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Microbial biofilm activity and physicochemical characterization of biodegradable and edible cups obtained from abdominal exoskeleton of an insect

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ABSTRACT

A contemporary focus in food industry is the use of edible bio-based products with properties such as antimicrobial and biodegradable to replace the synthetic harmful petroleum-based polymers. Among the natural polysaccharides, chitin has generated considerable research interest thanks to its biocompatibility and abundance. This study investigated the production of chitin bio-cups from abdominal exoskeleton of an insect as an alternative to synthetic materials in food processing industry. The physicochemical properties of the obtained chitin and chitosan cups were studied by FT-IR, TGA, XRD and SEM analyses. The purity of the extracted chitin was examined by chitinase digestive test. The microbial biofilm formation on the cups was tested and no growth was recorded for the common food pathogen bacteria (*Listeria monocytogenes*) and yeast (*Candida albicans*). Considering the antimicrobial, antioxidant, nontoxic and edible nature of chitin and chitosan, these cups can be suggested as an alternative bioplastic for food protection.

Industrial relevance: In recent years much research has focused on the use of nontoxic and edible biopolymers as film and coating material in food industry to eliminate the use of carcinogenic and harmful petroleum products. Among the biopolymers, chitin and its deacetylated form, chitosan, are attracting widespread interest thanks to their nontoxic, biodegradable and edible properties. Here in this study, we investigated the production of chitin bio-cups from abdominal exoskeleton of an insect as an alternative to synthetic materials in food processing industry.

1. Introduction

In recent years much research has focused on the use of nontoxic and edible biopolymers as film and coating material in food industry to eliminate the use of carcinogenic and harmful petroleum products (Reddy & Yang, 2013; Sharma & Luzinov, 2013; Zarate-Ramirez et al., 2014). Among the biopolymers, chitin and its deacetylated form, chitosan, are attracting widespread interest thanks to their nontoxic, biodegradable and edible properties (Guo et al., 2015; Shen & Kamdem, 2015). Chitin is an abundant polymer and it naturally occurs in many invertebrates including Arthropoda, Porifera, Mollusca and mushrooms (Ehrlich et al., 2007; Ehrlich et al., 2013; Ifuku et al., 2011; Lavall et al., 2007). Chitin and chitosan are commercially available generally in powder form and sometimes in flake or granulated forms (Ehrlich et al., 2010). Three-dimensional form of chitin has been isolated from only sponges up to now (Brunner et al., 2009; Ehrlich et al., 2010; Wysokowski et al., 2013). On the other hand, chitosan bioplastic in different shapes (cubic, oval, glass etc.) is produced by molding techniques by using gel form of chitosan (solved in acetic acid) (Fernandez & Ingber, 2014). However, chitin or chitosan based materials with a definite structure can be produced directly from the source organisms. In this respect, the phylum Arthropoda offers excellent choices. This phylum has more than one million species, and this figure makes up almost 80% percent of whole animal species. Despite the diversity in body shapes of organisms in this phylum, there is no study on production of natural three-dimensional chitin or chitosan materials from any arthropod species by keeping the original shape of the chitinous matrix.

Chitin is found in the insect cuticle together with proteins, minerals and pigments (Hackman, 1953). In extraction procedure, protein, mineral and pigment contents of cuticles are separated from the chitinous matrix using chemicals in mild conditions and three-dimensional chitin structure can be obtained from any part of the insect. It has been widely acknowledged that chitin is a nontoxic, biocompatible and biodegradable biopolymer with antitumor, antioxidant and antimicrobial activities(Younes & Rinaudo, 2015); these unique properties has made chitin a versatile

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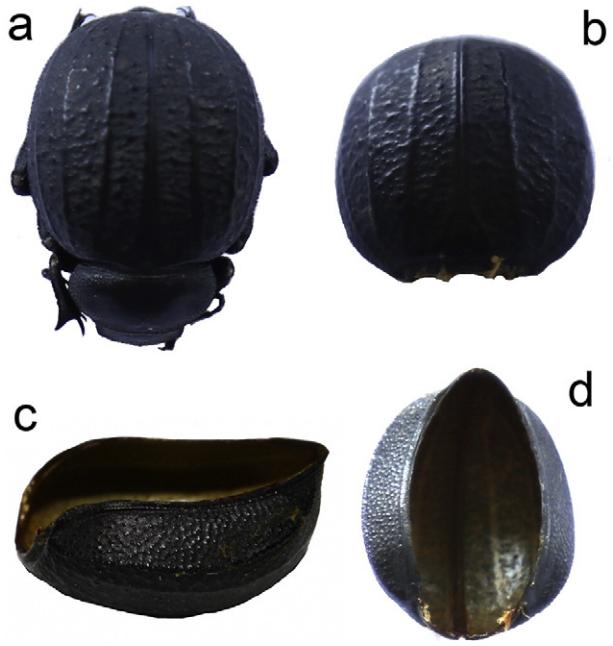

Fig. 1. *Pimelia* sp. (a. habitus, b, c, d. abdominal exoskeleton).

biopolymer for applications in various fields such as medicine, pharmacy, textile, agriculture, food industry and biotechnology (Aranaz et al., 2009; Rinaudo, 2006). Three-dimensional chitin isolates can be used more effectively in some specific areas than classical dust or flake form.

Pimelia is a genus of Tenebrionidae (Coleoptera) with saprophagous and flightless nature and it is distributed in all Mediterranean countries such as Turkey, Spain, France, Italy, Israel, Greece etc. They can live in desert and arid environments (Bruvo-Madaric et al., 2007). It is distributed around all the regions in Turkey except Black sea coast (Tezcan et al., 2004). This genus was selected in the present study due to its well-designed cup shaped abdominal exoskeleton.

L. monocytogenes is a ubiquitous food-borne pathogen which causes severe listeriosis infections in humans. For example, according to Centers for Disease Control and Prevention (CDCP) figures, every year 500 out of 2500 listeriosis cases end up dying of the victims. According to the data released by Food and Drug Administration (FDA), 5% of processed food is infected with billions of *L. monocytogenes* (Gombas et al., 2003). *C. albicans* is a fungal pathogen that is capable of attaching itself to the surfaces easily and forming biofilms. Particularly in the dairy industry it causes severe economic loses by deteriorating the products properties like shelf life, taste or aroma due to its high proteolytic activity (Srey et al., 2013). In the present study these two common pathogens were preferred as model organisms to test the usability of chitin and chitosan bio-cups in food preservation.

This present study describes the preparation of chitin and chitosan cups from an insect abdominal exoskeleton by keeping the original three-dimensional shape of the raw material. Identification of three-dimensional chitin and chitosan cups was performed with FT-IR, TGA, XRD and SEM analyses. Purity of the chitin cups was tested by chitinolytic activity test and chitinase enzyme hydrolyzed the chitin isolates. In addition, microbial biofilm activity of these chitin and chitosan cups was tested on common food pathogen bacteria (*L. monocytogenes*) and yeast (*C. albicans*).

2. Materials and methods

2.1. Sample collection and preparation of chitin and chitosan cups

The used samples of *Pimelia* sp. in the present study were collected in Gucunkaya, (Aksaray, Turkey) on 24-07-2015 (Fig. 1). First, the abdominal exoskeleton samples of *Pimelia* sp. were separated by hand and cleaned by distilled water many times. Then the samples were dried at 40 °C for 4 days. The dried samples were decolorized in 6% NaClO solution at 40 °C for 10 min. After decolorization, the samples were refluxed in 1% HCl solution at 35 °C for 2 h for demineralization. Lastly, the samples were refluxed in 1 M NaOH solution at 70 °C for 10 h for deproteinization. After each treatment, the samples were cleaned by distilled water reaching to neutral pH. The wet samples were dried at 40 °C for 5 days in an oven. The weight of the dried chitin cups was measured and used for the determination of the chitin content of the abdominal exoskeleton structure.

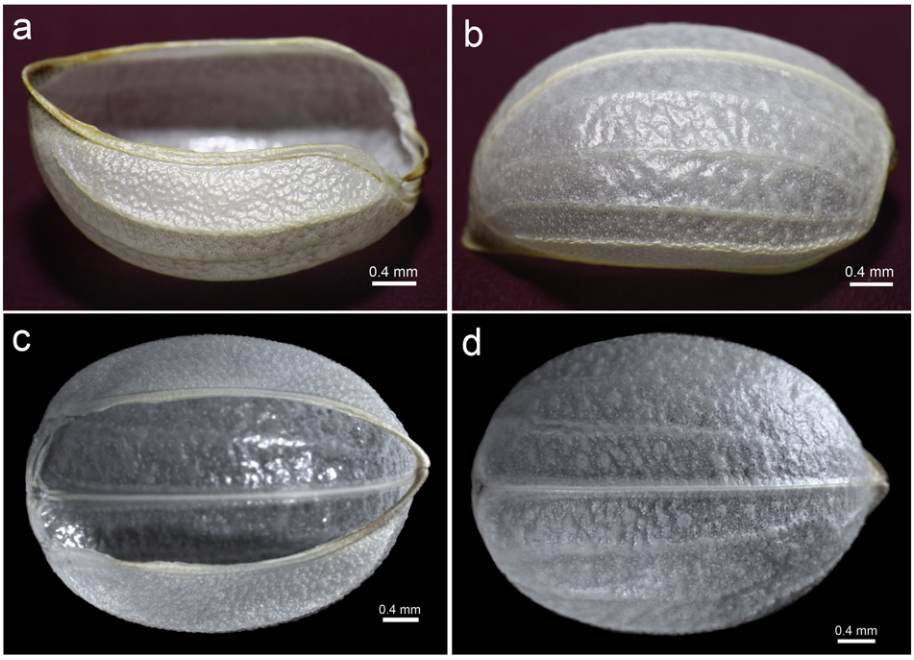

Fig. 2. Chitin and chitosan bio-cups obtained from abdominal exoskeleton of *Pimelia* sp. (a, b. chitin cup, c, d. chitosan cup).