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Chemical viz biological method for the extraction of chitin and chitosan from adults of the black soldier fly (*Hermetia illucens*)

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ABSTRACT

The black soldier fly -BSF- (*Hermitia illucens*) offers an environmentally responsible and sustainable substitute for chitin and chitosan, which are valuable polysaccharides with broad applications in pharmaceuticals, agriculture, and food technology. This study aims to compare chemical and biological techniques to isolate chitin and chitosan from adult flies, evaluating their antibacterial properties. In the chemical method, demineralization and deproteinization were achieved using HCl and NaOH, whereas the biological method employed protease-producing *Streptomyces* spp. for protein removal and *Lactobacillus acidophilus* for demineralization. Biological extraction produced higher yields of chitin (32%) and chitosan (26.6%) than chemical extraction (29% and 23%, respectively). Analysis using FTIR confirmed that distinctive functional groups were present and effective deproteinization, with biological samples retaining better structural integrity. XRD verified the existence of α-chitin in both techniques, exhibiting higher crystallinity in the chemical extracts, favoring industrial uses, and more native structures in the biological extracts, suitable for biomedical applications. SEM revealed fibrous and porous morphologies in chitin extracted by the biological method, while chitin extracted by the chemical method appeared denser. Biologically derived chitosan exhibited high antibacterial properties, particularly towards *Staphylococcus aureus* and *Klebsiella pneumoniae*. These findings highlight biological extraction as a sustainable strategy that improves yield, preserves bioactivity, and broadens the potential of BSF for advanced applications.

1. Introduction

Chitin and chitosan are natural biopolymers recognized for their significant biological activity and extensive applications in food, medicine, and industry. These biopolymers are predominantly sourced from crustaceans, fungi, and insects [1]. Chitosan obtained from insects has garnered significant interest as a cost-effective and eco-friendly alternative to traditional sources like crustaceans, presenting reduced allergic concerns compared to shellfish-derived chitosan [2]. Furthermore, BSF can be cultivated on organic waste, rendering their biomass a sustainable and environmentally acceptable resource for chitosan production [3]. The lack of seasonal constraints and the elevated reproductive rates of insects render them a dependable source throughout the year [4]. The process of extracting chitin and chitosan from insects comprises several essential stages: delipidation, deproteinization, demineralization, and deacetylation. Various methods—chemical, enzymatic, and microbial—have been devised to optimize the process, enhancing yield while preserving the structural integrity and functional qualities of chitosan [2]. The biological extraction of chitin provides an environmentally friendly approach utilizing enzymes for selective breakdown [5]. The application of biological technologies for the extraction of chitin and chitosan may reduce dependence on acidic and alkaline procedures that are less environmentally sustainable. Consequently, it is imperative to optimize biological processes for the production of chitin and its associated chemicals. Protease-producing bacteria and lactic acid have both been utilized in the processes of mineral and protein removal [6]. Protease-producing and lactic acid-producing bacteria were employed in a 10-day biological chitin extraction process for deproteinization and demineralization, respectively [7]. Nonetheless, the reaction time of the biological process and the residual protein concentrations are excessively elevated, and it is costlier than the chemical method. These characteristics limit the industrial usability of the technique [8]. The proteolytic protease from *Streptomyces* sp. comprises a blend of exo- and endo-proteases, containing a minimum of 10 proteolytic components. Due to its wide selectivity, it can cleave nearly any peptide bond and is employed when comprehensive or complete protein degradation is required [9].

The antibacterial capabilities of chitosan are particularly beneficial in addressing antibiotic-resistant infections [10,11]. Chitosan is a crucial compound in the pharmaceutical and biomedical sectors owing to its notable antibacterial characteristics [10]. Numerous studies have examined the antibacterial properties of chitosan produced from black soldier flies (BSF) [12,7,13]. This work

sought to compare biological and chemical approaches for the extraction of chitin and chitosan from BSF adults, define their physicochemical features, and evaluate their antibacterial effectiveness. This is, to our knowledge, the inaugural paper demonstrating the potential of *Streptomyces* spp. for the biological extraction of chitin from mature BSF.

2. Materials and Methods

2.1. Experimental insects

BSF adults were obtained from Egymag company in Banha, Qalubia Governorate, Egypt.

2.2. Bacterial isolates

Microbial strains employed in the antimicrobial assay are Gram-positive *L. acidophilus* and *S. aureus,* as well as the Gram-negative strains, *Pseudomonas aeruginosa, Salmonella* spp., and *K. pneumoniae* were obtained from the Department of Microbiology, the National Research Center, Dokki branch, and were kept at -20 °C until used.

Chitin was physiologically isolated using protease-producing *Streptomyces* species. The bacteria were extracted from ten grams of soil samples and subsequently introduced into 90 mL of sterilized NaCl solution (0.85% w/v). Streptomycetes were isolated by plating suspensions with the serial dilution method established by Hayakawa and Nonomura [14]. Petri dishes were prepared one day prior to plating and incubated overnight at 37 °C to remove moisture films from the agar surface [15]. Each plate received 0.1 mL of the diluted bacteria, which were subsequently spread using a sterile glass rod. Streptomycetes were isolated utilizing starch nitrate agar media [16]. The inoculation plates were incubated at 28 °C to facilitate the growth of slow-growing organisms, with Streptomycetes assessed after two to three weeks. Streptomycetes were isolated on agar slants identical in composition to the original plating medium. The isolates were further purified using starch nitrate agar. The inoculating plates were replicated and streaked on agar plates for purification. Single colonies were excised from agar slants and subcultured.

2.3. Extraction of Chitin and Chitosan

The process of extracting chitin and chitosan from insects typically involves a multi-step methodology comprising delipidation, demineralization, and the deacetylation of chitin to produce chitosan. Chemical and biological methods were employed in this study to extract chitin and chitosan from black soldier fly (BSF).

In both methodologies, deceased individuals were rinsed with tap water, followed by distilled water, and then dried in an oven at 80°C for 4 hours. They were subsequently manually pulverized

in a mill for 30 minutes. The resultant material was sieved through meshes with a 100 μm mesh size to eliminate contaminants.

Lipids may hinder subsequent processes; hence, the delipidation method is essential. Ravi *et al.* [17] reported the addition of approximately 50 mL of chloroform to a 50 g sample at 50 °C, followed by agitation for two hours. The mixture underwent filtration, after which the precipitate was isolated and dried in an oven at 80 °C for 2 hours.

2.4.1. Chemical extraction

2.4.1.1. Demineralization

Demineralization was conducted according to the method of Marei *et al.* [18] using a solid-to-liquid ratio of 1:12 with 5.0% HCl at ambient temperature for 2 hours. The precipitate was rinsed with distilled water until a neutral pH was achieved. The pH was assessed using a pH meter (HANNA Instruments, HI28197, Italy).

2.4.1.2. Deproteinization

Deproteinization involved treatment with 1 M NaOH (solid-to-liquid ratio of 1:12) for 3 hours at 90 °C with continuous stirring. The procedure was reiterated until the color was absent, following the methodology of Sagheer *et al.* [19]. The mixture was subsequently filtered, after which the precipitate (chitin) was rinsed with distilled water and stored at -20 °C for the deacetylation process.

2.4.1.3. Deacetylation

The transformation of chitin to chitosan was executed by a deacetylation process utilizing a potent alkaline solution. About one gram of chitin was mixed with 50 mL of a 50% sodium hydroxide solution and heated to 95°C while being swirled constantly. Following 4 hours of stirring, the solid was filtered and washed with distilled water until the filtrate reached neutrality. The resultant solid (chitosan) was dehydrated overnight at 80°C in an oven [20].

2.4.2. Biological extraction

2.4.2.1. Demineralization

The frozen stock culture was refreshed by putting 1 mL of L. acidophilus stock culture into 9 mL of sterile starch nitrate broth. A starter inoculum was prepared by transferring 10 mL of refreshed culture to 90 mL of starch nitrate broth in a 250 mL glass flask, followed by incubation at 37 ºC. We incorporated ten grams of desiccated adult powder into 250 mL of liquid medium and 100 mL of beginning inoculum. 100 mL of medium comprised KNO3, K2HPO4, MgSO4,

H2O, NaCl, CaCO3, and FeSO4. The pH was modified to 7.0. The fermentation occurred at 37 ºC with agitation at 50 rpm for 24 hours. Upon completion of the demineralization process, the sample was extracted from the broth and rinsed with running water until the effluent reached neutrality (pH 7.00) and subsequently drained. The demineralized material was subsequently stored in a freezer at -20 ºC for the ensuing procedure.

2.4.2.2. Deproteinization

The frozen stock culture of *Streptomyces* spp. was incubated for refreshing in starch nitrate broth at 37 °C for 24 h. We added ten grams of dead adults to 200 mL of inocula in a 250 mL glass flask in a shaking incubator (Lab-microbiology science lab, Egypt) at 120 rpm for 5 days. Upon completion of the deproteinization process, the sample was extracted from the broth, washed, drained, and stored at -20 ºC for the deacetylation process.

2.4.2.3. Deacetylation

This process was performed as mentioned previously in the chemical extraction.

2.5. Determination of percentage yield of chitin and chitosan

The chitin yield was determined from the dry weight using gravimetric measurements of the raw adult cuticle and the chitin extracted thereafter. The adult cuticle, chitin, and chitosan were desiccated to a stable weight to guarantee that moisture content did not influence the outcomes. Additionally, the chitosan yield was determined from the dry weight, computed as the difference between the weight of the raw adult cuticle and the chitosan produced, utilizing the following formulae [7]:

$$\text{Chitin yield (\%)} = \frac{a}{b} \times 100$$

where a is the obtained chitin weight (g) and b is the adult cuticle weight (g).

$$\text{Chitosan yield (\%)} = \frac{c}{d} \times 100$$

where c is the weight (g) of chitosan that was obtained, while d is the weight (g) of the adult cuticle.

2.6. Chitin and Chitosan characterization

2.6.1. Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra of pure chitin and chitosan samples were acquired using an ALPHA II Bruker spectrometer, with a resolution of 4 cm-1 and 32 accumulations. The data was processed using OPUS software. Each sample was measured thrice [7].

2.6.2. X-ray diffraction (XRD)

The pure chitin and chitosan samples underwent triple XRD studies with the Rigaku MiniFlex 600 diffractometer. Each sample was subsequently pulverized in a mortar before measurement. An Empyrean system equipped with a cobalt tube (PANalytical, Empyrean, Almelo, The Netherlands) was utilized to acquire XRD patterns. Data was collected using a PIXcel detector with a scan angle of 0 to 45°, at 40mA, 45kV, and a scan speed of 0.067335°s−1. The cobalt value was transformed into copper using High Score Plus (PANalytical) software. The intensity (counts) at 15.0° (ICr am, amorphous phase) and 19.3° ("ICr" 110) were utilized to calculate the crystallinity index (ICr) as per the specified formula [21].

$$\mathrm{ICr} = \frac{\mathrm{ICr110} - \mathrm{ICr\ am}}{\mathrm{ICr110}} * 100$$

6.3. Scanning electron microscopy (SEM)

The surface morphology of the chitin and chitosan samples was examined via SEM. SEM analysis yields high-resolution, three-dimensional pictures that provide a complete investigation of the surface morphology and microstructural attributes of the samples. Scanning Electron Microscopy (SEM) was conducted utilizing a Jeol JMS-700 EDS electron microscope following the deposition of a +1.5 nm Pt/PD 80/20 wt% coating on the chitin and chitosan samples via a Sputter Coater HP208 Cressington (Watford, England).

2.7. Antimicrobial testing

The antibacterial efficacy of chemically and biologically derived chitin and chitosan solutions was evaluated against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*P. aeruginosa, Salmonella spp,* and *K. pneumoniae*) employing the disc diffusion method as outlined by Arancibia *et al.* [22]. To prepare the samples (chitin or chitosan), 0.5 g of the sample was dissolved in 100 mL of a 1% acetic acid solution. Twenty milliliters of nutritious agar medium were dispensed into petri plates, which were thereafter allowed to solidify. Bacterial inocula were applied to agar plates using a swab. 20 µl of the sample solution was utilized to saturate holes with a diameter of 6 mm. The agar plates were incubated at 37 ˚C for 24 hours. The diameter of the inhibition zones against the examined microorganisms was utilized to assess antibacterial activity. The outcomes were documented in millimeters, and each test was conducted thrice.

2.8. Statistical Analysis

The SPSS statistical software package (SPSS 27.0 for Windows, SPSS Inc., Chicago, IL, USA) was utilized to provide the mean and standard deviation data for the triplicate trials. One-way

ANOVA with a significance threshold of $P < 0.05$ was employed to evaluate the statistical significance of the differences among data groups.

3. Result and discussion

3.1. Morphological characterization of *Streptomyces*

Streptomyces sp was successfully identified based on its distinctive colony morphology. The colonies exhibited deep-seated growth in the agar medium, abundant sporulation, and characteristic pigmentation. This isolation was typically round and convex, with a dry, powdery surface covered by dense spore masses. These morphological features are consistent with the general characteristics of the genus Streptomyces.

In this study, *Streptomyces* sp was used for the biological extraction of chitin and chitosan. *Streptomyces* reduced the time required for the deproteinization process to 24 hours compared to other types of bacteria. Biological extraction/deproteinization via fermentation of BSF or similar insect cuticle materials often requires several days to achieve high levels of protein removal. For example, Xiong *et al*. [23] reported that deproteinization with *Bacillus subtilis* S4 required several days in BSF puparia and adult shells. Similarly, Witono *et al*. [24] required about seven days for a complete biological deproteinization step. In addition, Tan *et al*. [25] applied acid + protease treatments over six days to remove protein from insect-derived material.

Streptomyces spp. is increasingly explored as biological agents for chitin deproteinization due to their ability to secrete extracellular proteases during fermentation. These enzymes hydrolyze protein contaminants bound to chitin in crustacean shells, insect cuticles, or fungal cell walls, offering a milder and more environmentally friendly alternative to harsh chemical methods [26]. During solid-state or submerged fermentation, *Streptomycetes* break down proteins through proteolysis while leaving the polysaccharide backbone of chitin largely intact, minimizing depolymerization and deacetylation [27]. The use of *Streptomyces* strains for deproteinizing insect cuticles has been reported less directly than for crustacean shells. However, *Streptomyces* spp. are well-documented producers of extracellular proteases and have been successfully used, either directly or through their enzymes, in bioprocesses for protein removal. Enzyme preparations from *Streptomyces* (e.g., *S. griseus*) and chitinolytic *Streptomyces* strains (e.g., *S. variabilis*, *S. parvulus*, *S.* sp. TH-11) represent promising candidates for insect cuticle deproteinization, with expected protein removal typically in the >70–90% range depending on strain and conditions [28].

3.2. Chitin and chitosan yield from BSF

The yields of chitin and chitosan extracted by chemical and biological techniques showed a discernible difference (Table 1). The biological extraction technique produced slightly higher yields, 32% for chitin and 26.6% for chitosan, compared to 29% and 23%, respectively, from the chemical method. These findings suggest that while both extraction approaches are effective for obtaining chitin and chitosan from BSF, the biological method offers an advantage in terms of higher product yields.

The biological extraction method applied by Kim *et al*. [29] produced a greater chitin yield (39.6%) from BSF pupal shell than the chemical extraction (33.3%). On the other hand, Legat *et al.* [7] reported how the chitin yields from chemical and biological extraction from BSF pupae were 10.18% and 11.85%, respectively. Through chemical treatment, a maximum chitosan yield of 6.58% was also attained. Numerous factors, including the specific methods employed, the properties of the starting material, and the optimization of extraction parameters, can be attributed to the observed variations in extraction yields by different investigators.

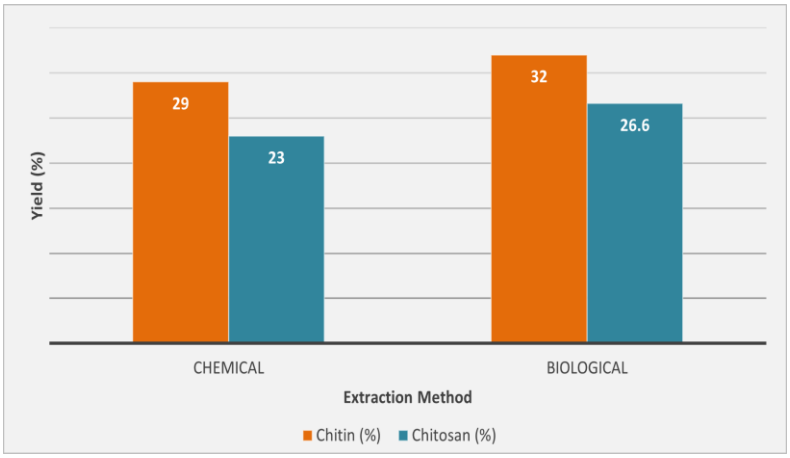


Figure 1: Chitin and chitosan yield using the chemical and biological extraction from *H. illucens*.

The enhanced performance of the biological methods could be attributed to their milder processing conditions, which may better retain the fundamental structure of the chitin molecules. Additionally, the target molecule may be separated more effectively and selectively if specific microbes or enzymes are used in the biological extraction process [30].

3.3. FTIR analysis:

For describing and contrasting the chemical makeup and structure of chitin and chitosan from various sources, FTIR is an effective tool. Its purity and structural integrity are confirmed by FTIR analysis. The chitin structure that was retrieved using chemical and biological approaches showed similarities (Figure 2). The vibration peaks of biological chitin were at 3450, 2350, 1650, and 1550 cm^{-1}, whereas those of chemical chitin were at 3400, 2300, 1661, and 1555 cm^{-1}.

Because of the hydroxyl groups in the chitin, the band at 3440 cm^{-1} was wide. The hydroxyl groups in chitin are located on the C3 and C6 positions of each N-acetylglucosamine unit, with the C6 being a primary hydroxyl and C3 a secondary hydroxyl. These groups are involved in extensive hydrogen bonding, which affects the physical properties of chitin. In terms of spectroscopic ranges, the O-H stretching in IR is around 3200-3600 cm^{-1}, typically around 3450 cm^{-1} [31,32].

The existence of methyl groups was suggested by the absorption band at 2355 cm^{-1}, which correlated with C–H stretching vibrations. Secondary amide I is C=O stretching vibrations were related to the band at 1650 cm^{-1}, whereas amide II is N–H bending vibrations were linked to the band at 1550 cm^{-1}.

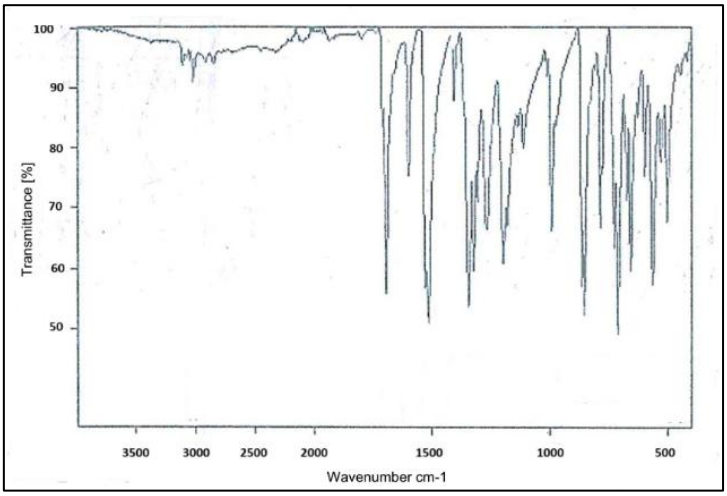
Zimri [33] identified identical distinctive peaks of chitin sourced from both BSF larvae and adult flies. Furthermore, Erdogan and Kaya [34] and Kaya *et al.* [35] utilized chitin derived from grasshoppers and *Vespa crabro* (wasp) at various developmental stages (larvae, pupae, and adults), respectively, achieving identical characteristic bands. Erdogan and Kaya [34] demonstrated that the characteristic chitin peaks of alpha (α)-chitin are observed at 1650, 1620, and 1550 cm^{-1}.

Tsurkan *et al.* [36] identified that chemical chitin samples had a glycosidic bond at 896 cm^{-1}, characteristic of alpha-chitin, which was not evident in the biological approach (Figure 2, A & B). No peaks were detected at 1540 cm^{-1} in any of the chitin samples. The lack of this band is attributed to the efficient elimination of protein impurities, signifying successful deproteinization.

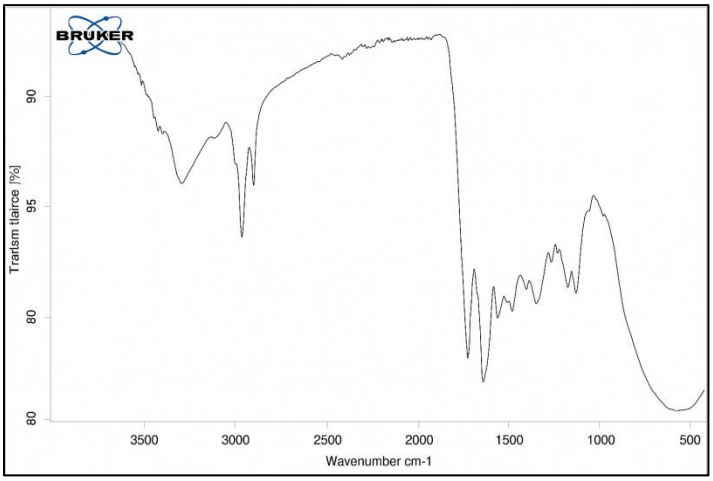
Additional prominent bands were identified using chemical techniques:1413 cm^{-1}, 1256 cm^{-1}, 1069 cm^{-1}, 1023 cm^{-1}, and 1159 cm^{-1}. The results obtained were consistent with Zimri [33] on larvae and adults of BSF flies.

Compared to the biological method, chemical extraction achieves more deproteinization and demineralization, which is reflected in the presence of sharper chitin peaks. In contrast, the biological method is a milder process that better preserves the native chitin structure. This reduced

intensity minimizes the risk of deacetylation, leading to more prominent amide I (1655 cm^{-1}) and amide II (1555 cm^{-1}) bands [37].



A: Chemical method



B: Biological method

Figure 2. Effect of extraction method on the FTIR spectra of chitin from *H. illucens*.

The characteristic bands of chitosan recovered from BSF using the biological technique were noted at 1650 cm^{-1} and 1587 cm^{-1}, while those extracted via the chemical method were recorded at 1647 cm^{-1} and 1587 cm^{-1} (Fig. 3, A & B).

The amide I (C=O) in the acetamide group (NHCOCH3) accounts for the characteristic peaks observed in biological and chemical samples at 1650 cm^{-1} and 1649 cm^{-1}, respectively. The amide II band (NH2) in the NHCOCH3 group had peak values at 1587 cm^{-1}. These results align with Zimri's [33] findings. The author indicated that chitosan exhibited peaks about 1650–1655

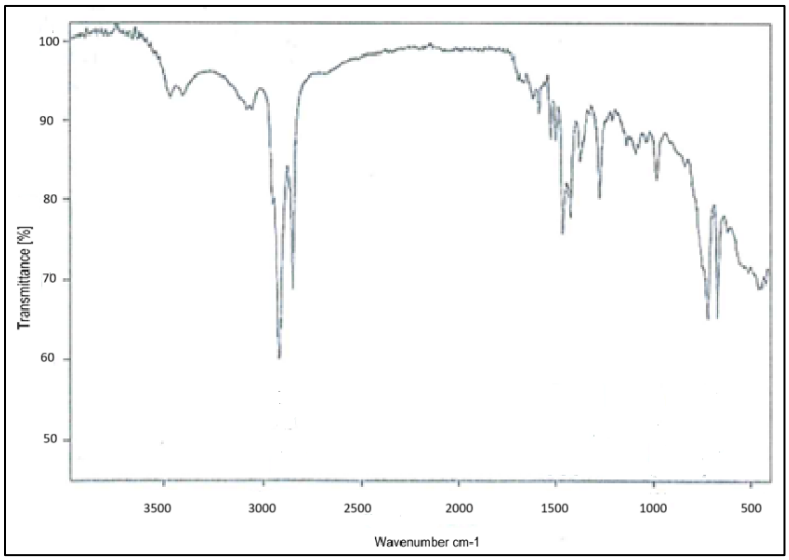
cm-1 and 1583–1590 cm^{-1}, corresponding to (C=O) in the NHCOCH3 group (amide I band) and (NH2) in the NHCOCH3 group (amide II band), respectively.

Broad absorption bands were observed in both methods at 3250–3750 cm^{-1}. The bands result from the symmetric stretching vibrations of the O-H and NH2 groups, attributed to the robust intermolecular hydrogen bonding in chitosan polysaccharides [7].

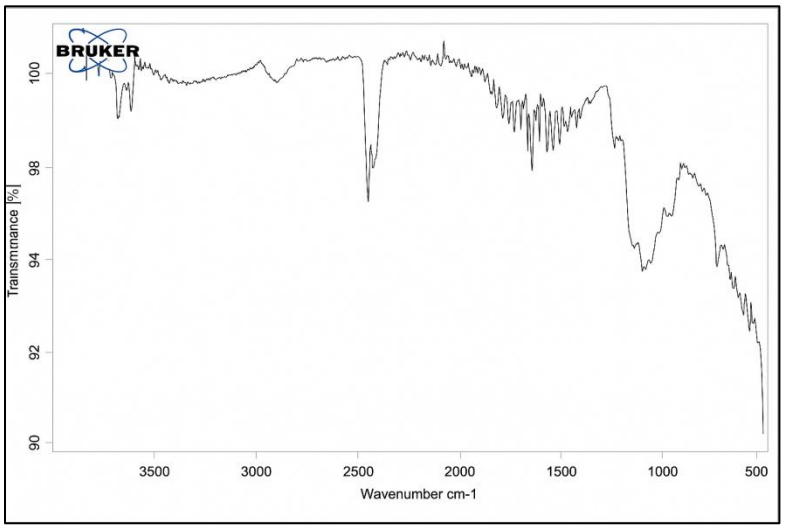
The peaks at 2350 cm^{-1} in biological extraction have been associated with both symmetric and asymmetric vibrations of the C-H groups in chitosan samples. The peak vanished in chitosan obtained using the chemical approach. In biological extraction, the peaks at 2350 cm^{-1} have been associated with both symmetric and asymmetric vibrations of the C-H groups in the chitosan samples. Tsurkan *et al*. [36] utilized chitosan derived from grasshopper species, and their results corroborate these findings.

The (1–4) glycosidic connections in the polysaccharide and the vibrations from the stretching of C-O-C in a glucose ring were identified as the sources of the peaks observed at around 1153 cm^{-1} and 1082 cm^{-1}, respectively. Eddya *et al.* [38], who utilized chitosan derived from shrimp shells, obtained analogous findings to these. The successful deacetylation was evidenced by the reduced intensity of the amide II absorption band relative to amide I.

The transformation of chitin to chitosan resulted in a significant reduction in the strength of the amide II absorption band at approximately 1550 cm^{-1}, coupled with a notable rise in the band near 1595 cm^{-1} associated with free amino ($–NH_2$) groups. This spectral shift unequivocally indicates the effective deacetylation of chitin and the resultant production of primary amine groups, a defining trait of chitosan. The FTIR spectra indicated a significant similarity in the chemical composition and bonding patterns of chitosan derived from both chemical and biological extraction methods (Figure 3). The results align with those of Erdogan and Kaya [34], who noted similar spectral characteristics in chitosan obtained from nymph and adult grasshoppers, hence corroborating the validity of the current findings.



A: Chemical method



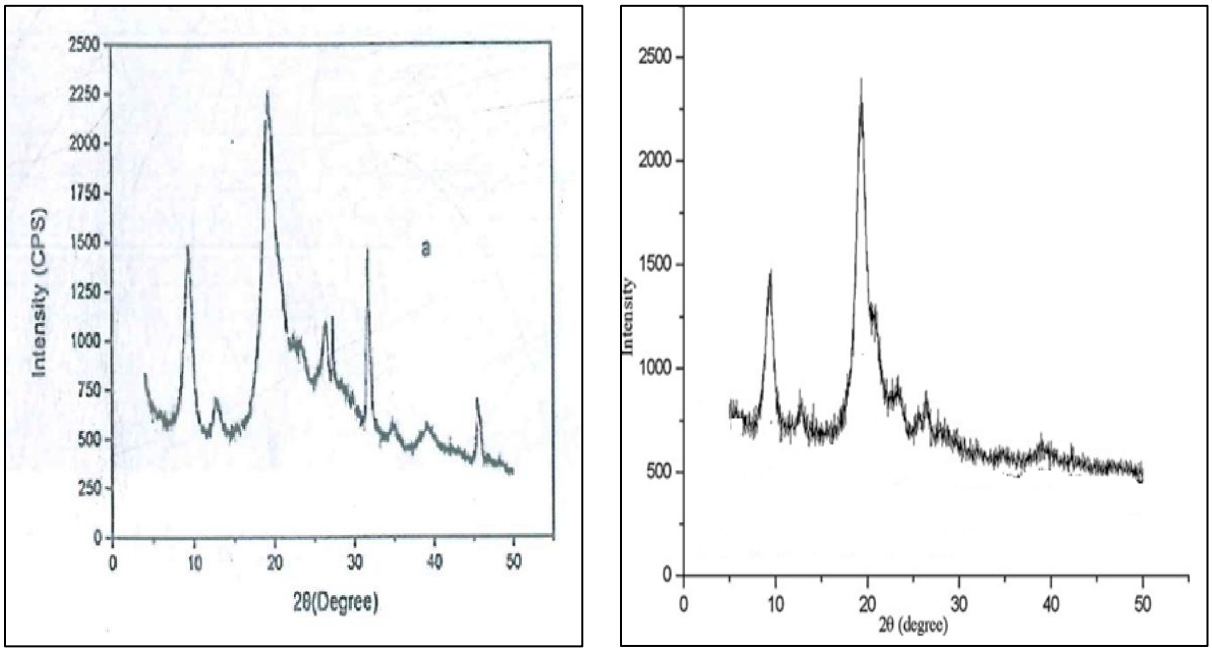
B: Biological method

Figure 3. Effect of extraction method on the FTIR spectra of chitosan from *H. illucens*.

3.4. XRD analysis

XRD of chitin is presented in Fig. 4 (A, B). Chemical and biological chitin revealed three or four minor peaks around 13° and 26°, as well as notably strong peaks at 9° and 19°, supporting the polymer's α-form [39,40]. In addition, biological chitin showed a weak peak around 23°. All chitin samples displayed diffraction peaks closely matching those previously documented for chitin derived from other insect species, occurring within the 9°–26° range [41] and for *H. illucens* itself [42].

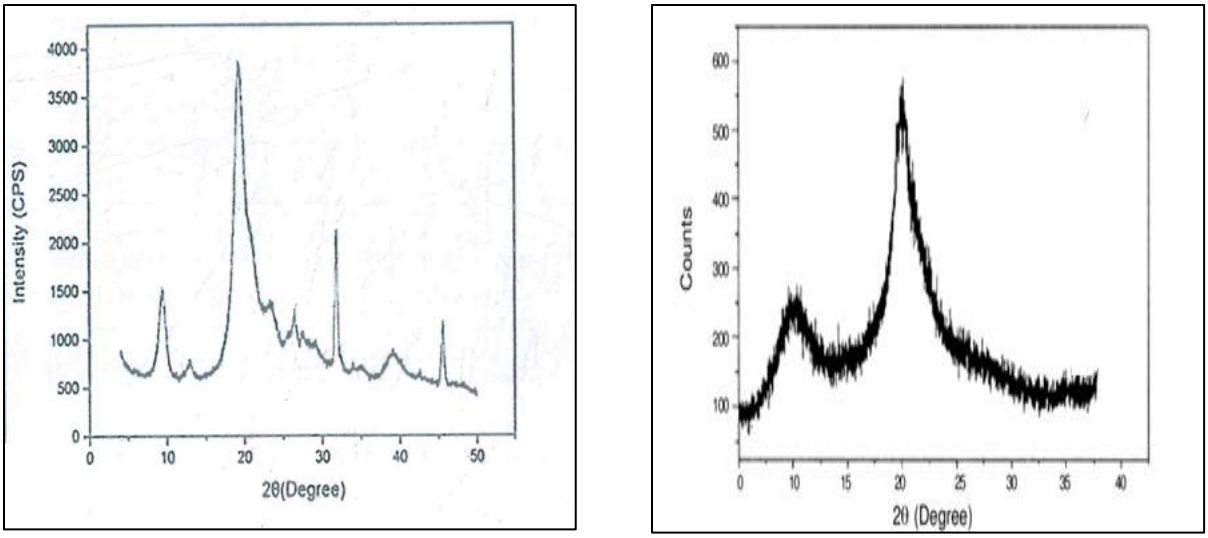
Generally, biological chitin (70%) had a slightly lower ICr than chemical chitin (77.8%) but retains natural structure. Also, peaks of biological chitin were broader than those of chemical chitin. These values are within the range of ICr chitin values (47-91%) reported [41]. Chitin has a variety of applications based on its crystallinity. Chemical chitin is better for industrial uses (e.g., chitosan production, where high crystallinity is desired). Biological chitin is preferred for biomedical applications such as tissue engineering, where native structure matters [43].



A: Chemical method B: Biological method

Figure 4. Effect of extraction method on the XRD analysis of chitin from *H. illucens*.

XRD patterns of chitosan extracted by both chemical and biological methods are demonstrated in Fig. 5 (A & B). Both samples had two sharp peaks in the XRD study, located at about 10° and 20°. These peaks were comparable to those found in chitosan derived from insects [18]. The disappearance of the 26.4° peak in Chitosan confirms successful demineralization.
Chitosan extracted by the biological method (68%) had a slightly lower ICr than chitosan extracted by the chemical method (74.8%) but retains natural structure. The All of the chitosan samples, including the commercial one, had ICr values between 74 and 67%, which were similar. Crystallinity Index (ICr): ~60–70%, lower than chitin due to partial disruption of crystalline regions during deacetylation [44].

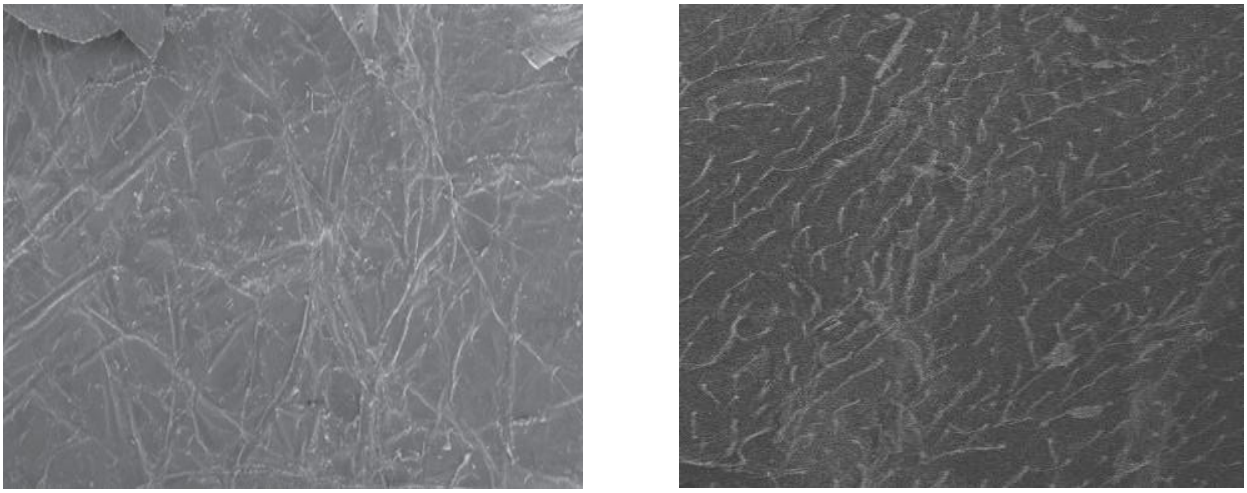


A: Chemical method B: Biological method

Figure 5. Effect of extraction method on the XRD analysis of chitosan from *H. illucens.*

3.5. Morphology of Chitin and Chitosan extracted from BSF by SEM

SEM analysis of the surface morphology of the chitin isolated from *H. illucens* using chemical and biological techniques is displayed in Fig. 6, A & B at 250× magnification. Both techniques produced chitin with the same form and rough surface morphology, but chitin extracted by the biological method had more fibers and pores.

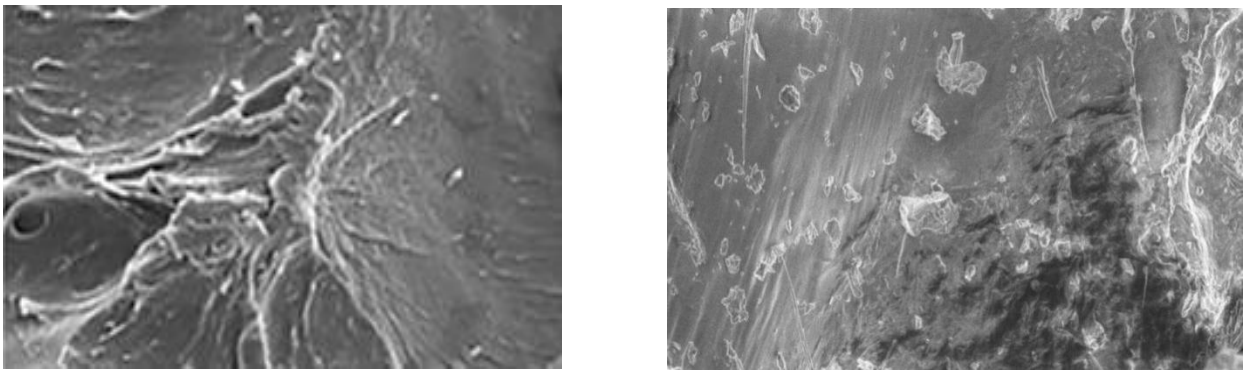


A: the chemical method B: the biological method

Figure 6. Effect of the extraction method on SEM images of the chitin extracted from the *H. illucens* (magnification 250×).

Similar observations were also reported by Waśko *et al.* [40], who assessed *H. illucens*-derived chitin larvae and flies. According to Kaya *et al.* [45], the Colorado potato beetle's larvae and adults both showed porous nanofiber structures, but the adults' chitin had a significantly greater quantity of pores. Chitin can be utilized for a variety of purposes depending on its morphology; for example, chitin with a fibrillary surface is appropriate for the textile industry, whereas chitin with a porous structure can be used for medication administration and tissue engineering [34].

The surface of the chitosan that was chemically and biologically isolated from BSF was rough and devoid of fibers and/or pores (Figure 7 A & B), indicating that the deacetylation process changed the chitin's structure to make it less fibrillated as well as more homogenous. After the solid polymer is reprecipitated from an acidic solution, chitosan nanofibers can be created, or the pre-existing chitin ones can be modified. The latter process may be more prevalent in this instance because adult chitin has a more complicated morphology [46].



A: chemical method B: Biological method

Figure 7. Effect of the extraction method on SEM images of the chitozan extracted from the *H. illucens* (magnification 250×).

3.6. Evaluation of the Antimicrobial Potential of Chitin and Chitosan

The antibacterial properties of chitin and chitosan solutions obtained through chemical and biological extraction methods were evaluated using the disc diffusion method against the Gram-positive bacterium *S. aureus* as well as the Gram-negative bacteria *P. aeruginosa*, *Salmonella* sp., and *K. pneumoniae* using the disc diffusion method. The data showing the antimicrobial activity in terms of inhibition zone diameter in millimeters is presented in Table 2.

Table 2: The antibacterial activity of chitin and chitosan extracted by biological and chemical methods as the inhibition zone diameter (mm) against the tested bacteria.

Sample	*P. aeruginosa*	*Salmonella spp.*	*K. pneumoniae*	*S. aureus*
	mean (mm) ± standard error			
Biologically extracted chitosan (A)	15 ± 0.0577[a]	16 ± 0.0441[a]	25 ± 0.0764[a]	25 ± 0.0333[a]
Chemically extracted chitosan (B)	15 ± 0.0289[a]	15 ± 0.0549[a]	15 ± 0.0625[b]	20 ± 0.0437[b]
Biologically extracted chitin (C)	10 ± 0.0289[b]	11 ± 0.0549[b]	15 ± 0.0404[b]	18 ± 0.0318[b]
Chemically extracted chitin (D)	none[c]	none[c]	11 ± 0.0348[c]	12 ± 0.0346[c]

Different letters in the same column indicate significant differences ($P \leq 0.05$).

Chitosan extracted by the biological method shows the highest antimicrobial activity against *S. aureus* (25 ± 0.0333 cm) and *K. pneumoniae* (25 ± 0.0764 cm). Chitosan extracted by the chemical method also shows significant activity against *S. aureus* (20 ± 0.0437 cm) but lower activity against *K. pneumoniae* (15 ± 0.0404 cm). Biologically extracted chitin had moderate activity against all tested bacteria, with the highest activity against *S. aureus* (18 ± 0.0318 cm). Chemically extracted Chitin showed no activity against *P. aeruginosa* and *Salmonella* spp., and moderate activity against *S. aureus* (12 ± 0.0346 cm) and *K. pneumoniae* (11 ± 0.0348 cm). In other words, biologically extracted chitosan and chitin generally exhibit better antimicrobial activity compared to their chemical counterparts. *S. aureus* appears to be the most susceptible bacterium. The results of Raafat and Sahl [47] are consistent with our findings. In contrast, *P. aeruginosa* and *Salmonella* sp. were less affected by chemically extracted chitin, showing no inhibition of growth.

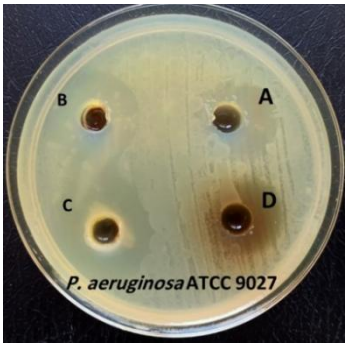


Plate: 1

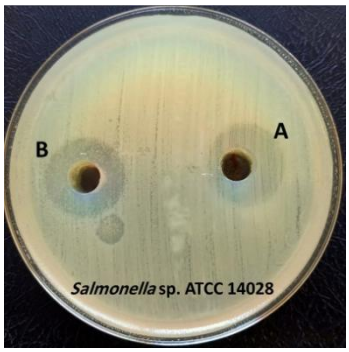


Plate: 2

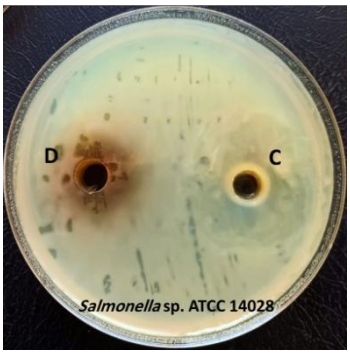


Plate: 3

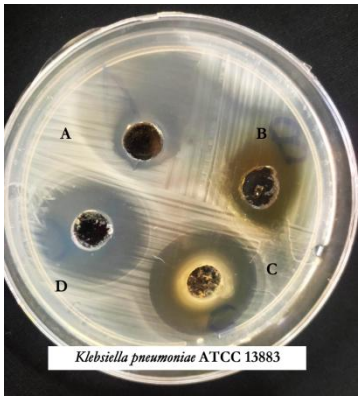


Plate: 4

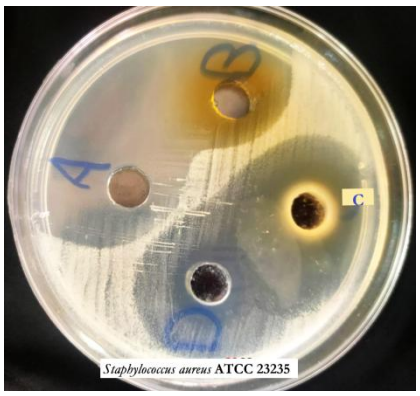


Plate: 5

Figure 8: The antibacterial activity of chitin and chitosan extracted by biological and chemical methods against the tested bacteria. Biologically extracted chitosan (A), Chemically extracted chitosan (B), Biologically extracted chitin (C), Chemically extracted chitin (D)

Prior studies [48,49,50] have shown that chitosan has greater antibacterial efficacy against Gram-positive bacteria compared to Gram-negative bacteria, likely due to differences in cell surface architecture. The antibacterial efficacy of chitosan is contingent upon the bacterial species and its physicochemical properties. The antibacterial properties of chitosan are contingent upon its pH [51]. Chitosan, possessing a higher concentration of positive charges due to its low pH, interacts with negatively charged bacterial cell membranes, hence augmenting its antibacterial effects [52]. The antibacterial activity of chitosan may be extracellular, intracellular, or both, contingent upon its molecular weight and structure [51].

4. Conclusion

Adult BSF was successfully used to extract chitin and chitosan. The biological extraction method enhanced the antibacterial activity of chitosan against pathogenic bacteria tested, suggesting their potential as an effective natural antibacterial agent from BSF regardless of the preparation method. *Streptomyces* sp. offers a systematic strategy to enhance protein removal efficiency compared to other bacterial strains, thereby emerging as a promising competitor to biological and conventional chemical extraction methods. For better commercial application, it is advised to look into additional potential biological activities of the BSF chitosan, such as antioxidant and anticancer properties. Chemical extraction often yields purer chitin and chitosan, but at risk of degradation, whereas biological methods provide an eco-friendlier alternative that retains the native properties of chitin and chitosan.

Ethical consideration

The study was authorized by the Faculty of Science's Ethics Committee at Benha University and carried out in compliance with the university's statement (Code: BUFS-REC-2025-458 Ent).

References

[1] K. U. Rehman, C. Hollah, K. Wiesotzki, V. Heinz, K. Aganovic, R. U. Rehman, J.-I. Petrusan, L. Zheng, J. Zhang, S. Sohail, et al., "Insect-derived chitin and chitosan: A still unexploited resource for the edible insect sector," Sustainability 15 (2023) 4864.

[2] Z. Mei, P. Kuzhir, G. Godeau, "Update on chitin and chitosan from insects: Sources, production, characterization, and biomedical applications," Biomimetics 9 (2024) 297.

[3] A. Mannucci, L. Panariello, L. Abenaim, M. B. Coltelli, A. Ranieri, B. Conti, M. Santin, A. Castagna, "From food waste to functional biopolymers: Characterization of chitin and chitosan produced from prepupae of black soldier fly reared with different food waste-based diets," Foods 13 (2024) 278.

[4] C. Jucker, D. Lupi, C. D. Moore, M. G. Leonardi, S. Savoldelli, "Nutrient recapture from insect farm waste: Bioconversion with Hermetia illucens (L.) (Diptera: Stratiomyidae)," Sustainability 12 (2020) 362.

[5] M. B. Kaczmarek, K. Struszczyk-Świta, X. Li, M. Szczęsna-Antczak, M. Daroch, "Enzymatic modifications of chitin, chitosan, and chitooligosaccharides," Frontiers in Bioengineering and Biotechnology 7 (2019) 243.

[6] C. T. Doan, T. N. Tran, V. B. Nguyen, T. P. K. Vo, A. D. Nguyen, S. L. Wang, "Chitin extraction from shrimp waste by liquid fermentation using an alkaline protease-producing strain, Brevibacillus parabrevis," International Journal of Biological Macromolecules 131 (2019) 706–715.

[7] M. K. Lagat, S. Were, F. Ndwigah, V. J. Kemboi, C. Kipkoech, C. M. Tanga, “Antimicrobial activity of chemically and biologically treated chitosan prepared from black soldier fly (Hermetia illucens) pupal shell waste,” Microorganisms 9 (2021) 2417.
[8] A. Percot, C. Viton, A. Domard, “Optimization of chitin extraction from shrimp shells,” Biomacromolecules 4 (2003) 12–18.
[9] Y. Narahashi, K. Shibuya, M. Yanagita, “Studies on proteolytic enzymes (Pronase) of Streptomyces griseus K-1. Separation of exo and endopeptidases of Pronase,” Journal of Biochemistry 64 (1968) 427–437.
[10] I. Aranaz, M. Mengíbar, R. Harris, I. Paños, B. Miralles, N. Acosta, G. Galed, Á. Heras, “Functional characterization of chitin and chitosan,” Current Chemical Biology 3 (2009) 203–230.
[11] D. Yan, Y. Li, Y. Liu, N. Li, X. Zhang, C. Yan, “Antimicrobial properties of chitosan and chitosan derivatives in the treatment of enteric infections,” Molecules 26 (2021) 7136.
[12] A. Jayanegara, R. P. Haryati, A. Nafisah, P. Suptijah, M. Ridla, E. B. Laconi, “Derivatization of chitin and chitosan from black soldier fly (Hermetia illucens) and their use as feed additives: an in vitro study,” Advances in Animal and Veterinary Sciences 8 (2020) 472–477.
[13] A. Khayrova, S. Lopatin, B. Shagdarova, O. Sinitsyna, A. Sinitsyn, V. Varlamov, “Evaluation of antibacterial and antifungal properties of low-molecular-weight chitosan extracted from Hermetia illucens relative to crab chitosan,” Molecules 27 (2022) 577.
[14] M. Hayakawa, H. Nonomura, “Humic acid vitamin agar, a new medium for the selective isolation of soil actinomycetes,” Journal of Fermentation and Bioengineering 65 (1987) 501–509.
[15] M. C. Shearer, “Methods for the isolation of non-streptomycetes actinomycetes,” Journal of Indian Microbiology 2 (1987) 91–97.
[16] S. A. Waksman, “The actinomycetes. Classification, identification, and descriptions of genera and species,” The Williams and Wilkins Co., Baltimore 1 (1961) 1–363.
[17] H. K. Ravi, A. Degrou, J. Costil, C. Trespeuch, F. Chemat, M. A. Vian, “Effect of devitalization techniques on the lipid, protein, antioxidant, and chitin fractions of black soldier fly (Hermetia illucens) larvae,” European Food Research and Technology 246 (2020) 2549–2568.
[18] N. H. Marei, E. A. El-Samie, T. Salah, G. R. Saad, A. H. M. Elwahy, “Isolation and characterization of chitosan from different local insects in Egypt,” International Journal of Biological Macromolecules 82 (2016) 871–877.
[19] F. A. A. Sagheer, M. A. Al-Sughayer, S. Muslim, M. Z. Elsabee, “Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf,” Carbohydrate Polymers 77 (2009) 410–419.

[20] L. Soetemans, M. Uyttebroek, L. Bastiaens, “Characteristics of chitin extracted from black soldier fly in different life stages,” International Journal of Biological Macromolecules 165 (2020) 3206–3214.

[21] M. Kaya, M. Mujtaba, H. Ehrlich, A. M. Salaberria, T. Baran, C. T. Amemiya, R. Galli, L. Akyuz, I. Sargin, J. Labidi, “On chemistry of γ-chitin,” Carbohydrate Polymers 241 (2020) 116254.

[22] M. Y. Arancibia, A. Alemán, M. M. Calvo, M. E. López-Caballero, P. Montero, M. C. Gómez-Guillén, “Antimicrobial and antioxidant chitosan solutions enriched with active shrimp (Litopenaeus vannamei) waste materials,” Food Hydrocolloids 35 (2014).

[23] A. Xiong, L. Ruan, K. Ye, Z. Huang, C. Yu, “Extraction of chitin from black soldier fly (Hermetia illucens) and its puparium by using biological treatment,” Life 13 (2023) 1424.

[24] J. R. Witono, A. Putra, et al., “A comprehensive analysis of chitin extraction from the black soldier fly: Evaluation of chemical and biological methods,” Periodica Polytechnica Chemical Engineering 68 (2024) 23714.

[25] Y. N. Tan, L. H. Wong, S. H. Ng, “Comparison of sustainable lipid and protein removal strategies from insect biomass for chitin extraction,” ACS Food Science and Technology 1 (2021) 357–367.

[26] K. C. Hoang, T. H. Lai, C. S. Lin, Y. T. Chen, C. Y. Liau, “The chitinolytic activities of Streptomyces sp. TH-11,” International Journal of Molecular Sciences 12 (2010) 56–65.

[27] J. Waldeck, G. Daum, B. Bisping, F. Meinhardt, “Isolation and molecular characterization of chitinase-deficient Bacillus licheniformis strains capable of deproteinization of shrimp shell waste to obtain highly viscous chitin,” Applied and Environmental Microbiology 72 (2006) 7879–7885.

[28] E. Kotb, A. H. Alabdalall, A. I. Alghamdi, et al., “Screening for chitin degrading bacteria in the environment of Saudi Arabia and characterization of the most potent chitinase from Streptomyces variabilis Am1,” Scientific Reports 13 (2023) 11723.

[29] S. Kim, J. Park, H. Lee, “Microbial fermentation for the extraction of chitin from black soldier fly,” Bioresource Technology 345 (2023) 126–134.

[30] M. N. Marzieh, F. Zahra, E. Tahereh, K. N. Sara, “Comparison of the physicochemical and structural characteristics of enzymatic produced chitin and commercial chitin,” International Journal of Biological Macromolecules 139 (2019) 270–276.

[31] J. Brugnerotto, J. Lizardi, F. M. Goycoolea, W. Argüelles-Monal, J. Desbrieres, M. Rinaudo, "An infrared spectroscopy study of the crystallinity of polysaccharides," Polymer 42 (2001) 3569–3580.
[33] M. N. Zimri, "Preparation and electrospinning of chitosan from waste black soldier fly biomass," University of the Western Cape, Cape Town, South Africa (2018).
[34] S. Erdogan, M. Kaya, "High similarity in physicochemical properties of chitin and chitosan from nymphs and adults of a grasshopper," International Journal of Biological Macromolecules 89 (2016) 118–126.
[35] M. Kaya, K. Sofi, I. Sargin, M. Mujtaba, "Changes in physicochemical properties of chitin at developmental stages (larvae, pupa, and adult) of Vespa crabro (wasp)," Carbohydrate Polymers 145 (2016) 64–70.
[36] M. V. Tsurkan, A. Voronkina, Y. Khrunyk, M. Wysokowski, I. Petrenko, H. Ehrlich, "Progress in chitin analytics," Carbohydrate Polymers 252 (2021) 117204.
[37] A. Poerio, T. Girardet, C. Petit, S. Fleutot, J. P. Jehl, E. Arab-Tehrany, F. Cleymand, "Comparison of the physicochemical properties of chitin extracted from cicada ornisloughs harvested in three different years and characterization of the resulting chitosan," Applied Sciences 11 (2021) 11278.
[38] M. Eddya, B. Tbib, K. El-Hami, "A comparison of chitosan properties after extraction from shrimp shells by diluted and concentrated acids," Heliyon 6 (2020) e03486.
[39] W. Sajomsang, P. Gonil, "Preparation and characterization of α-chitin from cicada sloughs," Materials Science and Engineering C 30 (2010) 357–363.
[40] A. Waśko, P. Bulak, M. Polak-Berecka, K. Nowak, C. Polakowski, A. Bieganowski, "The first report of the physicochemical structure of chitin isolated from Hermetia illucens," International Journal of Biological Macromolecules 92 (2016) 316–320.
[41] C. Y. Soon, Y. B. Tee, C. H. Tan, A. T. Rosnita, A. Khalina, "Extraction and physicochemical characterization of chitin and chitosan from Zophobas morio larvae in varying sodium hydroxide concentration," .
[42] D. Purkayastha, S. Sarkar, "Physicochemical structure analysis of chitin extracted from pupa exuviae and dead imago of wild black soldier fly (Hermetia illucens)," Journal of Polymers and the Environment 28 (2020) 445–457.

[43] T. Hahn, E. Tafi, A. Paul, R. Salvia, P. Falabella, S. Zibek, “Current state of chitin purification and chitosan production from insects,” Journal of Chemical Technology and Biotechnology (2020).

[44] S. Kumari, P. Rath, A. H. Kumar, T. N. Tiwari, “Extraction and characterization of chitin and chitosan from fishery waste by chemical method,” Environmental Technology and Innovation 3 (2015) 77–85.

[45] M. Kaya, T. Baran, S. Erdoğan, A. Menteş, M. A. Özüsağlam, Y. S. Çakmak, “Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (Leptinotarsa decemlineata),” Materials Science and Engineering C 45 (2014) 72–81.

[46] M. Triunfo, E. Tafi, A. Guarnieri, et al., “Characterization of chitin and chitosan derived from Hermetia illucens, a further step in a circular economy process,” Scientific Reports 12 (2022) 6613.

[47] D. Raafat, H. G. Sahl, “Chitosan and its antimicrobial potential – a critical literature survey,” Microbial Biotechnology 2 (2009) 186–201.

[48] B. P. Sudatta, V. Sugumar, R. Varma, P. Nigariga, “Extraction, characterization and antimicrobial activity of chitosan from pen shell (Pinna bicolor),” International Journal of Biological Macromolecules 163 (2020) 423–430.

[49] Y. C. Chung, Y. P. Su, C. C. Chen, G. Jia, H. L. Wang, J. G. Wu, J. G. Lin, “Relationship between antibacterial activity of chitosan and surface characteristics of cell wall,” Acta Pharmacologica Sinica 25 (2004) 932–936.

[50] H. K. No, N. Y. Park, S. H. Lee, S. P. Meyers, “Antibacterial activity of chitosans and chitosan oligomers with different molecular weights,” International Journal of Food Microbiology 74 (2002) 65–72.

[51] C. L. Ke, F. S. Deng, C. Y. Chuang, C. H. Lin, “Antimicrobial actions and applications of chitosan,” Polymers 13 (2021) 904.

[52] V. P. Varlamov, I. S. Mysyakina, “Chitosan in biology, microbiology, medicine, and agriculture,” Microbiology 87 (2018) 712–715.

الملخص العربي

المقارنة بين الطريقة الكيميائية والطريقة الحيوية لاستخلاص الكيتين والكيتوزان من الحشرات البالغة لذبابة الجندي الأسود (*Hermetia illucens*)

تعدّ ذبابة الجندي الأسود *(Hermetia illucens)* مصدرًا بديلًا مستدامًا وصديقًا للبيئة للحصول على الكيتين والكيتوزان، وهما من السكريات المتعددة ذات القيمة العالية والتطبيقات الواسعة في مجالات الصيدلة والزراعة وتكنولوجيا الأغذية. تهدف هذه الدراسة إلى مقارنة الطريقتين الكيميائية والحيوية في استخلاص الكيتين والكيتوزان من الحشرات البالغة، وتقييم خصائصهما المضادة للبكتيريا.

في الطريقة الكيميائية، تم إجراء عمليتي إزالة المعادن وإزالة البروتين باستخدام حمض الهيدروكلوريك (HCl) وهيدروكسيد الصوديوم (NaOH)، في حين استُخدمت في الطريقة الحيوية سلالات *Streptomyces spp.* المنتجة لإنزيم البروتييز لإزالة البروتين، وسلالة *Lactobacillus acidophilus* لإزالة المعادن.

أظهرت نتائج الاستخلاص الحيوي زيادة في نسب الإنتاج لكل من الكيتين (32%) والكيتوزان (26.6%) مقارنة بالاستخلاص الكيميائي (29% و23% على التوالي). أكّد تحليل الأشعة تحت الحمراء (FTIR) وجود المجموعات الوظيفية المميزة وفعالية عملية إزالة البروتين، مع احتفاظ العينات الحيوية بتكاملها البنيوي بدرجة أفضل. كما أثبت تحليل حيود الأشعة السينية (XRD) وجود الشكل البلوري-α كيتين في كلا الطريقتين، مع تبلور أعلى في العينات الكيميائية مما يجعلها ملائمة للاستخدامات الصناعية، بينما احتفظت العينات الحيوية ببنية أكثر طبيعية، ما يجعلها مناسبة للتطبيقات الطبية الحيوية.

وأخيرا، أظهرت صور المجهر الإلكتروني الماسح (SEM) أن الكيتين المستخلص بالطريقة الحيوية يتميز ببنية ليفية ومساميّة، في حين ظهر الكيتين الناتج من الطريقة الكيميائية أكثر كثافة. وعلاوة على ذلك، فقد أبرز الكيتوزان المستخلص حيويًا نشاطًا مضادًا قويًا للميكروبات، خاصة ضد *Staphylococcus aureus* و*Klebsiella pneumoniae*.

وبناءً على النتائج المتحصل عليها، فإن الاستخلاص الحيوي يُعدّ نهجًا مستدامًا يُحسّن من المردود، ويحافظ على النشاط الحيوي، ويوسّع من آفاق استخدام الكيتين والكيتوزان المستخلصين من ذبابة الجندي الأسود في التطبيقات المتقدمة.