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ISHQORIY ISHLOV BERISHNING IPAK FIBROINI TUZILISHIGA TA’SIRI
ВЛИЯНИЕ ЩЕЛОЧНОЙ ОБРАБОТКИ НА СТРУКТУРНЫЕ ОСОБЕННОСТИ ШЕЛКОВОГО ФИБРОИНА

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Annotatsiya. Ushbu tadqiqotda 3 %li kaliy gidroksid (KOH) eritmasi yordamida mikroto'lqinli ishlov berilgan ipak fibroinining strukturaviy o’zgarishi tizimli ravishda o'rganildi.Strukturaviy modifikatsiyalar rentgen difraksiyasi (XRD), Furye konvertatsiya infraqizil spektroskopiyasi (FTIR), ultrabinafsha ko'rinadigan (UV-Vis) spektroskopiyasi va molekulyar og'irlik tahlili yordamida tavsiflandi. Natijalar ishqoriy va mikroto'lqinli ishlov berish ipak fibroinining strukturaviy tuzilishiga sezilarli ta'sir ko'rsatganligini ko'rsatdi. Molekulyar og'irlikning 233 kDa ga pasayishi va kristallik va ikkilamchi tuzilishdagi o'zgarishlar o'rtasida yaqin bog'liqlik aniqlandi

Kalit so‘zlar: Ipak fibroini, KOH bilan ishlov berish, mikroto'lqinli nurlanish, rentgen difraksiyasi (XRD), Furye konvertatsiya infraqizil spektroskopiyasi (FTIR), molekulyar og‘irlik, ultrabinafsha spektroskopiyasi

Аннотация. В данном исследовании систематически изучалась структурная эволюция фиброина шелка, обработанного микроволнами с использованием 3%-ного раствора гидроксида калия (KOH).Структурные изменения были охарактеризованы с

помощью рентгеновской дифракции (XRD), инфракрасной спектроскопии с преобразованием Фурье (FTIR), ультрафиолетово-видимой спектроскопии (UV-Vis) и анализа молекулярной массы. Результаты показали, что щелочная и микроволновая обработка оказывают значительное влияние на структурную структуру фиброина шелка. Была обнаружена тесная корреляция между уменьшением молекулярной массы на 233 кДа и изменениями кристалличности и вторичной структуры.

Ключевые слова: фиброин шелка, обработка KOH, микроволновое облучение, рентгеновская дифракция (XRD), инфракрасная спектроскопия с преобразованием Фурье (FTIR), молекулярная масса

Abstract. In this study, the structural evolution of silk fibroin treated with microwaves using 3% potassium hydroxide (KOH) solution was systematically studied. The structural modifications were characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible (UV-Vis) spectroscopy, and molecular weight analysis. The results showed that alkaline and microwave treatments had a significant effect on the structural structure of silk fibroin. A close correlation was found between the decrease in molecular weight by 233 kDa and the changes in crystallinity and secondary structure. The sorption properties of the resulting fiber are expected to improve

Keywords: Silk fibroin,KOH treatment, microwave irradiation, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), molecular weight.

Introduction. Silk fibroin (SF), the main structural protein of Bombyx mori silk, has attracted considerable scientific attention due to its unique combination of high mechanical strength, excellent biocompatibility, and tunable physicochemical properties. [1–3].The molecular architecture of silk fibroin consists of a heavy chain (~390 kDa), a light chain (~26 kDa), and a P25 glycoprotein, which assemble into a stable complex through disulfide, hydrophobic interactions. The heavy chain mainly contains highly repetitive amino acid sequences such as -Gly–Ala–Gly–Ala–Gly–Gly–Ser-, which readily form β-sheet crystallites. These crystal domains are located in less ordered amorphous regions composed of tyrosine, valine, aspartic acid, and other non-repetitive amino acids. The coexistence of crystalline and amorphous regions provides silk fibroin with an excellent balance between thermal stability, mechanical strength, elasticity, stability, and biodegradability [2,4,5].

Literature review and methods.

Alkaline treatment is one of the most effective chemical approaches to tailor the molecular structure of silk fibroin, as hydroxide ions easily penetrate the amorphous regions of the protein matrix. As a result, peptide bonds are cleaved by hydrolysis and partial depolymerization occurs, resulting in a decrease in molecular weight and rearrangement of the remaining molecular chains. Therefore, the final structural properties of silk fibroin depend on factors such as exposure time, alkali concentration, temperature, and the internal organization of the protein

network [7–8].Microwave processing has recently emerged as an environmentally friendly and energy-efficient alternative to traditional thermal treatment. Unlike conductive heating, microwave radiation interacts with polar molecules such as water and amino acid side chains to generate bulk dielectric heating. As a result, it accelerates molecular mobility, enhances the diffusion of alkaline solution into the fiber, and promotes structural reorganization in a short time[6,9,10]. Therefore, combining alkaline treatment with microwave radiation is expected to lead to structural improvement of silk fibroin.

The structural changes of silk fibroin during chemical treatment are usually evaluated using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). XRD provides information on crystal polymorphs, diffraction peak positions, crystal packing, and crystallinity. In addition, complementary information on long-range crystal order and short-range molecular conformations can be obtained by combining XRD and FTIR, which allows for a complete understanding of the structural transformation processes [3,6,9,11].XRD and FTIR provide valuable structural information, but molecular weight determination plays an important role in determining the hydrolysis mechanism of silk fibroin. The decrease in molecular weight reflects the cleavage of the peptide chain caused by alkaline hydrolysis and provides quantitative evidence of depolymerization. Meanwhile, ultraviolet (UV–Vis) spectroscopy provides additional information on the changes associated with aromatic amino acid residues. Combining crystallinity analysis with molecular weight determination and UV spectroscopy allows for a more complete assessment of the relationship between molecular hydrolysis and structural change [3,7,10]

Required materials and equipment. Sodium carbonate (purity 99,9%), Fibrous waste of silk (Cleaned of additives. Urganch Bahmal, LLC, Urgench, Uzbekistan), KOH (purity 99,9%) was purchased from Chimreaktivinvest (Uzbekistan). Calcium chloride and ethyl alcohol (98%) were purchased from Fortek company (Uzbekistan). Bidistilled water is obtained from the “GFL 2104 Double distillation water still” device (Germany).Ultra-high frequency radiation was performed on a ME81ARW (Samsung) device at a frequency of 2450 MHz. UV-spectra were obtained on UV-1800 Shimadzu spectrometer, Thermostat spare parts (Assistant cat. № 3180) (Hamburg, Germany).Crystallinity degree of the samples were measured by powder x-raydiffraction (XRD, Rigaku, The SmartLab SE, Japan(Khorezm Mamun Akademy lab)), ATR-IR Fourier spectroscopy analyses were performed on a “FTIR 4600 JASCO” (Japan) spectrophotometer.

Obtaining fibroin fiber from natural silk fiber waste. Studies are aimed at using silkworm waste as a sorbent in the purification of vegetable oils. It was found that silk fiber waste contains up to 85-88% fibroin, up to 12-15% sericin and other substances. Conditions for obtaining fibroin from silk fiber waste were selected: sericin was dissolved in a NaHCO3 solution at 85-90°C for 45 minutes [12]. Fibroin fibers were removed from the solution and washed with distilled water to a neutral environment.

Obtaining “HF” from silk fibroin. Additional studies were conducted on the hydrolysis of fibroin fibers from silk fibers and their sorbent properties. Silk fiber fibroin was hydrolyzed under alkaline conditions and microwave irradiation (510 W) to obtain “HF” in the form of fibers [13]. The extraction of “HF” in an alkaline environment was carried out by immersion in a 3% KOH solution. In this case, it took 60 minutes to extract “HF” from fibroin fibers in a 3% KOH solution. Structural changes of “HF” fibers were studied. The fact that “HF” has ampholytic properties and the presence of multifunctional groups allows it to be used in various environmental conditions. To further increase the cracks and porosity in the “HF” particles, the “HF” powder was exposed to microwave(MW) radiation for 5 minutes after immersion in an alkaline medium and in a wet state. After the procedure, the sample was neutralized and washed with hot distilled water until the salt remained. As the pores on the “HF” surface increase, its sorption properties also increase.

Determination of molecular mass of “HF” sample obtained alkaline medium by MW rays by spectrophotometry .The measured mass of “HF” fiber was dissolved in a solution of CaCl2:C2H5OH:H2O (1:2:8 mol) at 65 ^{o}C. To determine the molecular mass of “HF” obtained under laboratory conditions, solutions with concentrations of 0.11, 0.125, 0.15, 0.19 and 0.25 g/L were prepared. The UV-1800 Shimadzu spectrophotometer showed that the maximum absorption wavelength of the prepared “HF” solution was 280 nm. The light absorption was determined in a 1 cm thick (l) cuvette[14].

Results and discussion

Silk fibroin fibers swell better in alkaline environments[15], so it was planned to treat the fibers with microwaves by immersing them in a KOH solution. As a result, polar substances in the fibers immersed in the alkaline solution are released from the fibers by microwave exposure, which at high temperatures causes cracks in the fibers and increases the porosity of the fibers. The number of sorption centers also increases.

The UV spectra of “HF” obtained were studied (Figure 1). The light absorption values of “HF” solutions at 280 nm were determined and the A/l and C correlation graphs were plotted (Figure 2). To determine the molecular mass of “HF”, it is necessary to determine the angular length (α) of the graph line A/l—C concerning the C axis. Molecular mass is determined by the following formula(1).

(1)
$$M = \frac{\epsilon}{tg\,\alpha}$$

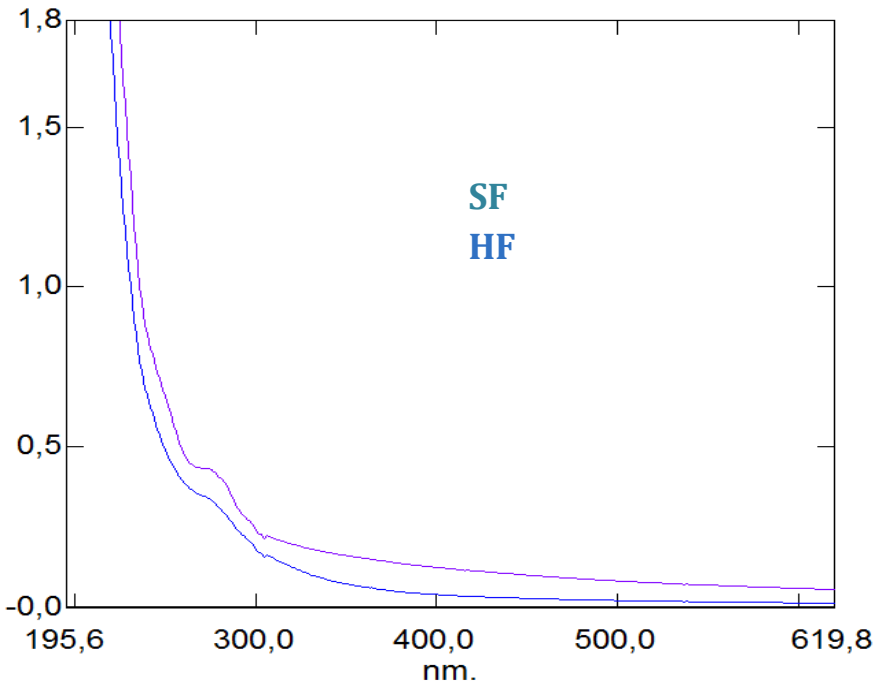


Figure 1: UV spectra of Silk fibroin(SF) and "HF" solution obtained under the influence of MW radiation

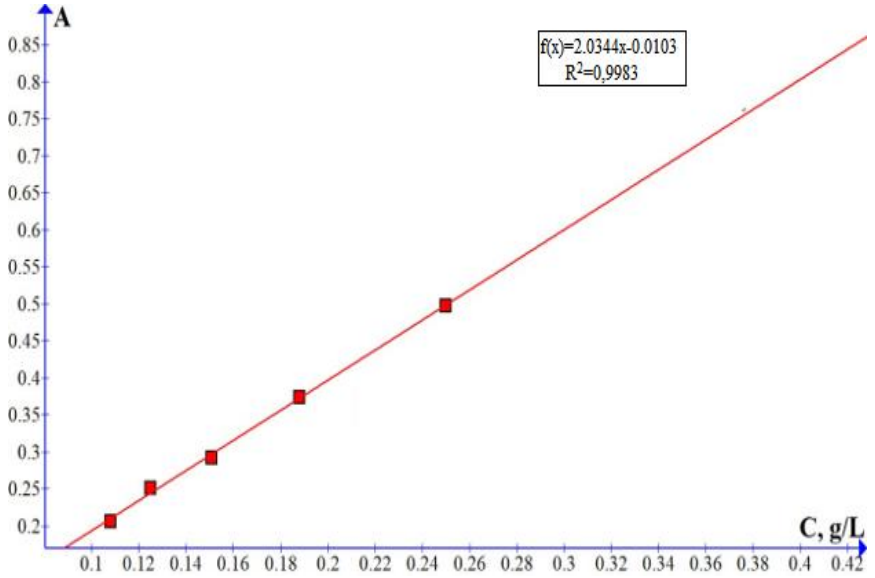


Figure 2. Graph of the dependence of A/l and C of the "HF" solution obtained under the influence of MW radiation

Based on the results obtained by spectrophotometry, it was determined that the molecular mass of "HF" obtained under the influence of MW rays in an alkaline medium (equation 1) is 233.3 kDa. The decrease in molecular weight can be explained by the hydrolysis process.

ATR-IR Fourier transform spectroscopy analyses were performed using a JASCO FTIR 4600 spectrophotometer and were conducted in the 4000–400 cm-1 range [16]. In this study, samples of silk fibroin waste fiber from which sericin was removed and fiber samples obtained by microwave irradiation hydrolyzing in an alkaline medium were analyzed together.

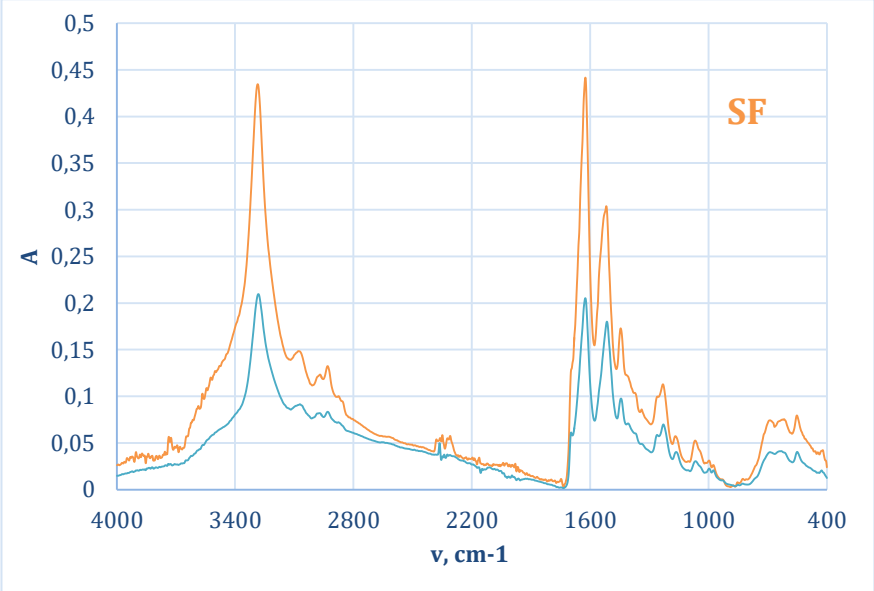


Figure 3. FTIR spectra of silk fibroin and alkali1ne hydrolyzed fibroin(by MW radiation)

The FTIR spectra of silk fibroin (IF) fiber and hydrolyzed fibroin (HF) samples were studied, which were immersed in a 3% KOH solution and then treated with microwave radiation, to determine the changes in their chemical structure. A broad absorption band was observed in the region of 3281–3285 cm-1 in both samples. These bands correspond to the O-H vibrations of the hydroxyl groups in the fibroin molecule, as well as the N-H stretching vibrations of the peptide bonds. After microwave irradiation of fibroin immersed in a KOH solution, the change in intensity in this band indicates the reorganization of the hydrogen bonding system.

The strong absorption band located around 1624 cm-1 corresponds to the Amide I region of fibroin, and the absence of a significant shift in this range indicates that the main polypeptide chain of fibroin is preserved. However, changes in the shape and intensity of the peak may indicate changes in the amorphous phases and β-sheet ratio.The absorption band observed in the range of 1514–1519 cm^{-1} belongs to the Amide II region, which is formed by N–H deformation and C–N stretching vibrations. A slight decrease in the intensity of this peak in the KOH-treated sample indicates a partial disruption of the hydrogen bonds around the peptide bonds. The absorption observed at 1443–1445 cm-1 corresponds to the deformation vibrations of the -CH2- groups and The preservation of this peak indicates that the main chain skeleton of the fibroin molecule is not damaged even after microwave irradiation. The absorption band in the range of 1229–1231 cm-1 corresponds to the Amide III band and is associated with C–N stretching and N–H deformation vibrations. In the literature, this band is considered an important parameter in assessing the secondary structure of fibroin, especially its crystal structure. Changes in this band after treatment with KOH and ultrahigh-frequency radiation indicate a partial reorientation of fibroin macromolecules.

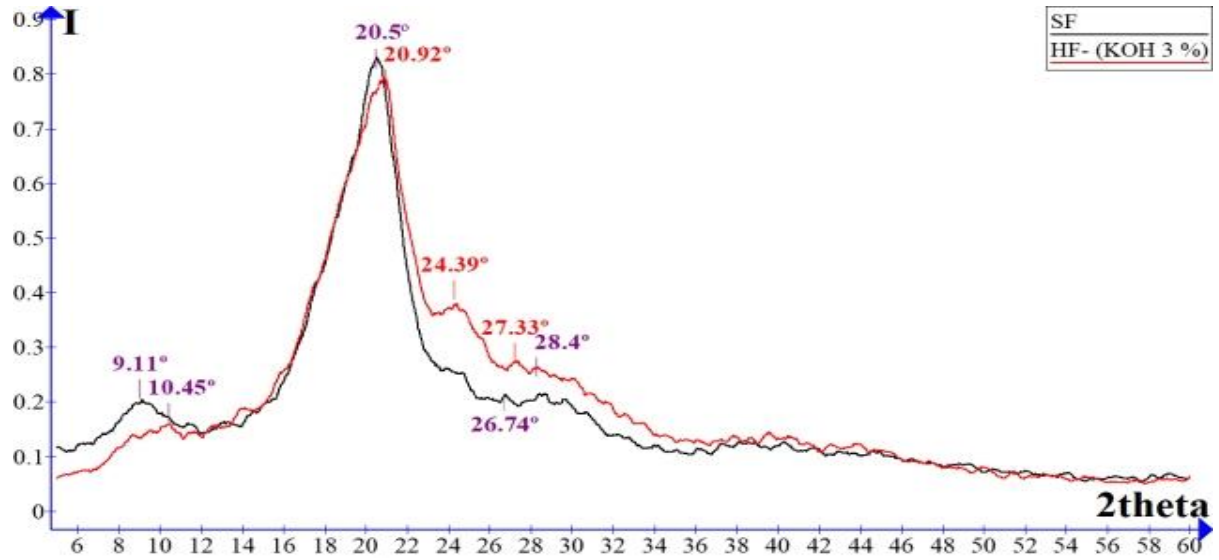


Figure 4. XRD patterns of SF and HF-(KOH 3%)

The XRD patterns of SF and HF-(KOH 3%) samples reveal the characteristic semi-crystalline structure of silk fibroin. The dominant diffraction peak located at 2θ = 20.5–20.9° corresponds to the β-sheet crystalline domains (Silk II structure). After treatment with 3% KOH followed by microwave irradiation, the main reflection shifted from 20.50° to 20.92° and exhibited a slight increase in intensity, indicating enhanced molecular packing and improved crystalline ordering. The calculated interplanar spacing decreased from 4.33 Å to 4.24 Å, suggesting a more compact arrangement of fibroin chains. Furthermore, additional reflections observed at 24.39°, 27.33°, and 28.40° indicate the development of more ordered crystalline regions. The reduction of the low-angle reflection around 9–10° implies a partial transformation of less ordered Silk I/amorphous domains into the thermodynamically more stable Silk II structure. These findings demonstrate that alkaline treatment combined with microwave irradiation promotes molecular rearrangement and increases the crystallinity of silk fibroin.

Conclution. Based on these considerations, the simultaneous application of potassium hydroxide (KOH) solution and microwave irradiation enhances the selective hydrolysis of amorphous regions, resulting in peptide chain cleavage and molecular weight reduction. The molecular weight of HF fiber was determined to be 233.3kDa by spectrophotometric method. The structural evolution of silk fibroin treated with KOH solution under microwave irradiation was analyzed by X-ray diffraction (XRD) analysis of crystalline and amorphous parts, which revealed that the crystallization degree of the sample was 38%. This also affects its sorption property. It was systematically studied by integrating Fourier transform infrared spectroscopy (FTIR), molecular weight determination and ultraviolet-visible spectroscopy (UV-Vis). It was assumed that fibroin was partially hydrolyzed, while maintaining its basic protein structure. Through processing, HF fiber, which has increased sorption centers than SF, can be used in various industrial sectors such as cottonseed oil dusting for various sorption purposes.

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