



Thermal analysis of Muga silk (*Antheraea assama*) Pupae protein

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Abstract

The Chemical behaviour of the pupae protein (*Antheraea assama*) were carried out by differential Scanning Calorimetry (DSC) and by Thermo Gravimetry (TG), Differential Thermo Gravimetry (DTG) and Differential Thermal Analysis (DTA). With the typical DSC thermogram of pupae protein of *A assama*, the glass transition (T_g) temperature, and heat capacity were determined. TG, DTG and DTA studies of pupae protein showed the decomposition temperature and exothermic peak temperature. In this study it was found that thermal decomposition of protein had taken place in three main degradation steps referred as initiation, propagation and carbonization in which weight changes in protein samples were recorded as a reaction temperature that is — deformation, degradation and decomposition.

Keywords : DSC, TG, DTG, DTA, initiation, propagation, carbonization, deformation, degradation, decomposition.

1. Introduction

Protein contains many amino-acids in various sequences and serves as useful model compounds to undergo decomposition mechanisms.

The silk fibrin and other silk protein were studied for the thermal degradation by using Thermo Gravimetric analysis and observed the impact of β -sheet, glass transition, crystallization and thermal stability (Lutz and Schreuer, 2009). The peak temperature of TG, DTG, DTA thermogram were studied for protein and found decomposition due to loss of water and carbon-di-oxide. In protein weight losses occur due to loss of water, which occurs in individual amino acid units by loss of one hydrogen atom from amide and hydroxyl group from carboxylic group. The amino acid which do not contain two carboxylic groups the weight loss was associated with

removal of carbon-di-oxide molecules (Lorant, 1965). Lorant (1965) also observed the temperature of large peaks on DTG curves for proteins like-egg albumin (290°C), casein (282°C), Human skin (302°C), Insulin (314°C) Trypsin (250°C).

Eri silk and Bombyx mori silk decomposition studied by (Rajkhowa *et al.*, 2009). They found decomposition started of lower temperature about 30°C and completely decomposed at 360°C. Eri silk under went three thermal decomposition stages approximately at 350°C to 400°C.

DSC thermogram of eri silk shows peaks below 100°C get distributed due to the dehydration. Eri silk protein showed multiple endothermic peak at approximately 310°C, 380°C and 450°C. In Bombyx mori silk protein two strong peak occurred at 310°C and 340°C.

Nagura *et al.*, (1985) reported that the sheets in the β -form crystal of the tussah silk fibroin

obtained by casting at 250°C and the temperature of the endothermic peak at 220°C.

2. Objective

This study is carried out to characterize muga silk pupae protein for thermal behaviour as it is widely used in bio-technological and bio-medical application due to its unique properties like non-toxicity, bio-compatibility and bio-degradability. It is most valuable material for uses such as textiles, bio-medical devices and used in various field like cosmetics, food additive, medical material, food powder, gel, fillers, ink, enzyme immobilizer, drug etc.

3. Materials

Silk worm pupae of *A assama* were collected from reeling centres. Samples were washed with de-ionized water and sun dried. The dried samples were ground to a fine powder in wiley mill to pass through a 90 mesh BS sieve.

3.1 Chemicals

All chemicals used were of analytical grade.

3.2 Methods

3.2.1 Extraction of protein

Fat free pupae powder were treated with 1.5% NaOH at 50-70°C for 2.5 hrs., filtered to obtain pupae powder solution. Isoelectric point was adjusted at pH 4.3-4.6, precipitated and then centrifuged. This precipitate is a protein sample used for the study.

3.2.2 Thermal Analysis

- Differential Scanning calorimetry (DSC) : DSC was done with a Perkin Elmer DSC-7 with kinetic software.
- Thermogravimetry (TG) : Differential Thermo Gravimetry (DTG) and Differential Thermal Analysis (DTA) were carried out by using Shimadzu Analyzer 30 in Nitrogen Atmosphere.

4. Results

Differential Scanning Calorimetry (DSC): The typical DSC thermogram of protein sample isolated from the pupae powder of *A assama* has been recorded from room temperature to 800°C and were presented in Table-I and Fig.-1.

Table-1 : Thermal analysis data of decomposition temperatures and Tg values of protein of *A assama* at heating rate 10°C min⁻¹.

Sample	Decomposition temperature °C	Tg °C	Exo-peak °C
Pupea protein (<i>A assama</i>)	67.49	232	338.86

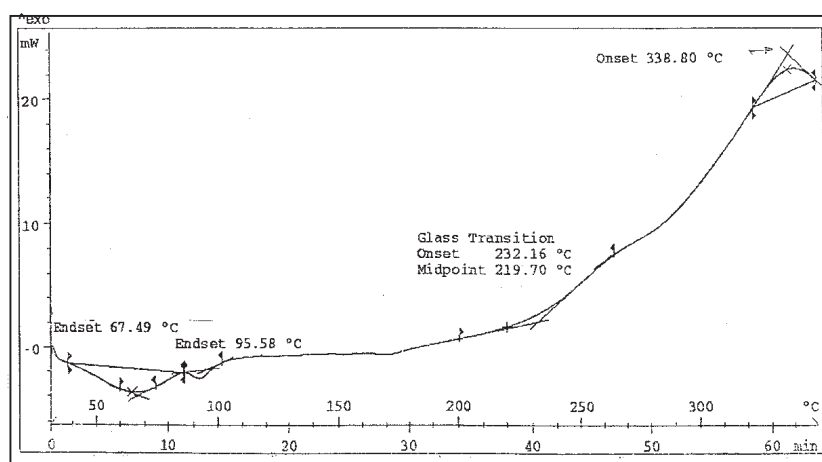


Fig. 1 : Thermal analysis of decomposition temperatures and Tg values of protein of *A assama* at heating rate 10°C min⁻¹.

The thermogram showed an exothermic peak at 67.49°C indicating the removal of absorbed moisture which was completed at 95.58°C. As the temperature increases, the protein showed noticeable thermal stability until 219.90°C. The glass transition temperature recorded at 232.16°C and the sample formed a prominent exothermic peak at 338.80°C. This exothermic peak might be attributed to the thermal decomposition of the

pupae protein with conformation (Freddi *et al.*, 1994).

Thermogravimetric (TG), Differential thermogravimetry (DTG) and differential thermal analysis (DTA).

The TG, DTG and DTA curves were presented in the Fig. 2. The decomposition temperature and exothermic peak temperature recorded in Table-2.

Table 2 : Thermal Analysis data, active deformation temperature and weight loss for protein.

Sample	Weight loss %			Active decomposition temperature °C		
	I ₀ C	II ₀ C	III ₀ C	60	122	328
Protein	5.0 (30-90)	18.7 (90-170)	40.9 (170-540)	(EXO)	(EXO)	(EXO)

I, II, III = (Pre, Second and third stages and the values in parentheses indicate temperature range in °C.

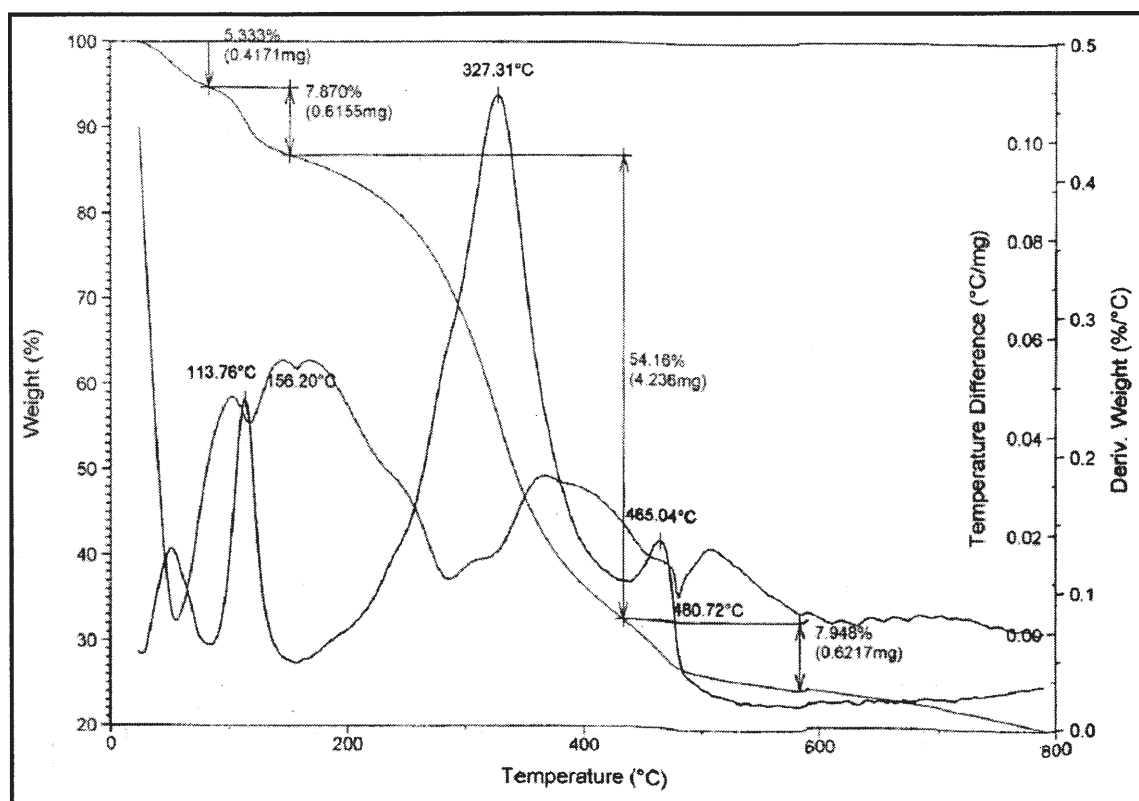


Fig. 2 : Thermal Analysis of active deformation temperature and weight loss for protein.

In this study the decomposition of proteins had taken place in three main degradation steps referred as initiation, propagation and carbonization in which the weight changes in protein sample were recorded as a function of temperature i.e., deformation, degradation and decomposition.

The TG curve for the pupae protein showed an initial small mass loss around 20^o-90^oC which was attributed to the removal of absorbed moisture.

In the second stage, a major weight loss took place in the temperature range 90-170^oC. The weight losses of the sample were found as 5%, 18.7% and 49.9% in the three stages as shown in the Table-2.

The DTG curve in Fig. 2 illustrated more clearly the differences in thermal decomposition behaviour of the sample. The DTG curve of the pupae protein had three peaks. The first peak was at around 51.67^oC, the second at 122.88^oC and the third peak at 326.89^oC. The peak at lower and higher temperature were used to measure thermal stability.

In Fig. 2 The DTA curve for protein showed Endothermic peaks associated with the decomposition of protein were observed at 60^oC, 140^oC, 286.02^oC, 468.47^oC and at 744.08^oC.

5. Discussion

Freddi *et al.*, (1994) studied the DSC curve of *A assama* silk Fibroin from room temperature to 400^oC. The first broad endothermic peak they found was below 100^oC and it was due to evaporation of water. As the temperature increased, the fiber showed noticeable thermal stability until above 200^oC. Two minor and broad endothermic transitions appeared at 230^o and 300^oC (shoulder form) followed by a prominent endothermic peak at 362^oC attributing to the thermal decomposition of silk fiber with β -conformation. The two minor endothermic peaks occurred above Tg (190-200^oC) and related to the molecular motion of the fibroin chain either in the amorphous and laterally ordered region (Tsukada *et al.*, 1992) or in crystalline region.

The DSC thermogram of the tussah silk fibroin exhibited a major single endothermic peak (355^oC) associated with thermal decomposition of the specimen as well as endothermic peak at (218^oC) and an exothermic peak at 226^oC, when film was immersed in methanol for 5 minutes, the exothermic peak at 226^oC had disappeared (Tsukada, 1986).

Magoshi and Nakamura (1976) had studied the glass transition, crystallization and α : β transition by means of DSC. The endothermic shift due to the glass transition at 162^oC was observed the endothermic peak due to the crystallization occurred at 230^oC.

In the present investigation, the DSC studies for *A assama* pupae protein endothermic peaks were found around 67^oC, 95^oC, 232^oC and might be attributed as transition from random coil to β -form and from α -helix to β -form. The exothermic peak for pupae protein was found at 338^oC, but for Tussah silk, it ranges from 218^oC-238^oC and were assigned to the crystallization of the amorphous region to β -form crystal (Magoshi and Magoshi, 1977).

Das and Saikia (2000) and Das *et al.*, (2001) obtained thermal curve (Tg & DTG) at the heating rate 20^oC min⁻¹ for *A assama* silk fiber. In the study, all the TG Curves showed an initial small mass loss at around 160^oC which could be attributed to the removal of absorbed water. In the second stage a major weight loss took place in the temperature range 150^oC to 390^oC while in the third stage the rest of the decomposition took place in the temperature ranges 390^oC to 640^oC. The weight loss was found to be more for silk fiber; in first stage (12.5%) and second stage (41.8%) then in the pupae protein of present study found in first stage (5%) second stage (18.7%) but in third stage decomposition was found more (48.9%) than in silk fiber (43%). The temperature ranges for pupae protein were (30^oC-90^oC, 90^oC-170^oC and final decomposition 170^oC-540^oC) than silk fiber protein (390^oC-640^oC). Therefore, it was found that silk fiber protein were thermally more stable than the pupae protein of present study.

6. Conclusion

Thermal analysis of protein obtained from pupae powder of *A assama* carried out to establish

the thermal stability of pupae protein. This protein has the potentiality to be used as value added commercial by-product from silk industry.

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