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Production and characterization of sunflower (*Helianthus annuus L.*) protein-enriched products obtained at pilot plant scale

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ABSTRACT

Sunflower protein concentrates with different content of phenolic compounds were produced at pilot plant scale from the by-product of oil manufacturing. The products obtained were characterized, and their physicochemical properties (i.e. surface hydrophobicity, thermal stability, and polypeptide composition) were evaluated at different storage conditions. All the procedures evaluated resulted in sunflower protein concentrates with high protein solubility (>60%) but with different chemical composition, color, and physicochemical properties. The products exhibited intense coloration and antioxidant properties due to their residual phenolic compounds content. The addition of an isoelectric precipitation step increased the protein content and the removal of phenolic compounds. The resulting concentrates exhibited high protein digestibility *in vitro*, even in the presence of phenolic compounds, and maintained high protein solubility for at least 6 months of storage. During this period, only structural changes in proteins were observed, as evidenced by their surface hydrophobicity. The results demonstrate that it is feasible to produce sunflower protein concentrates with high solubility on a pilot plant scale, using sunflower oilcake as starting material.

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1. Introduction

In the last few years there has been an increasing interest in searching protein sources with high nutritional value and/or adequate functionality, and in promoting their use as functional ingredients in food industry or as biopolymers in the formation of biodegradable materials (Damodaran & Paraf, 1997; Moure, Sineiro, Domínguez, & Parajó, 2006). The production of such proteins from agro-industrial by-products appears as a sustainable alternative for adding commercial value to these products, allowing their industrial exploitation (Moure et al., 2006). From this point of view, and considering that Argentina is the second largest world producer of sunflower oil, a joint project was established with the Argentine Society of Sunflower Producers (ASAGIR) to study alternative applications of sunflower pellet (ASAGIR, 2009). At present, sunflower oilcakes are almost completely used for animal feeding in spite of their high content of proteins (~32 g/100 g dry matter) and certain structural similarities with soy proteins, which are widely used as functional ingredients in food industry (González-Pérez & Vereijken, 2008; Molina, Petruccelli, & Añón, 2004). The main reason for this restricted use is the presence of relatively high amounts of phenolic compounds, especially chlorogenic and caffeic acids. They affect the quality of sunflower protein in several ways such as reducing the digestibility, altering the organoleptic properties, prolonging or shortening storage life and stability, and adversely altering the functional properties and behavior of sunflower protein in food systems (Bau, Mohtadi Nia, Mejean, & Debry, 1983; González-Pérez & Vereijken, 2008; Kroll, Rawel, & Rohn, 2003; Moure et al., 2006; Sastry & Narasinga Rao, 1990). While for many years these arguments supported studies aimed at improving the production of sunflower protein products free from phenolic compounds (González-Pérez & Vereijken, 2008; Karayannidou et al., 2007; Pickardt et al., 2009; Sripad & Narasinga Rao, 1987), in the last years there has been a tendency to keep or even add these compounds in view of their antioxidant action (Raskin et al., 2002).

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It is well known that the functionality of proteins depends on the structural and physicochemical characteristics of these molecules, and that these properties are in turn conditioned by the extraction procedure and the storage conditions, among other factors (Martins & Netto, 2006). Wagner, Sorgentini, and Añón (2000) and Añón, Sorgentini, and Wagner (2001) found marked differences in solubility and structural properties between soy protein isolates obtained in the laboratory and commercial isolates, which were attributed to the effects of the industrial process, such as drying at high protein concentrations or in the presence of salts. While some published studies have focused on the operating variables during the preparation of sunflower protein products at pilot plant scale (Castor-Normandin, Ducastaing, Prevot, & Raymond, 1984; Lawhon, Glass, Manak, & Lusas, 1982; Weisz, Schneider, Schweiggert, Kammerer, & Carle, 2010), the impact of different processes on the structural and functional properties of the products was not analyzed in spite of importance of such knowledge for redesigning some of these processes. In addition, proteins may also suffer changes in their properties according to the storage conditions and duration. Da Silva Pinto, Lajolo, and Genovese (2005) and Martins and Netto (2006) observed that the solubility of soy protein isolates diminished during storage at different temperatures and relative humidity (RH) values due to aggregation reactions (caused by the formation of disulfide bonds or by hydrophobic reactions) which conditioned the potential use of these products as functional ingredients. In contrast, the effect of storage on the physicochemical and functional properties of sunflower protein products has not been studied. The presence in these products of phenolic compounds that are difficult to completely eliminate (Salgado, Molina Ortiz, Petruccelli, & Mauri, 2011) and which are sensitive to oxidation, determines the importance of making detailed studies in these systems.

The aims of this study were: i) to obtain sunflower protein-enriched products at pilot plant scale with good yields, using sunflower oilcake as starting material, and ii) to characterize the structural, physicochemical and antioxidant properties, and the protein digestibility of these products at the end-process and during their storage under different conditions.

2. Materials and methods

2.1. Materials

Defatted sunflower (*Helianthus annuus L.*) oilcake was provided by Aceitera Santa Clara (Molinos Río de La Plata, Rosario, Argentina). This starting material was ground in a mill (Bühler Miag MLGV Variostuhl), and was passed through a screen with particle diameter of 1.19 mm, thus yielding the "milled sunflower oilcake".

2.2. Preparation of sunflower protein-enriched products

The processing conditions for preparing at pilot plant scale sunflower protein-enriched products with different contents of phenolic compounds were chosen on the basis of previous studies performed at laboratory scale (Salgado et al., 2011). Flow diagrams of the procedures used for preparing sunflower concentrates at pilot plant scale are shown in Fig. 1.

2.2.1. Preparation of sunflower protein concentrates

Aqueous dispersions (45 L) of the milled sunflower oilcake (67 g/L) were stirred for 1 h and their pH was adjusted to 9 with 3 mol/L NaOH. Solid-liquid separation was performed in a basket type centrifuge with filtering material (2100×g and 20 °C) and the supernatant was collected. The residue was subjected to a second extraction of proteins as described above. The supernatants of both extractions were pooled, the pH was adjusted to 9 with 3 mol/L NaOH, and the mixture was spray-dried using a Niro Atomiser spray drier with an inlet temperature of 170–190 °C and an outlet temperature of 80–90 °C (Niro Atomiser Production Minor,

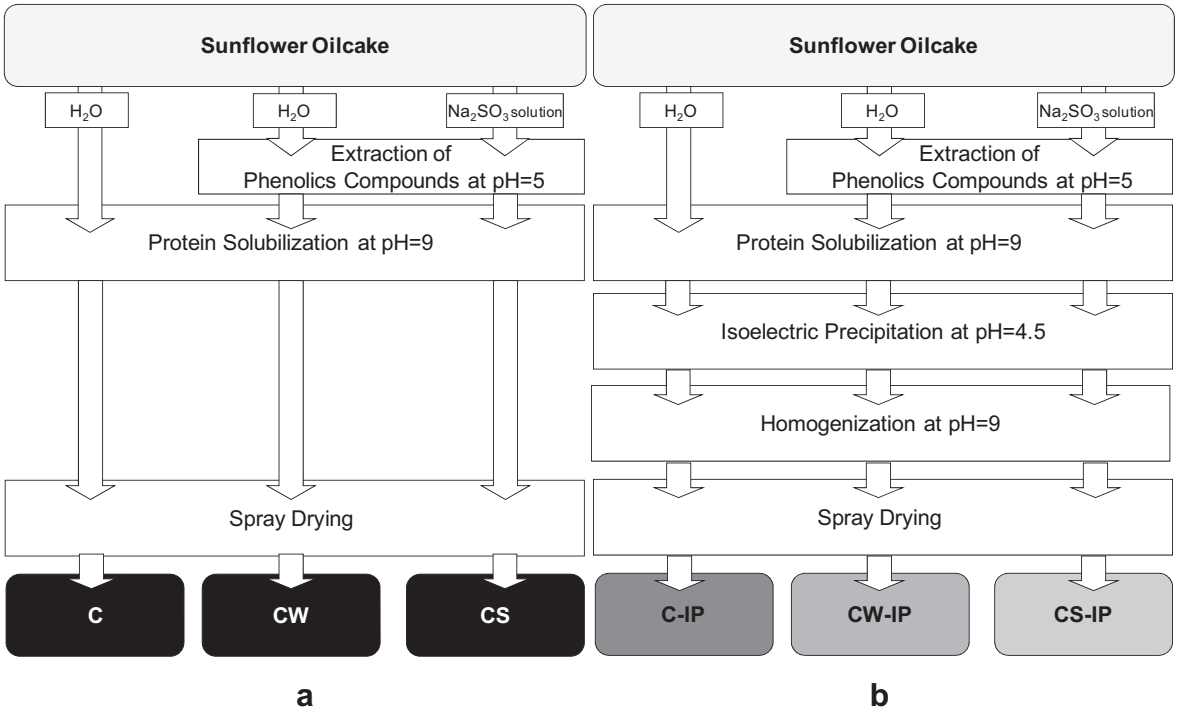

Fig. 1. Flow diagrams for pilot plant production of sunflower protein concentrates with (b) or without (a) protein isoelectric precipitation (**C-IP** and **C**) and with reduced content of phenolic compounds by extraction with water (**CW-IP** and **CW**) or 1 g/L Na_2SO_3 solution (**CS-IP** and **CS**), using sunflower oilcake as starting material.

Denmark). This resulted in a sunflower protein concentrate (**C**). Three replicates of **C** were prepared.

2.2.2. Preparation of sunflower protein concentrates with isoelectric precipitation

Aqueous dispersions (60 L) of the milled sunflower oilcake (67 g/L) were processed as described for protein concentrate (**C**), but the supernatants obtained after the protein extraction steps were mixed and subjected to isoelectric precipitation by adjusting the pH to 4.5 with 3 mol/L HCl. The mixture was stirred for 30 min and separation of the precipitate was carried out in a Westfalia centrifuge (Westfalia SAADH 205 model, Germany). The resulting precipitate was washed and was centrifuged once more. This washed precipitate was resuspended in water (approximately 0.5 L/kg precipitate). The suspension was passed through a Manton-Gaulin two-stages homogenizer (Gaulin Corp., USA) with 2×10^5 and 5×10^5 Pa in the first and second stage respectively, the pH was adjusted to 9 with 3 mol/L NaOH, and the solution was spray-dried using a Niro Atomiser spray drier with an inlet temperature of 170–190 °C and an outlet temperature of 80–90 °C (Niro Atomiser Production Minor, Denmark). This resulted in a sunflower protein concentrate obtained with isoelectric precipitation (**C-IP**). Three replicates of **C-IP** were prepared.

2.2.3. Preparation of sunflower protein concentrates with reduced content of phenolic compounds

To obtain sunflower protein concentrates with a lower content of phenolic compounds, the milled sunflower oilcake was subjected to two extractions at acid pH before extracting the proteins in alkaline medium. The milled sunflower oilcake (4 kg) was suspended in the extraction medium (67 g/L), the pH was adjusted to 5 with 3 mol/L HCl, and the suspension was stirred for 30 min (with verification of pH constancy every 10 min). Solid-liquid separation was performed in a basket type centrifuge with filtering material (2100×g at 20 °C), 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 as described for the preparation of the **C** product (see 2.2.1). The media used for extracting phenolic compounds were water (**W**) and 1 g/L Na_2SO_3 solution (**S**). The protein concentrates obtained with these media were named **CW** and **CS** respectively.

The **CW-IP** and **CS-IP** products were obtained by the addition of two serial phenolic extraction steps with water (**W**) or 1 g/L Na_2SO_3 solution (**S**) respectively, before the procedures described for the preparation of the **C-IP** product (protein solubilization at alkaline pH and isoelectric precipitation, see 2.2.2).

Three replicates of each protein product (**CW**, **CS**, **CW-IP** and **CS-IP**) were prepared.

2.3. Procedures yields

The protein recovery yields (g of proteins in the product/g of proteins in the milled sunflower oilcake) and the percent residual content of phenolic compounds (mg of phenolic compounds in the product/mg of phenolic compounds in the milled sunflower oilcake) were determined for each procedure assayed.

2.4. Chemical composition

Chemical composition of the milled sunflower oilcake and the sunflower protein concentrates obtained were determined. Moisture and ash values were determined by gravimetric measure (AOAC 935.29 and AOAC 923.03, 1995), respectively. The protein content was determined by the Kjeldahl method (AOAC 920.53, 1995) using 5.55 as nitrogen-to-protein conversion factor. Phenolic compounds were measured by UV spectrophotometry at 324 nm as described by Moore, McDermott, and Wood (1948), using chlorogenic acid (Chemika Fluka, Germany). The percent content of the other compounds (fiber, carbohydrates and lipids) were calculated by difference. All determinations were performed at least in duplicate.

2.5. Storage of sunflower protein concentrates obtained with isoelectric precipitation

Sunflower protein concentrates obtained at pilot plant scale (**C-IP**, **CW-IP**, **CS-IP**) were introduced into low density polyethylene bags and were stored for six months in two sets of environmental conditions: i) cold conditions: 4 °C and 64.3% RH, and ii) mild conditions: 20 °C and 58.9% RH. Protein solubility, surface hydrophobicity, degree of protein denaturation, polypeptide composition, and color of sunflower protein concentrates were determined at several storage times: 0, 30, 60, 90, 120 and 180 days.

2.6. Color

Color of sunflower protein concentrates were determined using a Minolta Chroma meter (CR 300, Minolta Chroma Co., Osaka, Japan). A CIE Lab color scale was used. To measure color parameters, protein samples were homogeneously dispersed on the white plate surface. Total color difference (ΔE) was calculated using the following equation:

$$\Delta E = \sqrt{\left(L_{\text{sample}} - L_{\text{standard}}\right)^2 + \left(a_{\text{sample}} - a_{\text{standard}}\right)^2 + \left(b_{\text{sample}} - b_{\text{standard}}\right)^2}$$

Values were expressed as the means of nine measurements on different areas of each sample for each replicate.

2.7. 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 (Molina et al., 2004). All assays were conducted in duplicate for each replicate.

2.8. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The polypeptide composition of the sunflower protein concentrates were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a separating gel (0.12 g polyacrylamide/mL) with a stacking gel (0.04 g polyacrylamide/mL) in a minilabs system (Bio-Rad Mini-Protean II Model) (Laemmli, 1970). Protein molecular weights were estimated using low MW markers (94, 67, 43, 30, 20.1 and 14.4 kDa) (Pharmacia, Amersham,

England). Gel images were analyzed with the Image J software (Bethesda, MD: US National Institute of Health).

2.9. Protein solubility at pH 8

Sunflower protein concentrates were dispersed (1 mg/mL) in distilled water for 30 min with agitation, then the mixture was adjusted to pH 8 with 1 mol/L NaOH and the dispersion was kept with constant agitation for a further 1 h. 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 (1976) method 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. Determinations were performed at least in duplicate for each replicate.

2.10. Surface hydrophobicity (Ho)

The surface hydrophobicity (Ho) was determined according to the method described by Kato and Nakai (1980) using a digital fluorimeter Perkin–Elmer model 2000 (Norwalk, CT, USA). All determinations were performed in duplicate for each replicate.

2.11. Antioxidant capacity (AC)

The $ABTS^{\bullet+}$ radical (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging capacity by antioxidant compounds was determined according to the method described by Re et al. (1999). Protein concentrates dissolved in 0.01 mol/L sodium phosphate buffer (pH 7.4) (1 mg protein/mL, 50 μL) were added to 950 μL of the solution containing the $ABTS^{\bullet+}$ The mixture was vortexed for 1 min and its absorbance at 734 nm was measured 10 min after the addition of the pre-formed radical.

2.12. In vitro protein digestibility

This was determined following the technique reported by Hsu, Vavak, Satterlee, and Miller (1977). The drop in pH was measured with a potentiometer after 10 min. Apparent *in vitro* digestibility (Y) was measured using the following equation: $Y = 210.464 - 18.103X$; where: X = pH of protein suspension immediately after digestion with the multienzymatic solution for 10 min. Results were expressed as percentage relative to the *in vitro* digestibility of casein, a protein usually employed as standard in studies on human feeding.

2.13. Statistical analysis

Results were expressed as mean ± standard deviation 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).

3. Results and discussion

3.1. Chemical composition and recoveries of sunflower protein concentrates

Chemical composition of the sunflower oilcake and the sunflower protein concentrates obtained at pilot plant, the protein yields and the efficacy of removal of phenolic compounds are presented in Table 1. The **C** product had the lowest protein content (41.4 g/100 g dry matter) and the highest amounts of ashes and phenolic compounds (5.4 g/100 g dry matter). This process recovered 41.3% of total proteins present in sunflower oilcake. The addition of successive extraction steps to eliminate phenolic compounds led to an increase in the protein content of **CW** and **CS** (~62–65 g/100 g dry matter) but diminished protein recovery (~25–30%). These concentrates (**CW** and **CS**) were characterized by significantly lower contents of phenolic compounds, as well as lower contents of ashes (and others) as compared to **C**, due to the solubilization of these components in the aqueous extraction media. This effect had been also observed during its processing at laboratory scale (Salgado et al., 2011).

Regarding the sunflower protein concentrates obtained with isoelectric precipitation, sample **C-IP** had 70.4 g protein/100 g dry matter and the highest contents of phenolic compounds (Table 1). Additional steps for removing these compounds (aqueous extraction procedures) produced concentrates (**CW-IP** and **CS-IP**) without significant differences in protein, ash or moisture content as compared to **C-IP**. Protein recovery values of concentrates obtained with isoelectric precipitation were lower than those of concentrates produced without this step, but were similar to values reported for other vegetable protein products (such as amaranth and sunflower protein concentrates; lupine and soy protein isolates) obtained under pilot plant conditions (Andrich, Carrara,

Table 1
Chemical composition of the sunflower oilcake and the sunflower protein concentrates obtained at pilot plant scale. Yields are expressed as protein recovery and phenols elimination for each procedure.

Sunflower samples[a]	Chemical composition (g/100 g)					Yields (%)	
	Proteins	Phenolic compounds	Ashes	Moisture	Others	Proteins recovery	Phenols elimination
Oilcake	31.7 ± 0.1^{a}	2.7 ± 0.1^{c}	8.0 ± 0.4^{c}	11.0 ± 0.9^{d}	57.6	–	–
C	41.4 ± 2.9^{b}	5.4 ± 0.3^{d}	11.4 ± 0.1^{e}	6.3 ± 0.1^{c}	41.8	41.3 ± 0.4^{d}	35.7 ± 0.5^{a}
CW	65.6 ± 0.5^{d}	1.9 ± 0.2^{a}	7.0 ± 0.1^{b}	$5.5 \pm 0.1^{b,c}$	25.5	25.9 ± 0.3^{c}	$91.0 \pm 1.6^{b,c}$
CS	62.1 ± 0.2^{c}	1.9 ± 0.2^{a}	8.8 ± 0.1^{d}	5.2 ± 0.3^{b}	27.1	28.8 ± 0.1^{c}	89.1 ± 1.2^{b}
C-IP	70.4 ± 0.8^{e}	2.5 ± 0.1^{b}	4.0 ± 0.1^{a}	$4.9 \pm 0.4^{a,b}$	23.1	16.6 ± 0.2^{b}	92.9 ± 0.6^{c}
CW-IP	70.1 ± 1.4^{e}	2.1 ± 0.1^{a}	4.5 ± 0.1^{a}	$5.7 \pm 0.1^{b,c}$	23.3	12.4 ± 0.3^{a}	95.4 ± 0.4^{d}
CS-IP	66.7 ± 0.8^{d}	1.8 ± 0.1^{a}	4.0 ± 0.4^{a}	4.1 ± 0.9^{a}	27.5	$14.3 \pm 0.2^{a,b}$	95.4 ± 0.2^{d}

Values in columns 'Proteins, Phenolic compounds, Ashes and Others' are expressed on dry basis.
Values in column 'Others' represents the content of lipids, carbohydrates and fibers was calculated by difference.
Values in column 'Yields' are expressed as percentage of proteins or phenolic compounds with respect to the amount present in the milled sunflower oilcake.
Reported values for each protein product are means ± standard deviation ($n = 3$ for proteins, ashes and moisture determinations and protein recovery; $n = 4$ for phenolic compounds determinations and phenols elimination). In columns, means followed by the same letter are not significantly different ($p > 0.05$) according to Fisher's test.
[a] Values in column 'Sunflower samples' represents sunflower protein concentrates with or without protein isoelectric precipitation (**C-IP** and **C**) and with reduced content of phenolic compounds by extraction with water (**CW-IP** and **CW**) or 1 g/L Na_2SO_3 solution (**CS-IP** and **CS**).

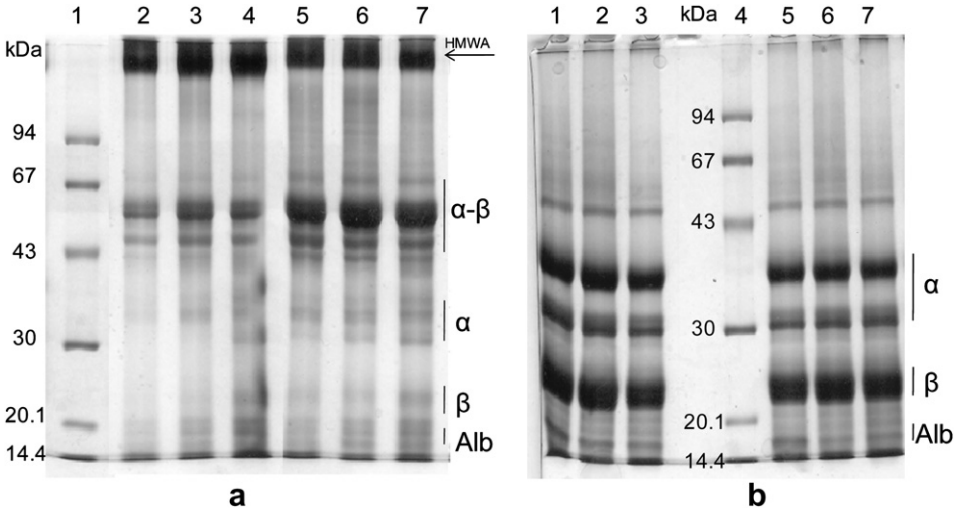

Fig. 2. SDS-PAGE electrophoretic patterns under non-reducing (a) or reducing conditions (b, β-mercaptoethanol added) of sunflower protein concentrates with or without protein isoelectric precipitation (**C-IP** and **C**) and with reduced content of phenolic compounds by extraction with water (**CW-IP** and **CW**) or 1 g/L Na_2SO_3 solution (**CS-IP** and **CS**), obtained at pilot plant scale. (a): **LWM** (lane 1), **C** (lane 2), **CW** (lane 3), **CS** (lane 4), **C-IP** (lane 5), **CW-IP** (lane 6), **CS-IP** (lane 7). (b): **C** (lane 1), **CW** (lane 2), **CS** (lane 3), **LMW** (lane 4), **C-IP** (lane 5), **CW-IP** (lane 6), **CS-IP** (lane 7). Nomenclature: α-β: subunits of sunflower globulins (11S); α and β: acidic and basic polypeptides respectively; Alb: sunflower albumins (2S); HMWA: aggregates of high molecular weight.

Castel, Netto, & Santiago, 2007; Castor-Normandin et al., 1984; D'Agostina et al., 2006; González, Remondetto, Coutaz, Santiago, & Bonaldo, 1995).

A comparison of the content of phenolic compounds of concentrates produced with isoelectric precipitation and their respective concentrates obtained without this step shows that only **C** and **C-IP** differed significantly, evidencing that phenolic compounds are also removed during this process. Extractions with either water or Na_2SO_3 aqueous solution were as effective as isoelectric precipitation for removing phenolic compounds (~90–93%), achieving a greater effect when both steps were combined (~95%). In addition, extractions with water or Na_2SO_3 aqueous solution were also effective for removing phytates (Gandhi, Jha, & Gupta, 2008; Saeed & Cheryan, 1988). In the present study, none of the procedures assayed resulted in the complete removal of phenolic compounds, possibly because the residual fraction interacted with proteins (Salgado et al., 2011). However, Weisz et al. (2010) could remove 99.4% of the phenolic compounds present in defatted sunflower flours without using thermal treatments, by combining mild-acidic protein extraction (Pickardt et al., 2009) with adsorption and ion exchange to produce light-colored sunflower protein isolate at pilot plant scale.

3.2. Structural properties of sunflower protein concentrates: polypeptide composition and degree of protein denaturation

To evaluate if the different extraction procedures used and/or the isoelectric precipitation step produces the removal of certain polypeptides, SDS-PAGE of the protein concentrates was performed (Fig. 2). Under non-reducing conditions all the protein products (Fig. 2.a) yielded similar electrophoretic patterns, and contained mainly the 11S globulins, with α-β subunits of molecular mass between 45 and 62 kDa. Under reducing conditions (Fig. 2.b) these α-β subunits were dissociated into acidic (α) and basic (β) polypeptides (30–40 kDa and 20–30 kDa, respectively), in agreement with a previous report by Molina et al. (2004). All the samples also contained aggregates of high molecular weight (Fig. 2.a HMWA, higher than 94 kDa) which did not enter the resolving gel, but disappeared completely from protein isolates under reducing conditions (Fig. 2.b) suggesting that they were stabilized by disulfide bonds.

Thermograms of all sunflower protein concentrates obtained by DSC showed a single endotherm with similar denaturation temperature (100–102 °C) but different denaturation enthalpies (Table 2). The energy required for denaturing proteins present in **C**, which had the highest phenolic content, was the lowest ($p < 0.05$). It seems that the removal of phenolic compounds by means of aqueous extraction or isoelectric precipitation likely favors the formation of other interactions that could stabilize these molecules, thus increasing their denaturation enthalpy.

3.3. Protein solubility and surface hydrophobicity of sunflower concentrates

Since high solubility is a frequent requisite for the use of a protein product as functional ingredient in food industry (Damodaran & Paraf, 1997), the protein solubility of sunflower concentrates obtained at pilot plant was measured (Fig. 3). While all the products analyzed exhibited a high protein solubility (at pH 8) (higher than 60%), protein concentrates obtained with isoelectric precipitation had solubility values higher than 80% that did not

Table 2
Denaturation temperatures and enthalpies (Td, ΔH) of sunflower protein concentrates obtained at pilot plant scale.

Sunflower protein products[a]	DSC	
	Td (°C)	ΔH (J/g protein)
C	102.3 ± 0.1^{b}	4.2 ± 0.2^{a}
CW	$100.7 \pm 1.0^{a,b}$	6.9 ± 0.2^{c}
CS	100.1 ± 0.1^{a}	6.9 ± 0.4^{c}
C-IP	100.1 ± 1.6^{a}	5.4 ± 0.3^{b}
CW-IP	102.3 ± 0.4^{b}	5.8 ± 0.3^{b}
CS-IP	$101.0 \pm 0.1^{a,b}$	5.4 ± 0.2^{b}

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

[a] Sunflower protein concentrates with or without protein isoelectric precipitation (**C-IP** and **C**) and with reduced content of phenolic compounds by extraction with water (**CW-IP** and **CW**) or 1 g/L Na_2SO_3 solution (**CS-IP** and **CS**).

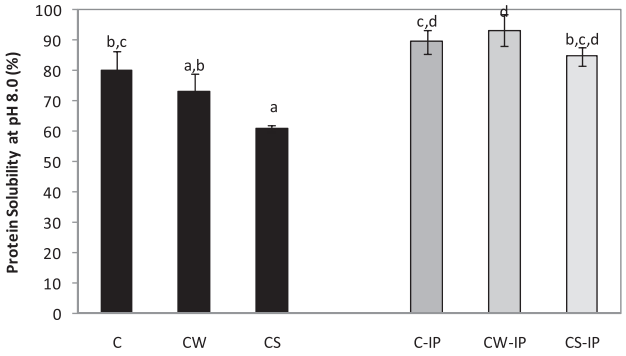

Fig. 3. Protein solubility (at pH 8.0) of sunflower protein concentrates with or without protein isoelectric precipitation (**C-IP** and **C**) and with reduced content of phenolic compounds by extraction with water (**CW-IP** and **CW**) or 1 g/L Na_2SO_3 solution (**CS-IP** and **CS**), obtained at pilot plant scale. Reported values for each protein product are means ± standard deviation ($n = 4$). Bars followed by the same letter are not significantly different ($p > 0.05$) according to Fisher's test.

differ significantly ($p > 0.05$) according to the phenolic content. In the case of protein concentrates produced without isoelectric precipitation, solubility diminished with phenolic content diminution ($p < 0.05$). These solubility values were significantly higher than those reported by other authors for sunflower protein concentrates and isolates obtained from laboratory-prepared flours (i.e. by milling defatted sunflower seeds without thermal treatments). Rodríguez Patino et al. (2007) reported 30% protein solubility at pH 8, while Bau et al. (1983) and Sripad and Narasinga Rao (1987) reported values between 50% and 55% at this pH.

The surface hydrophobicity (Ho) of the soluble protein fraction is a good parameter to predict its use as foaming agent and emulsifying agent (Damodaran & Paraf, 1997). Ho values of water soluble proteins are shown in Fig. 4. It can be observed that for both, concentrates obtained with or without isoelectric precipitation, the addition of phenolic compounds extraction steps resulted in an increased hydrophobicity of proteins ($p < 0.05$). Such increase was greater for samples treated with Na_2SO_3, which shows that this treatment leads to an increased exposure of the hydrophobic zones of proteins, possibly due to a dissociation of soluble aggregates, a modification of the oligomeric state (equilibria between helianthinin monomers, trimers and hexamers), or conformational changes. Proteins present in **C** samples exhibited a lower Ho than those present in **C-IP** ones, possibly due to the presence of phenolic compounds capable to interact with proteins through their

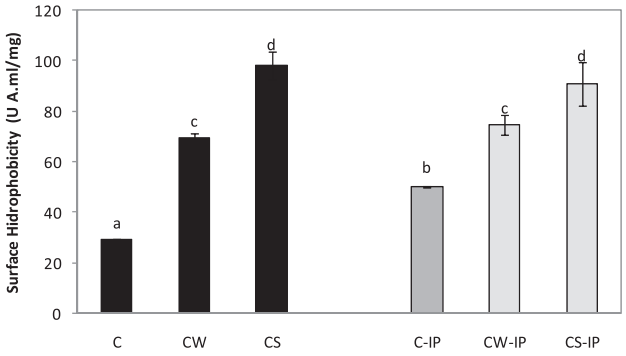

Fig. 4. Surface hydrophobicity of proteins present in sunflower protein concentrates with or without protein isoelectric precipitation (**C-IP** and **C**) and with reduced content of phenolic compounds by extraction with water (**CW-IP** and **CW**) or 1 g/L Na_2SO_3 solution (**CS-IP** and **CS**), obtained at pilot plant scale. Reported values for each protein product are means ± standard deviation ($n = 4$). Bars followed by the same letter are not significantly different ($p > 0.05$) according to Fisher's test.

Table 3
Hunter-Lab color parameters of the sunflower protein concentrates obtained at pilot plant scale.

Sunflower protein products[a]	Hunter-Lab color parameters			
	L	*a*	*b*	Δ*E*
C	52.8 ± 0.3^{c}	-8.29 ± 0.2^{a}	15.5 ± 0.1^{c}	47.3 ± 0.3^{c}
CW	54.5 ± 0.6^{d}	2.12 ± 0.1^{c}	16.4 ± 0.2^{d}	45.3 ± 0.5^{b}
CS	59.4 ± 0.3^{e}	5.79 ± 0.1^{e}	19.5 ± 0.3^{f}	42.2 ± 0.3^{a}
C-IP	42.3 ± 0.2^{a}	-1.7 ± 0.1^{b}	6.8 ± 0.1^{a}	55.2 ± 0.2^{e}
CW-IP	49.1 ± 0.4^{b}	4.7 ± 0.1^{d}	13.8 ± 0.1^{b}	49.9 ± 0.4^{d}
CS-IP	53.2 ± 0.8^{c}	7.9 ± 0.1^{f}	16.8 ± 0.1^{e}	47.3 ± 0.7^{c}

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

[a] Sunflower protein concentrates with or without protein isoelectric precipitation (**C-IP** and **C**) and with reduced content of phenolic compounds by extraction with water (**CW-IP** and **CW**) or 1 g/L Na_2SO_3 solution (**CS-IP** and **CS**).

hydrophobic zones (González-Pérez & Vereijken, 2008; Pierpoint, 1969; Sastry & Narasinga Rao, 1990). In contrast, **CW** and **CS** did not exhibit significant differences ($p > 0.05$) in Ho as compared to **CW-IP** and **CS-IP** respectively, possibly because removable phenolic compounds were eliminated from both types of preparations during the washing steps (with water or Na_2SO_3 solution) leaving the proteins with conformations that were not modified during isoelectric precipitation (Salgado et al., 2011).

3.4. Color of sunflower protein concentrates

Protein products obtained at pilot plant showed differences in coloration. Table 3 shows the Hunter-Lab color parameters and the color difference (Δ*E*) of the products. Samples **C** and **C-IP** had a greenish color (negative *a* values), which can be attributed to the oxidation of phenolic compounds to o-quinones during protein extraction in an alkaline medium (Saeed & Cheryan, 1988). Protein products subjected to extraction of phenolic compounds with water or Na_2SO_3 had a lighter tone (higher *L* values) and a more brownish color as evidenced by higher values of *a* and *b* parameters (Table 3). On the other hand, **CS** and **CS-IP** products had the lowest color levels (lowest Δ*E*). This can be attributed to the reducing environment generated by sulphite that protected phenolic compounds from air oxygen. The content of phenolic compounds is not the only factor that determines the color of the protein concentrates. Alkaline treatment prior to the removal of phenolic compounds induces a greenish color that cannot be eliminated

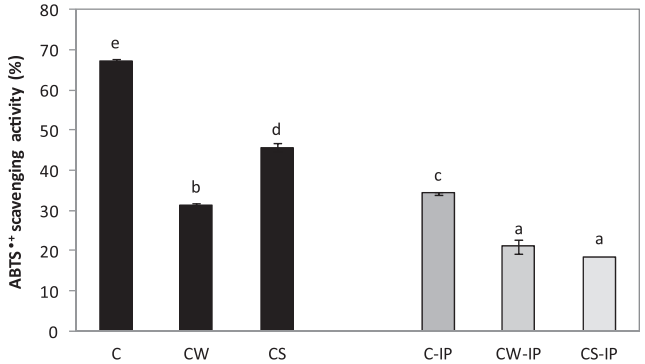

Fig. 5. Antioxidant capacity (measured as $ABTS^{\bullet+}$ scavenging capacity) of sunflower protein concentrates with or without protein isoelectric precipitation (**C-IP** and **C**) and with reduced content of phenolic compounds by extraction with water (**CW-IP** and **CW**) or 1 g/L Na_2SO_3 solution (**CS-IP** and **CS**), obtained at pilot plant scale. Reported values for each protein product are means ± standard deviation ($n = 4$). Bars followed by the same letter are not significantly different ($p > 0.05$) according to Fisher's test.

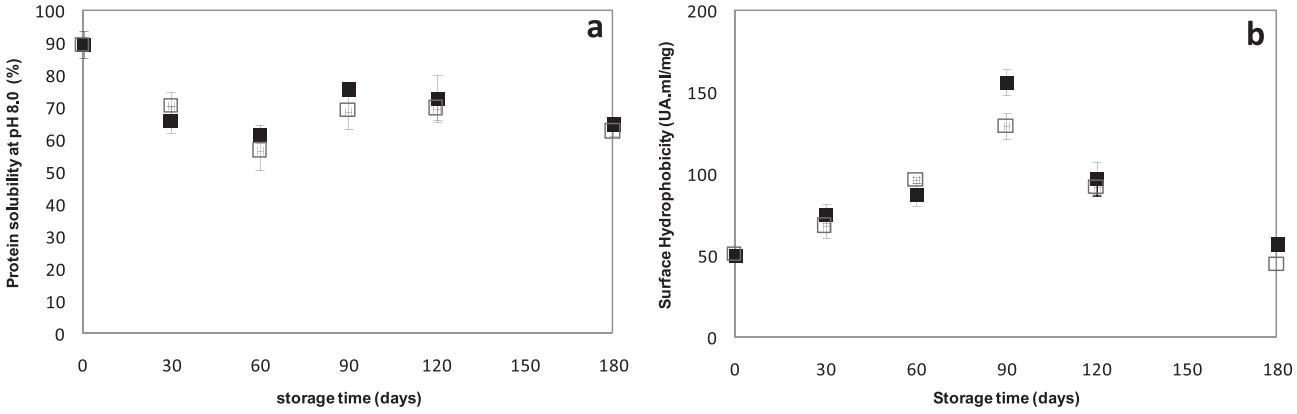

Fig. 6. a) Protein solubility at pH 8 and b) surface hydrophobicity of a sunflower protein concentrate (**C-IP**) obtained at the pilot plant scale as a function of storage time at 4 °C and 64.3 %RH (■), or at 20 °C and 58.9 %RH (□). Reported values for each protein product are means ± standard deviation ($n = 4$).

with further water extraction steps and does not correlate with the final content of phenolic compounds. The intense color of these protein products should be taken into account when considering their potential applications.

3.5. Antioxidant properties of sunflower protein concentrates

All protein products exhibited antioxidant capacity as measured by their $ABTS^{\bullet+}$ scavenging power (Fig. 5). **C** product had the highest phenolic content and antioxidant capacity. Interestingly, **CS** exhibited a higher antioxidant capacity than **CW** despite the low phenolic content of both products. This may be due to the fact that **CS** retains residual amounts of Na_2SO_3 (less than 50 mg/100 g), which has reducing capacity and may interfere in this determination. In every case the concentrates obtained with isoelectric precipitation had lower antioxidant capacity than their respective protein concentrates produced without this step ($p < 0.05$). These findings indicate that residual phenolic compounds, which cannot be removed because of their strong interaction with proteins (Salgado et al., 2011), confer antioxidant properties to the sunflower protein products. This fact constitutes an advantage because it widens the potential range of application of these protein products.

3.6. In vitro protein digestibility of sunflower protein concentrates obtained with isoelectric precipitation

The *in vitro* protein digestibility of the sunflower protein concentrates obtained with isoelectric precipitation was determined. Sample **C-IP**, which had the highest phenolic content, presented a 95.4% (±0.3) protein digestibility, using casein digestibility as a reference (100% digestibility). The concentrates with the lowest content of phenolic compounds (**CW-IP** and **CS-IP**) presented a slightly higher protein digestibility (97.4% ± 0.3) ($p < 0.05$). These results reveal that the presence of phenolic compounds reduces slightly the digestibility of sunflower proteins, as has been proposed by Kroll et al. (2003) and Rawel, Rohn, Kruse, and Kroll (2002). Notwithstanding, the digestibility values found in these studies are high and would not limit the use of these proteins for human feeding.

3.7. Stability of sunflower protein concentrates obtained with isoelectric precipitation during storage

The stability of sunflower concentrates obtained with isoelectric precipitation was evaluated for two different storage conditions: refrigerated or room temperature, both at intermediate relative humidity. As an example, Fig. 6.a and 6.b show the variation in protein solubility (at pH 8) and surface hydrophobicity of **C-IP** as a function of storage duration under the two conditions employed: 4 °C, 64.3% RH and 20 °C, 58.9% RH; the other samples (**CW-IP** and **CS-IP**) exhibited the same tendency. As shown in Fig. 6.a almost 90% of proteins present in concentrates were initially soluble in water at pH 8, but after one month of storage this value declined to 65% (average for **C-IP** and **CW-IP**) or 55% (for **CS-IP**), remaining almost constant during the remaining of the storage follow-up (6 months). Martins and Netto (2006) described a similar behavior for soy protein isolates, while Da Silva Pinto et al. (2005) also informed a reduction of water solubility for soy proteins but under more severe storage conditions (42 °C).

Regarding surface hydrophobicity, Fig. 6.b shows that Ho values increased during the first 3 months until reaching a maximum, but decreased during the following 3 months. These findings suggest that modifications of the association-dissociation state or conformational changes leading to exposure of hydrophobic residues may be taking place during the first period. In the second phase, in contrast, aggregation processes possibly through the formation of hydrophobic interactions or disulfide bonds may be causing a diminution of Ho values. As shown in both figures, there was no relationship between solubility and surface hydrophobicity, suggesting that the protein solubility decrease was due to conformational changes followed by protein aggregation, although such changes were not observed in thermograms obtained by DSC or in electrophoretic patterns (data not shown). There were not changes in the color of protein products during storage (not shown), in agreement with results reported by Hou and Chang (2004) regarding soy glycinin storage.

Notably, the properties of sunflower protein concentrates obtained with isoelectric precipitation did not vary significantly when storage conditions (T and RH) were changed, and since the samples studied had similar behavior regardless of phenolic compounds content of each concentrate, it can be speculated that such compounds do not affect the results of storage. The residual phenolic compounds, which would be interacting with proteins, are probably protected or less susceptible to oxidation along time than if they were stored free of proteins.

4. Conclusions

It was possible to successfully obtain at pilot plant scale sunflower protein concentrates with different content of phenolic compounds, high water solubility and antioxidant properties, starting from the residual sunflower oilcake. These protein products obtained through different processes also showed differences

in their chemical composition, phenolic content, color, surface hydrophobicity, and denaturation degree, which probably would be reflected in the functionality of these products. The presence of phenolic compounds only affected significantly the color and the antioxidant capacity of protein products. Sunflower protein concentrates obtained with isoelectric precipitation maintained high protein solubility until at least 6 month of storage, without differences according to the storage conditions assayed or the content of phenolic compounds of the protein products. These results, together with the good digestibility of sunflower proteins, allow reappraising the value of the residual sunflower oilcake as a source of proteins useful for the food industry. Moreover, considering that the equipment used in this study is similar to that should be used at industrial scale (basket type centrifuge with filtering material, self-desludging centrifuge, colloid mill and spray dryer), the protein products obtained in this work could be scaled up to commercial level.

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