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Trends in Food Science & Technology 105 (2020) 17–42

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects

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ARTICLE INFO

Keywords:
Insects
Chitin
Chitosan
Extraction
Characterization
Biological activities

ABSTRACT

Background: Insects are a living resource used for human nutrition, medicine, and industry. Several potential sources of proteins, peptides, and biopolymers, such as silk, chitin, and chitosan are utilized in industry and for biotechnology applications. Chitosan is an amino-polysaccharide derivative of chitin that consists of linear amino polysaccharides with D-glucosamine and N-acetyl-D-glucosamine units. Currently, the chief commercial sources of chitin and chitosan are crustacean shells that accumulate as a major waste product from the marine food industry. Existing chitin resources have some natural challenges, including insufficient supplies, seasonal availability, and environmental pollution. As an alternative, insects could be utilized as unconventional but feasible sources of chitin and chitosan.

Scope and approach: This review focuses on the recent sources of insect chitin and chitosan, particularly from the Lepidoptera, Coleoptera, Orthoptera, Hymenoptera, Diptera, Hemiptera, Dictyoptera, and Odonata orders. In addition, the extraction methods and physicochemical characteristics are discussed. Insect chitin and chitosan have numerous biological activities and could be used for food, biomedical, and industrial applications.

Key findings and conclusions: Recently, the invasive and harmful effects of insect species causing severe damage in agricultural crops has led to great economic losses globally. These dangerous species serve as potential sources of chitin and are underutilized worldwide. The conclusion of the present study provides better insight into the conversion of insect waste-derived chitin into value-added products as an alternative chitin source to address food security related challenges.

1. Introduction

Insects have been considered a valuable food source since ancient times, with ~2 billion people globally consuming 1900 different species of insects for human nourishment (Van Huis, 2013). Major insect consumers are in Southeast Asia, the Pacific, sub-Saharan Africa, and Latin America. In general, insects consist of 30–45% protein, 25–40% fat, and 10–15% chitin (Spranghers et al., 2017). Chitin is the second most abundant bioactive polysaccharide in nature following cellulose. Among the various components in insects, chitin is a significant biopolymer, and the extraction of chitin and chitosan from insects is more advantageous in terms of extraction methods, chemical consumption, time and yield compared to existing sources. However, the proportion of chitin varies in every species in relation to its life-cycle. Adult *Tenebrio molitor* and *Hermetia illucens* species contain up to 5% chitin (Mariño-Pérez, 2015), whereas the prepupa/pupa stages of black soldier flies, Tebo worms, Turkestan cockroaches, and house flies contain 21 g/kg, 11.1 g/kg, 6.7 g/kg and 11.9 g/kg of chitin, respectively, which represents 1.2%

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https://doi.org/10.1016/j.tifs.2020.08.016
Received 29 May 2020; Received in revised form 18 August 2020; Accepted 26 August 2020
Available online 4 September 2020

(Finke, 2013). Chitin is considered to be a fibre with defensive activity against microbes. While the chemical chitinase is found in human gastric juices, it has been found to be inactive. Chitin is therefore, is mostly hydrolysed by lysozymes and hydrochloric acid found in human saliva and the stomach (Adámková et al., 2017).

Recently, scientists have extracted chitin from *cicada quagmires*, silkworms, and honeybees and described the functional properties of chitosan from these sources (Ma, Xin, & Tan, 2015). They reported that chitosan from insect sources is promptly accessible because of their reproductive rate and their ease of cultivation. Similarly, the removal of chitosan from the original organism influences its biological activity, and the extraction of chitosan from insects can be practised utilizing moderate conditions instead of the rigid conditions required for extraction from marine crustaceans. The yield of chitosan material from insects is higher than from shellfish, and chitin and chitosan from insect species have been reported to have useful applications (Y. Zhao, Park, & Muzzarelli, 2010). For example, chitosan extracted from cicada slough, silkworm chrysalises, mealworms, and grasshopper species showed higher potential water holding capacity (594–795%) and fat binding capacity (275–645%) compared to shrimp shell chitosan. This property is a promising feature for food applications. Additionally, *C. molossus* L. consists of 33 g/100 g of chitin that demonstrates better mechanical properties, including tensile strength (62 mPa) and elongation at break (10.4%), for the production of a biodegradable film similar to that of commercial medical grade shrimp chitosan film (Ma et al., 2015). Further, chitin isolated from *Pterophylla beltrani* showed better anti-fungal activity against the entomopathogenic fungi *M. anisoplia* (Torres-Castillo et al., 2015).

These studies show the benefits of using insect-based chitin/chitosan in biomedical and food applications that have recently been reported. However, conventional ethnobiological information demonstrates that insects have been used as nourishment and as an indispensable ingredient for treatments of various diseases since ancient times. Insects as traditional medicine are frequently not revealed or reported to the world as are herbal medicines (Chakravorty, Meyer-Rochow, & Ghosh, 2011). Therefore, changing the natural waste from the biomass of catastrophic insects into valorization would provide global benefits. From 1998 to 2020, there have been approximately 67 research papers published and indexed in scientific journals and databases with the keywords “insect chitin and chitosan”. Their specific geographical distribution data are shown in Fig. 1. Most of the research has been performed in Turkey (28%), China (24%) and South Korea (7%), which correspond to 59% of all the published research studies, while 4% of the studies originated from Egypt, Iran, Russia, and Brazil, and 3% of the research was from Japan, Poland, Malaysia, and India. However, in Mexico, Spain, Slovakia, Italy, Thailand, Bulgaria, and Belgium, only one report was identified. Chitin and chitosan extracted from crustacean, fungal and mollusc sources and their applications in various fields have been comprehensively covered in multiple critical reviews (Abdel-Ghany & Salem, 2020Abdel-Ghany & Salem, 2020; Ahmed et al., 2019; Alishahi & Aïder, 2012; Arbia, Arbia, Adour, & Amrane, 2013; Ganesan et al., 2020; Gortari & Hours, 2013; Hamed, Özogul, & Regenstein, 2016; Kaur & Dhillon, 2015; Kurita, 2006; Mohan et al., 2019; R. A.; Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012; Rasti, Parivar, Baharara, Iranshahi, & Namvar, 2017; Shanmugam & Abirami, 2019). Although insect chitin and chitosan possess an enormous amount of biological value and several studies have been performed to review these values, there has not been a comprehensive review of their extraction, characterization, and bioactivity. The primary intent of this review is to explore the potential applications of insect chitin and chitosan. This study supports future developments in converting catastrophic species into commercialization.

2. Chemical extraction methods

Numerous methods have been proposed and used to extract pure chitin and chitosan from crustacean shell waste, insects, fungi, and molluscs. In general, both demineralization and deproteinization could be performed using appropriate chemical methods. These conventional chemical treatments (Fig. 2a) are used for the extraction of chitin and chitosan from insects because they are both simple and inexpensive techniques.

2.1. Delipidation (DL)

The amount of lipid present in the insect body could influence the chitin and chitosan content and affect the yield during extraction. However, 80% of fats are in the triacylglycerol form, so a delipidation/defatting process was performed before the deproteinization step. Nonetheless, the involvement of this method in insect chitin extraction was found to be limited. The usage of ethanol in delipidation at 121 °C for 20 min leads to the removal of organic aromatic compounds during protein extraction (Tedesco, Castrica, Tava, Panseri, & Balzaretti, 2020). Therefore, selecting an appropriate mixture of reagents in the delipidation process would provide a better quantity of chitin compared to the demineralization and deproteinization processes. For instance, 100 g of

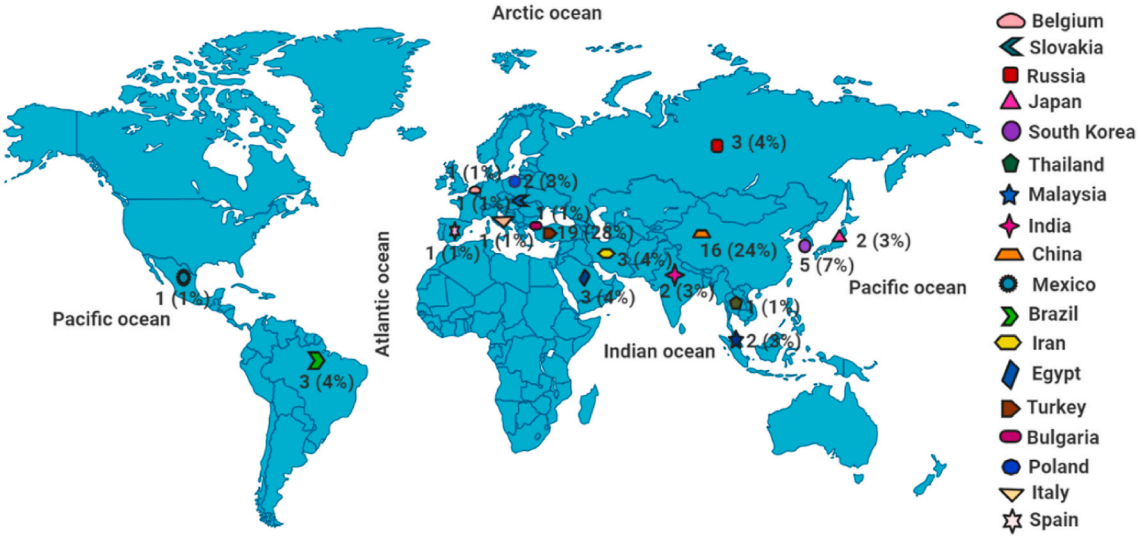

Fig. 1. The research distribution diagram of chitin and chitosan from insects.

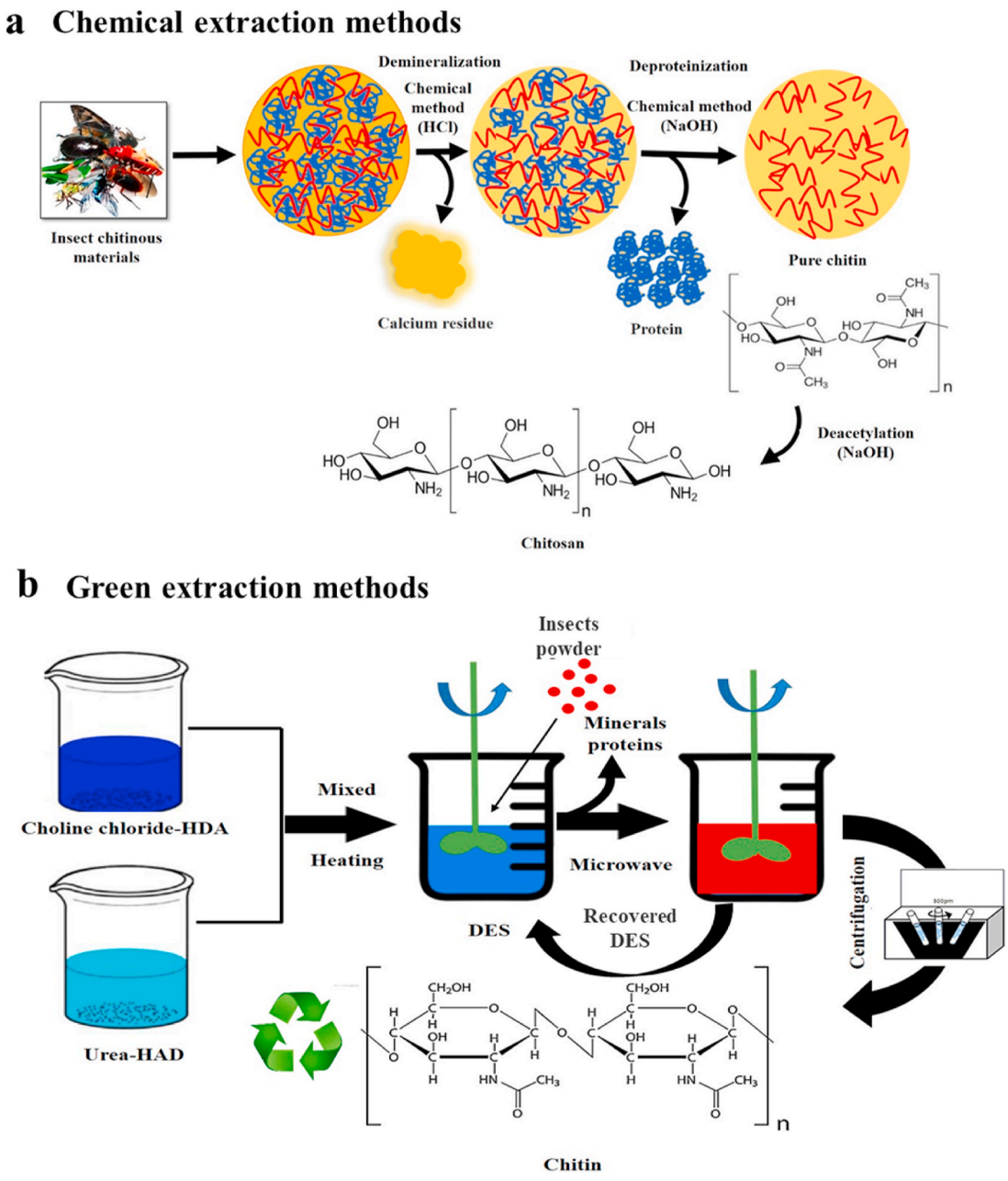

Fig. 2. Pictorial representation of a) Chemical extraction methods b) Green extraction methods of chitin and chitosan from insects. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Hermetia illucens larvae that was defatted with $CHCl_3$:CH_3OH (7:3 mixture, at 20 °C for 4 h) yielded 93 g of chitin-containing material (Khayrova, Lopatin, & Varlamov, 2019), whereas demineralization (using 2% HCl at 20 °C for 2 h) and deproteinization (5% NaOH at 50 °C for 2 h) yielded 58% and 46% of chitin, respectively. Although it was reported as a maximum yield, the biotechnology industries require a single step process and green technology for the removal of fat. For example, concentrated mineral acids are used to maximize the chitin yield in a single step process. Mineral acids such as phosphoric acid do not hydrolyse the chitin, unlike HCl and H_2SO_4. This process replaces multiple-step processes such as delipidation, demineralization, or deproteinization in chitin extraction.

2.2. Deproteinization (DP)

The deproteinization step is quite difficult due to the cleavage of the chemical bonds between the chitin and proteins. Chemical treatments are the first step in the removal of proteins. Generally, a wide range of chemicals have been used for the deproteinization of commercial chitin from shrimp, crab, lobster, and krill, and reaction conditions vary considerably between studies. The chemical extraction of chitin from insects is explained in Table 1. Furthermore, NaOH is the preferential inorganic base, and it is applied in various concentrations, ranging from 0.125 to 5.0 M (Kaya et al., 2014; Kaya, Erdogan, Mol, & Baran, 2015; M. W.; Kim, Song, Han, et al., 2017; Luo et al., 2019; Soon, Tee, Tan, Rosnita, & Khalina, 2018); at varying temperatures, up to ≥160 °C (Ibitoye et al., 2018; Kaya, Lelešius, et al., 2015; Kaya et al., 2016; Shin, Kim, & Shin, 2019; S.; Wu, 2011; Xia, Chen, & Wu, 2013); and at various treatment durations (from a few minutes up to a few days) (Luo et al., 2019; N. H. Marei, Abd El-Samie, Salah, Saad, & Elwahy, 2016; Mehranian, Pourabad, Bashir, & Taieban, 2017; Sajomsang & Gonil, 2010; Julliana Isabelle; Simionato, Villalobos, Bulla, Coró, & Garcia, 2014; Y.

Table 1
Extraction methods, characterization and biological activities of chitin and chitosan from insects.

Order/species	Deproteinization	Demineralization	Decoloration	Deacetylation	Yield (%)		Characterization	Physical properties/ Biological activities	References
					Chitin	Chitosan			
Lepidoptera									
Silk worm, *Bombyx mori*	1 M NaOH in 90 °C for 2 h	1 M HCl in 30 °C for 2 h	2% $KMnO_4$ for 2 h	60% NaOH in 100 °C for 8 h	NA	3.1	XRD, FT-IR, TGA, SEM	Rheological	Luo et al. (2019)
Bombyx mori	NaOH (1.0 mol L^{-1}) for 24 h at 80 °C	HCl (1.0 mol L^{-1}) for 20 min at 100 °C	NA	40 % wt NaOH and $NaBH_4$	NA	NA	FT-IR, ^{13}C NMR, DTG, SEM	Textile effluents treatment	Simionato et al. (2014)
Bombyx mori	NaOH (1.0 mol L^{-1}) for 24 h at 80 °C	HCl (1.0 mol L^{-1}) for 20 min at 100 °C	0.4% Na_2CO_3	40 % wt NaOH and $NaBH_4$	2.59	88.40	FT-IR, ^{13}C NMR, TGA, DTG, SEM	NA	Paulino et al. (2006)
Bombyx mori	NaOH (1.0 mol L^{-1}) for 24 h at 80 °C	HCl (1.0 mol L^{-1}) for 20 min at 100 °C	0.4% Na_2CO_3	NaOH (40 wt %), with $NaBH_4$ (0.83 g L^{-1})	2.59	88.40	^{13}C NMR, SEM	Textile wastewater treatment	Simionato et al. (2006)
Bombyx mori	1 N NaOH	1 N HCl	NA	NA	56	NA	XRD, ^{13}C CP/MAS NMR, SEM	NA	Zhang et al. (2011)
Bombyx mori	1 N NaOH at 80 °C for 36 or 24 h	1 N HCl at 100 °C for 20 min	NA	40 % wt NaOH and $NaBH_4$ for 4 h at 110 °C	15–20	NA	XRD, ^{13}C CP/MAS NMR, SEM	NA	Yang et al. (2000)
Flour moth, *Ephestia kuehniella*	1 M NaOH at 85 °C for 60 min	1 M HCl at 100 °C for 20 min	1% $KMnO_4$ for 60 min	NA	9.5–10.5	NA	FT-IR, EA, EDX, SEM	NA	Mehranian et al. (2017)
Pine caterpillar, *Dendrolimus punctatus*	5% NaOH at 7 0 °C for 10 h	3% HCl at 35 °C for 20 h	11% H_2O_2 at 85 °C for 2.5 h	55% NaOH at 100 °C for 6 h	NA	NA	NA	NA	Weixing (2008)
Butterfly, *Argynnis pandora*	2 M NaOH solution at 50 °C for 24 h	2 M HCl at 50 °C for 24 h	Distilled water, methanol, and chloroform (4:2:1) for 10 min	NA	Wing-22 OBP-8	NA	FT-IR, TGA, XRD, SEM	NA	Kaya et al. (2015a)
Hawk moth, *Clanis bilineata*	Flavourzyme hydrolysis at pH 6.5 and 50 °C	NA	NA	55% NaOH (w/w), 120 °C, and 4 h	NA	31.37	FT-IR	NA	Wu (2011)
Clanis bilineata	10% (w/v) NaOH at 60 °C for 24 h	7% (v/v) HCl at 25 °C for 24 h	NA	NA	NA	NA	FT-IR	Anti-oxidant Anti-ageing	Wu et al. (2013)
Clanis bilineata	10% (w/v) NaOH at 60 °C for 24 h	7% (v/v) HCl at 25 °C for 24	NA	55% NaOH (w/w), 120 °C, and 4 h	NA	95.8 96.2	HPLC, FT-IR	Anti-bacterial	Wu (2011)
Clanis bilineata	10% (w/v) NaOH at 60 °C for 24 h	7% (v/v) HCl at 25 °C for 24	NA	55% NaOH (w/w), 120 °C, and 4 h	NA	95.9	HPLC	Hypolipidemic	Xia et al. (2013)
Coleoptera									
Mealworm, *Tenebrio molitor*	1 M NaOH in 90 °C for 2 h	1 M HCl in 30 °C for 2 h	2% $KMnO_4$ for 2 h	60% NaOH in 100 °C for 8 h	NA	2.5	XRD, FT-IR, TGA, SEM	Rheological	Luo et al. (2019)
Tenebrio molitor	500 mL 5% NaOH at 95 °C for 3 h	3 h in 1500 mL 2 N HCl at 20 °C	NA	500 mL of NaOH at 95 or 105 °C for 3 h or 5 h	Dry-17.32 Wet-16.94	Dry-14.48 Wet-13.07	NA	NA	Song et al. (2018)
Comb-clawed beetles, *Omophlus* sp.	2 M NaOH for 20 h at 100 °C	2 M HCl for 4 h at 50 °C	Methanol–chloroform–water (2:1:4)	NA	NA	NA	SEM, XRD, TGA, FTIR	BSA adsorption capacities	Kaya et al., 2016a
White grub cockchafer, *Melolontha melolontha*	4 M NaOH at 150 °C for 18 h	50 mL of 4 M HCl solution at 75 °C for 2 h	Water, alcohol and chloroform (4:2:1) for 20 min	NA	13–14	NA	FT-IR, TGA, XRD, ESEM, EA	NA	Kaya et al. (2014b)
Melolontha sp.	1 M of NaOH for 20 h at 100 °C.	2 M HCl at 60 °C for 20 h	Distilled water, methanol, and chloroform (4:2:1) for 30 min	NA	Male-16.60 Female-15.66	NA	FT-IR, XRD, SEM, TGA	BSA adsorption capacities	Kaya et al. (2016b)
Water scavenger beetles, *Hydrophilus piceus*	100 mL of 1 M NaOH at 110 °C for 18 h	100 mL of 1 M HCl at 90 °C for 1 h	Chloroform, methanol, and water (1:2:4)	NA	19–20	74	FT-IR, TGA, XRD, SEM	NA	Kaya et al., 2014a
Colorado potato beetle, *Leptinotarsa decemlineata*	50 mL of 2 M NaOH at 80–90 °C for 16 h	100 mL of 2 M HCl at 65–75 °C for 2 h	Chloroform, methanol and water (in a ratio of 1:2:4) for 1 h	50% NaOH (w/v 1:20) at 100 °C for 3 h	Adult-20 Larvae-7	Adult- 72 Larvae-67	FT-IR, XRD, TGA, SEM	Antimicrobial Anti-oxidant	Kaya et al. (2014c)
Dung beetle, *Catharsius molossus*	4.0 M NaOH at 90 °C for 6 h	1.30 M HCl at 80 °C for 30 min	2% oxalic acid at 70 °C for 30 min	8 M NaOH at room temperature for 24 h	17	24	FT-IR, XRD, TGA, SEM	Rheological	Ma et al. (2015)
		1 M HCl	NA		5.0	NA	FT-IR, XRD, SEM	NA	

(*continued on next page*)

Table 1 (*continued*)

Order/species	Deproteinization	Demineralization	Decoloration	Deacetylation	Yield (%)		Characterization	Physical properties/ Biological activities	References
					Chitin	Chitosan			
Large ground beetle, *Calosoma rugosa*	1.0 M NaOH at100 °C for 8 h			50% NaOH (15 mL/g) at 100 °C for 8 h					Marei et al. (2016)
Calosoma rugosa	1.0 N NaOH	36.5% HCl	NA	50% NaOH at 100 °C for 8 h	NA	NA	FT-IR, XRD,	Anti-bacterial	Marei et al. (2019)
Dark black chafer beetle, *Holotrichia parallela*	1 M NaOH	1 M HCl for 30 min	1% KMnO4	NA	15	NA	FT-IR, XRD, SEM,	NA	Liu et al. (2012)
Mealworm Beetle, *Zophobas morio* and Rhinoceros Beetle, *Allomyrina dichotoma*	NaOH at 80 °C for 24 h	7% (v/v) HCl at 25 °C for 24 h	NA	55% (w/v) NaOH at 90 °C for 9 h	L-4.60 A-8.40 SW-3.90 L-10.53 P-12.70 A-14.20	80.00 78.33 83.33 83.37 83.37 75.00	FT-IR, XED	Anti-bacterial	Shin et al. (2019)
Zophobas morio	0.5 M, 1.0 M and 2.0 M NaOH in °C for 20 h	1.0 M of HCl in 35 °C	Glacial acetone for 30 min	50 wt % NaOH in 90 °C for 30 h	0.5 M-5.43 1.0 M-5.22 2.0 M-4.77	50 wt% −65.84, 70.88, 75.52	FT-IR, SEM, TGA, DSC, XRD	Anti-oxidant	Soon et al. (2018)
Dung beetle	2. 0–2. 5 mol•L-1 NaOH, 90–100 °C, for 4–5 h	0 8 mol L^{-1} HCl at 70 °C for 12 h	NA	10. 00–11. 25 mol L^{-1} NaOH for 3 h 130 °C	28.7	NA	NA	NA	Wang et al. (2013)
Lucanus cervus *Polyphylla fullo*	1 M NaOH in 90 °C for 14 h	1 M HCl in 90 °C for 1 h	chloroform-methanol-water (1:2:4, v:v)	NA	10.9 11.3	NA	XRD, FT-IR, TGA, SEM	NA	Kabalak et al. (2020)
Orthoptera									
Grasshopper	1 M NaOH in 90 °C for 2 h	1 M HCl in 30 °C for 2 h	2% $KMnO_4$ for 2 h	60% NaOH in 100 °C for 8 h	NA	5.7	XRD, FT-IR, TGA, SEM	Rheological	Luo et al. (2019)
Mexican katydid, *Pterophylla beltrani*	NA	NA	NA	NA	11.8	58.8	NA	Anti-oxidant	Torres-Castillo et al. (2015)
Moroccan locust, *Dociostaurus maroccanus*	2 M NaOH in 50 °C for 18 h	2 M HCl in 55 °C for 1 h	Methanol, chloroform and distilled water (in the ratio of 2:1:4)	60% NaOH in 150 °C for 4 h	Nymphs-12 Adults-14	Nymphs-77.38 Adults-81.69	FT-IR, TGA, XRD, ESEM	NA	Erdogan and Kaya (2016)
House cricket, *Brachytrupes portentosus*	1 M NaOH at 95 °C for 6 h	Oxalic acid for 3 h at room temperature	1% sodium hypochlorite for 3 h	50% (w/v) NaOH in 121 °C for 5 h	4.3–7.1	2.4–5.8	FT-IR, XRD, SEM	NA	Ibitoye et al. (2018)
Celes variabilis *Decticus verrucivorus* *Melanogryllus desertus* *Paracyptera labiata*	4 M NaOH for 20 h at 150 °C	4 M HCl at 75 °C for 2	NA	NA	4.71–11.84	NA	FT-IR, EA, TGA, XRD, SEM	NA	Kaya et al. (2015)
Calliptamus barbarus *Oedaleus decorus*	1 M NaOH at 80–90 °C for 21 h	1 M HCl at 100 °C for 30 min	Chloroform:methanol:distilled water solution (1:2:4) for 1 h	50% NaOH (w/v 1:15) at 130 °C for 2 h	20.5 16.5	70–75 74–76	FTIR, TGA, XRD, SEM	Anti-microbial Anti-oxidant	Kaya et al. (2015b)
Ailopus simulatrix *Ailopus strepens* *Duroniella fracta* *Duroniella laticornis* *Oedipoda miniata* *Oedipoda caerulescens* *Pyrgomorpha cognata*	2 M NaOH at 175 °C for 18 h	4 M HCl at 75 °C for 1 h	Chloroform:methanol:distilled water in the ratio of 1:2:4	NA	5.3 7.4 5.7 6.5 8.1 8.9 6.6	NA	ESEM, FT-IR,TGA, XRD	NA	Kaya et al. (2015c)
Two-spotted field crickets, *Gryllus bimaculatus*	1.25 M NaOH	2 N HCl	NA	50% NaOH (w/v)	A- 20.91 B-21.68 C-21.35 D-23.35	A-86.44 B-94.14 C-90.26 D-79.03	NA	NA	Kim et al., 2017a
Desert locust, *Schistocerca gregaria*	1.0 M NaOH at 100 °C for 8 h	1 M HCl	NA	50% NaOH (15 mL/g) at 100 °C for 8 h	12.2	NA	FT-IR, XRD, SEM	NA	Marei et al. (2016)
Schistocerca gregaria	1 M NaOH	1 N HCl	NA	50% NaOH	22.5	55	FT-IR, XRD	Wound healing	Marei et al. (2016)
		1 M HCl in 90 °C for 1 h		NA		NA	XRD, FT-IR, TGA, SEM	NA	

(*continued on next page*)

Table 1 (*continued*)

Order/species	Deproteinization	Demineralization	Decoloration	Deacetylation	Yield (%)		Characterization	Physical properties/ Biological activities	References
					Chitin	Chitosan			
Bradyporus sureyai *Gryllotalpa gryllotalpa*	1 M NaOH in 90 °C for 14 h		Chloroform-methanol-water (1:2:4, v: v)		9.8 10.1				Kabalak et al. (2020)
Hymenoptera									
European honey bee, *Apsis mellifera*	1 M NaOH at 80 °C	1 M HCl for 1 h	$KMnO_4$ with concentration of 1, 0.5, and 0.1% were used at 20 °C	NA	NA	NA	^{1}H NMR, FT-IR	NA	Draczynski (2008)
Apsis mellifera	2 M of NaOH and refluxed for 20 h at 100 °C	2 M HCl at 80 °C for 6 h	Distilled water (40 mL), methanol (20 mL) and chloroform (20 mL)	NA	Head-8.9 Thorax-6.79 Abdomen-8.61 Legs-13.25 Wings-7.64	NA	FT-IR, TGA, SEM	NA	Kaya et al. (2015d)
Apsis mellifera	1 M NaOH for 12 h at ambient temperature (20 °C)	1 N HCl	NA	NA	8.8	NA	FT-IR	NA	Tsaneva et al. (2018)
Apsis mellifera	1.0 M NaOH at100 °C for 8 h	1 M HCl	NA	50% NaOH (15 mL/g) at 100 °C for 8 h	2.5	NA	FT-IR, XRD, SEM	NA	Marei et al. (2016)
Apsis mellifera	1.0 N NaOH	36.5% HCl	NA	50% NaOH at 100 °C for 8 h	NA	NA	FT-IR, XRD,	Anti-bacterial	Marei et al. (2019)
Apsis mellifera	50% NaOH (ratio, 1 : 15) at 125 °C for 1 h	NA	30% H_2O_2 at 75 °C for 1 h	NA	30–40	16–25	NA	NA	Nemtsev et al. (2004)
Vespa crabro *Vespa orientalis* *Vespula germanica*	4 M NaOH at 150 °C for 18 h	2 M HCl solution at 75 °C for 2 h	Distilled water, methanol, and chloroform (4:2:1 ratio) for 2 h	NA	8.3 6.4 11.9	NA	FT-IR, TGA, XRD, EA, SEM	NA	Kaya et al. (2015d)
Vespa crabro	60 °C in 1 M NaOH for 16 h	1 M HCl (100 mL) at 50 °C for 6 h	Distilled water (40 mL), methanol (20 mL) and chloroform (10 mL) at room temperature	NA	Larvae-2.2 Pupa-6.2 Adult-10.3	NA	FT-IR, TGA, SEM	NA	Kaya et al. (2016c)
Vespa velutina	1 M NaOH (100 mL) at 60 °C for 8 h	1 M HCl (100 mL) at 50 °C for 3 h	100 mL 1% sodium hypochlorite solution	NA	11.7	NA	FT-IR, NMR, SEM, TGA	NA	Feás (2020)
Bumblebee, *Bombus terrestris*	1 M NaOH at 85 °C for 24 h	1 M HCl at 100 °C for 20 min	H_2O_2/33% HCl 9:1	NA	NA	NA	^{13}C CP/MAS- NMR, FT-IR, EA	NA	Majtán et al. (2007)
Diptera									
Housefly, *Musca domestica*	1 mol/l NaOH solution at 100 °C for 3 h	NA	NA	NaOH (50% w/v) at 125 °C for 4 h	NA	NA	FT-IR, XRD, TGA, DSC	NA	Zhang et al., 2011a
Musca domestica	500 mL of 1.25 N NaOH at 95 °C for 3 h	3 h in 500 mL of 2 N HCl solution at room temperature	NA	50% NaOH at 105 °C for 3 h	8.02	5.87	NA	NA	Kim et al. (2016)
Musca domestica	100 mL of 1 mol/L NaOH at 95 °C for 6 h	NA	10 mg/mL $KMnO_4$ for 4 h	400 mg/mL NaOH at 70 °C for 8 h	NA	NA	NA	Anti-oxidant Anti-tumour	Ai et al. (2008)
Black soldier fly, *Hermetia illucens*	1 M NaOH at 80 °C for 24 h	1 M HCl for 1 h	1% $KMnO_4$	NA	NA	NA	SEM, XRD, FT-IR, EA	NA	Waśko et al. (2016)
Hermetia illucens	1 M NaOH at 80 °C for 24 h	1 M HCl solution (250 mL) at 100 °C for 30 min	NA	1% potassium permanganate solution (100 lL) for 1 h	9 23	NA	FT-IR, NMR, XRD, TGA, SEM	NA	Purkayastha and Sarkar (2020)
Hermetia illucens	1 M NaOH 1 h at 80 °C	NA	NA	NA	8.5	NA	FT-IR	NA	D'Hondt et al. (2020)
Hermetia illucens Larvae Prepupa Puparium Adults	2 M NaOH at 50 °C for 18 h	2 M HCl at 55 °C for 1 h	NaClO at 80 °C for 4 h	NA	 3.6 3.1 14.1 2.9	NA	FT-IR, TGA, XRD, SEM	NA	Wang et al. (2013)

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Table 1 (*continued*)

Order/species	Deproteinization	Demineralization	Decoloration	Deacetylation	Yield (%)		Characterization	Physical properties/ Biological activities	References
					Chitin	Chitosan			
Hermetia illucens	NaOH at 90 °C for 3 h	HCl at 2 h	NA	NA	21.3	NA	NA	NA	Antonov et al. (2019)
Hermetia illucens	NaOH 50 °C for 2 h	2% HCl for 2 h at 20 °C	NA	NaOH at 100 °C for 2 h	7	32	NMR, FT-IR	NA	Khayrova et al. (2019)
Hermetia illucens	NA	2 N HCl for 24 h at 15 min	NA	NA	9	NA	NA	NA	Caligiani et al. (2018)
Musca domestica	5% NaOH at 95 °C for 6 h	1 mol/L HCl at room temperature for 3 h	0.3% $KMnO_4$ at room temperature for 4 h	NA	NA	NA	NA	Anti-bacterial	Jing et al. (2007)
Common fruit fly *Drosophila melanogaster*	NaOH (8% w:w) solution for 20 h at 70 °C	2 M HCl solution for 3 h at 40 °C	Methanol:chloroform:distilled water (in a ratio of 2:1:4) for 30 min	10 mL of NaOH solution (60%, w:w) for 48 h at 150 °C	7.85	70.91	TGA, SEM, FT-IR	NA	Kaya et al. (2016d)
Blowfly *Chrysomya megacephala*	NA	NA	sodium hypochlorite solution (0.5%, w/v) for 3 h	100 mL NaOH (1 mol/l) at 95 °C for 6 h	NA	26.2	EA, FT-IR, ^{13}C CP/ MAS NMR	Anti-oxidant	Song et al. (2013)
Hemiptera									
Cicada slough	1 M NaOH in 90 °C for 2 h	1 M HCl in 30 °C for 2 h	2% KMnO4 for 2 h	60% NaOH in 100 °C for 8 h	NA	28.2	XRD, FT-IR, TGA, SEM	Rheological	Luo et al. (2019)
Aquatic bug *Ranatra linearis*	100 mL of 1 M NaOH at 110 °C for 18 h	100 mL of 1 M HCl at 90 °C for 1 h	Chloroform, methanol, and water (1:2:4)	NA	15–16	70	FT-IR, TGA, XRD, SEM	NA	Kaya et al., 2014a
Cicada lodosi *Cicada mordoganensis* *Cicadatra platyptera* *Cicadatra atra* *Cicadatra hyaline* *Cicadivetta tibialis*	2 M NaOH solution at 100 °C for 20 h	2 M HCl for 2 h at 100 °C	Water, methanol, and chloroform mixed at the ratio of 4:2:1.	NA	4.97 6.49 8.84 6.70 5.51 5.88	NA	FT-IR, SEM,	NA	Mol et al. (2018)
Cicada slough	1 N NaOH at 80 °C for 36 h	1 N HCl at 100 °C for 20 min	6% sodium hypochlorite	NA	36.6	NA	EA, ATR-FTIR, ^{1}H NMR, CP/MAS NMR, XRD, TGA	NA	Sajomsang and Gonil (2010)
Cicada *Cryptotympana atrata*	1000 mL of 10% (w/ w) NaOH at 60 °C for 24 h	1000 mL of 7% (w/w) HCl at room temperature (~25 °C) for 24 h	NA	NaOH (55%, w/w) at 110 °C for 4 h	62.42	NA	FT-IR	Anti-bacterial	Wu et al. (2013)
Dictyoptera									
American cockroach, *Periplaneta americana*	1.25 N NaOH at 95 °C for 3 h	2 N HCl at room temperature for 3 h	NA	50% NaOH in 95 °C for 3 h	3.36	2.08	NA	NA	Kim et al. (2017b)
Periplaneta americana	4% of NaOH for 1 h	20 mL of 1% HCl for 24 h	50 mL of 2% NaOH solution for 1 h	NA	NA	0.024	FT-IR	NA	Wanule et al. (2014)
Periplaneta americana	4 M NaOH solution for 20 h at 150 °C	4 M HCl solution for 2 h at 75 °C	Water, methanol and chloroform (in the ratio of 4:2:1)for 4 h at 30 °C	NAs	Wings-18 Without wings-13	NA	ESEM, FT-IR, TGA, XRD	NA	Kaya et al. (2015b)
Blaberus giganteus	2 M NaOH at 90 °C for 9 h	NA	Chloroform:methanol:water (1:2:2) at room temperature for 1.5 h	NA	Wing-26.9 Dorsal pronotum-21.2	NA	FT-IR, TGA, SEM, AFM	Anti-bacterial Anti-fungal	Kaya et al. (2017)
Periplaneta americana *Blattella germanica*	1 M NaOH at 100 °C for 24 h	1% sodium hypochlorite solution (1%, w/v)	NA	50% NaOH at 100 °C for 4 h	Nymph-8.4 Adult-15 Nymph-5.4 Adult-6.2	Nymph-4 Adult-7.4 Nymph-2.6 Adult-2.8	FT-IR, XRD	Anti-bacterial Anti-fungal	Basseri (2019)
Periplaneta americana *Blattella germanica*	1 M NaOH at 80 °C for 24 h	1 M HCl at 100 °C for 30 min	NA	50% NaOH at 100 °C for 4 h	Nymph-4.4 Adult-14.8 Nymph-5.6 Adult-6.2	Nymph-3.6 Adult-11 Nymph-5 Adult-5.2		Anti-bacterial Anti-fungal	Basseri (2019)

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Table 1 (*continued*)

Order/species	Deproteinization	Demineralization	Decoloration	Deacetylation	Yield (%)		Characterization	Physical properties/ Biological activities	References
					Chitin	Chitosan			
Odonata									
Dragonfly, *Sympetrum fonscolombii*	1 M NaOH at 50 °C for 15 h	1 M HCl at room temperature for 1 h	Chloroform: methanol: distilled water (1: 2: 4, v/v)	NA	20.3	NA	FT-IR, SEM, XRD	NA	Kaya et al. (2016b)
Emperor dragonfly, *Anax imperator*	100 mL of 1 M NaOH at 110 °C for 18 h	100 mL of 1 M HCl at 90 °C for 1 h	Chloroform, methanol, and water (1:2:4)	NA	11–12	67	FT-IR, TGA, XRD, SEM	NA	Kaya et al., 2014a

^{13}C CP/MAS NMR: Cross polarization magic angle spinning nuclear magnetic resonance; **^{13}C NMR:** Carbon-13 nuclear magnetic resonance; **^{1}H NMR:** Proton nuclear magnetic resonance; **AFM:** Atomic force microscopy; **ATR-FT-IR:** Attenuated total reflectance fourier transform infrared spectroscopy; **DTG:** Differential thermal analysis; **EA:** Elemental analysis; **EDX:** Energy-dispective X-ray spectroscopy; **ESEM:** Environmental scanning electron microscopy; **FT-IR:** Fourier transform infrared spectroscopy; **H_2O_2:** Hydrogen peroxide; **HCl:** Hydrochloric acid; **HPLC:** High performance thin layer chromatography; **$KMnO_4$:** Potassium permanganate; **NA:** Not available; **Na_2CO_3:** Sodium carbonate; **$NaBH_4$:** Sodium borohydride; **NaOH:** Sodium hydroxide; **SEM:** Scanning electron microscopy; **TGA:** Thermogravimetric analysis; **XRD:** X-ray powder extraction.

S.; Song et al., 2018; Weixing, 2008). Alternative methods involving the use of enzymes such as alkaline proteases are emerging and represent a newfound method for protein extraction (Duong & Nghia, 2014; Guo, Sun, Zhang, & Mao, 2019). However, the amount of protein remaining is higher and it requires a longer reaction time than following chemical treatment (Hackman, 1954), meaning it is costlier compared to chemical treatment (Arbia et al., 2013). These problems mean that the enzymatic method of protein degradation is less likely to be applied (Younes et al., 2012).

2.3. Demineralization (DM)

The removal of minerals, mainly using calcium carbonate, is termed demineralization. In 1978, the process of commercial demineralization of chitin from crustacean shells was patented. This process is commonly achieved by acid treatment using sulphuric, hydrochloric, nitric, acetic, oxalic and formic acids (Al Sagheer, Al-Sughayer, Muslim, & Elsabee, 2009; Srinivasan, Kanayairam, & Ravichandran, 2018). In chitin extraction from insects, HCl has been found to be superior to all of these other acids (Ibitoye et al., 2018; Mehranian et al., 2017; Percot, Viton, & Domard, 2003; Shin et al., 2019; Julliana Isabelle; Simionato et al., 2014; Y. S.; Song et al., 2018). The demineralization process involves the breakdown of calcium carbonate into calcium chloride along with the release of carbon dioxide. An alternative method to this harsh chemical demineralization is the use of lactic acid fermentation. Jung et al. (2005) demonstrated the efficacy of lactic acid fermentation for the DM of crab shell waste with *Lactobacillus paracasei* KCTC-3074 compared with chemical treatments, such as 2 N HCl, 0.1 M EDTA, and 0%–10% lactic acid.

2.4. Decolourization (DC)

The decolourization step is usually essential for removing pigments and for obtaining a colourless product. These treatments are applied to chitin sources, regardless of the nature of the starting material. The residual protein and pigments are removed for further utilization, especially for biomedical or food applications (Rinaudo, 2006). Various decolouring agents have been used for decolourization of the chitin extracted from crustacean shells and insects.

2.5. Deacetylation (DA)

Deacetylation refers to the process of eliminating the acetyl groups attached to chitin and the substitution of reactive amino groups. The degree of deacetylation determines the percent of free amino groups within the structure and would therefore be helpful in distinguishing between chitin and chitosan. DDA is taken into consideration for chitosan as it influences the physicochemical and biological properties (Nessa et al., 2010), including the acid-base ratio, electrostatic characteristics, biodegradability, self-aggregation, sorption properties, and the ability to chelate metal ions (Hussain, Iman, & Maji, 2013). Chitin can be converted into chitosan using chemical methods (Philibert, Lee, & Fabien, 2017) at industrial scale due to the feasibility of mass production. For crustacean shell waste and insects, the chemical method of deacetylation uses alkali-NaOH (Anand, Kalaivani, Maruthupandy, Kumaraguru, & Suresh, 2014; N. H.; Marei et al., 2016; Paulino, Simionato, Garcia, & Nozaki, 2006; Y. S.; Song et al., 2018; Srinivasan et al., 2018; Torres-Castillo et al., 2015) or acids to deacetylate chitin. Since glycosidic bonds are highly vulnerable to acid, alkali is proposed to be a better chemical option (Hajji et al., 2014). Several factors during the deacetylation reaction can impact the characteristics of the resulting chitosan product. Temperature and processing time were the parameters that had the most significant impact on the DDA and molecular weight (Rege & Block, 1999).

Table 2
XRD peaks and crystalline index value (%) of chitin and chitosan from insects.

Species	Chitin		Chitosan		References
	XRD peaks at 2θ	CrI (%)	XRD peaks at 2θ	Major crystalline peak intensity	
Bradyporus (C.) sureyai	9.62, 12.5, 19.72, 23.74, 26.22, 27.8, 39.2	83.1	NA	NA	Kabalak et al. (2020)
Gryllotalpa gryllotalpa	9.44, 12.3, 19.41, 23.31, 26.2, 27.9, 39.0	80.6			
Polyphylla fullo	9.2, 12.4, 19.46, 23.50, 26.21, 28.1, 39.5	86.1			
Lucanus cervus	9.67, 12.40, 19.60, 23.41, 26.26, 39.1	85.2			
Omophlus sp	9.42, 12.72, 19.34, 20.84, 23.32, 26.44	82.9	NA	NA	Kaya et al., 2016a
Agabus bipustulatus	9.76, 12.76, 19.62, 21.10, 23.54, 26.48, 38.88	90.6	10.44, 19.86	1726	Kaya et al., 2014a
Anax imperator (larvae)	9.24, 12.94, 19.76, 21.36, 23.28, 26.74, 38.84	76.4	11.06, 20.06	1240	
Asellus aquaticus	9.46, 12.6, 19.48, 21, 23, 26.62, 39.11	77.2	10.3, 20.12	700	
Hydrophilus piceus	9.38, 12.9, 19.52, 20.82, 23.44, 26.7, 39.3	89.4	11.08, 19.74	753	
Notonecta glauca	9.54, 12.78 19.6, 21.08, 23.66, 26.96, 39.52	87.3	10.84, 20.38	1506	
Ranatra linearis	9.34, 12.38, 19.66, 20.88, 23.22, 26.56, 38.96	84.8	9.74, 20.24	833	
Leptinotarsa decemlineata	9.6, 13.22, 19.68, 21.42, 23.26, 26.7	76	9.38, 20.4	NA	Kaya et al. (2014c)
	9.66, 13.18, 19.48, 21.06, 23.16, 26.76	72	9.7, 20.2		
Melolontha sp	9.60, 12.78, 19.64, 20.70, 23.34, 26.06	79			Kaya et al. (2016b)
	9.44, 12.96, 19.48, 20.54, 23.50, 26.14	74.1	NA	NA	
Holotrichia parallela	9.2, 19.1, 12.6, 22.9, 26.2	89.05	NA	NA	Liu et al. (2012)
Schistocerca gregaria	NA	NA	9.3, 20.2, 24.4	69	Marei et al. (2016)
Apis mellifera			9.7, 20.3	59	
Calosoma rugosa			9.7, 20.3, 22.6	49	
Zophobas morio	NA	NA	10.62, 20.02	58.11	Shin et al. (2019)
Allomyrina dichotoma			10.74, 19.92	62.77	
Periplaneta americana	9.14, 19.58, 12.88, 20.98, 23.12, 26.8	86.7	NA	NA	Kaya et al. (2015b)
Hermetia illucens			NA	NA	Wang et al. (2013)
Larvae	9.30, 12.78, 19.26, 21.82, 23.31, 26.41	33.09			
Prepupa	9.38, 12.93, 19.33, 21.19, 23.42, 26.37	35.14			
Puparium	9.30, 12.67, 19.29, 20.77, 23.38, 26.45	68.44			
Adult	9.50, 12.82, 19.33, 20.81, 23.31, 26.34	87.92			
Hermetia illucens	9.3, 19.8, 23, 26.0	49.4	NA	NA	Purkayastha and Sarkar (2020)
Vespa crabro	9.64, 12.74, 19.38, 20.94, 23.92, 26.88	69.88	NA	NA	Kaya et al. (2015c)
Vespa orientalis	9.68, 12.72, 19.32, 21.6, 23.74, 26.86	53.92			
Vespula germanica	9.32, 12.92, 20.l0, 21.24, 23.16, 25.9	50			
Cicada sloughs	9.2, 12.6, 19.18, 20.68, 23.3, 26.48	89.7	NA	NA	Sajomsang and Gonil (2010)
Schistocerca gregaria	NA	NA	9.3, 20.2, 24.4	69	Marei et al. (2016)
Calosoma rugosa			9.7, 20.3, 22.6	49	
Apis mellifera			9.7, 20.3	59	
Argynnis pandora	9.3, 19.3, 12.84, 21.04, 22.9, 26.36	64	NA	NA	Kaya et al. (2015a)
	8.5, 19.3, 12.84, 21.14, 23.06, 26.66	66			
Sympetrum fonscolombii	9, 19	96.4	NA	NA	Kaya et al. (2016c)
Brachytrupes portentosus	9.4, 12.8, 17.1, 19.4, 21.1, 23.2, 26.3, 28.5, 35.0, 39.0	88.02	9.6, 19.6, 21.2, 12.4, 23.0, 26.2, 28.5, 35.0, 39.0	86.64	Ibitoye et al. (2018)
Dociostaurus maroccanus	9.56, 12.76, 19.72, 21.12, 23.96, 26.64	71	NA	NA	Erdogan and Kaya (2016)
	9.42, 12.86, 19.72, 21.56, 23.38, 26.66	74			
Calliptamus barbarus	9.26, 19.28, 21.24, 23.28, 26.36, 31.78	70.9	10.92, 20.08	NA	Kaya et al. (2015b)
Oedaleus decorus	9.6, 19.6, 21.1, 23.7, 26.64	76.8	10.08, 20.14		
Ailopus simulatrix	9.3, 12.7, 19.6, 21.1, 23.8, 26.6	76	NA	NA	Kaya et al. (2015c)
Ailopus strepens	9.5, 12.8, 19.6, 20.8, 23.8, 26.4	75			
Duroniella fracta	9.5, 12.6, 19.4, 20.9, 23.5, 26.8	72			
Duroniella laticornis	9.5, 12.8, 19.3, 20.7, 23.2, 26.5	71			
Oedipoda miniata	9.7, 12.9, 19.6, 21, 23.7, 26,8	74			
Oedipoda caerulescens	9.3, 12.7, 19.3, 20.7, 23.1, 26.9	74			
Pyrgomorpha cognata	9.4, 13.3, 19.6, 20.9, 23.4, 26,9	63			

2.6. Green extraction methods

The disadvantages of using the traditional chitin chemical extraction process include alterations in physicochemical properties, the use of expensive chemicals in the purification process and the release of toxic effluent wastewater into the environment. These challenges lead to the deterioration of environmental health (Dhillon, Kaur, Brar, & Verma, 2013) reduce the levels of valuable proteins that can be used as animal feed (Shirai et al., 2001). Therefore, green extraction methods (Fig. 2b) are gaining popularity due to their cleaner and more eco-friendly approaches (De Holanda & Netto, 2006).

The biological extraction process using microorganisms such as Lactobacillus (Rao, Munoz, & Stevens, 2000), *Pseudomonas aeruginosa* K-187 (Oh, Shih, Tzeng, & Wang, 2000) and *Bacillus subtilis* (Yang, Shih, Tzeng, & Wang, 2000) can be used to reduced chitin degradation and reduce impurities down to a satisfactory level for specific applications. For example, Khanafari & Sanatei (2008) examined chitin and chitosan isolated from shrimp waste by chemical and microbial methods, and the results showed that the microbial process was preferable to the chemical method. The microbial method required less time, a simple procedure,

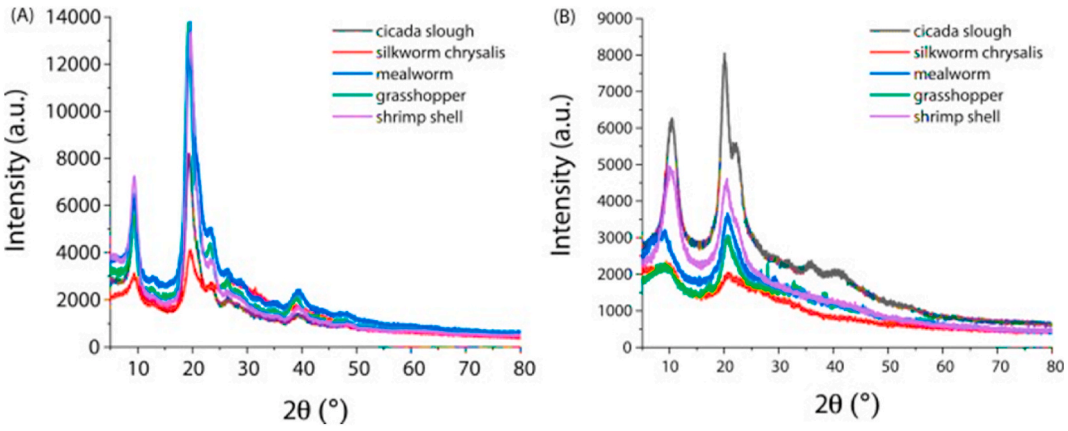

Fig. 3. XRD of (A) chitin and (B) chitosan extracted from five sources: cicada slough, silkworm chrysalis, mealworm, grasshopper and shrimp shells. Reprinted with permission (4873290806712) from Carbohydrate Polymers (Luo et al., 2019), copyright 2019 Elsevier.

Table 3
Elemental analysis (EA) results of the insect chitin.

Species	Chitin (%)				References
	Carbon (C)	Hydrogen (H)	Nitrogen (N)	CN ratio	
Bradyporus (C.) sureyai	46.6	7.7	5.3	8.8	Kabalak et al. (2020)
Gryllotalpa gryllotalpa	44.2	7.6	5.0	8.8	
Polyphylla fullo	45.4	7.5	5.1	8.9	
Lucanus cervus	45.9	7.6	5.3	8.5	
Melolontha melolontha	45.09	6.29	6.72	NA	Kaya et al. (2014b)
Holotrichia parallela	44.36	5.92	6.45	6.88	Liu et al. (2012)
Cicada sloughs	40.85	6.12	5.92	NA	Sajomsang and Gonil (2010)
Bumblebee	43.92	6.43	5.92	NA	Majtán et al. (2007)
Periplaneta americana	45.74	6.59	6.69	NA	Kaya et al. (2015b)
Hermetia illucens	39.74	5.46	6.00	6.62	Purkayastha and Sarkar (2020)
	43.74	5.82	6.14	7.12	
Hermetia illucens	35.23	5.11	3.73	9.45	Waśko et al. (2016)
	32.09	4.80	3.9	8.23	
Vespa crabro	46.62	6.42	6.85	NA	Kaya et al. (2015d)
Vespa orientalis	46.01	6.34	6.71	NA	
Vespula germanica	44.94	5.95	6.90	NA	
Argynnis pandora	44.89	6.53	6.62	NA	Kaya et al. (2015a)
	44.91	6.45	6.48		
Sympetrum fonscolombii	47.09	6.65	6.83	NA	Kaya et al. (2016c)
Brachytrupes portentosus	41.30	NA	6.022	6.858	Ibitoye et al. (2018)
Dociostaurus maroccanus	42.35	5.64	4.63	NA	Erdogan and Kaya (2016)
Celes variabilis	45.44	6.31	6.23	7.29	Kaya et al. (2015)
Decticus verrucivorus	45.05	6.56	6.34	7.01	
Melanogryllus desertus	48.90	6.88	6.08	8.04	
Paracyptera labiata	46.10	6.41	6.25	7.38	

low solvent consumption, and lower energy input. Although there is less research on the biological method of chitin extraction, it can replace the chemical methods that are overwhelmed with several disadvantages at the industrial scale. Other extraction methods have also been reported for chitin production, mainly from shrimp waste, including enzymatic (Gartner, Peláez, & López, 2010), microwave-assisted (Hongkulsup, Khutoryanskiy, & Niranjan, 2016) and ultrasonic-assisted (Valdez-Peña et al., 2010) and phytoextraction (Gopal et al., 2019).

Among all techniques, ionic liquids (ILs) are considered a promising volatile organic solvent for chitin production (Qin, Lu, Sun, & Rogers, 2010), although some specific ILs have some disadvantages, such as high cost and toxicity, which make them unsuitable for biological applications (Sharma, Mukesh, Mondal, & Prasad, 2013). Therefore, deep eutectic solvents (DES) are a green alternative to conventional methods of chitin production (Paiva et al., 2014). In comparison to traditional methods, DES possess more advantages, such as low or non-toxicity, lower cost, ease of synthesis and biodegradability (Q. Zhang, Vigier, Royer, & Jerome, 2012). DES extraction has been used for chitin production from shrimp (Huang, Zhao, Guo, Xue, & Mao, 2018) and lobster (Hong, Yuan, Yang, Zhu, & Lian, 2018; Zhu, Gu, Hong, & Lian, 2017), as well as in the insect *Hermetia illucens* (Zhou et al., 2019). Recently, Brigode et al. (2020) reported the production of chitin from *H. illucens* using acid detergent fibre and acid detergent lignin methods (ADF-ADL). Additional research is required to study green methods with smaller carbon-footprints for chitin and chitosan extraction from insects (Brigode et al., 2020).

3. Physico-chemical characterization

3.1. Extraction yield

Yield is one of the crucial features in the extraction of chitin and chitosan from insects. As stated in the earlier section, the insect chitin sources have a significant amount of protein content. Therefore, deproteinization using alkaline treatments like NaOH and KOH was carried out to recover high purity chitin. The efficiency of deproteinization process depends on various factors including temperature, concentration of NaOH, and reaction time (Kaya et al., 2014; Kaya, Erdogan, et al., 2015; Paulino et al., 2006). Use of high concentration of NaOH eliminates more protein molecules deposited on the chitin, but it decreases the yield of chitin (Soon et al., 2018). The yield of chitin and chitosan from insects are shown in Table 1. The dry weight (DW) basis of yield of chitin and chitosan extracted from various lepidopteran insects such as *Bombyx mori*, *Ephestia kuehniella*, *Dendrolimus punctatus*, *Argynnis pandora*, and *Clanis bilineata* were found to be 2.59–56%, 3.1–88.40%, 9.5–10.5%, 8–22% and 31.37–96.2% respectively (Kaya, Bitim, Mujtaba, & Koyuncu, 2015; Luo et al., 2019; Mehranian et al., 2017; Paulino et al., 2006; S.; Wu, 2011; Xia et al., 2013). Earlier studies have shown that the yields of chitin and chitosan from various marine sources including crab, *Scylla tranquebarica* (34.27% and 19.13%), *Portunus segnis* (19.6%), *Portunus pelagicus* (20%), shrimp, *Penaeus semisulcatus*

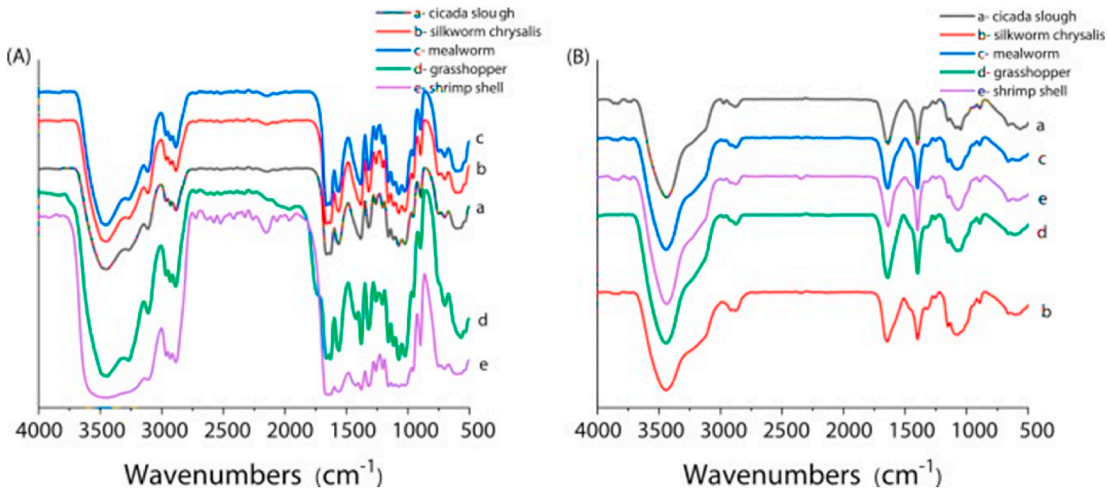

Fig. 4. FTIR spectrograms of (A) chitin and (B) chitosan extracted from five sources. Reprinted with permission (4873290806712) from Carbohydrate Polymers (Luo et al., 2019), copyright 2019 Elsevier.

(19.13%) *Penaeus monodon* (30% and 35%), *Parapenaeus longirostris* (24%) shell, 27.80% in the krill (*Euphausia superba*), 24.6% in the lobster (*Nephrops norvegicus*), 17.8% in the squilla and 31% in the squid (*Illex argentinus*) pen (Al Sagheer et al., 2009; Benhabiles et al., 2013; Cortizo, Berghoff, & Alessandrini, 2008; Hamdi et al., 2017; Sayari et al., 2016; Srinivasan et al., 2018; Thirunavukkarasu & Shanmugam, 2009; Wang et al., 2013) After the deproteinization, demineralization and decoloration it was found that the chitin and chitosan content of coleopteran insects like *Tenebrio molitor* (Luo et al., 2019), *Omophlus* sp (Kaya et al., 2016), *Melolontha melolontha* (Kaya, Baublys, et al., 2014; Kaya, Bulut, et al., 2016), *Hydrophilus piceus* (Kaya et al., 2014), *Leptinotarsa decemlineata* (Kaya et al., 2014), *Catharsius molossus* (Ma et al., 2015), *Calosoma rugose* (N. H. Marei et al., 2016), *Holotrichia parallela* (Liu et al., 2012), *Lucanus cervus*, *Polyphylla fullo* (Kabalak, Aracagök, & Torun, 2020), *Zophobas morio* (Shin et al., 2019), *Allomyrina dichotoma* and Dung beetle (Mingtang, 2004) was 17.32 and 14.48%, 13–16.60%, 19–20 and 74%, 7–20 and 67–72%, 17 and 24%, 5%, 15%, 10.9%, 11.3%, 3.90–8.40 and 78.33–83.33%, 12.70–14.20 and 75–83.37% and 28.7% of the dry weight respectively. The chitin and chitosan content of Odonata including *Sympetrum fonscolombii* and *Anax imperator* ranges between 20.3 and 67% DW (Kaya et al., 2014; Kaya et al., 2016). Besides, the chitin and chitosan content in cockroach, *Periplaneta americana* varied between 3.36 and 26.9% and 0.024–2.08%, respectively (Kaya, Baran, & Karaarslan, 2015; Kaya et al., 2017; M.-w.; Kim, Song, Han, et al., 2017; Wanule, Balkhande, Ratnakar, Kulkarni, & Bhowate, 2014). In comparison with this amount, *Ranatra linearis* had 15–16% of chitin, 6.70% in *Cicadatra atra*, 5.51% in *C. hyalina*, 36.6% in Cicada slough, 4.97% in *Cicada lodosi*, 6.49% in *C. mordoganensis*, 8.84% in *Cicadatra platyptera*, 5.88% in *C. tibialis* and 62.42% in *Cryptotympana atrata* (Mol, Kaya, Mujtaba, & Akyuz, 2018; Sajomsang & Gonil, 2010; S.-J.; Wu, Pan, Wang, & Wu, 2013). Further, grasshoppers, *Pterophylla beltrani*, locusts and crickets was reported as 11.8%, 20.5%, 16.5%, 5.3%, 7.4%, 5.7%, 6.5%, 8.1%, 8.9%, 6.6%, 22.5%, 12.2%, 12%, 14%, 4.3–7.1%, 4.71–11.84%, 20.91–23.35%, 9.8% and 10.1% of chitin in DW. While, chitosan content of the grasshoppers, *Pterophylla beltrani*, locusts and crickets was found to be 5.7%, 75%, 76%, 58.8%, 81.69%, 55%, 70.03–94.14% and 2.4–5.8% DW, respectively (Ibitoye et al., 2018; Kabalak et al., 2020; Kaya, Baran, & Karaarslan, 2015; M. W.; Kim, Song, Han, et al., 2017; Torres-Castillo et al., 2015). It was reported that the chitin and chitosan contents of hymenopteran species such as honey bee, *Apsis mellifera* (N. Marei, Elwahy, Salah, El Sherif, & Abd El-Samie, 2019; Nemtsev, Zueva, Khismatullin, Albulov, & Varlamov, 2004; Tsaneva et al., 2018) different varied from wasp species (Kaya, Bağrıaçık, Seyyar, & Baran, 2015; Kaya et al., 2016) and Bumblebee, *Bombus terrestris* (Majtán et al., 2007) ranged between 2.5 and 40%, 16–25% and 2.2–11.9% DW. Nevertheless, some species of housefly had low chitin including *Musca domestica*, black soldier fly, *Hermetia illucens*, and *Chrysomya megacephala* reported to be 8.02–5.87%, 3.1–23 and 32%, but *Drosophila melanogaster*, showed a low to high chitin yield of 7.85–70.91% (Antonov, Ivanov, Pastukhova, & Bovykina, 2019; D'Hondt et al., 2020; Kaya et al., 2016; Kim et al., 2016; Purkayastha & Sarkar, 2020; C.; Song, Yu, Zhang, Yang, & Zhang, 2013). The yield of the chitin and chitosan from insects are similar to the chitin extracted from crustacean shell waste. From the above discussed studies, it was concluded that chitin and chitosan from insects have alternative chitin sources.

3.2. Solubility

The solubility (1% of aqueous acetic acid) of chitosan extracted from different insect species was found to be high, ranging from 94.3% to 99.3%. Previous reports have found that the solubility of mussel, oyster shell, crab, pang scale, silver scale, prawn and conus shell chitin was 85.71%, 77.78%, 70.67%, 68%, 67.74%, 58.33% and 72.35%, respectively (Alabaraoye, Achilonu, & Hester, 2018; Mohan et al., 2019). The cohesive energy, associated with strong intermolecular interactions through hydrogen bonds in the crystalline state, is high, which makes the dissolution of chitin difficult (George & Roberts, 1992, pp. 249–267). Chitin is insoluble in many organic solvents, but chitosan is substantially soluble in dilute acidic solutions with pH $\leq$ 6.0 (Chang, Lin, Wu, & Tsai, 2015; Kumari, Annamareddy, Abanti, & Rath, 2017; Zargar, Asghari, & Dashti, 2015). The solubility of chitosan relies on the temperature, the alkali concentration, the ratio of the chitin in alkali solution, particle size, percentage of the degree of deacetylation (DD), M_w, and biological origin (Hossain & Iqbal, 2014; Samar, El-Kalyoubi, Khalaf, & Abd El-Razik, 2013). Based on the above factors, the solubility of insect chitosan is similar to that of crustacean shells, and the high solubility of insect chitosan should therefore be employed in many useful applications in the future.

3.3. Water binding capacity and fat binding capacity

Water binding capacity is the tendency of water to associate with hydrophilic substances. Fat binding capacity is a measure of the amount of oil absorbed per unit weight. The WBC and FBC of chitosan isolated from a cicada, silkworm chrysalis, mealworm, and grasshopper were noted to be 795-574%, 635-412%, 643-408%, and 594-275%, respectively (Luo et al., 2019). The values of the WBC and FBC of chitosan

Table 4
Surface morphology (SEM analysis) of insect chitin and chitosan.

Species	Surface morphology				References
	Chitin	Pore diameter	Chitosan	Pore diameter	
Bradyporus (C.) sureyai	Nanofiber and nanopore	10 μm	NA	NA	Kabalak et al. (2020)
Gryllotalpa gryllotalpa	Nanofiber and nanopore	12–17 μm	NA	NA	
Polyphylla fullo	Nanofiber and nanopore	4–5 μm	NA	NA	
Omophlus sp	Nanofiber with porous surface	150–400 nm	NA	NA	Kaya et al., 2016a
Melolontha melolontha	Nanofiber with porous surface	185–400 nm	NA	NA	Kaya et al., 2014b, 2016b
Ranatra linearis	Nanofiber	NA	Nanofibre	NA	Kaya et al., 2014a
Anax imperator	Nanofiber				
Hydrophilus piceus	Nanofiber				
Notonecta glauca	Nanofiber				
Agabus bipustulatus	Nanofiber				
Leptinotarsa decemlineata	Nanofiber	NA	Nanofibre	NA	Kaya et al. (2014c)
Catharsius molossus	NA	NA	Smooth surface	NA	Ma et al. (2015)
Cicada slough	NA	NA	Needle shape	NA	Luo et al. (2019)
Silkworm chrysalis			Reticular structure		
Mealworm			Irregular fibers		
Grasshopper			Rough structure		
Holotrichia parallela	Rough and thick surface	NA	NA	NA	Liu et al. (2012)
Schistocerca gregaria			Nanofibers with pores		Marei et al. (2016)
Apis mellifera			Rough surface without pores		
Calosoma rugosa			Nanofibers		
Zophobas morio	Smooth surface with tiny pores	NA	NA	NA	Soon et al. (2018)
Periplaneta americana	Oval nanopores without nanofibers	230–510 nm	NA	NA	Kaya et al. (2015b)
Blaberus giganteus	Nanofibers and pores	NA	NA	NA	Kaya et al. (2017)
Hermetia illucens		NA	NA	NA	Wang et al. (2013)
Larvae	Porous surface				
Prepupa	Rough surface with no holes				
Puparium	Rough surface with irregular holes				
Adult	Rough and flocculent				
Hermetia illucens	Honeycomb structure and no porosity	NA	NA	NA	Waśko et al. (2016)
Chrysomya megacephala	NA	NA	Fine regular fibril structure	NA	Song et al. (2013)
Cicada sloughs	Rougher morphology	NA	NA	NA	Sajomsang and Gonil (2010)
Cicadatra atra	Nanofibers with nanopores	NA	NA	NA	
Cicadatra hyalina	Nanofibrils and with rarely distributed pores				
Cicadatra platyptera	Fiberous and porous				
Cicada lodosi	Fibril bundles without pores				
Cicada mordoganensis	Fibril bundles without pores				
Cicadetta tibialis	Nanofibrils and with rarely distributed pores				
Honey bee		NA	NA	NA	Kaya et al. (2015d)
Wing	Regular rough surface				
Head	Highly fibrous and rarely porous				
Legs	highly fibrous and rarely porous				
Thorax	Overlapped scales				
Abdomen	Only porous without fibers				
Vespa crabro	Nanofibers and nanopores	100 and 200 nm	NA	NA	Kaya et al. (2015a)
Vespa orientalis	Nanofibers and nanopores	100 and 200 nm			
Vespula germanica	Nanofibers and nanopores	100 and 200 nm			
Vespa crabro	Nanofibrils and pores	NA	NA	NA	Kaya et al. (2016c)
Argynnis pandora	Overlapping scales, smooth porous, tubular structures with big pores, plane area with no pores, rough surface	20 μm	NA	NA	Kaya et al. (2015a)
Ephestia kuehniella	Pores and parallel nanofibers	5.2 μm	NA	NA	Mehranian et al. (2017)
Silkworm chrysalides	Fine loosely united leaves	NA	Porous structure	NA	Paulino et al. (2006)
Brachytrupes portentosus	Nanopores, thread-like fibrous	0.30–0.89 μm	Big pores and fibres	72.1 nm to 0.12 μm	Ibitoye et al. (2018)
Grasshopper	Porous with highly adherent nanofibers	180–260 nm	NA	NA	Kaya et al. (2015)
Calliptamus barbarus and *Oedaleus decorus*	Smooth surface	NA	porous and nanofibrillar structure	100–200	Kaya et al. (2015b)
Pyrgomorpha cognata	Nanofibres and nanopores	NA	NA	NA	Kaya et al. (2015c)
Oedipoda caerulescens	Nanofibres with no pores				
Oedipoda miniata	Nanofibres and nanopores				
Aiolopus strepens	Nanofibres and nanopores				
Aiolopus simulatrix	Nanopores and nanofibres				
Duroniella fracta	Nanopores and nanofibres				

(*continued on next page*)

Table 4 (*continued*)

Species	Surface morphology				References
	Chitin	Pore diameter	Chitosan	Pore diameter	
Duroniella laticornis *Schistocerca gregaria*	Nanopores and nanofibres Fibrous structure	NA	NA	NA	Marei et al. (2019)

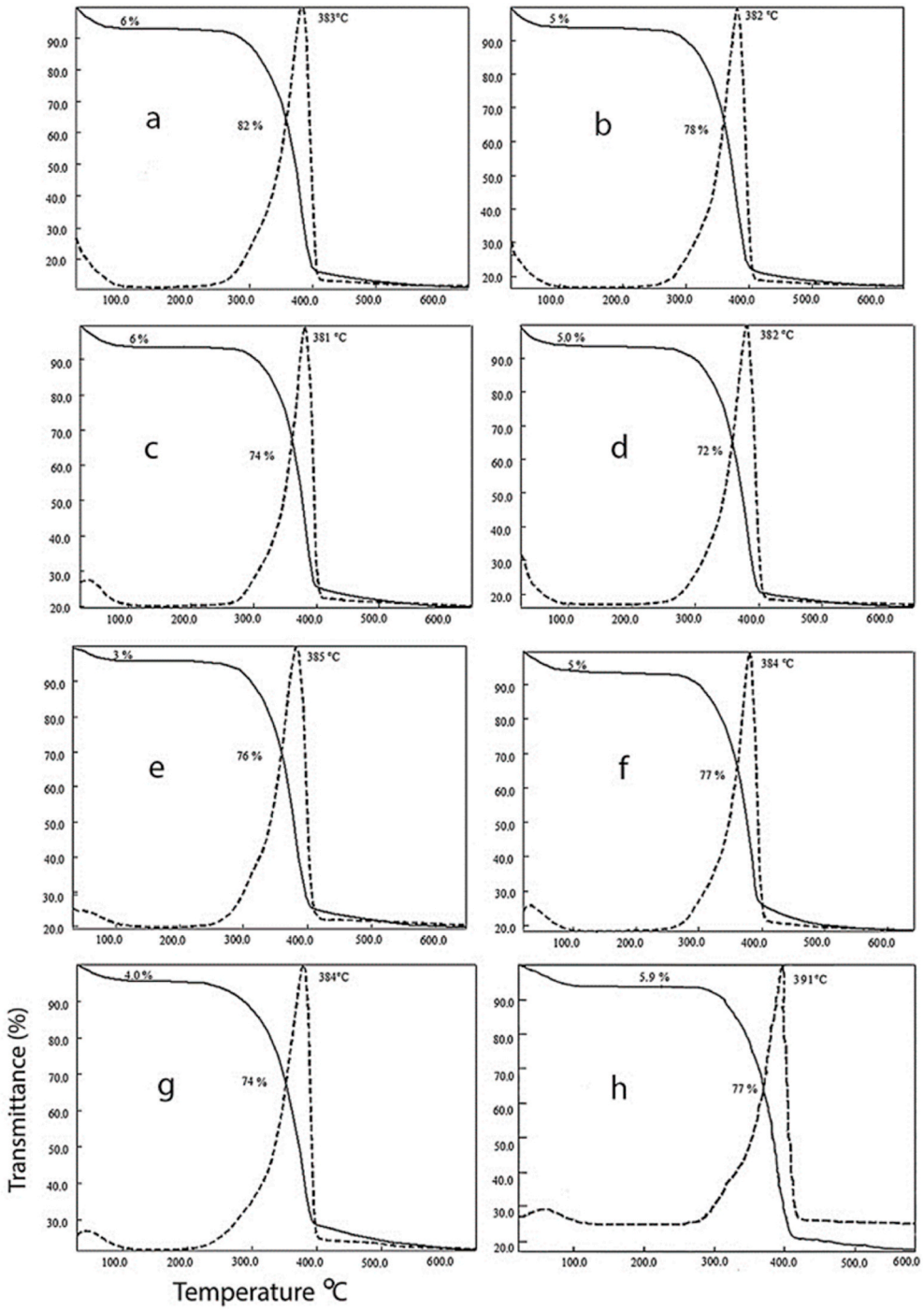

Fig. 6. TGA curves for chitins from seven grasshopper species (a. Chitin from *Ailopus simulatrix*, b. Chitin from *A. strepens*, c. Chitin from *Duroniella fracta*, d. Chitin from *D. laticornis*, e. Chitin from *Oedipoda miniata*, f. Chitin from *O. caerulescens*, g. Chitin from *Pyrgomorpha cognata* and h. Commercial chitin). Reprinted with permission (4873291045484) from International Journal of Biological Macromolecules (Kaya et al., 2014), copyright 2014 Elsevier.

Table 5
Thermogravimetric analysis (TGA) of insect chitin and chitosan.

Species	Chitin			Chitosan			References
	First mass loss (%)	Second mass loss (%)	DTG_{max} peak (°C)	First mass loss (%)	Second mass loss (%)	DTG_{max} peak (°C)	
Melolontha melolontha	4	78	380	NA	NA	NA	Kaya et al. (2014b)
Ranatra linearis	6	78	393	9	50	289	Kaya et al., 2014a
Anax imperator	6	75	387	9	87	295	
Hydrophilus piceus	5	73	386	3	59	288	
Notonecta glauca	7	73	385	8	61	308	
Agabus bipustulatus	5	71	384	6	67	296	
Asellus aquaticus	5	71	350	8	74	280	
Melolontha sp.	5.4	81.2	384.6	NA	NA	NA	Kaya et al. (2016b)
Bradyporus (C.) sureyai	5.2	72	382.4	NA	NA	NA	Kabalak et al. (2020)
Gryllotalpa gryllotalpa	6	70	374.6				
Polyphylla fullo	5.9	73	374.7				
Lucanus cervus	6.6	70	379.9				
Omophlus sp.	3.6	78.8	385.3	NA	NA	NA	Kaya et al., 2016a
Leptinotarsa	4	74	379	5	59	289	Kaya et al. (2014c)
decemlineata	3	48	307	5	59	292	
Periplaneta americana	5	76	389	NA	NA	NAS	Kaya et al. (2015b)
Blaberus giganteus				NA	NA	NA	Kaya et al. (2017)
Adult	6.44	71.69	401.7				
Larvae	5.96	71.37	374.1				
Hermetia illucens							
Larvae	4.42	69.48	372	NA	NA	NA	Wang et al. (2013)
Prepupa	6.74	71.16	373				
Puparium	8.52	71.25	371				
Adult	7.5	73.31	372				
Hermetia illucens							
BSFE	5	70	363	NA	NA	NA	Purkayastha and Sarkar (2020)
BSFI	6	80	371				
Hermetia illucens							
Larvae	2	62	389	NA	NA	NA	Waśko et al. (2016)
Imago	3	63	387				
Cicada sloughs	7.3	66.4	362	NA	NA	NA	Sajomsang and Gonil (2010)
Cicada atra	4.54	83.75	411.50				Mol et al. (2018)
Cicadatra hyalina	5.47	66.78	412.70				
Cicada lodosi	4.41	83.94	411.70				
Cicada mordoganensis	4.88	80.44	412.40				
Cicadatra platyptera	3.80	81.78	412.20				
Cicadivetta tibialis	4.04	73.49	402.30				
Honeybee							
Head	6	67	308	NA	NA	NA	Kaya et al. (2015d)
Thorax	4	56	360				
Abdomen	3	68	367				
Legs	5	68	359				
Wings	3	60	359				
Vespa crabro	6	73	383	NA	NA	NA	Kaya et al. (2015a)
Vespa orientalis	6	83	385				
Vespula germanica	6	76	385				
Vespa crabro							
Larvae	3.51	88.70	384.8	NA	NA	NA	Kaya et al. (2016c)
Pupa	2.7	69.9	381.7				
Adult	6.5	78.3	384.2				
Argynnis pandora							
Wings	4.8	76.7	386.9	NA	NA	NA	Kaya et al. (2015a)
Other body parts	4.9	82.2	389.6				
Sympetrum	2.9	73.2	369.2	NA	NA	NA	Kaya et al. (2016b)
fonscolombii							
Dociostaurus maroccanus							
Adult	4	77	386	5	62	308	Erdogan and Kaya (2016)
Nymph	4	82	383	7	59	302	
Celes variabilis	5	80	386	NA	NA	NA	Kaya et al. (2015c)
Decticus verrucivorus	3	87	388				
Melanogryllus desertus	5	94	385				
Paracyptera labiata	6	77	385				
Calliptamus barbarus	8	72	381	8	61	296	Kaya et al. (2015b)
Oedaleus decorus	6	77	390	9	57	305	
Ailopus simulatrix	6	82	383	NA	NA	NA	Kaya et al. (2015c)
Ailopus strepens	5	78	382				
Duroniella fracta	6	74	381				
Duroniella laticornis	5	72	382				
Oedipoda miniata	3	76	385				
Oedipoda caerulescens	5	77	384				
Pyrgomorpha cognata	4	74	384				

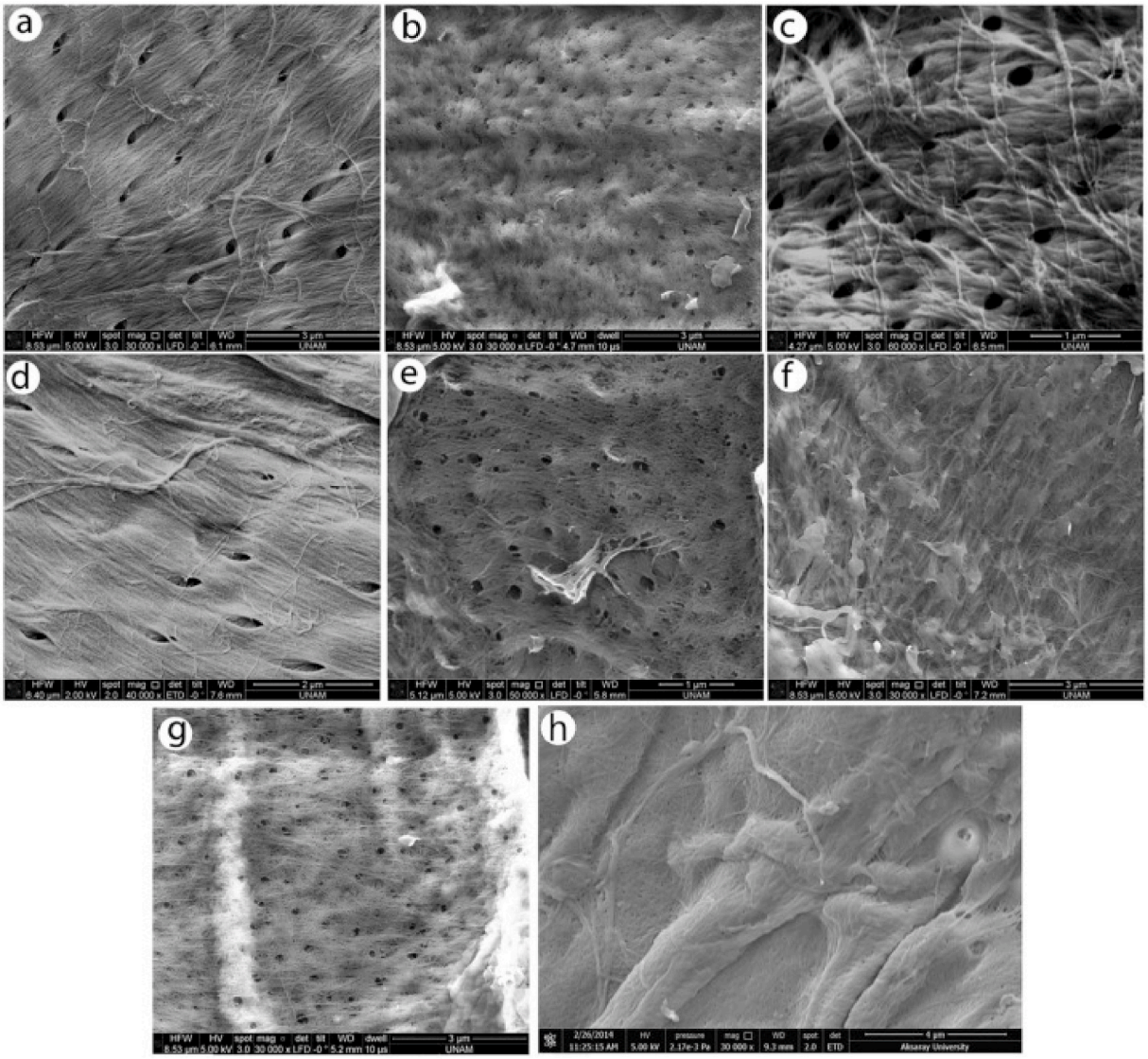

Fig. 5. ESEM photographs of chitins from seven grasshopper species at 3000–6000 × magnifications (a. Chitin from *Ailopus simulatrix*, b. Chitin from *A. strepens*, c. Chitin from *Duroniella fracta*, d. Chitin from *Duroniella laticornis*, e. Chitin from *Oedipoda miniata*, f. Chitin from *O. caerulescens*, g. Chitin from *Pyrgomorpha cognata* and h. Commercial chitin). Reprinted with permission (4873291045484) from International Journal of Biological Macromolecules (Kaya et al., 2014), copyright 2014 Elsevier.

extracted from *Schistocerca gregaria*, *Apis mellifera*, and *Calosoma rugosa* were 516-307%, 511-304%, and 506-300%, respectively (N. H. Marei et al., 2016). The WBC and FBC of chitosan from crab (*Chionoecetes opilio*) legs range from 355% to 611% and 217%–403% (No, Lee, & Meyers, 2000). The WBC and FBC, therefore, could vary based on differences in the crystallinity of the products, the amount of salt-forming groups, deproteinization and demineralization processes (Knorr, 1982; Kumari et al., 2017).

Table 6
Solid-state ^{13}C CP/MAS NMR spectral data of chitin and chitosan in different insect sources.

Sources	Chemical shift (ppm)										References
	C1	C2	C3	C4	C5	C6	C=O	C=C	G- C	CH_3	
Cicada sloughs chitin	104.2	55.3	73.5	83.3	75.8	61.0	173.8	NA	NA	23.0	Sajomsang and Gonil (2010)
Silkworm pupa exuviae chitin	104.4	55.4	73.6	83.4	75.9	61.1	173.5	NA	NA	23.0	Zhang et al. (2011)
Beetle larvae cuticles chitin	104.4	55.7	74.0	83.6	76.1	61.5	174.3	NA	NA	23.0	
Bumblebee cuticles chitin	103.9	54.9	73.1	82.7	75.5	60.6	173.3	NA	NA	22.3	Majtán et al. (2007)
Silkworm chrysalides chitin	104.5	55.6	73.8	83.5	76.1	61.4	NA	NA	NA	23.2	Paulino et al. (2006)
Blowfly larvae chitosan	104.47	56.78	75.14	85.31	75.14	60.41	NA	NA	NA	22.64	Song et al. (2013)
Black soldier fly chitin											Purkayastha and Sarkar (2020)
Imago	104.6	55.7	74.2	84.0	76.4	61.5	173.9	NA	NA	23.4	
Pupae exuviae	103.4	55.0	73.3	82.7	75.5	60.7	172.6	NA	NA	22.7	
Silkworm chrysalides chitin	104.5	55.6	73.8	83.5	76.1	61.4	NA	NA	NA	NA	Simionato et al. (2006)
Silkworm chrysalides chitosan	105.3	57.9	75.8	82.3	75.8	61.1	174.0	NA	NA	23.0	

3.4. Ash and moisture content

It is necessary to quantify the ash content in chitin and chitosan before beginning the demineralization process, and it is important to evaluate its efficiency for the elimination of calcium carbonate. The demineralization process results in products containing 31%–36% ash (Kaya, Erdogan, et al., 2015). A high-value grade of chitosan should have <1% ash content (Nessa et al., 2010). The ash content of chitin and chitosan from fish (1.2% and 1.0%), shrimp (0.03%), crab (2.5%), conus shell (1.2%), honeybees (9.2%), beetles (2.0%, 2.20% and 0.50%), locusts (1.6%), cicada slough (0.03% and 11.3%), silkworms (0.05%), grasshoppers (0.89%), housefly larvae (0.13%), house crickets (1.0%) and *Hermetia illucens* (3.3, 5.6 and 19%) were measured (Caligiani et al., 2018; Ibitoye et al., 2018; Kumari et al., 2017; N. H.; Marei et al., 2016; Purkayastha & Sarkar, 2020; Sajomsang & Gonil, 2010; A.-J.; Zhang et al., 2011). Low ash content could be a reason for the superior

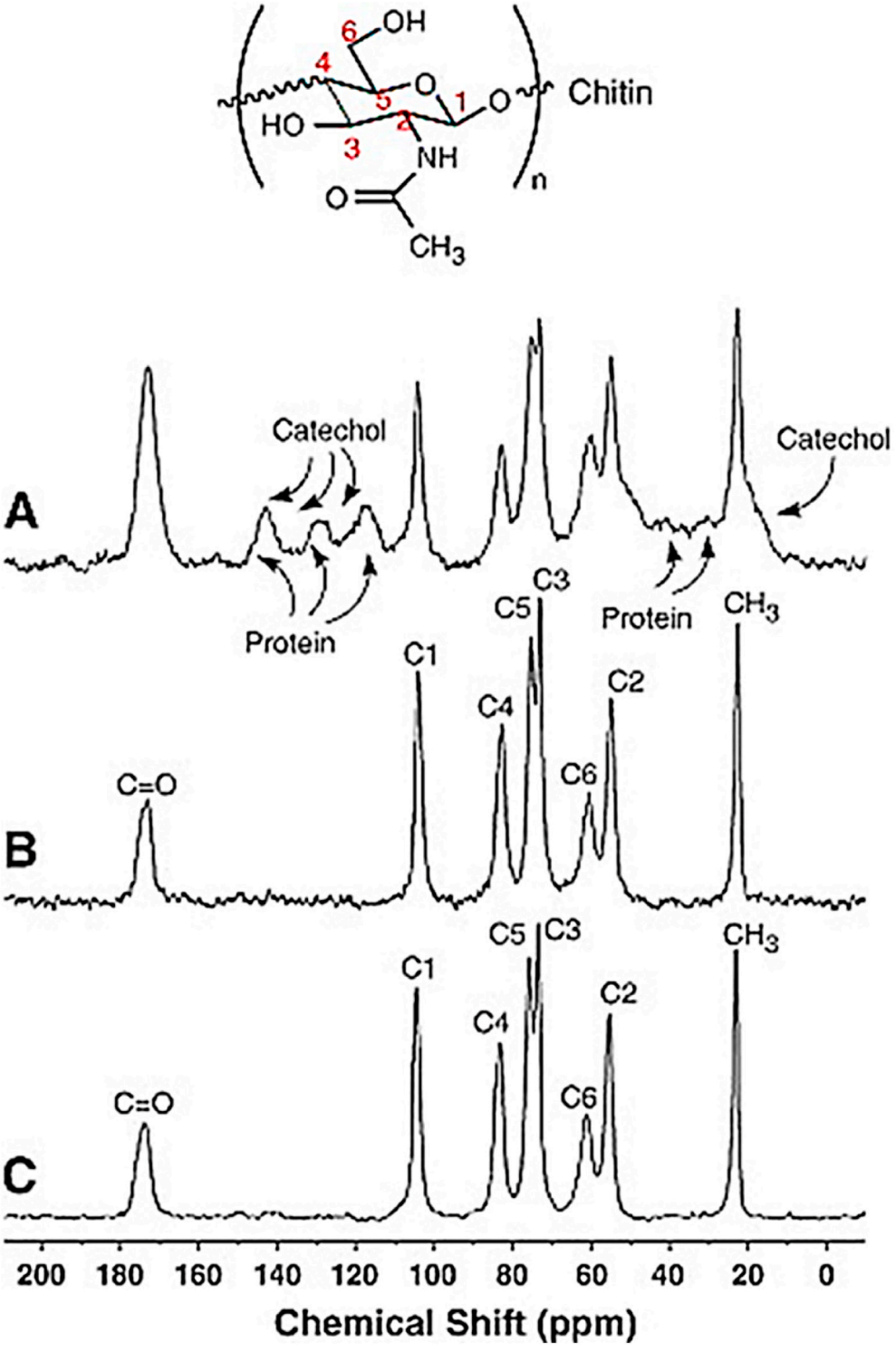

Fig. 7. ^{13}C CP/MAS NMR spectra of the cicada sloughs (A), chitin from cicada sloughs (B), and chitin from rice-field crab shells (C). Reprinted with permission (4873291128692) from Materials Science and Engineering C (Sajomsang & Gonil, 2010), copyright 2010 Elsevier.

solubility of chitosan (Kumar, Xavier, Lekshmi, Balange, & Gudipati, 2018). Furthermore, the moisture content can determine the performance of the powder when used in capsule/pill preparations. The moisture content of chitin and chitosan isolated from fish (13.8% and 3.0%), shrimp (0.0004%), crab (0.0048%), conus shell (6.5%), honeybee, beetles, locusts, cicada slough, silkworms, grasshoppers and house crickets were 17.6%, 8.8%, 14.1%, 7.12%, 0.18%, 0.07%, 0.19%, 1.8%, 8.7%,4% and 3.33%, respectively (Kumari et al., 2017; Liu et al., 2012; Luo et al., 2019; N. H.; Marei et al., 2016; Mohan et al., 2019). Importantly, the moisture content of chitosan is not dependent on the Mw or the DD (Cho, No, & Meyers, 1998).

3.5. Molecular weight (Mw)

The Mw of commercial chitosan is between 100 and 1200 kDa (Li, Dunn, Grandmaison, & Goosen, 1992). The molecular weight of chitin and chitosan differs based on the source and the extraction methods used. The average viscosity Mw of chitin from honeybees and grasshopper larvae and adults is 738.806 kDa, 7.2 kDa, and 5.6 kDa, respectively (Draczynski, 2008; Erdogan & Kaya, 2016). The Mw of the Orthopteran chitin varied between 5.2 and 6.8 kDa (Kaya, Baran, & Karaarslan, 2015). The Mw of chitosan extracted from Colorado potato beetle adults (Kaya et al., 2014) and larvae, grasshoppers (Luo et al., 2019), *Periplaneta americana*, *Hermetia illucens* and *Musca domestica* (Ai, Wang, Yang, Zhu, & Lei, 2008; Jing et al., 2007) were 2.722 kDa, 2.676 kDa, 4.5 kDa, 3.779 kDa, 4.090 kDa, 3.975 kDa, 3.989 kDa, 230.3 kDa, 15 kDa, 426 kDa, and 63 kDa, respectively. High molecular weight is responsible for the poor solubility of chitosan in water and its high solution viscosity, which limits its use in the cosmetics, agriculture and food industries. The lower molecular weight chitosan from shrimp shells demonstrates higher antibacterial activity (Du, Zhao, Dai, & Yang, 2009), as does the low molecular weight (25 kDa) chitin extracted from conus shell (Mohan et al., 2019). Chitosan has a moderate molecular weight and demonstrates higher anti-cholesterol activity (Kara & Stevens, 2002). The Mw of insect chitin and chitosan could be determined by viscometry methods (Draczynski, 2008; Erdogan & Kaya, 2016; Kaya et al., 2014; M. W.; Kim, Song, Han, et al., 2017) and high-performance liquid chromatography. The diverse Mw of chitin can be used in many useful ways. The low Mw chitin and chitosan from shrimp and insects have excellent antiseptic and anticancer properties useful for drug development.

3.6. Degree of deacetylation (DD)

The DD of chitin and chitosan is the significant parameter influencing the biological, physicochemical, and mechanical properties dependent on the method of extraction (Khan, Peh, & Ch'ng, 2002). The DD of chitosan was 94.9% in *Catharsius molossus*, 89%, 96% (Ma et al., 2015) and 95% in locusts, honeybees and beetles (N. H. Marei et al., 2016), 81.06% in *Zophobas morio* (Soon et al., 2018), 91.86% in *Periplaneta americana*, 42.47% in *Hermetia illucens* (Khayrova et al., 2019), and 83% and 90.3% in housefly larvae (Ai et al., 2008; A.-J.; Zhang et al., 2011); the DD of chitin was 133%, 86%, 121%, 120%, 117% and 86% in *Ranatra linearis*, *Anaz imperator*, *Hydrophilus piceus*, *Notoneeta glauca*, *Agabus bipustulatus* and *Asellus aquaticus*, respectively (Kaya et al., 2014). Several methods have been developed for the determination of DD in chitin and chitosan from insects. Among them, the potentiometric titration method (Ma et al., 2015), the conductometric titration method (Khayrova et al., 2019), the acid-base titration method (A.-J. Zhang et al., 2011) and the FT-IR (Kaya et al., 2014) are effective for perfectly soluble materials. The DD of chitosan from fish, shrimp, and crab shells was 75%, 78% and 70%, respectively (Kumari et al., 2017). Previous studies have suggested that a higher DD is a significant development of chitin that can be used in scaffolds and implantations in the biomedical field (Akpan, Gbenebor, & Adeosun, 2018).

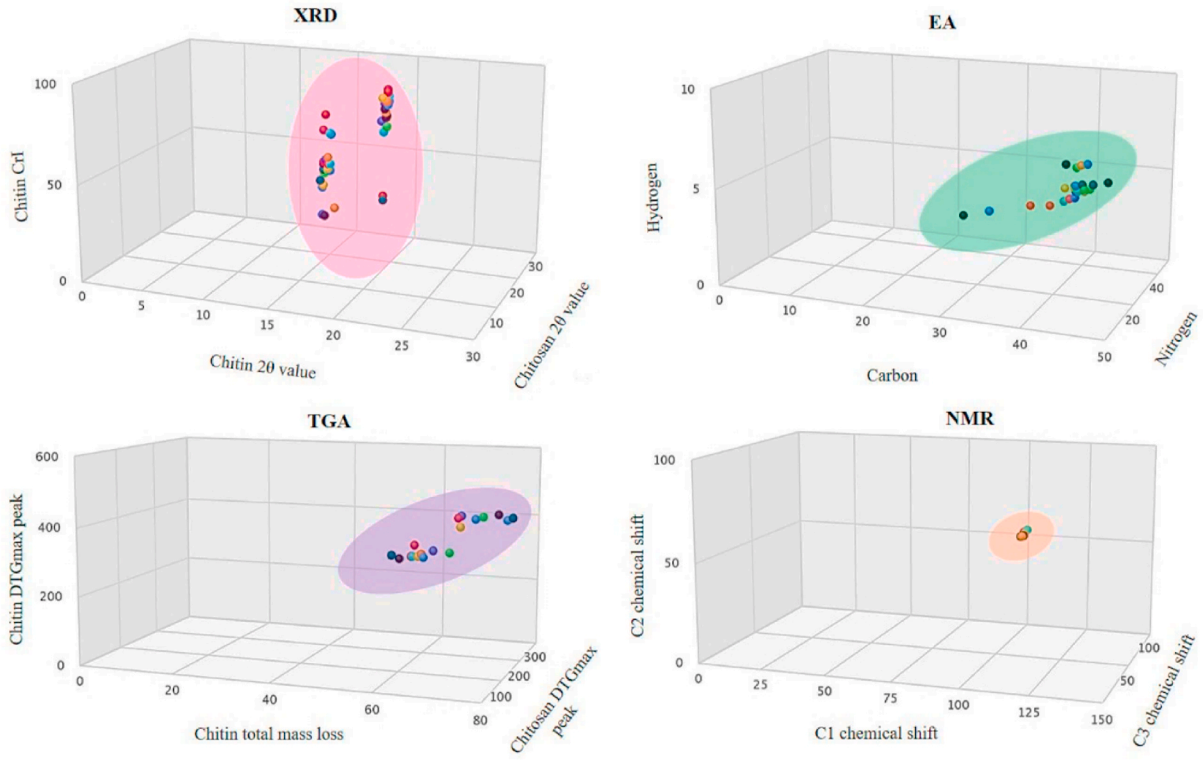

Fig. 8. 3D scatter plot of structural characterization studies (XRD, EA, TGA and NMR analysis) in insect chitin and chitosan.

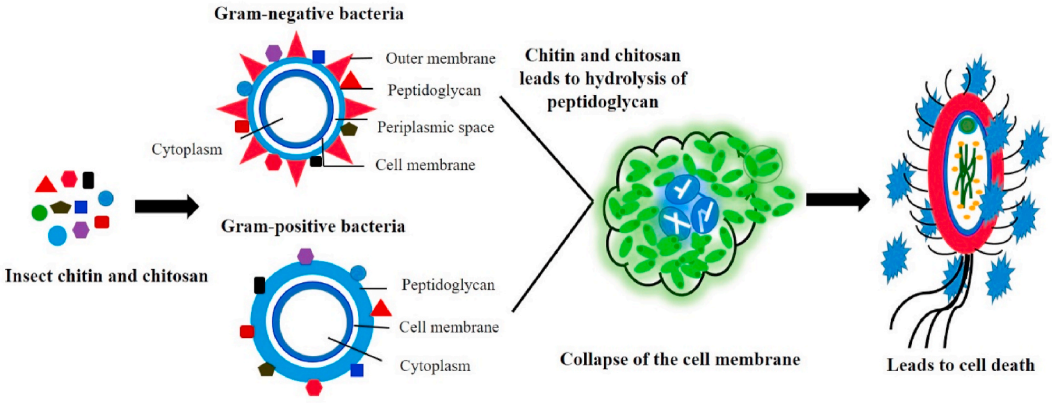

Fig. 9. Schematic representation of antibacterial mechanism of chitin and chitosan from insects.

4. Structural characterization

The structural characterization of insect chitin and chitosan was determined by X-ray diffraction, elemental analysis, Fourier transform infrared spectroscopy, scanning electron microscopy, thermogravimetric analysis, and nuclear magnetic resonance spectroscopy.

4.1. Crystalline properties

The CrI values of chitin and chitosan are significant in determining their potential application areas (Aranaz et al., 2009), as they depend on their crystalline and amorphous nature. This could be detected using X-ray diffraction. Nevertheless, the crystalline nature also represents the purity and size of the crystals in the biopolymer. As noted in previous studies, a low crystalline index (CrI %) was obtained in chitin from *Hermetia illucens* at the larval (33.05%) and prepupal (35.14%) stages. However, the puparium (68.4%) and adult (87.92%) stages of same species have also had high CrI recorded (Caligiani et al., 2018). High molarity (2 M) NaOH during the deproteinization process has been found to increase the amorphous nature and decrease the crystallites of insect chitin. Furthermore, the surface morphology of the obtained chitin had a lower CrI with an amorphous region with a porous surface compared to the higher CrI that had a rough and irregular surface (Table 2). According to Park et al. (2010), the CrI was measured as the ratio between the area of the crystalline contribution and the total area. Similarly, the total XRD peaks obtained from *Agabus bipustulatus* and *Brachytrupes portentosus* showed 7 and 10 distinct peaks at 2θ with the highest CrI of 90.6% and 88.02% (Ibitoye et al., 2018; Kaya et al., 2014). This finding also indicates the impurity of the chitin obtained from *B. portentosus* using N-6.02%. CrI values of chitosan from cicada slough, silkworm chrysalises, mealworms, grasshoppers and shrimp shells were observed to be 64.8%, 32.9%, 51.9%, 50.1% and 49.1%, respectively, and the crystallinity indices of shrimp shells, mealworms and grasshopper chitosan were similar (Luo et al., 2019) (Fig. 3). The chitosan extracted from crab and squilla exhibited two characteristic crystalline peaks at $2\theta = 10.3^{\circ}$ and 19.2° and $2\theta = 10.2^{\circ}$ and 19.5°, which were slightly shifted to a higher diffraction angle and showed semi-crystalline chitosan (Anand et al., 2014). *Vespa crabro, Vespa orientalis, Vespula germanica, Argynnis Pandora, Ailopus simulatrix* (Kaya, Baran, & Karaarslan, 2015; Kaya et al., 2016) exhibited 6 crystalline peaks and a CrI between 69 and 76%. Moreover, a high number of XRD peaks attributed to impurities (6.6–6.9% N-factor) have been found to be present in

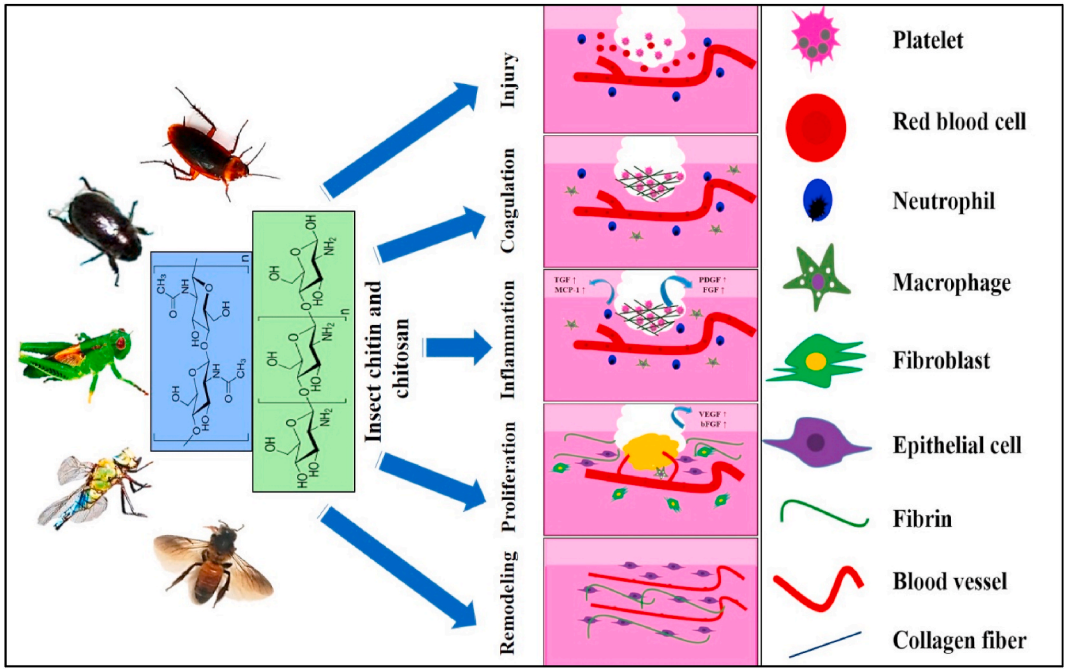

Fig. 10. Graphic representation of wound healing mechanism of insect chitin and chitosan.

insect chitin, which influences the degradation of the polysaccharides with DTG at ~383–386 °C. In addition, the chitosan showed 3 variant peaks demonstrated from *Schistocerca gregaria* and *Brachytrupes portentosus* in thread-like fibrous structures with a crystal size of ≥72.1 nm to 0.12 μm (Ibitoye et al., 2018; N. H.; Marei et al., 2016), which is large compared to other insect chitosan reported to date. Furthermore, all published literature reports the crystalline properties of insect chitin to be in the range of ≥60–90% CrI, although these numbers would differ based on the alkaline and acidification used in the extraction process. The chitin with the higher CrI value obtained from insects is an alternative chitin source that can be used in the biomedical field. The XRD patterns of the chitin and chitosan extracted from all insects species are also quite similar (Fig. 8).

4.2. Elemental analysis (EA)

Elemental analysis of chitin from different types of insects, including the carbon, nitrogen, hydrogen and carbon-nitrogen ratio are shown in Table 3. The percentage of C atoms from chitin originating from various insects ranged from 32.09% to 48.90%. The N content of chitin is a significant indicator of its purity, and the N content of pure (acetylated) chitin has been found to be 6.89%. Nitrogen content >6.89% (Liu et al., 2012; Majtán et al., 2007; Sajomsang & Gonil, 2010) shows that protein residues may still be present in the chitin sample, though nitrogen content <6.89% suggests that inorganic materials may not have been completely removed. The N% value of chitin from *Melolontha melolontha*, *Periplaneta americana*, *Vespa crabro*, *Argynnis pandora,* and *Sympetrum fonscolombii* was measured to be 6.72%, 6.69%, 6.85%, 6.62%, and 6.83%, respectively (Kaya, Bağrıaçık, et al., 2015; Kaya, Baublys, et al., 2014; Kaya, Bulut, et al., 2016). Additionally, the EA results for the chitin from crab was 6.03%, 42.9% and 5.65%; from crayfish was 6.09%, 42.88%, 6.02%; and from shrimp was 6.17%, 43.2%, 6.42% (Kaya, Baran, & Karaarslan, 2015). The N% values of the chitin from insects from different orders were very close to the theoretical value. The above studies show that chitin obtained from insects is of high purity. In this context, the elemental composition of the chitin and chitosan extracted from all insect species is similar (Fig. 8).

4.3. Fourier transform infrared spectroscopy

FT-IR spectroscopy is generally used for the identification of organic samples (Dukor, Story, & Marcott, 1999). There are three crystalline forms of chitin, which are *alpha, beta* and *gamma*, but there is little information about the *gamma* form (Jang, Kong, Jeong, Lee, & Nah, 2004). FT-IR spectra is helpful for differentiating between the α-form and the β-form using the presence or absence of the amide I band. In the α-form, the amide I band divides into two bands at approximately 1650 and 1620 cm^{-1} (Wang et al., 2013), while in the β-form, there is only one amide I band in the 1656 cm^{-1} region. Beta chitins are found in squid pens (Jang et al., 2004), and alpha chitin is found in the order Arthropoda (Al Sagheer et al., 2009; Sajomsang & Gonil, 2010). In the FT-IR spectra of the chitin and chitosan extracted from various insects (Fig. 4), such as *Holotrichia parallela* (Liu et al., 2012), *Zophobas morio* (Shin et al., 2019), *Periplaneta americana* (Kaya, Baran, & Karaarslan, 2015), *Hermetia illucens* (Waśko et al., 2016), and *Apis mellifera* (Kaya, Lelešius, et al., 2015), the amide I band is split at 1654 cm^{-1}, 1663 and 1618 cm^{-1}, 1647 and 1654 cm^{-1}, 1654 and 1621 cm^{-1}, 1654, 1617 and 1550 cm^{-1}, and 1656 cm^{-1}, respectively. The FT-IR spectra of the amide I band of the chitosan extracted from squilla, crab, conus shell, krill, lobster and shrimp is split at 1643 cm^{-1}, 1634 cm^{-1}, 1625 cm^{-1}, 1628 cm^{-1} and 1667 cm^{-1}, respectively (Anand et al., 2014; Mohan et al., 2019; Sayari et al., 2016; Srinivasan et al., 2018; Wang et al., 2013). These results show that the chitin and chitosan isolated from crustacean shell waste and insects are in the α-form.

4.4. Scanning electron microscopy

Scanning electron microscopy is an instrumental technique for the visual confirmation of the morphology and physical state of the surface of chitin. The surface morphology of insect chitin and chitosan differs according to the organisms from which they originate. Generally, insect chitin and chitosan may be classified into the following surface morphologies (Table 4): (I) nanofibre and nanopore, (II) nanofibre, (III) nanopores without nanofibres, (IV) nanofibres without nanopores, (V) smooth surface, and (VI) rough surface. Crickets (Kabalak et al., 2020), grasshoppers (Kaya, Bağrıaçık, et al., 2015), Orthopteran species (Kaya, Baran, & Karaarslan, 2015) (Fig. 5) and house cricket chitin (Ibitoye et al., 2018) show both nanofibre and nanopore structures. Aquatic bugs, water scavenger beetles, desert locust (Kaya et al., 2014) and Colorado potato beetle chitosan (N. H. Marei et al., 2016) have a nanofibrous structure. A few reports have shown that cockroach and black soldier fly chitin had nanopores without nanofibres and nanofibres (Kaya et al., 2014) without nanopore structures (Waśko et al., 2016). In addition, the chitin from Zophobas *morio* and *Holotrichia parallela* and the chitosan from *Catharsius molossus* had smooth and rough surface morphologies. In this context, Anand et al. (2014) reported that sponge and cauliflower leaf-like morphology was observed in crab and squilla chitin. The SEM analysis of conus chitin showed a microfibrillar crystalline structure and porosity (Mohan et al., 2019). The tightly arranged fibres were also observed in the chitin obtained from krill, shrimp and lobster shell (Al Sagheer et al., 2009; Wang et al., 2013). Furthermore, SEM analysis of the chitin and chitosan surface morphologies of *P. monodon* showed microfibril and porous structures (Srinivasan et al., 2018). Surface morphology is one of the vital properties that determines the effective use/application of chitin and chitosan. The nanofibre and nanopore forms of chitin and chitosan could be used in textiles, food and therapeutic applications (Aranaz et al., 2009; Synowiecki & Al-Khateeb, 2003).

4.5. Thermogravimetric analysis

The thermal stability of the chitin and chitosan from insects is measured in the mass losses found at two steps (Table 5; Fig. 6). The loss at the first step is attributed to the evaporation of water from the chitin and chitosan molecules, and the loss at the second step represents the degradation of the chitin and chitosan units (Ofem, 2015). Anand et al. (2014) reported in the TGA analysis of chitosan from crab and squilla that mass loss occurred three stages; the first mass loss occurred below 100 °C, followed by a second mass loss (252 °C, 269 °C, and 213 °C) and a third mass loss (367 °C, 384 °C and 350 °C). Ladchumananandasivam, da Rocha, Belarmino, and Galv (2012) demonstrated that decomposition occurs in the ranges of 50–100 °C and 400 °C−500 °C for shrimp and crab chitosan. For all the chitin samples from various insects, the first mass loss was noted to be between 2% and 8.52%, while the second mass loss ranged from 48% to 94% (Ladchumananandasivam et al., 2012). The maximum degradation temperatures (DTG_{max}) of chitin extracted from different insect orders ranged between 307 °C and 412.40 °C (Kaya, Baublys, et al., 2014; Kaya, Lelešius, et al., 2015; Kaya et al., 2016; Mol et al., 2018; Sajomsang & Gonil, 2010). The above findings concluded that insect chitin molecules could disintegrate at higher temperatures than chitosan molecules. This variance could be due to the N-acetylated polymer units of chitin molecules that are more stable than the amine polymer units of chitosan (Paulino et al., 2006). These results indicated that insect chitin molecules are more stable than insect chitosan units. Additionally, the thermal stability of chitin and chitosan extracted from all insect species is similar (Fig. 8).

4.6. Nuclear magnetic resonance spectroscopy

NMR spectroscopy is the most potent structural elucidation technique for organic compounds, and it functions using a magnetic field and

radiofrequency pulses transmitted at a particular resonant frequency to detect the signal of specific nuclei, including ^{1}H, ^{31}P, or ^{13}C, in the region of interest (Mandal, 2007). The solid-state ^{13}C NMR is useful for the structural characterization of carbohydrate polymers such as chitin and chitosan without damaging the samples. ^{13}C CP/MAS NMR spectroscopy could be used to determine the assignments of carbon chemical shifts of chitin and chitosan from various insect sources, as shown in Table 6. The ^{13}C CP/MAS NMR spectra of the cicada slough chitin spectrum contains eight well-defined peaks of C1–C6, CH_3 and C=O carbons, which are detected by a chemical shift ranging from 20 to 190 ppm (Fig. 7). The C1–C6 carbons displayed a chemical shift ranging from 50 to 110 ppm, while the methyl carbon and the carbonyl carbon showed a chemical shift of 23 ppm and 174 ppm, respectively (Sajomsang & Gonil, 2010). The ^{13}C CP/MAS NMR spectrum of the chitosan from blowfly larvae, *Chrysomya megacephala*, consists of seven well-defined peaks of C1 (δ 104.47), C2 (δ 56.78), C3 (δ 75.14), C4 (δ 85.31 and 80.97), C5 (δ 75.14), C6 (δ 60.41) and CH_3 (δ 22.64) and identified a weak methyl resonance (δ 22.64) representing a relatively high degree of acetylation (C. Song et al., 2013). This study indicated that highly deacetylated chitin and chitosan had more biological properties than less deacetylated chitin and chitosan (Heux, Brugnerotto, Desbrieres, Versali, & Rinaudo, 2000). Moreover, the chemical shifts in the NMR from the chitin and chitosan extracted from all insect species are similar (Fig. 8).

5. Biological activities

Insect chitin and chitosan have a broad spectrum of biological activities, such as antioxidant effects and antibacterial effects with substantial rheological properties, which could be used in the food industry to enhance food safety, shelf-life and quality control.

5.1. Antioxidant activity

Free radicals are produced by abnormal metabolic processes and cause extensive damage to living organisms, which may result in several diseases, such as cancer, inflammation, and neurodegenerative diseases (Halliwell, 2011; Moskovitz, Yim, & Chock, 2002). Commonly, free radicals are effectively removed by antioxidant enzymes in the body. Generally, naturally derived compounds have been used to treat free radical-mediated harmful effects in biological systems. Numerous studies have examined the antioxidant activities of chitin and chitosan from insects (Ai et al., 2008; Kaya, Bitim, et al., 2015; Kaya, Bulut, et al., 2016; C.; Song et al., 2013; Torres-Castillo et al., 2015; S.-J.; Wu et al., 2013). Chitosan from the adult Colorado potato beetle with low M_W has been reported to have a higher DPPH radical scavenging action at a concentration of 5 mg/mL, but chitosan obtained at the larvae stage of the same species displayed only 33.05% of the scavenging action with M_W. However, these chitosan showed similar action against the ferric ion reducing test. Furthermore, this study stated that a higher degree of acetylation (DA) had high antioxidant action, while the DA of the adult and larval Colorado potato beetle was 82% and 76%, respectively (Kaya et al., 2014). Additionally, no FRAP action was recorded in chitosan and colloidal chitin polymers derived from DNA fragmentation chitin from commercial shrimp shell (Kidibule, Santos-Moriano, Plou, & Fernández-Lobato, 2020); nonetheless, hydrolysis of the polymers improved FRAP action between 77% and >90%. In comparison with this result, chitosan derived from *C. barbarus* and *O. decorus* displayed lower reactions of 33.51%, and 33.26% in DPPH scavenging activity at a concentration of 5 mg/mL (Kaya, Bitim, et al., 2015). This action was less efficient compared to the housefly *Musca domestica*, which displayed the highest DPPH scavenging effect of 57.1% at a low concentration of 0.5 mg/mL (Ai et al., 2008). Furthermore, this outcome suggested that these two species, which can be catastrophic to food crops, could possibly be considered as a potential source of chitin and chitosan to be used in the food/feed industry for its antimicrobial properties.

5.2. Antibacterial activity

Recent findings have confirmed that insect chitin and chitosan possess significant antibacterial activity. In a few reports, shrimp and crab shell chitosan demonstrated better action against Gram-negative microbes than Gram-positive organisms (Chung et al., 2004). The possible mechanism for this difference could be the hydrolysis of peptidoglycan due to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membranes (Fig. 9). This interaction leads to the collapse of the cell membranes, escape of the intracellular components, and ultimately, to cell death (Chien, Yen, & Mau, 2016). However, chitosan from two grasshopper species, *C. barbarous* and *O. decorus*, showed a potential effect against both gram-positive and gram-negative microbes compared to standard antibiotics. The gram-positive bacteria were *L. garvieae, S. agalactiae, L. monocytogenes,* and *B. subtilis,* and the gram-negative bacteria, such as *Y. enterocolitica, V. alginolyticus,* and *S. enteritidis* showed minimal bactericidal concentrations (MBCs) of 0.32 mg/mL and 0.16 mg/mL for the chitosan derived from both grasshopper species (Kaya, Erdogan, et al., 2015). Similarly, chitooligosaccharide extracted from the cicada *Cryptotympana atrata* displayed maximum zones of inhibition against *B. subtilis, S. aureus*, and *E. coli* of 9.52 mm, 12.64 mm, and 10.79 mm, respectively. These chitooligosaccharides confirm the linkage of the β-1, 4-linked 2-amino-2-deoxy-d-glucopyranose (GlcN) and 2-acetamido-2--deoxy-d-glucopyranose (GlcNAc) (S.-J. Wu et al., 2013). This linkage has been found to be similar to that of COS from crustacean chitin (*Polybius henslowii* crab), which displayed a better inhibition against the fungi *Cryphonectria parasitica* at a concentration from 0.0125 to 0.1 mg/mL (Avelelas et al., 2019). However, chitooligosaccharides from *Clanis bilineata* indicated significant inhibitory action against *B. subtilis*, which was found to be similar to that of commercial chitosan (S. Wu, 2011). Furthermore, 4% deacetylated chitosan from *T. molitor* mealworm beetle larvae did not show any inhibitory effect against *S. aureus*, *B. cereus*, *L. monocytogenes,* or *E. coli*, but increasing the chitosan concentration to 8% resulted in 1–2 mm of inhibition. The crystallinity index (Cr I) value of *T. molitor* chitosan was 58.11% compared to that of fish waste chitosan, which ranged from 36 to 71% (Kumari et al., 2017). A chitin film developed from *B. giganteus* cockroach wing and the dorsal pronotum region limited biofilm formation by *A. baumannii* and *S. sonnei* bacteria. Furthermore, a 7-day incubation of the fungal strain *A. niger* on the surface of the chitin film demonstrated 7.6×10^{6} mL^{-1} spores, but the wing chitin film had 4.26×10^{6} mL^{-1} *A. niger* spores (Kaya et al., 2017). Nevertheless, ciprofloxacin loaded nanoparticles developed from chitosan derived from insects such as beetles (*Calosoma rugosa*) and honeybee (*Apis melifera*) exoskeletons displayed similar inhibition against Methicillin-resistant *Staphylococcus aureus* with an MIC of 0.14 μg/mL (N. Marei et al., 2019). This finding demonstrates that the antibacterial effects of insect chitosan can also be used as active edible packaging in food applications (Hamed et al., 2016; R.; Muzzarelli & Muzzarelli, 2005).

5.3. Rheological properties

Rheology is the study of flow and deformation of food materials and is a vital tool for characterizing the fundamental material properties, such as processing, handling, quality control, storage and sensory evaluation of food ingredients (Kutz, 2007). During food production and processing, several materials are often in liquid form. Polysaccharides are comprised of chain conformations and produce bio-macromolecular aggregates when scattering in the presence of water molecules, which could be due to the intermolecular hydrogen bonding. In most cases, biopolymers have pseudoplastic or non-Newtonian properties that aid in their applications in food production and pharmaceuticals. However, flow property is profoundly influenced by polysaccharide structural arrangements, the pH of the medium, the temperature applied to the system and the ionic concentrations of the external matter. Chitosan

derived from cicada slough, silkworm chrysalises, mealworms, and grasshoppers (prepared as a 2% solution with 1% aqueous acetic acid) exhibited a high shear rate and shear-thinning behaviour compared to shrimp shell chitosan with a sweeping decline in viscosity. Similarly, chitosan with a higher Mw possesses higher viscosity; for instance, shrimp shell chitosan, which has a Mw of 1.620×10^5 Da, showed high viscosity, and cicada slough, which possess a low Mw of 3.779×10^4 Da, had low viscosity (Luo et al., 2019). However, these two factors are highly influenced by the degree of acetylation (DD) and are decreased by the degree of deacetylation (DDA) (Liu et al., 2012). Alternately, biopolymers expressing shear-thinning behaviours demonstrate pseudoplastic fluid/non-Newtonian characteristic features in food applications. Decreasing the NaOH concentration to less than 50% in chitosan extraction reduces the DDA reaction and increasing the percent NaOH decreases viscosity. Similar results were obtained in the chitosan derived from housefly larvae extracted using 50% (w/v) at 125 °C for 4 h, which exhibited ~79% DDA with ~347 mPa.S viscosity and 60% NaOH in the extraction process had ~82% DDA with ~250 mPa.S viscosity (A.-J. Zhang et al., 2011). Additionally, 1 M NaOH at 80 °C with a varied time of 39, 44, 49, 54, 59, and 64 h showed a significant reduction in the intrinsic viscosity ranges from 30.6 to 18.9 ή from chitin obtained from honeybees (Draczynski, 2008). Furthermore, the quality of housefly larvae chitosan was equivalent to food-grade chitosan according to the Chinese Fishery Trade Standard SC/T3403-2004. Therefore, orthogonal experiments or optimization of multiple parameters in insect chitosan extraction could provide appropriate storage modulus (G') and loss modulus (G'') in food applications (Nishinari, 1997). Nevertheless, shrimp shell chitosan expressed more G'' with high viscous properties, and as a result of this characteristic, crustacean-derived chitosan is directly used in many food applications. In addition, insect chitosan solutions donate non-covalent cross-linking at a low level, which might be utilized as low viscosity chitosan (X. Zhang & Waymouth, 2017). In the future, the lower viscosity of insect chitosan could be used as a thickening and suspending material for the food industry.

5.4. Wound healing

Engineering skin substitutes provides a prospective source of advanced therapy to combat acute and chronic skin wounds. The wound healing process involves multiple consecutive reaction pathways, including haemostasis, aggregation, cell multiplication, and regeneration (Goldberg & Diegelmann, 2010). This process contains various cell types, including the extracellular matrix and cytokine mediators active in healing. The wound healing mechanisms of chitin and chitosan from insects are shown in Fig. 10. Recently, skin substitutes using biomaterials from natural materials have been used as wound dressings. For example, desert locust (*Schistocerca gregaria*) chitosan was tested for the wound remodelling process in a mouse model. A 9 mm wound created on the mouse's back displayed potential wound closure when treated with locust chitosan (N. H. Marei et al., 2016). This chitosan reduced the inflammatory necrosis on the skin cells after 5 days of treatment for up to 14 days. A similar healing process has been found with shrimp chitosan, but a higher count of dermis active angiogenesis was found using seeded locust chitosan. It was reported that 1–2% of chitosan from *P. niloticus* (freshwater crab) increased the thickness of the epidermis in wounded rats compared to a high concentration (3%) of chitosan applied to the wound (Amer & Attia, 2020). Furthermore, researchers stated that chitosan consists of glycan derivatives that might act as macrophage stimulating agents as well as initiating cytokine production from the macrophages. These two reactions amplify the wound healing process in the early phase (Ueno et al., 1999), and insect chitosan may therefore be a promising natural wound healing material.

5.5. Anti-tumour

Chitin and chitosan derived from insects have shown substantial anti-tumour activities. The *in vitro* inhibitory effect of chitosan from housefly *Musca domestica* larvae displayed 50.8% and 52.9% action against HeLa and S-180 tumour cells at 1 mg/mL in an MTT assay. Furthermore, this chitosan could chelate ferrous ions *in vitro*, which is considered an effective pro-oxidant found in the food system that induces cell proliferation. It was noted that native and inoculated larvae of *Musca domestica* extract demonstrated antitumour action against the human colon cancer cell line CT26 with an inhibition rate of 62–89% at 500 and 1000 μg/mL of extract. However, this wholesome extract also showed the presence of peptidoglycan as an active ingredient and exhibited antitumour action (Hou, Shi, Zhai, & Le, 2007). In contrast, lower concentrations (400 μg/mL and 200 μg/mL) of chitosan from *P. longirostris* (shrimp) displayed >50% cytotoxic activity against Human larynx carcinoma (Hep2) cells and Human embryo rhabdomyosarcoma (Rd) cells (Ganesan et al., 2020). Nevertheless, chitosan nanoparticles (CNPs) demonstrated competent action at low concentrations. For example, 80 and 100 μg/mL of CNP from *Musca domestica*, *Lucilia sericata*, and *Chrysomya albiceps* exhibited productive anticancer activity against human liver carcinoma (HepG-2) and human colon carcinoma (HCT-116) cell lines. These CNPs reported an IC_{50} value of 37.3–74.3 μg/mL, with the most potent inhibition recorded from *C. albiceps* CNP (Hasaballah, 2019). Hence, insect chitosan could serve as alternative therapeutic agents for the treatment of tumours.

5.6. Anti-ageing

Ageing is a natural process that affects most biological activities and seems to be a consequence of the cumulative action of various types of stressors. Evidence shows that oxidative stress from ROS, telomere attrition, a decline in DNA repair and protein turnover systems serve as significant causes of ageing (Kirkwood, 2005; Vijg & Campisi, 2008). Oxidative stress is caused by the disparity between ROS production and ROS removal in the biosystem, which leads to oxidative injury to cells and tissues and alterations in their morphology and function, resulting in ageing and age-related disorders, such as cognitive deficits and Parkinson's disease (Shan et al., 2009). The anti-ageing activities of chitin and chitosan from insects are rarely reported. Wu et al. (2016) reported that different concentrations of water-soluble chitosan of *Clanis bilineata* larva skin were intragastrically administered to D-gal-induced mice at 42 days. The results indicated that the administration of chitosan significantly increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) and decreased malondialdehyde (MDA) in the brains and sera of the mice. This finding suggests that *Clanis bilineata* chitosan could be used as an effective antioxidant an anti-ageing medicine. In comparison with insect chitin, crustacean chitin, chitin-nanofibrils and chitin-hyaluronan nanoparticles have been reported to increase the creation of fibroblasts, inhibit IL-8 and TNF-α release, and trigger antioxidant enzyme release from the skin layer in addition to their skin-hydrating properties (Morganti et al., 2013). However, further innovative mechanisms are required to explain the anti-ageing activity of insect chitin and chitosan.

5.7. Hypolipidaemic activity

Hyperlipidaemia, characterized by high levels of fats in the blood and the impairment of lipid metabolism, is a major cause of atherosclerosis and subsequent related cardiovascular diseases (Ahmad & Beg, 2013; Navar-Boggan et al., 2015; Prasad & Kalra, 1993). In recent years, many studies have focused on the reduction of serum lipid levels and the absorption of fat in the intestinal tract to reduce chronic diseases (A.-J. Zhang et al., 2011). Hence, the antihyperlipidaemic activity of many bioactive components from natural materials such as polysaccharides are novel possible hyperlipidaemic agents (Knopp, 1999). Insect chitosan and its derivatives have a lowering effect on plasma cholesterol, which plays a vital role in the prevention and treatment of cardiovascular disease, although minimal investigations have examined these

effects of insect chitin and chitosan (Anraku et al., 2010; Lamiaa & Barakat, 2011). Xia et al. (2013) stated that chitooligosaccharides (COS) from *Clanis bilineata* fed rats at 6 weeks had the ability to prevent increases in body weight and to lower plasma triacylglycerol (TC), total cholesterol (TG), and plasma low-density lipoprotein cholesterol (LDL-C) levels. These results showed that insect COS could be used as alternative hypolipidaemic drugs. Other chitin sources, such as fungal, crustaceans and sponges have also been reported to have hypolipidaemic actions. These chitins downregulated adipogenesis and adipocyte-specific gene promoters by modulating adenosine monophosphate-activated protein kinase (AMPK) and aquaporin-7 (Kong, Kim, Bak, Byun, & Kim, 2011). Further investigation is required to examine the AMPK signalling pathway to confirm the anti-hyperlipidaemic activity of insect chitin.

5.8. Industrial application

Chitosan is a biodegradable cationic biopolymer that could aid in the decrease of metal pollutants from industrial effluents through the adsorption and chelation of particles through productive electrostatic activity (Evans, Davids, MacRae, & Amirbahman, 2002). This action could act in the agglutination of colloidal particles. The use of chitin and chitosan from shrimp as an adsorbent agent has been widely investigated for the removal of azo dyes from the textile industry (Duarte, Ferreira, Marvao, & Rocha, 2002; Szyguła, Guibal, Ruiz, & Sastre, 2008). The chitin and chitosan from silkworm chrysalides at concentrations of 50 mg/L and 21.3 mg/L reduced the amount of the anthraquinone dye and residual aluminium (Al) in textile industry effluents by 6 and 70 h. The study indicated that adsorption quality is higher in insect chitosan than in insect chitin (Julliana I Simionato, Paulino, Garcia, & Nozaki, 2006; Julliana Isabelle Simionato et al., 2014).

6. Shortcomings and possible technical solutions

Extracting chitin from insect biomass is undoubtedly more challenging compared to marine sources. Even though green technologies or process optimization may lead to high quantity products, it is evident that this could only be accomplished through extensive research. Research related to understanding the feasibility of the techniques and variances in proximate composition and processing conditions should continue to be explored in this field. For example, untreated larvae, including blanched and dried larvae, did not exhibit chitin due to their high-fat content (3–20%) (Khayrova et al., 2019). However, at this stage, the use of phosphoric acid in chitin extraction might not be useful due to the hydrophobic repulsion that occurs on the cell wall of the insect (Mba, Kansci, Viau, Rougerie, & Genot, 2019). Similarly, the amount of pigment in the insect cell could influence chitin extraction. It was reported that melanin covalently binds to chitin at the pupae or late-stage of insects and blocks the extraction of chitin using organic acids (H_3PO_4). Therefore, these challenges should be rectified using depigmentation processes or by choosing non-pigmented insects for chitin extraction. These challenges again necessitate multiple-steps for chitin extraction, and in order to scale-up and lessen the extraction procedures, it is required to develop novel/innovative technologies. Recently, an electrochemical technique was identified to minimize the multiple-downstream methods used for the removal of lipids, proteins and pigments from marine organism-based chitin (Nowacki et al., 2020). Two primary steps involved in this method use catholyte and anolyte treatments in two chambers within the same system. It was engaged at a high pH (12.5) of the electro-alkali in the cathode chamber (at 70 °C for 16 V, 1.5 A), which lysed the cell walls and partially degraded the lipids, proteins and pigments. It was reported that the chitinous skeleton was removed from the interlayer spaces of *Cirrhipathes* sp (black coral) during this step. Moreover, deep eutectic solvents (DES), also known as novel ionic liquids that are comprised of hydrogen bond donors (HBDs) and acceptors (HBAs), could be suitable for insect chitin extraction. Some common HBAs are betaine, HCl, ChCl, etc., and HBDs such as urea, ethylene glycol and glycerol have been used at minimum temperatures of 50–90 °C. HBDs and HBAs have been applied to skimmed black soldier flies (*Hermetia illucens*) and showed efficient results (Zhou et al., 2019). Chitin extracted by DES was found to have a high purity (74–91.345) and yield (12.71–26%) compared to the conventional acid/alkali method (purity 91% and yield 6.5%). It was observed that the best efficiency of deproteinization was obtained by using highly acidic solvent in a HBD at a high temperature (80–90 °C) in the extraction system, which leads to increased protein removal of approximately 3%–10% (Zhou et al., 2019). However, DES-integrated with microwaves showed better deproteinization efficiency (88–93% rate of removal) in shrimp chitin (D. Zhao et al., 2019; Y. Zhao et al., 2010). Therefore, the integrated method using microwave, autoclaving, and enzymatic treatments would be appropriate to simplify the chitin extraction process from insects. In addition, designing a suitable electrochemical system with the involvement of electrolysis would be useful for scaling up the quantity of chitin obtainable from insects.

7. Challenges and opportunities

Globally, industrial chitin/chitosan producers rely upon marine-derived sources for its production. Major commercial plants for chitin/chitosan production are located in various countries, including Europe (https://mealfoodeurope.com), USA (https://tidalvisionusa.com), India (http://thahirachemicals.com/profile.html), and France (https://chitosanlab.com). Most of these industries use the exoskeletons of shrimp, crab, squid bone, or fungi, etc., for large scale chitin production. Therefore, various strategies are required to extend the commercialization of insect chitin/chitosan conversion at industrial levels. A few industries, such as Sfly®, utilize *Hermetia illucens* larvae for high-quality chitin/chitosan (http://sflyproteins.com/sfly-products/) production. This demonstrates the lack of technology transfer in the scaling up of insect chitin, which needs to be addressed. Some challenges involved in the extraction of chitin from insect sources are (1) Insect collection: The gathering of catastrophic species (locusts, crickets, termites, etc.) would require specific techniques, but they are not consistently available throughout the year. Similarly, a suitable processing method should be adopted to retain the chitin proportion until its extraction, which leads to additional requirements ideal for various species. Therefore, the cost of conversion of biomass into a useable form for extraction could exceed unit operational costs. (2) Extraction: Process optimization is crucial for insect chitin extraction. While increased alkaline (NaOH/KOH) concentrations could negatively affect the total quantity of chitin extracted, the same condition favours a deproteinization process. Similarly, few concentrated acids (H_3PO_4) showed hydrophobic repulsion on the insect exoskeleton, but some (HCl and H_2SO_4) are found to hydrolyse chitin. Therefore, identifying efficient solvent mixtures appropriate for insect species are required for mass production. Therefore, technological innovations are essential to deviate from the conventional downstream processes using single components. Positively: Insects have been used as a meal in Europe that has received significant attention due to its high protein content (https://mealfoodeurope.com/en/tecnologia/). Meanwhile, industries are breeding and insects for high quality and quantity. Cricket flies as baking ingredients (https://thecricketbakery.com/) and mealworms in snacks (https://www.diewurmfarm.at) are a few examples of cultured insects in food applications. Meanwhile, these insects are consumed as wholesome food/feed in various parts of the world and obtained approved by the European Commission as a novel food (EC, Regulation (EC) No 1069/2009), Regulation (EU) 2016/759) (EC,), demonstrating that insect chitin could have direct applications in the food system without any regulatory issues.

8. Future perspectives

Globally, the market for chitin and chitosan is growing steadily. Due

to the pandemic disease COVID-19, there is an increase in the demand for biopolymer materials for healthcare, personal care, packaging, and coating materials, and this emerging situation has increased the demand for biomaterials for biomedical, food, and pharmaceutical applications. The projected statistics show the market size for chitin/chitosan will grow up to 162.7 thousand MT, mainly derived from 15.6% of chitosan with a growth rate of 17.6% in the following years (Newswire, 2019). Furthermore, in-depth research has been conducted on chitin/chitosan applications, including scaffolds in tissue engineering (wound healing), drug release encapsulation, food packaging, coating, 3D scaffolds, and hydrogels from marine-invertebrate waste, with less focus on insect chitin. Therefore, studies of 3D chitin and chitosan from insect shells are needed for biomedical applications. Additionally, food security issues are another alarming problem due to the devastation of food-crops by locust (grasshopper) waves. Though agricultural scientists are working on measures for controlling these pests, converting waste into valorization would be a significant technique for its prevention.

Therefore, the future direction of research should focus on the destruction of catastrophic species into a value-added product that could replace the existing biopolymers and increase the opportunities in this field. Further studies are required to optimize the production process for higher yield using electrochemical methods or integrated approaches such as ultrasonication and microwave. Innovative insect rearing methods would also produce a constant supply of specific species/stages of insects for industrial needs. Methods with cost-effective and straightforward synthesis approaches could be required for large-scale production of insect chitin. Therefore, up-scaling efficiency, insect species selectivity, and stability in real-time applications need to be explored.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgement

This research did not receive any specific grants from funding agencies in the public, commercial, or non-profit sectors. Authors sincerely thank Department of Zoology, Sri Vasavi College, Erode-TN, India and Fiji National University for supporting and providing essential facilities needed for this study.

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