein fractions in SSC are negatively affected by phenolic compounds. This study compared the functional and nutritional properties of phenolised and dephenolised protein fractions. Dephenolisation using ethanol extraction reduced the total phenolic content significantly and improved the purity and yield of proteins. Significant improvements were recorded in functional traits: water-holding capacity, oil-holding capacity, emulsifying activity and foaming capacity, after the removal of phenolics. Nutritionally, dephenolised fractions had higher purities and yields due to exposure of active sites that were being held internally within phenolic complexes. Of all fractions, globulin and albumin elicited better functional and nutritional performance upon dephenolisation. Overall, these findings confirm dephenolisation as an appropriate pretreatment to enhance protein functionality in SSC, supporting its application in sustainable food formulation and protein-enriched products.

Keywords: Sunflower seed cake, Protein fractions, Dephenolisation, Phenolic compounds, Functional properties, Nutritional quality.

INTRODUCTION

Global demand for sustainable, plant-based proteins has reinforced research into agro-industrial byproducts as alternative protein sources. De-oiled sunflower seed cake (SSC), a major residue of the sunflower oil industry, is rich in protein, majorly the globulin helianthinin protein fraction, fiber and antioxidant phenolics particularly chlorogenic acid, making it an attractive raw material for food-grade protein recovery and valorisation [1]. Largely valued as animal feed, SSC contains substantial levels of protein (often 20-40% on dry-basis, depending on dehulling, oil extraction method and processing) along with appreciable amounts of bioactive compounds [2]. However, the natural co-occurrence of phenolic compounds with proteins in sunflower residues creates both opportunities and challenges, for example, phenolics can impart antioxidant functionality and potentially beneficial bioactivity, yet they also form strong non-covalent and covalent complexes with proteins that reduce protein solubility, digestibility, colour acceptability and some functional properties essential for food applications [3].

Therefore, unraveling the full potential of SSC as a human food ingredient, requires exploration to address these phenolic interactions. Dephenolisation wherein is the removal or reduction in phenolic compounds prior to protein extraction or during processing has emerged as a promising strategy. When phenolics are removed or reduced, protein fractions tend to have improved functional properties and sometimes enhanced digestibility and bioavailability [4]. A recent example is work by Vartiya *et al.* [5] found that dephenolised sunflower protein isolate had significantly reduced phenolic content with concomitant increases in crude protein and protein yield. Furthermore, optimisation-based extraction of proteins from de-oiled sunflower meal shows that controlling pH, temperature and extraction time with phenolic removal can yield isolates with high protein purity, high recovery and good functional performance.

In the nutritional aspects phenolic compounds exhibit dual characteristics. Many phenolics such as chlorogenic acid, have antioxidant, antimicrobial and health-promoting properties. On the contrary, excessive presence of phenolics can reduce protein digestibility by forming protein-phenol complex, enz-

This is an open access journal, and articles are distributed under the terms of the Attribution 4.0 International (CC BY 4.0) License. This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit the author for the original creation. You must give appropriate credit, provide a link to the license, and indicate if changes were made.

yme inhibition and interacting with mineral absorption, thus negatively affecting the sensory properties [6]. Studies on animal models revealed that protein fractions extracted from sunflower seed with low residual phenolic content can support growth and health efficiency comparable to conventional protein sources, such as casein [7]. According to Sen & Bhattacharyya [7] a sunflower seed protein fraction extracted using isopropanol was shown to have very low residual chlorogenic acid (0.07%) and animals fed with it exhibited similar growth and food compared to casein-fed control groups.

Functionally, the food-product applications depend critically on protein solubility, emulsification capacity, foaming, water holding, gelation, and other techno-functional properties. The presence of phenolics tends to reduce the performance and can impart dark colour or off-flavours. Hence, dephenolisation strategies such as solvent washes, Soxhlet extraction, or other "green" techniques have been explored [8]. Despite these advances, there remain significant gaps. Thus, these dual effects have prompted the recent work comparing "phenolised" SSC-P and "dephenolised" SSC-DP protein fractions to identify how the two differently treated protein fractions shape the nutritional value and functional properties. To the best of our knowledge, the extraction and characterisation of sunflower seed cake protein fractions have not been explored to date. Therefore, the present work is designed to fill the gaps related to sunflower seed cake protein fractionation, followed by their functional characterisation.

EXPERIMENTAL

Raw material: The sunflower seed cake was purchased from Annapurna Ladki Ghana oil, Maharashtra, India. A total of 5 kg of sunflower seed cake was used for this study.

Preparation of sunflower seed cake: The sunflower seed cake was grinded finely in a mechanical grinder and then subjected to defatting by Soxhlet extraction using hexane for 8 h to get the defatted meal. It was then dried at 40 ºC in a tray dryer to remove the residual solvent. Finally, the defatted seed cake was stored in air tight bags for further analysis.

Chemical analysis of sunflower seed cake: The moisture, protein, ash, lipid and crude fibre content of the sunflower seed cake was performed using the standard AOAC method [9]. The carbohydrate content was estimated by subtracting the sum of percentage of moisture, ash, fiber, protein ash, lipid and crude fibre.

Dephenolisation of sunflower seed cake: Dephenolisation of sunflower seed cake was performed as per Đermanović *et al.* [10] with slight modifications. Defatted sunflower seed cake was dispersed in 60% (v/v) ethanol at a meal to solvent ratio of 1:20 (w/v) and stirred using shaking incubator for 2 h. The suspension was filtered and extraction was repeated twice followed by drying of dephenolised seed cake at 30 ºC overnight.

Determination of total phenolic compounds: Total phenols were analysed utilizing Folin-Ciocalteu (FC) reagent. The seed cake extract of 1 mL was combined with 5 mL (10%) FC reagent and 4 mL 7.5% (w/v) Na_2CO_3 and left undisturbed for incubation. The absorbance was measured at 765 nm. The findings were obtained as gallic acid equivalents [11].

Osborne fractionation of sunflower seed cake protein: Protein fractions, *i.e.*, albumin, globulin, prolamin and glutelin were extracted form defatted sunflower seed cake using the method of Kaur *et al.* [12] with slight modifications. Defatted sunflower seed cake powder was dissolved in deionised water at a ratio of 1:10 (w/v) (pH-7.0) and kept in a magnetic stirrer for overnight mixing. The solution was then centrifuged at 6000rpm for 20 min. the supernatant was decanted and saved as albumin-1. Following the similar procedure, albumin-2 and albumin-3 were also collected. Three stage extraction was conducted in order to extract the maximum amount of protein. Similarly, globulin-1,2 and 3, prolamin-1,2 and 3 and glutelin -1,2 and 3 were extracted using 0.5 N NaCl, 70% aqueous ethanol and 0.2% NaOH solution, respectively. The rest of the procedure for the extraction of globulin, prolamin and glutelin was the same as for albumin. After extraction, all the protein solutions were kept in a refrigerator at 4 ºC for further analysis.

Preparation of protein powder: To prepare protein powder, supernatants collected from albumin, globulin, prolamin and glutelin were separately freeze-dried and kept under refrigerated condition in air-tight bags for further analysis.

Determination of yield and protein content: The yield of each fraction of protein extracted from sunflower seed cake was determined according to eqn. 1:

$$\text{Yield (\%)} = \frac{P}{S} \times 100 \quad (1)$$

where, P = weight of particular protein fraction (g); S = dry weight of sunflower seed cake taken for protein extraction (g).

The protein content of each fraction was estimated using the Kjeldahl apparatus.

Functional properties

Water holding capacity (WHC) and oil holding capacity (OBC): The water and oil holding capacity of SSC fractions was determined as per Mathews *et al.* [13] with some modifications. The readings were recorded in triplicates and the results were expressed as either as g of water or sunflower oil per g of sunflower seed cake.

Foaming capacity: The foaming capacity of protein fractions were determined as per Sert *et al.* [14] with minor modifications. Deionised water was added to each protein fraction (0.5 g), bringing the total volume to 50 mL (V_2). The dispersion was homogenised for 2 min at 10,000 × g. The samples were then immediately transferred into a measuring cylinder and the total volume (V1) was measured. The foaming capacity was calculated according to the following formula:

$$\text{Foaming capacity (\%)} = \frac{V_1 - V_2}{V_2} \times 100 \quad (2)$$

where V_1 = volume of protein sample solution (mL) and V_2 = volume of foam (mL).

Emulsifying capacity: The emulsifying capacity (EC) was determined according to a method described by Tounkara *et al.* [15] with some modifications. A 20 mL of protein solution was mixed with 2 mL of soyabean oil and homogenised in an electric blender for 5 min. The sample was then centrifuged at 3000 rpm for 10 min and the supernatant was left undistur-

bed to separate into emulsified layers. The EC was calculated using eqn. 3:

$$\text{Emulsification capacity (\%)} = \frac{V_1 - V_2}{w} \times 100 \quad (3)$$

where V_1 = volume of oil mixed to prepare emulsion (mL); V_2 = volume of oil released after centrifugation (mL); W = sample weight (g, db).

Particle size: The protein fractions were analysed for particle size distribution using a laser light particle size analyzer (Shimadzu SALD-2300, Shimadzu Corporation, Japan) as per Deb *et al.* [16] with slight modifications. An amount of 0.5 g of albumin, globulin, glutelin protein sample was individually dissolved in 9.9 mL of aqueous ethanol and 0.1 g of prolamin protein sample was dissolved in 9.9 mL of deionised water. After preparation of the sample, protein fractions were vortexed for 5 min. Finally, the prepared sample was added to a cuvette until the refractive index reached in between 20 and 40%.

FTIR analysis: FT-IR analysis was performed at room temperature by using a Perkin-Elmer spectrometer (Lambda 750s; Perkin-Elmer Instruments, USA). It was conducted as per the method of Kyomugasho *et al.* [17]. In brief, 1 g of sample was mixed with 100 mg of KBr dics and ground under infrared lamp irradiation. The mixture was then compressed into a transparent sheet with appropriate diameter and thickness by using a tablet press and then subjected to FT-IR scan at 4000-400 cm^{-1}.

Surface hydrophobicity: The surface hydrophobicity was measured according to the SDS-binding method given by Yao *et al.* [18] with slight modifications. A 1 mL of SDS solution (0, 0.02, 0.04, 0.06, 0.08 and 0.1 mmol/L) was taken and added to 20 mL of chloroform and 5 mL of 0.24 g/L methylene blue solution. The solution was centrifuged at 3457 g for 20 min. The mixture of SDS and methylene blue to the bottom was collected. Absorbance was taken at the wavelength of 655 nm. The surface hydrophobicity of the protein fractions was expressed as 1 mg of protein combined with SDS (μg/mg). About 10 mg of sample was dissolved in 40 mL of 0.1 mmol/L SDS solution and stirred for 4 h at room temperature. The mixture was centrifuged at 3500 g for 10 min and the supernatant was mixed with 20 mL of chloroform. The collected chloroform was added along with 5 mL of 0.24 g/L methylene blue solution. The mixture was mixed and centrifuged at 1500 g for 20 min. The mixture of SDS and methylene blue at the bottom was taken for the absorbance taken at the wavelength of 655 nm. Surface hydrophobicity was calculated using the following eqn. 4:

$$\text{Surface hydrophobicity} = \frac{A_1 - A_2}{A_3} \quad (4)$$

where A_1 is the total SDS quantity, A_2 is the unbound SDS quality and A_3 is the sample quantity.

Free and total sulphydryl content: Free and total sulphydryl content was calculated according to Ellman's reagent (DTNB) colorimetry, to measure the free and total sulphydryl of protein [19]. The sample (0.05 g) was mixed with in 4 mL of 8 mol/L urea tris-gly solution (pH 8) and centrifuged at 10414 g for 15 min. The supernatant was obtained and protein content was determined by biuret method. Each Protein fractions sample (1 mL) was added to 5 mL of 8 mol/L urea tris-gly solution (pH 8) and 8 mL of 4 mg/mL DTNB and placed in a water bath at 25 ºC for 25 min. Absorbance (B) was taken at the wavelength of 412 nm and blank control was replaced by the protein sample solution with 8 mol/L urea tris-gly solution (pH 8). Free sulphydryl content was calculated according to the following formula:

$$\text{FSC } (\mu\text{mol/g}) = 73.53 \times \frac{A_{412} \times D}{\rho} \quad (5)$$

where FSC is the free sulphydryl content; D is the dilution factor (D = 6.04 for free sulphydryl content), ρ is the mass concentration of the protein sample (mg/mL), 73.53 is the molar extinction coefficient of Ellman reagent [L/(mol cm)].

The liquid protein sample (0.6 mL) was added with 3 mL of 8 mol/L urea Tri–Gly solution (including 40 mmol/L DTT with pH 8). The mixture was placed in a water bath at 25 ºC for 1 h and added with 6 mL 12% TCA. The mixture was placed again in the water bath at 25 ºC for 1 h and then centrifuged at 3214 g for 10 min. The precipitate was washed four times with 12% TCA and dissolved in 9 mL of 8 mol/L urea Tris–Gly solution. The mixture was added with 0.09 mL of4 mg/mL DTNB solution and placed in a water bath for 25 min at 25 ºC. Absorbance (B1) was recorded at the wavelength of 412 nm.

$$\text{TSC } (\mu\text{mol/g}) = 73.51 \text{B1} \frac{D}{\rho}$$

where TSC is the free sulphydryl content and D is the dilution factor (D = 16.15 for total sulphydryl content determination).

Statistical analysis: All the experiments were performed in triplicate. One-way analysis of variance (ANOVA) was performed to check the variation in proper ties. Simultaneously, Duncan's multiple range tests (SPSS version 16.0, IBM, USA) were applied to check the homogeneity among the protein fractions at a probability factor of 0.05.

RESULTS AND DISCUSSION

Proximate analysis: The proximate composition of SSC-P and SSC-DP is given in Table-1. A significant variation ($p < 0.05$) was observed between the two types in most of the compositional parameters, indicating the impact of dephenolisation on the nutritional profile. As expected, the phenolic content reduced drastically in SSC-DP (0.67 ± 0.14 mgGAE/g) compared to SSC-P (5.02 ± 0.02 mgGAE/g). This significant reduction confirms the effectiveness of dephenolisation in removing the phenolic compounds. Their high presence may reduce protein digestibility and mineral bioavailability [20]. Hence, dephenolisation can improve the overall digestibility of SSC. In terms of protein content, SSC-DP exhibited significantly higher value (38.71 ± 0.19%) compared to SSC-P (34.02 ± 1.29). This increase is likely because phenolised cakes retain phenolic-protein complexes, which reduce protein extractability and purity. The presence of phenolic compounds like chlorogenic acid, a dominant phenolic compound in SSC [21] binds to protein *via* hydrophobic interactions forming non-covalent complexes [22]. Furthermore,

TABLE-1
PHYSICO-CHEMICAL DATA OF SSC-P AND SSC-DP

Composition	SSC-P	SSC-DP
Moisture (%)	9.34 ± 0.61^a	9.24 ± 0.23^b
Ash (%)	7.92 ± 0.76^a	7.84 ± 0.1^b
Fiber (%)	17.23 ± 0.02^a	17.01 ± 0.02^b
Protein (%)	34.02 ± 1.29^b	38.71 ± 0.19^a
Fat (%)	2.21 ± 0.39^a	1.81 ± 0.05^b
Carbohydrates (%)	24.06 ± 0.34^a	24.04 ± 0.40b
Phenolic content (mgGAE/g extract)	5.02 ± 0.02^a	0.67 ± 0.14b

Superscripts (a-b) within a row shows significant differences ($p < 0.05$) between native and dephenolised samples.

washing with solvents like ethanol removes ~96% of phenols from the meal. Hence, dissolving phenolics, effectively and breaking the hydrophobic seals between phenolics and proteins [10]. The presence of high protein content in sunflower seed cake indicate that sunflower seeds or sunflower seed cake can be used to develop new value-added products [23,24]. The fat content showed a statistically significant decrease after the dephenolisation process. The lower value of fat for SSC-DP may be attributed primarily to the solvent-based extraction process employed during dephenolisation, which may co-extract lipid soluble components along with phenolic compounds [25]. Moreover, phenolic compounds can form complexes with lipids, potentially stabilizing or protecting them from oxidation. Their removal may lead to minor reduction in fat content. Crude fiber and ash content were slightly but significantly reduced in SSC-DP compared to SSC-P, possibly due to partial leaching or breakdown of cell wall- bound phenolic-fiber complexes [25]. Moisture and carbohydrate contents showed only minor differences, though statistically significant, indicating limited impact of dephenolisation on these components of SSC.

Protein yield and content of dephenolised and phenolised sunflower seed cake protein: Protein yields and total protein content of different fractions of native and dephenolised SSC are shown in Tables 2 and 3, respectively. Among all fractions, globulins represented the predominant protein class, contributing 65.01% and 69.07% of the total protein in native and dephenolised SSC, respectively. The higher proportion of globulin in dephenolised SSC suggests improved extractability of salt-soluble proteins following phenolic removal. The albumin fraction was the second major component, making up about 17.02 and 17.41% in native and dephenolised SSC, respectively. Dephenolisation resulted in a slight increase in yield with the albumin fraction from 5.36 g in the native form to 5.80 g. This indicates that with dephenolisation, there is enhanced solubilisation of water-soluble proteins. Globulins, particularly 11S helianthinin, are the predominant storage proteins in sunflower seeds. These phenolic compounds are known to form strong hydrophobic and hydrogen bonds with seed proteins especially, globulins and hinder the extractability and relative proportion of the globulin protein fraction. Karefyllakis *et al.* [26] have demonstrated that solvent based dephenolisation effectively disrupts these complexes, resulting in increased proportion of globulins. Moreover, globulins recovered from SSC-DP exhibit superior functional properties like enhanced emulsification, foaming, solubility compared to those from phenolic rich SSC. The findings suggest that phenolic removal not only improves the purity of protein besides also enriches the globulin content by minimizing interference from non-proteinaceous polyphenolic constituents. A combination of albumin and globulin protein together showed that SSC had the highest proportion of albumin and globulins. While globulins constitute approximately 60-70% of the total seed protein, albumin-specifically 2S albumin make up around 30% of the protein content [27]. Although less abundant than globulins, albumins stand out for their pronounced functional properties. As per Janssen *et al.*'s report [28] albumin fractions were found to exhibit higher foaming capacity and foam stability than globulin fractions thus making them valuable for food applications requiring aeration and stability in foamed systems. In addition, SSC albumins are rich in sulphur containing amino acids and methionine, contributing to its favourable amino acid profile, making them more digestible with a high biological value and digestibility compared to legume proteins [29]. The minor fractions included prolamins and glutelins, which together contributed less of the total protein content in both samples. However, no significant difference was seen in the protein content and yield of prolamin and glutelin fractions

TABLE-2
PROTEIN FRACTIONS, YIELD AND PROTEIN CONTENT OF PHENOLISED SUNFLOWER SEED CAKE

Protein fractions	Extract I (g)	Extract II (g)	Extract III (g)	Total yield (g)	Total protein (%)
Albumin	2.20 ± 0.60^a	1.82 ± 0.51^b	1.34 ± 0.06^c	5.36 ± 0.38^e	17.02^x
Globulin	8.75 ± 0.09^a	7.32 ± 0.04^b	3.55 ± 0.06^c	19.62 ± 0.18^d	65.01^w
Prolamin	1.32 ± 0.01^a	1.01 ± 0.01^b	0.45 ± 0.01^c	2.78 ± 0.01^f	9.2^y
Glutelin	1.21 ± 0.009^a	0.97 ± 0.01^b	0.21 ± 0.01^c	2.39 ± 0.009^g	7.9^z

Superscripts (a-c) within a row;(d-g) and (w-z) within the column shows significant differences ($p < 0.05$) between phenolised protein fractions.

TABLE-3
PROTEIN FRACTIONS, YIELD AND PROTEIN CONTENT OF DEPHENOLISED SUNFLOWER SEED CAKE

Protein fractions	Extract I (g)	Extract II (g)	Extract III (g)	Total yield (g)	Total protein (%)
Albumin	2.30 ± 0.03^a	1.94 ± 0.12^b	1.56 ± 0.03^c	5.80 ± 0.38^e	17.41^x
Globulin	9.04 ± 0.11^a	8.10 ± 0.34^b	3.78 ± 0.32^c	20.92 ± 0.18^d	69.07^w
Prolamin	1.30 ± 0.21^a	1.14 ± 0.03^b	0.60 ± 0.14^c	3.04 ± 0.20^f	8.04^y
Glutelin	1.74 ± 0.29^a	0.98 ± 0.26^b	0.34 ± 0.31^c	3.06 ± 0.19^f	6.18^z

Superscripts (a-c) within a row; (d-f) and (w-z) within the column shows significant differences ($p < 0.05$) between dephenolised protein fractions.

of SSC-P and SSC-DP. This is largely due to their low native abundance in SSC. While globulins and albumins constitute the bulk of SSC proteins, glutelin and prolamin together constitute less than 10% of the total protein content [30]. Almost all proteins derived from seed cakes showed such a low quantity of prolamin and glutelin content and globulin as the typically predominant protein fraction [31-33].

Functional properties of phenolised and dephenolised sunflower seed cake protein

Water holding capacity and oil holding capacity: The WHC and OHC of SSC protein fractions is significantly influenced by their interaction with polyphenols and the data is shown in Fig. 1a-b. In SSC-DP, the WHC and OHC of albumin were 4.16 ± 0.04 g/g and 2.14 ± 0.03 g/g, respectively, which were significantly higher when compared to SSC-P (2.82 ± 0.05 g/g and 1.89 ± 0.04 g/g, respectively). Globulin also recorded high WHC and OHC values of 3.65 ± 0.02 g/g and 3.16 ± 0.02 g/g in SSC-DP compared to 2.45 ± 0.05 g/g and 2.14 ± 0.04 g/g in SSC-P, respectively. Glutelin and prolamin fractions had relatively lower values but followed the same trend, with improved hydration and oil-binding properties after dephenolisation. These results indicate WHC increased significantly after dephenolisation. The phenolic compounds are abundant in the untreated sample and they tend to form non-covalent and covalent complexes with proteins, thereby reducing the exposure of hydrophilic site necessary for efficient water absorption [34]. Through dephenolisation process, these polyphenol-protein interactions are disrupted leading to structural modifications including decreased crystallinity and increased flexibility of protein chains [35]. Hence, causing enhancement in the WHC of proteins by exposing polar groups and increasing their interaction with water molecules. Among the four protein fractions, albumin demonstrated the highest WHC, attributing to its high solubility and abundance of hydrophilic residues. Globulin follows showing significant improvement after removal of phenols due to reduced aggregation and improved solubility. Glutelin although structurally rigid and require alkaline conditions for its solubilisation, shows moderate improvement after dephenolisation. In contrast, prolamin characterised by inherent hydrophobicity remains largely unaffected. This pattern reinforces the importance of dephenolisation and remains same for both SSC-P and SSC-DP although with a significant difference.

Oil holding capacity is protein's ability to entrap lipids. The OHC is enhanced by hydrophobic amino acid residues, porous protein structure and surface interactions. The protein-polyphenol complexes occlude hydrophobic amino acid residue sites and reduce the porosity, impairing lipid retention. Dephenolisation removes the bounded phenolics and hence the protein networks become more open and flexible. These structural changes expose the lipid binding sites, substantially enhancing OHC. Among protein fractions, globulins were found to possess the maximum OHC due to more accessible hydrophobic patches and enhanced structure after dephenolisation. Albumins, despite being more hydrophilic displayed improved OHC post-dephenolisation. Glutelin gained modest OHC due to structural rigidity, while prolamins being insoluble and tightly packed, retain poor oil binding capacity even after dephenolisation, therefore, the relative OHC improved significantly.

Foaming capacity: The foaming capacity are critical for their application in aerated food systems like bakery products. The comparative analysis for foaming capacity of SSC-P and SSC-DP protein fractions are shown in Fig. 1c. Albumins, being water soluble and relatively small, exhibited the highest foaming capacity (21.19%). Globulin also exhibited moderate foaming performance with enhanced interfacial adsorption, whereas glutelin and prolamins being more hydrophobic and less soluble reduced the surface activity and exhibited less foaming performance. It can be seen that in both cases the protein fractions presented a stark difference and a higher value for SSC-DP. The noticeable difference is obviously due to dephenolisation, which improves the foaming behaviour of SSC proteins. The removal of phenolic compounds reduces the protein aggregation and allows more protein molecules to interact freely with the air-water interface. This results in stronger and more elastic interfacial films that stabilize better over a period of time [36]. Furthermore, this improvement can be attribute to the reduced protein-polyphenol interactions and increased solubility. On the other hand, in SSC-P the polyphenols bind to protein side chains, particularly on globulin and glutelin, due to their higher content of aromatic and hydrophobic residues, which have a greater affinity for polyphenols, impairing their ability to unfold and adsorb at the air-water interface. The absolute values improve markedly following dephenolisation in SSC-P and SSC-DP protein fractions.

Emulsification capacity: Fig. 1d showed the emulsification capacity of phenolised and dephenolised SSC protein. Albumin again demonstrated the maximum emulsifying capacity of 60.04 ± 0.14%, followed by globulin with an emulsifying capacity of 49.92 ± 0.18%, glutelin 41.91 ± 0.24% and prolamin 40.05 ± 0.31% in SSC-DP. Corresponding values in SSC-P are 58.55 ± 0.15%, 47.92 ± 0.08%, 40.23 ± 0.17% and 39.81 ± 0.06%, respectively. SSC-P reduced interfacial activity due to protein-phenol complex formation, which hampers the protein unfolding and adsorption at the oil-water interface, thereby decreasing both emulsification capacity and emulsion stability. Dephenolisation disrupts these complexes, increasing solubility, structural flexibility and ability to form robust interfacial films. Li *et al.* [8] employed green solvent defatting and reported that sunflower protein isolate displayed an improved structural looseness and solubility, suggesting enhanced emulsifier function. Another study conducted by Huang *et al.* [37] conducted a comparative analysis of two sunflower protein concentrates, *e.g.* one prepared through chlorogenic acid removal using isoelectric precipitation followed by ultrafiltration (AI-SPC), and the other obtained *via* alkaline extraction coupled with ultrafiltration (AU-SPC). Their study revealed that AU-SPC exhibited superior emulsifying capacity and greater emulsion stability compared to AI-SPC. It also presented that smaller oil droplet sizes, smoother surface morphology and higher protein recovery and purity.

Albumin fraction innately highly soluble showed the highest enhancement in emulsifying performance, by almost doubling the performance post-dephenolisation. Globulins, although less soluble in water, stand to gain form better disper-

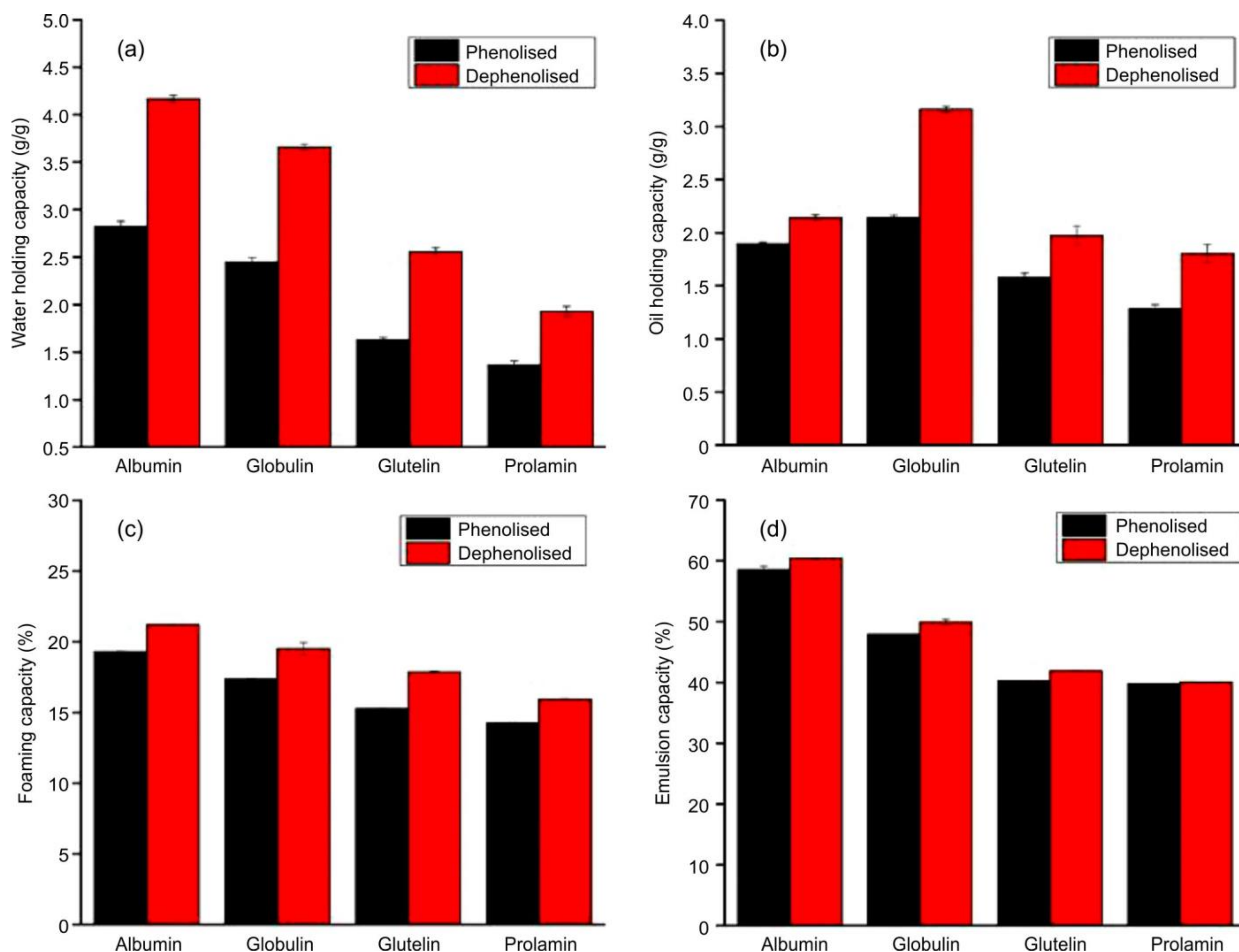


Fig. 1. Functional properties of phenolised and dephenolised sunflower seedcake protein isolate (a) water holding capacity, (b) oil holding capacity, (c) foaming capacity and (d) emulsification capacity

sion and less agglomeration. Glutelin showed modest improvements, while prolamins exhibited little to no enhancement in emulsification performance; similar to the trend observed for foaming capacity, this pattern persisted after dephenolisation, although the absolute emulsifying capacity (EC) values increased.

Particle size: The particle size of protein fractions in SSC is greatly affected by the presence of phenolic compounds (Fig. 2a). A significant decrease in the particle size was observed after dephenolisation. Phenolics like chlorogenic, prior to dephenolisation, create non-covalent (hydrophobic and hydrogen bonds) and covalent (quinone-mediated) interactions with protein molecules, resulting in aggregation and increased particle size [38]. These interactions lower solubility and modify the functional activity of proteins by hiding reactive groups. Within the fractions, globulins (886.26 ± 4.20 µm) and glutelins (746.81 ± 2.45 µm) have the highest phenolic binding because of their hydrophobic residues and restricted solubility, leading to bigger and irregular aggregates and albumins (595.05 ± 4.45 µm) are relatively dispersed, while prolamins (624.62 ± 2.39 µm) have medium aggregation. Following dephenolisation, disruption of protein-phenol crosslinks results in decrease of particle size from the extraction of phenolics. This decrease of binding forces triggers disaggregation and protein structure unfolding with elevated hydration and dispersion. Recent studies further confirm these findings. According to Prignet *et al.* [39] ethanol-mediated dephenolisation of sunflower seed cake proteins significantly improved the protein dispersion. Similarly, optimised ethanol extraction shifted the molecular weight profiles toward smaller aggregates [40]. Further Zhang *et al.* [41] conducted a comparative study on canola and soybean proteins and reported that fractionation improved protein solubility and reduced the average particle size. Consequently, the average hydrodynamic diameter measured by dynamic light scattering became significantly smaller, producing fractions with more uniform particle sizes.

Among the fractions, albumins experience a minor particle size decrease due to their already high solubility and limited phenolic interaction. Globulins have a huge size reduction upon dephenolisation, suggesting the breaking of phenolic crosslinks and better salt solubility. Prolamins, being less polar, experience moderate decrements, whereas glutelins have the most significant particle size decline as a result of the disruption [40]. Therefore, the expected particle size hierarchy after dephenolisation globulin < glutelin < prolamin < albumin.

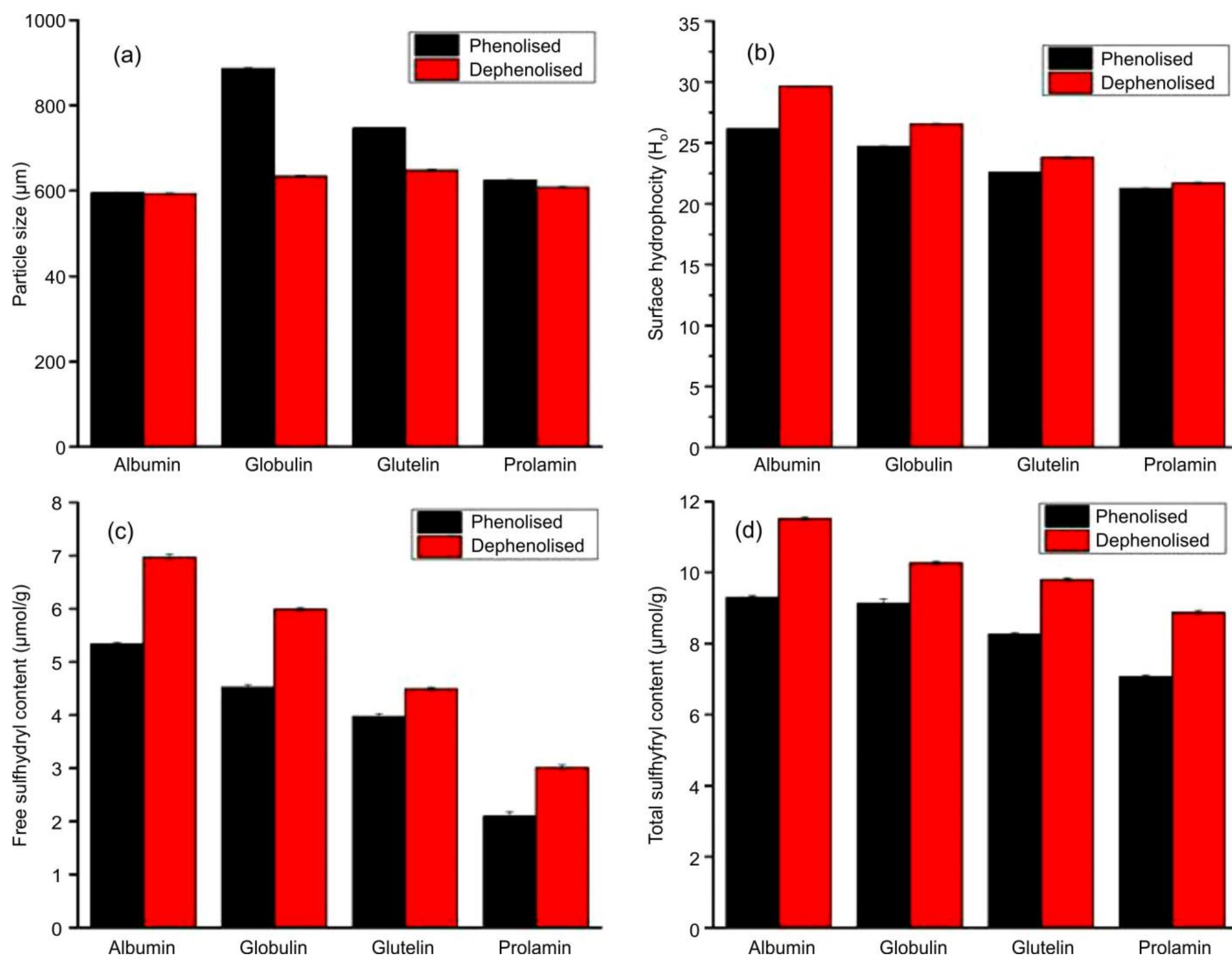


Fig. 2. Surface hydrophobicity, free and total sulphydryl content and particle size of phenolised and dephenolised sunflower seed cake protein isolate (a) particle size, (b) surface hydrophobicity, (c) free sulphydryl content and (d) total sulphydryl content

Surface hydrophobicity: The surface hydrophobicity (H_o) reflects the extent of exposed non-polar amino acid residues in proteins, which directly influences solubility, emulsification and foaming behaviour. Fig. 2b summarizes the effect of dephenolisation on the surface hydrophobicity of SSC protein fractions. A significant difference was observed between phenolised and dephenolised fractions across all proteins. Among the fractions, albumin exhibited the highest surface hydrophobicity (26.11), followed by globulin, glutelin and prolamin. This trend remained consistent for both SSC-P and SSC-DP samples, although the magnitude of hydrophobicity differed significantly. Dephenolisation markedly modified the protein structure by disrupting covalent and non-covalent interactions formed between phenolic compounds and amino acid side chains. This treatment facilitated the restoration of native conformations and exposed previously masked hydrophobic regions. Albumin showed the highest increase in surface hydrophobicity, likely due to its relatively flexible tertiary structure, which allowed extensive unfolding and re-exposure of non-polar residues once phenolics were removed. In contrast, globulin exhibited only a moderate increase in hydrophobicity, indicating partial refolding and limited exposure of hydrophobic sites. This behaviour can be attributed to its more compact quaternary structure, which restricts extensive conformational rearrangement. Glutelin and prolamin, being salt-soluble and alcohol-soluble fractions, respectively, displayed comparatively smaller increases in hydrophobicity after dephenolisation. Their limited response suggests that these fractions underwent only partial tertiary structure restoration, consistent with their inherently rigid or aggregated nature. These findings align with Kaur & Prasad [12] and Akharume *et al.* [42] where phenolic interactions were found to either conceal or expose hydrophobic residues based on protein structure and the nature of phenol–protein binding.

Free and total sulphydryl content: Fig. 2c-d shows the free and total sulphydryl content of the protein fractions and it can be observed that phenolised protein fractions have less sulphydryl groups, which suggests the oxidative coupling and protein crosslinking, leading to decreased solubility and flexibility but enhanced structural rigidity. On the other hand, dephenolised protein fractions have higher sulphydryl content implying restored thiol functionality, improved emulsifying and foaming properties and greater reactivity toward struct-

ural modifications [43]. Albumin is water soluble and rich in reactive cysteine residues; hence phenolics can easily mask them. The phenolised fraction of albumin exhibit the lowest total and free -SH content due to its compact globular nature and strong interactions with phenolics. Upon dephenolisation it shows significant increase in free –SH groups due to the release of bound thiols and partial unfolding. Globulins form large quaternary structures; phenolics may oxidize accessible thiols on the surface. Hence, it was followed by albumin and an increase in the total and free -SH content was observed reflecting reversible phenol–protein interactions. Glutelin has a high cysteine content and tends to polymerize easily under oxidative conditions. Followed by albumin and globulin, an increase in total –SH content was measured in glutelin after dephenolisation, suggesting partial unfolding and exposure of buried cysteine residues. Prolamins are rich in cysteine and glutamine; phenolic oxidation promotes –S–S– formation. Dephenolisation increased both free and total -SH bonds in prolamin fraction due to breakdown of disulphide-linked aggregates. Hence, the overall trend follows, albumin < globulin < prolamin < glutelin. This trend is consistent with earlier reports [44,45] and also concluded that the phenolic–protein coupling, through covalent thiol–quinone reactions or hydrophobic binding, effectively blocks accessible cysteine residues and promotes inter-protein cross-linking.

FTIR analysis: Fig. 3 represents the FTIR spectra of phenolised and dephenolised protein fractions. The spectra revealed consistent amide-I (~1700-1600 cm^{-1}), amide-II (~1600-1500 cm^{-1}) and amide-III (~1400-1200 cm^{-1}) bands, for each globulin, albumin, glutelin and prolamin. Phenolised fractions showed systematic downshifts and broadening of the amide-I band and increased O–H/N–H stretching band width (~3400-3200 cm^{-1}), consistent with stronger H-bonding and partial unfolding due to protein–phenolic interactions; most profound effects were found in globulin followed by glutelin and least in prolamin. Phenolised globulin exhibited a more intense and broadened amide-I band, suggesting disruption of ordered α-helical structures and an increase in β-sheet or aggregated conformations [46]. Dephenolised globulin showed sharper, better-resolved peaks, reflecting a more native conformation. Dephenolised albumin retained higher structural integrity, showing less alteration in the fingerprint region (1200-900 cm^{-1}). Glutelin, a more hydrophobic fraction, displayed broader peaks overall whereas phenolised glutelin revealed greater reductions in amide-I intensity and an increase in aromatic ring vibrations around 1600-1510 cm^{-1}, consistent with the bound phenolic groups. The dephenolised form had more distinct secondary structure signatures, implying removal of phenolic interferences [47]. Prolamin, rich in non-polar residues, showed minimal shifts in the main Amide bands, but phenolisation caused a significant increase in the C–H stretching intensities (2950-2920 cm^{-1}), indicating increased hydrophobic clustering. Dephenolised prolamin displayed clearer amide-III and distinct peaks in the fingerprint region, reflecting improved structural clarity [48]. Furthermore, the dephenolised fractions exhibit sharper amide I/II peaks and a relative recovery of fingerprint-region features (1200-900 cm^{-1}), indicating removal of phenolic adducts and partial restoration of native secondary-structure peaks. The relative sensitivity followed the order globulin > glutelin > albumin > prolamin, consistent with globulin's larger surface exposed binding sites and hydrophobic domains that favour phenolic binding; this fraction-specific pattern agrees with recent studies showing that phenolic removal reduces surface hydrophilicity and restores clearer FTIR secondary-structure signals in sunflower protein isolates [30]. Thus, the FTIR indicates that phenolic complexation induces conformational loosening and increased hydrogen bonding, while dephenolisation partially reverses these modifications and clarifies the secondary-structure features.

Conclusion

The present study demonstrated that dephenolisation significantly enhances both the functional and nutritional properties of SSC protein fractions. The removal of phenolic compounds effectively improved protein purity and overall

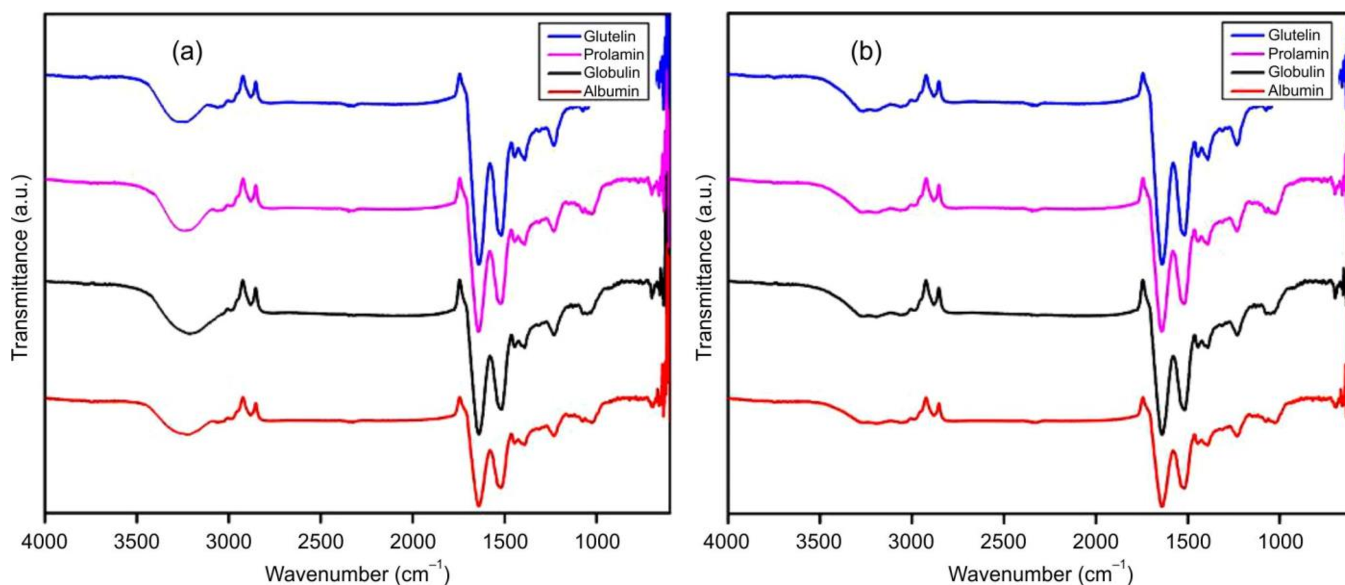


Fig. 3. FTIR spectra of phenolised (a) and dephenolised (b) sunflower seed cake protein

digestibility of the protein's fractions albumin, globulin, prolamin and glutelin. Functional characteristics such as WHC and OHC, emulsifying activity and foaming ability were notably improved after dephenolisation, indicating the reduction of protein-phenol interactions enhanced protein flexibility and surface activity. Among all fractions, globulin and albumin showed the most remarkable enhancement in functional attributes, suggesting their superior potential for food formulation applications. Nutritionally, phenolic removal facilitates enzyme accessibility and minimizes anti-nutritional intervention. These enhancements emphasize dephenolisation as an efficient strategy to upgrade the quality of plant-derived proteins from oilseed by-products containing anti nutritional compounds such as phenols and the value of SSC as a cost-effective, sustainable and nutrient-rich protein source which can be utilised in the development of functional and fortified food products. Altogether, this comparative analysis reinforces dephenolisation as an effective way to alleviate the negative effects of phenolic compounds but additionally enhances the techno-functional performance and nutritional potential of sunflower seed cake protein fractions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

REFERENCES

1. G. Francesca, C. Costanza, N. Federica and P. Laura, *Food Funct.*, **16**, 1992 (2025); https://doi.org/10.1039/D4FO04530K
2. F. Girotto, M. Merlino, G. Giovanelli, C. Condurso and L. Piazza, *Int. J. Food Sci. Technol.*, **59**, 4784 (2024); https://doi.org/10.1111/ijfs.17208
3. M. Cisneros-Yupanqui, V.I. Chalova, H.R. Kalaydzhiev, D. Mihaylova, A.I. Krastanov and A. Lante, *Food Bioprocess Technol.*, **14**, 1962 (2021); https://doi.org/10.1007/s11947-021-02695-y
4. B.G. Subaşı, F. Casanova, E. Capanoglu, F. Ajalloueian, J.J. Sloth and M.A. Mohammadifar, *Food Biosci.*, **38**, 100749 (2020); https://doi.org/10.1016/j.fbio.2020.100749
5. P. Vartiya, P. Kashyap, B. Raj, M. Sharma, M. Adnan, S.A. Ashraf and R. Mehra, *J. Agric. Food Res.*, **22**, 102088 (2025); https://doi.org/10.1016/j.jafr.2025.102088
6. A. Michalska-Ciechanowska, J. Brzezowska, K. Lech, K. Masztalerz, M. Korzeniowska, A. Zambrowicz and M. Szoltysik, *Foods*, **13**, 1433 (2024); https://doi.org/10.3390/foods13101433
7. M. Sen and D.K. Bhattacharyya, *Plant Foods Hum. Nutr.*, **55**, 265 (2000); https://doi.org/10.1023/A:1008152201872
8. Z. Li, X. Huang, Y. Hu, S. Ma, K. Gafurov, F. Gu, Q. Guo and Q. Wang, *Int. J. Biol. Macromol.*, **310**, 143552 (2025); https://doi.org/10.1016/j.ijbiomac.2025.143552
9. AOAC, Official Methods of Analysis of AOAC International, Rockville, MD, USA: AOAC International, edn. 20 (2016).
10. B. Dermanovc, J. Vujetic, T. Sedlar, D. Dragojlovic, L. Popovic, P. Kojic, P. Jovanov and B. Saric, *Foods*, **14**, 1762 (2025); https://doi.org/10.3390/foods14101762
11. I. Zardo, N.P. Rodrigues, J.R. Sarkis and L.D. Marczak, *J. Sci. Food Agric.*, **100**, 578 (2020); https://doi.org/10.1002/jsfa.10051
12. R. Kaur and K. Prasad, *Int. J. Food Sci. Technol.*, **57**, 3990 (2022); https://doi.org/10.1111/ijfs.15769
13. A. Mathews, A.D.S. Tangirala, N. Thirunavookarasu S, S. Kumar, A. Anandharaj and A. Rawson, *Food Chem. Adv.*, **2**, 100326 (2023); https://doi.org/10.1016/j.focha.2023.100326
14. D. Sert, H. Rohm and S. Struck, *Foods*, **11**, 4029 (2022); https://doi.org/10.3390/foods11244029
15. F. Tounkara, T. Amza, C. Lagnika, G.W. Le and Y.H. Shi, *Songklanakarin J. Sci. Technol.*, **35**, 159 (2013).
16. S. Deb, Y. Kumar and D.C. Saxena, *Food Chem. X*, **13**, 100205 (2022); https://doi.org/10.1016/j.fochx.2022.100205
17. C. Kyomugasho, S. Christiaens, A. Shpigelman, A.M. Van Loey and M.E. Hendrickx, *Food Chem.*, **176**, 82 (2015); https://doi.org/10.1016/j.foodchem.2014.12.033
18. G.L. Yao, W. He, Y.G. Wu, J. Chen, X.W. Hu and J. Yu, *Int. J. Food Prop.*, **22**, 1283 (2019); https://doi.org/10.1080/10942912.2019.1642355
19. S. Žilić, G. Akıllıoğlu, A. Serpen, M. Barać and V. Gökmen, *Food Res. Int.*, **49**, 1 (2012); https://doi.org/10.1016/j.foodres.2012.06.031
20. F. Shahidi and M. Naczk, Phenolics in Food and Nutraceuticals, CRC Press (2003).
21. G.M. Weisz, D.R. Kammerer and R. Carle, *Food Chem.*, **115**, 758 (2009); https://doi.org/10.1016/j.foodchem.2008.12.074
22. B. Saricaoglu, H. Yılmaz, B.G. Subaşı and E. Capanoglu, *Food Res. Int.*, **164**, 112345 (2023); https://doi.org/10.1016/j.foodres.2022.112345
23. A. Blicharz-Kania, A. Pecyna, B. Zdybel, D. Andrejko and A. Marczuk, *Sci. Rep.*, **13**, 10864 (2023); https://doi.org/10.1038/s41598-023-38094-w
24. H. Rohm, S. Morejón Caraballo, A. Salvador, S. Mendo, E. Llorca, S. Cattaneo, I. De Noni, S. Struck, R. Foschino and I. Hernando, *Foods*, **14**, 1489 (2025); https://doi.org/10.3390/foods14091489
25. W. Jia, K. Kyriakopoulou, B. Roelofs, M. Ndiaye, J.-P. Vincken, J.K. Keppler and A.J. van der Goot, *Food Chem.*, **362**, 130204 (2021); https://doi.org/10.1016/j.foodchem.2021.130204
26. D. Karefyllakis, S. Altunkaya, C.C. Berton-Carabin, A.J. van der Goot and C.V. Nikiforidis, *Food Hydrocoll.*, **73**, 326 (2017); https://doi.org/10.1016/j.foodhyd.2017.07.018
27. L.T.D. Alves, P. Fronza, I. Gonçalves, W.A. da Silva, L.S. Oliveira and A.S. Franca, *Polymers*, **16**, 1905 (2024); https://doi.org/10.3390/polym16131905
28. F. Janssen, V. Monterde and A.G. Wouters, *Compr. Rev. Food Sci. Food Saf.*, **22**, 1517 (2023); https://doi.org/10.1111/1541-4337.13120
29. R. Kaur and G. Ghoshal, *Adv. Colloid Interface Sci.*, **306**, 102725 (2022); https://doi.org/10.1016/j.cis.2022.102725
30. M.A. Malik, H.K. Sharma and C.S. Saini, *J. Food Sci. Technol.*, **53**, 3455 (2016); https://doi.org/10.1007/s13197-016-2320-y
31. U.C. Jha, H. Nayyar, S.K. Parida, R. Deshmukh, E.J. von Wettberg and K.H. Siddique, *Int. J. Mol. Sci.*, **23**, 7710 (2022); https://doi.org/10.3390/ijms23147710
32. K. Kotecka-Majchrzak, A. Sumara, E. Fornal and M. Montowska, *J. Sci. Food Agric.*, **101**, 297 (2021); https://doi.org/10.1002/jsfa.10643
33. M. Borchani, H. Yaich, F. Abbès, C. Blecker, S. Besbes, H. Attia and M. Masmoudi, *Waste Biomass Valoriz.*, **12**, 1749 (2021); https://doi.org/10.1007/s12649-020-01111-4
34. Y. Feng, C. Jin, S. Lv, H. Zhang, F. Ren and J. Wang, *Antioxidants*, **12**, 1577 (2023); https://doi.org/10.3390/antiox12081577
35. R. Kaur, G. Ghoshal and S. Chauhan, *J. Food Meas. Charact.*, **18**, 3708 (2024); https://doi.org/10.1007/s11694-024-02443-8
36. P. Kashyap, C. Singh Riar and N. Jindal, *Biomass Convers. Biorefin.*, **14**, 4883 (2024); https://doi.org/10.1007/s13399-022-02740-6
37. X. Huang, Y. Li, C. Cui and D. Sun-Waterhouse, *Food Chem.*, **439**, 138090 (2024); https://doi.org/10.1016/j.foodchem.2023.138090

38. B. Masoumi, M. Tabibiazar, Z. Golchinfar, M. Mohammadifar and H. Hamishehkar, *Crit. Rev. Food Sci. Nutr.*, **64**, 3539 (2024); https://doi.org/10.1080/10408398.2022.2132376
39. S. Prigent, Ph.D. Thesis, Interactions of Phenolic Compounds with Globular Proteins and Their Effects on Food-Related Functional Properties, Wageningen University, Netherlands, pp. 131-133 (2005).
40. I.D. Nwachukwu and R.E. Aluko, *Food Chem.*, **255**, 216 (2018); https://doi.org/10.1016/j.foodchem.2018.02.068
41. Q. Zhang, Z. Cheng, Y. Wang and L. Fu, *Crit. Rev. Food Sci. Nutr.*, **61**, 3589 (2021); https://doi.org/10.1080/10408398.2020.1803199
42. F.U. Akharume, R.E. Aluko and A.A. Adedeji, *Compr. Rev. Food Sci. Food Saf.*, **20**, 198 (2021); https://doi.org/10.1111/1541-4337.12688
43. D. Tazeddinova, M.R. Rahman, S.B. Hamdan, M.M. Matin, M.B. Bakri and M.M. Rahman, *BioResources*, **17**, 7110 (2022).
44. F. Shahidi and C.S. Dissanayaka, *Food Prod. Process. Nutr.*, **5**, 2 (2023); https://doi.org/10.1186/s43014-022-00121-0
45. H. Yilmaz, B. Gultekin Subasi, H.U. Celebioglu, T. Ozdal and E. Capanoglu, *Front. Nutr.*, **9**, 914118 (2022); https://doi.org/10.3389/fnut.2022.914118
46. K. Schild, F.D. Sönnichsen, D. Martin, V.M. Garamus, A.J. Van der Goot, K. Schwarz and J.K. Keppler, *Food Chem.*, **426**, 136496 (2023); https://doi.org/10.1016/j.foodchem.2023.136496
47. E. Torres-Sánchez, B. Hernández-Ledesma and L.F. Gutiérrez, *Foods*, **12**, 2401 (2023); https://doi.org/10.3390/foods12122401
48. J.T. Lembe, Ph.D. Thesis, Hemi-synthesis and Biological Activity of Naturally Occurring Compounds from Two Cameroonian Medical Plants: *Hypodaphnis zenkeri* Engl. Stapf. (Lauraceae) and *Staudtia kamerunensis* warb. (Myristicaceae), University of Johannesburg, South Africa (2024).