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ARTICLE

Differential Scanning Calorimetry (DSC) Studies on the Thermal Properties of Peanut Proteins

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Studies related to the functional and thermal properties of peanut proteins are limited if compared with other vegetable protein sources. The aim of this work was to study the thermal denaturation of peanut protein isolates (PPI) by DSC. The thermal profile of PPI showed two endothermic peaks (assigned to denaturation of arachin and conarachin fractions). The thermal stability of arachin and conarachin increased when water content decreased, and a critical water level was found for both fractions. The effect of protein denaturants was studied. Low contents of urea stabilized protein fractions, but lower T_d values were found with increasing concentrations. ΔH values of arachin were affected by urea. SDS affected ΔH values and thermal stability of conarachin; the arachin fraction showed higher resistance to SDS-induced denaturation. DTT addition did not affect conarachin stability, although enthalpy values decreased significantly. On the other hand, arachin was greatly affected by DTT. In summary, thermal denaturation parameters of PPI were sensitive to water content, indicating that polar groups of arachin and conarachin contribute to structure stabilization. Urea addition mainly affected the structure of the arachin fraction, which was attributed to its higher surface hydrophobicity. Results obtained from SDS and DTT suggest that hydrophobic interactions and disulfide bonds play an important role in structure maintenance of arachin and conarachin.

KEYWORDS: Peanut; proteins; thermal denaturation; DSC; protein denaturants

INTRODUCTION

Vegetable proteins are major components in the diet of food-producing animals and are increasingly important in human nutrition. In recent years, the addition of legume proteins to food has increased significantly because their high nutritional value and functional properties make them desirable for food. Soybean, rapeseed, cottonseed, sunflowerseed, and peanut are the greatest sources of protein meal and represent 69, 12.4, 6.9, 5.3, and 2.8% of the world production of protein meal (*1*), respectively. These meals are interesting sources of vegetable proteins that can be used in the food industry.

Although peanut protein is an underutilized ingredient, it can be a good alternative in food formulation. The protein cake residue from peanut oil extraction is frequently used as an animal feed; from this defatted meal, protein concentrates and isolates can be prepared. Their use as food ingredients could be of major importance because peanut proteins have high essential amino acid content and they constitute an important low-cost protein source (*2*). On the other hand, protein concentrates and isolates could improve the sensorial attributes of food.

Protein denaturation involves structural or conformational changes from native structure without alteration of the amino acid sequence (*3*). The native-to-denatured change in the protein state is a cooperative phenomenon that is accompanied by significant heat uptake, seen as an endothermic peak in the differential scanning calorimetry (DSC) thermogram (*4*). Furthermore, during heating, changes in proteins may be endothermic, such as rupture of hydrogen bonds, or exothermic, such as breaking up of hydrophobic forces and aggregation of proteins (*5*). Thermoanalytical methods such as DSC are considered to be convenient and reliable for studying such changes.

Legume and legume foods are routinely subjected to various thermal treatments during harvesting, processing, and preparation (*6*). These processes can induce loss of proteins' native structure, which in turn can affect their functionality (*7*). Despite the importance that thermal and functional properties have in food processing, studies in this field related to peanut proteins are limited. Therefore, it becomes very important to understand the structural changes caused by the thermal process in order to recognize their effect on the functional properties involved in food elaboration. In addition, the role of chemical forces in stabilizing the conformation of peanut protein needs to be clarified to confirm the usefulness of peanut protein as a potential food ingredient. The aim of this work was to study the thermal denaturation of peanut proteins by DSC. The influence of water content and several protein denaturants on the thermal behavior of proteins was analyzed to assess the interactions that stabilize the peanut protein structure,

MATERIALS AND METHODS

Preparation of Peanut Protein Isolates (PPI). Peanut seeds (*Arachis hypogaea* L. cv. Tegua Runner type), provided by Lorenzati, Ruetsch y Cía. S.A (Ticino, Córdoba, Argentina), were milled and

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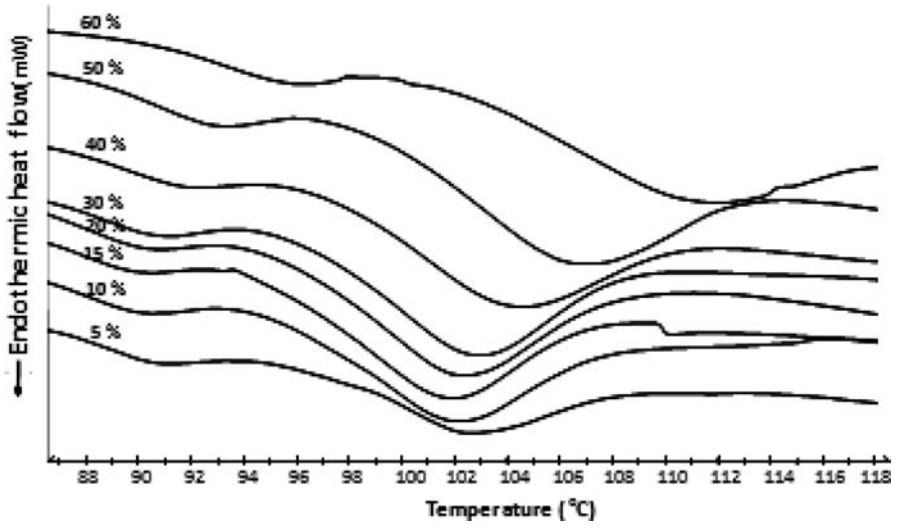

Figure 1. DSC profiles comprising 5−60% w/w of PPI in water.

suspended in hexane (1:20 w/v) to extract the lipids. The suspension was left at room temperature for 4 h until hexane was removed and another amount of organic solvent (in the same proportion) was added. This new suspension was again left at room temperature for 4 h. The entire process was repeated until four changes of solvent had been made. PPI were prepared using the method described by Yu et al. (*2*). The defatted peanut meal obtained was dispersed (1:20 w/v) in distilled water and adjusted to pH 10 with 1.0 N NaOH. The peanut flour suspension was stirred at room temperature for 1 h and then centrifuged at 3000*g* for 15 min. The supernatant was collected and adjusted to pH 4.5 (the isoelectric pH of peanut proteins) with 1.0 N HCl. The suspension was centrifuged at 3000*g* for 15 min. The precipitate obtained (PPI) was suspended (1:10 w/v) in water and dialyzed against distilled water to eliminate residual salts. The dialyzed suspension was lyophilized. Protein content of PPI (N × 6.25) was 79.33 ± 0.32% as determined by the Kjeldahl method (*8*).

Differential Scanning Calorimetry. Thermal characteristics of PPI were analyzed using a differential scanning calorimeter (DSC-821e, Mettler Toledo, Switzerland). The equipment was calibrated with indium and zinc, and an empty, sealed but pierced, aluminum pan was used for reference. Onset temperature (T_o), peak or denaturation temperature (T_d), denaturation enthalpy (ΔH), and cooperativity, represented by the width at half-peak height ($\Delta T_{1/2}$), were determined using the software STARe version 9.0x (Mettler Toledo).

Kinetic Parameters of Denaturation. The kinetic parameters in the denaturation process were analyzed using the dynamic method of Ozawa (*9*). The relationship between the temperature and the reaction rate was considered to follow the Arrhenius expression. Therefore, activation energies (E_a) were calculated from the slopes of the linear regressions between $\ln(\beta/T_d^2)$ and $1/T_d$ (β = heating rate). Hermetically sealed aluminum pans were prepared to contain 3 mg of PPI suspended in distilled water (20% w/v). Samples were incubated at room temperature for 24 h.

The samples were scanned at different heating rates, 2.5, 5, 10, 15, 20, and 25 °C/min, and denaturation temperatures (T_d) were determined.

Water Content Effect on Protein Denaturation. Protein isolates (3 mg) were placed in aluminum pans, and different amounts of water were added to obtain PPI/water suspensions of 5, 10, 15, 20, 30, 40, 50, and 60% w/w. Pans were hermetically sealed, incubated at room temperature for 24 h, and scanned at 10 °C/min from 30 to 120 °C.

Effect of Additives on Thermal Denaturation. The influence of urea, sodium dodecyl sulfate (SDS), and dithiotreitol (DTT) on thermal properties of PPI was analyzed. These compounds can disturb protein structure and therefore its thermal characteristics. Structure denaturants were dissolved in 0.05 M sodium phosphate buffer (pH 7) at different concentrations. PPI (3 mg) were placed in aluminum pans, and 15 μL of solution buffers containing the additives were added. Pans were hermetically sealed, incubated at room temperature for 24 h, and scanned at 10 °C/min from 30 to 120 °C.

Statistical Analysis. Results were expressed as mean of two replicates ± SD. The data obtained were statistically treated by variance analysis, whereas the means were compared by the LSD Fisher test at a significance level of 0.05 in both cases using INFOSTAT statistical software (*10*).

RESULTS AND DISCUSSION

Determination of Kinetic Parameters of Denaturation. Figure 1 shows DSC profiles of PPI with different water contents. Around 90% of peanut proteins are globulins, which can be classified into two main fractions: arachin (11S) and conarachin (7S) (*11*). The DSC profile (**Figure 1**) shows two endothermic peaks at 102 and 91 °C, which can be attributed to thermal denaturation of arachin and conarachin fractions, respectively (*12*). As mentioned before, activation energies of the denaturation process were obtained according to the Ozawa method. **Figure 2** shows a plot of $\ln(\beta/T_d^2)$ versus $1/T_d$ for analyzed proteins. The E_a value obtained for the conarachin fraction was 57.31 kcal/mol, with a correlation coefficient of 0.94. The E_a value for the arachin fraction was 98.91 kcal/mol (and the correlation coefficient was 0.96). According to Laidler and Bunting (*13*), activation energies of thermal denaturation of proteins ranged between 40.8 kcal/mol (trypsin) and 129.0 kcal/mol (ovalbumin). Therefore, the transition energy values reflect the denaturation of peanut proteins.

Effect of Water Content on Protein Denaturation. As mentioned, the effect of water on peanut protein denaturation is shown in **Figure 1**. Thermal parameters obtained from these thermograms are shown in **Table 1**. Water plays a major role in food systems and affects protein conformations (*14*). T_o and T_d values of protein fractions increased, whereas water content decreased. Similar results were obtained with wheat (*15*, *16*), fababean (*17*), and sunflower (*18*) protein isolates.

Figure 3 shows the relationship between denaturation temperature and denaturation enthalpy with the water content of the system (expressed as g of water/g of PPI). Both arachin and conarachin had a critical water level: below that, there was an inverse relationship between T_d and water content of the samples. The critical water level depends on the protein studied, but in the present work, both protein fractions showed similar values (around 2.3 g/g, **Figure 3**). Critical water content is related to protein hydration, so that denaturation temperature increases only when available water belongs to the first hydration shell (i.e., water joined to the protein) or the second hydration shell (i.e., water structured by the protein). These water molecules, which

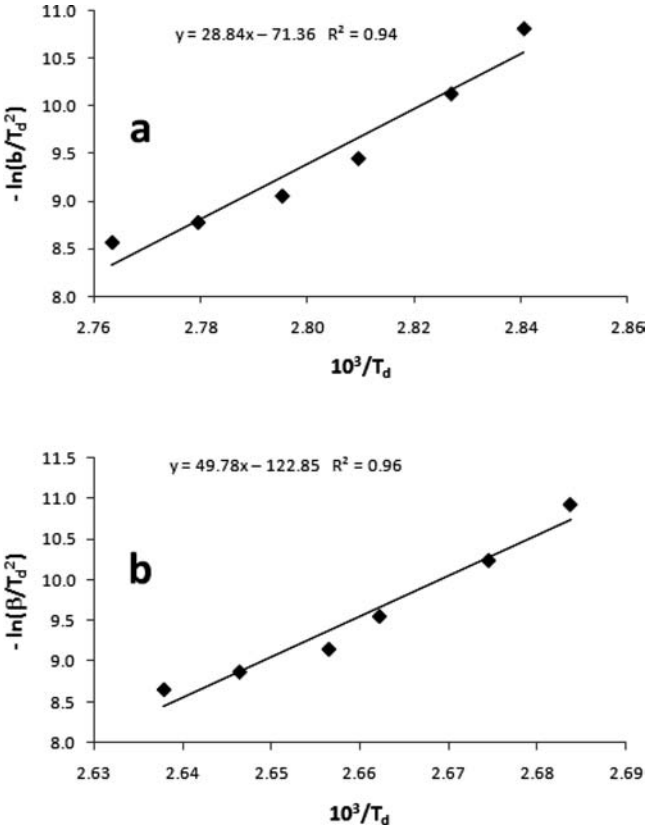

Figure 2. Linear regression between $\ln(\beta/T_d^2)$ and $1/T_d$ for peanut proteins: (**a**) conarachin fraction; (**b**) arachin fraction. The equations and correlation coefficients (R^2) are shown in each case.

Table 1. Onset Temperature (T_o), Denaturation Temperature (T_d), Denaturation Enthalpy (ΔH), and Width at Half-Peak Height ($\Delta T_{1/2}$) of PPI/Water Suspensions[a]

g of water/g of PPI	T_d (°C)	ΔH (J/g of PPI)	$\Delta T_{1/2}$ (°C)
	Conarachin Endothermic Peak		
20.0	89.41 ± 0.04 a	0.76 ± 0.01 a	4.03 ± 0.35 ab
10.0	89.53 ± 0.08 ab	0.80 ± 0.06 ab	3.82 ± 0.13 a
6.7	89.76 ± 0.19 ab	0.76 ± 0.09 a	3.83 ± 0.08 a
5.0	89.64 ± 0.40 ab	1.07 ± 0.15 bc	4.08 ± 0.13 ab
3.3	90.23 ± 0.35 b	1.16 ± 0.07 c	3.95 ± 0.09 ab
2.5	91.11 ± 0.59 c	1.28 ± 0.21 c	4.27 ± 0.25 bc
2.0	92.96 ± 0.36 d	1.26 ± 0.06 c	4.28 ± 0.01 bc
1.7	95.84 ± 0.33 e	1.21 ± 0.23 c	4.54 ± 0.01 c
	Arachin Endothermic Peak		
20.0	101.09 ± 0.15 a	3.89 ± 1.14 a	6.14 ± 0.34 a
10.0	101.39 ± 0.04 a	9.14 ± 0.41 b	6.97 ± 0.23 b
6.7	101.47 ± 0.08 a	10.86 ± 2.68 bcd	7.39 ± 0.57 b
5.0	101.68 ± 0.28 a	12.21 ± 1.39 cd	7.29 ± 0.30 b
3.3	102.19 ± 0.11 a	12.84 ± 0.58 d	7.33 ± 0.21 b
2.5	103.49 ± 0.47 b	9.83 ± 0.25 bc	6.91 ± 0.05 b
2.0	106.67 ± 0.48 c	11.10 ± 0.93 bcd	8.26 ± 0.08 c
1.7	111.56 ± 1.15 d	8.63 ± 0.93 b	9.24 ± 0.21 d

[a] Values are means ± SD of two independent determinations. Values followed by different letters within a column are significantly different ($p < 0.05$).

are associated with the proteins, require more energy to mobilize and contribute to the denaturation process (*14*).

There was a continuous lowering in ΔH values as water content decreased from a critical water level for the arachin fraction. In addition, when moisture contents were lower than the critical water level, the enthalpy decreased but the denaturation temperature increased, although the opposite effect should have been observed (*19*). Rouilly et al. (*18*), analyzing the sunflower oil cake (residue from the solvent extraction of oil), described the noncovalent interactions that maintain protein structure as being essentially hydrogenous and ionic interactions, because the oleaginous globulins were in a hydrophobic environment (in the seed and during the extraction process). Therefore, polar groups are localized in the heart of the globular structure, and proteins are reacting to the presence of water. The outcome is that protein structure should be stabilized in an anhydrous environment. Consequently, these authors concluded that the decrease of the enthalpy with increasing T_d could be caused by a pressure increase in the pans or by a greater extent of exothermic coagulation with the temperature increase.

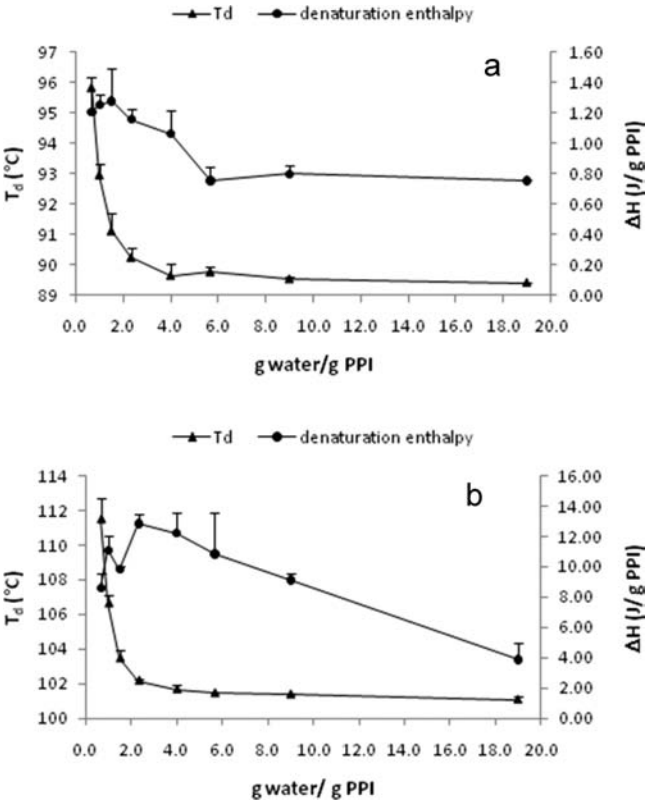

Figure 3. Relationships between denaturation temperature (T_d) and denaturation enthalpy (ΔH) with water content of the system (expressed as g of water/g of PPI) for conarachin (**a**) and arachin (**b**) protein fractions.

From this critical point, a decrease in denaturation enthalpy values with a rise in available water is observed (**Figure 3**), indicating a lower energy requirement to protein structure destabilization. Both arachin and conarachin fractions showed a significant increase in $\Delta T_{1/2}$ values as water content decreased (**Table 1**), which is indicative of less cooperativity in the denaturation process.

Effect of Protein Denaturants. *Urea.* To gain knowledge referred to the main interactions existing in thermal protein denaturation, the influence of some molecules (which are able to disturb protein structure) on PPI thermal behavior was analyzed. **Figure 4** shows the effect of adding different urea concentrations on some thermal parameters. T_d values of the conarachin fraction showed an initial increase but then decreased as urea concentration increased. The thermal stability of the arachin fraction was reduced starting at 4 M urea. Urea is commonly used to denature proteins, but it can also stabilize protein structure. Chan and Ma (*20*) found a rise in T_d values of

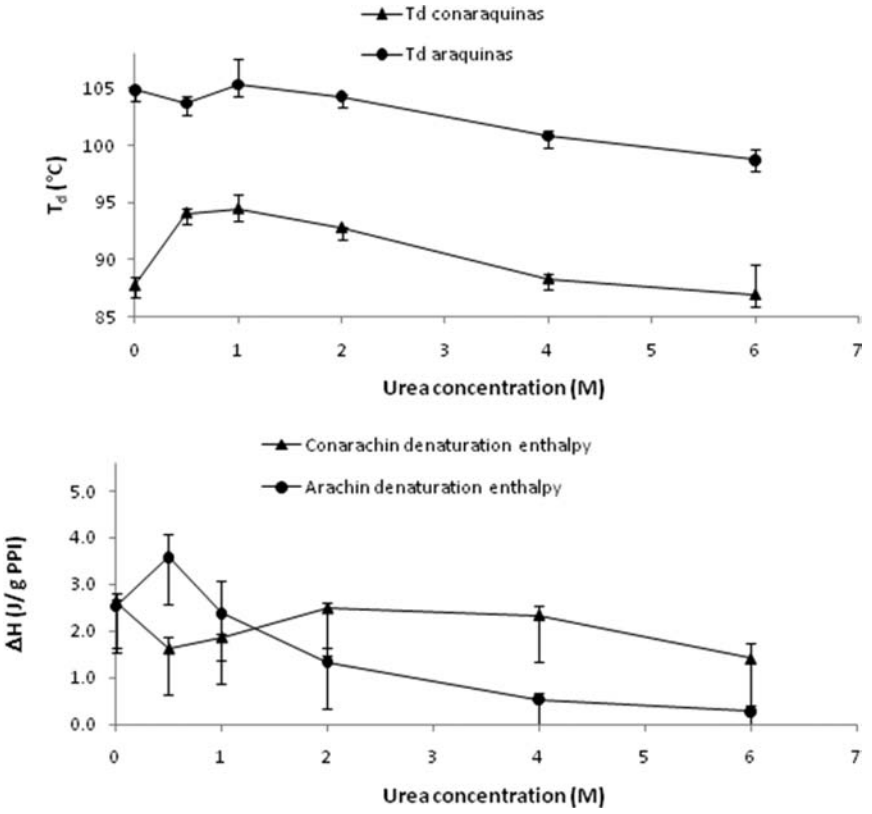

Figure 4. Effect of urea concentration on (**a**) denaturation temperature and (**b**) denaturation enthalpy (ΔH) of arachin and conarachin fractions.

flaxseed protein with increasing urea concentration; these authors suggested that protein may assume a specific conformation which is more heat-stable than the native protein.

The denaturation enthalpy values of the conarachin fraction were not affected by urea addition. On the other hand, the ΔH values of arachin decreased with urea concentration, which implied a decrease in the energy requirement for denaturation, in accordance with other authors (*21*, *22*).

According to Kinsella (*23*), urea allows protein unfolding through the weakening of hydrophobic interactions. In addition, urea increases the "permittivity" of water for the apolar residues, causing loss of protein structure and thermal stability (*24*). Recently, Stumpe and Grubmüller (*25*) showed that, aside from interference on the hydrogen bond structure of water, urea molecules are located at the protein surface; in particular, they accumulate close to less polar residues and the backbone, inducing the displacement of water molecules and facilitating protein unfolding due to exposure of apolar groups. As a consequence, urea can be considered to denature proteins by interfacing between water and the natively buried parts of the protein.

Monteiro and Prakash (*26*) found that arachin has higher values of hydrophobicity than conarachin. Therefore, the former proteins have a large proportion of less polar residues at their surface, which can interact with urea molecules. These lead to lower ΔH values of arachin fractions, as already mentioned.

Sodium Dodecyl Sulfate. To further examine hydrophobic interactions, the influence of SDS on the denaturation parameters of PPI was studied (**Table 2**). SDS is an anionic surfactant that can exert a stabilizing as well as a destabilizing effect on proteins, depending on its concentration (*14*). This detergent joins to proteins by means of interactions between sulfate groups and positively charged lateral chains and between alkyl chains and hydrophobic lateral chains (*27*). SDS addition caused an increase of $\Delta T_{1/2}$ and a severe decrease of the denaturation enthalpy values of conarachin. The thermal stability of this fraction was significantly affected starting from 20 mM SDS, in accordance with studies in flaxseed and oat proteins (*20*, *24*). These results indicate previous denaturation and a less stable protein structure. An important number of protein molecules change to a new state, which contributes, to a lesser degree, to the unfolding process, causing a significant decrease in calorimetry enthalpy (*28*). For the arachin fraction, T_d values were significantly lower only to 40 mM SDS. Hegg and Löfqvist (*29*) have shown that, at specific levels, SDS increases T_d in some proteins, due to the formation of a bridge between a charged group in one loop of a protein polypeptide chain and a hydrophobic region in another.

Table 2. Effect of SDS Concentration on Thermal Denaturation Parameters of PPI[a]

SDS concentration (mM)	T_d (°C)	ΔH (J/g of PPI)	$\Delta T_{1/2}$ (°C)
	Conarachin Endothermic Peak		
0 (control)	88.47 ± 0.47 b	2.39 ± 0.12 b	5.19 ± 0.25 a
5	87.03 ± 1.18 ab	1.50 ± 0.25 a	5.56 ± 0.21 a
10	87.17 ± 0.70 ab	1.14 ± 0.06 a	6.27 ± 0.01 b
20	85.15 ± 0.48 a	1.18 ± 0.00 a	6.23 ± 0.04 b
40	no thermal response		
	Arachin Endothermic Peak		
0 (control)	104.34 ± 0.11 b	3.73 ± 0.34 b	5.87 ± 0.00 ab
5	103.81 ± 0.04 b	3.63 ± 0.52 b	6.16 ± 0.14 b
10	103.72 ± 0.35 b	4.16 ± 0.05 b	6.59 ± 0.01 c
20	104.11 ± 0.02 b	2.02 ± 0.05 a	5.51 ± 0.18 a
40	101.81 ± 0.45 a	2.44 ± 0.02 a	7.65 ± 0.25 d

[a] Values corresponding to conarachin and arachin endothermic peaks are shown. Values are means ± SD of two independent determinations. Values followed by different letters within a column are significantly different ($p < 0.05$).

Table 3. Effect of DTT Concentration on Thermal Properties of Peanut Proteins[a]

DTT concentration (mM)	T_d (°C)	ΔH (J/g of PPI)	$\Delta T_{1/2}$ (°C)
	Conarachin Endothermic Peak		
0	87.73 ± 0.76 a	2.63 ± 0.03 b	6.07 ± 0.10 c
10	89.37 ± 0.33 b	1.80 ± 0.08 a	4.62 ± 0.11 a
20	90.81 ± 0.36 c	1.53 ± 0.27 a	4.95 ± 0.14 b
50	88.93 ± 0.15 b	1.45 ± 0.04 a	4.56 ± 0.09 a
100	88.60 ± 0.43 ab	1.42 ± 0.25 a	5.12 ± 0.13 b
	Arachin Endothermic Peak		
0	104.89 ± 0.05 c	3.88 ± 0.10 c	6.03 ± 0.41 c
10	101.50 ± 0.57 b	2.59 ± 0.24 b	5.76 ± 0.30 bc
20	101.75 ± 0.35 b	1.27 ± 0.15 a	5.56 ± 0.27 abc
50	99.74 ± 0.15 a	2.21 ± 0.35 b	5.32 ± 0.21 ab
100	99.29 ± 0.55 a	0.98 ± 0.11 a	4.97 ± 0.00 a

[a] Denaturation parameters related to arachin and conarachin fractions are shown. Values are means ± SD of two independent determinations. Values followed by different letters within a column are significantly different ($p < 0.05$).

Besides, arachin ΔH values decreased significantly starting from 20 mM SDS. This protein fraction has a molecular weight of ~350 kDa and is composed of six different subunits, classified into two groups of hydrophilic (S_1, S_2, and S_3) and hydrophobic subunits (S_4, S_5, and S_6) (*30*, *31*). According to Yamada et al. (*31*), heating can increase the hydrophobicity in the surface region of the arachin molecule. As a result, the higher resistance of this protein fraction to SDS-induced denaturation can be attributed, to a greater extent, to hydrophobic interactions between its proteins.

Dithiotreitol. According to Basha and Cherry (*32*), both arachin and conarachin can be dissociated in subunits using SDS and reducing agents. **Table 3** shows the effect of DTT on the thermal behavior of PPI. DTT is a reducer agent capable of reducing disulfide bonds to sulfhydryl groups and so causing protein destabilization. T_d significantly increased up to 20 mM DTT for the conarachin fraction; higher additive concentration did not affect protein stability. A similar result was obtained by Tang (*22*) when studying buckwheat proteins. On the other hand, Chan and Ma (*20*) showed that the thermal stability of flaxseed proteins was affected by the reduction of the disulfide bonds by DTT. Conarachin ΔH values decreased significantly with DTT addition. Disulfide bond breakdown requires a lot of energy; therefore, the rupture of these bonds before thermal denaturation reduces markedly the energy needed to unfold the residual protein structure (*33*).

When the disulfide bonds in arachin are reduced, polypeptides of lower molecular weight appear (*34*). This is not expected when SDS or urea is added to PPI. Thermal stability (T_d) and ΔH values of arachin were greatly decreased by DTT, suggesting that the polypeptides of lower molecular mass generated may also exhibit significant DSC response.

For both protein fractions, a great decrease in $\Delta T_{1/2}$ values was also observed. Protein denaturation implies cooperative transitions in which various bonds are broken simultaneously. Therefore, the lower $\Delta T_{1/2}$ values obtained after DTT addition would indicate a reduction in the number of disulfide bonds.

Conclusion. The thermal profile of peanut protein isolate showed two endothermic peaks, which were assigned to peanut protein denaturation. The major one occurred at higher temperatures and was attributed to arachin unfolding. Hence, this protein fraction has a more ordered structure and a higher thermal stability than conarachin.

The thermal denaturation parameters of peanut proteins were sensitive to the water content of the system, indicating that polar groups of arachin and conarachin contribute to structure stabilization. The addition of protein denaturants allowed us to assess the significance of interactions in the maintenance of peanut proteins' native structure. Urea concentration mainly affected the structure of the arachin fraction, which was attributed to the higher surface hydrophobicity of this protein fraction. SDS addition mainly affected the conarachin fraction, whereas arachin parameters were modified only at higher SDS values. On the other hand, DTT addition affected both protein fractions. These results suggest that hydrophobic interactions and disulfide bonds play an important role in maintaining the arachin and conarachin structures.

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