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ORIGINAL ARTICLE

Influence of organic solvents on the structural and thermal characteristics of silk protein from the web of *Orthaga exvinacea* Hampson (*Lepidoptera*: *Pyralidae*)

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Abstract The silk protein from the web of *Orthaga exvinacea* was isolated, purified, and casted into films. This film was treated separately with methanol, acetone, ethyl acetate, and isopropyl alcohol in 50 % concentration for about 30 min. The treated films were thus dried in a desiccator and subjected to FTIR and TG-DTA analysis. The structural studies revealed that the organic solvents induce conformatory changes in the protein film, especially the most sensitive amide I (1650 cm^{-1}) band. This band had shifted to lower wavenumber (1633–1636 cm^{-1}). Furthermore, the conformatory characteristics associated with amide I band also changed from random coil to β-sheet. Generally, β-sheet contributes strength to the protein film. Among the treated films, film treated with acetone showed much thermal stability. Moreover, the film treated with methanol had shown two different temperatures of maximum degradation. It is concluded that in addition to β-sheet content, various other factors such as various processing conditions and structural organization of protein may influence the stability of the films.

Keywords FTIR · *Orthaga exvinacea* · Silk protein · TG-DTA · Silk film · Silken web

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Introduction

Silk, a common textile fiber, is produced by various silkworms. Moths belonging to different species are actively involved in the production of silk. Silk filaments obtained from the cocoons of silk moths consist of two different proteins. The core of the filament is formed by a fibrous protein, fibroin. Fibroin is a structural protein, characteristically rich in glycine, alanine, and serine [1]. Another protein, sericin, forms a sticky layer around the fibroin filament [2, 3]. Silk fibroin is a textile commodity dating back several centuries. Before the invention of synthetic materials, silk sutures were used in surgery. Decades of research on silk fibroin has manifested in applications of this material in various fields, such as biomedical [4], tissue engineering [5], and cosmetics.

The insolubility of fibroin in water is owing to the presence of highly hydrophobic amino acid residues [6]. Different processing methods for silk fibers and proteins have extended its utility to fields other than textiles. It was reported that the dispersion of silk fiber readily occurs in a solvent containing bivalent ions [7, 8]. However, the pure solution of silk fibroin is obtained by dialysis [9], and it is unstable to the fluctuation in temperature and mechanical agitation [10].

Silk fibroin can be processed into various forms such as gels [11], films, fibers [12], powders [13], sponges, and hydrogels [14]. Films of silk fibroin are produced primarily by solution casting methods [8, 15]. The conformational characteristics of the films are influenced by many factors such as the concentration of the solution, the type of solvent, and the drying temperature [16]. The secondary structural characteristics of fibroin can be influenced by low dielectric organic solvents that convert random coils of silk fibroin into a β-sheet conformation [17–20]. It was reported that methanol had improved the mechanical properties of silk fibroin film.

Some of the attractive features such as high mechanical strength, biodegradability, biocompatibility, microbial resistance, thermal stability, and non-toxicity [21] have made the fibroin protein as the most promising biomaterial in the biomedical fields.

Both silk proteins have a vast array of applications in biomedical fields [22]. Nowaday's exploration is also put forth into silks synthesized by many other insects. In this study, the silken web of the mango leaf webber, *Orthaga exvinacea*, a pyralid moth, was used. Silk protein isolated from the web of *O. exvinacea* is rich in the following amino acids: serine, histidine, alanine, and aspartic acid [23]. Furthermore, investigations of this pyralid silk are necessary for its use in different fields, and this research is aimed at investigating the effects of organic solvents on the silk fibroin films that were prepared from the web of *O. exvinacea*. The structural and thermal properties of the treated films were studied using FTIR and TG-DTA techniques.

Experimental

Experimental animal

Silken webs of *O. exvinacea* were collected the culture of this insects reared in the laboratory at a temperature and relative humidity of about 27 ± 2 °C and 70–80 %, respectively.

Degumming, dissolution, purification, and casting of the silk protein extracted from the web of the mango leaf webber *O. exvinacea* was done according to the method described by Sajitha and Gokuldas (2014) [23].

Treatment of the film prepared from the web protein with organic solvents

The films were treated separately using organic solvents, methanol, isopropyl alcohol, acetone, and ethyl acetate. These organic solvents were diluted with suitable solvents to 50 % (*v*/*v*) viz., methanol and isopropyl alcohol with water, acetone with methanol, and ethyl acetate with hexane. Films of area 10 cm^2 were transferred separately into Petri dishes containing the organic solvents and immersed in it for 30 min. After treatment, the solvents were drained off and films were dried in a desiccator containing silica gel for further analysis.

The structural and thermal characterization of films treated with organic solvents

The structural studies of the films were conducted using FTIR spectroscopy (JASCO-4100). The spectra were taken at room temperature and the wavenumber ranges between 4000 and 400 cm^{-1}. The transmittance infrared spectra were recorded at a resolution of 4 cm^{-1} with 32 scans per sample. Whereas thermal properties of the protein films (1.5 mg) were studied by heating the films non-isothermally using Perkin Elmer STA-6000 from 40 to 500 °C by increasing the temperature of 10 °C for every min. The whole process was done under inert atmosphere, which was furnished by nitrogen (200 ml/min.)

Results

Structural and thermal characterization of films of web protein treated with organic solvents

FTIR structural characterization

The FTIR spectroscopic method has been employed to elucidate the structural characteristics of protein films treated with different organic solvents. The spectra (Fig. 1) of protein films revealed the extent of influence of organic solvents on its structure. The analysis focused mainly on the shift in frequency of amide I and II bands in films treated with organic solvents.

The spectrum of film treated with isopropyl alcohol (Fig. 1) showed intense absorption band at 1633 cm^{-1}, which is the amide I band. The amide II band of the film came at a frequency of 1530 cm^{-1}. The amide III band of the film was seen at 1243 cm^{-1}. The absorption band at 629 cm^{-1} represented amide IV region. The amide A and B bands were also seen in the spectrum, which were at 3277 and 3080 cm^{-1}, respectively. Besides the amide bands, a few other absorption bands have also been noticed in this film. They were at 2965,

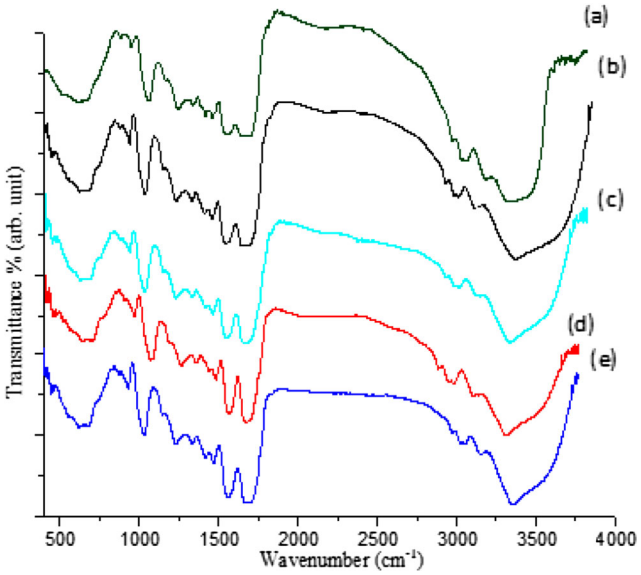

Fig. 1 FTIR Spectra of the web protein films of *O. exvinacea* treated with different organic solvents **a** untreated film, **b** isopropyl alcohol, **c** acetone, **d** ethyl acetate, and **e** methanol

2937, 2138, 2879, 1452, 1402, 1334, 1170, 1064, 963, and 899 cm^{-1}.

In web protein film treated with ethyl acetate (Fig. 1), the amide I band appeared at 1636 cm^{-1}, which is slightly higher in wavenumber than that of film treated with isopropyl alcohol. The spectrum of methanol-treated film exhibited broad amide I band at 1635 cm^{-1} (Fig. 1). Anyhow, the frequency of amide I band of both acetone and isopropyl alcohol-treated films were same. The rest of the amide bands in all films were almost similar. Moreover, all films possess additional bands also, which have lesser significance in the case of protein film. Compared to the spectrum of the untreated film [23], the films treated with organic solvents had shown different frequencies in amide I region.

In order to know the thermal stability of protein films treated with organic solvents, the treated films (methanol, isopropyl alcohol, and acetone) were subjected to TG-DTA analysis. The TG-DTA curves of the proteins (Figs. 2 and 3) gave detailed information about the thermal degradation by change from room temperature to 500 °C.

The isopropyl alcohol-treated film (Fig. 2) showed 8 % of weight loss at 100 °C. In between 100 and 200 °C, a weight loss of about 11 % was noticed. However, a remarkable weight loss of the film has been observed in the range of 250 to 350 °C. About 43 % of weight loss occurred in the above temperature range and showed the maximum rate of thermal degradation at 301.08 °C (Fig. 3). At 500 °C, 99 % of the film was charred. The residual weight of the protein at the above temperature was found to be around 1 % (Fig. 2).

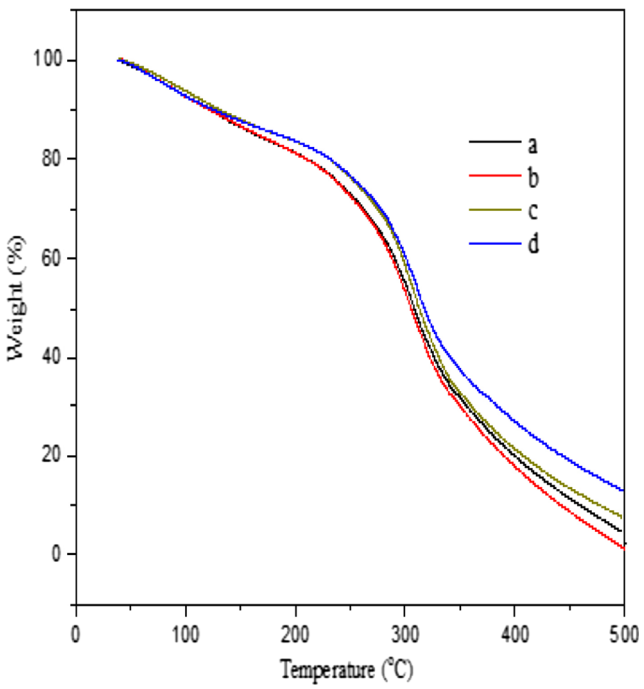

Fig. 2 Thermogravimetric curves of *O. exvinacea* web protein films treated with different organic solvents **a** untreated film, **b** isopropyl alcohol, **c** methanol, and **d** acetone

The methanol-treated film (Fig. 2) also showed a similar thermal behavior. However, the DTG curve displayed maximum rate of thermal decomposition of the protein at two different temperatures, i.e., one at 300.41 °C and the other at 306.86 °C (Fig. 3). About 7 % of the film remained unaffected at 500 °C.

Like other treated films, acetone-treated film (Fig. 2) also exhibited a loss of weight at 100 °C. But, the temperature in the range of 250 to 350 °C, the film had undergone a loss of about 39 %. The film shows that rate of maximum thermal degradation of the protein took place at a temperature of 307.85 °C (Fig. 3). TG of the film had recorded almost 12 % of residual weight at 500 °C.

On a comparison of the changes taking place between the untreated and solvent-treated films, it was seen that the organic solvent-treated films and pure film [23] showed almost same percentage of weight loss at 100 °C. Nevertheless, the maximum loss of weight of all treated films occurred in range of 300 to 350 °C, and methanol-treated film had shown maximum weight loss in the above temperature range than other protein films. Another peculiar characteristic shown by methanol-treated film was that the maximum rate of thermal degradation of this film occurred at two different temperatures (Fig. 3), whereas in the other films, the above degradation occurred only at one temperature. It was observed that the methanol and acetone-treated films lost half of their weights at 311 °C. Among all the protein films, the most important feature noticed was that the acetone-treated film showed a high residual weight of 12 % at 500 °C (Fig. 2), whereas the others had a residual weight lower than 10 % of the weight of the films.

Discussions

The most important component which decides the structure of proteins are amide I and II, which appears in the range of 1700–1600 cm^{-1} and 1575–1480 cm^{-1}, respectively [24–28]. The amide I arise due to C=O stretching and N-H bending vibration in the protein back bone [29]. According to Dong et al. [30, 31], the amide I band appearing at 1650 ± 2 cm^{-1} is attributed to the random coil conformation of protein. In the non-treated film of web protein of *O. exvinacea*, amide I band contribute random coil conformation [23]. After the treatment of the films with organic solvents, the amide I band had shifted to a lower wavenumber, and this contributes β-sheet conformation [30, 31] to the protein.

Prasong et al. [32] carried out studies on the effect of organic solvents such as ethyl acetate, methanol, ethanol, and acetone on the silk fibroin film of *B. mori*. The outcome of the experiment was different from the result obtained for the protein film of *O. exvinacea*. An interesting thing noted was that although acetone was able to induce conformational transition

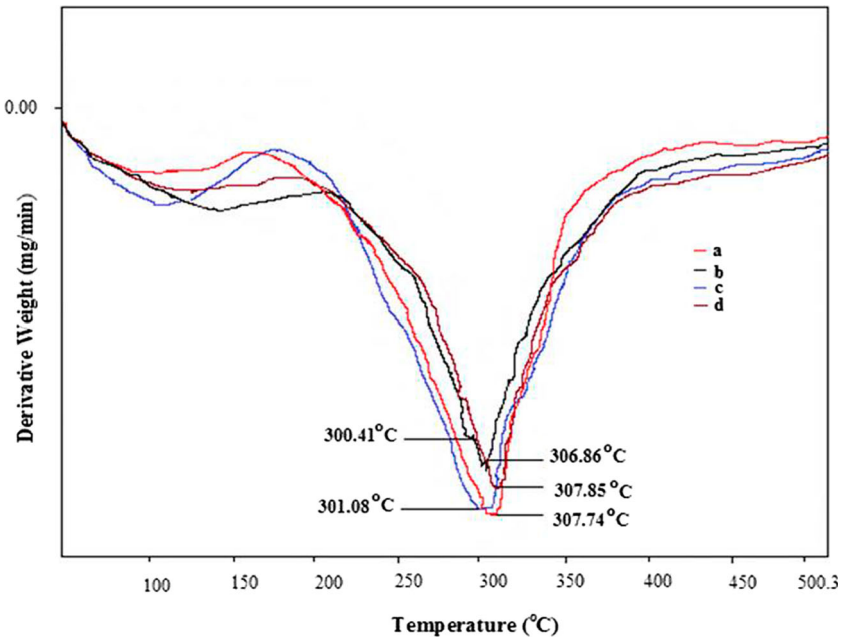

Fig. 3 DTG curves of films of web protein treated with organic solvents **a** untreated web protein, **b** methanol, **c** isopropyl alcohol, and **d** acetone

in protein film of *O. exvinacea*, the same was incapable of inducing any structural characteristics in protein films of *B. mori*. This was evident from its FTIR spectra, where there was a shift in amide I band from higher to lower wavenumber. In *O. exvinacea*, the thermal characteristics of protein film changed on treating with organic solvents, and comparable events have been reported in *B. mori* [32] also, where the organic solvents except acetone, increased the thermal stability of silk fibroin film. This result thus strongly suggests that β-sheet structure in the protein film is the primary cause of its thermal stability.

A change in the structural characteristics under the influence of organic solvents was noticed in the FTIR spectra of protein film of *O. exvinacea*. It had resemblance with structural changes made out in the silk fibroin film of tussah silk fibroin treated with methanol [33]. In this case, the transition from α-helix to β-sheet structure took place. In *O. exvinacea*, the organic solvents altered the conformation contributed by amide I only. In the case of tussah silk fibroin film, on the other hand, methanol influenced both amide I and III bands [33].

In *O. exvinacea*, methanol had changed the conformation of protein from random coil to β-sheet, thereby increasing the mechanical strength of the film. A similar event was reported in *A. yamamai*, where ethanol changed both random coil and α-helical structure of silk fibroin film to β-sheet [34]. It may be inferred that the stability and strength of film is improved due to the increase in the content of β-sheet in the protein.

In *O. exvinacea*, methanol had affected the amide I band of the protein and shifted the absorption band from 1650 to 1635 cm^{-1} (Fig. 1). It has been reported that, methanol brought a structural transition in amide II band of eri silk fibroin, where the already existing α-helical structure changed to β-sheet [35]. However, the change in the thermal characteristics after treatment with methanol found in the case of *O. exvinacea* had not been reported in eri silk film [35]. The result thus suggests that different silks respond differently to organic solvents. It may or may not alter the thermal stability of silk protein. Furthermore, the stability of protein film may be affected by a number of factors such as the overall structural characteristics of a protein, composition of protein, and processing conditions.

From the aforesaid, it becomes clear that the response of the silk protein films prepared from the web of the mango leaf webber, *O. exvinacea*, a pyralid moth, to various organic solvents is different from that of other silk films. Extensive research works have been done on the rearing of mulberry and non-mulberry silk moths, the production of silk, and the properties of these silks for application in textile industry as well as in biomedical applications. The extent of use of silk in other fields such as cosmetics and tissue engineering is increasing day by day. In this context, further research on this silk produced by a pyralid moth that could be reared easily and inexpensively is absolutely essential for testing its suitability for applications in various fields.

Conclusions

From FTIR spectral studies, it was concluded that treatment with organic solvents induced the formation of β-sheet in the protein films. But the DTG studies showed that the thermal stability of the treated films were comparatively lower. It may be concluded that a number of factors such as processing

conditions, change in overall structural characteristics after treating with organic solvents, reorganization of protein, and composition of protein. influences the stability of the film. Sometimes the proportion of β-sheet contributed by the amide I band may be much lower, and in such conditions, a change in conformation may not have a prominent effect on its thermal stability.

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