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Functional Food Ingredients Based on Sunflower Protein Concentrates Naturally Enriched with Antioxidant Phenolic Compounds

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Abstract The functional properties of three sunflower protein concentrates having different content of phenolic compounds (mainly chlorogenic and caffeic acids) obtained from sunflower oil cake, a by-product of the oil industry, were evaluated. Sunflower protein concentrates exhibited high water solubility and moderate water-imbibing and water-holding capacities. It was possible to obtain foams and emulsions of different stability at different pH and ionic strength from these protein concentrates, as well as self-supporting gels produced by thermal induction. The presence of phenolic compounds not only conferred antioxidant activity and changed the color of protein products, but also reduced the water imbibing capacity of sunflower protein concentrates, the stability of the emulsions obtained, and the hardness of protein gels. In contrast, phenolic compounds did not modify the water holding capacity, their water solubility or their foaming properties. These results suggest that these protein products may be used as functional ingredients in the food industry.

Keywords Sunflower protein concentrates · Phenolic compounds · Functional properties · Solubility · WIC · WHC · Emulsions · Foams · Gelation

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Introduction

Functional properties of proteins include solubility, water or oil absorption and/or holding, viscosity, foam and emulsion formation and stabilization, and ability to form masses, fibers and gels. Such properties are fundamentally related to the physical, chemical and structural/conformational properties of proteins, which in turn depend on the raw material from which they were obtained and on the processes employed for their isolation. As these properties affect the behavior of proteins in food systems during processing, storage, preparation and consumption, they are of vast technological importance [1]. Given the great interest in elucidating the molecular mechanisms involved in these properties, simple model systems are used to measure specific parameters that give information about the contribution of proteins to the property being considered. Although these determinations do not reflect the behavior of proteins in the end-product, since such products are more complex systems, this knowledge together with measures of the structure–function relationships constitute the bases for rational design of protein ingredients with specific functionality to be used in the development of foods with desired characteristics [2].

Sunflower oil cake, a by-product of the oil industry, is an alternative and economic source of proteins with good nutritional quality. At variance with soy protein isolates, widely used as functional ingredients in the food industry [3], there has been a limited use of sunflower protein concentrates and isolates due to their high content of phenolic compounds, mainly chlorogenic and caffeic acids (1.4–5.8%) [4]. These compounds, which can interact with proteins, were considered to reduce solubility and digestibility of proteins and to affect their color and shelf-life [5]. For many years these arguments led to attempts to develop

processes for obtaining sunflower protein products free from phenolic compounds [4], and to improve their functionality through the structural modification of proteins by means of physical, chemical or enzymatic treatments [6–8].

We have previously reported the preparation of sunflower protein products (concentrates and isolates) with different contents of phenolic compounds, by different methodologies on laboratory and pilot plant scales [9, 10]. All the extraction procedures resulted in protein products with high water solubility (>60%) and in vitro protein digestibility (>95%), but with different chemical composition and physicochemical properties. The complete removal of phenolic compounds was not achieved because of their association with proteins [9]. The residual phenolic compounds endowed antioxidant properties to the protein products, and influenced their color, being more dependent on the conditions used in the preparation process than on the amount of phenolic compounds in the product [10]. Although strong coloration could limit potential applications for these proteins, it also could be neutral or beneficial for other applications, such as plastics used for agriculture [11].

There is a current tendency to keep phenolic compounds in food, or even to incorporate them into its formulation, due to their antioxidant properties and their benefits for disease prevention and aging retardation [12]. Considering this trend and that high water solubility is a frequent prerequisite for good functional properties, it is important to evaluate whether the different sunflower protein products exhibit an adequate functionality to be considered as functional ingredients for the food industry.

The aims of the present study were to assess the functional properties of sunflower protein concentrates with different contents of phenolic compounds and determine the structure–function relationship of sunflower proteins.

Materials and Methods

Materials

Defatted sunflower (*Helianthus annuus* L.) oil cake was provided by Aceitera Santa Clara (Molinos Río de La Plata, Rosario, Argentina). Their proximate composition was (on a dry basis) 31.7% protein, 8.0% ashes, 2.7% phenolic compounds, 1.0% lipids and 56.6% carbohydrates and fibers. This starting material was ground in a mill (Bühler Miag MLGV Variostuhl), and sieved through a 1.19 mm screen to yield the “milled sunflower oil cake”.

Preparation of Sunflower Protein Concentrates

Aqueous dispersions (60 L) of the milled sunflower oil cake (67 g/L) were stirred for 1 h after the pH was adjusted to 9 with 3 M NaOH. Solid–liquid separation was performed in a basket type centrifuge with filtering material at 2,100×*g* and 20 °C for 30 min. The supernatant was collected and the pellet residue was subjected to a second extraction of proteins as described above. The supernatants obtained after the two protein extraction steps were mixed and an isoelectric precipitation was performed by addition of 3 M HCl until pH 4.5 was reached. After 30 min of incubation at room temperature (~20 °C) with agitation, the insoluble proteins were separated using a Westfalia centrifuge (Westfalia SAADH 205 model, Germany). The isoelectric precipitate was washed once with water at pH 4.5 and centrifuged again; finally it was resuspended in water, i.e. approximately 0.5 L/kg precipitate. The resulting suspension was passed through a Manton–Gaulin two-stage homogenizer (Gaulin Corp., USA), at a pressure of 2×10^5 and of 5×10^5 Pa in the first and second stage respectively. The pH was adjusted to 9 with 3 M NaOH, and the solution was spray-dried at 170–190/80–90 °C (inlet/outlet temperatures) using a Niro Atomizer spray dryer (Niro Atomizer Production Minor, Denmark). The sunflower protein concentrate obtained by isoelectric precipitation as described above was named C + IP [10].

To obtain sunflower protein concentrates with a low phenolic compounds content, the milled sunflower oil cake was subjected to two sequential extractions with water (W) or 1 g/L Na_2SO_3 aqueous solution (S) at acid pH (pH = 5.0), followed by protein extraction in an alkaline medium at pH 9.0 and isoelectric precipitation at pH = 4.5, to get either CW + IP or CS + IP products, respectively [10]. Briefly, to eliminate phenolic compounds, the milled sunflower oil cake (4 kg) was suspended in the extraction medium (67 g/L), the pH was adjusted to 5 with 3 M HCl, and the suspension was stirred for 30 min (during this time period pH was checked every 10 min and kept at 5). Solid–liquid separation was performed in a basket type centrifuge with filtering material at 2,100×*g* and 20 °C for 30 min, and the precipitate obtained was subjected to a new extraction of phenolic compounds under the same conditions. After two extractions, the residue was subjected to protein extraction at alkaline pH and isoelectric precipitation as described above for the preparation of the C + IP product. Three independent preparations of each protein treatment (C + IP, CW + IP and CS + IP) were made.

Characterization of Sunflower Protein Concentrates

Chemical Composition

Moisture content was analyzed following the AOAC Method 935.29 [13]. Total nitrogen was obtained by Kjeldahl (AOAC 920.53, [13]) and transformed into protein content by multiplying by the conversion factor 5.55

[9]. Phenolic compounds were measured by UV spectrophotometry at 324 nm as described by Moores et al. [14], using chlorogenic acid (CGA, Chemika Fluka, Germany) as the standard. All determinations were performed at least in duplicate for each replicate.

Differential Scanning Calorimetry (DSC)

A TA Instrument DSC Q100 V9.8 Build 296 (New Castle, Del., USA) was used for these studies. Hermetically sealed aluminum pans containing 10–15 mg of sunflower protein concentrates dispersed in distilled water (0.2 g/mL) were prepared and scanned at 10 °C/min over the range of 20–120 °C [15]. All assays were conducted in duplicate for each replicate.

Surface Hydrophobicity (Ho)

The surface hydrophobicity (Ho) of sunflower proteins dispersed in distilled water or in the buffer solutions used for evaluate emulsifying and foaming properties was determined according to the method described by Kato and Nakai [16] using a digital fluorimeter Perkin–Elmer model 2000 (Norwalk, CT, USA). All determinations were performed in duplicate for each replicate.

Functional Properties Dependent on Protein–Water Interactions

Protein Solubility in Water

Sunflower protein concentrates were dispersed (1 g/L) in distilled water for 30 min with magnetic agitation, then the pH of the mixture was adjusted to 8 with 1 M NaOH and the dispersion was agitated for an additional 1 h at 20 °C. The dispersion was centrifuged at 23,700×g for 15 min at 20 °C (Avanti J-25, Beckman Coulter, California, USA). Soluble proteins were determined in the supernatant by the Bradford method [17] using bovine serum albumin (Sigma-Aldrich Chemical Co., St. Louis, USA) as standard. Results were expressed as percentage of the original protein content in the starting material determined by the Kjeldahl method [13]. Determinations were performed at least in duplicate for each replicate.

Water Imbibing Capacity (WIC)

The WIC of sunflower protein concentrates was measured in a Baummann apparatus according to a method modified by Sorgentini et al. [18]. The method determines the spontaneous uptake of liquid water by a protein powder at a given temperature. Wetted filter paper is placed in a funnel connected to a graduated water pipette filled with distilled water (1 mL). A sample of spray-dried concentrate (50 mg) was screened and placed as a thin layer on the filter paper while closing the funnel mouth with a hermetic lid. For each concentrate, the water uptake (q in mL H_2O imbibed/g of dry sample) over time (t in s) was recorded at 20 °C until equilibrium was reached. Practical equilibrium conditions were attained when uptake readings in the plateau zone repeated within 0.01 mL. The water uptake curves were described mathematically by Eq. 1, as was proposed by Pilosof et al. [2].

$$q = \frac{\mathrm{WIC} \cdot t}{t_{\mathrm{eq}/2} + t} \quad (1)$$

The water imbibing capacity (WIC in mL H_2O imbibed/g of dry sample) and the imbibing time ($t_{\mathrm{eq}/2}$ in s) were obtained by nonlinear regression using the program Microcal Origin 6.0 (Microcal Software Inc., USA). Measurements were performed in duplicate.

Water Holding Capacity (WHC)

The WHC of sunflower protein concentrates were determined under the action of a centrifugal force. Samples were suspended in distilled water (at 10% w/v) and stirred every 10 min with vortexing for 1 h at 20 °C. Subsequently the supernatant was separated from the precipitate by centrifugation at 9,000×g for 20 min at 20 °C (A15, B. Braun Biotech International, USA). The weight of the pellet and the supernatant-protein content were determined. The WHC (mL H_2O/g protein concentrate) was calculated using Eq. 2, as was proposed by Pilosof et al. [2].

$$\mathrm{WHC} = \frac{m_2 - (m_1 - m_3)}{m_1 \cdot \delta} \quad (2)$$

where, m_1 is the weight of the dry sample (g), m_2 is the weight of the pellet (g), m_3 is the weight of the soluble protein from the supernatant (g) and δ is the density of water at room temperature (1 g/mL). Triplicate determinations were made for each sample.

Functional Properties Dependent on Protein–Surface Interactions

Preparation of Samples

The following buffer solutions were used to disperse the sunflower protein concentrates 0.1 M sodium phosphate buffer pH 3 and 80 mM NaCl; 0.1 M sodium phosphate buffer pH 7 and 80 mM NaCl; 0.1 M sodium phosphate buffer pH 3 and 540 mM NaCl; 0.1 M sodium phosphate buffer pH 7 and 540 mM NaCl. Dispersions were stirred continuously for an hour and centrifuged at 23,700×g for 15 min at 20 °C (Avanti J-25, Beckman Coulter, California, USA).

Protein concentration in the supernatants was quantified by the Bradford method [17], and adjusted to the same protein concentration of 1 g/L by appropriate dilution with the corresponding buffer system. Surface hydrophobicity (Ho) of proteins dispersed in buffer solutions was measured as previously described. Aqueous solutions of a commercial soy protein isolate (SPI, Supro 500E, Solae Company, Brazil) and bovine serum albumin (BSA, Sigma-Aldrich Chemical Co., St. Louis, USA) were used as controls of emulsifying and foaming process, respectively.

Emulsifying Properties

Oil-in-water emulsions (1:4 v/v) were prepared by homogenizing 4 mL of refined sunflower oil (Natura, Aceitera General Deheza, Argentina) and 12 mL of the protein solution (1 g/L) with a homogenizer ULTRA-TURRAX T25 (IKA-Werke GmbH & Co., Germany; rotor diameter = 0.8 mm) at 20,000 rpm for 60 s. Emulsions were performed in duplicate.

The emulsifying activity index (EAI) was determined according to the technique described by Pearce et al. [19]. After emulsion preparation, four aliquots (1 mL) were immediately diluted 250-fold in 0.1 M sodium phosphate buffer pH 7 containing 100 mM NaCl and 0.1% w/v SDS, and the absorbance was measured at 500 nm (Beckman DU 650 Spectrophotometer, Germany). EAI (m^2/g) was defined by Pearce et al. [19] as:

$$\mathrm{EAI} = \frac{2 \cdot 2.303 \cdot \mathrm{Abs}_{500} \cdot D}{L \cdot \Phi \cdot [P] \cdot 1000} \tag{3}$$

where, Abs_{500} is the absorbance at 500 nm, D is the dilution factor (250), L is the path length (0.01 m), Φ is the oil volume fraction (0.25) and $[P]$ is the protein concentration in the solution before emulsification (g/L). Measurements were performed in quadruplicate for each emulsion obtained.

Morphology of the droplets was observed by optical microscopy. Each emulsion (20 µL) was placed on a slide with a cover and immediately examined with a microscope equipped with a Leica DC100 camera (Bensheim, Germany).

Stability of the emulsions was analyzed by dynamic light scattering measurements using a vertical scan analyzer QuickScan (Beckman-Coulter Inc., USA) [20]. Samples were loaded into a cylindrical glass measurement cell and the backscattering percentage profiles (%BS) all along the tube were immediately monitored every 1 min for 1 h as a function of the sample height (total height, 60 mm approximately). Then the cells were allowed to stand undisturbed for 24 h at room temperature (20 °C) and another individual %BS measurement was done. The measured %BS values were plotted against time to facilitate a kinetics analysis. Initial value of backscattering along the tube at time zero ($\%\mathrm{BS}_0$) was used to evaluate the emulsifying capacity. In the bottom of the tube (15–20 mm height) the kinetics of destabilization was determined resulting in an average time value ($t_{1/2}$, s) defined as the time for which $\%\mathrm{BS} = \%\mathrm{BS}_0/2$ [20]. While at the top of the tube (45–50 mm height) the kinetics of creaming was determined. The cream destabilization percentage (%CD) was defined by Palazolo et al. [20] as:

$$\%\mathrm{CD} = \frac{\%\mathrm{BS}_{24\,\mathrm{h}} - \%\mathrm{BS}_0}{\%\mathrm{BS}_0}\,100 \tag{4}$$

where, $\%\mathrm{BS}_0$ is the initial value of backscattering and $\%\mathrm{BS}_{24\ \mathrm{h}}$ is the backscattering at 24 h, both at the top of the tube (45–50 mm height). The determinations were made in duplicate.

Foaming Properties

Measurements were made in a graduated glass column (3 cm × 30 cm) having a glass frit plate (G4 type, 5–15 µm) at the bottom. The column had electrodes coupled to the bottom for conductivity measurement of protein dispersion [21]. A volume of protein solution sufficient to cover the electrodes (6 mL) was added to the column, and the foam was generated by bubbling N_2 (flow rate 1.33 mL/s) for 30 s. Temporal variation of the conductivity of the protein solution was recorded. As the conductivity of the solution is inversely proportional to the volume of liquid incorporated into foam, it was possible to know its variation with time [21, 22]. From these curves the following parameters were determined: the maximum volume of liquid incorporated into the foam (V_{max} in mL), the initial rate of liquid incorporation to the foam (v_o in mL/min), and the time for half-drainage of the liquid that was incorporated to the foam at the end of the bubbling period ($t_{1/2}$ in s). Photographs of the foam were taken with a digital camera (iLook 1321v2, Genius, Taiwan) to study their morphology. Measurements were replicated five times.

Functional Properties Dependent on Protein–Protein Interactions

Gelation

The least gelation concentration (LGC) was determined according to the method of Adebowale et al. [22] with slight modifications. Aqueous suspensions of sunflower protein concentrates were prepared with different concentrations (7.5, 10, 12.5 and 15% w/v) at pH 8 and room temperature. Test tubes containing 5 mL of each suspension were placed in a water bath at 100 °C for 5, 10, 15, 20

and 25 min. The samples were then cooled immediately in a water bath at 4 °C and kept refrigerated at the same temperature for 24 h. LGC was the lowest concentration at which the sample did not fall or slip when the test tube was inverted [22]. The determinations were made in duplicate.

Statistical Analysis

Results were expressed as means ± standard deviations and were analyzed by analysis of variance (ANOVA). Means were tested with the Fisher's least significant difference test for paired comparison, with a significance level $\alpha = 0.05$, using the Statgraphics Plus version 5.1 software (Statgraphics, USA).

Results and Discussion

Characterization of Sunflower Protein Concentrates

Sunflower protein concentrates had similar protein concentrations (≈70% on dry basis, Table 1), represented mostly by globulins and to a lesser extent, albumins and high molecular weight aggregates [10]. These protein products presented thermograms (by DSC, not shown) with a single endotherm at similar denaturation temperatures (≈100 °C) and enthalpies (≈5.5 J/g protein) (Table 1). These enthalpy values were lower than those reported for sunflower oil cakes (9.4 J/g protein) [9] and native sunflower protein isolates (14.5 J/g protein) [4]. These results suggest that proteins were partially denatured during oil extraction due to high temperatures (150–170 °C), pressures (50 kg/m^2), and organic solvent (*n*-hexane) treatments and during the extraction procedures used to obtain protein concentrates, which includes self-desludging centrifugation, colloid milling and spray drying.

Sunflower protein concentrates had different contents of phenolic compounds (Table 1). Over 90% of the phenolic compounds initially present in the sunflower oil cake were removed during the washing steps before protein extraction or in the isoelectric precipitation step. For the C + IP procedure, isoelectric precipitation step removed ~93% of phenolic compounds, whereas in CW + IP and CS + IP procedures, the removal of phenolic compounds occurred mainly in washing steps (~90%, regardless the type of solvent used) and the contribution of the isoelectric precipitation step was only ~5% (data not shown). Although the different procedures assayed to remove phenolic compounds have similar efficiencies, the final color tone of different protein concentrates was more dependent on the conditions used in the preparation process than on the amount of phenolic compounds in the product. Sample C + IP had a greenish color, which can be attributed to the oxidation of phenolic compounds to *o*-quinones during protein extraction in an alkaline medium [5–7, 9]. Protein products subjected to extraction of phenolic compounds with water (CW + IP) or sodium sulfite (CS + IP) had a lighter tone and a more brownish color [9, 10].

While the phenolic content of sunflower protein concentrates did not affect the denaturation degree detected by DSC ($p > 0.05$) as mentioned above, it had a significant influence on the surface hydrophobicity of proteins (Ho) ($p < 0.05$), since the latter increased as the phenolic content of concentrates decreased (see Table 1). This increased surface hydrophobicity indicates that treatments for eliminating phenolic compounds produced conformational changes in proteins that led to an increased exposure of their hydrophobic zones.

Functional Properties Dependent on Protein–Water Interactions

Hydration properties (water solubility, WIC and WHC) of sunflower protein concentrates are shown in Table 2. In agreement with our previous findings [9, 10], all sunflower protein concentrates exhibited high protein solubility in water (greater than 80%) regardless of the concentration of phenolic compounds and the different surface hydrophobicity of proteins (see Table 1) ($p > 0.05$). These values were significantly higher than those reported by other authors for sunflower protein products obtained from laboratory-prepared flours (i.e. by milling defatted sunflower

Table 1 Protein and phenolic content, surface hydrophobicity in water (Ho), denaturation temperature (T_d) and denaturation enthalpy (ΔH) of sunflower protein concentrates

Sample[A]	Protein content (%)	Phenolic content (%)	Ho (UA.ml/mg)	T_d (°C)	ΔH (J/g)
C + IP	70.4 ± 0.8^b	2.5 ± 0.1^c	50.4 ± 0.3^a	100.1 ± 1.6^a	5.4 ± 0.3^a
CW + IP	70.1 ± 1.4^b	2.1 ± 0.1^b	74.6 ± 4.0^b	102.3 ± 0.4^b	5.8 ± 0.3^a
CS + IP	66.7 ± 0.8^a	1.8 ± 0.1^a	91.1 ± 8.6^c	101.0 ± 0.1^{a,b}	5.4 ± 0.2^a

Reported values for each protein product are means ± standard deviations ($n = 2$). In columns, means followed by the same letter are not significantly different ($p \geq 0.05$) according to Fisher's test

[A] Sunflower protein concentrate with isoelectric precipitation (C + IP) and with reduced content of phenolic compounds by extraction with water (CW + IP) or 1 g/L Na_2SO_3 solution (CS + IP)

Table 2 Functional properties dependent on protein–water interactions: water solubility, water imbibing capacity (WIC) and water holding capacity (WHC) of sunflower protein concentrates with different concentration of phenolic compounds

Sample[A]	Water solubility (%)	WIC (ml H_2O/g)	$t_{eq/2}$ (s)	WHC (ml H_2O/g)
C + IP	89.5 ± 4.0[a]	1.9 ± 0.1[a]	172 ± 49[a]	6.1 ± 0.9[a]
CW + IP	93.3 ± 5.3[a]	2.2 ± 0.1[b]	354 ± 50[b]	5.0 ± 0.9[a]
CS + IP	84.8 ± 3.1[a]	2.9 ± 0.3[c]	554 ± 14[c]	5.2 ± 0.4[a]

Reported values for each protein product are means ± standard deviations ($n = 3$). In columns, means followed by the same letter are not significantly different ($p \geq 0.05$) according to Fisher's test

[A] Sunflower protein concentrate with isoelectric precipitation (C + IP) and with reduced content of phenolic compounds by extraction with water (CW + IP) or 1 g/L Na_2SO_3 solution (CS + IP)

seeds without thermal treatments). For example, Rodríguez Patino et al. [8] reported 30% water solubility, while Bau et al. [23] and Sripad et al. [24] reported values between 50 and 55%. The differences between the values reported by these authors and the ones presented in this work can be attributed to different sunflower varieties that can have distinct polypeptide composition, and to changes in the aggregation–dissociation state causes by diverse physical and chemical treatments during sunflower oil cake and protein concentrates preparations.

The water-imbibing capacity (WIC) and imbibing time ($t_{eq/2}$) values of sunflower protein concentrates were obtained by modeling the water uptake curves with Eq. 1 ($r^2 > 0.97$), and are shown in Table 2. The WIC and $t_{eq/2}$ parameters increased after the phenolic compounds were partially removed, and the highest values were reached when these compounds were extracted with sodium sulfite (CS + IP) ($p < 0.05$). The increments of both parameters could be attributed to conformational changes of proteins during the extraction procedures. Hydrophilic groups, as consequence of these conformational changes, are exposed and accessible for interaction with water, in agreement with the decrease in the Ho values (Table 1). The WIC values of sunflower samples varied between 1.9 and 2.9 mL H_2O/g dry solids. These values are similar to those reported for amaranth and quinoa protein isolates (1.7–2.8 mL H_2O/g dry solids) [25], but lower than those reported for rice protein isolates (2.9–4.3 mL H_2O/g dry solids) [26] and soy protein isolates (4.2–12.8 mL H_2O/g dry solids) [27]. The $t_{eq/2}$ values presented in this work (3–9 min) are similar to those found in the literature. According to Petruccelli et al. [27], the WIC is determined mainly by the content and the level of hydration of the insoluble fraction of a protein isolate. Our results show that all the sunflower protein concentrates exhibit low water imbibing capacity, which is consistent with their high values of water solubility.

Sunflower protein concentrates analyzed in the present study exhibited WHC values between 5.0 and 6.1 mL H_2O/g concentrate (Table 2), with no significant differences between them ($p > 0.05$) in spite of their dissimilar phenolic content and the different surface hydrophobicity of their proteins. These values are higher than those reported by other authors for sunflower protein isolates (0.8–3.9 mL H_2O/g isolate) [28] and are within the range of values reported for lupin, rice and quinoa protein isolates (1.4–7.2 mL H_2O/g isolate) [25, 26, 29, 30]. Petruccelli et al. [27] reported WHC values lower than 5 mL H_2O/g isolate for native soy protein isolates, as well as values between 20 and 25 mL H_2O/g isolate for the same isolates after thermal treatment. These authors also observed a correlation between high water solubility and low WHC values, in agreement with the findings of the present study.

Functional Properties Dependent on Protein–Surface Interactions

The ability of solutions of sunflower protein concentrates (1 mg protein/mL buffer) to form and stabilize emulsions and foams was studied. Since pH and ionic strength changes can modify the structure of sunflower proteins [4, 15], also affecting their Ho, these proteins are likely to exhibit a different functional behavior depending on the medium used. For this reason, 0.1 M sodium phosphate buffers with pH 3 or 7 and with 80 or 540 mM NaCl were used to simulate the limit values of pH and ionic strength frequently found in food [4].

The emulsifying properties of the sunflower protein concentrates were also evaluated (Figs. 1, 2). The emulsifying capacity was determined from the emulsifying activity index (EAI) (Fig. 1a). This property was not affected ($p > 0.05$) by differences in composition of sunflower protein concentrates but was significantly affected ($p < 0.05$) by the characteristics of the medium (pH and ionic strength). As an example, images obtained by optical microscopy of CS + IP emulsions (undiluted 10×) (Fig. 1b) showed that initial distributions of drop size were polydisperse and depended on the pH and ionic strength of the medium.

Sunflower protein concentrates at pH 3 and 540 mM NaCl exhibited the lowest EAI values (Fig. 1a), indicating that the same protein mass produced a smaller interfacial area, thus resulting in a lower number of drops of greater size (Fig. 1b). Under these conditions (pH 3 and 540 mM NaCl) helianthinin is in its monomeric form (2S, $\alpha\beta$ subunits) [4, 15]. This fact, together with the high Ho (430–2430 UA ml/mg) and low flexibility of helianthinin could interfere with the rearrangement of macromolecules in the interface and with the formation of an interfacial film.

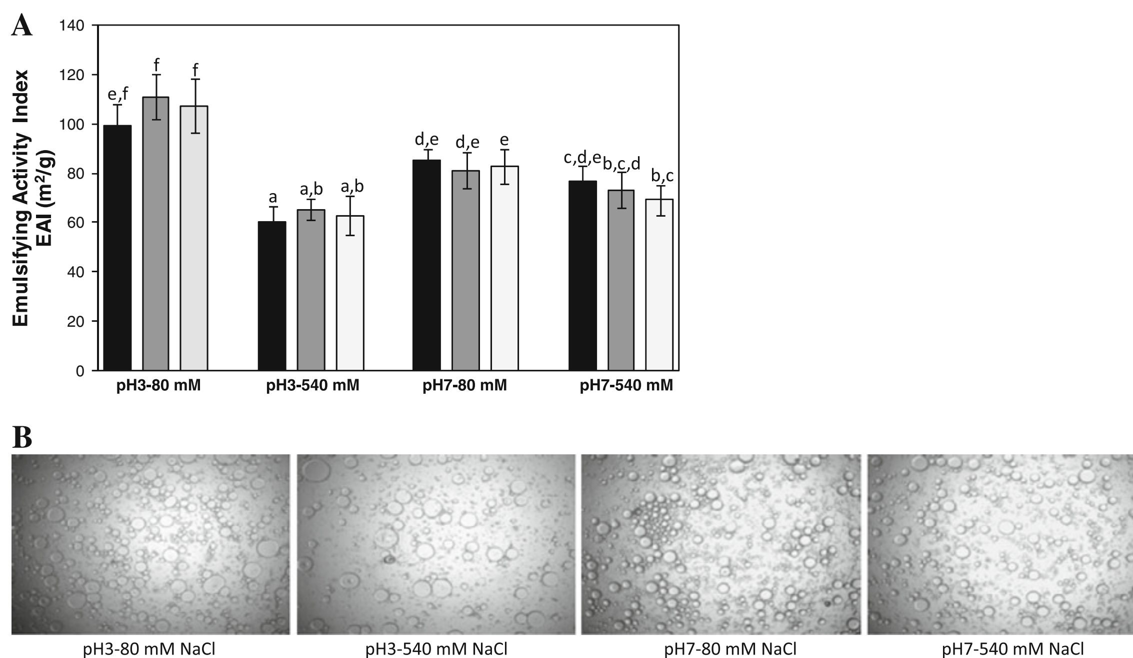

Fig. 1 Ability to form emulsions of sunflower protein concentrates. **a** Emulsifying activity index (EAI) of (*left bar*) C + IP, (*middle bar*) CW + IP and (*right bar*) CS + IP as function of pH and ionic strength. **b** Optical microscopy (10×) of oil-in-water (O/W) emulsions obtained with CS + IP under different conditions of pH and ionic strength. Reported values for each protein product are means ± standard deviations ($n = 4$). Bars followed by the same letter are not significantly different ($p \geq 0.05$) according to Fisher's test

The ability to form emulsions was also evaluated through $\%BS_o$ values. It has been reported that high $\%BS_o$ values indicate a higher drop density in the emulsion [20]. In all the cases studied here the $\%BS_o$ values varied between 54 and 60% (not shown), without any statistically significant differences between them ($p > 0.05$). The differences found when comparing EAI values were not reflected in $\%BS_o$ values.

Stability of emulsions prepared from sunflower protein concentrates was evaluated through $t_{1/2}$ values (Fig. 2a). At neutral pH (pH 7 and 80 mM NaCl and pH 7 and 540 mM NaCl) and with an acidic medium with high ionic strength (pH 3 and 540 mM NaCl), the stability of the emulsions was higher for the samples with lower phenolic content ($p < 0.05$), indicating that the presence of such compounds has a negative impact on this property. A direct relationship between emulsion stability ($t_{1/2}$) and Ho values of proteins (for each medium evaluated) was also observed ($r^2 > 0.90$; Fig. 2b) and was in agreement with data reported by Kato et al. [16] for other proteins. However, such relationship was not unequivocal, indicating that other important factors may be involved in the destabilization process, such as presence of aggregates, net charge, and denaturation degree, whose influence is more difficult to quantify. Emulsions obtained from samples treated with sodium sulfite (CS + IP) were the most stable, indicating that disulfide bonds play an important role in their stabilization. The higher Ho values of these samples as compared to samples not treated with sulfite (C + IP and CW + IP) could also contribute to this phenomenon. All the emulsions obtained at pH 3 and 540 mM NaCl were very unstable (Fig. 2a). The lower stability in this medium can be attributed to the fact that sunflower proteins in their 2S conformation cannot form an interfacial film with adequate properties, and may also be due to differences in protein charge at this pH. Sunflower protein concentrates yielded stable emulsions at low ionic strength (80 mM NaCl), with a $t_{1/2}$ slightly higher at acid pH ($p > 0.05$) (Fig. 2a), probably due to a net positive charge of the protein that would help to keep the oil drops separated from each other by electrostatic repulsion. These results agree with those of González-Pérez et al. [4], who evaluated the stability of emulsions prepared from a sunflower protein isolate with very low phenolic content. In the present study all the emulsions evaluated had a cream destabilization percentage (%CD) between 5 and 15% (data not shown). Notably, this parameter followed the same tendency as the $t_{1/2}$.

Emulsions made from sunflower protein concentrates had characteristics similar to those made from an aqueous solution of a commercial soy protein isolate (1 mg/mL; $BS_o = 57\%$; $t_{1/2} = 2218$ s; $\%CD = 13\%$), the later being an ingredient frequently used in the food industry as an emulsifying agent.

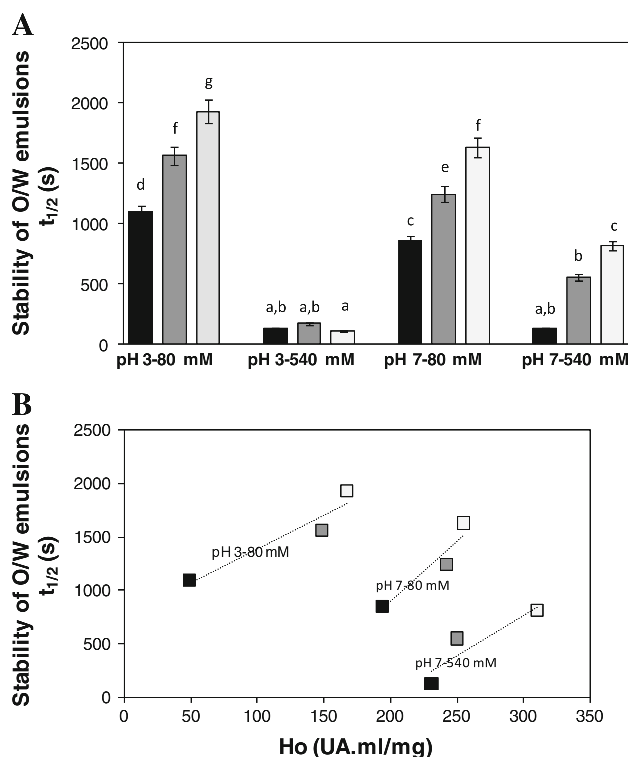

Fig. 2 Capacity to stabilize emulsions of sunflower protein concentrates. **a** Stability ($t_{1/2}$) of oil-in-water (O/W) emulsions obtained with (*left bar*) C + IP, (*middle bar*) CW + IP and (*right bar*) CS + IP as function of pH and ionic strength. **b** Relationships between emulsions stability ($t_{1/2}$) and surface hydrophobicity (Ho) of (*left bar*) C + IP, (*middle bar*) CW + IP and (*right bar*) CS + IP under different conditions of pH and ionic strength. Reported values for each protein product are means ± standard deviations ($n = 2$). Bars followed by the same letter are not significantly different ($p \geq 0.05$) according to Fisher's test

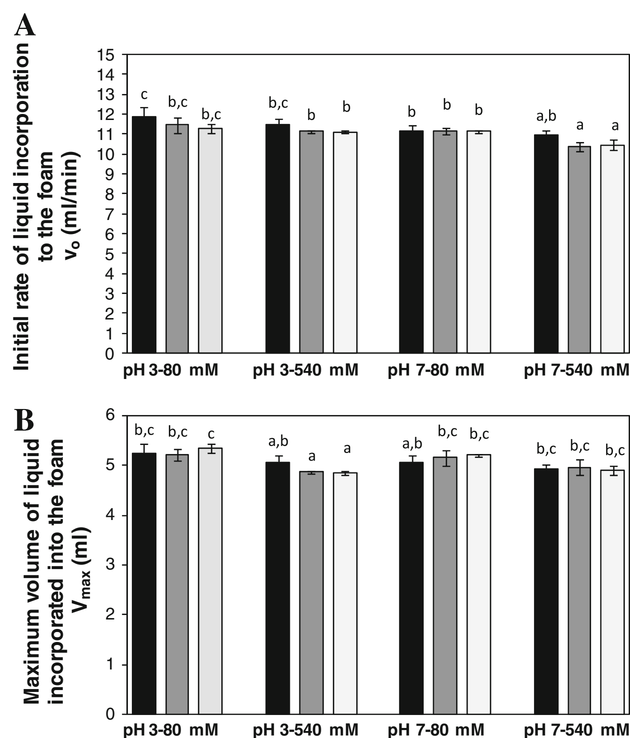

Fig. 3 Ability to form foams of sunflower protein concentrates—(*left bar*) C + IP, (*middle bar*) CW + IP and (*right bar*) CS + IP—under different pH and ionic strength conditions. **a** Initial rate of liquid incorporation to the foam (v_o). **b** Maximum volume of liquid incorporated into the foam (V_{max}). Reported values for each protein product are means ± standard deviations ($n = 5$). Bars followed by the same letter are not significantly different ($p \geq 0.05$) according to Fisher's test

The foaming capacity of sunflower protein concentrates was evaluated by measuring the initial rate of liquid incorporation to the foam (v_o) and the maximum volume of liquid incorporated into the foam (V_{max}) (Fig. 3a, b, respectively). Samples did not show significant differences in v_o and V_{max} values ($p > 0.05$) regardless of their chemical composition or the pH and ionic strength of solution medium used in the foaming assays. These results indicate that proteins present in these samples migrated rapidly to the interface (high v_o values ≈ 10–12 mL/min) and incorporated a great volume of liquid in the foam (V_{max} ≈ 5 mL).

An analysis of $t_{1/2}$ values (Fig. 4a) revealed that only the pH affected significantly ($p < 0.05$) the stability of the foams, which was higher at neutral pH ($t_{1/2}$ ≈ 85–125 s) than at acid pH ($t_{1/2}$ ≈ 55–72 s). At neutral pH, the stability of foams increased slightly with decreasing ionic strength of the medium ($p > 0.05$). It is known that sunflower proteins form 15–18S soluble aggregates at pH 7 and 80 mM NaCl, and that these aggregates may constitute an interfacial protein film with better characteristics than the films formed by proteins in the hexameric conformation (11S, at pH 7 and 540 mM NaCl) [4, 15]. At pH 3, regardless of ionic strength, sunflower proteins acquire positive charge and a monomeric conformation (2S or $\alpha\beta$ subunits) [4, 15], these molecules being smaller and having a lower capacity to form a resistant interfacial protein film.

Figure 4b shows as an example the destabilization of foams obtained with C + IP in medium with low ionic strength (pH 3 and 80 mM NaCl, pH 7 and 80 mM NaCl). At the end of bubbling, foams presented an homogeneous distribution with a great number of small spherical bubbles. While all the destabilization mechanisms occur simultaneously and synergically, liquid drain and bubble floating predominate in recently formed foams. Over time, the characteristics of the foams changed: remaining bubbles increased their size, became polyhedral, and acquired heterogeneous distribution. During this phase the predominating mechanisms are those of disproportion and collapse [1]. These mechanisms were more important in destabilizing foams formed at pH 3 and 80 mM NaCl. This

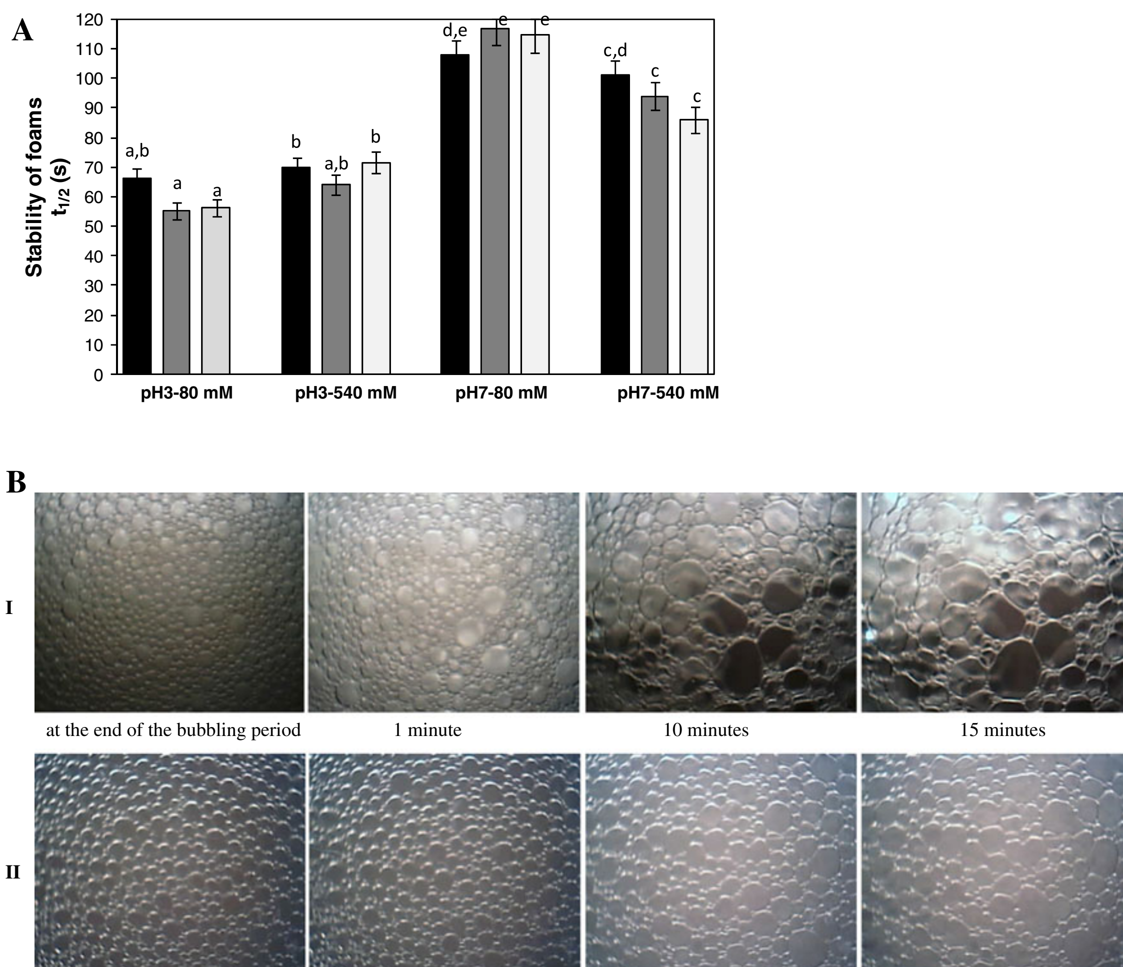

Fig. 4 Capacity to stabilize foams of sunflower protein concentrates. **a** Stability ($t_{1/2}$) of foams obtained with (*left bar*) C + IP, (*middle bar*) CW + IP and (*right bar*) CS + IP as function of pH and ionic strength. **b** Appearance of C + IP foams formed at *I* pH 3–80 mM NaCl and *II* pH 7–80 mM NaCl), at different times after bubbling. Reported values for each protein product are means ± standard deviation ($n = 5$). Bars followed by the same letter are not significantly different ($p \geq 0.05$) according to Fisher's test

result disagrees with observations by González-Pérez et al. [4], who obtained foams by the whipping method from a sunflower protein isolate with very low content of phenolic compounds.

No relationship was observed between foaming parameters and the physicochemical properties of sunflower proteins, including Ho. This result is in agreement with other published works that suggest that foaming ability is related to macromolecule average hydrophobicity and net charge density [1, 16].

Foams studied here exhibited similar characteristics to those obtained from rice protein isolates (at pH 9 $V_{max} = 5.1$ mL, $t_{1/2} = 91$ s; at pH 3 $V_{max} = 4.7$ mL, $t_{1/2} = 58$ s) but had more volume and were more stable than those foams formed from a commercial soy protein isolate ($V_{max} = 3.0$ mL and $t_{1/2} = 46$ s) [26], and also these characteristics were comparable to those of bovine serum albumin foams (1 mg protein/mL of water) ($v_o = 9.7$ mL/min, $V_{max} = 4.7$ mL and $t_{1/2} = 92$ s) which constitutes the commonly used control.

Functional Properties Dependent on Protein–Protein Interactions

The least gelation concentration (LGC) of sunflower protein concentrates that produces a self-supporting gel by thermal induction at 100 °C was determined (Fig. 5a). As expected, an inverse correlation was observed between gelation time and the LGC. The presence of phenolic compounds did not affect gel formation, as the only difference among the concentrates behavior was the LGC for 5 min treatment, being highest for C + IP concentrate. As several plant proteins with a structure similar to that of sunflower proteins such as soy and amaranth proteins exhibit good gelation properties [31, 32], sunflower proteins would be expected to present such properties also.

The lowest concentration required to form gels from amaranth protein isolates is 7% [32], while that for soy isolates varies between 10 and 14% [31]. However, controversial data exist in the literature regarding the gelation capacity of sunflower proteins. On one hand, Sosulski et al. [33] and Sánchez et al. [34] reported that sunflower proteins lack gelation capacity, however, on the other hand, González-Pérez et al. [4] reported the formation of gels by thermal induction (at 98 °C) from sunflower protein dispersions (10% w/v), although such gels were weaker than those obtained under the same conditions from soy glycinins (G′: 500 and 5,000 Pa, respectively).

Hardness of obtained gels depended on protein concentration, type of protein concentrate, and heating time.

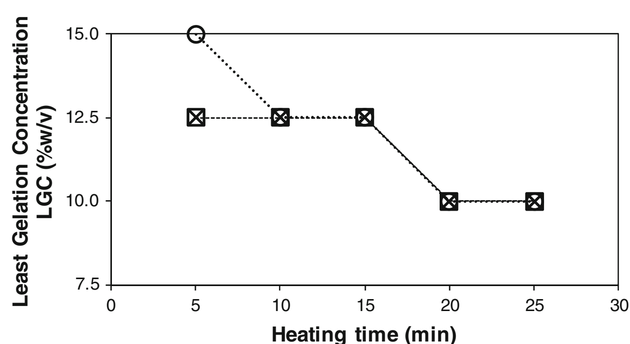

Fig. 5 Gelation properties of sunflower protein concentrates. Least gelation concentration of (*circles*) C + IP, (*multiplication signs*) CW + IP and (*squares*) CS + IP as a function of heating time

The increase of protein concentration over 10% and/or prolongation of heating time period produced more resistant and rigid gels than the ones obtained at protein concentrations below this value and/or were heated for a shorter time (Table 3). This finding can be explained by the high protein concentration interaction between polypeptide chains being facilitated, in addition increasing the heating time period favors opening of the protein structure which also has an effect on the probability of interaction between polypeptide chains.

Protein concentrates used in this work were partially denatured and did not present significant differences in their denaturation degree ($p > 0.05$) (see Table 1); nevertheless, a particular behavior was observed—gels obtained with the C + IP were weaker than those obtained with protein concentrates with reduced phenolic content (CW + IP and CS + IP) (Table 3). This fact indicates that phenolic compounds interfere with protein matrix formation, yielding a weaker gel. In general, the stability of gels in an alkaline medium is mainly related to the formation of disulfide bonds and, to a lesser extent, with hydrogen bonds and hydrophobic interactions. Disulfide bonds increase the hardness of the protein matrix, whereas non-covalent interactions help to maintain its structure [31, 32]. Therefore, it could be hypothesized that C + IP gels are weaker than those made from CW + IP and CS + IP, since the first ones possesses a higher concentration of phenolic compounds able to interact with the sulfhydryl groups of proteins [35], thus reducing the chances for disulfide bond formation.

Table 3 Gelation properties of sunflower protein concentrates

Sample[a]	Concentration (% w/v)	Heating time (min)					
		0	5	10	15	20	25
C + IP	7.5	–	–	–	–	–	–
	10.0	–	–	–	–	+	++
	12.5	–	–	–	+	++	++
	15.0	–	+	++	+++	++++	++++
CW + IP	7.5	–	–	–	–	–	–
	10.0	–	–	–	–	+	++
	12.5	–	–	+	+++	++++	++++
	15.0	–	+	+++	++++	+++++	+++++
CS + IP	7.5	–	–	–	–	–	–
	10.0	–	–	–	–	+	+++
	12.5	–	–	+	+++	+++++	+++++
	15.0	–	+	+++	++++	+++++	+++++

Effect of heating time (at 100 °C) and sample concentration on the appearance of gels formed for C + IP, CW + IP and CS + IP. Results of duplicate determinations

– no gelation, + very weak gel, ++ weak gel, +++ gel medium, ++++ firm gel, +++++ very firm gel

[a] Sunflower protein concentrate with isoelectric precipitation (C + IP) and with reduced content of phenolic compounds by extraction with water (CW + IP) or 1 g/L Na_2SO_3 solution (CS + IP)

Potential Applications of Sunflower Protein Concentrates

The functional behavior of protein concentrates shown in this work, together with the good nutritional and antioxidant properties shown in previous studies [9, 10], indicate the potential of sunflower protein concentrates to be used as high quality functional food ingredients for different applications. The high water solubility values of sunflower protein concentrates allow their use in the formulation of beverages, whereas their moderate WHC values are similar to values corresponding to commercial soy protein isolates used as thickening agents. Stability of emulsions and foams prepared with sunflower protein concentrates showed themselves to be dependent on the type of concentrates and on the medium conditions, and some of them were comparable to the ones prepared from soybean commercial protein isolates and bovine serum albumin. Therefore, sunflower protein concentrates could be use also as emulsifying and foaming agents. Sunflower protein concentrates also showed a gelation capacity in agreement with published work using both purified sunflower proteins and laboratory enriched protein products obtained from sunflower seeds. Although these results are preliminary, they are comparable with commercial proteins used as gelling agents in different applications.

Conclusion

Sunflower protein concentrates naturally enriched with phenolic compounds—obtained from a byproduct of the oil industry—show a broad range of functional properties and have therefore a great potential to be used as high quality functional food ingredients for different applications. After selection of the target product in which they can be used as additives, further studies should be performed to establish their functionality performance in the presence of other food components.

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