Studies on Indian Silk. I. Macrocharacterization and Analysis of Amino Acid Composition

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ABSTRACT: This first in a series of articles characterized the different varieties of Indian silk for their macrostructural parameters, such as filament length, degumming loss, denier, cross section, moisture regain, and intrinsic viscosity, for example. The results of amino acid analysis using a reverse-phase technique were also reported. Five Indian silk varieties—two mulberry (bivoltine and crossbreed) and three nonmulberry (tasar, muga, and eri)—were investigated. The differences existing between the different varieties and the extent of lengthwise variations within a cocoon in the dimensional and macrostructural parameters were discussed. It was observed that denier of the filament decreases considerably from the outer to the inner layers, whereas density showed an increasing trend in all the varieties. Both the mulberry silks demonstrated lower moisture regain. Electron micrographs of all the nonmulberry varieties showed microvoids in their cross section. Fraction studies showed the development of mushroom structure on the tips. In both types of mulberry silk, glycine, alanine, and serine constitute about 82% of the amino acids present. On the other hand, in nonmulberry silks, these constitute about 73% with a high proportion of alanine. The nonmulberry varieties showed a substantial proportion of amino acids with bulky side groups. Similarly, the higher hydrophilic to hydrophobic amino acid ratio (9.06–9.85) for nonmulberry silks, compared against that of the mulberry varieties (5.29–6.22), was shown to be responsible for the higher moisture content of nonmulberry silks. Cystine and methionine were present in all the varieties. The higher intrinsic viscosity of nonmulberry varieties suggested their higher molecular weight. Through amino acid analysis, it was shown that there is no difference in chemical architecture between the outer and the inner layers of cocoons. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 1080–1097, 2004

Key words: silk; macrostructure; amino acid; denier; cross section

INTRODUCTION

Humans have had a historic and cherished association with silk. The lustre, handle, and draping qualities of silk fibers are definitely superior to those of other textile fibers.[1] Because of this, efforts are ongoing to manufacture silklike fibers. Silk is an animal fiber secreted as a continuous filament by the silkworm, and consists essentially of the fibrous protein, fibroin, and in the raw state is coated with the gummy protein sericin. Fibroin and sericin constitute up to 95% of the raw fiber. The remaining part consists of other proteins, waxes, fats, salts, and ash.[2] It is the only natural fiber available in continuous filament form.

India produces all four varieties of silk: *Bombyx mori* (mulberry), *Antheraea mylitta/Antheraea prolei* (tasar), *Antheraea assama* (muga), and *Phylisomia ricini* (eri). The silk worm species and the rearing and reeling conditions affect the quality of cocoons and the fiber. These factors may result in noticeable morphological and structural changes among different varieties and along the filament length within a variety.

Investigations conducted on different varieties of silk fibers in the last few years have revealed remarkable differences in their structure and properties. Iizuka et al.[3–5] demonstrated that properties such as tenacity and dynamic modulus increased as the denier of the filament decreased, whereas the elongation decreased as the denier decreased. In a study on the chemical composition of silk, Lucas et al.[6] showed that the amino acid composition of mulberry and nonmulberry silk varieties is different and this influenced the physical properties.

In the early 1950s, Lucas et al.[7] used acid hydrolysis followed by ion-exchange chromatography to separate and identify the amino acids. These workers characterized the amino acid composition of both sericin and fibroin of *Bombyx mori* and *Antheraea* silks. They also determined the amino acid sequence. In later studies, Lucas et al.[8,9] determined the composition and the sequence of amino acids in the amorphous fractions of the *Bombyx mori* fibroin.

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TABLE I
Specifications of Cocoons

Type of silk	Voltinism	Color	Shape	Average cocoon weight[a] (g)
Mulberry (bivoltine)	Bivoltine	White	Oval	0.64
Mulberry (crossbreed)	Multivoltine	Yellow	Oval	0.61
Tasar	Bivoltine	Brown	Oval	3.44
Muga	Multivoltine	Golden yellow	Oval	1.05
Eri	Multivoltine	Creamish white	Oval	0.84

[a] Average of 10 cocoons.

The four main varieties of Indian silks—mulberry, tasar, muga, and eri—were previously investigated by Dhavalikar.[10] This study revealed that glycine, alanine, and serine form the major components in mulberry and nonmulberry varieties. The composition of these three amino acids was shown as the highest in eri silk (84.26%) followed by mulberry (82.8%) and tasar (72.06%). Muga was shown to have the least, with 67.77%. In a study on chemical composition and physical properties of *Antheraea assama* silk, Freddi et al.[11] reported a high alanine content of 42.62% and very low arginine content (2.83%), although cystine (0.34%) was present and the other sulfur-containing amino acid, methionine, was not detected in their analysis.

Shaw[12–14] investigated the amino acid composition of the residues obtained by treatment of various silk fibroins with acid, alkali, and hydrogen peroxide by column chromatography, discussed in relation to the structure and the effect of amino acid composition and the residues derived from them on the structure. The above studies also included the analysis of the amino acid composition of Japanese *Bombyx mori* fibroins treated with enzymes such as trypsin using paper chromatography. The results of the above studies indicated that the fibroins of *Bombyx mori* type are characterized by the presence of glycine, alanine, and serine in the crystalline regions. In their work on selective acid hydrolysis of Indian silk, Nadiger et al.[15] showed that in the crystalline regions, the composition of the above three amino acids was about 91% for mulberry, about 81–82% for tasar and muga, and about 77% for eri.

Most of the studies were confined to mulberry and to some extent tasar silks. Information on muga silk is limited and not much work has been reported on eri silk in this respect. It was therefore considered of interest to characterize the different varieties of Indian silk in detail and analyze the differences, if any, more systematically and scientifically. In the present study, the emphasis was on macroproperties and cross-sectional topology. An attempt was also made to analyze the amino acid composition of different silks using reverse-phase HPLC technique.

EXPERIMENTAL

Materials

The raw materials, cocoons of mulberry, tasar, muga, and eri, were chosen for the present study. The general typical specifications are presented in Table I.

Sample preparation

Cooking

Mulberry cocoons were cooked using water at boil for 10 min. Because tasar cocoons have a hard and compact shell, normal cooking procedures cannot be used; hence they were cooked in water containing 10 wt % ethylenediamine at 80°C for 50 min to soften the shell.[16] Muga cocoons were cooked in the same way as mulberry except for the addition of soda ash (5 wt %) in the water bath. Eri cocoons are open-mouthed and were not subjected to cooking treatment to avoid entanglement.

Reeling

The cooked cocoons of mulberry and muga were wet reeled, whereas tasar cocoons were dry reeled on a laboratory wrap reel. Several leas of continuous filaments were collected while reeling with enough care to avoid breaks and entanglements during reeling. Successive leas of 110 m length were cut and numbered sequentially, where the outermost layer was designated as 1. In the case of eri, the filaments were carefully hand-separated layer by layer so as not to damage the fibers.

Evaluation

Average filament length

The filament length (in meters) was measured on a laboratory wrap reel, whereas the individual cocoons were reeled separately. An average of 20 readings were taken and the average filament length was determined.

Degumming loss (%)

The filament leas were degummed with 25% Marseilles soap (w/w) at the boil for 90 min, at a liquor ratio of 1 : 40. Degummed samples were washed thoroughly, dried, and conditioned for 24 h under standard conditions of 27 ± 2°C and 65% relative humidity. The percentage loss in weight was taken as the degumming loss (%).

Linear density (denier)

For mulberry, tasar, and muga, each layer of 110 m was weighed on an electronic balance and its denier was calculated. For eri, a 5-m filament length was collected after degumming and its denier was determined using the following expression:

$$\text{Denier} = \frac{9000W}{L}$$

where L is the length of the filament in meters and W is the weight in grams.

In each variety, 20 readings were taken and their average was calculated.

Scanning electron microscopy (SEM) studies

Longitudinal and cross-sectional shape. The filament cross section and the longitudinal shape of different varieties of silk were observed under a scanning electron microscope. Average cross-sectional dimensions (in μm) were measured.

Fracture studies. For this, in the first case, a twisted bundle of filaments was dipped in liquid nitrogen for 10 s and broken. The broken tip was observed for the presence of fibrillation, the presence of voids, and so forth. In the second case, the filaments were treated with 6*N* HCl for 12 h, washed thoroughly, and conditioned. These filaments were broken after dipping in liquid nitrogen for 10 s and the tip fracture behavior was studied.

Density

The density (in g/cm^3) was measured in the Davenport density gradient column. The column was prepared using mixtures of carbon tetrachloride (density 1.599 g/cm^3) and *n*-heptane (density 0.68 g/cm^3) in different proportions and was calibrated with standard glass floats. The samples were allowed to settle in the column for 24 h. The density of the samples was determined from the calibration curve obtained by measuring the column height of the floats versus the density. The average was taken from five readings.

Moisture regain

The moisture regain of silk fiber samples was determined on a Saratorius moisture analyzer under standard conditions of 25 ± 2°C and 65 ± 2% relative humidity. The average was taken from five readings.

Determination of intrinsic viscosity

The intrinsic viscosity measurement of different varieties of silk was done using a Ubbelohde viscometer (Cannon–Ubbelohde, State College, PA). For this procedure a 0.5% solution of silk in 9*M* lithium bromide solution was prepared. The measurement was carried out in a water bath at a constant temperature of 25°C.

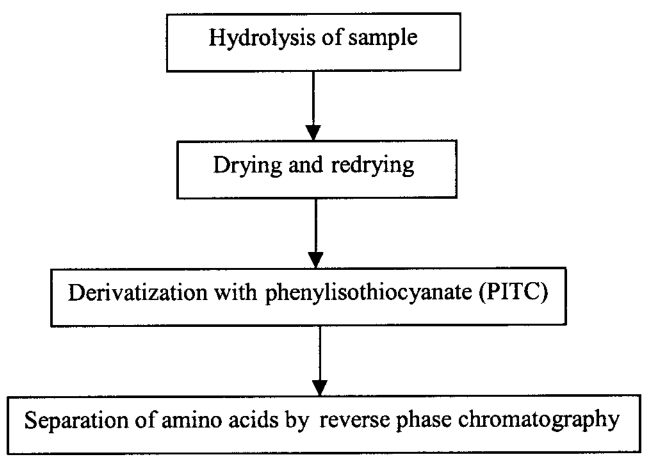
First, the solvent flow time (t_0) for 25 mL volume was measured. Similarly, 25 mL of silk solution was taken in a viscometer and its flow time (t) was measured. The average of five readings was taken for all cases. The solution was then progressively diluted and the flow time was measured at each concentration (g/dL). The intrinsic viscosity [IV = (η_{sp}/C)] was determined by plotting (η_{sp}/C) versus concentration C, and extrapolating the value to zero, where $\eta_{sp} = (t - t_0)/t_0$.

Amino acid analysis using HPLC

Chemicals

The following chemicals were used for amino acid analysis: phenylisothiocyanate (PITC; Sigma, St. Louis, MO), amino acid standard (Pierce H), constant boiling hydrochloric acid (Merck, Darmstadt, Germany), triethylamine (Merck), acetonitrile and methanol (HPLC-grade from Merck), sodium acetate trihydrate (Merck), orthophosphoric acid (Merck), water (HPLC-grade; Merck), and phenol (HPLC-grade; Merck).

The steps involved in the amino acid analysis of silk are represented in the following schematic diagram:



Hydrolysis of silk

The hydrolysis of the silk proteins was carried out on a Waters Pico-Tag WorkStation (Waters Chromatography Division/Millipore, Milford, MA). Weighed samples of silk fiber (10 μg) were taken in 50 × 6-mm sample tubes that were then placed in the reaction vials. The reaction was carried out by constant boiling of 6*N* HCl containing 1% (v/v) phenol vapors under oxygen-free conditions at a temperature of 110°C for 24 h.

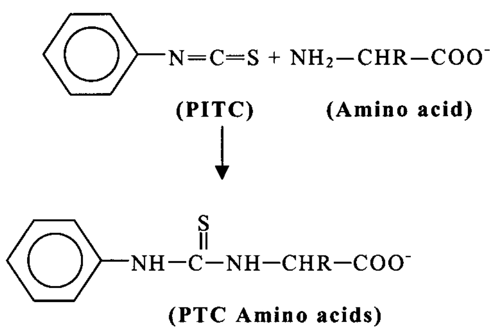
Drying and redrying

The samples were dried in vacuum after hydrolysis for 15 min. The redrying solution made up of 2 : 2 : 1 mixture of ethanol : water : tryethylamine was added to each sample tube and the samples were dried again in vacuum for 60 min.

Derivatization of amino acids with PITC

The derivatization of the hydrolyzed samples was carried out to produce phenylthiocarbamyl (PTC) derivatives of amino acids, which were then analyzed by HPLC. The derivatization reagent was freshly made and consisted of methanol : triethylamine : water : phenylisothiocyanate (PITC) in the ratio of 7 : 1 : 1 : 1. Into each tube, 20 μL of this reagent was added. The reaction of free amino acids with PITC was essentially complete in 20 min at ambient temperature. After the reaction, the derivatized samples were dried under vacuum.

Derivatization with PITC produces phenylthiocarbamyl amino acids as follows:



Separation of amino acids by reverse-phase chromatography

The chromatographic separations were carried out using the Waters Pico-Tag Amino Acid Analysis System containing a Waters Pico-Tag Free Amino acid column. Detection of the PTC amino acids was done at a fixed wavelength of 254 nm. The temperature of the column was maintained at 38°C. The separation of all 18 amino acids was accomplished within 12 min of run time.

The solvent system used consisted of two eluents, designated simply A and B. Eluent A was prepared with the following solvents: water (1 L), sodium acetate trihydrate (19 g/L), triethylamine 0.5 mL/L), and glacial acetic acid (pH 6.4); 940 mL of the above solvent mixture was mixed with 60 mL of acetonitrile. Eluent B was prepared with the following solvents: water (600 mL) and acetonitrile (400 mL).

TABLE II
Average Filament Length of Different Varieties of Silk

Sample	Type of silk	Average filament length (Mts)[a]
1	Mulberry (bivoltine)	1200
2	Mulberry (crossbreed)	700
3	Tasar	700
4	Muga	450
5	Eri	450

[a] Average of 20 cocoons.

RESULTS AND DISCUSSION

Average filament length

The data on average filament length are presented in Table II. It may be observed that, among the mulberry varieties, the bivoltine cocoon yields the highest average length of silk (1200 m) during reeling compared to the mulberry crossbreed variety (700 m). This is expected, given that the bivoltine cocoons have a compact shell, weigh more, and thus contain a greater amount of silk. In addition, it was observed that the waste generated during reeling is minimal because of less filament breakage. The mulberry (crossbreed) cocoons have a weaker shell, weigh less, and have a flossy layer. This results in more wastage in the initial stages of reeling, thus reducing the average length of the filament.

On the other hand, among the nonmulberry varieties, tasar shows the greatest length (700 m). Tasar cocoons have a very hard and compact shell; hence, their degumming was done in a solution containing ethylenediamine. It was observed that more breakage occurred during reeling in the initial stages until the true end of the filament was identified. In muga, the average filament length varied from 400 to 450 m. Because eri cocoons were open mouthed and unsuitable for cooking and reeling, the individual layers were carefully taken out from the cocoons by hand, degummed, and the average length was measured. The length varied from 400 to 450 m.

Degumming loss

Degumming loss represents the amount of sericin present. One expects that the sericin should be present in equal amounts throughout the length; the basic purpose of sericin is to keep the brins together, al-

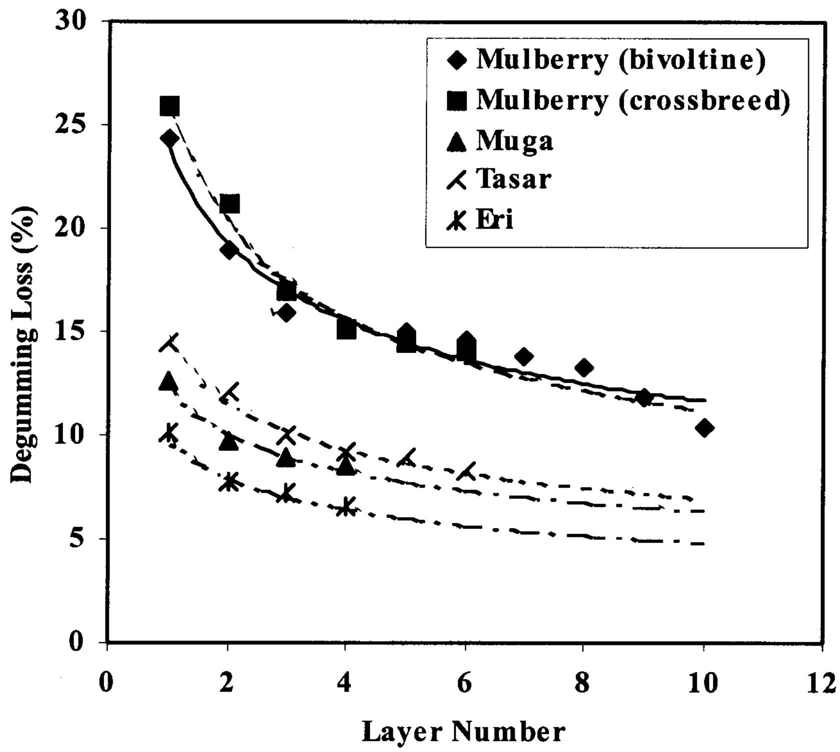


Figure 1 Degumming loss along the layers.

though interestingly it does not. The average degumming loss (%) values decreased to 10.39 from 24.38 for mulberry (bivoltine), to 14.09 from 25.93 for mulberry (crossbreed), to 8.57 from 12.69 for muga, to 8.24 from 14.42 for tasar, and to 6.47 from 10.10 for eri, as one moves to the inner layers from the outer layers (Fig. 1).

The degumming loss, in general, was found to be greater in both the mulberry varieties than in the three nonmulberry varieties of silk. In the case of mulberry, cooking involved a mild boiling treatment for a short duration, which does not remove appreciable quantities of sericin during cooking, most of which comes out during degumming. However, variation in sericin content from layer to layer may be observed, which is a natural sericin distribution along the layers, particularly for the mulberry variety.

Muga and tasar may also have been expected to behave in a similar fashion, although they did not. To facilitate reeling, the cooking involved soda ash and ethylenediamine in the bath; the vigorous nature of the method resulted in most of the sericin being removed during cooking itself, thereby showing a marginal reduction from the outer to the innermost layers. It is interesting to note that in eri there was a low sericin content, which decresed from the outer to the inner layers, despite the fact that it was not even subjected to cooking.

It appears that the silkworm initially delivers a greater amount of sericin to protect itself from unfavorable environmental conditions, by making the outer layer of the cocoon shell tough. The reduction in degumming loss in subsequent layers means a low sericin content in the inner layers. Because the purpose is to keep the brins together and make them adhere to the body of the cocoon, the sericin level does not change all that significantly in subsequent layers. In that eri is open mouthed, the intention may be to allow insects an easy escape; the outer layers are not especially hard, so the overall release of sericin is low. This is also the reason that the eri cocoons are flossy in nature.

Filament denier

The progressive change in the denier of the silk filament within the cocoon is shown in Figure 2. It is evident from the figure that denier of the filament decreases from the outer to the inner layer of cocoons in all cases. The denier decreases to 1.3 from 4.6 for mulberry (bivoltine), to 1.7 from 2.9 for mulberry (crossbreed), to 3.9 from 6.6 for muga, to 4.7 from 10.7 for tasar, and to 2.3 from 3.6 for eri cocoons. This, no doubt, is a substantial reduction within the cocoon. In percentage terms, the decrease is greater for the mulberry (bivoltine) variety than for the other varieties (Table III). This decrease in denier from the outer to the innermost layer of filament, which is a natural phenomena, may be attributed to the gradual decrease in the concentration of aqueous silk solution during

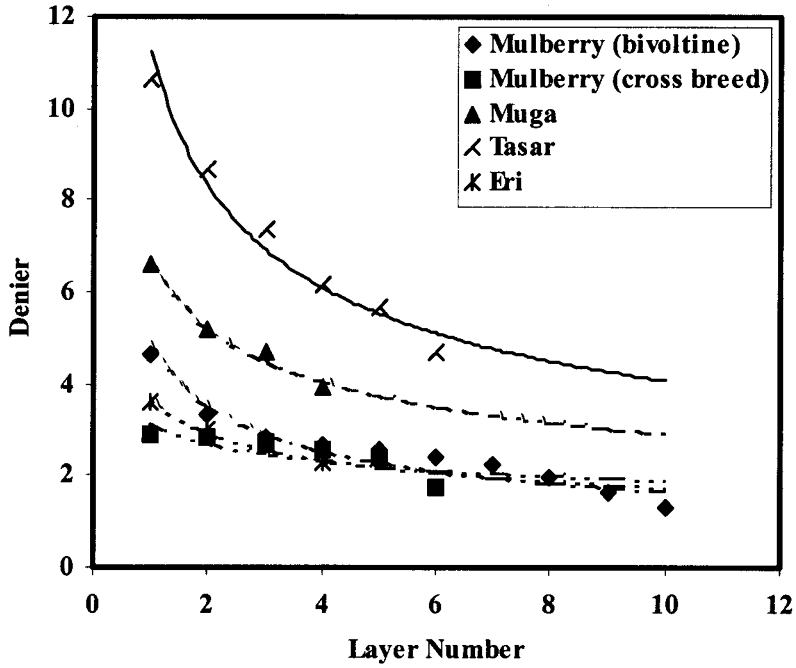


Figure 2 Change of denier along the layers.

the spinning process from an initial polymer solution concentration of about 30 to 15% in the *Bombyx mori* (mulberry) silk worm.[17] A similar phenomenon is also proposed for other nonmulberry varieties. Between the different varieties, mulberry (crossbreed) and eri are the finest and tasar is the coarsest. It is interesting to note that denier reduction in the crossbreed variety is the least. This genetically modified variety seems to be more uniform in this respect. However, it must be emphasized that the lengths of reeled filament in different varieties are different. Probably a better way to look at this change would be in terms of percentage reduction in denier per 100 m of length. The percentage change in denier in all the varieties is more or less comparable when calculated per 100 m length with the value for mulberry (bivoltine) at around 6.5%, for mulberry (crossbreed) at 6.25%, for tasar at 8.4%, for muga at 9.3%, and for eri at 8.2%. In general, one may say that in nonmulberry varieties, the reduction/100 m is higher (Table III).

TABLE III
Percentage Change in Denier along the Layers

Variety	Outer layer	Inner layer	Percentage reduction
Mulberry (bivoltine)	4.6	1.3	71.73
Mulberry (crossbreed)	2.9	1.7	41.37
Tasar	10.7	4.7	56.07
Muga	6.6	3.9	40.90
Eri	3.6	2.3	36.11

Cross section and longitudinal shape

The cross-sectional views of different varieties of silk obtained using a scanning electron microscope are presented in Figures 3 and 4. It may be observed that, in this respect, the mulberry and nonmulberry silks exhibit an altogether different cross-sectional morphology. The mulberry silks show a more or less triangular cross section and a smooth surface. Among the nonmulberry varieties, tasar and muga exhibit an elongated rectangular or a wedge-shape cross section and a large cross-sectional area. The average dimensions are also listed in Table IV. The eri silk has a more or less triangular shape. Tasar, muga, and eri all have striations on their surface compared to the smooth surface of mulberry (Fig. 5). It may be noted that there is a decrease in the cross-sectional areas if one compares the outer and the inner layers: this is true with all the varieties and is a direct result of the progressively deceasing denier along the filament length.

Density

The density values of different varieties of silk are presented in Table V. Both mulberry varieties show higher density values compared to those of nonmulberry silks. To gain more insight, the fiber cross sections were studied under SEM at high magnification. It may be observed that mulberry varieties do not exhibit pores or voids in their cross section and have a compact structure, whereas tasar, muga, and eri silks show the presence of voids (Fig. 6). This could be one

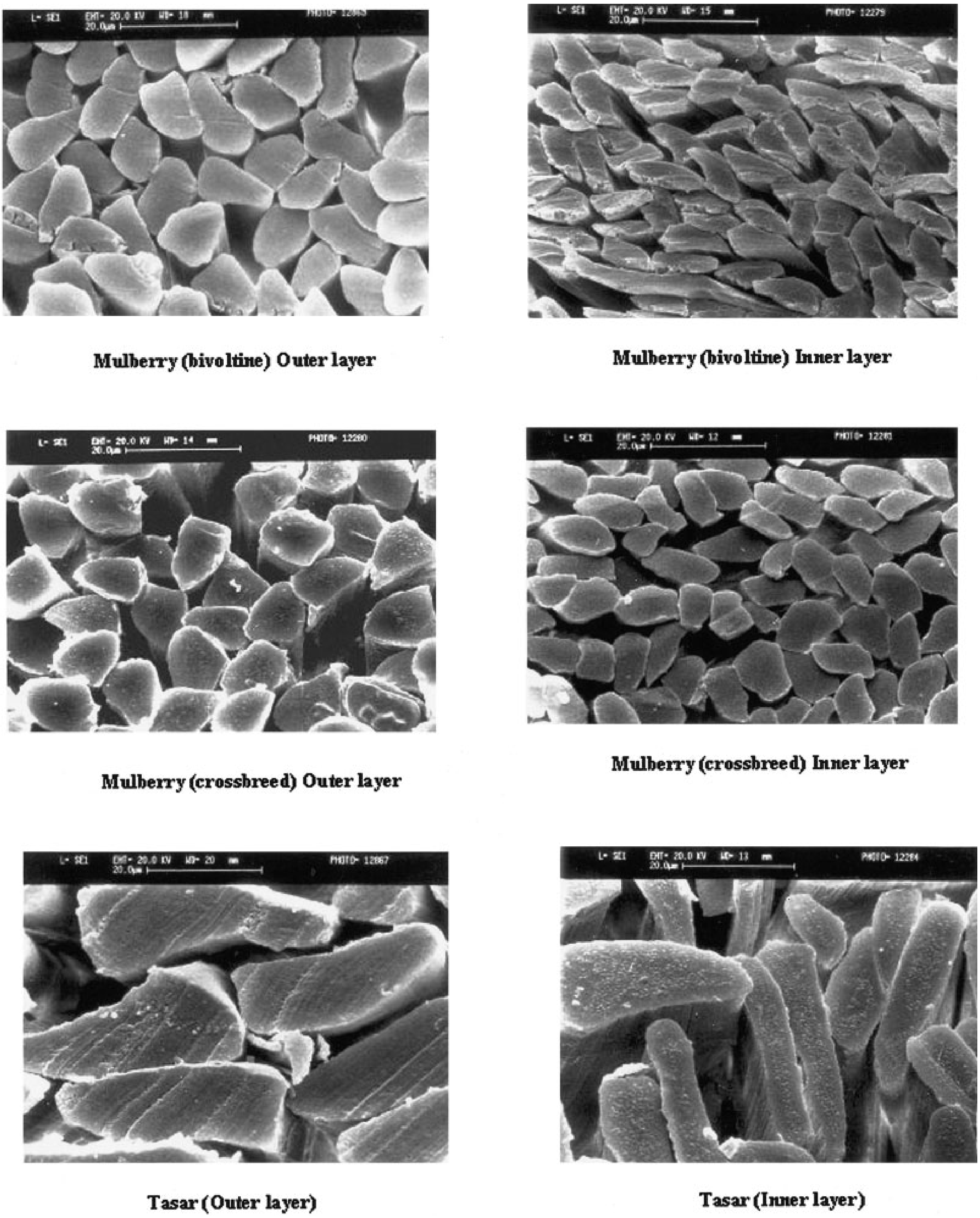


Figure 3 Cross section of silk fibers.

of the reasons for the higher density of mulberry varieties. The higher density values of mulberry silks also indicate a higher degree of order and compact molecular packing compared to those of nonmulberry silks. The mulberry (bivoltine) variety shows higher density than that of the mulberry (crossbreed) variety.

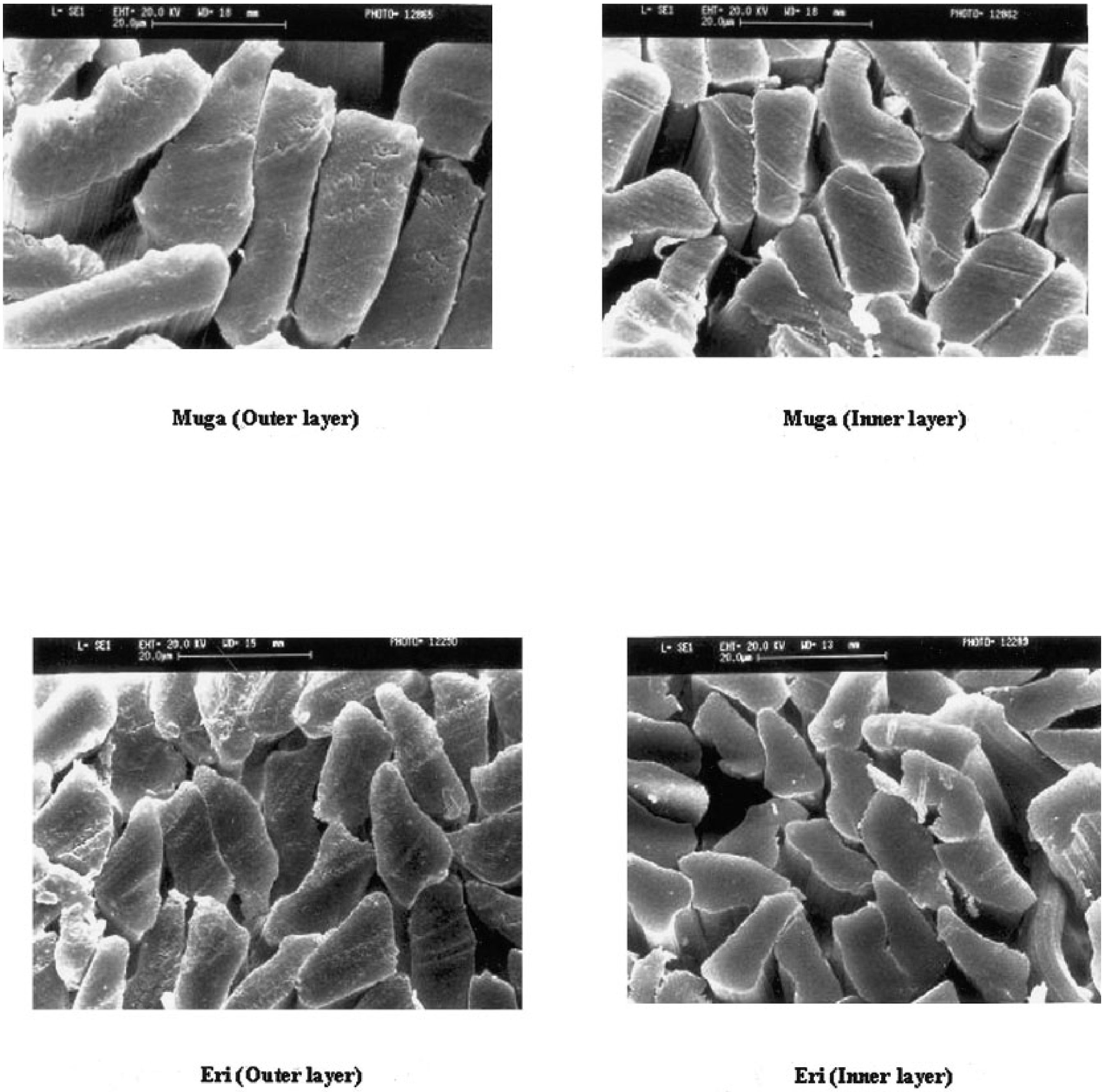


Figure 4 Cross section of silk fibers.

It is interesting to note an increasing trend in density values from the outer to the inner layers for all the varieties, which definitely suggests a possible increase in the degree of crystallinity and crystallite orientation as one moves from the outer to the innermost layers. Among the three nonmulberry silks, muga exhibits the highest density, followed by tasar and eri.

Fracture studies

To gain greater insight into the mechanism of fiber failure, the fiber bundles were twisted, immersed in liquid nitrogen, bent quickly, and pulled. This led to a catastrophic failure. The fractured tips were studied under SEM. Figure 7 shows typical fractures observed with different varieties.

It is interesting to note that, in general, the fractures represent a typical viscoelastic failure showing smaller or larger mushroom-type ends covering the broken tip. The mushroom formation suggests reversion of extended chains immediately after snapping to form a typical structure. However, in the case of tasar the fracture is more like a brittle failure, possibly suggesting poor interfibrillar interaction. Muga and eri also

TABLE IV
Average Cross-Sectional Dimensions of Silk Fibers

	Outer layers (μm)			Inner layers (μm)		
Variety	Major axis (a)	Minor axis (b)	a/b	Major axis (a)	Minor axis (b)	a/b
Mulberry (bivoltine)	20.5	15.3	1.34	12.5	3.5	3.57
Mulberry (crossbreed)	21.6	16.5	1.30	15.7	3.8	4.13
Tasar	43.8	25.6	1.71	34.6	12.4	2.79
Muga	35.7	27.4	1.30	26.5	10.5	2.52
Eri	20.4	16.8	1.21	13.6	4.1	3.31

show some tendencies to fibrillate before breaking, which is not the case with mulberry variety. Bhat and Nadiger[18] found that treatment with 6*N* HCl could preferentially dissolve the amorphous portions in silk. To see whether the fracture mechanism changes, the fibers were treated in 6*N* HCl for 12 h, washed, dried, immersed in liquid nitrogen, and broken. One may note that after the treatment, in the case of tasar and

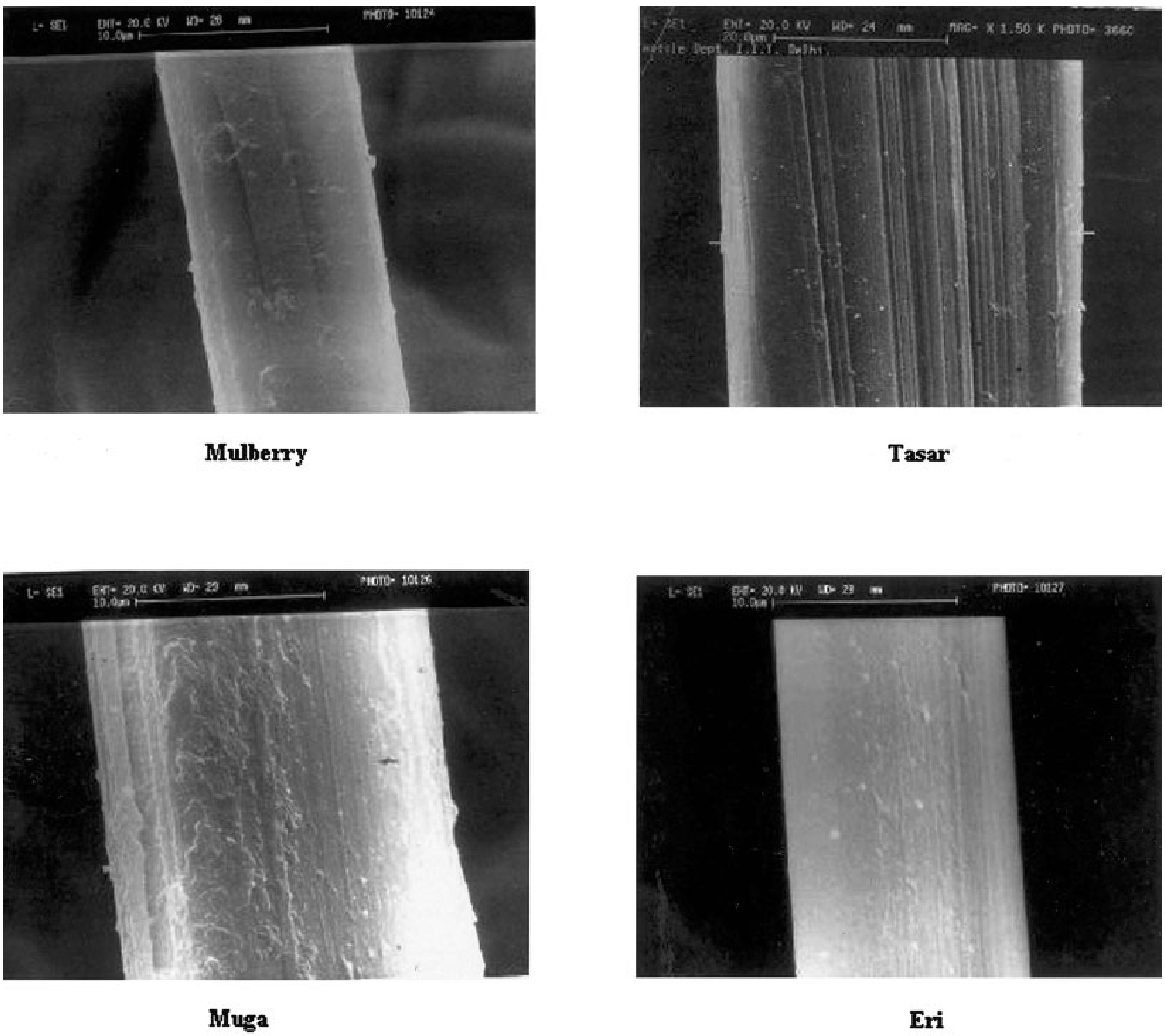


Figure 5 Longitudinal view of silk fibers (outer layers).

TABLE V
Density ρ Values for Different Varieties of Silk

Type of silk	ρ (g/cm^3)		
	Outer layer	Middle layer	Inner layer
Mulberry (bivoltine)	1.350	1.361	1.365
Mulberry (crossbreed)	1.342	1.35	1.356
Tasar	1.30	1.33	1.34
Muga	1.332	1.34	1.348
Eri	1.28	1.29	1.295

eri the fractures are more brittle and, in general, formation of the mushroom-type structure is reduced (Fig. 8). Surprisingly, one may notice development of very large hole in the cross section. Although limited data do not allow a definitive hypothesis, this phenomenon suggests a weaker core in these fibers, which becomes even weaker after HCl treatment. In these two fibers the fibrillar structure is also more clearly visible. Some additional deposits on the surface of all these fibers may be attributable to precipitation of some of the dissolved fibroin.

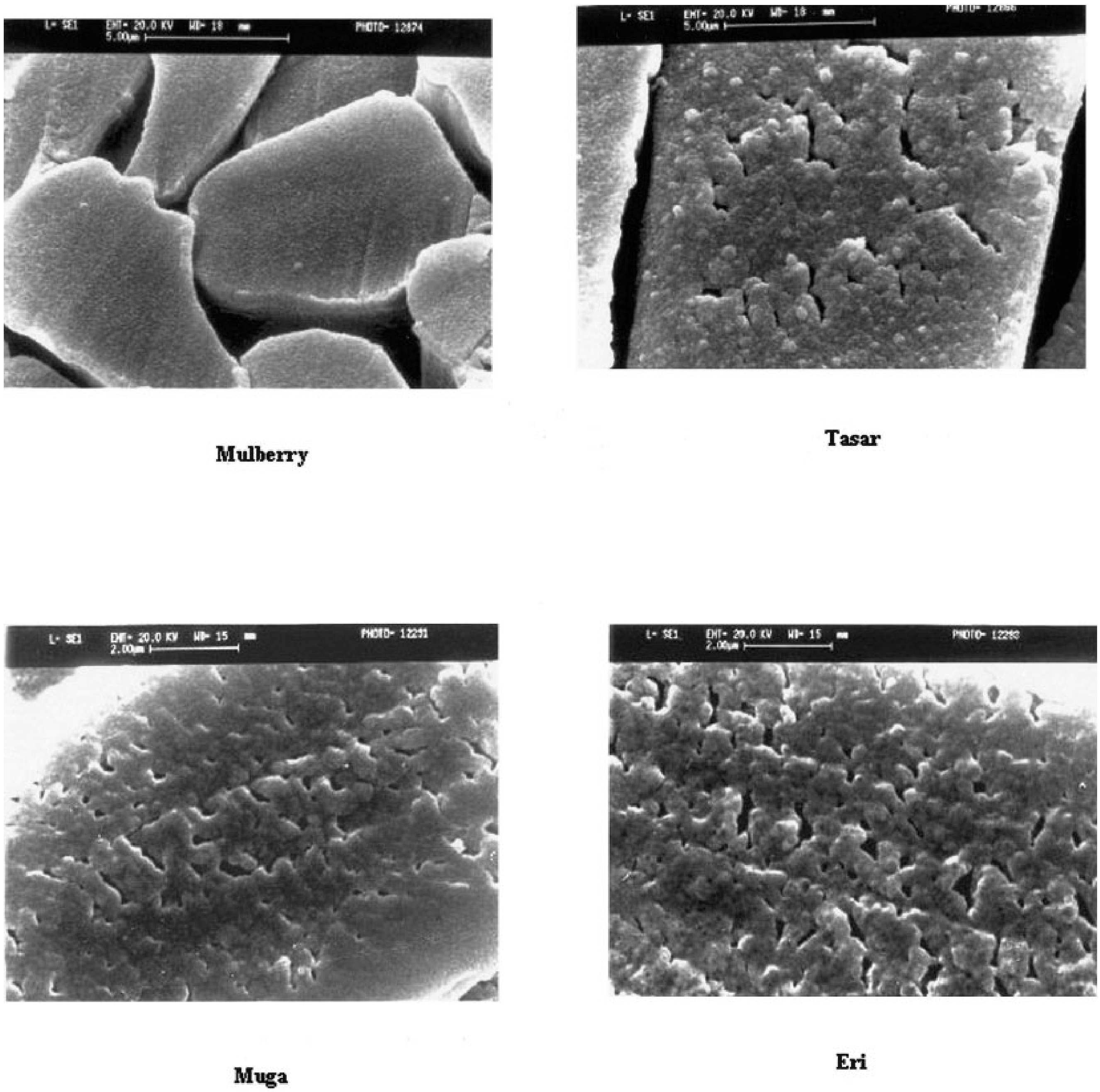
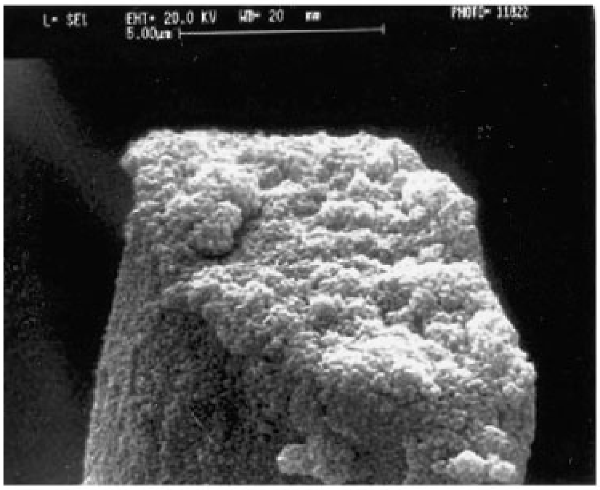
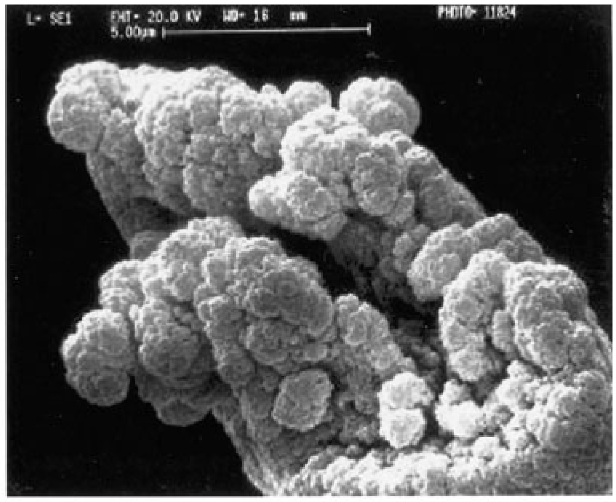


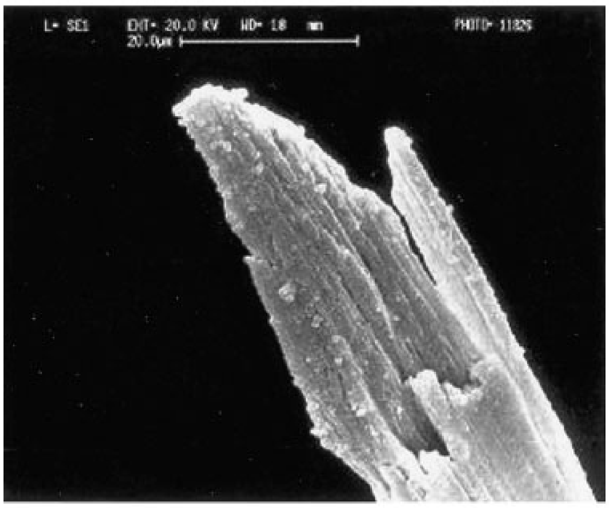
Figure 6 Presence of voids in silk fibers.



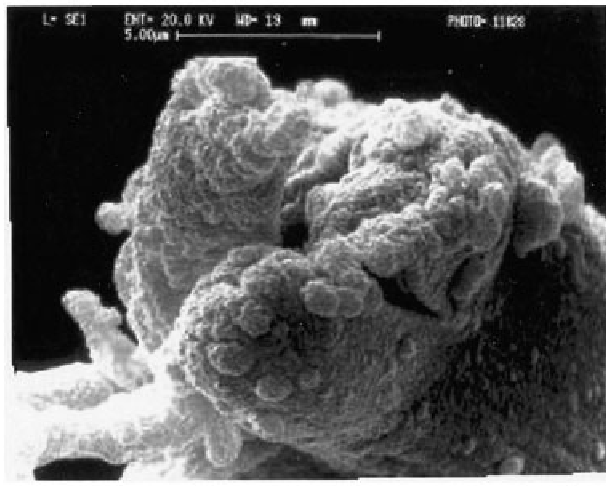
Mulberry (bivoltine)



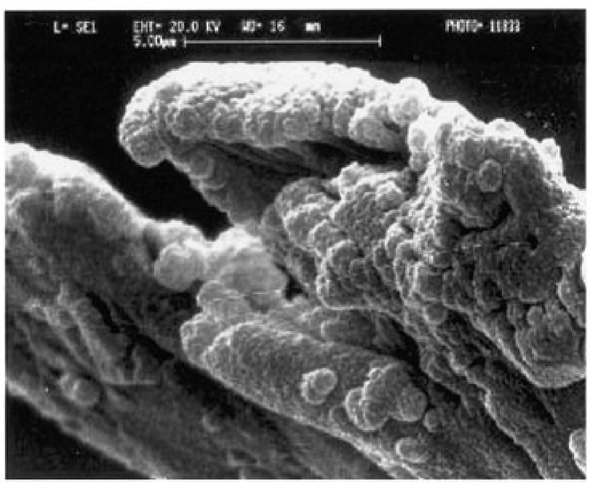
Mulberry (crossbreed)



Tasar



Muga



Eri

Figure 7 Typical tip fracture of silk fibers.

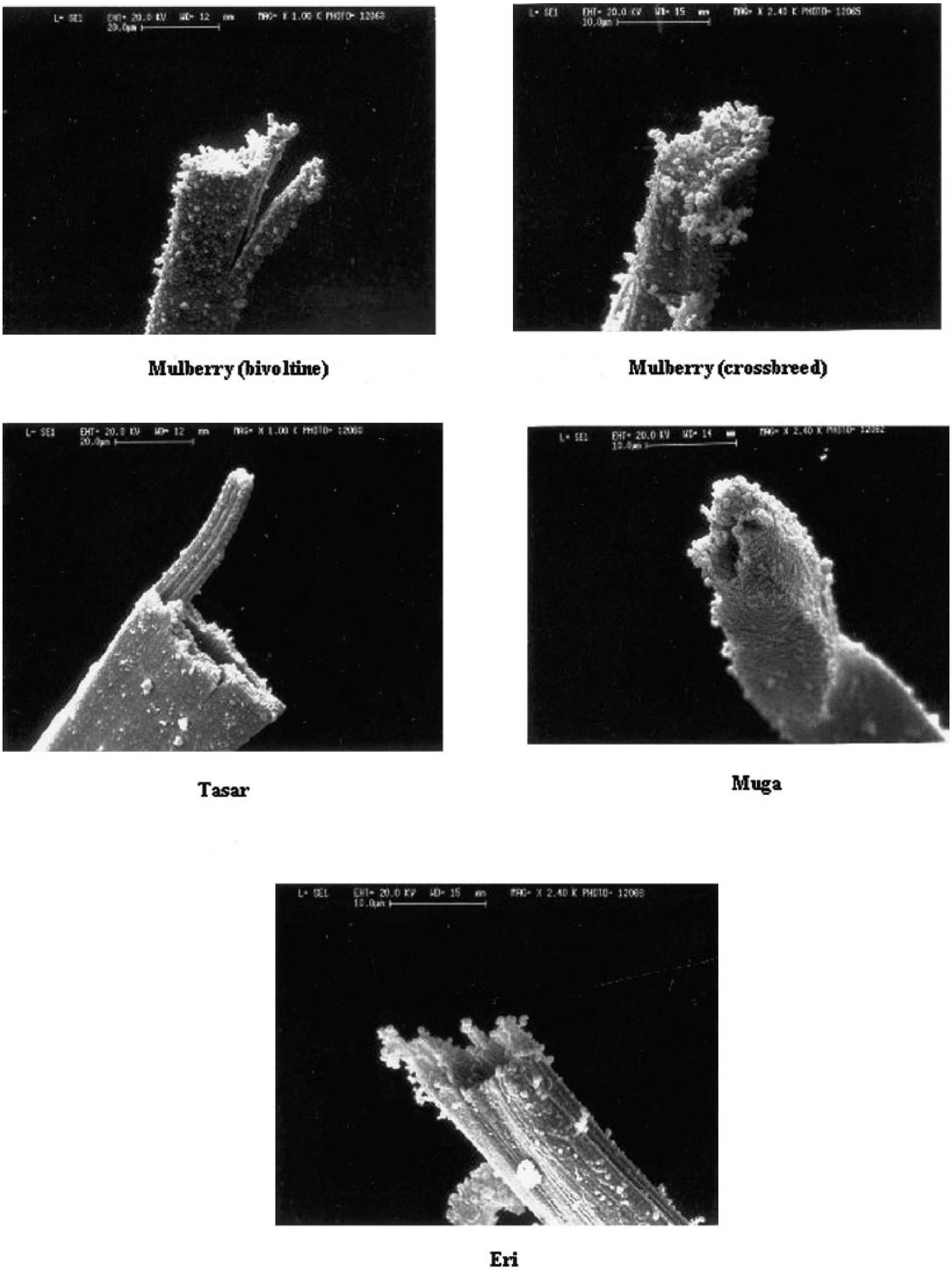


Figure 8 Typical tip fracture of silk fibers treated with 6*N* HCl.

TABLE VI
Moisture Regain (%) of Silk Fibers

Variety	Outer layer	Inner layer	Percentage change
Mulberry (bivoltine)	8.52	8.14	4.46
Mulberry (crossbreed)	8.63	8.28	4.05
Tasar	10.76	10.27	4.55
Muga	9.82	9.47	3.56
Eri	10.21	9.79	4.11

Moisture regain

The results of the moisture regain of different varieties of silk fibers determined under standard conditions are presented in Table VI. All three nonmulberry silk fibers show higher moisture regain values compared to those of the mulberry varieties. Among these, tasar shows the highest value (10.76%), followed by eri (10.21%) and muga (9.82%) for the outer layers. On the other hand, mulberry bivoltine and crossbreed varieties show lower values of 8.52 and 8.63%, respectively. The higher moisture regain of nonmulberry silks suggests that all three nonmulberry silk varieties may consist of a higher ratio of hydrophilic to hydrophobic amino acid residues in their chemical architecture compared to that of the mulberry varieties. Interestingly, moisture regain of the inner layers is about 4.0–4.5% less compared to that of the outer layers, suggesting compactness of the inner layers.

Amino acid composition and analysis

The main objective of this study was to establish the differences, if any, in the chemical architecture of the different silk fibroins of Indian origin. The first part of the study entailed the determination of the amino acid composition of the five varieties and implications thereof.

For calibration, the Pierce H standard of amino acids was used. This was also derivatized using a procedure previously described and analyzed on HPLC. The gradient profile was so chosen as to optimize maximum separation in minimum time. The separation of the amino acids is shown in Figure 9. It may be noticed that all the amino acids are well resolved.

The silk fibroin samples, however, were first hydrolyzed then derivatized and run on the HPLC. Figure 10 depicts the typical the chromatogram obtained for mulberry (bivoltine) silk. It may be observed that there are three major peaks: serine, glycine, and alanine. Among the other major amino acids present are tyrosine and valine. One may also notice that the first two peaks of aspartic and glutamic acids are more prominent compared to those of basic amino acids such as hystidine, arginine, and so forth. The crossbreed variety also shows a similar chromatogram. However, to quantitatively analyze the differences and similarities the amino acid compositions of all the varieties were determined (Table VII).

It may be observed from the results that, in both types of Mulberry silk, glycine, alanine, and serine together constitute about 82%, of which about 10% is serine. Tyrosine and valine may be considered next to these at about 5.5 and 2.5%, respectively. It may be inferred from the data that overall composition of acidic amino groups (i.e., aspartic and glutamic acids) in the mulberry variety is greater than that of the basic amino acids. In fact the ratio of basic to acidic amino acids taken together is 0.65 for the bivoltine and 0.75 for the crossbreed variety (Table VIII).

The other important aspect is the composition of amino acids with bulkier side groups. The presence of bulky side groups can hamper close packing of molecules and hinder crystallization process. In general, a large portion of the mulberry fibroin is made up of simple amino acids such as glycine and alanine, suggesting a favorable condition for crystallization. The ratio of bulky/nonbulky amino acids in these two varieties is about 0.17–0.18 (Table VIII). The ratio of hydrophilic/hydrophobic groups in both varieties suggests that the fibers are basically hydrophilic in character, although the crossbreed variety has a slightly higher value at 0.29. Lucas et al.[6] suggested that the presence of small amounts of basic amino acids in the fibroins of *Bombyx mori* (mulberry) account for the lack of crosslinking of the salt linkage type. These ratios can give an idea of the reactivity/affinity of silk toward different dyes and chemicals.

Figure 11 shows a typical chromatogram of eri. Apart from the major peaks of glycine, alanine, and serine, a substantially high peak of aspartic acid and very significant peaks of histidine and arginine are also evident. This is a major departure from what was seen in the mulberry varieties. The other two nonmulberry silk varieties also show similar chromatograms. For quantitative analysis one may refer to Table VII, where the amino acid compositions of all the varieties are listed. Compared to the mulberry silks, the total amount of glycine, alanine, and serine constitute about 73% in the nonmulberry variety, less by about 10%. As was seen by earlier workers, all the nonmulberry silks exhibit a high proportion of alanine compared to that in the mulberry variety. The proportion of alanine is about 34% in tasar, 36% in eri, and 35% in muga. This value is consistent but is lower than that reported by Freddi et al.,[11] particularly for muga (~ 44%). On the other hand, the glycine content in these varieties is about 27–29%, which is lower than that found in the mulberry varieties (~ 43%). The glycine/alanine ratio, therefore, is very different for mulberry varieties; that is, it is 1.5 for mulberry and <1 for nonmulberry, averaging around 0.8. It is well known that these two amino acids form the major part of the crystalline

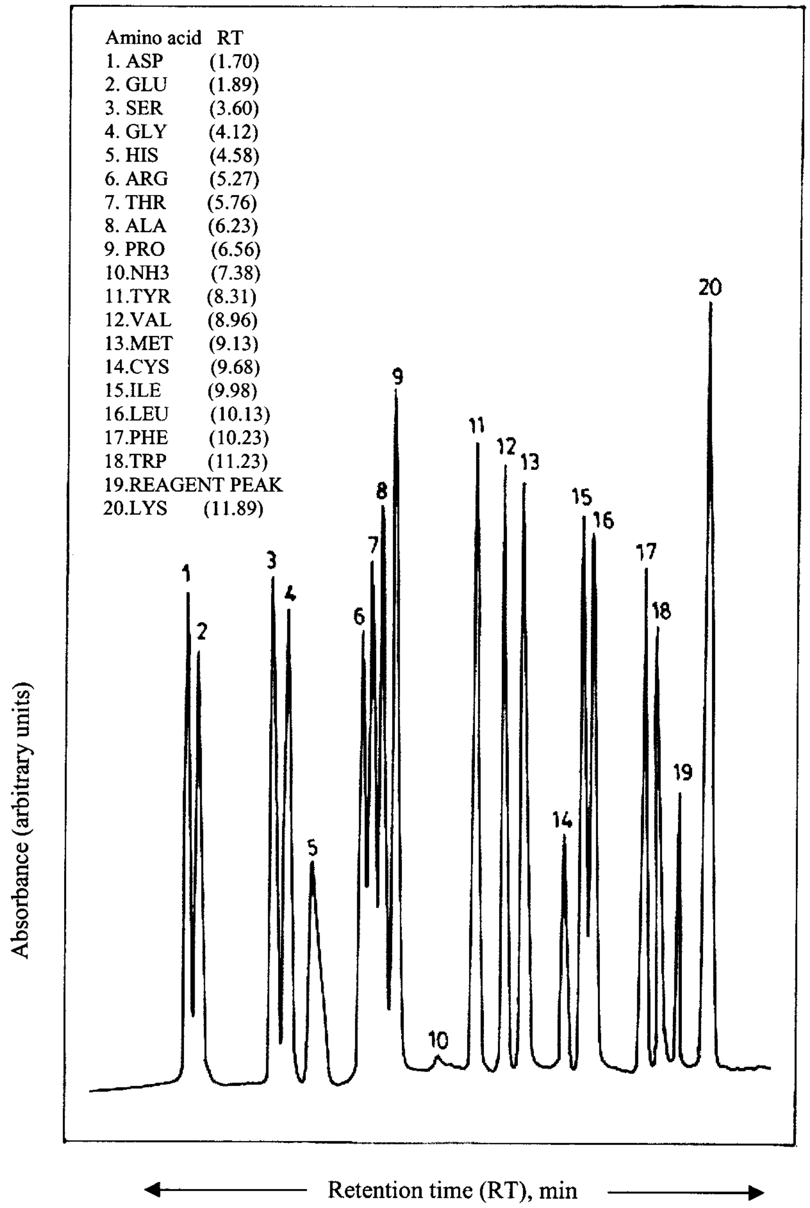


Figure 9 Separation of amino acid standards (Pierce H).

region and thus a change in this ratio should definitely lead to a different crystallographic form. In addition, the nonmulberry varieties have a substantial proportion of amino acids with bulky side groups, especially aspartic acid (4–6%) and arginine (4–5%), which means that not only the acidic but also basic amino acid levels are greater. Incidentally, nonmulberry silks show a higher ratio of basic to acidic amino acids: for instance, 0.97 for tasar, 1.24 for muga, and 1.3 for eri, compared to 0.65 for mulberry (bivoltine) and 0.75 for mulberry (crossbreed) (Table VIII). Not only this, the ratio of bulky/nonbulky amino acids is also high here, ranging from 0.24 to 0.32. Similarly, the ratio of hydrophilic to hydrophobic is also high for all three nonmulberry silks.

It is interesting to note the presence of sulfur-containing amino acids (i.e., cystine and methionene) in all the varieties of silk. Methionene content in nonmulberry silks is slightly higher (0.28–0.34%) compared to that found in mulberry varieties (0.11–0.19%), whereas the cystine content is comparable. Previous workers reported that the presence of cystine may play some role in determination of molecular weight.[11]

A glance at Table VII reveals that composition of various amino acids varies substantially, some as high as 40% and others as low as 0.1%. In such a situation, simple ratios may not afford a very clear picture of the actual influence these amino acids may have on the properties and reactivity. To make the analysis more

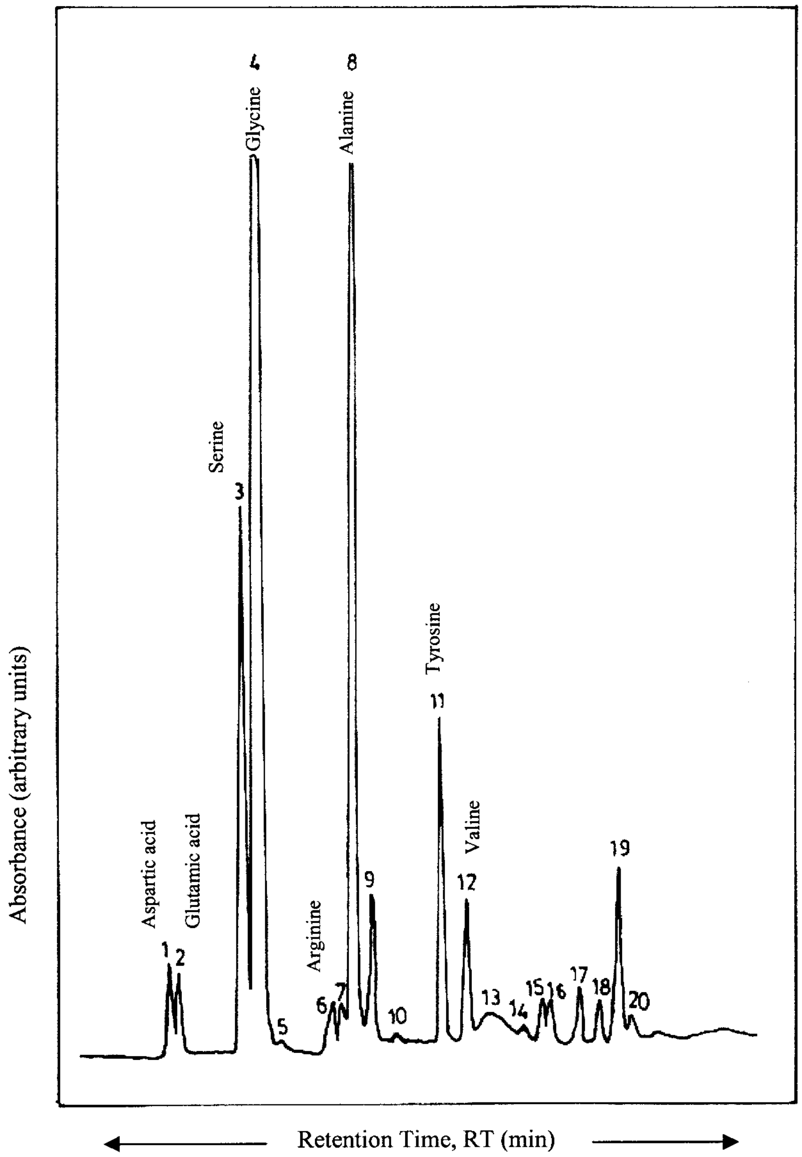


Figure 10 Separation of amino acids of mulberry (bivoltine) silk fibers.

meaningful, impact factors (F_i) pertaining to these ratios was calculated as

$$F_i = R_{XY}(X + Y)$$

where X and Y represent the composition of a particular group of amino acids (for instance, if the composition of basic amino acid is X mol % and that of acidic is Y mol %, then R_{XY} is the ratio of their composition). This factor gives the actual impact of these differences on structure and thus on properties. These values are presented in Table IX.

Morphology and chemical architecture

One may note that the impact factors are distinctly different for mulberry and nonmulberry varieties. For instance, the impact factor of bulky/nonbulky amino acids for mulberry varieties is about 18, whereas for nonmulberry varieties it ranges from 24 to 33. It is imperative that bulkier amino acids will not be a part of the crystalline region because of steric hindrance. Other authors also hypothesized that amino acids with bulky side groups are confined to amorphous areas.[14] This means that the microstructural morphology of these silks is likely to be different. Similarly, the impact factor for glycine/alanine is also different for mulberry, about 113, as against 51–53 for the nonmulberry varieties. The higher alanine content of the nonmulberry varieties points to distinctly different crystallographic diffraction patterns. While working on these silk varieties, the authors observed indications to this effect in a work reported earlier[19]: it was observed that nonmulberry silks such as tasar, muga, and eri

TABLE VII
Amino Acid Composition of Different Varieties of Silk Fibers

Amino acid	Amino acid composition (mol %)				
	Mulberry (bivoltine)	Mulberry (crossbreed)	Tasar	Muga	Eri
Aspartic acid	1.64	1.49	6.12	4.97	3.89
Glutamic acid	1.77	1.53	1.27	1.36	1.31
Serine	10.38	10.85	9.87	9.11	8.89
Glycine	43.45	43.73	27.65	28.41	29.35
Hystidine	0.13	0.15	0.78	0.72	0.75
Arginine	1.13	1.16	4.99	4.72	4.12
Threonine	0.92	0.76	0.26	0.21	0.18
Alanine	27.56	28.36	34.12	34.72	36.33
Proline	0.79	0.76	2.21	2.18	2.07
Tyrosine	5.58	5.76	6.82	5.12	5.84
Valine	2.37	2.89	1.72	1.5	1.32
Methionine	0.19	0.11	0.28	0.32	0.34
Cystine	0.13	0.12	0.15	0.12	0.11
Isoleusine	0.75	0.78	0.61	0.51	0.45
Leucine	0.73	0.75	0.78	0.71	0.69
Phenylalanine	0.14	0.18	0.34	0.28	0.23
Tryptophan	0.73	0.75	1.26	2.18	1.68
Lysine	0.23	0.25	0.17	0.24	0.23

exhibited higher extension-at-break and lower tenacity and initial modulus compared to those of mulberry silk. Whether the lower density of nonmulberry silks is attributable to the high proportion of bulky side groups or the presence of microvoids, and thus higher amorphous content, is nothing less than a hazardous guess at this juncture. Possibly, both these factors may be contributing to some extent.

Effect on moisture regain

The higher impact factors of hydrophilic/hydrophobic amino acids points toward the nonmulberry variety being significantly more hydrophilic. It may be noted that the moisture regain values of mulberry silk varieties are lower (~ 8.5%) compared with those of tasar (10.7%), muga (9.8%), and eri (10.2%). This compares well with the hydrophilic/hydrophobic ratio (Table VIII) and corresponding impact factor (Table IX). Surprisingly, the moisture regain values were found to be slightly lower for the inner layers compared to those of the outer layers, irrespective of the type of silk, although this change is small but consistent. Does this mean that the chemical architecture of fibroin changes along the layers? This is most unlikely, although for verification, the amino acid analysis of the innermost layer of mulberry silk was also done. It is quite evident from the results that there is no change in the amino acid composition (Table X). This difference in moisture regain within a variety may be attributable to compact structural morphology along the filament length. An increase in density along the filament length may lead to a slight reduction in accessibility of hydrophilic sites and hence a slight reduction in moisture regain.

TABLE VIII
Various Amino Acid Ratios[a]

Ratio	Mulberry (bivoltine)	Mulberry (crossbreed)	Tasar	Muga	Eri
Basic/acidic	0.65	0.75	0.97	1.24	1.30
Hydrophilic/hydrophobic	0.28	0.29	0.44	0.38	0.35
Bulky/nonbulky side groups	0.17	0.18	0.33	0.28	0.24
Glycine/alanine	1.58	1.54	0.81	0.82	0.80

[a] Basic amino acids: arginine, lysine, histidine, tryptophan; acidic amino acids: aspartic acid, glutamic acid; hydrophilic: serine, threonine, aspartic acid, glutamic acid, tyrosine, arginine, lysine, Histidine; hydrophobic: glycine, alanine, cystine, proline, tryptophan, valine, isoleucine, phenylalanine, methionine, tryptophan; amino acids with bulky side groups: aspartic acid; glutamic acid, tyrosine, phenylalanine, leucine, isoleucine, valine, arginine, cystine, methionine, lysine, tryptophan; amino acids with nonbulky side groups: glycine, alanine, serine, threonine, proline, histidine.

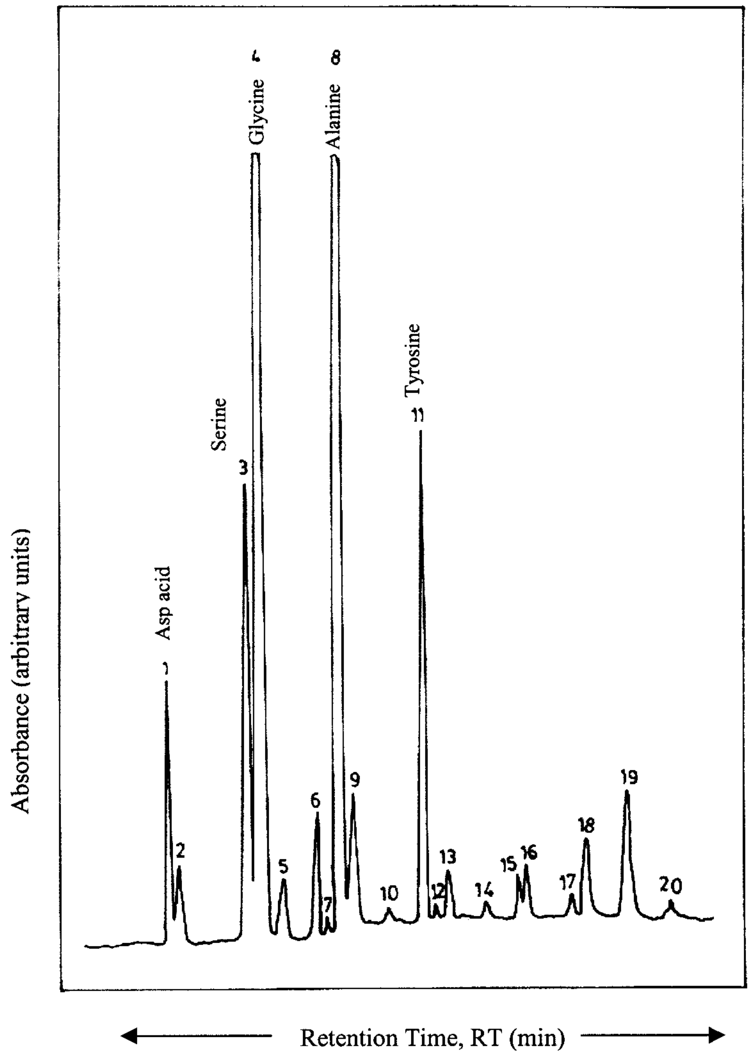


Figure 11 Separation of amino acids of eri silk fibers.

Intrinsic viscosity

The molecular weight of macromolecules also plays a very significant role in governing the structure and properties of fibers. Determination of molecular weight is a difficult exercise in silk as was reported by previous workers. On the other hand, intrinsic viscosity of the fibroin is easier to determine and can give a fairly good idea about the molecular weight. The intrinsic viscosity values are listed in Table XI. It is interesting to note that the nonmulberry silks show a very high value (0.69–0.76 dL/g) compared to that of mulberry (0.19–0.25 dL/g). This certainly indicates the higher molecular weight of nonmulberry varieties. Could this be attributed to the presence of cystine? This is unlikely because the cystine content in all the varieties is comparable (Table VII), which suggests the higher molecular chain length of the nonmulberry variety. Higher molecular weight coupled with more bulky side groups hints toward fibers with higher extension at break because these would tend to inhibit

TABLE IX
Impact Factors of Various Amino Acid Ratios

Ratio	Mulberry (bivoltine)	Mulberry (crossbreed)	Tasar	Muga	Eri
Bulky/nonbulky side groups	18.0	18.0	33.0	28.0	24.0
Glycine/alanine	113.4	112.8	51.3	53.1	53.9
Hydrophilic/hydrophobic	28.0	29.0	44.0	38.0	35.0

the formation of highly ordered and oriented structure in the fibers. To resubstantiate that there is no change in the chemical architecture within the same variety, the intrinsic viscosity of the inner layer of mulberry (bivoltine) was also determined. There was no change in the intrinsic viscosity as one goes from the outer to the inner layers (Table XI), meaning thereby that, although physical and microstructural properties may change along the length of silk fiber, the chemistry remains the same.

TABLE XI
Intrinsic Viscosity of Different Varieties of Silk

Variety	Layer	Intrinsic viscosity (dL/g)
Mulberry (bivoltine)	Outer	0.25
Mulberry (crossbreed)	Outer	0.19
Tasar	Outer	0.71
Muga	Outer	0.76
Eri	Outer	0.69
Mulberry (bivoltine)	Inner	0.25

CONCLUSIONS

The reelable filament length in the bivoltine variety is more than 1000 m; others have approximately one third to two thirds of this value. The cross-sectional shape and size of different varieties vary, the coarsest among which is tasar. Interestingly the properties, such as denier, cross section, and density, change significantly from the outer to the inner layers, given that the area or denier decreases and density increases. The nonmulberry silks have microvoids, thereby having lower density. Indications are that interfibrillar interactions are weaker in the nonmulberry variety. Higher moisture regain in the nonmulberry variety indicates more hydrophilic groups. The moisture regain also decreases to some extent in all the varieties as one moves from the outer to the inner layers. Despite being genetically different, the chemical architectures of tasar, muga, and eri are very similar with respect to amino acid composition, and thus a broad division—mulberry and nonmulberry—is justified. This is substantiated from various parameters such as the ratios of bulky/nonbulky, hydrophilic/hydrophobic, glycine/alanine, and also from different parameters, such as moisture regain and intrinsic viscosity, because all these parameters differ significantly for the mulberry and the nonmulberry varieties. In general the nonmulberry varieties are characterized by higher amounts of amino acids with bulky groups and hydrophilic groups and have higher content of alanine, aspartic acid, and arginine. The molecular weights are also higher. All the silk varieties are characterized by the presence of sulfur-containing amino acids. Within a variety, the amino acid composition and the molecular weight do not change from the outer to the inner layers.

TABLE X
Amino Acid Composition of Mulberry (Bivoltine) Silk Fibers

Amino acid	Outer layer	Inner layer
Aspartic acid	1.64	1.69
Glutamic acid	1.77	1.71
Serine	10.38	10.45
Glycine	43.45	43.13
Hystidine	0.13	0.16
Arginine	1.13	1.08
Threonine	0.92	0.88
Alanine	27.56	26.92
Proline	0.79	0.73
Tyrosine	5.58	5.62
Valine	2.37	2.46
Methionine	0.19	0.13
Cystine	0.13	0.12
Isoleusine	0.75	0.81
Leucine	0.73	0.76
Phenylalanine	0.14	0.16
Tryptophan	0.73	0.80
Lysine	0.23	0.21

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