T.R.

AKSARAY UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

DEPARTMENT OF BIOTECHNOLOGY AND MOLECULAR BIOLOGY

CHITIN ISOLATION AND PHYSIOCHIMICAL CHARACTERIZATION FROM CORNEAL LENSE OF *SYMPETRUM FONSCOLOMBII* (DRAGONFLY)

MASTER OF SCIENCE THIESES

Ivan AL-JAF

SUPERVISER

Assoc. Prof. Dr. MURAT KAYA

AKSARAY, 2016

T.R.

AKSARAY UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

DEPARTMENT OF BIOTECHNOLOGY AND MOLECULAR BIOLOGY

CHITIN ISOLATION AND PHYSIOCHIMICAL CHARACTERIZATION FROM CORNEAL LENSE OF *SYMPETRUM FONSCOLOMBII* (DRAGONFLY)

MASTER OF SCIENCE THIESES

Ivan AL-JAF

SUPERVISER

Assoc. Prof. Dr. MURAT KAYA

AKSARAY, 2016

AKSARAY UNIVERSITY, INSTITUTE OF SCIENCE AND TECHNOLOGY APPROVAL

Aksaray Universty, institute of Technology, M.Sc. The entitled "CHITIN ISOLATION AND PHYSIOCHIMICAL CHARACTERIZATION FROM CORNEAL LENSE OF *SYMPETRUM FONSCOLOMBII* (DRAGONFLY)" prepared by (Ivan AL-JAF), student no: 142330801, student at Biotechnology and Molecular Biology Faculty of Science, was certified by all the majority jury members, whose signatures are given below, with success.

Thesis advisor: **Assoc. Prof. Dr. Murat KAYA**
Aksaray University

Jury members: **Assoc. Prof. Dr. Yavuz Selim ÇAKMAK**
Aksaray University

Jury members: **Asst. Prof. Dr. Cemil İŞLEK**
Nigde University

Delivery Data: 03 May 2016
Defense Data: 01 June 2016

DECLARATION

The inspiration for this present study was taken from the gap in the literature regarding the said topic. Throughout this research the issue has been tackled through certain analytical techniques. The whole study was presented in pattern including introduction, material and methods and the results and discussion. Previous literature was used as supporting tool in present study. Whole the study was conducted under the supervision of Assoc. Prof. Dr. Murat KAYA, at the Faculty of Science and Letters.

Ivan AL-JAF

PREFACE

This thesis was made as a completion of master degree of Biotechnology and Molecular Biology. This thesis focused on chitin extraction from the compound eye lenses of Dragonfly for the first time and characterize the physiochemical properties of this chitin. All the Dragonflies were collected on the campus of Aksaray University. The results of the analyses proved that this chitin is totally pure and has a new type of surface morphology i.e., the outer surface has long fibers and the inner surface has short fibers. Dragonfly is an insect belonging to the order Odonata, characterized by large multifaceted eyes which have nearly 30.000 ommatidia, two pairs of strong transparent wings, long abdomen and three pairs of legs. Our study focused on dragonfly eye lens because it is easy to collect from wetland environments and the insect has larger compound eyes in relation to its head. Also the possible role of chitin polymer in the function of such a specialized body part of an insect was investigated.

This thesis study was done in General Biology Laboratory situated at Science and Technology Research and Application Center, Aksaray University, Turkey.

ACKNOWLEDGMENTS

I am very thankful to Assoc. Prof. Dr. Murat KAYA for being my supervisor and supporting me step by step. He give me all the essential information that can make my thesis strong and useful.

Also i am very thankful to Dr. Idris for his help all the time during my research.

After that i would like to thanks all my friends especially Bahar Akyuz, Esra Bulut, Karwan Sofi, and Muhammad Mujtaba who helped me a lot during my whole thesis.

The authors thanks to Assoc. Prof. Dr. Nurten HACET for species identification of the dragonfly and Asst. Prof. Dr. Sevil ERDOGAN for helping us during sample collection.

I am cardinally thankful to my father (Mohammad Mahmood) for his support in every manner which is the only reason for finishing my master studies.

A big role has been played by my daughter (Sevan) throughout my study that she lived far away from me for long time. Now as her mother I am proud of my daughter and appreciate her tolerance. Love you my dear Sevan.

Last but not the least i would like to thanks all Turkish people who helped me a lote time by time. I really found Turkey as my second home country.

Especially I did not have a chance to meet my mother because she died when I was two year old. I am dedicating this thesis to my late mother (Thuraya Najm Aldeen).

IVAN AL-JAF

LIST OF CONTENTS

Page

ÖZET

SYMPETRUM FONSCOLOMBII (DRAGONFLY)' NİN KORNEAL LENS YAPISINDAN KİTİN İZOLASYONU VE FİZİKOKİMYASAL KARAKTERİZASYONU

Kitin, eklembacaklıların göz bileşenlerinde görme sisteminin bir parçası olarak hizmet vermektedir. Böyle son derece özelleşmiş vücut bölgelerindeki kitinin kalitesi daha detaylı inceleme gerektirmektedir. Yusufçuk böceğinin (*Sympetrum fonscolombii*) bileşik gözlerinin korneal (ommatidial) lensinde bulunan kitin, klasik metod kullanılarak izole edilmiştir. Korneal lenslerinin kitin içeriği oldukça yüksek olduğu belirlenmiştir (% 20.3±0.85). Korneal lens kitinin FT-IR analizi sonuçları, tüm eklembacaklı türlerinde bulunan α-formda olduğunu göstermiştir. Taramalı elektron mikroskobu ile elde edilen yüzey morfolojisi analizi sonuçları, korneal lens kitininin pürüzsüz iç kısmı kısa kitin nanofibriller ile eş merkezli ince tabakalardan yapılmış olan kitin oluşumuna sahipken korneal lens kitininin dış kısmı, papiller yapıların düzenli diziler ile uzun kitin fibrillerin oluştuğunu göstermiştir. Kitinaz ile enzimatik sindirim çalışmaları, elementel analiz sonuçları ve asetilasyon derecesi değeri korneal lenslerden elde edilen kitin örneklerinin saflığını göstermiştir. Korneal lens kitinin maksimum bozunma sıcaklık değeri 369.2°C' de gözlenmiştir. X-ışını analizi, korneal lens kitininin yüksek kristallik indeksine (% 96.4) sahip olduğunu göstermiştir. Böceğin bileşik gözlerinin ommatidial' de bulunan kitinin belirlenmesi böceğin görüşünü anlama ve kitin tabanlı optik malzeme tasarım çalışmalarına yarar sağlayabilir.

Anahtar Kelimeler: Böcek görüşü, Bileşik gözler, Ommatidia.

ABSTRACT

CHITIN ISOLATION AND PHYSIOCHIMICAL CHARACTERIZATION FROM CORNEAL LENSE OF *SYMPETRUM FONSCOLOMBII* (DRAGONFLY)

Chitin in the compound eyes of arthropods serves as a part of the visual system. The quality of chitin in such highly specialised body parts deserves more detailed examination. Chitin in the corneal (ommatidial) lenses of dragonfly (*Sympetrum fonscolombii*) compound eyes was isolated by using the classical chemical method. The chitin content of the corneal lenses was determined to be quite high (20.3±0.85%). The FT-IR analysis showed that corneal lens chitin was in the α-form as found in all arthropod species where mechanical strength is required. The surface morphology analysis by scanning electron microscopy revealed that the outer part of corneal lenses consisted of long chitin fibrils with regular arrays of papillary structures while the smoother inner part had concentric lamellated chitin formation with shorter chitin nanofibrils. Chitinase enzymatic digestion studies, elemental analysis results and the degree of acetylation value showed the purity of chitin samples from corneal lens. The maximum degradation temperature value of the corneal lens chitin was observed at 369.2 ºC. X-ray analysis revealed that corneal lens chitin has high crystallinity index (CrI); 96.4%. Identification of chitin found in ommaditia of insect compound eyes can provide insights into insect vision and chitin-based optical material design studies.

Keywords: Insect vision, Compound eyes, Ommatidium**.**

LIST OF FIGURES

INDEX OF ICONS

°C	Degrees Celsius
g	Gram
I_{am}	Amorphous peak
I_{110}	Maximum intensity
M	Molar
mg	Milligram
mM	Millimolar
mL	Milliliter
µg	Microgram
nm	Nanometer
rpm	Revolution Per Minute
α	Alfa
β	Beta
ɣ	Gama
%	Percentage

ABBREVIATIONS INDEX

C	Carbon
^{13}CNMR	Carbon-13 Nuclear Magnetic Resonance
CrI	Crystallinity Index
DA	Degree of Acetylation
DSC	Diferansiyel Scanning Calorimetry
DTG_{max}	Temperature of Maximum Degredation
E.C.	Enzyme Number
ESI-MS	Electrospray Ionization Mass Spectrometry
FESEM	Field Emission Scanning Electron Microscopy
FT-IR	Fourier Transform Infrared Spectroscopy
HCl	Hydrochloric acid
N	Nitrogen
NaOH	Sodium hydroxide
SEM	Scanning Electron Microscopy
TGA	Thermogravimitric Analysis
UV	Ultraviyolet
UV-Vis	Ultraviolet–visible Spectroscopy
XRD	X-Ray Diffraction

1. INTRODUCTION

The phylum Arthropoda possesses remarkably sophisticated visual systems consisting of compound eyes and/or ocelli (Friedrich, 2006; Harzsch and Hafner, 2006). The architecture of arthropod eyes has captivated much attention and consequently has inspired many morphological (Meyer and Labhart, 1993), optical (Toh and Okamura, 2007) and artificial compound eye design studies (Duparré and Wippermann, 2006; Jeong et al., 2006; Song et al., 2013). The histological studies on the arthropod compound eyes (Alagboso et al., 2014) revealed that they have organic and inorganic contents such as protein and chitin fibrils, pigments and calcium carbonate or phosphate. It is already known that chitin in the cornea functions as a part of the visual system and mechanical support Alagboso et al., (2014). But there is no focus on corneal chitin itself. Especially, it is not known what the percentage of chitin in the cornea is, and also what distinctive physicochemical properties (surface morphology, degree of acetylation (DA), thermal stability, crystallinity etc.) chitin of corneal lens exhibits.

Chitin is an optically transparent natural biopolymer. It exhibits unique characteristics such as biocompatibility, biodegradability, antitumor, antioxidant and antimicrobial properties. Thanks to these properties, it has wide applications in optics, medicine, pharmacy, food processing industry, textile, agriculture, wastewater treatment (Jayakumar et al., 2010). Physicochemical properties of chitin extracted from the shells of shrimp, crayfish, crab and krill are well documented in the literature (Salaberria et al., 2015). In recent years, properties of chitin from the exoskeleton of different insect species and the cell wall of mushrooms have been reported (Rinaudo, 2006). Still some other recent studies characterized chitin from sponges (Ehrlich et al., 2013; Wysokowski et al., 2013) and anthozoan species (Bo et al., 2012). All these studies showed that chitin differs in its physicochemical properties depending on the organism.

Composition of chitinous matrix in the exoskeleton of an individual organism can exhibit regional differences depending on the function of the body part. Here in the present study, we wondered if there are any differences between the corneal chitin and the earlier defined chitin samples from various sources. If so, since translucent chitin in the cornea of compound eyes serves as a part of the visual system, the identification the properties of this chitin can benefit the studies on development of chitin-based optical materials.

Dragonfly is a common insect in the order Odonata inhabiting wetlands (Johansson and Suhling, 2004). Being a predator preying on the wing, the dragonfly is equipped with remarkably large pair of compound eyes to have better visual resolution. In each compound eye of an adult dragonfly there are nearly 30.000 repeating visual hexagonal units called ommatidia (Sherk, 1978). The outer part of the each unit consists of a transparent cornea. The lenses in the cornea are considered to be continuous with the chitinous envelope of the head of the insect (Toh and Okamura, 2007). This present study focuses on the characteristics of chitin isolated from the corneal lenses of a dragonfly (*Sympetrum fonscolombii*) compound eye. We preferred studying on compound eyes of a dragonfly on the basis of (1) the ease of sample collection from wetland environments; (2) the larger compound eyes of the insect in relation to its head and (3) the possible role of chitin polymer in the function of a much specialized body part of an insect with interesting optical properties i.e., the compound eyes of a predatory insect.

This study was aimed to identify the characteristics of the chitin found in ommaditia (corneal lens) which is highly related to the vision and to present evidence for possible differences between the corneal lens chitin and the chitin samples reported in previous studies. It was also wondered what the chitin content of corneal lenses is, which play a vital role in the vision of an insect.

2. LITERATURE REVIEW

Several studies have been reported about chitin extraction from different sources such as the exoskeletons of insects, crustacean, some type of bacteria, green algae, cell wall of fungi, and in fresh water sponge (Ehrlich et al., 2013). As it is well known the chitin content different from each source depend on physiochemical properties for isolation of chitin. In each study a huge variation was determined in terms of chitin content and other analytical characterizations such as Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Thermogravimitric Analysis (TGA) etc.

Chitin was extracted beetle (larva cuticle) using the classical chemical techniques. Isolated chitin was analyzed by X-ray and SEM. The result showed that this chitin was in α-form and exhibited a higher affinity for chitinase compared to shrimp chitin (Zhang et al., 2000).

Silkworm chrysalides chitin employed for the production pure chitin and chitosan. In this study chitin was extracted using classic techniques (demineralization, deproteinisation and decalorization). Produced chitin was characterized by SEM, FT-IR, TGA and differential scanning calorimetry (DSC) (Paulino et al., 2006).

The shell-like chitin structure was prepared from the bumblebee corpses using different chemical treatments. The bumblebee chitin was compared with crustacean (shrimp) chitin by using normal analytical procedures (FT-IR, Confocal microscopy and others). Additionally it was revealed that bumblebee chitin had a 5% lower DA then that of shrimp chitin (Majtan et al., 2007).

Hossein Tajik et al studies *Artemia urmiana* cyst shell for its chitin content using normal chemical process which used for other crustacean species. The result show that the *A. urmiana* cyst shell is rich source of chitin (29.3-34.5%) when compared with commercial chitin. Analytical results showed the purity of chitin (Tajik et al., 2008) Schiffman and Schauer (2009) used normal techniques to get the chitin from lady butterfly in larvae stage and the results evaluated and compared to that of crab Shell α-chitin. The FT-IR, XRD and Field Emission Scanning Electron Microscopy (FESEM) showed that the obtained chitin was in α-form.

Sajomsang and Gonil (2010) used different chemical treatments to extract chitin from *Cicada sloughs*. The *C. sloughs* chitin content was recorded higher than that of the chitin content of rice-field crap shell. Isolated chitin was characterized using elemental analysis, FT-IR, proton nuclear magnetic resonance spectroscopy, XRD and TGA. Analytical results showed that *C. sloughs* chitin is in α-form. DA was recorded in the range of 97% to 102%.

In this study chitin nanofibers of mushroom were isolated from the cell walls of five different type of mushroom. After the chemical treatments the obtained chitin exhibited uniform structure with long fiber. The different analytical techniques such as FT-IR, TGA, XRD and elemental analyses show that α-chitin crystal structure was maintained and glucans remained on the nanofiber surface (Ifuku et al., 2011).

Liu et. al. (2012) extracted chitin from adult *Holotrichia parallela*. Extracted chitin was in α-form. Also this chitin compared with commercial α-chitin from shrimp, by using FT-IR, SEM, XRD, and elemental analysis.

Antarctic krill recognized as a target for commercial fishing. In this study the crystalline structure and thermal properties of Antarctic krill chitin characterized by FT-IR, SEM, XRD and TGA. Result show that Antarctic chitin was in α-chitin, and was composed of small, stable and uniform microcrystals. The DA was 86%. There was no result about chitin content (Wang et al., 2013).

Ehrlich et. al. (2013) extracted chitin is from the skeleton fiber of the freshwater sponge (*Spongilla lacustris*). Results of analytical techniques (^{13}C solid state NMR, FT-IR, Raman, NEXAFS, Electrospray Ionization Mass Spectrometry (ESI-MS), Morgan-Elson assay and Calcoflour White Staining) showed that this sponge chitin was similar to α-chitin.

Kaya et. al. (2013) studied *Daphnia magna* resting egg for its chitin content. The chitin contents was determined as (18-21%), and the FT-IR, TGA, XRD, elemental analysis and SEM, showed that this chitin in α-form.

In this study chitin was extracted from crustacean. Atomic force microscopy and Scanning transmission electron microscopy, show chitin nanofibers with width between 3 and 4 nm. The DA, crystal structure as well as length and width of the native chitin micro fibrils in the crustacean was successfully preserved (Mushi et al., 2014).

In this study results revealed difference between α-chitin from shrimp wastes and crab shells, and β-chitin in cuttlefish bone. FT-IR, XRD analysis indicated that α-

chitins are more crystalline polymorph than that of β-chitins. Also CNMR, IR determined the DA (Hajji et al., 2014).

Ospina Alvarez et. al. (2014) extracted chitin from mushrooms (*Ganoder malucidum)*. Obtained chitin was characterized by XRD, FT-IR and TGA. Similarities were found in the characteristic result of *G. malucidum* chitin.

In the same regard in eggs of *Ceriodaphnia quadrangula* the chitin content was recorded in the range of 16-17%. The obtained chitin was characterized by using FT-IR, TGA and SEM. The crystalline index (CrI) was recorded as 74%. DTG max value of chitin was determined as 381°C. The surface morphology revealed that the chitin consist of nanofibre structure (Kaya et al., 2014a).

Spiders are widely spread including more than 44,000 species. In present study two species of spiders were selected as *Geolycosa vultuosa* and *Hogna radiate*. The chitin content was determined as 8-8.5%, for *G. vultuosa* and 6.5-7% for *H. radiate*. Physicochemical properties of chitin isolated from both the spiders were determined by using different analytical techniques such as FT-IR, TGA and SEM. Chitin surface morphology of *G. vultuosa* showed two different pore structures, the size of first one ranged between 190-240 nm and it was rarely sequenced, while the second one it size ranged between 11-32 nm and it was tightly sequenced. But *H. radiate* has normal (classical) surface morphology. Acetylation degree of both chitin samples recorded as 97% for *G. voltuosa* and 99% for *H. radiate* (Kaya et al., 2014b).

In the present study the chitin extracted from fish scales, after subjecting to the different chemical materials (base and acid). The obtain chitin was characterized by using different technique such as XRD, Spectral analysis, elemental analysis, FT-IR, SEM and DSC. The fish scales chitin founded in α-form (Kumari et al., 2015).

Wysokowski et. al. (2015) observed web like chitin structure in marine sponge. It is known as is the basic element of the microtubular scaffold-like skeleton of these organisms. Analytical tools such as SEM, TGA, and FT-IR and electron diffraction was used to analyze the isolated chitin.

In this study chitin was isolated from different body part of honey bee (head, thorax, abdomen, legs and wings). A big difference was recorded in the chitin content of each parts. Highest chitin content was observed for legs (13,25%) while the lowest chitin content was recorded for thorax (6,79%). The surface morphology for each part was analyzed through SEM. SEM results revealed five different surface

morphologies including three different surface morphologies only in abdomen (Kaya et al., 2015c).

There are several studies regarding the extraction of chitin from numerous sources in previous studies, but still there is no specific and accurate study on the chitin extraction from eye lens of insects. But in current study for first time chitin was extracted from the compound corneal lens of dragonfly. All analytical result showed that this chitin was pure and in α-form.

3. MATERIAL AND METHODS

3.1 Materials

Chitinase (from *Streptomyces griseus*; E.C. 3.2.1.14), shrimp shell chitin (pcode: 1001416772) sodium hydrogenphosphate, sodium dihydrogenphosphate, sodium carbonate, sodium hydroxide (NaOH), and ethanol was obtained from Sigma-Aldrich. Potassium hexacyanoferrate(III), methanol, and chloroform and HCl (Hydrochloric acid) were purchased from Merck.

3.2 Sample Collection and Dissection of Corneal Lens

One-hundred individuals of adult *Sympetrum fonscolombii* (Selys, 1840) were collected on the campus of Aksaray University in July 2015. The picture of the insect is given in Figure 3.1. The dragonflies were kept at −40 ºC for 24 h. The corneal lenses of the dragon flies were dissected out under a stereo microscope by using a simple needle. The surrounding tissues were removed carefully and separated corneal lenses were subject to chitin extraction procedure.

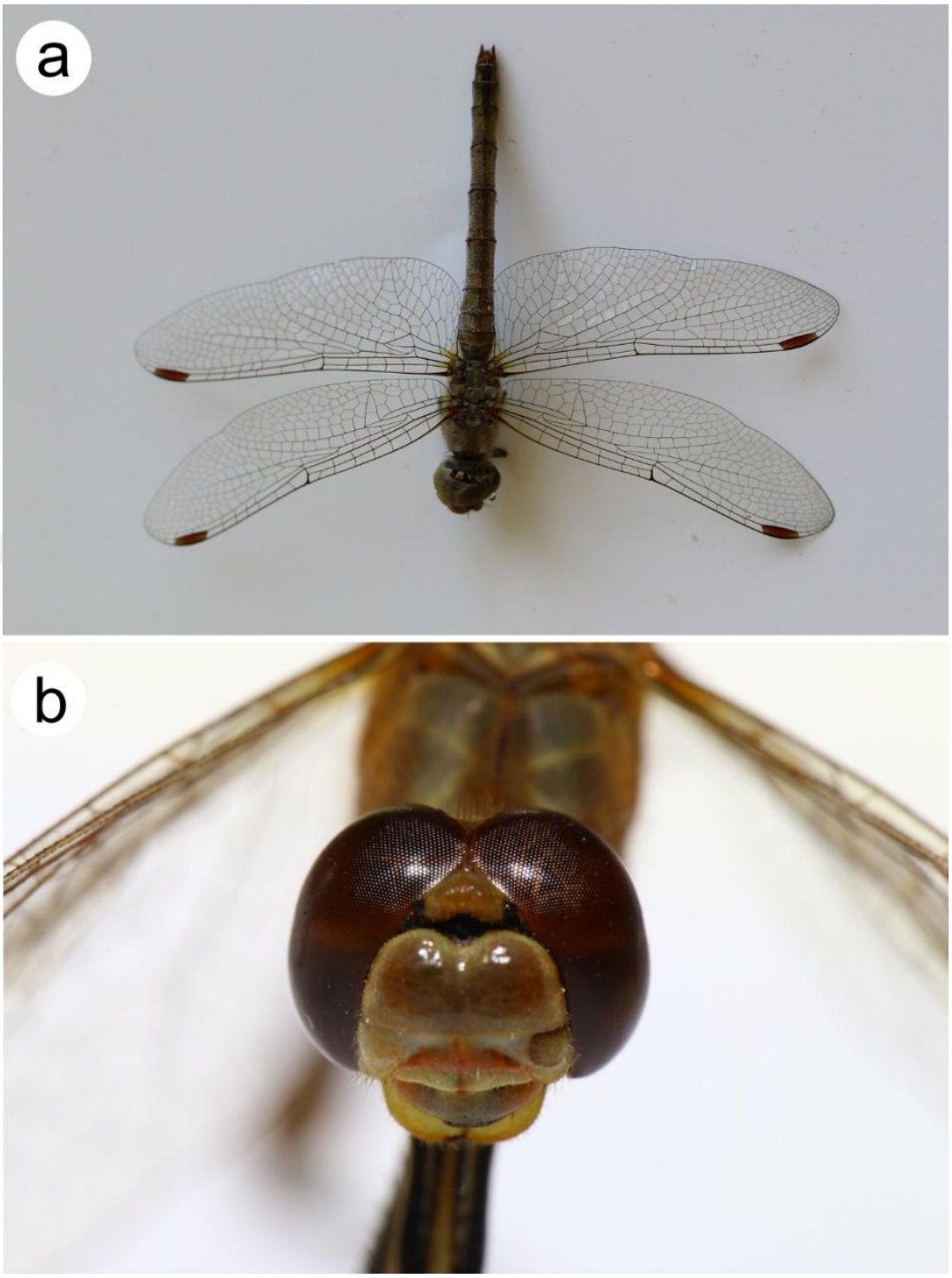

Figure 3. 1: (a) Whole body of dragonfly, (b) Head of dragonfly with a compound eye lens.

3.3 Chitin Isolation

The corneal lenses of dragonfly specimens (*Sympetrum fonscolombii*) were treated with 1M HCl solution at room temperature for 1 h for removal of minerals. Then the samples were filtered, washed and rinsed with distilled water. This was followed by the deproteinization treatment in 1M NaOH solution at 50 ºC for 15 h. Subsequently, the samples were thoroughly rinsed in distilled water until neutrality. Finally, the samples were treated with a mix solution of chloroform, methanol and distilled water (1: 2: 4, v/v) for decolourisation. After filtering and rinsing with distilled water, the extracted chitin samples were dried at room temperature.

3.4 Instrumentation

FT-IR spectra of corneal lens chitin were recorded with a Perkin Elmer 100 FT-IR Spectrometer 2.5. SEM images of corneal chitin samples were recorded with a Scanning Electron Microscope EVO LS 10 ZEISS. Thermogravimetric analysis of the samples were conducted in a TGA system (EXSTAR S11 7300) in an inert nitrogen atmosphere. Elemental analysis of the chitin samples was performed in an Elemental analyzer Flash 2000. From the results of elemental analysis the DA of the chitin samples were calculated using the equation given (Xu et al., 1996).

$$DA = [(C/N - 5.14) / 1.72] x 100\% \quad (1)$$

A UV-vis spectrophotometer (Shimadzu Model 1601, Tokyo, Japan) was used in absorbance measurements. Three types of microscopes (scanning electron, binocular and stereo light microscopes) were used for imaging. X-Ray diffractogram of corneal lens chitin was recorded on a Bruker AXS D8 XRD diffractometer (Bruker; Germany) at 2θ angle from 3° to 90°. The crystalline index (Siracusa, #2366) of corneal lens chitin was calculated using the formula given below (Focher et al., 1990);

$$CrI_{110}\ (\%) = [(I_{110} - I_{am}) / I110] \times 100 \quad (2)$$

Where I_{am} is the amorphous peak and I_{110} is the maximum intensity at $2\theta \cong 19°$.

3.5 Chitinolytic Activity on Corneal Lens Chitin

Chitinolytic activity analysis was conducted to show the purity of chitin sample from corneal lens. Enzymatic hydrolysis of corneal lens chitin isolate and commercial

shrimp shell chitin was conducted by following the similar method reported previously by Kaya et al. (2015b). The amount of enzymatic digestion products, i.e., reducing sugars, was assayed by using potassiumferricyanide assay. The reagent to be used in potassium ferricyanide assay was prepared by dissolving potassium hexacyanoferrate(III) (0.025 g) in 500 mL of 0.5 M sodium carbonate solution. The substrate of chitinase; i.e., colloidal chitin, was prepared by dissolving the chitin isolate from corneal lens or shrimp shell chitin (50 mg) in cold concentrated HCl (1 mL; 37%) with vigorous stirring in an ice-water bath. Once reaching homogeneous dispersion, the suspension was kept at 4°C for 24 h. Upon addition of 4 mL of ethanol water mixture (1:1, v:v), the mixture was stirred vigorously for 5 min, dialysed against first distilled water then 0.1 M phosphate buffer at pH 7.0. Then, colloidal chitin solutions were stored at 4°C in a fridge. After being resuspended with vigorous stirring, 1 mL of the colloidal chitin solution was incubated with 0.5 mL of 100 mM chitinase solution buffered at pH 7.0 at room temperature for 72h. After the incubation the solution was heated in hot water bath for 5 min to terminate the enzymatic reaction, then it was centrifuged at 10.000 rpm for 5 min to separate colloidal particles suspended in the mixture. For determinations of reducing sugar concentration liberated in the enzymatic digestion by ferricyanide assay, equal volumes of the supernatant and the reagent solution were mixed, heated in boiling water for 20 min, and finally centrifuged at 10.000 rpm for 5 min. UV-vis spectrophotometer absorbance measurements for the supernatant solution were performed at 420 nm and the amounts of the hydrolysis products were determined UV-vis spectrophotometeric method based on the absorbance of ferricyanide ions (Imoto and Yagishita, 1971).

4. RESULTS AND DISCUSSION

4.1 Chitin Content of Dragonfly Corneal Lenses

The chitin content of dragonfly corneal lenses was determined as 0.85±20.3% on dry weight basis. The mass losses after demineralization, deproteinization and decolourisation treatments were calculated as 11.6%, 64.8% and 3.3%. In a previous study it was revealed that *Cicada sloughs* contained 36.6% chitin and 39.8% protein besides moisture, ash lipid and lipoprotein (Gonil and Sajomsang, 2012). It appears that the corneal lenses of dragonfly compound eyes contained more protein fibrils and therefore less amount of chitin compared to the whole protective cuticle of *C. sloughs*. Earlier studies also revealed that the chitin composition of an organism can vary throughout the whole body relative to the function of the chitinous matrix in the body (Kaya et al., 2015a; Kaya et al., 2015b). Particularly, a previous study on corneal lens secretion in *Drosophila melanogaster* revealed that in the fully developed lens, lens materials, which contain chitin fibrils and protein, turn over continuously without disturbing the normal optic path (Yoon et al., 1997). In corneal lenses chitin fibrils combined with proteinaceous material to form an optical structure with mechanical strength and optical properties.

4.2 FT-IR Spectra Analysis

The infra-red absorption spectra of natural corneal lenses and the chitin material isolated from the lenses were recorded in the region 4000–625 cm^{-1} (Fig. 4.1). The interpretation of the spectrumof the isolated chitin revealed that the corneal lensesconsisted of chitin and this chitin had typical crystalline α-form as found in the cuticle of the insects. The bands appeared at 3433 (ν O-H), 3266 (ν N-H), 2919 (ν COCH3), 2853 (ν C-H), 1654 (carbonyl stretching of secondary amide; amide I band; ν C-O), 1622 (the amide II stretching of N-H ν C=O of N-acetyl group), 1550 (N-H bending vibrations of secondary amides; amide II band; δ N-H of N-acetyl

group) and 1306 (amide III stretching of C-N) cm^{-1} can be assigned to thebandscorresponding tothe spectrum of α chitin (Liu et al., 2012). Considering the peak assignments reported in previously published works, it is clear that corneal lens chitin had crystalline α form. Particularly, the amide I band of α chitin splits into sub-bands; and the most prominent of these are the amide I bands at 1654 and 1622 cm^{-1} which are assigned to amide I stretching of C=O. Previous chitin isolation studies from insect cuticles demonstrated that cuticular integument of insects consists of α chitin (Liu et al., 2012; Majtán et al., 2007). This observation is in agreement with the literature reports suggesting that corneal lens in the compound eyes of the insects was continuous with the surrounding the head cuticle chitin (Alagboso et al., 2014; Toh and Okamura, 2007). Corneal lens chitin of dragonfly occurred as α allomorph as found in other body parts where mechanical strength is required. In contrast to α polymorph, β chitin offers roughness together with flexibility to the organisms such as squid pens (Ianiro et al., 2014).

As seen in Fig. 4.1, a completely different the spectrum was recorded for pristine corneal lens, demonstrating the chemically complex nature of the lens matrix. Still, some bands with high intensity and strength were observed. Chitin fibrils in many organisms are found together with protein fibrils and inorganic material. To remove organic content and inorganic salts from the chitinous matrix in purification process, the sample is usually subjected to chemical or biological treatments. Compared to relatively milder conditions in biological method, chemical method requires harsh conditions such as high acidic or alkaline media and high temperatures depending on the nature of the raw material. Under harsh conditions chitin sample may undergo partial deacetylation and hydrolysis causing changes in physicochemical nature of the final product (Arbia et al., 2013a; Arbia et al., 2013b). Hence, it is possible that corneal lens chitin isolates produced by biological method based on enzymatic hydrolysis can exhibit different physicochemical characteristics.

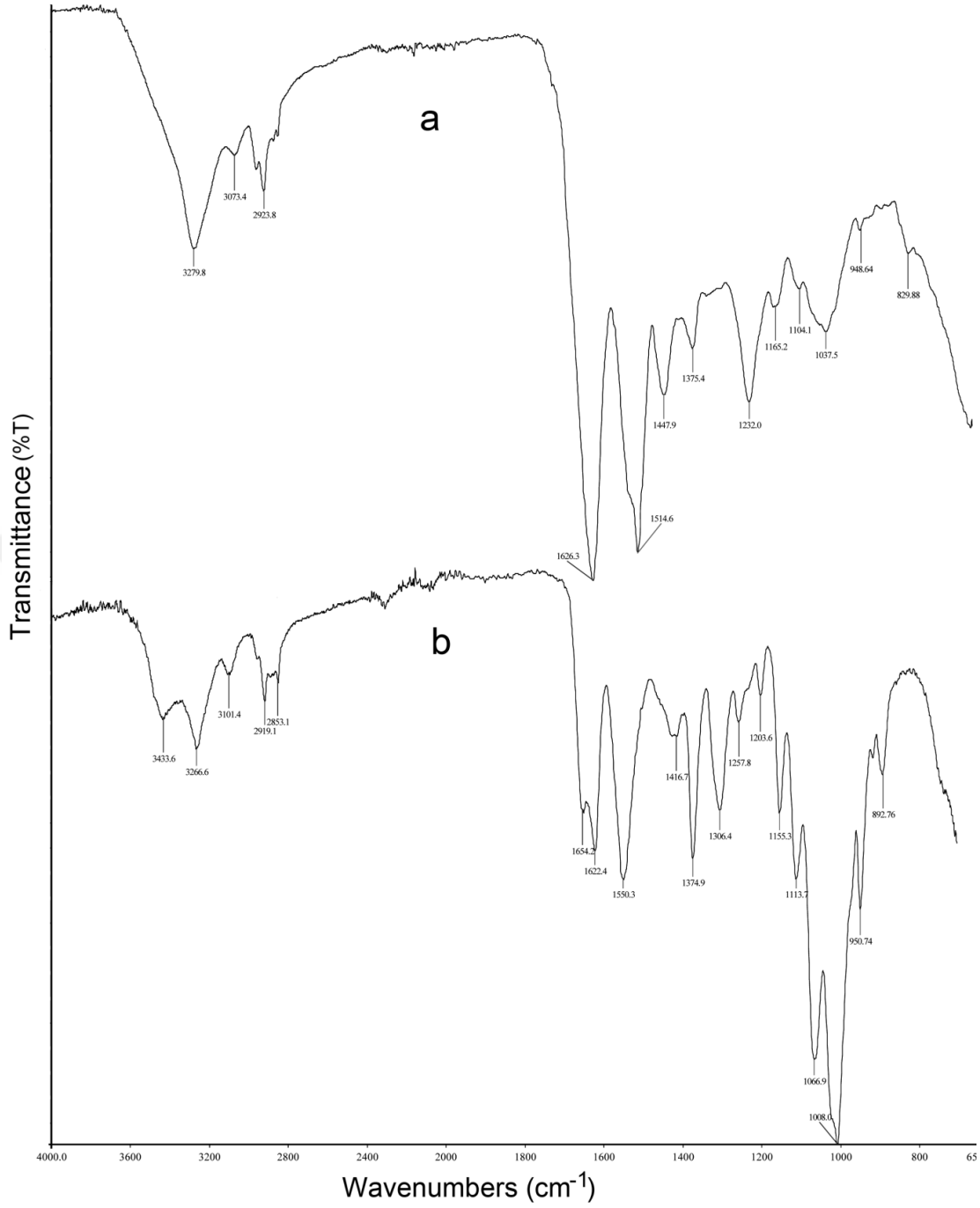

Figure 4. 1: FT-IR spectra of (a) corneal lens from dragonfly compound eyes, (b) corneal lens chitin obtained from dragonfly compound eyes.

4.3 Light and SEM Microscopy Analysis

The light and SEM microscopy images of dragonfly pristine corneal lenses are presented in Fig. 4.2. The compound eyes have a diameter of 1.1-1.5 mm and a height of 0.7-1 mm and are composed of hexagonal facets. The measure varied depending on the samples from different individuals. At the microscale, hexagonal

protuberances with sizes of 23-29 μm can be seen clearly on the surface of each ommatidia (Fig. 4.3b, d).

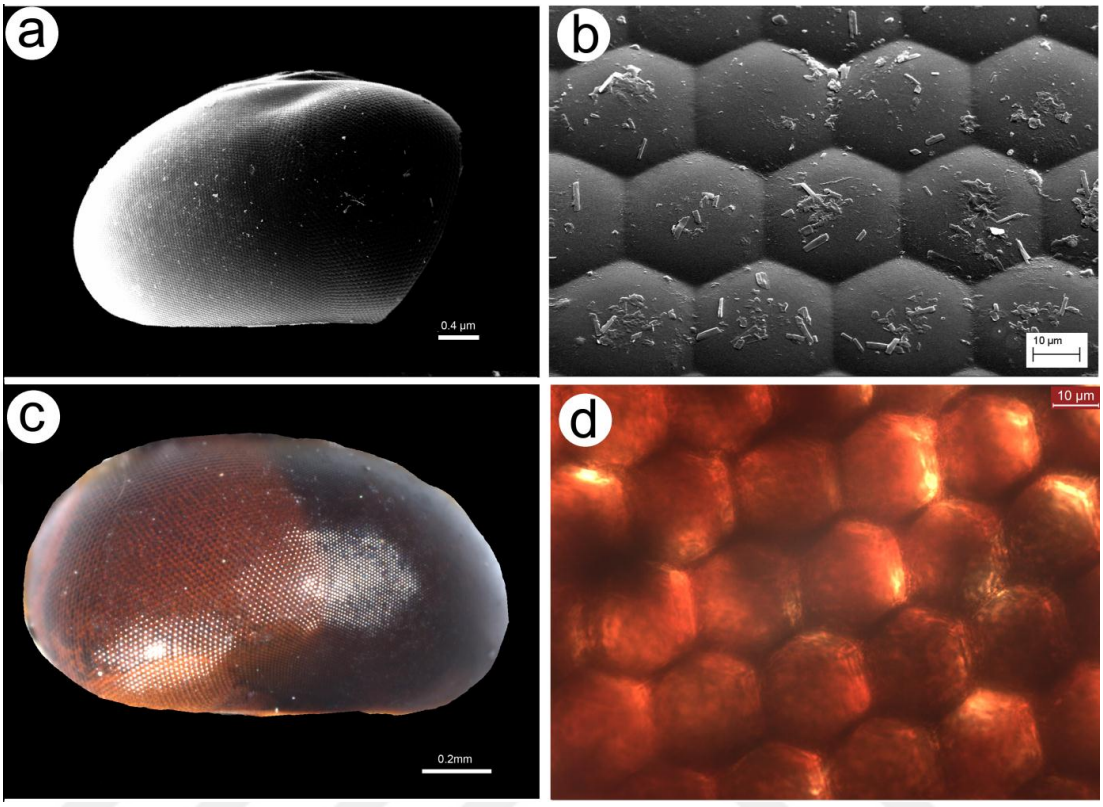

Figure 4. 2: Dragonfly corneal lens (a,b. SEM images and c,d. binocular light microscopy images).

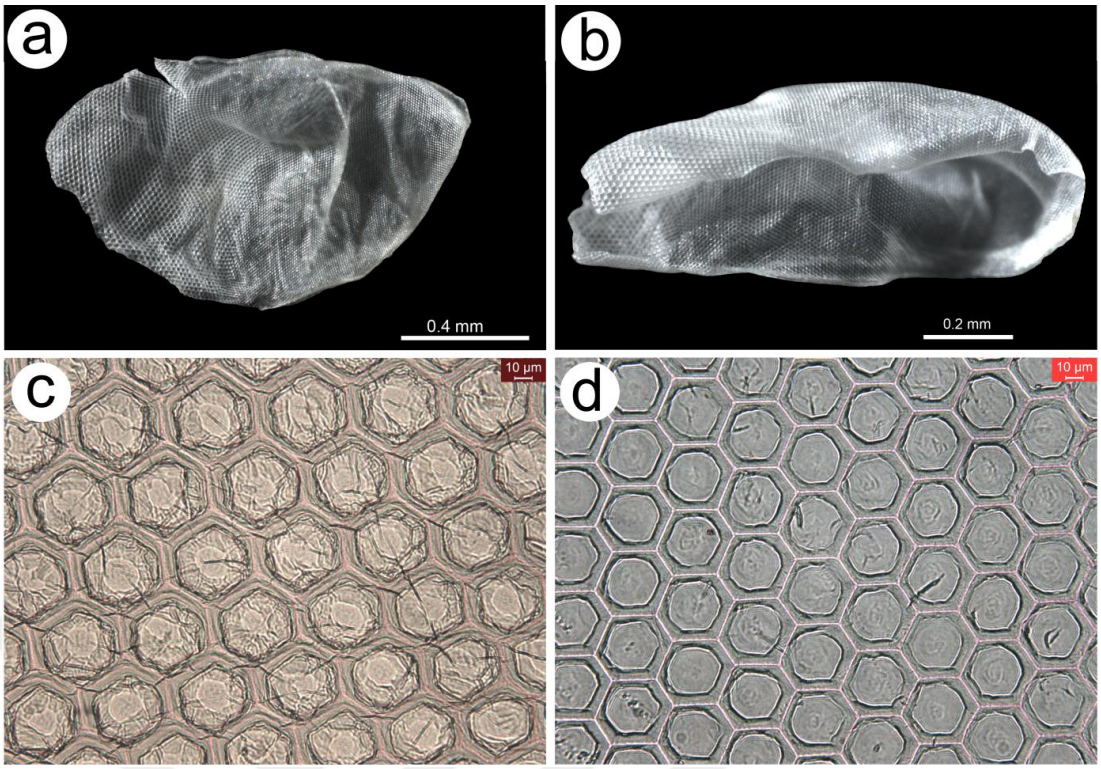

Figure 4. 3: Stereo and binocular light microscopy pictures of dragonfly corneal lens chitin (a,b. corneal chitin, c. outer part of corneal chitin and d. inner part of corneal chitin).

Upon the extraction procedure, fine microstructures i.e., ommaditial facets on the corneal lens chitin were preserved whereas nanoscale pattern of conical protrusions disappeared. Nano-sized nipple arrays on intact corneal lens surface were observed in earlier studies (Boseman et al., 2013; Kryuchkov et al., 2011). These ordered and organized microstructures on the eyes of the insects were reported to have both anti-reflective (Rahul et al., 2012) and anti-fogging properties (Darmanin and Guittard, 2015). In addition, transparency of the corneal lenses should be due to the regular arrays and sizes of these papillary structures (Rahul et al., 2012). However, it appears that chemical treatment of corneal lens during the chitin isolation procedure was responsible from the loss of nipple structures on the dragon fly corneal lenses. The isolated chitin samples from corneal lens cannot be expected to have anti-reflective or anti-wetting properties like the intact corneal lenses.

On the other hand, the SEM and light microscopy images of chitin specimens from the corneal lenses demonstrated that each corneal lens kept its typical hexagonal shape even after the chitin purification treatments and the periodic structure of the

lenses were not disrupted (Fig. 4.4a, b). At the microscale, the outer surface of the corneal lens chitin still had the papillary structures (Figs. 4.4c, 4.5a,b) while the inner surface had smooth texture with hexagonal shape (Figs. 4.4d, 4.4c,d). In addition, the internal structure of the lens composed of concentric lamellar chitin formations as clearly seen in Fig. 3d. At the nanoscale, a reticular arrangement of long chitin fibrils was observed on the outer surface (12-26 nm) (Fig. 4.5). On the other hand, the inner part consisted of reticular shorter and thicker chitin fibrils (21-37 nm) (Fig.4.5b).

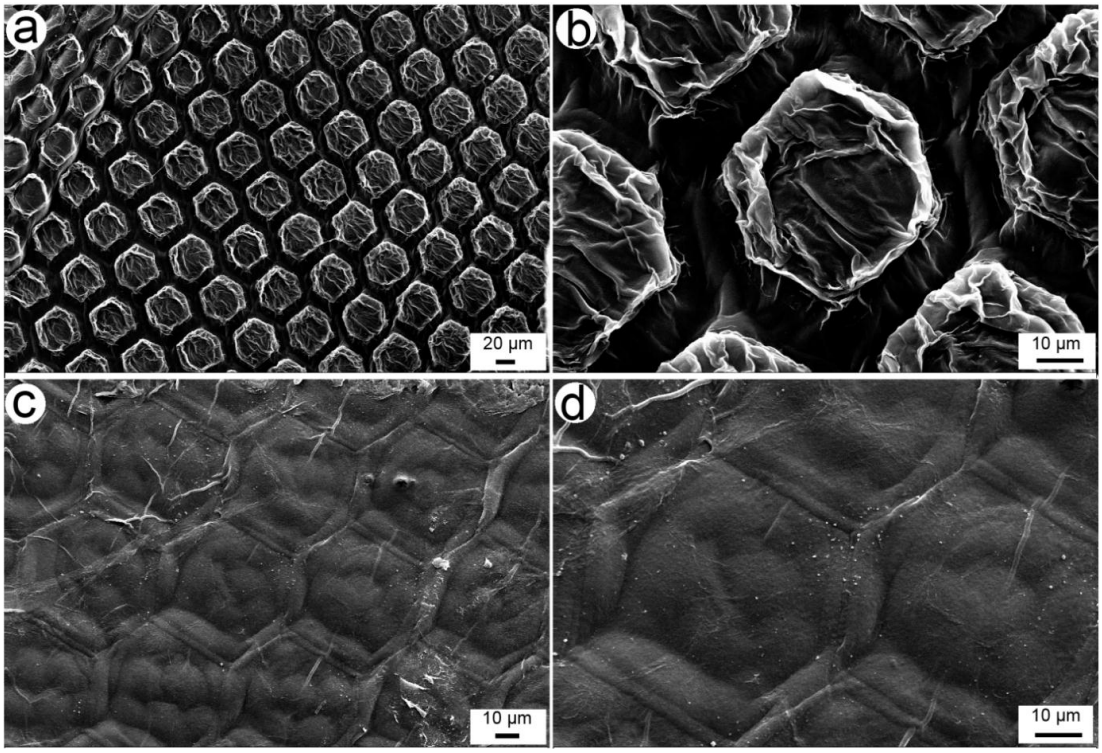

Figure 4. 4: Mikro boyutta yusufçuk böceğinin korneal lens kitinin SEM resimleri (a, b. korneanın dış kısmı ve c,d. iç kısmı).

Chitin surface morphology depends on source and it is very variable (Kaya et al., 2015c). Some extracted chitin samples have smooth surface without fibres or pores and this type generally occurs in fungal chitins (Yen and Mau, 2007). Some chitin samples from crustacean and insect species have nanofibres and nanopores (Mushi et al., 2014; Paulino et al., 2006; Yen et al., 2009) but here the chitin from dragonfly corneal lenses has micropapillar hexagonal shape and it is a new surface morphology. This chitin has only nanofibres without pores on its surface. It is also important to say that outer part has long fibres and inner part has short fibres. This

distinct chitin organization may have a role in the optical function of chitin in corneal lenses of dragonfly.

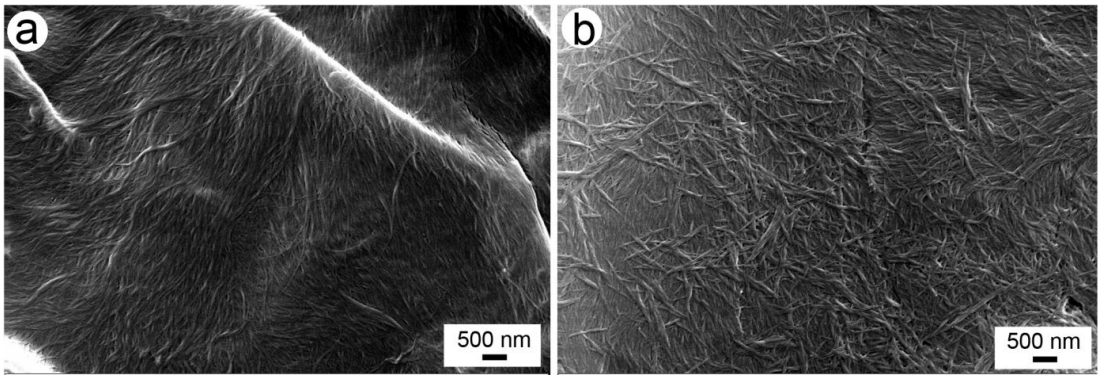

Figure 4. 5: SEM pictures of dragonfly corneal lens chitin in nano size (a. outer part of cornea and b. inner part of cornea).

4.4 Thermogravimetric Analysis

Thermal decomposition of chitin isolate from dragonfly corneal lens was examined and TGA results are presented in Fig. 4.6. Thermal decomposition of corneal chitin isolate occurred in two steps. In total 76.1% of the sample was degraded and 23.9% ash residue after pyrolysis was recorded. Mass loss, 2.9%, was observed between 25–100ºC, which corresponds to evaporation of water molecules. The presence of water can be attributed to the hygroscopic nature of chitin (Chandumpai et al., 2004). Mass loss (73.2%) between 100-750ºC was because of degradation of polymeric material. The maximum degradation temperature (DTG_{max}) value of the corneal lens chitin was observed at 369.2 ºC. This result was close the reports in previous chitin extraction studies (Juárez-de la Rosa et al., 2012; Kaya et al., 2013). Also, in a review study by Wysokowski et al. (2015) comparison of thermal stability of α-chitins from various sources including krill, crab, shrimp, spider and sponges was listed. Thermal degradation of chitin isolates was reported in a range of 220–530°C. The list also had records for β and γ chitin isolates from different sources and it appeared that α chitin isolates were relatively more thermally stable than the other allomorphs.

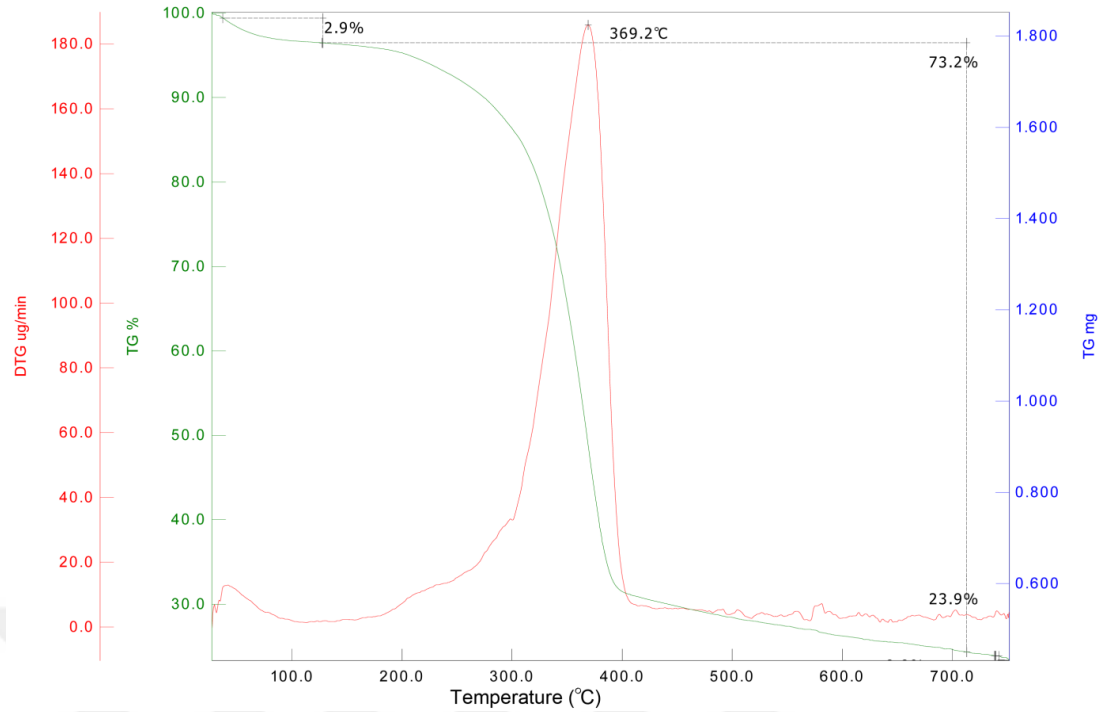

Figure 4. 6: TGA analysis of dragonfly corneal lens chitin.

4.5 Elemental Analysis

The N content and acetylation degree of the extracted chitin give its purity. In other words, when proteins, lipids and pigments found in the chitinous matrix are removed effectively by a harsh chemical treatment, the N content of chitin isolate becomes closer to the theoretical value (6.89%). On the other hand, during the extraction procedure hot alkali treatment leads to deacetylation of chitin sample resulting in higher N content. With corneal lenses the conventional chitin extraction method used in this study resulted in N, C and H contents 6.83, 47.09 and 6.65%. According to Liu et al. (2012), the N content of pure chitin is known to be 6.89% and DA is 100%. Here in the present study, N content of the corneal lens chitin was determined very close to theoretical value. This relatively lower N content can be attributed to the residual proteinaceous materials in the purified chitin samples (Majtán et al., 2007).

The DA value of the corneal lens chitin was calculated as 102% from the elemental analysis results. Although this value was found very close to the theoretical value, it is already known that DA of any chitin sample can vary depending on the raw material source and the method followed in isolation of chitin (Majtán et al., 2007). Considering the elemental analysis result and DA value, it can be concluded that the corneal lens chitin was extracted efficiently. However this higher value of DA can be ascribed to the hygroscopic nature of corneal lens chitin. As it was revealed by TGA,

corneal lens chitin had 2.9% water content. It seems that because of the presence of water in the chitin sample we recorded higher acetylation degree for corneal lens chitin.

4.6 Crystallinity of Corneal Lens Chitin

According to literature reports (Hongkulsup et al., 2015; Marei et al., 2016), two major peaks around 9 and 19° are characteristics of chitin and its derivatives. Here in the present study these major peaks were observed, indicating that chitin was successfully isolated from the corneal lens (Fig. 4.7). Additionally, the weak peaks at 13, 21 and 26° demonstrate the purity of chitin sample (Sayari et al., 2016). The CrI of corneal lens chitin was calculated as 96.4%, which was among the highest CrI values ever recorded for chitin. CrI values of chitin isolates from some organisms were reported very low (e.g., 54%, 58%) (Zhang et al., 2000). On the other hand, high CrI values were reported for chitin samples from various organisms (around 90%) (Cárdenas et al., 2004; Liu et al., 2012; Sajomsang ve Gonil, 2010). Highest CrI value of corneal lens chitin could be related with the function of chitin in visual system of the insect. Also, considering these results, this high crystalline structure of chitin in the corneal lens could provide some insights for optical studies based on chitinous materials.

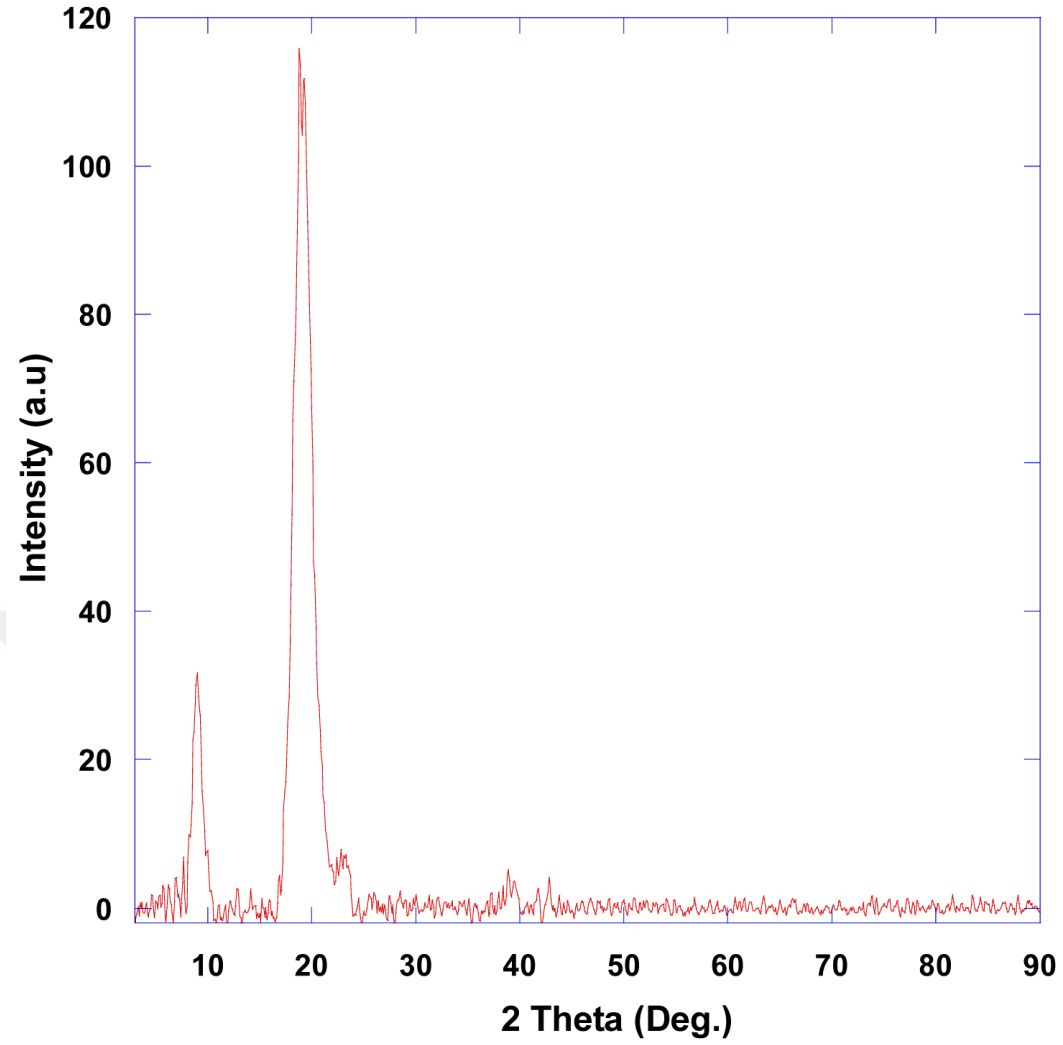

Figure 4. 7: XRD profile of dragonfly corneal lens chitin.

4.7 Chitinase Hydrolytic Activity on Corneal Lens Chitin

Chitinolytic activity on corneal lens chitin and commercial chitin from shrimp was assayed colourimetrically by potassium ferricyanide assay. The concentration of hydrolysatesreleased in enzymatic hydrolysis of commercial chitin and chitin from corneal lens were assayed and the quantification of hydrolytic products were determined; reducing sugar concentration (µM) 144.99 (commercial chitin) and 135.09 (corneal lens chitin). The enzyme chitinase exhibited relatively higherchitinolytic activity on chitin from shrimp shell. Digestion rate of commercial chitin wasdetermined to be 95.4%, whereas 88.7% was recorded for corneal lens chitin, indicating that the method used for isolation of chitin was effective at purifying chitin from corneal lenses of dragonfly compound eyes.

5. CONCLUSIONS

Presence of chitin in the corneal lens of insect compound eyes is known and has been referred to in many studies support (Alagboso et al., 2014; Chandran et al., 2016; Watson and Watson, 2004). However, there are no detailed reports on the characteristics of corneal lens (ommatidial) chitin. In this present study chitin isolated from the corneal lens of dragonfly compound eyes was examined and relatively high chitin content was recorded. As widely found in other arthropods, corneal chitin has alpha allomorph. Thermal stability of corneal lens chitin was observed close to those of chitin samples from various organisms. However, a very high CrI was recorded; 96.4%. Surface characteristics of corneal lens chitin were examined by different microscopy i.e., stereo, binocular and SEM. The morphological examination revealed that the outer surface of the corneal lens chitin had the protuberance structures with longer chitin fibrils and the inner part consisted of a network of short chitin fibrils forming concentric formation. All these findings can provide some insights into optical, histological, bio-mimetic and bio-inspired studies on compound eyes of arthropod species.

REFERENCES

Alagboso, F.I., Reisecker, C., Hild, S. and Ziegler, A. 2014. Ultrastructure and mineral composition of the cornea cuticle in the compound eyes of a supralittoral and a marine isopod, Journal of Structural Biology, 187, 2, 158-173.

Arbia, W., Adour, L., Amrane, A. and Lounici, H. 2013a. Optimization of medium composition for enhanced chitin extraction from Parapenaeus longirostris by Lactobacillus helveticus using response surface methodology, Food Hydrocolloids, 31, 2, 392-403.

Arbia, W., Arbia, L., Adour, L. and Amrane, A. 2013b. Chitin extraction from crustacean shells using biological methods-A review, Food Technology and Biotechnology, 51, 1, 12.

Bo, M., Bavestrello, G., Kurek, D., Paasch, S., Brunner, E., Born, R., Galli, R., Stelling, A.L., Sivkov, V.N., Petrova, O.V., Vyalikh, D., Kummer, K., Molodtsov, S.L., Nowak, D., Nowak, J. and Ehrlich, H. 2012. Isolation and identification of chitin in the black coral Parantipathes larix (Anthozoa: Cnidaria), International Journal of Biological Macromolecules, 51, 1–2, 129-137.

Boseman, A., Nowlin, K., Ashraf, S., Yang, J. and LaJeunesse, D. 2013. Ultrastructural analysis of wild type and mutant Drosophila melanogaster using helium ion microscopy, Micron, 51, 26-35.

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

Chandran, R., Williams, L., Hung, A., Nowlin, K. ve LaJeunesse, D. 2016. SEM characterization of anatomical variation in chitin organization in insect and arthropod cuticles, Micron, 82, 74-85.

Chandumpai, A., Singhpibulporn, N., Faroongsarng, D. and Sornprasit, P. 2004. Preparation and physico-chemical characterization of chitin and chitosan from the pens of the squid species, Loligo lessoniana and Loligo formosana. Carbohydrate Polymers, 58, 4, 467-474.

Darmanin, T. and Guittard, F. 2015. Superhydrophobic and superoleophobic properties in nature, Materials Today, 18, 5, 273-285.

Duparré, J. and Wippermann, F. 2006. Micro-optical artificial compound eyes, Bioinspiration & biomimetics, 1, 1, R1.

Ehrlich, H., Kaluzhnaya, O.V., Brunner, E., Tsurkan, M.V., Ereskovsky, A., Ilan, M., Tabachnick, K.R., Bazhenov, V.V., Paasch, S., Kammer, M., Born, R., Stelling, A., Galli, R., Belikov, S., Petrova, O.V., Sivkov, V.V., Vyalikh, D., Hunoldt, S. and Wörheide, G. 2013. Identification and first insights into the structure and biosynthesis of chitin from the freshwater sponge Spongilla lacustris, Journal of Structural Biology, 183, 3, 474-483.

Focher, B., Beltrame, P., Naggi, A. and Torri, G. 1990. Alkaline N-deacetylation of chitin enhanced by flash treatments. Reaction kinetics and structure modifications, Carbohydrate Polymers, 12, 4, 405-418.

Friedrich, M. 2006. Ancient mechanisms of visual sense organ development based on comparison of the gene networks controlling larval eye, ocellus, and compound eye specification in Drosophila, Arthropod Structure & Development, 35, 4, 357-378.

Hajji, S., Younes, I., Ghorbel-Bellaaj, O., Hajji, R., Rinaudo, M., Nasri, M. and Jellouli, K., 2014. Structural differences between chitin and chitosan extracted from three different marine sources, International Journal of Biological Macromolecules, 65, pp.298-306.

Harzsch, S. and Hafner, G. 2006. Evolution of eye development in arthropods: Phylogenetic aspects, Arthropod Structure & Development, 35, 4, 319-340.

Hongkulsup, C., Khutoryanskiy, V.V. and Niranjan, K. 2015. Enzyme assisted extraction of chitin from shrimp shells (Litopenaeus vannamei), Journal of Chemical Technology and Biotechnology.

Ianiro, A., Di Giosia, M., Fermani, S., Samorì, C., Barbalinardo, M., Valle, F., Pellegrini, G., Biscarini, F., Zerbetto, F., Calvaresi, M. and Falini, G. 2014. Customizing Properties of β-Chitin in Squid Pen (Gladius) by Chemical Treatments, Marine Drugs, 12, 12, 5979-5992.

Ifuku, S., Nomura, R., Morimoto, M. and Saimoto, H. 2011. Preparation of Chitin Nanofibers from Mushrooms, Materials 4, 1417-1425.

Imoto, T. and Yagishita, K. 1971. A simple activity measurement of lysozyme.

Jayakumar, R., Menon, D., Manzoor, K., Nair, S. and Tamura, H. 2010. Biomedical applications of chitin and chitosan based nanomaterials—A short review, Carbohydrate Polymers, 82, 2, 227-232.

Jeong, K.-H., Kim, J. and Lee, L.P. 2006. Biologically inspired artificial compound eyes, Science, 312, 5773, 557-561.

Johansson, F. and Suhling, F. 2004. Behaviour and growth of dragonfly larvae along a permanent to temporary water habitat gradient, Ecological Entomology, 29, 2, 196-202.

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

Kaya, M., Sargin, I., Tozak, K.Ö., Baran, T., Erdogan, S. and Sezen, G. 2013. Chitin extraction and characterization from Daphnia magna resting eggs, International Journal of Biological Macromolecules, 61, 0, 459-464.

Kaya, M., Baran, T., Saman, I., Ozusaglam, M. A., Cakmak, Y. S. and Menteş, A. 2014a. Physicochemical characterization of chitin and chitosan obtained from resting eggs of Ceriodaphnia quadrangula (Branchiopoda: Cladocera: Daphniidae), Journal of Crustacean Biology, 34, 2, 283-288.

Kaya, M., Seyyar, O., Baran, T., Erdoğan, S. and Kar, M., 2014b. A physicochemical characterization of fully acetylated chitin structure isolated from two spider species: With new surface morphology, International Journal of Biological Macromolecules 65, 553–558.

Kaya, M., Bitim, B., Mujtaba, M. and Koyuncu, T. 2015a. Surface morphology of chitin highly related with the isolated body part of butterfly (Argynnis pandora), International Journal of Biological Macromolecules, 81, 443-449.

Kaya, M., Lelesius, E., Nagrockaite, R., Sargin, I., Arslan, G., Mol, A., Baran, T., Can, E. and Bitim, B. 2015b. Differentiations of chitin content and surface morphologies of chitins extracted from male and female grasshopper species, PLoS One, 10, 1, e0115531.

Kaya, M., Mujtaba, M., Bulut, E., Akyuz, B., Zelencova, L. and Sofi, K. 2015c. Fluctuation in physicochemical properties of chitins extracted from different body parts of honeybee, Carbohydrate Polymers, 132, 9-16.

Kryuchkov, M., Katanaev, V.L., Enin, G.A., Sergeev, A., Timchenko, A.A. and Serdyuk, I.N. 2011. Analysis of micro-and nano-structures of the corneal surface of Drosophila and its mutants by atomic force microscopy and optical diffraction, PloS one, 6, 7, e22237.

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

Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., Jiang, C. and Yang, Q. 2012. Extraction and characterization of chitin from the beetle Holotrichia parallela motschulsky, Molecules, 17, 4, 4604-4611.

Majtán, J., Bíliková, K., Markovič, O., Gróf, J., Kogan, G. and Šimúth, J. 2007. Isolation and characterization of chitin from bumblebee (Bombus terrestris), International Journal of Biological Macromolecules, 40, 3, 237-241

Marei, N.H., El-Samie, E.A., Salah, T., Saad, G.R. and Elwahy, A.H. 2016. Isolation and characterization of chitosan from different local insects in Egypt, International journal of biological macromolecules, 82, 871-877.

Meyer, E.P. and Labhart, T. 1993. Morphological specializations of dorsal rim ommatidia in the compound eye of dragonflies and damselfies (Odonata), Cell and tissue research, 272, 1, 17-22.

Mushi, N.E., Butchosa, N., Salajkova, M., Zhou, Q. and Berglund, L.A. 2014. Nanostructured membranes based on native chitin nanofibers prepared by mild process, Carbohydrate Polymers, 112, 255-263.

Ospina Álvarez, S.P., Ramírez Cadavid, D.A., Escobar Sierra, D.M., Ossa Orozco, C.P., Rojas Vahos, D.F., Zapata Ocampo, P. and Atehortúa, L. 2014. Comparison of Extraction Methods of Chitin from Ganoderma lucidum Mushroom Obtained in Submerged Culture, Hindawi Publishing Corporation BioMed Research International Volume, 169071, 7.

Paulino, A.T., Simionato, J.I., Garcia, J.C. and Nozaki, J. 2006. Characterization of chitosan and chitin produced from silkworm chrysalides, Carbohydrate Polymers, 64, 1, 98-103.

Rahul, D., Stefan, F., Meyer-Rochow, V.B., Yasemin, Ö., Saeed, H. and Dietmar, K. 2012. Studying nanostructured nipple arrays of moth eye facets helps to design better thin film solar cells, Bioinspiration & Biomimetics, 7, 1, 016003.

Rinaudo, M. 2006. Chitin and chitosan: Properties and applications, Progress in Polymer Science, 31, 7, 603-632.

Sajomsang, W. and Gonil, P. 2010. Preparation and characterization of α-chitin from cicada sloughs, Materials Science and Engineering: C, 30, 3, 357-363.

Salaberria, A.M., Diaz, R.H., Labidi, J. and Fernandes, S.C.M. 2015. Preparing valuable renewable nanocomposite films based exclusively on oceanic biomass – Chitin nanofillers and chitosan, Reactive and Functional Polymers, 89, 31-39.

Sayari, N., Sila, A., Abdelmalek, B.E., Abdallah, R.B., Ellouz-Chaabouni, S., Bougatef, A. and Balti, R. 2016. Chitin and chitosan from the Norway lobster by-products: Antimicrobial and anti-proliferative activities, International Journal of Biological Macromolecules, 87, 163-171.

Schiffman, J. D. and Schauer, C. L 2009. Solid state characterization of α-chitin from Vanessa cardui Linnaeus wings. Materials Science and Engineering 29, 1370–1374.

Sherk, T.E. 1978. Development of the compound eyes of dragonflies (Odonata), III. Adult compound eyes. J Exp Zool, 203, 1, 61-80.

Song, Y.M., Xie, Y., Malyarchuk, V., Xiao, J., Jung, I., Choi, K.-J., Liu, Z., Park, H., Lu, C., Kim, R.-H., Li, R., Crozier, K.B., Huang, Y. and Rogers, J.A. 2013.

Digital cameras with designs inspired by the arthropod eye, Nature, 497, 7447, 95-99.

Tajik, H., Moradi, M., Rohani, S.M.R., Erfani, A.M. and Jalali, F.S.S., 2008. Preparation of Chitosan from Brine Shrimp (Artemia urmiana) Cyst Shells and Effects of Different Chemical Processing Sequences on the Physicochemical and Functional Properties of the Product, Molecules 13, 1263-1274.

Toh, Y. and Okamura, J.Y. 2007. Morphological and optical properties of the corneal lens and retinal structure in the posterior large stemma of the tiger beetle larva, Vision Res, 47, 13, 1756-68.

Wang, Y., Chang, Y., Yu, L., Zhang, C., Xu, X., Xue, Y., Li, Z. and Xue, C., 2013. Crystalline structure and thermal property characterization of chitin from Antarctic krill (Euphausia superba), Carbohydrate Polymers 92, 90–97.

Watson, G.S. and Watson, J.A. 2004. Natural nano-structures on insects—possible functions of ordered arrays characterized by atomic force microscopy, Applied Surface Science, 235, 1–2, 139-144.

Wysokowski, M., Bazhenov, V.V., Tsurkan, M.V., Galli, R., Stelling, A.L., Stöcker, H., Kaiser, S., Niederschlag, E., Gärtner, G., Behm, T., Ilan, M., Petrenko, A.Y., Jesionowski, T. and Ehrlich, H. 2013. Isolation and identification of chitin in three-dimensional skeleton of Aplysina fistularis marine sponge, International Journal of Biological Macromolecules, 62, 94-100.

Wysokowski, M., Petrenko, I., Stelling, A., Stawski, D., Jesionowski, T. and Ehrlich, H. 2015. Poriferan Chitin as a Versatile Template for Extreme Biomimetics, Polymers, 7, 2, 235.

Xu, J., McCarthy, S.P., Gross, R.A. and Kaplan, D.L. 1996. Chitosan Film Acylation and Effects on Biodegradability, Macromolecules, 29, 10, 3436-3440.

Yen, M.T. and Mau, J.L. 2007. Physico-chemical characterization of fungal chitosan from shiitake stipes, Lwt-Food Science and Technology, 40, 3, 472-479.

Yen, M.T., Yang, J.H. and Mau, J.L. 2009. Physicochemical characterization of chitin and chitosan from crab shells, Carbohydrate Polymers, 75, 1, 15-21.

Yoon, C.S., Hirosawa, K. and Suzuki, E. 1997. Corneal lens secretion in newly emerged Drosophila melanogaster examined by electron microscope autoradiography, J Electron Microsc (Tokyo), 46, 3, 243-6.

Zhang, M., Haga, A., Sekiguchi, H. and Hirano, S. 2000. Structure of insect chitin isolated from beetle larva cuticle and silkworm (Bombyx mori) pupa exuvia, International Journal of Biological Macromolecules, 27, 1, 99-105.

CURRICULUM VITAE

Name and Surname **:** Ivan AL_JAF

Birth Date and Place **:** 05.06.1990 Baghdad / IRAQ

E-mail address **:** evanaljaf2@gmail.com

EDUCATION

Bachelor's Degree: In university of Sulimani, 2009-2013
Department of Biology
Al Sulaymaneyah/ IRAQ

Master degree: Aksaray University, 2014-2016
Department of Biotechnology and Molecular Biology

Aksaray/ TURKEY

DISSERTATION

Kaya, M., Sargin, I., Al-jaf, I., Erdogan, S. and Arslan, G., 2016. Characteristics of corneal lens chitin in dragonfly compound eyes, International Journal of Biological Macromolecules, DOI: 10.1016/j.ijbiomac.2016.04.056