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Fluctuation in physicochemical properties of chitins extracted from different body parts of honeybee

Murat Kaya [a,*], Muhammad Mujtaba [a], Esra Bulut [a], Bahar Akyuz [a], Laura Zelencova [a,b], Karwan Sofi [a]

[a] Department of Biotechnology and Molecular Biology, Faculty of Science and Letters, Aksaray University, 68100 Aksaray, Turkey
[b] Department of Biology, Vytautas Magnus University, 44404 Kaunas, Lithuania

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ABSTRACT

It is well known that physicochemical properties of chitin are related with the extraction method. Recently, it was revealed that some physicochemical properties of chitin are also related with taxonomical relationship. For the first time in this study, it was tested how these properties of chitin are affected by different body parts of one organism. The chitins were extracted from five different body parts (head, thorax, abdomen, legs and wings) of honeybee. These chitins were physicochemically characterized and differences among these body parts were identified. Highest chitin content was observed in legs (13.25%) while the lowest from thorax (6.79%). The surface morphologies of the isolated chitin structures from five different body parts were analyzed with SEM, as a result, five different types of surface morphologies were recorded. However, three different types of surface morphologies were observed only in abdomen. Maximum degradation temperatures (DTG_{max}) of thorax, abdomen, legs and wings were recorded between 359 and 367 °C while DTG_{max} value of head chitin was found as 308 °C.

1. Introduction

As is known, physicochemical properties of chitin are highly affected by extraction method and source (Aranaz et al., 2009). Generally chitin isolation was made from exoskeletons of different marine sources like crabs, shrimps and lobsters and also from whole body of different organisms like mushroom, insects, sponges and corals (Al Sagheer, Al-Sughayer, Muslim, & Elsabee, 2009; Ehrlich et al., 2013; Ifuku, Nomura, Morimoto, & Saimoto, 2011; Juárez-de la Rosa, Quintana, Ardisson, Yáñez-Limón, & Alvarado-Gil, 2011; Liu et al., 2012). Unlike the previous studies, Kaya and Baran (2015) extracted chitin separately from wings and the other body parts of cockroach using the same method. And they observed different surface morphologies for each body part. These results demonstrated a variation in the physicochemical properties of isolated chitins from different body parts of organisms. In that study by Kaya and Baran (2015), they extracted and compared chitin from only two different body parts but here in this study, five different body parts of honeybee were tested for determining variations in the physicochemical properties of extracted chitin.

Inspiration for conducting the present study was obtained from some reviewers who are always making complaints about chitin surface morphology. Some reviewers support that surface morphology of chitin is not related with the source. The surface morphology is highly affected by the method. Here in our study, we tried to check out that is there any difference in the surface morphologies of chitins isolated from different body parts by using the same method.

Globally, bee keeping is being practiced extensively but due to certain reasons every year a great number of honeybees were lost. Some of these reasons are irregular use of pesticides, fertilizers, loss of habitat, declination of floral diversity, and erosion. For example in Poland every year about 200 ton honeybees are vanishing. While in Russia this rate was ranged from 6000 to 10,000 in the year of 2002. Until now researchers focused upon chitin extraction from whole body of different insects, larva cuticle of beetle and silk worm (*Bombyx mori*) exoskeleton of pupa, honeybees (*Apis mellifera*), bumblebee (*Bombus terrestris*) (Draczynski, 2008; Majtan et al., 2007; Nemtsev, Zueva, Khismatullin, Albulov, & Varlamov, 2004; Zhang, Haga, Sekiguchi, & Hirano, 2000). These dead bees have a great environmental concern as it is a big waste. It can be utilized as an alternative source of chitin.

Chitin is present in Arthropoda, mushrooms, sponges and diatoms in the form of a bio composite, like chitin–mineral, chitin–protein and also chitin–pigments (Ehrlich, 2010; Ehrlich

* Corresponding author. Tel.: +90 382 288 2184; fax: +90 382 288 2125.
E-mail address: muratkaya3806@yahoo.com (M. Kaya).

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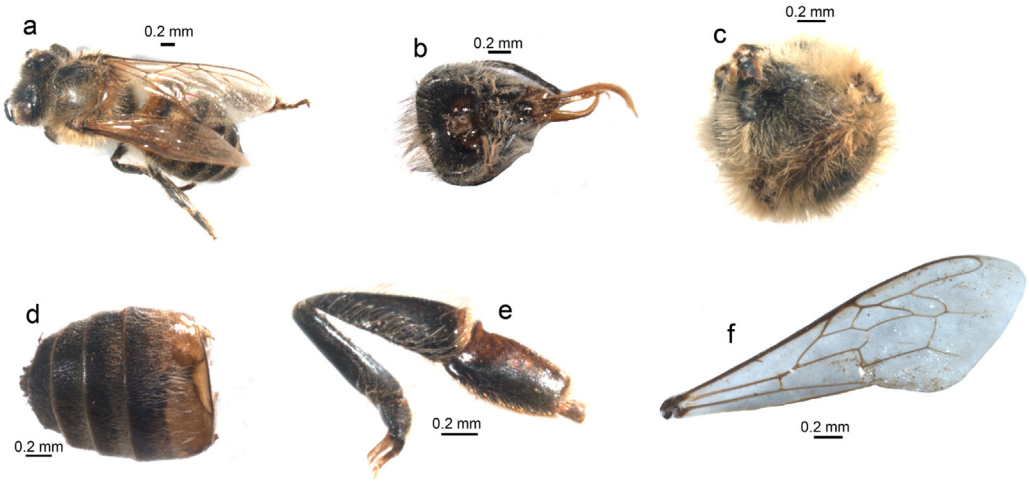

Fig. 1. Honeybee (*Apis mellifera*): (a) whole body shape, (b) head, (c) thorax, (d) abdomen, (e) leg and (f) wing.

et al., 2010a). Hydrogen fluoride and hydrogen peroxide are used for extraction of pure chitin from different sources (Brunner et al., 2009) while these harsh treatment highly affect the physico-chemical and structural properties of chitin (Ehrlich et al., 2010b). Consequently, chitin with changes in physico-chemical properties due to these treatments can be applicable in different areas such as tissue engineering, medicine, agriculture and water treatment (Ehrlich et al., 2010c).

In the present study, we focused upon investigating variations in different physicochemical properties of isolated chitin from separated body parts i.e. (head, thorax, abdomen, leg, and wing) of honeybee. These properties included chitin content (%), surface morphology and thermal stability. The isolated chitins were characterized by FTIR, SEM and TGA analysis. Obtained results were compared among these five body parts.

2. Materials and methods

2.1. Samples collection

Dead honeybees were collected from a private company during a hard winter. Collected bees were washed several times with distilled water and oven dried for a week at 50 °C. Honeybees were separated into five different body parts i.e. head, thorax, abdomen, leg and wing (Fig. 1). All the separated body parts were grinded in a pestle–mortar for obtaining a fine powder.

2.2. Isolation of chitin

For demineralization of each body part, 10 g of dry sample (weighed through assay balance) was treated with 2 M HCl solution (100 ml) at 80 °C for 6 h. The demineralization step was tailed by rinsing with distilled water until all the neutrality was reached. For deproteinization, the demineralized sample was treated with 2 M of NaOH and refluxed for 20 h. at 100 °C. Deproteinized chitin product was rinsed for many times to achieve the neutral pH. After deproteinization for removal of lipids and pigments the extracts were kept in a mixture of distilled water (40 ml), methanol (20 ml) and chloroform (20 ml) and stirred for 40 min at 250 rpm. Then the solution was filtered and washed with distilled water for a couple of times. Finally, the obtained chitins were put at room temperature for drying up. The dry samples were weighted and used for determination of chitin content.

2.3. Characterization

The structural analysis of chitin was carried out through scanning electron microscopy. FT-IR spectroscopy was used to determine the type and purity of chitins. TG and DTG curves of honeybee chitins were analyzed by using an S11 EXSTAR 7300 at a heating rate of 10 °C/min.

Table 1
FT-IR spectrum data of chitins isolated from five different body parts of honeybee (*Apis mellifera*).

Functional group and vibration modes	FT-IR bands (cm^{-1})				
	Head	Thorax	Abdomen	Legs	Wings
O—H stretching	3441	3434	3441	3434	3441
N—H stretching	3256–3101	3256–3098	3257–3098	3261–3101	3262–3098
CH_3 sym. stretch and CH_2asym. Stretch	2923	2962	2919	2920	2920
CH_3 sym. Stretch	2850	2878	2851	2851	2851
C=O secondary amide stretch	1654	1654	1656	1653	1658
C=O secondary amide stretch	1620	1620	1621	1620	1621
N—H bend, C—N stretch	1552	1553	1554	1552	1553
CH_2 ending and CH_3 deformation	1418	1422	1425	1420	1431
CH bend, CH_3 sym. deformation	1376	1376	1376	1376	1376
CH_2 wagging	1307	1308	1309	1308	1309
Asymmetric bridge oxygen stretching	1153	1155	1155	1156	1154
Asymmetric in-phase ring stretching mode	1115	1115	1114	1114	1113
C—O—C asym. stretch in phase ring	1068	1068	1070	1067	1060
C—O asym. stretch in phase ring	1008	1010	1012	1008	1010
CH_3 wagging	951	952	952	951	952
CH ring stretching	893	895	896	895	896