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DSC study on the thermal properties of sunflower proteins according to their water content

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Abstract

The use of sunflower protein isolate for the manufacturing of biodegradable materials by thermomechanical means requires a characterization of its thermal properties. After modelling of its hydration properties, DSC analysis shows a relaxation phenomenon at 60°C and makes it possible, despite the changes in state of the absorbed water, to determine its glass transition temperature. The evolution of this temperature according to the water content of the samples is then modelled and compared to that of more common proteins. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Sunflower; Proteins; Relaxation

1. Introduction

The substitution of biodegradable polymers of natural origin for plastic packaging has been developing fast for about the last 10 years [1]. And even if their ecological advantages are at times questioned [2], the use of renewable raw materials must become a priority for, on the one hand, compensating for the exhaustion of fossil fuels [3] and, on the other hand, to make a better use of our natural environment. The research carried out in this area has already obtained interesting results. Polysaccharides, notably starch [4], can easily be set, but the strong sensitivity to water of the materials formed greatly limits their applications. Proteins, on the other hand, seem more promising: their complex chemical structure causes many different interactions to occur which makes them less soluble, even insoluble [5]. Their most common setting process, casting (drying of the protein solution), is not easily industrializable [6]. Research therefore focuses on thermo-mechanic means (extrusion, thermoforming) which require good knowledge of the thermal properties of the proteins before transformation.

Research carried out on the stability of food polymers [7] has shown that they have an amorphous structure in which water acts as a plasticizer. Their properties are therefore linked to water activity (a_w), which conditions their hydration [8], as well as to the temperature and pressure occurring during transformation [9,10]. The combined effect of these three factors can therefore cause the polymer to change from a metastable glassy state to rubbery one, characterized by a general increase of the free volume, disorder and mobility of the polymer chains [11]. This glass transition, strongly dependant on the chemical structure of the biopolymers brings about a sudden change in their thermal, mechanical and dielectric properties and, in particular, a sudden drop in their viscosity which is an essential parameter in the setting.

Traditionally, it is the discontinuous variation of the calorific capacity (C_p) of amorphous materials according to temperature which is measured by differential scanning calorimetry (DSC). Another more sensitive technique consists in measuring the evolution, during temperature increase, of the viscoelastic properties of materials under oscillatory stress (DMTA). However, with both methods, the measurements are disturbed by the presence of absorbed water. At high temperature, its evaporation [12] causes an important modification of the thermal properties (endothermal phenomenon and modification of calorific capacity) and mechanical properties (viscoelastic characteristic modification) of materials. At low temperature, freezable water [13] can change state and disturb measurements. Furthermore, DMTA is essentially used for biopolymer already set and of which the structure has already been modified. Indeed, measurements on powders [14] cannot be taken under hermetic conditions. However, glass transitions of many proteins such as soya globulins [15], wheat gluten [16] and maize zein [17] have been studied and modelled using these techniques.

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Table 1
Average composition (3 determinations per characteristic) of the sunflower cake and of the sunflower protein islated

In (%)	Sunflower oil cake	ISFP	Determination method
Moisture	10 ± 2	6 ± 1	105°C/24 h
Ash	7.6 ± 0.4	2.4 ± 0.4	Incineration (525°C/5 h)
Proteins	34.4 ± 1	90 ± 1	Kjedhal, Rapid-N
Lipids	1 ± 0.5	0.6 ± 0.4	Soxhlet extraction (hexane)
Cellulose	22.3 ± 2	0	ADF/NDF
Lignin	5.2 ± 1	1.7 ± 1	ADF/NDF

Another thermal phenomenon, identified for food proteins, must also be taken into account: denaturation. This modification of quaternary, tertiary and secondary macromolecular structures [18] has an influence on the interactions between chains and therefore on their mobility. Linked to the thermal history of proteins, as with the relaxation observed in polysaccharides [19] its study provides additional information about the structure of the biopolymer-water system.

The possibilities for the use of sunflower proteins for the manufacture of films have already been demonstrated [20]. The objective of this work is therefore to study by DSC the unknown thermal properties of these proteins, according to their humidity rate. The observation of all the possible thermal events for the water–protein system helps to obtain a better understanding of the glass transition phenomenon and a good thermal characterization.

2. Materials and methods

2.1. Material

The protein isolate used is obtained by alkaline extraction from sunflower oil cake (compositions reported in Table 1) on a pilot scale. The protocol followed uses 1 kg of oil cake with 201 of aqueous sodium hydroxide solution (pH 12). This is stirred at 50°C for 20 min following the protocol defined by Leyris [21]. After centrifugation, the soluble proteins are precipitated at their isoelectric point by adding concentrated sulphuric acid. A second centrifugation then allows their separation from the aqueous phase. The third stage consists in drying at 50°C, followed by a grinding step with a conventional knives grinder. The isolate thus obtained contains 90% of mass proteins compared to dry matter (determined by Kjedhal and Rapid-N analysis of nitrogen level (protein level = nitrogen level × 6.25)).

The salts used for the conditioning of the humidity-controlled containers were provided by Aldrich (St Quentin Fallavier, France), and are of analytical purity.

2.2. Experimental part

2.2.1. Conditioning of the protein isolate samples

Isolate samples of different humidity levels are prepared by conditioning in humidity-controlled hermetic containers. Each of these containers holds a saturated saline solution which imposes a relative humidity determined at the head space above it. The salts used: KOH, $MgCl_2$, K_2CO_3, $Na_2Cr_2O_7$, NH_4NO_3, NaCl, $(NH_4)_2SO_4$, KCl, KNO_3, K_2SO_4 allow relative humidities of 8, 33, 43, 54, 62, 75, 80, 85, 92 and 97% respectively to be obtained.

The protein isolate samples are dried under vacuum (5 mmHg) at 60°C for 15 days in a desiccator containing P_2O_5. They are then placed in the different humidity-controlled containers. Thymol is added to the containers with relative humidities over 62%, in order to avoid the development of mold on the proteins during the conditioning [22]. The proteins are then brought to their humidity equilibrium level, that is to say when their mass no longer varies over a period of 24 h. This phase requires one to three weeks, according to the humidity level considered.

To determine the different water contents, 1 g of each sample is collected in triplicate and is dried in an oven at 105°C during 24 h. The evaporated mass is then divided by the mass of dry matter to obtain the humidity rate.

2.2.2. Carrying out of the DSC measures

The studies are carried out with a power modulation DSC device Pyris 1 (Perkin Elmer), equipped with an Intracooler cooling system. The purge gas used is nitrogen at a flow rate of 20 ml/min. Indium ($T_f = 156, 6$°C) and distilled water ($T_f = 0$°C) are used for temperature calibration. The treatment of the data obtained is carried out using the Pyris software (Perkin Elmer).

Two types of hermetic capsules are used in this study: aluminium capsules and O-ring sealed stainless steel capsules. The latter are resistant to high pressure (40 bars) and are therefore better adapted for studying low hydrated biopolymers at high temperatures. Whichever the type of capsule used, DSC analysis is performed on samples from about 10 mg. Each humidity level is tested in triplicate.

2.2.2.1. Relaxation/denaturation study. The measures are carried out in a first scan, between 25 and 90°C. Given the low temperatures and therefore low pressures, the use of steel capsules proved unnecessary. Aluminium sealed capsules are sufficient. The temperature increase rate used here is 5°C/min. Furthermore, the relaxation is broad enough to expect no leveling which could result from a too slow heating speed.

2.2.2.2. Determination of the glass transition temperature (T_g). Most of the analysis performed on proteins, whether purified or not, uses relatively low heating speeds. Temperature increase rates found in literature are of 5°C/min [15,17,23] or 10°C/min [16,24,25], on a scale of temperature ranging from −30°C to 180°C and more.

Unlike previous studies on vegetable proteins, the temperature increase rate used is 20°C/min. Contrary to generally accepted ideas, the adoption of this heating

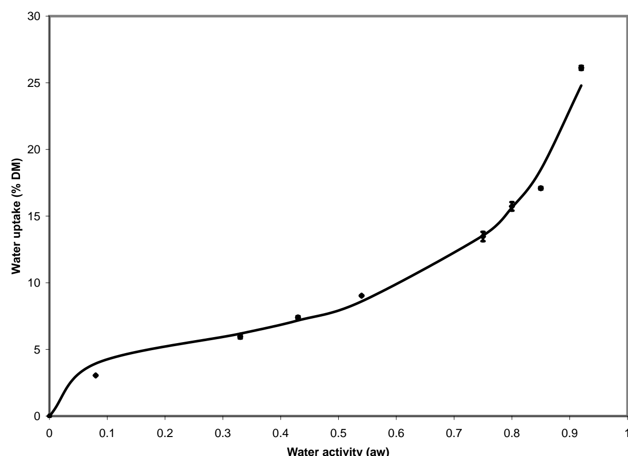

Fig. 1. Water uptake isotherm (g per 100 g of dry matter) of sunflower proteins at 25°C. The continous line is the GAB modelling.

speed corresponds to the phenomenon we will try to quantify. A high increase rate helps to better observe changes in C_p related to weak amplitude transitions, which is the case with biopolymer glass transitions. Furthermore, in preliminary tests, we saw that a relatively high heating speed minimizes water evaporation, which remains the main parasite phenomenon in the T_g determination of samples with intermediate humidity levels. A relatively high increase rate of temperature also helps to reduce alterations in protein structure which can occur when the heating speed used is low.

T_g is determined during the second increase in temperature, so as to eliminate the effects of the thermal history of the sample [24,25]. The value of T_g is taken at the point of inflexion of the observed transition by calculating the second derivative (the value corresponds to the nullification of the second derivative).

T_g changes according to the water content of proteins has been the subject of mathematic modelling. In literature, there are mainly three models: the equations of Couchman–Karasz [26], Gordon–Taylor [27] and Kwei [28].

The most often cited of these three models in literature is the Couchman–Karasz thermodynamic model (1978). It is based on the thermodynamics laws of mixtures and allows a prediction to be made about the effect of a plasticizer on proteins by calculating the new T_g:

$$T_g = \frac{X_p \delta C_{p_p} T_{g_p} + X_d \delta C_{p_d} T_{g_d}}{X_p \delta C_{p_p} + X_d C_{p_d}}$$

where 'X' represents mass fraction (or possibly molar fraction) and 'ΔC_p' the variation of mass (or molar) calorific capacity observed at glass transition temperature T_g. Letter 'p' refers to the polymer (in this case protein) and letter 'd' to the diluent, that is to say to the plasticizer (water, sugar, alcohols…).

Beck et al. [29] have used this equation to predict the plasticizing effect of water on maize zein, and Di Gioia [17] to predict the total T_g value of maize gluten from his zein and glutenin fractions. Similarly, Gontard and Ring [30] have used this equation to study wheat gluten plastification with a water–glycerol mixture.

Following the Couchman–Karasz theoretical model, the Gordon–Taylor model allows a prediction to be made about T_g in the case of an addition of plasticizer to a polymer. This has the advantage of requiring only the experimental determination of a constant called K (and not the knowledge of the ΔC_p). This equation is as follows:

$$T_g = \frac{W_p T_{g_p} + K W_d T_{g_d}}{W_p + K W_d}$$

As for the Kwei equation, it comes from the previous model to which is added a term representing the influence of specific interactions able to stabilize or destabilize the mixture:

$$T_g = \frac{W_p T_{g_p} + K W_d T_{g_d}}{W_p + K W_d} + Q W_p W_d$$

These three equations have been compared in this study to model the glass transition temperature variations of sunflower proteins according to their water contents.

3. Results and discussion

3.1. Sorption isotherm

The determination of the sorption isotherms makes it possible to define the hydration capacities of a compound [7] and generally characterizes foodstuff stability in variable atmospheric humidity conditions. The isotherm obtained for sunflower proteins (Fig. 1) has a sigmoidal shape, comparable to that of hydrophilic polymers. This type of curve is characteristic of the presence of bound and non-bound water [31] and helps make sure that there will be no phase separation during a thermo-mechanical transformation [32].

Two mathematical description of water uptake: BET and GAB [33], based on particular sorption theory, are frequently used. However, the fitting ability of the GAB model is often much better [34], so this is the equation used to describe the mass of water absorbed by 1 g of dry matter:

$$m = \frac{m_m\, C\, K\, a_w}{(1 - K\, a_w)[1 + (C - 1)\, K\, a_w]}$$

where m_m, C and K are empirical constants and for water activity a_w varying from 0 to 0.9. Mathematical solving of the equation gives, for sunflower proteins, $m_m = 4.6$ g/g, $C = 55.4$ and $K = 0.89$ with a correlation coefficient of 0.97.

If we compare this uptake isotherm to those proposed in literature [8] and shown in Fig. 2 for various biopolymers used for the creation of materials, sunflower proteins have a

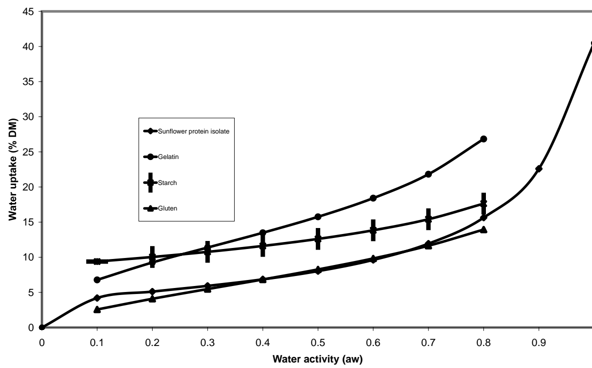

Fig. 2. Comparison of modelled water sorption isotherms of different biopolymers at 25°C (datas from Iglesias [8]).

behavior closer to that of wheat gluten, more hydrophobic [6], than to that of gelatin or starch.

3.2. Relaxation

All the curves obtained in DSC for relative humidities of 6–17% show in the first scan an endothermic peak at about 60°C (correponding temperatures and enthalpies are shown in Fig. 3). This could be the result of several phenomena, all linked to the breakdown in weak energy interactions.

The first is denaturation [18]. It corresponds to a modification in the three-dimensional organization of protein structure. The values of the temperature of protein denaturation are between 50 and 100°C. For sunflower globulins 11S, this temperature is equal to 95°C (measured in solution) [35]. Of course this temperature varies with protein hydration up to a level value called denaturation temperature for hydration levels above 150% [36]. This denaturation phenomenon does therefore not match the results obtained: a single peak at 60°C whatever the relative humidity of the proteins. Denaturation apparently happens during the different stages of the extraction process (drying, basic extraction conditions).

The second phenomenon, identified during glass transition measurements and linked to the thermal history of the polymers, is relaxation [37]. Following this phenomenon, when the cooling speed (or even dehydration speed) is much lower than the heating speed, the curve presents an endotherm at temperatures close to the glass transition temperature, corresponding to relaxation of the stresses generated during cooling [38]. Even though this phenomenon does disappear in the second scan, relaxation cannot explain the appearance of the corresponding peak always at the same temperature.

The last phenomenon which could be put forward and which is comparable to relaxation has been identified for low hydrated polysaccharides [19]. It is the rupture of a complex network of hydrogen bonds stabilized by the presence of water molecules and which can rapidly restore itself. It consists in an endotherm with a temperature varying between 50 and 70°C for moisture contents between 5 and 25% and whose relatively narrow peak indicates a cooperative thermal disruption of interactions in the hydrogen bonded network.

The origin of the phenomenon observed would therefore be the setting up of an organization within the polypeptide chains formed during the drying or conditioning of the samples and maintained by weak interactions, notably by hydrogen bonds. The endothermic peak therefore corresponds to the necessary energy for relaxation of these stresses.

A comparative study of relaxation enthalpy evolution and uptake isotherm helps to obtain more information about water–protein isolate relationships. Indeed it seems that the first phase of water uptake, for water contents of between 0 and about 6% (content corresponding to the point of inflexion of the uptake isotherm), corresponds to

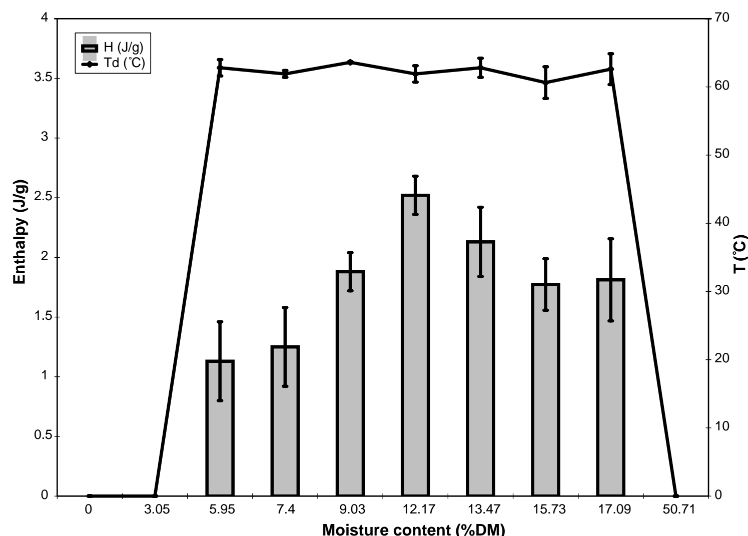

Fig. 3. Evolution of sunflower proteins relaxation temperature and of the enthalpy associated according to their water content (g per 100 g of dry matter).

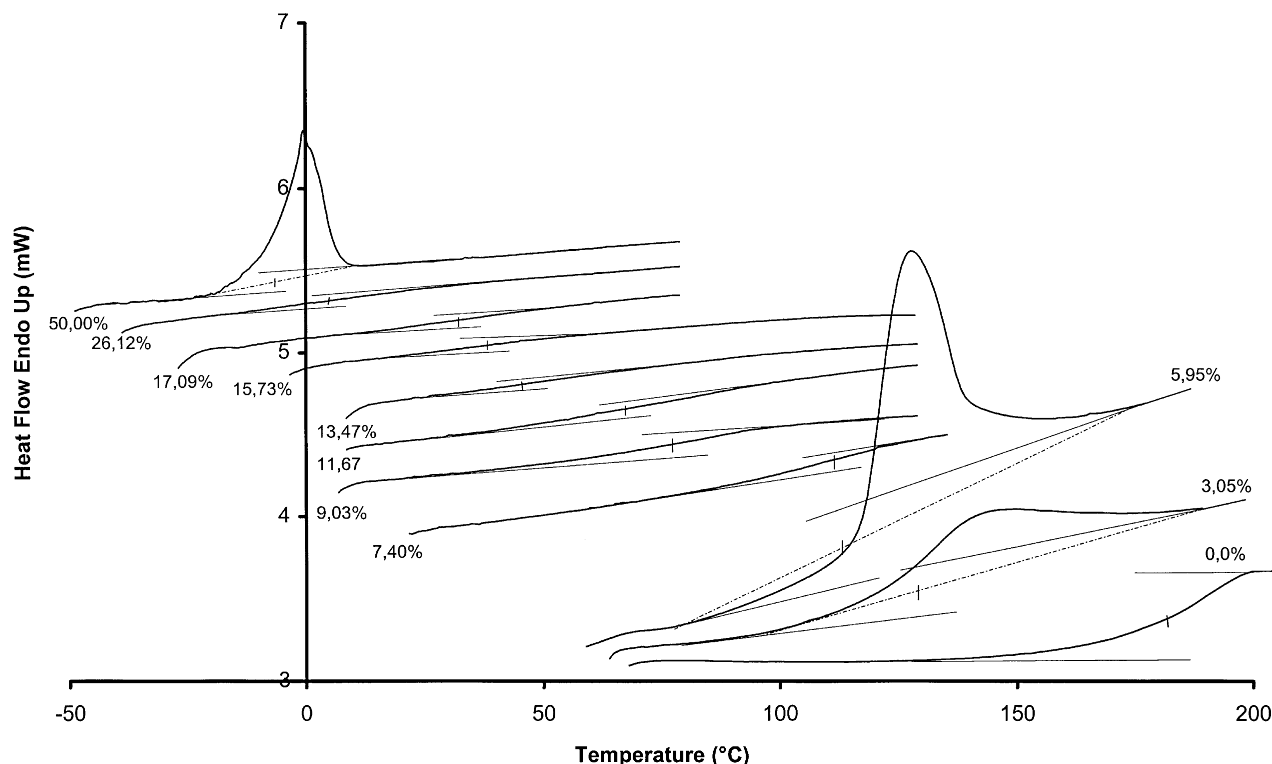

Fig. 4. Evolution of the glass transition temperature of the sunflower protein isolated according to its water content.

the hydration of charged groups [39]. Moreover, the energy of these dipole-ion interactions is superior to those of dipole–dipole interactions [40], the only observable thermal phenomena, which are actually often confused, are therefore water evaporation above 130°C (characteristic of the intensity of the interactions) and glass transition.

For hydration levels between 5 and 12%, the chains are organized by the stabilizing effect of water. Hydrogen interactions are then created [19], maintaining this organization. As water quantity increases, these interactions spread and relaxation enthalpy increases. For water content of between 12 and 20%, content corresponding to $a_w = 0.9$ and therefore to the radical change in hydration process [34], these interactions are diluted by the water molecules absorbed. More and more water–polymer interactions are then formed, to the detriment of polymer–polymer interactions, and enthalpy decreases. Finally, for higher water levels, all polymer–polymer interactions responsible for this relaxation disappear. The system suffers no more stress.

3.3. Determination of glass transition temperature

T_g is measured during the second scan, the only purpose of the first scan being to relax the stresses in the protein network which are characterized by the relaxation peak [41]. However, it must not be the cause of a matter dehydration, even weak, before measuring glass transition temperature. That is why the capsules are hermetic and the cooling rate slow enough for the matter to reabsorb water which it may have lost during the first scan.

Unlike the soy protein isolate that has two separated glass transitions for 11S and 7S fractions [15], the thermograms of sunflower protein isolate show a single T_g, indicating a complete denaturation of the structure during the extraction process [42].

In the case of dry proteins, there is no peak in the change of state of water (evaporation): it is therefore easy to determine T_g. On the other hand, for humidity levels other than 0, two phenomena are observed: the presence of a water evaporation peak [43], for humidity levels above 2%, and a frozen water fusion peak [13,44] for the highest water levels (26, 50%). The problem linked to water evaporation is partly resolved by adopting a temperature increase rate high enough to ‘concentrate’ the phenomenon in a relatively narrow peak. The chances of being in a situation where glass transition is masked by this state change of water are thus reduced. Concerning the second phenomenon mentioned above, it would clearly be illusory to try to determine a T_g below water freezing temperature on a sample containing free water. Below this temperature, the free water in the protein network is indeed frozen, it therefore cannot participate in the phenomenon of protein chain plastification [42].

Precautions taken for T_g determination are therefore different according to the water content of the matter studied. A major difficulty is in particular met for proteins with a water content of between 5 and 8%; glass transition is

Table 2
Evolution of glass transition temperature of sunflower proteins according to their water content

Enclosure R.H. (%)	Water content (% DM)	T_g (°C)
*****	0	180.8
8	3.05	129.5
33	5.95	113.1
43	7.40	111.9
54	9.03	77.8
62	11.67	68.4
75	13.47	46.1
80	15.73	39.6
85	17.09	33.8
92	26.12	5.3

Table 3
Couchman–Karasz, Gordon–Taylor and Kwei model coefficients

Coefficient (Model)	Coefficient value
K (Gordon–Taylor)	5.16
K (Kwei)	5.19
Q (Kwei)	0.0004
ΔC_p (Couchman–Karasz)	0.38 J g^{-1} °C^{-1}
T_g (Gordon–Taylor)	452.0 K
T_g (Kwei)	452.1 K
T_g (Couchman–Karasz)	452.0 K

indeed masked by the water evaporation peak. In this case, we have used the tangent method. There is indeed a variation in the base line before and after water evaporation which results, on the one hand from the transition of interest to us, and on the other hand from the change in calorific capacity of 'dry' matter compared to wet matter. However, T_g determination remains possible. This is later confirmed by correct integration of T_g values determined in these conditions into 'classically' obtained values. On the other hand, ΔC_p values are obviously overestimated. It is important to recall that this water evaporation phenomenon [43] cannot be avoided: any transition taking place between 90 and 130°C is extremely difficult to measure. Furthermore, glass transition cannot be measured for a water content of 50%. In that case, the 'frozen' water fusion peak completely masks the phenomenon. On the other hand, for humidity level of 26%, the free water fusion peak is absent and T_g is perfectly visible and measurable, which confirms the idea that this humidity level still corresponds to bound water. The value obtained by the second derivative method is 5°C. For protein isolate samples with humidity levels of 8–26%, T_g is easily measured: glass transition and water evaporation are quite distinct in this zone. All the curves observed are shown in Fig. 4, and the corresponding T_g values in Table 2.

Globally, we observed an important drop in glass transition temperature as water content of the proteins increased. This result is comparable to that obtained by many authors with other types of proteins such as elastin [45,23], wheat gluten [15,25,46,47], their two main fractions, glutenins [23,25] and gliadins [25,48,49], and also maize zein [49,50].

The T_g values obtained were modelled by the equations of Couchman–Karasz, Gordon–Taylor and Kwei. The coefficients calculated for the different models are shown together in Table 3. Here, the three models give equivalent results with a correlation coefficient compared to the experimental T_gs of 0.9946. The different curves obtained are shown in Fig. 4: T_g drops from 179°C to about 0°C when the humidity level of the protein passes from 0 to 21%. The Kwei model does not fit better than the Gordon Taylor model. Indeed, coefficient Q in the first of these two models is very weak, which leads to neglecting the term QW_pW_d. However, according to certain authors, this term indicates the importance of secondary interactions which may exist between the protein network and water. It would therefore seem in this case that these are almost non-existent here, and thus that sunflower proteins have a weak affinity for water. This result would present a great interest for the manufacture of materials made from sunflower proteins. Indeed, we know well that the hydrophilic character of most polymers is the main obstacle for their use as materials in many applications. Nevertheless, we point out that a previous study carried out on wheat gluten attributed to it a coefficient of $Q = 600$, which led the authors to conclude that wheat gluten has a hydrophilic character [51]. Given the similarity of the water uptake isotherms of these two proteins, we can only be cautious in our conclusions until more advanced investigation are carried out to determine more clearly the hydrophobic character of sunflower proteins.

The Couchman–Karasz equation gives a value of $\Delta C_p = 0.38$ J g^{-1} °C^{-1} for sunflower proteins, value identical to the 0.381 J g^{-1} °C^{-1} (average of three measurements) obtained experimentally. This value is close to the ΔC_p found by different authors for other types of proteins such as wheat gluten, $\Delta C_p = 0.4$ J g^{-1} °C^{-1} [41] or $\Delta C_p = 0.39$ J g^{-1} °C^{-1} [52], γ-gliadins $\Delta C_p = 0.4$ J g^{-1} °C^{-1} [24], and also maize zein $\Delta C_p = 0.365$ J g^{-1} °C^{-1} [16].

4. Conclusions

The thermal properties of sunflower proteins were measured taking into account the problems presented by the state changes of the water contained in each sample. The results of this study show the plasticizing character of the water contained in the protein network, and its influence on the temperature of glass transition of sunflower proteins. Furthermore, T_g modelling confirms a good homogeneity of experimental values. This result is a major first step towards the creation of a thermo-mechanical process for making sunflower protein based materials. However, we think it would be interesting to complete this work with a study of the influence of pressure, which is a determining parameter in the state of matter during thermo-mechanical modifications.

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