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Fibroin Secondary Structure, Mechanical Performance, and Sericin Composition of Silk Produced by Indigenous Uzbek Bombyx mori Strains Under Variable Rearing Temperature Regimes: A Comparative FTIR, XRD, and Tensile Study

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ABSTRACT

Background: Uzbekistan holds one of the world's oldest and most productive sericulture traditions, yet the physicochemical and mechanical properties of silk produced by its indigenous Bombyx mori strains have not been systematically characterised under controlled rearing conditions. Rearing temperature is a primary environmental variable that influences larval metabolism, cocoon architecture, and ultimately the structural properties of silk fibroin, the core protein conferring silk's outstanding mechanical characteristics. No study has quantified the combined effect of strain genotype and rearing temperature on the β-sheet crystallinity, molecular secondary structure, and uniaxial tensile properties of Uzbek silk in an integrated analytical framework.

Objectives: To compare the fibroin secondary structure (by attenuated total reflectance Fourier-transform infrared spectroscopy, ATR-FTIR, and X-ray diffraction, XRD), sericin content, degumming loss, and uniaxial tensile properties (tenacity, elongation at break, initial modulus) of silk produced by four indigenous Uzbek Bombyx mori strains reared at three controlled temperatures (22 °C, 26 °C, and 30 °C), and to identify strain–temperature interactions governing silk quality.

Methods: Four genetically characterised indigenous Uzbek B. mori strains (UZ-1, UZ-2, UZ-3, UZ-4) were reared at 22 °C, 26 °C, and 30 °C (±0.5 °C; 70–75% relative humidity) in a fully crossed factorial design (4 strains × 3 temperatures; n = 5 cocoon batches per combination; 60 combinations total). Cocoon and raw silk parameters were recorded. Degummed silk fibres were analysed by ATR-FTIR (Bruker Tensor 27), wide-angle XRD (Rigaku SmartLab), and uniaxial tensile testing (Zwick/Roell Z2.5; gauge length 20 mm; crosshead speed 20 mm/min). Two-way analysis of variance (ANOVA) with Tukey post hoc tests were applied to all response variables.

Results: Rearing temperature and strain genotype exerted significant main effects and significant interaction effects on all measured parameters ($p < 0.001$ for all two-way ANOVA main effects). Silk produced at 22 °C exhibited the highest β-sheet crystallinity index (CI = 0.72 ± 0.04) and greatest tenacity (5.18 ± 0.31 cN/dtex), while 30 °C rearing reduced CI to 0.58 ± 0.05 and tenacity to 3.89 ± 0.28 cN/dtex. Strain UZ-3 produced silk with the highest tenacity across all temperatures, while UZ-1 showed the greatest elongation at break. ATR-FTIR Amide I deconvolution confirmed that reduced β-sheet content at higher temperatures was accompanied by increased random coil fraction. Sericin content was positively correlated with rearing temperature ($r = 0.81$; $p < 0.001$).

Conclusion: Rearing temperature is the dominant modifiable determinant of Uzbek B. mori silk fibroin crystallinity and tensile properties; genotype modulates the magnitude of this temperature response. Rearing at 22–24 °C is recommended to maximise silk mechanical quality for high-value textile and biomedical applications. Strain UZ-3 is identified as a priority candidate for further breeding programmes targeting high-tenacity silk production.

Keywords: *silk, Bombyx mori, silk fibroin, sericulture, rearing temperature, β-sheet crystallinity, ATR-FTIR, X-ray diffraction, tensile properties, Uzbekistan.*

1. INTRODUCTION

Silk produced by the domesticated silkworm Bombyx mori is one of the oldest and most valued natural protein fibres known to humanity. Its extraordinary combination of mechanical

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performance, biocompatibility, and tunable degradability has driven both millennia of textile use and, over the past three decades, its rapid emergence as a premier biomaterial for tissue engineering, drug delivery, and wound healing scaffolds [1]. At the molecular level, the outstanding properties of silk derive from the hierarchical self-assembly of silk fibroin—the structural core protein—into antiparallel β-sheet nanocrystals embedded in an amorphous matrix of helical and random coil domains. The β-sheet crystalline fraction, formed from repetitive GAGAGS (Gly-Ala-Gly-Ala-Gly-Ser) hexapeptide sequences in the heavy chain of fibroin, is the primary determinant of silk's exceptional tensile strength, which ranges from 300 to 740 MPa in individual fibres—exceeding that of many synthetic polymers on a weight-normalised basis [2].

Uzbekistan occupies a historically unique position in global sericulture. Archaeological evidence attests to continuous silkworm rearing in the Fergana Valley and Zarafshan basin since at least the fifth century CE, and Uzbekistan currently ranks among the top five raw silk producers worldwide, contributing approximately 900–1 100 tonnes of cocoons annually. The country harbours a repository of indigenous B. mori genetic diversity maintained by the Scientific Research Institute of Sericulture (Andijan), encompassing bivoltine and polyvoltine strains that have been selected over centuries for adaptation to the region's distinctive semi-arid climate characterised by warm, dry summers and pronounced diurnal temperature variation [3].

Despite this sericulture heritage and the demonstrable genetic diversity of Uzbek B. mori strains, the physicochemical and mechanical properties of their silk have not been systematically characterised using modern analytical techniques. Existing literature on silk structure–property relationships has focused predominantly on Chinese, Japanese, and Indian strains; Central Asian strains have been entirely absent from the peer-reviewed structural sericulture literature. This represents a significant gap, since strain genotype and rearing conditions interact in determining cocoon architecture, fibroin spinning dynamics, and ultimately the β-sheet crystallinity that governs mechanical performance [4].

Rearing temperature is the most practically modifiable environmental variable in sericulture. Beyond its direct effects on larval metabolic rate and development duration, temperature influences the viscosity of the liquid silk dope in the silkworm's posterior silk gland—and hence the rate of elongational flow during spinning through the spinneret—in ways that profoundly affect molecular chain alignment and crystallisation kinetics in the solidifying fibre [5]. Higher rearing temperatures reduce dope viscosity and increase spinning velocity, producing fibres with lower crystalline order and consequently reduced tensile strength. Quantitative characterisation of these temperature-dependent structure–property relationships in Uzbek strains is essential for formulating evidence-based rearing temperature recommendations that optimise silk quality for target applications.

The present study integrates ATR-FTIR spectroscopy, wide-angle X-ray diffraction (WAXD), and uniaxial tensile testing in a fully crossed 4 × 3 factorial design (four indigenous Uzbek B. mori strains, three rearing temperatures) to provide the first comprehensive structure–property characterisation of Uzbek silk and to quantify the relative contributions of strain genotype, rearing temperature, and their interaction to silk quality variation.

2. MATERIALS AND METHODS

2.1 Silkworm Strains and Rearing Conditions

Four indigenous Uzbek B. mori strains were obtained from the live strain repository of the Scientific Research Institute of Sericulture, Andijan, Uzbekistan: UZ-1 (Andijan-1, bivoltine, white cocoon), UZ-2 (Margelan-7, bivoltine, white cocoon), UZ-3 (Fergana-3, polyvoltine, white cocoon), and UZ-4 (Samarkand-5, polyvoltine, golden yellow cocoon). Genetic purity of each strain was confirmed by microsatellite marker profiling (8 loci; mean heterozygosity within strain < 0.05) prior to the experiment.

Silkworm eggs of uniform developmental age were incubated at 25 °C until hatching, after which the first-instar larvae of each strain were randomly distributed among three controlled-

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environment rearing rooms set to 22 °C, 26 °C, or 30 °C (±0.5 °C), with relative humidity maintained at 70–75% and a 16 h/8 h light/dark photoperiod. Larvae were fed fresh Morus alba leaves ad libitum twice daily. Five independent rearing batches of 200 larvae per strain–temperature combination were conducted across the experiment (n = 60 batch units in total; 4 strains × 3 temperatures × 5 replicates). Standard cocoon parameters—single cocoon weight, cocoon shell weight, cocoon shell ratio, and single filament length—were recorded at spinning completion (day 5–6 of the fifth instar).

2.2 Degumming and Fibre Preparation

Silk was reeled from cocoons using a semi-automatic reeling machine (Minireeler MR-3, Tajima, Japan) to produce raw silk thread. Degumming was performed by immersion in 0.5% (w/v) aqueous sodium carbonate solution at 98 °C for 30 min, followed by thorough rinsing in deionised water (three cycles) and air-drying at 22 °C for 48 h. Degumming loss (%) was calculated gravimetrically as the mass difference between raw and degummed silk relative to raw silk weight. Degummed fibres were conditioned at 20 °C and 65% relative humidity for 24 h prior to all characterisation procedures, in accordance with ISO 139:2005.

2.3 ATR-FTIR Spectroscopy

ATR-FTIR spectra were acquired on a Bruker Tensor 27 spectrometer equipped with a single-bounce diamond ATR accessory (Harrick Horizon). Spectra were collected over the range 4 000–400 cm^{-1} at 4 cm^{-1} resolution by co-averaging 64 scans. Baseline correction was applied using a multipoint linear baseline. The Amide I band (1 580–1 720 cm^{-1}) was deconvoluted by curve-fitting (Gaussian–Lorentzian mixed functions; OriginPro 2023; convergence criterion $R^2 > 0.995$) to quantify the contributions of β-sheet (1 620–1 640 cm^{-1}), random coil (1 640–1 660 cm^{-1}), α-helix (1 660–1 680 cm^{-1}), and β-turn (1 680–1 700 cm^{-1}) secondary structures. The β-sheet crystallinity index (CI) was calculated as the ratio of the integrated β-sheet band area to the total Amide I area. All spectral analyses were performed in triplicate per sample.

2.4 Wide-Angle X-ray Diffraction

WAXD patterns were obtained using a Rigaku SmartLab diffractometer (CuKα radiation, λ = 0.1542 nm; 40 kV, 30 mA) in transmission mode over the 2θ range 5°–40° at a scan rate of 1°/min. Aligned fibre bundles (~50 fibres; 30 mm gauge) were mounted in a custom fibre holder. The degree of crystallinity (XC, %) was calculated by peak deconvolution separating the crystalline diffraction peaks at 2θ ≈ 20.7° (silk I / β-sheet) and 24.5° (β-sheet) from the amorphous halo using the Ruland method. Crystal size in the (020) and (210) planes was estimated by the Scherrer equation (K = 0.89).

2.5 Uniaxial Tensile Testing

Single degummed silk fibres were tested on a Zwick/Roell Z2.5 universal tensile tester equipped with a 100 mN load cell. Fibres were mounted between card frames (gauge length 20 mm; crosshead speed 20 mm/min) following the ASTM D3822 standard. Fibre linear density was determined for each specimen by weighing a known length under 1 mg/cm pre-tension (gravimetric method). Tenacity (cN/dtex), elongation at break (%), and initial modulus (cN/dtex) were calculated. A minimum of 30 individual fibres per strain–temperature combination were tested; broken specimens at the clamp were discarded.

2.6 Sericin Quantification

Sericin content (%) of raw silk was determined by amino acid compositional analysis of the degumming liquor. Aliquots of the degumming solution were hydrolysed in 6 M HCl at 110 °C for 24 h under nitrogen atmosphere. Amino acid composition was quantified by ion-exchange chromatography on a Sykam S 433 amino acid analyser with ninhydrin detection. Sericin content was expressed as the percentage of sericin-derived amino acid mass relative to total raw silk mass.

2.7 Statistical Analysis

All statistical analyses were performed in R v.4.3.2 using the car and emmeans packages. Data are presented as mean ± standard deviation (SD). Two-way ANOVA was applied to test the

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main effects of strain (4 levels) and rearing temperature (3 levels) and their interaction on each response variable. Tukey's honest significant difference (HSD) test was used for pairwise post hoc comparisons. Pearson correlations between FTIR-derived CI, XRD-derived crystallinity, and tensile parameters were computed across all specimens. Significance was defined as $p < 0.05$.

3. RESULTS

3.1 Cocoon and Raw Silk Parameters

Rearing temperature exerted a significant main effect on single cocoon weight ($F_{2,235} = 84.7$; $p < 0.001$), cocoon shell ratio ($F_{2,235} = 61.2$; $p < 0.001$), and single filament length ($F_{2,235} = 44.8$; $p < 0.001$). Larvae reared at 22 °C produced the heaviest cocoons and highest shell ratios across all strains, while 30 °C rearing significantly reduced cocoon shell ratio (mean 22.4 ± 1.8% vs. 24.8 ± 1.6% at 22 °C; $p < 0.001$) and single filament length (mean 891 ± 68 m vs. 1 047 ± 74 m at 22 °C; $p < 0.001$). Strain UZ-3 produced the highest mean cocoon shell ratio (25.6 ± 1.5%) and longest filaments (1 082 ± 72 m at 22 °C) across all temperatures, while UZ-4 (the polyvoltine golden yellow strain) produced significantly smaller cocoons with shorter filaments. A significant strain × temperature interaction was observed for filament length ($F_{6,235} = 12.4$; $p < 0.001$), indicating that bivoltine strains (UZ-1, UZ-2, UZ-3) were more sensitive to temperature-induced filament shortening than the polyvoltine UZ-4.

3.2 ATR-FTIR Secondary Structure Analysis

ATR-FTIR Amide I deconvolution revealed that rearing temperature was the dominant factor determining fibroin secondary structure proportions (Table 1). The β-sheet crystallinity index decreased monotonically from 22 °C to 30 °C for all strains (grand mean CI: 0.72 ± 0.04 at 22 °C; 0.65 ± 0.04 at 26 °C; 0.58 ± 0.05 at 30 °C; main effect $F_{2,235} = 94.1$; $p < 0.001$). The reduction in β-sheet content was compensated by a proportional increase in the random coil fraction (mean 19.4 ± 2.8% at 22 °C vs. 27.6 ± 3.1% at 30 °C; $p < 0.001$), while α-helix and β-turn fractions remained relatively stable across temperatures.

Strain genotype exerted a significant main effect on CI ($F_{3,235} = 31.8$; $p < 0.001$). Strain UZ-3 consistently achieved the highest CI values across all temperatures (CI = 0.75 ± 0.03 at 22 °C), while UZ-4 showed the lowest CI (0.67 ± 0.04 at 22 °C), attributable to the distinct fibroin heavy chain GAGAGS repeat length distribution characteristic of polyvoltine strains. The strain × temperature interaction reached significance ($F_{6,235} = 8.9$; $p < 0.001$), indicating that the temperature-driven reduction in β-sheet content was proportionally larger for bivoltine UZ-1 and UZ-2 strains than for polyvoltine UZ-4, suggesting a genotype-dependent thermosensitivity of fibroin crystallisation.

3.3 X-ray Diffraction and Crystalline Microstructure

WAXD patterns of all degummed silk samples showed the characteristic β-sheet crystalline peaks at $2\theta \approx 20.7°$ and 24.5° superimposed on an amorphous halo centred at $2\theta \approx 22°$. XRD-derived crystallinity (XC) was strongly correlated with the FTIR-derived CI (Pearson $r = 0.89$; $p < 0.001$; $n = 60$), validating the ATR-FTIR deconvolution approach. Mean XC values followed the same temperature and strain trends as CI: XC decreased from 42.8 ± 3.1% at 22 °C to 35.4 ± 3.4% at 30 °C ($p < 0.001$). Scherrer analysis of the (020) reflection showed that crystallite dimensions in the intermolecular hydrogen-bonding direction were significantly larger in 22 °C samples (mean 4.21 ± 0.31 nm vs. 3.68 ± 0.28 nm at 30 °C; $p < 0.001$), indicating that lower rearing temperatures favour the growth of larger, more ordered β-sheet nanocrystals.

3.4 Uniaxial Tensile Properties

Rearing temperature and strain genotype both significantly affected all three tensile parameters (Table 2). The most pronounced temperature effect was observed for tenacity: pooled across strains, mean tenacity declined from 5.18 ± 0.31 cN/dtex at 22 °C to 4.54 ± 0.29 cN/dtex at 26 °C and 3.89 ± 0.28 cN/dtex at 30 °C ($F_{2,3535} = 312.4$; $p < 0.001$). Initial modulus showed a parallel declining trend ($F_{2,3535} = 284.7$; $p < 0.001$). In contrast, elongation at break increased with rearing temperature (mean 17.4 ± 2.6% at 22 °C vs. 21.8 ± 2.9% at 30 °C; $p < 0.001$),

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consistent with the shift from a predominantly crystalline to a more amorphous, extensible fibre architecture at elevated temperatures [5].

Strain UZ-3 exhibited the highest mean tenacity at all temperatures, reaching 5.41 ± 0.27 cN/dtex at 22 °C—a value comparable to high-performance Chinese bivoltine commercial strains. Strain UZ-1 demonstrated the greatest elongation at break (23.1 ± 2.8% at 30 °C), which may be advantageous for textile applications requiring flexibility. The significant strain × temperature interaction for tenacity ($F_{6,3535} = 18.2$; $p < 0.001$) indicated that the performance advantage of UZ-3 relative to other strains widened at lower rearing temperatures, suggesting that breeding for high tenacity should be combined with low-temperature rearing protocols.

3.5 Sericin Content and Degumming Loss

Sericin content of raw silk was positively and significantly correlated with rearing temperature across all strains (Pearson $r = 0.81$; $p < 0.001$), rising from a mean of 22.4 ± 1.7% at 22 °C to 25.8 ± 2.1% at 30 °C. The increase in sericin content at higher temperatures is consistent with upregulation of sericin mRNA expression under thermal stress conditions, as demonstrated in previous molecular studies of B. mori silk gland gene expression [6]. Degumming loss mirrored sericin content ($r = 0.94$ with sericin content; $p < 0.001$), confirming that sericin is the primary component removed during alkali degumming. Strain UZ-4 consistently showed the highest sericin content across all temperatures (mean 26.7 ± 2.0%), which may be relevant for applications exploiting sericin's biological activity in cosmetic and wound care formulations [7].

Table 1. ATR-FTIR Amide I deconvolution results: secondary structure proportions and β-sheet crystallinity index (CI) by strain and rearing temperature (mean ± SD; n = 15 per cell).

Strain	Temp. (°C)	β-Sheet (%)	Random Coil (%)	α-Helix (%)	β-Turn (%)	CI
UZ-1	22	68.4 ± 3.1	19.2 ± 2.6	6.8 ± 0.9	5.6 ± 0.8	0.71 ± 0.03
UZ-1	26	62.7 ± 3.4	24.1 ± 2.9	7.2 ± 1.0	6.0 ± 0.9	0.64 ± 0.04
UZ-1	30	55.8 ± 4.1	29.3 ± 3.3	8.1 ± 1.1	6.8 ± 1.0	0.57 ± 0.05
UZ-3	22	72.1 ± 2.8	16.4 ± 2.3	6.2 ± 0.8	5.3 ± 0.7	0.75 ± 0.03
UZ-3	26	65.9 ± 3.2	21.4 ± 2.7	6.8 ± 0.9	5.9 ± 0.8	0.68 ± 0.04
UZ-3	30	58.4 ± 3.9	26.8 ± 3.1	7.9 ± 1.0	6.9 ± 0.9	0.60 ± 0.04
UZ-4	22	65.1 ± 3.4	22.6 ± 2.8	6.9 ± 1.0	5.4 ± 0.8	0.67 ± 0.04
UZ-4	30	54.2 ± 4.3	30.8 ± 3.5	8.4 ± 1.2	6.6 ± 1.0	0.55 ± 0.05

CI: β-sheet crystallinity index (ratio of β-sheet Amide I area to total Amide I area). Full dataset for all four strains and three temperatures available in Supplementary Table S1.

Table 2. Uniaxial tensile properties of degummed silk by strain and rearing temperature (mean ± SD; n = 30 fibres per cell).

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Strain	Temp. (°C)	Linear Density (dtex)	Tenacity (cN/dtex)	Elongation at Break (%)	Initial Modulus (cN/dtex)
UZ-1	22	1.38 ± 0.06	4.98 ± 0.28	18.6 ± 2.4	78.4 ± 5.2
UZ-1	26	1.41 ± 0.07	4.37 ± 0.26	20.9 ± 2.6	70.1 ± 4.8
UZ-1	30	1.44 ± 0.08	3.72 ± 0.25	23.1 ± 2.8	61.7 ± 4.4
UZ-3	22	1.29 ± 0.05	5.41 ± 0.27	16.2 ± 2.1	84.6 ± 5.7
UZ-3	26	1.32 ± 0.05	4.79 ± 0.24	18.4 ± 2.3	76.3 ± 5.1
UZ-3	30	1.35 ± 0.06	4.12 ± 0.26	20.7 ± 2.6	67.4 ± 4.9
UZ-4	22	1.52 ± 0.08	4.84 ± 0.31	17.9 ± 2.3	76.8 ± 5.4
UZ-4	30	1.58 ± 0.09	3.91 ± 0.29	22.4 ± 2.7	62.1 ± 4.7

Full dataset for all four strains and three temperatures with pairwise Tukey HSD comparisons available in Supplementary Table S2.

4. DISCUSSION

This study provides the first systematic physicochemical and mechanical characterisation of silk produced by indigenous Uzbek B. mori strains and establishes that rearing temperature is the primary modifiable determinant of silk fibroin β-sheet crystallinity and tensile properties, with strain genotype serving as an important secondary modulator of both the absolute property values and the sensitivity of silk quality to temperature perturbation.

The mechanistic basis of the temperature–crystallinity relationship observed here is consistent with the spinning kinetics model proposed by Shao and Vollrath [5], who demonstrated that the extensional flow experienced by silk fibroin dope during passage through the silkworm's drawing zone is the critical crystallisation trigger, and that temperature modulates this process by altering dope viscosity. At lower rearing temperatures, the higher dope viscosity in the silk gland imposes greater elongational stress on unfolding fibroin chains during spinning, promoting β-sheet nucleation and crystal growth. The larger crystallite dimensions measured by XRD Scherrer analysis at 22 °C (4.21 nm vs. 3.68 nm at 30 °C) directly reflect this enhanced crystal maturation. Importantly, the elongation at break increased with temperature despite the reduction in crystallinity, because a higher amorphous fraction provides greater chain mobility and extensibility before network fracture—a classic crystallinity–extensibility trade-off in semicrystalline polymer fibres.

The performance advantage of strain UZ-3 (tenacity 5.41 cN/dtex at 22 °C) is noteworthy and warrants mechanistic investigation. Preliminary GAGAGS repeat number analysis by PCR amplification of the fibroin heavy chain (FibH) variable region suggests that UZ-3 carries a longer mean GAGAGS repeat array than the other three strains (data not shown; full genomic characterisation is in progress). Longer GAGAGS arrays increase the length of individual β-sheet-forming sequence blocks, enabling larger and more thermally stable crystalline domains [2]. This genotypic feature, combined with low rearing temperature, appears to produce a

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synergistic enhancement of β-sheet order, explaining the disproportionate tenacity advantage of UZ-3 at 22 °C relative to 30 °C. If confirmed by full FibH sequencing, UZ-3's repeat length advantage would provide a rational molecular basis for its selection as a priority parent strain in precision breeding programmes.

The positive correlation between rearing temperature and sericin content ($r = 0.81$) is consistent with transcriptomic studies of B. mori silk glands showing upregulation of sericin-1 and sericin-2 mRNA under moderate heat stress conditions [6]. Sericin is synthesised in the middle silk gland and functions as the adhesive matrix coating the paired fibroin brins; its elevated deposition at higher temperatures may represent a compensatory mechanism to maintain cocoon structural integrity when individual fibroin brins carry lower crystalline order. From a processing perspective, higher sericin content at 30 °C increases degumming loss and the volume of sericin-rich wastewater requiring treatment. However, the elevated sericin yield from high-temperature cocoons—particularly from the high-sericin UZ-4 strain—could be exploited commercially as a sericin by-product for cosmetic moisturisers, wound hydrogels, and protein film applications [7].

Our results carry direct practical implications for Uzbek sericulture management. The country's semi-arid summer climate frequently pushes field rearing temperatures above 26–28 °C in the Fergana Valley during the principal spring harvest (April–May), with peak daily temperatures reaching 32–34 °C in late May and June. The strong negative effect of temperatures above 26 °C on tenacity demonstrated here suggests that Uzbek silk currently produced under field conditions in the late spring and summer may be operating at a significant quality deficit relative to its genetic potential. Controlled-environment rearing facilities maintaining 22–24 °C for high-value silk production—which is technically and economically feasible for the premium textile and biomedical markets—could recover 20–25% of the tenacity deficit associated with warm-season field rearing. For the biomedical market, where precise control of fibroin β-sheet crystallinity is essential for tuning scaffold degradation rates and mechanical properties, temperature-controlled rearing offers an accessible means to standardise raw material quality [1].

Several limitations of this study should be acknowledged. First, the study was conducted over a single rearing season; inter-seasonal variation in leaf quality (which affects larval nutrition and silk gland biochemistry) was not controlled and may introduce additional variance in silk properties. Second, only single-end uniaxial tensile properties were measured; multi-axial and cyclic fatigue properties, which are relevant for biomedical applications, require dedicated testing protocols. Third, molecular weight distribution and GAGAGS repeat number of fibroin heavy chains were not fully characterised in all strains; their inclusion in future studies would enable a more complete genotype–property structure–function model. Fourth, the study did not examine how the temperature-induced structural changes affect the performance of silk-derived biomaterials such as fibroin hydrogels or porous scaffolds, which are increasingly of interest for the Uzbek bioeconomy [8].

5. CONCLUSION

This study establishes that rearing temperature is the dominant controllable determinant of silk fibroin β-sheet crystallinity, crystallite dimensions, tenacity, and initial modulus in indigenous Uzbek B. mori strains, with a rearing temperature increase from 22 °C to 30 °C reducing mean β-sheet crystallinity index from 0.72 to 0.58 and tenacity from 5.18 to 3.89 cN/dtex across all strains. Strain genotype significantly modulates both absolute silk quality and the magnitude of the temperature response, with the bivoltine strain UZ-3 (Fergana-3) demonstrating the highest tenacity and β-sheet crystallinity across all conditions and emerging as a priority candidate for high-performance silk production and targeted breeding programmes.

The integrated analytical framework combining ATR-FTIR secondary structure analysis, wide-angle XRD crystallinity quantification, and uniaxial tensile testing provides a replicable characterisation platform for the Uzbek sericulture industry and generates baseline data essential for quality benchmarking against commercial Chinese and Japanese standards. From an applied

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perspective, three recommendations emerge from this work: (i) controlled-environment rearing at 22–24 °C should be adopted for premium silk destined for high-value textile or biomedical applications; (ii) strain UZ-3 should be prioritised for breeding programme development targeting high-tenacity silk; and (iii) the elevated sericin yield of strain UZ-4 at higher temperatures represents an underexploited bioeconomy opportunity warranting dedicated extraction and valorisation studies.

Priority directions for future research include: full genomic characterisation of the fibroin heavy chain GAGAGS repeat architecture in all indigenous Uzbek strains; transcriptomic profiling of the silk gland response to temperature in UZ-3 relative to lower-performing strains; evaluation of silk fibroin scaffolds derived from temperature-controlled UZ-3 cocoons for bone and cartilage tissue engineering applications; assessment of sericin from UZ-4 as a functional cosmetic and wound care ingredient; and, critically, field-scale evaluation of controlled-environment rearing economics in the context of the Uzbek national sericulture programme.

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