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Nanostructured active chitosan-based films for food packaging applications: Effect of graphene stacks on mechanical properties

Christian Demitri *, Vincenzo Maria De Benedictis, Marta Madaghiele, Carola Esposito Corcione, Alfonso Maffezzoli

University of Salento, Department of Engineering for Innovation, Via Monteroni, Campus Ecotekne, 73100 Lecce, Italy

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ABSTRACT

Bioactive food-preserving materials are based on the use of a natural antimicrobial compound loaded in a carrier material, which is able to trigger its release when requested and to modulate the rate of release, thus using either toxic or inhibitory properties against pathogens or bacteria due to food decomposition. In this study, the Schiff base formation for chitosan functionalization was achieved by the reaction of chitosan with cinnamaldehyde at different concentrations. Cinnamaldehyde is an aromatic α,β-unsaturated aldehyde, and the major component in essential oils from some cinnamon species. It has been shown to exert antimicrobial action against a large number of microorganisms including bacteria, yeasts, and mould. The formation of the Schiff base is reversible under suitable conditions, and this might allow the release of the active cinnamaldehyde from chitosan, used as the carrier. The reaction kinetics was investigated by means of rheological measurements, while infrared spectroscopy was used to assess the efficacy of the functionalization. The addition of nanometric graphene stacks to the cinnamaldehyde-functionalized chitosan films was evaluated with the aim to increase the mechanical properties of the film. Finally, the films were tested for antifungal properties with bread slices against a selected mould line.

1. Introduction

Recently, different authors have been focused the attention on the development of natural innovative and active materials for food packaging applications [1]. It is well known that there are different processes involved in extending the shelf life of food. These involve controlled moisture transfer between inner and external environment, high permeability to certain substances, temperature control, structural reinforcement of food and coat flavor compounds, reduction of oxygen partial pressure in the package, extended release of organic agents like antimicrobial substances, antioxidants [2]. Active packagings are barriers that perform interactive action with their contents, releasing protective substances for the food or absorbing others that accelerate the deterioration process. Active packagings are divided into absorbers, reactors and releasers [3]. Absorbers absorb the harmful substances such as ethylene, oxygen and moisture [4]. Reactors provide a reaction, exothermic or endothermic, which allows the heating or cooling of the product contained therein. The releasers release active substances for the preservation of the product [5]. The increased perception of consumers towards products in which chemical preservatives have been removed has shifted the focus in using natural active agents. It is known that the essential oils extracted from plants and spices are excellent antioxidants and antimicrobials [6]. These compounds are often incorporated in the package by impregnation of the film, or by means of functionalization of the polymer substrate. To this aim, different techniques are commonly used in order to obtain functionalized films of incorporating natural active agents into natural polymers. Even if many types of synthetic polymer formulations have been industrially manufactured, natural polymers, especially polysaccharides, are growing in interest in a range of technological fields [7–12]. Between the polysaccharides, chitosan has been widely studied due to its excellent film-forming nature [13], antimicrobial properties, physical and mechanical properties, its biocompatibility and biodegradability [14]. Chitosan is a natural polymer obtained by deacetylation of chitin. It is a linear polycationic polysaccharide soluble only in acidic environment (pKa 6.5). The aim of this study is to develop an innovative method to achieve an improvement in food safety by controlling the proliferation of both native or food-spoilage microorganisms by reducing the use of synthetic preservatives.

* Corresponding author.
E-mail address: christian.demitri@unisalento.it (C. Demitri).

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These active systems are based on the use of natural antifungal agents incorporated in carrier materials or devices, that trigger the release once necessary, control the rate of release, having either lethal or inhibitory actions against pathogens or microorganisms comprised in foodstuffs. This study is focused on the modification of chitosan with cinnamaldehyde and nanometric sonicated expanded graphite stacks (EGS). Cinnamaldehyde is an aromatic α,β-unsaturated aldehyde, and the major component in essential oils from some cinnamon species. In literature there are many studies focused on active packaging, for example cassava starch and gliadin [15], functionalized with cinnamaldehyde, which showed both antimicrobial and fungicide effectiveness of cinnamaldehyde [16]. It has been shown to exert antimicrobial and antifungal action against a wide number of microorganisms including bacteria, yeasts, and mould [17]. The functionalization of chitosan occurs via Schiff base formation [18]. The essential aspect of this reaction mechanism is its reversibility. Schiff base is reversible under suitable conditions, and this might allow the release of the active cinnamaldehyde from chitosan, used as the carrier [19]. Moreover the addition of nanometric stacks was evaluated in terms of enhancement of the mechanical properties.

2. Materials and methods

2.1. Materials

Chitosan (CS) with low molecular weight (50–190 kDa, deacetylation degree: 75–85%), cinnamaldehyde (CA) and glacial acetic acid were purchased from Sigma Aldrich (Milan, Italy) and used as received. Expandable graphite (GIC) was supplied by Anthracite Industries (Sunbury, USA).

2.2. Preparation of EG

Expanded graphite (EG) was gained by expansion and exfoliation of GIC by heating up to 600 °C for 2 min. EG particles were then added to chitosan solution in order to obtain an EGS-chitosan dispersion [20].

2.3. Preparation of a Schiff base of EGS-chitosan dispersion

The modified EGS-chitosan dispersion *via* Schiff base was obtained by suspending 2 g of CS powder in 100 ml of acetic acid solution (0.1 M). The flasks were immersed in a thermostatic bath at 25 °C and the suspension was stirred gently for two hours with a mechanical stirrer until complete dissolution. Then the EGS dry powders at different concentrations (1.5%, 3%, 6% w/w of dry polymer) were added to chitosan solution under constant stirring. The modification of chitosan *via* Schiff base was achieved by the reaction with cinnamaldehyde at different concentrations (0.1%, 0.25%, 0.5% w/w of dry polymer) under controlled temperature (25 °C). The gels were cast in Teflon trays and dried at 25 °C to obtain films.

2.4. Characterization of the Schiff base

The study of kinetics reaction was followed via viscosity measurements made with a parallel plate rheometer (ARES, Scientific Rheometric) at 25 °C, from 0.1 to 100 s^{-1}. For each CA concentration, independent measurements were performed in triplicate, and the results were expressed as the average, including standard deviation.

A Fourier transform infrared spectrometer (FTIR-6300 Jasco, Easton, MD, USA) was used to assess the efficacy of the functionalization. These analyses were carried out on the samples without any modification at a resolution of 4 cm^{-1}, by 64 scans, at transmittance range from 4000 cm^{-1} to 400 cm^{-1}.

2.5. Morphology: SEM, TEM and X-ray

SEM micrographs were made using a Jeol JSM-6550F instrument. Transmission electron microscopy (TEM) images were taken by a Hitachi H-9000NAR model instrument operated at an accelerating voltage of 100 kV. Samples were prepared by placing a single drop of the suspensions (diluted in deionized water and sonicated before use) onto carbon coated copper grids, dried in air and loaded into the electron microscope chamber.

Wide Angle X-ray diffraction (WAXD) patterns were collected on a PW 1729 Philips, using Cu Kα radiation in reflection mode (λ = 0.154 nm). Samples of natural graphite (NG), expandable graphite (GIC), expanded graphite (EG), expanded and sonicated graphite (EGS) were step-scanned at room temperature from values of 2θ ranging from 1.3–60°. The samples were held in the diffractometer using a socket glass sample holder.

2.6. Mechanical tests

The mechanical properties of the CSCA_EGS specimens were investigated with Z1.0 TH material testing machine (Zwick Roell, Germany), equipped with a 100 N load cell. Specimens for uniaxial tensile tests were cut into strips 10.0 mm wide and 60 mm long, from films with 0.3 mm of thickness, and mounted between the clamps of the tensile tester. The upper clamp was connected to the load cell and to the movable crosshead. Tests were performed under displacement control, with a displacement rate of 1 mm/min. The elastic modulus (E), fracture strength (σ) and elongation at break (ε) were elaborated and recorded by means of TestXpert II software. The elastic modulus was calculated as the initial slope of the stress–strain curve. Three samples from each group were tested to obtain average and standard deviation values of E, σ and ε.

2.7. Preliminary antifungal activity tests

Rhizopus stolonifer was isolated and grown for 2 weeks on agar plates (potato dextrose). Spores were scrubbed from the agar surface plate by means of a glass rod. Then a small volume of sterile solution containing a surfactant (0.5% v/v, Triton X-100) was added. The obtained suspension was gently shaken for 15 s to break any conidial chains and then filtered to remove mycelial fragments. The concentration of the spore was determined using a haemocytometer and adjusted with sterile water to ca. $5 \cdot 10^5$ spores/ml, and stored on ice until use. *In vitro* tests were carried out by inoculating fresh cut white bread slices with a suspension of the selected spores, prepared as described above. Cinnamaldehyde-functionalized EGS-chitosan films with different CA concentrations were used to wrap the slices after each inoculation. In order to prevent water evaporation, each sample was further stored in dark conditions (37 °C) in sealed polyethylene bags.

2.8. Statistical analysis

Two-way analysis of variance (ANOVA), performed by means of StatView software, was used to assess the effect of both cynnamaldehyde (CA) and expanded graphite (EGS) concentrations on the mechanical properties of the films. Fisher's Protected Least Significant Difference (PLSD) tests were applied to compare sets of data. Significance was accepted with $p < 0.05$.