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Incorporation of sporopollenin enhances acid–base durability, hydrophobicity, and mechanical, antifungal and antioxidant properties of chitosan films

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ABSTRACT

Sporopollenin-chitosan blend films were produced for the first time. Sporopollenin is a robust structural component of plant pollens exhibiting excellent features such as nontoxicity, biodegradability, biocompatibility, high thermal stability, durability to strong acid and base solutions and homogeneity in size. To benefit from these advantages, sporopollenin samples obtained from *Betula pendula* (silver birch) were incorporated into chitosan film at different concentration; 10, 20 and 40 mg in 100 mL chitosan gel (1%). Stereo microscopy, FT-IR and TG/DTG analyses showed that sporopollenin was successfully incorporated into the chitosan matrix. Incorporation of sporopollenin in gradually increasing amount into chitosan films was found advantageous in (1) enhancement in chemical durability of the films, (2) increment of hydrophobicity, (3) boosting the mechanical properties, (4) improvement of antifungal and (5) antioxidant activities. This study revealed that sporopollenin can be suggested as an effective blend material for biodegradable edible chitosan film production.

Introduction

Petroleum-derived films pose serious risks for the environment due to their non-degradable nature and there is also a growing number of reports on their carcinogenic effects in humans [1,2]. Biodegradable edible films have emerged as an alternative to synthetic films for food and pharmaceutical applications in recent years [3–6]. Proteins [7,8], lipids [6], plant extracts and polysaccharides have been widely used for the production of biodegradable films [9,10]. Chitosan, a polysaccharide derivative, has become as a versatile material in edible film production technology [2,11].

Chitosan is easily obtained through the deacetylation of chitinous wastes of shrimp, crab, crayfish, and squid used in food industry [12]. Chitosan has applications in many fields of science thanks to its nontoxic, biocompatible, biodegradable, antimicrobial, antitumoral and antioxidant properties [2]. It can be easily turned into gel (film) by dissolving in week acids and it can be used directly or blended with different materials. Up to now many studies have been conducted on the applications of chitosan-based films in pharmacy, medical and food industries; chitosan–chitin nanofiber blend film [13], chitosan–poly(vinyl alcohol) film [14], chitosan–fish gelatin film [15], and quinoa protein–chitosan–sunflower oil film [16]. However, considering the literature review, there have been no studies carried out on chitosan-sporopollenin blend films.

Sporopollenin is typically obtained in a microcapsule form through chemical treatment (acid, base and chloroform etc.) ofplant pollens which results in removal of genetic materials and

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proteins. Sporopollenin is a cross-linked biopolymer having (1) extraordinary chemical resistance, (2) high thermal stability, (3) non-allergic after removing the internal materials and (4) can remain intact in nature for thousands of years. In some earlier studies, sporopollenin samples were used in food industry as microcapsules to mask the taste of cod liver oil, sunflower oil and fish oil [17,18]. In these studies, the obtained sporopollenin capsules were performed to healthy volunteers. These studies showed that sporopollenin samples are safety for human consumption. Additionally, sporopollenin samples (as a natural material) were already used as microcapsules in cosmetic, pharmaceutical and food industries [19,20]. According to a mini review [21], sporopollenin is known as "diamond of the plant world" [22] and "probably one of the most extraordinary resistant materials known in the organic world" [23]. The most important feature is the homogeneous size of sporopollenin derived from plants of the same species or from different species [24,25]. In the present study pollens of *Betula pendula* (warty birch or silver birch) belonging to Betulaceae was used due to ease in collection and abundant availability.

Glycerol is one of the most commonly used plasticizers in edible film technology due to its nontoxic nature [9]. Here in the present study, glycerol was used as a plasticizer for production of chitosan based sporopollenin films.

The aim of the current study was to elaborate the chitosan film incorporating different concentrations of sporopollenin. The newly obtained chitosan-sporopollenin blend films were characterized by means of different techniques (FT-IR, stereo microscopy, TGA, transparency, mechanical and contact angle analysis) in addition to the assessment of their antifungal and antioxidant activities.

Material and methods

Material

Commercial medium molecular weight chitosan from Sigma-Aldrich (Code number: 448877) was used in the study. The sporopollenin used in the study was obtained from *B. pendula* (silver birch) pollens by following the method reported by Baran et al. [26]. In literature there some sporopollenin extraction protocols from *Lycopodium clavatum* spores [27] and sunflower pollen grains [28]. Those protocols generally include refluxing in acetone for defatting, removal of internal cellulosic intine layer and proteins with hydrochloric acid or phosphoric acid, hot alkaline treatment with sodium or potassium hydroxide solutions and washing with ethanol and water. However, it appeared that there is no a universal method for sporopollenin extraction. As pollens differ in structure and morphology, so does sporopollenin extraction procedure. In this study we followed a procedure reported in our previous study to extract sporopollenin microcapsules from pollen grains of *B. pendula*. In sporopollenin extraction procedure pollen grains of *B. pendula* were treated in mineral acid solution (6 M HCl) at 60 °C for 1 h, alkaline and chloroform–methanol solutions. Following the washing with distilled water, acid-treated pollens were heated at 90 °C in 4 M NaOH solution for 5 h. The acid and base treatments were repeated under same conditions. As a final step, the samples were kept in chloroform–methanol solution (1:1, v:v) at room temperature for 20 min. Finally the sporopollenin samples were washed extensively with distilled and dried at room temperature. All other chemicals were purchased from Sigma-Aldrich.

Preparation of the films

Four different types of films were produced. For each film sample, 10 mg of chitosan was dissolved in 1% acetic acid (10 mL). 8 mg of glycerol was added to each chitosan solution as a plasticizer. Chitosan solution without sporopollenin was used as control and labeled as CS. Sporopollenin was added into three chitosan solutions as 10 mg, 20 mg and 40 mg. Other samples were labeled as CS10, CS20 and CS40 having 10, 20 and 40 mg of sporopollenin respectively. Following the addition of glycerol and sporopollenin into the chitosan solutions, the samples were homogenized at 26,000 rpm for 10 min with a homogenizer (Heidolph, Silent Crusher M). The homogenized samples were poured into plastic petri dishes and allowed to dry at 30 °C for 48 h. The produced films are shown in Fig. 1. Film thickness measurements were recorded with a digital micrometer (Mitutoyo, China). For each film, the measurements were carried out for six different parts and then the average values were calculated.

Stereo microscopy

The surface morphologies of the films were examined on a LEICA Z6 APO stereo microscope at ambient conditions.

Fourier transform infrared spectroscopy (FT-IR)

Infra-red spectra of the films were recorded on a Perkin-Elmer ATR FT-IR spectrometer over the range of 4000–650 cm^{-1}. Also, FT-IR spectra of chitosan, glycerol and the sporopollenin were recorded to make comparisons with those of film samples. After each analysis, the instrument was baselined in oxygen atmosphere. For each film, three different samples were scanned in the transmittance range of 0–100% (number of scans: 3).

Thermogravimetric analysis (TG/DTG)

TG/DTG analysis was carried out to investigate the thermal stability of chitosan and chitosan-sporopollenin films using TGA/SDTA 851 Mettler Toledo instrument. For each analysis ~5 mg specimen was used. Samples were heated at a constant rate of 10 °C min^{-1} from room temperature to 900 °C under a nitrogen atmosphere of 20 mL min^{-1}.

Film solubility

Solubility of the films was determined gravimetrically at different pHs. The samples were cut into small pieces (5 × 5 mm

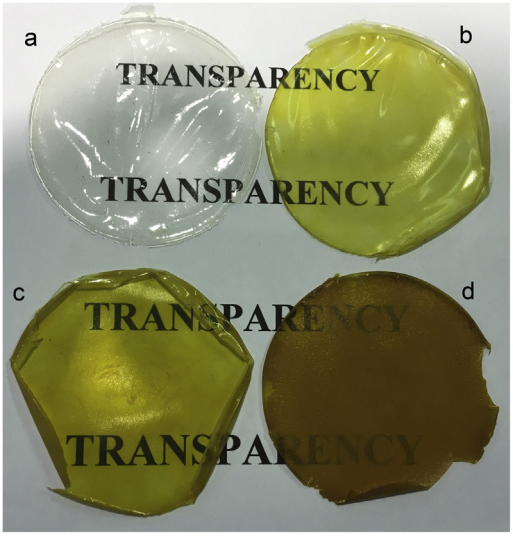

Fig. 1. Visual transparency images of pure chitosan film (a) and sporopollenin-chitosan blend films; (b) CS10, (c) CS20, (d) CS40.

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