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A Comprehensive Analysis of Chitin Extraction from the Black Soldier Fly for Chitosan Production

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Abstract

Chitin, a versatile biopolymer with applications in biomedicine, antimicrobial agents, and cosmetics, can be sustainably sourced from the black soldier fly (BSF). This alternative is gaining popularity because it not only provides a sustainable supply of raw materials but also possesses chitin properties. Consequently, this broadens the potential applications of chitin in the development of derivative products with varied characteristics. The BSF undergoes complete metamorphosis, consisting of four stages: egg, larva, pupa, and fly. While the extraction method is commonly used to isolate chitin from other sources, a modified approach is necessary for the BSF due to the unique chitin-binding elements present in its biological structures. Given the high fat content in BSF and its metamorphosis stages, separating the fat prior to the extraction process is crucial. Co-fermentation, a biologically driven extraction technique, offers a cost-effective, environmentally friendly alternative with potential for high chitin yields. These findings underscore the potential of BSF as a sustainable chitin source and emphasize the significance of optimizing extraction processes to produce high-quality chitin and chitosan products. There remains considerable scope for future research, particularly in areas such as the identification of effective bleaching agents, optimizing conditions for maximum chitin extraction from BSF, and refining the extraction process to enhance cost-effectiveness.

Keywords

black soldier fly, bleaching, chitin extraction, defatting, demineralization, deproteination

1 Introduction

The prominence of non-renewable fossil-based materials has sparked widespread discussion [1], primarily due to the inevitable depletion of resources such as natural gas, crude oil, olefins and aromatics [2, 3]. Consequently, there is a growing focus on materials derived from biomass (biobased) for research and development [4]. Biobased materials are increasingly attractive for research investigation as they promote sustainability, including the 3R principles of reusing, reducing, and recycling, while mitigating the limitations to fossil-based materials [5, 6]. Their applications span a wide array of sectors, including construction materials, bioenergy, and food industries, among others [7].

One of the versatile biobased materials is chitosan, which is derived from the deacetylation of chitin [8, 9]. The term "chitin" originates from "tunic", meaning protective shell [10]. Chitin, the second most common polysaccharide after cellulose [11], is prevalent in nature, occurring naturally in the exoskeletons of crustaceans [12], crabs [13], shrimp [14], and partially in fungi [15]. It is also referred to as N-acetyl-2-aminocellulose or 2-acetamido-2-deoxycellulose with its formula is $[C_8H_{15}NO_6]_n$. The structure of chitin consists of a series of N-acetyl-β-glucosaminyl units joined by β-(1,4) linkages (Fig. 1) [16–18].

Chitin comprises 47.29% carbon, 6.45% hydrogen, 6.89% nitrogen, and 39.37% oxygen. It naturally exists in

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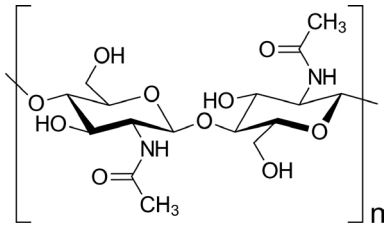

Fig. 1 The structure of chitin

three forms: α-chitin, β-chitin, and γ-chitin [19–21]. These forms differ in the orientation of their microfibrils: α-chitin has an antiparallel chain arrangement, β-chitin has a parallel chain arrangement, and γ-chitin combines both parallel and antiparallel chains [22]. Chitin is characterized by its white, hard, and dense appearance, and it is a nitrogenous polysaccharide [23]. However, its applicability is limited due to its semi-crystalline structure and numerous intermolecular hydrogen bonds, which render it insoluble in most solvents [24].

Meanwhile, chitosan is a derivative obtained through the deacetylation of chitin (Fig. 2). It is characterized by its biocompatibility, biodegradability and non-toxic nature [25]. Typically, chitin undergoes further processing to yield chitosan prior to its application. Chitosan finds wide-ranging applications across various sectors, such as biomedicine, antimicrobial agents, and as a hydrating agent [26].

Chitosan possesses significant biomedical application [27] owing to its polycationic characteristics [28] which enable enables the formation of ionic and hydrogen bonds with drug molecules [24]. Due to its biocompatibility, chitosan serves as an effective drug delivery system, particularly in topical applications [29]. Notably, chitosan holds promise in cancer treatment [30], by facilitating the targeted delivery of cancer drugs and demonstrates antiproliferative effects, thereby reducing cell viability. Additionally, chitosan also supports epithelial cell and collagen regeneration, as well as fibroblast production, further augmenting its therapeutic potential [31].

Besides biomedical applications, chitin and chitosan find widely extensive use as antimicrobial and antifungal agents [30]. Chitosan, in particular, exhibits antimicrobial properties due to its positive charge on the C-2 atom in its glucosamine monomer [32]. These properties are most

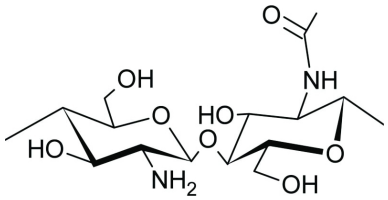

Fig. 2 The structure of chitosan

pronounced under acidic conditions (pH below 6–6.5), as the solubility of chitosan decreases at higher pH levels [33]. Chitosan can interact with cell membranes, altering their permeability. Furthermore, its antimicrobial activity is influenced by factors such as the degree of chitosan polymerization, environmental conditions, and medium composition for bacteria/microbes/fungi [32]. Examples of chitosan derivatives for antifungal use include N-carboxymethyl chitosan (NCMC) [34], and oligomeric chitosans (pentamer and heptamer) [35]. Chitosan is also employed as an antifungal agent in agriculture, inhibiting fungi like *F. oxysporum* in soil during seed planting [36, 37]. For antibacterial applications, chitosan derivatives such as diethylaminoethyl chitin, diethylaminoethyl chitosan, and triethylaminoethyl chitin are commonly employed [38, 39]. Notably, triethylaminoethyl chitin inhibits growth and kills bacteria like *Staphylococcus aureus* and *E. coli* within 2 h at a 500 ppm concentration, showing its ability as rapid bactericidal activity [40]. Furthermore, chitosan is also widely utilized in the cosmetic industry [41], particularly amidst the growing interest in green cosmetic products [42]. Chitosan can serve as a versatile material for various cosmetic formulations, including skin and hair care, moisturizing agents, sunscreens, and more [43–46].

Most chitin is conventionally sourced from marine organisms [47]. However, the utilization of chitin derived from insects, particularly the black soldier fly (BSF), presents a promising avenue for sustainable chitin sourcing. The utilization of BSF offers significant advantages as it enables the straightforward cultivation of insects by converting organic waste into high-value products. Employing BSF as the primary organism for chitin extraction not only facilitates the creation of valuable materials but also addresses pressing environmental concerns. Moreover, while chitin and chitosan sourced from crustaceans are extensively utilized worldwide, the availability of crustaceans is often seasonal and not consistently reliable. Consequently, the potential to produce chitin and chitosan using BSF as the primary material offers an alternative approach, complementing rather than replacing current production methods reliant on crustaceans [15]. Therefore, the potential to produce chitin and chitosan using BSF as the main material presents an alternative avenue, although it does not intend to replace current production methods utilizing crustaceans. Consequently, these production methods can operate simultaneously to meet the growing demand for chitosan in the global market in the near future.

In general, the production of chitin entails a multi-stage process. Initially, the biobased material containing chitin undergoes drying to reduce its moisture content [48]. Subsequently, the material is subjected to demineralization using acids such as formic acid [49] or HCl [50], with demineralization typically lasting approximately 1 h. Following demineralization, the material is washed to neutral pH using demineralized water. The demineralized and dried material is then deproteinized using NaOH [51] or KOH [52]. The demineralized material is subsequently deproteinized using NaOH to remove protein present in the material [47]. Next, the deproteinized material undergoes further processing called bleaching, involving hydrogen peroxide as a bleaching agent. This is followed by thorough washing and drying [53]. Section 3 will provide a comprehensive overview of the entire extraction process, delving into the intricacies of each stage and their respective significance in chitin production.

The results of chitin extraction from crustaceans and the BSF can indeed manifest disparities, as evidenced by recent research findings. Studies have indicated that the chitin extraction from the BSF can yield as much as 21.3% from adult specimens, including dead specimens, surpassing the yields obtained from certain crustaceans. Moreover, since chitin can be converted into chitosan, recent discoveries suggest that the final product derived from BSF chitosan demonstrates a higher adsorption capacity compared to chitin from crustaceans [54]. This observation underscores significant potential for further exploration and potential industrial-scale production of chitin and chitosan from the BSF. Understanding why the chitosan product derived from the BSF demonstrates superior properties compared to that from crustaceans could herald advancements in chitin and chitosan production processes.

This review paper will delve into the specifics of chitin extraction from the BSF, providing an introduction to the BSF and elucidating the primary and additional processes entailed in extracting chitin from this creature. The BSF has demonstrated significant chitin potential throughout its lifecycle. Previous research has extensively analyzed the surface morphology of chitin from different BSF stages, revealing varying surface morphology [51]. Specifically, larvae, prepupae, pupae, shedding, and cocoon materials exhibited hexagonal honeycomb-like surfaces with varying hexagon size, whereas the fly material has showcased irregular surface patterns [51].

2 BSF overview

2.1 Lifecycle

The BSF, a member of the subfamily *Hermetiinae* within the order *Diptera*, originally hails from the America and the Neotropics region [55–58]. Notably, BSF larvae exhibit remarkable capabilities in recycling animal tissue and processing a diverse array of waste materials, including domestic waste, chicken, pig/cow manure, and even human excreta [58, 59]. Unlike other fly species, BSF is not attracted to human food or living environments [56].

The BSF undergoes a complete metamorphosis, progressing through four distinct stages: egg, larva, pupa, and adult fly [58, 59]. The larval and pupal stages typically span a longer duration, while the egg and adult fly stages are relatively shorter [40]. A female adult BSF can lay approximately 320–620 eggs per day [60], with eggs hatching within 8–20 h, and the majority hatching within four days. Interestingly, merely 1 g of fly eggs can yield 30–40 kg of maggots (larvae) [61]. This hatching process is influenced by relative humidity and temperature levels in its environment.

Once hatched, a BSF larva begins consuming various organic materials to fuel its growth. As it develops, its exoskeleton undergoes a noticeable transformation, becoming harder, thicker and dark in color. Newly hatched larvae typically measure between 2 to 5 mm, with subsequent shedding of their skin. Larvae possess a mouth at the anterior and an anus at the posterior end, as well as a respiratory system located at the posterior. They also feature segmented muscles, a digestive system, intestines, and a pair of salivary glands situated between the head and tail regions. Larvae continue to molt as they reach lengths of 10 mm and 15–20 mm. Upon completing their final molt, larvae transition to the pupal stage [62, 63]. Pupation usually takes 4 days, depending upon ambient temperature. Pupae have a hard outer skin known as puparium. The transformation from pupae to adult flies typically takes 4–6 days. Newly emerged adult flies lack mouths and digestive systems, resulting in a markedly short lifespan [64]. Flies possess antennae consisting of 8 flagellomeres, with their longest part [65]. They engage in activities such as flying, mating, and egg-laying. Typically, flies are often kept in mesh cages ranging from 4 to 20 m^3, providing access to sunlight/LED lighting and plants. Their lifespan typically ranges between 6–8 days, although this duration may decrease in unsupportive environmental conditions [40].

It is crucial to highlight the remarkable breeding capabilities of the BSF. This species reproduces rapidly and requires minimal space for breeding. Moreover, their food source can be obtained from abundant organic waste, which is often readily available at low cost or even for free. Establishing a suitable breeding environment for BSFs is relatively straightforward, with the primary requirement being to ensure that the environment meets the specific needs of each life stage of the BSF. The unused byproducts of BSF production, such as cocoons or sheddings, contain a substantial amount of chitin derived from their exoskeletons. These byproducts are plentiful due to the rapid reproduction rate of BSF. In comparison, materials sourced from crustaceans are noted for their somewhat seasonal production, limiting their availability to certain months. Conversely, BSF can thrive year-round without the need to wait for specific seasons. This continuous availability makes BSF a reliable and sustainable source for chitin extraction, offering significant advantages over traditional sources.

2.2 BSF compositions

Throughout the BSF life stages, they contain various components such as protein, fat, fiber, carbon/ash, and chitin. Chitin, found predominantly in their exoskeletons, can constitute up to 35% of the material extracted from this part of the BSF [66, 67]. The composition of a BSF fly is presented in Table 1 [68]. Research conducted by Soetemans et al. analyzed chitin content at different BSF life cycle stages, with a comparative analysis presented in Table 2 [51]. Based on the composition data, the fly and pupal stages of the BSF have significant potential for yielding chitin compounds. While the larval and prepupal stages contain chitin, it is in comparatively lower quantities. The shedding (molted larval skin) and the cocoon (hard pupal covering) have the highest chitin content. Due to their compositions, particularly the high protein content (approximately 32–44%), BSF prepupae are often used as animal feed [63].

Table 1 Composition of BSF in dry basis [68]

Parameter	Value (g kg^{-1})	Composition (%)
Crude protein	431	45.3
Crude fat	386	40.5
Crude fiber	41	4.3
Ash	27	2.8
Chitin	67	7.1

Table 2 Chitin composition in various stages of BSF life cycle on a dry basis [51]

Life stages	Chitin (%)*	Chitin (%)**
Larvae	9.5	7.8
Prepupae	9.1	10.9
Pupae	10.3	10.7
Fly	5.6	8.4
Shedding	31.1	23.7
Cocoon	23.8	22.4

* Chitin from the gravimetric method
** Chitin from glucosamine measurement

3 BSF chitin extraction

3.1 Pre-treatment

The pre-treatment process is crucial for optimizing the chitin extraction from the BSF. Various pre-treatment methods have been explored to enhance the efficiency and yield of chitin extraction. These methods typically involve size reduction (grinding) and drying. Researchers employed a drying process at 105 °C for 48 h for BSF material [51]. The dried particles were subsequently crushed with a mortar and passed through a wire mesh, resulting in the following particle size distribution as follows: 25% > 2 mm, 49% between 2 and 1 mm, 17% between 1 and 0.5 mm, and 9% < 0.5 mm, corresponding to a mesh range of approximately 8–32 mesh [51]. In contrast, Guarnieri et al. dried BSF material at 60 °C for 48 h, then finely ground it with a laboratory blender before being used for chitin extraction [49]. Similarly, by Triunfo et al., drying was conducted at 60 °C for 24–48 h for both cocoon and BSF materials. The dried material was subsequently crushed using a mortar [53]. Meanwhile, Khayrova et al. adjusted the particle size of BSF-based material to a finer range of 35–70 mesh before using it for the extraction process [52]. Due to its size, the yield from Khayrova et al. [52] is higher than that from Soetemans et al. [51], reaching approximately 57% with a 35–70 mesh. However, it is important to note that the result from Khayrova et al. [52] was achieved without a defatting process. Accounting for defatting, the chitin yield would be around 17–19%.

3.2 Demineralization

The demineralization method is a crucial step in the extraction process aimed at removing inorganic materials, particularly calcium carbonate, from the chitin-containing material [48]. This step can be accomplished through either chemical or biological methods [50]. Chemically, demineralization is typically achieved using acids such as HCl, formic acid, H_2SO_4, HNO_3, or acetic acid. In a standard chemical demineralization process, the chitin-containing material is mixed with 1 M HCl at a 1:10 (w/v) ratio

and stirred for 1 h at 30 °C. Following this step, the demineralized solid material is thoroughly washed with deionized water until it reaches a neutral pH [51]. The chemical demineralization process is illustrated in Fig. 3.

In a biological approach to demineralization, lactic acid bacteria are employed to remove inorganic materials from chitin-containing substrates. Commonly used strains include *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* [69]. The biological demineralization process commences with the incubation of a fermentative culture medium (sample, water, and bacterial broth) for 7 days at 30 °C under environmental conditions of 5% CO_2. Afterward, the fermentative culture medium is centrifuged at 3000 rpm for 5 min to separate the solid from the liquid medium. The remaining solids are then washed with deionized water until achieving a neutral pH [69], similar to the chemical approach. The biological demineralization process is illustrated in Fig. 4.

The success of the demineralization process is evaluated through ash content analysis, which measures the amount of inorganic material remaining after the chitin is degraded at high temperatures. Demineralized products typically have an ash content of around 31–36% [21]. This process is crucial as it directly impacts the quality of the subsequent chitosan product; residual inorganic material can significantly affect the viscosity and solubility of chitosan [48].

Researchers have employed various factors, such as solid-to-acid ratio, demineralization time, and type of acid used to optimize the demineralization process. For instance, Soetemans et al. [51] utilized a solid-to-liquid ratio of 1:10 and 1 M HCl to demineralize BSF cocoon, larva, and fly materials. Marei et al. [48], on the other hand, employed a solid-to-liquid ratio of 1:15 and 1 M HCl. Sagheer et al. [50] utilized a solid-to-liquid ratio of 1:40 and experimented with HCl concentrations ranging from 0.25 to 3 M. Triunfo et al. [53] opted for formic acid for demineralization. Additionally, Khayrova et al. [70] utilized a 1% HCl concentration for demineralization with a solid-to-liquid ratio of 1:10 (w/v), resulting in a 74% demineralization yield. These variations in demineralization parameters underscore the flexibility and potential for optimization in the demineralization process, allowing researchers to tailor the method to specific research objectives and material characteristics.

3.3 Deproteinization

The deproteinization method constitutes a critical series of chitin extraction steps, aimed at removing proteins from the chitin-containing material [53]. This step can be achieved through either chemical or biological methods [50]. Chemically, deproteinization is typically performed using bases such as NaOH and KOH [70]. The deproteinization process involves stirring the chitin-containing material

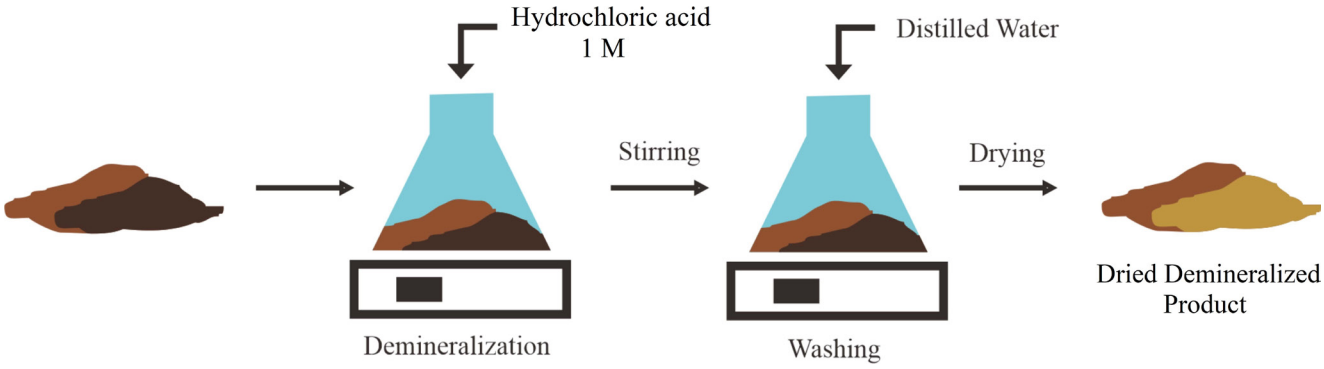

Fig. 3 Demineralization through chemical ways

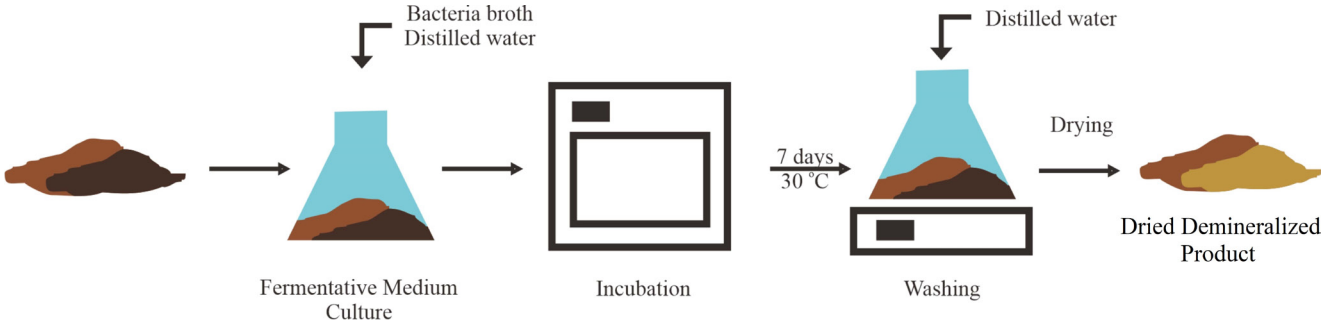

Fig. 4 Demineralization through biological ways

(which can also be solid material from demineralization) mixed with 1 M NaOH at a 1:25 (w/v) ratio for 1 h at 80 °C [51]. Subsequently, the solid material is washed until it reaches a neutral pH using 96% ethanol and deionized water [51]. This chemical deproteinization process ensures the removal of proteins, enhancing the purity of the extracted chitin. The procedure is illustrated in Fig. 5.

Biological methods for deproteinization utilize microorganisms to break down and remove proteins. These methods are more environmentally friendly but may require longer processing times compared to chemical methods. Protease-producing bacteria can be used for deproteinization process, include *Pseudomonas aeruginosa*, *Pseudomonas maltophilia*, *Serratia marcescens* and *Bacillus subtilis* [71]. The biological deproteinization starts with a fermentation process of the mixture (sample, sugar solution, and bacterial broth/inoculum) for 7 days at 30 °C with a shaking speed of 180 rpm [71]. The biological deproteinization process is depicted in Fig. 6.

The deproteinization process, a crucial step in chitin extraction, varies significantly across different studies in terms of solid-to-liquid ratio, deproteinization time, process temperature, and the type of base used. For instance, Soetemans et al. [51] used a solid-to-liquid ratio of 1:25 with 1 M NaOH (80 °C; 1 h) to deproteinize BSF cocoon, larva, and fly materials. Marei et al. [48] performed deproteinization using 1 M NaOH (100 °C; 8 h). Sagheer et al. [50] utilized a solid-to-liquid ratio of 1:20 with 1 M NaOH (70 °C; 1 day). Khayrova et al. [52] employed KOH for deproteinization and achieved a 77% deproteinization yield. These variations underscore the adaptability and optimization potential of the deproteinization process, which can be tailored to specific research objectives and material characteristics.

Indeed, an increasingly favored approach in chitin extraction is the shift towards environmentally friendly techniques, particularly those utilizing microorganisms. This transition is driven by the negative environmental impacts associated with chemical processes, which often leave residual chemicals harmful to the environment and entail additional costs for chemical procurement. Moreover, since chitin derivatives are extensively used in medicine and cosmetics, the presence of chemical residues can significantly compromise the quality and safety of the final products. Therefore, microbial-based techniques not only enhance environmental sustainability but also ensure the integrity and suitability of chitin derivatives for medical and cosmetic applications.

One promising approach developed by Xiong et al. involves utilizing of *Acetobacter pasteurianus* AS1.41 and *Bacillus subtilis* S4 to remove minerals and proteins from samples [66]. This microbial-based method offers potential advantages in terms of economic viability and environmental friendliness in the long term. The observed

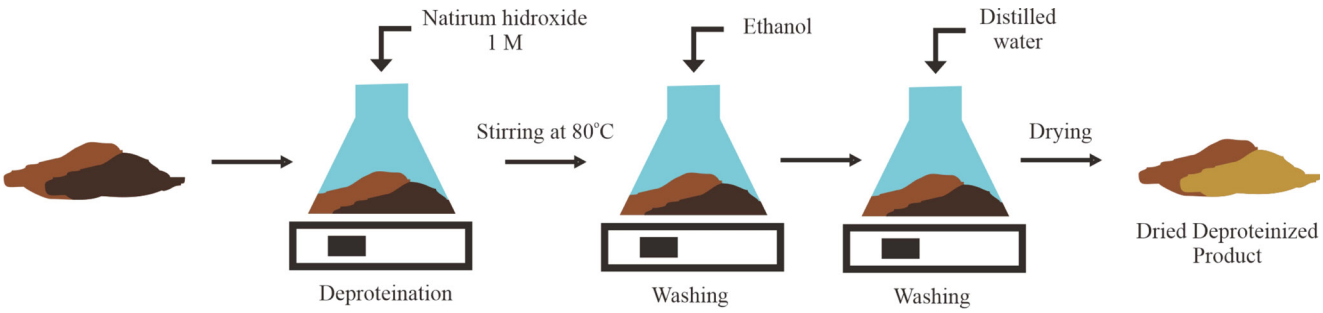

Fig. 5 Deproteinization through chemical ways

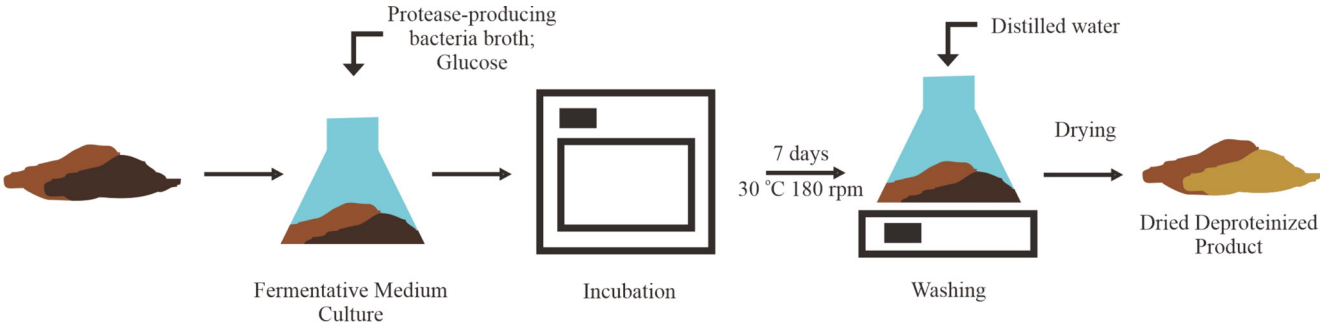

Fig. 6 Deproteinization through biological ways

chitin yield, ranging from 47.31 to 59.9% [66], reflects the effectiveness of this approach. However, a notable drawback of this extraction method is the time required to obtain the chitin, which typically spans roughly five days. Despite these limitations, the overall benefits in terms of sustainability and efficiency make microbial-based techniques giving an attractive option for chitin extraction.

3.4 Bleaching (depigmentation)

Bleaching, also referred to as depigmentation, is an essential step in chitin extraction aimed at removing pigments from insect exoskeletons. The pigmentation in insects, including the BSF, is primarily due to melanins and ommochromes. Melanins in BSF are classified based on their water solubility, typically eumelanins or semiquinones in nature [72–74]. Ommochromes, characterized by a phenoxazine structure, are another significant group of pigments found in BSF, categorized further into dimers and oligomers of kynurenine. These pigments not only determine the coloration of BSF but also hold potential as renewable pigment sources for future applications [75, 76].

The necessity of a bleaching step in chitin extraction depends on the desired color of the final product [77]. Several methods can be employed for the bleaching process, with many of them utilize different biomass excluding BSF. These methods include the use of compounds such as acetone and ethanol mixture [78, 79], chloroform plus alcohol solutions [21, 80–82], P_2O_5 [77, 83, 84], $KMnO_4$ [85–90], oxalic acid [85, 91, 92], NaClO [78, 79, 93–95], H_2O_2 [14, 47, 53, 96–101], and H_2O_2 with added NaOH [102] or make use of a solvent such as acetone [78, 103] or ethyl acetate [14].

In research focusing on chitin extraction from BSF, the use of bleaching methods has been relatively limited. H_2O_2 has been used as a bleaching agent to produce chitin on the BSF [98]. For example, a 3% of H_2O_2 was put into the bleaching process in 90 °C for 60 min, resulting in a significantly whiter chitin compared to methods using microbes. Attempts to introduce additional agents such as $MgSO_4$ and NaOH at varying temperatures yielded similar color outcomes to those achieved using only H_2O_2. Another study used 30% of H_2O_2 for 48 h, with no report about the color change from pupal shell to chitin. An analysis of unbleached and bleached chitin was made in *Hermetia illucens* using 5% (v/v) H_2O_2 for approximately 30–60 min of bleaching while the temperature was 90 °C, showing that bleaching significantly improved the color of chitin and chitosan, transforming them from dark brown (attributed to the physical characteristics of the larvae, pupal exuviae and dead adults) to white and colorless. Importantly, the yield of decolorization remained unchanged, as confirmed through characterization analyses using Fourier-Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD) [53].

The decolorization, or bleaching, process of chitin extracted from BSF typically involves treating the chitin obtained from demineralization and deproteinization with a solution of H_2O_2 at a specific concentration. This step usually takes around 1 h to 48 h, depending on the color requirement for the chitosan product. After this process, the bleached chitin is filtered, washed with distilled water to remove the impurities and H_2O_2 and dried at 60 °C to obtain chitin with reduced coloration. A schematic depiction of the bleaching process can be seen in Fig. 7.

To date, research efforts concerning different bleaching agents and optimizing the bleaching process for chitin extraction have been scarce. While H_2O_2 is commonly used for bleaching chitin, its high-temperature requirements can result in decomposition into other chemical substances [104]. Therefore, new research efforts should consider varying the temperature to investigate its influence on the color and structure of chitin post-extraction. Moreover, the bleaching process effect on proximate analysis, including lipids, proteins, or ash content, may vary based on the processing step or temperature variations. Additionally, simultaneous bleaching with other procedures, such as the deproteinization process, could optimize

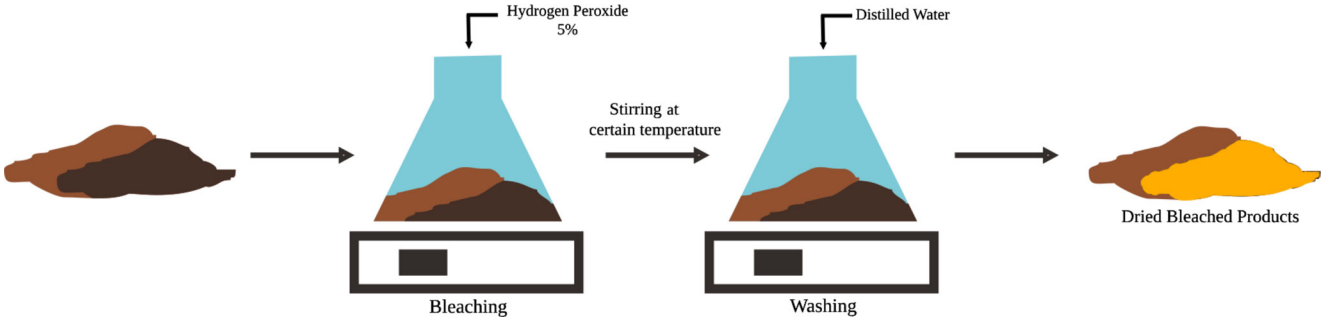

Fig. 7 Bleaching process for chitin extraction

the overall extraction process. This approach is supported by with previous research that utilized NaOH as an additional chemical substance in the H_2O_2 system [102]. These considerations can enhance the efficiency and effectiveness of chitin extraction processes while minimizing potential drawbacks associated with high-temperature bleaching.

3.5 Defatting

In the larval stage of the BSF, conducting a defatting treatment prior to chitin extraction is essential to prevent potential interference with the subsequent deproteinization process. This precaution is particularly crucial due to the saponification reaction that occurs between alkali agents used in the chemical deproteination process and fats present in the material. Consequently, most researchers prioritize defatting as a preparatory step before deproteinization to ensure optimal results. Defatting involves the extraction of fat from the BSF material, with the extracted fat serving various purposes or contributing to enhancing chitin purity before or after extraction. Several defatting methodologies are employed in chitin extraction processes. One approach involves utilizing KOH and NaOH, which can concurrently facilitate defatting and deproteinization. However, adjustments are often necessary to optimize the deproteination process in such cases [105]. Alternatively, lipid extraction can occur just before the primary chitin extraction process (demineralization and deproteinization). Common solvents such as ethanol [106], hexane [107] or petroleum ether [72] offer options for lipid extraction, albeit with safety considerations due to flammability and toxicity, particularly hexane or petroleum ether. Furthermore, employing a supercritical method presents another viable option for defatting. For instance, supercritical carbon dioxide has been utilized for this purpose, involving a 2 h treatment under high pressure (372 bar) at 65 °C [108].

3.6 The sequential step for extraction

As mentioned in Section 2.2, the BSF exhibits varying compositions of fat, protein, ash, and color throughout its life cycle. It is necessary to ensure the attainment of satisfactory chitin quality, the optimization of processes such as demineralization, deproteinization, defatting, or bleaching, particularly in their sequence. Several studies have been conducted to explore and refine these processes to enhance chitin extraction efficiency. One such study adopted a systematic approach to extract fat from BSF larvae, prepupae, and pupae, followed by protein separation [109]. This separation yielded solids that could be further demineralized and deproteinized to obtain chitin from larvae, prepupae, and pupae, respectively, at 3.85%, 4.72%, and 6.31% per dry biomass weight. In addition, research by Pedrazzani et al. [110] focused on chitin extraction from BSF larvae and puparia, employing both enzymatic and chemical processes. The study explored variations in process repetition, combinations of enzymatic and chemical methods, and the inclusion of initial mechanical or ultrasonication treatments. The results revealed varying chitin yields across different extraction methodologies, with the highest yield achieved through a two-stage chemical extraction process combined with enzymatic extraction (referred to as three-stage chemical and enzymatic extraction), yielding 77.9 g/100 g of dry matter extract on a proximate basis. Consequently, these findings underscore the importance of selecting appropriate process treatments to optimize chitin production from BSF. The diverse outcomes observed across different extraction methodologies highlight the potential for further refinement and optimization, offering promising avenues for future research in enhancing chitin extraction efficiency from BSF. To date, state of the art of chitin extraction from BSF in any life-stages treatment can be seen in Table A1 [51, 52, 54, 66, 70, 72, 98, 99, 108, 109, 111–123].

4 Conclusion and future research

BSFs represent a valuable resource for the extraction of chitin, which can be further processed into chitosan. Chitin content is found throughout the life cycle of black soldier flies, so it can be said that BSF is a source of chitin that is cheap and easy to obtain. Demineralization and deproteinization are two typical techniques used to extract chitin from various sources. However, when it comes to BSF, additional steps such as degreasing and decolorization are necessary to ensure the chitosan product meets the desired standards.

Future research in this area focuses on optimizing the chitin extraction process by determining the most effective sequence of steps, the possibility of combining certain processes, and exploring low-energy methods or the transition from chemical to biological techniques. This shows a shift towards more sustainable and environmentally friendly extraction methods. By perfecting and optimizing the chitin extraction process from BSF, the development of chitosan-based products will have the opportunity to develop further.

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Appendix

Table A1 State of the art of chitin extraction from BSF basis material

Sample	Pretreatment	Treatment						Yield (%)	Literature
		Demineralization		Deproteination		Additional step			
		Chemical	Conditions	Chemical	Conditions	Chemical	Conditions		
Larvae	Drying 105 °C, 48 h, then sieving process	HCl 1 M	1:10 (m/v), 1 h, room temperature	NaOH 1 M	1:25 (w/v), 1 h, 80 °C	–	–	7.8	[51]
Prepupae								10.9	
Pupae								10.7	
Shed-ding								23.7	
Cocoon								22.4	
Flies								8.4	
Larvae	Blaching	HCl 2%	20 °C, 2 h	NaOH 5% (w/w)	50 °C, 2 h	Defatting process using $CHCl_3$-CH_3OH	20 °C, 4 h	46	[70]
Pupal exuviae	Cleaned with ethanol and water, dryed at 60 °C, grounded	HCl 1 M	Room temperature, 1 h	NaOH	80 °C, 24 h	H_2O_2 depigmentation	80 °C, 2.5 h	–	[111]
						Water depigmentation	100 °C, 24 h		
						$KMnO_4$ 1% depigmentation	80 °C, 20 min		
						Oxalic acid 4%	80 °C, 1 h		
Larvae	Lyophilised, grinding	HCl 1 M	100 °C, 1 h	NaOH 1 M	80 °C, 24 h	–	–	3.85	[109]
Prepupae								4.72	
Pupae								6.31	

Table A1 State of the art of chitin extraction from BSF basis material (continued)

Sample	Pretreatment	Treatment						Yield (%)	Literature
		Demineralization		Deproteination		Additional step			
		Chemical	Conditions	Chemical	Conditions	Chemical	Conditions		
Prepupae	Grinding	HCl 2 N	Room temperature, 24 h	NaOH 1 M	40 °C, 1 h	Lipid extraction with petroleum ether	40 °C, 1 h	11.7	[72]
Larvae	Washing, drying, grinding	HCl 2 M	55 °C, 1 h	NaOH 2 M	50 °C, 18 h	NaClO depigmentation	40 mL, 10 times	3.6	[112]
Prepupae								3.1	
Puparium								14.1	
Adult flies								2.9	
Pupal exuviae	Grinding until 20 mesh	HCl 1 M	100 °C, 30 min	NaOH 1 M	80 °C, 24 h	$KMnO_4$ 1% depigmentation	1 h	9	[113]
Imago								23	
Pupal exuviae	Washing, drying in sunlight, grinding	HCl 1 M	Boiled, 2 h	NaOH 1 M	Boiled, 4 h	–	–	–	[114]
Pupal shell	Washing, drying at 65 °C, grinding	–	–	–	–	$NaHCO_3$ 0.5% H_2O_2 30% bleaching	1:10 (w/v), 75 °C 48 h	–	[99]
Pupal exuviae	Crushing	H_3PO_4 H_2SO_4 HNO_3 HCl CH_3COOH HCOOH	40 or 70 °C, 2 h	NaOH (optimum in 2 M and 1.25 M)	50-90 °C, 1–4 h	Using ten different solutions for bleaching	–	14 (optimum condition)	[98]
Pupal shell	Grinding with blender	HCl 1 M	Boiled, 2 h	NaOH 1 M	Boiled, 4 h	–	–	–	[115]
Pre-pupae	Lipid removal	HCl 1 M	100 °C, 1 h	KOH 1 M	80 °C, 2 h	–	–	–	[116]
Pupae	Drying	CH_3COOH	Room temperature, 72 h	Na_3PO_4 buffer	37 °C, 72 h	–	–	65–70 (with impurities)	[108]
Larvae	–	HCl 1 M	1:100 (w/v), 95 °C, 1 h	NaOH 1 M	80 °C, 24 h	–	–	–	[117]
Pupal exuviae	Grinding until 35–70 mesh	HCl 1%	Room temperature, 2 h	KOH 5% (w/v)	50 °C, 2 h	Deffating process, using stronger KOH and NaOH	–	30	[52]
Dead imagoes								9	
Pupal exuviae	Drying 60 °C, 24 h	HCl 1 M	Room temperature, 1 h	NaOH 1 M	80 °C, 24 h	$KMnO_4$ 1% depigmentation	–	–	[118]
Dead imagoes									
Puparia	Drying 60 °C, grinding	HCl 5%	Room temperature	NaOH 2%	70–90 °C, 2 h	H_2O_2 30% bleaching	1:20 (w:v), 90 °C, 45 min	23.82	[66]
Adult flies								11.99	
Larvae	–	–	–	–	–	–	–	5	[119]

Table A1 State of the art of chitin extraction from BSF basis material (continued)

Sample	Pretreatment	Treatment						Yield (%)	Literature
		Demineralization		Deproteination		Additional step			
		Chemical	Conditions	Chemical	Conditions	Chemical	Conditions		
Prepupae	Washing, boiling for 10 minutes	HCl	100 °C, 20 min	NaOH	80 °C	NaOCl 0.315%	–	18.05	[120]
Pupae shells	–	HCl 1 M	50 °C, 100 min	NaOH	85 °C, 10 h	Bleaching process	–	13.5	[121]
Adult flies								5	
Flake	Drying 50 °C, grinding	HCl 1 M	100 °C, 30 min	NaOH 1 M	24 h	–	–	22	[122]
Puparia								25	
Adult flies								7	
Larvae								3	
Prepupae	Cleaning, drying 65 °C, grinding	HCl 1 M	1:10 (w/v), room temperature, 2 h	NaOH 1 M	80 °C, 6 h	$KMnO_4$ 1% depigmentation	1:30 w/v, 4 h, room temperature	5.4	[123]
Pupal exuviae								18.8	
Imagoes								11.8	
Dead flies	Grinding, washing	HCl 5%	Room temperature, 2 h	NaOH 5%	100 °C, 3 h	–	–	21.3	[54]