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Thermal studies on protein isolates of white lupin seeds (*Lupinus albus*)

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Abstract This study used TG, DSC, and SDS-PAGE techniques to study protein isolates (PIs) in the powder form obtained from lupin seeds flour *Lupinus albus*. Different methods of preparing PIs were tested, resulting in final products that were different only in relation to the yield and protein content. The results of the protein analysis by SDS-PAGE showed that the same protein fractions were present in the lupin seeds and in the obtained PIs. This result shows that the process of extraction was not damaging to the composition of the original protein. On the other hand, the results of the thermal analysis (DSC and TG–DTG curves) obtained for the different PIs, led to the detection of changes in the protein conformation through the ΔH values, which in general decreased with increasing values of pH and ionic strength in the experimental conditions of extraction.

Keywords *Lupinus albus* · TG · DSC · Protein isolate · SDS-PAGE

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Introduction

Legumes have been consumed for a long time, essentially in the form of grain. Nowadays, these plants are also utilized in other forms such as flour, concentrates, and protein isolates [1, 2]. The consumption of legumes and their components is growing due to, among other factors, several recent studies indicating beneficial effects to human health of legume when compared to animal protein consumption, and the availability of varieties with low contents of anti-nutritional factors [3, 4].

Lupin is a more versatile legume than soybean, since it grows better in low fertility soils and in regions with mild winters and/or colder and wetter summers. This legume has been consumed by humans for more than 3,000 years in the Mediterranean region [5]. The protein-rich lupin has an amino-acid profile similar to that of soybean and a high potential to be exploited in the manufacture of food products [1, 6]. Nevertheless, the use of protein isolates by the food industry is not only conditioned by their nutritive quality, but also by other factors affecting their functional properties, such as solubility and emulsifying properties [6].

The increasing use of lupin proteins by the food industry is stimulated mainly by the beneficial effects of these macromolecules and their major globulin components (beta- and alpha-conglutins) on human health and

well-being (hypocholesterolaemic, anti-atherogenic, hypotensive, and hypoglycaemic activities) [4–6]. However, this use is hindered by the yet limited knowledge available on the consequences of industrial processing on the quality of the lupin protein isolates obtained, and its technological and functional properties.

Protein isolate extraction and food processing usually involve heat treatment but there are only a few reports analysing the thermal behavior of lupin flour and their protein isolates. In fact, when promoting lupin-based foods for inclusion in a daily diet, the thermal damage suffered during processing is of relevance to the bioactive and nutritional quality of the food product [7]. Hence, more information is needed for a better understanding of the changes that occur in those molecules during heat treatment. Thermal analysis, an important set of techniques used in quality control of food processing [8, 9], including thermogravimetry-derivative and thermogravimetry (TG–DTG) and differential scanning calorimetry (DSC), was used to that purpose. These techniques have previously been applied to the study of isolated and purified proteins [10, 11].

The aim of this study was to assess the effects of salt and pH during the isolation procedure of protein that affect conformational structure and stability of the resultant proteins in powder form. Although these effects are mainly studied in solution, they still prevail in the powder. As this is the form that will be used when these proteins are added to food this information becomes relevant.

This study describes the molecular and thermal characteristics of protein isolates obtained from white lupin (*Lupinus albus*) grown in Brazil. Different methods of protein extraction were tested and the results compared. DSC techniques were used here to identify the white lupin protein denaturation temperature and to help in its quality control.

Experiment

Materials

The mature seeds of a sweet variety of white lupin were obtained from IAPAR (Agronomic Institute of Paraná), Londrina, PR, Brazil. Alkaloids were removed by soaking the seeds in water at 50 °C, three times a day, for 5 days. Afterwards the seeds were oven dried at 50 °C, manually decorticated, and pulverized in a hammer grinder with a 0.4 mm sieve. The resulting lupin flour was defatted with hexane (1:5 w/v) for 4 h, under constant shaking, and oven dried at 50 °C to remove the solvent residues. Finally, the flour was homogenized, stored in polyethylene bags, and frozen (−18 °C) until analysis.

Methods

Chemical composition

Moisture, ashes, fibers, crude fat, and protein ($N \times 6.25$) content from lupin seed flour (LSF) were determined according to AOAC [12] (standard methods 925.10, 923.03, 920.86, 920.85, and 960.52, respectively). Moisture content was determined by drying the samples (105 °C), and ashes were by incineration (550 °C), both to a constant weight. Crude fiber content of the defatted and dehulled samples was determined by decomposing the starches with acid and the protein with alkali and then filtering. The crude fat was determined by the Soxhlet method. Crude protein ($N \times 6.25$) was determined by the micro-Kjeldahl method. All analyses were performed in triplicate, and the results presented as mean ± standard deviation on a dry weight basis.

Influence of pH and NaCl concentration on protein solubilization

The percentage of soluble protein in the isolate was measured according to Wang and Kinsella [13] with modifications. In brief, solubility was measured over the pH range of 1.0–12.0, in solutions without or with NaCl (concentrations varying from 0 to 1.0 mol/L). LSF sample dispersions (1:20 w/v) were adjusted and monitored to each pH level by adding HCl or NaOH (0.1 N) at room temperature, and stirring for 60 min. Each sample was then centrifuged at 10,000 *g* for 60 min. Nitrogen content (mg) in the supernatant was determined by the Kjeldahl method (960.52) [12] and $N \times 6.25$ was used to convert nitrogen to protein. Solubility was expressed as the percentage of total nitrogen of the original sample that was present in the soluble fraction.

Preparation of protein isolates (PIs)

PIs were obtained from LSF as described by Liadakis et al. [14] with modifications on the extraction step. In brief, LSF was suspended in distilled water (1:20 w/v), with or without the addition of Na_2SO_3 (0.25%) or NaCl (0.3 or 0.5 mol/L), and the pH adjusted and monitored between 7.0 and 11.0 by adding drops of 0.5 mol/L NaOH. The suspension was stirred for 30 min at room temperature, and then centrifuged at 10,000 *g* for 30 min. The supernatant volume obtained during samples extraction with 0.5 mol/L NaCl at pH 10.0 and 11.0 was further subjected to ultrafiltration (pore size 5 kDa) and the permeate was discarded to reduce the initial volume to a few milliliters. The supernatants were then adjusted to pH 4.5 or 5.0 for isoelectric precipitation (pI) of the protein, by adding

Table 1 Chemical composition of dehulled and defatted lupin seed flour (LSF) *Lupinus albus*

Components	Mean value/%[a]
Moisture	8.10 ± 0.30
Protein	49.88 ± 1.31
Ether extract (crude fat)	2.94 ± 0.08
Fibers	16.10 ± 0.43
Mineral residue (ash)	1.54 ± 0.05

[a] Mean ± standard deviation

0.1 mol/L HCl. Finally, they were centrifuged at 10,000 *g* for 30 min. The protein pellet obtained was resuspended in water, pH adjusted to 7.0 with the addition of 0.5 mol/L NaOH, freeze-dried, and homogenized.

At the end of each extraction, the lyophilized protein isolate was weighed and yield was calculated as a percentage of the protein in the isolate relative to the amount of protein present in the lupin seed flour (see Table 1) used for extraction.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out on LSF protein isolates and ground lupin seed samples. The dried samples were dissolved in 0.2 M phosphate buffer (pH 7.5) at a final concentration of 1.2 mg mL^{-1}, were denatured in a sample buffer (0.125 M Tris–HCl, pH 6.8, 10% glycerol [v/v], 2% SDS [w/v], 5% 2-mercaptoethanol [v/v], 0.02% bromophenol blue [w/v]). The denatured samples were boiled for 5 min and immediately cooled on ice before being analysed in a discontinuous SDS-PAGE. The electrophoresis was carried out by the method of Laemmli [15] with a 4.0% concentration gel and a 12.5% separating gel containing 0.1% SDS. Samples were electrophoresed at 25 mA before entering the separating gel, where they were electrophoresed at 30 mA constant current for 5 h. Full-size slab gels (16 × 18 × 1.5 cm) were run using a vertical electrophoresis apparatus (SE 600 Ruby, GE Healthcare, Piscataway, NJ, USA). Total protein in the gels was stained with Coomassie brilliant blue R. A molecular size ladder (Pageruler™ unstained protein ladder, Fermentas), with markers from 10 to 200 kDa, was used as reference. The molecular weights of polypeptide bands were estimated with the Kodak Digital Science 1D (2.0.3) (Eastman Kodak Co, Rochester, NY, USA).

Thermal analysis

Simultaneous TG–DTG and DSC curves were obtained from a SDT 2960 and a DSC 2910 calorimeters (TA Instruments, New Castle, DE, USA). The enthalpy of indium was used for the calibration of temperature and heat capacity (m.p.: 156.6 °C, $\Delta H_f = 28.45$ J g^{-1}). The PI sample sizes were ~4.0 mg (±0.05 mg) of protein in PI and were analysed by TG–DTG in an α-alumina crucible at heating rates of 10 °C min^{-1} and under nitrogen flow (100 mL min^{-1}). DSC curves were obtained from ~4.0 mg (±0.05 mg) of protein in PI samples, sealed in an aluminum crucible (covered) under a nitrogen flow (50 mL min^{-1}) and at a heating rate of 10 °C min^{-1} from 20 to 140 °C. The TG/DTG and DSC results for protein isolates are the average of at least three runs. Denaturation temperatures and enthalpy values were noted.

Statistical analysis

Statistical analyses using one-way analysis of variance (ANOVA) were performed to test the significance of differences ($P < 0.05$) between extraction conditions. When the interaction was significant, the Tukey multiple comparison test was used as a post hoc test. The statistical analyses were performed using the SPSS software, version 13.0 for Windows (SPSS, Inc., Chicago, Ill., U.S.A.).

Results and discussion

Chemical composition

The average values of the chemical composition of LSF are shown in Table 1.

When compared to the protein values obtained from whole lupin by Martinez-Villaluenga et al. [16], (30.6%) and Lqari et al. [17], (33.8%), the ones reported in our dehulled and defatted lupin (Table 1) were higher (49.88%). Other high protein contents in defatted samples were reported [18, 19]. Therefore, we may consider these two procedures (dehulling and defatting) as being necessary to optimize the process of obtaining PIs. The works previously mentioned indicate fat contents of 14.6 and 13.6%, respectively, on full fat flour. In our case, the flour under study was defatted, and the values obtained (2.94%) were similar to the ones obtained by Neves et al. [18] and Rodrigues-Ambriz et al. [19] also on defatted flours. These identical and low values seem to demonstrate the efficiency of the defatting process. This is of critical importance since, when obtaining PIs, during the extraction of the protein fat can result in emulsions, hindering the attainment of PIs. As expected, the LSF fiber content observed in our study was lower than the one obtained from whole seeds (e.g., Martinez-Villaluenga et al. [16]) and similar to those obtained on a dehulled Turkish *L. albus* variety [20].

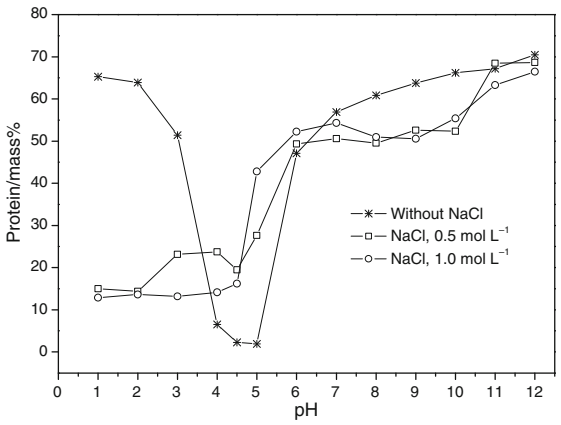

Fig. 1 LSF protein solubility curves achieved for $1 \leq pH \leq 12$ and NaCl concentration varying from 0 to 1.0 mol/L, $T = 25 \pm 3$ °C

Influence of pH and NaCl concentration on protein solubilization

The lupin seed flour (LSF) protein solubility curves in distilled water or NaCl solution (0.5 and 1.0 mol/L) are presented in Fig. 1. The solubility varies with the pH values and salt concentration, being higher at extremes of pH and in the presence of the salt.

Previous works pointed out the effect of salt concentration on the protein solubilization. In fact, El-Adawy et al. [21] reported an increase in protein solubility of Egyptian bitter (*L. termis*) and sweet (*L. albus*) lupin samples as a response to sodium chloride concentration. The solubility was higher at the region of the isoelectric point when salt was present in the extracting medium. The same phenomenon was observed for the major *L. albus* protein, globulin [22]. However, this study shows that solubility is influenced not only by salt concentration, but also by pH. Finally, and according to Fig. 1, a solubility minimum was observed at pH 5.0 (without salt) at the isoelectric point (pI), as previously observed with *L. termis* and *L. albus* [21] and *L. albus* globulin [22]. The addition of NaCl resulted in a change of the solubility profile, leading to an increase in solubility at the isoelectric region.

Experimental proceedings to extract PIs were based on the solubility curves. Seeking to optimize the process, several proceedings were also adopted from the literature: a solution of 0.3 mol/L NaCl with pH adjustments ranging from 7.0 to 10.0 [18], and at extreme pH values, Na_2SO_3 was added [17].

Obtaining of protein isolates (PIs)

Table 2 shows average values of the extraction yield and protein content for PIs obtained from LSF. This table shows that the lowest yield value was obtained when extraction was performed with NaCl at pH 7.0–10.0. By using the same conditions above mentioned and increasing the pH value to 11.0, and NaCl to 0.5 mol/L, the yield was improved to 40.85% and the protein content increased to 90.63%. While extracting at pH 9.0 and pI 4.5, Rodrigues-Ambriz et al. [19] obtained from *L. campestris* a PI with a protein content of 93.2%, but no yield value was mentioned.

The extraction of PI at pH 11.0 with the addition of 0.25% Na_2SO_3 led to a higher yield (53.19%), but also to lower protein content (79.75%). El-Adawy et al. [21] reported a protein content higher than the ones obtained at this study (~91%) for PIs from *L. termis* and *L. albus* extracted at pH 9.0, using 0.25% Na_2SO_3. However, Lqari et al. [17] recorded 83.9% protein on a PI extracted at pH 10.5 with 0.25% Na_2SO_3. This value increased to 87.4% when the extraction was carried at pH 12.0, without the salt.

Table 2 also reveals that PIs extracted from material retained after ultrafiltration by solubilization with 0.5 mol/L NaCl at pH 10.0 and 11.0 solution, and precipitation at pH 5.0, and protein isolate extracted with 0.3 mol/L NaCl at pH 10.0 and precipitation at pH 4.5, presented higher protein content than those obtained under other experimental conditions, but with intermediate yields. Isoelectric precipitation of lupin proteins can vary between pH 4.5 and 6.5, depending on the subunits of the proteins [23, 24]. On this study, the use of pI 5.0 was based on the results obtained from the protein solubility curves (see Fig. 1), where minimum solubility was observed.

During isoelectric precipitation, the ionic strength has promoted the "salting out" effect in the protein isolate extracted with NaCl in alkaline pH (10.0 and 11.0), resulting in purer PIs but with lower yield values when compared with PIs extracted without NaCl. These results show that at higher levels of pH and in the absence of salt, we can extract higher masses of PI but with lower protein contents. Isolation without salt adding was less efficient with less "salting out" effect and more salt formed by neutralization of NaOH present in the precipitate. Fontanari et al. [11], in similar procedure for guava seed protein isolate, reported higher levels of ash in PIs obtained at higher pHs without NaCl adding.

Thermal behavior of PIs

The TG–DTG curves recorded for PIs extracted at pH 10.0 and 11.0 (0.5 mol/L NaCl) presented in Fig. 2 are representative of those obtained for all the PIs.

The TG curves show two stages of well-defined mass loss. The first stage was attributed to the removal of water over the temperature interval of 30–150 °C, with a loss of ~6% of the mass. This stage is associated with the process

Table 2 Yield, protein content, denaturation enthalpy and peak temperature for *L. albus* PIs obtained under different extraction conditions

Protein isolate/PI	pI	Yield/%	Protein content/%	ΔH/J/g	Tp/°C
pH 7.0 NaCl 0.3 mol/L	4.5	31.01 ± 0.95^{f}	$87.85 \pm 0.53^{b,c}$	335.7 ± 0.4^{a}	70.0 ± 0.5^{a}
pH 8.0 NaCl 0.3 mol/L	4.5	$32.58 \pm 0{,}86^{f}$	$83.69 \pm 1.11^{c,d}$	325.5 ± 0.6^{b}	65.7 ± 0.7^{b}
pH 9.0 NaCl 0.3 mol/L	4.5	$33.89 \pm 0.86^{e,f}$	89.20 ± 0.94^{b}	314.5 ± 0.7^{c}	66.5 ± 0.7^{b}
pH 10.0 NaCl 0.3 mol/L	4.5	$35.94 \pm 0.79^{e,f}$	$90.10 \pm 0.62^{a,b}$	$313.3 \pm 0.9^{c,d}$	$68.8 \pm 0.6^{a,b}$
pH 10.5 Na_2SO_3 0.25%	4.5	$48.31 \pm 0{,}92^{c}$	80.39 ± 1.19^{d}	310.8 ± 1.0^{d}	$68.4 \pm 0.8^{a,b}$
pH 11 Na_2SO_3 0.25%	4.5	$53.19 \pm 0.89^{a,b}$	79.75 ± 0.37^{d}	na	na
pH 10.0 (H_2O)	5.0	52.39 ± 0.70^{b}	85.82 ± 1.22^{c}	276.9 ± 0.9^{e}	$69.3 \pm 0.4^{a,b}$
pH 11.0 (H_2O)	5.0	56.08 ± 0.54^{a}	$86.33 \pm 1.22^{b,c}$	274.8 ± 1.2^{e}	$69.2 \pm 0.5^{a,b}$
pH 10.0 NaCl 0.5 mol/L[a]	5.0	$34.77 \pm 0.92^{e,f}$	92.41 ± 0.40^{a}	277.2 ± 0.8^{e}	$69.9 \pm 0.7^{a,b}$
pH 11.0 NaCl 0.5 mol/L[a]	5.0	40.85 ± 0.86^{d}	$90.63 \pm 0.63^{a,b}$	250.3 ± 0.9^{f}	66.6 ± 0.6^{b}

[a] Ultrafiltration undertaken before isoelectric precipitation

pI isoelectric point, *na* not analysed

Means in the same column not sharing common *superscript letters* are significantly different ($P < 0.05$)

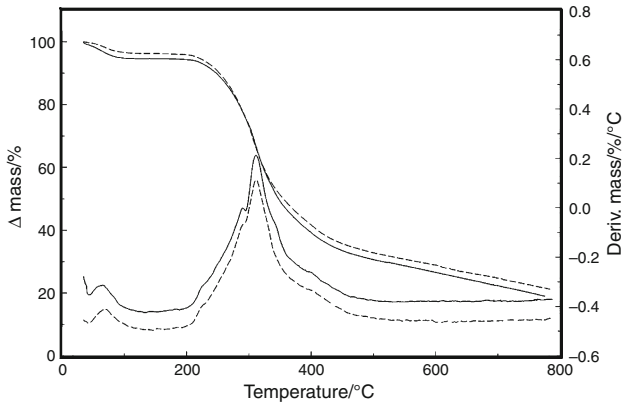

Fig. 2 TG–DTG curves of the PI compounds extracted at pH 10.0 (*solid curve*) and 11.0 (*dashed curve*); 4 mg of protein under a dynamic nitrogen atmosphere (100 mL/min), heating rate 10 °C/min

of protein denaturation [25] and agrees with the results presented in Table 2, from the DSC technique. The second stage takes place in the temperature interval of 208–800 °C, where the initial temperature of this stage suggests a high thermal stability of this fraction (T = 195–208 °C). Such a temperature is important for the food industry, allowing the use of proteins at higher temperatures than the conventional ones. This second stage corresponds to the thermal decomposition of the protein fraction. The TG curve was generated in a nitrogen atmosphere, but even so, carbonized matter was produced at the final temperature (800 °C).

The temperature peak (T_p) and enthalpy denaturation (ΔH, J g^{-1}) values for the PIs tested by DSC curves recorded at the range of 30–140 °C are presented in Table 2. The DSC curves (see Fig. 3) showed the presence of only one broad endothermic peak between 63 and 74 °C, corresponding to the denaturation temperature (T_p) of these PIs analyzed in the powder form. This single peak can be the convolution of various contributors, as several protein fractions are expected in the isolates.

Sousa et al. [26] studying another species of lupin (*Lupinus luteus*) found that the protein isolate in solution presented three endothermic peaks (96.9, 103.85, and 111.85 °C) being the major characteristic central peak assigned to globulin. When the major protein fraction (globulin) was compared to the whole protein isolate, they observed the presence of two endothermic peaks, that were attributed to the denaturation of the 7S (102.95 °C) and 11S (109.05 °C) fractions. All these experiments were performed using the protein in solution.

Xu and Mohamed [27] reported for lupin flour the presence a middle point of vitreous transition (63 °C) being assigned to pro gelatinization process and exothermic transitions (146 and 153 °C) attributed to aggregates in this temperature range. They also compared the lupin flour with gluten (wheat protein) and reported for wheat protein only one middle peak in the region of 63 °C, temperature close to that found in this study.

Sirtori et al. [28] observed for the *Lupinus angustifolius* protein, the presence of two endothermic peaks when analyzed in a pH 7.0 solution. The peak of the first transition occurred around 71.49 °C, was attributed to the denaturation fraction Vicilin (β-conglutin) and the second peak, which occurred around 90.92 °C, was attributed to the denaturation of legumin fraction (α-conglutin).

Works on behavior thermal of proteins are performed in solution or powder dry, thus obtaining different results, according to the variables used as pH, ionic strength, dispersion m:v ratio, among others [29–31]. The study with PI in dry powder to observe the endothermic transition process after the removal of water (see Figs. 2, 3a, b) for the denaturation of the protein, typically presents a large peak

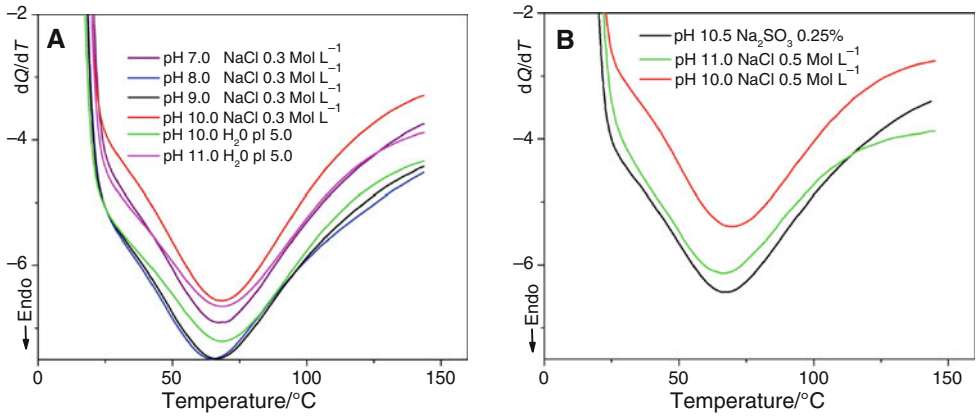

Fig. 3 DSC thermograms for: **a** PIs without and with 0.3 mol/L NaCl and **b** PIs extracted with 0.25% Na_2SO_3 and 0.5 mol/L NaCl, plus ultrafiltration before isoelectric precipitation

in the region of denaturation, offering so a new characterization method for vegetable proteins [32].

Table 2 also shows that the highest value of ΔH, the energy required to completely denature the PI, was obtained with the PI extracted at neutral pH and in the presence of salts (NaCl). Hence, the use of salts in the extraction process can alter protein conformation, and thus its thermal stability. The stability and the conformation of proteins are controlled by the balance between the polar and non-polar side chains of amino acid residues from proteins [25]. Therefore, the quantity of salt present in the medium (the ionic strength) can lead to greater or lower molecular stability. Based on Table 2 data, we observe that at pH 10.0, when the concentration of NaCl rises from 0.3 to 0.5 mol/L, the heat of denaturation falls $\sim$12%. This suggests that the extraction conditions have influenced the PI stability, even though the DSC experiments were not performed in the aqueous medium.

The PIs extracted at pH 10.0 and 11.0 without salts, showed decreased values of ΔH. The same was observed for extraction at pH 10 and 11 with NaCl 0.5 mol/L, demonstrating that the pH variation and ionic strength had influence on the value of enthalpy. In fact, a pH further from neutrality led to lower values of ΔH, suggesting that at an alkaline environment, the protein structure is altered, provoking its denaturation. Similar results were found for rice globulin [33], flax seed proteins [25], and guava seed protein isolates and their glutelin fractions [10, 11]. These results suggest that, in PIs extracted at a pH close to the pI and with enthalpy near a neutral pH (7), the proteins remain in their original state, maintaining a folded conformation.

DSC curves for the PIs obtained from LSF are show in Fig. 3a, b. The curves were recorded in the range of 30 up to 140 °C and reveal the presence of an endothermic peak in the region of 63–74 °C, corresponding to the denaturation temperature (T_p) of these PIs.

Figure 3a reveals that: (i) the addition of 0.3 mol/L NaCl led to higher values of ΔH, when compared to the PIs extracted without salt. At low concentrations, the salt can thus provoke a stabilizing effect, attributed to electrostatic induction or alteration of the structure of water around the protein, leading to the hydration of protein molecules and increased stabilization [33]; (ii) at alkaline environment, denaturation required less energy (ΔH) to complete the endothermic process, suggesting the promotion of denaturation at pH values further from neutrality. The same relationship was observed for the PIs extracted with 0.3 mol/L NaCl.

Figure 3b shows the DSC curves of PIs extracted in 0.5 mol/L NaCl, plus ultrafiltration, and of the PI extracted with 0.25% Na_2SO_3. From this Figure and the data presented in Table 2, it can be established that: (i) the different salts used had distinct effects, with Na_2SO_3 as exhibiting a greater peak area, and consequently leading to a higher ΔH to complete denaturation compared with NaCl; (ii) the PI extracted at pH 10.0 showed a greater area and value of enthalpy ($\sim$277 J g^{-1}) than the PI extracted at pH 11.0 ($\sim$250 J g^{-1}), revealing that pH affects the process of protein denaturation; (iii) the PI extracted at pH 10 showed a lower value of enthalpy when compared with PI at pH 10.0 from Fig. 3a, suggesting that ionic strength (0.3–0.5 mol/L) has an effect on the process of protein denaturation.

SDS-PAGE

The electrophoretic profiles of lupin proteins that comprise the subunits of the bulk storage proteins are shown on Fig. 4. On this gel and in all the samples analysed, eight distinct and strongly reactive electrophoretic bands can be identified between $\sim$71 and 14 kDa. These polypeptide bands were analysed with Kodak Digital Science 1D software and the identified bands had estimated molecular weights of about 71, 65, 51, 48, 44, 33, 19, and 14 kDa.

Conditions used to extract PIs may contribute to alterations and loss of protein fractions present in the whole seed. For instance, the pH of the extracting solution may

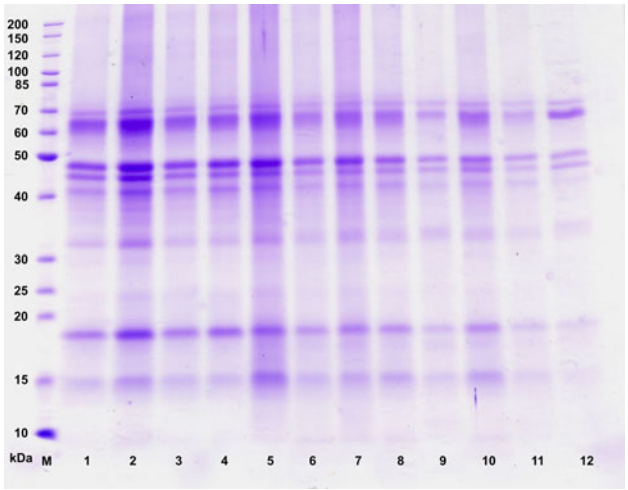

Fig. 4 SDS-PAGE of proteins from white lupin protein isolates (WLPI) and seeds. The lanes identified along the bottom of the gel contained the following samples: *M* molecular weight markers with sizes marked on the left, *1* WLPI extracted with 0.3 M NaCl at pH 7.0, *2* WLPI extracted with 0.3 M NaCl at pH 8.0, *3* WLPI extracted with 0.3 M NaCl at pH 9.0, *4* WLPI extracted with 0.3 M NaCl at pH 10.0, *5* WLPI extracted with H_2O plus Na_2SO_3 (0.25% w/v) at pH 11.0, *6* WLPI extracted with H_2O at pH 10.0, *7* WLPI extracted with H_2O at pH 11.0, *8* WLPI extracted with 0.5 M NaCl at pH 11.0 (ultrafiltration before isoelectric precipitation), *9* Proteins from alkaloid-free white lupin flour, *10* WLPI extracted with 0.5 M NaCl at pH 10.0 (ultrafiltration before isoelectric precipitation), *11* Proteins from LSF, *12* Proteins from Portuguese whole white lupin flour

influence the structural degradation of the native protein. According to Yu et al. [34] this degradation is common at pH 2.0, where lower proportions of high-molecular-weight polypeptides are obtained than at neutral pH. In light of these facts, protein electrophoreses were carried out in order to analyse possible differences between the band patterns of the lupin storage proteins present in whole flour and the PIs obtained by various methods of extraction.

Lupin seeds contain albumins and globulins in an approximate ratio of 1:9. The globulins comprise the 7S beta-conglutin (vicilin-like) and 11*S* alpha-conglutin (legumin-like), and two minor components, gamma- and delta-conglutin [5]. In the case of *L. albus*: (i) beta-conglutin, the major globulin component, is formed by 10–12 major types of subunit (~15–72 kDa) and a considerable number of minor subunits, with no disulfide bonds [5, 6, 35, 36]; (ii) alpha-conglutin is composed of four main types of subunit (53, 60, 66, and 70 kDa), as well as a number of minor subunits. In the presence of a reducing agent (e.g., 2-mercaptoethanol), each of these four main subunits is split into a heavier (31, 36, 42, and 46 kDa, respectively) and a lighter polypeptide chain (19 kDa), the latter common to all four subunits [36]; (iii) gamma-conglutin, a minor globulin component, is composed of one main type of subunit (42–43 kDa) containing two polypeptide chains (~18 and 30 kDa), linked by disulfide bonds and, possibly, other minor subunits [37]; and finally, (iv) delta-conglutin, another minor globulin component, is described as formed by two subunits (~13 and 21.6 kDa), the latter formed by two protomers linked by disulphide bonds [35, 38].

The protein migration patterns in SDS-PAGE from all the Brazilian white lupin flour and PI samples under study were identical, presenting eight electrophoretic bands with molecular masses ranging from ~14 to 71 kDa. These migration patterns were similar to those produced by whole white lupin flour sample from a Portuguese cultivar tested in our work. Similar results were also obtained by Neves et al. [22] and Melo et al. [36]. The absence of differences recorded between the protein migration patterns of any of our samples, irrespective of the extraction method used, suggests that the experimental conditions used to obtain the protein isolates did not alter the white lupin native protein profile.

On the other hand, the results of the thermal analysis (DSC and TG–DTG curves) obtained for the different PIs, led to the detection of changes in the protein conformation through the ΔH values, which in general decreased with increasing values of pH and ionic concentration in the extraction experimental conditions.

Conclusions

Different methods of preparing PIs were tested, resulting in final products that were different only in relation to the yield and protein content. The results of the protein analysis by SDS-PAGE showed that the same protein fractions were present in the lupin seeds and in the obtained PIs. This result shows that the process of extraction had no damaging effect to the composition of the original protein. On the other hand, the results of the thermal analysis (DSC and TG–DTG curves) obtained for the different PIs, led to the detection of changes in the protein conformation through the ΔH values, which in general decreased with increasing values of pH and ionic concentration in the extraction experimental conditions.

The use of industrially obtained white lupin protein isolates (or their major globulins) as functional foods must take into consideration the potential damage of the extraction processes on the nutritive value and nutraceutical properties of the proteins. It is important the obtaining from lupin flours the existing protein fractions and of PIs with a high protein content, associated with a good rate of extraction, without major damaging the protein structure. Therefore, it is suggested that the alkaline extraction (pH 10.0) in the presence of NaCl (0.3 mol/L), followed by isoelectric precipitation at pI 4.5, is the best method for obtaining a purified protein isolate.

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