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Fractioning of the sunflower flour components: Physical, chemical and nutritional evaluation of the fractions

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ABSTRACT

Reduced phenolic content products were obtained from defatted sunflower flour using a process designed to make an integral use of the components. In order to eliminate the phenolic compounds, the flour was extracted at pH 5 with extractor solutions: 70 mL/100 mL ethanol, 0.1 g/100 mL sodium bisulfite and a 70:30 mixture of the two. The raw material, protein isolate and fibrous concentrate were chemically characterized. The protein isolates were evaluated for protein extraction yield, protein solubility, heat stability and nutritional properties (chemical score, digestibility, PDCAAS). The fibrous concentrates from the extraction with bisulfite presented 60.84 g/100 g fiber and 35.67 g/100 g protein. The protein isolates result in protein contents above 92.00 g/100 g and phenolic compounds content below 0.45 g/100 g. All showed elevated protein solubility (>84.22%) and *in vitro* digestibility (>90.00%). The residual phenolic compounds content interfered with the digestibility and coloration of the isolates. The feasibility of the process for the prior extraction of the phenolic compounds to obtain high protein and nutritional value products was demonstrated. The mixture of bisulfite and ethanol was the most promising to obtain the isolate, and bisulfite solution was the best for the co-production of the fibrous concentrate.

1. Introduction

The sunflower (*Helianthus annuus* L.) is one of the four major predominant oleaginous cultures in the world, widely cultivated on the five continents (USDA, 2016). The growth of this culture in the world is to a great extent linked to the adopstion of systems that make complete use of the seed, since this results in environmental gains as well as promoting the amplification and sustainability of the culture. Thus, the total and effective use of the byproducts results in the economic valorization of the whole productive chain (Pedroche, 2015). The potential of the oil extraction residue includes: an elevated protein content (40–50 g/100 g), the fact that it is not genetically modified organism (GMO) and is rarely allergenic. All these factors indicate sunflower bran as a raw material for human consumption (Gassmann, 1983; González-Pérez & Vereijken, 2007; Wildermuth, Young, & Were, 2016). However, the obstacle for its use consists of the elevated phenolic compounds content (1–4 g/100 g), being predominantly chlorogenic acid. The phenolic compounds confer a dark green color on the bran and also bind to the proteins, causing an alteration in their functional properties and undesirable organoleptic characteristics. Currently, the bran resulting from the oil extraction, it is exclusively destined to animal feeding (González-Pérez et al., 2002; Pedrosa et al., 2000; Sodini & Canella, 1977; Weisz, Kammerer, & Carle, 2009).

Therefore, there is a constant search for technologies to extract the phenolic compounds from sunflower byproducts, which are technically and economically feasible for industry, since to date there is no consensus concerning the best process to be employed (Wildermuth et al., 2016). It is desirable that the extraction of the phenolic compounds occurs concomitantly with an elevated protein yield, and that the technological properties that make its

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application in foods feasible be maintained (González-Pérez & Vereijken, 2007). The strategies explored to obtain protein isolates propose the use of mixtures of organic solvents, saline solutions and/or reducing agents, before the alkaline extraction of the proteins (González-Pérez et al., 2002; Pickardt et al., 2009; Salgado, Ortiz, Petruccelli, & Mauri, 2011). Other methodologies use a combination of a slightly acid protein extraction with the adsorption of the phenolic compounds in resin (Pickardt, Hager, Eisner, Carle, & Kammerer, 2011; Weisz, Schneider, Schweiggert, Kammerer, & Carle, 2010).

The aim of the present work consists of the complete use of the byproducts resulting from the extraction of sunflower oil, by fractioning the major components of the flour, producing the following fractions: protein isolate, fibrous concentrate and an extract rich in phenolic compounds. Hence the procedures were selected aiming an elevated yield of protein extraction and a reduced phenolic content, presenting color characteristics, technological and nutritional properties appropriate to be used in human diet.

2. Materials and methods

2.1. Materials

Sunflower dehulled grain (*Helianthus annuus* L.) were provided by the company Giroil Agroindústria Ltda (Santo Ângelo, Rio Grande do Sul, Brazil). The oil was extracted in two steps: 1) cold extraction in a mechanical press (Carver Press, USA), and 2) hexane to extract the residual oil. The material was subsequently ground and homogenized (Retsch ZM 200, Germany) to obtain the sunflower flour used in the following procedures.

2.2. Obtaining of the fibrous concentrate (C-FC) and conventional protein isolate (C-I)

The conditions used were based on the methodology of Salgado, Molina Ortiz, Petruccelli, and Mauri. (2011) modified, as shown in Fig. 1. Preliminary studies pointed to the efficiency of the mixture in the proportion adopted in this work. The flour was dispersed in water (1:10 w/v), the pH adjusted to 9 (1 mol/L NaOH) and the mixture agitated for 1 h with monitoring of the pH value. It was then centrifuged at 11000×*g* for 20 min at 20 °C (Sorvall RC-26 Plus, USA), the supernatant reserved and the concentrate re-extracted. The supernatants were mixed and submitted to isoelectric precipitation (pH 4.5/1 mol/L HCL) of the proteins, leaving to rest for 1 h before centrifugation (11000×*g* for 20 min at 4 °C). The pH values of the final fibrous concentrate (C-FC) and final protein isolate (C-I) were adjusted to 7 (1 mol/L NaOH) and freeze dried. The processes was carried out in duplicate for validation of the yield results.

2.2.1. Treatment to reduce the phenolic contents and obtain the fractionated components

The efficiencies of the following 3 extraction systems were evaluated in order to obtain protein isolates with low phenolic contents: 1) 70 mL/100 mL ethanol (E), 2) 0.1 g/100 mL sodium bisulfite in water (B), and 3) a mixture of 70 mL/100 mL ethanol with 0.1 g/100 mL sodium bisulfite (70:30) (M). The fibrous concentrates and protein isolates generated were denominated: E-FC and E-I = sunflower fibrous concentrate and protein isolate extracted with 70 mL/100 mL ethanol; B-FC and B-I = sunflower fibrous concentrate and protein isolate extracted with 0.1 g/100 mL sodium bisulfite; M-FC and M-I = sunflower fibrous concentrate and protein isolate extracted with 70 mL/100 mL ethanol and 0.1 g/100 mL sodium bisulfite (70:30). For each extractor system, the defatted sunflower flour was submitted to 2 sequential extractions (1:10 w/v) at pH 5, and agitated for 1 h with monitoring of the pH value. After the extractions, the residue from each system was submitted to an alkaline protein extraction process (1:10 w/v) obtaining 3 products: 1) fibrous concentrate, 2) phenolic compounds rich extract, and 3) protein isolate, by the same procedure described to obtain the C-I. The processes were carried out in

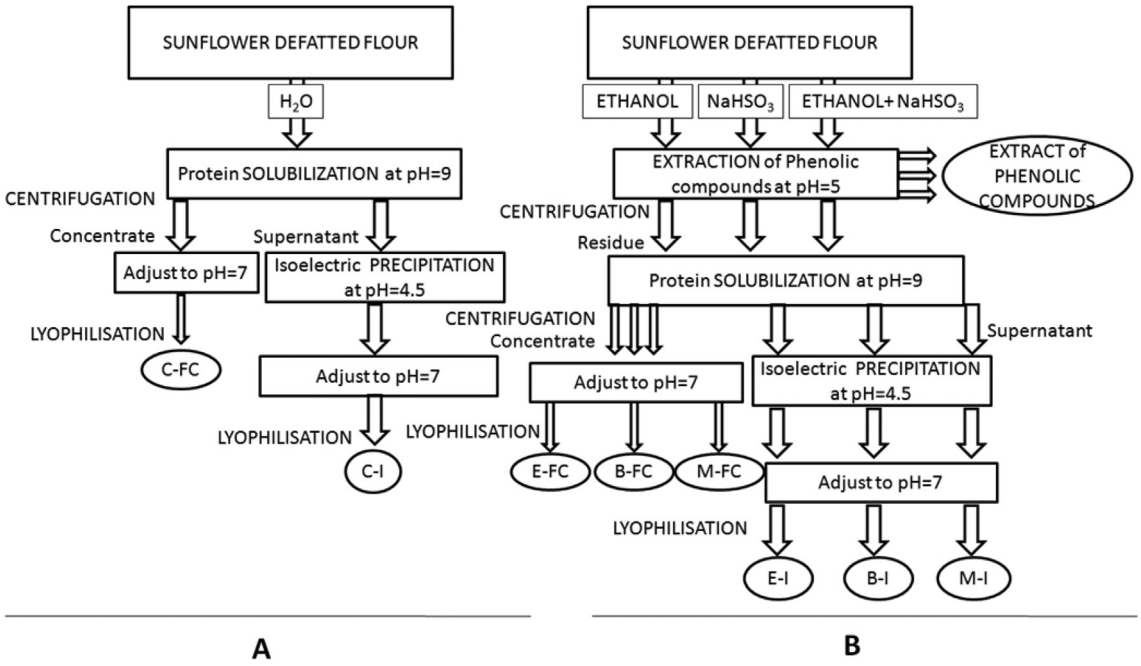

Fig. 1. Flowcharts for obtaining the sunflower products without (**A**) and with (**B**) extraction of phenolic compounds. C-FC and C-I = sunflower fibrous concentrate and conventional protein isolate; E-FC and E-I = sunflower fibrous concentrate and protein isolate extracted with 70 mL/100 mL ethanol; B-FC and B-I = sunflower fibrous concentrate and protein isolate extracted with 0.1 g/100 mL sodium bisulfite; M-FC and M-I = sunflower fibrous concentrate and protein isolate extracted with 70 mL/100 mL ethanol and 0.1 g/100 mL sodium bisulfite (70:30).

duplicate.

2.3. Protein yield and elimination of phenolic compounds

The protein extraction yield (g protein in protein isolate/g protein in flour) and the percent residual phenolic compounds expressed as chlorogenic acid (CGA) in the isolates (g CGA in protein isolate/g CGA in flour) were determined for each process. CGA was used to determine the residual phenolic compounds content since it is the predominant phenolic compound in sunflower (Weisz et al., 2009).

2.4. Determination of the chemical composition

The moisture (AOAC, 925.09), ash (AOAC, 923.03), dietary fiber (AOAC, 985.29) and protein contents, the latter determined by the Kjeldahl method with a conversion factor of 5.75 (AOAC, 960.52), were determined according to Latimer, 2012. The lipid content (AOCS Ai 3–75) according to Firestone (2013). The total phenolic content of the flour was determined by spectrophotometry at 750 nm (Varian Cary 50, USA), using chlorogenic acid (Sigma-Aldrich Co., St. Louis, USA) as the standard, according to Kim, Jeong, and Lee (2003). The chlorogenic acid content was determined by HPLC-DAD at 324 nm (Shimadzu Corporation, Tokyo, Japan), as described by Tfouni et al. (2012).

2.5. Color

The color of the sunflower protein isolates was determined in triplicate by the CIE L*a*b* color system using a CR 300 Minolta Choma Meter (Minolta Chroma Co., Osaka, Japan).

2.6. Protein solubility in water

This was determined at pH 7 according to Pilosof (2000). Samples were dispersed in distilled water (0.40 mg/mL) and agitated for 2 h at 30 °C. They were then centrifuged (2000×*g*/30 min/25 °C) and filtered. Soluble protein was determined in the filtered (Whatman nº 1) supernatant by the Kjeldahl method (AOAC, 960.52). The results were expressed as the percentage of soluble protein in relation to the protein content in the initial sample.

2.7. Differential scanning calorimetry (DSC)

The TA model 2010 DSC instrument (New Castle, USA) was used to study the thermal stability according to Añón (2000). Samples of approximately 10 mg were dispersed in distilled water at 10% (w/w) in hermetically closed pans. The heating rate was 10 °C/min in the interval from 20 to 160 °C.

2.8. Determination of the amino acid profile

The amino acid profiles of the flour and protein isolate were determined in a RP-HPLC with a UV detector at 254 nm (Shimadzu Corporation, Tokyo, Japan), equipped with a Luna/Phenomenex C18 column (250 mm × 4.6 mm × 5 μ; Phenomenex Inc., Torrence, USA). The amino acids were identified and quantified using an external standard (Pierce/PN 20088), as described by Hagen, Geada, and Augustin (1989) and White, Hart, and Fry (1986). Tryptophan was determined separately according to Spies (1967).

2.9. Nutritional value

The nutritional evaluation of the sunflower flour and protein products was carried out by *in vitro* digestibility, calculation the chemical score of the amino acids and the PDCAAS (protein digestibility corrected amino acid score). The PDCAAS was calculated according to Henley and Kuster (1994) with modifications, using the *in vitro* digestibility of the proteins.

The *in vitro* digestibility was determined according to Argyri, Miller, Glahn, Zhu, and Kapsokefalou (2007). The pH values of the flour (0.05 g/mL) and protein isolates (0.01 g/mL), dispersed in water, were adjusted to 2.8 (6 mol/L HCl) and 0.5 mL of a pepsin suspension (4 g pepsin in 100 mL 0.1 mol/L HCl) added and incubated for 2 h in a water bath at 37 °C with agitation. The pH value was then adjusted to 5.7 (5 mol/L NaOH) and 2.50 mL of a pancreatin solution containing bile salts (0.20 g enzyme plus 1.20 g bile salts in 100 mL 0.1 mol/L $NaHCO_3$) added, followed by incubation as above (2 h/agitation/37 °C). The samples were then centrifuged (9000×*g*/15 min/4 °C) and the digested nitrogen determined in the supernatant by the Kjeldahl method. The result was expressed in percent soluble nitrogen in relation to the amount of initial sample.

2.10. Statistical analysis

The results were expressed as the mean ± standard deviation and evaluated by the analysis of variance (ANOVA) and Tukey's test to verify the difference between the means, with a level of significance of $\alpha = 0.05$, using the XLSTAT program version 2012.6.03 (Addinsoft, France).

3. Results and discussion

3.1. Chemical composition, protein extraction yield and elimination of phenolic compounds

Table 1 shows the chemical composition and protein extraction yield. The use of dehulled grain to produce the flour contributed to a reduction in the total dietary fiber content, resulting in an increase in the contents of the other components, principally the protein content (61.06 g/100 g dry matter).

The fibrous concentrates obtained with the different extraction systems presented an interesting nutritional composition, due to the elevated proportions of the components of greater interest for the food industry, as also for consumption, such as protein and fiber. Fiber contents above 35.15 g/100 g (dry matter) and protein contents of up to 44.30 g/100 g (dry matter) were obtained. B-FC showed the highest fibrous concentration, probably due to the reduced ash content, which caused a concentration of the other components (Van Soest, Robertson, & Lewis, 1991). The residual mineral content was lower in the B-FC sample, probably due to the ionic strength of the sodium bisulfite solution, which led the ions to the phenolic extract. Fact also observed in the mineral content of B-I that was also proportionally lower amongst the protein isolates (Salgado et al., 2011).

The protein recovery yields from the isolates varied from 43.24% to 60.40%, E-I showing the lowest yield (43.24%) and protein content (92.08 g/100 g). However, all the isolates submitted to a prior extraction with the different extraction solutions showed a reduction in their phenolic compounds contents, as shown in Table 2. Similar characteristics showing lower protein yields in the production of protein concentrates from sunflower seeds when using 70 mL/100 mL ethanol were observed by Salgado et al. (2011). The other samples showed similar protein yields in the range from 55.67% to 60.40%, including the C-I. However, as expected, sample C-I showed the highest chlorogenic acid content (0.45 g/100 g dry matter) since it was not submitted to a prior extraction of phenolic compounds, before the alkaline treatment.

The protein isolates B-I and M-I showed no differences with

Table 1
Chemical composition of the sunflower whole grain, dehulled grain and flour used to produce the protein isolates and fibrous concentrates. Yield are expressed as protein recovery for each production process of the protein isolates.

Sunflower samples[A]	Chemical composition (g/100 g)						Yield (%)B
	Lipids[*]	Proteins[*]	Ashes[*]	Moisture	Dietary Fibers[*]	Carbohydrates[*,**]	Protein recovery
Whole grain	39.99 ± 0.36^{b}	18.36 ± 0.11^{g}	3.21 ± 0.01^{e,f,g}	7.82 ± 0.04^{a}	36.08 ± 0.07^{c}	2.06	NA
Dehulled grain	57.68 ± 0.44^{a}	25.99 ± 0.49^{f}	3.94 ± 0.02^{e,f}	6.03 ± 0.04^{b,c,d,e}	6.73 ± 0.11^{e}	5.66	NA
Flour	1.70 ± 0.28^{c}	61.06 ± 0.33^{c}	8.63 ± 0.04^{d}	9.88 ± 0.05^{a}	15.93 ± 0.05^{d}	12.68	NA
Protein isolates							
C-I	ND	92.68 ± 2.02^{b}	2.26 ± 0.08^{g}	5.44 ± 0.37^{c,d,e,f}	ND	–	60.40 ± 3.01a
E-I	ND	92.08 ± 1.10^{b}	4.00 ± 0.25^{e,f}	3.54 ± 1.64^{f,g}	ND	–	43.24 ± 1.67b
B-I	ND	94.81 ± 0.82^{a,b}	2.48 ± 0.04^{f,g}	3.34 ± 0.27^{g}	ND	–	55.67 ± 1.20a
M-I	ND	95.69 ± 2.30^{a}	3.86 ± 0.84^{e,f}	4.15 ± 1.71^{f,g}	ND	–	58.90 ± 0.75a
Fibrous concentrates							
C-FC	ND	23.01 ± 0.80^{f}	21.99 ± 1.94^{a}	6.92 ± 0.12^{b,c}	43.62 ± 0.49^{b}	–	–
E-FC	ND	44.30 ± 0.33^{d}	15.92 ± 0.18^{c}	4.81 ± 0.68^{d,e,f,g}	35.15 ± 0.79^{c}	–	–
B-FC	ND	35.67 ± 1.39^{e}	4.74 ± 1.06^{e}	4.60 ± 0.07^{e,f,g}	60.84 ± 0.21^{a}	–	–
M-FC	ND	37.87 ± 0.29^{e}	17.83 ± 0.15^{b}	6.77 ± 0.39^{b,c,d}	43.10 ± 0.44^{b}	–	–

[*]Values of chemical composition are expressed on dry basis. Protein (N = 5.75). ND = not determined. (n = 2), NA = not applicable.
[**] The content of carbohydrates was calculated by difference.
a, b, c, d, e, f, g Values in columns followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's test.
[A] Abbreviations in column represents: C-FC and C-I = sunflower fibrous concentrate and conventional protein isolate, E-FC and E-I = sunflower fibrous concentrate and protein isolate extracted with 70 mL/100 mL ethanol, B-FC and B-I = sunflower fibrous concentrate and protein isolate extracted with 0.1 g/100 mL sodium bisulfite; M-FC and M-I = sunflower fibrous concentrate and protein isolate extracted with 70 mL/100 mL ethanol and 0.1 g/100 mL sodium bisulfite (70:30).
[B]Yield (%) = protein recovery for each process considering protein content in flour as 100%.

Table 2
Chlorogenic acid (CGA) content of flour and protein isolates and its elimination of the protein isolates with respect to the amount present in the flour.

Sunflower samples[A]	CGA (g/100 g)	CGA Elimination (%)
Flour	2.46 ± 0.11^{a}	NA
Protein isolates		
C-I	0.45 ± 0.00^{b}	80.86 ± 0.00^{b}
E-I	0.07 ± 0.00^{c}	97.01 ± 0.15^{a}
B-I	0.09 ± 0.01^{c}	96.53 ± 0.22^{a}
M-I	0.08 ± 0.00^{c}	96.71 ± 0.07^{a}

Values of CGA are expressed on dry basis; NA = not applicable.
[a,b,c] Reported values for flour and protein isolates are means ± standard deviation (n = 2). In the columns, means followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's test.
[A] Abbreviations in column represents: C-I = sunflower conventional protein isolate, E-I = sunflower protein isolate extracted with 70 mL/100 mL ethanol, B-I = sunflower protein isolate extracted with 0.1 g/100 mL sodium bisulfite; M-I = protein isolate extracted with 70 mL/100 mL ethanol and 0.1 g/100 mL sodium bisulfite (70:30).

respect to protein content, yield and residual phenolic compounds, showing that the mixture of an organic solvent (ethanol) with a reducing agent (sodium bisulfite) caused combined effects which led to better results. Other studies employing different methods, such as that of Pickardt et al. (2011) obtained protein isolates with elevated protein contents (99.40 g/100 g dry matter), applying a saline system (2 mol/L) in a slightly acid medium (pH 6), with a protein yield of 58.00%). They used a polymeric resin to adsorb and hence reduce the phenolic compounds content, resulting in a residual of 0.26 g/100 g in dry matter of chlorogenic acid. Thus, washing steps were necessary to desalinize the product obtained, since a high saline concentration was used in order to favor protein extraction (Pickardt et al., 2009).

The values found in other works differed from this with respect to both the protein yield and residual phenolic content, due to the different raw materials and methodologies employed (González-Pérez et al., 2002; Kabirullah & Wills, 1981; Pickardt, Eisner, Kammerer, & Carle, 2015; Salgado et al., 2011, 2012; Shchekoldina & Aider, 2012). The differences can be attributed to the use of different solutions, extraction times and agitation intensities between the processes (Salgado et al., 2011, 2012).

The total phenolic content of the flour as determined by the Folin-Ciocalteu assay was 4.00 (±0.01) g/100 g dry matter, expressed as equivalents of chlorogenic acid. In the present study, the chlorogenic acid determined by HPLC corresponded to approximately 62% of the total phenolic compounds, since the flour contained 2.46 (±0.11) g/100 g dry matter (Table 2). These values for the total phenolic compounds are comparable to those found by Weisz et al. (2009) in defatted sunflower bran (~4.20 g/100 g). In this work, the phenolic compounds profile was discriminated and chlorogenic acid was predominant in all the fractions in the different sunflower varieties analyzed. Based on data in the literature, chlorogenic acid was selected to express the extraction yield of phenolic compounds (González-Pérez et al., 2002; Weisz et al., 2009).

Despite the elevated phenolic compounds extraction yields, none of the extraction processes completely removed the phenolic compounds, probably due to interactions with the proteins (Salgado et al., 2012). González-Pérez et al. (2002) obtained the complete removal of the chlorogenic and caffeic acids using 80 mL/100 mL methanol, and other phenolic compounds elimination methods used adsorption resins (Weisz et al., 2010) and combinations of mild acid extraction methods with adsorption on ion exchange resins, leading to the removal of more than 99.40% (Pickardt et al., 2015).

3.2. Color of sunflower protein isolates

The color parameters observed for the protein isolates presented differences (Table 3) between them, which can not be directly correlated to the residual content of phenolic compounds (Table 2). The greenish color (-a) of the sunflower protein products has been attributed to the presence of phenolic compounds. However, other papers in the literature also failed to find a direct proportionality of the residual content of phenolic compounds and color parameters, as occurred in the present study (Pickardt et al., 2015; Salgado et al., 2011). The lighter color (L*) presented by the flour was probably because it had not been submitted to the alkaline extraction process, despite its higher total phenolic compounds content (4.00 g/100 g dry matter) (Wildermuth et al., 2016). The flour showed a lighter coloration (L*) in comparison to the

Table 3
Color parameters of the sunflower flour and protein isolates.

Sunflower samples[A]	Color parameters		
	L^*	a^*	b^*
Flour	85.16 ± 0.06[a]	0.25 ± 0.04[d]	8.11 ± 0.14d
Protein isolates			
C-I	51.66 ± 0.12[e]	−7.02 ± 0.03[e]	2.21 ± 0.02e
E-I	66.88 ± 0.38[b]	1.16 ± 0.08[c]	10.23 ± 0.33c
B-I	62.27 ± 0.09[d]	5.17 ± 0.07[a]	12.22 ± 0.07b
M-I	65.11 ± 0.35[c]	3.73 ± 0,02[b]	12.92 ± 0.06a

[a, b, c, d, e] Values in columns followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's test. ($n = 3$).
L^* (lightness), $a^* = (+a$ = reddish, -a = greenish), $b^* = (+b$ = yellowish, -b = bluish).
[A] Abbreviations in column represents: C-I = sunflower protein isolates obtained without extraction of phenolic compounds, E-I = protein isolate extracted with 70 mL/100 mL ethanol, B-I = protein isolate extracted with 0.1 g/100 mL sodium bisulfite, M-I = protein isolate extracted with 70 mL/100 mL ethanol and 0.1 g/100 mL sodium bisulfite (70:30).

isolates because the procedure that caused the oxidation of its phenolic compounds was not applied. Amongst the protein isolates E-I was the lightest (greater L^*) and B-I the darkest, with a slightly reddish hue (greater a^*) when compared to M-I. The protein isolate C-I presented a greenish hue (negative value for a^*), an undesirable characteristic, since the color is considered to be an important item for the technological application of the isolates. The dark green color can be attributed to the presence of phenolic compounds oxidized during the alkaline protein extraction (Saeed & Cheryan, 1988). Thus, Budryn and Rachwal-Rosiak (2013) stated the need to remove the phenolic compounds to obtain a high quality protein without green pigmentation, due to the oxidation of the phenolic compounds and their covalent binding to the proteins.

However, due to the appeal of the phenolic compounds in relation to their antioxidant capacity and resulting benefits, it might be reasonable to search for a balance between the antioxidant capacity and the color of the protein products (Salgado et al., 2011; Wildermuth et al., 2016).

3.3. Solubility and thermal stability of sunflower protein isolates

The results obtained (Fig. 2) show the elevated water solubility of the isolates, with values between 78.49% and 96.66%. On applying various types of extraction solutions, Rahma and Rao (1981) observed that organic solvents reduced the solubility of protein isolates, a fact demonstrated by samples E-I and M-I. The solubility of the flour was much lower than that of the isolates (40.71%), possibly due to the involvement of the proteins with the other components of the matrix. The protein solubility may be affected by the presence of other components, such as carbohydrates. The charged groups of protein side chain, such as Ɛ-amino lysine, may complex to carbohydrates or reducing sugars, affecting treducing the solubility (Venktesh & Prakash, 1993).

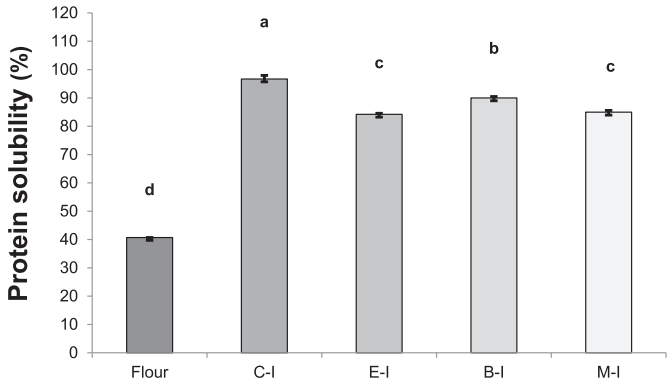

Fig. 2. Protein solubility (pH 7) of sunflower flour and protein isolates obtained without (C-I) and with extraction of phenolic compounds: E-I = protein isolate extracted with 70 mL/100 mL ethanol, B-I = protein isolate extracted with 0.1 g/100 mL sodium bisulfite, M-I = protein isolate extracted with 70 mL/100 mL ethanol and 0.1 g/100 mL sodium bisulfite (70:30). Reported values for each samples are means ± standard deviation ($n = 3$). Bars followed by different letter means significantly difference ($p < 0.05$) according to Tukey's test.

Studies have shown that non-covalent bonding between phenolic compounds and globular proteins does not alter the solubility in a pronounced way, whereas covalent bonds can affect this properties of the protein (Prigent et al., 2003). Distinct values for protein solubility were found in the literature, from approximately 10%–80%. These results must reflect the degree of protein denaturation as a consequence of the procedures adopted to extract the phenolic compounds and/or proteins (González-Pérez et al., 2002; Salgado et al., 2011; Pickardt et al., 2009; Pickardt et al., 2015).

The protein denaturation temperatures of the isolates obtained in this work varied between 108.7 °C and 113.0 °C, slightly above the value found for the globulin heliantinin (~105.0 °C), the major protein in sunflower, at a pH value between 6 and 8 (Molina, Petrucelli & Añón, 2004). The sample E-I presented the lowest denaturation temperature (108.7 °C), and also the lowest solubility. On the other hand, the lowest denaturation enthalpy was registered for the sample M-I, the others being slightly higher and showing no difference between them (data not shown). The protein solubility and the protein denaturation enthalpy showed that the processing conditions were mild and did not cause extensive protein denaturation.

3.4. Nutritional evaluation

The sunflower flour and products obtained presented an adequate essential amino acid balance, except for lysine, according to the FAO/WHO/ONU standard (2007). The limiting amino acids in the flour were lysine (0.75) and the sulfur amino acids (0.88) (Table 4). The results showed that the processes used to obtain the isolates reduced the lysine contents even more (0.56–0.65). This reduction was probably caused by the alkali in the protein extraction step (Bagnis, 1984). Although lysine is an essential amino acid and indication of nutritional quality, this does not prevent the sunflower from being considered as a source of good quality protein, and complement other protein sources where lysine is in excess (Conde, Escobar, Jiménez, Rodríguez, & Patino, 2005).

There is controversy concerning the influence of phenolic compounds on protein digestibility. Some authors found reduced *in vitro* digestibility, explaining that the bonding between phenolic compounds and the sunflower proteins could inhibit the proteolytic digestive enzymes (Bau, Mohtadi-Nia, Mejean, & Debry, 1983; Synge, 1975). However, studies with animals have shown that the presence of chlorogenic acid does not affect the *in vivo* protein digestibility (Eklund, 1975; Treviño et al., 1998). Salgado et al. (2012) showed elevated *in vitro* digestibility, where samples of protein concentrate containing 2.50 g/100 g phenolic compounds, presented 95.40% digestibility. The samples evaluated in the present study also showed elevated *in vitro* digestibility (Table 4), all the values for the protein isolates being above 90%. The best value for digestibility was presented by E-I (95.32%), and the flour presented the lowest protein digestibility (83.13%), probably due to the interaction of the proteins with other matrix components and also the elevated phenolic compounds content (4.00 g/100 g dry matter). It was shown that the phenolic compounds content had a slight effect on digestibility, since sample C-I, which had 0.45 g/100 g in dry matter chlorogenic acid residual, presented reduced

Table 4
Essential amino acid composition, digestibility and PDCAAS of the sunflower flour and protein isolates.

Amino acids (mg/g protein)	FAO (reference)[A]	Flour		Protein isolates[B]							
				C-I		E-I		B-I		M-I	
		AA	CS	AA	CS	AA	CS	AA	CS	AA	CS
Histidine	19.00	24.81	1.65	26.22	1.75	25.43	1.70	25.36	1.69	26.70	1.78
Isoleucine	28.00	41.65	1.39	45.67	1.52	44.60	1.49	42.68	1.42	46.74	1.56
Leucine	66.00	61.83	1.05	66.04	1.12	63.71	1.08	62.93	1.07	66.68	1.13
Lysine	58.00	33.83	0.75	29.05	0.65	26.85	0.60	25.35	0.56	28.93	0.64
Methionine + cysteine	25.00	19.40	0.88	31.33	1.42	30.30	1.38	27.29	1.24	30.50	1.39
Phenylalanine + tyrosine	63.00	72.05	1.90	80.73	2.12	82.36	2.17	75.90	2.00	84.18	2.22
Threonine	34.00	39.20	1.70	39.49	1.72	36.83	1.60	33.09	1.44	39.19	1.70
Tryptophan	11.00	10.90	1.82	13.49	2.25	14.38	2.40	14.71	2.45	13.51	2.25
Valine	35.00	50.67	1.30	55.97	1.44	53.09	1.36	50.56	1.30	56,72	1.45
Digestibility (%)		83.13 ± 0.82[c]		90.66 ± 2.64[b]		95.32 ± 1.13[a]		91.72 ± 2.17[a,b]		91.62 ± 1.93a,b	
PDCAAS		0.63 ± 0.00[a]		0.59 ± 0.00[b]		0.57 ± 0.00[c]		0.52 ± 0.00[d]		0.59 ± 0.00b	

[A] FAO (reference) recommended essential amino acids for adults by FAO/WHO/ONU, 2007.
AA = amino acids. CS = chemical score. PDCAAS = protein digestibility corrected amino acids score. ($n = 2$).
[a, b, c] Digestibility values ($n = 4$) in row followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's test.
[B] C-I = sunflower protein isolates obtained without extraction of phenolic compounds, E-I = protein isolate extracted with 70 mL/100 mL ethanol, B-I = protein isolate extracted with 0.1 g/100 mL sodium bisulfite, M-I = protein isolate extracted with 70 mL/100 mL ethanol and 0.1 g/100 mL sodium bisulfite (70:30).

digestibility compared to the other isolates (90.66%).

When the protein digestibility is associated with a lysine limitation as determined by the chemical score of the amino acids in the isolates, this is reflected in the values calculated for PDCAAS. The flour showed the highest value (0.63) amongst the samples, the lowest result was obtained for the sample B-I (0.52). The other samples showed no significant differences.

4. Conclusions

The industrial production of oil from dehulled sunflower seeds generates, as by-product, a flour rich in proteins and dietary fiber which can be destined to human food, once it's phenolic compounds are reduced. The proposed methodology allowed the removal of the flour's phenolic compounds, and the integral use of the defatted flour main components. The proposed technique enabled a sustainable use of the flour, without production of inorganic residues and waste of raw material. All protein isolates showed low phenolic contents and high yield of protein recovery, however the best extractive solution was the mixture composed by sodium bisulfite with ethanol. The fibrous concentrates besides the dietary fibers content, as main component, presented a considerable protein content as well. The most effective fiber's recovery extraction was observed by sodium bisulfite solution. Phenolic compounds once oxidized, even in reduced amounts in the protein isolates, affected their coloration. Nevertheless, the composition characteristics and nutritional properties presented by the obtained products were shown to be appropriate for their application as a food ingredient.

Conflict of interest

The authors declare that there are no conflicts of interest.

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