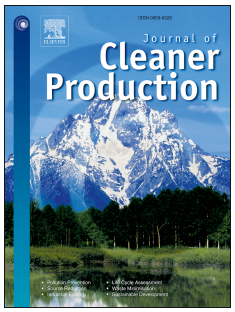

Isolation and physicochemical properties of chitin polymer from insect farm side stream as a new source of renewable biopolymer

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Isolation and physicochemical properties of chitin polymer from insect farm side stream as a new source of renewable biopolymer

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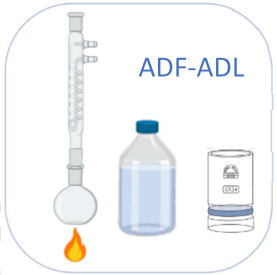
Hermetia illucens

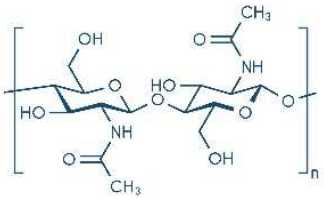
Insect farm

Fish Food

Side stream

Chitin Extraction





chitin

Side Stream Biomass Valorisation

Potential applications

Chito oligosaccharide

Biomaterials

Isolation and physicochemical properties of chitin polymer from insect farm side stream as a new source of renewable biopolymer

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ABSTRACT

This study aims to valorize chitin polymer from the side stream of an insect farm and to determine the chitin content and its physicochemical properties obtained from different processing steps in the insect farm (Adult Black Soldier Fly insect, Puparia, and Flake). We used an acid-base method (using 1M HCl and 1M NaOH) as a conventional technique and the acid detergent fiber (ADF) with acid detergent lignin (ADL) methods. The chitin samples are then characterized for thermal stability (TGA-DTA), crystallinity (XRD), chemical compounds (FTIR), and C/N content, and the results were compared to the commercial shrimp chitin. The Puparia had the highest chitin content of 21-33%, followed by the Flake 20-28% and the Adult insect with 7-13% chitin, depending on the extraction method. The chitin yield from ADF-ADL method was on par with the conventional method, while the ADF results were approximately 3-10% higher than the ADF-ADL results. The insect farm side stream is an abundant rich source of high-quality chitin with physiochemical properties comparable with the commercially available shrimp derived chitin.

Keyword: Black soldier Fly (BSF); *Hermetia illucens*; chitin; acid detergent fiber (ADF); acid detergent lignin (ADL)

1 Introduction

Black soldier fly BSF (*Hermetia illucens*) is an efficient converter of organic waste as the largest municipal waste. BSF larvae with a fast growth rate can rapidly decompose organic waste and convert it into economically valuable biomass of 41-44% protein and 15-49% fat (Jucker et al., 2017; Jucker et al., 2020), with reduced bad odor and eliminated potential diseases. This multi-beneficial breeding insect has attracted much attention in the last decade. The high-quality protein-rich insect biomass has increasingly become a sustainable feed for farmed fish industries such as tuna and salmon, where the feed is normally sourced from wild fish stocks. Insect cuticles, shells of crabs, lobsters, shrimp, fungal, yeast cell walls, and marine sponges contain α-chitin as a stable, resistant, and abundant biopolymer arranged in anti-parallel strands (Hou et al., 2016). The presence of the chitin along with the protein and lipid can affect the nutrients digestibility; therefore there is a chitin rich side stream from the insect farms, which is a biopolymer with a versatile range of applications such as biochemical and biomedical applications (Dutta et al., 2012; Shavandi et al., 2016a, b).

Chitin and its derivative chitosan are conventionally produced from shrimp or crab shells, and there is a large number of publications regarding their modification, function, and physiochemical properties (El Knidri et al., 2018; Shavandi et al., 2015). Nevertheless, the chitin generated as a side stream from the insect farms has not yet been well investigated and characterized. While there are several reports on the content and properties of various insect-derived chitin (Purkayastha and Sarkar, 2020; Wang et al., 2020), there is a lack of precise isolation and characterization of chitin, and the side stream fraction from the insect farms has not yet been investigated to valorize the biopolymer. In addition, biopolymers extracted from the insects can have physicochemical properties different than the chitin normally derived from shrimp and crab, which can affect its final applications. For example, pupae shells and adult insects reported having a medium molecular weight, lower than some crustacean groups, such as lobster, crab shells, and fungi but higher than shrimp (Zimri, 2018).

Chitin isolation method could influence the final properties of an extracted chitin in terms of purity, crystallinity, thermal and mechanical stability. The insect chitin is covalently bonded to cuticular protein

during the sclerotization process, which can make its determination erroneous using normal gravimetric measurements (Ishimaru et al., 2016).

This study aims to isolate and characterize the physicochemical properties of different chitin fractions obtained from the Adult insect, Puparia, and Flakes generated after separating the oil. To our knowledge this is the first study on the valorization of chitin biopolymer from an insect farm side stream residue (flakes) which are generated after removing of the oil. This oil extraction process from the insect might affect the physicochemical properties of the chitin, and there is a need to investigate the yield of chitin and its physiochemical properties. For this purpose, the conventional chitin isolation method, acid-base (HCl and NaOH) is compared with a two-step method based on the measurement of acid detergent fiber (ADF) and acid detergent lignin (ADL) where the remaining amino acid in the chitin samples is removed, and the chitin content can be accurately determined.

2 Materials and Methods

Black Soldier Fly samples of the exoskeleton, pupa exuviae (**Puparia**), Flakes obtained after the oil extraction (**Flakes**), and intact insects (**Adult insect**) were provided by ProteinFarm (A company based in Flanders, Belgium) where insects were reared on plant waste and food industry waste streams. The samples stored in tight plastic containers in a -20ºC freezer before the experiment. Commercial shrimp chitin (C7170), which used as the benchmark and hydrochloric acid (HCl) were purchased from Sigma (Sigma Chemical, St. Louis, MO, USA). Sodium hydroxide (NaOH) were purchased from BDH (Leuven, Belgium) and hexane, high purity Cetrimonium bromide (CTAB), and sulfuric acid were from VWR.

2.1 Conventional chitin extraction with acid and base

The samples (Adult insect, Flake, and Puparia) were dried in an oven at 50ºC overnight then ground by a Waring lab grinder (Waring Commercial, CT, USA) and samples with 0.5 mm particle size were kept in an airtight container at 4ºC. The preparation of chitin from insect samples was carried out as described by Liu et al (Liu, S. et al., 2012). Briefly, the powdered samples (5g) were added slowly to 250 mL of 1M HCl, the solution temperature was brought to 100°C and was stirred at that temperature for 30 min to remove pigments and minerals. The samples were washed to neutrality and then treated with 250 mL of

1M NaOH for deproteinization for 24 hours. The light-yellow chitin samples were obtained after washing with distilled water until neutrality and then dried in an oven at 50ºC. The obtained dried chitin was weighed to determine the yield.

2.2 ADF, ADL, and ADF-ADL chitin isolation methods

The ADF and ADL methods were performed following the method previously reported (Hahn et al., 2018). Briefly, after defatting the ground samples with hexane, 1g of dried sample was suspended in 100 mL of a 0.5 M H_2SO_4 with 20g/L of CTAB in a 250 mL round bottom flask. The suspension was boiled under reflux for one hour and then vacuum filtered using a dried and weighed 50 mL filter crucible (VitraPOR® glass filters) with a pore size of 40 μm. The retentate was soaked in distilled water (80ºC, 50 mL), then vacuum filtered and washed with distilled water twice following by one step wash with 50 mL of acetone and then dried overnight. The ADF content was calculated as the residual weight of biomass in relation to the initial weight of the dry sample before lipid extraction. To determine the ADL content, the crucible was put in a 150 mL beaker, and the filter cake was covered with 25mL of 12M H_2SO_4 followed by stirring and after acid permeated through the filter an additional 25 mL of the acid was added again after 30 minutes. After 3 hours, the suspension was vacuum filtrated and washed until neutrality with 80ºC water. The crucible was weighed after drying overnight. Eventually, the crucible was subjected to 550ºC heating in a furnace for 4.5 hours and weighed after cooling. The ADL content was calculated according to ADF values, and by subtracting the ADF from the ADL values, the ADF-ADL was calculated. The ADF and ADL values were determined for all samples with a minimum of three replicates, and the values were compared with the values obtained from the conventional acid-base method.

2.3 Carbon and Nitrogen (C/N) analysis and crystallinity

Carbon and Nitrogen (C/N) analysis was done using a Eurovector EA3000 Analyzer. The distribution of the crystal structure in chitin matrix was evaluated using an X-Ray Diffractometer (XRD; Bruker ecoD8 advance) in the range of $5°<2\Theta<70°$ with Cu Kα irradiation (k=0.154060 nm). The diffractometer

performed at 40 keV and 25 mhA and continuous scan with a step size of 0.019 and scan step time of 96.00s. The crystalline index value (CrI) was calculated via the following equation:

$$CrL = [\frac{(I_{110} - I_{am})}{I_{110}}] \times 100 \qquad (1)$$

where I_{110} is the maximum intensity at 2 = 20◦, and I_{am} is the intensity of amorphous diffraction at 2 = 13 (Waśko et al., 2016).

The functional groups of the samples were identified using Fourier transform infrared spectroscopy (FT-IR; Bruker Vertex 70) over the region 400–4,000 cm^{-1} with 2 cm^{-1} spectral resolution using the KBr pellet technique (Kumar et al., 2012). The spectra characterization was performed using previously recorded references for chemical bonds, and the peaks were assigned to the correlated chemical bonds.

Thermogravimetric analysis (TGA-DTA) of samples was carried out using a TA instrument (Netzsch STA 409 PC coupled with Netzsch QMS 403C) from 30ºC to 1000°C at 10°C/min heating rate under oxygen flow (60mL/min) using 50 mg of the samples. Scanning electron microscope (SEM) (Hitachi SU-70) was used to examine the microstructure and microscopic characteristics of the surface of the chitin samples.

2.4 Statistical analysis

The statistical analyses were performed using three independent samples, and the results were reported as mean ± standard error of the means. Data were analyzed as repeated measurement analysis of variance (ANOVA) to investigate the effects of the source and the methods used for chitin isolation on the measured yield of chitin. Significant differences between means and multiple comparisons between means were analyzed by Tukey's test using Minitab 19 Statistical Software (Minitab®, PA, United States).

3 Results and Discussion

3.1 Effect of isolation method and the source of sample

Acid detergent fiber has been used to determine the cellulose and lignin residuals after treatment with sulfuric acid and CTAB, however considering the structural similarity of chitin to cellulose, this method can be adopted for chitin extraction (Hahn et al., 2018). The ADF-ADL method was

adopted in this study to consider the content of quinone and catechol in the insect samples. Figure 1 shows an overview of the chitin content obtained from different samples subjected to the treatment methods. The ADF method showed the highest chitin content values in all the samples and was significantly higher than the ADF-ADL and acid-base method in the case of Puparia chitin. While there was no significant difference between the yield of chitin from ADF-ADL and the conventional acid-base method. The large gap between the Puparia chitin obtained from ADF and acid-based method compared to the ADF-ADL could be related to the high content of catechol linked to chitin and protein due to sclerotization, which is the hardening process of insect exoskeleton through protein-protein or protein-chitin cross-linking (Hahn et al., 2018; Rubin et al., 2010). While ADF results do not specify the source of chitin either free or in a complex packed structure, the ADL treatment step can correct for this entangled chitin resulting in values that are closer to the values obtained by the Acid-base method. The observed chitin value of 20% and higher for the Puparia samples are in alignment with the previously reported results by T Hahn et al. The Adult insect samples had the lowest chitin content of 7-13%, which is following the existence of other components such as protein and lipid in the insect body. The obtained results for adult insect is aligned with the values of 106 g/kg and 86 g/kg previously reported by T Hahn et al. (Hahn et al., 2018) using the ADF method and Diner S et al. (Diener et al., 2009) using the formic acid method respectively. In the case of chitosan, a maximum chitosan yield of 2% reported for adult insect-chitosan and 11% for pupae shells-chitosan. This chitosan yield was obtained 15.40% from shrimp, 14% from krill, and 23% from crawfish (Leke-Aladekoba, 2018).

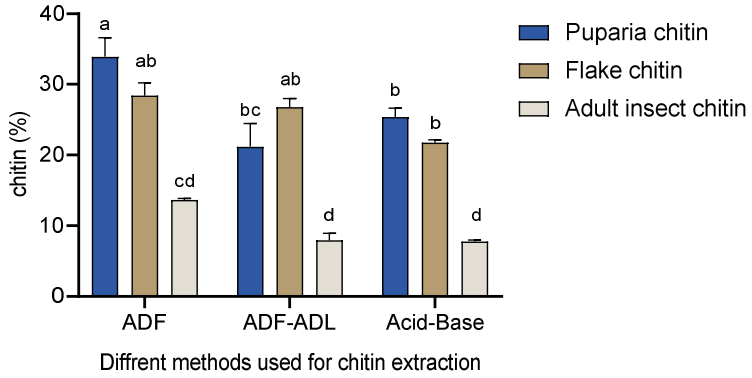


Figure 1. The yield of chitin obtained from different fractions of Puparia, Flake, and Adult insect using the three different isolation methods. [a-d] bars that do not share a letter are significantly different at $p<0.05$.

3.2 Chemical bonds (FTIR)

The chemical composition of the chitin samples was characterized (Figure 2) using FTIR analysis, and the major characteristic peaks are listed in Table 1. There was a close similarity between the chemical composition and bonding of chitin from the Puparia, Adult insect, and the commercial chitin, which is aligned with the previously reported study (Waśko et al., 2016). Chitin can be found in α, β, and γ crystalline form based on the source of the chitin. The α-crystal form of chitin has three peaks around 1660 cm^{-1} and 1620 cm^{-1}, and 1550 cm^{-1} (Focher et al., 1992). While the β-crystal form of chitin has only weak intramolecular hydrogen bonds around 1650 cm^{-1} (Cárdenas et al., 2004). Puparia, Flake, and Adult insect samples showed three peaks around 1660-1665 cm^{-1}, 1620-1627 cm^{-1}, and 1558-1560 cm^{-1} which attributed to the C=O secondary amide stretch (Amide I), C=O secondary amide stretch (Amide I), and N-H bend and C-N stretch (Amide II), respectively. These results indicated that the chitins extracted from the black soldier fly have the α-crystal form, like the commercial chitin extracted from shrimp. Wang *et al* reported a similar result on the crystal origin of isolated chitin in different stages of black soldier fly (larvae, prepupa, puparium, and adults) (Wang et al., 2020). The peaks around

1650 cm^{-1}, 1620 $cm^{-1,}$ and 1550 cm^{-1} were attributed to amide I of C-O, the amide II of N=H, and amide III of C=N stretching, respectively (Elshaarawy et al., 2016). The absence of a band at 1540 cm^{-1} demonstrated no protein residues in the chitins, indicating the successful deproteinization process (Morin and Dufresne, 2002).

The FTIR results of Puparia and Adult insect chitosan exhibited similarity to those of commercial shrimp chitosan, and chitosan extracts from insects such as potato beetle, grasshoppers, silkworm chrysalides and two orthoptera species (Leke-Aladekoba, 2018). Determining the physicochemical characteristics of chitin and chitosan extracts from pupae shells and the adult insect showed that these extracts were similar in terms of quality and purity as commercially produced chitin and chitosan from shrimp (Leke-Aladekoba, 2018).

Table 1. FTIR bands of the chitin isolated from black soldier fly fractions (Puparia, Flake, and Adult insect) and the commercial chitin.

Functional groups and vibration mode	**Classification**	**Pupria**	**Flake**	**Adult insect**	**Commercial chitin**
O–H hydroxyl stretching		3440	3445	3440	3441
N–H secondary amine asym. stretch	Amide	3271-3106	3270-3103	3269-3100	3268-3100
CH3 sym. stretch and CH2 asym. stretch	Aliphatic compounds	2927	2930	2929	2932
CH3 sym. stretch	Aliphatic compound	2888	2890	2886	2884
C O secondary amide stretch	Amide I	1665	1661	1660	1654
C O secondary amide stretch	Amide I	1620	1621	1623	1627
N–H bend, C–N	stretch Amide II	1558	1559	1559	1560
CH2 ending and CH3 deformation	–	–	1424	–	–
CH bend, CH3 sym. deformation	–	–	1380	–	–
CH2 wagging	Amide III, components of protein	1320	1322	1322	1318
C–O–C asym. stretch in phase ring	Saccharide rings	1074	1079	1075	1070
C–O asym. stretch in phase ring	–	1030	1031	1028	1024
CH ring stretching Saccharide rings	Saccharide rings	897	898	895	896

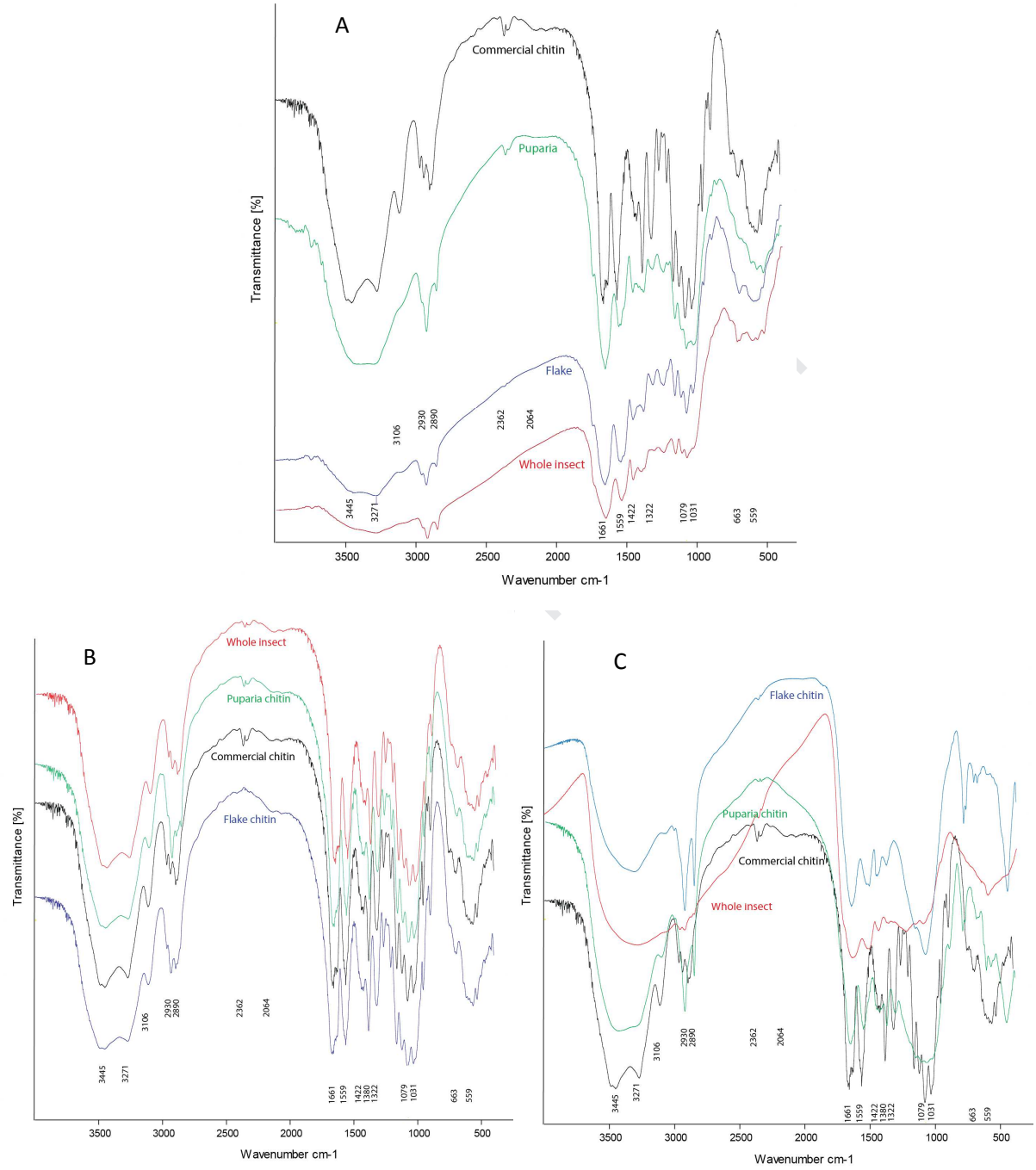


Figure 2. FTIR spectra of untreated samples (A), chitin samples obtained through acid-base method (B), and ADF-ADL method (C).

3.3 Thermal properties

Thermogravimetric analysis has been performed to investigate the thermal stability and degradation rate of extracted chitin from the samples (Figure S1 in supporting information). The

TGA revealed two stages of degradation for all samples. In the first degradation stage, the weight loss could be attributed to the evaporation of moisture existed in the samples (Ifuku et al., 2010), which occurred between 0-150°C. In the first degradation stage, the mass loss of Puparia, Flake, Adult insect, and commercial chitin was between 0.5-9.67% (Table 3) for both chitin isolation method. No stable intermediate was formed during the thermal decomposition due to the single-stage decomposition. The second degradation stage occurred between 180-450°C, and the second mass loss of 55.83% - 67.95% were recorded for Puparia, Flake, Adult insect, and commercial chitin. The second stage of degradation was assigned to the decomposition of sugar structure, loss of water from sugar ring, and degradation of both acetylated and deacetylated chitin components (Shankar et al., 2015).

Wang *et al.* reported a similar result for the thermal stability of chitin isolated from different developmental stages of black soldier fly. The first and second stages of degradation started at 0-150°C, 150-400°C, and the maximum thermal degradation temperature of around 373°C for different developmental stages black soldier fly has been reported (Wang et al., 2020).

Figure (S1) indicates the differential thermal analysis (DTA) result of the samples for both ADF-ADL and acid-based methods. The maximum degradation temperatures (DTGmax) was recorded 366.1°C and 321.35 for Puparia chitin obtained from the acid-based and the ADF-ADL methods, respectively.

There was not any significant difference in the thermal stability of all insect chitin samples. All three chitin samples obtained from the acid-based method exhibited a higher maximum degradation temperature (DTGmax) compared to the commercial chitin (Table 2), indicating higher thermal stability of isolated chitin from BSF than the commercially existed chitin (Table 2).

There was no significant difference between the degradation rate of chitin extracted by the ADF-ADL method in comparison with the acid-base method indicating that the ADF-ADL method can be used as an effective method for isolation of chitin with high purity.

Table 2. Thermal properties of chitin isolated from Puparia, Flake, Adult insect, and commercial chitin.

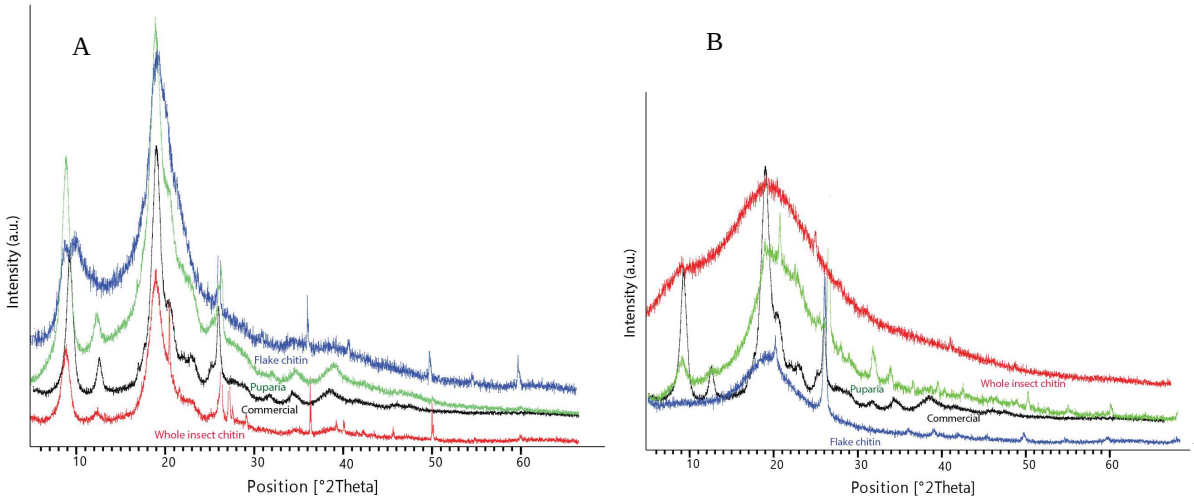
Sample	First degradation mass loss (0-150ºC)		Second degradation mass loss (260-450ºC)		Maximum degradation temperatures (DTG max)	
	Acid-base	ADF-ADL	Acid-base	ADF-ADL	Acid-base	ADF-ADL
Puparia	7.39 %	0.5 %	64.74 %	67.95 %	366.1°C	321.35 °C
Flake	9.67 %	3.32 %	51.75 %	66.35 %	356.6 °C	310.73 °C
Adult insect	8.52 %	8.62 %	61.87 %	55.58 %	356.7 °C	312.25 °C
Commercial chitin	6.00 %		65.69 %		345.4 °C	

3.4 Crystallinity (XRD)

The XRD patterns of the chitin samples obtained from the two different extraction methods are presented in Figure 3 A and B. Chitin samples from the acid-base method showed significant sharp peaks of α crystalline structure of the chitin at 9 and 19 º and minor peaks at 12, 23 and 26 º similar to the commercial chitin sample (black line) (Juárez-de la Rosa et al., 2012; Waśko et al., 2016). For the ADF-ADL samples, only the Puparia chitin showed a pattern closer to the commercial sample. While the Flake and the Adult insect chitin patterns showed the peak at 19 º however, the peaks were broad, indicating to amorphous characteristics of these samples.

As shown, all insect-derived chitins had a lower CI compared to the commercial shrimp chitin, and ADF-ADL samples except Puparia chitin had significantly lower crystallinity compared to the samples obtained from the acid-base method. The CI observed in this study is in alignment with the previously reported works (Waśko et al., 2016; Zimri, 2018). Zimri et al. reported CI values of 49.6%, 50.3%, and 60.1% for Adult BSF-chitin, pupae shells-chitin, and commercial chitin, respectively. Moreover, the CI index value for chitins extracted from brown shrimp, pink

shrimp, crab, and crayfish was 64%, 66.6%, 59.86%, and 56.94%, respectively. Low crystallinity chitin obtained from the insect can provide desirable sorption properties and high heavy-metal-removal efficiency due to low resistance to diffusion (Aranaz et al., 2009; Zimri, 2018). The result of this study also showed that chitin fractions obtained via different steps at an Insect farm (Adult insect, Puparia, and Flakes) have different crystalline properties. In another study CI of 35% reported for BSF larval chitin and 24.9% for BSF imago chitin which is lower than other insect species with CI ranges from 40% to 80% depending on the species, growth phase, sex and isolation method (Waśko et al., 2016). For example, chitin from beetle *Holotrichia parallela* had a similar high crystallinity (~ 89%) to the tested commercial chitin from shrimp (Liu, S. et al., 2012).



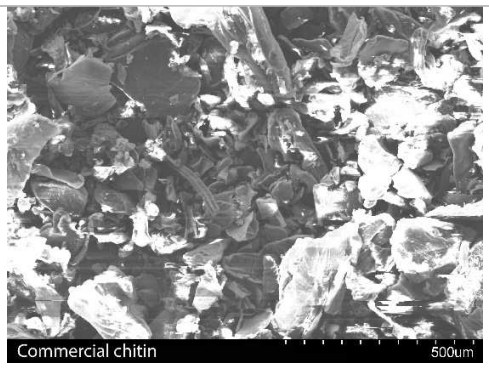
Crystallinity (%)			
Commercial	Flake	Puparia	Adult insect
86.8			
Acid-base	61.1	74.1	77.8
ADF ADL	50.0	70.8	39.0

Figure 3. X-ray diffraction patterns and crystallinity of the chitin samples obtained from Insects (Puparia, Flake, and Adult insect) using different chitin isolation methods of Acid-base (A) and ADF-ADL (B) and the commercial chitin.

3.5 Morphological properties (SEM)

Scanning electron microscopy of the chitin samples is shown in Figure 4, indicating the heterogeneous particle size distribution for the commercial, Adult insect, and Puparia chitin samples. While a rough, packed Flake structure was observed for the Flake chitin sample. At higher magnification (inset in Figure 4), the Flake samples show a repeating honeycomb structure, similar morphology for BSF exoskeleton that has previously reported (Waśko et al., 2016).

SEM analysis of surface morphologies of pupae shells-chitin revealed that it is a tightly packed structure with repeating circular and hexagonal units in a honeycomb-like arrangement, while the adult insect chitin consists of loosely packed oval units separated by circular-shaped structures with repeating arrangements of fibers. Both chitins are fibrous with no porosity. The commercial shrimp chitin did not exhibit nanofibrillar structure nor pores but showed layers of crumbling Flakes with a smooth surface (Leke-Aladekoba, 2018). In the case of chitins isolated using the ADF-ADL method, the Puparia and Flake samples were more homogeneous, fluffy powder compared to the samples obtained from the Acid-Base technique. The commercially obtained chitosan from shrimp reported having slightly rougher than the adult insect-chitosan (Leke-Aladekoba, 2018).



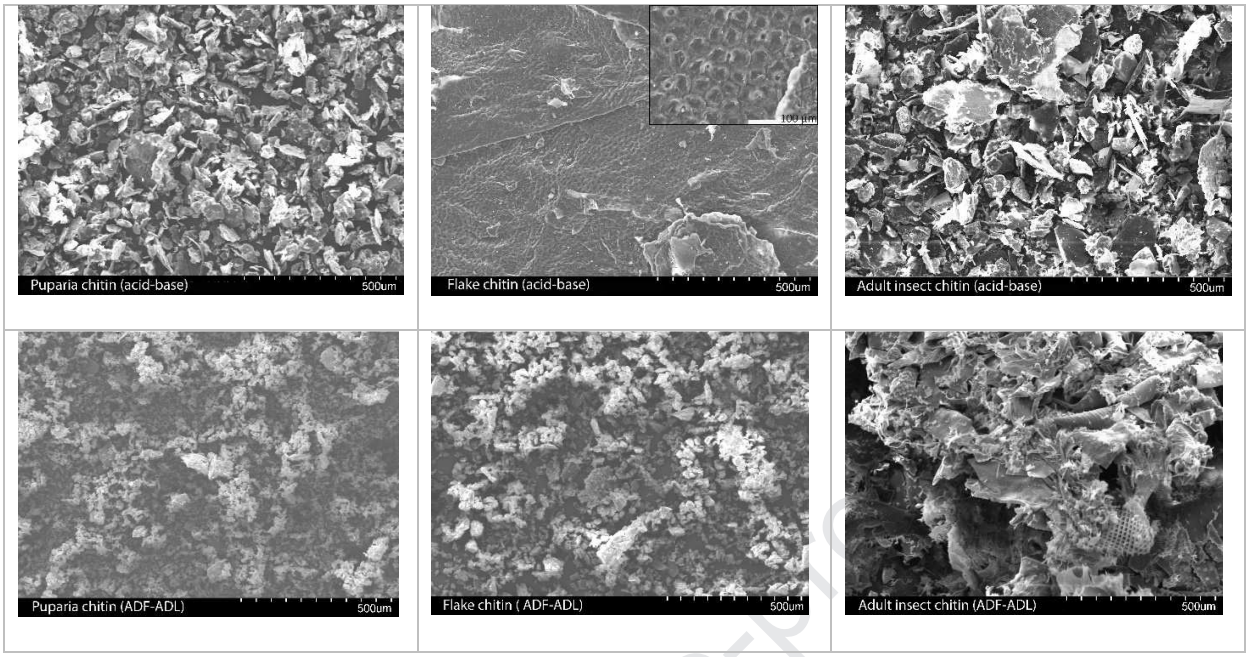


Figure 4. The morphological properties of commercial chitin and chitin samples obtained from the black soldier fly insect farm (Adult insect, Puparia, and Flakes) using a scanning electron microscope.

3.6 C and N analysis

Based on the elemental analysis, for the acid-base samples C and N content of 40.3% and 5.6% obtained for pupae shells-chitin, 42.1% and 5.8% for adult insect-chitin and 44.8% and 6.5% for commercial shrimp chitin, respectively. For the samples from the ADF-ADL method, C and N content of 29.7% and 3.4% for pupae shells chitin, 38.9 and 4.5 for Flake and 55.3 and 13.1 for the adult insect chitin was obtained.

The percentage of N content present in the adult insect-chitin was higher than that of pupae shells chitin, Flake, and commercial shrimp chitin, and both were observed to be lower than the amount of 6.89%, which is the reference N content typical for fully acetylated chitin, these results are in agreement with the previously reported data by Leke-Aladekoba (Leke-Aladekoba, 2018). The degree of acetylation (DA) of chitin is highly affected by the analytical methods and also knowing that any protein or mineral residual in the isolated chitin sample may affect in the results, the calculation of DA based on elemental analysis was not performed in this study. Thus,

a comprehensive study will be performed in our future work to determine DA of the chitin samples from the insect farm using a sensitive and precise technique such as H NMR (Kasaai, 2010) to obtain acceptable and reasonable result for the DA.

The previous reports on the chitin extraction from insects in comparison with our results (in bold) have been listed in Table 3.

Table 3. A summary of the previous reports on the chitin properties obtained from various insects.

Insect source	**Isolation method**	**Crystalline form**	**Chitin content (%)**	**MW (kDa), DA (%)**	**Thermal properties**		**Crystalline index % value (CrI)**	**Ref**
					DTG max[1] (°C)	**Weight loss**		
bumblebee (*Bombus terrestris*)	Chemical method (HCl, NaOH)	α-chitin		NA, 87.3%				(Majtán et al., 2007)
Spider *(Geolycosa vultuosa)*	Chemical method (HCl, NaOH)	α-chitin	8–8.5	NA, 97%	381	6 % (0-150 °C) 74 % (150-600°C)	78.6	(Kaya et al., 2014)
Wolf spider *(Hogna radiata)*		α-chitin	6.5–7	NA, 99%	378	7 % (0-150 °C) 74 % (150-600°C)	58.9	
Aiolopus simulatrix	Chemical method (HCl, NaOH)	α-chitin	5.3	5.3 kDa, NA	383	6 % (0-150 °C) 82 % (150-600°C)	76	(Kaya et al., 2015b)
Pyrgomorpha cognata			6.6	5.5 kDa, NA	384	4 % (0-150 °C) 74 % (150-600°C)	63	
Green shield bug *(Palomena prasina)*	Chemical method (HCl, NaOH)	α-chitin	10.8	NA, 83.7%	386	5 % (0-150 °C) 75 % (150-600°C)	84.9	(Kaya et al., 2015a)
Larvae BSF	Chemical method (HCl, NaOH)	α-chitin	3.6	–	372	4.42 % (0-150 °C) 69.48 % (150-400°C)	33.09	(Wang et al., 2020)
Prepupa BSF		α-chitin	3.1	–	373	6.74 % (0-150 °C) 71.16 % (150-400°C)	35.14	
Puparium BSF		α-chitin	14.1	–	371	8.52 % (0-150 °C) 71.25 % (150-400°C)	68.44	
Adults insect BSF			2.9	–	372	7.5 % (0-150 °C) 73.31 % (150-400°C)	87.92	
Larvae (BSF)	Chemical method (HCl, NaOH)	α-chitin	–	NA, 250 %	389	2-3 % (0-122°C) 62-64% (122-450 °C)	35	(Waśko et al., 2016)
Adult		α-chitin	–	NA, 179 %	387		24.9	
beetle larva cuticle	Chemical method (HCl, NaOH)	α-chitin	15–20	–	–	–	47	(Zhang et al., 2000)

[1] Maximum decomposition temperature

silkworm (*Bombyx mori*) pupa exuvia			α-chitin			–	–	56	
Wasp *(Vespa crabro)* larvae		Chemical method (HCl, NaOH)	α-chitin	2.2	–	384.8	3.51 % (30-180°C) 88.70 % (180-400°C)	–	(Kaya et al., 2016)
pupa			α-chitin	6.2	–	381.7	2.7 % (30-180°C) 69.9 % (180-400°C)	–	
adult			α-chitin	10.3	–	382.4	6.1 % (30-180°C) 88.7 % (180-400°C)	–	
Bombyx mori larva		Three steps extraction	α-chitin	Purity grade: 93.3	>87%	369	First degradation (<100 °C)	66	(Huet et al., 2020)
BSF (*Hermetia illucens*)		Three different extraction method	α-chitin	9	–		–	–	(Caligiani et al., 2018)
House cricket		Chemical method (HCl, NaOH)	α-chitin	4.3-7.1	NA, 108.1 %		–	88.02	(Caligiani et al., 2018)
Lucanidae		Chemical method (HCl, NaOH)	γ-chitin	524kDa,	–		–	–	(Jang et al., 2004)
Beetle Motschulsky (*Holotrichia parallela*		Chemical method (HCl, NaOH)	α-chitin	15	100.16 %		–	89.05	(Liu, Shaofang et al., 2012)
Millipede (Spirobolida)		Chemical method (HCl, NaOH)	α-chitin	35.7	–		–	–	(Achur, 2018)
Cicada Slogh (shell of insect Cryptotympana pustulata Fabricius)		Chemical method (HCl, NaOH)	α-chitin	36.6	EA:102.3 % HNMR: 90.8 Cp/MAS NMR: 96.8	362	7.3 %, (0-150°C) 66.4 % (180-400°C)	82.7	(Sajomsang and Gonil, 2010)
Wild BSF (*Hermetia illucens*)	Pupa Exuviae	Chemical method (HCl, NaOH)	α-chitin	9	NA, 115%	371	5-6 % (0-200°C) 70 % (200-450°C)	25.20	(Purkayastha and Sarkar, 2020)
	Dead Imago		α-chitin	23	NA, 86%	363	5-6 % (0-200°C) 80 % (200-450°C)	49.4	
Black soldier fly BSF (*Hermetia illucens*)	Puparia	Chemical method (HCl, NaOH)	α-chitin	25.39 ± 2.43	–	366.1 °C	7.39 % (0-150°C) 64.7 % (180-450°C)	74.1	Present study
	Flakes			20.69 ± 2.47	–	356.6 °C	9.67 % (0-150°C) 51.7 % (180-450°C)	61.1	
	Adult insect			7.75 ± 0.49	–	356.7 °C	8.52 % (0-150°C) 61.8 % (180-450°C)	77.8	
	Puparia	ADF-ADL	α-chitin	21.19 ± 5.71	–	321.35 °C	0.5 % (0-150°C) 67.9 % (180-450°C)	70.8	
	Flakes			26.78 ± 2.17	–	310.73 °C	3.32 % (0-150°C) 66.3 % (180-450°C)	50.0	
	Adult insect			7.94 ± 1.92	–	312.25 °C	8.62% (0-150°C) 55.5 % (180-450°C)	39.0	

4 Conclusion

This study is the first report highlighting the valorization of biopolymer from an insect farm and evaluate the physicochemical properties of the isolated chitin. The insect farm side streams of Puparia (insect shell) and Flakes contain more than 20% of chitin, which had lower crystallinity compared to the commercial shrimp chitin. Puparia had the highest chitin content indicating that Puparia can be valorized as a rich source of chitin. Moreover, isolated chitin through the ADF-ADL method had more homogenous surface morphology compared to the acid-based method. The present result demonstrated that chitin from BSF has specific physicochemical properties that can be used in different applications such as wastewater treatment, textile industry, and biomedical application. Moreover, the ADF-ADL method can be used to extract chitin from different sources instead of conventional extraction methods. However, the inclusion of CTAB in the ADF-ADL method may increase the final production cost of the chitin and further investigation is required to valorize chitin from insect farms side streams with an eco-friendlier method, using no or fewer chemicals for the process.

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Conflict of interest

The authors declare no conflict of interest.

References

Achur, R.N., 2018. Isolation and characterization of chitin from Millipede (Spirobolida). The Journal of Basic and Applied Zoology 79(1), 30.

Aranaz, I., Mengíbar, M., Harris, R., Paños, I., Miralles, B., Acosta, N., Galed, G., Heras, Á., 2009. Functional characterization of chitin and chitosan. Current Chemical Biology 3(2), 203-230.

Caligiani, A., Marseglia, A., Leni, G., Baldassarre, S., Maistrello, L., Dossena, A., Sforza, S., 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.

Cárdenas, G., Cabrera, G., Taboada, E., Miranda, S.P., 2004. Chitin characterization by SEM, FTIR, XRD, and 13C cross polarization/mass angle spinning NMR. Journal of Applied Polymer Science 93(4), 1876-1885.

Diener, S., Zurbrugg, C., Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste management & research : the journal of the International Solid Wastes and Public Cleansing Association, ISWA 27(6), 603-610.

Dutta, J., Tripathi, S., Dutta, P.K., 2012. Progress in antimicrobial activities of chitin, chitosan and its oligosaccharides: a systematic study needs for food applications. Food science and technology international = Ciencia y tecnologia de los alimentos internacional 18(1), 3-34.

El Knidri, H., Belaabed, R., Addaou, A., Laajeb, A., Lahsini, A., 2018. Extraction, chemical modification and characterization of chitin and chitosan. International Journal of Biological Macromolecules 120, 1181-1189.

Elshaarawy, R.F., Refaee, A.A., El-Sawi, E.A., 2016. Pharmacological performance of novel poly-(ionic liquid)-grafted chitosan-N-salicylidene Schiff bases and their complexes. Carbohydrate polymers 146, 376-387.

Focher, B., Naggi, A., Torri, G., Cosani, A., Terbojevich, M., 1992. Chitosans from Euphausia superba. 2: Characterization of solid state structure. Carbohydrate Polymers 18(1), 43-49.

Hahn, T., Roth, A., Febel, E., Fijalkowska, M., Schmitt, E., Arsiwalla, T., Zibek, S., 2018. New methods for high-accuracy insect chitin measurement. Journal of the science of food and agriculture 98(13), 5069-5073.

Hou, Y., Shavandi, A., Carne, A., Bekhit, A.A., Ng, T.B., Cheung, R.C.F., Bekhit, A.E.-d.A., 2016. Marine shells: Potential opportunities for extraction of functional and health-promoting materials. Critical Reviews in Environmental Science and Technology 46(11-12), 1047-1116.

Huet, G., Hadad, C., Husson, E., Laclef, S., Lambertyn, V., Farias, M.A., Jamali, A., Courty, M., Alayoubi, R., Gosselin, I., 2020. Straightforward extraction and selective bioconversion of high purity chitin from Bombyx eri larva: Toward an integrated insect biorefinery. Carbohydrate polymers 228, 115382.

Ifuku, S., Nogi, M., Yoshioka, M., Morimoto, M., Yano, H., Saimoto, H., 2010. Fibrillation of dried chitin into 10–20 nm nanofibers by a simple grinding method under acidic conditions. Carbohydrate Polymers 81(1), 134-139.

Ishimaru, Y., Tomonari, S., Matsuoka, Y., Watanabe, T., Miyawaki, K., Bando, T., Tomioka, K., Ohuchi, H., Noji, S., Mito, T., 2016. TGF-beta signaling in insects regulates metamorphosis via juvenile hormone biosynthesis. Proceedings of the National Academy of Sciences of the United States of America 113(20), 5634-5639.

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 42(14), 3423-3432.

Juárez-de la Rosa, B.A., Quintana, P., Ardisson, P.L., Yáñez-Limón, J.M., Alvarado-Gil, J.J., 2012. Effects of thermal treatments on the structure of two black coral species chitinous exoskeleton. Journal of Materials Science 47(2), 990-998.

Jucker, C., Erba, D., Leonardi, M.G., Lupi, D., Savoldelli, S., 2017. Assessment of Vegetable and Fruit Substrates as Potential Rearing Media for Hermetia illucens (Diptera: Stratiomyidae) Larvae. Environmental Entomology 46(6), 1415-1423.
Jucker, C., Lupi, D., Moore, C.D., Leonardi, M.G., Savoldelli, S., 2020. Nutrient Recapture from Insect Farm Waste: Bioconversion with Hermetia illucens (L.) (Diptera: Stratiomyidae). Sustainability 12(1), 362.
Kasaai, M.R., 2010. Determination of the degree of N-acetylation for chitin and chitosan by various NMR spectroscopy techniques: A review. Carbohydrate Polymers 79(4), 801-810.
Kaya, M., Baublys, V., Šatkauskienė, I., Akyuz, B., Bulut, E., Tubelytė, V., 2015a. First chitin extraction from Plumatella repens (Bryozoa) with comparison to chitins of insect and fungal origin. International journal of biological macromolecules 79, 126-132.
Kaya, M., Erdogan, S., Mol, A., Baran, T., 2015b. Comparison of chitin structures isolated from seven Orthoptera species. International journal of biological macromolecules 72, 797-805.
Kaya, M., Seyyar, O., Baran, T., Erdoğan, S., Kar, M., 2014. A physicochemical characterization of fully acetylated chitin structure isolated from two spider species: With new surface morphology. International journal of biological macromolecules 65, 553-558.
Kaya, M., Sofi, K., Sargin, I., Mujtaba, M., 2016. Changes in physicochemical properties of chitin at developmental stages (larvae, pupa and adult) of Vespa crabro (wasp). Carbohydrate polymers 145, 64-70.
Kumar, G.S., Thamizhavel, A., Girija, E.K., 2012. Microwave conversion of eggshells into flower-like hydroxyapatite nanostructure for biomedical applications. Materials Letters 76(0), 198-200.
Leke-Aladekoba, A.A., 2018. Comparison of extraction methods and characterisation of chitin and chitosan with antimicrobial and antioxidant properties from black soldier fly (hermetia illucens) meal. NovaScotia.
Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., Jiang, C., Yang, Q., 2012. Extraction and characterization of chitin from the beetle Holotrichia parallela Motschulsky. Molecules (Basel, Switzerland) 17(4), 4604-4611.
Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., Jiang, C., Yang, Q., 2012. Extraction and characterization of chitin from the beetle Holotrichia parallela Motschulsky. Molecules 17(4), 4604-4611.
Majtán, J., Bíliková, K., Markovič, O., Gróf, J., Kogan, G., Šimúth, J., 2007. Isolation and characterization of chitin from bumblebee (Bombus terrestris). International journal of biological macromolecules 40(3), 237-241.
Morin, A., Dufresne, A., 2002. Nanocomposites of chitin whiskers from Riftia tubes and poly (caprolactone). Macromolecules 35(6), 2190-2199.
Purkayastha, D., Sarkar, S., 2020. 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(2), 445-457.
Rubin, D.J., Miserez, A., Waite, J.H., 2010. Chapter 3 - Diverse Strategies of Protein Sclerotization in Marine Invertebrates: Structure–Property Relationships in Natural Biomaterials, in: Casas, J., Simpson, S.J. (Eds.), Advances in Insect Physiology. Academic Press, pp. 75-133.
Sajomsang, W., Gonil, P., 2010. Preparation and characterization of α-chitin from cicada sloughs. Materials Science and Engineering: C 30(3), 357-363.
Shankar, S., Reddy, J.P., Rhim, J.-W., Kim, H.-Y., 2015. Preparation, characterization, and antimicrobial activity of chitin nanofibrils reinforced carrageenan nanocomposite films. Carbohydrate polymers 117, 468-475.
Shavandi, A., Bekhit, A.E.-D.A., Ali, M.A., Sun, Z., 2015. Bio-mimetic composite scaffold from mussel shells, squid pen and crab chitosan for bone tissue engineering. International Journal of Biological Macromolecules 80, 445-454.
Shavandi, A., Bekhit, A.E.-D.A., Sun, Z., Ali, M.A., 2016a. Bio-scaffolds produced from irradiated squid pen and crab chitosan with hydroxyapatite/β-tricalcium phosphate for bone-tissue engineering. International Journal of Biological Macromolecules 93, 1446-1456.

Shavandi, A., Bekhit, A.E.-D.A., Sun, Z., Ali, M.A., 2016b. Injectable gel from squid pen chitosan for bone tissue engineering applications. Journal of Sol-Gel Science and Technology 77(3), 675-687.
Wang, H., ur Rehman, K., Feng, W., Yang, D., ur Rehman, R., Cai, M., Zhang, J., Yu, Z., Zheng, L., 2020. Physicochemical structure of chitin in the developing stages of black soldier fly. International Journal of Biological Macromolecules.
Waśko, A., Bulak, P., Polak-Berecka, M., Nowak, K., Polakowski, C., Bieganowski, A., 2016. The first report of the physicochemical structure of chitin isolated from Hermetia illucens. International Journal of Biological Macromolecules 92, 316-320.
Zhang, M., Haga, A., Sekiguchi, H., Hirano, S., 2000. Structure of insect chitin isolated from beetle larva cuticle and silkworm (Bombyx mori) pupa exuvia. International Journal of Biological Macromolecules 27(1), 99-105.
Zimri, M.N., 2018. Preparation and electrospinning of chitosan from waste Black Soldier Fly biomass, Department of Chemistry. University of the Western Cape Western Cape South Africa, p. 148.

Highlights:

The isolation of chitin polymer from the side stream of an insect farm was explored.

The Puparia fraction had the highest chitin content of 21-33%.

The flakes as the insect farm side stream are a rich source of chitin (20-28%),

the insect samples showed lower crystallinity compared to the commercial shrimp chitin.

The ADF-ADL method can be used instead of acid-based technique for chitin determination.

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Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Frederic Verwilghen and Emmanuel Baeten are working for ProteinFarm company who provided the insect sidestream sample materials for this research and contributed to the revesion of the text.