RGe ISSN: 2177-3246

USING MORE ENVIRONMENTALLY FRIENDLY METHODS TO EXTRACT CHITIN FROM BLACK SOLDIER FLY LARVAE

UTILIZAÇÃO DE MÉTODOS MAIS SUSTENTÁVEIS PARA A EXTRAÇÃO DE QUITINA DE LARVAS DA MOSCA-SOLDADO-NEGRA

USO DE MÉTODOS MÁS SOSTENIBLES PARA LA EXTRACCIÓN DE QUITINA DE LARVAS DE MOSCA SOLDADO NEGRA

10.56238/revgeov17n5-088

Viviane Ferreira Andrade[1], Vinícius Pimentel Silva[2], Marcia Cristina Campos de Oliveira[3]

ABSTRACT

The main sources of the polysaccharide chitin are crustacean shells generated as waste from fishing activities. However, the extraction of chitin by this methodology is highly expensive and polluting process. In order, to minimize the use of chemical agents the present work had as main objective to seek new matrices for chitin extraction, which allow methodologies that uses eco-friendly chemical agents. The chosen matrix was the larvae of the Hermetia Illucens L. and the process for extracting the polysaccharide was designed taking into account the physical property of chitin to be soluble in a warm aqueous system. The methodology followed with the extraction of oil from the larvae of H. Illucens through maceration with dichloromethane and later the larvae were subjected to hot extraction in an autoclave with controlled pressure and temperature, with water as solvent. The aqueous extract was cooled, and ethanol cool was added, which favored the precipitation of the polysaccharide. The polysaccharide was evaluated by FTIR and NMR and by comparing the data in the literature, it is concluded that the proposed methodology was efficient to extract γ-chitin.

Keywords: Matrix. Larvae. *Hermetia illucens L*. Extraction. Polysaccharide.

RESUMO

As principais fontes do polissacarídeo quitina são as carapaças de crustáceos geradas como resíduos das atividades pesqueiras. Entretanto, a extração de quitina por essa metodologia é um processo altamente caro e poluente. Com o objetivo de minimizar o uso de agentes químicos, o presente trabalho teve como principal objetivo buscar novas matrizes para a extração de quitina, que permitam metodologias utilizando agentes químicos ambientalmente sustentáveis. A matriz escolhida foi a larva de *Hermetia illucens* L., e o processo de extração do polissacarídeo foi desenvolvido levando em consideração a

[1] Universidade Federal Rural do Rio de Janeiro. Brazil. E-mail: vivianeandradequimica@ufrrj.br
Orcid: https://orcid.org/0009-0005-0488-571X Lattes: http://lattes.cnpq.br/9123546110655790
[2] Department of Animal Nutrition and Pastures. Universidade Federal Rural do Rio de Janeiro. Brazil.
Orcid: https://orcid.org/0000-0002-5424-2094 Lattes: http://lattes.cnpq.br/1899895022524077
[3] Organic Department. Universidade Federal Rural do Rio de Janeir. Brazil. E-mail: mccdeo@ufrrj.br
Orcid: https://orcid.org/0000-0002-0923-0254

propriedade física da quitina de ser solúvel em sistema aquoso aquecido. A metodologia consistiu inicialmente na extração do óleo das larvas de *H. illucens* por meio de maceração com diclorometano e, posteriormente, as larvas foram submetidas à extração a quente em autoclave com pressão e temperatura controladas, utilizando água como solvente. O extrato aquoso foi resfriado e adicionou-se etanol frio, o que favoreceu a precipitação do polissacarídeo. O polissacarídeo foi avaliado por FTIR e RMN e, pela comparação dos dados com a literatura, concluiu-se que a metodologia proposta foi eficiente para extrair γ-quitina.

Palavras-chave: Matriz. Larvas. *Hermetia illucens* L. Extração. Polissacarídeo.

RESUMEN

Las principales fuentes del polisacárido quitina son los caparazones de crustáceos generados como residuos de las actividades pesqueras. Sin embargo, la extracción de quitina mediante esta metodología es un proceso altamente costoso y contaminante. Con el fin de minimizar el uso de agentes químicos, el presente trabajo tuvo como principal objetivo buscar nuevas matrices para la extracción de quitina que permitan metodologías utilizando agentes químicos ambientalmente sostenibles. La matriz elegida fue la larva de *Hermetia illucens* L., y el proceso de extracción del polisacárido fue diseñado teniendo en cuenta la propiedad física de la quitina de ser soluble en un sistema acuoso caliente. La metodología consistió inicialmente en la extracción del aceite de las larvas de *H. illucens* mediante maceración con diclorometano y, posteriormente, las larvas fueron sometidas a extracción en caliente en autoclave con presión y temperatura controladas, utilizando agua como solvente. El extracto acuoso fue enfriado y se añadió etanol frío, lo que favoreció la precipitación del polisacárido. El polisacárido fue evaluado mediante FTIR y RMN y, al comparar los datos con la literatura, se concluyó que la metodología propuesta fue eficiente para extraer γ-quitina.

Palabras clave: Matriz. Larvas. *Hermetia illucens* L. Extracción. Polisacárido.

1 INTRODUCTION

Chitin is the second most abundant biopolymer in nature and is a fundamental structural component in the exoskeleton of arthropods, the cell walls of fungi, and some marine organisms [1-5]. It is a polysaccharide composed of N-acetyl-D-glucosamine units, whose scientific and technological relevance lies in its biodegradable, biocompatible, and bioactive properties [6]. Traditionally, chitin is obtained from fishing industry waste, especially from crustaceans such as shrimp and crabs [7]. However, this extraction route has limitations related to the seasonality of the raw material, the extraction methodology is not eco-friendly and expensive [8-11].

In this context, insects have emerged as an alternative and sustainable source of chitin, especially due to their high feed conversion efficiency, large-scale cultivation capacity, and adaptability to low-value organic substrates. Among the species studied, the black soldier fly (Hermetia illucens) stands out for its rapid life cycle, high accumulation of larval biomass, and remarkable capacity for bioconversion of organic waste [12-15]. These attributes make its larvae a promising raw material for obtaining chitin, with the potential to meet industrial demands in a more sustainable manner [16-20].

Several approaches have been proposed for the extraction of chitin from H. illucens larvae, including conventional chemical methods (based on acid-base treatments), enzymatic techniques, and more environmentally benign strategies, such as the use of alternative solvents or ultrasound-assisted extraction. The choice of extraction method directly impacts the purity, yield, and structure of the chitin obtained, influencing its applicability in different sectors [6,21].

This article aims to present a critical analysis of the main methods of chitin extraction from black soldier fly larvae, discussing their efficiencies, limitations, and potential environmental impacts, with a view to contributing to the advancement of the use of insects as an alternative source of biopolymers and comparing it with the proposed method.

1.1 SOURCES OF CHITIN

Chitin is a nitrogenous biopolymer widely distributed among living beings, playing a structural role in various organisms of the Animalia, Fungi, and Protista kingdoms. It occurs predominantly in the exoskeleton of arthropods, such as insects and crustaceans, as well as in the structural matrix of mollusks, annelids, and coelenterates. It is also found in the cell walls of diatom algae and filamentous fungi belonging to the phyla Ascomycota, Zygomycota, Basidiomycota, and Deuteromycota11. Fungi such as Mucor rouxii and Aspergillus niger, as well as cell wall components of other filamentous fungi, have been explored as alternative

sources of high-purity chitin [4,22]. From an ecological and technological point of view, marine and terrestrial organisms have significant potential as chitin sources. Among mollusks, the following stand out, oyster shell, squid pen,andkrill, which have varying concentrations of the biopolymer. Crustaceans such as shrimp, crabs, and lobsters still represent the main industrial source due to the availability of fishing industry waste.

Recently, insects such as silkworms (Bombyx mori), diptera, and butterflies have attracted growing interest, especially due to their lower environmental impact and the specific properties of the chitin obtained, such as thermal stability, polymorphism, degree of crystallinity, and extraction yield. These factors directly influence the viability of chitin application in sectors such as pharmaceuticals, biomedicine, and sustainable packaging [23,24]. Although most of the chitin currently used still comes from crustacean exoskeletons, with contents ranging from 15% to 40% depending on the species, season, and extraction methodology [25], alternative sources such as insects are increasingly being considered by the biopolymer industry [26].

The black soldier fly (Hermetia illucens L., Diptera: Stratiomyidae) is a synanthropic species native to the tropical, subtropical, and temperate regions of the American continent, currently distributed cosmopolitanly between latitudes 40° South and 45° North [27]. This species is known for its ecologically relevant role in the conversion of organic waste of plant and animal origin, being widely used in the bioconversion of urban and agro-industrial waste [12,13].

The species has a holometabolous life cycle, comprising five stages: egg, larva, pre-pupa, pupa, and adult. This complete development occurs in approximately 38 to 45 days, depending on environmental conditions [13]. Females lay between 320 and 1000 eggs, usually arranged in narrow rows on dry substrates and close to food sources, in order to protect them from predators and ensure the availability of nutrients during the larval stage [14,28].

The larval stage is considered the most relevant for industrial and research applications, as it is highly efficient in converting organic waste into biomass rich in proteins and lipids, compared to other insect species [14,15]. This high nutritional density, combined with the potential for recovering biocomposites of interest, such as chitin, positions H. illucens as a strategic resource for the development of sustainable technologies in biorefineries and in the production of biopolymers.

The chemical composition of H. illucens larvae varies considerably depending on factors such as species, stage of development, diet, and environmental conditions of breeding. Studies indicate that insects, in general, have chitin contents between 5% and 25%,

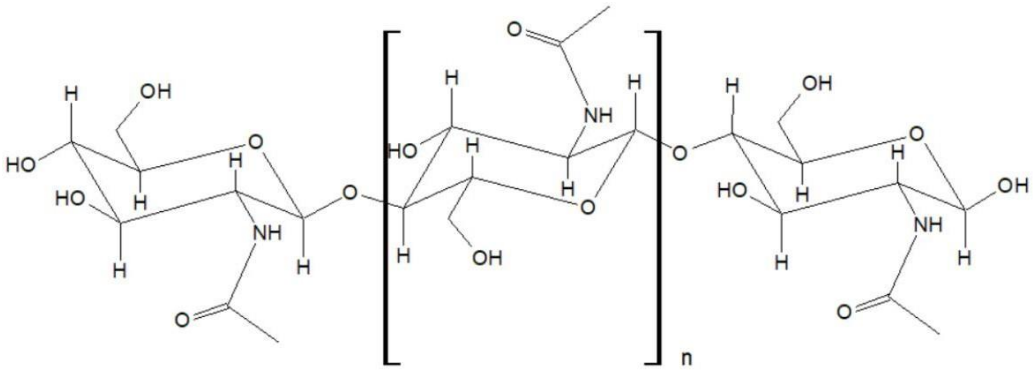
protein between 30% and 60%, lipids between 10% and 25%, and minerals between 2% and 10%. In the specific case of black soldier fly larvae, the composition per 100 g of dry matter can contain between 30 and 53 g of protein, 20 to 41 g of lipids, and 2 to 9 g of chitin [16-20].

1.1.1 Chemical Structure of Chitin and Derivation of Chitosan

Chitin is a naturally occurring linear aminopolysaccharide composed of repeating units of N-acetyl-D- glucosamine ($C_8H_{13}O_5N$), linked by β-(1→4) [2], glycosidic bridges [22]. The structure of chitin is similar to that of cellulose, with the substitution of the hydroxyl group at the C-2 position by an acetamide group, conferring specific properties, such as greater rigidity and chemical resistance. As illustrated in Figure 1, chitin consists of macromolecules that are insoluble in water and highly resistant to acids, bases, and most organic solvents, although it is soluble in fluorinated solvents such as hexafluoroisopropanol and hexafluoroacetone [29].

Figure 1

Molecular structure of chitin

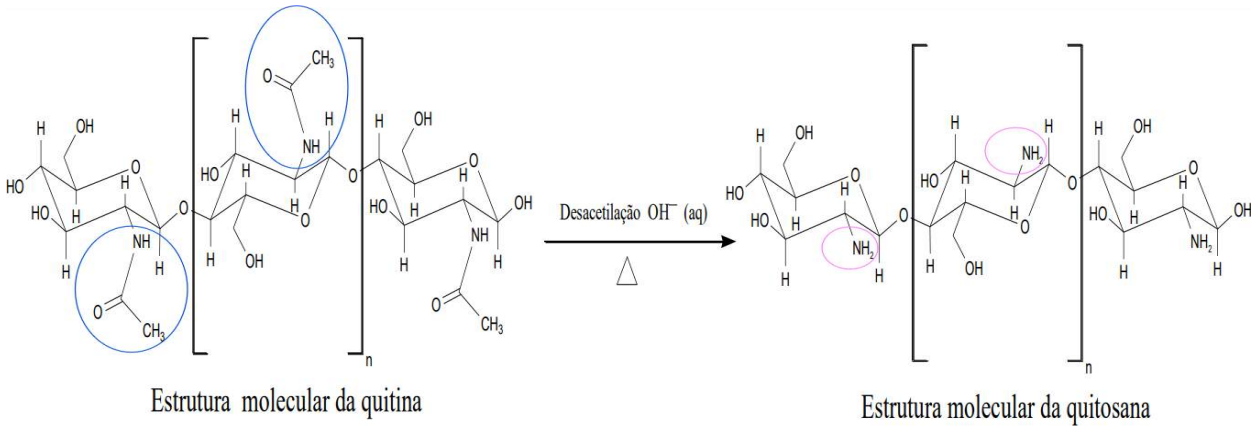


In nature, chitin occurs in the form of organized crystalline microfibrils, composing highly ordered structures. In its purest form, the material is a white or yellowish solid with high biocompatibility, bioabsorbability, and biodegradability properties [2,30,31]. Chitin is estimated to be one of the most abundant nitrogenous polymers on the planet, with an annual production in the biosphere estimated between 10^{11} and 10^{14} tons [32].

Chitosan is obtained from the partial deacetylation of chitin, involving the removal of 50% or more of the acetyl groups in an alkaline medium, resulting in a copolymer consisting of β-(1→4)-2-amino-2-deoxy-D-glucopyranose and β-(1→4)-2-N-acetyl-2-deoxy-D-glucopyranose units (Figure 2)[14,15]. This modification makes chitosan soluble in acidic media and amplifies its technological applicability [33].

Figure 2

Process of chitin hydrolysis to form chitosan

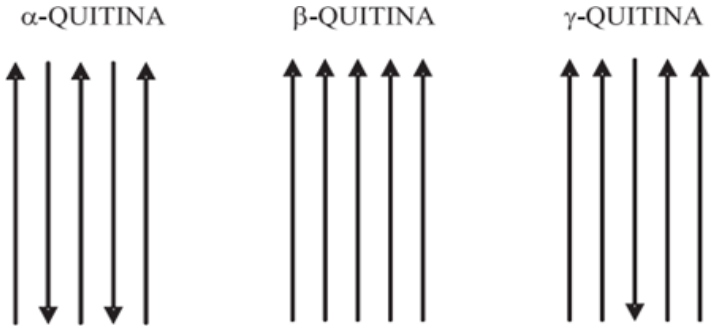


1.1.2 Structural Polymorphism of Chitin

Chitin has three main allotropic or polymorphic forms: α-, β-, and γ-chitin, differentiated by the arrangement of polymer chains within the crystalline domains2(Figure 3). These structural variations directly influence physicochemical properties such as packing density, crystallinity, and mechanical strength [34]. The α-chitin form, predominant in crustaceans and insects, is characterized by an antiparallel packing of polymer chains in adjacent lamellae, which favors the formation of extensive networks of intra- and interlamellar hydrogen bonds. This arrangement confers high thermal stability and low chemical reactivity. In contrast, β-chitin, common in cephalopods such as squids, has a parallel packing of chains, resulting in a less dense structure that is more susceptible to interaction with solvents and chemical reagents2. γ-chitin, in turn, is less studied and occurs less frequently. It has a hybrid organization, composed of lamellae alternating with parallel and antiparallel chains. This form has been described in specific biological structures, such as the fibers of the Ptinus beetle cocoon, in the stomach of Loligo, and in insects such as grasshoppers, cockroaches, and larvae of Antheraea pernyi and Phymatocera aterrima [2,30,35]. Polymorphic morphology also influences the mechanical and pharmacotechnical behavior of chitin. Studies show that both α- and β-chitin have high compaction strength and rapid dissolution, desirable characteristics for fast-release oral tablet formulations [35,36]. Thus, the biological origin and crystalline form of chitin are determining factors in the selection of raw materials for specific applications [34,37].

Figure 3

Schematic representation of the polymorphic structures of chitin, with the arrows representing the polymer chains in the direction from the non-reducing to the reducing end. Source: Campana-Filho, 2007[2]



1.1.3 Extraction of Chitin from Hermetia illucens Larvae

Chitin is a biopolymer of great scientific and industrial relevance and obtaining it from alternative sources has been intensively explored in recent years. This chapter discusses the main strategies for extracting chitin from Hermetia illucens (Black Soldier Fly) larvae, a promising source due to its high productivity, low cost, and sustainability. The main objective of the study is to develop an efficient and environmentally safe method for extracting this biopolymer, with an emphasis on reducing the use of toxic reagents and simplifying the process steps. Conventional chemical approaches are analyzed, as well as emerging methods based on clean technologies, such as the use of deep eutectic solvents, thermal hydrolysis, and microbial fermentation. The choice of the MSN larval stage is justified by its high availability and potential for industrial waste utilization. The expected results aim not only to enable a sustainable extraction protocol but also to enhance the value of insects as a biotechnological platform for the production of functional materials, in line with the principles of green chemistry and circular economy.

1.1.4 Extraction of Chitin from Black Soldier Fly (BSF) Larvae Using the Conventional Method

The methodology for extracting chitin from the pupal exuviae of the black soldier fly (BSF) was carried out through the sequential steps of demineralization, deproteinization, and bleaching [21]. Initially, 1 g of pupal exuviae was subjected to demineralization in 15 mL of acid at varying concentrations using a Heidolph Synthesis 1 device. For control purposes, a sample was incubated only with water at 70 °C, without the addition of acid, in order to establish a reference point. After the incubation period, the suspension was centrifuged at 4696 g for 10 minutes, and the supernatant was discarded. The remaining biomass was

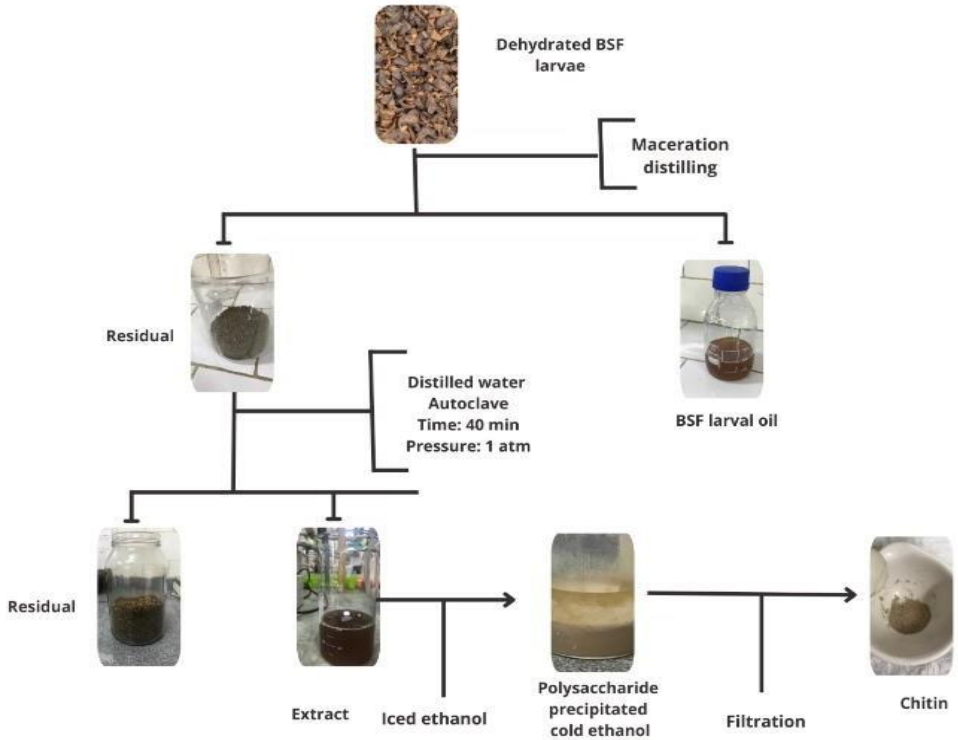
washed with 15 mL of distilled water and subjected to further centrifugation under the same conditions. The residue was then dried in an oven at 105 °C overnight, followed by determination of the ash content. In the subsequent step, 5 g of previously demineralized pupal exuviae were added to 50 mL of heated sodium hydroxide (NaOH) solution, under constant stirring at 300 rpm. After the treatment time, the mixture was cooled in an ice bath. The deproteinized material was separated by vacuum filtration using a glass funnel equipped with an integrated filter membrane with a porosity between 100 and 150 µm, and then dried in an oven at 105 °C. Finally, the third step consisted of incubating the pupal exuviae of in a 2.5 M NaOH solution, at a liquid/solid ratio of 10:1, under agitation at 500 rpm and a temperature of 90 °C for 2 hours. At the end, the material was filtered, washed with water heated to 80°C, and dried in an oven at 105 °C. Additionally, an alternative methodological approach was considered, based on modified protocols, which consisted of altering the experimental conditions of the conventional steps. Mineral extraction was performed using 0.5 mol L^{-1} formic acid at a ratio of 1:10 (m/v), under constant stirring for 1 hour at room temperature. The material was then subjected to multiple washes with distilled water until a neutral pH was obtained, and subsequently dried in an oven at 60°C. The deproteinization step was conducted with a 2 mol L^{-1} sodium hydroxide (NaOH) solution, maintaining a ratio of 1:10 (m/v), at a temperature of 80 °C for 2 hours, under continuous stirring. After the alkaline treatment, the same washing and drying conditions described in the previous step were used. Finally, the chitinous biomass was bleached using 5% (v/v) hydrogen peroxide (H_2O_2) in an aqueous medium, heated to 90°C for 30 to 60 minutes, with gentle stirring. At the end, the material was filtered using filter paper, washed with distilled water, and dried in an oven [21].

2 EXPERIMENTAL PART

The extraction of chitin from BSF larvae was performed in two steps, first the extraction of lipids based on the physical properties of these biomolecules, that is, their solubility in non-polar or low-polar organic solvents and their property of being insoluble in water, and then the extraction of chitin by autoclaving as shown in Figure 4.

Figure 4

Chitin extraction scheme from BSF larvae



2.1 EXTRACTION OF OIL FROM BSF LARVAE

The dehydrated BSF larvae were provided by the Animal Nutrition Laboratory "Prof. Mário Pinheiro" of the Department of Animal Nutrition and Pastures of the Animal Science Institute of the Federal Rural University of Rio de Janeiro (LABNUTRI). The extraction of oil from BSF larvae was the starting point for this work. The larvae were divided into three vials, each containing 300 grams of larvae and one litre of extraction solvent. The extraction method uses maceration with organic solvents anhydrous ethyl ether, dichloromethane and hexane for 24 hours under refrigeration in a domestic refrigerator. The solutions were subjected to simple filtration and finally distilled under reduced pressure in a rotary evaporator.

The oil extracted from the BSF larvae was analyzed by gas chromatography coupled to a mass spectrometer, GCMS-QP2010 Plus (Shimadzu). The column oven was programmed as follows: initial temperature 180 ºC (5 min), 260 ºC (10 ºC min-1), 300 ºC (2 ºC for 2 min -1), 300 ºC (30 min -1). A 220ºC injector was used on a VF-5ms column (30x0.25x0.25) with a flow rate of 0.91 ml/min-1 and dichloromethane as carrier gas.

2.2 EXTRACTION OF CHITIN FROM BSF LARVAE

After extraction of oil from BSF larvae, qualitative and quantitative characterization of chitin was performed by aqueous extraction in an autoclave and precipitation in ethanol-PA.

The first step of the process consisted of placing 600 ml of distilled water and 100 grams of the sample in an Erlenmeyer flask. The flasks were properly sealed and placed in an autoclave at 1.00 atm. pressure for 40 minutes. After this period, they were allowed to stand until they reached room temperature and were subjected to simple filtration.

In the second step, an amorphous solid was precipitated by adding ice-cold PA-ethanol to the aqueous extracts obtained and allowing them to rest for 24 hours in a refrigerated environment. The solid was separated by decantation and the solvent residue was removed by evaporation in a water bath until a dark brown solid was obtained.

The infrared spectrum of chitin was obtained with a VERTEX 70 instrument in the spectral range of 4000 - 400 cm-1, in the form of transmittance vs. wave number (s - symmetric stretching; asymmetric stretching; δ - deformation; ω - oscillation).

The NMR 13C spectrum was obtained with a Bruker high-resolution quadrupole/electrospray ionization time-of-flight instrument (microTOF II, Bruker Daltonics Billerica, MA) at a frequency of 8000 Hz.

3 RESULT AND DISCUSSION

3.1 ANALYSIS OF THE EXPERIMENTAL RESULTS FOR THE EXTRACTION OF OIL FROM BSF LARVAE

It is necessary to extract the oil from the larvae in order to use the method for extracting chitin from BSF larvae. The extraction of lipids from BSF larvae was based on the physical properties of these biomolecules, i.e. their solubility in non-polar or low-polar organic solvents. The extractions were carried out by maceration with three different extraction solvents: anhydrous ethyl ether, hexane and dichloromethane (Table 1).

The extraction of oil from BSF larvae was highlighted by the solvent dichloromethane, which gave better oil yields in addition to solvent recovery (70%), favouring the bioeconomy of the method.

Table 1

Results of MSN larval oils extracted with different solvents

Density	Yields (%)	Extraction time
0.81 g/ml[a]	15,7%[a]	24H[a]
0.81 g/ml[b]	27,3%[b]	24H[b]
0.81 g/ml[c]	8,7%[c]	

		24H[c]
0.94 g/ml[d]	28,3%[d]	24H[d]
1,06 g/ml[e]	11,0%[e]	24H[e]
0,92 g/ml[f]	4,2 %[f]	24H[f]

[a] Anhydrous ether. [b] First extraction with hexane. [c] Second extraction with hexane. [d] First extraction with dichloromethane [e] Second extraction with dichloromethane [f] Replica with dichloromethane.

3.2 ANALYSIS OF MSN OILS BY GC-MS

Analysis of the oil extracted with dichloromethane from BSF larvae was carried out using gas chromatography coupled to a mass spectrometer and revealed the presence of triacylglyceride. Structural elucidation of the major

component, glyceryl tridodecanoate, was possible by correlating the retention time associated with the peak generated in the chromatogram (Figure 5A) and the fragmentation patterns of the mass spectrum (Figure 5B) with the compounds cataloged in the library National Institute of Standards and Technology (NIST).

Figure 5

Gas chromatography with mass spectrometry of oil from BSF lavae: A) chromatogram of a oil solution; B) masss espectrum

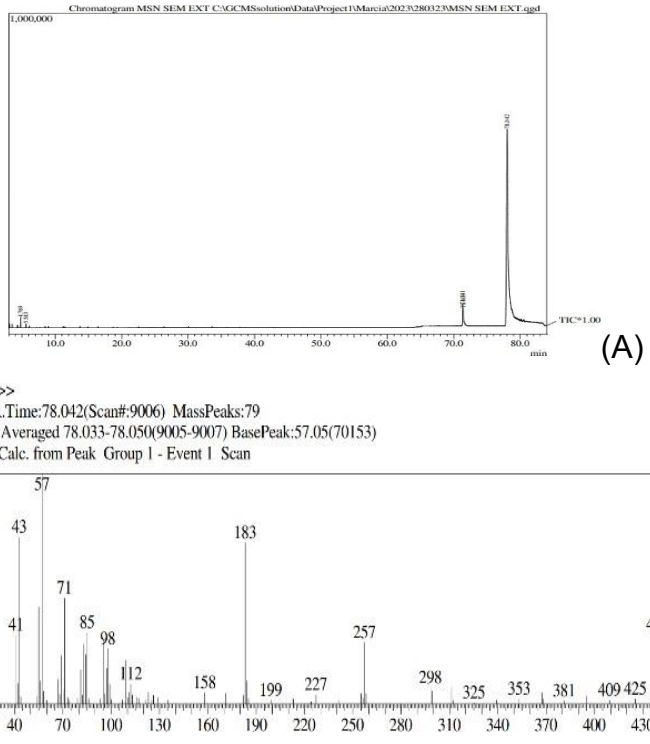
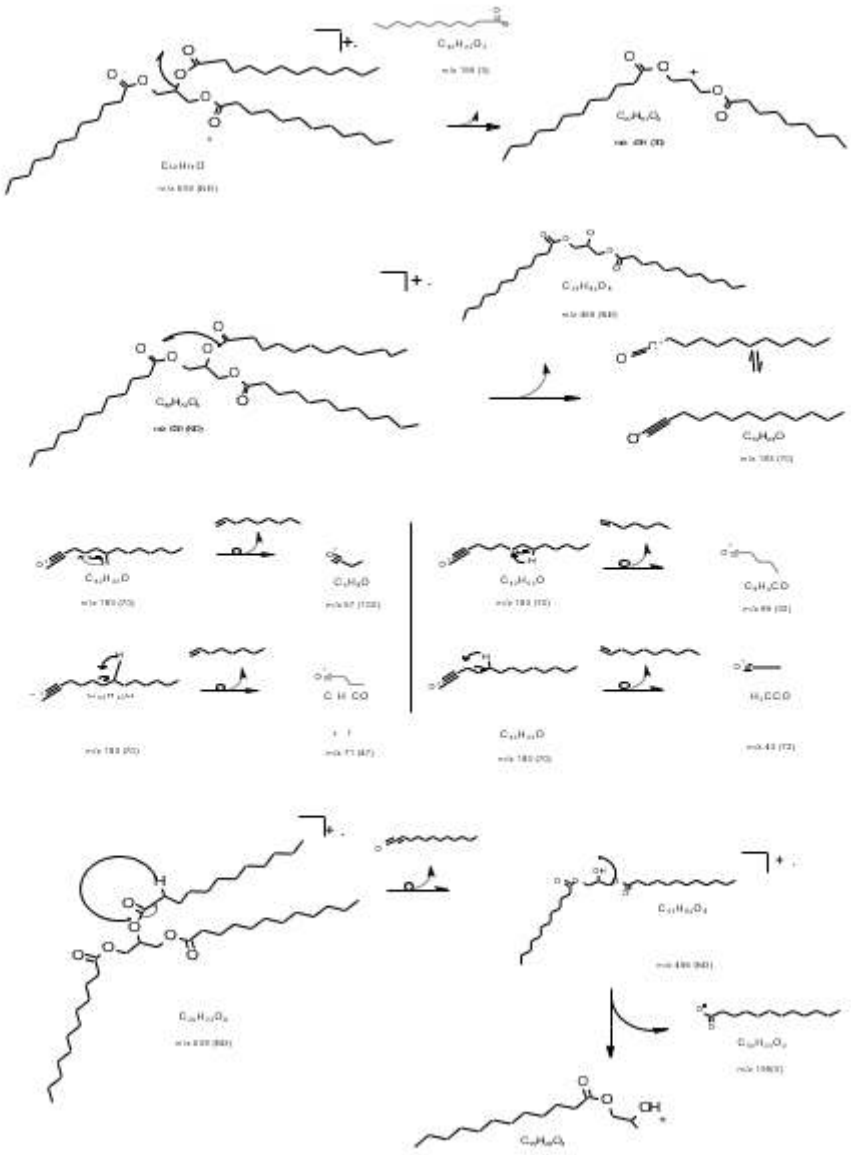


Figure 6 shows a fragmentation proposal to justify the main peaks detected in the mass spectrum of the majority component detected with the GC. The m/z 183 (70%) corresponds to the C12H23O of an acyl unit, m/z 257 (30%) the ion containing this unit linked to part of the glycerol (C15H29O3+) and the peak at m/z 439 (30%) represents an ion with two dodecanoyl units with part of the glycerol (C27H51O4+). The other peaks detected in the MS can be explained by additional fragmentations.

Figure 6

Proposed fragmentation of Dodecanoic acid, 1,2,3-propanetrilic ester



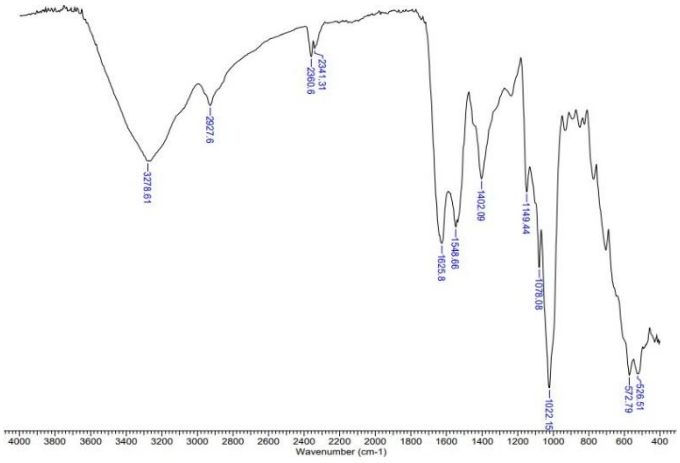
3.3 ANALYSIS OF THE POLYSACCHARIDE CHITIN BY MEANS OF THE INFRARED SPECTRUM

Extraction of chitin from BSF larvae by the autoclave method yielded 4% after extraction of oil with dichloromethane solvent. The infrared spectrum of chitin in the 4000-400 cm-1 region showed a band at 3278.61cm-1 characteristic of the axial stretching of the -OH group in intermolecular hydrogen bonding superimposed on the axial stretching of the N-H group. The band at 2927.6 cm-1 is an axial stretching of CH2 and CH3. The bands at 2360.6 cm-1 and 2341.31 cm-1 are associated with the angular deformation of N-H showing a change in values due to intermolecular hydrogen bonding. The stretching band at 1625.8 cm-1 of amide I and the stretching band at 1548.66 cm-1 of amide II. In addition, the band at 1022.15 cm-1 refers to the stretching of the C-O group of the primary alcohol and the stretching band at 1078.08 cm-1 of the secondary alcohol (Figure 7).

The three allomorphic forms of chitin can be rapidly determined by FT-IR spectroscopy. FT-IR studies show that the amide band of α-chitin has two bands (1660 and 1620 cm-1), whereas β-chitin has a single band at 1640 cm- 1 due to hydrogen bonds between the molecules [38, 39]. The γ-chitin shows that the band of the amide I is partially split into two bands, one of which is slightly weaker at 1654 cm-1 and the other more intense at 1621 cm-1[40, 41]. The results obtained showed that the polysaccharide isolated from BSF larvae is γ-chitin.

Figure 7

Infrared spectrum of chitin solids in the 4000-400 cm field -1



3.4 NMR ANALYSIS OF CHITIN POLYSACCHARIDE

Solid-state 13C nuclear magnetic resonance spectrometry (13C NMR) is an analytical tool used to determine the molecular structure of a molecule by detecting even small changes in its structure. It is therefore a useful tool for identifying the allomorphs of chitin[12].

The analysis of the 13C NMR spectrum of the chitin extracted from the BSF showed signals at 174.46 ppm for the carbonyl group, 102.79 for C1, 81. 85 ppm for (C4) and 72.34 ppm for (C3 and C5), the signal at 60.21 ppm for C6, 54.16 and 52.29 ppm for (C2 and C2'), and 24.58 and 18.25 ppm for the methyl group in different chemical environments (C8 and C8') (Figure 8).

As discussed in the literature, the resonance of chitin showed similarity in almost all peaks and chemical shifts of the three forms of its polyformism [43,44]. The 13C NMR of chitin from BSF larvae was consistent with that reported in the literature [45,46], confirming that the polysaccharide found in the sample was γ-chitin (Table 2).

Figure 8

13C solid-state NMR of chitin extracted from BSF larvae.

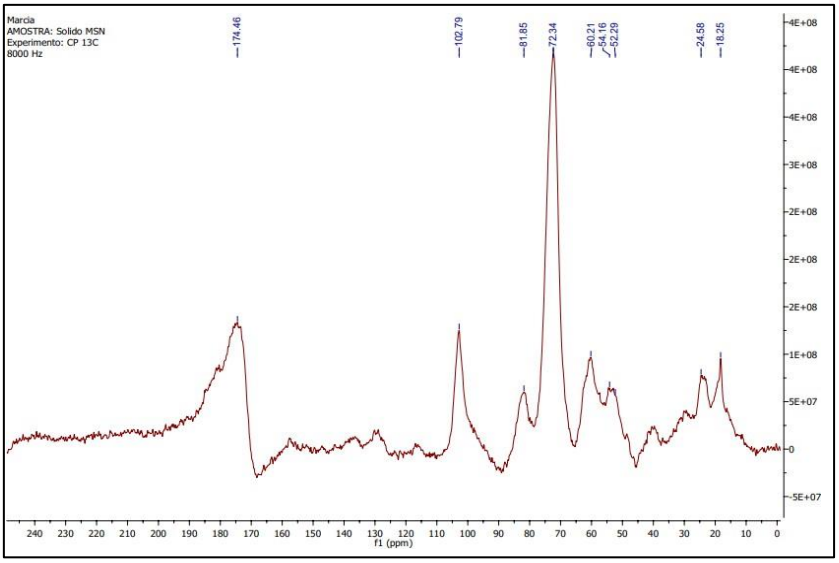


Table 2

NMR data for Chitin from BSF and literature date [15,16].

Carbon	Chitin (BSF) δc	Bahía, et. Al[15] δc	Xesus, et. Al[16] δc
C-7	174.4	173.1	173.2
C-1	102.8	104.2	103.4
C-4	81.9	85.0	81.9
C-3,5	72.3	75.6	14.2
C-6	60.2	59.7	60.7
C-2	54.2	55.6	55.1
C-8	24.6	23.0	21.6

4 CONCLUSION

In conclusion, the most effective and efficient method was the extraction of oil from BSF larvae by maceration with an organic solvent, followed by extraction of chitin using water and an autoclave at controlled pressure and temperature. The solid-liquid extraction of oil from the MSN larva by maceration with organic extraction solvents was characterised by the dichloromethane solvent, which gave the most effective result, taking into account the time of the process and the reuse of the solvent. The remaceration with organic solvents is not feasible because the cost and time spent do not compensate for the yield obtained in comparison with the first extraction. The quantitative and qualitative characterisation of the chitin polysaccharide by extraction with water and autoclaving of the BSF larvae waste, followed by the addition of ethanol, showed the method to be favourable for the bioeconomy. When extracting chitin from MSN larvae using an autoclave, the most effective ratio was 100 grams of residue to 500 ml of water, taking into account the volume of the container. For precipitation of the polysaccharide, the range of 100% to 300% ethanol in relation to the volume of water extract remained safe for the method.

REFERENCES

1. Hamed, I., Özogul, F., & Regenstein, J. M. (2016). Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. Trends in Food Science & Technology, 48, 40–50. https://doi.org/10.1016/j.tifs.2015.11.007

2. Campana-Filho, S. P., et al. (2007). Extraction, structures, and properties of alpha- and beta-chitin. Química Nova, 30(3), 644–650. https://doi.org/10.1590/S0100-40422007000300026

3. Ben Aoun, R., et al. (2024). Towards a greener future: Exploring the challenges of extraction of chitin and chitosan as bioactive polysaccharides. Materials Today Communications, 39, 108761. https://doi.org/10.1016/j.mtcomm.2024.108761

4. Kurita, K. (2006). Chitin and chitosan: Functional biopolymers from marine crustaceans. Marine Biotechnology, 8(3), 203–226. https://doi.org/10.1007/s10126-005-0097-5

5. Alabaraoye, E., Achilonu, M., & Hester, R. (2018). Biopolymer (Chitin) from various marine seashell wastes: Isolation and characterization. Journal of Polymers and the Environment, 26(6), 2207–2218. https://doi.org/10.1007/s10924-017-1118-y

6. Triunfo, M., et al. (2022). Characterization of chitin and chitosan derived from Hermetia illucens, a further step in a circular economy process. Scientific Reports, 12(1), 6613. https://doi.org/10.1038/s41598-022-10423-5

7. Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. Progress in Polymer Science, 31(7), 603–632. https://doi.org/10.1016/j.progpolymsci.2006.06.002

8. Pinto, A. S. (2014). Optimization of processes for obtaining chitin and chitosan from the

exoskeleton of Amazonian shrimp (Macrobrachium amazonicum, Heller, 1863) [Dissertação de mestrado, Federal University of Pará]. https://ppgcta.propesp.ufpa.br/ARQUIVOS/dissertacoes/2014/Andrea%20Pinto.pdf

9. Global Chitin Market Size. (2024). Chemical & Material Research. Verified Market Research. https://www.verifiedmarketresearch.com/product/chitin-market/

10. Basawa, R., et al. (2023). Repurposing chitin-rich seafood waste for warm-water fish farming. Heliyon, 9(7), e18197. https://doi.org/10.1016/j.heliyon.2023.e18197

11. Moura, C., Muszinski, P., Schimidt, C., Almeida, J., & Pinto, L. (2007). Chitin and chitosan produced from shrimp and crab waste: Evaluation of the process on a pilot scale. Vetor - Journal of Exact Sciences and Engineering, 16(1), 37–45. https://periodicos.furg.br/vetor/article/download/294/85

12. Silva, G. D. P., & Hesselberg, T. (2020). A review of the use of Black Soldier Fly larvae, Hermetia illucens (Diptera: Stratiomyidae), to compost organic waste in tropical regions. Neotropical Entomology, 49(2), 151–162. https://doi.org/10.1007/s13744-019-00719-z

13. Spinelli, R., et al. (2018). Using Black Soldier Flies (Hermetia illucens) to bioconvert waste from the livestock production chain: A life cycle assessment case study. In Waste Management and the Environment IX (pp. 47–58). https://doi.org/10.2495/WM180051

14. Santos, S. S., et al. (2021). Protein and lauric oil production from agricultural waste bioconversion by Hermetia illucens larvae. Revista Virtual de Química, 13(4), 959–968. https://doi.org/10.21577/1984-6835.20210028

15. Tomberlin, J. K., & Sheppard, D. C. (2002). Factors influencing mating and oviposition of Black Soldier Flies (Diptera: Stratiomyidae) in a colony. Journal of Entomological Science, 37(4), 345–352. https://doi.org/10.18474/0749-8004-37.4.345

16. Bosch, G., et al. (2014). Protein quality of insects as potential ingredients for dog and cat foods. Journal of Nutritional Science, 3, e29. https://doi.org/10.1017/jns.2014.23

17. Bußler, S., et al. (2016). Recovery and techno-functionality of flours and proteins from two edible insect species: Mealworm (Tenebrio molitor) and Black Soldier Fly (Hermetia illucens) larvae. Heliyon, 2(12), e00218. https://doi.org/10.1016/j.heliyon.2016.e00218

18. Caligiani, A., et al. (2018). Composition of Black Soldier Fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. Food Research International, 105, 812–820. https://doi.org/10.1016/j.foodres.2017.12.012

19. Finke, M. D. (2013). Complete nutrient content of four species of feeder insects. Zoo Biology, 32(1), 27–36. https://doi.org/10.1002/zoo.21012

20. Meneguz, M., et al. (2018). Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of Black Soldier Fly (Hermetia illucens) larvae. Journal of the Science of Food and Agriculture, 98(15), 5776–5784. https://doi.org/10.1002/jsfa.9127

21. Hahn, T., et al. (2022). Purification of chitin from pupal exuviae of the black soldier fly. Waste and Biomass Valorization, 13(4), 1993–2008. https://doi.org/10.1007/s12649-021-01645-1

22. Aoum, R. B., et al. (2024). Towards a greener future: Exploring the challenges of extraction of chitin and chitosan as bioactive polysaccharides. Materials Today Communications, 39, 108761. https://doi.org/10.1016/j.mtcomm.2024.108761

23. Zainol Abidin, N. A., et al. (2020). The potential of insects as alternative sources of chitin: A review on chemical extraction methods from various sources. International Journal of Molecular Sciences, 21(12), 4978. https://doi.org/10.3390/ijms21124978

24. Mei, Z., Kuzhir, P., & Godeau, G. (2024). Update on chitin and chitosan from insects: Sources, production, characterization, and biomedical applications. Biomimetics, 9(5), 297. https://doi.org/10.3390/biomimetics9050297

25. Yan, N., & Chen, X. (2015). Sustainability: Don't waste seafood waste. Nature, 524(7564), 155–157. https://doi.org/10.1038/524155a

26. Mersmann, L., Souza, V. G. L., & Fernando, A. L. (2025). Green processes for chitin and chitosan production from insects: Current state, challenges, and opportunities. Polymers, 17(9), 1185. https://doi.org/10.3390/polym17091185

27. Devic, E., & Fahmi, M. R. (2013). Technical handbook of domestication and production of Diptera Black Soldier Fly (BSFL) Hermetia illucens, Stratiomyidae. In Biology of Hermetia illucens (chap. 1, pp. 2–10). https://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers17-11/010063336.pdf

28. Tomberlin, J. K., Adler, P. H., & Myers, H. M. (2019). Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environmental Entomology, 38(3), 930–934. https://doi.org/10.1603/022.038.0347

29. Hayes, M., et al. (2008). Mining marine shellfish wastes for bioactive molecules: Chitin and chitosan – Part A: Extraction methods. Biotechnology Journal, 3(7), 871–877. https://doi.org/10.1002/biot.200700207

30. Ramírez, M. Á., et al. (2010). Chitin and its derivatives as biopolymers with potential agricultural applications. Biotechnology Applied, 27(4), 270–276.

31. Dutta, P. K., Dutta, J., & Tripathi, V. S. (2004). Chitin and chitosan: Chemistry, properties and applications. Journal of Scientific & Industrial Research, 63, 20–31.

32. Bastiaens, L., et al. (2019). Sources of chitin and chitosan and their isolation. In Chitin and chitosan: Properties and applications (pp. 1–34). Wiley. https://doi.org/10.1002/9781119450467.ch1

33. Rinaudo, M., & Pérez, S. (2019). From chitin to chitosan: Structural analysis, processes, and solubility in acidic media. In Glycopedia – From Chitin to Chitosan (chap. 4). Glycopedia.eu. https://www.glycopedia.eu/From-Chitin-to-Chitosan

34. Joseph, S. M., et al. (2021). A review on source-specific chemistry, functionality, and applications of chitin and chitosan. Carbohydrate Polymers Technologies and Applications, 2, 100036. https://doi.org/10.1016/j.carpta.2021.100036

35. Kaya, M., et al. (2017). On chemistry of γ-chitin. Carbohydrate Polymers, 176, 177–186. https://doi.org/10.1016/j.carbpol.2017.08.076

36. Al-Hmoud, L., et al. (2020). Influence of chitin source and polymorphism on powder-compression and compaction: Application in drug delivery. Molecules, 25(22), 5269. https://doi.org/10.3390/molecules25225269

37. Tovar-Jimenez, G. I., et al. (2020). Chapter 5 - Chitin blends, interpenetrating polymer networks, gels, composites, and nanocomposites for adsorption systems: Environmental remediation and protein purification. In Handbook of Chitin and Chitosan (Vol. 3, pp. 135–175). https://doi.org/10.1016/B978-0-12-817966-6.00005-4

38. Tsurkan, M. V., et al. (2021). Progress in chitin analytics. ELSEVIER, 1-21.

39. Darmon, S., & Rudall, K. (1950). Infra-red and X-ray studies of chitin. Discussions of the Faraday Society, 9, 251-260.

40. Jang, M. K., Kong, B. G., Jeong, Y. I., Lee, C. H., & Nah, J. W. (2004). Physicochemical characterization of α-chitin, β-chitin, and γ-chitin separated from natural resources. Journal of Polymer Science Part A: Polymer Chemistry, 322-337.

41. Kaya, M., et al. (2017). On chemistry of γ-chitin. ELSEVIER, 177-186.

42. Tsurkan, M. V., et al. (2021). Progress in chitin analytics. ELSEVIER, 1-21.

43. Fernando, L. D., et al. (2021). Structural polymorphism of chitin and chitosan in fungal cell wall from solid-state NMR and principal component analysis. Frontiers in Molecular Biosciences, 1-12.

44. De Velde, K. V., & Kiekens, P. (2004). Structure analysis and degree of substitution of chitin, chitosan and dibutyrylchitin by FT-IR spectroscopy and solid state 13C NMR. ELSEVIER, 409-416.

45. Abdelmalek, B. E., et al. (2017). α-Chitin and chitosan from squid gladius: Biological activities of chitosan and its application as clarifying agent for apple juice. ELSEVIER, 953-962.

46. Feás, X., et al. (2020). Extraction and physicochemical characterization of chitin derived from the Asian hornet, Vespa velutina Lepeletier 1836 (Hym.: Vespidae). Molecules, 25, 384.