Research Note

Antimicrobial Activity of Allyl Isothiocyanate Used To Coat Biodegradable Composite Films as Affected by Storage and Handling Conditions†

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ABSTRACT

We evaluated the effects of storage and handling conditions on the antimicrobial activity of biodegradable composite films (polylactic acid and sugar beet pulp) coated with allyl isothiocyanate (AIT). Polylactic acid and chitosan were incorporated with AIT and used to coat one side of the film. The films were subjected to different storage conditions (storage time, storage temperature, and packed or unpacked) and handling conditions (washing, abrasion, and air blowing), and the antimicrobial activity of the films against Salmonella Stanley in tryptic soy broth was determined. The films (8.16 μl of AIT per cm² of surface area) significantly (P < 0.05) inhibited the growth of Salmonella during 24 h of incubation at 22 °C, while the populations of Salmonella in controls increased from ca. 4 to over 8 log CFU/ml, indicating a minimum inactivation of 4 log CFU/ml on films in comparison to the growth on controls. Statistical analyses indicated that storage time, storage temperature, and surface abrasion affected the antimicrobial activity of the films significantly (P < 0.05). However, the differences in microbial reduction between those conditions were less than 0.5 log cycle. The results suggest that the films’ antimicrobial properties are stable under practical storage and handling conditions and that these antimicrobial films have potential applications in food packaging.

The demand for minimally processed, easily prepared, and ready-to-eat “fresh” food products, globalization of food trade, and distribution from centralized processing pose major challenges for food packaging. Supermarkets and consumers ask for foods that are pathogen free and of good quality throughout the entire shelf-life period (2). As consumer demands increase on the performance of food packaging for improved safety, quality, and extended shelf life, the protective functions of traditional packaging and packaging materials may not meet those demands. There have been marked developments in recent years in polymeric and edible packaging films with antimicrobial agents incorporated for improving the preservation of packaged foods (5, 8–10). These films possess the potential for improving the microbial stability of food by acting on the food surface.

Our previous study (12) demonstrated that coats of nisin and allyl isothiocyanate (AIT) on biodegradable composite films consisting of polyester and sugar beet exhibited strong antimicrobial activities against foodborne pathogens such as Salmonella and Listeria monocytogenes. However, before those films are applied in the food industry, it is necessary to answer the question of what happens to the antimicrobial activity of the films on their journey from film making to final use in food packaging. In real situations, packaging materials are stored in a warehouse after production and before use. In most cases, packaging materials would be transferred from one location to another. According to FDA requirements (21 CFR 110.35) (7), food packaging materials have to be cleaned by water, steam, or a sanitizer solution and dried by air in or off line before being used to package foods. When a packaging machine is used, packaging materials (films) usually are inserted between rollers and run on belts, which could cause surface abrasion and affect the surface integrity of the film. Numerous articles have been published on the development of antimicrobial films or their potential applications in the food industry. However, very limited information is available on how the storage or handling conditions of those films affect their antimicrobial activity. Therefore, it is necessary to determine how storage and handling conditions affect the antimicrobial activities of antimicrobial packaging materials.

The objective of this study was to investigate the antimicrobial activities of AIT used to coat the surface of biodegradable composite films consisting of polylactic acid (PLA) and sugar beet pulp under simulated storage and transport conditions (storage temperature, storage time, and...
packed or unpacked) and handling conditions (water washing, air blowing, and abrasion).

**MATERIALS AND METHODS**

**Chemicals.** The PLA (polymer 4060D) used for composite films and surface coating was obtained from Nature Works LLC (Minnetonka, MN). Methylene chloride was purchased from Fisher Scientific (Barrington, IL). Chitosan (150 kDa, 75 to 85% deacetylation) and AIT (>95% purity) were purchased from Sigma Aldrich (St. Louis, MO). Acetic acid was purchased from Mallinckrodt (St. Louis, MO).

**Preparation of biodegradable composite films.** The biodegradable composite films, consisting of PLA and sugar beet pulp, were prepared as described in our previous study (12).

**Preparation of antimicrobial coating solutions.** Two antimicrobial coating solutions were prepared, PLA plus AIT (PLA + AIT) and chitosan + AIT. The PLA + AIT coating solution was prepared by adding 200 mg of PLA resin to 10 ml of methylene chloride with 200 µl of AIT, and the chitosan + AIT coating solution was prepared by adding 200 mg of chitosan powder to 10 ml of acetic acid solution (1%, vol/vol) with 200 µl of AIT. These mixtures were stirred with a magnetic stir bar overnight until the polymers were completely dissolved.

**Preparation of antimicrobial films.** The composite film was cut to fit a glass petri dish (5 cm by 1.5 cm). Four milliliters of each coating solution was spread on the circular film in the petri dish so that an even coating (~0.05-mm thickness) was formed on one side of the substrates. Each dish was placed in a chemical hood for 24 h, allowing the solvent or water to evaporate, and then films were peeled from the dishes and stored in a plastic bag until time of use (within 3 days). Each film had 19.6 cm² of total coated surface area. The amount of AIT in the coated films was calculated to be 8.16 µl/cm² of surface area.

**Preparation of inoculum.** Salmonella Stanley H0558, which was associated with several outbreaks of food contamination, was selected for this study. Salmonella Stanley was obtained from the culture collection of the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA. The culture was maintained in tryptic soy broth (TSB; BD, Franklin Lakes, NJ) at 2°C and transferred bimonthly. Prior to inoculum preparation, Salmonella Stanley was grown in TSB aerobically at 37°C for 16 to 18 h.

**Storage tests.** The film samples were divided into four groups: (i) packed in polyethylene bag (15 cm by 10 cm), sealed, and stored at 4°C; (ii) packed in polyethylene bags, sealed, and stored at 22°C; (iii) placed on a plate and stored at 4°C; and (iv) placed on a plate and stored at 22°C. All films were stored for 4 weeks and sampled at 0, 2, and 4 weeks for their antimicrobial activity against Salmonella in TSB broth, as described in "Antibacterial tests" below.

**Water washing, abrasion, and air blowing of films.** To simulate washing, abrasion, and air blowing conditions during film cleaning and packaging, the following tests were conducted at room temperature.

Film samples were immersed in water (200 ml) for 2 min. The washed films were then held vertically under a chemical hood overnight to allow water to evaporate. The washed or unwashed films were used for antibacterial tests.

Two devices were set up in a chemical hood. Film samples were placed in a glass petri dish with the antimicrobial layer up. For abrasion, 10 small magnetic stirring bars (1 cm long) were loaded onto the surface of the film and the film placed on a stirring plate with stirring at 550 rpm for 4 h. For air blowing, a funnel was set 1 cm above the films to ensure even distribution of air on films. The end of the funnel was connected to a compressed-air source with a flow rate of 150 cc/min. The films were air blown for 4 h. The treated and nontreated films (controls) were used for antibacterial tests.

**Antibacterial tests.** The antimicrobial activities of coated films after each treatment were tested using a liquid release method as described by Jin and Zhang (11). Each film sample was cut into 10 pieces, and both sides of the film sample were exposed to UV light (germicidal ultraviolet irradiator, Atlantic Ultraviolet Corporation, Hauppauge, NY) for 5 min at 8 mW/cm² to eliminate possible microbial contamination. Ten pieces of film samples were randomly placed in two test tubes; each test tube contained five pieces of film (9.8 cm² of total coated surface area) and 10 ml of TSB inoculated with overnight cultures of Salmonella Stanley with cell concentrations of ca. 1 × 10⁸ CFU/ml. The test tubes were held at room temperature (22°C) and shaken at 100 rpm. The inoculated medium was sampled (1 ml) at 24 and 48 h. Samples were serially diluted with sterile 0.1% peptone water and surface plated (100 µl per plate and two plates per dilution) onto tryptic soy agar (BD). All plates were incubated at 37°C, and CFU were counted after 24 h. Inoculated medium without film samples served as controls. The antimicrobial activity of each film was expressed as the ability to reduce the growth of Salmonella after 24 and 48 h in comparison with the growth on controls and was calculated as Na - Nf, where Na is the log CFU per milliliter from each control and Nf is the log CFU per milliliter from each treated sample at 24 or 48 h.

**Statistical analysis.** All experiments were conducted independently twice using three film samples per treatment, according to the experimental design, for a total of six data points per treatment. Data were pooled and analyzed using analysis of variance (ANOVA) with SAS version 9.1 software (SAS Institute, Cary, NC). Duncan’s multiple range test was used to determine the significant differences of mean values (n = 6). Significance was defined at P ≤ 0.05.

**RESULTS AND DISCUSSION**

There were no significant differences between the levels of microbial inactivation of Salmonella Stanley at 24 h and at 48 h for all the samples. Hence, the results at 24 h are reported in Tables 1 and 2. The effects of storage conditions on antimicrobial activity of films coated with PLA + AIT or chitosan + AIT are shown in Table 1. ANOVA analysis indicates that the most significant factor was storage time, followed by storage temperature and whether or not the film was stored in pouches (data not shown). Except for the samples stored at 22°C in bags for 2 weeks, higher storage temperature or longer storage time reduced microbial inactivation of films. However, there was less than 0.5 log CFU/ml difference between 0 and 4 weeks storage times or 4 and 22°C storage temperatures.

The microbial inactivation before and after water washing was 5.42 and 5.24 log CFU/ml for PLA + AIT and 5.20 and 5.19 log CFU/ml for chitosan + AIT coatings,
TABLE 1. Microbial inactivation of Salmonella by antimicrobial coatings as affected by storage conditions

<table>
<thead>
<tr>
<th>Coating</th>
<th>Storage time (wk)</th>
<th>4°C</th>
<th>22°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In air</td>
<td>In bag</td>
<td>In air</td>
</tr>
<tr>
<td>PLA + AIT</td>
<td>0</td>
<td>5.61 ± 0.05 a</td>
<td>5.61 ± 0.05 ab</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.63 ± 0.07 a</td>
<td>5.67 ± 0.01 a</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.54 ± 0.04 b</td>
<td>5.58 ± 0.04 bc</td>
</tr>
<tr>
<td>Chitosan + AIT</td>
<td>0</td>
<td>5.62 ± 0.04 A</td>
<td>5.62 ± 0.04 A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.65 ± 0.03 A</td>
<td>5.64 ± 0.13 A</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.48 ± 0.14 B</td>
<td>5.48 ± 0.05 b</td>
</tr>
</tbody>
</table>

* Microbial inactivation equals log CFU per milliliter of control minus log CFU per milliliter of treated film samples in TSB incubated at 22°C for 24 h. Data are means ± standard deviations (n = 6). Means in the same column and coating with different letters are significantly different (P < 0.05).

respectively. Washing did not have statistically significant effects on the antimicrobial activity of each film (P > 0.05).

Abrasion significantly affected the antimicrobial activity of films with both coatings; the microbial inactivation was decreased from 4.57 to 4.04 and from 4.35 to 4.15 log cycles for PLA + AIT and chitosan + AIT coatings, respectively (Table 2). This result was expected as the antimicrobial activity of films relied on the thin layer on the film surface. After abrasion, the active surface layer was damaged to some degree; therefore, the antimicrobial activity was reduced. However, similar to the storage tests, the difference in microbial reduction before and after abrasion was ca. 0.5 log cycle.

Table 2 also lists the microbial inactivation of antimicrobial films before and after air blowing. It was expected that AIT would be sensitive to air blowing because it is a volatile compound. However, only AIT in the chitosan coating was significantly affected. This may be due to the different coating materials and methods, which need to be further studied.

AIT is a major essential oil component of cruciferous plants, such as cabbage, broccoli, mustard, and horseradish. It has long been used as a pungent food flavoring agent (14). However, the application of AIT in foods has been limited because of its high volatility, strong pungency, poor water solubility, and easy decomposition by reactions with many natural food nucleophiles, such as amines, amino acids, proteins, alcohols, and sulfites (3, 4, 6). Hence, direct addition of AIT to foods would significantly reduce its antimicrobial effectiveness. These limitations could be effectively overcome by physically entrapping AIT molecules within an inert biopolymer matrix.

There are various ways to incorporate antimicrobial compounds into polymers. Antimicrobial compounds, such as volatile essential oils, that cannot tolerate the high temperatures used in polymer-processing methods like extrusion and injection molding are often either used as a coat that is applied to the film material after forming or are added to cast films (1). In this study, the antimicrobial layer was used as a coat on pre-extruded films, which provided several advantages over the one-step method, including (i) less concern for the loss of antimicrobial activity during thermal film making; (ii) reduced amount of antimicrobial compounds used in packaging material, since only the outer layer plays an antimicrobial role; and (iii) avoidance of "filler effect" that could breech the integrity of the plastic films and cause structural changes, and minimal impact on the physical or mechanical properties of the base materials. In our previous study, PLA + AIT or chitosan + AIT coatings on the composite films only caused minor structural changes (12), while the inclusion of EDTA and nisin directly into PLA blends significantly reduced the mechanical properties of the films and caused them to become less flexible (13).

The results from this study demonstrate that the films with PLA + AIT and chitosan + AIT coatings can inhibit the growth of Salmonella by 4 log or more in comparison to its handling conditions had statistically significant effects on

TABLE 2. Microbial inactivation of Salmonella by films as affected by surface abrasion and air blowing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of sampling</th>
<th>PLA + AIT</th>
<th>Chitosan + AIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrasion</td>
<td>Before treatment</td>
<td>4.57 ± 0.26 a</td>
<td>4.35 ± 0.15 a</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>4.04 ± 0.09 b</td>
<td>4.15 ± 0.06 b</td>
</tr>
<tr>
<td>Air blowing</td>
<td>Before treatment</td>
<td>4.49 ± 0.04 A</td>
<td>5.51 ± 0.06 A</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>4.46 ± 0.05 A</td>
<td>5.09 ± 0.05 b</td>
</tr>
</tbody>
</table>

* Microbial inactivation equals log CFU per milliliter of control minus log CFU per milliliter of treated film samples in TSB incubated at 22°C for 24 h. Data are means ± standard deviations (n = 6). Means in the same column and coating with different letters are significantly different (P < 0.05).
the antimicrobial activity of the films, the difference between the maximal and minimal microbial inactivation was less than 0.5 log cycle, which could be negligible in industrial applications. Therefore, we conclude that the antimicrobial properties of the films developed in this study would be stable under practical storage and handling conditions. These films can be used for food packaging and allow migration of AIT into the food or the headspace inside the package to inhibit the growth of microorganisms. Further investigation of these films in real food systems will be conducted. To the best of our knowledge, there are limited data in the literature regarding the effects of storage or handling conditions on antimicrobial films. Therefore, the results from this study could be a useful reference for others in this research area.

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