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Silk Beyond Fabric : Exploring the Diversity of Tasar Byproducts

Edited by :
**Dr. Karmabeer Jena, Dr. Jay Prakash Pandey,
Dr. Jitendra Singh and Dr. N.B. Chowdary**

CSB-Central Tasar Research and Training Institute
**Central Silk Board, Ministry of Textiles, Govt. of India
Ranchi-835303, Jharkhand**

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Dr. N.B.Chowdary
Director,
CSB-Central Tasar Research and Training Institute,
Central Silk Board, Ministry of Textiles
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Editors :

Dr. Karmabeer Jena

Dr. Jay Prakash Pandey

Dr. Jitendra Singh

Dr. N.B.Chowdary

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Ranchi-835303, Jharkhand

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Member Secretary

केन्द्रीय रेशम बोर्ड / Central Silk Board
वस्त्र मंत्रालय / Ministry of Textiles
भारत सरकार / Govt. of India

FOREWORD

I'm delighted to announce the release of the book titled "Silk Beyond Fabric: Exploring the Diversity of Tasar Byproducts" on the occasion of 75 years of Central Silk Board. Recently, CSB-Central Tasar Research and Training Institute (CSB-CTRTI) has achieved significant breakthroughs in the realm of tasar byproducts. Alongside the primary product, silkworm cocoons, the sericulture industry yields a range of secondary products such as pupae, exuviae, faeces, fibre waste, cooking water, and grainage waste. These byproducts hold considerable potential in sectors like pharmaceuticals and cosmetics. By strategically harnessing these secondary and waste products, sericulture can extend its impact beyond textiles, thereby enriching the entire tasar silk value chain. To address rising production costs, collaborative research initiatives have been launched to explore the utilization of these byproducts. Innovations include advancements like cocoonase, sericin extraction, use of pupae for human and animal consumption, industrial applications of chitin, and production of *Cordyceps* from seri-waste. These initiatives aim to bolster the involvement of primary producers throughout the tasar value chain.

The collective efforts in research and development, alongside recent initiatives, are poised to enhance sustainable livelihoods, particularly benefiting women and tribal communities. I'm pleased that CSB-CTRTI is prioritizing the utilization of tasar silk waste, such as pupae for fish feed, *Cordyceps* production, chitosan preparation, and pigment extraction. Additionally, methods like using cocoon cooking wastewater for sericin extraction and cocoonase for softening cocoons during reeling are proving effective. This approach not only boosts profits by approximately 30-40% but also ensures responsible management and disposal of silk waste. From my perspective, sericulture byproducts represent substantial potential for generating profitable employment and livelihood opportunities across the silk value chain. I commend everyone involved in this sector for their commitment to sharing vital information and research insights. The publication of this book documenting tasar byproduct research will undoubtedly catalyse the development of research strategies, facilitate efficient technology transfer, and foster valuable partnerships.

I believe that collaborative learning about byproducts and joint planning with esteemed organizations will forge strong connections between CSB and other stakeholders in the sector. This collaboration promises to accelerate R&D efforts and significantly contribute to the advancement of the tasar silk industry in the coming years. I appreciate Dr. N.B. Chowdary, Director of CSB-CTRTI, Ranchi, and his team for their meticulous documentation of the byproduct concept.

I am confident that this book will effectively achieve its objectives by consolidating R&D insights on tasar byproducts into a cohesive platform. I extend my best wishes to the researchers and all collaborators involved in their comprehensive efforts on this critical subject matter.

P. SIVAKUMAR

के.रे.बो.-केन्द्रीय तसर अनुसंधान एवं प्रशिक्षण संस्थान
(केन्द्रीय रेशम बोर्ड, वस्त्र मंत्रालय, भारत सरकार)
पिस्का-नगड़ी, राँची - 835 303 (झारखण्ड)
CSB-Central Tasar Research & Training Institute
(Central Silk Board, Ministry of Textiles, Govt. of India)

Preface

"Silk Beyond Fabric: Exploring the Diversity of Tasar Byproducts" goes beyond the traditional view of Tasar silk as just a luxurious textile, diving into its diverse and often overlooked byproducts. This exploration highlights the dedication and creativity of researchers who have uncovered the many potential uses of these byproducts across various industries. Chapter 1 provides a comprehensive introduction to the byproducts of Tasar silk production, showcasing their applications beyond textiles and setting the stage for a deeper exploration of these materials. Chapter 2 examines the waste from Tasar food plants, turning what is often discarded into opportunities for innovation. This biomass, crucial to silkworm farming, is explored for its potential in medicinal research, blending traditional knowledge with modern science to promote sustainable practices and health advancements. Chapter 3 focuses on cocoonase, an enzyme with transformative applications beyond its role in silk production. Its diverse industrial uses are discussed, highlighting its potential across various sectors. Chapter 4 reveals the nutritional value of silkworm pupae, presenting them as a sustainable source of nutrients for both human consumption and animal feed. The chapter emphasizes the bioactive compounds in pupae as a solution to global food security and sustainable agriculture challenges. Chapter 5 highlights the applications of sericin and fibroin beyond silk production. These natural proteins are valuable in cosmetics, biomedical engineering, and sustainable materials, showcasing their impact on various industries. Chapter 6 explores chitosan nanoparticles derived from Tasar silkworm pupae, emphasizing their potential in biomedical and environmental applications. These nanoparticles offer controlled drug delivery and environmental remediation solutions, underscoring their versatility and eco-friendly nature. Chapter 7 introduces an innovative use of Vanya silkworm refuse for cultivating *Cordyceps* fungi. This method not only converts waste into valuable medicinal products but also promotes economic opportunities and ecological sustainability. Chapter 8 delves into the biomedical applications of silk proteins, focusing on their roles in drug delivery systems, tissue engineering, and wound healing. Sericin and fibroin are highlighted as crucial components driving advancements in medical technologies. Chapter 9 addresses the waste generated during Tasar silk production, exploring innovative approaches to managing this waste in response to the growing demand for sustainable practices.

"Silk Beyond Fabric" illuminates the intersection of tradition, innovation, and sustainability in the Tasar silk industry. It celebrates the rich heritage of Tasar silk while inspiring further exploration and collaboration in leveraging its diverse byproducts for a sustainable future.

Director

Phone: 0651-2960015, 2775628, 2775035 **Email**: ctrticsb@gmail.com, ctrtiran.csb@nic.in

About the Editors

Dr. Karmabeer Jena, Scientist-D received M.Sc. (Zoology, Sp-Biochemistry) degree from Utkal University, Bhubaneswar, M.Phil. (Life Science, Sp-Physiology and Biochemistry) degree from Sambalpur University, Sambalpur. He completed his Ph.D. in Zoology at Utkal University, where his research centered on oxidative stress and antioxidant defense mechanisms in bivalves. His work on the effects of pollutants on oxidative stress and antioxidant systems in bivalves identified these organisms as ideal pollution biomarkers. This significant contribution earned him the Junior Scientist of the Year Award in 2007 from the National Environmental Science Academy, New Delhi.After his doctoral studies, Dr. Jena pursued post-doctoral research at National Taiwan University, Taipei. In 2010, he joined the Central Silk Board as a Scientist-B at the CSB-Central Tasar Research and Training Institute (CSB-CTRTI) in Ranchi. His research at CSB-CTRTI focuses on the transformation of silk waste into valuable byproducts, and exploring their potential applications. Dr. Jena has published over 45 papers in national and international journals. His major research interests include the isolation and molecular characterization of sericin and chitosan from Tasar silk waste, highlighting their promising pharmaceutical and cosmeceutical applications.

Dr. Jay Prakash Pandey, Scientist-D is presently working at CSB-Central Tasar Research & Training Institute Ranchi. He has published around 65 research articles in various Journals, published 08 books, attended 52 scientific events, conference seminars, symposiums etc., and delivered 15 invited lectures in various forums at National and International levels. His key areas of research are Molecular Insect Physiology, Tasar Silkworm Biotechnology and By-product utilization. In the recent past, his research focus has been on whole genome sequencing of tasar silkworm and detailed characterization of cocoonase enzyme. He is also associated with various projects for the implementation of outcomes at the field level. In addition, he has established excellent public relations with numerous organizations and is instrumental in performing 8 MOUs & MOAs with prestigious organizations His published research got excellent citations in various National and International Journals/books/book-chapters/proceedings. Additionally, he has contributed in 15 R&D Projects as PI and CO-PI, and has initiated field oriented research projects and collaborations. Presently, he is engaged in 03 research projects as PI and Co-PI in collaboration with research institutes. He is associated with the Editorial committee of various books and has also published a Memoir on 60 years remarkable history of CSB-CTRTI Ranchi and prepared the documentary on the glorious contributions of the Institute. Besides this, he has also contributed in technology-oriented videos, print media, digital media and social media for better visibility of R&D activities which was appreciated at various levels.

Dr. Jitendra Singh, Scientist-D received M.Sc. (Environmental Science) degree from P.G. College Ghazipur, U.P and Ph.D. in Environmental Science SHUATS, Prayagraj, UP, where his research centered on heavy metals and its behaviour in soil and vegetables and Climate change & Pest Dynamics. After his doctoral he has joined as Research Associate in 2011 in NICRA project in National research Centre for Integrated Pest Management. In 2015, he joined the Central Silk Board as a Scientist-B at the CSB-Central Tasar Research and Training Institute (CSB-CTRTI) in Ranchi. He has received Young scientist Award in 2012 from Bioved Research Society, Prayagraj for outstanding contribution in the field of Environmental Science. Dr. Singh has published over 50 research papers in national and international journals, 08 books and more than 15 book chapters.He major contribution for development of 03 web enabled decision support system for forewarning & management pest in agricultural crop. His major research interests include the climate change & pest dynamics, Low cost & eco-friendly technologies for pest management, directional approach for pest management and waste management in tasar culture.

Dr. N.B.Chowdary is the Director of the CSB-Central Tasar Research and Training Institute (CSB-CTRTI), Central Silk Board (CSB), Ministry of Textiles, Govt. of India, Ranchi, Jharkhand. As the only institute in the world dedicated to research and developmental activities in the tasar silk sector, CTRTI plays a crucial role in supporting ten central and northern states of India. Dr. Chowdary's expertise lies in plant pathology and microbiology. He oversees practical, field-oriented research at CSB-CTRTI Ranchi and provides an expert guidance to scientists. His leadership has led to the launch of several field-based research initiatives, and he has fostered collaborations with research organizations, universities, corporations, and NGOs. Notably, Dr. Chowdary has authored over 200 publications in national and international journals, books, and conference proceedings. His contributions extend beyond research, as he has also developed numerous training modules to enhance knowledge and skills in the sericulture domain.

Contributors

Dr. N.B. Chowdary
CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Dr. K. Sathyanarayana
Dr. Kalam Agricultural College, Arrabari, Kishanganj - 855107, Bihar

Smt. Susmita Das
CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Dr. D.M. Pandey
Department of Biochemical Engineering,
Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand

Dr. Jitendra Singh
CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Sri. Mohammed Muzeruddin Baig
CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Sri. Ashu Kumar
CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Dr. Basanta Kumar Das
ICAR-Central Inland Fisheries Research Institute Barrackpore, Kolkata-700 120, West Bengal

Dr. K. Jayaram Kumar
Department of Pharmacy, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand

Dr. Jay Prakash Pandey
CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Dr. Karmabeer Jena
CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Dr. Trishana Bal
Department of Pharmacy, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand

Dr. Jyotirmayee Pradhan
Kuntala Kumari Sabat Women's College, Balasore-756001, Odisha

Dr. Divya Rajawat
CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Dr. Sruthy K.S

CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Smt. Ananta Sinha

CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Miss Shazia Mumtaz

CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Miss Sutapa Satpathi

Department of Pharmaceutical Sciences & Technology, Birla Institute of Technology, Mesra, Ranchi-835215

Miss Bidyutlata Patra

Kuntala Kumari Sabat Women's College, Balasore-756001, Odisha

Miss Aruna Rani

CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Miss Stuti Ananta

CSB-Central Tasar Research and Training Institute, Central Silk Board, Ministry of Textiles Govt. of India, Ranchi-835303, Jharkhand

Smt. Barsha Baisakhi

Kuntala Kumari Sabat Women's College, Balasore-756001, Odisha

Miss Debasmita Mohanty

Kuntala Kumari Sabat Women's College, Balasore-756001, Odisha

1. Unveiling the Hidden Treasures: Byproducts of Tasar Silk Sector

N.B. Chowdary

CONTENTS

INTRODUCTION

India holds a unique distinction as the sole producer of all types of silk: mulberry, eri, tasar, and the distinct golden-yellow muga silk. Tasar silkworms, specifically the wild tropical species *Antheraea mylitta*, feed on a variety of primary and secondary food plants. Primary sources include trees like Arjun (*Terminalia arjuna*), Asan (*T. tomentosa*), and Sal (*Shorea robusta*), while secondary plants include *Lagerstroemia parviflora, L. speciosa, Zizyphus mauritiana*, and others. These plants thrive in humid, dense forests found predominantly in the Central and Southern plateaus of Central India, spanning states like Jharkhand, Chhattisgarh, Bihar, Madhya Pradesh, Odisha, West Bengal, Maharashtra, Uttar Pradesh, and Andhra Pradesh. In contrast, the temperate species *Antheraea proylei* feeds on various oak species such as *Quercus incana, Q. himalayana, Q. semicarpifolia, Q. griffithii,* and *Q. serrata.* Tasar culture is one of the very important industry in India. In tasar sericulture, alongside silk fibre, various byproducts and wastes are generated. Recently, there has been a significant emphasis on developing and utilizing these byproducts across the tasar silk value chain. The aim is to increase the benefits for primary producers. Efforts are ongoing to maximize the use of these byproducts, not only to improve the economic viability of sericulture but also to explore their potential in diverse industries such as agriculture, bioenergy, pharmaceuticals, and cosmetics. This approach supports sustainability and enhances the overall value proposition of tasar silk production.

The extracts from the bark of the arjuna plant, used as food for tasar silkworms, exhibit various pharmacological properties such as inotropic, antiplatelet, antioxidant, antiatherogenic, blood pressure-lowering, hypolipidemic, anti-ischemic, and antihypertrophic effects. As reported chemical components of arjuna include glycosides, flavonoids, β-sitosterol, tannins, triterpenoids, and steroids, making it attractive to pharmaceutical industries. During tasar silkworm rearing, significant waste is generated, including uneaten leaves, larval litter (comprising 60% of their food intake), and discarded exuviae from larvae

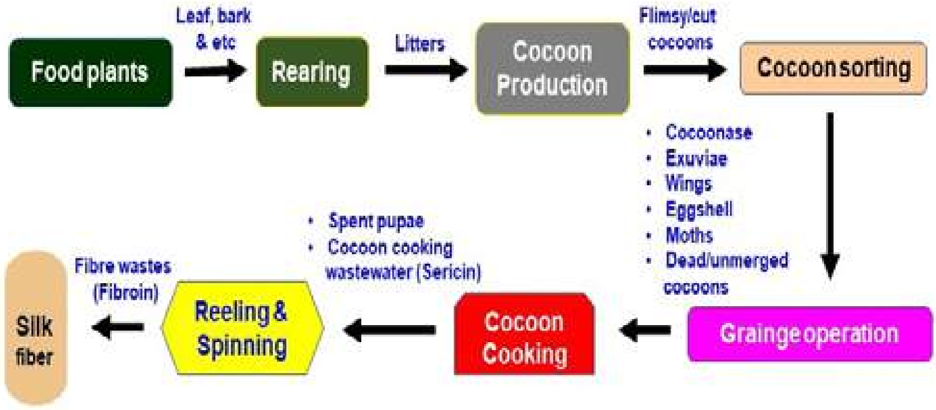

Figure 1: Schematic representation of silk waste generation

molting. Among these, larval litter is particularly valuable as an organic fertilizer due to its high nitrogen, phosphorus, and potassium content. However, direct application of fresh litter is less effective, so converting it into compost or vermicompost is recommended for optimal agricultural benefits. Similarly, field waste from rearing can be efficiently used to produce briquettes with a high calorific value. Cocoonase, a proteolytic enzyme produced by silk moths during pupa-to-adult transition, selectively breaks down sericin while preserving fibroin. Standardized processes for cocoonase collection have been developed, and variants are being trialed for commercial-scale use, aiming to produce organic silk with desired softness, luster, and natural color.

Figure 2: Various tasar byproducts generated throughout the value chain

In recent years, CSB-CTRTI has achieved significant milestones in exploring tasar byproducts. Apart from the primary product, which is silkworm cocoons, sericulture also yields various sub-products and secondary products including pupae, exuviae, faeces, fibre waste, and cooking water. The potential uses of these byproducts in pharmaceuticals and cosmetics are substantial. Therefore, enhancing the engagement of primary producers throughout the tasar silk value chain involves effective processing methods for these secondary and waste products. Increasing production costs, collaborative research efforts have focused on utilizing byproducts such as cocoonase, sericin, pupae for human and animal consumption, chitin for industrial applications, and using seri-waste for *cordyceps* production. These initiatives aim to broaden the participation of primary producers in the tasar silk industry, thereby contributing to its sustainable growth.

Efforts in research and development, along with recent initiatives, have paved the way for establishing micro-entrepreneurship opportunities through the utilization of byproducts in the tasar sector. This focus not only promotes sustainable livelihoods, particularly for women and tribal communities but also enhances profitability and proper disposal practices. CSB-CTRTI has been at the forefront, exploring the potential of tasar silk waste such as pupae for fish feed, *Cordyceps* production, chitosan, and pigments. Additionally, they are utilizing cocoon cooking wastewater to extract sericin and cocoonase to soften cocoons for reeling, which can increase profits by 30-40%.

The tasar sector's byproducts offer significant potential to generate lucrative employment and livelihood opportunities throughout the silk value chain. Documenting research on tasar byproducts in a comprehensive book format is crucial. It will aid in developing research strategies, facilitating technology transfer, fostering private partnerships, and promoting mutual learning and collaboration among CSB, other organizations, and sector partners. This collaborative approach aims to accelerate research and development, ultimately boosting the tasar silk industry in the years ahead.

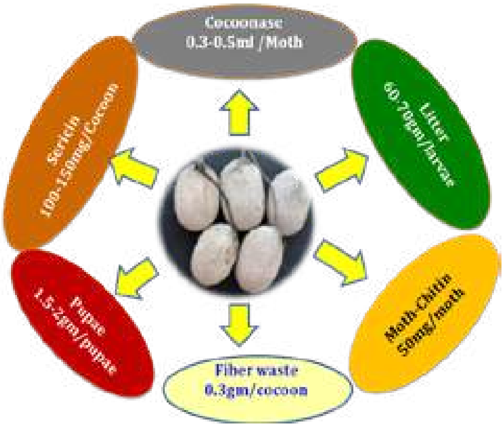

Figure 3: Quantification of various tasar byproducts generated during production cycle

The researchers from CSB-CTRTI focused documentation of by-product research aims to consolidate information into a single platform. This book will serve as a valuable resource for researchers and stakeholders involved in holistic research on tasar byproducts, facilitating informed decision-making and further advancements in the field.Top of FormBottom of Form

ARJUNA BARK

Various extracts of the stem bark of *Terminalia arjuna* plant have revealed to possess several pharmacological properties including inotropic, anti-ischemic, antioxidant, blood pressure lowering, antiplatelet, hypolipidemic, antiatherogenic, and antihypertrophic. The major chemical constituents of arjuna *viz.,* triterpinoids, glycosides, flavonoids, tannins, β-sitosterol, steroids etc., are attracting the pharmaceutical industries.

SAL SEEDS

Figure 4: Sal seeds

Sal, is a major source of income generation in rural and tribal areas. It has multifaceted importance, with various parts of the tree serving essential roles in ecology, economy, culture, and traditional medicine. Every part of the sal tree illustrates its significance whether be its timber, leaves, seed, bark, flower, or resin. The sal timber is highly valued for its durability, strength, and resistance to pests and decay. It is one of the most important hardwoods in India and Southeast Asia. The timber is extensively used in construction for building houses, making furniture, doors, window frames, and flooring. It is also used for crafting boats and railway sleepers due to its ability to withstand heavy loads and adverse weather conditions. The Sal leaves contribute to nutrient cycling and soil fertility as they decompose. They provide organic matter to the forest floor, supporting plant growth and maintaining soil structure. In some regions, Sal leaves are used as plates (patravali) for serving food during religious ceremonies and festivals. This practice has cultural significance and reflects the tree's role in local customs and traditions. Moreover, the Sal seeds are a valuable source of Sal seed oil, which is used in soap making, candle production, and as a lubricant. The seeds also have potential uses in traditional medicine. Sal seeds play a vital role in the regeneration and propagation of *Shorea robusta* forests. Much more need to be explored in this field.

COCOOONASE

Figure 5: Cooking with cocoonase

Cocoonase is a proteolytic enzyme exuded by sericigenous insects during pupal-adult metamorphosis, which softens the cocoon to allow the moth to emerge. This enzyme remains an underutilized by-product in the silk industry. Silk fibres produced by silkworms consist of fibroin proteins enclosed in sericin protein. To extract silk from cocoons, it is crucial to remove sericin. Currently, in the tasar silk industry, sericin removal (known as cooking, softening, or degumming) primarily occurs under highly alkaline conditions (pH 9.8 to 11.5), which can alter the natural color and softness of tasar silk. There is a critical need to develop enzyme-based, eco-friendly techniques for cocoon softening to preserve the natural color and soft texture of tasar silk. Cocoonase targets the glue protein sericin without affecting fibroin, thereby preserving the natural color, luster, and texture of tasar silk yarn. This process adds significant value to tasar silk. Molecular characterization has identified active cocoonase variants, paving the way for their potential use in silk processing. Cocoonase obtained from various sources, including natural and recombinant DNA methods, has been characterized for its enzymatic activities. Moreover, studies on the post-translational modification of recombinant cocoonase have compared its activity with native cocoonase and its variants. Overall, cocoonase enzymes play a crucial role in the silk industry by transforming raw silkworm cocoons into high-quality silk fibres suitable for diverse applications. Ongoing research suggests that these enzymes could find new applications in biomedicine, cosmetics, and biotechnology, highlighting their versatility and importance in modern industrial processes.

SILK SERICIN

Silk sericin, a natural polymer produced by silkworms, envelops and binds two fibroin filaments within the silk thread of cocoons. During silk production, sericin is typically removed through a process known as degumming. Sericin is recognized as a biocompatible and biodegradable material with additional properties such as antioxidants, gel-forming ability, anti-tumor effects, wound healing promotion, moisture retention, and skin adhesion. These attributes have led to its widespread application in the medical, pharmaceutical, and cosmetics sectors.

Figure 6: Tasar sericin (before and after purification)

Recently, CSB-CTRTI Ranchi has developed a prototype unit for extracting sericin from tasar cocoon cooking wastewater. This extracted sericin holds potential for various cosmetic and pharmaceutical applications. Silk fibroin, the inner core of silk, provides mechanical strength, while sericin acts as a protective coating. Silk fibroin is also highly regarded for its biocompatibility, gradual degradation, low immunogenicity, adaptability, excellent mechanical properties, controllable porosity, and permeability to oxygen and water. As a result, it is extensively researched for biomedical applications such as wound healing and treatment of conditions like psoriasis.

Despite the promising attributes of sericin, challenges persist in optimizing its extraction, purification, and application processes. Researchers are actively exploring methods to enhance its functional properties and ensure compatibility across various industrial and biomedical uses.

Sericin transcends its role as a mere binding agent in silk production; it represents a versatile biomaterial poised to contribute significantly to sustainable materials and biomedical innovations as scientific knowledge and technological capabilities continue to advance.Top of Form

SILK FIBROIN

Silk fibroin, synthesized from the posterior part of the silk gland, is the core protein of silk fibres. It is a biopolymer characterized by a complex hierarchical structure that gives silk its strength, flexibility, and lustrous appearance. Composed primarily of repeating amino acid sequences like glycine, alanine, and serine, silk fibroin allows the fibres to combine remarkable light weight properties with exceptional tensile strength, often surpassing that of steel relative to its weight.

In addition to its traditional use in textiles, silk fibroin has garnered significant attention in biomedical applications. Its biocompatibility and biodegradability make it suitable for tissue engineering and drug delivery systems. Researchers are exploring its potential in creating scaffolds for wound healing, repairing damaged tissues, and developing biodegradable implants. Silk fibroin's versatility in forming films, hydrogels, and nanoparticles further expands its applications in medicine and beyond.

Ongoing research aims to enhance silk fibroin's properties through genetic engineering of silkworms to produce tailored silk variants and advancements in processing techniques to improve scalability and versatility. Silk fibroin embodies nature's elegant design coupled with human innovation, bridging ancient craftsmanship with modern scientific advancements. Its diverse applications across industries highlight its potential to contribute to sustainable materials, advanced therapies, and functional textiles, paving the way for a future where natural proteins play a crucial role in addressing global challenges.Top of Form

SILKWORM PUPAE

Silkworm pupae (SWP), a substantial by-product of the silk reeling industry, possess high nutritional and therapeutic value, making them suitable as an alternative dietary supplement. Approximately 2 kg of dry SWP is produced for every 1 kg of raw silk. Silkworm pupae contain a variety of biological components that are valuable as feed for humans and animals, as well as for medicinal purposes and as crop fertilizer.

Recent advancements have demonstrated the potential of SWP in various applications. Tasar waste pupae have been successfully developed into fish feed, with ongoing research exploring their use as poultry feed and for extracting antioxidants. Additionally, *Cordyceps*, a fungus found in Himalayan alpine pastures, exhibits a wide range of health benefits including immunomodulatory, anti-inflammatory, anti-tumor, and antioxidant properties. Cultivation of *Cordyceps militaris* fruiting bodies using substrates such as silkworm egg and pupae powder has shown promise for medicinal purposes.

CONCLUSION

Tasar sericulture yields a series of sub-products and secondary products such as bark-gums-seeds tasar host plants, tasar silkworm cocoonase enzyme, pupae, exuviae, faeces, fibre waste, cooking water, and grainage waste. The major waste generated during rearing includes an excess of uneaten leaves, larval litter (60% of ingested food), and exuviae of the moulted larvae. Among these litter can be utilized, as an excellent organic fertilizer as the litter or feaces are an excellent source of nitrogen, phosphorous, and potash. Several collaborative researches were taken up in the field of by-product utilization *viz.*, cocoonase, sericin, pupae utilization for human and animal consumption, chitin for industrial use, fish feed, seri-waste for *Cordyceps* production, etc. More focus needed on tasar food plants arjun, asan and sal based byproducts as well as other byproducts. Byproducts generated during tasar silk production offer substantial opportunities across multiple industries. Ongoing research and development efforts are exploring innovative applications that not only enhance the sustainability of sericulture but also contribute to broader ecological and economic benefits. These initiatives highlight the potential of tasar silk byproducts to play a pivotal role in sustainable agriculture, animal nutrition, pharmaceuticals, and beyond, shaping a more diverse and resilient future.

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2. Valorizing Tasar Food Plant Waste: From Byproduct to Beneficial Medicinal Resources

J.P. Pandey, Aruna Rani, K. Jena, Divya Rajawat, K. J. Kumar and N.B. Chowdary

CONTENTS

INTRODUCTION

As a general sericulture practice, different types of economically important sericigenous insects are utilized for silk production *i.e. Antheraea mylitta, A. assamensis, A. pernyi, A. proylei, A. frithi, Samia ricini* and *Bombyx mori*. Tasar sericulture is one of the very important areas of research and development. There are a series of soil-to-silk steps to produce quality tasar silk fibre. During this process, various types of byproducts and waste materials gets generated. Recently, focus has been given to the utilization of various byproducts across the tasar silk value chain to increase the share of primary producers and minimize the pollution load. CSB-CTRTI has taken various initiatives in the field of tasar by-product utilization. Tasar sericulture yields a series of sub-products and secondary products such as bark-gums-seeds of tasar host plants, tasar silkworm cocoonase, pupae, exuviae, faeces, fibre waste, cocoon cooking waste water, and grainage waste. The possible use of these products in Ayurvedic medicine, pharmaceuticals, cosmetics, etc are enormous. Interestingly practicing tasar sericulture for non-textile uses through appropriate processing methods from the secondary and waste products can help the economic upliftment of the tasar silk sector. The major waste generated during rearing includes an excess of uneaten leaves, larval litter (60% of ingested food), and exuviae of the moulted larvae. Among these litter can be utilized, as an excellent organic fertilizer as the litter or feaces are an excellent source of nitrogen, phosphorous, and potash. As a direct application of fresh litter is less effective, it may be recommended to convert it into compost or vermin compost. Similarly, rearing field waste can be effectively utilized in briquette preparation, with high calorific value. Although, several collaborative researches were taken up in the field of by-product utilization *viz.*, cocoonase, sericin, pupae utilization for human and animal consumption, chitin for industrial use, fish feed, seri-waste for *Cordyceps* production, etc. More focus is also needed to be drawn on tasar food plants arjun, asan and sal based based byproducts. It is needful to mention that various extracts of the stem/bark of *Terminalia arjuna* plant have revealed to possess several pharmacological properties including inotropic, anti-ischemic, antioxidant, blood pressure lowering, antiplatelet, hypolipidemic, antiatherogenic, and antihypertrophic. The major chemical constituents of arjuna *viz.*, triterpinoids, glycosides, flavonoids, tannins, β-sitosterol, steroids etc., are attracting the pharmaceutical industries. In this chapter preliminary initiatives, present status, and prospective utility of some tasar byproducts has been mentioned with diverse possibilities of valuable remnants, utilisation, as well as problems in by-product.

UNIQUE FEATURES OF THE TASAR SILK INDUSTRY

Tropical tasar silk is produced only in India. Tasar silk industry provides livelihood to nearly 3.5 lakh families and has socio-economic relevance along with favour to ecology and environment. It harbours high potential for gainful rural employment and remunerative income to the tribal populace. Some of the key advantages for this industry are availability of the abundant nature-grown plants, and the high market demand for tasar silk domestic as well as global, profitable traditional occupation that requires the least investment to get a good return. Moreover rural workforces can be utilized gainfully (Table 1). Figure 1 depicts the various stages of the life cycle of tasar silkworm in detail.

Figure 1: Various life stages of tasar silkworm *Antheraea mylitta* Drury. 1. Eggs, 2 & 3. Larvae, 4. Cocoons, 5. Moth, 6. Cocoon garlands, 7 & 8. Emerging adults

DISTINCTIVE POTENTIAL AND BIO-PROSPECTING IN TASAR BYPRODUCTS

Effective extraction of useful products from tasar industry waste, moth, antennae, wings, scale, and dead pupae will offer additional income to the tasar farmers. Insect fat body works analogous to the liver; hence isolation of useful bio-molecules from the fat body of tasar silkworm pupae/moth is another excellent area of exploration. Identification of useful/

novel microbes/proteolytic enzymes from tasar silkworms and their prospective utilization in product diversification is another potential area of tasar industry bio-prospecting. Various byproducts of tasar silk industry and its prospective utilization in bio-prospecting are listed in Table 1. Functional characterization of silkworm enzymes, proteins, and product diversification needs to be explored in light of bio-prospecting. Several silkworm proteins and enzymes are proteolytic and have huge future applications in the biomedical field. Various prospective applications of byproducts from tasar silk industry are depicted in Figure 2.

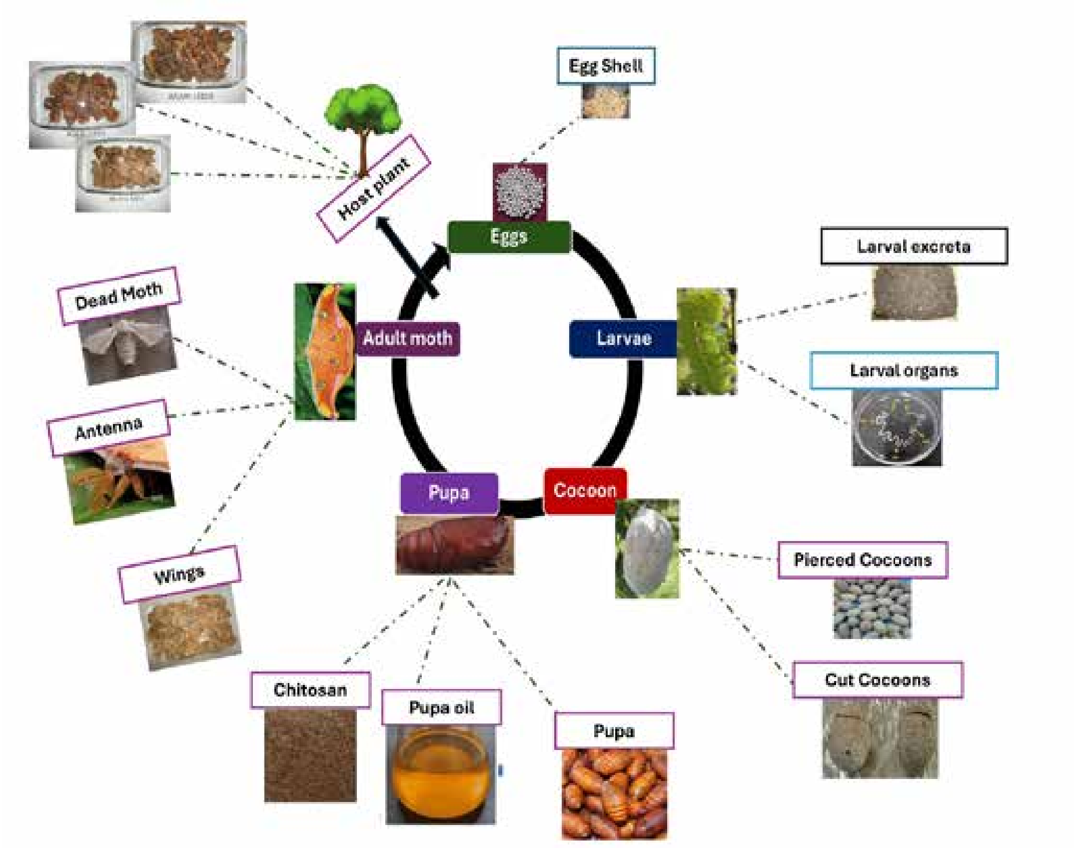

Figure 2: Various prospective applications of byproducts from tasar silk industry

SIGNIFICANCE OF BYPRODUCTS OBTAINED FROM HOST PLANTS

Silkworms are known for their remarkable ability to produce silk, with different species selecting varied host plants to support their lifecycle. The choice of host plants impacts the silkworms' health and silk production and offers significant byproducts that can be utilized in various ways. For instance, mulberry leaves, rich in vitamins and proteins, are valuable byproducts in sericulture. Excess or leftover leaves are often used as Animal feed. *Terminalia arjuna*, a critical host plant for tasar silkworms, is renowned for its medicinal properties its bark decoction is traditionally used in the Indian subcontinent to treat anginal pain, hypertension, congestive heart failure, and dyslipidemia. Furthermore, surplus leaves from these plants can be employed in vermicomposting, biogas production, and as nutrient-rich feed sources. This chapter explores the diverse and beneficial byproducts derived from silkworm host plants, emphasizing their potential applications in different fields. By understanding and utilizing these byproducts, we can enhance the economic viability of sericulture and promote sustainable agricultural practices.

Table-1: List of various byproducts of tasar silk industry and its prospective utilization in bio-prospecting

S. No.	Various types of byproducts	Prospective areas of Bio-prospecting
1	Sericin isolation from tasar waste	Bio-active molecules, nano-particle, hydro-gels, cosmetics, anti-cancer, biomedical, textiles, etc.
2	Cocoonase isolation from emerging moths	Proteolytic, bio-medical, organic silk, anti-inflammatory.
3	Protein powder isolation from waste pupae	Source of protein, nutrient, amino-acid, bio-polymer, immuno-adjuvant, anti-diabetic etc.
4	Preparation of compost from silkworm Litters	Biomedial, microbial culture, edible natural colors, medicinal etc.
5	Isolation of fibroin from silk waste	Biomaterials, pharma-industry
6	Isolation of chitin and useful proteins from waste moths	Chitin, protein, sensory and endocrine
7	Extraction of chitin and anti-ageing protein from waste puparium	Anti-ageing, chitin, etc.
8	Extraction of bio-molecules from Larval Exuvae	Anti-canceric, fluroscent pigments
9	Extraction of useful proteins from Eggs-of meconium	Anti-septic, Anti-microbial, antifungal
10	Extraction of pupal Pigments	Metabolic enhancer
11	Cementing material from calcium oxalate anal secretion	Anti-microbial, anti-fungal, etc.
12	*Terminalia arjuna* bark	Anticoagulant, bioactive compound
13	*Shorea robusta* Sal seed	Pharmaceutical properties
14	Pupal oil	Antioxidant, anti-diabetic, neuroprotective
15	Chitosan	Wound dressing, food packaging

INTRINSIC WORTH OF *W*

Terminalia arjuna is one of the very valuable plants that has many crucial merits. The plant grows into a big tree, loves water but can tolerate very dry soil. Grow under water stress and waste land too hence survival percentage is good. Arjuna is one of the trees that produce oxygen in abundance and is known as an air purification plant. It increases the oxygen level and helps reduce numerous respiratory problems hence the perfect tree for air purification (Fig. 3). During the rearing of tasar silkworms, a huge quantity of tasar food plant arjuna waste is generated. More importantly, various extracts/powders and tablets based on the stem bark of the *Terminalia arjuna* plant have been revealed to possess several pharmacological properties including inotropic, anti-ischemic, antioxidant, blood pressure lowering, antiplatelet, hypolipidemic, antiatherogenic, and antihypertrophic. The major chemical constituents of arjuna *viz.*, triterpenoids, glycosides, flavonoids, tannins, β-sitosterol, steroids, etc., are attracting the pharmaceutical industries. The Arjuna tree (*Terminalia arjuna*) holds significant pharmacological importance due to its wide range of therapeutic properties. Known for its potent antimicrobial activity, extracts from the Arjuna tree have demonstrated significant efficacy against various gram-negative bacteria, including *Escherichia coli, Klebsiella aerogenes, Proteus vulgaris*, and *Pseudomonas aeruginosa*. In studies involving 34 plant species from 18 different families, *Terminalia arjuna* stood out for its robust antibacterial properties. Moreover, the tree's extracts have shown promise in the field of oncology, with evidence suggesting that they can increase the lifespan of experimental animals with cancer. The cardioprotective qualities of the Arjuna tree are well-documented in ancient medicine, where its use for treating cardiac diseases has been recorded for centuries. Additionally, *Terminalia arjuna* exhibits lipid-lowering activity, which is beneficial in managing cholesterol levels. Its antifungal, antioxidant, and anti-inflammatory properties further underscore its versatility and effectiveness in various therapeutic applications. Thus, the Arjuna tree is a valuable natural resource in both traditional and modern medicine, contributing to health and wellness across multiple dimensions. CSB-CTRTI has taken initiatives to prepare the *T. arjuna* bark tablet in collaboration with the Department of Pharmacy, BIT Mesra, Ranchi (Figure 3).

SHOREA ROBUSTA AND ITS DIVERSE APPLICATIONS

Shorea robusta commonly known as sal, is a non timber forest product of India. Sal is a major source of income generation in rural and tribal areas. Sal tree (*Shorea robusta*) is esteemed for its multifaceted importance, with various parts of the tree serving essential roles in ecology, economy, culture, and traditional medicine. Every part of the sal tree illustrates its significance whether be its timber, leaves, seed, bark, flower, or resin. The sal timber is highly valued for its durability, strength, and resistance to pests and decay. It is one of the most important hardwoods in India and Southeast Asia. The timber is extensively used in construction for building houses, making furniture, doors, window frames, and flooring. It is also used for crafting boats and railway sleepers due to its ability to withstand heavy loads and adverse weather conditions. The sal leaves contribute to nutrient cycling and soil fertility as they decompose. They provide organic matter to the forest floor, supporting plant growth and maintaining soil structure. In some regions, Sal leaves are used as plates (patravali) for serving food during religious ceremonies and festivals. This practice has cultural significance and reflects the tree's role in local customs and traditions. Moreover, the Sal seeds are a valuable source of Sal seed oil, which is used in soap making, candle production, and as a lubricant. The seeds also have potential uses in traditional medicine. Sal seeds play a vital role in the regeneration and propagation of *Shorea robusta* forests (Table 2). The diverse application of Sal tree is depicted in Figure 4.

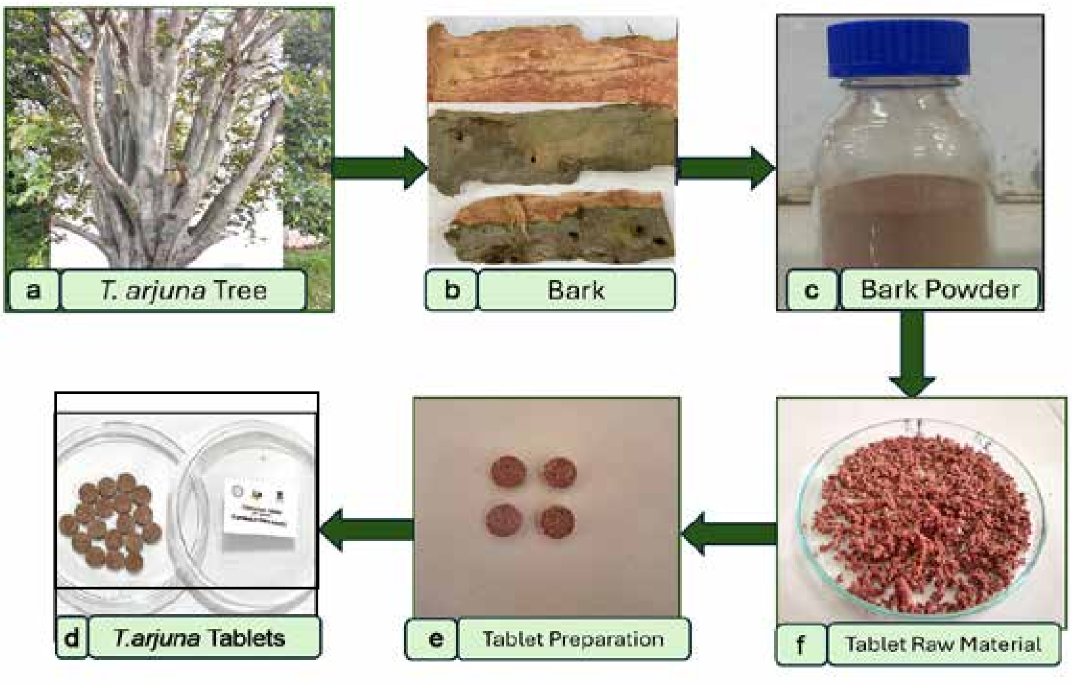

Figure-3: (a-d) Detailed depiction of the steps involved in tablet formation using *T. arjuna* bark powder.

They are dispersed by wildlife, contributing to the expansion and diversity of Sal forests. The bark of the Sal tree has medicinal properties and is used in traditional medicine systems like Ayurveda. It is believed to have anti-inflammatory, antimicrobial, and wound-healing properties. Sal bark extracts are used in religious rituals and ceremonies, symbolizing purification and spiritual cleansing. Sal flowers are revered in Hindu and Buddhist traditions. They are offered to deities in temples and used in ceremonial garlands during religious festivals. Sal flowers attract pollinators such as bees and butterflies, supporting biodiversity and the health of forest ecosystems. Sal resin, also known as dammar, is used in varnishes, lacquers, and incense. It has adhesive properties and is used in the production of paints and as a sealing agent in ceramics. Sal resin has traditional uses in local craftsmanship and is valued for its cultural and economic contributions to rural livelihoods.

Shorea Robusta Seed

In the state of Jharkhand, *Shorea robusta* seeds, commonly known as Sal seeds, hold significant importance across various facets of life, contributing to the state's economy, ecology, culture, and livelihoods. The seeds of *Shorea robusta*, commonly known as Sal seeds (Figure 5), exhibit distinctive morphological features typical of their species. Here are some key characteristics:

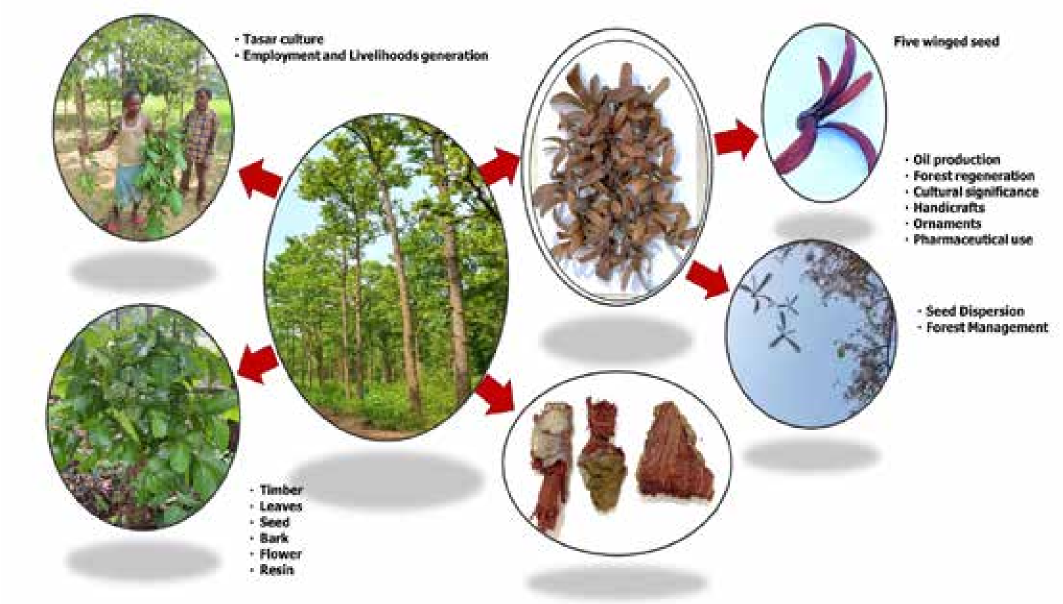

Figure 4: Diverse applications of sal tree (*Shorea robusta*) in nature.

1. **Shape and size**: Sal seeds are generally ovoid or ellipsoid in shape, with a pointed tip at one end and a rounded base. They can vary in size, typically ranging from 1.5 to 3 centimetres in length.

2. **Colour**: When mature, Sal seeds are typically dark brown or black in color. The seed coat is hard and durable, protecting the seed within.

3. **Texture**: The seed coat is smooth and glossy, contributing to its durability and resistance to environmental conditions.

4. **Hilum and micropyle**: The hilum (scar) is usually prominent and located near the pointed end of the seed. It marks the point where the seed was attached to the fruit or pod. The micropyle, a small pore or opening in the seed coat, is also present and allows for water uptake during germination.

5. **Endosperm and embryo**: Inside the hard seed coat, Sal seeds contain a large, oily endosperm that serves as a nutrient reserve for the developing embryo. The embryo itself is small and located at one end of the seed.

6. **Germination**: Sal seeds have orthodox seeds which means they can be dried to low moisture content without damage and stored for extended periods. Germination typically begins when conditions are favourable.

Figure 5: Five-winged seed of *Shorea robusta*.

Table 2: Various uses of sal *Shorea robusta* seed are described as under.

Contribution of sal seed in various aspects	
Economic contribution	**Oil production:** Sal seeds are a vital source of sal seed oil, which is extracted and used in various industrial applications. The oil is particularly valued in soap manufacturing for its cleansing properties and is also utilized in the production of candles and as a lubricant. The processing and sale of sal seed oil provide income opportunities for local communities and support small-scale industries in the region.
Cultural and traditional uses	**Cultural significance:** Sal seeds hold cultural significance among indigenous communities in Jharkhand. They are used in religious ceremonies and rituals, symbolizing prosperity and auspiciousness. The seeds are sometimes included in traditional handicrafts and ornaments, reflecting their cultural value and aesthetic appeal.
Socio-economic impact	**Livelihoods:** The collection and trade of sal seeds provide livelihood opportunities for many rural communities in Jharkhand. Forest-dependent communities engage in seed collection and processing, contributing to their income and livelihood security. Collection of tasar cocoons is also source of income for villagers. **Income generation:** Sal seeds contribute to income generation and economic diversification in rural areas, supporting sustainable forest management practices and enhancing the socio-economic well-being of local populations.
Environmental conservation	**Forest management:** Sustainable harvesting of sal seeds promotes forest conservation practices in Jharkhand. It encourages the preservation of sal forests, which are essential for maintaining ecological balance, carbon sequestration, and mitigating climate change impacts.

Medicinal use of sal seed	Sal seeds, derived from the *Shorea robusta* tree, possess several medicinal properties that have been recognized and utilized in traditional medicine systems like Ayurveda. Here are some of the medicinal properties and uses of sal seeds. **Anti-inflammatory properties**: Sal seeds are known to have anti-inflammatory properties. In Ayurvedic medicine, extracts from sal seeds are used to reduce inflammation and swelling associated with various conditions. **Antioxidant activity**: Sal seeds contain antioxidants that help in scavenging free radicals and reducing oxidative stress in the body. This property is beneficial in maintaining overall health and preventing chronic diseases. **Digestive benefits**: In traditional medicine, sal seed extracts are used to improve digestion and alleviate gastrointestinal disorders such as indigestion, bloating, and diarrhea. **Cardiovascular health**: Sal seeds are believed to have cardio-protective effects. They may help in maintaining healthy cholesterol levels and promoting cardiovascular well-being. **Anti-diabetic properties**: Some studies suggest that sal seed extracts may have hypoglycemic effects, helping to regulate blood sugar levels and manage diabetes. **Wound healing**: Sal seed oil, extracted from the seeds, is used topically in traditional medicine to promote wound healing and skin regeneration. It has moisturizing properties and helps in soothing skin irritations. **Antimicrobial activity**: Sal seed extracts exhibit antimicrobial properties against certain bacteria and fungi. This makes them useful in treating infections and promoting overall immune health.
	Anti-allergic effects: Sal seed extracts are used traditionally to alleviate allergic reactions and respiratory conditions by reducing inflammation and calming immune responses. **Sal butter**: Sal butter is derived from the seeds of *Shorea robusta* and is used in the cosmetic industry for its moisturizing and emollient properties. It is often included in skincare products such as lotions and creams.

Ecological importance	**Forest regeneration**: *Shorea robusta* seeds are crucial for the natural regeneration and propagation of sal forests. Dispersed by wildlife such as birds and mammals, these seeds play a vital role in replenishing and expanding forest cover. Sal forests are essential ecosystems that support biodiversity, provide habitat for wildlife, and contribute to soil fertility and watershed management. **Food and nutrition:** In some regions, Sal seeds are consumed after roasting. They serve as a nutritious snack and are sometimes used in local cuisines, contributing to food security and dietary diversity.

Thus, *S. robusta* seeds, play a crucial role in the economic development, cultural heritage, and environmental sustainability. Their diverse uses and contributions underscore the importance of conserving Sal forests and promoting sustainable livelihoods based on forest resources in the state.

BIO-PROSPECTING OF SILK BYPRODUCTS

Silk produced by *A. mylitta* is unique attributable to silk fabric quality and luster. As tasar culture involves various biological and bimolecular processes to extract the tasar silk and other useful products having high economic value, therefore, it is felt that the tasar silk industry has vast possibilities for bio-prospecting. Effective extraction of useful/best products from tasar industry waste, moth, antennae, wings, scale, and dead pupae will offer supplementary earnings to the tasar farmers. It is reported that the insect fat body works analogously to the liver; hence isolation of useful products from the fat body of tasar silkworm pupae/moth is another excellent area of exploration. Perusal literature survey suggests that diverse R&D work was conducted by researchers worldwide (Table-3).

Table 3: Depicting the diverse R&D work conducted worldwide on tasar byproducts

S. No.	Diverse R&D work conducted worldwide on tasar by-product
1.	Potential applications of silk sericin, a natural protein from textile industry byproducts
2.	Uranium recovery from dilute aqueous solutions using silk fibroin
3.	Optimization of the recovery of carotenoids from tomato processing wastes: application on textile dyeing and assessment of its antioxidant activity.
4.	Viability and proliferation of L929, tumour, and hybridoma cells in the culture media containing sericin protein as a supplement or serum substitute,
5.	Processing and characterization of silk sericin from *Bombyx mori* and its application in biomaterials and biomedicines.
6.	Natural protein fibre dissolution in ionic liquids.
7.	Wild silk-induced asthma and inhalation allergies caused by wild and tussah silk-filled bed quilts and silk waste allergen in the bedroom.
8.	Reported enhancement of the bleaching and degradation of textile wastewaters by Gliding arc discharge plasma in the presence of TiO_2 catalyst.

S. No.	Diverse R&D work conducted worldwide on tasar by-product
9.	Hypoglycaemic effects of functional tri-peptides from silk in differentiated adipocytes and streptozotocin-induced diabetic mice.
10.	Nightly asthma is caused by allergens in silk-filled bed quilts and clinical and immunologic.
11.	Pretreatment of silk-dyeing industrial wastewater by UASB reactor.
12.	Controlled fabrication of silk protein sericin-mediated hierarchical hybrid flowers and their excellent adsorption capability of heavy metal ions of Pb(II), Cd(II), and Hg(II).
13.	Hydrothermal production and characterization of protein and amino acids from silk waste.
14.	Fabrication and features of a methylene green-mediating sensor for hydrogen peroxide based on regenerated silk fibroin as immobilization matrix for peroxidase.
15.	Development of silk contact anaphylaxis and silk-hydrogel lenses for light-emitting diodes.
16.	Comparison of the properties of *B. mori* silk cocoons against sericin-fibroin re-gummed bio-composite sheets.
17.	The potential of 2D cross-linked sericin membranes with improved bio-stability for skin tissue engineering and reported preparation and characterization of ethanol-treated silk fibroin dense membranes for biomaterials application using waste silk fibres as raw material.
18.	Equilibrium and kinetic adsorption study of a cationic dye by a natural adsorbent--silkworm pupa, Bio-sorption of Cu (II) and Pb (II) ions from aqueous solution by natural spider silk.
19.	Amperometric new methylene blue N-mediating sensor for hydrogen peroxide based on regenerated silk fibroin as an immobilization matrix for peroxidase.
20.	Organic micro-pollutant removal in liquid-phase using carbonized silk cotton hull.
21.	An axial distribution of seeding, proliferation, and osteogenic differentiation of MC3T3-E1 cells and rat bone marrow-derived mesenchymal stem cells across a 3D Thai silk fibroin/gelatin/hydroxyapatite scaffold in a perfusion bioreactor.
22.	Physical and biological characterization of sericin-loaded copolymer liposomes stabilized by polyvinyl alcohol.
23.	Utilization of modified silk cotton hull waste as an adsorbent for the removal of textile dye (reactive blue MR) from aqueous solution.
24.	Electricity from the silk cocoon membrane and harvested electricity from human hair.
25.	Human amniotic epithelial cells combined with silk fibroin scaffold in the repair of spinal cord injury.

S. No.	Diverse R&D work conducted worldwide on tasar by-product
26.	Natural silk protein sericin for regenerative medicine and reported an injectable, photo-luminescent, cell-adhesive 3D hydrogel.
27.	Silk-induced asthma in children: a report of 64 cases.
28.	A novel sodium N-fatty acyl amino acid surfactant using silkworm pupae as stock material.
29.	"Wild silk" asthma-an ever-current inhalation allergy to silk waste.
30.	Regeneration of high-quality silk fibroin fibre by wet spinning from $CaCl_2$-formic acid solvent.
31.	Immobilized L-asparaginase on the micro-particles of the natural silk sericin protein and its characters.
32.	In view of the aforesaid Bio-prospecting of silk products and byproducts, firm PNA needs to be established and executed to better utilization of tasar byproducts.

PROBLEMS AND PROSPECTS IN BY-PRODUCT UTILIZATION

Although, the tasar sector by-product has great potential to generate remunerative employment and livelihood opportunities across their silk value chain the establishment of micro-entrepreneurship avenues on by-product utilization in the tasar sector is a big challenge due to scattered and unorganized market. The creation of sustainable livelihood through byproducts is not yet established. Although, various serious efforts have been taken by CSB-CTRTI Ranchi on tasar silk waste such as pupae (for Fish feed, production of *cordyceps*, preparation of chitosan and pigments), cocoon cooking wastewater for sericin and cocoonase for softening cocoons. Although this can add to the profit to the tune of 30-40% besides their proper disposal. Efficient exploitation of byproducts at the field level/ market level is yet to be established. Therefore, tasar by-product research for developing research strategies, effective technology transfer, private partnerships, etc. is very much needed. Mutual learning on byproducts and joint planning with reputed organizations would help to build linkages amongst the CSB & other organizations and partners in the sector, for accelerated R&D growth and development of the tasar silk industry, in the years to come. Precise byproducts and their goals on R&D information at one platform will be helpful for researchers and other associates for holistic research on tasar byproducts.

COMPREHENSIVE POTENTIAL OF TASAR PRODUCTS AND BYPRODUCTS

The multifaceted potential of Tasar silk beyond its traditional textile applications is huge. Tasar silk offers a wealth of valuable byproducts that are both intriguing and promising in various fields. This is high time to focus on following key aspects:

- The major chemical constituents of arjuna *viz.*, triterpenoids, glycosides, flavonoids, tannins, β-sitosterol, steroids, etc., are attracting the pharmaceutical industries. CSB-CTRTI has taken the initiative to prepare the *T. arjuna* bark tablet in collaboration of the Department of Pharmacy BIT Mesra Ranchi.

- Comprehensive glance into the byproducts within the tasar sector by emphasizing their possible applications beyond conventional textile purposes.
- Comprehensive exploration of cocoonase, revealing not only its fundamental biological role but also its burgeoning applications in various industries which underscores the transformative potential of this enzyme derived from tasar silk industry byproducts and multifaceted roles and promising applications of cocoonase, derived from the tasar silk industry byproducts.
- Tasar silk has long been revered for its luxurious fabric, but beyond its threads lies a wealth of untapped potential in the form of byproducts. Among these, cocoonase stands out as a valuable protease with copious functions that extend far beyond its initial biological role in silk production. Cocoonase enzyme has captured the attention of researchers and industries alike due to its diverse applications across various sectors including bio-medical.
- The natural proteins sericin and fibroin take center and their versatile applications beyond silk production need to be explored in depth which explores expansive applications of sericin and fibroin, natural proteins extracted from silk, beyond their traditional role in textile production.
- These proteins exhibit unique properties such as bio-compatibility, moisture retention, and antioxidant activity, making them valuable in various industries. In cosmetics, sericin's ability to hydrate and protect the skin has led to its inclusion in moisturizers, anti-aging creams, and hair care products.
- Exploration of silkworm pupae a nutritional wealth often overlooked in food sciences. We need to uncover the nutritional richness of silkworm pupae, presenting them as a sustainable source of nutrients with implications for both human consumption and animal feed.
- The hidden potential and diverse applications of silkworm pupae, presenting them as a sustainable source of nutrients with profound implications for human and animal nutrition. Silkworms have long been synonymous with the production of exquisite silk, yet their pupae represent a rich, yet underutilized resource.

CONCLUSION

Tasar silk industry byproducts are very crucial in the present scenario. As tasar culture involves various biological and bio-molecular processes to extract the tasar silk and other useful products having high economic value, therefore, there are vast possibilities in bio-prospecting of tasar silk products and byproducts. Deep discussion and target-oriented research in this area are imperative need for the sustainable development of the tasar industry and its beneficiaries. Several byproducts such as *T. arjuna* bark, sal seeds and various other parts of sal, waste silk, cocoons, fecal pellets, pupae, moth, puparium, larval exuvae, eggs, fibre wastes, etc., need to be utilized on a priority basis. Effective and easy technologies need to be developed with a complete value chain and linkage, it will offer additional revenue to the tasar farmers in the future.

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3. Tasar silkworm *Antheraea mylitta* cocoonase: A valuable trypsin-like endopeptidase with various function

J.P. Pandey, Aruna Rani, K. Jena, D.M. Pandey and N.B. Chowdary

CONTENTS

INTRODUCTION

During pupal-adult metamorphosis, sericigenous insects exude proteolytic enzyme 'cocoonase' which makes the anterior portion of cocoon soft and the moth comes out from cocoon shell. This enzyme is one of the un-utilized by-product of silk industry. However, various landmarks in the field of tasar byproducts have been achieved, besides the main product like silkworm cocoons, sericulture yields a series of sub-products and secondary products such as pupae, exuviae, faeces, fibre waste, cocoon cooking waste water etc., grainage waste and their possible use in pharmaceuticals, cosmetics are enormous. Practicing sericulture for non-textile uses through appropriate processing methods from the secondary and waste products can help increase the share of primary producers across the tasar silk value chain. Various collaborative research projects focusing on by-product utilization such as cocoonase, pupae utilization for human and animal consumption, sericin, seri-waste for *Cordyceps* production, chitin for industrial use, etc. were undertaken in light of the rising cost of production.

Silkworm is a holometabolous insect having four life-stages evidently distinguished from each other egg, larva, pupa, and adult. Dramatic morphological modifications from larva to adult occur in the pupa by an exceptionally regulated metabolism, which includes the degradation, remodeling, and regeneration of tissues. A cocoon protects a dormant silkworm pupa from a number of unfavourable circumstances, such as desiccation and predators, but an adult moth must escape from its cocoon after going through a metamorphosis. The silkworm cocoon is mostly made of the two proteins fibroin and sericin. The posterior silk gland secretes fibroin, a fibrous protein. The middle silk gland secretes a globular protein called sericin, which serves as an adhesive to hold the fibres of fibroin together. Cocoonase, designated as a protease is produced by silk moth during pupal-adult emergence, which hydrolyses sericin without affecting fibroin and facilitate its escape from the cocoon during emergence. An adult moth during emergence releases around 600-900µl of cocoonase dropwise steadily, and this release procedure continues for 2-4 hours. It acts upon the glue protein sericin which holds the silk fibroin coils at one end of the cocoon. This natural process wanes the mechanical strength of the cocoon and enables the adult silk moth to emerge. It is an essential protease for breaking the sealed cocoon of the Lepidoptera insects. This natural mechanism originates a novel idea to potentially utilize this proteolytic enzyme cocoonase to soften the tasar cocoons. The cocoon cooked in cocoonase retains the natural beautiful unique colour of tasar silk, besides providing the soft texture and luster to the tasar silk yarn. The investigation on cocoonase has been mostly focused on the Indian tasar silkworm (*A. mylitta*), Polyphemus moth (*A. polyphemus*), *B. mori* and the Chinese Tussah silkmoth (*A. pernyi*). Interestingly, cocoonase is also reported to be secreted in non-sericigenous insects in butterflies such as *Heliconius melpomene, Danaus plexippus etc.* where cocoonase is used in pollen digestion. *Heliconius melpomene* salivary glands do not express cocoonase, indicating that butterflies, like moths, directly release this digesting enzyme from the proboscis. Additionally, cocoonase has potential commercial relevance for a variety of uses relevant to protease activity. Furthermore, to counteract the seasonal limitations in cocoonase production and collection, extensive efforts have been made to guarantee its consistent availability in larger quantities throughout the year. Using diverse expression systems and methodologies, this has been accomplished by employing molecular cloning to express the cocoonase gene. Moreover, several explored and unexplored questions are need to be answered:

- Whether cocoonase of various sericigenous insects and prominent ecoraces of *A. mylitta* are identical?
- Whether the cocoonase activity is similar among native, recombinant and analogues forms?
- What are structural variations among various forms of cocoonase and which is the most active cocoonase?
- Is there any post-translational modification in recombinant cocoonase?
- How the cocoon cooking in cocoonase retains the natural color of tasar silk? Whether it is related to silk pigments?
- What are the pigments which get washed during cocoon cooking in alkali solution?
- Whether modification in silk surface using cocoonase is causing positive impact on comfort, quality, durability, properties, beautiful color and tensile strength of silk?
- What is the mechanism of action of cocoonase/analogue in softening of cocoons and how it is different from existing methods (containing soap, H_2O_2 and alkali) that sluice natural unique color of tasar silk?

INITIATIVES ON COCOONASE RESEARCH

Cocoonase enzyme is an un-utilized by-product of silk industry. It was the year 1964, when Kafatos and Williams studied the enzymatic mechanism and collected the cocoonase using a capillary tube from the face of a newly emerged *A. pernyi* moth. They also showed the moth (*A. mylitta*) beginning to expand its wings after escaping through the hole formed in the anterior end of cocoon. During 1969-1970, the cocoonase collection method of Kafatos and Williams (1964) was slightly modified. Instead of capillary tubes, glass tubes bent in V shape at an angle of 45° were used for *A. mylitta* cocoonase collection. The investigators have also tried to collect the cocoonase by dissecting the galea and collecting enzymes in crystal form. But this work was stopped probably due to difficulty in the cocoonase collection procedure and the unavailability of emerging moths throughout the year. Subsequently, property of cocoonase was also studied. The cocoonase explorers were able to collect the cocoonase in small quantities as these cocoonase collection methods were difficult and labour intensive, therefore, in the last five decades cocoonase inspite of being a crucial by-product of tasar industry could not be effectively utilized.

Until now, several investigators have carried out various basic and molecular studies without much correlation with softening of cocoons. Such as investigation of the secretory organs of cocoonase, rapid determination of cocoonase contents in the maxilla of silk moth breeds by dot blotting has been carried out. Additionally, cloning and expression of the cocoonase gene have been also carried out. Moreover, the evaluation of eukaryotic expression and biological activities of the expressed product has been also carried out. Further, the expression of cocoonase in *B. mori* cells by using a recombinant *Baculovirus,* and its bioactivity assay was also studied. However, there is no well-established cocoonase or its analogue based technology for tasar silk processing.

ENZYME BASED METHODS IN COCOONS SOFTENING

The silk fibre produced by silkworm is a composite material formed by fibroin proteins surrounded by a sericin protein. To obtain the silk from cocoons, sericin removal is necessary. Predominantly, tasar cocoon sericin removal (cooking/ softening/ degumming) is being carried out in highly alkaline (pH 9.8 to 11.5) conditions which influence the natural unique colour and softness of tasar silk. But as a ruling practice, cooking of tasar cocoon is generally used to perform in alkaline solution by using soap, soda, H_2O_2, alkali etc. which adversely affects the natural colour and softness of tasar silk. Moreover, various enzymes have been tried for cocoon cooking and degumming of silk. However, cocoonase is known to act on sericin without affecting the fibroin which softens the cocoon. This natural mechanism originates a novel idea to potentially utilize this proteolytic enzyme cocoonase to soften tasar cocoons. The possible efficacy of cocoonase in softening of tasar cocoon has been investigated. The results revealed that the primary target of cocoonase is the glue protein sericin and not fibroin. Cocoon cooking in cocoonase is used to preserve the natural beautiful unique tasar silk colour, soft texture and luster of tasar silk yarn. It offers a value addition to tasar silk. Moreover, the cocoonase variants identified through molecular characterization showed potential utilization in silk processing. Additionally, the comparative evaluation of enzyme activities among cocoonase of various sericigenous insects through molecular characterization has also been carried out. Further, for effective utilization of cocoonase, the active cocoonase variant has also been identified. The variation in silk content and commercial characters of various ecoraces of tasar silkworm has also been reported. However, fibroin-sericin ratio and cocoonase content may differ in prominent ecoraces which leads to difficulties during silk processing. Although cocoonase from Daba ecoraces has been characterized using specific enzyme activities.

COCOONASE ENZYME SECRETION AND ACTIVATION

The epidermal cells located on the maxillary galeae of the moth produce an inactive form of the cocoonase enzyme, known as a zymogen. Zymogens are proteins that are initially synthesized and folded into an inactive precursor form. Activation of the zymogen occurs at a specific serine residue, converting it into its active form, cocoonase. This activation process resembles the mechanism observed in mammalian trypsin. After activation, the enzyme is deposited onto the surface of the galeae in a dry form. Subsequently, it is dissolved by the buffer generated by the labial gland. Before this dissolution, the dry enzyme deposit can be extracted almost completely in a pure form from the galeae, with minimal contamination by peptides. During a specific developmental stage, the cells that produce the zymogen in the galeae undergo significant proliferation and become polyploid. This process marks the appearance of zymogen that can be stained cytologically. The timing of this development seems to be controlled at the post-transcriptional level. In mature moths, a fluid is secreted that moistens the anterior end of the cocoon. This fluid triggers the rapid breakdown of sericin, which softens the moistened area. This softening allows the delicate moth to push aside the unravelled fibroin threads, thereby creating a sufficiently large opening through which it can emerge.

CHEMISTRY OF COCOONASE

Cocoonase is a serine protease (EC:3.4.21.4) enzyme that shares chemical, physical, and catalytic similarities with trypsin. The fluid it secretes is clear, colourless, and has a basic pH ranging from 8.3 to 8.7. Cocoonase has a molecular weight of approximately 26kDa. Its isoelectric point (pI) is above 9.5. It exhibits high stability against autodigestion at neutral and slightly alkaline pH levels but is prone to deactivation at low pH levels. The cocoonase gets denatured easily and irreversibly by heat. It gets rapidly inactivated at a pH range that is mildly acidic pH 3 to 4. Cocoonase also exhibits esterase, thrombolytic, and fibrinolytic activity. The catalytic triad Ser-His-Asp is located in the middle of the active site cleft and is utilized by all serine proteases to hydrolyze peptide bonds. The specificity pocket is made up of binding sites close to this triad, this area and its surrounding loops help to select the preferred substrates of enzymes. Cocoonase besides hydrolyzing its natural substrate sericin, also acts upon other substrates such as gelatin, acid-denatured hemoglobin, oxidized ribonuclease, BAEE. For inhibition, the typical trypsin inhibitors were found to be capable of inhibiting cocoonase.

MOLECULAR CLONING AND EXPRESSION OF COCOONASE GENE

The advancement of recombinant technology has led to the cloning and expression of cocoonase gene in various expression systems. Application of this technique provides a scope to industrially produce cocoonase on a large scale. This also ensures its consistent availability throughout the year and in large quantities. This has been achieved through molecular cloning and expression of the cocoonase gene using various systems, such as expressing the *B. mori* cocoonase gene in *Pichia pastoris*, Baculovirus expression vectors, and *E. coli* expression systems. The goal of expressing cocoonase is to enable its use in silk degumming, a process that relies on enzyme-based methods for ecofriendly degumming. Traditional industrial methods for removing sericin from silk involve chemical treatments with alkaline solutions, which can degrade fibroin and thus diminish silk quality. In contrast, cocoonase-based degumming preserves the quality of silk fibres by selectively targeting sericin removal while maintaining the integrity of fibroin.

CORE R&D THEME ON COCOONASE

During the eclosion stage generally sericigenous insects secretes the proteolytic enzyme cocoonase which helps in softening the anterior portion of the cocoon shell and facilitates the emergence of moths by making an opening near the peduncle region. The natural process of *A. mylitta* cocoonase secretion has been successfully investigated, followed by cocoonase characterization, molecular sculpt preparation, and various R&D initiatives on cocoonase have been carried out. Additionally, cocoonase collection, purification methods, have been developed and standardized (Fig.1). Further, it was also observed that silk content, fibroin-sericin ratio and cocoonase content of various sericigenous insects vary markedly. Moreover, it was observed that chemical-based degumming adversely affects the tasar silk fibres. Hence, there was a need to explore a new eco-friendly technique for tasar cocoon cooking. The cocoonase/analogue based cocoon cooking method might be a probable solution to avoid chemical-based degumming. The enzymes are known for specific and mild action hence it is expected that enzyme-based cocoon softening is useful in preserving natural color and softness of tasar silk in an eco-friendly and organic manner. It is reported that, several sericigenous insects including *A. mylitta* exude a proteolytic enzyme cocoonase as they near the final stages of their metamorphosis. This proteolytic enzyme makes the anterior portion of the cocoon soft which facilitates the moths' exit from cocoon. This natural phenomenon generates an idea, "if cocoonase has the capacity to soften the anterior portion of the cocoon during adult emergence, then it can be utilized for cooking of tasar cocoons". Interestingly, it is proven that cocoonase directly acts on the sericin protein without affecting the fibroin protein. It evidently indicates that, sericin is an excellent natural substrate of cocoonase. The cocoonase based cocoon cooking retains the natural, beautiful unique tasar silk color, softness and luster of tasar silk yarn.

INITIATIVES ON *A. MYLITTA* COCOONASE

Antheraea mylitta is one of the industrially important species of silkworm which habitats in the tropical region of India. Various studies on *A. mylitta* cocoonase have been conducted during the collaborative research starting from exploring the natural process of cocoonase secretion, its collection, purification, characterization, preparation of molecular sculpt, and the potential of cocoonase in degumming. Various initiatives have been taken on cocoonase depiction (Fig.1 & 2). Earlier report suggest that during emergence *A. mylitta* exudes 600-900 µl of cocoonase with a concentration of 221µg/ml. The moth emerges after cocoonase secretion and forms a hole in anterior portion of cocoon during emergence (Fig.3a-Fig.3b). The elemental-mapping/profiling of secreted cocoonase, suitable condition for specific activity of cocoonase, utilization of purified native cocoonase for cocoon softening, and degumming report also suggests that the cocoonase can be reused at least once after degumming one batch of cocoon. It is also reported that around 2150 ml of cocoonase was collected from 3000 tasar silkworm during emergence. The specific stage of pupae was identified for cocoonase secretion and its collection. The cocoons after harvest were collected and cocoons were cut open to take the pupae out of the cocoon thereafter these pupae were allowed to get ready for emergence which was monitored based on changes in integument colour. The integument of the pupae changes from its initial reddish-brown colour to black as it approaches closer to adult emergence (Fig.4). Before emergence, pupae were transferred to cocoonase collection set-up for cocoonase secretion and collection. Maldi Tof (MS and MSMS) of cocoonase (MS 1320.477) was similar to that of the cocoonase/proteolytic enzyme of other sericigenous insects. Molecular matching of the cocoonase of *Antheraea* species was also carried out to determine potential natural variants. Further, a specific time for cocoonase expression has been determined based on a sequential change in the concentration of secreted cocoonase. The observed changes in native cocoonase concentration over time are interesting. The collection phases with the

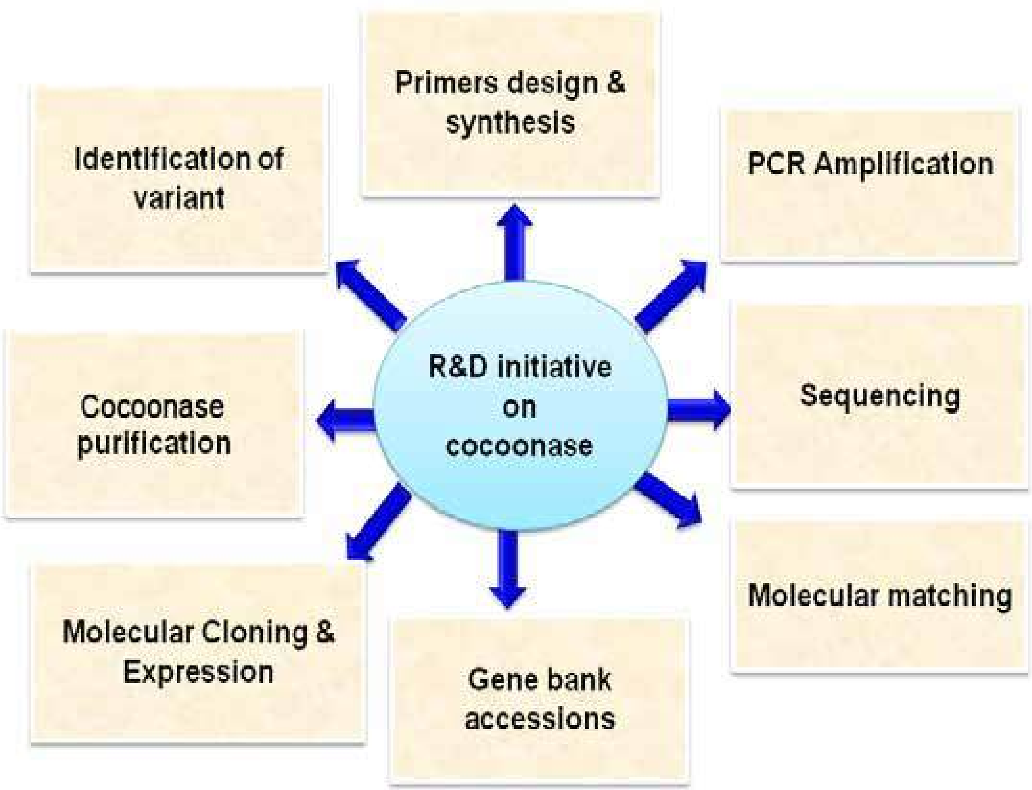

Figure 1: Showing R&D initiatives on cocoonase.

highest concentration were early, mid, and late. The first thirty minutes were observed to secrete a greater secretion of cocoonase. During the first 30 minutes around 260-300 µl of cocoonase were secreted during this time while the last 60 to 120 minutes was observed to release around 200-300 µl of cocoonase. The collected cocoonase was purified using Sephadex G-50 and the molecular weight of cocoonase upon 12% SDS-PAGE separation was found to be 26kDa (Fig.5a). Further, the concentration of different components present in liquid cocoonase was analysed using an element analyzer. The carbon to nitrogen (C/N) ratio in cocoonase was observed to be 17.04. It was also observed that the cocoonase of sericigenous insect and the *Fusarium oxysporum* trypsin sequences were observed to be strikingly similar.

Moreover, CSB-CTRTI developed a technique to extract cocoonase from freshly pierced cocoons and standardized the protocol for cocoon degumming using the extracted cocoonase from the pierced cocoon (Fig.5b) by incubating at various concentrations, pH, and temperatures suitable for cocoon softening. The dilution of cocoonase was done using tris-buffer and this also served as a control in degumming experiment. It was discovered that for Daba bi-voltine cocoon cooking in cocoonase at 1:5 dilutions in Tris buffer at pH 8.5-9.0 and incubation of 24-36 hours, the temperature range of 35-40°C was more favourable. For degumming cocoons were initially boiled in water for 30 minutes. They were then cooked in cocoonase (1:5) at 35-40°C, which resulted in a significantly higher cooking efficiency with 50-55% silk recovery. The leftover cocoonase solution can be utilized once more after adding 10% fresh cocoonase in addition. It was observed that cocoonase mildly softens the cocoons leading to the retention of the natural colour of tasar silk which looks glazy, soft and alluring (Fig.5c). A comparative study of Scanning Electron Microscopy (SEM) of silk thread also confirmed the cocoonase-induced favorable modification on silk surface that might be responsible for its novelty in contrast to the chemical method. Based on the earlier studies it is confirmed that sericin present in cocoons is the best substrate for cocoonase which can be utilized to soften the cocoons by targeting the glue protein sericin as natural substrate and it degums the sericin without affecting the fibroin to produce comparatively more comfortable, durable and organic silk. Thus, the cocoonase based product diversification modules will constructively modify and value the silk product. Further, it will also enhance the product quality to benefit primary silk producers. Additionally, cocoonase being designated as an endopeptidase and sharing its resemblance with trypsin can find several applications in silk industry including the biomedical field. Therefore, to meet the industrial requirement of cocoonase in large-scale degumming, the use of recombinant cocoonase can suffice the purpose. It has been revealed that cocoonase exhibits both fibrinolytic and thrombolytic potential. However, further research is required to substantiate this finding before it can be used in a viable pharmaceutical context as a viable antithrombotic agent. Moreover, apart from this, many more industrial applications related to cocoonase need to be explored and worked out.

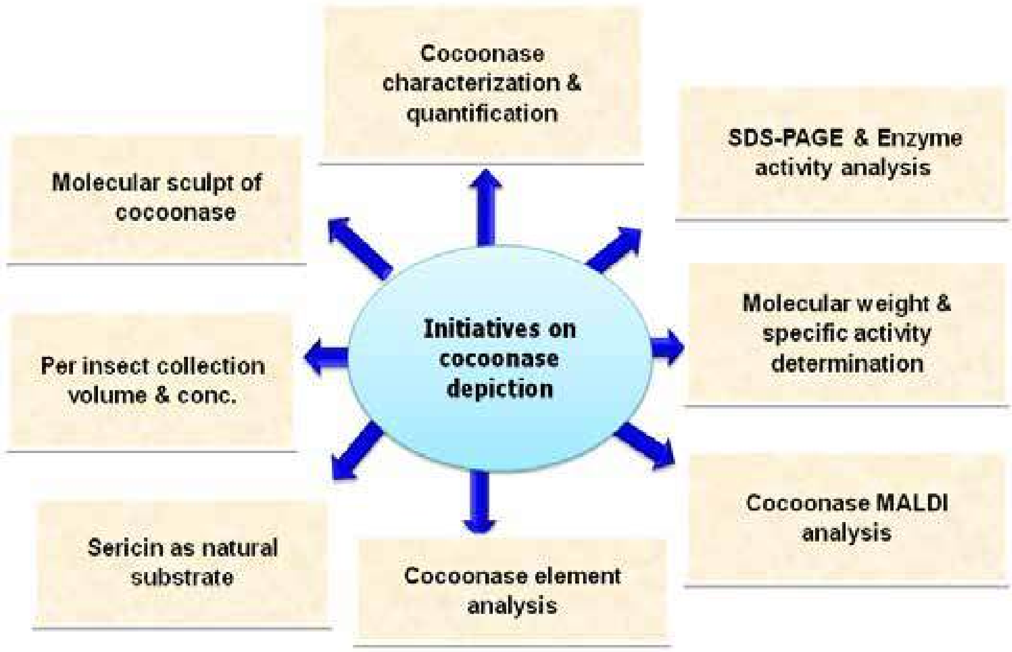

Figure 2: Various initiatives on cocoonase depiction.

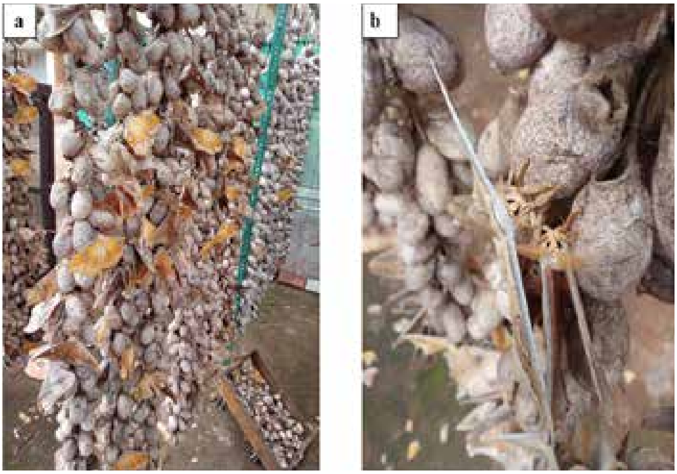

Figure 3: (a) Emergence of moth after cocoonase secretion (b) Hole in anterior portion during emergence.

Figure 4: Changes in integument colour of pupae from natural red-brownish to black colour.

Figure-5: (a) Purified cocoonase (26kDa) separation on 12% SDS-PAGE. (b) Pierced cocoon after moth emergence (c) Cocoon softening using cocoonase and reeling of silk fibre.

CONCLUSION

The exploration of cocoonase has illuminated its pivotal role in the moth emergence by softening the anterior region of cocoon thereby facilitating its emergence. The present book chapter accomplishes its goals and leads to convey cocoonase based R&D information at one platform. This chapter also illustrates and unveils the deep insights of cocoonase enzyme and emphasizes on its protease activity validating its cocoon softening potential and its prospective application in the silk industry. Moreover, numerous other potential industrial applications of cocoonase remain to be investigated and developed.

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4. Silkworm Pupae: A Nutritional Wealth

K. Jena, S. Ananta, S. Das, J. Singh,
B. K. Das and N.B.Chowdary

CONTENTS

INTRODUCTION

Sericulture is an age-old practice, involving cultivation of silkworms to produce raw silk. Silk is an integral part of the textile industry contributing about 0.2% of the total textile production of the world. There are different species of silkworms that are reared for different varieties of silk, namely Mulberry silkworms (*Bombyx mori)*, Tropical tasar silkworms (*Antheraea mylitta*), Oak tasar silkworms (*Antheraea proyeli*), Muga silkworms (*Antheraea assamensis)* and Eri silkworm (*Samia ricini*).

B. mori are well known as the mulberry silkworms that solely feed on mulberry leaves. They are the commercially domesticated silkworms that contribute about 90% of the world's silk production. In India, Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Jammu & Kashmir are the major mulberry silk producing states. Also, mulberry silk is produced in different parts of world namely China, India, Uzbekistan, Thailand, Brazil, Vietnam, North Korea and Turkey.

Silkworms other than mulberry are called non-mulberry silkworms or vanya silkworms. The commercially important natural vanya silks are Eri silk; Tasar silk; Oak tasar silkworms and Muga silk. Eri silkworm mainly feed on castor oil leaves. It produces an off-white or brick-red colored silk. In India, major eri silk producing states are Meghalaya, Assam, Nagaland, Manipur, Mizoram and Arunachal Pradesh. Eri silk is preferred by people in China, Japan, Nepal and Bhutan, due to its non-violent origins. The domesticated species, *Samia ricini* is multivoltine, while the wild species are usually trivoltine.

Similarly, tropical tasar silkworms (*A. mylitta*) belong to the family Saturniidae of the order Lepidoptera. These worms are bi and tri-voltine. They feed mainly on food plants *Terminalia arjuna* (Arjun), *Terminalia tomentosa* (Asan) and *Shorea robusta* (Sal) produce copperish/ grey color coarse silk. In India it is produced in the states of Jharkhand, Bihar, Chhattisgarh, Madhya Pradesh Orissa, West Bengal and Andhra Pradesh. Oak Tasar silkworms (*A. proyeli*) belong to the order Lepidoptera and feed on the oak tree to produce finer tasar silk. In India oak tasar silkworm are reared mainly in Assam, Manipur, Himachal Pradesh, Jammu & Kashmir and Uttrakhand. The world's largest producer of oak tasar is China, which are reared from the *A. pernyi* variety of silkworm.

Muga silkworm (*A. assamensis*) belongs to the Saturniidae family of the order Lepidoptera. Its 99% of production occurs in the Assam and regions of North-Eastern India. Muga silkworm feed mostly on Soalu (*Litsea monopetala)* and Som (*Persea bombycina)* leaves and produces a strong and glossy golden-yellow silk thread. *A. assamensis* is the multivoltine silkworms with five or six generations per year.

Silkworms are the commercially important insects, and its lifecycle passes through four distinctive phases-egg, larvae, pupae and adult (moth). Silkworm completes its life cycle faster in summer as compared to winter season. The silkworm life cycle starts when female moth lays egg after mating. The eggs of silkworm hatches in 8 to 10 days and silkworm larva are produced, where silkworm feed on its host plants and moults 4 times. After completing its moulting, larva spin cocoons and is transformed into pupae. The pupae undergo internal modifications and metamorphosis into an adult moth. The adult stage completes silkworm life cycle. The moth emerges out of the cocoon by softening the cocoon shells secreting proteolytic in enzyme called cocoonase. This is the reproductive stage where male and female moth mates and the female lay egg, so its life cycle continues.

SILK PRODUCTION

Silk production originates from China, and till date it is the largest producer of silk in the world. According to the International Sericulture Commission (ISC), the major silk producing countries in the world are China, India, Uzbekistan, Thailand, Brazil, Vietnam, North Korea and Turkey. China is the World's leading countries to produce silk and supply to the world market. India and Uzbekistan are the world's second and third country respectively. Some other countries involved in the production of silk are, Zambia, Botswana, Nigeria, , Kenya, Zimbabwe, Nepal Malaysia, Bangladesh, Colombia, Japan, Egypt Bulgaria, Uganda Romania, Bolivia, etc. The major silk consuming countries in the world are India, China, USA, Italy, Japan, Germany, France, United Kingdom, Switzerland, Viet Nam, U.A.E, Korea, etc.

India is the second largest producer of raw silk in the world. India produced 36,582 metric tons of annual silk in year 2023-24 (CSB Report). About 97% of the raw silk is produced in the Indian states of Karnataka, Andhra Pradesh, Assam, Tamil Nadu, West Bengal and Jharkhand. India is the only country in the world to produce all varieties of silk.

SILKWORM PUPAE

Silkworm pupae are one of the major byproducts of the silk industry. The main part of the cocoon is the silkworm pupae, equaling about 80% of the weight of the fresh cocoon and 50% of weight of the dry cocoon. During silk processing, pupae were killed by drying in hot air oven. For removal of threads, cocoons were boiling in water or soaked in soda/ soap/detergent. The pupae obtained after the reeling process is called spent pupae (Fig-1), usually discarded in the environment as waste. To produce 1 kg of raw silk, approximately 8 kg of wet pupae and almost 2 kg of dry pupae are generated as waste. These pupae are the rich sources of proteins, essential amino acids, pupae oil, carbohydrate, vitamins and minerals.

Table-1: Production statistics of mulberry and non- mulberry silk in India

RAW SILK PRODUCTION STATISTICS (Unit: MT)					
Years	Mulberry	Tasar	Eri	Muga	Total
2000-01	14,432	237	1,089	99	15,857
2001-02	15,842	249	1,160	100	17,351
2002-03	14,617	284	1,316	102	16,319
2003-04	13,970	315	1,352	105	15,742
2004-05	14,620	322	1,448	110	16,500
2005-06	15,445	308	1,442	110	17,305
2006-07	16,525	350	1,485	115	18,475
2007-08	16,245	428	1,530	117	18,320
2008-09	15,610	603	2,038	119	18,370
2009-10	16,322	803	2,460	105	19,690
2010-11	16,360	1,166	2,760	124	20,410
2011-12	18,272	1,590	3,072	126	23,060
2012-13	18,715	1,729	3,116	119	23,679
2013-14	19,476	2,619	4,237	148	26,480
2014-15	21,390	2,434	4,726	158	28,708
2015-16	20,478	2,819	5,060	166	28,523
2016-17	21,273	3,268	5,637	170	30,348
2017-18	22,066	2,988	6,661	192	31,906
2018-19	25,344	2,981	6,910	233	35,468
2019-20	25,239	3,136	7,204	241	35,820
2020-21	23,896	2,689	6,946	239	33,770
2021-22	25,818	1,466	7,364	255	34,903
2022-23	27,654	1318	7349	261	36,582
2023-24	29,892	1586	7183	252	38,913

Silkworm pupae contain 50-60% crude protein, 20-30% fat, 8-10% carbohydrate, 5-10% amino acids (Out of 21 amino acids, 18 amino acids are present, of which 8 are essential for humans), Vitamin A, B1, B2, B5, B6, B7, B9, B12, C, D, E, K and minerals such as calcium, phosphorus, copper, and iron. These essential nutrients make silkworm pupae more nutritive. 100 g of dried silkworm pupae can meet 75% of a person's daily protein needs. The percentage of total fat content by dry weight is 32.2%. The ratio of saturated to monosaturated and unsaturated fatty acids in silkworm pupae oil is 28.8%, 27.7%, and 43.6%, respectively. Silkworm pupae oil has a polyunsaturated fatty acid (PUFA), monosaturated fatty acid (MUFA), and saturated fatty acid (SFA) ratio of 1.6:1:1. Silkworm pupae are novel sources of protein and lipids.

Silkworm pupae are considered as highly nutritious food as they are rich source of protein, oil, carbohydrate, vitamins and minerals. They are regarded as a future food as it has high food conversion rate. Beside they have lower negative impact on the environment. Silkworms are not only used as food but it's byproducts have many potential applications in the field of agriculture, chemical, cosmetic (skin and hair products), biomedical science, pharmaceutical, food and textile industries.

UTILIZATION OF SILKWORM PUPAE

After reeling, silkworm pupae are considered waste and often discarded in the environment. Silkworm pupae are rich in protein source can be used as supplement in ruminants' diet, due to its favorable protein profile containing essential and non-essential amino acids. Silkworm pupae meal can replace fish meal and help significant increase in bodyweight against the control. Better technical and economic performance for silkworm pupae meal compared to other feeds (feed consumed and number of eggs produced ratio, egg size, egg shell thickness, grading of eggs, yolk color, mortality rate, feed cost, and cost per dozen eggs) has been reported earlier.

Since silkworm pupae are rich source of nutrition it is consumed as food by human beings. Silkworm pupae in terms of protein, fat, vitamins, and calories, are comparable to meat and superior to the protein found in soyabeans, fish, or beef. They have also been used to improve lactation in tribal women. In some countries like Korea, China, Japan, Thailand and Northeastern states of India, the silkworm pupae are used as delicious and nutritious food. Japan Aerospace Exploration Agency [JAXA] has reported a pupal recipe during 36th Scientific Assembly of the Committee on Space Research [COSPAR] as astronaut food. In countries such as China, Japan, Hong-Kong and Korea, silkworm pupae are included in bakery products (cakes), soups and sauce. They are sold as healthy commercial foods that are sterilized and vacuum dried.

Proteins from silkworm pupae are utilized in specialized diets for diabetic and cardiac patients because they are simple to digest and help lower cholesterol and blood sugar levels by providing additional energy. The biomedical and pharmaceutical industry uses Serra peptidase, a type of silkworm protein, to treat oral surgery, tonsillectomy, and acute sinusitis with anti-inflammatory and anti-tumefacient effects. The pupae are utilized in the synthesis of vitamins A, E, and K as well as in the treatment of neurological, ophthalmic, anti-bacterial, and anti-histaminic conditions as well as pancreatitis, leukocytopenia, and liver hepatitis. The silkworm pupae are used as a base material for the cultivation of medicinal-grade mushrooms that fight cancer and boost the immune system.

Pupae oil containing linolenic acid, the building block of human DHA (docosahexaenoic acid), have a significant impact on human intelligence, memory enhancement, eye-sight protection, and acts as a preventative chemical against hyperlipoidemia. Pupal oil successfully manages triglycerides, aid fatty livers, safeguards the liver after alcohol use, enhances blood quality and vascular domain, effectively softens blood vessels, lower blood pressure, and avoid arteriosclerosis and thrombosis. Pupal oil improves the functions of insulin-producing beta cells, restores the fatty acid desaturase activity of cells in diabetic patients, and has a notable hypoglycemic impact free from recurrence by allowing the prostaglandins to maintain equilibrium with the effects of preventing prostate disorders.

The silkworm pupae oil can be used in cosmetic industry including hair, face and body products. The same pupae oil is used to make soaps, printing inks, PVC plasticizers, lubricants, dyes, and varnishes for the textile and leather industries. The saponification values of the soap made from pupal oil closely resemble those of the oils used to make soap. The pupal oil had saponification values of 203. The pupal oil, which has a fishy smell and a dark brown color, can be used with linseed oil (25:75) to make paints and varnishes. Moreover, sterols extracted from pupae oil are used to make candles.

Silkworm pupae chitin can potentially fight off bacteria like *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Aspergillus niger*, and fungi like *Trichophyton equinum*. Chitin is also utilized as a biocompatible membrane to control bleeding during major surgeries, as well as an immuno-adjuvant (antiviral agent), bacteriostatic, fungistatic, and anti-sordes agent. Chitin and chitosan are being used in a variety of biomedical applications, including wound dressings, drug delivery, tissue scaffolds, tissue engineering, anticoagulant vascular grafts sutures, cellular functions, regulation of blood lipids and glucose by artificial kidney membrane, antiapoptotic, antioxidant, hepatoprotective activity, and as anticancer agent. It is also used to increase the shelf life of fruits.

Since silkworm pupae are rich source of protein, nitrogen and essential minerals such as calcium and phosphorus it can be used as organic manure and a good nutrient source. Silkworm pupae can be used in different forms *i.e.*, raw silkworm pupae, silkworm pupae powder and de-oiled silkworm pupae powder as manure. The use of pupae can help plants with greater root and shoot weight. The pupae fat, its residue formed during the pupae oil's extraction, pupae excreta can also be used as natural organic fertilizer.

WORK PROGRESS AT CSB-CTRTI ON TASAR PUPAE

CSB-Central Tasar Research and Training Institute, is the leading institute deals with R&D activities of tasar culture. In recent past CSB-CTRTI has initiated multi-institutional collaborative research work on utilization of tasar pupae (Fig-1).

Figure 1: Tasar silkworm pupae

Tasar Pupae : A Cheap Source Of Nutrition

Tasar pupae are considered as a cheap source of nutrition as they contain higher level of proteins, fat, carbohydrate and minerals The nutritional composition analysis of *A. mylitta* pupae shows higher crude protein (55-63%), crude fat (18-25%), carbohydrate (0.5-1.5%) and energy level (430-470 kcal) on a dry matter basis. Tasar pupae contain amino acids that are essential for human body which can be used in supplements as amino acids enriched foods. The pupae protein of *A. mylitta* contains glycine, alanine, serine, proline, valine, threonine, cysteine, leucine, asparagine, aspartic acid, lysine, glutamic acid, methionine, histidine, ethionine, phenyl alanine, arginine, citrulline, tyrosine, beta 3-4 dihydroxy phenyl alanine and tryptophan. Also, tasar pupae contains both saturated as well as unsaturated fatty acids, namely, lauric acid, mystic acid, palmitic acid, palmitoleic acid, linolenic acid, linoleic acid, stearic acid eicosanoic acid and behenic acid.

Vitamins and minerals are required for the proper working of the human body. It helps buid-up immunity and act as an antioxidant enzyme cofactor. Vitamins and minerals are needed in small amount for the well-functioning of metabolic processes that enable us to get energy from proteins, carbohydrate and fats. Tasar pupae contain both water-soluble vitamins *i.e.*, niacin, pyridoxine, pantothenic acid, biotin, thiamine, riboflavin, folic acid and cyanocobalamin and fat-soluble vitamins *i.e.*, vit D, tocopherol, vit K. Among the minerals, zinc, iron, manganese, magnesium, calcium, copper, sodium and potassium are present in trace amount.

Due to its high nutritional value, tasar pupae and its products are used as a valuable food products and dietary supplements. It can help eradicate malnutrition and protein deficiency. Interestingly, silkworm pupae have also been considered as possible food source by astronauts as space food on long-term missions. On the other hand, large quantity of pupae of the sericulture industry is discarded as a waste. These pupae express great future potential to be used commercially and economically for the benefit of mankind.

Source Of Feed

More than half of the expense of raising poultry and aquaculture is attributable to feed costs. There is a significant gap between the availability and demand of concentrate ingredients for feeding the country’s livestock due to the lack of feed resources. Hence, the available feed resources particularly spent silkworm needs to be utilized very judiciously in addition to exploring the unconventional feed resources in livestock feeding. The cost of the oil seeds, oil cakes/meals have been increased considerably in the recent past. Meeting the consumer demand for more animal products is depending upon to a major extent on the availability of appropriate cost effective and safe animal feeds. It has become necessary to explore alternate feed supplements from non-edible sources to bridge the gap between availability and requirement of animal feeds.

Tasar silkworm pupae are the major waste of silk industry, after recovery of silk fibre from cocoons. As silkworm pupae are rich source of proteins, high value essential oils, vitamins, minerals and antioxidants, this facilitates its usage as feed ingredient for fish and poultry. This helps create additional demand for sericulture industry producing lower cost feeds. The low cost of feed for fish and poultry will help generate extra income to the stake holders at one hand and helps reduce disposal of pupae in open environment, converting waste into wealth.

Many trails and research are going on for the better utilization of silkworm pupae. Silkworm pupae are easily spoiled due to their high moisture content; as a result, they are usually sun-dried and grounded to powder. Moreover, defatted silkworm pupae have a high protein content and are less perishable, making them a good choice for making fish feed. In this text, in collaboration with ICAR-CIFRI, Barrackpore, CTRTI has developed fish feeds (RESHMEEN) by using tasar pupae (Fig-2). Further, preparation of poultry feed are in pipe line with CSIR-CIFTRI, Mysore.

Figure 2: Fish feed 'Reshmeen' prepared from spent tasar pupae in collaboration with ICAR-CIFRI, Barrackpore

Source Of Chitin And Chitosan

Silk industry by- product wastes are serious threat to the environment. These wastes are biodegradable but their degradation takes a long time. Approximately 5000 MT of tasar pupae are accessible per year. They are discarded as waste which can be a promising source of biopolymer. This waste can be used in extraction of chitin and chitosan that can be modified into products of economic importance. Chitin and its derivatives are non-toxic,

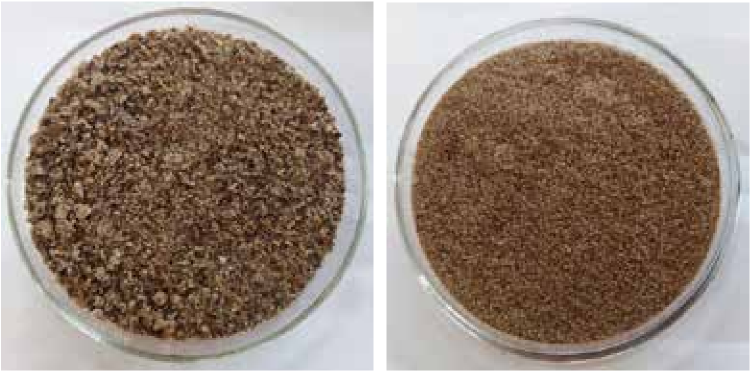

Figure 3: Chitin and Chitosan extracted from tasar pupae

renewable, biocompatible, and biodegradable substances having a variety of biological activities, including anti-cancer, antioxidant, antibacterial, and anti-coagulant.

The chitosan obtained from tasar silkworm pupae has several advantages over chitosan obtained from other sources (Fig-3). Firstly, silkworm pupae are abundant and widely available in India. Secondly, the chitosan obtained from tasar pupae has a high degree of deacetylation compared to other sources, which makes it more soluble and biologically active. Finally, the use of tasar pupae for chitosan production provides generous use of pupae that provide an additional income for silk farmers, contributing to rural development.

Chitin is a naturally occurring biopolymer synthesized from units of *N*-acetyl-D-glucosamine linked by β-(1→4)-linkages. Chitin has a highly crystalline structure and the main reason for its poor solubility and low processing. Therefore, chitin is deacetylated to obtain chitosan under alkaline condition. Chitosan is a versatile biomaterial because of its non-toxicity and diverse bioactivities. Unlike chitin, chitosan is water soluble in acidic media or under specific conditions at neutral pH, allowing for extensive growth in solution and hydrogel domains. These biopolymers can be used for a variety of innovative purposes, such as biotransformation to create food items with additional value, protecting food from microbial destruction, creating biodegradable sheets, recovering waste from food processing discards, and purifying water. Further, chitosan prepared from tasar silkworm pupae have higher fluorescence intensity than shrimp chitosan (Fig-4), which may used in bio imaging techniques. The optically active nature of chitosan can be employed successfully in the fields of optical sensors, biolabeling, bioimaging, and cancer research.

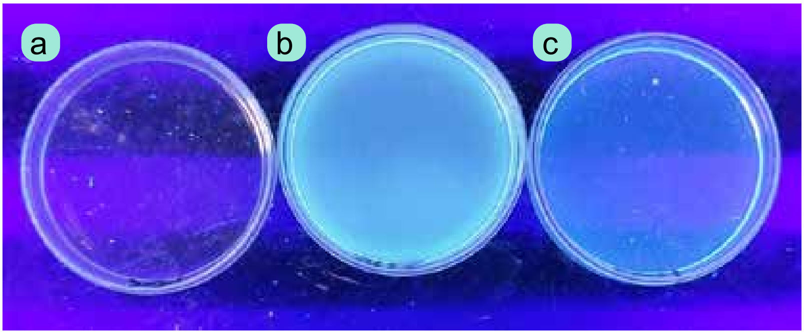

Figure 4: Chitosan illuminated in UV light: a. only methanol, b. silkworm pupae chitosan and c. shrimp chitosan

Chitosan does not show any cytotoxity which makes it an ideal source of coating material of fruits. To enhance the post harvesting self-life of banana, it was coated with chitosan. The result shows that silkworm pupae chitosan coating delays the ripening of banana when compared with control and commercially available shrimp chitosan (Fig-5).

Further pupae chitosan is a natural biopolymer, which is used in preparation of Chitosan based sheets and mats. These scaffolds are environment friendly, biodegradable, non-toxic and economical. It can be used in the biomedical industry especially in the wound healing applications. The natural biopolymer can be combined with synthetic polymer such as carboxymethyl cellulose (CMC)/poly vinyl alcohol (PVA). These synthetic biopolymers have film forming abilities and are non-toxic, biodegradable and biocompatible. Also, in order to enhance the textural qualities of films and improve its mechanical properties cross linkers and plasticizers are used. Glycerol has received the greatest attention and is

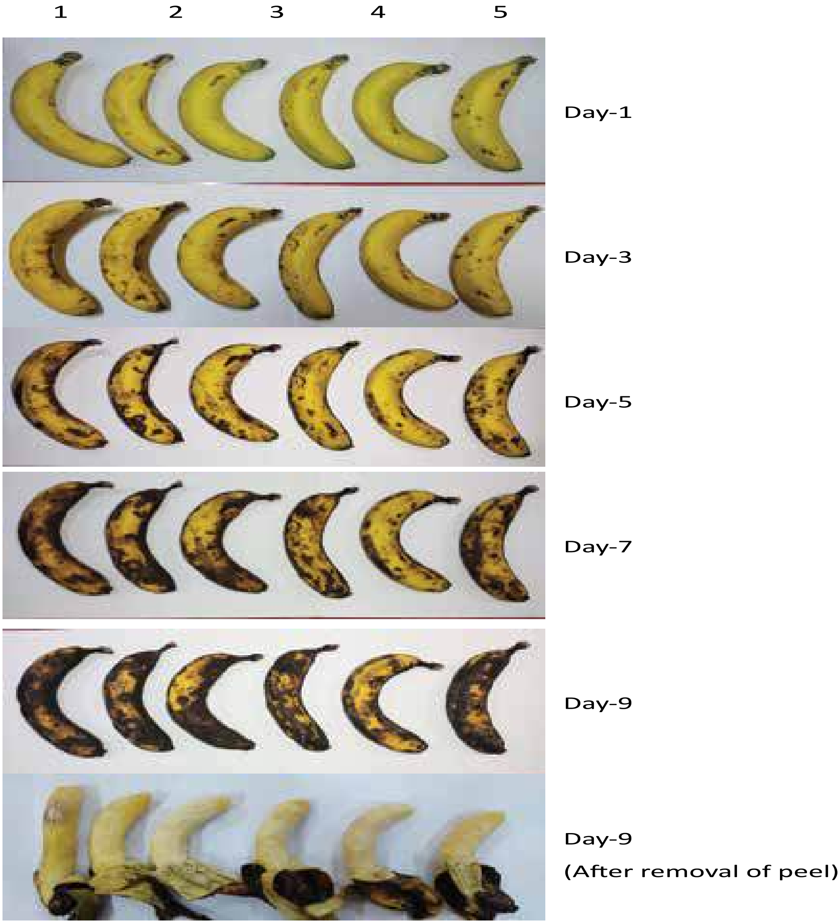

Figure 5: 1. Control (DW), 2. 1% Acetic Acid, 3.0.25 % Chitosan (Tasar),4. 0.5 % Chitosan (Tasar), 5. 1 % Chitosan (Tasar), 6. 1 % Chitosan (Shrimp)

a commonly used plasticizing component in carbohydrate films. Thus, the development of wound dressings capable of wound healing is of great medical importance. The initial work is under progress.

Source Of Pigment & Antioxidants

Tasar pupae contain bioactive molecules. These bioactive molecules are extracted using organic solvents from the silkworm pupae. The bioactive components identified in pupae have a wide range of pharmacological roles, including anticancer, antioxidant, hepatoprotective, antibacterial, antiapoptotic, and immunomodulatory properties. This expands greater possibilities for the utilization of silkworm pupae in biomedical research industries. The bioactive molecule in silkworm pupae *i.e.*, antioxidant compounds are extracted from silkworm pupae by Soxhlet extraction method using n-hexane and ethyl acetate as a solvent (Fig. 6). They are studied for its antioxidant (DPPH and H_2O_2) properties. The fraction shows free radical scavenging activity in a concentration dependent manner. As silkworm pupae contains polyphenolic and flavonoid compounds, the scavenging potential of the pupae extract may be due to the presence of these polyphenols and flavonoids.

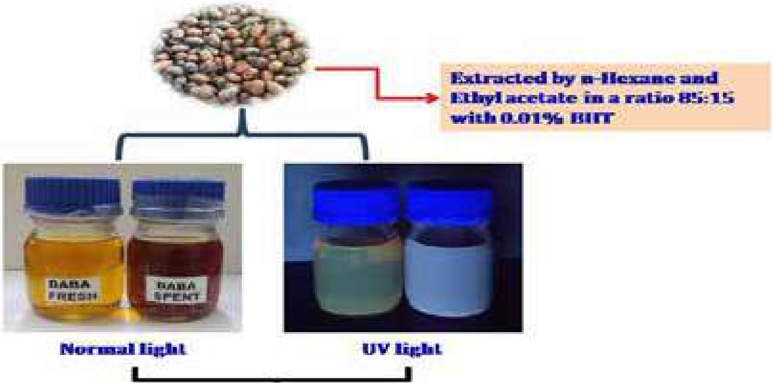

Figure 6: Tasar pupae oil

UTILIZATION OF SILKWORM EXUVIAE

Exuviae are the shed outer skins or covering of the silkworm (Fig-7). When silkworm undergoes metamorphosis, it shed its old skin or exoskeleton to take new size and shape. According to estimates, 159 MT of exuviae are produced from 1586 MT of raw silk. Silkworm exuviae is a rich sources of chitin (approx 40%) and chitosan, and are often discarded as waste in the open environment. Exuviae is a type of silk waste produced both during the extraction of fibre and during the grainage operation.

Figure 7: Tasar silkworm exuviae

The earlier studies shows the usage of silkworm exuviae as a low cost dye biosorbents by adsorption technique. Exuviae are rich sources of chitosan, which act as a biodegradable polymer. Due to its biocompatibility with other substances and lack of toxicity, it is utilised as cellulose alternatives to build biocomposite materials. The proximate composition analysis of exuviae shows it to be good source of chitin and minerals such as calcium, magnesium, manganese, copper, zinc and sodium. The methanolic extract of exuviae shows glowing activity when viewed under UV light (Fig-8). It shows great antioxidant activity (free radical scavenging assay) and anti-microbial activity against *Candida albicans, Bacillus subtilis*, *Enterococcus farcalis*, *Pseudomonas aeruginosa, Lactobacillus acidophilus*, and *Streptococcus mutans.*

Figure 8: Methanolic extract of exuviae glowing under UV-Vis light

The silkworm exuviae shows a good antioxidant activity due to the presence of phenolic acids and flavonoids. The phenolic acid profiling of exuviae indicates gentisic acid, p-coumaric acid, protocatechuic acid, gallic acid and ferulic acid are in larger amount as compared to other phenolic acids. Similarly, the flavonoids profiling of exuviae shows the presence of luteolin, catechin, epicatechin, quercetin, umbelliferone, epigallocatechin and myricetin in major amounts. In addition to scavenging free radicals, flavonoids and polyphenolic compounds also have biological effects that include antiviral, anti-inflammatory, antibacterial, and antiallergic activities. The presence of antioxidant activity in exuviae of silkworm indicates its ability to protect cells against the damaging effects of reactive oxygen species (ROS) by protecting body from wide range of diseases including heart disease, cancer and neurodegenerative disorders. It can help neutralize ROS and prevent their formation, thereby reducing the risk of oxidative damage.

The silkworms exuviae extract have biological potential. These glowing compounds shows cytotoxic effect against breast cancer cell lines *viz.* MCF7, BT474 and SKBR3. The WST-1 assay was used to assess how methanolic extract of exuviae affects the cellular proliferation and viability. When exposed to methanolic extract, the WST assay showed that breast cancer cells' ability to proliferate was inhibited in a dose and time-dependent manner. This might be because the methanolic extract contains polyphenolic constituents.

Silkworm exuviae are excellent source of chitin and chitosan. Chitin is extracted from silkworm exuviae by chemical extraction method *i.e.*, deproteinization (10% NaOH) and demineralization (7% HCl). Further, chitosan was prepared from the chitin by a process called de-acetylation (55% NaOH). Chitosan is the N-deacetylated form of chitin, a linear polysaccharide made up of ß-(1, 4)-linked 2-acetamido-2-deoxy-D-glucose. Chitosan are widely used and are more versatile than chitin due to reactive free amino group and solubility.

The silkworm exuviae chitosan are studied for its functional properties including Water binding capacity (WBC) and Fat binding capacity (FBC) which shows good WBC and FBC when compared with the commercially available shrimp chitosan. The isolated chitosan morphology was captured by Field emission electron microscopy (Fig-9), having flat surface.

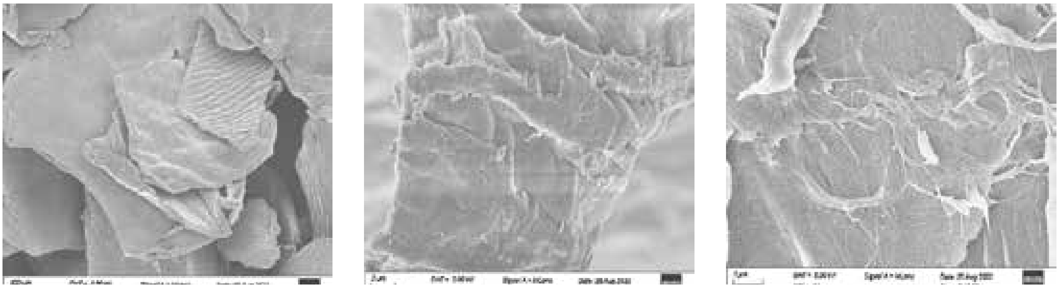

Figure 9: FESEM image of exuviae chitosan

CONCLUSION

Silkworm pupae represent an exceptional source of nutrition, rich in proteins, essential amino acids, vitamins, and minerals. Their unique nutritional profile positions them as a valuable addition to diets, particularly in regions where they are a traditional food source. Beyond their health benefits, incorporating silkworm pupae into modern diets could also offer sustainable solutions to global food security challenges. As we continue to explore and embrace diverse food sources, the potential of silkworm pupae underscores the importance of integrating traditional knowledge with contemporary nutrition science to address the evolving needs of a growing population. The exploration of silkworm pupae as a nutritional powerhouse is not just a testament to their value but also an invitation to rethink and diversify our dietary practices for a healthier and more sustainable future.

ACKNOWLEDGMENTS

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5. Exploring Sericin and Fibroin: Natural Proteins and Their Diverse Applications

K. Jena, A. Sinha, J.P. Pandey, T. Bal, K. Sathyanarayana and N. B. Chowdary

CONTENTS

INTRODUCTION

Sericulture is an agricultural sector that is also known as a silk farming method in which silkworms are reared to produce cocoon. The sericulture industry encompasses various stages from plantation and seed production to rearing, reeling, weaving of silk, and collection of byproducts. This entire process significantly contributes to generating employment and livelihoods for rural and tribal communities on a large scale. It serves as a crucial economic activity, offering sustainable income opportunities and supporting local economies in rural

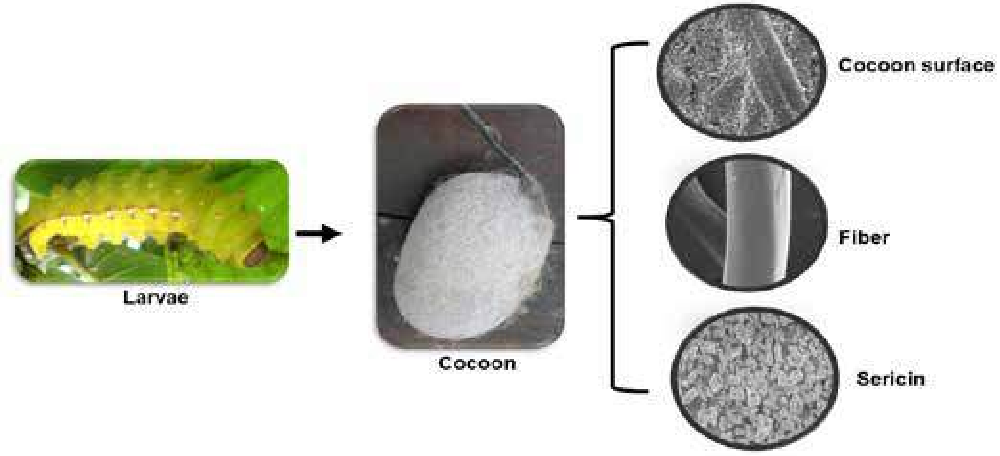

Figure 1: Tasar silkworm and scanning images of cocoon surface, fibre and sericin

areas. This sector not only provides employment directly through tasks like silk production and weaving but also indirectly through associated activities such as plantation, silkworm rearing, and the processing of silk byproducts. This holistic approach ensures that the sericulture industry plays a vital role in uplifting rural and tribal livelihoods, fostering economic growth, and promoting socio-economic development in these communities.

With the advancement of Science and Technology, the textile industry has experienced significant growth in the quality of product development. Every stage of the manufacturing process begins with sourcing raw materials, followed by producing high-quality fibres and yarns, achieving vibrant and durable colors, and innovatively designing and printing fabrics to create the final textile products.

Silk is an unique textile component because of its inherent affinity for colours, dazzling color, natural brilliance, smooth feeling, sheen, softness, low weight, flexibility, strength, elasticity, fitness, high absorption, resilience, elegance, and grace. Hence, it is regarded as the Queen of Textiles and is a marvellous gift of nature to society. Scientists have been intrigued by silk for many years, and thanks to improvements in analytical methods and molecular biology tools, a new understanding of these polymers is now emerging. It is possible to weave this naturalistic protein fibre into fabrics.

SILK PROTEIN

Silk, a natural fibre primarily composed of fibroin and sericin, is produced by silkworms. Sericin serves as a binder for the filaments that form cocoons, providing structural integrity. On the other hand, fibroin is the essential component of the fibre and finds extensive use in the textile industry. During silk processing, sericin is typically discarded as waste, separated from fibroin to obtain a bright, soft, and manageable silk thread. Globally, over 50,000 tons per year of sericin are disposed of in wastewater during the degumming process, contributing to environmental pollution due to the high oxygen demand from decomposing microorganisms and chemicals used in the process. Further due to its unique properties, efforts have been ongoing to recover sericin and utilize it as a natural biopolymer in various sectors.

SYNTHESIS OF SILK PROTEIN

The silkworm forms its cocoon by moving its head in a figure-8 or S pattern, which involves rhythmic bending of its body. The silk glands, located along its digestive canal, are specialized exocrine glands divided into three distinct regions: the anterior silk gland (ASG), middle silk gland (MSG), and posterior silk gland (PSG). Each region serves specific functions in silk production. The ASG, forming the excretory duct, consists of approximately 200 cells. The MSG, about 7 cm long, secretes sericin, a protein that surrounds the fibroin produced by the PSG. The PSG, measuring about 15 cm, secretes fibroin, which forms the core of the silk thread. Silk gland development begins during embryonic stages and continues through out larval growth. In the MSG, different areas secrete inner, middle, and outer layers of sericin, which immediately coat the fibroin. The resulting silk thread can measure between 900 to 1500 meters in length, offering protection against environmental factors and predators such as birds and insects. The cocoon itself is oval-shaped with varying thickness at its ends. Cooconase protease enzyme secreted by the moth dissolves these ends, facilitating the moth's emergence. The composition of the cocoon is primarily fibroin and sericin (approximately 97%), with minor components including lipids, waxes (0.4 - 0.8%), inorganic salts (0.7-1.0%), and pigment (0.2-0.5%). During the transition from larvae to pupae, the silk gland undergoes significant morphological and functional changes, eventually degenerating completely during the pupal stage.

SERICIN A GLUE PROTEIN: ITS EXTRACTION, PROPERTIES AND APPLICATIONS

Sericin is a water-soluble protein known for its hydrophilic and adhesive characteristics. It has a globular structure that plays a crucial role in adhering silk filaments together, thereby maintaining the structural integrity of the cocoon. Sericin comprises 18 amino acids, with notable concentrations of serine (20-40%), aspartic acid (11-23%), and glycine (12-20%). These compounds contribute significantly to its functional properties within the cocoon structure.

Table-1: Comparative amino acid% (polar, non-polar, aromatic, positive and negative charged) of *B. mori* and *A. mylitta*

	B. mori	*A. mylitta*
Polar uncharged	49.58	33.77
Non-Polar	20.96	20.64
Aromatic	5.95	6.60
Positively charged	9.08	12.36
Negatively charged	14.43	26.63

The molecular weight of sericin range from 6-245kDa and above, which depend on their extraction process (alkaline, acidic, enzymes), and other factors such as pressure, temperature and the processing time. Based on the solubility in water, sericin has been classified into three fractions (A, B & C). The outermost layer of the cocoon contains sericin A, which is more soluble in warm water and contains 17.2% nitrogen with serine, threonine, glycine, and aspartic acid as the main amino acids. Sericin B, which includes 16.8% nitrogen and an addition of tryptophan and is made up of the same amino acids as sericin A, is located in the intermediate layer. Sericin C, the final fraction, is located in the innermost layer next to fibroin; it is insoluble in hot water and has reduced nitrogen content (16.6%). Fraction C contains proline in addition to the amino acids present in sericins A and B.

Extraction Methods Of Sericin From Silk Cocoons

Since sericin is hydrophilic and soluble in water, can be extracted from silk, unlike fibroin, which is hydrophobic and insoluble in water. Detergents/soaps, sodium carbonate and sodium bicarbonate solution are used in the degumming process by the silk industry. The technology that successfully removes sericin from the cocoons enables the recovery of pure fibroin for use in the textile industry. During degumming, the recovered sericin is severely damaged due to use of detergents/soaps and high alkaline chemicals, and losing physio-chemical and biological characteristics and reducing in molecular weight. In addition, sericin and soap are very difficult to separate. Therefore, sericin may contain soap, which limits its biomedical and pharmaceutical applications. Various degumming techniques, such as thermal degumming, chemical degumming, and enzymatic degumming, have been developed to facilitate the recovery of sericin rather than fibroin. These techniques are sometimes used to produce sericin with appropriate utility and properties. In the heating method cocoons are often heated/boiled in water, which interact with the hydroxyl group of polar amino acids to further separate sericin and fibroin, because high temperature and pressure causes unstability of hydrogen bonds between the hydroxyl groups. Sericin is extracted from silk using acids (tartaric, citric, succinic, etc.) or bases (sodium carbonate, sodium silicate, sodium hydrosulphite, sodium phosphate) which hydrolyze sericin by rupturing peptide bonds of the amino acid into small molecules. Sericin is then released into the alkaline or acidic solution, where it is highly soluble. For example, The COOH group present in sericin molecule is changed by sodium carbonate into -$COONa^+$, which enhances its solubility due to the high hydration of Na^+. Sericin has also been extracted using proteolytic enzymes, such as alcalase, savinase, degummase, papain, and trypsin. These enzymes cause the hydrolysis of the peptide bonds between the amino group (NH_2) of the neighbouring amino acids and the carboxyl group of lysine/arginine.

Table-1: Adopted extraction process for separation of sericin from cocoons

Extraction methods	Approach	Advantages	Limitations
Conventional	Detergents / Soaps and sodium bicarbonate, hydrogen peroxide	Effective and widely acceptable	• Sericin is highly degraded. • Recovery is difficult and it is not environment friendly.
Chemical	Acidic solutions (e.g,citric, tartaric, succinic acid) Urea (with or without mercaptoethanol)	Sericin is less degraded than when using alkaline solutions Effective	• Sericin is degraded • Purification steps are needed to remove the chemical impurities.Toxic to cells • Difficult to separate high quality sericin
Enzymatic	Proteolytic enzymes (e.g, papain, pancreatin, alcalase, trypsin, deggumase etc	• Effective • Environmental friendly/ no effluent problems	▪ No purification steps are required ▪ Not economic
Heat& Pressure	Boiled in high temperature and pressure	• Simple low cost • Environmental friendly/ no effluent problems	▪ Sericin is degraded (when used at high temperatures) ▪ Damages fibroin, removes only the outer layer of sericin

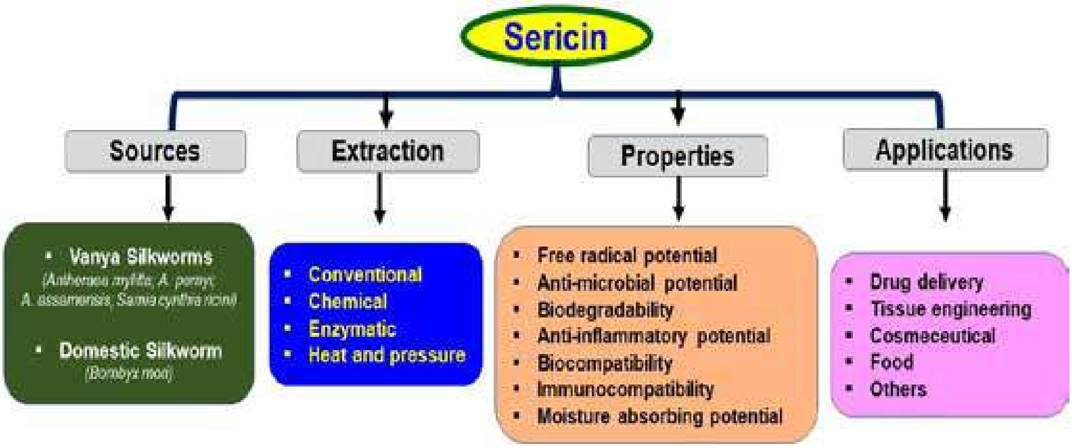

Figure 2: Schematic diagram of sericin sources, extraction methods, properties, and applications

Properties

Biocompatibility

The primary criteria for every biomedical material are biocompatibility. Whenever a biomaterial comes into contact with the human body, it should not have any negative impacts (e.g., immune response). As sericin is an immunologically inactive protein, its biocompatibility has been proven in a variety of studies. Sericin's safety for cells is demonstrated by the fact that it does not cause cytotoxicity when used in the culture media of various cell lines. Moreover, sericin does not induce immunological responses.

Biodegradability

An organism can convert a biodegradable biomaterial into other substances via a variety of biological processes. The majority of the time, it is preferable to use biodegradable biomaterials to prepared biomedical products because they are only kept in the body until they are needed and are then expelled gradually and naturally once they have degraded. When biodegradable biomaterials are utilised to treat wounds, is an advantage. By using a biodegradable wound dressing, the patient will experience less pain and discomfort and less harm to the newly produced tissue because the dressing won't need to be changed or removed from the wound site. Sericin is a biodegradable polymer, and proteolytic enzymes, such as chymotrypsin, papain, proteinase K, protease XIV, matrix metalloproteinases, and collagenase, operate on the amorphous hydrophilic portions of the heavy and light chains of silk to mediate its destruction both in vitro and in vivo. Amino acids, which are the byproducts of sericin breakdown, are absorbed by the body without evoking an immunological response.

Anti-Inflammatory activity

Inflammation is a crucial stage in the healing process, where phagocytosis removes dead tissues and pollutants from the wound site. It's also a phase where inflammatory cells release growth factors and cytokines to attract cells necessary for tissue regeneration.

However, this phase requires control because unregulated inflammatory cytokines can lead to excessive expression of metalloproteinases, which degrade the extracellular matrix. Therefore, biomaterials used in wound healing must possess the ability to modulate the inflammatory response effectively.

Antibacterial activity

Biomaterial having potential to eliminates germs or inhibits their development or capacity for multiplication, it has antibacterial characteristics. The management of infectious diseases has become more and more difficult for healthcare systems in recent years. For instance, one of the most serious issues in wound care is the emergence of infections throughout the healing process. Microorganisms at the wound site impede the usual healing process from taking place, leading to additional more serious local and systemic problems. As reported cysteine, an uncharged polar amino acid is responsible for sericin's antibacterial activity due to the presence of sulfhydryl groups. These sulfhydryl groups can then create extremely reactive compounds by weakly hydrogen bonding with oxygen or nitrogen. These compounds have an impact on a variety of enzymatic processes and metabolic processes in bacteria. Sericin has been shown to have antibacterial activity against both Gram-positive and Gram-negative bacteria. Sericin has antimicrobial actions against bacteria like *Escherichia coli* and *Staphylococcus aureus.*

Free radical scavenging potential

Reactive oxygen species (ROS) are naturally produced during biological metabolism and are typically regulated by the cell's antioxidant defense systems. Imbalances in ROS levels can lead to oxidative stress, causing damage to cells and tissues, and contributing to various diseases such as cirrhosis, cancer, ischemic reperfusion injury and etc. Sericin is known for its potent antioxidant properties. It effectively inhibits lipid peroxidation and scavenges ROS, thereby protecting cells from oxidative damage. Sericin also modulates the activity of important antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase. The antioxidant activity of sericin can be attributed to its high content of amino acids such as serine and threonine, whose hydroxyl groups chelate trace metals like iron and copper, thereby neutralizing free radicals. Additionally, sericin's antioxidant potential may also stem from pigment molecules such as flavonoids and carotenoids associated with its structure. Moreover, sericin plays a role in protecting against UV radiation by maintaining redox balance and absorbing UV light under wavelengths below 200 nm. Its composition rich in hydrogen, oxygen, and nitrogen contributes to these protective properties against oxidative stress and UV-induced damage.

Biomedical And Pharmaceutical Applications of Sericin

Commonly, naturally derived polymers such as cellulose, alginate, sericin, chitosan, collagen, agarose, keratin etc. are preferred as they have several advantages over synthetic polymers (poly (anhydride), poly (glycolic acid) (PGA), poly (caprolactone) (PCL), poly (lactic-co-glycolic acid) (PLGA), poly (lactic acid) (PLA), etc.). Non- toxicity, biocompatibility and biodegradability are the most important biological properties of these polymers.

Drug delivery

Various materials such as scaffolds, films, hydrogels, fibres, capsules, spheres, foams, and microneedles are employed for local and systemic drug delivery. Sericin stands out as a promising carrier material due to its amphiphilic nature. This characteristic includes polar side chains and hydrophobic domains, enabling sericin to effectively bind charged medicinal molecules as well as hydrophobic and hydrophilic drugs. Sericin also possesses advantageous properties such as a prolonged half-life in vivo and robust moisture absorption and desorption capabilities. These attributes are highly beneficial for its application in drug delivery systems.

Tissue engineering

Sericin is a biomaterial that has been used for the regeneration of bone, skin, cartilage and adipose tissues. Sericin improves osteoblast cell (MC3T3-E1) proliferation by up to 135% when compared to the untreated reference. However, the best typical combination of biomaterials utilized to create scaffolds for bone tissue regeneration is the combination of sericin with hydroxyapatite or other calcium phosphate-based materials, since these biomaterials make it possible to simulate the formation of bone matrixes. Further, silk protein has been widely used in ancient times sealing wounds and damaged skin. In addition, sericin also stimulate cell migration and proliferation, which seem to be directly accelerated wound healing properties. It is an ideal biomolecule for the preparation of wound dressings and bioartificial skin grafts.

Agricultural application of sericin

Sericin offers diverse agricultural applications. It enhances seed germination and seedling growth by forming a protective coating that retains moisture and nutrients, creating optimal initial growth conditions. Studies have explored its potential as a biopesticide, showing insecticidal effects against agricultural pests, which may reduce reliance on synthetic pesticides and their environmental impact. In soil, sericin improves structure, water retention, and nutrient availability, thereby boosting overall soil health and crop productivity. It serves as an effective carrier for fertilizers and micronutrients, adhering to plant surfaces and gradually releasing nutrients to enhance uptake efficiency and minimize fertilizer runoff. Biodegradable films derived from sericin function as mulch in agriculture, aiding in weed control, moisture retention, and soil temperature regulation, while breaking down without harmful residues. Sericin's amino acids and peptides stimulate plant growth and development, making it a promising candidate for enhancing crop yield and quality through foliar sprays or root applications.

Other applications

Sericin exhibits beneficial effects on lipid and sugar metabolism, particularly evident in studies involving mice fed high-fat diets. When 4% sericin was added daily to their food for 5 weeks, there were no significant changes in nutritional intake, body weight, or body fat. However, sericin supplementation did lower levels of phospholipids, cholesterol, free unsaturated fats, triglycerides, very low-density lipoproteins (VLDL), and low-density lipoprotein (LDL). Furthermore, sericin has been shown to have hypoglycemic effects in Type II diabetic mice. It significantly reduced fasting blood glucose levels, fasting plasma insulin, and glycosylated serum protein levels. Sericin also improved oral glucose and insulin tolerance and enhanced antioxidative activities. These effects contribute to regulating insulin and lipid metabolism, normalizing blood sugar levels, controlling insulin secretion, and reducing inflammation. These properties suggest sericin could be developed as a functional food with notable hypoglycemic benefits. Recent research indicates that sericin can protect against liver injury in Type II diabetes. Dietary supplementation with sericin improved liver size and function in diabetic rats compared to controls. Sericin achieves this by regulating glycogen synthesis, accelerating glycolysis, and inhibiting gluconeogenesis. These effects are attributed to its antioxidant capacity and ability to reduce inflammatory responses, further supporting its potential for developing blood sugar-lowering food products. In addition to its medicinal uses, sericin is widely employed in personal care products like face creams, shampoos, and hair treatments. This is due to its elasticity, moisturizing properties, hypoallergenic nature, cleansing abilities, and effects on anti-aging and reducing wrinkles.

WORK PROGRESSED AT CSB-CENTRAL TASAR RESEARCH AND TRAINING INSTITUTE ON THE EXTRACTION OF TASAR SERICIN

CSB-CTRTI is an R&D institute under the aegis of the Central Silk Board, Ministry of Textiles, Govt of India to conduct R & D to cater to the needs of the Tasar silk Industry (both tropical and temperate). In the recent past, CSB-CTRTI has significantly progressed in the extraction of sericin from tasar silk cocoons as well as from cocoon cooking waste water.

Figure 3: Schematic diagram of sericin isolated from tasar cocoons and fibre waste

Isolation And Characterisation Of Sericin From Cocoons Of Different Ecoraces Of Tasar Silkworm

Tasar silk is produced by tasar silkworms, *A. mylitta*, a wild insect species. *A. mylitta* is distributed in many states of India. *A. mylitta* populations are located in different regions are commonly called as ecoraces. A total of 44 ecological variation of tasar silkworms have been recorded in 17 Indian states. Among them Daba, Raily, and Modal are major commercial tasar silkworm ecoraces found in Jharkhand, Chhattisgarh and Odisha, respectively. Tasar ecorace cocoons vary in size, shape, colour, volume and silk content. These different characters indicate different chemical properties of molecules that can affect sericin's biological potential. The results showed that sericin is a wide molecular range protein with higher thermal stability and a C: N ratio. It has been observed that sericin isolated from Raily cocoons has more free radical scavenging, anti-tyrosinase and anti-elastase potential compared to Modal and Daba ecorace, which may be due to more aromatic and phenolic content.

Isolation And Characterisation Of Sericin From Cocoons Harvested From Different Food Plants Of Tasar Silkworm

A. mylitta is polyphagous in nature, primarily feeding on *Terminalia tomentosa, T.arjuna* and *Shorea robusta*, and to a lesser extent about approximately two dozen of secondary food plants. Previous reports have shown that different tasar food plants have different nutritional values. High level of protein content was found in *T.tomentosa*. Similarly, *T. arjuna* and *T. tomentosa* were higher in total mineral content and lower in crude fibre content. However, carbohydrates, ascorbic acid and phenolic content are estimated higher in *S. robusta*. Higher phenolic content, C:N ratio, aromatic and non-polar amino acids, higher antioxidant, anti-elastase, anti-tyrosinase, anti-GST activity were observed in sericin isolated from cocoons of silkworm fed on food plant *S. robusta,* when compared to *T.arjuna* and *T. tomentosa*.

Isolation And Characterisation Of Sericin From Tasar Silk Fibre Waste

Approximately, 30-40% of fibre waste is being generated during silk processing, which contains 5-7% of inner layer sericin. The fibre silk wastes were deflossing waste (formed before reeling process for easy operation), reeling waste (feeding after exhaustion and mending breakage) and basin waste (left over residues *i.e.*, unreelable innermost shell layer). In India, the total production of tasar silk during 2023-24 was 1586 MT, which produces about 476 to 634 MT of TSFW. Fibre wastes were being used for producing spun silk and fabrics. In addition, silk effluents contain sericin an important protein that can be used in the preparation of drugs, cosmetics and biomaterials. The results showed that the recovered sericin has a wide range of molecular weight 11–245 kDa, higher C:N ratio and thermal stability. In addition, the isolated sericin can scavenge free radicals, inhibit tyrosinase and elastase activity which can be used important ingredient for cosmetics as well as pharmaceutics.

Isolation And Characterisation Of Sericin From Tasar Cocoon Cooking Waste Water

In India, the total production of tasar cocoon in the year 2023-24 was 1586 MT (CSB, Annual Report). Further, from 1586 MT approximately 127-159 MT of tasar sericin was discarded as waste (from our earlier observation 8-10% / gm cocoon shell). Further, in cocoon cooking process 20 ml of fresh water is consumed per cocoon, which means 3.16×10^7 lit. of degumming water has been generated, which is going as waste and chief source of sericin. So large scale purification unit for separation of tasar sericin from cocoon cooking waste water is highly necessary before application in various fields. In this context, CSB-CTRTI has developed a prototype sericin purification unit (fig-4) on separation of tasar sericin from cocoon cooking waste water.

It has been observed that physico-chemical nature of purified tasar sericin having similarity with available standard sericin. However, the biological potential of tasar sericin are higher than the commercially available sericin, and thus it can be used as a component of cosmetics as well as pharmaceutics. However, application of tasar sericin for foods, drugs and skin care products must be tested invivo.

Tasar sericin (Before purification)

Tasar sericin (After purification)

Figure 4: CSB-CTRTI developed sericin purification unit and sericin (before and after purification)

Sericin is a natural protein of varying molecular weights produced by silkworms, constituting 17-25% of their cocoons. This water-soluble glycoprotein contains hydrophobic and hydrophilic amino acids, contributing to its diverse biological properties such as antibacterial, antioxidant, anticancer, anti-tyrosinase, anti-inflammatory, and anti-aging effects. Sericin has found numerous applications, including its combination with other biomaterials utilized in the food industry. Notably, sericin-based coatings and films have demonstrated effectiveness in preserving fruits and vegetables. These coatings have potential applications in post-harvest storage to prolong the shelf life and quality of perishable produce. Moving forward, there is promising scope for further development of sericin-based coating materials for fruits, vegetables and seeds enhancing their storage conditions and reducing spoilage. This underscores sericin's versatility and potential in agricultural and food technology innovations.

FIBROIN A CORE ELEMENT OF SILK FIBRE: ITS PROPERTIES AND APPLICATIONS

Silk is among the oldest known animal fibres, primarily produced by insects such as silkworms to form cocoons. It is renowned for its luxurious sheen and allure, often referred to as the queen of textiles. Silk comprises high molecular weight organic polymers with alternating hydrophobic and hydrophilic peptide sequences, making it a fibrous protein polymer. Its composition, structure, and properties vary depending on its origin and purpose. Commercially, silk mainly comes from the filaments spun by silkworms, particularly the mulberry silk produced by *Bombyx mori*. There are also wild silks like tasar (*Antheraea mylitta*), oak tasar (*Antheraea proylei*), eri (*Philosamia ricini*), and muga (*Antheraea assamensis*), which differ from domesticated silks in color, size, and texture. Spider silks and other insect-produced silks are notable for their glycine-rich composition, distinctive synthesis and processing methods, as well as their exceptional strength and flexibility. These silks represent a unique category within the realm of natural fibres, characterized by their specialized properties and applications.

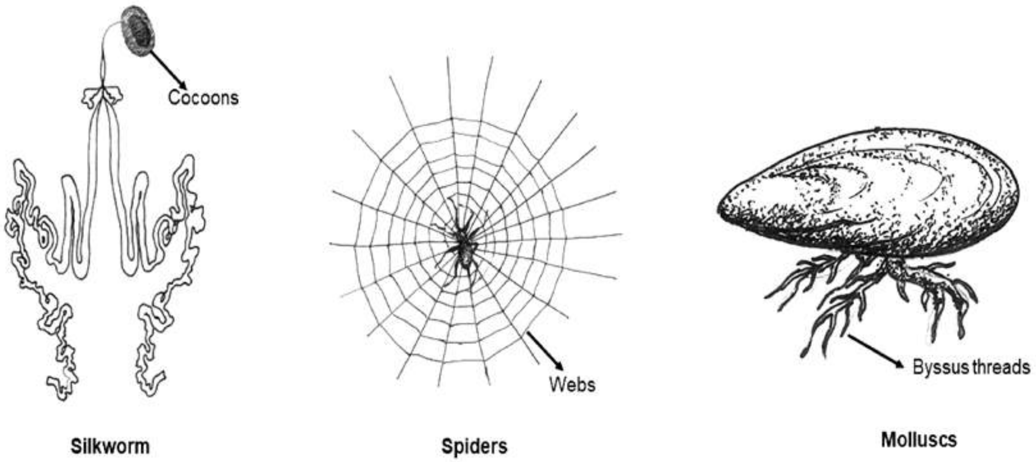

Figure 5: Production of fibres by different animals

Composition Of Silk

Silk filaments are composed primarily of two proteins: fibroin, which forms the core of the fibre, and sericin, which acts as a binding agent around the fibroin. Morphologically, silk is a straightforward fibre consisting of two compact, endless threads spun by the silkworm, encapsulated in a layer of sericin (silk gum). In addition to fibroin and sericin, silk contains small quantities of wax, minerals, pigments, and other components, making up approximately 2-5% of its total weight. Fibroin constitutes 70%-80% of the filament, while sericin accounts for 17%-25%. Silkworms produce silk by extruding liquid fibre from two silk glands, which converge in the spinneret located in their heads. In the spinneret, the two fibres merge and are bound together by sericin, forming a continuous filament. Both sericin and fibroin proteins exhibit valuable properties and have been found to possess various biological functions, contributing to their significance in both industrial and biomedical applications.

Amino Acid Composition

The amino acid composition varies between different types of silk. Both mulberry and non-mulberry silks contain three major amino acids: serine, glycine, and alanine. In mulberry silk, these amino acids typically make up around 82% of the total composition, with serine

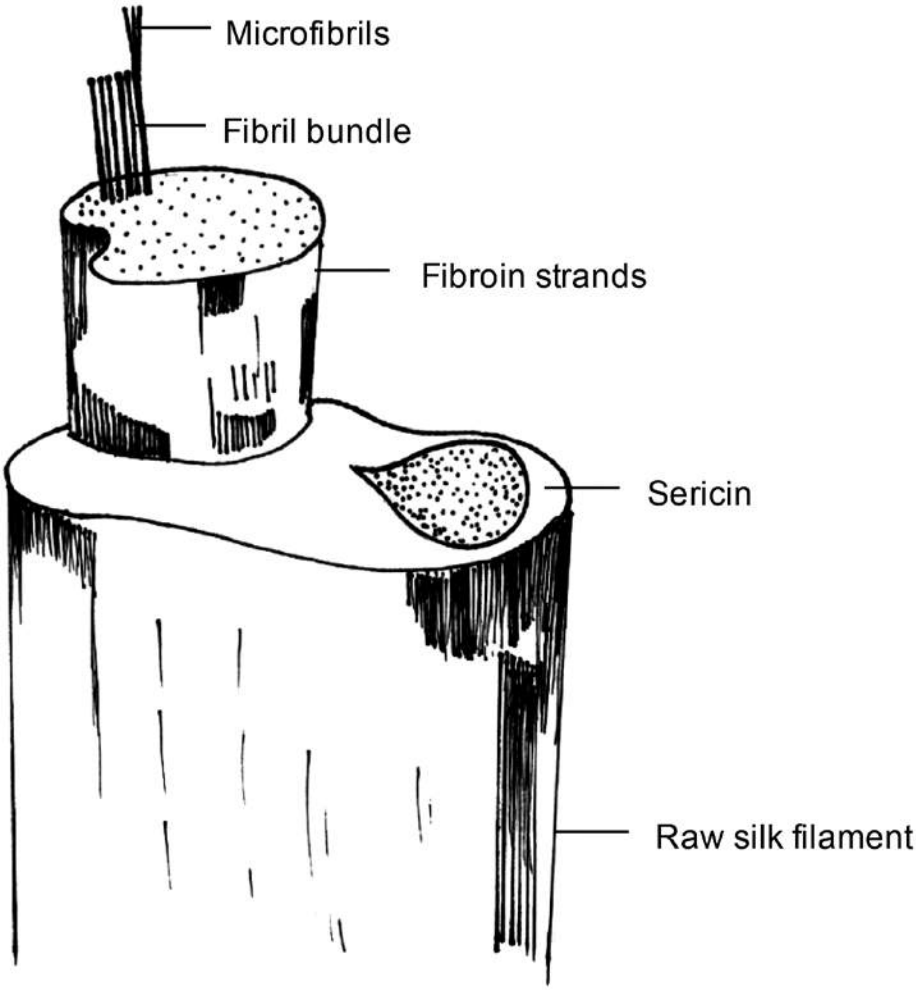

Figure 6: Structure of silk fibre

alone accounting for up to 10%. Non-mulberry silks generally have a lower total percentage of glycine, alanine, and serine, around 73%, which is approximately 10% less than mulberry silk. Vanya silks, such as tasar, eri, and muga, show a higher proportion of alanine compared to mulberry silk. Alanine constitutes about 34% in tasar, 36% in eri, and 35% in muga. On the other hand, glycine content in these varieties ranges from 27% to 29%, lower than the 43% found in mulberry silk. Non-mulberry silks also exhibit a significant presence of amino acids with bulky side groups, particularly aspartic acid (4-6%) and arginine (4-5%). This indicates higher levels of both acidic and basic amino acids compared to mulberry silk. Interestingly, all types of silk contain sulfur-containing amino acids such as cystine and methionine. Non-mulberry silks tend to have slightly higher methionine content (0.28-0.34%) compared to mulberry silk (0.11-0.19%).

Table-1: Amino acid composition of different varieties of silk

Amino acid composition (%)						
Amino acid	Mulberry silkworm	Vanya silkworms			Other invertebrates	
	Bombyx mori	*Antheraea mylitta*	*Antheraea assamensis*	*Samia ricini*	Spider	Mussel
Aspartic acid	1.64	6.12	4.97	3.89	2.5	3.6-18
Glutamic acid	1.77	1.27	1.36	1.31	8.6	0.6-2.3
Serine	10.38	9.87	6.11	8.89	3.6	1-4.3
Glycine	43.45	27.65	28.41	29.35	30.2	13.7-29
Hystidine	0.13	0.78	0.72	0.75	1.1	1-4.8
Arginine	1.13	4.99	4.72	4.12	6.8	3.1-9
Threonine	0.92	0.26	0.21	0.18	2.5	-
Alanine	27.56	34.12	34.72	36.33	24.3	1-2.9
Proline	0.79	2.21	2.18	2.07	3.6	3.6-8
Tyrosine	5.58	6.82	5.12	5.84	0.1	0.2-19.2
Valine	2.37	1.72	1.5	1.32	2.4	-
Cystine	0.13	0.15	0.12	0.11	1.1	2.9
Isolusine	0.75	0.61	0.51	0.45	1.1	-
Leusine	0.73	0.78	0.71	0.69	4.1	-
Phenylalanine	0.14	0.34	0.28	0.23	1.1	-
Tryptophan	0.73	1.26	2.18	1.68	1.8	6.0
Lysine	0.23	0.17	0.24	0.23	0.4	4-19.8
Metheonine	0.19	0.28	0.32	0.34	-	-
Dopa	-	-	-	-	-	3.2-30.4

Applications Of Silk

Textile

Silk is renowned for its comfort in warm weather and its ability to retain warmth in cold weather due to its low conductivity, which traps warm air close to the skin. Its versatility extends across various uses in daily life. Silk is commonly utilized in clothing such as shirts, formal dresses, high-fashion attire, stoles, shawls, pyjamas, skirt suits, including wedding gowns. Its elegant sheen, soft texture, and graceful drape make it ideal for decorative purposes, often seen in accessories like handbags and headbands. In home decor, silk's lustre and texture are highly valued, making it a popular choice for items such as sheets, bedding, curtains, and cushions. Additionally, silk's excellent insulation properties make it suitable for warmth clothing. These diverse applications highlight silk's unique qualities and enduring appeal in both fashion and home furnishings.

Bio-medical field

Silk fibroin has various biomedical applications due to its excellent characteristics such as biocompatibility, slow degradability, and impressive mechanical properties. These attributes have enabled its use in diverse fields including drug delivery, gene therapy, wound healing, and bone regeneration. The ability to manipulate its molecular structure and morphology through versatile processing techniques and surface modifications further enhances its utility in biomaterials and tissue engineering. Silk fibroin can be fabricated into various formats such as films, fibres, nets, meshes, membranes, yarns, and sponges. These formats have demonstrated capability in supporting stem cell adhesion, proliferation, and differentiation both in laboratory settings (*in vitro*) and within living organisms (*in vivo*). Particularly, the application of 3D silk fibroin scaffolds in stem cell-based tissue engineering has broadened the scope of silk-based biomaterials for creating tissues like bone, ligament, cartilage, and skin.

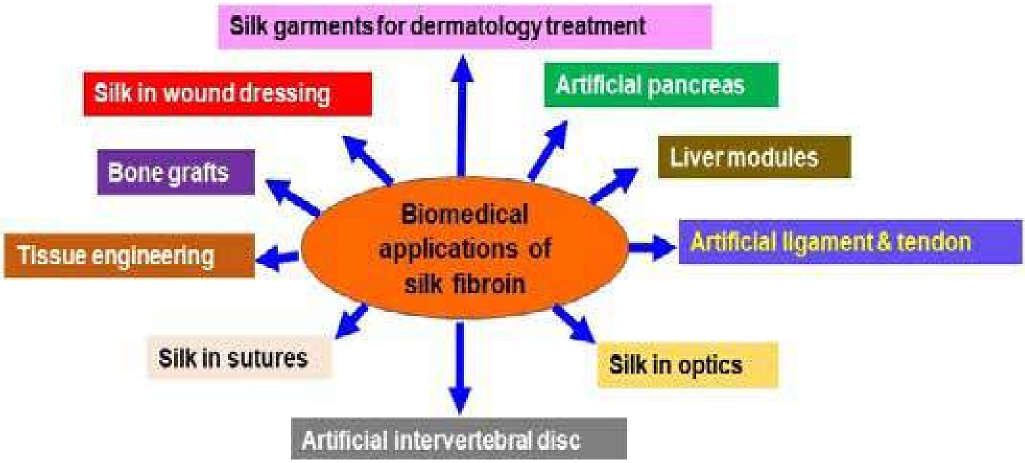

Figure 7: Schematic representation for applications of fibroin in the biomedical field

Surgical sutures

Silk fibroin is a widely used suture material due to its desirable combination of high strength, low bacterial adherence, and biodegradable, it degrades slowly enough to provide sufficient support to tissues during the critical initial phases of wound healing. This gradual degradation aligns with the natural healing process and helps maintain tissue integrity. Typically, silk is braided when used as sutures, which makes it prone to inducing inflammatory reactions. In contrast, non-braided sutures usually reduce the incidence of inflammatory reactions because their smoother surface lacks grooves that can harbor inflammatory-causing substances, mainly immune cells like neutrophils, lymphocytes, fibroblasts, histiocytes, and giant cells. Overall, while both braided and non-braided sutures have their respective uses and advantages, the smoother surface and reduced inflammatory potential of non-braided sutures make them a preferred choice in many surgical applications where tissue reaction and healing outcomes are paramount. Ongoing advancements in silk fibroin technology continue to optimize its properties, paving the way for enhanced surgical outcomes and patient care. As such, silk fibroin represents a promising and effective choice for modern surgical sutures, integrating natural biocompatibility with advanced biomedical engineering principles

Bone regeneration

As a specific kind of connective tissue, bone is mainly made up of hydroxyapatite and type I collagen. The strength and hierarchical structure of bone tissue are enhanced by this nanocomposite structure. Silk fibroin hydrogel-derived scaffolds for bone tissue engineering have gained popularity because of their strong mechanical strength, high toughness, good biocompatibility, and slow biodegradability. Supplementary, Its versatility allows for the incorporation of bioactive molecules and growth factors, further enhancing its regenerative potential. Recent advancements in silk fibroin technology, such as electrospinning and 3D printing, have enabled the fabrication of scaffolds with tailored architectures and mechanical properties suited for specific bone defects. In vivo studies have demonstrated the efficacy of silk fibroin-based scaffolds in promoting new bone formation and integration with surrounding tissues. Challenges remain in optimizing degradation rates and ensuring long-term stability and functionality in clinical applications. Nonetheless, silk fibroin holds great promise as a biomaterial for bone regeneration, offering a sustainable and biocompatible solution to address critical orthopaedic challenges and improve patient outcomes.

Cartilage regeneration

Cartilage is an avascular connective tissue that provides support and is made up of intercellular substances and chondrocytes. Silk fibroin's inherent biocompatibility, biodegradability, and mechanical properties make it well-suited for mimicking the extracellular matrix of cartilage. This biomaterial supports the adhesion, proliferation, and differentiation of chondrocytes, the specialized cells responsible for cartilage maintenance and repair. Silk fibroin scaffolds can be engineered with controlled porosity, structure, and mechanical strength, facilitating the formation of new cartilage tissue. Furthermore, silk fibroin's ability to incorporate bioactive molecules and growth factors enhances its regenerative potential by promoting chondrogenic differentiation and extracellular matrix synthesis. In vivo studies have demonstrated the efficacy of silk fibroin-based constructs in promoting cartilage regeneration and functional recovery in animal models. Challenges in optimizing scaffold properties, such as degradation kinetics and long-term stability, remain areas of active research. Nevertheless, silk fibroin represents a promising biomaterial for cartilage regeneration, offering a versatile and biocompatible platform to address the clinical challenges of cartilage defects and osteoarthritis.

Nerve regeneration

Both peripheral and central nerve lesions have been repaired with silk fibroin hydrogels. Research highlighted methacrylate-silk fibroin hydrogels filled with basic fibroblast growth factor (bFGF), which enhanced neuronal mitochondrial function, inhibited glial cell proliferation, and encouraged neurite repair. Further, conductive biocomposite hydrogels using graphene oxide (GO) nanosheets and silk shown to support Schwann cell survival and proliferation, and the higher conductivity promoted the functional behavior of Schwann cells. The hydrogel demonstrated a strong ability to guide axonal sprouting and encourage directional outgrowth of axons.

Dental applications

The use of silk biomaterials in dentistry is limited to suture materials and dental tissue regeneration, despite their long history in biomedical applications. Innovative nanocomposites made of silk proteins and silica have been developed, combining the advantageous qualities of the two ingredients. Silk has been employed for tissue regeneration either alone or as composite materials with other materials. By mixing hydroxyapatite nanoparticles with silk solutions, similar composites known as silk–hydroxyapatite composites have been created. Dentin and tooth enamel are made primarily of minerals like hydroxyapatite, which also gives these tissues their hardness and resilience. Dentin matrix protein 1 (DMP1), phosphoproteins, osteonectin, and other organic macromolecules regulate the nucleation of hydroxyapatite and the crystal structure of the material. In dentin, DMP1 is crucial for regulating the hydroxyapatite crystals' nucleation, development, and shape. Ameloblasts, osteoblasts, osteocytes, and cementoblasts are among the cells of other hard tissues that contain it as well. A biomimetic approach has been employed to synthesize biominerals, utilizing a blend of DMP-1 and spider silk. In order to achieve this, a new spider-like domain and a DMP-1 domain were cloned, expressed, and utilized for the self-assembly and hydroxyapatite nucleation processes. The combination of the silk domain's distinctive properties and the DMP1 domain's capacity to nucleate hydroxyapatite presents a great deal of promise for tissue engineering of biomineralized tissues.

Wound healing

As a biomaterial for wound dressings, silk fibroin (SF) has proven to have excellent properties, including gas permeability and the ability to maintain a moist environment. It has also been shown to improve cell growth, proliferation, and migration of various cell lines involved in the various stages of the wound healing process. Fibroin matrices expedite the synthesis of collagen, re-epithelialization, wound contraction, cellular adhesion, and angiogenesis. SF-based biomaterials have shown good cell adhesion and fibroblast proliferation, with improved neovascularization, faster and better tissue healing, and complete regeneration in a rat model in both in vitro and in vivo experiments on wound healing. Several investigations revealed how silk fibroin affected various cell lines and the molecular signaling involved in the healing of wounds. For instance, it is known that growth factors and cytokines are essential for wound healing. Research has also shown how silk fibroin functions to suppress the elevated proinflammatory cytokines during the wound's inflammatory phase, protecting cells and tissues as the wound heals. Additionally, an acellular goat-dermal matrix was added to silk fibroin protein to create a hybrid skin graft that would promote wound healing. Research highlighted that silk fibroin scaffolds in conjunction with elastin to replicate the extracellular matrix in the healing of burn injuries. Elastin/fibroin scaffolds encouraged the development of human fibroblasts and showed signs of faster wound healing and re-epithelialization.

As materials for composites

Silk fibroin exhibits exceptional biocompatibility, biodegradability, and mechanical properties, making it an ideal candidate for enhancing the performance and functionality of composite materials. The inherent strength and flexibility of silk fibroin fibres contribute to the mechanical reinforcement of composites while maintaining lightweight characteristics. Its ability to be processed into different forms, such as films, fibres, and hydrogels, enables the fabrication of composites with tailored structures and functionalities. Silk fibroin also offers opportunities for biofunctionalization, allowing for the incorporation of bioactive molecules and nanoparticles to impart additional properties, such as antimicrobial activity or enhanced biocompatibility. Moreover, the sustainability of silk fibroin production aligns with increasing demands for environmentally friendly materials in composite applications. This abstract discusses recent advancements in silk fibroin-based composites, including their use in biomedical implants, tissue engineering scaffolds, and structural materials. Challenges in optimizing processing techniques, improving interfacial interactions, and ensuring long-term stability in composite structures are also addressed. Overall, silk fibroin emerges as a promising material for developing advanced composites that combine superior mechanical properties with biocompatibility, paving the way for innovative applications across diverse industries.

CONCLUSION

Sericin and fibroin represent natural proteins with remarkable properties that have found diverse applications across various fields. Sericin, often discarded during silk processing, has shown promise in skincare, wound healing, and biomedical coatings due to its biocompatibility and antioxidant properties. Fibroin, the structural core of silk fibres, is utilized extensively in tissue engineering, drug delivery systems, and advanced textiles, owing to its biodegradability, mechanical strength, and versatility in forming various biomaterial formats.

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6. *Cordyceps*: Biochemical Insights, Cultivation Techniques, and its Significance

Sruthy K.S., M. M. Baig, K. Jena, S. Mumtaz and N. B. Chowdary

CONTENTS

INTRODUCTION

The name *Cordyceps* is derived from two Greek words "kordyle," meaning "club," and the Latin word "ceps," meaning "head." The genus *Cordyceps*, with over 700 known species, belongs to the Ascomycota phylum, class Pyrenomycetes, order Hypocreales, and family Clavicipitaceae. Each species of *Cordyceps* targets a specific host, usually insect caterpillars. The most extensively studied species, *Ophiocordyceps sinensis* (formerly *Cordyceps sinensis*) has been documented by Tibetan doctors in the past 14 century. Among 700 identified species in the genus *Cordyceps*, approximately 20 infest fungi in the genus Elaphomyces. The others parasitize insects and arthropods belonging to orders Arachnida, Hymenoptera, Isoptera, Coleoptera, Hemiptera, and Lepidoptera. Many species of this genus have been explored for their medicinal properties. The most important species are, *C. militaris, O. sinensis, C. sobolifera, C. ophioglossoides, C. cicadicola* and *C. liangshanesis*.

An unidentified author originally mentioned *Cordyceps* as a therapeutic fungus in a book called Sheng Nung Bon Cas Chien (The Classic Herbal of the Divine Plowman) circa 101 BC. *C. sinensis* is regarded as warm and appealing in Chinese medicine. It enters the kidney and lung channels and is usually taken in doses of 3 to 9 grams. The eighteenth century saw the first Western accounts of the health advantages of the *Cordyceps* fungus. The first account of this Himalayan aphrodisiac was written by a French priest, Perennin Jean Baptiste du Halde, who wrote about it while he was a guest at the court of an emperor in China. The term *Cordyceps* first appears in a medical literature, the New Compilation of Materia Medica, in 1757. In traditional Chinese medicine, *Cordyceps* have long been recognized and utilized to treat a wide range of illnesses, alleviate weariness, and prolong lifespan. A review of the literature revealed that *Cordyceps* possesses a broad range of pharmacological characteristics, including antibiotic, hepatoprotective, nephroprotective, antioxidant, anti-tumor, anti-metastatic, anti-cancer, anti-inflammatory, and hypoglycemic and hypocholesterolemic effects. Numerous bioactive substances, such as polysaccharides, adenosine, cordycepin, ergosterol, sterol, protein, and amino acids, as well as vitamins E, K B1, B2, and B12, are responsible for these attributes. Nucleosides, mono-, di-, and oligosaccharides, as well as macro- and microelements (K, Na, Fe, Cu, Mn, Ca, Mg, Zn, Pi, Si, Ni, Se, Al, Sr, Ti, Ga, V, Cr, and Zr) are also present in it. From the mycelia, culture supernatants, and fruiting bodies of *Cordyceps*, more than 20 pharmacologically bioactive substances and different extracts employing solvents (water, ethanol, methanol, and ethyl acetate) have been described.

Cordyceps sinensis is the more famous and expensive fungus, it is relatively rare and difficult to cultivate. In contrast*, C. militaris* is found worldwide and can form fruiting bodies on unprocessed rice grain. The host range of *Cordyceps* is narrow, confines within a single species or closely related species for most of the *Cordyceps* species. The highly sophisticated mechanisms of survival on the specific host species end up in the production of many metabolites that are economically important in many ways. *Cordyceps* species are diverse and thrive primarily in humid temperate and tropical forests. They are widely distributed across North America, Europe, and East and Southeast Asian countries, particularly in China, Nepal, Japan, Bhutan, Vietnam, Korea, and Thailand. Additionally, various *Cordyceps* species have been discovered in different habitats worldwide, indicating their global distribution. China, Korea, Japan, Taiwan, and other eastern Asian nations use a variety of natural *Cordyceps* sp. as traditional medicines. The common name for *Cordyceps sinensis* is "Yarsu Gumba." The locals in the Indian Himalayas also refer to this fungus as "Keera Jhar" (insect herb), "Keeda ghass," "Jeevan buti," "Chyou kira," "Sanjeevani buti," or "Himalayan Viagra." Currently, plenty of *Cordyceps* based products are available in the global market. A few of them are, OM™ Maitake, marketed by Yukiguni Maitake CO., LTD, Japan, Nutricafe-organic, CaféCeps® Packets and Gold Nutrition. The *Cordyceps* products are globally distributed in various regions such as USA, UK, Japan and Germany.

PROCESS OF GROWTH AND MULTIPLICATION ON HOST

Cordyceps spp. target host from various Lepidoptera families, particularly the larvae of the *Thitarodes armoricanus* commonly known as Himalayan ghost moth, The insects can be infected at different life stages, including larvae, pupae, and adults. Infection begins when fungal conidia disperse onto the insect cuticle. After getting in touch with cuticle, spores attach to the insect's outer surface and germinate. The process of germination completes within few hours. Conidia secrete peroxidases and superoxide dismutase which function as protective enzymes and various hydrolytic enzymes like chitinases, proteases, and lipases to aid in germination. Soon the conidia forms a germ tube with an appressorium, which is a flattened, disk-like structure at the tip of germ tube. The exoskeleton will be penetrated by appressorium and the fungi enters the hemocoel through a combination of enzymatic action and mechanical pressure. During multiplication and growth, the fungus produces toxic secondary metabolites within the host's body, leading to the host's death. The fungal hyphae then consume the insect, spreading throughout all visceral organs. Ultimately, the host tissue is replaced by fungal mycelium, leaving only the exocuticle to support the fungal stroma.

These fungi form a stroma (fruiting body) on infected arthropods. The color of the stroma varies by species and includes a stipe, which is embedded in the soil or dead trees. The fertile region of the stroma is located at the tip and resembles a head. Spores formed via sexual reproduction are contained within sacs housed in perithecia. Ascospores and asci are microscopic structures, with each perithecium contributing to the stroma's small blade-like shape. A fruiting body forms in the summer following infection on the insect body in the winter, and then the spores take to the air. The elongated stroma, which frequently protrudes from the host's head, has a branching or cylindrical appearance. The fruiting body is covered with many ascospore-containing infectious perithecia. Several species of *Cordyceps* may be grown on artificial media, and some can even be grown without soil.

BIOACTIVE COMPOUNDS FROM *CORDYCEPS*

Bioactive compounds are molecules found in plant or animal-based foods that provide regular energy intake and offer various therapeutic benefits, such as combating metabolic disorders, inflammation, and chronic diseases. Even in small amounts, these compounds meet basic nutritional needs and provide significant health advantages. Epidemiological studies show that consuming foods high in bioactive compounds, including antioxidants, vitamins, flavonoids, and carotenoids, positively impacts human health.

The genus *Cordyceps* is rich in a wide variety of chemical compounds and their derivatives, classified as secondary metabolites. The diversity of these compounds makes them particularly fascinating for therapeutic and pharmacological research. Many Primary and

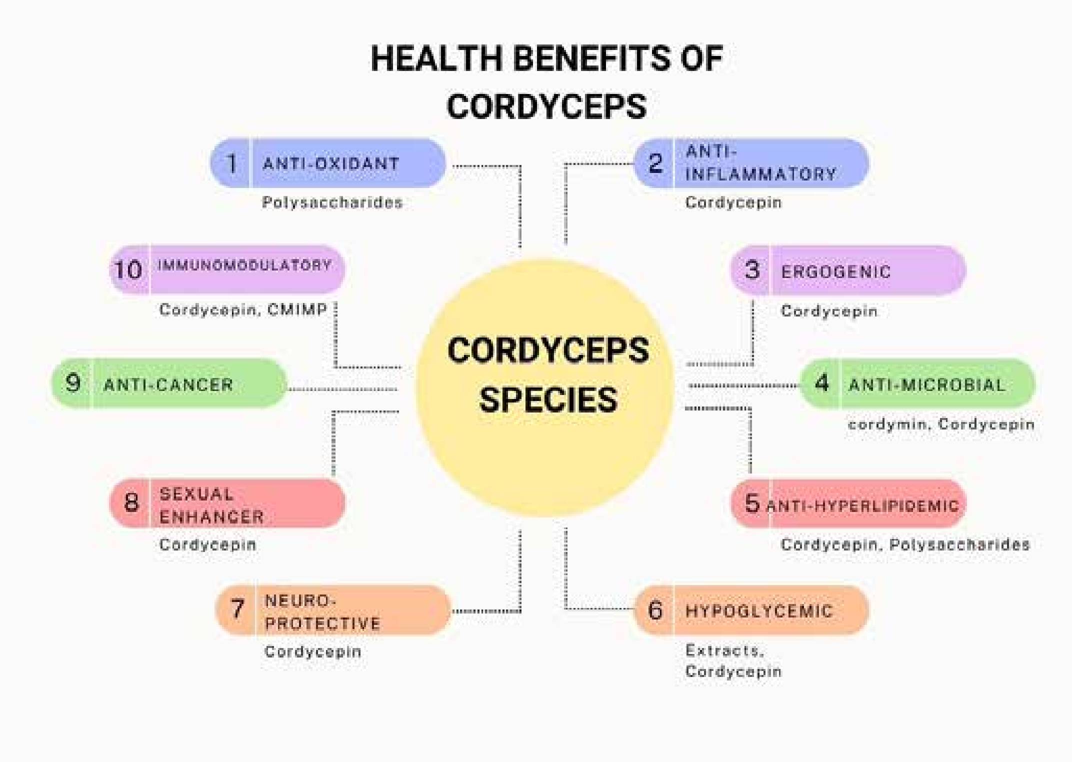

Figure 1: Potential health benefits of *Cordyceps* and responsible component

secondary metabolites including nucleosides, proteins, fats, flavonoids, polysaccharides, sterols, proteoglucans,cyclic peptides, amphinol, bioxanthracenes, cordycepin, cordycepic acid, phenolics, polyketides, and alkaloids.

Among these compounds, cordycepin secures first position in terms of medicinal properties. Although *Cordyceps* is rich in medicinal properties, the two significant pharmacologically active substances that were extracted from the fruiting bodies and culture supernatant of *Cordyceps* are cordycepin and cordycepic acid. The primary bioactive ingredient in *Cordyceps*, cordycepin, was first isolated from *C. militaris*; cordycepic acid, an isomer of quinic acid, is later recognized as d-mannitol. These days, cordycepin is added to other medications and used as a raw material in injections. Cordycepin aids in lowering blood levels of triglycerides, low-density lipoprotein, and cholesterol overall. Moreover, its impact on glucose metabolism may control blood glucose levels. Because cordycepin can obstruct RNA synthesis, it also possesses anti-inflammatory and anticancer effects.

Cordycepin

The basic structure of cordycepin includes a purine molecule linked to a ribose sugar moiety. The absence of 3′-hydroxyl group is the only structural difference between adenosine and cordycepin. Due to this reason, it is regarded as a highly potent bioactive component with essential properties for nutraceutical applications. The concentration of cordycepin in *C. militaris*, approximately 2–3 mg/kg, is insufficient to meet commercial demand. Consequently, various synthetic and semi-synthetic methods have been developed for its production. This is possible by substituting the OH group with an H group at the 3′-position of the ribofuranosyl moiety, resulting in an adenosine analogue.

Due to potent health hazards of other antidiabetic medicines, interventions for new alternatives are inevitable. Some studies have indicated that *C. militaris* extract significantly reduces blood glucose levels by boosting glucose metabolism and protecting against diabetic nephropathy. The antidiabetic activity of cordycepin is not completely elucidated. However, literatures show that, cordycepin is involved in suppression of diabetic regulating genes such as 11β-HSD1 and PPARλ, neutralizing NF-κb-dependent inflammatory responses, suppressing cell renal fibrosis, apoptosis, and rescued cell autophagy.

Cordycepin is also linked with reduction of hyperlipidaemia in human. Previous investigations have indicated that cordycepin's high efficacy in lipid reduction can be attributed to its structural similarity to adenosine, that is an AMPK activator. Similarly, giving cordycepin was able to successfully lower the build-up of total cholesterol, triglycerides, and low-density lipoprotein cholesterol. Cordycepin may also be a useful nutraceutical for lowering hyperlipidemia brought on by high-fat diets. Other than these two major advantages, cordycepin also possess various nutraceutical qualities such as, anti-inflammatory, immunomodulatory, anti-osteoporosis, anti-arthritic, antioxidant/antiaging, and anti-Malarial effect. In 6-OHDA-treated cells, cordycepin was discovered to substantially increase the amounts of antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase. Furthermore, research revealed that the strong antioxidant properties of cordycepin shield adrenal pheochromocytoma (PC12) cells from the neurotoxicity caused by 6-OHDA. Moreover, it has been demonstrated that protein-bound polysaccharide containing cordycepin lowers lipid peroxidation and an increase the antioxidant activity enzymes in the liver.

D-Mannitol And Other Polysaccharides

D-mannitol is a polyhydric alcohol that is produced from *C. militaris.* The only difference between D-sorbitol and D-mannitol, which are both linear six-carbon polyols, is in the orientation of the C_2-OH group. D-mannitol has important uses in fine chemicals, food, medicine, and textiles because of its distinct physical and chemical properties. Cordycepic acid is the usual term for D-mannitol derived from *C. militaris*. In the osmoregulation and regulation of metabolic pathways, *C. militaris* uses it as a repository of carbohydrates and as a transporter of other substances. Due to its osmotic activity, D-mannitol is a diuretic and anti-edematous medication that can be utilized in clinical settings. Previous reports also state that, D- mannitol helps to protect heart and used as an adjunctive therapy for acute promyelocytic leukaemia.

D-mannitol is currently produced using three main methods: chemical synthesis, biosynthesis, and fermentation. D-mannitol is synthesised chemically using D-fructose, D-glucose, and D-mannose as starting materials. Stepwise crystallization is then used to obtain the final product following catalytic hydrogenation. Unfortunately, chemical synthesis has a limited range of applications due to its high energy requirements and harmful byproducts. In addition, a wide variety of natural species, including higher plants, algae, lichens, bacteria, and fungi, are capable of synthesizing D-mannitol. Nevertheless, D-mannitol is the principal photosynthetic product and energy storage molecule in photosynthetic organisms, and it is challenging to raise its concentration to a significant level without impairing the organisms' ability to develop. Therefore, in recent decades, more effective methods have been used to generate D-mannitol via ecologically benign biosynthetic pathways using lactic acid bacteria, filamentous fungus, and *Escherichia coli*.

C. militaris also produces other carbohydrates, known as polysaccharides, which have more complex structures than D-mannitol. These polysaccharides can be secondary intracellular (IPS) or extracellular (EPS) metabolites, depending on their location within the hyphae of *C. militaris*. The polysaccharides extracted from *C. militaris* mycelium have a unique chemical composition. The chemical structure of these polysaccharides is influenced by the types of monosaccharides they contain, their linear sequence, spatial arrangement, the positions of glycosidic linkages, and the degree of chain branching (cross-linking). Significant variations have been observed in the monosaccharide composition and chemical structure of the polysaccharide fraction in *C. militaris*, depending on whether it is growing in its natural habitat or cultured in a laboratory. The three primary monosaccharides that form saccharide polymers are mannose, glucose, and galactose. Additionally, research on *C. militaris* polysaccharides has identified other monosaccharides, including xylose, rhamnose, and arabinose.

Amino Acids And Carotenoids

In addition to all protein amino acids, there are two amino acids namely GABA (Gamma - Amino Butyric Acid) and ergothioneine found in *C. militaris.* The human body uses glutamic acid, sometimes known as glutamate, to biosynthesize GABA, a non-protein amino acid. GABA is an inhibitory neurotransmitter that acts on the cerebellum, hippocampus, hypothalamus, striatum, and spinal cord, among other areas of the nervous system. Subtypes A, B, and C of GABAergic receptors are inhibited by GABA. Sleep, memory, learning, and emotional processes like stress and anxiety are all regulated by it. Moreover, GABA has myorelaxant and anticonvulsant properties (Bo. Ergothioneine (2-thiol-L-histidine-betaine) is an amino acid L-histidine's sulfur analog that dissolves in water and has a portion of the betaine molecule connected to it. Ergothioneine supplementation (at doses of 5 mg/day and 25 mg/day) was observed to correspond with a small decrease in the level of oxidative stress indicators in healthy volunteers aged 21 to 35. Ergothioneine's antioxidant action is likely to be more significant in circumstances that increase the risk of producing oxygen free radicals, such as inflammatory disorders or physical exertion.

Numerous fungal species, including *C. militaris*, have been shown to contain carotenoids in their fruiting bodies, including derivatives of xanthophyll. The striking yellow-orange hue of *C. militaris* fruiting bodies is a result of carotenoids. The primary xanthophylls found in *C. militaris* fruiting bodies are lutein, zeaxanthin, lycopene, and β-carotene. The content of β-carotene and lycopene in *C. militaris* is reported to be 0.328 mg/g and 0.277 mg/g, respectively. It addition to these, a novel class of carotenoids, namely cordyxanthins, are present in the fruiting bodies of *C. militaris*. In *C. militaris* fruiting bodies, four cordyxanthins have been found; these are numbered cordyxanthin I–IV. The concentrations of cordyxanthin I, II, III, and IV in the fungal fruiting bodies were 0.289 mg/g, 0.235 mg/g, 0.401 mg/g, and 0.175 mg/g respectively. Cortixanthin II is one of the prominent cordyxanthins that merits notice since, in contrast to most carotenoids, it is found in one six-membered and one five-membered carbon structure.

Table 1. Different bioactive compounds from *Cordyceps* and their health benefits

Compound	Function
Cordycepin	Inhibition of purine biosynthesis pathway, Facilitate premature termination of transcription, interferes in mTOR signal transduction, Anti metastasis, Induce apoptosis, anti-fungal and anti-malaria activity, Anti Diabetic/Hypoglycemic, Steroidogenesis and Anti-aging,
D-Mannitol	Diuretic and anti-edematous drug
Polysaccharides	Immunostimulatory, antioxidant, antitumor, hypoglycemic, anti-inflammatory, hypolipidemic, and hepatoprotective potential
Amino acids GABA	Inhibitory neurotransmitter in the central nervous system, Myorelaxant and anticonvulsant activities
Carotenoids	Protective effect on eye structures, improves cognitive functions, decreases cortisol levels and associated stress symptoms and induces antioxidant activities
Phenolic Compounds	Strong antioxidant potential and protect biomolecules like proteins including enzymes, lipids or nucleic acids from oxidative damage
Cordycepic Acid	Treatment of livre fibrosis
Sterols and Fatty Acid	Important part of vitamin D_2,

Cordyceps Sinensis

Cordyceps sinensis (Berk) Sacc., also known as *Ophiocordyceps sinensis* is the noble species of *Cordyceps* family, with plethora of metabolites that possess excellent medicinal and pharmaceutical properties. The fungus commonly known as caterpillar fungus, is the real hero documented in the Tibetan reports by doctors dated back in the 14^{th} century. Following the SARS outbreak in China in 2003, the value of fungi experienced a significant surge. This increase in value led to a heightened demand, causing severe price hikes.

Dong ChongXia Cao is a renowned Chinese medicine. In Tibetan, it is often called “yarsa gumba,” with “yarsa” meaning winter and “gumba” meaning summer. The term “gumba” can also be replaced with “gunba” or “gonba.” In the Himalayan region, this fungus is known as “keera jhar” (insect herb).

The months of April until August are when harvesting typically occurs. It can only be found growing at altitude of roughly 3800 meters above sea level, in the chilly alpine meadows of the Himalayan Mountains. About 1500 years ago, Yak herders in Tibet, the surrounding regions of Sikkim, and Nepal discovered this Himalayan aphrodisiac. They noticed that their Yak got more vigorous after consuming this fungus. A thousand years or so later, the Qing Dynasty’s emperor’s physician discovered something and combined it with their own intelligence to create potent aphrodisiacs.

In the new classification of genus *Cordyceps*, a new family, *Ophiocordycipitaceae*, has been established. This is based on the observation, as compared to *Cordyceps* spp., *O. sinensis* colonies exhibit distinct colony characteristics. Most *Cordyceps* species feature fleshy, brightly colored stromata. Whereas, most species in genus *OphioCordyceps* produce dark pigments and have tough to pliable stromata, and often have aperithecial apices. This includes *C. sinensis*, which has been reclassified to *Ophiocordyceps* and is now known as *O. sinensis*. The *Cordycipitaceae* family, on the other hand, has been validated based on the type species *C. militaris*.

Now, coming to the anamorphs (asexual reproductive stage), total 22 names encompassing 13 genera linked with the anamorph of *C. sinensis* have been reported. However, *Hirsutella sinensis* is the mostly reported anamorph of *C. sinensis.* DNA sequences and microcyclic conidiation have proved that, *H. sinensis* as the anamorph of *C. sinensis*. Additionally, it has been shown that *C. multiaxialis* and *C. nepalensis* possess ITS sequences that are nearly or identical to those of *C. sinensis*.

In natural *O. sinensis*, there is a high diversity in the fungal community structure. Significant genetic divergence in *O. sinensis* has been observed, with greater divergence among isolates from southern China compared to isolates from northern China. The genetic similarity indices, varies from 0.282 to 0.782, display a high level of diversity among natural *C. sinensis* samples. Additionally, 141 markers were identified from 180 individual samples of native *C. sinensis* from 18 populations, with 99.3% of these markers being polymorphic. Further, based on genetic distance, the 18 populations can be divided into five groups, with grouping patterns that align with geographic distributions along the latitudinal gradient. Diversity in medicinal effects among *C. sinensis* may be due to the presence of genetically differentiated chemotypes in the morphotaxon.

Proximate composition of the fermented mycelia of *C. sinensis* differed significantly from that of the corpus and fruiting body of wild DongChongXiaCao in terms of protein, fat, carbohydrate, and moisture content. At 39.4%, the carbohydrate content of fermented mycelia is noticeably higher than that of the corpus (24.20%) and fruiting body (24.9%) of wild DongChongXiaCao. In addition, the protein and lipid contents of fermented mycelia are lower (14.8% and 6.63%, respectively) than those of the wild DongChongXiaCao corpus (29.1% and 8.62%) and fruiting body (30.4% and 9.09%).

Several volatile compounds have been identified in *H. sinensis* mycelia cultured using both solid-state media (SSM) and submerged fermentation (SF). The primary categories of these compounds are phenols, acids, and alkanes. Butylated hydroxytoluene was the most prevalent volatile compound, comprising 47.38% of the total volatiles in SSM and 46.12% in SF cultures. The availability of wild or natural *C. sinensis* is declining due to careless harvesting, constraints on geography, and poor weather for its growth. Fermentation is a reliable solution for scarcity of *C. sinensis*. Several strains including *C. sinensis* 762, *C. sinensis* CCRC36421, *C. sinensis* Cs-HK1 and *C. sinensis* CS001 has been successfully cultivated in *in vitro.* Depending on the fungal species and growth conditions, the biomass and EPS yields differed widely, from 10 to 54 g/L and <1.0 to >40 g/L, respectively. Previous examples of optimising temperature, starting pH, carbon and nitrogen levels, minerals, and surfactants (Tween 80) for submerged culture conditions of *C. sinensis* (Cs-HK1) are also available.

Cordyceps Militaris

For lab culture, *C. militaris* is a better choice than *C. sinensis*. Numerous bioactive substances are produced by *C. militaris*, such as polysaccharides, cordycepin, amino acids, cordycepic acid, sterols, ergosterol, myriocin, superoxide dismutase (SOD), multivitamins, bioxanthracenes, naphthaquinone, hypoxanthine, lectin, dipicolinic acid, and cordymin. The biology of *C. sinensis* life cycle, which includes a prolonged symbiosis between the fungus and the larva from autumn to spring, its endemic occurrence, unusual growth pattern contributes to its limited availability and high price. Enough natural habitat is not available to harvest this species, especially in Asia, the US, and Europe, to supply the rising demand for raw materials for the manufacturing of nutraceuticals, functional foods, and nutritional supplements. Because the bioactive compounds in *C. militaris* grown *in vitro* are qualitatively and quantitatively identical to those in *C. sinensis* fruiting bodies, *C. militaris* is used as a substitute for *C. sinensis*.

Coming to the macroscopic and microscopic characters, the stroma of the fungus is club shaped containing fertile and vegetative segments. The fertile part, with 10–30 × 5–12 mm dimension, is eroded by ostioles from the orange perithecia and is reddish to darker orange in colour. The sterile section measures 30–4 x 5–10 mm and is yellow, light or red orange, sometimes speckled with orange. Ovoid, semi-submerged perithecia with dimensions of 250-400 x 550–700 *µm*. Asci 350-400 × 3–4 *µm*, cylindrical, eight-spored, and capable of dividing into multiple distinct spores.

In a recent report, six insects were used for multiplication of *C. militaris.* The insect host used were, *B. mori, G. bimaculatus, T. molitor, Caelifera, P. brevitarsis* and *A. dichotoma.* The maximum quantity of cordycepin was produced by the cultivation on *Allomyrina dichotoma* followed by *B. mori* pupae. The amount of cordycepin in the fruiting body affects the value of the *Cordyceps*. In the Indian market, the cost of *C. militaris* dried fruiting bodies ranges from Rs. 1-4 lakhs per kilogram. One can obtain cordycepin even from the mycelium. This mushroom is becoming more and more in demand in India. Even while China and other South Asian nations are mass-producing them in laboratories using cereal-based media, growing them on insect media—namely, Vanya silkworms—will result in higher quantities of cordycepin.

CORDYCEPS MILITARIS AS AN ALTERNATIVE TO *C. SINENSIS*

1. **Conservation concerns**: The indiscriminate collection of *C. sinensis* from the wild has led to significant reductions in its populations. This has resulted in *C. sinensis* being placed on the IUCN Red List, highlighting its endangered status and the urgent need for conservation efforts.

2. **Bioactive compounds**: *C. militaris* produces many bioactive compounds similar to those found in *C. sinensis*. These compounds include cordycepin, adenosine, polysaccharides, amino acids, cordycepic acid, ergosterol, sterols, multivitamins, superoxide dismutase, myriocin, bioxanthracenes, hypoxanthine, naphthaquinone, dipicolinic acid, lectin, and cordymin. This similarity makes *C. militaris* a viable alternative in terms of medicinal and nutritional benefits.

3. **Cultivation and marketing**: *C. militaris* is more suitable for cultivation and marketing than *C. sinensis*. It can be easily cultivated under controlled conditions, making it more accessible and sustainable. This contrasts with *C. sinensis*, which is challenging to cultivate artificially and primarily relies on wild harvesting.

4. **Higher active ingredient levels**: Several research papers have reported that *C. militaris* products contain higher levels of active ingredients than those found in *C. sinensis*. This means that *C. militaris* not only matches but potentially exceeds the bioactive efficacy of C. *sinensis*, providing enhanced health benefits.

5. **Stability and maintenance**: *C. sinensis* strains tend to be very unstable and difficult to maintain under artificial cultivation conditions. In contrast, *C. militaris* exhibits greater stability and is easier to cultivate, making it a more practical choice for large-scale production.

6. **Affordability**: The price of *C. militaris* is generally more affordable than that of *C. sinensis.* This cost-effectiveness makes *C. militaris* products more accessible to a broader range of consumers, promoting widespread use and acceptance.

In summary, *C. militaris* presents a sustainable, potent, and economical alternative to *C. sinensis*. Its ease of cultivation, higher levels of active ingredients, and affordability make it a superior choice for both producers and consumers, while also alleviating pressure on the endangered *C. sinensis* populations.

PRODUCTION AND MULTIPLICATION OF *CORDYCEPS MILITARIS*

Due to its unusual growing habitat high elevation regions and host specificity, wild *C. militaris* is rarely observed in nature. Scientists and the business community have taken notice of the increased production of *C. militaris* due to the high demand for the substance in the medicinal and cosmeceutical industries. These requirements led to the development and optimization of *Cordyceps* cultivation in laboratory, which closes the gap between supply and demand and keeps natural *Cordyceps* from going extinct due to overharvesting. Furthermore, the expeditious cultivation of *C. militaris* was considered significant because of its capacity to yield particular biological components quickly, its simplicity of scaling up production, and its short cultivation time.

Rice substrates have been widely employed by researchers to cultivate *C. militaris* in experimental settings. Mycelia development and fruiting body yield are strongly impacted by the porosity of the fruiting medium, which is determined by grain size and the water-to-grain ratio during preparation. According to Yue (2010), there is an ideal rice to water ratio of 1:1 to 1:1.35 for stroma growth and production, however this might change depending on the rice cultivar and its glutinous quality. Cultivating *C. militaris* usually involves using husked rice, and utilizing whole rice grains will maximize the amount of fruiting bodies produced (Wen et al., 2008). Furthermore, *C. militaris* stromata is also produced using various organic materials such bean powder, maize grain, corncobs, cotton seed coats, pieces of sunflower floral disks, jowar, millet, and wheat grain. Brown rice, malt, and soybean are also far superior nutrition sources for *C. militaris* than chemical media. Just 36 of the species that have been described for artificial cultivation to produce fruiting bodies. Only *C. militaris* has been commercially cultivated out of all the artificially cultivated species. The commercial development has concentrated on *C. militaris* due to its superior medicinal qualities and low production time.

Solid State Fermentation

In order to artificially cultivate *C. militaris* on a solid substrate, such as rice or grains, the technique of solid-state fermentation is frequently utilized to produce fruiting bodies. However, cultivating *C. militaris* to form fruiting bodies requires several months, and maintaining consistent quality in the final product is challenging. This long cultivation period and quality control difficulty pose significant hurdles for large-scale production. Additionally, the large-scale extraction of bioactive compounds from *C. militaris* fruiting bodies is typically a time-consuming and labor-intensive process. Due to these challenges, solid-state fermentation is not a suitable method for the large-scale industrial production of *C. militaris* biological ingredients.

Despite these limitations, the fruiting bodies of *C. militaris* retain high economic value, particularly in specific consumer markets. In regions where traditional Chinese medicine is popular, *C. militaris* fruiting bodies are highly sought after for their medicinal properties and are often used in Chinese herbal remedies and medicinal diets. This traditional consumption type of *C. militaris* continues to be prized for its health benefits and maintains a strong market presence, providing economic opportunities despite the challenges associated with its cultivation and extraction processes.

In solid-state fermentation, the choice of substrate is crucial for the successful cultivation of *C. militaris* and the production of its specific bioactive ingredients. A variety of substrates can be utilized for this purpose, including wheat, oats, and rice. Among these, rice stands out as the most used substrate for several reasons. Rice is rich in nutrients essential for the growth and development of *C. militaris*. It provides an abundance of amino acids, which are the building blocks of proteins and play a vital role in the organism's metabolic processes. Additionally, rice contains multiple B vitamins, which are crucial for energy production and the overall functioning of the mycelium. Mineral elements present in rice, such as iron, magnesium, and zinc, further support the growth and health of *C. militaris* cultures.

Another advantage of using rice as a substrate is its status as a cash crop. Rice is widely cultivated and readily available, which helps to keep production costs relatively low. This economic aspect makes rice an attractive option for large-scale cultivation of *C. militaris*, as it can be sourced easily and affordably. The use of rice in solid-state fermentation not only supports robust growth of *C. militaris* but also enhances the yield of valuable bioactive compounds. These compounds, such as cordycepin, adenosine, and various polysaccharides, have significant medicinal and nutritional benefits, making the end product highly desirable in various markets, including pharmaceuticals, dietary supplements, and traditional medicine. Overall, the selection of rice as a substrate for the solid-state fermentation of *C. militaris* combines nutritional adequacy, economic efficiency, and practice.

Liquid Culture

The alternative method for cultivating *C. militaris* is liquid culture. Liquid culture is more suitable for large scale production/industrial scale production, due to its higher productivity, short duration for culturing, less requirement of area, and less laborious. As compared to the traditional methods, which need 60 days to accomplish full growth, *Cordyceps militaris* can be cultivated within 15 days, allowing it to thrive in liquid as mycelia without producing fruiting bodies. To make the extraction procedure easier, bioactive chemicals are released from mycelia and gather in the culture broth. It is possible to conduct liquid culture without the requirement for controlled relative humidity and light/dark conditions. As a result, liquid culture is seen to be a potential method for cultivating *C. militaris* that can yield valuable and effective compounds for use in industrial settings.

Liquid culture in *C. militaris* can be performed in two ways,

1. Submerged fermentation: It is a method to produce various biomolecules. In this method, the components are submerged in liquid and the substrate will be in free flowing state. Continuous agitation is mandatory in submerged fermentation to dissolve oxygen and media components. As compared to the surface culture, availability of large quantity of oxygen is vital and pivotal in submerged cultures. Here also the target molecule is directly harvested from the liquid broth after cultivation.

2. Surface culture: In surface culture, the fermentation system will be in a static state after inoculation. As agitation is completely avoided, the culture tends to form a biofilm on the surface/top of the culture media. The biomolecules secreted from the microorganism will be suspended in the liquid broth, which will be harvested later. Production of vinegar is a classic example of surface culture.

As scale up is challenging in surface culture also, submerged fermentation is being explored by majority of the research communities in *Cordyceps* production.

Production Systems In *C. Militaris*

There are two major industrially viable production systems available for producing *C. militaris.*

1. Fed-batch culture

2. Repeated batch culture

In batch fermentation, all the media components and inoculum are loaded into the fermenter at a time and harvest the product after the fermentation is over. However, this method has several disadvantages, including instability of the culture conditions, depletion of nutrients and accumulation of hazardous byproducts that stop the culture from growing. To combat with these undesirable qualities of batch culture, fed-batch system is introduced. In fed-batch mode, nutrients are continuously added to improve efficiency of the production process. The recent study claims that, continuous feeding of nitrogen in *C. militaris* fed batch system increased the production of cordycepin from 208.8 to 346.1 mg/L.

Repeated batch culture is a cost-effective, sustainable, and environmentally benign fermentation technique, can even be applied as powerful strategy for specific items during manufacturing. In this method, the spent media component is replaced with fresh media after each batch. However, the microbial inoculum remains the same and reused for several batches. As the initial inoculum concentration is high, the time for fermentation can be saved, as the duration of lag phase of cell growth is minimum. According to a prior study, by reducing the length of the lag phase through repeated-batch fermentation, the biomass of *C. militaris* can sustain a production level of >85% of that of the first cycle for at least four repetitions. According to another investigation, *C. militaris* could produce up to 5.713 g/L of exopolysaccharide (EPS), with a productivity of 476 mg/L/day in the second run. Furthermore, Zheng et al. (2019) devised a plan to enhance EPS production by combining repeated-batch fermentation with two-stage foam fractionation.

Silkworms As Host For *Cordyceps*

Several researchers attempted production of *C. militaris*. As a natural host, silkworm pupae provide energy and nutrients for the growth and development of *C. militaris*, facilitating cordycepin synthesis. It has been proven that the lipids found in silkworm pupae enhance the synthesis of cordycepin. The primary lipid constituents of silkworm pupae are α-linolenic acid, linoleic acid, oleic acid, and palmitic acid. Research has indicated that the presence of oleic acid in the lipids of silkworm pupae can increase the output of cordycepin by increasing the expression levels of the cns1-2 genes.

Chinese producers employ silkworm-based liquid medium for fermentation, to which minerals and carbohydrates are added to promote the rapid growth of the mycelium. Given that *Cordyceps* is found in nature, growing on insects, this decision appears reasonable. Dried silkworm carcasses are a by-product of China's silkworm industry. So, this method of growing *Cordyceps* in China is particularly economical and effective because to the low cost of the silkworm and its continuous supply. Hong et al. (2018), injected *C. militaris* into alive silkworm pupae. The incidence of infection was maximum after 11 days and the concentration of more than 2×10^5 colony-forming unit (cfu).

Figure 2 : *Cordyceps militaris* stroma emerges from tasar silkworm pupa

As reported inoculated *B. mori*, with *C. militaris* to develop techniques to produce stromata on a large scale. The team used 5th instar larvae and pupae and the results were compared. Pupae exhibited a higher infection rate than the larvae did. In the silkworm pupae, the stromata formed and grew more effectively. At several locations on the pupal body, a conidial suspension was injected. On the other hand, there was little variation in infection rates between the sites. The injection site had no effect on the development or expansion of the stromata.

Different components of silkworm support growth of *Cordyceps* and production of cordycepin. Crude proteins in silkworm pupae have a greater capacity to stimulate the synthesis of cordycepin than do lipids. Crude protein and globulin both have comparable promoting effects. Furthermore, the stage of cordycepin synthesis is prolonged for 6 days by supplementing with 3.31 g/L globulin. After globulin supplementation, transcriptome study of *C. militaris* metabolic pathway showed that upregulating energy and glucose metabolism was essential for extending the synthesis stage of cordycepin to 2.5 times. The primary functional component controlling synthesis of cordycepin is silkworm pupae globulin, which has the potential to be used as a raw material for cordycepin production on an industrial scale.

Table 2. *Cordyceps* production on Silkworms

Species of silkworm	*Cordyceps* species	Bioactive produced	Reference
Bombyx mori	*Cordyceps militaris*	Cordycepin	Aramwit et al., 2014
Bombyx mori	*C. militaris NBRC100741*	Cordycepin	Kato et al., 2021
Not specified	*C. militaris*	Cordycepin	Kang et al., 2017
B. mori	*C. militaris*	cordycepin	Wang et al., 2019
B. mori	*Cordyceps cateniobliqua Bm1*	-	Zhu et al., 2022

National Level

Scanty of literature is available related to *Cordyceps*. Majority of work done in India is survey-based. But a lot of individual cultivators are growing this mushroom using the conventional rice-based medium. When *Cordyceps* is cultivated on this medium, the amount of cordycepin in the fruiting bodies will be low. *C. sinensis* is a traditional aphrodisiac and tonic in North Sikkim and is used to treat eighteen ailments in the Indian state of Sikkim. Scientific data has long supported its use as an aphrodisiac. It is known as Himalayan Viagra or Himalayan aphrodisiac abroad. In the alpine regions of Sikkim Himalaya, Uttaranchal, Himachal Pradesh, Western Nepal, Tibet, and Bhutan, this Himalayan aphrodisiac is readily accessible. It was discovered that this fungus is traditionally utilized in the Lachung and Lachen area of North Sikkim to treat both male and female sexual dysfunction, to improve overall health and appetite, and to lengthen life. The Bhutia communities use Chang, a locally produced alcohol, as an aphrodisiac. They put one piece of *C. sinensis* in a cup of Chang and leave it for an hour. They consume it in the morning and evening. Some people substitute hot water for booze. They said that it is more powerful than ginseng and that it is also used to cure liver and renal problems, cancer, exhaustion, and chronic pain.

The eri silkworm *(Samia ricini*) is one of the many insects that the native people of northeast India consume as sustenance. The pupae and pre-pupae of eri silkworm are prized in northeast India; nevertheless, only local shops sell them after they are harvested, and the harvesting process is still informal. The proximate composition of eri silkworm pre-pupae and pupae raised on tapioca or castor was equivalent, according to nutrient analysis, and it was a good source of minerals, fat (8%), and protein (16 %). Leucine was the limiting amino acid in both eri pre-pupae and pupae protein, which had amino acid scores of 99 and 100, respectively. Pre-pupae and pupae had a net protein utilisation (NPU) of 41 compared to 62 in casein. Defatted eri silkworm meal has a high protein content (75%) and contains 44% of all important amino acids, making it a great option for producing protein concentrate isolates with improved protein quality for use in animal feeding.

According to the latest data available in the sericulture statistics, Vanya Sericulture, which includes tasar, muga, and eri, produced 8928 MT of raw silk from 7349 MT of eri, 1318 MT of tasar, and 261 MT of muga (CSB Report 2022-23). The tribal people have been the primary practitioners of tasar sericulture from ancient times, it has a traditional heritage significance in India. 1318 MT of tasar raw silk are produced in India overall (2022–23). This makes it clear that a significant number of cocoons are treated each year in India to produce silk, and the resulting reeled pupae are then discarded as garbage. Moth tissues were discarded after oviposition and mother moth investigation. Massive amounts of male moths are also tossed in addition to this. When combined with technological intervention, these wastes/refuses have the potential to yield significant financial value through the growth of the highly valuable *Cordyceps* fungus. The vast amount of waste produced by the Vanya silk industry has potential value. The industry as well as the entrepreneurs stand to gain from the by-product (*Cordyceps*) created from the vanya refuses.

INITIATIVES BY THE CSB-CTRTI

CSB-Central Tasar Research and Training Institute (CSB-CTRTI) is a premier institute that meets the R&D needs of the tropical (tasar) and temperate (oak) sectors. The institute's ultimate goal is to raise the socio-economic standing of the parties involved in tasar culture through research and development. The suitability of *Cordyceps* on tasar silkworm (*Antheraea mylitta* Drury) and pupae of other silkworm muga (*Antheraea assamensis* Helfer) and Eri (*Samia ricini*) was tested in 2020 by CSB-CTRTI, Ranchi. *Cordyceps militaris* DMRO 1163 was produced on silk waste. Among the substrates tested, tasar egg, tasar pupa, tasar adult moth powder, oak tasar egg, oak tasar adult, eri pupa and muga pupa were found to be suitable for producing *Cordyceps*. It was found that the ideal temperatures and humidity ranges were 20–22 ˚C and 80–85% RH. Moreover, rice has the highest biological efficiency percentage of all the substrates studied. Following rice in descending order by biological efficiency percentage are wheat, ragi, barley, oats, and soybeans. Free radicals can be scavenged by extract from *Cordyceps*. Further, gentistic acid, coumaric acid, cinnamic acid, ferulic acid, and gallic acid were the primary phenolic components identified following phenolic and flavonoid profiling of *Cordyceps*. Similarly, the main flavonoids found in *Cordyceps* fruiting bodies were naringenin, catechin, and epicatechin.

Figure 3: Vanya Silkworm refuses used as substrate for *Cordyceps militaris* DMRO 1163 mass production. A-C: Eri silkworm cocoons, pupae and moths respectively. D-F: Muga stifled cocoons, pupae and rejected eggs respectively. G-I: Tasar silk moths, pupae and rejected eggs respectively.

Figure 4: *Cordyceps militaris* DMRO 1163 production over vanya silkworm refuses. a:Liquid spawn ready for inoculation; b: Inoculation under LAF; c: Mycelial run initiation; d: Complete mycelial run; e: pin head initiation; f-i: Growth of fruiting bodies.

Challenges

1. Any metallic chemical element that is dangerous or poisonous at low concentrations and has a comparatively high density is referred to as a heavy metal. Cadmium (Cd), Mercury (Hg), arsenic (As), chromium (Cr), lead (Pb), thallium (Tl), and other elements are examples of heavy metals. The Earth's crust naturally contains heavy metals. They cannot be destroyed or degraded. They enter our bodies through food, drinking water, and the air to a limited degree. Certain heavy metals, like copper, zinc, and selenium, are necessary as trace elements to keep the human body's metabolism running smoothly. Higher doses, meanwhile, have the potential to cause toxicity. Recently, lead contamination in *C. sinensis* was reported and Chinese Food and Drug Administration given alert on the same. So, heavy metal content needs to be analysed thoroughly in the fruiting bodies of *Cordyceps*.

2. Given the live nature of silkworms, utilization of the same highlight ethical conundrums with their decision to grow *C. militaris*. Some individuals might consider it unethical to utilize silkworms for this purpose because they are effectively being exploited as hosts for the growth of another organism.

3. To manage the supply chain for the production of *C. militaris* and silkworm pupae, distributors, silkworm farmers, and growers of *Cordyceps* must collaborate. Ensuring the timely delivery of healthy silkworm pupae to *Cordyceps* farms while maintaining quality requirements may provide a logistical challenge.

4. Depending on the location and extent of *C. militaris* and silkworm pupae growth, regulations and restrictions might need to be addressed. Following laws relating to the environment, animal welfare, and food safety may make operations more complex and expensive.

PROSPECTS OF *CORDYCEPS* CULTIVATION

The cultivation of *C. militaris* is a field with significant potential for growth and innovation. By leveraging advancements in biotechnology and integrating sustainable practices, the industry can achieve greater efficiency, productivity, and environmental responsibility. Below is an elaboration on key prospects based on the given points.

ENHANCING EFFICIENCY AND PRODUCTIVITY THROUGH BIOTECHNOLOGY

Advances in biotechnology, such as genetic engineering and tissue culture techniques, hold promise for improving the cultivation of *C. militaris*. By manipulating the genetic makeup of *C. militaris*, researchers can potentially enhance desirable traits such as faster growth rates, higher yield, and improved bioactive compound production. Tissue culture techniques can also play a crucial role by enabling the mass production of high-quality fungal cultures in a controlled environment, ensuring consistency, and reducing the risk of contamination. These biotechnological approaches can make the cultivation process more efficient and cost-effective.

Reducing Resource Consumption And Waste

Future developments in *C. militaris* cultivation are likely to focus on sustainability. Innovations aimed at reducing resource consumption and minimizing waste generation will be essential. For instance, optimizing the use of silkworm pupae as a substrate by recycling and reusing waste materials can significantly lower the environmental impact. Additionally, exploring alternative substrates, such as agricultural byproducts or other organic waste, can reduce reliance on specific inputs and promote a more sustainable cultivation practice. Implementing energy-efficient technologies and processes will also contribute to reducing the overall environmental footprint of *C. militaris* production.

Integrating Cultivation With Other Agricultural And Industrial Processes

Integrating *C. militaris* cultivation with other agricultural or industrial processes can create synergies and promote circular economy principles. For example, utilizing silkworm pupae waste from the silk industry as a feedstock for *C. militaris* cultivation can turn a waste product into a valuable resource. Similarly, agricultural residues, such as crop stalks or husks, can be used as substrates, thereby reducing waste and creating additional revenue streams for farmers. Such integration can enhance the sustainability of both industries and contribute to a more resource-efficient and circular economy.

Products Developed

Cordyceps Green tea

This is ancient method of consuming *Cordyceps*. Benefit of drinking *Cordyceps* tea is that it has a better bioavailability and expedites the body's absorption of biomolecules. Preparing *Cordyceps* tea is similar to that of green tea. How to use *Cordyceps* tea is given below,

- Put six to eight fruiting bodies of *Cordyceps* in 200-250 ml of water.
- Boil the *Cordyceps* bodies in water for 1 minute.
- After a minute of a vigorous boiling, reduce the heat to a simmer for an additional 14 to 15 minutes, then cover the pot with a lid. According to research, using this procedure to prepare *Cordyceps* tea extracts the most water-soluble components from the root into the water. Serve and drink *Cordyceps* tea preferably after the meals and consume the fruiting bodies.
- Mask the mushroom earthy smell, squeeze few drops of lemon or mint.
- Take *Cordyceps* tea twice a day for full effects.

Cordyceps powder

While *Cordyceps* are dried and placed in capsule form, hot-water extract can also be machine dried, crushed, and combined with *Cordyceps* fruit bodies to form a powder. The dosage and efficacy of *Cordyceps* powders are the same as those of *Cordyceps* capsules. In addition to other specific compounds like triterpenes, ergosterols, and cordycepin, the majority of experts believe that a *Cordyceps* powder that mentions 25% beta-glucans (1:1

hot water extract) is a good product. The majority of *Cordyceps* powders that are sold on the market may or may not contain ground fruit bodies or other fillers.

CONCLUSION

In conclusion, *Cordyceps* fungi, particularly *C. militaris* and *C. sinensis*, represent a valuable resource in contemporary healthcare and nutrition. Their unique biochemical composition, including cordycepin and polysaccharides, underpins their therapeutic versatility, spanning from immune modulation to disease prevention and management. The cultivation of *Cordyceps* on silkworms has the potential to revolutionized production capabilities, ensuring consistent quality and supply of *Cordyceps*-derived products worldwide. In silk producing countries like India, mass production of *Cordyceps* on silkworm is highly feasible. Research initiatives are already taken by Central Silk Board to utilize this opportunity. As ongoing research continues to unveil new facets of *Cordyceps* biology and pharmacology, their role in integrative medicine and sustainable agriculture is poised to expand further. By harnessing the medicinal potential of *Cordyceps* fungi through innovative cultivation practices and rigorous scientific inquiry, we can unlock new avenues for improving human health and well-being in the 21st century and beyond. However, there is scanty publications related to mass production of *Cordyceps* on silkworms. It is need of the hour to utilize this opportunity and unveil the scientific facts supporting the mass production of *Cordyceps* on silkworms.

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7. Chitosan: A Green Revolution for Sustainable Development

J. Pradhan, B. Baisakhi, B. Patra, B. K. Das,
K. Jena, S. Ananta and D. Mohanty

CONTENTS

INTRODUCTION

After cellulose, chitin is the second most prevalent polysaccharide in nature and the source of chitosan, a naturally occurring biopolymer. It is made up of N-acetyl-D-glucosamine and β-(1→4)-linked D-glucosamine units that are dispersed at random. Because of its special qualities, including antibacterial activity, non-toxicity, biodegradability, and biocompatibility, chitosan is a desirable material for various industrial and biomedical uses. The main method for obtaining chitosan is by deacetylating chitin, which is present in the exoskeletons of crustaceans like lobsters, crabs, and shrimp. Additionally, the cell walls of some fungi and insects can provide chitin. Chitin must be treated with an alkaline solution-typically sodium hydroxide-to eliminate the acetyl groups before it can be converted to chitosan. The degree of deacetylation (DD) determines chitosan's solubility and functional properties. The availability of chitin as a byproduct presents a sustainable and economical source for chitosan production, contributing to waste reduction and environmental sustainability.

Acetyl groups are removed during a process known as deacetylation, which turns chitin into chitosan. The solubility and characteristics of chitosan are influenced by the varying DD. More glucosamine units result from more DD, which boosts solubility in acidic solutions. Food preservation and medical applications benefit from chitosan's antibacterial qualities against a variety of bacteria, fungi, and viruses. As the amino groups are protonated, chitosan dissolves in acidic solutions but remains insoluble in water. Its use in a variety of sectors depends on this feature. Chitosan solutions' viscosity varies according to its molecular weight and concentration, which can impact how well it works in various formulations. Since chitosan contains free amino groups, it is simple to modify chemically to create derivatives with unique qualities for particular uses. Strong, flexible films that are helpful for packaging, particularly for food products, can be formed from chitosan. Because of these qualities, chitosan is a substance that can be used in a wide range of fields, including environmental protection, medical, and the food and agriculture industries.

Because of its biodegradability, safe and effective production methods, and renewable supply, chitosan embodies the ideals of green chemistry. Its numerous eco-friendly applications demonstrate how much it may contribute to sustainable development in a variety of industries. Chitosan is predicted to play a bigger role in advancing sustainability and green chemistry as science and technology develop, spurring inventions that will benefit the environment and society as a whole. Chitosan is a desirable material for a variety of applications intended to promote sustainability and lessen ecological footprints because of its biodegradability and favourable environmental effects.

Tasar silk pupae are used to produce chitosan, which encourages sustainability by turning waste into useful biopolymers. This strategy benefits the silk industry economically in addition to reducing waste to the environment. Furthermore, in accordance with the principles of green chemistry, the extraction and processing of chitosan from tasar silk pupae are generally more environmentally friendly than those of synthetic polymers.

HISTORIC BENCHMARK RELATED TO CHITOSAN

Historical accounts state that Charles Rouget is credited with discovering chitosan in 1859, whereas Henri Braconnot is credited with discovering chitin in mushrooms in 1811 which marked the beginning of the study of chitin isolation and characterisation. He researched specific fungus species and applied an aqueous alkali treatment, which resulted in the extraction of a material he called "fungine." By examining the exoskeleton of the silkworm species *Bombyx mori*, Lassaigne proved that chitin's structure included nitrogen in 1843. Chitosan was developed in 1859 through the application of hot potassium hydroxide to chitin. Ledderhose discovered in 1878 that chitin includes substances like acetic acid and glucosamine. The name "chitosan" was initially used by Felix Hoppe-Seyler in 1894. Because of its distinct structure, inertness, antibacterial qualities, flexibility, and varied physical and chemical characteristics, chitosan has come to be known as a green biopolymer.

SOURCE OF CHITOSAN

Major Natural Sources

Chitin is a highly prevalent biopolymer that serves as the primary structural element in the exoskeletons of insects and molluscs, the cell walls of certain fungi, and the shells of crustaceans (crab, shrimp, and lobster). It is less usual for certain types of fungi, including zygomycetes, to contain chitosan. Many species include chitin in addition to other macromolecules. Nevertheless, chitin or chitosan is not present in the structure of higher animals or plants. The main industrial feedstock for the large-scale synthesis of chitin and chitosan has been the leftovers from shrimp and crab shells. Marine creatures are said to have 20–30% chitin, 30–40% proteins, 0–14% lipids, and 30–50% minerals. Chitosan is derived from a variety of natural sources, with crustacean shells being the most prominent. Fungal sources, insect exoskeletons, and squid pens provide alternative options that contribute to the sustainable and versatile production of this valuable biopolymer.

Crustacean Shells

The exoskeletons of crustaceans, including shrimp, crabs, and lobsters, are the most prevalent and important source of chitosan from a commercial standpoint. As a plentiful by-product of the seafood industry, chitin provides an affordable and sustainable source for the synthesis of chitosan.

Fungal Sources

Certain fungi, particularly those in the Zygomycetes class, produce chitin in their cell walls. Examples include species from the genera Mucor and Rhizopus. Fungal-derived chitosan can be produced in a controlled environment, independent of seasonal and geographic variations that affect crustacean availability. It also avoids the allergenic concerns associated with crustacean sources.

Marine Sources

Squid pens, which are internal shells of squids, are another source of chitin. The chitosan derived from squid pens is known for its high purity and unique properties. This source is less common but represents a valuable use of marine biomass that might otherwise be discarded.

Insect Exoskeletons

Insect exoskeletons are emerging sources of chitosan. Insects such as beetles and certain arthropods also contain chitin in their exoskeletons. This source is currently less exploited but holds potential for future chitosan production. Insect farming is gaining attention for its low environmental impact and high efficiency in biomass conversion, making it a promising area for sustainable chitosan sourcing.

Tasar Silkworm Pupae: Source Of Chitosan

Most insects, such as silkworms, beetles, and grasshoppers, are significant providers of chitosan. One of the main byproducts of the reeling business is silkworm pupae. Since chitin lines the exoskeleton and internal organs of silkworm pupae, including the spiracle and tracheae, chrysalids are utilized as a substitute source of chitin; as a result, chitosan is more frequently used in industrial settings than chitin. It was discovered that the amount of chitin in silkworms varied by sex and between races. The chitin content of male and multivoltine silkworms was higher than that of univoltine and female insects, respectively. Over the last few decades, mulberry silkworms have been the subject of much research and use in biomedicine. Although widely distributed across the nation in a variety of geoclimatic situations, the non-mulberry silkworm, *Antherea mylitta*, is rarely reported despite its ability to produce biomaterials. This chapter centres on the possible antibacterial and antioxidant properties of chitosan and chitosan nanoparticles derived from *A. mylitta*, as a natural biomaterial source.

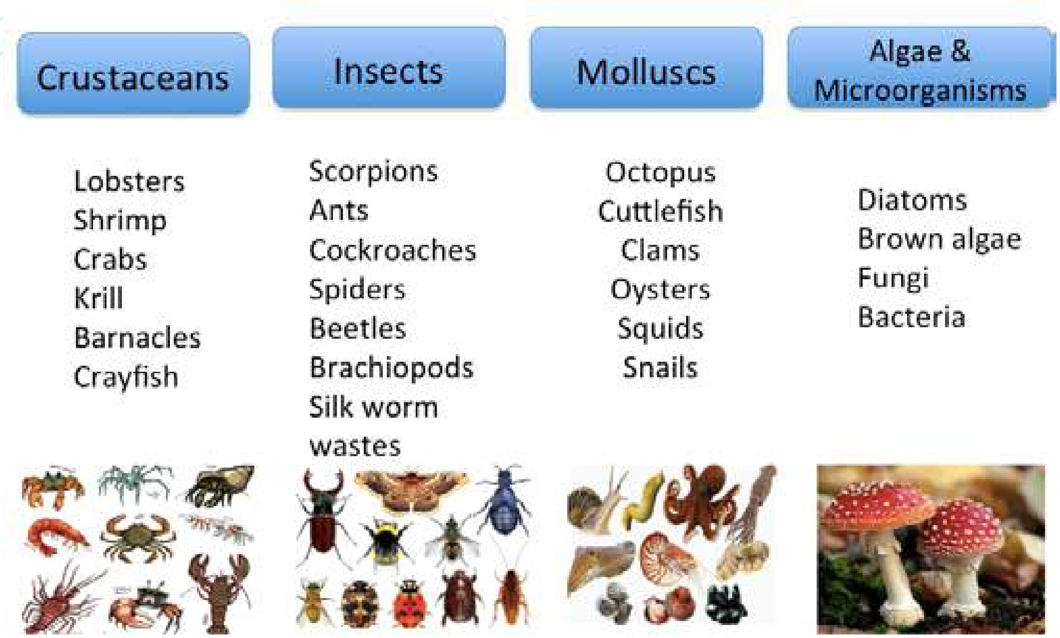

Figure 1: Sources of Chitosan

STRUCTURAL COMPOSITION OF CHITIN AND CHITOSAN

Three crystalline forms of chitin may be found in nature: α-, β-, and γ-chitin. These forms differ in their degree of hydration, unit cell size, and number of chitin chains per cell, and hence have different physicochemical properties. The differences between these polymorphs are associated with the reciprocal arrangement of the chains in crystalline areas. A set of two parallel strands and a single antiparallel strand alternate in γ form, β form, and γ form arrangements, respectively. Of all the chitin types, α-chitin is the most common and extractable, found in the cell walls of fungi and crustaceans.

The only structural difference between chitin and cellulose (poly-(1-4)-N-acetyl-D-glucosamine) is that chitin has one acetyl amine group instead of one hydroxyl group on each monomer. Chitosan, the ultimate form of chitin, is a modified polycationic polysaccharide polymer that occurs naturally and has an average molecular weight ranging from 1 to 5×10^5 Da. N-acetyl-D-glucosamine and D-glucosamine, the two monosaccharides that make up this compound, are joined by β (1→4) glycosidic links (Fig. 2). Reactive functional groups found in chitosan include amino groups and primary and secondary hydroxyl groups, which are located at locations C-2, C-3, and C-6, respectively. Whereas chitosan is insoluble in neutral and alkaline pH in aqueous solution, chitin is insoluble in organic solvents. It is, however, easily soluble in diluted acids (pH<6–6.5), including acetic, formic, succinic, lactic, and malic acids, as well as in diluted HCL. This is because chitosan has main amino groups, which make it a strong base. Strong hydrogen bonds between the acetamide, hydroxyl, and carbonyl groups generate its crystalline structure and keep chitosan from dissolving in water or other organic solvents.

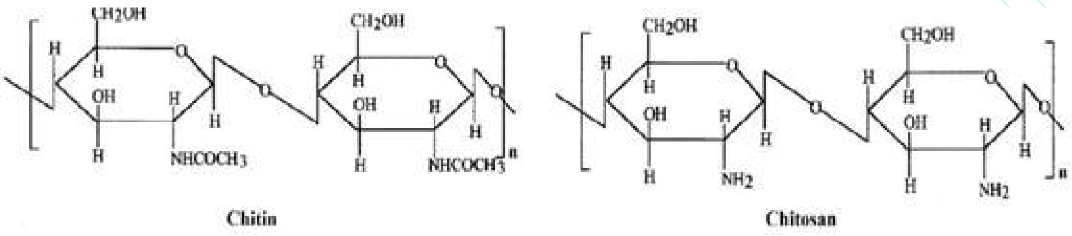

Figure 2: Structure of Chitin and Chitosan

EXTRACTION METHODS

Conventional methods of extracting chitosan from chitin involve a series of chemical treatments to remove minerals, proteins, and acetyl groups (Fig. 3). While these methods are effective and widely used, ongoing research aims to develop greener, more sustainable alternatives to reduce the environmental footprint and improve the overall efficiency of the extraction process.

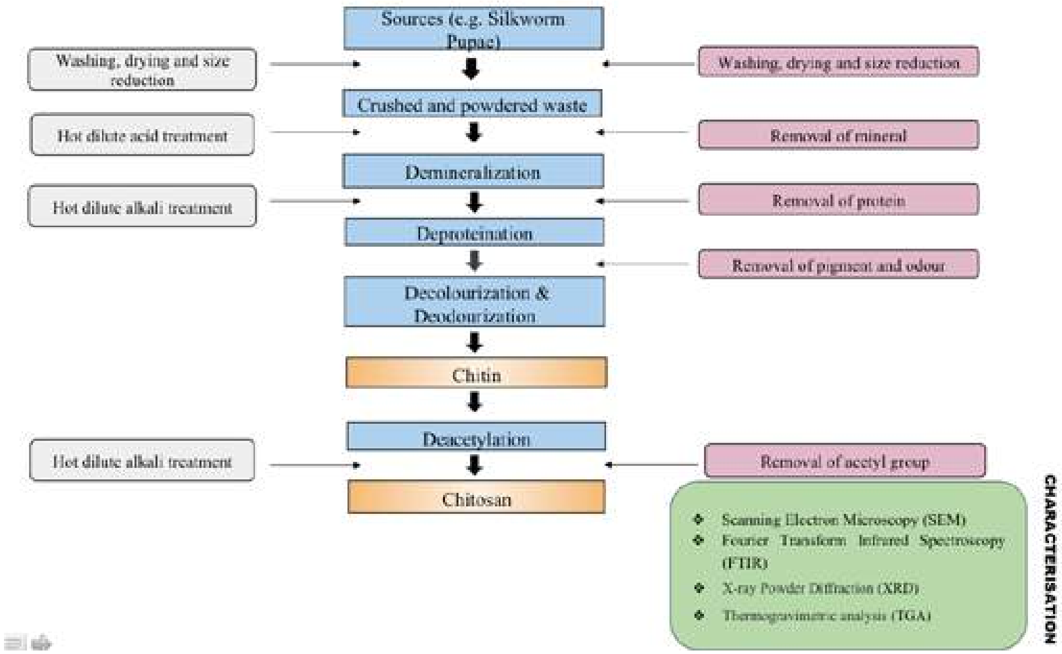

Figure 3: Conventional method of extraction of chitosan

The initial step is demineralization, where the cleaned sample is treated with dilute hydrochloric acid to remove minerals, primarily calcium carbonate. Then in deproteinization, the demineralized samples are treated with sodium hydroxide to remove proteins and other organic impurities. The chitin obtained from the previous steps is subjected to concentrated sodium hydroxide treatment at elevated temperatures to convert it into chitosan is the deacetylation process. The resulting chitosan is washed with water and sometimes neutralized with dilute acid to remove any residual sodium hydroxide and other impurities. It is then filtered, dried, and ground into a fine powder. The key factors in conventional methods are concentration of chemicals, temperature and time, which determine the efficiency of demineralization, deproteinization and DD of chitosan. The conventional methods aim to maximize the yield of chitosan while maintaining high purity and desirable physicochemical properties. Conventional extraction methods involve the use of strong acids and bases, which can pose environmental and safety challenges. Proper handling, neutralization, and disposal of chemical wastes are essential to minimize environmental impact. Innovations in the extraction processes of chitosan focus on improving efficiency, reducing environmental impact, and enhancing the quality of the final product. Enzymatic methods, microwave and ultrasonic-assisted extractions, supercritical fluid extraction, and the use of ionic liquids and green solvents represent significant advancements over conventional chemical methods. Additionally, biotechnological approaches offer promising alternatives for sustainable and scalable chitosan production. These innovations contribute to the broader goals of green chemistry and sustainable development.

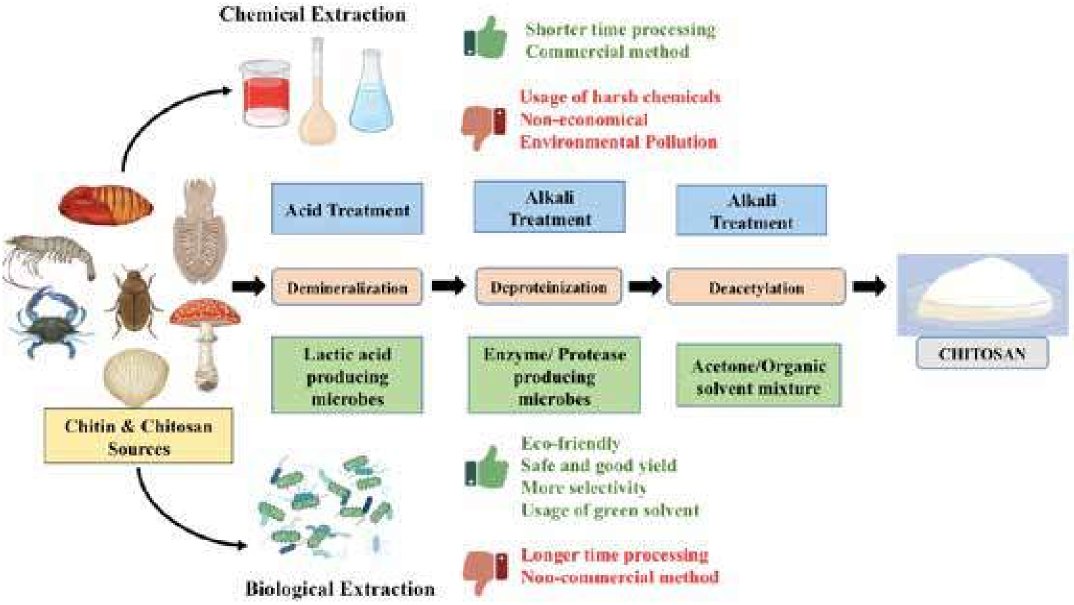

Figure 4: Various methods used for extraction of chitosan

PROPERTIES OF CHITOSAN

Physicochemical Properties Of Chitosan

The components of chitin and chitosan consist of thousands of D-glucosamine residues connected by β-(1-4) bonds. The amount of N-acetyl-D-glucosamine units (15–50%) in chitosan is determined by the degree of chitin deacetylation. Chitin and chitosan have different biological roles because they have different amino groups and have different nitrogen contents (5–8%). The majority of chitosan's physical and chemical attributes, such as its solubility, molecular weight, degree of deacetylation, and viscosity, are derived from these attributes.

Molecular weight

The source material and the techniques used to prepare and extract it affect the molecular weight of chitosan. Chitosan's solubility, viscosity, and biological characteristics are influenced by a broad range of molecular weights. Chitosan's molecular weight can be classified as high, medium, or low according to its range. Chitosan becomes less soluble and more viscous as its molecular weight increases, which frequently renders it less useful for a range of industrial uses. Low molecular weight chitosan is recommended for usage in biological and industrial applications due to its superior stability and solubility. Because of its superior solubility and stability, low molecular-weight chitosan is recommended for use in biological and industrial applications.

Degree of deacetylation

One of the main variables affecting the physicochemical properties, activity, and use of chitosan is its degree of deacetylation. It has to do with how amino groups are arranged throughout the polymer chain. The amino group in the polymer chain of chitosan is what gives it its positive charge in an acidic environment. Consequently, the degree of deacetylation has a major influence on the solubility and viscosity level. The polymers chitin and chitosan can be distinguished from one another based on the degree of deacetylation. The effective conversion of chitin into chitosan is usually indicated by a deacetylation level greater than 50%.

Solubility

While chitosan is insoluble in neutral or alkaline solvents, it can dissolve in acidic solvents. Although chitin is normally insoluble in liquids, chitin can be deacetylated to produce soluble chitosan, which has primary amino groups with a pKa of 6.5. Chitosan becomes soluble when it dissolves in acidic solutions because the amine groups get protonated and take on a positive charge. Nevertheless, these groups lose their charge and chitosan becomes insoluble as the pH rises to 6 or above. In addition to pH, chitosan's solubility is affected by temperature, polymer crystallinity, molecular weight, and the degree of deacetylation.

Viscosity

The viscosity of chitosan is influenced by its degree of deacetylation and molecular weight. Higher deacetylation increases viscosity, while lower molecular weight decreases it. Additionally, particle size and storage time can also impact viscosity. Viscosity serves as a key indicator of polymer stability in solution, as a decrease is noted during polymer storage attributed to polymer degradation.

Film formation

Chitosan can form films and membranes, which are useful in various applications.

Mechanical properties

Chitosan exhibits good mechanical strength and flexibility.

Biological Properties of Chitosan

Biocompatibility

Because chitosan is so biocompatible, it can be used in pharmaceutical and medical settings. However, the preparation process (residual proteins could cause allergy reactions) and the DD (biocompatibility increases with DD increase) determine whether or not chitosan is compatible with physiological media. In vitro, chitosan was found to be more cytocompatibility than chitin. The interaction between cells and chitosan rises as the quantity of positive ions does, and this tends to enhance biocompatibility.

Biodegradability

Chitosan is naturally broken down by lysozyme and other proteases, which allows it to be safely broken down in biological systems. Three of the eight human chitinases that have been identified thus far have demonstrated enzymatic activity on chitosan. Chitosan biodegrades to produce oligosaccharides of varying lengths that are non-toxic.

Bio-adhesion

Chitosan has excellent bio-adhesive properties, allowing it to adhere to mucosal surfaces and enhance drug delivery systems. In an acidic environment, it carries positive charges as a result of amino group protonation. This enables it to bind with negative residues in mucin, enhancing its mucoadhesive properties.

Non-toxicity

Chitosan is non-toxic to human cells, which supports its use in food and biomedical applications.

Antimicrobial activity

The strong antibacterial and antifungal characteristics of chitosan make it useful against a wide range of infections. The mechanisms take advantage of chitosan's polycationic properties. According to the main theory put forth, chitosan causes intracellular components to leak by interfering with cellular permeability. It interacts with the anionic elements of the cell membrane to cause this, which ultimately leads to cell death. Chitosan entering the cell and attaching itself to DNA to stop replication and kill the cell is another possible mechanism. Furthermore, microbial growth is inhibited by chitosan's high capacity to chelate metal ions (Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, and Cu^{2+}) at pH levels higher than its pKa value. Chitosan can stop the growth of microorganisms by covering their surface with a thick polymer layer that prevents them from taking in oxygen and nutrients.

Hematostatic property

The hemostatic property of chitosan refers to its ability to promote blood clotting and stop bleeding. Chitosan's positive charge allows it to interact with negatively charged cell membranes and blood components, facilitating platelet adhesion and aggregation, which accelerates the clotting process. Additionally, chitosan can form a physical barrier at wound sites, further aiding in hemostasis by sealing the wound and reducing blood flow.

Immune system modulation

Chitosan can enhance the immune system by activating macrophages, stimulating the production of cytokines, and promoting the activity of other immune cells. These interactions help to boost the body's defence mechanisms, facilitating faster wound healing, and providing protection against infections.

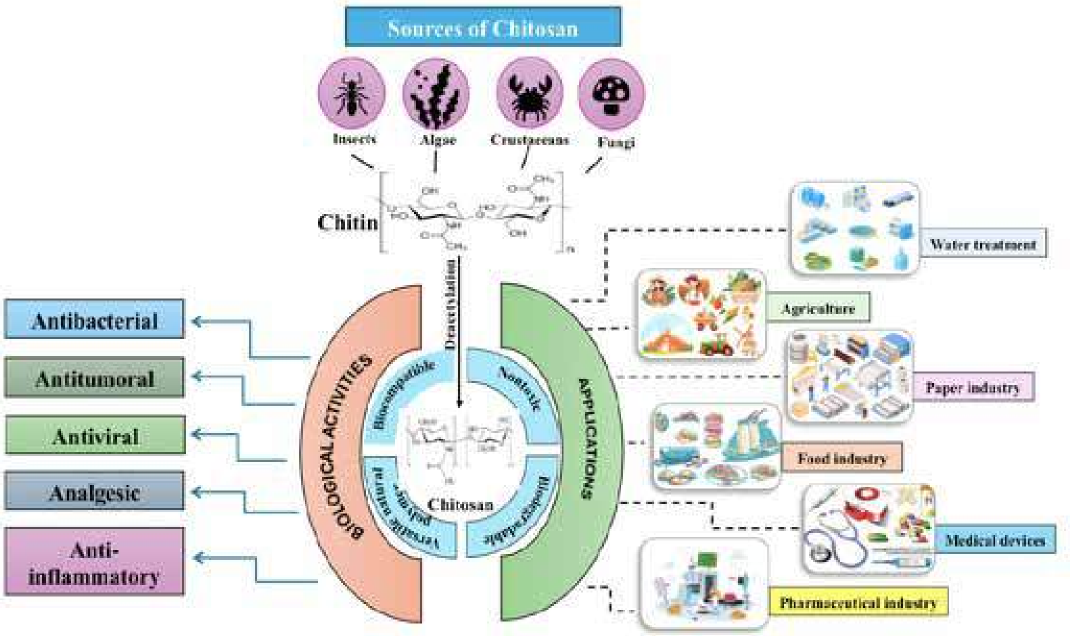

Figure 6: Biosynthesis of Chitosan from various sources and it's application

APPLICATIONS IN VARIOUS INDUSTRIES

AGRICULTURE

Chitosan is increasingly utilized as a biopesticide and fertilizer due to its natural origin and environmental advantages. It stimulates the production of natural defense compounds in plants, enhancing their resistance to pathogens and pests. Chitosan can also repel insects and inhibit their growth, reducing crop damage. Additionally, it disrupts the cell membranes of fungi and bacteria, causing their death and preventing infections.

Chitosan contributes to plant growth and soil health by:

a) **Improving soil structure**: It enhances soil aeration and water retention.

b) **Enhancing nutrient absorption**: It boosts plants' ability to absorb nutrients, promoting better growth.

c) **Promoting growth**: It acts as a natural growth promoter, aiding in seed germination and root development.

By using chitosan in agriculture, farmers can reduce their reliance on chemical pesticides and fertilizers, leading to more sustainable and eco-friendly farming practices.

Food Industry

Because of its ability to form films and act as a barrier, chitosan is a great raw material for coatings or films that are edible. Chitosan is the perfect material to utilize as a biodegradable antimicrobial packaging material because of its natural antibacterial and antifungal qualities as well as its capacity to create films. This works as a good preservative and coating substance to extend the shelf life and quality of perishable goods. It can also make them much easier to store.

Drug delivery systems

With its biocompatibility, biodegradability, and capacity to improve drug absorption and regulate release, chitosan is a flexible and efficient drug delivery medium. Many drug delivery applications have made use of chitosan, such as ocular drug delivery, oral drug delivery, pulmonary drug delivery, nasal drug delivery, mucosal drug delivery, gene delivery, buccal delivery, vaccine distribution, and cancer therapy. Chitosan can be used to transport drugs in a variety of ways, including hydrogels, films, nanoparticles, and nanofibres. Aerogel, a porous, extremely light substance whose qualities rely on the precursor materials, material mixing ratio, production technique, and additives, is another way that chitosan is utilized.

Wound dressings and tissue engineering

Due to the haemostatics and antibacterial qualities, biocompatibility, and capacity to promote tissue regeneration, chitosan is an adaptable substance for wound healing. Its ability to promote the creation of type IV collagen, activate fibroblasts and polymorphonuclear cells, produce cytokines, and encourage giant cell migration is all related to its efficacy in wound healing. The gel-like layer that chitosan creates keeps the wound wet, which is essential for the best possible healing outcome. It strengthens new tissue by promoting the deposition of collagen, a crucial structural component in wound healing. Furthermore, lysozyme and other body fluid enzymes break down chitosan into chito-oligomers, which stimulate macrophages and promote collagen deposition, hastening the healing of wounds. Wounds are covered and shielded with chitosan-based dressings, which promote the best possible healing environment. Chitosan hydrogels have antibacterial properties and maintain moisture in wounds. Chitosan scaffolds are used in tissue engineering to promote the creation of new tissue and facilitate the healing of deeper wounds.

Water Treatment

Chitosan is a highly effective and environmentally friendly material for water treatment. Its ability to adsorb pollutants, flocculate suspended particles, and chelate heavy metals makes it a versatile and valuable tool in improving water quality. Chitosan effectively adsorbs and removes heavy metals such as lead, mercury, and cadmium from contaminated water. It can also adsorb dyes from textile and industrial wastewater, reducing color and chemical load. Additionally, chitosan removes various organic pollutants, including pesticides and pharmaceuticals, and is used in purifying drinking water by removing impurities and contaminants, thereby improving water quality and safety. Chitosan can be formed into beads or granules for easy handling and efficient pollutant removal. Chitosan-based membranes are used for filtration and purification processes. Chitosan is often combined with other materials, such as activated carbon or graphene oxide, to enhance its adsorption capacity and mechanical properties

ENVIRONMENTAL IMPACT AND SUSTAINABILITY

Waste Reduction

Chitosan is a biopolymer derived from chitin, which is abundant in the exoskeletons of insects and crustaceans such as shrimp, crabs, and lobsters as well as in fungi. Its production and application play a significant role in waste reduction and promote sustainable practices across various industries.

Source of Chitosan

The primary source of chitosan is chitin, which is extracted from the shells of crustaceans. These shells are often discarded as waste in the seafood industry, leading to environmental concerns and disposal challenges.

Waste Conversion

By converting this otherwise discarded material into valuable chitosan, the process significantly reduces the volume of waste. This not only addresses waste management issues but also adds value to seafood byproducts.

Eco-friendly Alternatives

Chitosan stands out as an eco-friendly alternative across multiple industries. Its natural origin, biodegradability, and versatility make it a sustainable option that contributes to waste reduction and environmental conservation. By incorporating chitosan into products and processes, industries can move towards more sustainable practices, benefiting both the environment and society.

LIFECYCLE ASSESSMENT OF CHITOSAN

Lifecycle Assessment (LCA) of chitosan evaluates the environmental impacts of a product from raw material extraction to disposal. For chitosan, the LCA typically includes:

Raw Material Extraction

Chitosan is derived from chitin, primarily sourced from the exoskeletons of crustaceans like shrimp and crabs. These shells are byproducts of the seafood industry, reducing waste and providing a renewable raw material.

Production Process

The production involves demineralization, deproteinization, and deacetylation of chitin to produce chitosan. While these processes consume energy and chemicals, advancements in green chemistry aim to minimize environmental impact.

Usage Phase

Chitosan's biodegradability and non-toxic nature contribute to its low environmental impact during use. Its applications in agriculture, water treatment, and medicine further enhance its eco-friendly profile.

End-of-Life

Being biodegradable, chitosan decomposes naturally, reducing landfill accumulation and environmental persistence compared to synthetic polymers.

ENVIRONMENTAL FOOTPRINT OF CHITOSAN

Carbon Footprint

Chitosan production generally has a lower carbon footprint compared to synthetic polymers, especially when sourced from seafood industry byproducts. Energy use and emissions during production can be optimized to further reduce the carbon footprint.

Water Usage

The water usage in chitosan production can be significant due to the various washing and processing stages. However, advancements in recycling process water can mitigate this impact.

Chemical Use

The production process involves chemicals for demineralization and deproteinization. Using environmentally friendly alternatives and optimizing processes can reduce the chemical footprint.

CHALLENGES AND FUTURE DIRECTIONS

Despite its promising applications across various industries, several challenges must be addressed to fully realize its potential. This chapter explores these challenges and outlines future directions for research and development in chitosan technology.

Technical and Economic Challenges

Source variability and quality control

Chitosan quality can vary depending on the source and processing of chitin. Factors such as the type of crustacean, geographic origin, and extraction methods can influence the final product's properties. Ensuring consistent quality and properties of chitosan is essential for its widespread application. Standardizing production processes and establishing quality control measures are crucial.

Cost of production

The cost of producing high-quality chitosan can be relatively high compared to synthetic polymers. Cost-effective production methods are needed to make chitosan more competitive in the market.

Scaling up

Large-scale production poses technical and economic challenges. Developing efficient, scalable production techniques is necessary to meet industrial demands.

Environmental and Regulatory Issues

Sustainability

Although chitosan is derived from renewable resources, its production involves the use of chemicals that can impact the environment. Developing greener production methods is crucial.

Regulatory approvals

Navigating the regulatory landscape for new applications, especially in food and pharmaceuticals, can be time-consuming and costly. Ensuring compliance with international standards is necessary for market acceptance.

Challenges in research and development

However, advancing the research and development (R&D) of chitosan to fully exploit its potential involves overcoming several scientific and technical challenges. Advancing the research and development of chitosan involves addressing significant challenges related to production, customization, regulatory compliance, and economic viability. Future R&D efforts should focus on innovative and sustainable production techniques, advanced functionalization, and interdisciplinary collaboration to enhance chitosan's properties and broaden its application spectrum. By overcoming these challenges, chitosan can significantly contribute to sustainable development and innovation across various industries.

Policy and regulation

The development and commercialization of chitosan face significant challenges, including technical, economic, and regulatory hurdles. Addressing these challenges requires coordinated efforts involving research and development, supportive policies, and streamlined regulatory frameworks. By fostering innovation, promoting sustainability, and harmonizing regulations, the potential of chitosan can be fully realized, benefiting various industries and contributing to a more sustainable future. Chitosan-based products, particularly in food, pharmaceuticals, and medical applications, must meet stringent regulatory requirements. Navigating these regulations can be complex and resource-intensive. Extensive testing and clinical trials are required to demonstrate the safety and efficacy of chitosan for new applications, adding to the time and cost of development.

Future directions

While chitosan presents numerous opportunities across various sectors, addressing the challenges related to its production, customization, and regulatory compliance is essential. Future research and development efforts should focus on sustainable production techniques, advanced applications, and functionalization to enhance its properties and broaden its use. By overcoming these challenges, chitosan can play a pivotal role in promoting sustainability and innovation in materials science.

Innovative production techniques

Advancements in green chemistry can lead to more sustainable and environmentally friendly production methods. Research into alternative solvents and catalysts is ongoing. Utilizing biotechnology, such as microbial fermentation, for chitosan production can potentially lower costs and reduce environmental impact.

Advanced applications

Chitosan's biocompatibility makes it ideal for developing advanced medical applications, including drug delivery systems, tissue engineering, and wound healing. Continued research in these areas can unlock new therapeutic potentials. Chitosan's role in sustainable agriculture, such as bio-fertilizers and natural pesticides, is promising. Enhancing its effectiveness and understanding its interactions with plants can lead to more widespread use.

Functionalization and composites

Chemical and physical modifications can improve chitosan's properties and expand its applications. Functionalized chitosan derivatives with enhanced solubility, antimicrobial properties, and mechanical strength are areas of active research. Combining chitosan with other materials (e.g., nanoparticles, polymers) can create composites with superior properties for applications in packaging, construction, and electronics.

Sustainability and circular economy

Developing methods to utilize chitin waste from various industries (not just seafood) can enhance the sustainability of chitosan production. Integrating chitosan into circular economy models ensures efficient resource utilization. Comprehensive life cycle assessments (LCA) of chitosan products can help identify areas for improvement in sustainability and guide the development of eco-friendly practices.

Regulatory and market development

Establishing global standards for chitosan quality and production can facilitate international trade and market expansion. Collaborative efforts between industry, academia, and regulatory bodies are necessary. Educating consumers about the benefits of chitosan-based products can drive demand and support market growth. Public awareness campaigns and transparent labeling can play a significant role.

CONCLUSION

Chitosan, with its biocompatibility, biodegradability, and versatility, holds significant promise across various industries, from biomedicine to agriculture. However, to fully harness its potential, it is essential to address the existing challenges related to production, standardization, cost, and regulatory compliance. Further research is crucial to overcoming these obstacles. Advances in extraction and processing technologies can lead to more efficient and environmentally friendly production methods. Interdisciplinary research can facilitate the development of tailored chitosan formulations for specific applications, enhancing its functionality and performance. Chitosan derived from tasar silk pupae presents a promising alternative to traditional sources. Its unique properties and wide range of applications, coupled with the sustainability benefits, make it an attractive option for various industries. Further research and development in this area can enhance the efficiency of the extraction process and expand the potential uses of this biopolymer, contributing to a more sustainable future. In conclusion, by fostering an environment that encourages research and sustainable practices, the full potential of chitosan can be realized. This will not only lead to technological and industrial advancements but also contribute to environmental sustainability and the development of safer, more effective products across various sectors.

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8. Tasar Silk Proteins as Smart Biomaterial for Pharmaceutical and Biomedical applications

T. Bal, K. Jena, A. Sinha & S. Satpathi

CONTENTS

INTRODUCTION

Natural biomaterials are recently being explored increasingly due to their ability to mimic extracellular matrix (ECM). The major composition of ECM is glycosaminoglycans and proteins mainly the collagen and fibronectin which are principally responsible for formation of tissues in human body. The collagen and fibronectin form a 3D network which very well imitates the ECM of the cells, helping in quick tissue regrowth in case of major wounds and critical conditions like burns or wounds related to diabetes like diabetes foot ulcers. In case of bed ridden patients, development of critical bed sores is a common phenomenon as shown in Figure 1, with tremendous dead tissues which cannot be easily regenerated, as such situation demands some external graft materials, which can act as scaffold for supporting the wounded tissue with prompt healing and also regenerating new tissues effectively in a short span of time. Management of critical wounds conditions become difficult to manage as usually grafts are obtained from other parts of the body to refill the lost tissues for healing and filling purpose, but physically and financially debilitated patients cannot handle such medical treatment. Thus, to have a cost-effective treatment, development of some magical biomaterials possessing properties of self-healing which on repeated applications, can easily help in provoking cells through the wounded area for rapid recovery and activate healing spontaneously.

Biomaterials are sourced either from natural or synthetic origin and their main purpose is to act as scaffold which can mimic and interact with the living cells and can be a good choice as medical devices or implants viz heart valves, stents, artificial joints, hearing loss implants, dental implants, helping in rapid tissue regeneration in wound healing. Other advantages possessed by biomaterials is their enormous potential to be utilized as drug delivery platforms as various nano-formulations or even modifications by formation of hydrogels to deliver macromolecular drugs viz proteins in the form of DNA or SiRNAs or peptides or even drug moieties which cannot cross the blood brain barrier in case of sensitive areas like brain. Also, these biomaterials play a successful role as controlled or sustained delivery systems for very potent drugs as well as can be used for fabrication of

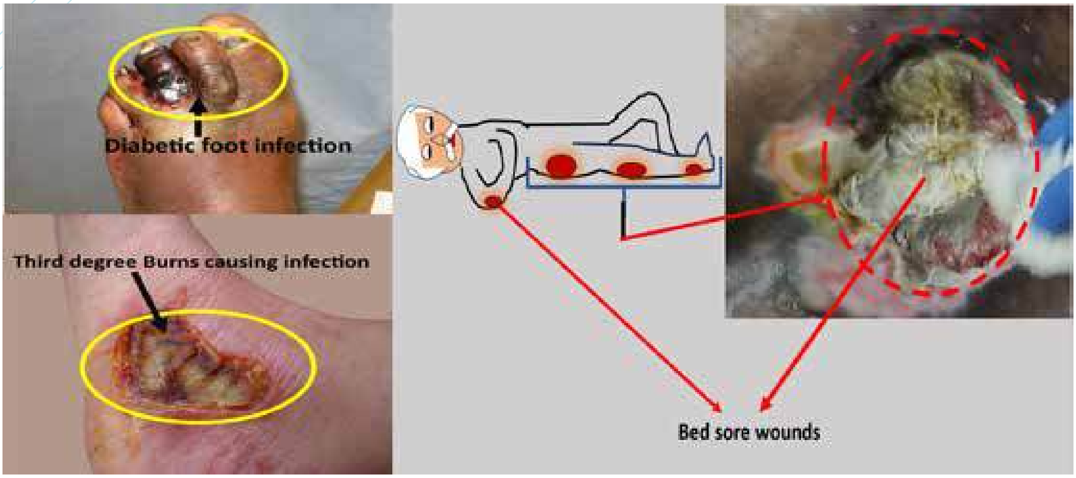

Figure 1: Clinical case of critical bed sores, burns and diabetic foot infection

nanoparticle biosensors. Egyptians were the pioneers in utilizing biomaterials in the form of animal tendon like fibres in the form of sutures for wound healing. Biomaterials from natural sources have the ideal properties by being biocompatible, biodegradable but less stable with the possibility of generating immunogenic reactions, whereas synthetic materials suffer from poor biocompatibility and degradability issues. These issues trigger for fabrication of a very stable, biocompatible, biodegradable and non-immunogenic biomaterial for tissue regeneration and drug delivery applications. Silk proteins obtained from either mulberry source like *Bombyx mori* or non-mulberry source like *Antheraea mylitta*, *Antheraea assamensis*, *Samia ricini* etc. are promising natural biomaterial, but the sequence of polypeptides and the types of amino acids in both the varieties are completely different which also alters their properties. Silk proteins mainly fibroin from non-mulberry source *A. mylitta* are most appropriate for tissue regeneration as compared to the mulberry variety due to the tripeptide combination of Arginyl-Glycyl-Aspartic acid (RGD) sequence which helps in rapid cell proliferation and cell attachment process . These properties of *A. mylitta* makes it an interesting research platform and thus the current chapter provides a deep insight of *A. mylitta* silk proteins as an effective smart biomaterial with its huge potential in tissue regeneration applications, drug formulations.

A. MYLITTA SILK PROTEINS AS EFFECTIVE BIOMATERIAL FOR TISSUE REGENERATION

Tissue regeneration in any wound initiates with hemostasis, and inflammation, where hemostats with anti-inflammatory properties act to heal the wound rapidly, this is followed by proliferation, remodelling and maturation as shown in Figure 2, where self-healing polymers with tissue regrowth properties termed as biomaterials play a key role in tissue engineering.

Silk has been used for ages in medical industries as a suture material by virtue of its cell compatibility and cell attachment properties with escalated tensile strength making silk as an excellent biomaterial scaffold or medical implants for tissue regeneration especially in case of healing of variety of wounds. Silk is composed of two main proteins namely sericin

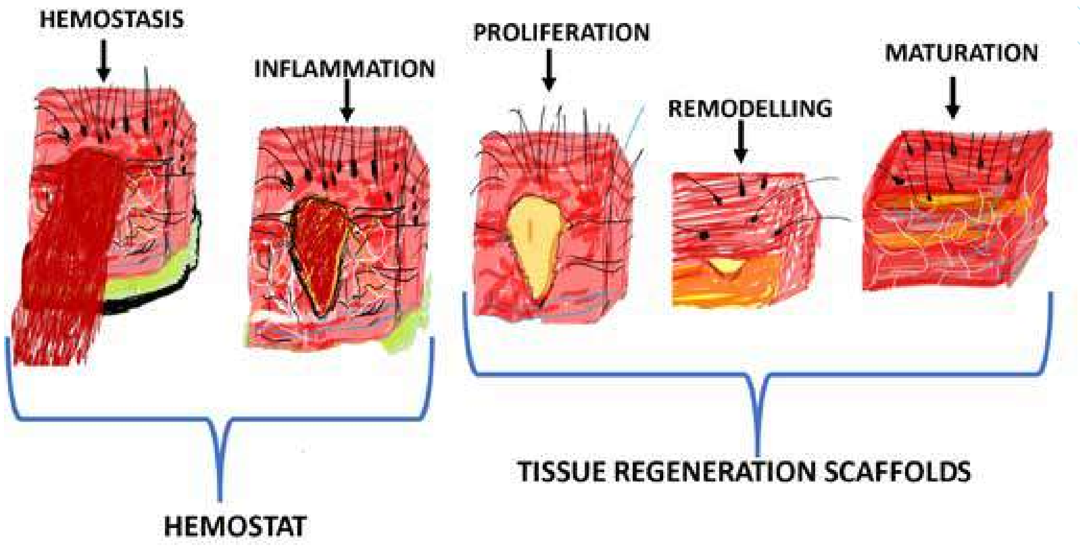

Figure 2: Wound healing stages with appropriate applied materials for healing

which mainly constitutes 20-30% of silk proteins containing 17-18 types of amino acids having functional groups mainly carboxyl, hydroxyl groups, with high content of amino acids like serine, glycine, aspartic acid and fibroin which is crystalline in nature. Sericin of non-mulberry silk mainly *A.mylitta* shows very effective results in inhibiting UV induced-apoptosis in human skin keratinocytes. Non-mulberry silk proteins have higher antioxidant and UV-protection abilities with added potential of being resistant to extreme climatic conditions by virtue of its wild nature. Sericin from *A.mylitta* silk are more hydrophilic in nature making them more appropriate for tissue regeneration process and also enables fast collagen production. But this high hydrophilic property creates problem for sericin to be formulated alone which triggers the use of other polymers in combination either in the form of graft copolymers, or hydrogels etc. for tissue regeneration with improved mechanical properties.

A work was done on *A.mylitta* silk proteins mainly fibroin and formulated in the form of a cardiac patch which almost mimicked similar to fibronectin which is a principle component of extracellular matrix of cardiomyocytes, thus the prepared scaffold showed much better cell adhesion, cell metabolic activity, better response to extracellular stimuli. It was also seen that cells produces connexin 43 exhibiting sarcomeres which connect with each other and produced electrical signals resulting in simultaneous beating of cardiomyocytes in postnatal rat.

In another study , a very porous scaffold was prepared by combining *A.mylitta* silk fibroin along with chitosan by freeze drying technique, using the protein-polysaccharide ratio from 90:10 to 50:50 , and all the grades showed a very high swelling percentage and when incubated with MG-63 cell line to check the bone cell compatibility, showed escalated growth with excellent cell adhesion and increased Fluoride production from day 3 to day 7 which helps in enhanced activity of osteoblasts. Moreover, the scaffold showed tremendous healing in a rabbit tibia bone defect model.

A major highlight of the research on *A.mylitta* silk proteins was in the field of corneal tissue regeneration where a thin film of *A.mylitta* silk fibroin was used as a substitute for amniotic membrane and the amniotic membrane is currently being used for corneal regeneration. It was observed that that the epithelial cells and keratocytes from rat corneal explants showed excellent growth in presence of the silk scaffold which was mechanically strong and robust. The corneal cells preserved their phenotypes and preserved cytokeratin and vimentin which helps in maintaining cellular integrity in presence of the silk film. Moreover the fibroin film when implanted as a cornea replacement in the rabbit model without cornea, showed no signs of neovascularization or any inflammation in the eyes with perfect tear formation and intraocular pressure maintenance, which demonstrated the fact that *A.mylitta* silk fibroin can be an excellent alternative for corneal scaffold.

Also, *A.mylitta* silk fibroin was processed using ionic liquids for the regeneration of cartilage using mesenchymal stem cells. *A.mylitta* silk fibroin were used for fabrication of hydrogels. The silk fibroin were dissolved in ionic liquid (IL),1-butyl-3-methyl imidazolium acetate(BMI-Ac) solvent and transferred into polystyrene molds and kept at 4 0C overnight for the elation process which was concluded by placing these molds in ethanol which resulted in the formation of A.mylitta hydrogels. The prepared hydrogels were further processed for complete removal of BMIAc by immersing the hydrogel in ethanol for 24 hours followed by Soxhlet's extraction in ethanol for another 5days. After the BMIAc removal, the hydrogel scaffold was treated with methanol-water mixture (80/20 vol%) for 30minutes to obtain the beta sheets of the hydrogel.

In another study, a 3D porous scaffold was fabricated utilizing *A.mylitta* silk fibroin from the silk glands of the insect and applied the scaffold for the differentiation of rat bone marrow cells in adipocytes and osteocytes. The silk gland of the insect was separated and fibroin was obtained by dissolving in sodium dodecyl sulfate and later dialyzed the fibroin solution to remove the excess surfactant and finally obtained the concentrated fibroin by combining with 30% polyethylene glycol with dialysis membrane. The 3D porous scaffold of the fibroin was obtained by freeze drying a 6% fibroin solution followed by lyophilization and later treated with ethanol for 30 minutes to develop β-sheet to make the scaffold water insoluble. The 3D scaffold showed very less immunogenicity with very low inflammation when compared to FDA approved *Bombyx mori* silk fibroin scaffolds. Moreover, the study revealed that due to highly porous nature of the scaffold, the cells easily migrated which impacted their rapid proliferation and obvious cell attachment.

In another study, 3D *A.mylitta* silk fibroin construct model loaded with hepatocarcinoma cells (HepR21) for screening of potent anticancer drugs was prepared where they overexpressed the HepR2 cells with Hyaluronan binding protein 1(HABP1) known as HepR21, to check the effect of hyaluronan in tumor cells and these HepR21 cells are insensitive to reactive oxygen species (ROS) making them more cell friendly. This showed that such 3D scaffolds helped in screening of potent drugs which can help in triggering the overexpression of HABP1 in the HepR2 cells to prevent usage of any animal model.

Another 3D scaffold of *A.mylitta* silk fibroin was fabricated as breast cancer model. The 3D scaffold was incubated with human breast cancer cell line, breast adenocarcinoma

MDA-MB-231, where the cells appropriately grew with sufficient tissue formation and cell attachment showing the appropriateness of the scaffold for cell viability.

In another study, *A.mylitta* silk fibroin solution in ionic liquid (IL),1-butyl-3-methyl imidazolium acetate(BMI-Ac) of different concentration of 5, 7 & 10% w/v was prepared and electrospinned to obtain nanofibre mat which were further coated with green synthesized silver nanoparticles prepared with *Tridax procumbens* leaf extract to provide antimicrobial potential to the prepared nanofibrous mat. The prepared *A.mylitta* silk fibroin nanofibrous mat showed good cytocompatibility and cell adhesion *invitro* with L929 cell lines which explored the use of the prepared scaffold as an effective platform for tissue regeneration.

Another study involved a 3D scaffold fabrication from fibroin of *A.mylitta* silk using freeze drying method. Fibroin was isolated from the silk gland and dissolved in sodium dodecyl sulfate (SDS) and removed the excess surfactant from the fibroin solution by dialysis method and later fabricated 3D scaffolds for growing human neural progenitor (hNP) cells, which play a key role for neural tissue regeneration and act as effective treatment therapies for central and peripheral nervous system.

Also, fabricated a 3D scaffold from *A.mylitta* silk fibroin using freeze drying technique and the scaffold was evaluated for providing appropriate environment for growing feline fibroblast cells of AH-927.The scaffold showed excellent growth of cells with greater pore size, with high cytocompatibility, indicating the material to be an excellent platform for bioengineering and tissue regrowth applications.

In another study, a hydrogel was prepared composed of *A.mylitta* silk sericin and chitosan which was crosslinked with genipin and utilized it as a dermal substitute. The prepared scaffold was sufficiently porous, where cell adhered effectively showing no inflammation with zero response of inflammatory cytokines tumor necrosis factor (TNF-α) and interleukin (IL-1) with hemocompatible properties.

A nanofibre matrix composed of *A.mylitta* silk sericin combined with Chitosan and Polyvinyl alcohol indicated its potential application for skin tissue regeneration. The nanofibre mat loaded with antibiotic Cephalexin hydrate enhanced the process of wound healing prohibiting any secondary infections.

Moreover, *A.mylitta* silk sericin showed huge potential as antioxidant, anti-elastase and anti-tyrosinase and it was concluded that the biomaterial can be an effective cosmeceutical component.

Also, *A.mylitta* silk sericin hydrogels sheets without polyethylene glycol was prepared which were crosslinked with citric acid. The prepared hydrogel sheet showed tremendous cell proliferation with NIH-3T3 cell fibroblasts indicating the prepared material can be an appropriate material for wound healing and skin tissue regeneration.

Although research has been conducted with *A.mylitta* silk proteins in the field of tissue regeneration but still there are lot more to be explored and fabricated with *A.mylitta* silk proteins in the form of composite systems which can be utilized not only for soft tissue regeneration but also for hard tissues like bone.

A.MYLITTA SILK PROTEINS AS EFFECTIVE BIOMATERIAL FOR DRUG DELIVERY

Natural biomaterial plays a significant role in design of varied dosage forms by virtue of their biocompatibility and zero immune responses leading to toxicities. Target oriented deliveries are of more interest as they can deliver minimum quantity of drug which are very potent to the desired location without causing unwanted distribution of drugs in other body compartments. Nano-deliveries are appropriate and safe as targeted systems and they can be fabricated using synthetic or natural biomaterials, while natural moieties like gelatin, collagen, albumin etc. are more preferred due to their cytocompatibility and Silk is one such biomaterial having all the ideal properties overcoming the loop holes of synthetic biomaterials of biodegradability with very high stability and increased mechanical strength. Non-mulberry silk proteins have different types of amino acids with different functional groups and thus can bind to the specific receptor of the cell surface , and thus they can serve as advantageous scaffolds for targeted delivery of drugs and offers many advantages over the synthetic counterparts. *A.mylitta* silk proteins are enriched with RGD making them more cell adherent and compatible and thus they are fabricated in diverse formulations for drugs viz nanoparticles, hydrogels, micellar systems etc.

In a study, nanoparticles of *A.mylitta* silk fibroin conjugated with folic acid and Doxorubicin was loaded into the formed nano systems and the systems were evaluated for *invitro* drug release and effect of drug loaded nanoparticles on human breast adecarcinoma cell line(MDA-MB-231). The results indicated that folic acid decorated nanoparticles loaded with doxorubicin was very effective in circulating within the intracellular spaces of blood capillaries without agglomerating indicating the formulation to be very stable. Moreover, the cellular uptake of folate-conjugated nanoparticles was higher and the formulation showed higher release at pH 4.5 due to less bonding between the doxorubicin and the amine group of the fibroin nanoparticles.

In another study, nanoparticles was prepared using *A.mylitta* silk sericin in presence of Dimethyl sulfoxide as desolvating agent. The nanoparticles loaded with Vascular endothelial growth factor (VEGF) showed release for more than 3 weeks without any initial burst, indicating the prepared nanoparticles can be significant tool in cancer diagnosis and drug delivery.

In another significant study, it was concluded that the fabricated self-assembled micellar nanostructures using sericin of *A.mylitta* silk combined with pluronic F-127 and F-87, had the potential of entrapping both hydrophilic and hydrophobic drugs. The researchers used hydrophilic drug moiety inulin and hydrophobic drug Paclitaxel for the experiment and the formulation very well delivered the drugs to the targeted site.

A.mylitta silk proteins have lot of significant properties which can be used for different formulations as in case of diabetes, targeted release in cancer etc., but lot more is to be explored for new and innovative formulations like using 3D technique which can help in fabrication of artificial skin, or any other organs, and even can be used for fabrication of drug formulations which can be of benefit to mankind.

CONCLUSION

Biomaterials can serve as a boon for serving mankind and can create new evolution for management of serious ailments and diseases. *A.mylitta* silk proteins are such biomaterials with versatile properties which can trigger the physiological responses and help in replenishing dead tissues as well as result in targeting specific cell receptors leading to release of highly potent drugs as in case of brain disorders, chemotherapeutic agents etc. The nonmulberry *A.mylitta* silk proteins with sufficient mechanical strength can be utilized for different wound dressings with very prompt healing properties as compared mulberry *Bombyx mori* silk proteins. A lot of research is to be carried out before *A.mylitta* silk proteins can be used for commercial tissue engineering applications and products as well as clinical trials. *A.mylitta* silk proteins will contribute in fabrication of very sophisticated different disease models which can help in avoiding animal studies, also serve as significant scaffold for bone tissue regeneration, thereby helping in mineral resorption and vascularization as well as investigating potent therapeutic drugs in innovative formulations.

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9. Tasar Silk Fibre Waste: An Overview

A. Kumar, J.P. Pandey and N.B. Chowdary

CONTENTS

INTRODUCTION

Silk is known to be queen of the textile fibres also it is the only fibre available in filament form. It is also available in spun form which is generally prepared from silk wastes. Apart from silk available in filament form which is also called reeled silk, there is also huge demand of silk available in the spun form across India as well as world. It is known for its luxurious luster, beauty, and elegance, and delivers qualities incomparable with any other fibre. Silk is mainly classified in mulberry and non-mulberry silk, out of which mulberry is domesticated variety and non-mulberry is wild variety and mainly reared in outdoor area. Tasar is one of the wild silk varieties and both tropical and temperate tasar silks are available in India. Tropical tasar belongs to the species of *Antheraea mylitta* D which is mainly reared in 10 states of India in which around 3.5 lakh families are involved. Out of all 10 states Jharkhand holds number one position with around 60-70% of total production in India. Unwinding of filament from cocoons is called as reeling, but during reeling of tasar cocoons, only 55 to 65% raw silk is obtained and remaining 35-45% goes into waste in various form. Also, the cocoon which is unreelable as well as pierced cocoons by-product of grainage activities also considered as waste as it does not compete with the quality of reelable cocoons. So, a good quantum of silk waste is generated during reeling in addition to unreelable cocoons and peduncles. At present, these tasar silk wastes are utilized using conventional spinning method by using takli and matka to produce spun yarn. But amount of spun yarn produced by this method is very low and there is also a limitation for yarn count *i.e.* up to 15 Nm. To overcome this problem Trivedi spinning wheel, N.R. Das spinning Wheel and Chowdhary Spinning Wheel were developed but that was not commercially successful. But there is some established method which now people are using because the utilization of tasar silk waste is very important as it gives additional profit to the reelers.

During the process of Tasar silk reeling & post-reeling process the different varieties of silk fibre waste is obtained as byproducts are: -

TYPES OF TASAR SILK FIBRE WASTE

Cocoon waste/Unreelable cocoons

- Pierced/Cut cocoons
- Damaged cocoons
- Stained/Double cocoons etc.

Waste generated during Reeling

- De-flossing waste
- Reeling waste
- Basin waste

Post-Reeling Waste

- Re-reeling waste
- Winding, warping & weaving waste

Tasar Peduncle

METHOD OF UTILIZATION OF SILK FIBRE WASTE

Methods of utilization of tasar silk wastes depends upon the type of the waste. As unreelable cocoons needs different treatment from that given to silk waste generated during reeling & post-reeling process. The first step of using silk wastes is the classification of the material into various varieties. These are the various methods for different types of wastes-

A. Cocoon wastes & some part of basin wastes are generally converted into Ghicha, Katia & Jhuri Yarn

B. Other wastes like reeling, de-flossing & post-reeling waste converted to spun silk yarn

C. Tasar peduncle is also converted to yarn by spinning method and yarn made out of it is called Balkal yarn

COCOON WASTES & BASIN WASTES

There are some cocoons which are called unreelable because of some defects are pierced cocoons, pin hole cocoons, double cocoons, stained cocoons, cut cocoons etc. they are not suitable for reeling process and called cocoon wastes. Basin waste is the innermost layer of cocoon which is also called pellade.

Methods of Conversion

Earthen Pitcher (Matka)

Ghicha, and Jhuri yarns are produced using Earthen Pitcher popularly known as Matka. Ghicha yarn is made from un-reeleble cocoons as well as basin waste. For conversion of un-reelable cocoons to Ghicha yarn, firstly it is cooked/softened using either sodium carbonate

i) ii) iii)

Figure 1: -Various types of Unreelable cocoons & Basin waste; i) Pierced Cocoons ii) Double cocoon iii) Basin waste

and sodium bi-carbonate or Hydrogen peroxide and sunlight soap. Thereafter reeler/spinner withdraws strand of fibres from softened cocoons in semi moist condition by hand and rubs on the back of the Earthen Pitcher (Matka) to insert little twist or false twist to impart cohesion between fibres, prepared yarns is continuously lays down on any paper above floor (Figure 2). After spinning process, layers of ghicha yarn converted into hank form by re- reeling process. Re-reeling process is a process to convert bobbins or layers of yarn into hank of 1.5 meter circumference and remove the faults present in the yarn. Around to 80-100 g of Ghicha yarn can be produced per day or in eight hours. Similarly, Jhuri yarn is produced by using Matka from reeling basin wastes following the above spinning method.

Figure 2: Ghicha yarn preparation using Earthen Pitcher (Matka)

Motorized Spinning Machine (MSM)

Due to limitation of yarn fineness, quality and productivity in production of ghicha yarn, CSB-CSTRI has developed Motorized cum Pedal operated Spinning Machine for improvement of quality and productivity. It is a 27.50 cm long and 25 cm high iron framed machine which works on ring & spindle principle. Spindle has been placed horizontally and it is operated by single-phase motor of 0.25 HP capacity. The machine has mechanized traversing system to perform uniform distribution of yarn over the bobbin. This is a portable model and finer count of yarn (up to 40^s Metric count) with uniform twist can be spun using this device. Tasar unreelable cocoons can be used in this machine for preparation of spun yarn commonly known as Katia yarn, the average production from this machine is about 150-200 g per day or in 8 hours. Other than ring and traveller mechanism; flyer twisting technique is also available in the field which can produce low twisted yarn (Figure 3b). Both types of machines can run by pedal, electrical motor and solar power system.

(a) Ring & traveller (b) Flyer (c) Katia Yarn

Figure 3: Motorized Spinning Machine (MSM)

REELING & POST-REELING WASTES

These wastes are produced during deflosing, reeling and post-reeling process (Figure 4). Deflosing wastes are generated before reeling process during a very important step called as deflossing, objective of this process is to find out the single end of individual continuous filament from each cocoons for easy operation of reeling process. The waste generated during reeling on account of feeding of cocoons after exhaustion of running cocoons and during mending of breakages is called as reeling waste. And post-reeling wastes consists of waste generated during knotting in different stages of silk yarn or fabric production *i.e.* re-reeling, winding, throwing and weaving, wet processing etc. These wastes are required to be opened by mechanical opening machine for further utilization.

i. ii. iii. iv.

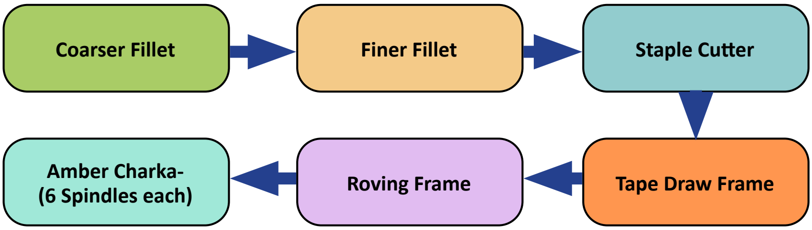
Figure 4: -Various types of reeling & post-reeling waste; i) deflossing waste ii) reeling waste iii) re-reeling/winding/weaving waste & iv) Warping/drawing waste

Amber Charka

Amber Charkha is very popular for producing cotton and polyester/cotton blended yarns in Khadi sector of India. All types of silk wastes can also be processed in these machineries successfully (Figure 5), There are series of machines used for this purpose namely one no of coarser fillet, finer fillet, Staple cutter, tape draw frame and roving frame each are required. The details of machineries for Amber charkha spinning process is shown in the flow chart-1.

Amber Charkha Amber Charkha Spun Yarn

Figure 5: Spun yarn preparation through Amber Charkha



Flow Chart: 1 Process sequence of Amber Charkha spinning

Although the tasar silk waste can be spun by Amber Charkha set of machineries along with improvement of quality characteristics but due to very low productivity; mill spinning process is followed for producing superior quality yarns.

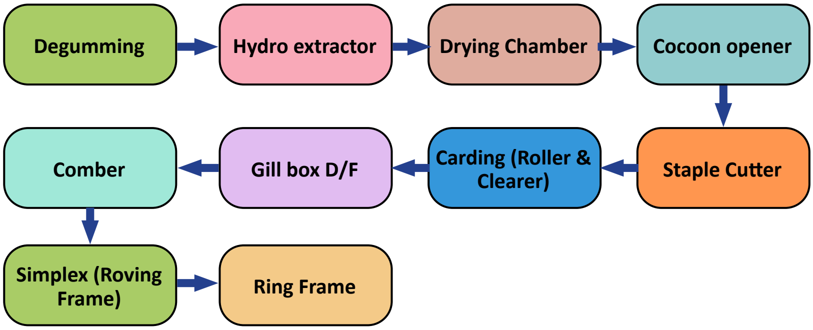
Mill Spinning Process

Tasar silk waste can be effectively utilized for production of various spun yarn by mill spinning process. There are mainly two different processes available in the field which is Italian and Japanese system. In Italian system, Roller and Clearer Card, Gill Box Draw Frame, Comber, Speed Frame and Ring Frame are the main machineries required in addition to degumming, drying and cocoon opener.

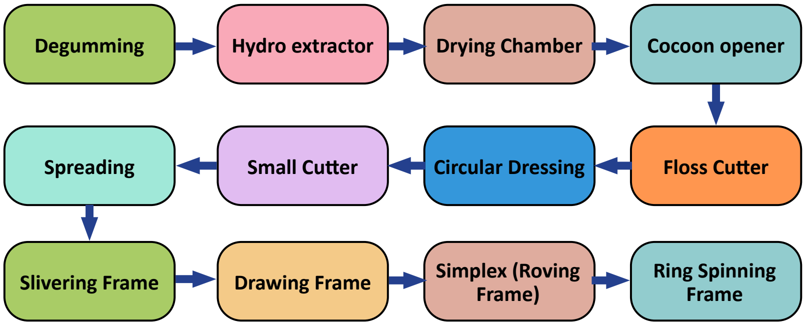
Similarly, in case of Japanese system, Floss Cutter, Circular Dressing, Small Cutter, Spreading, Slivering Frame, Drawing Frame, Roving Frame and Ring Frame are the set of machines which are required. But yarn realization is low for Mill spinning process *i.e.* about 50%, due to noil (short fibres) elimination during combing operation for Italian system and dressing operation in case of Japanese system. The noil eliminated during processing can be utilized for spun yarn production following short staple cotton spinning process with yarn count ranging from 10 Nm to 40 Nm. The spun yarns produced by Ring Frame are processed through Cone Winder, followed by post spinning processes. However, the realization is less as compared to manual process, but the quality characteristics in terms of uniformity and tensile are quite better.

TASAR PEDUNCLE

A small quantity of silk wastes which is very rich in protein and calcium oxalate is obtained from tasar cocoon peduncles which are generally cut and separated before cooking of cocoons for reeling purpose (Figure 6). These peduncles degummed/cooked using sodium carbonate for one hour and get converted into peduncle silk waste and these wastes



Flow Chart: 2: Details of machineries for Italian System



Flow Chart-3: Details of machineries for Japanese System

converted to balkal yarn either by Amber Charkha set of machineries or by Mill Spinning process.

Tasar Peduncles Degumming

Matured tasar silkworm before the formation of cocoons makes peduncle as a support on their food plant. The peduncles also can be converted to silk yarn called balkal yarn or peduncle fibres can be blended with wool or other fibre for preparation of blended yarn. As it facilitates support for spinning of cocoons; so it is very tough and vigorous alkaline boiling is required. For 1 kg of peduncles, about 10 litters water is taken. Sodium carbonate of about 10 g/l being dissolved in water and the peduncles are boiled for 60 minute duration followed by neutralization, hot washing and cold washing in order to remove presence of alkali. After drying, the peduncles are beaten by wooden hammer for pre-opening and removal of cuticles as well as other impurities. These pre opened peduncles need to be processed through coarser fillet (2 times) followed by finer fillet (2 times) for proper opening and individualization of fibres. And then converted to balkal yarn either by Amber Charkha set of machineries or by Mill Spinning process.

APPLICATIONS OF SILK WASTE

Apparel & Home Textiles

Silk exhibits good absorbency, it can store up to 33% of water by weight without feeling damp which makes it comfortable to wear in warm weather. Also, silk waste like balkal yarn can be blended with wool which provides warmth in the winter. Tasar silk waste is

Peduncles | Peduncles during Degumming | Peduncles after Degumming | Balkal Yarn

Figure 6: Steps involved in processing of peduncles

generally used for clothing such as Shawls, bandi, Kurta, Sarees, Suits, Chaddar, etc. It is also used in many furnishing applications. It is used for upholstery, window curtains (blended with another fibre), rugs/carpets, quilts and wall hangings. It can also be used in handbags headbands and scarves. Because of its lustre and texture, it is also used for home decoration.

Medical & Healthcare

In recent years, there are various work reported on use of silk/silk waste in medical field because of its biocompatibility & biodegradable nature. Silk/silk waste consists of two proteins namely sericin & fibroin, both are explored for various biomedical work like, development of sutures, wound healing related product drug delivery agent, and tissue-engineering etc.

Composites

Silk fibre offers great mechanical properties. Which has created demand to use it in fibre reinforced composites for different applications. But composites made of silk fibre becomes costly because of high price of silk filament. To overcome this problem use of silk/tasar silk waste in fibre reinforced composite became popular. Which enables composites to be cost effective. Some study on Tasar silk waste/Jute/Grewia-optiva fibres-reinforced epoxy laminates were done in which inclusion of tasar silk waste increases tensile, flexural and wear performances of composites. The inclusion of the tasar silk waste fibres can contribute significantly to the impact resistance by ensuring either or both a sufficient strength of the composite and a good deformability of the composite.

Nonwovens

Silk non-woven fabrics can be developed from tasar silk reeling waste and waste generated during weaving preparatory and weaving process. At present, these wastes are used for manufacturing of Ghicha, Katia, jhuri & spun silk yarn, besides hand spun yarn. This waste can be more effectively utilized for development of silk non-woven fabrics for diversified end uses. Other wastes like pierced and cut cocoons may also be used for production of nonwoven fabrics. The web formation by air laid method and bonding by chemical/needle punching may be attempted for production of non-woven webs.

CONCLUSION

So, there are various types of tasar silk fibre waste generated from cocoon harvesting/ grainage to reeling & post-reeling processes like unreelable cocoons, peduncles, deflossing, reeling, basin & post reeling waste etc. Around 35-45% of cocoon shell content goes into waste in various form during reeling process, also lakhs of cocoons becomes un-reelable either due to grainage activities or due to various reasons involved during rearing of cocoons. But the good thing is these wastes can be processed using various traditional as well as technological method/machineries. Traditional method involves conversion by earthen pitcher (Matka) or Takli whereas technological method involves conversion by motorized spinning machine, Amber charkha & Mill Spinning. Use of method for conversion depends upon type & quantum of waste, skill of spinner and availability of machineries. Therefore, utilization of these technology/method can give extra income to the reelers/spinners along with their regular income which they are getting from production of reeled yarn.

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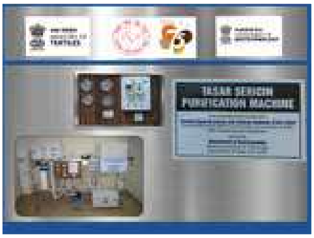
Fabrication of Tasar Sericin Separating Machine

Year: 2015

Year: 2016-2018

Year: 2021

Year: 2022



Year: 2023

Re-usable water | Metal waste | Purified Sericin

TRADEMARK

CSB-Central Tasar Research and Training Institute

Central Silk Board, Ministry of Textiles, Govt. of India

Ranchi-835303, Jharkhand

ctrtiran.csb@nic.in / ctrticsb@gmail.com www.ctrtiran.res.in