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Modified chitosan: A step toward improving the properties of antibacterial food packages

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ABSTRACT

Current needs for minimally processed food products are spurring the development of antibacterial packages and necessitate new antibacterial additives capable of merging with plastic bulk material. We present a new approach that can potentially improve the physical properties of antibacterial packaging by enhancing the compatibility between the main polymeric ingredient of the package and an antibacterial additive. Enhance compatibility of chitosan, a polysaccharide with known antibacterial properties, was obtained by conjugating polyethylene glycol (PEG) onto chitosan to create PEGylated chitosan. We show that the PEG conjugation does not hamper the antibacterial properties of the chitosan. Moreover, the PEGylated chitosan film exhibited antibacterial activity which was absent in the native chitosan film. Improved compatibility with polyethylene films, leading to better transparency compared to films compounded with native chitosan, is demonstrated. The thermal behavior of the film is governed by the bulk polyethylene due to the small concentration of the PEGylated chitosan, yet the footprint of the additive is detectable. The addition of PEGylated chitosan decreases the Young modulus, which was assumed to be responsible for the compatibilizing effect of the grafted PEG side chains.

1. Introduction

Increasingly, customers are demanding fresh, minimally processed food products. Several areas are rapidly developing as a results of these trends, one example being active packaging (Suppakul, Miltz, Sonneveld, & Bigger, 2003). Active packaging involves the interaction between the package, product and environment in order to prolong product shelf life or enhance its safety, while maintaining its nutritional quality (Suppakul et al., 2003). One class of active packages are antimicrobial packages used to reduce the growth rate and limit the maximum population of microorganisms (Hun, 2000). Most current research in this field focuses on finding ways to combine preservation products and antibacterial additives traditionally used in plastic packaging in the food industry (Cooksey, 2005; Han & Floros, 1997; Oussalah, Caillet, Salmieri, Saucier, & Lacroix, 2004; Perez-Perez, Reralado-Gonzalez, Rodriguez-Rodriguez, Barbosa-Rodriguez, & Villasenor-Ortega, 2006; Weng & Hotchkiss, 1993). Due to their limited compatibility with plastic bulk materials and fast migration arising from their low molecular weight, the use of such products and additives in food packaging is limited (Perez-Perez et al., 2006). Inorganic materials such as silver and copper ions and ammonium based chemicals were found to be effective antibacterial agents, yet concerns regarding their safety have been raised in the last decade.

Some limitations of low molecular weight additives may be overcome by utilizing synthetic or natural antibacterial polymers (Dutta, Tripathi, Mehrotra, & Dutta, 2009; Friedman

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& Juneja, 2010; No, Meyers, Prinyawiwatkul, & Xu, 2007b; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003; Varga & Gonzalea-Martinez, 2010). One such antibacterial polymer is chitosan, a linear polysaccharide derived from the de-acetylation of chitin, the main component of the exoskeleton of crustaceans. Chitosan has been widely studied and used for a broad range of biological applications due to its biodegradability, biocompatibility, low toxicity to mammalian cells, haemostatic activity, anti-tumor activity, wound healing acceleration, and antimicrobial activity (Tang et al., 2010). Chitosan's anti-bacterial activity is believed to be a result of its polyelectrolyte attribute, in particular the presence of positively charged amino groups ($-NH_3+$) located at the C-2 positions in the glucose monomers with a $pK_a \approx 6.3$. The exact mechanism of these actions is still under debate although several mechanisms have been proposed. The most common hypothesis deals with membrane permeability changes occurring upon cationic–anionic interactions that alter bacterial surface morphology (No, Meyers, Prinyawiwatkul, & Xu, 2007a). These interactions change membrane permeability, which promote cell death. Chitosan's activity was found to be effective against both gram-negative and gram-positive bacteria (Tang et al., 2010).

Developing active packages based on common plastic bulk materials such as polyolefin and chitosan is not a trivial task, mainly due to the poor compatibility between hydrophobic synthetic polymers and the hydrophilic chitosan that might damage the material properties (Mir, Yasin, Halley, Siddiqi, & Nicholson, 2011; Prasanna & Sailaja, 2011). Park, Marsh, and Dawson (2010) have incorporated chitosan into low density polyethylene (LDPE) films and demonstrated good antibacterial activity with chitosan concentrations above 1.4% for both Gram-negative and Gram-positive bacteria. However, decrease in tensile strength and in percent elongation as chitosan concentration increased was observed. Several researchers have proposed the addition of a third component to act as a compatibilizer in order to improve film properties. One approach uses a cross-linking process based on peroxide (Mir et al., 2011) or epoxy-functionalized LDPE (Prasanna & Sailaja, 2011). In these studies the compatibilizer active group undergoes reactive blending with the hydroxyl and amide groups of chitosan, leading to better mechanical properties. Since the antibacterial activity was not reported, it is hard to evaluate the effect of the process on this parameter. Rogovina, Alexanyan, and Prut (2011) produced blends of chitin and chitosan with LDPE, as well as ternary blends with polyethylene oxide (PEO) as an additional component. They have reported achieving good mechanical properties; however, antifungal properties were harmed by the PEO addition. Recently, Martínez-Camacho et al. (2013) incorporated ethylene-co-acrylic acid (EAA) molecules to chitosan/polyethylene (PE) mixtures in order to improve chitosan's compatibility and ensure homogeneous dispersion of it in the PE film. Good mechanical and antibacterial activity was demonstrated. Another approach, in which the chitosan salt, chitosonium acetate, was extruded with ethylene based copolymers, ethylene methyl acrylate and ethylene vinyl acetate, was shown to demonstrate antibacterial activity in all samples (Massouda, Visioli, Green, & Joerger, 2012). The mechanical and thermal characteristics of the films were not studied.

We note that chitosan is not a thermoplastic polymer and does not melt during blending (Correlo et al., 2005). As a result, the above mentioned active films are, in fact, composites in which the chitosan is dispersed as filler rather than true polymeric blends.

The current research aimed to develop, synthesize and characterize a new antibacterial material based on chitosan with grafted polyethylene glycol (PEG) chains. Our working hypothesis was that the grafted PEG chains will induce better homogeneous dispersion of chitosan in plastic bulk material due to their low melting temperature of around 70 °C. We report on the synthesis, thermal behavior, and mechanical properties of the additive and of films compounded with it and verify that the antibacterial properties of this new polymer are not hampered, compared to native chitosan. Antibacterial activity was also demonstrated in the PEGylated chitosan based film. In addition, we demonstrate that the chemical modification improves the dispersion of chitosan particles within polyethylene films.

2. Experimental

2.1. Materials

Chitosan with medium molecular weight was purchased from Sigma, Israel. 2-Iminothiolane was purchased from Proteo-Chem, Denver, USA. Polyethylene glycol di-acrylate (PEG-DA) was synthesized in Prof. Seliktar's lab in the Department of Biomedical Engineering, Technion, Israel according to a known procedure (Almany & Seliktar, 2005). Low density polyethylene (LDPE), Ipethene 320, was obtained from Carmel Olefins, Israel. Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and Tris(2-carboxyethyl)phosphine hydrochloride solution (TCEP) 0.5 M were purchased from Sigma and used without further purification. Nutrient Broth medium (NB) was purchased from Fluka, Israel.

2.2. Synthesis of PEGylated chitosan

The synthesis of PEGylated chitosan (chitosan with grafted side chains) was carried out as a two-step procedure involving the synthesis of thiolated chitosan, followed by the conjugation of polyethylene glycol di-acrylate (PEG-DA) to the thiolated chitosan through the sulfide end group. This conjugation, termed the Michael type addition reaction, occurs between molecules carrying an electronegative vinyl end group such as acrylate and electronegative neighboring groups such as sulfide.

The process of synthesizing thiolated chitosan was adopted from Bernkop-Schnurch, Hornof, and Zoidl (2003). In brief, 1 g of chitosan was dissolved in 1 l of 2% acetic–acid aqueous solution. 0.4 g of 2-iminothiolane was added to the solution while the pH was adjusted to 6.3. The reaction was allowed to proceed for 24 h. The final product was dialyzed using a cellulose tube having a 12–14 kDa molecular weight cutoff against 5 mM HCl, two times against 5 mM HCl containing 1% NaCl, once against 5 mM HCl, and finally, once against 1 mM HCl. The final sample was lyophilized by drying a frozen aqueous polymer solution at −30 °C and 0.01 mbar and stored at 4 °C until further use.