

Title Natural antimicrobial lysozyme-chitosan composite films for food preservation
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Abstract

There has been increasing interest in antimicrobial edible packaging materials. Lysozyme is an antimicrobial enzyme found in many natural systems, however, its limited antimicrobial efficacy against gram-negative bacteria restricts its potential application in food industry. The antimicrobial spectrum of lysozyme may be enhanced when it is used with other substances, such as chitosan that is known for its natural antimicrobial function and as a carrier of other functional ingredients. Our objectives were to develop semi-permeable chitosan-lysozyme composite films, to evaluate their basic film properties, and to examine their antimicrobial capability. Two percent chitosan solution in 1% acetic acid was mixed and homogenized with 10% lysozyme solution in concentration of 0, 20, 60 and 100% (w/w chitosan). Films were prepared by solvent evaporation. Barrier and mechanical properties of films were evaluated. Surface and internal structure of films were examined using a scanning electron microscopy. Lysozyme releasing rates were determined by measuring turbidity changes in *Micrococcus lysodeikticus* suspension exposed to each film. *Streptococcus faecalis* and *Escherichia coli* were used for antimicrobial activity measurements. We observed that water vapor permeability of the films was not affected by lysozyme incorporation, while the tensile strength and percent elongation values decreased with increasing lysozyme incorporation. SEM images suggested that lysozyme was homogeneously distributed throughout the film matrix. Lysozyme was logarithmically released from the film matrix and release rates were increased with increasing concentration of lysozyme. All lysozyme containing films effectively inhibited the growth of both test microorganisms. This study demonstrated that a chitosan film matrix could effectively carry a high concentration of lysozyme without causing significant loss in the film functionality. The enhanced antimicrobial activity of lysozyme with chitosan and the synergistic effect of chitosan-lysozyme conjugation may broaden the application of lysozyme in ensuring food safety and quality.