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Structural and Thermal Investigations of Biomimetically Grown Casein–Soy Hybrid Protein Fibers

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Abstract A hybrid protein fiber from different protein sources such as casein and soybean using wet-spinning technique was prepared. The casein/soybean hybrid fibers were synthesized at different weight ratios such as 100/0 (casein), 75/25, 50/50, 25/75, and 0/100 (soy) and characterized. Electron microscopic analysis confirmed the growth of pure and hybrid fibers and shows an increased surface roughness as the soy concentration increases in the hybrid fibers. Infrared spectra did not exhibit any significant changes in the functional groups between pure and hybrid fibers. X-ray diffraction pattern indicates slight increase in the diffraction peak values of hybrid fibers compared with the neat fibers. Thermal analyses show a moderate increase in the thermal stability of hybrid fibers when compared with the pure fibers. These results implicitly indicate that the casein and soy proteins are homogeneous in the hybrid fiber form. It has been demonstrated that the hybrid fiber with ≥50 wt.% casein content exhibits better morphology and increased thermal stability, which has scope for application in technical and medical industries.

Keywords Fiber · Spinning · Thermal stability · Structure · Composite · Electron microscopy

Introduction

Many of the living world's structural materials are based on self-assembled fibers. Biomaterials such as skin, bone, or tendon become attractive models for new materials

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since they have undergone a long process of evolutionary optimization [1]. Biosynthesis is not only able to control the sequence, composition, and conformation of protein fibers, but is unmatched in its capacity to make complex composite structures through the seamless integration of protein synthesis, mineralization and self-assembly [2]. Biopolymers used for production of fibers can fall under two general classes namely plant-based carbohydrate polymers such as cellulose and animal-based protein/peptide polymers such as collagen, wool, or silk [1]. Protein fibers are fundamental building blocks of life playing an essential role in motility, elasticity, scaffolding, stabilization, and the protection of cells, tissues, and organisms. Despite nearly a century of research into the assembly mechanisms and structures of fibrous proteins, only limited information is still available [3]. Progress is being made to employ protein fibers as performance molecules in a range of technical applications in filter, membrane, paper, textile, and leather fabrication as well as medical applications.

It is necessary to assemble proteins under controlled conditions for defined man-made processes in order to utilize protein fibers in medical and technical applications. Considerable efforts have therefore been made to investigate fiber assembly in vitro. Different approaches such as self-assembly [4, 5], peptide engineering [6, 7], wet-spinning [8, 9], and electrospinning [10, 11] of proteins have been investigated to build up protein fibers in a prescribed manner so as to produce significant quantities. The properties of fibers made according to these processes vary widely, depending partly on the composition of the protein itself and partly on the conditions of treatment. The manufacture of protein fibers is still a largely empirical operation.

The mechanism by which the fiber formed from a protein source such as silk may be applied for making fibers from other protein sources too. Wet-spinning is a highly complex technique that aims to mimic the natural process of spider silk production [3]. Studies of natural examples offer a number of potentially useful lessons on the materials chemistry and materials engineering of fiber production. Proteins with very different properties such as wool or silk are derived from animals. The unmatched toughness of spider silk has made this protein of particular interest and research has been carried out with the aim of producing high-performance fibers that can be employed in several technical and industrial applications [3]. Although the properties of silk are superior to those of wool or cotton, its spread has been restricted due to the limited availability and high prices. If other proteins could be “regenerated” into a fibrous form using the principles of nature, then protein fibers could be produced that would have similar or improved properties, enabling them to compete with wool and silk. Casein fiber extruded from milk protein has the feel of silk [12]. Conversely, protein fiber extruded from soybean milk has wool feel [13]. Although several studies exist on the development of blend casein or soy protein fibers with synthetic polymers [14–18], the feasibility of developing a hybrid protein fiber from different protein sources such as casein and soybean was examined for the first time using wet-spinning technique. The basis for producing such fibers is the fact that the proposed hybrid protein fiber would have properties of both silk and wool, which will have enormous applications. A major application would be in the field of textiles where currently fabrics containing both wool and silk feel and properties are not available. It is also envisaged that the proposed hybrid fibers will be applicable in the field of biomedical engineering for preparing biomaterials such as sutures, scaffolds, etc. The developed hybrid protein fibers have been analyzed for structural and thermal characteristics.

Experimental

Materials

Casein soluble in alkali was obtained from Loba Chemie Pvt. Ltd., India. Soybean flour, Type 1, not roasted, ~52% protein (85+% dispersible) and 1% fat was obtained from Sigma, USA. All the other chemicals used were of analytical grade.

Extraction of Protein from Soybean Flour

An acid leach extraction was performed to extract the protein from soy flour [19]. In 25 ml of water, hydrochloric acid was continuously added to form a 0.276% HCl solution. To this, 3.74 g of soy flour was added. The resulting soy water mixture has a pH of about 4.4. The mixture was then placed in a decanting centrifuge with 5,000 rpm for 15 min. The wet cake yielded a soy protein concentrate, which was dried at room temperature and powdered.

Preparation of Protein Solution

A 20% protein solution was prepared by adding 5 g of the protein powder into an aqueous solvent composed of 15 g urea, 0.5 g sodium sulfite, and 25 ml distilled water at room temperature. The pH was adjusted to 11.5 by the addition of sodium hydroxide stock solution. The casein and soybean protein solutions were prepared by the above method separately and mixed at various ratios for fiber extraction. The casein/soybean (C/S) protein weight ratios for solution spinning were 100/0 (casein), 75/25 (C/S (75/25 wt.%)), 50/50 (C/S (50/50 wt.%)), 25/75 (C/S (25/75 wt.%)), and 0/100 (soy).

Fiber Spinning

Wet-spinning process was employed to synthesize the casein/soy hybrid fibers mimicking the spider silk production [3]. The fiber spinning line, as represented in Fig. 1, included the spinning apparatus, coagulation, cross-linking, and washing bath. Spinning was carried out using a conventional syringe with a needle having 0.5±0.02 mm inner diameter and 0.9± 0.03 mm outer diameter. Various compositions of casein/soy protein solutions were filled in the syringe and dispersed through the needle into the coagulation bath for fiber spinning. The coagulation bath contained 1 M sulphuric acid saturated with sodium sulphate. The bath was maintained at 50 °C. The coagulated fibers were kept into a cross-linking bath, which contained 8% formaldehyde in water, for 3 h at room temperature. Then the cross-linked fibers were washed and dried.

Analysis of Structural Properties

The samples were mounted on aluminum stubs using double-sided adhesive tapes and coated with gold in Edwards E-306 sputter coater, UK. The micrographs for the grain surface and cross-section were obtained by operating the FEI Quanta 200 series scanning electron microscope (SEM), USA at high vacuum with an accelerating voltage of 12 kV in different lower and higher magnification levels. The wide angle X-ray diffraction analysis

Fig. 1 Process flow diagram showing the fiber spinning line

of the developed fibers was carried out at room temperature. The samples were tested using Rigaku Miniplex powder wide angle X-ray diffractometer, Japan. The scanning 2θ range was from 5° to 50°. Fourier transformed infrared (FT-IR) spectra of the developed fibers were recorded using a Perkin Elmer Spectrum RX-I FT-IR system, USA. The samples were ground with KBr and FT-IR spectra were recorded over a wide wavelength or wave number as a function of percent transmittance for the developed fibers in this study.

Analysis of Thermal Properties

The developed fiber samples were tested using a differential scanning calorimeter (DSC) Q200 analyzer, TA Instruments, USA. The samples were heated up to 400 °C in nitrogen atmosphere at a rate of 10 °C/min. The nitrogen flow was maintained at a level of 50 ml/min. The samples were also tested using Netzsch STA 409 C/CD thermo gravimetric analyzer (TGA), Germany. The samples were heated up to 1,200 °C in nitrogen atmosphere at a rate of 10 °C/min. The nitrogen flow was maintained at a level of 80 ml/min.

Results and Discussion

Scanning Electron Microscopic Analysis

The morphology of the developed fibers were investigated using scanning electron microscope at various magnifications. Lower magnification micrographs (×250) of the protein fibers derived from casein, soy and their hybrids are shown in Fig. 2. All the fibers exhibit diameter in the range of 100 to 250 μm. It is seen that pure casein fiber displays circular and cylindrical shape with very smooth surface (Fig. 2a). On the other hand, soy fiber shows an irregular and rough surface (Fig. 2b). It is clearly seen that the roughness

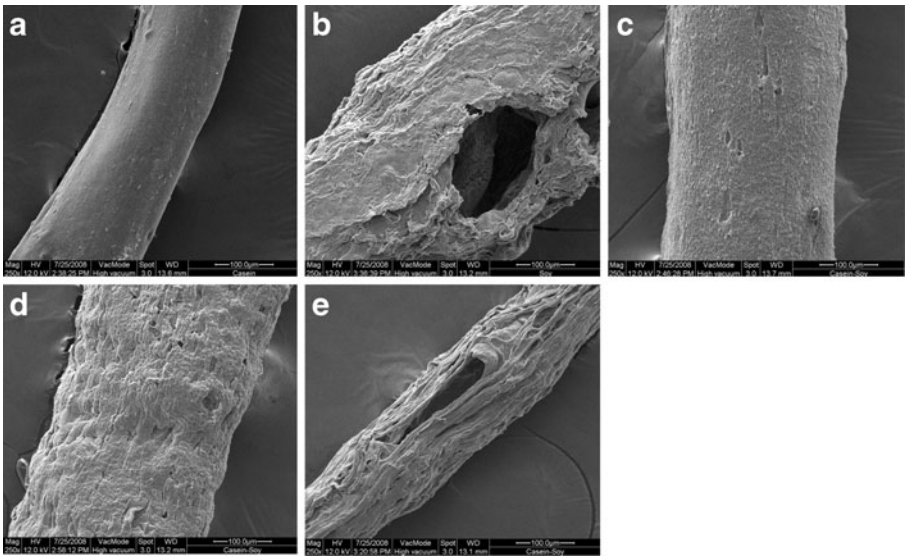

Fig. 2 Scanning electron micrographs of the protein fibers and their hybrids at a magnification of ×250; **a** casein; **b** soy; **c** C/S (75/25 wt.%); **d** C/S (50/50 wt.%); **e** C/S (25/75 wt.%)

increases as the soy concentration increases in the blended fibers as inferred from Fig. 2c–e. However, Fig. 2c displays fiber with fairly smoother surface among all the blended fibers, which may be due to the presence of higher casein content in the hybrid fiber. The soy fibers tend to have a hollow core as clearly seen from Fig. 2e. Such hollow fiber is also observed in Fig. 2d, which may be due to the presence of higher soy content in the fiber spinning solution. It is generally known that the shape of the solution-spun fibers relates to the coagulation rate; a higher rate results in non-circular forms [20, 21]. Therefore, the hollowness and non-circularity of soy fibers implies that its coagulation rate is faster than that of casein.

Higher magnification micrographs of the protein fibers derived from casein, soy, and their hybrids are shown in Fig. 3. Sectional view of pure casein fiber shows adjoined foldings of several layers of casein macromolecules (Fig. 3a), which may be due to the choice of spinning technique and the diameter of the needle. This observation is in agreement with the earlier report [18]. When casein is blended with soy at a ratio of 75/25 wt.%, the folding of casein layers is reduced. On the other hand, an individual fibril protruding from the casein/soy blend fiber (50/50 wt.%) is seen in Fig. 3d. When the casein content is reduced to 25 wt.%, several longitudinal fibrils fused together to form a single fiber as evident from Fig. 3e. Nevertheless, pure soy fiber exhibits fibrils in sheathe form fused together to form a fiber with coarse surface, as seen in Fig. 3b. These results are in agreement with the lower magnification results and support the reasoning. Hence, scanning electron micrographs of the blended fibers indirectly suggest that casein and soy proteins are compatible in the selected weight ratios and solution spinning conditions employed in this study.

Wide Angle X-ray Diffraction

Wide angle X-ray diffraction spectra of casein, soy and their hybrid fibers are shown in Fig. 4 after curve smoothening. The original spectra did not show any noticeable diffraction

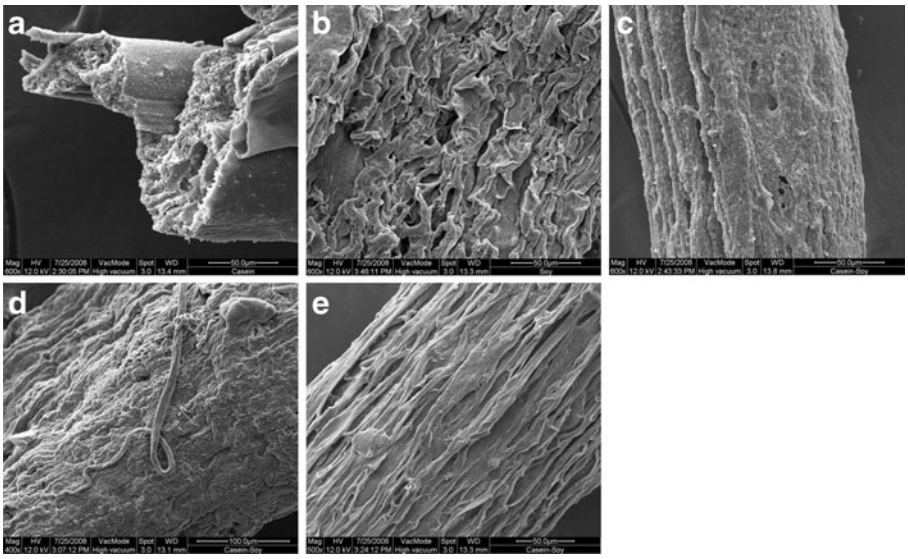

Fig. 3 Scanning electron micrographs of the protein fibers and their hybrids at higher magnifications; **a** casein; **b** soy; **c** C/S (75/25 wt.%); **d** C/S (50/50 wt.%); **e)**C/S (25/75 wt.%)

peaks in all the fibers. This may be due to the fact that all the samples are milk protein fibers, which are complex in nature and cannot be crystallized easily. Besides, the large side chain groups hinder the fitting of milk protein molecules into crystal lattices. Hence, the curves were smoothened and peaks were obtained using Origin software. The broad diffraction peak of the pure casein and soy fibers is observed at 22.2° and 22.5°, respectively. This indicates that there is no significant difference between the structure of the both the milk protein fibers. The diffraction peak of casein fiber is in agreement with the earlier reports [18, 22]. When the pure protein fibers are blended, there seems to be little shift in the diffraction peaks owing to the fact that both constituents are milk proteins. The diffraction peaks of the C/S fiber blends such as 75/25, 50/50, and 25/75 wt.% are 25.2°, 23.24°, and 23.88°, respectively. It is seen that there is slight increase in the diffraction peak values of blend fibers compared with the pure casein and soy fiber. The shift in the diffraction peak indicates the possible interaction between casein and soy proteins and implicitly shows that the hybrid fibers are homogeneous. It is envisaged that soy protein could have packed in the amorphous regions of casein when the casein composition is high (C/S 75/25 wt.%) thereby resulting in positive shift in diffraction peak. However, such packing is inferior when the casein composition is decreasing in the hybrid fiber leading to small shift in diffraction peak. This is in agreement with the scanning electron microscopic analysis.

Fourier Transform Infrared Spectroscopy

FT-IR spectra of the casein, soy and select hybrid fibers are shown in Fig. 5. Characteristic bands found in the infrared spectra of proteins and polypeptides include the Amide I and Amide II. These arise from the amide bonds that link the amino acids. The absorption associated with the Amide I band (ranging from 1,600 to 1,700 cm^{-1}) leads to stretching vibrations of the C=O bond of the amide while the absorption associated with the Amide II

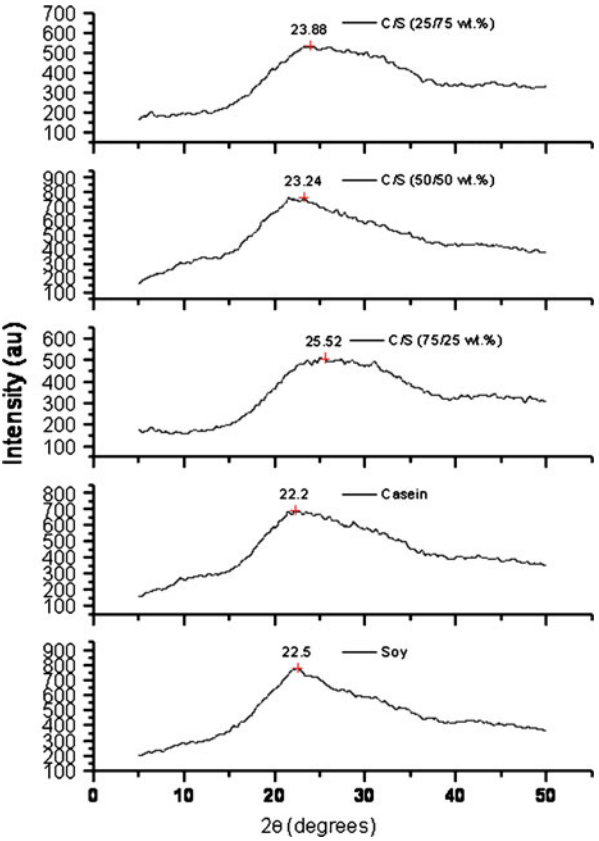

Fig. 4 Wide angle X-ray diffraction spectra of the protein fibers and their hybrids after curve smoothening

band (ranging from 1,520 to 1,550 cm^{-1}) leads primarily to bending vibrations of the N–H bond. The Amide III band is usually weak but can be found in the range of 1,200 to 1,350 cm^{-1} interval, mainly associated with the C–N stretching and in-plane N–H deformation modes of the peptide group [23, 24]. It is seen from Fig. 5 that neat casein and soy fibers show characteristic absorption peaks associated with proteins. Peaks at 1,650 cm^{-1} referred to C=O stretching vibration of Amide I band coupled to the in-plane N–H bending and C–N starching modes, 1,540 cm^{-1} due to the N–H bending vibration of Amide II band and 1,050 cm^{-1} on account of C–N stretching vibration. Broad peak at 3,300 cm^{-1} may be due to the O–H or N–H stretching vibration. These assignments are in agreement with those reported earlier for casein and soy protein materials [16, 22, 25]. The FT-IR spectra of hybrid protein fibers are relatively similar compared to that of neat casein and soy fibers. This indirectly indicates that the selected proteins are miscible in the experimental condition and produce hybrid fibers.

Differential Scanning Calorimetric Analysis

The thermal properties of the developed hybrid protein fibers were investigated using DSC along with pure protein fibers. The DSC curves of the casein, soy and their hybrid fibers are shown in Fig. 6. It is seen that all the fibers exhibit peaks in the region of 80 to 100 °C,

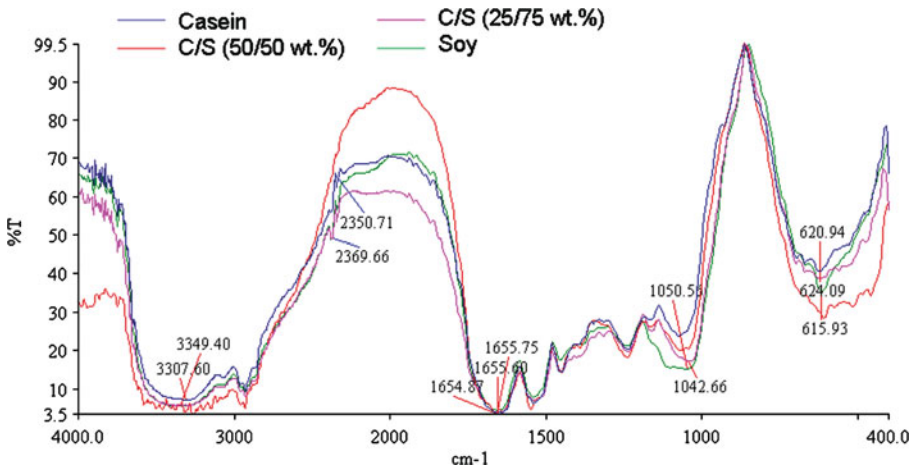

Fig. 5 FT-IR spectra of the casein, soy, and select hybrid protein fibers

which are endothermic in nature. This may be due to the evaporation of moisture present in the fibers. In addition, it is seen that several overlapping melting and decomposition peaks are found between 220 and 350 °C for all the fibers. This is in agreement with the previous reports [15, 22]. It is observed that the melting endotherm for pure casein and soy fiber is around 285 °C. It is interesting to note that the hybrid fibers with 75 wt.% protein component have almost similar melting isotherm to that of corresponding pure protein counterparts. On the other hand, C/S hybrid (50/50 wt.%) fiber exhibits a significant

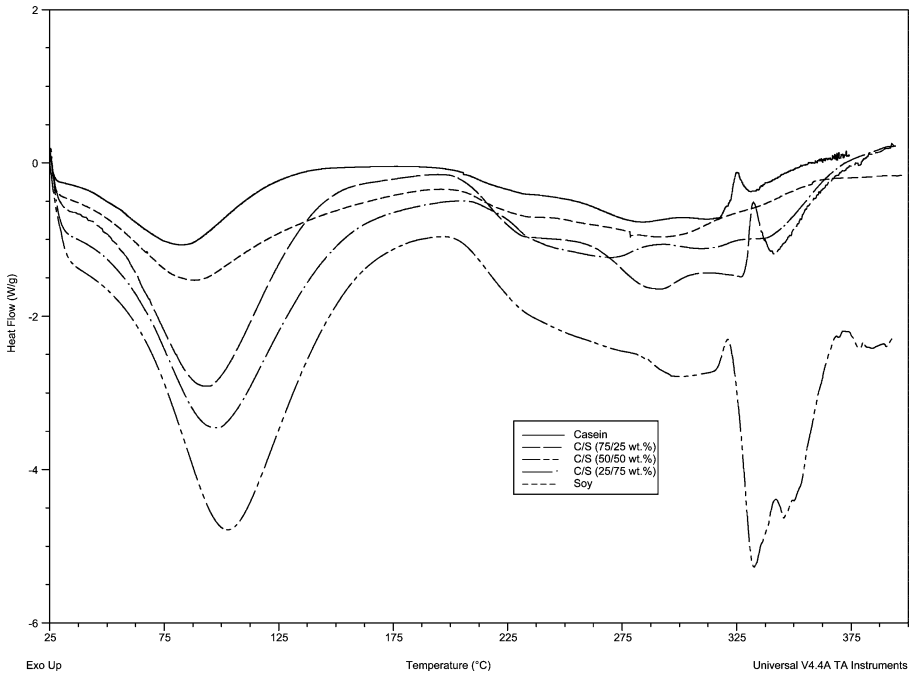

Fig. 6 DSC curves of the protein fibers and their hybrids

endothermic peak around 330 °C, which indicates the initiation of decomposition. This suggests possible weak interactions between the casein and soy proteins leading to homogeneous mixing during the preparation of hybrid fibers. In general, all the protein fibers developed in this study did not exhibit any significant exothermic peaks, indicating their low degree of crystallinity.

Thermogravimetric Analysis

Thermogravimetric analysis of pure and hybrid protein fibers are shown in Fig. 7. The TGA curves of all the fibers indicate two-stage weight loss. The first stage, ranging from room temperature to 100 °C, corresponds to loss of residual and absorbed moisture with a weight loss about 10%. The second stage starts at 200 °C and continues up to 500 °C. This may be due to the decomposition of protein macromolecular structure. The weight loss of pure casein fiber is 57%, on the other hand, it is only 46% for pure soy fiber [26]. The composite fibers show slightly higher percentage weight loss compared to that of pure casein fiber when there is more than or equal to 50 wt.% casein content. On the other hand, when the soy composition is 75 wt.%, the weight loss in the corresponding hybrid fiber is slightly reduced to 55%. Similar trends were obtained for inflection points. The inflection points

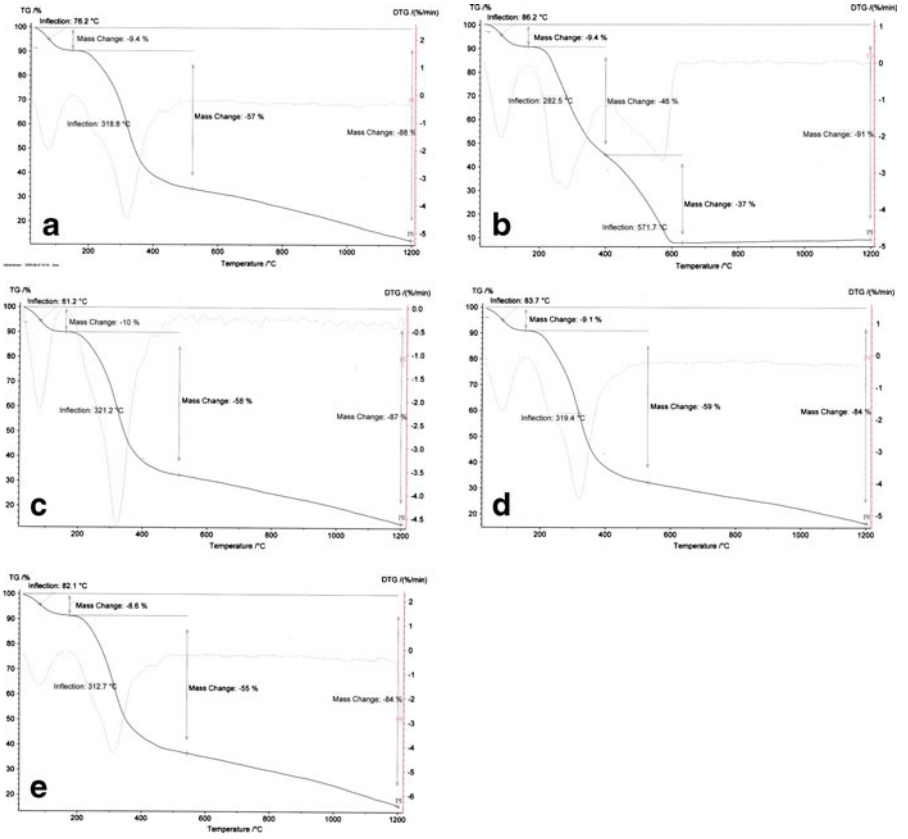

Fig. 7 TGA curves of the protein fibers and their hybrids; **a** casein; **b** soy; (**c**) C/S (75/25 wt.%); **d** C/S (50/50 wt.%); **e** C/S (25/75 wt.%)

during decomposition of pure casein and soy fibers are 319 and 283 °C, respectively [27]. As noted above during weight loss, the hybrid fibers exhibit slightly higher inflection points to that of pure casein fibers when the casein content is ≥50 wt.%. Conversely, when the soy composition is higher, the inflection point of the C/S hybrid (25/75 wt.%) fiber is reduced to 313 °C. This indirectly shows possible weak interactions between casein and soy proteins during the preparation of hybrid fiber. These results are in agreement with DSC results obtained in this study. In general, all the hybrid fibers show similar or improved thermal stability compared with pure casein or soy fibers.

Conclusions

Casein and soy proteins are widely used in the manufacturing of protein fibers. These fibers have been blended individually with various other fibers to enhance their property. Casein fibers have a silky feel while soy has woolly effect. Hence, an attempt has been made to develop a hybrid fiber from casein and soy protein sources and analyze their structural and thermal properties. Scanning electron microscopic analysis reveal that the casein fiber has circular and cylindrical shape with very smooth surface whereas soy fiber exhibits a non-circular and rough surface. The surface roughness of the fibers increases as the soy concentration increases in the hybrid fibers. C/S (75/25 wt.%) fiber showed fairly smoother surface among all the hybrid fibers. Wide angle X-ray diffraction spectra of neat casein and soy fibers did not show any significant difference between their structures. There seems to be slight increase in the diffraction peak values of composite fibers compared to the pure casein and soy fibers. FT-IR spectra of the casein and soy fibers show characteristic absorption peaks associated with proteins. The FT-IR spectra of hybrid protein fibers are relatively similar compared to that of neat casein and soy fibers indicating their homogeneous nature. The DSC curves of the pure casein and soy fibers in comparison to hybrid fibers suggest possible weak interactions between them. This was supported by TGA results in terms of weight loss and inflection point data. This indirectly shows the uniform mixing of casein and soy proteins during the preparation of hybrid fiber. In conclusion, casein/soy hybrid fiber with ≥50 wt.% casein content exhibits better morphology and increased thermal stability, which can be considered for various technical and medical applications. Nevertheless, it should be mentioned that semi-technical level trials need to be carried out in order to ascertain the mechanical and bulk properties of the hybrid fibers before attempting for any applications.

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