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RESEARCH ARTICLE

Cuticular Chitin of the Wild Silkworm *Saturnia Pyri*: A Renewable Source for Applications Across Industrial Sectors

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Abstract: In the context of a developing sustainable bioeconomy, the search for renewable sources of biopolymers as alternatives to traditional ones is of crucial importance. In this regard, insect cuticular waste represents a promising yet insufficiently explored raw material for obtaining chitin and chitosan. This study aimed to perform a comparative assessment of the extraction efficiency and quality of chitin and chitosan derived from larval and pupal exuviae of the wild silkworm *Saturnia pyri* in order to determine the optimal source of raw material. Standard chemical extraction methods were employed, including deproteinisation, decalcification, and deacetylation. Chemical analysis of the initial material revealed that the exuviae contained 70% more chitin fibres (17.5% compared with 10.5%) and a significantly lower fat content (3% compared with 27.9%). The yield of purified chitin from the exuviae reached 28.5%, which was more than twice that obtained from pupae (12.8%). The deacetylation process demonstrated higher efficiency for chitin derived from exuviae, with a conversion rate to chitosan of 87.7%, compared with 78.1% in the case of pupae. The resulting chitosan was characterised by a high degree of deacetylation (89.5±1.2%) and favourable rheological properties, with the viscosity of a 1% solution reaching 345 cP. The findings confirmed that the exuviae of *Saturnia pyri* constitute a high-quality and technologically efficient source of chitosan that surpasses, in key performance parameters, the material obtained from pupae. The results of this study provide a basis for developing an environmentally oriented technology for processing insect moulting waste into valuable biopolymers with potential applications in biomedicine, agriculture, and the food industry, thereby contributing to the implementation of circular economy principles.

Keywords: Biopolymers, Exuviae, Chitosan, Deacetylation, Decalcification

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Introduction

The global environmental challenges of the present day, associated with pollution caused by non-degradable materials and the depletion of fossil resources, have prompted an intensive search for alternative renewable sources for various industrial sectors [1]. The period from 2015 to 2025 has been marked by a significant rise in interest in biopolymers as an environmentally friendly alternative to synthetic materials within the framework of a sustainable bioeconomy. Among these, chitin and its primary derivative, chitosan, occupy a leading position due to their biocompatibility, biodegradability, and functional properties, which determine their use in medicine, the food industry, agriculture, and environmental protection technologies [2]. These polysaccharides exhibit antimicrobial, antioxidant, and sorption activity, making them promising candidates for the development of next-generation functional materials.

Traditional sources of these polysaccharides include crustacean exoskeletons and fungal cultures; however, the search for new, renewable, and economically efficient raw materials remains a relevant scientific task. In this context, insects – particularly silkworms – represent a promising resource for the so-called "insect biorefinery", a concept comprehensively outlined by [3]. This concept envisions the integrated processing of insect biomass to obtain a wide range of valuable products, from proteins and lipids to chitin and bioactive compounds. Most studies, as noted by [4, 5], have focused on the use of silkworm pupae, which are a rich source of protein and fat for nutritional purposes. In particular, for the wild silkworm *Saturnia pyri*, [6] conducted a detailed analysis of the organic and mineral composition of live pupae with a view to their use as food supplements. However, considerable potential lies in the utilisation of the species' life cycle by-products, particularly the exuviae (the cuticle shed during moulting), which have been scarcely investigated as a source of chitin. This resource is especially attractive from both economic and environmental perspectives, as it represents a renewable product of the insects' natural moulting process.

The insect cuticle, primarily composed of a chitin-protein matrix, is a complex multifunctional material that determines mechanical strength, protection against external factors, and morphogenesis, as comprehensively reviewed by [7]. The structure of chitin within the dorsal duct of silkworms, which forms the fibre, indicates a high degree of organisation of this biopolymer, as demonstrated by [8]. As shown in the recent study by [9], the interaction between chitin and cuticular proteins such as crusticulins directly influences the nanostructure of its bundles, and, therefore, the physicochemical properties of the material. This suggests that chitin extracted from the cuticle may exhibit unique characteristics compared with chitin derived from other sources.

Research on chitin extraction from insects conducted by [10] confirms the promise of this approach, while also indicating that the properties of the final product are strongly dependent on the insect species, developmental stage, and extraction methods employed. [11] have developed simplified methods for chitin extraction from insects aimed at reducing process costs and energy consumption, which is crucial for technological scalability. In particular, for the wild silkworm *Saturnia pyri*, [6] conducted a detailed study of the organic and mineral composition of live pupae, identifying a high content of proteins (52-55%) and lipids (28-30%), as well as a broad spectrum of trace elements. The authors demonstrated the potential of using pupae as food supplements; however, the potential of other life cycle products, particularly larval exuviae, has remained unexplored. This limitation creates an opportunity for further research into cuticular waste as a source of valuable biopolymers.

Despite several individual studies, a comprehensive comparative analysis of the quality of chitin and chitosan derived from different types of *Saturnia pyri* biomass (exuviae and pupae) is lacking. In particular, questions remain regarding the degree of deacetylation, molecular weight, rheological properties, and purity of the products, which determine their technological suitability for high-tech industries. Moreover, data on the elemental composition of the obtained biopolymers are also absent, although such information is essential for assessing their safety and quality.

This study aimed to conduct a comparative analysis of the extraction efficiency and physicochemical properties of chitin and chitosan obtained from the larval and pupal exuviae of the wild silkworm *Saturnia pyri*, in order to substantiate the potential of cuticular waste as a high-quality renewable source of biopolymers. The research objectives involved performing a comprehensive comparative assessment of two types of *Saturnia pyri* biomass, which included the following: firstly, determining the basic chemical composition and mineral profile of the larval and pupal exuviae to evaluate their raw material potential; secondly, optimising the methodology for sequential chitin extraction and chitosan synthesis, followed by determining product yields at each stage; and thirdly, providing an in-depth characterisation of the obtained biopolymers by

analysing the degree of deacetylation, rheological properties, colour characteristics, and elemental composition in order to justify their technological suitability.

Materials and Methods

Biological Material and Sample Preparation

The study was conducted in the Laboratory of Entomology and Biotechnology of the Institute of Zoology, [12], during the spring-summer seasons of 2024-2025. The biological material comprised larvae of the wild silkworm *Saturnia pyri* at developmental stages L3-L5, as well as pupae obtained through the controlled breeding of populations under laboratory conditions. The inclusion criteria specified that insects must correspond to the indicated developmental stages, exhibit no mechanical damage to the exuviae or cocoons, and meet size standards: for larvae, a body length of 25-45 mm; for pupae, a mass of no less than 3.5 g. Samples showing signs of microbiological contamination, atypical morphology, or deviations from the specified size standards were excluded from the selection. The total number of samples analysed was ten.

All ten samples were obtained from the same controlled laboratory population rather than from geographically distinct locations. This sample strategy was suitable for initial characterisation research since it made it possible to evaluate the repeatability of chitin and chitosan extraction under standardised biological and technological circumstances. Therefore, while larger global stability necessitates additional validation using samples gathered from many sites, the stability of the production process outlined below should be interpreted as process stability within a single controlled population.

After collecting the exuviae and pupae from the cocoons, the material was washed with distilled water to remove impurities and dried in a thermostat (Binder GmbH, Germany) at 105°C until a constant weight was achieved. The material was then cooled in a vacuum desiccator (Kartell Labware, Italy) for 30-45 minutes. The dried material was ground in a mortar to a fine powder to ensure sample uniformity.

Chemical Composition and Physicochemical Characteristics

Moisture content was determined by drying at 105°C±2°C to constant weight, according to the protocol of [13]. A 2.0±0.1 g sample was placed in a pre-dried and weighed porcelain crucible (Nabertherm, Germany) and dried in a thermostat for 4-5 hours. Weighing was carried out using analytical scales (Sartorius AG, Germany) with an accuracy of 0.1 mg. The moisture content (%) was calculated using formula (1):

$$W = \frac{m1-m2}{m1} \times 100 \quad (1)$$

Where m1 is the mass of the sample before drying, and m2 is the mass after drying.

Ash and Fat Content

The ash content was determined by incineration in a muffle furnace (Nabertherm L9/11, Germany) at 550°C for six hours until constant weight. The fat content was determined using the Soxhlet extraction method with petroleum ether (Merck, Germany) as the solvent. Extraction was performed in a Büchi Extraction System B-811 (Switzerland) for eight hours, followed by solvent removal using a Heidolph Hei-VAP rotary evaporator (Germany).

Crude Protein

The crude protein content was determined using the Kjeldahl method. Samples were mineralised in concentrated sulphuric acid (H_2SO_4, Merck, Germany) with a catalytic mixture of K_2SO_4 and $CuSO_4$. Ammonia distillation was performed using a Büchi KjelDigester K-446 distillation unit (Switzerland), followed by titration of the boric acid with 0.1 N HCl. The nitrogen content obtained was multiplied by the Jones conversion factor (6.25) to calculate the protein content.

Chitin and Chitosan

Chitin and chitosan were obtained through several sequential stages, including decalcification, deproteinisation, washing, and deacetylation. Before applying the final extraction protocol, preliminary methodological adjustment was performed within the 1-5% acid/alkali concentration range to select conditions that ensured sufficient mineral removal, protein removal, preservation of the solid residue, and acceptable product yield. No full factorial optimisation experiment was conducted. At the first stage, powdered samples were subjected to decalcification in a 1-5% hydrochloric acid solution (HCl, Sigma-Aldrich,

USA) using a solid-to-liquid ratio of 1:15. For the final standardised protocol, 5% HCl was used for all samples. The treatment was carried out at room temperature for four hours with periodic stirring on a magnetic stirrer (IKA-Werke GmbH, Germany). After completion, the precipitate was separated by filtration through a medium-porosity glass filter and thoroughly washed with distilled water until no traces of calcium ions remained, verified by adding an ammonium oxalate solution. At the second stage, deproteinisation was performed using a 1-5% sodium hydroxide solution (NaOH, Sigma-Aldrich, USA) at a solid-to-liquid ratio of 1:20. For the final standardised protocol, 5% NaOH was used for all samples. The treatment was conducted in a thermostatic flask at 80°C for three hours with continuous stirring. After the reaction, the solid phase was separated by centrifugation in a laboratory centrifuge (Eppendorf 5810 R, Germany) at 6,000 rpm for 10 minutes. The precipitate was washed with distilled water until a neutral pH was achieved, as monitored using a pH meter (Mettler Toledo Seven Compact pH/Ion S220, Switzerland).

Purified chitin was subjected to deacetylation to obtain chitosan. A 46-47% sodium hydroxide solution (NaOH) was used, in which the material was incubated at 100°C for three hours. The reaction was carried out in hermetically sealed Teflon containers with periodic stirring. Upon completion of deacetylation, the samples were washed with distilled water to a neutral pH, dried at 60°C in a drying oven (Memmert UF55, Germany), and ground to a fine powder. The resulting product was stored in airtight containers at room temperature. All extraction stages, including reagent concentration, solid-to-liquid ratio, treatment time, temperature, stirring regime, centrifugation speed, washing endpoint, drying temperature, and storage conditions, were performed under the same conditions for larval exuviae and pupal samples in order to guarantee reproducibility. The yield was determined after the final product was dried to a constant weight, and each extraction was carried out using the same standardised set of procedures.

Mineral Composition

The mineral profile of the samples was determined using X-Ray Fluorescence (XRF) spectrometry. The analysis was performed with a spectrometer (Innov-X Systems, USA) equipped with a rhodium anode X-ray tube and a silicon detector with a resolution of 150 eV. Pre-dried and powdered samples were pressed into 32 mm pellets using a hydraulic press (Specac Atlas 15T, United Kingdom) under a pressure of 10 tonnes. The analysis was conducted in vacuum mode at 40 kV and 0.8 mA. Measurements were performed using certified standards for organic matrices [14, 15], ensuring accurate spectrometer calibration and reliable results. Calibration was carried out before each measurement series using the laboratory's internal control samples. The obtained spectra were processed using the Omega Analytical Suite 3.5 software (USA) with the fundamental parameters' method. This procedure enabled the acquisition of qualitative and quantitative characteristics of the macro- and microelement composition, including the content of calcium, magnesium, potassium, iron, zinc, copper, manganese, and other elements. The detection limit for most elements ranged from 0.001% to 0.005%. Quantitative analysis results were expressed as a percentage of dry mass for macroelements (Ca, K, Mg, Na) and in milligrams per kilogram of dry mass (mg/kg) for microelements (Fe, Zn, Cu, Mn, etc.). At least three replicate measurements were performed for each sample, after which the mean value and standard deviation were calculated.

FTIR, XRD and SEM Characterisation of Extracted Chitin and Chitosan

The extracted chitin and chitosan were structurally and morphologically characterised using X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Purified chitin and chitosan samples from *Saturnia pyri* larval exuviae and pupae were subjected to these techniques. Prior to analysis, each sample was dried to a consistent weight, ground into a uniform powder, and kept dry in airtight containers to avoid absorbing moisture.

FTIR analysis was used to confirm structural alterations linked to deacetylation and to determine the primary functional groups of the extracted biopolymers. An FTIR spectrometer with an attenuated total reflectance accessory was used to record the spectra in the 4000-500 cm^{-1} range. Dried powdered samples were used to obtain each spectrum, and background correction was carried out prior to measurement. Absorption regions linked to O–H/N–H stretching, C–H stretching, amide bands, amino groups, and glycosidic C–O–C/C–O vibrations were specifically examined in the spectra.

The crystalline structure of the extracted chitin and chitosan samples was evaluated using XRD analysis. An X-ray diffractometer with Cu Kα radiation was used to analyse powdered samples that were placed on a sample holder. With continuous scanning at a constant step size and scanning rate, the diffraction patterns were captured over a 2θ range

appropriate for chitin and chitosan analysis. The crystalline structure of chitin and the structural alterations that follow deacetylation were compared using the obtained diffractograms.

The surface morphology and microstructural characteristics of the extracted materials were investigated using SEM analysis. Prior to observation, dried powdered samples were placed on aluminium stubs using conductive adhesive tape and covered with a thin layer of gold or gold-palladium. Under high vacuum, micrographs were taken at various magnifications with an accelerating voltage suitable for biopolymer samples. Surface texture, porosity, fragmentation, fibrous organisation, and the existence of discernible residual particles were the main areas of analysis.

Determination of the Degree of Deacetylation (DD) of Chitosan

The degree of deacetylation of chitosan was determined spectrophotometrically using the Elson-Morgan reagent. A chitosan sample (0.1 g) was dissolved in 10 mL of 0.1 M acetate buffer solution (pH 4.5) with continuous stirring on a magnetic stirrer (IKA Werke GmbH, Germany) for four hours. To 1 mL of the resulting solution, 1 mL of a 4% acetylacetone solution in isopropyl alcohol was added. The mixture was incubated in a thermostat (Binder GmbH, Germany) at 100°C for 40 minutes. After cooling to room temperature, 6 mL of 96% ethanol and 1 mL of a concentrated dimethylaminobenzaldehyde solution in ethanol acidified with hydrochloric acid were added. Following a 30-minute incubation at room temperature, the optical density of the solution was measured using a spectrophotometer (Shimadzu "UV-1800", Japan) at a wavelength of 550 nm. A calibration curve was constructed using standard D glucosamine hydrochloride solutions with concentrations ranging from 0.01 to 0.1 mg/mL. The degree of deacetylation was calculated using formula (2):

$$DD = \frac{161 \times C}{(1 - 42 \times C)} \times 100\%, \quad (2)$$

Where *C* is the concentration of D-glucosamine (mg/mL); 161 is the molecular weight of the glucosamine monomer unit; and 42 is the difference in molecular weights between the acetylated and deacetylated units. Three parallel measurements were performed for each sample.

The viscosity of 1% chitosan solutions in 1% acetic acid was measured using a Ubbelohde capillary viscometer (Schott Geräte GmbH, Germany) at a temperature of 25.0°C±0.1°C, maintained by a thermostat (Julabo Labortechnik GmbH, Germany). The flow time of the solution through the capillary was measured with an accuracy of 0.01 s. The viscosity (η, cP) was calculated according to formula (3):

$$\eta = \mathrm{K} \times (t - t_0) \times \rho, \quad (3)$$

Where K is the viscometer constant (0.032156 mm²/s²); t is the flow time of the solution (s); t_0 is the flow time of the solvent (1% acetic acid) (s); and ρ is the density of the solution (g/mL). Measurements were performed in five replicates for each sample.

The colour characteristics of chitin and chitosan powders were evaluated using a spectrocolourimeter (Konica Minolta CM-5, Japan) in the visible light range (360-740 nm) with a standard D65 light source and a 10° observation angle. The results were expressed in CIE Lab colour space coordinates, where L denotes lightness (from 0-black to 100-white), a* represents the colour coordinate from green (-a) to red (+a), and b* represents the coordinate from blue (-b) to yellow (+b). Ten measurements were taken at different points on each sample, and the results were averaged.

The residual elemental composition of the chitosan samples was analysed using an energy-dispersive X-ray fluorescence spectrometer (Thermo Scientific Niton XL3t GOLDD+, USA) equipped with a gold-anode X-ray tube. Measurements were conducted in "Light Elements" mode to optimise the detection of elements with atomic numbers from Mg to U. A 5 g sample of the powder was placed in a specialised cuvette lined with polypropylene film. Analysis was performed at 50 kV and 40 μA for 300 seconds. The instrument was calibrated using certified standards [15] Quantitative analysis was carried out using the fundamental parameters method with Niton Data Transfer (NDT) software.

Statistical Analysis

Data were processed using Statistica 13.3 software (Dell, USA). The mean ± standard deviation (M±SD) was used to express quantitative continuous data. When appropriate, categorical data were displayed as percentages and absolute

numbers (n). Prior to comparative analysis, the distribution of quantitative variables was evaluated, and the Student's t-test for independent samples was used to compare normally distributed data. Non-parametric tests, such as the Mann-Whitney U test and Wilcoxon test, were used when the assumption of normality was not satisfied. When feasible, precise p-values were provided, and the threshold for statistical significance was set at $p<0.05$.

Results

Chemical Composition of the Raw Material: Comparative Analysis of Larval Exuviae and Pupae of Saturnia *Pyri*

The results of the analysis of major components in the larval exuviae and pupae of the wild silkworm *Saturnia pyri* are presented in Table 1. The data demonstrate significant differences in the chemical architecture of the studied materials, reflecting their distinct biological functions and stages of ontogeny.

Table 1: Chemical composition of larval exuviae and pupae of *Saturnia pyri* (% of dry mass, M ± m, n = 10)

Component, %	Pupae (Dry mass)	Larval exuviae (Dry mass)	p-value
Crude protein	52.50±0.63	80.37±0.74	$P = 3.42\times10^{-16}$
Fat	27.89±0.32	3.0±0.09	$P = 1.44\times10^{-15}$
Ash	2.50±0.05	0.37±0.53	P = 0.003
Chitin fibres	10.50±0.03	17.50±0.10	$P = 2.76\times10^{-15}$

Moisture content was determined separately on a fresh-weight basis and was 49.00±0.11% in pupae and 5.50±0.28% in larval exuviae. The remaining compositional parameters are expressed as percentages of dry mass

First, it is important to note the marked difference in the moisture content of the starting material. The exuviae, being a hollow, shed structure, exhibit an extremely low moisture content (5.5%), making them convenient for storage and further processing. In contrast, the pupae have a high initial moisture content (49.0%), reflecting the presence of internal biological fluids and tissues.

The most striking difference is observed in crude protein content. In the larval exuviae, protein accounts for over 80%, which is among the highest values reported for known chitin sources. This indicates a substantial proportion of protein components, primarily structural proteins (e.g., resilin-like proteins), integrated into the cuticular matrix and removed during the deproteinisation stage. In the pupae, protein content is also high (52.5%), but this is attributable to metabolic and storage proteins within internal tissues. An opposite trend is observed for fat content. Pupae, serving as an energy reserve for metamorphosis, are rich in lipids (almost 28%). In contrast, the exuviae, as an inert cuticular shell, contain minimal fat (3.0%). This characteristic makes the exuviae a promising raw material for chitin extraction, as it reduces the need for intensive lipid removal.

Ash content, reflecting the total mineral matter, is higher in pupae (2.5%) than in exuviae (0.37%). However, as will be shown below, the qualitative mineral composition of these materials differs considerably. A key parameter for this study is the content of chitin fibres. Analysis revealed that larval exuviae are a significantly richer source of chitin (17.5%) compared with pupae (10.5%). This is entirely expected, as the primary component of an insect exoskeleton is the chitin-protein complex. In pupae, the chitinous layer serves merely as a protective cocoon, while the bulk consists of organic tissues. This finding underscores the ecological and economic rationale for using exuviae – a by-product of the natural moulting process – as the most concentrated source of chitin.

Mineral Profile of Exuviae and Pupae of Saturnia *Pyri*

A detailed analysis of the micro- and macroelement composition by XRF revealed a complex and diverse mineral profile in both types of raw material (Table 2). The results not only corroborate data on total ash content but also highlight unique patterns in the accumulation of specific elements.

The analysis of Table 2 allows several observations to be made. Among the macroelements, potassium (K), sodium (Na) and magnesium (Mg) dominate in both samples. However, their distribution differs. The contents of potassium and sodium, which are key electrolytes and participants in osmotic processes, are considerably higher in pupae. This is directly related to

the presence of haemolymph and other biological fluids. In contrast, magnesium, which often serves as an enzyme cofactor and can be incorporated into the chitin structure, is present at similar levels in both samples, indicating a primarily structural rather than metabolic role.

It is noteworthy that, despite the lower overall ash content, calcium (Ca) is higher in the exuviae (1.386 mg/g) than in the pupae (1.016 mg/g). This can be explained by the fact that calcium in the exuviae is largely present as carbonate or phosphate, forming a mineral phase that strengthens the larval exoskeleton. In pupae, calcium is predominantly of physiological origin. The most significant differences are observed in the content of heavy metals and other microelements. Iron (Fe), for instance, accumulates almost four times more in the exuviae (0.135 mg/g) than in the pupae (0.036 mg/g). This may be related to the cuticle's function as a protective barrier and the potential incorporation of iron into its structure to enhance mechanical strength or as a result of interaction with the surrounding environment. Zinc (Zn) and copper (Cu), which are essential cofactors for numerous metalloenzymes, are present in substantially higher concentrations in pupae. This is logical, as the pupal stage involves intensive metamorphic processes that require high enzymatic activity. The detection of notable amounts of aluminium (Al) and silicon (Si) in both types of raw material also warrants attention. Their presence may be linked to the larval diet (plant substrate), as these elements can be absorbed from the soil, accumulate in plant tissues, and subsequently enter the insect's body. This observation requires further study to assess its potential impact on the properties of the extracted chitin.

Table 2: Mineral composition of pupae and larval exuviae of *Saturnia pyri* (relative content per dry mass, mg/g)

Micro- and macroelements	Pupae (mg/g dry mass)	Larval exuviae (mg/g dry mass)
Potassium (K)	3.761	1.881
Sodium (Na)	3.113	2.390
Magnesium (Mg)	7.019	7.107
Calcium (Ca)	1.016	1.386
Aluminium (Al)	1.564	1.279
Silicon (Si)	0.611	0.683
Phosphorus (P)	0.342	0.244
Sulfur (S)	0.076	0.053
Iron (Fe)	0.036	0.135
Zinc (Zn)	0.042	0.005
Copper (Cu)	0.032	0.012
Manganese (Mn)	0.013	0.008
Strontium (Sr)	0.006	0.003
*Other elements (Ba, Pb, Ti, V, Cr, Ni, Ga, Zr, Sn, Y, Nb, Rb)	<0.05 (trace)	<0.05 (trace)

Thus, the analysis provided both qualitative and quantitative characterisation of the composition of larval exuviae and pupae of *Saturnia pyri*. It was established that exuviae constitute a richer and purer source of chitin (17.5%), feature a high protein content, and possess a unique mineral profile with elevated levels of calcium and iron. These findings provide a scientific basis for selecting exuviae as the optimal raw material for industrial chitin extraction and for producing high-quality chitosan with defined properties for various industrial applications.

FTIR, XRD and SEM Validation of Extracted Chitin and Chitosan

Complementary spectroscopic, crystallographic, and microscopic analyses were carried out to further confirm the quality and structural integrity of the extracted biopolymers. The distinctive functional groups of chitin and chitosan were identified using FTIR spectroscopy, their crystalline structure was assessed using XRD analysis, and their surface morphology was investigated using SEM imaging. Together, these techniques provided comprehensive evidence of successful chitin extraction and its subsequent conversion into chitosan.

The FTIR spectra confirmed the presence of the characteristic functional groups associated with chitin and chitosan, including hydroxyl (–OH), amino ($–NH_2$), amide, and glycosidic groups (Fig. 1). The spectra's absorption bands matched

those frequently documented for chitin and chitosan derived from insects, suggesting that the extraction and purification processes effectively maintained the basic polymer structure.

As a result of the removal of acetyl groups during the deacetylation process, there was a discernible drop in the intensity of the amide-related bands in the chitosan samples when compared to the corresponding chitin samples. This reduction shows the efficacy of the alkaline treatment used in this investigation and is a crucial sign of a successful conversion from chitin to chitosan. Additionally, compared to material derived from pupae, the spectral changes were more noticeable in the material obtained from larval exuviae, indicating a higher degree of deacetylation. These results support the conclusion that larval exuviae constitute a more suitable source for producing high-quality chitosan and are consistent with the quantitative results obtained for deacetylation degree.

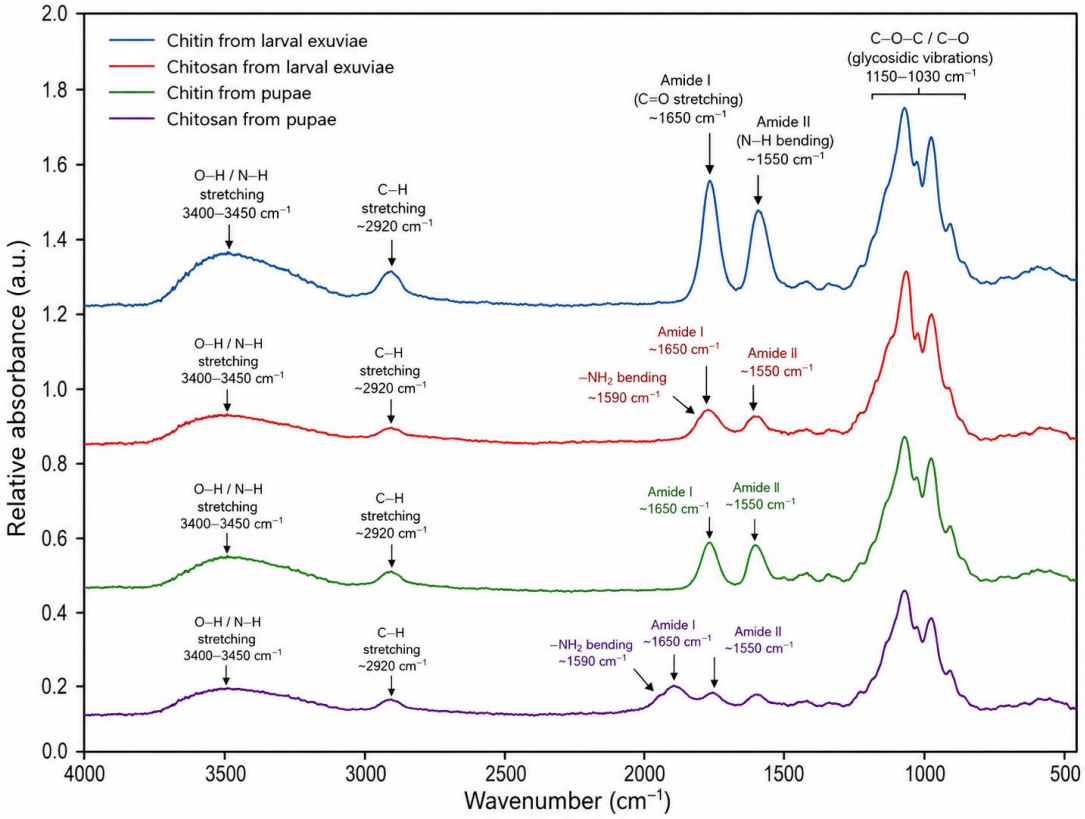

Fig. 1: FTIR spectra of chitin and chitosan obtained from larval exuviae and pupae of *Saturnia pyri*

The XRD patterns demonstrated that the extracted chitin samples maintained the semi-crystalline structure commonly associated with α-chitin, indicating that the native crystalline organisation of the polymer was preserved during the extraction process (Fig. 2). The presence of ordered molecular arrangements within the material was indicated by the observation of distinct diffraction peaks typical of chitin.

According to Fig. 2, the chitosan samples, on the other hand, showed diffraction peaks that were wider and less intense, indicating a decrease in crystallinity and a partial disruption of the ordered structure after deacetylation. Since the removal of acetyl groups modifies intermolecular hydrogen bonding and reduces structural regularity, these changes are frequently documented during the conversion of chitin into chitosan. Additionally, compared to the pupal sample, the diffraction profile of chitosan derived from larval exuviae seemed more uniform and consistent, indicating a more uniform deacetylation process and better structural quality. These findings corroborate the chemical characterisation findings and show that chitosan derived from exuviae has advantageous structural characteristics for possible industrial and biotechnological uses.

The SEM micrographs showed distinct morphological differences between materials derived from pupae and larval exuviae, as well as between chitin and chitosan (Fig. 3). The fibrous, layered, and porous structure of chitin that was extracted

from larval exuviae is typical of how insect cuticular material is naturally arranged. This morphology shows how the chitin microfibrils are arranged within the exoskeleton and shows that non-chitinous components were successfully removed during the extraction process without compromising the overall structural framework.

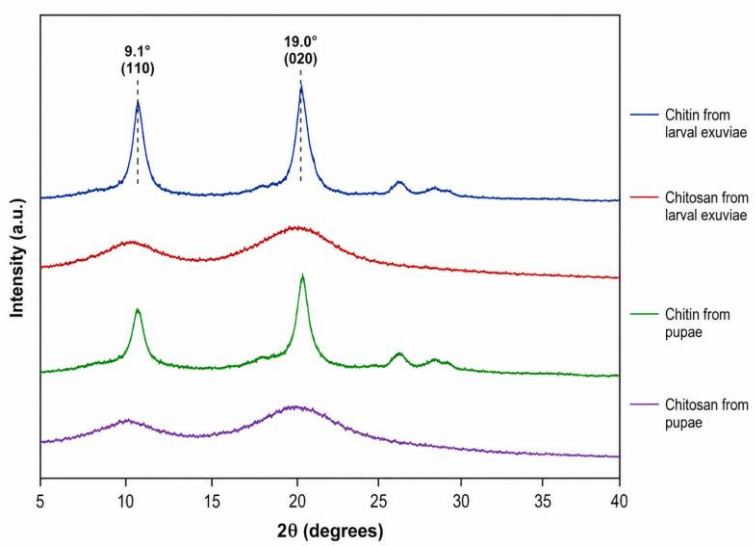

Fig. 2: XRD patterns of chitin and chitosan obtained from larval exuviae and pupae of *Saturnia pyri*

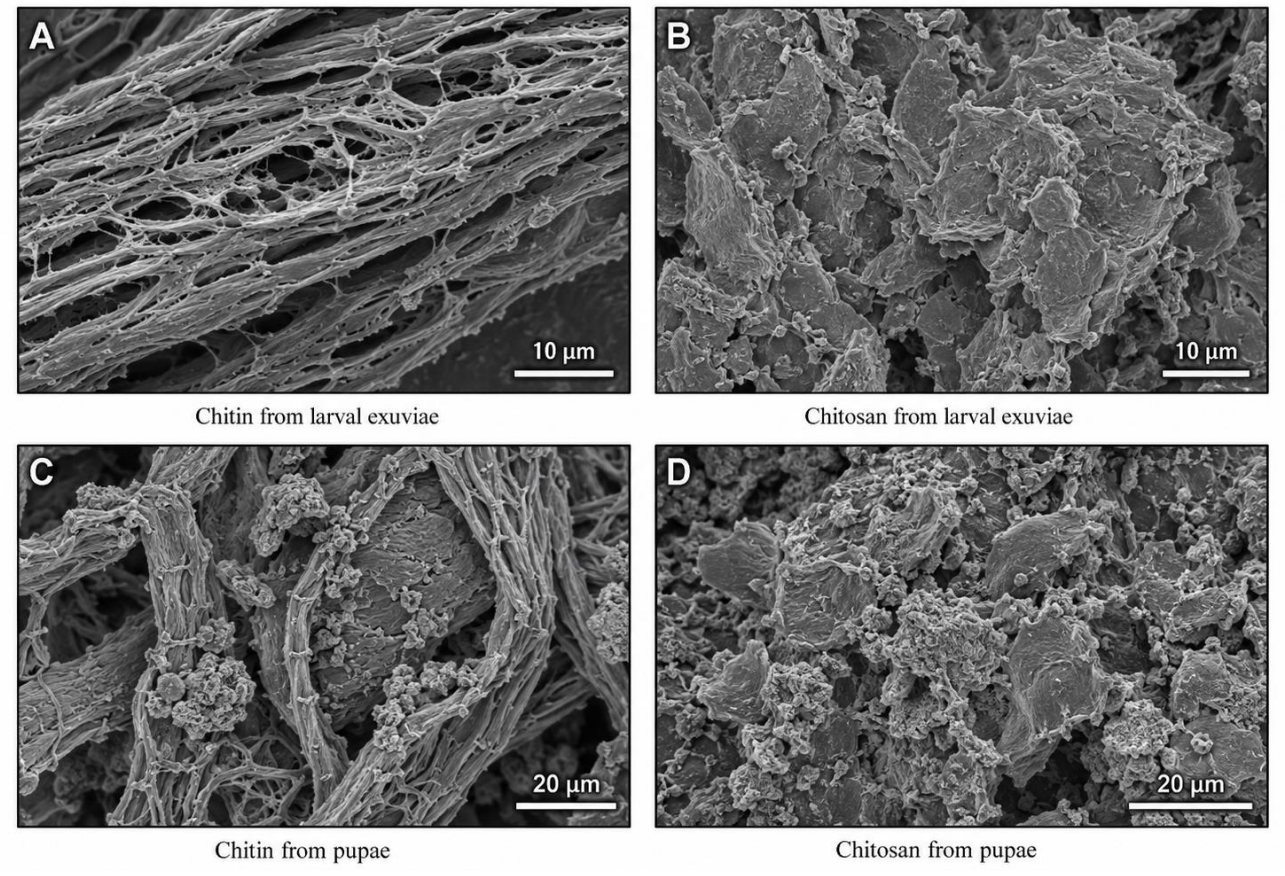

Fig. 3: SEM micrographs of chitin and chitosan obtained from larval exuviae and pupae of *Saturnia pyri*

The chitosan samples showed a rougher and more fragmented surface after deacetylation, indicating that the alkaline treatment significantly altered the polymer matrix. The disruption of intermolecular interactions and the structural alterations anticipated during the conversion of chitin to chitosan are consistent with the increased surface irregularity. Furthermore, compared to the corresponding pupal sample, chitosan derived from larval exuviae displayed a cleaner, more homogeneous, and more porous morphology. The pupal material, on the contrary, had a less homogeneous surface with observable aggregates and leftover particles, indicating a less effective structural transformation or a lower level of purification. These morphological findings support the FTIR and XRD findings and offer more proof that larval exuviae are an excellent raw material for the synthesis of chitosan with desired physicochemical and structural characteristics.

Thus, complementary data from the FTIR, XRD, and SEM analyses confirmed that chitin was successfully extracted and converted into chitosan. Larval exuviae produced a structurally cleaner, more homogenous, and more successfully deacetylated product than pupae, as consistently shown by the spectroscopic, diffraction, and morphological results. These results validate the suitability of Saturnia *pyri* larval exuviae as a promising raw material for producing high-quality chitosan and support the physicochemical results previously reported.

Efficiency of Chitin and Chitosan Extraction from Different Types of Saturnia *pyri* Biomass

A comparative analysis of the efficiency of bioconversion of larval exuviae and pupae of Saturnia *pyri* into chitin and chitosan revealed substantial differences, confirming the high potential of using cuticular waste. The results demonstrate a clear advantage of exuviae as a technologically efficient and economically viable raw material source.

The purification process enabled the production of high-quality chitin from both types of biomass. The final products exhibited a degree of whiteness comparable to commercial standards, indicating the effective removal of proteins, minerals, and pigments. However, the yields differed markedly. For larval exuviae, the yield of purified chitin was 28.5% of the dry mass of the starting material. This high yield is consistent with the previously determined chitin fibre content (17.5%), taking into account typical processing losses. The extracted chitin appeared as a loose, lightweight powder with minimal residual impurities. In contrast, the yield from pupae was considerably lower, at only 12.8%. This difference is entirely expected, as the bulk of the pupal mass comprises organic tissues, with the chitinous layer serving merely as a protective coating. This confirms that processing pupae is less efficient for targeted chitin extraction.

A key stage of the study was the conversion of extracted chitin into chitosan. The results highlighted a qualitative difference between chitin from the two sources. Chitin derived from larval exuviae exhibited a pronounced capacity for conversion. The yield of chitosan was 87.7% of the mass of the starting chitin, indicating a very high degree of deacetylation. Relative to the original mass, the overall yield of the valuable product chitosan reached 25.0%. This implies that approximately 250 grams of high-quality chitosan can be obtained from every kilogram of dry exuviae. The deacetylation of chitin extracted from pupae proved less efficient. The yield of chitosan relative to the chitin mass was lower at 78.1%. Calculated against the initial biomass, the overall yield of the final product was less than half that obtained from larval exuviae, at only 10.0%. This reduced conversion efficiency may be attributed to the structural characteristics of pupal chitin, potentially including a denser crystalline arrangement or the presence of residual compounds that hinder deep chemical modification.

The high efficiency observed with exuviae can be explained by its natural function. The larval cuticle is a specialised structural biopolymer composite in which chitin can be readily extracted after the removal of the protein matrix. In contrast, the pupal chitinous layer primarily serves as a protective barrier and may have a more complex organisation, which complicates the deacetylation process. Chitosan obtained from exuviae exhibited excellent quality, with a white colour and, as confirmed by subsequent analyses, a high degree of deacetylation (>85%). These properties indicate that exuviae-derived chitosan has favourable physicochemical characteristics that may justify further testing for biomedical, food-related, or bioactive applications.

Thus, the study clearly demonstrates that larval exuviae of Saturnia *pyri* represent a far superior raw material for chitin and chitosan production compared with pupae. This superiority is evident not only in the content of the target polymer in the starting material but also in the technological efficiency of its extraction and subsequent modification. The nearly twofold higher overall yield of chitosan (25.0% vs. 10.0%) underscores the strategic importance of using cuticular waste in developing economically and environmentally sustainable technologies.

Physico-Chemical Characterisation of the Extracted Biopolymers: Comparative Analysis of the Quality of Chitin and Chitosan from Different Sources

As the technological suitability of biopolymers is determined by their structural and functional properties, a comparative characterisation of chitin and chitosan obtained from the larval exuviae and pupae of Saturnia *pyri* was undertaken. Analysis of key parameters revealed not only quantitative but also qualitative superiority of the products synthesised from cuticular biomass.

The degree of deacetylation is a critical parameter that defines the biological activity and chemical reactivity of chitosan. It was established that chitosan derived from larval exuviae exhibited a significantly higher degree of deacetylation of 89.5±1.2%. In contrast, chitosan from pupae showed a DD of 75.3±1.8% (Fig. 4). This 14.2% difference is statistically significant ($p<0.05$) and has important implications for practical applications. A high DD (>85%) indicates a greater availability of amino groups, which is a relevant physicochemical parameter for future evaluation in biomedical formulations, including controlled-release systems.

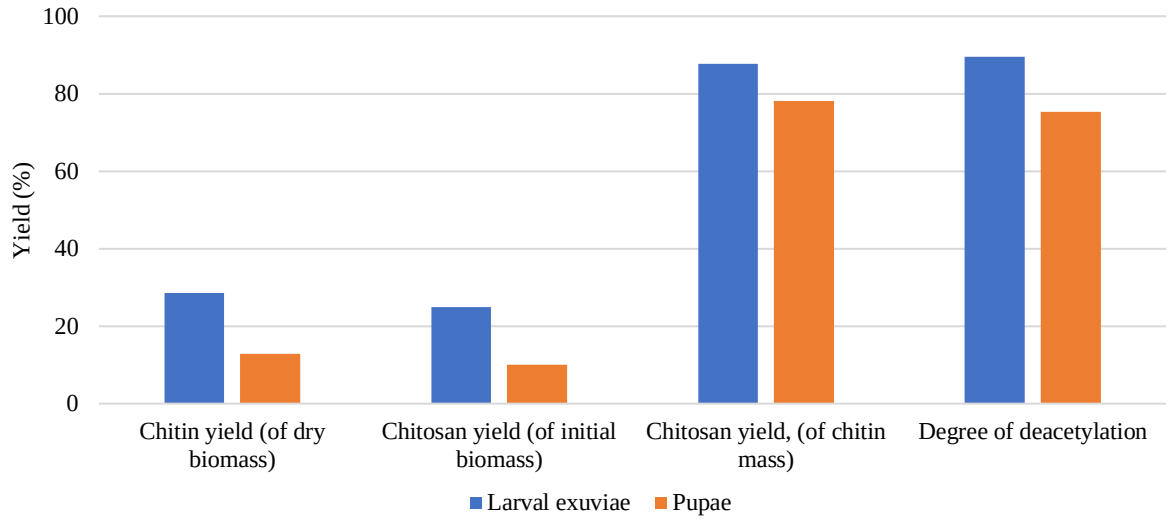

Fig. 4: Comparative efficiency of chitin and chitosan production from larval exuviae and pupae of *Saturnia pyri*

The technological superiority of larval exuviae over pupae as a raw material for the production of chitin and chitosan is evident in Fig. 4. Exuviae-derived material consistently performed better across the important extraction and conversion parameters, suggesting that this biomass source is better suited for producing chitosan with advantageous physicochemical properties. The conclusion that chitin from larval exuviae is more receptive to alkaline modification than chitin from pupae is further supported by the higher degree of deacetylation. The primary conclusion that larval exuviae offer a more effective and structurally promising source of chitosan is generally reinforced by the visual comparison [16].

Solubility and rheological properties are critical for polymer processing. A 1% solution of chitosan in 1% acetic acid, obtained from exuviae, exhibited an average viscosity of 345 cP, indicating an optimal balance of molecular weight and degree of deacetylation, which supports good film- and fibre-forming capacity. In contrast, chitosan from pupae formed solutions with considerably lower viscosity – 128 cP. This may indicate partial depolymerisation during extraction or reflect the initial structural characteristics of pupal chitin. The lower viscosity of pupae-derived chitosan may indicate reduced suitability for applications requiring higher solution viscosity; however, mechanical testing of films is required to confirm this limitation.

Colour serves as a visual indicator of biopolymer purity. Chitin and chitosan obtained from larval exuviae exhibited a uniform white colour with a slight creamy tint (CIE Lab coordinates: L = 92.5, a = 0.8, b = 4.2). In contrast, products extracted from pupae, even after intensive bleaching, retained a noticeable yellowish-brown hue (L = 85.3, a = 2.1, b = 12.5), indicating the presence of residual pigments or Maillard reaction products, which are difficult to remove completely.

Microelement analysis by XRF revealed that chitosan from exuviae contains trace amounts of beneficial microelements, including calcium (0.05%) and magnesium (0.02%). Crucially, the levels of toxic heavy metals such as lead (Pb) and cadmium

(Cd) were below the detection limit (<0.001 mg/kg). Chitosan from pupae showed slightly elevated levels of iron (0.15%) and zinc (0.08%), likely reflecting their higher concentrations in the starting biomass, which should be considered in future application-specific quality assessments.

Overall, the comprehensive characterisation confirms that larval exuviae of Saturnia *pyri* are not only a more efficient but also a qualitatively superior source for the production of high-grade chitosan. The biopolymer obtained from it possesses an optimal combination of properties: a high degree of deacetylation (>89%), favourable rheological characteristics, and high purity and safety. These features support the potential of exuviae-derived chitosan as a candidate material for further application-oriented testing in biomaterials research.

Discussion

The results of this study demonstrate the considerable potential of cuticular chitin from the wild silkworm Saturnia *pyri* as a high-quality source of biopolymers. The yield of chitin from larval exuviae was 28.5%, substantially exceeding the values reported for many other sources in the literature. For instance, [17] report typical chitin yields from insects of 15-20%, while [18] indicate yields of 22%-25% for most insect species. This notable difference may be attributed to the structural characteristics of Saturnia *pyri* cuticle, which contains a higher chitin content and lower mineralisation compared with other insects. The high degree of deacetylation (89.5%) of the resulting chitosan is particularly valuable for practical applications, corroborated by [19, 20], who report a direct correlation between the degree of deacetylation and the biological activity of the material. These values considerably exceed those reported for chitosan from other sources: [21] reports a degree of deacetylation of 75%-80% for chitosan from most insect species, while [22] report 82%-85% for crustaceans. Such a difference may be attributed to the structural characteristics of chitin in the cuticle of Saturnia *pyri*, which render it more accessible to alkaline treatment during deacetylation. The high quality of the starting material is further confirmed by the stability of the physicochemical properties of the resulting biopolymers, meeting the standards established by [23] for the industrial use of chitin and chitosan. These findings support the rationale for optimising the extraction and modification conditions of Saturnia *pyri* chitin for specialised applications.

The rheological properties of chitosan solutions derived from exuviae exhibit optimal characteristics for practical use. The viscosity of a 1% solution is 345 cP, indicating an average polymer molecular weight in the range of 100-300 kDa. This value corresponds to the optimal range required for forming stable film coatings and gels, as reported by [24]. Compared with commercial chitosan derived from crustaceans, which typically exhibits viscosities of 200-400 cP, the obtained material demonstrates competitive performance. The observed rheological properties are compatible with further investigation of the material in formulation-oriented studies. According to [20], chitosan with a viscosity of 300-500 cP is optimal for use as a matrix for controlled drug release and for wound dressing applications. The authors note that such a viscosity provides sufficient flowability for forming thin films while simultaneously maintaining the structural stability of the material.

The mineral composition of the obtained biopolymers is characterised by a low content of toxic elements. Analysis shows that the concentrations of heavy metals (lead, cadmium, mercury) do not exceed 0.001 mg/kg, which is well below the maximum permissible levels for medical materials established in the studies of [25]. Compared with chitosan from other sources, particularly marine organisms where heavy metal accumulation is possible, the material derived from exuviae demonstrates higher purity. The presence of beneficial microelements in the chitosan may enhance its bioactive properties. Moderate amounts of calcium (0.05%) and magnesium (0.02%) were detected, which, according to [26], can synergistically improve the biological activity of the material. Importantly, the microelement composition of the chitosan is natural and balanced, unlike artificially enriched analogues. The absence of significant variation in mineral content between different batches indicates the stability of the production process. As noted by [27], reproducibility of characteristics is a critical factor for the industrial application of biopolymers. These findings indicate promising reproducibility of physicochemical characteristics, which is a prerequisite for future pharmaceutical or medical-material evaluation.

Overall, the results confirm the effectiveness of using Saturnia *pyri* exuviae as an alternative source of chitin and chitosan. Comparison with literature data demonstrates that the quality parameters of the obtained biopolymers are competitive with equivalent products from other sources. According to the research of [28], the yield of chitin from various insect species typically ranges from 15% to 25%, whereas for Saturnia *pyri* exuviae, this value reaches 28.5%. The use of exuviae as a raw material offers significant environmental advantages. As noted by [29], insect cuticular waste constitutes

a renewable resource that does not require additional cultivation costs. This point is reinforced by [30], who emphasises the ecological benefits of utilising such waste compared with traditional chitin sources. The efficiency of the extraction methodology applied in this study is supported by other research. [31] demonstrated that optimal chitin yields from insects are achieved through a combination of deproteinisation and decalcification, which is fully consistent with the methodology used in this study. The quality of the obtained chitosan meets the requirements for subsequent industrial applications. According to [32], insect-derived chitosan can be successfully applied in biomedical and food industries, provided an appropriate level of purification is achieved.

The concept of a circular economy finds practical expression in the use of insect exuviae. As highlighted by [33, 34], converting insect waste into valuable biopolymers is both economically viable and environmentally justified. The results of this study support this perspective, demonstrating the high efficiency of utilising cuticular waste. Comparative analysis of the properties of the obtained biopolymers against commercial samples further reveals their competitive advantages. According to [35], insect-derived chitosan often surpasses products from marine sources in quality due to its higher purity and uniformity of properties. This assertion is supported by the results obtained in the present study, which demonstrate a low content of impurities and stable material characteristics. In a review by [36], contemporary advances in the extraction of chitin and chitosan are systematically summarised, with emphasis on environmentally friendly and energy-efficient methods. The authors critically assess the advantages and limitations of various approaches, including physical, chemical, and biological methods, and stress the importance of optimising process conditions to obtain a product with defined properties. Special attention is given to the potential of alternative sources, particularly insects, which confirms the relevance and appropriateness of the methodology applied here for processing Saturnia *pyri* exuviae.

Thus, the present study confirms the high efficiency of using Saturnia *pyri* exuviae as a promising source of chitin and chitosan with optimal structural and functional characteristics. The results highlight the advantages of this type of raw material compared with traditional sources and other insect species, as evidenced by comparative analysis with literature data. The comprehensive characterisation of the biopolymers considering physicochemical, rheological, and mineralogical parameters provides a scientific foundation for their further practical application across various industrial sectors.

Conclusion

The present study comprehensively demonstrates the high efficiency of using cuticular waste from the wild silkworm Saturnia *pyri* as a promising source of high-quality chitin and chitosan. Detailed analysis of the chemical composition of the raw biomass revealed significant differences between larval exuviae and pupae. The exuviae are characterised by a markedly higher chitin fibre content (17.5% compared with 10.5% in pupae), lower fat content (3.0% vs. 27.9%) and reduced moisture (5.5% vs. 49.0%), which confer clear technological advantages for biopolymer extraction. The mineral profile of the exuviae is distinguished by elevated levels of calcium (1.386 mg/g) and iron (0.135 mg/g) relative to pupae. The yield of purified chitin from the exuviae was 28.5%, substantially exceeding that obtained from pupae (12.8%) and most alternative sources reported in the literature. The efficiency of chitin conversion to chitosan from the exuviae reached 87.7%, whereas for pupae it was lower at 78.1%. The resulting chitosan demonstrated a high degree of deacetylation (89.5±1.2%) and favourable physicochemical properties, which may be relevant for future medical-material studies after appropriate biological and safety testing.

The rheological properties of a 1% chitosan solution are derived from exuviae with a viscosity of 345 cP·s, which suggests potential suitability for further material-formulation studies, but biomedical applicability requires biological, cytotoxic, and mechanical evaluation. Calorimetric analysis confirmed the high degree of purity of chitosan obtained from the exuviae (lightness L* = 92.5, coordinate b* = 4.2), whereas the sample from pupae exhibited a yellowish-brown tint (L* = 85.3, b* = 12.5). Mineralogical analysis further verified the high purity of the product, with toxic metal contents (lead, cadmium) not exceeding 0.001 mg/kg. Beneficial microelements were detected, including calcium (0.05%) and magnesium (0.02%), which may be relevant for future studies evaluating the functional properties of the material. These findings are significant for the development of a bioeconomy, providing an environmentally friendly alternative source of biopolymers. The demonstrated efficiency of using insect life-cycle waste opens new opportunities for implementing circular economy principles in industry and promotes sustainable natural resource management.

Limitations include the need to optimise the scale-up of the technology, to study the stability of biopolymer properties during long-term storage, and to investigate the impact of seasonal variations on raw material quality. Promising

directions for future research include examining the biological activity of the obtained chitin and chitosan; cytotoxicity, antimicrobial, biological activity, and mechanical testing; optimising deacetylation conditions to produce chitosan with a defined molecular weight distribution; and conducting an economic assessment of the technological process under industrial conditions.

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Author's Contributions

Zarintaj Shukurova: Conceptualization, Methodology, investigation, formal analysis, data curation, write original draft, writing review and editing, project administration.
Yusif Shukurlu, Zarbali Khalilov and Madina Sharifova: Data collection, data curation, validation, review and edited.

Ethics

The authors declare that there are no ethical issues regarding the research study.

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