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Beyond Silk: Exploring the Value of Silkworm Pupae

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A B S T R A C T

For centuries, sericulture has revolved around silk production, with the mulberry silkworm as its core. However, silkworm pupae long considered a by-product are now gaining recognition for their remarkable value beyond silk. Rich in high-quality proteins, essential fatty acids, and bioactive compounds, silkworm pupae offer significant potential as a sustainable alternative protein source for human consumption and animal feed, particularly in aquaculture. Also, silkworm pupae offer promising benefits for human, acting as natural α- glucosidase inhibitor, helping to lower post-meal blood glucose levels. Their oil, characterized by a favorable unsaturated fatty acid profile and antioxidant properties, is gaining traction in the food, cosmetic, and pharmaceutical industries. Recent research has also uncovered bioactive peptides in pupae with promising health benefits, opening new doors in the nutraceutical and biomedical sectors.

Introduction

Among the vast diversity of insects in the order lepidoptera, the silkworm stands out as a species that has profoundly shaped human civilization, especially in the context of textile production. There are five different types of silkworms *viz*; mulberry, muga, eri, tropical tasar, oak tasar, and India is the only country that rears all the five silkworms in different parts of India. being holometabolous insect undergoes complete metamorphosis consisting of four distinct developmental stage such as; egg, larva, pupa and adult moth. Among these, the pupal stage is the third and the transitional phase where the silkworm undergoes major physiological transformation inside a protective silk cocoon spun during the final larval instar. The silkworm pupa accounts for 60% of the dried cocoon weight (Rao, 1994). In the context of sericulture, the focus primarily lies in harvesting the silk filament that makes up the cocoon is typically subjected to heat (a process known as stifling) which kills the pupa inside and prevents it from emerging as a moth and breaking the silk thread. As a result, silkworm pupae are produced in large quantities as a by-product of the silk-reeling industry.

While the pupal stage itself plays no direct role in silk production, its importance in the sericulture industry is multifaceted and growing steadily, once considered waste, silkworm pupae are now organized as a valuable resource due to their rich nutritional, biochemical and industrial properties. Silkworm pupae, once viewed as a byproduct of silk production, are emerging as a valuable resource with multiple applications that reach far beyond sericulture (Wu *et al*., 2021; Hăbeanu *et al*., 2023). These pupae, which were formerly abandoned after reeling, are now recognized for their tremendous nutritional, industrial, and environmental potential. Silkworm pupae, which are rich in high-quality proteins (up to 76%), important fatty acids including linoleic and alpha-linolenic acids, and a variety of vitamins and minerals, provide a sustainable alternative to traditional protein sources for both human and animal consumption. They are already used as a delicacy in places like Northeast India, and their uses worldwide range from the extraction of chrysalis oil for cosmetics and medications to the use of them as feed for poultry and aquaculture (Mishra *et al.,* 2003; Zhou *et al.,* 2022; Sadat *et al.,* 2022). Furthermore, its bioactive compounds—including antioxidants like as lutein and hexoxanthin have showed promise in benefiting human health and increasing cattle productivity (Sheikh *et al.,* 2018; Altomare *et al.,* 2020)). The environmental benefits are equally compelling: utilizing silkworm pupae reduces organic waste from the silk industry and contributes to circular bioeconomy models. As the global demand for sustainable protein sources intensifies, silkworm pupae stand out as a "goldmine waste" a once-overlooked by-product now poised to revolutionize food systems, biomedicine, and green technologies (Bharat *et al.,* 2024).

Components of Silkworm Pupae

Silkworm pupae are used as food insects, especially in China, where they have been eaten for over 2000 years (Feng *e al.,* 2018). Although there are many different kinds of silkworm pupae, *Bombyx mori* , *Antheraea pernyi, Antheraea yamamai, Samia ricini, Antheraea mylitta, Antheraea roylei*, and other species are currently the most commonly utilized commercial silkworm pupae for study (Sheik, 2018; Shukurova, 2021 and Abdoli *et al.,* 2022).

Proteins

The pupae of silkworms are nutrient-rich. Together with minerals, vitamins, polyphenolic compounds, and numerous other nutrients, the most prevalent substances are protein, fat, and sugar (Rao, 1994). *Bombyx mori* is the most prevalent dry matter in silkworm pupae and has a high protein content of 55.6% dry weight. Multiple to several dozen amino acids are found in biologically active peptides, which have a range of physiological roles. The pharmacological actions of silkworm pupae can be carried out by a range of physiologically active peptides that are produced when these pupae proteins are hydrolyzed. With the exception of Eri silkworm pupae, all species of silkworm pupae have proteins with a nearly identical amino acid content, with 18 amino acids making up each species. Out of these, eight essential amino acids satisfy WHO, FAO, and UNU guidelines. Ten amino acids that are not necessary for human health are also present. Phe and Pro levels in pupae are greater than in hen eggs (Zhou and Han, 2006). As such, silkworm pupae are regarded as an essential nutrient and a high-quality source of protein (Altomare *et al.,* 2020). The amino acid makeup of the proteins found in the various types of silkworm pupae is summarized in Table 1.

Minerals

Minerals have a crucial part in living beings. They are available in many different forms in silkworm pupae. Silkworm pupae contain up to 25 distinct kinds of minerals, some of which may carry out specific physiological tasks for the body (Longvah *et al.,* 2012 and Shukurova, 2021). The kind and concentration of minerals in pupae can differ based on the kind of pupa and the conditions in which it was raised (Zhou and Han, 2006). Notably, silkworm pupae have a very low sodium-to-potassium (Na:K) ratio. The prediction of non-communicable diseases by Na:K implies that eating silkworm pupae may lower the risk of developing non-communicable diseases (Ying *et al.,* 2022 and Lanca *et al.,* 2018). Cardiovascular disease, hypertension, stroke, and other conditions are examples of non-communicable diseases (Lim *et al.,* 2012 and Rodrigues *et al.,* 2014).

Additionally, some pupae have high levels of selenium, which can be found in the protein of the pupae. The prevention of cancer and protection against oxidative stress are two significant functions of selenium-rich pupae (Hu *et al.,* 2005 and Liu *et al.,* 2010). The concentration of eight minerals in the three types of pupae is listed in Table 2, and it is evident that the pupae have larger levels of phosphorus, calcium, and magnesium.

Silkworm pupae oil

Pupae oil is the second most abundant substance in silkworm pupae, behind protein. The Eri silkworm pupae, which have the highest oil content of the four species, contain 26.2% of the oil. In addition to being high in oils, silkworm pupae also have high concentrations of unsaturated fatty acids, particularly polyunsaturated fatty acids, which are highly nutritious as a source of edible oil. The fatty acid composition of the various types of silkworm pupae oil is summarized in Table 3.A nutrient-rich by-product of the sericulture industry, silkworm pupa oil is mostly made from the pupae of mulberries (*Bombyx mori*) and non-mulberry species like *Philosamia ricini* (eri). It is becoming more and more important in the food, pharmaceutical, and cosmetic industries. The oil stands out for having a high concentration of polyunsaturated fatty acids (PUFAs), particularly alpha-linolenic acid (ω-3), which can make up as much as 44% of the total fatty acid profile. Oleic acid (ω-9) and linoleic acid (ω-6) are also present. (Arasakumar *et al.,* 2021). Due to its high omega-3 content, the oil is being investigated as a functional food ingredient and is even used in snacks that are higher in protein. It has potential for use in antibacterial, anti-diabetic, and anti-obesity pharmacological formulations. Because of its emollient and antioxidant qualities, it is very useful in cosmetics, especially in anti-aging and moisturizing products. As a result of its high saponification value and oxidative stability, silkworm pupa oil is also being researched for application in bio-based polymers, biolubricants, and soap manufacture (Bharath *et al.,* 2024; Mahesh *et al.,* 2015).

Additional Components of Silkworm Pupae

In addition to the substances listed above, silkworm pupae are abundant in vitamins. VA, for instance, can reach 5 mg/g. The vitamins VA, VB1, VB2, VB3, VB5, VB7, VB9, VB12, VC, and VE are the primary vitamins found in silkworm pupae (Paul and Dey, 2014; and Sadat *et al.,* 2022). Moreover, silkworm pupae contain five tocopherols and phospholipids.... Tocopherols come in five different forms: α-tocopherol, β-tocopherol, γ-tocopherol, γ-tocotrienol, and σ-tocopherol. Pupae of silkworms also contain flavonoids and polyphenols. The pupae of the silkworm *Antheraea assamensis* contained 10 mg/g of polyphenols and 20 mg/g of flavonoids, respectively (Deori *et al.,* 2014). The two primary categories of sugars found in silkworm pupae are chitosan and chitin, along with separated and purified polysaccharides, all of which have biological activity (Ali *et al.,* 2018, Battampara *et al.,* 2020 and Ali *et al.,* 2022). Silkworm pupae contain chitosan and chitin, particularly carboxymethyl chitosan, which have considerable physiological action but are not cytotoxic (Zhu *et al.,* 2018 and Zhu *et al.,* 2020).

Nutritional and food application

The need for sustainable food sources is growing. Edible insects have therefore long been a mainstay of the diets of people throughout Asia, Africa, and Latin America (de Castro *et al.,* 2018), and they have recently been a significant topic of scientific research. The silkworm is a farmed insect raised for its silk on a vast scale. According to Ratcliffe *et al.,* (2011) and Yang *et al.,* (2009), silkworm and its metabolites are highly valuable in terms of nutrition, medicine, and economics. Asian countries such as Japan, Korea, India, and particularly China regularly use silkworm pupae as food (Zhou and Han, 2006).

Numerous studies demonstrate that silkworm pupae have a nutritional profile that is well-balanced, making them fit for human consumption. In general, silkworm pupae are a healthy source of fat, protein, vitamins, and minerals. They include more of the three calorigenic nutrients *viz*., protein, fat, and carbohydrates than traditional foods, with up to 230 kcal per 100 g. Table. 4 shows the values of important nutrients in silkworm pupae in comparison to other typical diets.

Pupae of silkworms have a protein level of roughly 21.5%, which is higher than that of most common animal products. It has been observed that silkworm pupae have a protein content of 49% to 54% on a dry-weight basis (Longvah *et al.,* 2011; Nowak *et al.,* 2016). Due to their high concentration of necessary amino acids, silkworm pupae proteins are regarded as complete proteins. Silkworm actually has all the amino acids the human body needs, in the right amounts, according to the FAO/World Health Organization (WHO) guidelines (Köhler *et al.,* 2019; Ni *et al.,* 2003; Wang *et al.,* 2009; Yang *et al.,* 2009; Zhou and Han, 2006). When silkworm pupae eat mulberry leaves, they also absorb some antinutrients. Tannic acid, alkaloids, flavonoids, saponin, oxalate, phytate (72.89–110.16 mg/g), and phytin phosphorus (20.54–31.03 mg/g) are examples of these antinutrients. These antinutrients do, however, exist in trace amounts and are within the range of human tolerance. Accordingly, silkworm pupae are safe for ingestion by humans (Omotoso, 2015). All things considered, silkworm pupae are a rich source of vitamins, minerals, lipids, and protein—all of which are essential parts of the human diet.

As Human Food

The growth of the insect industry has accelerated since the significance of insects as the last unexplored biological resource was re-examined in recent years. Because of its high nutritional content, silkworm pupae can improve health by adding beneficial bioactive substances to the diets of both humans and animals. Even though dry matter contains a larger percentage of the protein, the oil structure also suggests using this by-product in the culinary and medical sectors. A number of metabolic illnesses have been linked to elevated blood pressure, blood glucose, and cellular oxidation. Intracellular reactive oxygen species trigger an immune response by controlling many signaling pathways that combat inflammation. Unbalanced reactive oxygen species can harm cells, which can result in heart disease. Levels of poly unsaturated fatty acids are favorably connected with reactive oxygen species generation (Petermann *et al.,* 2022). Humans can eat silkworm pupae in three different forms: whole, pupal oil, and pupal powder. Because silkworm pupae have a high concentration of necessary amino acids, their proteins are regarded as complete proteins (Sharma *et al.,* 2022).

A normal biscuit mix was combined with three (3) different pupae concentrations (15, 20%, and 25%) to create three distinct formulations. Pupae incorporation's impact on the biscuit's technological, nutritional, and sensory qualities was assessed. The sensory evaluation's findings showed that the biscuit formulations including pupae had favorable acceptance scores in the majority of the assessed qualities (color, texture, sweetness, scent, appearance, residual taste, and global acceptance), with scores higher than 6. The standard biscuit scored the highest in the sensory assessment. When 15% silkworm pupae were added to the recipe, the protein content (17.65%) produced a snack that was categorized as having a high protein content, indicating the biscuit's potential for nutritional benefits (Torres *et al.,* 2022). At the 36th scientific meeting of the committee on space research, Masamichi Yamashita, a researcher with the Japan Aerospace Exploration Agency, published a recipe for pupa biscuits. Three to six grams of silkworm pupal powder, 200 grams of rice powder, and three hundred cubic centimeters of soymilk, soy sauce, and salt make up this dish. These materials can be mixed with water by astronauts and then broken up into little pieces, which is not very appetizing but seems to be healthful (Priyadharshini and Swathiga, 2021). Because of its taste, flavor, and cultural significance, the indigenous peoples of northeastern India eat 200 kinds of edible insects, of which silkworm pupae comprise one of the main categories. Silkworms are raised in Northeastern India for both trade and human consumption.

The oil from Eri silkworm pupae is especially high in α-linolenic acid. According to nutritional and toxicological research, eri silkworm oil is safe and nutritionally equivalent to popular vegetable oils like sunflower oil, but because of its high α-linolenic acid content, it also has additional health benefits. In order to address India's acute edible oil scarcity and give marginal farmers who raise silkworms' extra revenue, eri pupae represent a possible supply of oil that may be extracted for use in the food and feed industry. It is clear that there is a great deal of potential for collecting various discarded silkworms for use in food, medicine, and business, in addition to eri silkworm pupae oil (Longvah *et al.,* 2012). Combining silkworm pupa powder intake with swimming exercise can minimize fat pads. Reduced fat pads were thought to help prevent weight gain (Ryu, 2014).

As Animal Feed

The silkworm pupae meal doesn't seem to contain any harmful substances. Birds fed diets containing silkworm meal had mortality rates in poultry that are comparable to those of animals fed more traditional protein sources (Dutta *et al.,* 2012).

Ruminants

Silkworm pupae meal is a very high-protein food that can be added to ruminant diets because of its favourable amino acid profile and extremely undegradable protein content. It is offered to the animal in the form of meal, and not as whole pupae. Its high oil content limits its suitability as ruminant feed. As a result, when silkworm pupae are fed in large quantities, fat extraction from their food is a crucial requirement. Silkworm meal can safely replace 33% of groundnut cake (GNC) in fattening diets for Jersey calves without compromising performance, making the diet less expensive. Additionally, they discovered that the groundnut cake diet had a lower protein digestibility than the silkworm meal-based diet (Ullah *et al.,* 2017).

To assess the impact of varying inclusion levels of defatted silkworm pupae meal (DSWP) on rumen fermentation, microbial protein synthesis, and nutrient utilization in cattle fed a diet based on finger millet straw (FMS), a two-phase study was carried out. At 0 (T0), 10 (T1), 20 (T2), and 30% (T3), four isonitrogenous concentrate mixtures (CM) were made using DSWP in place of soybean meal (SBM) protein. In phase I, four crossbred steers were used in a rumen fermentation experiment in a 4 × 4 Latin square design to examine the impact of varying DSWP levels on rumen fermentation. Rumen fermentation parameters as pH, ammonia nitrogen (NH_3-N), and total volatile fatty acids (VFA) did not differ significantly ($P > 0.05$) across the experimental groups. 20 crossbred cattle (311.2 ± 4.81 kg) participated in the digestibility trial in phase II. They were split into four experimental groups of five animals each using a completely randomized design to examine the impact of various rations (T0, T1, T2, and T3) on microbial protein synthesis and nutrient utilization. The experimental groups did not differ significantly in terms of microbial protein synthesis, excretion of urine purine derivatives, or nutrient intake and digestibility. Furthermore, there was no discernible ($P > 0.05$) alteration in the animals' blood biochemical markers after feeding DSWP. Thus, DSWP can replace SBM in cow rations up to 30% without influencing rumen fermentation pattern or nutrient utilization (Rashmi *et al.,* 2022). When defatted Tasar silkworm pupae are given to sheep with wheat straw and molasses, the crude protein digestibility has been determined to be approximately 70%. According to a study done on crossbred calves in the pre ruminant stage, silkworm pupae meal can effectively replace fish meal up to 50% of the time without negatively affecting the crossbred calves' feed intake, nutritional utilization, growth, or health (Sahib *et al.,* 2023).

Poultry

One emerging way to improve the sustainability of poultry feeds is to incorporate insect meal into their meals (Elahi *et al.,* 2022). No potentially dangerous ingredients seem to be present in silk worm poultry meal. Compared to chickens fed other traditional protein sources, those fed diets containing SWPM show similar mortality rates (Dutta, 2007). According to a different study, SWPM inclusion percentages in chicken feed normally fall between 5 -10% (Valarie *et al.,* 2015). Ecdysteroid, a hormone involved in the pupae's transformation, is responsible for the growth-promoting impact observed in developing chicks (Fagoonee, 1983). Similarly, the amino acid present in silkworm pupae may be the cause of enhanced development performance in birds fed Silkworm pupa meal (Rahman *et al.,* 1996; Khatun *et al.,* 2003). Silkworm pupa meal can partially replace traditional protein sources (fishmeal or soybean meal) in poultry diets due to its high nutritional value, high-quality natural protein levels, and improved digestibility. Furthermore, effective utilization of silkworm pupa meal following appropriate processing and appropriate use of this valuable resource could significantly reduce feed prices with no impact on chicken growth performance.

The tests conducted by a number of researchers to substitute silkworm meal for traditional protein sources in chicken diets are compiled in table 5 as follows.

Swine

There isn't much information available about incorporating silkworm pupae meal into pig diets. Given the high protein needs of pigs, most pig farmers supplement soybean meal in growing phases 1, 2, and 3 with fish meal. When it becomes necessary to substitute more sustainable sources of protein and oil for traditionally fed nutrients, problems occur.

Interestingly, the protein in pupae is better than that in fish and soy. Although the higher oil content may cause a negative reaction, the high protein content of the pupal powder makes it a suggested meal for pigs (Trivedy *et al.,* 2008).

A study by Choudhury *et al.,* (2021) found that feeding 2% and 4% of Muga silkworm pupa meal to Large White Yorkshire grower pigs improved overall production performance while reducing production expenditures. According to Coll *et al.,* (1992), referenced by Sheikh *et al.,* (2018), non-defatted silkworm pupae meal could be used in place of some soy oil meal in the diets of growing and finishing pigs without having a major impact on carcass characteristics or growth performance.

However, when the substitution rate surpassed 50%, a detrimental impact on consumption was observed. However, a better feed conversion rate made up for the lower intake. To evaluate the change of specific blood parameters as reference markers for health status, Medhi (2011) conducted research on finishing cross-breed pigs. There were no discernible differences. Further research can be done on the swine and pupa meal.

Aquaculture

The global aquaculture industry is facing increasing pressure to identify sustainable and cost-effective alternatives to traditional fish meal, which is becoming scarce and expensive due to overfishing and environmental constraints.

In this context, silkworm pupae a by-product of the silk reeling industry have emerged as a promising protein-rich feed ingredient for aquaculture. Fishmeal and dried silkworm pupae have similar nutritional values, but dried silkworm pupae are far less expensive.

It has a crude protein content of 52–72 percent, but the deoiled meal has a protein content of 65–80%. The use of SWP as a dietary protein source for Catla (Catla catla) fingerlings revealed that a diet made entirely of SWP improved growth performance and could fully substitute fish meal as a source of protein for Catla (Catla catla) fingerlings.

According to a study conducted to determine how diets including various protein sources affected the growth and metabolism of tropical catfish (*Clarias batrachus*), dried SWP are a superior source of protein that contributes to adequate growth. The use of silkworm pupae meal in feed for ornamental fish is also feasible (Swamy and Devaraj, 1994).

When compared to the traditional feed mixture of rice bran and mustard oil cake (1:3), the addition of de-oiled SWP to fish feed demonstrated superior growth and conversion (Nandeesha *et al.,* 1990). A comparable study found that giving *Cyprinus carpio* a diet consisting of 10% pupae and 15% fishmeal produced comparatively superior growth when fishmeal was partially or completely replaced by 10, 15, 20, and 25% de-oiled pupae in a feeding trial (Nandeesha *et al.,* 1990).

Shrimp (*Metapenaeus monoceros*) feeding tests showed that substituting silkworm pupae meal for fish meal decreased digestive efficiency (Venkatesh *et al.,* 1986; Karthick *et al.,* 2019). However, substituting SWP for fishmeal in giant freshwater prawns (*Macrobrachium rosenbergii*) had no negative effects on their ability to produce, and 8.6% by weight was the appropriate level of silkworm pupa meal (Joshi *et al.,* 1980, Karthick *et al.,* 2019, and Jeyaprakashsabari and Anand, 2021). A combination of soymeal (29%, DM basis) and SWP meal (16.9%, DM basis) could completely replace fishmeal and also improve survival and growth performance in juvenile abalone (*Haliotis discus*), according to Cho's study on the dietary effects of substituting animal and/or plant protein sources for fishmeal on the growth and body composition of juvenile abalone. Therefore, it can be said that shellfish species can be successfully raised with SWP, which can replace up to 30–40% of fish meal without compromising growth performance.

Biomedical and Pharmaceutical Potential

The active components found in silkworm pupae serve a number of pharmacological purposes and are highly effective in treating a wide range of illnesses. Experiments conducted in vitro and in vivo have demonstrated the potent pharmacological effects of silkworm pupae.

Antitumor, antioxidant, antibacterial, antiapoptotic, hypotensive, lipid- and blood-sugar-regulating, immunomodulatory, and hepatoprotective properties are a few examples of these actions.

As Anticancer

Finding natural antitumor medications would be a preferable choice because the current treatments for cancer primarily involve radiation and chemotherapy, both of which have negative effects on the body. Silkworm pupae's protein hydrolysates and amino acids were revealed to have anticancer properties and to be cytotoxic to human stomach, breast, and liver cancer cells in in vitro experiments (Hu *et al.,* 2005, Chukiatsiri *et al.,* 2020). Anticancer properties were discovered in both silkworm pupa oil and protein. Because silkworm pupae proteins alter the cancer cells' cycle of division and trigger the synthesis of apoptotic factors to encourage apoptosis, they function as anticancer drugs. The silkworm pupae as anticancer activity is done through inducing apoptosis in the cancerous cell. The mechanism by which silkworm pupae in cancer cells cause apoptosis (Fig3.):

a) Mitochondrial Pathway (Intrinsic Pathway)
b) Death receptor pathway (Extrinsic Pathway)
c) Inhibition of anti-apoptotic proteins

As Anti-bacterial

Silkworm pupae have been used in medicine for a long time, but their antibacterial capabilities have only recently been recognized. First, it was discovered that silkworm pupa oil possesses antimicrobial properties. Silkworm pupa oil's antibacterial activity was assessed using the minimum inhibitory concentration (MIC) method. It was found to significantly inhibit the development of a Staphylococcus sciuri strain CD97, with the best effect at 110 µL/mL (Dev *et al.,* 2017).

In addition, the antibacterial action of hot-pressed extracted silkworm pupa oil was discovered to be more evident in Gram-positive bacteria (Lee *et al.,* 2021). Silkworm pupae-derived chitosan exhibited higher antibacterial and antifungal activity compared to commercially available chitosan, with the fastest bacterial suppression taking place at 1–2 hours. An antibacterial compound found in silkworm pupae may be used to treat diseases and reduce the overuse of antibiotics (Battampara *et al.,* 2020). The mechanism of chitosan being explained through the figure 2, chitosan being cationic (positively charged), binds with the negatively charged bacteria and damages cell, RNA or DNA of the bacteria.

As Anti-oxidant

These days, several techniques are used to separate and extract a range of peptides and polyphenols with antioxidant properties from silkworm pupae. In vitro tests have demonstrated that these compounds are effective at scavenging intracellularly generated ROS (reactive oxygen species) as well as DPPH (1,1-Diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfoic acid)) free radicals (Zhang *et al.,* 2021; Cermeño *et al.,* 2022 and Lee *et al.,* 2021).

Depending on the extraction technique, antioxidant activity can change. A study on the use of microwave-assisted extraction to extract silkworm pupa oil discovered that, in addition to increasing the oil's yield, this method also raised the oil's total phenolic content, which in turn produced stronger antioxidant activity than that of the conventional extraction method (Hu *et al.,* 2017).

Silkworm pupae include phenolic compounds, peptides, and unsaturated fatty acids that have antioxidant activity. By employing silkworm pupae, it may be feasible to create foods or medications with antioxidant qualities. Natural antioxidants have a significant role in disease prevention due to their permeability, hydrophilicity, and multifactorial interaction with the biological environment. Synthetic antioxidants, however, might disrupt the biological processes of other nutrients in the body (Hrebień, 2021, and Liu, 2022).

Because of this, people usually believe that natural antioxidants are safer than synthetic ones. These compounds may have a safer and greater effect because they are natural antioxidants. The mechanism of silkworm pupae like any other anti-oxidant it neutralizes the oxidative stress by:

Free Radical Scavenging

Antioxidants neutralize free radicals by donating an electron or hydrogen atom.

Hydrogen Atom Transfer (HAT)

Antioxidants donate a hydrogen atom to stabilize free radicals.

Metal Chelation

Some antioxidants bind transition metals (e.g., Fe^{2+}, Cu^{2+}), preventing them from catalysing free radical formation via Fenton reactions

As Hepatoprotective

Silkworm pupae oil has been shown in animal experiments to protect against stomach ulcers caused by ethanol and hydrochloric acid. The oil from silkworm pupae raised the pH in the mice's stomachs and decreased the area of gastric secretions and ulcers. Silkworm pupae oil reduced blood levels of IL-6, IL-12, TNF-α, IFN-γ, MTL, and GT while raising serum levels of SOD, CAT, GSH-Px, SST, and VIP in mice with stomach ulcers. EGF, EGFR, VEGF, and eNOS expression was upregulated, while NF-κB, Bcl-2, COX-2, and iNOS expression was downregulated (Long *et al.,* 2019).

According to this data, using silkworm pupae oil helps mice's inflammatory reactions and oxidative damage. By blocking the oxidative stress-mediated NF-κB signaling pathway, silkworm pupae oil also decreased acetaminophen-induced acute liver injury, alcohol-induced hepatotoxicity, and oxidative stress in mice (Long *et al.,* 202 and Cha *et al.,* 2012). Pupae of silkworms may be used as a possible source of medication to treat stomach ulcers and avoid rapid liver damage.

Table.1 Amino acid content in different silkworm varieties (Zhou et al., 2022; Tomotake et al., 2010; and Longvah et al., 2011)

Amino Acid (g/100 g of Protein)	Antheraea assamensis	*Eri* Silkworm Pupae	Bombyx mori	*Antheraea pernyi*	Hen Egg
Asp	9.1	9.89	10.9	6.41	8.92
Thr	3.9	4.75	5.4	4.64	4.47
Ser	3.7	5.25	4.7	4.64	6.72
Glu	9.5	12.9	14.9	12.74	12.13
Gly	3.6	4.94	4.6	4.42	3.02
Ala	3.9	6.05	5.5	6.26	5.03
Cys	1.4	0.53	1.4	1.5	1.90
Val	4.7	5.36	5.6	6.63	5.42
Met	3.4	2.31	4.6	1.47	2.81
Ile	3.4	4.42	5.7	7.95	4.88
Leu	6.2	6.63	8.3	3.24	8.11
Tyr	5.6	6.4	5.4	2.06	3.81
Phe	4.6	5.24	5.1	8.10	4.82
Lys	6.1	6.54	7.5	4.54	6.59
His	2.7	2.67	2.5	2.94	2.09
Arg	4.7	4.41	6.8	4.12	5.70
Pro	7.0	6.46	4.0	12.22	3.38
Trp	1.5	NA	0.9	4.05	1.72

Table.2 Minerals of different silkworm varieties (Zhou *et al.,* 2022)

Minerals (mg/100 g Dry Weight)	*Bombyx mori*	*Eri* Silkworm Pupae	*Antheraea pernyi*
Phosphorus	474	584	272
Iron	26	24	4
Calcium	158	74.2	63
Zinc	23	7.24	3.57
Copper	0.15	1.75	0.73
Magnesium	207	178	154
Manganese	0.71	2.54	NA
Chromium	1.69	NA	9.84

Table.3 Fatty acid composition of different varieties of silkworm pupae oil (Hu *et al.,* 2017; Wang *et al.,* 2020 and Venkatesh *et al.,* 2020)

Fatty Acids (Percentage of Fatty Acids)	*Eri* Silkworm Pupae	Mulberry Silkworm Pupae	*Antheraea pernyi*	*Sunflower Oil*
Myristic acid (C14:0)	ND	0.18	NA	NA
Palmitic acid (C16:0)	26.98	23.18	17.25	5.6
Palmitoleic acid (C16:1)	1.82	1.07	1.14	NA
Stearic acid (C18:0)	4.73	4.69	2.23	2.2
Oleic acid (C18:1)	15.89	28.32	29.15	25.1
Linoleic acid (C18:2)	5.49	3.88	7.14	66.2
α-Linolenic acid (C18:3)	44.73	38.25	40.28	NA
Saturated fatty acids	31.71	28.05	19.48	7.8
Monounsaturated fatty acids	17.71	29.39	30.29	25.1
Polyunsaturated fatty acids	50.22	42.13	47.42	66.2
Myristic acid (C14:0)	ND	0.18	NA	NA

Table.4 Essential nutrients for 100-g silkworm pupae and other everyday foods (Wu *et al.,* 2021)

Name	Edible part (%)	Energy (kcal)	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	VA (µg)	B1 (mg)	B2 (mg)	B3 (mg)	VE (mg)	Na (mg)	Ca (mg)	Fe (mg)
Silkworm pupae	100	230	57.5	21.5	13	6.70	0	0.07	2.23	2.2	9.89	140.2	81	2.6
Egg (white part)	87	138	75.8	12.7	9	1.50	310	0.09	0.31	0.2	1.23	94.7	48	2
Milk	100	54	89.8	3	3.2	3.40	24	0.03	0.14	0.1	0.21	37.2	104	0.3
Chicken	66	167	69	19.3	9.4	1.30	48	0.05	0.09	5.6	0.67	63.3	9	1.4
Pork (lean meat)	100	143	71	20.3	6.2	1.50	44	0.54	0.1	5.3	0.34	57.5	6	3
Sea shrimp	51	79	79.3	16.8	0.6	1.50	0	0.01	0.05	1.9	2.79	302.2	146	3
Tilapia	55	98	76	18.4	1.5	2.80	0	0.11	0.17	3.3	1.91	19.8	12	0.

Table.5 Studies conducted on the diet of silkworm pupae in different countries

Country	Trial	Result	Reference
India	Substitution 0–50% of fishmeal	50% replacement of fishmeal with SWPM had no adverse effect on carcass characteristics	Qadri, 2015
Bangladesh	Broiler chicken: Diet (6% FM+ 0% SWP, 4% FM+2% SWP, 2% FM +4% SWP, 0% FM+6% SWP)	Increased growth rate with increasing levels of SWP in the diet. Feed intake decreased significantly and FCR increased significantly at 28 and 42 days of age	Khatun et al., 2003
Thailand	Replacement 0–100% fishmeal (0–20% Diet)	A mixture of defatted and as such basis SWPM replaced fishmeal at 10% inclusion level had little effect in broiler development with no adverse effect on	Jintasataporn, 2012
Nigeria	Replacement 0–75% Fishmeal	Cheaper silkworm caterpillar meal can be an excellent substitute for fish meal in broiler starter diets	Ijaiya and Eko, 2009
Pakistan	Substitution 0–100% of soybean meal	Replacement of soybean meal with silkworm meal did not affect broiler performance and carcass quality	Rafiullah, 2006

Table.6 Case studies

Author	Year	Outcome
Rashmi et. al	2022	The effect of different inclusion levels of defatted silkworm pupae meal (DSWP) on rumen fermentation, microbial protein synthesis and nutrient utilisation in cattle fed on finger millet straw (FMS)-based diet. The DSWP is a better meal for cattle than soyabean meal at a cheaper price
Andrzej	2019	the effect of different inclusion levels of defatted silkworm pupae meal (DSWP) on rumen fermentation, microbial protein synthesis and nutrient utilisation in cattle fed on finger millet straw (FMS)-based diet.
Qazi et. al	2023	The experiment revealed that Fish meal (FM) can be replaced successfully by Silkworm pupa meal (SWPM) upto 50% level without any adverse effect on feed intake, nutrient utilization and growth of the crossbred cattle calves.

Figure.1 Components of Silkworm Pupa

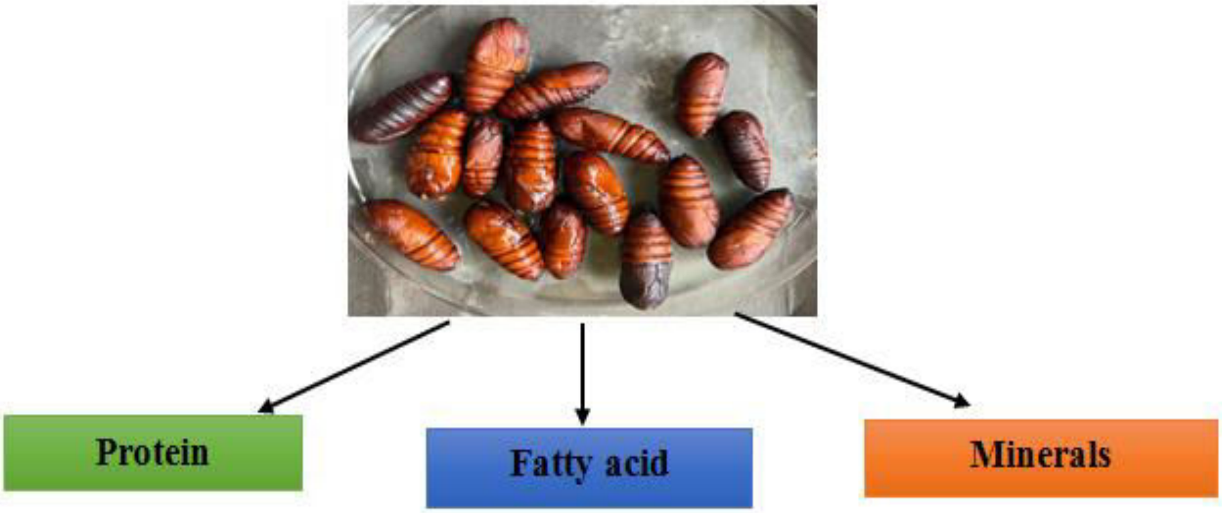

Figure.2 Silkworm poultry meal and nutrition value. (Valarie *et al*., 2015)

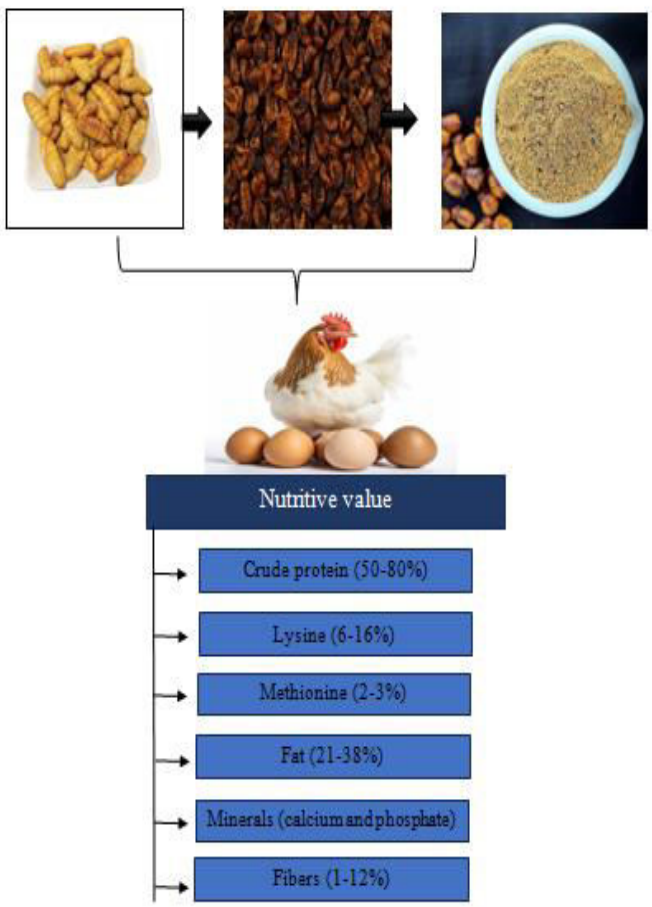

Figure.3 Mechanism of Apoptosis

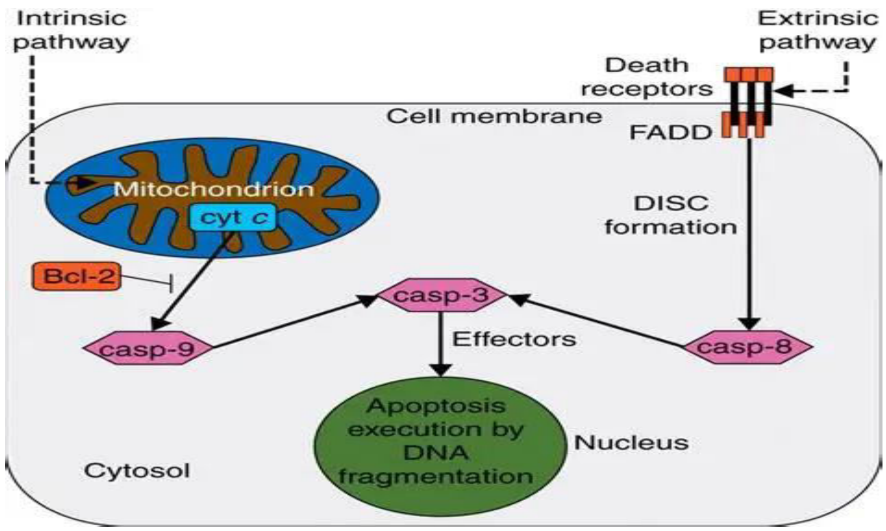

Figure.4 Mechanism of chitosan as antibacterial

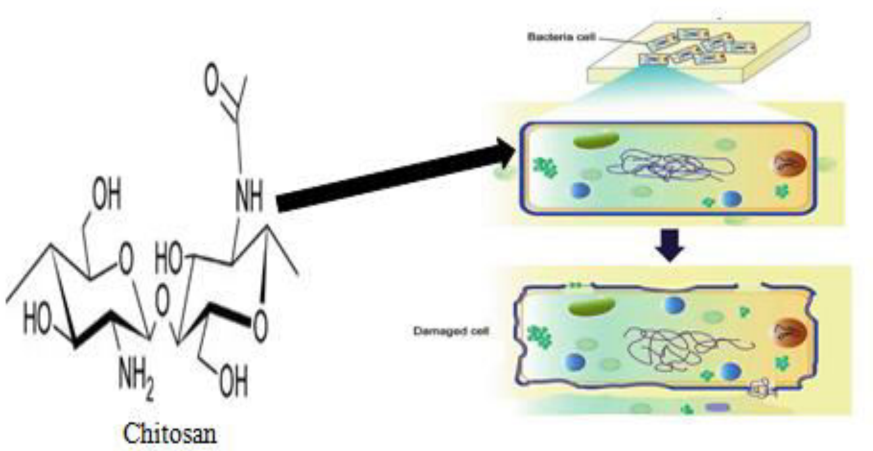

Figure.5 Mechanism as antioxidant of silkworm pupae

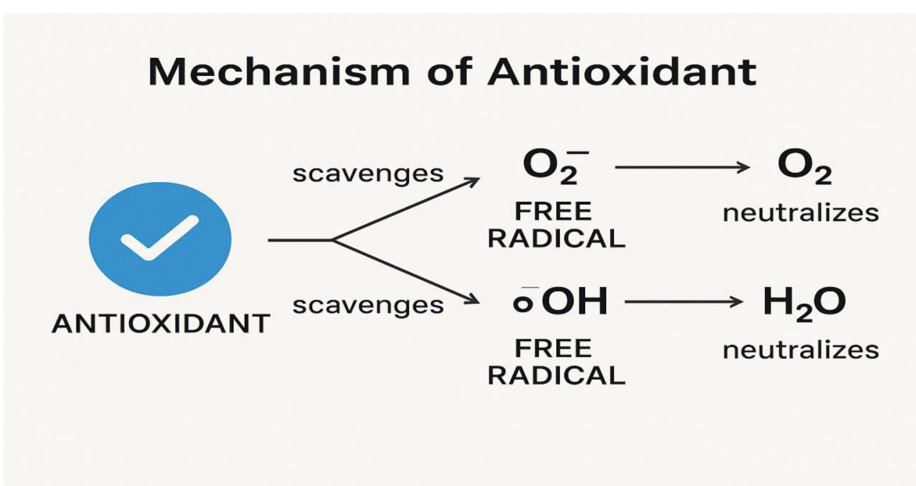

Figure.6 Different products of silkworm pupae available in market

Other Important Pharmacological Functions of Silkworm Pupae

Silkworm pupae also contain antifatigue (Lee *et al.,* 2019, Kim *et al.,* 2016), antiaging (*Manosroi et al.,* 2010), and antigenotoxic properties in addition to the pharmacological ones mentioned above (Deori *et al.,* 2014) and alcohol detoxifying (Kwon *et al.,* 2012) actions and prevent fibroblasts from proliferating (Zhu *et al.,* 2020). Additionally, silkworm pupae can be used as bioreactors to express heterologous proteins, which is crucial for the creation of recombinant proteins and vaccines (Chen *et al.,* 2006). The oil content obtained from the silkworm pupa can be prominently used to produce biodiesel (Natarajan and Marimuthu, 2023; Jaiswal and Banerjee, 2021). The silkworm pupae due to their high fat content (over 30%), are used as chrysalis oil to obtain cosmetic products (cream, soap, lotion, emulsion).A Field experiment conducted to evaluate the silkworm pupae compost (Seri-compost) on French bean yield showed that all the Seri-compost treatment was a very good than the other inorganic.

Silkworm pupae used as eco-friendly base for mass production of *Bacillus thuringiensis* (Patil *et al.,* 2013). Dongchunghacho is a fungus living inside the body of the larva or the image of insects in winter and later grows outside the body in the form of mushroom in summer. It is not only an animal but also a plant and known to have a miraculous energy. It has been called 'a mystic miraculous medicine for eternal youth' or 'a legendary elixir of life' for a long time (Kang-Sun Ryu, 2011).

- ✓ Traditionally considered waste or low value animal feed, silkworm pupae are now increasingly recognized for their high nutritional profile, unique bioactive compounds and versatile application
- ✓ In the pharmaceutical sector, extracts like chitosan, antimicrobial and antioxidant oils have opened new pathways for natural wound healing, anti-inflammatory creams and even potential anticancer therapies
- ✓ In the food industry, silkworm pupae offer a sustainable and highly nutritious alternative protein source for humans
- ✓ In animal husbandry and aquaculture, silkworm pupae meal provides a promising replacement for increasingly expensive and unsustainable fishmeal and soybean meal.
- ✓ Silkworm pupae represent a zero-waste opportunity in sericulture.

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Author Contributions

Toko Naan: Conceived the review topic; Rubi Sut: Grammer corrections and spacing management; Toko Naniya: Alignment and other editing done the author

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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