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Protein extracts from de-oiled sunflower cake: Structural, physico-chemical and functional properties after removal of phenolics

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Abstract

The effects of dephenolization on structural, physico-chemical and functional properties of sunflower protein isolate obtained from de-oiled sunflower cake were investigated. Proximate analysis showed that the moisture and crude protein content for sunflower dephenolized protein (SPI-DP) increased by 2 and 7% compared to the natural sunflower protein (SPI-N) samples, while the ash content and phenolic compounds decreased 1 and 5%, respectively. Powder of SPI-DP had a yellowish color and analysis using scanning electron microscopy showed a rough and spongy surface compared to SPI-N. The isoelectric point of SPI-N and SPI-DP were observed at pH 4.37 and 4.82, respectively. Analysis of mineral composition showed a lowered amount of minerals (except for Se and Sr), as a result of phenolic removal. Secondary structures of the protein did not change but lower hydrophilicity was observed after phenolic removal using Fourier transform infrared spectroscopy. No difference was observed using differential scanning calorimetry on the glass transition and denaturation temperatures. Higher values for foam capacity and foam stability were observed for SPI-DP compared to SPI-N. Interfacial rheological measurements showed that structure strength at the air/water interface increased after phenolic removal.

Keywords: sunflower cake, de-phenolized sunflower (*Helianthus annuus*) protein, foaming properties, interfacial rheology.
1. Introduction

Increasing population requires increased food production. Sustainable and economical food production is one of the most crucial challenges to be solved for the present and next generations (Foley et al., 2011). There is a strong interest in the industry and academy to manage and process food wastes and by-products (Ravindran and Jaiswal, 2016). Biological conversions, extraction and purification of desired compounds from food by-products/side streams of fruit, meat and dairy production are of importance for “zero waste” (Mirabella et al., 2014; Otles and Kartal, 2018). The outcomes could be energy and biogas (Xu et al., 2019), valuable enzymes (Uçkun et al., 2014), antioxidants (Stockhammer et al., 2009), carbohydrates and derivatives (Yilmaz-Çelebioğlu et al., 2012), lipids and derivatives (Dahiya et al., 2015) and proteins (Boland et al., 2013).

Proteins are a valuable nutrition component with specific physico-chemical and functional properties (Foegeding and Davis, 2011). Protein modifications to improve shelf life and structural stability, bioavailability and sensorial, physical and chemical quality would be beneficial (Alavi et al., 2018; Babaei et al., 2019; Çakır Fuller, 2015; García-Moreno et al., 2016). Animal-based proteins are the most used due to their better functional properties (Foegeding and Davis, 2011). Despite being the most favorable protein sources (cheaper and easy to obtained compared to vegetal proteins), animal based proteins have some drawbacks. Due to some reasons like animal welfare issues, increasing veganism/vegetarianism, religious prohibitions for believers and diet concerns have led to a tendency to avoid consuming animal based foods. Extracting and purifying more plant based proteins may be desirable to replace animal based proteins (Chung et al., 2018; Gonzalez-Perez and Vereijken, 2007).

Sunflower seeds are one of the most global crops for vegetable oil production (USDA, 2018). De-oiled sunflower cake is a valuable by-product and used as a proteinaceous animal feed, without any toxic compounds (Gonzalez-Perez and Vereijken, 2007; Sari et al., 2015).

Sunflower protein consists of two major protein groups: 11S globulin (helianthinin) and 2S albumin with a ratio of 2:1 (Mazhar et al., 1998). Sunflower seeds also contain phenolic compounds, predominantly chlorogenic acid which has a high antioxidant capacity (Salgado et al., 2012). However, during the protein extraction process, simultaneous polyphenol oxidation and phenolic-protein interactions lead to a dark green color. The nutritional value and functionality also decrease (Weisz et al., 2010). Previously, González Pérez and Vereijken (2007), Malik et al. (2016) and Pickardt et al. (2009) investigated the characterization and functional properties of sunflower protein isolate (SPI) obtained from sunflower seeds. Conde et al. (2005) and Dabbour et al. (2018) improved the isolation efficiency using an enzymatic
treatment of de-oiled sunflower cake. According to the extraction protocol and raw material, physico-chemical properties as well as functionalities of SPI from fresh sunflower seed or industrial de-oiled cake may possibly be different (Gonzalez-Perez and Vereijken, 2007). The objective of the present study was to investigate the physico-chemical and functional properties of SPI. The physico-chemical properties of natural (N) and de-phenolized (DP) SPI were analyzed. The thermal characteristics were investigated using differential scanning calorimetry (DSC) and the secondary structure using Fourier transform infrared spectroscopy (FTIR). And the interfacial viscoelasticity and foaming properties of SPI-N and SPI-DP were explored as well as their possible applications in foods or biomaterials.

2. Materials and methods

2.1. Isolation of protein

De-oiled sunflower cake was kindly donated by the Olin Edirne Oil Co. (Edirne, Turkey). Sunflower seeds were cleaned and shells were cracked. Seeds were heated to \( \sim 110 \) °C, pre-pressed and treated with hexane. The cake was further pressed and sunflower oil was extracted. After deoiling, the fresh cake was transferred from the factory to the laboratory in 3 h, dried at 45 °C and stored at 4 °C until protein extraction (for a maximum of 2 months). For a single extraction batch, 50 g dried meal was ground with a stainless steel grinder (Delonghi KG49, Treviso, Italy) for 30 sec until the flakes would pass through a sieve of 0.5 mm mesh size. SPI-DP was obtained by suspending the dried meal in 1000 mL 60% methanol and washing 4 times for a total of 8 h to remove phenolic compounds. Phenolic free meal was dried at 30 °C overnight in an oven, for residual methanol evaporation in the cake (Malik et al., 2017). SPI-N was obtained by suspending the meal in 1000 mL distilled water. pH was then adjusted to 8 with 1 N NaOH and left on a magnetic stirrer for 1 h and cooled on ice. Ultrasound treatment (Branson Digital Sonifier SFX 550, St. Louis, MO, USA) at 20 kHz (using 100% power of 550 W) with a 0.5-inch probe, maximum temperature of 30 °C, 10 min was applied. The probe ultrasonication device was equipped with a temperature control system to stop sonication when the preset maximum temperature was reached. Time of 10 sec on, 10 sec off, was applied to increase the protein yield using a protocol adopted from Dabbour et al. (2018). After protein extraction, the slurry was centrifuged at 10,000 x g for 20 min at 24 °C (Thermo Scientific Sorvall RC 6 Plus Centrifuge, Fisher Scientific, Waltham, MA, USA). Supernatant was collected and the cake was discarded. pH of this supernatant was adjusted to 4.5 with 1 N HCl for isoelectric precipitation and the solution was left 30 min (Malik et al., 2017). Then the
solution was centrifuged one more time with the previous conditions. Supernatant was
discarded, all precipitates were collected, washed twice with distilled water and freeze-dried
(Christ Beta 1-8 LSCplus, Osterode am Harz, Germany). All the chemicals used were of
analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Physico-chemical characterization

2.2.1. Chemical composition

Moisture, crude protein and ash contents of SPI, were determined according to AOAC
standards (2006), (method numbers 925.1, 981.10 and 923.03, respectively). After overnight at
105 °C, moisture content was measured using a Shimadzu MOC63u UniBloc Moisture
Analyzer (Kyoto, Japan). A ST 20000 ash oven (T = 600 °C) from Sistek Electronics (Ankara,
Turkey) was used to determine the ash content. Crude protein amount was determined using an
Elementar rapid MAX N exceed (Elementar, Langenselbold, Germany) using the Dumas
method and 5.7 (Claughton and Pearce, 1989) was used as the nitrogen conversion factor. The
amount of total phenolic compounds (TPC) was determined with Folin-Ciocalteau reagent
based on the method of Spanos and Wrolstad (1990). Phenolics in the 100 µl sample were
oxidized with 5 ml 0.2 N Folin-Ciocalteau reactive agent in a stabilized alkaline environment
with 4 ml 6% Na₂CO₃ for 2 h. Absorbance was measured at 765 nm against blank using Optima
SP-3000 nano spectrophotometer (Tokyo, Japan). TPC was reported as “mg gallic acid
equivalent (GAE) 100 g⁻¹ dry weight (dw)”.

2.2.2. Color measurement and macro imaging

The color values and macro images of powdered SPI were obtained with a
VideometerLab 2 (Videometer A/S, Herlev, Denmark). This is a hyperspectral imaging
system which enables the measurement of the sample’s surface color. Radiometric calibration
was done with a white and a dark targets. A geometric target was also used for geometric
calibration which enables obtaining the hyperspectral images of non-uniform shaped and/or
roughened surface samples. The Videometer has been used to analyze the surface color
changes of foods after processing (Rabeler & Feyissa, 2018). For each sample, the light setup
of the device was adjusted with standardized targets before the measurement. The
hyperspectral images were analyzed using MATLAB software (R2017a, The Mathworks Inc.,
Natick, MA, USA). \(L^*a^*b^*\) values were obtained based on CIELAB color space where \(L^*\) defines the lightness, \(a^*\) indicates green to red color and \(b^*\) indicates blue to yellow color.

2.2.3. Amino acid composition

Samples were hydrolyzed with 6 M HCl (10 mg:1 ml) for 18 h at 110 °C for amino acid profile analysis. Samples were cooled to room temperature (20 ± 1 °C), diluted 3 times with the HCl. Each 100 µl sample mixture was further diluted with 1.5 ml 1 M NaCO₃ and filtered using a 0.2 µm syringe filter (Q-max PTFE, Ø 13 mm, Frisenette ApS, Knebel, Denmark). Filtrate was derivatized using a Phenomenex® EZ:FaastTM Amino Acid Analysis kit (Torrance, CA, USA) and analyzed with an LC-(APCI)-MS (Agilent 1100, Agilent Technology, Santa Clara, CA, USA) using the method proposed by Safitri et al. (2017).

2.2.4. Mineral composition

Inductively coupled plasma mass spectrometry (Thermo iCAPq ICPMS, Thermo Electron, Fisher Scientific, Waltham, MA, USA) after microwave-assisted digestion (Multiwave 3000, Anton Paar, Graz, Austria) with concentrated nitric acid (SPS Science, Paris, France) was used for mineral composition analysis. Digested samples were diluted with ultrapure water (Millipore, Milford, MA, USA) before analysis. Quantification was done using external linear calibrations with yttrium as an internal standard. All calibration standards and the internal standard were prepared from element-specific certified stock solutions (SPS Science). For quality assurance, a certified reference material (DORM-4 fish protein, National Research Council of Canada, Ottawa, ON, Canada) was used.

2.3. Scanning electron microscopy (SEM)

SPI surface imaging was done using a Thermo Scientific FEI Quanta 3D FEG (Fisher Scientific). A small amount of sample was placed on the sample holder with a double-sided adhesive carbon tape. To obtain good image quality, samples were coated with gold with a sputter time of 8 sec and 40 mA using a Q150T Quorum (Quorum Technology Ltd., Lewes, UK).

2.4. FTIR spectra

A Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA), based on a Universal Attenuated Total Reflectance sensor 125 (UATR-FTIR) was used to measure the
FTIR spectra of powdered SPI samples in the transmission mode over a range of 4000 – 500 cm\(^{-1}\). The spectra were plotted as percentage transmittance (\%T) as a function of wavenumber (cm\(^{-1}\)). Automatic signals were obtained for every 4 scans (resolution of 0.25 cm\(^{-1}\)).

2.5. Measurement of \(\zeta\)-Potential

The \(\zeta\)-potential measurements were done using a Zetasizer Nano ZS (Malvern Instruments, Ltd., Malvern, Worcestershire, UK) coupled with an autotitrator (MPT-2, Autotitrator, Malvern Instruments Ltd.) to determine the surface charge of the protein as a function of the pH. To adjust the pH of the solution (from 2.0 – 8.0), 0.1 M NaOH and 0.1 M HNO\(_3\) were used. Measurements were done at 25 °C. Protein solutions were prepared at 0.2% final concentration at pH 7.0 using 0.05 M imidazole buffer and left to dissolve overnight on a magnetic stirrer at room temperature. For measurements, DTS-1070 disposable folded capillary cells (Malvern Instruments, Ltd.) were used. \(\zeta\)-Potential was calculated by the software with the instrument using Henry’s equation:

\[
\zeta = \frac{3\eta \mu}{2\varepsilon f(\kappa R_H)}
\]  

where \(\eta\) is the solution viscosity (Pa s\(^{-1}\)), \(\mu\) is the electrophoretic mobility (V Pa\(^{-1}\) s\(^{-1}\)), \(\varepsilon\) is the medium dielectric constant (\(\varepsilon = 80\)) and \(f(\kappa R_H)\) is the Henry’s function. \(\kappa\) is the Debye length (nm) and \(R_H\) is the particle radius (nm) and the \(R_H\) is accepted as 1.5 due to the aqueous medium used for the analysis, referred to as the Smoluchowski approximation. Isoelectric point (pI) was determined by the intersection on the x-axe at 0 mV. The values between –2.2 and +1.8 mV (dotted lines) were not possible to calculate properly due to the presence of aggregates.

2.6. Differential scanning calorimetry (DSC)

Thermal analysis of SPI was done using a TA differential scanning calorimeter (DSC 250, TA Instruments, New Castle, DE, USA) equipped with Trios software and Refrigerated Cooling System 90. The cell was purged with 50 ml min\(^{-1}\) of dry nitrogen and the instrument was automatically calibrated using directly an indium standard (\(T_{\text{peak}} = +156.5^\circ\text{C}, \text{DH} = 28.5\text{ J/g}\)) and a sapphire standard for specific heat capacity, provided by the company. Protein isolates were placed in the desiccator (the desiccant was LiCl) to stabilize its moisture content at \(\sim 10\%\) for 48 h. Approximately, 3.5 mg sample was weighed into an aluminum pan and hermetically sealed. An empty hermetically sealed identical pan was used as the reference. The scanning temperature was raised from -20 to +150 °C with a temperature modulation program.
operated by the software, with 5 °C min⁻¹ scan rate. No sample loss was observed by weighing the pans before and after scanning. Total heat flow was shown as W g⁻¹. Glass transition temperature (Tg), denaturation temperature (Td) and enthalpy of thermal degradations were obtained with a graphical analysis by the Trios software. Experiments were replicated three times.

2.7. Foaming properties

SPI samples were dissolved in 30 ml 0.05 M imidazole buffer (pH 7) to obtain a 2% crude protein solution and left overnight on a magnetic stirrer. pH was adjusted with 0.1 M NaOH or 0.1 M HCl. Foam was obtained using an Ultra-Turrax homogenizer (IKA – Yellowline, DI 25 Basic, Staufen im Breisgau, Germany) at 9,000 rpm min⁻¹. The solution was transferred into a 100 ml graduated cylinder immediately after whipping was completed and the tip was sealed with parafilm to minimize air flow. Foaming capacity (FC) and foaming stability (FS) were calculated based on the equations of Jarpa-Parra et al. (2015) and Malik et al. (2017), as shown below:

\[
FC (%) = \frac{V_f - V_i}{V_i} \times 100
\]

\[
FS (%) = \frac{V_{foam} (t_n)}{V_f (t_0)} \times 100
\]

where \( V_f \) is the volume of the protein solution after mixing, \( V_i \) is the volume of the protein solution before mixing, \( V_{foam} \) is the foam volume at any time \( (t_n) \) and \( V_f (t_0) \) is the foam volume immediately after mixing at time zero \( (t_0) \).

2.8. Interfacial properties at the air/water interface

Interfacial rheological properties of SPI were observed using a double wall ring (DWR) accessory for a stress-controlled DHR-2 rheometer (TA Instruments). The trough of DWR was placed on a temperature controlled Peltier plate. Prior to each set of measurements the “Oscillatory Mapping” for the “DWR Geometry” was done using the “Precision Mapping” option. The DWR trough was loaded with 18 - 20 ml 2% SPI solution which was dissolved overnight in 0.05 M imidazole buffer (pH 7.0). When the sample was ready for measurement, the ring was lowered until the first surface contact with the sample was seen. The ring was soaked in the sample at ~500 μm depth to locate the
edges of the DWR precisely at the air–water interface. Storage modulus ($G'$) and complex modulus ($G^*$) coupled with the axial force were obtained as a function of time with an oscillation time procedure ran 60 min. Strain sweep and frequency sweep tests were done to determine the linear region of the oscillation. Strain and frequency were chosen as 0.1% and 1.0 Hz, respectively. All tests were done at 25 °C.

2.9. Statistical analysis

The mean differences ± standard deviation (SD) of the chemical composition, color, ζ-potential, DSC measurements and foaming properties after phenolic compounds removal were tested. Statistical tests were done using one-way analysis of variance test (ANOVA, the level of significance was indicated by $p < 0.05$) followed by Tukey’s test using the Statistical Package for the Social Sciences software (SPSS 22.0, SPSS Inc., Chicago, IL, USA). Data run for statistical analysis were triplicate observations.

3. Results and discussion

3.1. Physico-chemical characterization

3.1.1. Chemical composition

Proximate compositions of SPI-N and SPI-DP are shown in Table 1 ($p < 0.05$). After removal of phenolic compounds, moisture contents of SPI-DP was increased compared with the SPI-N. At the same time, ash content was decreased with phenolics removal. Malik and Saini (2016) reported a moisture content of SPI-N ~9% without any change after the removal of phenolics on sunflower seeds. The authors reported an ash value ~4.6%, which decreased to 3.4% after phenolic removal. The protein content of the crude isolate increased after phenolics removal which was statistically significant ($p < 0.05$). Similar results were also reported in another study of Malik and Saini (2017); it was indicated that protein contents of SPI before and after phenolic removal were ~84 and 89%, respectively. Pickardt et al. (2011) have reported a crude protein content of SPI-N changing between 95.0 and 99.4%. Probably, this variation in protein content could be attributed to different protein isolation and phenolic removal/recovery methods (Pickardt et al., 2015). TPC was found ~5.05 GAE 100 g$^{-1}$ (dry basis). After methanol washing, total phenolic content decreased to ~0.1 GAE 100 g$^{-1}$ (dry basis), meaning that 98% of the initial phenolics present in the SPI-N were removed. Pickardt et al. (2015) reported a reduction of phenolic compounds between 90 and 99%. Salgado et al. (2011) found a reduction
between 29 – 94% whereas Malik and Saini (2017) reported this value as ~92%. According to Romani et al. (2017), different results depend on varying factors such as agricultural conditions or genetic factors.

### 3.1.2. Color measurement and macro imaging

One of the most important challenges to use SPI-N for human nutrition is its strong dark green color (Shchekoldina and Aider, 2014). Macro images of SPI-N and SPI-DP powder from de-oiled sunflower cake were obtained with a hyperspectral imaging system as shown in Fig. 1(A) and 1(B), respectively. SPI-N powder (Fig. 1 A) looks more homogenous compared to SPI-DP powder (Fig. 1 B). At the same time, \(L^*, a^*, b^*\) values were obtained as 33.1 ± 6, -4.7 ± 2, 10.9 ± 3 for SPI-N and 47.5 ± 8, 3.7 ± 2, 18.4 ± 3 for SPI-DP, respectively. The dark green color of SPI-N powder is probably due to the protein extraction process, simultaneous polyphenol oxidation and phenolic-protein interaction (Weisz et al., 2010). Consequently, increases in \(L^*\) (~14%), \(a^*\) (~7%) and \(b^*\) (~8%) were attributed to the de-phenolization process in SPI-D sample.

### 3.1.3. Amino acid composition

Amino acid compositions of SPI-N and SPI-DP are shown in Table 2. Removing phenolic compounds did not cause a significant difference in amino acid contents between SPI-N and SPI-DP. Lysine content of both SPI-N and SPI-DP, as the limiting essential amino acid for SPI, were below the minimum proposed level, which is 24 residues of 1000 residues (FAO, 1981). C-C represents the cystine, the joint form of two cysteine molecules. Similar results were also observed by Ivanova et al. (2013) and Villanueva et al. (2010) for SPI.

### 3.1.4. Mineral composition

Mineral composition of SPI-N and SPI-DP are shown in Table 3. The amount of Co, following by Na, Mg, K, Mn and Zn decreased whereas Se and Sr slightly increased for SPI-DP, compared to SPI-N. This was mainly because phenolic compounds and minerals bind with phytic acid and consequently, methanol washing had a dephenolization and consequently also a demineralization effect on SPI. Phytic acid is a natural substance existing in beans, seeds and nuts and considered as an anti-nutritional agent (Parmar et al., 2017; Maga, 1982). Phytic acid reduction of protein isolates during food processing also leads to demineralization (Miller et al., 1986). Parmar (2017) indicated that, most of the elements have a correlation with the color.
For example, light colored beans have a lower Zn content compared to darker colored beans. This observation is supported by the results in the present study, where the Zn content of SPI-N was 41.3 mg Kg\(^{-1}\) whereas it was lower in SPI-DP (8.4 mg Kg\(^{-1}\)).

3.2. Scanning electron microscopy (SEM)

SEM images of SPI-N and SPI-DP are shown in Fig. 2a and 2b, respectively. After phenolics removal, clear differences appeared between two proteins. Powder of SPI-N (Fig. 2a) looks smooth with sharp edges and some roughened cluster, as indicated with red arrows. On the other hand, SPI-DP (Fig. 2b) has a roughened surface with the presence of large pores. According to Karefyllakis et al. (2017) and Ozdal et al. (2013), phenolic compounds react with the side chains of amino acids and creating bridges between protein subunits. Phenolic removal has a visible roughening effect on the surface. On the other hand, protein-protein interactions might increase and lose elasticity at the interface (Karefyllakis et al., 2017). The smooth/roughened surface forms of the grinds might depend on the presence/absence of polyphenolic compounds.

3.3. FTIR spectra

FTIR spectra of SPI-N and SPI-DP are presented in Fig. 3. SPI-N and SPI-DP showed all five characteristic bands: Amide A broad band represents the N-H stretching coupled with H bonds while Amide B represents CH asymmetric and symmetric stretching, Amide I represents C=O stretching, Amide II represents N-H bending and C-N stretching and Amide III represents N-H bending and C-H stretching-ether functional group (Abedinia et al., 2017; Rodsamran and Sothornvit, 2018; Shanesazzadeh et al., 2018). Similar peak wavenumbers for SPI-N were also reported by Shanesazzadeh et al. (2018). The Amide I and Amide II regions are the most sensitive to secondary structure conformational changes (Shevkani et al., 2015). De-phenolization in general is expected to make the protein structure more ordered due to formation of more structured elements (\(\alpha\)-helix and \(\beta\)-strand) compared with presence of more random coils in SPI-N. The interaction with phenolic compounds leads the protein to be more unordered (Malik et al., 2016). However, no major differences based on the intensity differences of the spectra were observed in \(\beta\)-sheet or \(\alpha\)-helix content of SPI-N and SPI-DP in FTIR, similar to the study of Malik et al. (2016). It is probably because the secondary structures of proteins are mainly a consequence of non-covalent forces such as van der Waals forces, hydrophobic interactions, or hydrogen bonds (Cooper, 1999). In general, it has been reported
that in some protein isolates peaks belonging to Amide I region, i.e. 1600-1700 cm$^{-1}$, can be attributed to those around 1638, 1654, 1663 and 1680 cm$^{-1}$ corresponding to β-sheet, α-helix, β turn and anti-parallel β-sheets (β-A) conformations, respectively. Therefore, the peak in the amide I band at 1634 cm$^{-1}$ for both SPI-N and SPI-DP is correlated with the β-sheet content of the protein isolate. From the other side, having the same frequencies of absorption bands in random coil and helix structures as found by other researchers (Srouf et al., 2017) can be another reason for observing no differences in the corresponding peaks. However a broader peak was observed for SPI-N at ~3285 cm$^{-1}$ compared with SPI-DP. Which may represent a decreased hydrophilicity of SPI-DP after methanol treatment, which led to the removal of phenolics. Other researchers have also shown that SPI-DP shows lower hydrophilicity compared with SPI-N (Malik et al., 2016). Decreased hydrophilicity of SPI-DP might be due to removal of hydroxyl and carboxyl groups of phenolic compounds (Malik et al., 2016).

3.4. Measurement of ζ-potential

ζ-potential results are presented in Fig. 4. The isoelectric point (pI) of SPI-N and SPI-DP were observed to be pH 4.4 ± 0.1 and 4.8 ± 0.19, respectively (p < 0.05). Difference of pI of SPI-N (~ 0.5 units) could be attributed to the presence of high amount of phenolic compounds (Kroll et al., 2003). Both proteins were insoluble in the pH range of 4-5 while above and below this interval, charges vary between -30 and +10 mV. According to González-Pérez et al. (2002, 2003) and Pickardt et al. (2009) pI of SPI (i.e., helianthinin), was between pH 4–5. Recently, Karefyllakis et al. (2019) worked on protein isolates obtained from different fractions of sunflower meal (cake fraction, pure protein, fiber-based mixtures with and without phenolic compounds) and reported the pI value between pH 4–5, with a variation of the net charge below pI to -30 and above pI to +30 mV. Shanesazzadeh et al. (2018) observed a pI of SPI-N close to 4.8. The origin of raw material, different extraction protocols and the removal of phenolic compounds affect the purity of the protein isolate causing a shift in the ζ-potential (González-Pérez et al., 2002; Pickardt et al., 2015, 2011; Weisz et al., 2010).

3.5. DSC

DSC thermograms of SPI-N and SPI-DP are presented in Fig. 5. SPI-N and SPI-DP showed two endothermic peaks. The first one, which can be attributed to the Tg, was found at 37.8 and 37.2°C for SPI-N and SPI-DP. The value difference for Tg was not statistically significant (p < 0.05). The second peak can be attributed to the protein denaturation. SPI-N
denaturation enthalpy, onset point and peak temperature were obtained at 2.03 J g\(^{-1}\), 108.9 and 118.8°C, respectively. On the other hand, SPI-DP showed a denaturation enthalpy, onset point and peak temperature at 1.51 J g\(^{-1}\), 113.2 and 120.1°C, respectively. The peak temperatures of the two protein isolates were not statistically different, however, enthalpy and onset point differences were statistically different (p < 0.05). Molina et al. (2004) observed the denaturation temperature as 105.5°C for 11S in aqueous media at pH 7. The same authors found a denaturation temperature of SPI-N at pH 7.0 and ~100°C. González Pérez (2003) reported the denaturation temperature of pure helianthinin between 65 – 90°C, at pH 7.0. Thermal properties are highly dependent on moisture content and these differences are attributed to the presence of varying amounts of water (Rouilly et al., 2003).

3.6. Foaming Properties

Foaming is the generation of air bubbles throughout the mixture with the aid of a whipping or aeration process (Dickinson, 2010). As a surface active agent, proteins decrease the surface tension and build a viscoelastic region at the air/water interface (Foegeding and Davis, 2011). Foaming is one of the most important functional properties of proteins and influenced by the presence of phenolic compounds (Ozdal et al., 2013). Foam stability (FS) and foam capacity (FC) were presented in Fig. 6. FC of SPI-DP was found ~34% higher than SPI-N at pH 7.0. Malik and Saini (2017) obtained FC values for SPI-N and SPI-DP at 43 ± 0.5 and 48 ± 0.6%, respectively, whereas Shchekoldina and Aider (2014) reported the FC of SPI-DP as 49.0%. SPI-DP presented higher FS compared to SPI-N. In the first 100 min, stability of SPI-N and SPI-DP foams was similar. However, at the end of 180 min, the FC of SPI-DP was ~38% higher than SPI-N. Similar results were reported by Dabbour et al. (2018), Malik and Saini (2017) and Salgado et al. (2012). According to the authors, SPI-DP had higher foam stability than SPI-N after 60 min, mainly due to protein-protein interaction (Karefyllakis et al., 2017; Ozdal et al., 2013; Shahidi and Senadheera, 2018).

3.7. Interfacial properties at the air/water interface

Proteins, due to their amphiphilic nature, accumulate and develop a viscoelastic network at the air-water interface (Poirier et al., 2018). Generally, the presence of non-covalently bound phenolic compounds improve foaming properties at the interface (Jiang et al., 2018). The storage modulus (G') at the air-water interface was observed for SPI-N and SPI-DP during 60 min in Fig. 7 (A). After 20 min, SPI-DP started to develop a viscoelastic region at the interface.
and it was stabilized after ∼40 min. On the other hand, for SPI-N no $G'$ development was observed. In Fig. 7 (B), the complex modulus ($G'$) development with increased axial force over 60 min time sweep was shown. When the axial force was applied gradually below the yield stress, the $G'$ development was observed over time at the SPI-DP air-water interface, as the total resistance to deformation. Similar to that of $G'$, no $G'$ development was obtained at the SPI-N air-water interface.

4. Conclusions

The structural, physico-chemical and functional properties of proteins from de-oiled sunflower cake after phenolic removal (SPI-DP), compared to natural sunflower protein (SPI-N) were determined. After the removal of phenolics, moisture and crude protein contents increased for SPI-DP, while total ash and phenolic compounds decreased. Powder of SPI-DP had a yellowish color with a roughened and perforated surface compared to SPI-N. In terms of amino acid composition, FTIR pattern and thermal analysis, no significant differences were observed between SPI-N and SPI-DP. The isoelectric point was observed as pH 4.37 and 4.82 for SPI-N and SPI-DP, respectively, due to the acidic nature of phenolic compounds. SPI-DP built a viscoelastic region at the air/water interface and showed better foaming properties compared to SPI-N. SPI-DP have interesting physico-chemical and functional properties for possible applications in foods as well as biomaterials.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

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Table 1. Chemical composition of natural (SPI-N) and de-phenolized (SPI-DP) sunflower protein isolates.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Total Phenolics*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI-N</td>
<td>3.16 ± 0.11</td>
<td>1.52 ± 0.45</td>
<td>88.18 ± 1.21</td>
<td>5.05 ± 0.60</td>
</tr>
<tr>
<td>SPN-DP</td>
<td>5.12 ± 0.18</td>
<td>0.64 ± 0.14</td>
<td>95.46 ± 0.42</td>
<td>0.09 ± 0.04</td>
</tr>
</tbody>
</table>

*Total phenolic content is defined as mg gallic acid equivalent (GAE)/100 g dry weight.

Fig. 1. Macro images of SPI-N (a) and SPI-DP (b). Samples were prepared in 30 mm diameter circles and analyzed with Videometer.
Table 2. Amino acid composition of SPI-N and SPI-DP

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>SPI-N (g / 100 g)</th>
<th>SPI-DP (g / 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>12.43</td>
<td>12.56</td>
</tr>
<tr>
<td>Ser</td>
<td>4.65</td>
<td>4.81</td>
</tr>
<tr>
<td>Hyp</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>Gly</td>
<td>4.48</td>
<td>4.52</td>
</tr>
<tr>
<td>Thr</td>
<td>3.78</td>
<td>4.06</td>
</tr>
<tr>
<td>Ala</td>
<td>3.46</td>
<td>3.68</td>
</tr>
<tr>
<td>Pro</td>
<td>3.93</td>
<td>4.38</td>
</tr>
<tr>
<td>Met</td>
<td>1.05</td>
<td>1.79</td>
</tr>
<tr>
<td>Asp</td>
<td>10.27</td>
<td>11.65</td>
</tr>
<tr>
<td>Val</td>
<td>10.87</td>
<td>12.03</td>
</tr>
<tr>
<td>His</td>
<td>2.53</td>
<td>2.86</td>
</tr>
<tr>
<td>Lys</td>
<td>2.92</td>
<td>3.14</td>
</tr>
<tr>
<td>Glu</td>
<td>31.54</td>
<td>30.25</td>
</tr>
<tr>
<td>Leu</td>
<td>6.16</td>
<td>6.81</td>
</tr>
<tr>
<td>Phe</td>
<td>5.44</td>
<td>5.83</td>
</tr>
<tr>
<td>Ile</td>
<td>6.23</td>
<td>7.53</td>
</tr>
<tr>
<td>C-C</td>
<td>0.80</td>
<td>0.93</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.23</td>
<td>2.65</td>
</tr>
</tbody>
</table>
Table 3. Mineral composition of SPI-N and SPI-DP.

<table>
<thead>
<tr>
<th>Empty Cell</th>
<th>(mg kg(^{-1}))</th>
<th>SPI-N</th>
<th>SPI-DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major elements(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>5300</td>
<td>1600</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>350</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>1030</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>1370</td>
<td></td>
<td>1120</td>
</tr>
<tr>
<td>Minor elements(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.35</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>3.9</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>152</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>1.7</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>61.4</td>
<td>42.5</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>41.3</td>
<td></td>
<td>8.4</td>
</tr>
<tr>
<td>Trace elements and other heavy metals(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As</td>
<td>0.1</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>0.2</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>17.3</td>
<td>19.7</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.3</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) SDS <2%.

\(^b\) SDS <5%.
Fig. 2. SEM images of SPI-N (a) and SPI-DP (b). Red arrows in (a) indicate smooth and sharp edged protein clusters.

Fig. 3. FTIR spectra of SPI-N and SPI-DP
Fig. 4. ζ-potential as a function of pH for SPI-N (□) and SPI-DP (●). The values between –2.2 and +1.8 mV (dotted lines) were not possible due to the presence of aggregates. Small error bars overlapped with the symbols.

Fig. 5. DSC thermograms for SPI-N and SPI-DP samples.
Fig. 6. Foam stability (%) as a function of time (min) and foam capacity (insert) for SPI-N and SPI-DP.

Fig. 7. (A) Storage modulus ($G'$) as a function of time; (B) complex modulus ($G^*$) and axial force as a function of time at the air-water interface. Strain = 0.1%, $f$ = 1.0 Hz, $T$ = 25 °C.
References


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