METHODS OF EXTRACTING SERICIN FROM NATURAL SILK WASTE.

Khozhaniyazov Azamat Ruzimbaevich [1]
Matyakubova Dinara Shuxratovna [1]
Oygul Razzoqberganovna Karimova [1]
Abdullayeva Xafiza Davronovna [1]
Baltaeva Mukhabbat Matnazarovna [2]
[1] Urgench branch of the Tashkent Medical Academy
[2] Urgench State University
Login: x.azamat1992@gmail.com
dinarashuxratovna090588@gmail.com
oygul7713@gmail.com
abdullayevaxafiza355@gmail.com
https://doi.org/10.5281/zenodo.15031103

Abstract

An indispensable element in enhancing the technological efficacy of the silk industry lies in the refinement of cocoon raw material processing, particularly in augmenting the yield of raw silk. Equally significant is the comprehensive utilization of byproducts stemming from natural silk production, given that the current waste output per kilogram of raw silk exceeds one kilogram. Merely a third of the total fibrous waste derived from natural silk is repurposed for silk yarn production, with the majority being exported overseas due to the absence of efficient disposal techniques and insufficient manufacturing capacity. This underscores the technical, economic, and scientific viability of advancing endeavors in integrated waste management, encompassing the creation of biologically active compounds, novel protein and non protein derivatives from natural silk remnants, and their application across diverse sectors of the domestic economy. This trajectory appears to be a promising avenue, setting the stage for the pertinence of the chosen research focus.

Keywords: cocoon, sericin, outer layer, hydrolysis, mulberry, composition, glycine, waste of natural silk.

Introduction

At present, in order to solve problems related to the utilization of cocoon winding waste and the creation of a waste-free technology for processing cocoons, we have conducted research on obtaining biologically active substances from cocoon winding production waste [1-5]. The purpose of the work is the comprehensive use of all components of cocoon raw materials, the production of which has so far required very significant material and labor resources, the creation of resource-saving and waste-free technologies, the development of a method for isolating biologically active substances from cocoon winding production waste and the study of their use in medicine. Theoretical research. The cocoon consists of five layers. Each layer is woven from double silk thread (70-80%), fastened with silk glue (20-25%). Another 2-3% of the cocoon is accounted for by waxes (0.4-0.8%), carbohydrates (1.2-1.6%), pigments (0.2-0.4%). Double silk thread (mulberry) consists of water-insoluble protein fibroin, the structure of which has already been well studied. As for the “glue”, until recently the ideas about its chemical structure were very vague. It was known for sure that this substance is of protein origin. It was even given the name sericin. Sericin covers the surface of natural silk threads with a thin layer and is a high-molecular compound - a biopolymer related to proteins of the β -structure. Unlike fibroin, sericin is easily soluble in water. The high content of polar groups capable of hydration and the low order in the arrangement of chains with weak intermolecular action give sericin properties similar to animal glue, which is why it was called silk glue. Initially, when the worm's glands secrete silk threads, fibrillar sericin is formed. But later, under the influence of secretion, a large number of potassium compounds and under the influence of air, moisture, and pressure changes, its thin surface layer is destroyed. This is facilitated by the presence of a large number of active amino acid groups and resembles the formation of the surface layer of the amorphous coating of tree bark, consisting of short fibrils. The amorphous coating of the silk thread is so thin and loose that even at normal temperatures it dissolves, albeit slowly, in water. In all cases, in treated and untreated cocoons, changes mainly occur in the upper sericin part and do not affect the internal fibrillar parts, and do not affect the ordered structure. The sericin component is located on the surface of the threads, its macromolecules are less fibrous than fibroin macromolecules. As a result, sericin is easily removed during separation and almost without destroying fibroin [6]. Silk biopolymers are widely represented and find application in pharmaceuticals, medicine and electronics, including the creation of drug carriers, artificial tissues, flexible electronic diagnostic devices and implantable optical systems [7,8]. There are known works [9-14] aimed at the utilization of silk waste to obtain sericin powder from them by extraction at high temperature, where alkali or acid is used as solvents. A number of studies are being conducted in the laboratory of the Tashkent branch of the Uzbek Research Institute of Natural Fibers on the isolation and use of silkworm sericin [15-17]. Experimental study. Complete isolation of sericin from cocoon shells presents significant difficulties, because during the isolation of sericin, fibroin can also dissolve. The chemical properties of fibroin and sericin are very similar. As a result of sericin removal in different ways, the properties of silk change. When unwinding cocoons to obtain silk raw materials, their proteins, which have a high ability to absorb water and swell, as well as dispersed proteins (i.e. sericin) are dissolved and removed, while inorganic substances and pigments also dissolve, turning into colloidal solutions. Sericin obtained as a result of de-gluing silk is not individual, but is a mixture of at least two substances that can be separated by fractional precipitation. Separation of sericin solutions into fractions is carried out either by salting out with ammonium sulfate, or by precipitation with alcohol, or by acidifying the solutions to the isoelectric point of sericin. The structure of sericin has not yet been fully studied and awaits further study. Knowledge of the structure of sericin is necessary for silk processing. For example, in order to finally solve the problems of cocoon unwinding, processing and unwinding of raw silk, it is first necessary to study the degree of sericin dissolution, the isoelectric point of sericin, the changes that sericin undergoes, and other issues. Only in this case is it possible to continue rational cocoon steaming and thread unwinding, which will make it possible to achieve optimal flow of silk processing production processes. Complete removal of sericin from cocoon winding production waste presents significant difficulties, because fibroin can also dissolve during sericin extraction. The chemical properties of fibroin and sericin are very similar. As a result of sericin extraction in different ways, the properties of silk change. When waste is boiled, its proteins, which have a high ability to absorb water and swell, as well as dispersed proteins (i.e. sericin) dissolve and are removed. Inorganic substances and pigments also dissolve, turning into colloidal solutions. Several methods for sericin extraction are given below. Method of extraction with ethyl alcohol. Fibrous waste from cocoon winding production is boiled in distilled water for 60 minutes at a module of 1:50. In a solution of sericin layers ethyl alcohol is gradually added to the cocoon shell, and in this process two different types of sericin are separately precipitated. One, which has a comparatively high degree of solubility in water, and is mainly found in the outer layers of the cocoon shell, is called sericin "A". The other, which has a comparatively lower degree of solubility in water, and is mainly found in the inner layers of the cocoon shell, is called sericin "B". The principle of isolation is based on the different degrees of solubility in alcohol of these two types of sericin. The method of isolation with ammonium sulfide salts. Solid ammonium sulfide is added to the sericin solution in an amount of 15% of the weight of the sericin solution, and the sericin isolated in this way is called sericin "A". Then ammonium sulfide is added again to the filtered solution, and the sericin isolated is called sericin "B". Take 4-6 grams of cocoon-winding fibrous waste and place it in 200 ml of distilled water and, using a reverse water cooling system to prevent the amount of water from decreasing, boil it for 30 minutes and then filter it. At this point, in addition to scattered sericin micelles, the filtered solution also contains a small amount of electrolytes, pigments and other organic compounds. Before the solution has completely cooled, an equal volume of saturated ammonium sulfide solution is slowly added to the walls of the vessel. When the saturation reaches approximately 50%, then sericin “A” will coagulate earlier and float on the surface of the solution, while sericin “B” will gradually precipitate and will consistently fall as a sediment to the bottom of the vessel. After standing for ten hours, two types of sericin can be seen very clearly separated. These two types of sericin can be separated based on the shape of the sediment and

places of concentration. Sericin "A" is slightly transparent in appearance, with high polymerization, has a large sediment in the form of pieces floating on the surface of the solution. Sericin "B" is a white powder deposited on the bottom. If it is necessary to select these two types of sericin separately, you can add a saturated solution of ammonium sulfide to the solution until saturation is 1/3. The sericin curdled at this point will be sericin "A". If after filtration you continue to add a saturated solution of ammonium sulfide, another sediment appears, a significant part of which will be sericin "B". Then wash in sulfuric ether and methylcarbanol. sulfuric ether and dry in a vacuum low-temperature dryer. Isoelectric method of sericin isolation. Acetic acid is added dropwise to the sericin solution until the pH reaches 4.1. At this point, sericin "B" thickens and precipitates. After concentration of the filtered solution, 50% alcohol is added and the thickened sericin will be sericin "A". The chemical composition of the obtained sericin is determined. Sericin consists of five chemical elements: carbon, hydrogen, oxygen, nitrogen and sulfur. However, its molecular formula has not yet been established. The results of the analyses given in Table 1 gave different molecular compositions. These discrepancies are caused by the multiple cocoons and the greater difficulty in obtaining pure sericin.

IBMSCR | Volume 5, Issue 03, March

Table 1.

Chemical composition of silkworm sericin

Name of elements	Quantity, %
Carbon	44.32 – 46.29
Hydrogen	5.72 – 6.42
Nitrogen	16.44 – 18.30
Oxygen	30.35 – 32.50
Sulfur	0.15

Until now the group – the amino acid obtained after hydrolysis of sericin was not completely the same. In table 2 research data are provided, By definition composition amino acids, carried out in cocoons of the L-46 and L-2 breeds 8 . IN In L-46 cocoons, sericin does not contain sulfur, and in fibroin it is present, as for example, the presence of methion. However, in L-28 cocoons it is the opposite. This discrepancy is caused by different varieties of cocoons.

The main products of sericin hydrolysis

		Л-28 %	Vietnamese breed %	Chinese breed %
Glycine	0.2	1.5	1,2	3.93
Alanine	5.0	9.2	9.2	3.53
Leucine	-	4.8	5.0	0.40
Asparagine	-	2.8	2.5	3.91
Proline	-	3.0	2.5	0.35
Propion	6.6	5.4	5.8	5.99
Glutamine	-	1.8	2.0	3.00
Tyrosine	5.0	1.0	2,3	3.2
Phenylalanine	-	0.3	0.6	0.49
Arginyl	4.0	-	-	-
Histidine	traces	-	-	-

As for the main products of sericin hydrolysis of different types of silk raw materials, then there are also some discrepancies, which are indicated in Table 2. Based on the results analysis indicated V table 2 Can see, What some difference V content amino acids called a different varieties silk cheese or breed. Even even with the same type of cocoons there is a difference in the composition of sericin external And internal layers shells IN table 3 show content amino groups after hydrolysis sericin internal And external layers shells.

Table 2

Comparative data of amino acids obtained after hydrolysis

Name amino acids	The amount of amino acids in the sericin of the cocoon shell, %	
	outer layers	inner layers
Glycine	29.90	5.77
Alanine	9.21	8.49
Leucine	2.30	0.73
Asparagine	2.74	6.81
Glutamine	2.94	traces
Propion	6.33	2.56
Phenylalanine	2.60	2.66
Tyrosine	2.85	5.25

IN s e r i c i n e on the outside layers o h e a n d s the glycine content is higher than in the sericin of the inner layers of the shell . Content sericin V layers cocoon shells are very common It happens uneven V the heterogeneity of cocoon varieties in the shell layers. In silk The content of sericin in raw materials also varies because of different ways unwinding, steaming cocoons. Basically, the least in Chinese varieties, relatively many V ours, most of all in Vietnamese varieties. In further research learned sericin content in the outer, inner and middle layers of the shell cocoon. Results experiments are presented in Table 4.

Table 3

The influence of climate and feed type on sericin content

Cocoon breed	District	Feed mulberry	Sericin content in cocoon shell layers, %
L-46	warm	Low cut off	21.82
Chinese	cold	High cut off	20.55
Vietnamese	warm	Low cut off	21.04

From tables 5 it is visible, What cocoons caterpillars fed low cropped mulberries contain more sericin than cocoons caterpillars fed high cropped mulberry. The cocoons of caterpillars grown in warm regions contain more sericin than the cocoons of caterpillars grown in cold regions. Summarizing the results of all the above experiments, the following conclusions can be made. The following factors influence the quantitative content of sericin in natural silk waste:cocoon breed; layers of the cocoon shell. The amount of sericin gradually decreases from the outer layers to the inner layers of the shell;

- district feedings . Cocoons caterpillars raised in warm regions contain more sericin than cocoons caterpillars, fed V cold areas;
- type stern. Cocoons caterpillars, low- fed cropped mulberry contain usually more sericin, how cocoons caterpillars , high - fed trimmed mulberry.

Conclusions. The paper presents methods for extracting sericin from cocoons mulberry silkworm. Three methods of isolation are given: with ethyl alcohol, salts sulfurous ammonium And isoelectric method. Conducted analysis elemental composition of sericin. The influence of various factors on the quantitative content of sericin has been established.

List of references:

1. S.D. Komilova. Pillachilik sanoati chiqindilaridan kompleks foydalanish. J. Agro biznes inform. № 05/136. 2018 y. 28-29 b.

2. SH. Belgibaeva, Z.K. Galimova, S.D. Komilova. Kachestvo pechati na bumage, soderjaщey otxodы kokonomotalnogo proizvodstva. Respublika ilmiy - amaliy anjumani maqolalar toʻplami.TTESI, 2017 y. 12-13 dekabr. 345 b.

3. S.D.Komilova, A.S.Fedunina, S.Valiev, X.Yalgashev. The research on the extraction of fatty substances from non- textile residues of the cocoon milling production. International Journal of Academic Research and Development. Impact Faktor: RJIF 5.22. v.3, Issue 2, 2018. p. 1748-1749.

4. S.D. Komilova, B.S. Tulyaganov. Ipakchilik sanoatining rivojlanishi. J. Agro biznes inform. № 02/145. 2019 y. 26 b. 5. S.D. Komilova. B.S. Tulyaganov. Tabiiy ipakning shifobaxsh xususiyatlari. J. Agro biznes inform. № 04/148. 2019 y. 20 b.

6. Takasu Y., Yamada H., Tsubouchi K. Isolation of three main sericin components from the cocoon of the silkworm. Biochi. Biotechnol. Biochem. 2002, v 66..2715-2718.

7. Kundu B., Kurland N.E. Isolation and processing of silk proteins for biomedical applications. Int. JournalofBiol. Macromolecules. 2014, v.70, pp. 70-74.

8. Ishmatov A.B. Vliyanie kolichestva ostatochnogo seritsina na kachestvo shelka-sыrsa.-Izv. Vuzov. Texnologiya teks. promыshlennosti. 201yo2, № 3, s. 31-34.

9. Ishmatov A.B., YAminova Z.A. i dr. Obosnovanie rejimov polucheniya seritsina v vide poroshka dlya prigotovleniya shlixtы.-Texnologiya tekstilnoy promыshlennosti. 2015. № 6, s. 79-80.

10. http: // www. find Patent.ru/patent /182/ 1826999/ html. Poluchenie poroshka iz naturalnogo shelka. Semenov N.I., YAnukovich V.P.

11. http: //findPatent /201/ 2011697. html. Cposob polucheniya poroshka iz naturalnogo shelka. Karpov A.M., Kolinko S.I., Voronov V.I.

12. Terada S. et al. Preparation jf silk protein sericin as mutagenic factor for better mammalian cell culture. J. Biosci. Bioeng. 2005.

13. Mooneu A.C., Robertson H.M., Wanner K.W. Neonate silkworm larvae are attracted to mulberry leaves with con-specific feeding damage. J.Chem. Ecol. 2009.

14. Yanagihara K, et al Effect of the silk protein sericin on the production of adenovirus-based gene-therapy vectors . BiotechnolApplBiochem. 2006.

15. Komilova S.D., Tashkenbekova M.J. Seritsin va uning xossalari. J.Agrobiznes . №3.2020.

16. Komilova S.D., Fedyunina A.S. Izuchenie sposoba sushki seritsina. “Toʻqimachilik tolalarini chuqur qayta ishlashning muammolari va echimlari”. Respublika ilmiy-texnkaviy anjumani, Margʻilon, 19-20 oktyabr, 2020 yil. 34 b.

17. Komilova S.D., Fedyunina A.S. Izuchenie rastvorimosti seritsina. “Toʻqimachilik tolalarini chuqur qayta ishlashning muammolari va yechimlari”. Respublika ilmiy-texnkaviy anjumani, Margʻilon, 19-20 oktyabr, 2020 yil. 36 b.

IBMSCR | Volume 5, Issue 03, March