Alternative Proteins – Thermal Characterization

Dr. Ligia de Souza, Life Sciences Division

What Are Alternative Proteins?

Plant-based proteins have been present in the human diet for centuries. Edible seeds, such as beans, lentils, peas, and their products, as well as oilseeds including pumpkin and sunflower seeds, are examples of traditional protein sources [1]. Plant-based protein is not the full picture in the alternative protein market, though: algae, microorganisms, cultured meat, and insects are also considered to be protein sources. However, becoming a new product in the market is a long process. In addition to having suitable functional and organoleptic properties, any substitute to animal-based protein needs to be efficiently produced, so as to make processing and formulation feasible [2].

The increased use of alternative protein is being pushed by three main forces: 1) sustainability, given the environmental impact of the livestock; 2) interest in adopting healthier diets in order to avoid chronic diseases; and 3) concerns about animal welfare. The concept of alternative proteins is therefore intrinsically related to sustainability and the environmental impact of production. Additionally, respect for the cultural and social behavior of each population around the world has to be taken into consideration when establishing this concept [2].

What Are Proteins?

Proteins are responsible for several different functions inside a living cell, including transport, structure, metabolic, and immunologic activities. They are macromolecular structures built from the combination of 21 different α-amino acids. The regular repetition of the amino acids sequence will make these long chains turn over on themselves, forming the secondary structure of the proteins. The spatial arrangement of the secondary structures will favor their folding into tertiary (tridimensional) structures, which can then interact in a protein complex, forming the quaternary structures. The functional activity of proteins is dependent on their tridimensional conformation. However, this complex and fragile structure can be damaged by mechanical, chemical or thermal stress. Any conformational change in the protein structure is called denaturation. Depending on how the protein is processed, denaturation can be complete and irreversible.

Extracting the protein from its natural source and purifying it involves different mechanical, thermal and chemical processes that can destroy the protein structure. The protein state, i.e., native or denatured, will influence its functional properties, such as solubility, emulsification and ability to form solid structures such as gels and fibers and, consequently, its application in the food industry as a functional ingredient [3].

Thermal Characterization of Proteins

Dynamic Scanning Calorimetry (DSC) has been applied to investigate the thermodynamic properties of food components, including enthalpy and heat capacity changes, glass transitions and melting temperatures, and the thermal stability of proteins, carbohydrates, and lipids [4, 5]. Focusing on proteins, the application of classical calorimetry provided valuable information regarding the influence of concentration, pH, and ionic strength on the enthalpy of the protein denaturation. Complementary thermogravimetric analysis (TGA) can be applied to investigate the water (moisture) content, the thermal stability, or the decomposition temperature, as well as the mineral concentration by determining the ash content [6, 7].

In this study, DSC was used to characterize the denaturation temperature of a plant-based protein from sunflower seeds. Helianthus annuus L. is the cultivated sunflower species. The dehulled seed is composed of between 47% and 65% lipids, and between 20% and 40% protein, being primarily used as a source of edible oil. Depending on the oil extraction conditions, the remaining solid material, called sunflower meal, will have only denatured protein without any application besides the fortification of food products or animal feed. The product analyzed here is claimed to have been mildly processed and has a protein content of 60%, according to specifications given by the producer. It is intended to be used as an alternative to animal protein in bakery products and emulsion preparations [6]. The protein

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Wittelsbacherstraße 42 · 95100 Selb · Germany
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was dispersed in distilled water at a final concentration of 15% (w/v)*. A sample mass of 25 mg of dispersion, containing 3.75 mg protein, was analyzed in a closed cold-weldable Al crucible that can withstand a slight overpressure that occurs during the measurement (also called "low-pressure crucible"). The heating rate was 5 K/min and nitrogen was chosen as the atmosphere. The water content and thermal stability of this protein was determined using TGA. 10 mg of samples were analyzed in open aluminum oxide crucibles under a nitrogen gas atmosphere. The test parameters are summarized in table 1.

Measurement Results

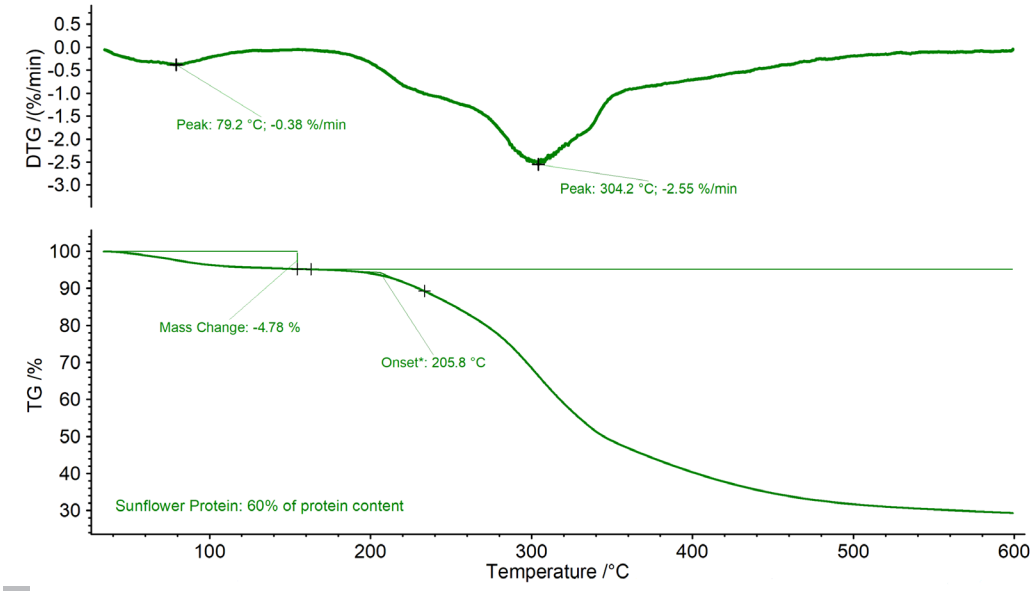
Figure 1 shows the thermogravimetric measurement. The DTG curve of the sunflower protein extract exhibits an initial mass-loss step of about 5% below 100°C. The onset of thermal degradation was detected at 206°C. Typically for plant protein, the moisture content of the dried isolates varies from 1.5% to 7.6%, depending on the source of the protein [7]. The presence of water can be confirmed via evolved gas analysis, e.g., FT-IR. In addition, FT-IR analysis of the evolved gases can also identify typical substances released due to the thermal decomposition of proteins and amino acids, such as H_2O, CO_2, NH_3 (ammonia), H_2S (hydrogen sulfide), and cyclic compounds rich in amide, carboxylic acid, and primary and secondary amine bonds [9].

Denaturation of a protein is an endothermic effect resulting from the exposure of the hydrophobic groups to the aqueous medium. Therefore, a heat absorption peak is often observed in the DSC curve, and its maximum is referred to in literature as the melting/transition temperature (T_m). Depending on the characteristics of the protein and the conditions of the medium, the thermal denaturation can be reversible or irreversible [10]. The reversibility of the denaturation can be seen via the second heating of a DSC analysis; if the second heating curve is similar to the first, this indicates that the denaturation undergone by the protein was reversible.

*weight per volume

Table 1 Measurement conditions

Method	Protein Mass	Crucible	Heating Rate	Atmosphere
TGA	10 mg	Aluminum oxide (Al_2O_3), open	5 K/min	N_2 (20 ml/min)
DSC	3.75 mg	Aluminum (Al), low pressure	5 K/min	N_2 (20 ml/min)



1 TGA curve of sunflower protein (bottom curve) and its first derivative DTG (upper curve). Sample mass: 9.9 mg; crucibles: Al_2O_3 open; heating rate: 5 K/min; atmosphere: N_2 at 20 ml/min.

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The DSC analysis of sunflower protein shows that its denaturation occurs in the range of 91°C to 102°C, with T_m at 98.9°C (green curve in figure 2). The denaturation process is not reversible, as can be seen in the second heating curve (purple), which does not show any endothermic effect. The temperature range of the denaturation is in accordance with the literature value of 99.7°C [11].

Conclusion

In this study, a plant-based protein intended as an alternative to animal protein for vegan food formulations was thermally characterized. Thermogravimetric analysis was employed to determine the water content of the dried sunflower protein extract and assess its thermal stability. Differential scanning calorimetry was utilized to examine the transition temperature and detect any native protein in the sample. The DSC profile indicated that the processing conditions were mild enough to preserve the protein, making it suitable for use as a functional food ingredient. The combination of DSC and TGA proved effective in evaluating the efficiency of the extraction process and the extracted protein's potential for industrial use. These techniques also help characterize food components and predict the shelf life of individual ingredients and formulations.

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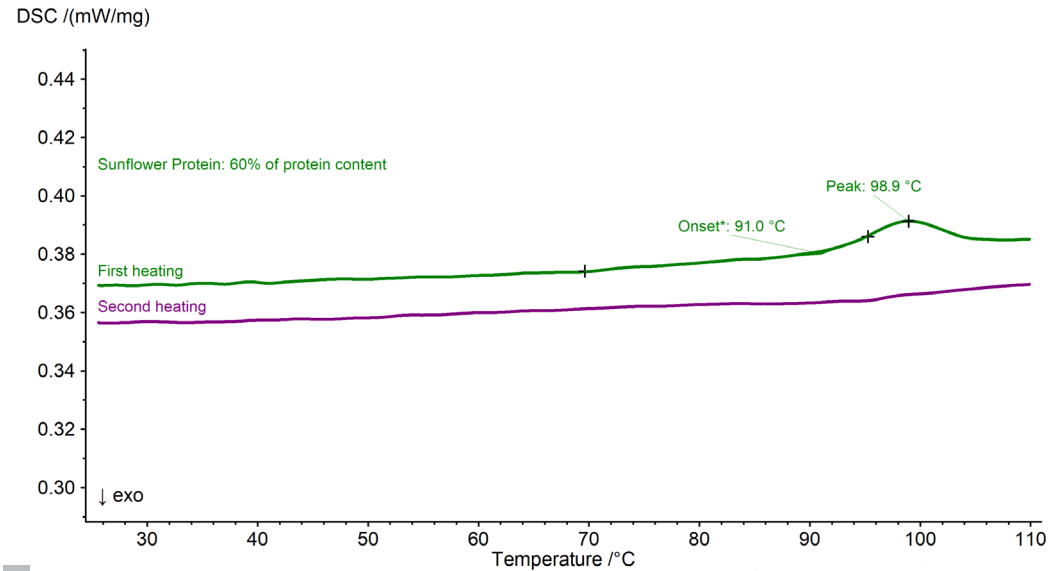

2 DSC curve of sunflower protein. First heating (green curve) and second heating (purple curve). Sample mass: 23.4 mg (3.5 mg protein); crucibles: low-pressure aluminum; heating rate: 5 K/min; atmosphere: N_2 at 20 ml/min.

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