**Valorization of *Bombyx mori* Pupae as a Sustainable Source of Functional Protein: Advances in Extraction Technologies, Nutritional Profiling, and Application Potential**

**Abstract**

The increasing global demand for sustainable and nutritionally rich protein sources has brought attention to insect-derived proteins, with Bombyx mori pupae emerging as a promising candidate. Traditionally considered a sericultural by-product, these pupae are abundant in high-quality proteins, essential amino acids, bioactive compounds, and beneficial lipids. This review comprehensively explores the nutritional potential, innovative protein extraction methods, functional and bioactive properties, and diverse applications of B. mori pupae in food, nutraceutical, feed, and biomedical sectors. Emphasis is placed on advanced green technologies such as enzymatic hydrolysis, membrane filtration, and deep eutectic solvents that enhance protein yield and maintain bioactivity. Regulatory frameworks, safety assessments, and consumer acceptance are discussed in relation to global commercialization. Furthermore, the environmental and economic benefits, including waste valorization and contributions to circular bioeconomy models, are examined. Addressing key challenges such as standardization, allergenicity, and policy alignment is crucial to unlocking the full potential of silkworm pupae protein as a sustainable and functional alternative in the global protein landscape.

**1.Introduction**

The food system of the whole world receives more and more pressure to find the solutions to satisfying the nutritional demands of an increasing population, and at the same time limiting the food system impact on the environment. Animals raised as a source of protein are shown to have excessively connected emissions to greenhouse gasses, land, and water usage, and consequential wastage on the ecological environment (FAO, 2017).

As a reaction, academia and industry are under investigation, using sustainable yet cost-effective and nutritionally available alternatives of conventional proteins. The proteins of insects are among them, and have become a valuable solution. Specifically, domesticated silkworm (Bombyx mori), which is mostly produced to employ papillae, provides a rather precious by-product in a form of pupae with a high level of nutrient and an especially rich source of biological resources that is mostly ignored. The production of sericulture waste products produces silkworm pupae in large quantities. These pupae usually are wasted after silk reeling, fed to animals, or extracted into low-value oil. Though, their composition richness, especially high-quality proteins, essential amino acids, lipids, and micronutrients designates them as a possible raw material of the value-added food and nutraceutical formulations (Zhou & Han, 2006; Rumpold & Schluter, 2013). The silkworm pupae aside being protein-rich is also a source of biologically active peptides and other biofunctional molecules, which potentially have antioxidative, antimicrobial, antihypertensive and immunomodulatory effects (Yi et al., 2010; Park et al., 2014). Here the possibility of valorization that entails the processing of agricultural or industrial by-products into better products is a combination that best describes what can be covered by the sustainable use of B. mori pupae. Via upgrading of silkworm pupae, the sericulture sector is capable of improving its material efficiency, waste management problems, and generation of circular bioeconomy. Moreover, this type of bioconversion works in favor of local economies most often located in rural areas, predominantly in developing countries such as India, China and Thailand, where sericulture contributes to the foundations of agro-based economic activities (Reddy et al., 2016).From a nutritional perspective, pupae proteins compare favorably with other animal and plant protein sources. Their amino acid profile is well-balanced, digestibility is high (up to 95%), and the presence of omega-3 and omega-6 fatty acids makes them suitable for both human and animal consumption (Feng et al., 2018; Longvah et al., 2011).The objective of the present review will thus be to synthesize, in detail, the existing body of knowledge on the protein potential of silkworm pupae, in terms of nutrition composition, protein extraction methods, and functional and bioactive properties, applications in foods and feeds as well as socioeconomical and environmental repercussions of its use. Safety, allergenicity, regulations and market acceptance are also discussed in the review as the issues that have to be resolved to enjoy commercial feasibility of the pupae-based protein formulation. By implementing this integrative manner, the article aims at bringing into view the multipronged worth of Bombyx mori pupae not only as a waste but creating a new, renewable and nutritionally powerful payoff of functional proteins capable of transforming the protein supply chain worldwide.

**2. Nutritional Composition of *Bombyx mori* Pupae**

Bombyx mori pupae has a very high nutritional content, therefore, making it a considered candidate of developing some rich food products and nutraceuticals. Silkworm pupa is composed of about 55-65 percent crude protein on dry weight which is not lower or even higher than traditionally used proteins like soybean and casein (Zhou & Han, 2006). The fraction of proteins also has all the nine essential amino acids in substantial amounts especially lysine, leucine, isoleucine and valine that plays a critical role in human development and muscle sustenance (Yi et al., 2010).

This amino acid content exceeds the requirement set by the world health organization in the nutrition of human beings. Besides protein with a good quality, silkworm pupae contain large amounts of lipids and constitute 20-30% of dry matter. Most of these lipids are constituted of unsaturated fatty acids that include linoleic acid, oleic acid and alpha-linolenic acid that are known to aid in cardiovascular health (Longvah et al., 2011). Micronutrients, as well, are present in considerable quantities in a pupa and, in addition, improve the nutritional value of these products, as this includes iron, zinc, calcium, phosphorus, and B-complex vitamins (Rumpold & Schlüter, 2013). It contains dietary fiber due to the presence of chitin, a polysaccharide contained in the exoskeleton making partial removal or alteration necessary before it can be consumed as human food. Also, pupal extracts contain bioactive components like flavonoids, peptides, and sterols that are expected to cause antioxidant and immunomodulatory effects (Park et al., 2014). In comparison to all other insect protein and even classical animal products, B. mori pupae are indeed protein dense and readily digestible. Pupal protein can be reported as digestible (85-95%), based on method of processing (Feng et al., 2018). This good bioavailability makes it appropriate to those in the vulnerable population such as children, the aged, and those with high protein needs like athletes. In general, nutritional profile of Bombyx mori pupae suggests its possible feasibility in high-protein food formulations, supplement, and therapeutic foods. Further analyses of these compositions and standardization work are critical in an attempt to optimise their usage within regulated food systems.

**3. Pre-treatment and Processing of Silkworm Pupae**

Bombyx mori pupae are important sources of protein powder to be used as human food and as industrial resource, efficient pre-treatment of pupae, processing of the pupae is among the essential development to achieve high-quality and safe resource. Choice of suitable processing methods affects the nutritional integrity, functional property and microbial safety and shelf life of the end product. Important stages are drying, defatting, grinding and sieving- all of which are being optimized depending on the purposes of use and compliance to regulations.

**Drying Techniques**

Silkworm pupae are usually about 70 -80% moisture, an amount that has to be brought down so that they do not get spoilt by microbial growth, making them easy to handle and store. Sun drying has been applied massively but because it is cheap, it is highly weather sensitive; this aspect makes it prone to contamination by airborne impurities and bugs (Longvah et al., 2011). With controlled temperatures (usually between 60-80°C), oven-drying is preferred as it is more standardized whereby moisture can be reduced to less than 10% as required in the later milling and storage of the material (Zhou & Han, 2006). The other refined technique is the freeze-drying (lyophilization) which does not degrade heat-sensitive bioactive compounds and instead retain the protein integrity, but it is energy-intensive and unsuitable when large volume processes are involved (Yi et al., 2010).

**Defatting Methods**

Defatting is an important step in pre-processing in order to increase the concentration of protein and the efficiency of extraction. The silkworm pupae have 20 to 30 percent of lipid content which is largely made up polyunsaturated fatty acids. Solvent extraction, especially the ones that entail food-grade hexane or petroleum ether, has been used extensively in laboratory-level explorations, where it is often desirable to strip off lipids as efficiently as possible (Rumpold & Schluter, 2013). Nevertheless, the toxicity of residual solvents has motivated the search of alternative approaches, including supercritical CO 2 extraction that allows a solvent-free defatting with negligible oxidation (Jayanegara et al., 2017). Another green method that can be selected (less effective than by using chemical solvents, but may work at industrial level) is mechanical pressing (cold pressing or hot pressing).

**Grinding and Sieving**

After defatting, the pupae are often ground in the form of fine powders by hammer mill, ball mill, or any other mechanical grinders. The use of the resulting powder in foods strongly depends on the particle size of the powder since it influences its solubility, dispersion, and functional properties (Kim et al., 2019). Sieving The size of the particles can then be made uniform, either after the protein has been solubilized or in the dry state, often using the process of sieving, when the protein is to be used in beverages, protein shakes or encapsulated nutraceuticals.

**Thermal and Microbial Safety Considerations**

Processing is also placed within the context of safety issues (microbial contamination, heavy metals and anti-nutritional compounds like tannin or phytic acid, microbial contamination, and heavy metals) that sometimes apply in the case of insect-based products. The microbial contaminants and allergenic proteins can be efficiently killed by heat treatment in the course of drying or post-processing procedures, such as roasting, but the thermolabile amino acids and peptides might be destroyed by excessive heat (Feng et al., 2018). In the case of food-grade usage, it should be done according to the HACCP procedures, and Good Manufacturing Practices (GMP). Moreover, hydrolysis of chitin or degradation of exoskeletal molecular components can be performed by enzymatic or (mild) chemical treatment, which enhances digestibility and palatability of the protein powder. Such steps as blanching or steaming may be employed beforehand in order to minimize the enzymatic browning and enhance the stability of the product.

**4. Protein Extraction Techniques**

Successful extraction of Bombyx mori pupae high quality protein is dependent on the choice of extraction strategies that will yield the largest possible volume of protein without altering the functional qualities. In classical aqueous extraction, defatted and fine flour pupal powder is dispersed in water (1:10 or 1:20 in weight by volume) under slight agitation at 40 to 50 C degrees.

Water only liberates water soluble peptides and small globular proteins but offers limited total yield (≤ 35 %) due to large quantity of the pupal protein trapped by the chitin protein matrix (Yi et al., 2013). In order to disassociate these bonds, researchers would use mildly alkaline extraction (pH 9-11 using 0.05-0.1M NaOH or KOH). Alkalinity denatures structural proteins and destroys the hydrogen networks and increases the extensiveness of solubilisation in 60-80 per cent without extreme racemization and lysin alanine formation when extraction temperatures and times are regulated (Zhou and Han,2006). The solubilised protein is recovered subsequently in an isoelectric precipitation at pH 4.3-4.8 as a cream-coloured curd of 80-90 % purity, giving the alkaline/Isoelectric route the current industrial reference point. Enzymatic hydrolysis is used by many groups in order to make nutritional quality even higher and to produce bioactive peptides. Endo proteases (alkalase, flavoenzyme, neutrase or the bromelain) are added (15 % E/S) on the alkaline extract or to minced pupae at 45-55 C. In addition to adding up to 25 % soluble nitrogen, controlled hydrolysis (degree of hydrolysis 10-20 %), expected to promote ACE inhibitory, antioxidant, and antimicrobial properties of peptides released (Feng et al., 2018; Kim et al., 2021). More importantly, addition of enzymes through enzymatic hydrolysis can partially deacetylate chitin to decrease insoluble fractions, and make them more digestible. Downstream processes That concentrate protein and fractionate peptides by molecular weight include membrane-based separations (ultrafiltration (UF) and diafiltration) which are being increasingly used. Protein concentration can be >85 % dry basis by use of 10-50 kDa cut off polysulfone or ceramic membranes to sequester residual salts and small molecule contaminants (Sun Waterhouse et., 2014). UF retentate when combined with low vacuum evaporation, the protein powder that comes out is easily spray dried which has high solubility and low microbial content. Green and intensified extraction technologies are also on the rise as determined by the imperatives of sustainability. The use of ultrasound assisted extraction (UAE) can speed up the transfer of mass due to acoustic cavitation; the extraction of protein with a 20 kHz probe at 400 W can increase its yield by 15-20 % compared to the traditional alkaline extraction with no significant loss of essential amino acids (Li et al., 2022). Solubilisation (detergent free) of hydrophobic pupal proteins with change of dielectric constant of water to that of an organic solvent by sub critical water extraction (150-180 C, 5-7 MPa) sterilises the slurry (Qin et al) expressions high-rock types hyper-pointers emissions.An effort has been made to replace the petroleum-derived solvents, with deep eutectic solvents (DES) made of both choline chloride and glycerol or lactic acid offering a tunable, biodegradable solvent capable of destabilizing chitin-protein interactions and selective recovery tests reporting recovery of >80 % free of significantly co-extracted pigments (Rahman et al., 2024). Also, newer high-pressure (400 MPa) or pulsed electric field (20 kV cm-1) pretreatments further destabilize cuticular components, halving the extraction duration and enhancing functional capabilities, including foaming and emulsification (Seo et al.,). Combined these developments create a trend toward cleaner more energy efficient bioeconomy processes that are in line with circular bioeconomy objectives.

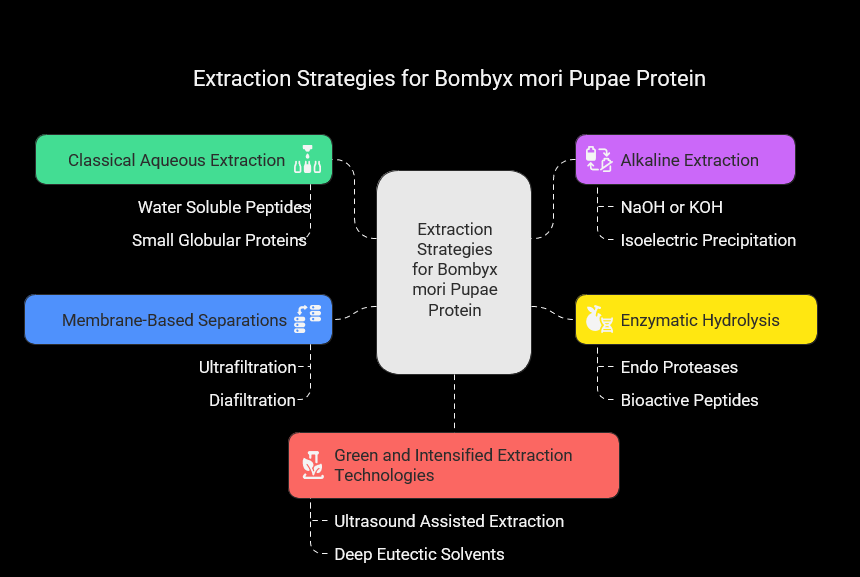
The combination of enzymatic hydrolysis with membrane fractionation or combination of ultrasound and DES provides synergistic yield, functionality, and environmental benefits opening the scaling of production of silkworm pupae protein ingredients to food, nutraceutical, and feed end users. The extraction strategies for Bombyx mori pupae protein is given as picture in Figure 1.

Figure 1: Protein Extraction Strategies

**5. Functional and Bioactive Properties of *Bombyx mori* Pupae Protein**

Bioactive and functional characteristics of proteins of Bombyx mori pupae are essential in their usefulness in food, nutraceutical as well as feed applications. Not only do these properties influence their techno-functional characteristics within different matrices but also Once in the GI they influence their physiological efficacy when present as consumed. Silkworm pupa protein powders or hydrolysates exhibit potential functions, such as, solubility, emulsifying power, foaming capacity, and gelation, all which are equal to or bigger than standard protein isolates like casein and soy (Yi et al., 2013).

**Functional Properties**

The main functional indicator, solubility, will greatly impact the protein use in beverages, high-moisture food systems. The silkworm pupae protein is dependent on the solubility with pH, ionic strength and extraction procedure. Enzymatic hydrolysis usually enhances solubility through exposing of a hydrophilic group and depolymerization of large-molecular-weight proteins to small peptides (Kim et al., 2021).

It has been demonstrated that the proteins isolated using the alkaline extraction procedure followed by membrane ultrafiltration process remain soluble in 85 % at the neutral pH (Sun-Waterhouse et al., 2014). Other important properties include emulsification and foaming capacity which enabled it to be added into mayonnaise like emulsions, desserts and protein bars. Amphiphilic characteristics of pupal proteins allow them to form oil-water interface and can form small emulsions with better creaming stability. Research studies have reported that proteins (hydrolyzed B. mori protein) could produce a stable foam with a maximum of 120% percent of foaming capacity (Zhang et al., 2020), making it viable in aerated foods. Textural and sensory meat analogue product and bakery item properties are vital to water and oil holding capacities. The oil-holding capacities of 2.1-2.5 g/g and water-holding capacities up to 4.2 g/g of protein isolates of B. mori fall within the optimum range of meat extenders and protein-fortified baked products (Feng et al., 2018).

**Bioactive Properties**

Besides their nutrition and functional properties, proteins of silkworm pupae have bioactive potentials that provide the reason to become useful in health benefiting foods. Protein in B. mori is broken down due to enzyme hydrolysis to result in peptides that have reported antioxidant, antihypertensive as well as antimicrobial properties. As an example, leucine, proline, and tyrosine-containing peptides have already been found to exhibit an equal or better radical-scavenging capacity than commercially available synthetic antioxidants such as BHT (Kim et al., 2021). Besides, ACE-inhibitory peptides isolated with pupae protein hydrolysates have antihypertensive properties, the mechanism of their action described by disturbing the mechanism of the renin-angiotensin system (Yi et al., 2013). The mechanism of action of such peptides is that they antagonize the angiotensin-converting enzyme that controls vasoconstriction hence promoting cardiovascular health. Immune-enhancing and cholesterol-lowering effects have also been shown in emerging evidence though the same must further be validated in in vivo and clinical studies. Immunomodulatory activity may also be the result of chitin and its derivatives that are found in small proportions (Park et al., 2014). All in all, these results allow developing B. mori-derived protein supplements as nutraceutical components to be not only sources of macronutrients but also functional and therapeutic additions. To sum up, the complex functional and bioactive characteristics of the protein of Bombyx mori pupae outline their enormous potential in the development of high-value food and health value-added products. How to optimally process the material to preserve or boost these properties is an important unexplored field of research and industrial scale-up.

**6. Applications of *Bombyx mori* Pupae Protein Powder**

Functional versatility and nutritional richness of the Bombyx mori pupae protein powder have already opened avenues in different sectors and especially in food, nutraceutical, feed, and biomedical sectors.

It has an auspicious amino acid composition, easy absorption, and bioactivity, which makes it an excellent component to develop products that would improve human and animal health and also encourage sustainable utilization of resources.

**6.1. Food Applications**

The food industry is becoming more familiar with usage of insect-based proteins as a healthy source of proteins in foods, especially protein fortified food like bars, drinks, soup, and snacks. As a source of protein, the protein powder associated with silkworm pupae can be inserted into formulations of high protein content aimed at athletes, the aging population, or people with protein-energy malnutrition (Rumpold & Schlüter, 2013). It is an emulsifier and foaming substance that enables it to be used in baked products, mayonnaise fillers, and whipped toppings (Kim et al., 2019). Moreover, its high solubility in enzyme-digested form qualifies it well when it comes to protein enriched drinks like functional smoothies or ready-to-sip meal replacers (Zhang et al., 2020). Due to the content of its bioactive peptides, the protein powder could be processed into nutraceutical preparations with blood pressure regulation, immune modulating, and antioxidant aids. Roast or steamed pupae are already traditional food in Asia. By switching to standardized protein powders, this can be readily accepted by the Western markets since this sensory element can be altered by the use of flavor masking and microencapsulation methods by altering appearance and aroma (Feng et al., 2018).

**6.2. Nutraceutical and Therapeutic Use**

B. mori protein hydrolysates used in bioactive peptides have great potential in physiological activity that includes angiotensin-converting enzyme (ACE) inhibition, antimicrobial and free radical scavenging. These properties allow them to be included in nutraceutical capsules, functional foods, and even products of medical nutrition therapy to manage cardiovascular disease or stress due to oxidation (Kim et al., 2021). Membrane ultrafiltration or chromatographic advanced extraction and fractionation processes are able to allow the isolation of particular peptides of characterized bioactivities so that they might be utilized in the production of specific health supplements. Besides, a low allergenic tendency and lack of frequent food allergens also imply that silkworm pupae protein can be used in hypoallergenic preparations after additional clinical confirmation.

**6.3. Animal Feed and Pet Food**

In addition to human food, the protein of happening of the Bombyx mori is used as an ideal high-protein feed in aquaculture, poultry, and pet food markets. The amino acids profile is similar to fish meal and so is its digestibility, suggesting that it can serve as an alternative of sustainable source of protein to carnivorous species of fish and shrimps (Longvah et al., 2011).

Feed trials have shown better growth rates, the ratio of feed intake as compared to the gain, and immune response in livestock and fish species against diets supplemented with pupa powder of silk worms (Zhou et al., 2016). Besides, the omega-3 and omega-6 fatty acids in lipid fraction of pupae help in the enrichment of animals products like eggs and meat.

**6.4. Cosmeceuticals and Biomedicine**

Recent studies indicate that the applications of hydrolysates of B. mori pupae protein in skin can be attributed to the antioxidant and the collagen boosting properties. The peptides can be added to anti-aging, moisturizing creams, or wound healing gels (Park et al., 2014). Moreover, the use of films and hydrogels formed using pupal protein isolates as biodegradable wound dressing is under consideration because of their bio compatibilities and their hydro retaining capabilities. To conclude, *Bombyx mori* pupae protein powder is a highly viable, sustainable and biologically active ingredient to various sectors. Its market potential can be realized through strategic integration with the state-of-the-art food processing and formulation technology; regulatory clearance; and consumer education. The application of pupae protein powder of *Bombyx mori* is simplified and given as image in Figure 2.

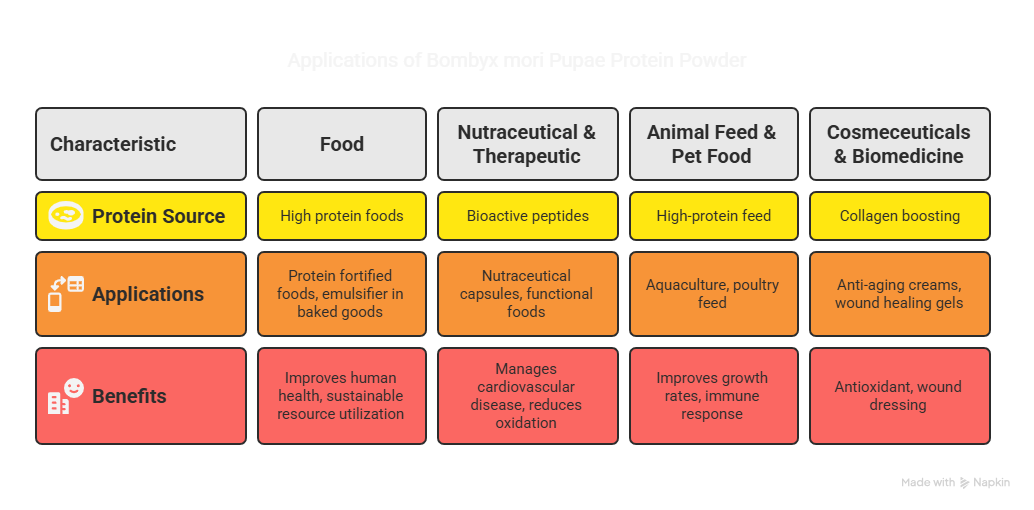


Figure 2: Application of *Bombyx mori* pupae protein powder

**7. Regulatory Framework, Safety Assessment, and Consumer Acceptance**

Commercialization of silkworm (Bombyx mori) pupae protein as food or nutraceutical ingredient will need to rely on its nutrition and functional properties as well as regulatory frameworks, sufficient safety evaluations and consumer acceptance. Although Asian culture has a history that long predates the practice of entomophagy (insect consumption) the recent development of insect-based food in the form of powdered proteins to serve new markets is subject to intense scrutiny as to its legal and consumer safety.

**7.1. Regulatory Status**

Regulation between insect-based products changes greatly, depending on the jurisdiction. Silkworm pupae are categorized as novel foods in the European Union so the pre-market safety approval must be granted under the EU Regulation 2015/2283. The European Food Safety Authority (EFSA) approved dried yellow mealworm (Tenebrio molitor) and dried yellow mealworm ground, in 2021, as the first permitted insect as food and several silkworm pupae-based applications are being evaluated (EFSA, 2021). Data on composition, processing, stability, allergenicity and toxicology have to be provided by the applicants. In India where sericulture is extensive, insect proteins are not yet officially approved as human food by the Food Safety and Standards Authority of India (FSSAI) but animal feed mixtures containing pupae are allowed. Silkworm pupae are already accepted in China, Thailand and Korea as traditional foods and have minimal regulatory hurdle to allow human consumption (Feng et al., 2018). According to the United States Food and Drug Administration (FDA), insects fall under the food ingredients as long as they are capable of meeting the established food safety. But organoleptic admissibility has not been generally recognized as safe (GRAS) regarding silkworm pupae protein isolates.

**7.2. Safety and Allergenicity**

The analysis of safety of pupal protein powders is critical through toxicological and microbiological analysis. Research results have continued to indicate that B. mori pupa is not bound by any serious toxin or harmful bacteria as long as it is properly processed (Zhou & Han, 2006). Thermal treatment like boiling, roasting, drying etc. can be very effective in destroying microbial contaminants whereas enzymatic hydrolysis can be used to reduce allergenicity. Nevertheless, cross-reactivity with shellfish allergens (e.g. tropomyosin and arginine kinase) was reported such that some consumers might have reactions to them (Jeong et al., 2016). The proteins are arthropod-conserved, and sensitized patients show allergic reactions. Therefore, before releasing to the markets, labeling, clinical testing and educating the population is imperative. In addition, heavy metals, pesticides and biogenic amines should be checked during safety evaluation, where pupae are not obtained using standardized sources. Under EU and FDA regulatory pathways, the analysis of comprehensive safety profiling, acute and chronic toxicity, genotoxicity, teratogenicity, and others are done.

**7.3. Consumer Acceptance**

Consumer acceptance of food products derived through insects rely on different factors, among these are culture, familiarity, perceived, and sensory experience. The silkworm pupae are very acceptable in countries such as Thailand, China and Korea. As a contrast, the Western consumers commonly feel disgusted or unfamiliar with insect consumption, with awareness of sustainability and health advantages leading to increased acceptance (Verbeke et al., 2015). Converting pupae to odor-neutral and taste-masked protein powders improves acceptability manyfold because the visual and sensory signals that relate to insect whole insects are absent.

The use of educational campaigns such as certification labels (e.g., high-protein sustainable source), culinary innovation (e.g., to be used in bakery or smoothie) can be used to make their use more common place. Recent research has demonstrated that as long as people present product marketing under the concept of clean protein or eco-friendly, consumer interest in insect-based products increases dramatically (Tan et al., 2016). In short, the potential scientific value of using silkworm pupae protein has been well established but the technology is not able to be universally adopted before being regulated with harmonization among different jurisdictions, subjected to strict safety validation, and extensive repositioning by initiating strategic communications to eliminate any psychological and market hurdle.

**8. Environmental and Economic Impacts of Silkworm Pupae Protein Production**

Valorization of Bombyx mori pupa pupae into protein powder will support the objectives of a circular bioeconomy, and sustainable food systems. Silk industry waste product, silkworm pupa are a nutrient rich and underused but low-cost source of biomass that can be processed into high-value protein at a low environmental cost. In this part, the benefits of using pupae protein with regard to the environment and its economic feasibility over the use of other conventional sources of protein are discussed.

**8.1. Environmental Benefits**

Silkworm pupae are waste of cocoon reeling process- in this case the adult larvae are killed and the cocoon is then detached. Raw cocoons are obtained in the range of 1kg and have a dry pupae weight of 250 300 g (Rumpold & Schluter, 2013). This by-product which is usually discarded or utilized as poor-quality feed is a source of valuable protein which does not necessitate any extra land, water, or feed to supplement the sericulture. Compared to animals that are used as a source of proteins like beef or poultry insect farming (including sericulture) has much lower greenhouse gas (GHG) emissions, water footprint, and land use. As an example, the production of beef protein releases up to 100 kg CO 2 -equivalent per kg protein whereas an estimated value is less than 10 kg CO 2 -eq/kg of pupae protein (van Huis et al., 2013). Further, silkworms are monogastric and ectothermic hence turn their feed to biomass more efficiently and without emission of methane. Carbon sequestration can also be done by silkworm rearing due to the mulberry plant. Mulberry plants that are deep rooted sequester atmosphere CO 2, as well as add to soil organic carbon and suppress erosion. The inclusion of pupae use in this scheme increases its overall carbon-neutral balance and helps sericulture waste-to-value transitions (Kumar et al., 2022).

**8.2. Waste Reduction and Circular Economy**

Aqueous extract of the reeling waste or de-gummed pupae or defective batches can be used in the production of protein thereby minimizing the amount of solid waste generated during the process of silk production. Developing closed-loop sericulture models, where both the primary product (silk) and the secondary product (pupae protein, lipids, chitin) are harvested, such models of zero-waste sericulture can be achieved.

After being extracted, defatted pupae cake might be converted into organic manure or biogas (Feng et al., 2018). The biodegradable films together with water purification and wound healing uses can also be applied to pupal cuticle chitin and chitosan, with all contributing to resource recovery. Therefore, there is an opportunity of integrated biorefinery based utilization of silkworm pupae valorization.

**8.3. Economic Viability and Market Potential**

Economically, this will offer an alternative in the form of a second source of income to sericulture farmers and processors on the one hand due to use of silkworm pupae as a source of protein. As the prices of traditional animal proteins increase, insect proteins present a low cost alternative. It was estimated that the production of the pupae protein powder would be cheaper than the production of whey or soy protein isolate, especially in silk-producing areas where the raw input is abundant (Kim et al., 2019). Besides, the world market of sustainable and functional proteins will exceed USD 70 billion by 2030, and insect protein will continue to take a larger portion of the overall market. One of these markets is nutraceutical, aquafeed, pet food, and sports nutrition. With the right processing and marketing as a brand, we may exploit premium health-conscious consumers both in the Asian market and the Western market with silkworm pupae protein (Rumpold & Schl goes cluescenter contributed to the Western World, 2013). The state programs promoting rural entrepreneurship, innovations in bioeconomy (the example of the DBT-BIRAC and NECTAR programs in India) and EU Horizon projects on novel food also develop an attractive environment to scale silkworm-based protein businesses. To sum up, environmental and economic benefits of silkworm pupae protein allow regarding it as a sustainable, low-emission, and scalable source of proteins to solve global protein shortages, and improve the resource efficiency of the sericulture industry.

**9. Future Prospects and Research Gaps**

Although the processing and use of Bombyx mori pupae protein is rather advanced, there exists some essential research gaps and future research opportunities that would have to be filled in order to realize its full potential in food, nutraceutical, and bio-based sectors. Interdisciplinary research, policy reform, and technological innovation is required to take silkworm pupae protein out of the niche product, and into the mainstream, sustainable protein alternative.

**9.1. Optimization of Extraction and Purification Technologies**

The most frequently used processes are alkaline extraction and enzymatic hydrolysis but they are also scarcely standardized to be applied on industrial scale. Enzyme specificity, reaction time, pH, and temperature are other variables that have to be optimized taking into consideration both the maximum yield and attainment of favorable functional and sensorial properties. The eco-friendlier technologies such as deep eutectic solvents (DES) and subcritical water extraction have more in-depth scalability study, techno-economic analysis, and assuring food safety prior to implementation in the commercial market (Rahman et al., 2024).

**9.2. Molecular Characterization and Functional Peptide Profiling**

The protein that silkworm pupae comes with offers bioactive potential that is promising but under-exploited. Development of sophisticated proteomics and peptide sequencing technologies are necessary to isolate, characterize and synthesize bioactive peptides of given health value (e.g. ACE inhibition, anti-inflammatory, antimicrobial). In future work, high-throughput analysis, e.g., using LC-MS/MS and MALDI-TOF, together with bioinformatics modeling to model the functional behavior of these peptides, in vivo and in vitro, should be carried out predictively and confirmatorily. Furthermore, structure-function associations amid amino acid numbering and biological action need to be expressed concerning health designations and feasible legislative endorsement in the nutraceutical sector.

**9.3. Toxicological and Allergenicity Studies**

Silkworm pupae is sold and consumed widely in Asia but little extensive clinical testing has been done, particularly on Western populations. Available safety evidence is founded on animal and small trials evidence. There is a need in the future to achieve double-blind, placebo-controlled human studies to examine digestibility, the potential to cause allergic reactions, and long-term safety. A major obstacle to acceptance in another country would be cross-reactivity with crustacean allergens, and specific immunological studies and allergen-exclusion measures like protein chemistry manipulation or exclusive hydrolysis should be conducted on this point (Jeong et al., 2016).

**9.4. Regulatory Harmonization and Consumer Education**

The international trade and investment are limited by the existing differences in the laws regarding insect protein in different countries. Hence, alignment in the standards of food safety, especially among the EU, the US, India and ASEAN countries should be established. Formation of the codified standards at Codex Alimentarius, Insect-consideration in GRAS list development, and integration into national food safety regulatory framework will help instill the consumer confidence and economic expansion of the industry. Similar activity must be undertaken in the area of consumer behavior, packaging design and marketing. It is important that research should work on positioning insect protein as clean-label, functional and eco-friendly to counter psychological aversion to insect protein, especially in the West (Tan et al., 2016).

**9.5. Integration with Biorefineries and Sustainable Development Goals (SDGs)**

The value of silkworm pupae within a broader context should be discussed in terms of circular bioeconomy and SDGs. Multistreaming, such as protein as food, chitin as a biomaterial, and lipids as biodiesel, fits the integrated biorefineries schemes, increasing profitability and lowering wastes. A combination of sericulture, entomology, biochemistry, and engineering research is important to devise such a model at the village, district, or industrial level (Kumar et al., 2022).

Lastly, the life cycle analysis (LCA) and carbon foot print models in the context of protein production with sericulture should be established to authenticate the claims of environmental sustainability, and be able to get green funding and carbon credits.

**10. Conclusion**

The conversion of the highly plentiful, protein rich by-product of sericulture, Bombyx mori pupae into valuable protein powder, is a notable approach as a solution to the rising demand of sustainable, functional, and alternative sources of protein. Silkworm pupae protein powder as a source of essential amino acids and digestible peptides and bioactive compounds exhibit excellent functional characteristics of solubility, emulsification, and antioxidant properties, which makes it useful to be used in food, nutraceutical, animal feed, and biomedical markets. Newer technology in protein extraction and purification, such as using enzyme based hydrolysis and membrane-based separation, or using green methods, such as deep eutectic solvents are allowing cleaner and more efficient isolation of high quality protein isolates. Moreover, functional peptides, and health consequences and consumers preferences research are growing at a dizzying rate which is broadening the scientific foundation of product development. On environmental level, valorization of silkworm pupae is an approach to the circular economy of bioeconomy construct because it utilizes agricultural biomass, minimizes waste, and decreases greenhouse gas emissions in comparison to traditional livestock proteins. Economically, it has twin advantages of both raising returns of silk rearing and acquiring high-growth markets like sports nutrition, functional foods and aquafeeds. Nevertheless, the end of the day success will demand the solution of such key barriers like the standardization of extraction procedures, clarification of the allergenicity issue, uniformity in global food legislation, and the development of awareness among people. Additional interaction between disciplines and stakeholders will be needed to continue growing this bioresource beyond local innovation to global nutrition solution. To conclude, silkworm pupae protein powder has a transformative potential to shape the sustainable development and food security, as well as the healthy nation through its contribution as a potential source of a superior protein of the future, not only as an alternative, but as a better protein source.

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