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Thermal denaturation of sunflower globulins in low moisture conditions

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Abstract

DSC analysis in pressure resisting pans of sunflower oil cake makes appear the endothermic peak of sunflower globulins denaturation. Its temperature decreases from 189.5 to 119.9 °C while the corresponding enthalpy increases from 2.6 to 3.3 J/g of sample, or from 6.7 to 12.2 J/g of dry protein, when the samples moisture content varies from 0 to 30.0% of the total weight. The plot of the denaturation temperature versus the moisture content is not linear but has a rounded global shape and seems to follow the hydration behavior of the proteins, modeled with the sorption isotherm.

As it can be seen on scanning electron microscopy (SEM) micrographs, protein corpuscles "melt" after such a thermal treatment and large aggregates form by coagulation.

Moisture dependence of the "fusion" temperature of native proteic organization, in low moisture conditions, offers so a new characterization method for the use of vegetable proteins in agro-materials.

Keywords: Sunflower oil cake; Denaturation; Protein; Globulin; DSC

1. Introduction

The thermal analysis of proteins is experiencing a new expansion due to the emergence of new agro-materials. There is indeed an increasing need to substitute synthetic plastics by materials both biodegradable and renewable. In this context, the physical and chemical properties of plant or animal proteins prove to be particularly advantageous in the manufacture of films [1] and molded objects [2].

By analogy with petrochemical polymers and subsequently by taking into account the setting process through the standard technology of plastic industry, protein glass transition is being widely studied by DSC and DMTA.

In particular, considering that it is accepted that biopolymers have an amorphous structure in which water acts as a plasticizer [3], the variations in their glass transition temperature according to their water content are being modeled [4].

The thermal properties of soy globulins [5], wheat gluten [6], maize zein [7] or sunflower proteins [8] have thus already been described. This glass transition is not however the only interesting thermal event for the use of proteins in the field of materials.

Thermal denaturation of protein (modification of the protein native structure by breaking the non-covalent interactions), widely studied for their alimentary properties [9,10], provokes a radical change in their physical and chemical properties, more particularly in their

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solubility, and can have a determinative influence on their eventual molding [11].

This DSC study of sunflower oil cake and protein isolate samples at different humidity levels shows on the one hand that, even though it has up to now mainly been studied in solutions or suspensions, the endothermic denaturation peak can be observed through DSC in low humidity conditions (moisture content under 30%) by using sealed capsules.

It brings, on the other hand, a thermal characterization of sunflower globulins (Helianthinin) which possess valuable properties for the manufacture of films [12] or for the molding of compound materials by direct transformation of the oil cake [13,14].

2. Experimental

2.1. Materials

The sunflower oil cake, residue from the solvent extraction of oil, was provided by the company "Toulousaine de Céréales" (Toulouse, France).

The protein isolate is obtained by alkaline extraction of the sunflower oil cake according to the protocol described in an earlier study [8] (mass compositions are given in Table 1).

The salts used for sample conditioning were provided by the company Aldrich (St. Quentin Fallavier, France) and are of analytic purity.

2.2. Conditioning of the samples

The oil cake and protein isolate are grinded in an IKA MF10 (Staufen, Germany) grinder, equipped with a 1 mm grid. Samples weighing a few grams are then dried up for 15 days at 60 °C in a vacuum desiccator containing P_2O_5, and then conditioned in humidity controlled containers.

Each container holds a saturated saline solution determining the relative humidity of the head-space. The salts used, KOH, $MgCl_2$, NH_4NO_3, NaCl, KCl, KNO_3 and K_2SO_4 allow relative humidity of respectively 8, 43, 62, 75, 85, 92 and 97% to be obtained. Stability of their humidity is achieved when their mass varies no more than 1% in 24 h.

The dry matter of the different samples is then evaluated three times by drying in an oven at 105 °C during 24 h. The evaporated mass is then compared to the mass of wet matter to determine its water content.

2.3. DSC Analysis

This study is performed on a Pyris 1 power compensation calorimeter (Perkin-Elmer) fitted with an intracooler cooling system. The purge gas used is nitrogen of analytic quality at a flow rate of 20 ml/min. Temperature and energy calibration is carried out with indium ($T_f = 156.6$ °C) and distilled water ($T_f = 0$ °C) before the beginning of the tests.

All analysis are performed with hermetic 60 μl stainless steel capsules fitted out with O-rings resistant to an internal pressure of 40 bar (Perkin-Elmer). Reference cell is empty.

They are carried out at a heating speed of 20 °C/min from 25 °C and stopped at 200 °C. The sample mass is around 15 mg and all measurements are done in triplicate. Peak integration is realized with a sigmoid base line.

Dry samples analysis are obtained in filling pans with oil cake at a known moisture content. The pans are then placed in the vacuum desiccator for 48 h and

Table 1
Average composition (three determinations per characteristic) of sunflower oil cake and of sunflower protein isolate (alkaline extraction [17])

Characteristics	Sunflower oil cake	Proteic isolate	Determination method
Moisture (%)	10 ± 1	6 ± 1	Oven drying 105 °C/24h
Ash (%)	7.6 ± 0.4	2.4 ± 0.4	Incineration (525 °C/5h)
Proteins (%)	34.4 ± 1 (38.6% DM)	90 ± 1	Kjedhal, rapid-N
Lipids (%)	1 ± 0.5	0.6 ± 0.4	Soxhlet extraction (hexane)
Lignin (%)	5.2 ± 1	1.7 ± 1	ADF/NDF
Cellulose (%)	22.3 ± 2	–	ADF/NDF
Hemicelluloses (%)	18.5 ± 1	–	ADF/NDF
Total	99.0 ± 6.9	100.7 ± 3.8	

immediately sealed after cooling. Sample mass are calculated according to the initial moisture content.

2.4. Scanning electron microscopy

The observation is carried out on a scanning electron microscope LEO 435VP (Cambridge, UK).

At the end of the DSC analysis, the samples are cooled down at a cooling rate of 30 °C/min, extracted from the pan and then dried at 60 °C for 48 h in a vacuum desiccator containing P_2O_5, before being metallized and observed.

3. Results

The DSC curves of the sunflower oil cake, which composition is reported in Table 1 show several remarkable characteristics (Fig. 1). First of all, for moisture contents between 3 and 20%, a small endothermic peak appears around 70 °C. Already noticed on polysaccharides [15], this relaxation peak is discussed in a previous work on the thermal properties of sunflower proteins [8]. All curves have a rising base line due to the increase of the calorific capacity of native protein with the temperature [16] and the increase of the water pressure which leads to a slight dehydration of the samples. Finally, all the curves comprise an endothermic peak, the temperature of which varies between 189 and 120 °C when sample moisture varies from 0 to 30% approximately. However, considering the shape of the curves, peak integration is particularly difficult; when moisture content increases, the enthalpy of the phenomenon corresponding to this peak slightly increases (Table 2). If the capsule is cooled down to 25 °C for 1 h after the first rise of the temperature, the endothermic peak does not appear anymore when heating is resumed. The phenomenon observed between 189 and 120 °C is therefore irreversible, as generally normal for denaturation.

Additional tests with standard aluminum capsules, have proved that these traditional analysis conditions do not make it possible to observe the phenomenon. In fact, if the pan is not hermetically sealed, the water evaporation peak at temperatures below those of the endotherm observed makes it disappear, and the sealed aluminum pans withstand only 2 bar of pressure.

A similar DSC study, carried out on protein isolate samples extracted in an alkaline environment (composition reported in Table 1), shows that the phenomenon completely disappears. The origin of the peak seems therefore to be linked to the native organization of sunflower proteins, since their thermal stability is not affected by oil extraction [17]. Actually, the analysis

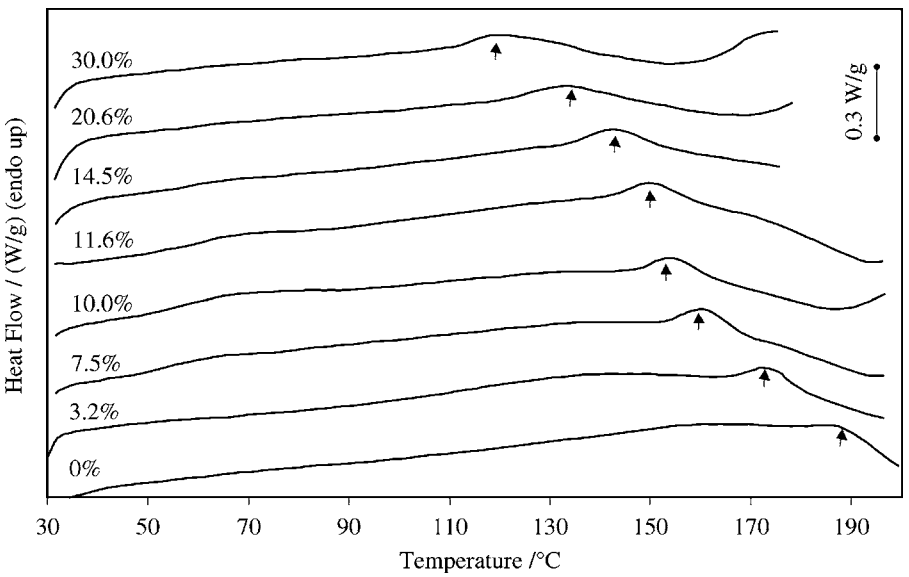

Fig. 1. DSC curves in pressure- resistant pans of the sunflower oil cake samples at different moisture contents (percentage of total weight).

Table 2
Average temperature, saturated water vapor pressure at this temperature, average enthalpy reported to the sample mass, to the mass of dry matter and to the mass of dry protein (Table 1) of the denaturation peak observed on DSC scans of sunflower oil cake, in pressure resistant pans, according to its water content

Moisture content (%)	Peak temperature (°C)	Water vapor pressure (bar)	Enthalpy (J/g_{sample})	Enthalpy (J/g_{DM})	Enthalpy ($J/g_{protein}$)
0	189.5 ± 1.6	–	2.6 ± 0.1	2.6	6.7
3.2	172.8 ± 0.7	8.5	2.9 ± 0.1	3.0	7.8
7.5	160.4 ± 0.3	6.2	2.8 ± 0.1	3.0	7.8
10.0	154.4 ± 0.7	5.3	3.0 ± 0.1	3.3	8.6
11.6	150.2 ± 0.2	4.8	2.7 ± 0.3	3.0	7.9
14.5	143.1 ± 0.9	3.9	3.2 ± 0.2	3.7	9.7
20.6	133.4 ± 0.1	2.9	3.3 ± 0.1	4.2	10.8
30.0	119.9 ± 0.5	2.0	3.3 ± 0.2	4.7	12.2

of a 5% isolate suspension does not give evidence of a denaturation peak in the standard temperature range of 60–90 °C [18]. It seems therefore that the phenomenon observed is due to the denaturation of sunflower proteins, particularly of globulins which account for approximately 50–65% of the protein mass of sunflower seeds [19] and that the extraction process causes the unfolding of proteins. Considering the macromolecular structure of these globulins (Fig. 4a) and the low hydration level of the samples, the word "fusion" seems more appropriate.

Experiments at different heating speeds (Table 3) show that the slower the heating rate, the lower the temperature and peak enthalpy. This result reminds us that temperature calibration must be carried out with the same temperature increase rate as the analysis. Experience shows that capsules do not always withstand the high pressures induced by water evaporation, even if the 15.5 bar water vapor pressure at 200 °C is less than the 40 bar pans limit. It may be due to the conjugated effects of the air compression in the pan, of the possible degradation of hemicelluloses above 160 °C and of the seals irregularities.

Table 3
Influence of the heating rate on the average temperature and enthalpy of the denaturation peak

Heating rate (°C/min)	Peak temperature (°C)	Peak enthalpy (J/g)
5	147.9 ± 0.4	2.4 ± 0.1
10	151.6 ± 1.3	2.8 ± 0.1
20	154.4 ± 0.7	3.0 ± 0.1

Moisture content of the samples was 10.0%.

4. Discussion

Sunflower proteins being used in animal food only, their thermal properties have therefore only been briefly mentioned in Bull's study of protein thermal stability [20]. Thus, the denaturation of 11S sunflower globulins takes place at a temperature of 95 °C (in solution). The temperature of the peak observed here varies between 189.5 and 120 °C, when the hydration level of the oil cake varies from 0 to 30% and could reasonably be extrapolated to 95 °C in humidity conditions exceeding 100%. The results obtained are then in the same range as those for ovalbumin [21] or for soy globulins [22]. And even if the measured hydration rate is that of the oil cake, and not of the protein alone, the sorption isotherms (Fig. 2) are quite identical and the moisture content of the globulins in the oil cake is the same as the oil cake one. The difference for a_w between 0.1 and 0.6 can be attributed to the carbohydrates, notably hemicelluloses. The shape of the plot of the denaturation temperature according to the water content is rounded (Fig. 3) as that of protein glass transition temperature [8]. The linear fit obtained by Kitabatake et al. [21] and Fujita and Noda [22] appears only for moisture content comprised between 3.2 and 14.5%, corresponding on the isotherm (Fig. 2) to the hydration of polar residue [23].

The denaturation enthalpy increases with the moisture content of the samples (Table 2). Yet thermodynamically, the enthalpy increases with the temperature [16]. And the oleaginous globulins having been in the seed and, during the extraction process, in an

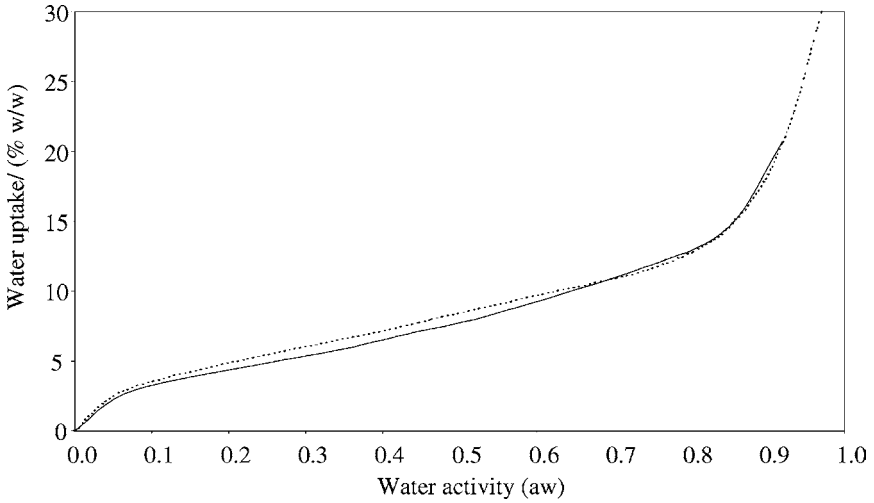

Fig. 2. Water uptake isotherm of sunflower oil cake (dotted line) and protein isolate (continuous line) at 25 °C.

hydrophobic environment, polar groups are localized in the heart of the globular structure. The non-covalent interactions which maintain it are thus essentially hydrogenous and ionic interactions [10] reacting to the presence of water. The structure should be thus stabilized in an anhydrous environment. The decrease of the enthalpy could then be caused by the pressure increase in the pans (Table 2), or by a bigger extent of exothermic coagulation with the temperature increase. The protein coagulation following their denaturation [24], already responsible for the phenomenon irreversibility [18] and maybe for the decrease in enthalpy while the heating rate increases (Table 3), can be observed through scanning electron microscopy (SEM) observations of oil cake samples.

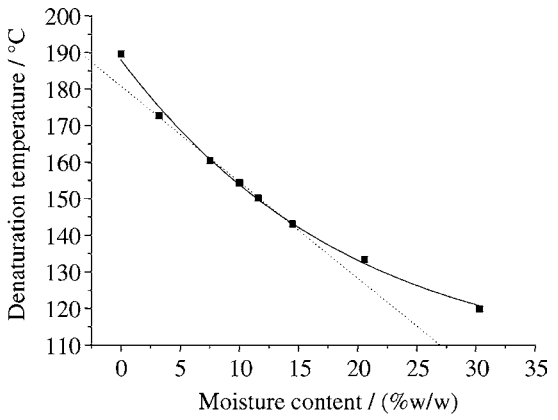

Fig. 3. Rounded shape of the plot of denaturation temperature of the sunflower globulins according to their water content.

First of all, the native protein corpuscles appear clearly before the thermal treatment (Fig. 4a). Their spherical shape is characteristic of an important number of non-polar residues on its surface [23] and their diameter varies from 2 to 6 μm. Following denaturation, some corpuscles are still visible but seem opened or emptied (Fig. 4b) and aggregates of sizes up to 10–15 μm appear (Fig. 4c). After the rupture of the interactions between polar residues inside the corpuscles leading to a molten state (molten globule state) in which the protein's secondary native structure is maintained, the thermal treatment induces rapidly the separation of existing disulphide bonds and/or activation of free sulfhydryl groups which can later form new intermolecular disulphide bonds [24]. This complex phenomenon can lead to other association/dissociation reactions between proteins [25], as well as to the formation of either soluble or insoluble complexes between proteins and phenolic residues [26]. The creation of these new bonds, covalent or not, leads to the formation of aggregates. Their surface is composed of the polar groups which were previously hidden inside the corpuscles. The physical and chemical properties

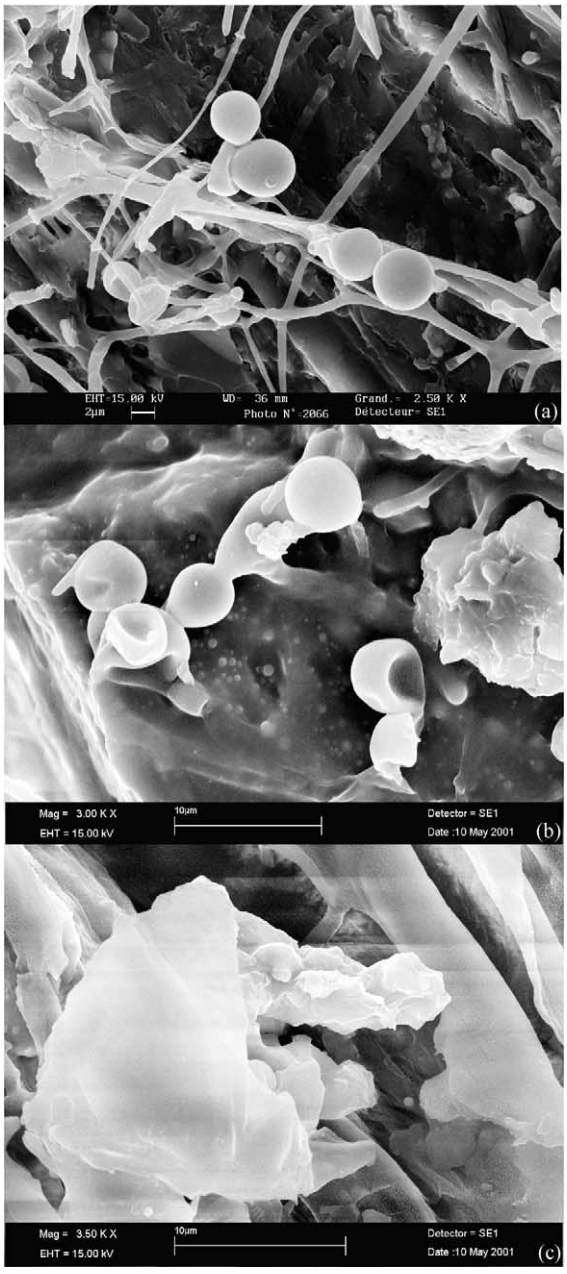

Fig. 4. SEM micrographs of the sunflower oil cake samples: (a) proteic corpuscles before the heat treatment; (b) after the endothermic event; and (c) formed aggregates.

of the proteins are thus completely modified, especially their solubility [27], in a way which can be advantageous in the manufacture of agro-materials [13].

5. Conclusions

The use of pressure resisting hermetic capsules makes it possible to show by DSC the denaturation of sunflower native globulins in low humidity conditions (hydration rate under 30%). The phenomenon is actually rather similar to a “fusion” but is irreversible, because of the following coagulation, which is observed through a scanning electron microscope. The moisture dependence of the denaturation temperature is of capital importance for the utilization of sunflower oil cake as a material base, set up by injection [28,13] or by thermal molding [14], and would maybe be modeled in the future.

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