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ORIGINAL PAPER

# Characterization of Soybean Proteins–Fatty Acid Systems

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**Abstract** This study investigated, with the aim of obtaining more flexible and hydrophobic proteins, the attachment of a low chain fatty acid, decanoic acid, to alkaline and acid soybean proteins; and the effect on their conformational and functional properties. The extent of esterification was high at acid pH and also increased with heating. Protein solubility decreased, mainly at the highest temperature (60 °C). Increasing levels of fatty acid formed a complex with a slightly more soluble protein with less surface hydrophobicity. Esterified proteins exhibited aggregation/dissociation and were stabilized by different protein subunits belonging to 7S and 11S globulins. Denaturation of these soybean protein fractions (7S and 11S) were also detected in these complexes. The highest level of fatty acids favored formation of a more ordered protein structure.

**Keywords** Soybean proteins · Decanoic acid · Esterification · Protein structural changes · Thermal behavior

## Introduction

Proteins are essential compounds of the human diet, especially animal proteins. In addition, these proteins are sometimes associated with fat components and related to cardiac diseases [1]. At present, vegetal proteins such as leguminous ones constitute a good alternative in our diets due to their high accessibility, low cost, and adequate nutritional value. In addition, soybeans contain (30–40%) a higher amount of proteins than cereals (15–20%) with a high content of lysine as a limiting amino acid [2]. Soybean proteins are utilized in numerous food products, such as drinks, desserts and mayonnaise. Functional properties of these proteins, such as gelation [3, 4], emulsification [5] and foaming [6] have been widely studied by our group and around the world by several authors. Those functional properties were improved by changes in pH, protein concentration, salt addition, thermal and high pressure treatments. Structural changes in soybean proteins due to chemical modification such as deamidation were also performed [7].

Modifications that increase the molecular flexibility of proteins, such as succinylation, are necessary to improve surface activity and therefore foaming and emulsification properties [8]. Proteins of cottonseed and soybean glycinin were succinylated [8–10] while pea proteins were acetylated [11]. Esterification of proteins might produce structural and functional modifications. Esterification of gelatine with fatty acid enhanced surface hydrophobicity and modified emulsion stability [12]. Kim and Kinsella [13] studied succinylation of soy glycinin. Up to a certain degree of succinylation, hydrophobicity and viscosity of glycinin increased due to partial dissociation and unfolding of the oligomeric structure of this globulin.

No studies on the improvement of hydrophobicity of soybean proteins through fatty acid attachment were performed. An industrial application of highly hydrophobic proteins would be in edible films for food. Films constitute a barrier against oxygen and moisture from the environment.

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The objective of this work was to study esterification of soybean proteins with low chain fatty acids under different conditions such as pH, fatty acid concentration and temperature of treatment. Structure, solubility, surface hydrophobicity and thermal properties of soybean proteins–fatty acid systems were also studied.

## Materials and Methods

### Preparation of Soy Protein Isolates

Soy protein isolate was prepared from defatted flour manufactured by Bunge, Ceval S.A. (Brazil). An alkaline extraction from the flour (pH 8.0), followed by precipitation at the isoelectric point ($pI = 4.5$) was carried out according to Puppo et al. [3]. The isoelectric precipitate was dispersed in distilled water and adjusted to pH 8.0 with 2N NaOH to obtain alkaline isolate (P8) or to pH 2.5 with 2N HCl to obtain acid isolate (P2.5). Dispersions obtained were lyophilized. The protein contents of isolates, determined by Kjeldahl method, were 82.4 ± 0.4% for P8 and 75.7 ± 0.4% for P2.5 ($N \times 6.25$).

### Soybean Protein–Fatty Acid Complex Formation

#### *Effect of pH and Temperature*

Two soybean protein isolates of different pH (P8 and P2.5) and a low chain fatty acid ($C_{10}$), capric acid ($C_{10}H_{20}O_2$) (99%, Anedra S.A., Argentina), were utilized. Aqueous dispersions (5%, w/v) of mixes containing 0.2 g $C_{10}$/g protein were prepared. Dispersions were stirred in a shaker at 40 and 60 °C overnight and centrifuged at 3,000×*g* for 15 min at 4 °C. Mixes were filtered using the vacuum filtration procedure. An aliquot of supernatant (P8 + $C_{10}$ (**S**) and P2.5 + $C_{10}$ (**S**)) was used for determining esterification percentage. Supernatant (S) and precipitate (P) were freeze dried before analysis.

#### *Effect of Fatty Acid Concentration*

Isolate P8 was treated with two different quantities of $C_{10}$: 0.2 and 0.4 g $C_{10}$/g protein. Samples were named as P8 + 0.2$C_{10}$ and P8 + 0.4$C_{10}$, respectively. Aqueous dispersions of the mixes at 5% w/v were prepared. Dispersions were stirred in a shaker at 60 °C overnight and centrifuged at 3,000×*g* for 15 min at 4 °C. Mixes were filtered using the vacuum filtration procedure. An aliquot of supernatant was used for determining the esterification percentage. Supernatant (S) and precipitate (P) were freeze dried before analysis.

### Determination of Esterification Percentage

Determination of esterification percentage was performed using the method of Djagny et al. [12] with modifications. An aliquot of 5 g of supernatant that contained the protein–fatty acid system was mixed with 50 mL of petroleum ether. The oil–water system was stirred to favor migration of free fatty acid to the organic phase. An aliquot of 10 mL of the organic phase (upper phase) was carefully transferred to an Erlenmeyer flask. Free fatty acid was titrated with 0.08605N NaOH. The content of free fatty acid was calculated. Considering total and free fatty acid, the fatty acid incorporated in the protein was calculated using Eq. 1:

$$(\text{g FA}_\text{T} - \text{ g FA}_\text{F}) \times 100/\text{g FA}_\text{T} \quad (1)$$

where $FA_T$ is the total content and $FA_F$ the free content of fatty acid.

### Characterization of Protein–Fatty Acid Systems

#### *Protein Solubility*

Protein–fatty acid samples were dissolved in distilled water (1% w/v) and stirred for 1 h at 30 °C. Every 15 min, samples were stirred in vortex to enhance solubility. After that, centrifugation was carried out at 10,000×*g* for 30 min at 15 °C. The protein content was determined applying the Lowry method [14].

#### *Protein Surface Hydrophobicity*

Surface hydrophobicity ($H_0$) of samples P8n, P8, P8 + 0.2$C_{10}$ and P8 + 0.4$C_{10}$ were determined by fluorescence measurements, following the method proposed by Cardamone and Puri [15]. The ammonium 1-anilino-8-naphthalene-sulfonate (ANS, Aldrich Chemical Co.) fluorescent probe was utilized. Samples were dissolved in water at final protein concentration from 0.03 to 0.10 mg/mL. Fresh solution of ANS (10 mM) in water was prepared. The ANS solution was incorporated to a cuvette containing water, used as a blank; and to the cuvettes containing the different protein samples. The emission spectrum was obtained, after incorporation of ANS at concentrations between 0 and 100 μM. Measurements were performed in a PerkinElmer Luminescence Spectrometer LS50B (PerkinElmer, MA, USA). Excitation wavelength was 350 nm, while emission was assayed between 370 and 600 nm. At the same ANS concentration, a resulting emission spectrum was obtained by subtracting to each sample spectrum, the corresponding of their blank. Fluorescence intensity (FI) detected at 470 nm, wavelength of the maximum emission of the protein–ANS complex, was the maximum fluorescence

intensity ($FI_{max}$). Saturation curve was obtained on a graph with $FI_{max}$ as a function of the ANS concentration. Experimental data were adjusted with Eq. 2:

$$FI = FI_{max}[ANS]/(1/K_a + [ANS]) \quad (2)$$

where $FI_{max}$ is the maximum fluorescence intensity for the protein concentration used, i.e., when surface protein is completely saturated with ANS; and $K_a$ is the association constant of the protein–ANS complex. Surface hydrophobicity, $H_0$, is proportional to the maximum fluorescence intensity, and it was calculated as:

$$H_0 = FI_{max}/C \quad (3)$$

where $C$ is the protein concentration (mg/mL), determined by the Lowry method [14].

### Protein Composition

#### *Native-PAGE*

Electrophoretic mobility of the proteins was analyzed by native electrophoresis. Lyophilized samples were dissolved (5 mg/mL) in a pH 6.8 buffer containing 0.25 M Tris-base, 50% glycerol. Samples were centrifuged at 15,800×*g* for 5 min at 20 °C in an IEC micro centrifuge (Centra MP4R, International Equipment Company). Supernatants were analyzed by native electrophoresis (30–40 μg of protein/lane).

Native electrophoresis was performed in a 6% polyacrylamide running gel. A non-dissociating buffer system containing 1.5 M Tris-base, pH 8.8 for the separating gel and 0.125 M Tris-base, 0.96 M glycine pH 8.3, for the running buffer was used.

#### *SDS-PAGE*

Denaturizing electrophoresis was achieved in the presence of a dissociating agent such as sodium dodecyl sulfate (SDS) and for reducing conditions, $\beta$-mercaptoethanol was utilized. Proteins were extracted for 2 h at 20 °C with a pH 6.8 buffer containing 0.25 M Tris-base, 0.2% SDS, 50% glycerol. For dissociating conditions, 5% of $\beta$-mercaptoethanol was incorporated in the sample buffer. In all cases, samples were heated at 100 °C for 1 min. These assays were performed using a 12% continuous running gel with a 4% stacking gel. A dissociating buffer system was used, containing 1.5 M Tris-base, 0.5% SDS, pH 8.8 for the separating gel; and 0.125 M Tris-base, 0.96 M glycine, and 0.5% SDS, pH 8.3, for the running buffer. Low MW markers (Pharmacia calibration kit) used included phosphorylase-b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

Electrophoresis was performed in a Mini Protean II (BIO-RAD) at a constant voltage of 200 V with a PowerPack 300 (Bio-Rad, Richmond, CA, USA). Gels were stained with Coomassie Brilliant Blue R-250.

### Statistical Analysis

Assays were performed in duplicate. Results were subjected to a one-way analysis of variance according to the general linear model procedure with least-square means effects. Multiple range tests were applied to determine which means were significantly different according to Fisher's least significant differences (LSD). Statistical analysis was carried out using Statgraphics plus, 2.1 software version (Statistical Graphics Corp., Princeton, NJ, USA).

## Results and Discussion

### Esterification Process

Esterification or formation of the protein–fatty acid complex was achieved using a low chain fatty acid, as decanoic acid ($C_{10}$) commonly named capric acid. The selection of $C_{10}$ was based in the fact that fatty acid should be soluble in protein dispersion media, in order to better characterize the complex with techniques normally used for proteins. Results of esterification percentage are shown in Table 1. Esterification was higher at pH 2.5 than at pH 8, indicating that the attachment of fatty acid was favored in acid conditions. Esterification is a process catalyzed by acids [16]. Considering that denaturation temperatures ($T_d$) of 7S and 11S soybean globulins of pH 8, are 79 and 90 °C, respectively [17]; we decided to increase the esterification temperature at a value lower than the $T_d$ of 7S fraction. The increase in heat treatment to 60 °C was considered to enhance fatty acid–protein attachment without protein denaturation. In this way, changes in protein structure would be due only to the effect of fatty acid attachment. Esterification percentages increased with temperature, in both isolates, independently of pH (Table 1). Nevertheless, at 60 °C, the esterification percentage was higher at pH 8 than at pH 2.5. At acidic pH, the heating treatment (60 °C) not only provoked protein denaturation, also produced a certain degree of aggregation [17]. If a protein is aggregated, a lower number of active sites is available for fatty acid attachment; consequently a low degree of esterification can be achieved.

Once protein pH (pH 8) and temperature of treatment (60 °C) had been chosen according to the highest values of esterification percentage, different content of fatty acid

**Table 1** Esterification percentage ($E$ %) of soybean proteins at different pHs: alkaline (P8) and acid (P2.5), and different temperatures: 40 and 60 °C

| | 40 °C | | 60 °C | |
|---|---|---|---|---|
| | P 8 + $C_{10}$ | P 2.5 + $C_{10}$ | P 8 + $C_{10}$ | P 2.5 + $C_{10}$ |
| $E$ (%)[a] | 44.8 ± 2.6[a] | 52.8 ± 3.1[b] | 92.2 ± 2.7[c] | 70.3 ± 2.9[d] |

Fatty acid content: 0.2 g/g protein

[a] *Different letters* in the same row indicate significant differences ($P \leq 0.05$)

(capric acid, $C_{10}$) were assayed in those conditions: 0.2 and 0.4 g $C_{10}$/g protein. A new set of experiments was performed and the increase in the decanoic acid level from 0.2 to 0.4 g/g protein produced a rise in the esterification percentage from 55 ± 0.0 to 67 ± 2.7%. Differences in the esterification percentage may be attributed to the difficulty in observing the end point in the titration process.

## Solubility and Hydrophobicity of Soybean Proteins–Fatty Acid Systems

### *Effect of pH and Temperature*

Solubility percentage values of different protein–fatty acid systems obtained at 40 and 60 °C, are shown in Table 2. As analyzed in a previous work [3], the solubility of alkaline soybean protein isolate (P8) was higher than values obtained for the acid protein (P2.5), at both temperatures. In addition, we observed that P8 did not change its solubility with heating at 60 °C, while for P2.5 solubility decreased due to partial denaturation and aggregation of these proteins under acid conditions [3, 17]. Treatment of proteins with fatty acid at 40 °C produced an alkaline complex of lower solubility (P8 + $C_{10}$ (S)). Solubility of the acid P–FA system (P2.5 + $C_{10}$ (S)) did not change the protein solubility. It is supposed that in precipitates there is no protein; therefore, protein solubility was low in those fractions (<20%). At 60 °C, the solubility decrease of both P–FA systems was considerable, especially under acid conditions. In P8 + $C_{10}$ (P) solubility was very low, indicating that no soluble protein was found. At acidic pH, the lower solubility of the complex compared to the precipitate, would indicate that the presence of capric acid decreased protein solubility at that temperature or at that higher amount of protein precipitated with $C_{10}$.

**Table 2** Solubility percentage ($S$ %) of samples treated at different pH and temperatures. Soybean protein isolates: P8 (alkaline), P2.5 (acid)

| $S$ (%)[a] 40 (°C) | | $S$ (%)[a] 60 (°C) | |
|---|---|---|---|
| P8 | P2.5 | P8 | P2.5 |
| 78.3 ± 2.75[a] | 71.4 ± 3.11[b] | 81.0 ± 0.85[a] | 66.3 ± 3.11[c] |
| $S$ (%)[a] 40 (°C) | | $S$ (%)[a] 60 (°C) | |
| P8 + $C_{10}$ (S) | P2.5 + $C_{10}$ (S) | P8 + $C_{10}$ (S) | P2.5 + $C_{10}$ (S) |
| 39.5 ± 1.20[d] | 70.7 ± 0.56[b] | 30.7 ± 1.17[e] | 10.1 ± 0.86[f] |
| $S$ (%)[a] 40 (°C) | | $S$ (%)[a] 60 (°C) | |
| P8 + $C_{10}$ (P) | P2.5 + $C_{10}$ (P) | P8 + $C_{10}$ (P) | P2.5 + $C_{10}$ (P) |
| 3.7 ± 0.4 [g] | 17.6 ± 0.07 [h] | 3.9 ± 0.7[i] | 30.7 ± 1.77[e] |

Soybean protein–capric acid systems (P + $C_{10}$): P8 + $C_{10}$ (S), P2.5 + $C_{10}$ (S) (soluble fraction); P8 + $C_{10}$ (P), P2.5 + $C_{10}$ (P) (precipitate)

Fatty acid content: 0.2 g/g protein

[a] *Different letters* indicate significant differences ($P \leq 0.05$)

### *Effect of Fatty Acid Content*

No differences in protein solubility between native isolate (P8n) and isolate heated at 60 °C (P8), were observed (Table 3). This behavior is attributed to the fact that thermal treatment was performed at a temperature below denaturation temperatures of 7S and 11S soy globulins. Esterification with $C_{10}$ significantly decreased protein solubility suggesting the formation of a complex with low soluble protein structure. The increase of $C_{10}$ content allowed a slight increase in protein solubility. In addition, the protein content of precipitate was almost negligible, especially with 0.4 g $C_{10}$/g protein.

### *Surface Hydrophobicity ($H_0$) and Solubility (S)*

Results of ($H_0$) were analyzed in comparison with solubility values (Fig. 1). Surface hydrophobicity of native isolate (P8n) increased with thermal treatment (P8), suggesting that the protein partially unfolds with the exposure to media of hydrophobic groups. The incorporation of low levels of $C_{10}$ (0.2 g/g protein) produced a slight decrease in protein hydrophobicity, while with a high content of fatty acid, $H_0$ significantly decreased, indicating a partial refolding with fewer hydrophobic groups at the protein surface. At low level of $C_{10}$, the diminution in $H_0$ was accompanied by a great decrease in solubility, suggesting the burial of groups along with aggregation phenomenon. However, with high level of $C_{10}$ a high content of hydrophilic groups were disposed to protein surface, favoring a more soluble structure. These results suggest that decanoic acid favored protein–fatty acid complex formation with changes in protein structure; favoring a certain degree of protein fold with a slight increase in protein solubility.

**Table 3** Solubility (*S*) of isolates and proteins treated at 60 °C with different contents of fatty acids

| | Isolate | | Soluble fraction | | Precipitate | |
|---|---|---|---|---|---|---|
| | P8n | P8 | P8 + 0.2$C_{10}$ | P8 + 0.4$C_{10}$ | P8 + 0.2$C_{10}$ | P8 + 0.4$C_{10}$ |
| **S (%)**[a] | 82 ± 0.8[a] | 80 ± 1.4[a] | 6.5 ± 0.3[b] | 12 ± 0.5[c] | 1.3 ± 0.1[d] | 0.8 ± 0.1[e] |

*P8n* native soybean protein isolate, *P8* soybean protein isolate treated at 60 °C, *P8 + 0.2C10* isolate treated with 0.2 g $C_{10}$/g protein, *P8 + 0.4C10* isolate treated with 0.4 g $C_{10}$/g protein

[a] *Different letters* in the same row indicate significant differences ($P \leq 0.05$)

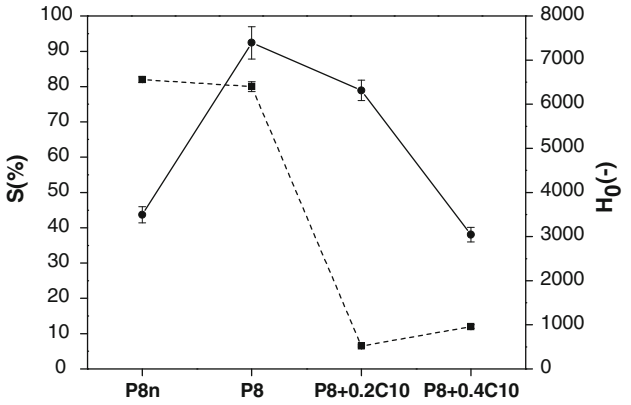


**Fig. 1** Solubility (*S*) and surface hydrophobicity ($H_0$) of protein–fatty acid complex. *P8n* native isolate, *P8* heated isolate. Heating conditions: 60 °C-overnight. Fatty acid levels: 0.2 and 0.4 g $C_{10}$/g protein (P8 + 0.2$C_{10}$, P8 + 0.4$C_{10}$)

## Protein Polypeptides of Soybean Protein–Fatty Acid Systems

### *Effect of pH and Temperature*

Native electrophoresis (N-PAGE) allows us to analyze mobility and the charge/mass ratio of proteins. Figure 2 shows the N-PAGE of P8 and P2.5 isolates and the esterified systems P8 + $C_{10}$ y P2.5 + $C_{10}$ obtained at 40 °C.

Non-thermal treated isolates, P8n and P2.5n, presented different electrophoretic profiles (Fig. 2a). P8n showed two bands of low mobility, corresponding to 7S and 11S globulins. In a previous work we identified the first band belonging to 7S and the second, to 11S fractions [18]. In P2.5n, 11S was absent, possible due to the fact that it was aggregated and did not enter the gel. Heating at 40 °C provoked an increase in the bands intensity, favoring changes in protein structure, and consequently in their solubility. Esterification with capric acid, produced conformational changes in soybean proteins, mainly in P2.5 where $C_{10}$ enhanced protein aggregation. At P8, the intensity of the 11S band increased while for 7S it decreased, suggesting changes in the structure of the proteins, mainly in 11S globulin. In addition, 11S augmented molecular mobility, possibly due to the reduction in

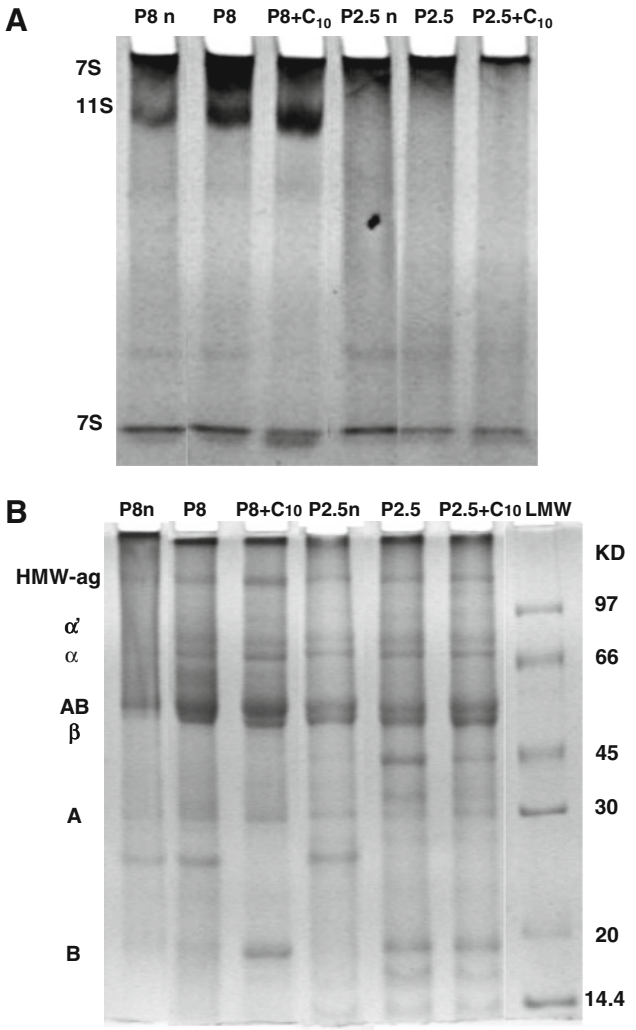


**Fig. 2** Electrophoresis of soybean protein samples: **a** Native-PAGE, **b** SDS-PAGE. *P8n* alkaline isolate, *P2.5n* acid isolate. *P8*, *P2.5* heated isolates. Heating conditions: 40 °C overnight. Fatty acid level: 0.2 g $C_{10}$/g protein.

molecular mass and/or an increase in the protein charge due to the presence of $C_{10}$.

Electrophoresis in denaturing conditions, SDS-PAGE is an adequate technique for analyzing peptides that compose the structure of native proteins or aggregates. Soybean proteins are conformed mainly by two globulins, *β*-conglycinin (7S fraction) and glycinin (11S fraction). Globulin

11S consists of two apposed hexagonal rings, each containing three subunits associated by hydrophobic interactions, containing pairs of disulfide-linked acidic, A (35–37 kDa) and basic, B (20 kDa) polypeptides [19]. The 7S globulin is a trimeric glycoprotein composed of three subunits: α (57 kDa), α′ (58 kDa) and β (42 kDa), associated by hydrophobic bonds [20]. Native isolate, P8n, presented a great proportion of high molecular weight aggregates that did not enter the gel, and a small quantity of AB-11S subunit (Fig. 2b). Acid isolate, P2.5n, presented higher proportion of HMW aggregates (HMW-ag) than P8n, probably formed by α′, α-7S y B-11S. The presence of AB-11S and β-7S subunits was detected in this acid soluble fraction. Heating at 40 °C disrupted some aggregates: P8 showed mainly the HMW-ag, AB-11S subunit and β-7S subunit, the last one being absent in P8n. These aggregates might be formed by proteins that are not present in the soluble fraction: α′, α-7S and A, B-11S polypeptides. At pH 2.5, the heating process also favored dissociation/aggregation phenomena. Insoluble aggregates dissociated and a band of 45 kDa and B-11S subunit appeared in the soluble fraction, while the A-11S subunit disappeared. Esterification of P8 increased the proportion of soluble aggregates of molecular mass higher than 97 kDa (HMW-ag) and the α-7S subunit. In addition, the subunit of 25 kDa disappeared and the B-11S subunit (20 kDa) was released. At pH 2.5, the presence of $C_{10}$ did not significantly modify the electrophoretic profile, only a lower quantity of the 45-kDa peptide was detected.

*Effect of Fatty Acid Content*

Figure 3a shows N-PAGE of P8 samples treated at 60 °C with different content of $C_{10}$. Content of aggregates decreased and the proportion of 7S, and especially 11S, increased with the addition of $C_{10}$. Results indicate that high molecular mass aggregates dissociate leading to an increase in both 7S and 11S fractions, especially at high $C_{10}$ contents (0.4 g/g protein).

In the presence of SDS (Fig. 3b) different proteins were extracted. With respect to the native protein (P8n), the B-11S band appeared and the intensity of the A-11S band increased with thermal treatment at 60 °C (P8). This behavior suggests that both, A-11S and B-11S polypeptides were released from aggregates. As in the case of treatment at 40 °C, the 25-kDa peptide disappeared from the soluble fraction (P8 + 0.2$C_{10}$ (S) and P8 + 0.4$C_{10}$ (S)), due to an esterification process. No significant differences were observed in electrophoretic profiles of systems with distinct content of $C_{10}$. The intensity of the bands was slightly pronounced for P8 + 0.4$C_{10}$ (S). In insoluble fractions, it is believed only free fatty acid can be found . Nevertheless, some protein was present, mainly for P8 + 0.2$C_{10}$ (P)

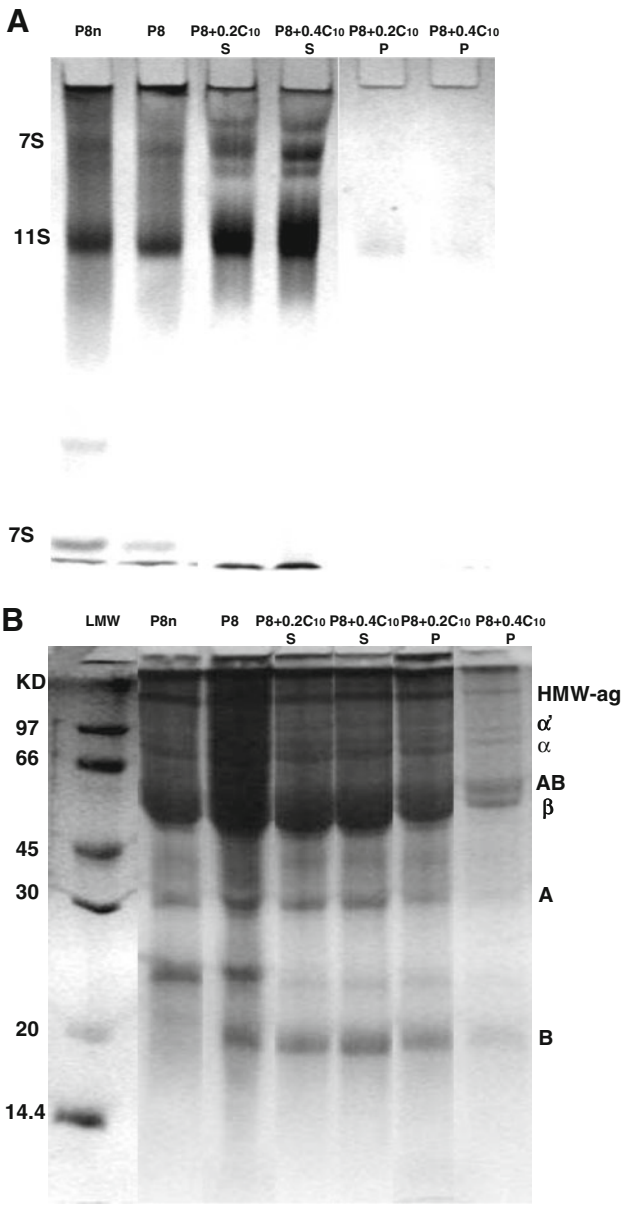


**Fig. 3** Electrophoresis of soybean protein samples: **a** Native-PAGE, **b** SDS-PAGE. *P8n*: native isolate, *P8* heated isolate. Heating conditions: 60 °C-overnight. Fatty acid levels: 0.2 and 0.4 g $C_{10}$/g protein. *S* soluble fraction (protein–fatty acid complex), *P* precipitate (free fatty acid)

sample, as we can also deduce from the solubility values (Table 3). In both precipitates, a decrease in intensity of bands corresponding to A-11S and B-11S polypeptides was observed; suggesting that these proteins were stabilizing the protein–fatty acid systems. From these results we can deduce that 0.2 g of $C_{10}$ was not a sufficient amount of fatty acid to esterify 1 g of proteins.

Figure 4 shows the effect of β-mercaptoethanol on protein extracts. No significant differences in protein composition were observed between samples. All samples,

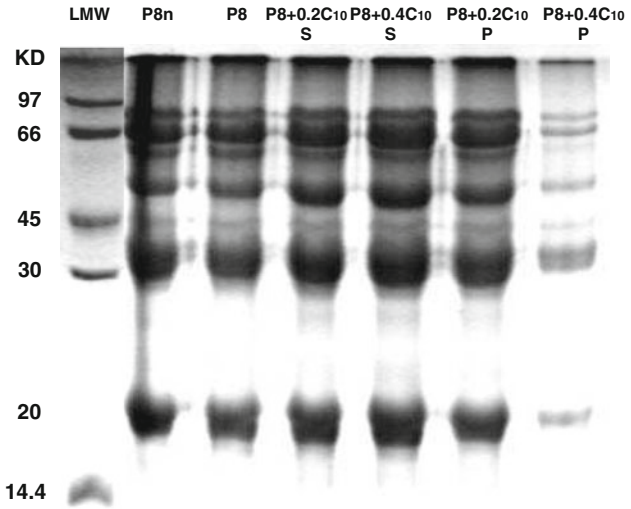


**Fig. 4** Electrophoresis of soybean protein samples in the presence of α-mercaptoethanol (SDS-PAGE). *P8n* native isolate, *P8* heated isolate. Heating conditions: 60 °C-overnight. Fatty acid levels: 0.2 and 0.4 g $C_{10}$/g protein. *S* soluble fraction (protein–fatty acid complex), *P* precipitate (free fatty acid)

were formed by subunits corresponding to both globulins: $\alpha'$, $\alpha$ and $\beta$-7S; and A-11S and B-11S polypeptides.

## Thermal Properties of Soybean Protein–Fatty Acid Systems

Native isolate (P8n) presented the two endotherms corresponding to soybean proteins, the first at 79.7 °C belonging to 7S globulin and the other at 94.7 °C for the 11S fraction (Fig. 5a). Heating at 60 °C provoked partial denaturation of 7S (P8). This effect could be attributed to the long duration of the thermal treatment (overnight) utilized in protein–fatty acid formation. The presence of fatty acid promoted total and partial denaturation for 7S and 11S, respectively (P8 + 0.2$C_{10}$ (S), P8 + 0.4$C_{10}$ (S)).

Figure 5b shows DSC curves corresponding to precipitates. At low contents of $C_{10}$ (0.2 g/g protein), almost no fatty acid was detected; although a small endotherm at approximately 96 °C was observed, belonging to the low quantity of 11S that precipitated. Adding 0.4 g $C_{10}$/g protein, a precipitate with an endotherm at 31.3 °C but no 11S peak, was observed. Decanoic acid, $C_{10}$, has a melting point of 31.5 °C, suggesting, great proportion of free $C_{10}$ in the precipitate.

These results, together with electrophoretic data, indicate that with high levels of $C_{10}$, all protein was esterified or forming the complex with fatty acid.

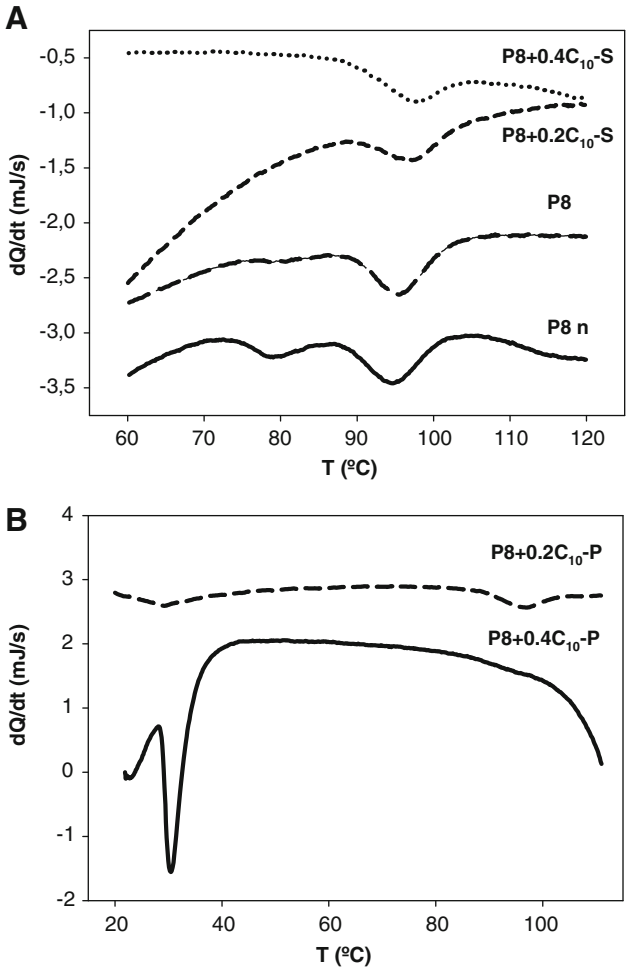


**Fig. 5** Differential scanning calorimetry (DSC) of soybean protein samples. *P8n* native isolate, *P8* heated isolate. Heating conditions: 60 °C-overnight. Fatty acid levels: 0.2 and 0.4 g $C_{10}$/g protein. *S* soluble fraction (protein–fatty acid complex), *P* precipitate (free fatty acid)

Peak temperatures ($T_p$) and denaturation enthalpy ($\Delta H$) are shown in Table 4. Addition of $C_{10}$ provoked a slight shift of 11S peak temperature ($Tp_2$) to high values. At level of 0.2 g $C_{10}$/g protein, $Tp_1$ (7S) was not detected and $\Delta H_2$ (11S) resulted slightly lower than value obtained for P8. These results confirm total and partial denaturation of 7S and 11S, respectively; shown in Fig. 5a. On the other hand, at 0.4 g $C_{10}$, $\Delta H$ increased almost twice the amount obtained for 0.2 g $C_{10}$ (8.6 mJ/mg), suggesting a renaturation process or an ordering of molecular bonds that leads in an endotherm of higher area.

These results confirm data obtained from surface hydrophobicity and solubility: the protein recovered a certain degree of native form, burring hydrophobic groups and leading to a more soluble protein.

**Table 4** Peak temperatures ($Tp_1$ and $Tp_2$) and denaturation enthalpy ($\Delta H_1$ and $\Delta H_2$) of isolates and proteins treated at 60 °C with different content of fatty acid

| Sample | $Tp_1$ (°C)[a] | $\Delta H_1$ (mJ/mg)[a] | $Tp_2$ (°C)[a] | $\Delta H_2$ (mJ/mg)[a] |
|---|---|---|---|---|
| P8n | 79.7 ± 0.95[a] | 1.44 ± 0.03[a] | 94.7 ± 0.18[a] | 4.85 ± 0.02[a] |
| P8 | 79.7 ± 0.90[a] | 0.33 ± 0.14[b] | 95.2 ± 0.49[a] | 6.05 ± 0.32[b] |
| P8 + 0.2$C_{10}$ (S) | – | – | 97.2 ± 0.49[b] | 4.15 ± 0.23[a] |
| P8 + 0.4$C_{10}$ (S) | – | – | 97.2 ± 0.01[b] | 8.61 ± 0.78[c] |
| P8 + 0.2$C_{10}$ (P) | – | – | 96.0 ± 0.35[a] | 4.07 ± 0.08[a] |
| P8 + 0.4$C_{10}$ (P) | 31.3 ± 1.05[b] | 38.7 ± 0.25[c] | – | – |

*P8n* native soybean protein isolate, *P8* soybean protein isolate treated at 60 °C, *P8 + 0.2C10* isolate treated with 0.2 g $C_{10}$/g protein, *P8 + 0.4C10* isolate treated with 0.4 g $C_{10}$/g protein. *S* soluble fraction, *P* precipitate

[a] *Different letters* in the same column indicate significant differences ($P \leq 0.05$)

## Conclusions

Formation of hydrophobic system between soybean proteins and low chain fatty acid, capric acid ($C_{10}$) was achieved. Esterification percentages were higher at acid than at alkaline pHs, and increased with thermal treatment and fatty acid content. The presence of $C_{10}$ in the complex affected protein solubility, which was dependent on pH and thermal treatment. A significant decrease in protein solubility of both complexes was observed at the highest temperature (60 °C). The esterification process modified the protein structure, through dissociation of different subunits of 7S and 11S globulins and aggregation of low molecular mass peptides. At pH 8, the hydrophobic protein–fatty acid complex contained 7S and 11S globulins totally and partially denatured, respectively. High levels of $C_{10}$ (0.4 g/g protein) favored formation of a system with a certain degree of native structure, confirmed by the increase in solubility and denaturation enthalpy and the decrease in surface hydrophobicity.

Nevertheless, to confirm protein–fatty acid bonds, more complex studies should be performed using methods such as FT-Raman and/or FT-IR.

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