1 **Physiochemical Properties and Food Application of Antimicrobial PLA**

2 **Film**

3
4 Haiyan Gao^a, Xiangjun Fang^a, Hangjun Chen^a, Yanhong Qin^a, Feifei Xu^a, Tony Z. Jin^b

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6 ^a Institute of Food Science, Zhejiang Academy of Agricultural Sciences, Key Laboratory of
7 Fruits and Vegetables Postharvest and Processing Technology Research of Zhejiang Province,
8 Hangzhou, 310021, China

9 ^b Eastern Regional Research Center, Agricultural Research Service, U.S. Department of
10 Agriculture, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA.

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20 Authors for correspondence:

21 Jin: Tel.: 215-836-6904; Fax: 215-233-6559; E-mail: tony.jin@ars.usda.gov.

22 Gao: [Tel: 0086-571-86404378](tel:0086-571-86404378); Fax: 0086-571-86406661; E-mail: spsghy@163.com.

Abstract

Antimicrobial polylactic acid (PLA) films releasing allyl isothiocyanate (AIT) vapor were developed. The physicochemical properties and water vapor and gas permeability characteristics of the films were investigated, and the antimicrobial properties in vapor phase in food application were evaluated. The results reveal that the resultant antimicrobial (AM) PLA films had less strength, less stiffness and more flexibility, a lower O₂ and CO₂ transmission rate, and a higher UV absorption rate than the pure PLA films. Avrami's equation showed the best fit with experimental release data ($R^2 > 0.91$) and indicated the release kinetics of AIT from the AM PLA film could be a combined mechanism of diffusion and first-order model. AM film treatment (4 x 8 cm film in 1 liter box) significantly ($p < 0.05$) inhibited total aerobic bacterial growth in fresh pak choi stored at 4 and 10°C for 15 days. The AM film treated pak choi had less color change and chlorophyll loss than the control samples during storage. The AM PLA film has the potential to be utilized for extending the shelf-life of various perishable food products.

Key words: antimicrobial packaging, essential oil, controlled release, polylactic acid, shelf life, fresh produce.

Chemical compounds studied in this article

Allyl isothiocyanate (PubChem CID: 5971); Polylactic acid (PubChem CID: 3081262); Methylene chloride (PubChem CID: 6344); Tributyl citrate (PubChem CID: 6507).

1. Introduction

The demand for safe, minimally processed, ‘fresh’ food products presents major challenges for the food-packaging industry to develop packaging materials which will be useful for maintaining the safety and quality of foods. The use of antimicrobial packaging is one of the approaches used to extend the shelf-life of minimally processed foods and reduce the risk of pathogen contamination. There is growing interest in the use of natural and renewable materials to produce antimicrobial food packaging. Plant extracts and biopolymers have been widely studied and reviewed for their food applications (Burt, 2004; Negi, 2012; Tharanathan, 2003).

Allyl isothiocyanate (AIT) is one of plant essential oils (EO). It is considered a natural antimicrobial agent for food preservation and is known for its volatility and strong antimicrobial activity. It is capable of inhibiting the growth of fungal and bacterial pathogens on plant seeds, fresh produce, bread, meat, and cheese (Lee et al. 2009; Li et al. 2007; Nadarajah et al. 2005; Nielsen and Rios 2000). Like all essential oils, however, large amounts of AIT in the liquid phase must be directly applied on foods in order to achieve the desired antimicrobial effect. This, along with the volatility and strong, pungent odor associated with AIT, could negatively affect the organoleptic properties of food. Numerous studies have shown that AIT can be incorporated into biopolymers such as chitosan to make edible food coatings or packaging films in order to extend the shelf-life and reduce pathogenic contamination in various types of food, such as cantaloupe (Chen et al. 2012), ready-to-eat meat (Guo et al. 2015; 2014a; 2014b), shrimp (Guo et al 2013a; 2013b), and tomatoes (Jin and Gurtler 2012). Other alternative methods which could increase the

antimicrobial efficacy of AIT in foods by using the lowest concentrations possible need to be developed. The use of essential oils by vapor contact has been proposed as an effective approach. Several studies have demonstrated that essential oils applied in vapor phase could be effective against foodborne pathogens and spoilage microorganisms at relatively lower concentrations than when applied in liquid phase, resulting in a less detrimental effect on the sensory attributes of food (Inouye et al. 2006; López et al. 2007; Nedorostova et al. 2009; Nielsen and Rios 2000; Suppakul et al. 2003; Tyagi and Malik 2011).

Polylactic acid (PLA) polymers, which are derived from renewable resources such as corn, wood residues or other biomass, are of current interest not only because of the need to ultimately replace many petroleum-based polymers but also because of their potentially useful physical and mechanical characteristics. PLA polymers have been used in food packaging for a decade and recently PLA was also used as an antimicrobial carrier for antimicrobial packaging and coating. Jin and Zhang (2008) incorporated nisin into the PLA polymer and studied the retention of nisin activity and the antimicrobial effectiveness of the nisin/PLA film against foodborne pathogens in BHI Broth, orange juice, and liquid egg white. In another study, Jin and Niemira (2011) developed a PLA coating solution containing multiple antimicrobials to reduce *E. coli* O157:H7 or *S. stanley* populations on whole fresh apples. Glass jars or PEF bottles were coated with a mixture of polylactic acid (PLA) polymer and antimicrobial compounds and the efficacy of bottle coatings in inactivating foodborne pathogens and spoilage microorganisms in liquid egg white and fruit juice were investigated (Jin et al. 2014; Jin and Gurtler 2011). Ray et al. (2012) developed gaseous chlorine dioxide-releasing antimicrobial films by incorporating sodium chlorite and citric

acid powder into PLA polymer. However, limited information is available regarding antimicrobial PLA films with AIT and their physicochemical and antimicrobial properties.

The objectives of this study were to 1) develop antimicrobial packaging materials incorporated with EOs; 2) evaluate their physicochemical properties; 3) and investigate their antimicrobial properties in vapor phase in food application. AIT was used as a model for EOs and pak choi (*Brassica rapa* L. ssp. *chinensis*) was used as a model for food application.

2. Materials and Methods

2.1 Preparation of PLA films

PLA films were prepared according to the solvent-casting method used by Jin and Zhang (2008) with some modifications. One gram of PLA resin (Natureworks, Minnetonka, MN, USA) was dispersed in 20 mL of methylene chloride (Fisher Scientific, Fairlawn, NJ, USA). This mixture was stirred by a magnetic bar until the polymer was totally dissolved. Predetermined amounts of tributyl citrate (TBC, Aladdin Industrial Corporation, Shanghai, China) and allyl isothiocyanate (AIT, Aldrich Chemical Co., Milwaukee, WI, USA) were added into the PLA solution and mixed. The solution was distributed into Teflon Petri dishes 10 cm in diameter and 1.5 cm deep (Performance Plastics, Wayne, NJ, USA). The methylene chloride was allowed to evaporate at 25°C under a chemical hood for 24 h to ensure no solvent residual in the films. After 24h, the films were peeled from the dishes, placed in a sealed bag, and stored at 4°C until time of use. The thickness of PLA film was determined using a spiral micrometer (Yiyu Mechanical Technology Co., Ltd., Shanghai, China). Measurements were made at 20 different spots and averaged.

2.2 Mechanical properties of PLA films

Tensile strength at break and percentage elongation of PLA films were determined using TA.XT *plus* Texture Analyzer (Stable Micro system, Surrey, U.K.). The film size was 10 mm×150 mm, and the crosshead speed was 20 mm/min. Other test parameters were set as follows: pre-test speed 1.0 mm/s, test speed 1.5 mm/s, and post-test speed 10.0 mm/s, trigger force 0.049 N, distance 500 mm. Three replicates were performed for each sample.

2.3 Scanning Electron Microscopy

The film samples were cut with surgical scissors into 3 × 5 mm pieces and mounted directly on specimen stubs with double-sided adhesive tape. The mounted film samples were vacuum-coated with gold sputtering layer. Digital images of topographical features of the film strips were collected using scanning electron microscopy (TM3000, Hitachi Limited, Japan) and Image J analysis software (Image J, National Institutes of Health, USA).

2.4 Differential Scanning Calorimetry (DSC)

DSC was performed on the film samples using DSC 1 (Mettler Toledo, Schwerzenbach, Switzerland). The samples (0.5 mg) were crimp-sealed in 40 µL stainless steel pans. The instrument was purged with nitrogen. Thermal scanning was performed by heating the sample from 20°C to 200°C at scanning rate of 10°C/min, then cooled from 200°C to 20°C. Thermal parameters were determined using the instrument software.

2.5 Fourier transform infrared (FT-IR) spectroscopy and UV-VIS spectra of PLA films

Approximately 2 mg of PLA films and 25 mg of dry potassium bromide (KBr) crystal were mixed and ground into small granules less than 2 μm in diameter before the FT-IR analysis. Attenuated total reflection (ATR) FT-IR spectra were obtained using a Bruker Tensor 27 FTIR spectrometer (Bruker Optics, Billerica, MA, USA) equipped with an ATR accessory (ZnSe crystal, 45°) in the frequency range of 4000-400 cm^{-1} . Spectra were collected with a resolution of 4 cm^{-1} , and an average of 32 scans for each sample.

UV-visible spectra (UV-VIS) of PLA films were determined using a Cintra10e UV-VIS-NIR spectrophotometer (GBC Scientific Equipment Pty Ltd., Melbourne, Australia) in the wavelength range of 200-900 nm.

2.6 Gas and water vapor permeability

Oxygen (O_2) and carbon dioxide (CO_2) permeability of film samples was measured using a Gas Permeability Tester (Lab BTY-B1 Model, Labthink, China). Film samples with a size of 120 $\times$ 120 mm were prepared and conditioned a desiccator with anhydrous CaCl_2 for 24 h before use. The test chamber was set at $23 \pm 2.0^\circ\text{C}$ with relative humidity of $50 \pm 5\%$. Three replicates were conducted for each sample.

Water vapor permeability (WVP) was measured using ASTM (1993) procedures. Briefly, deionized water was filled into round mouth conical plastic cups with a diameter of 60 mm and a height of 90 mm. Then, films with a thickness of about 50 μm were placed over the top of the cups and sealed on cup edge. There was a 4 mm distance between the film and water

surface. The cups were placed in a temperature chamber at $40 \pm 1.0^{\circ}\text{C}$ and $90 \pm 2\%$ RH for 48 h and then the weight losses were measured. The result of water vapor permeability was calculated by the following equation:

$$\text{WVP} = G/tA,$$

where G is the weight change in grams; t is the duration of test in days and A is the test area in m^2 . Six replicates were conducted for each WVP measurement and the results were averaged and reported as $\text{g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$.

2.7 Allyl-isothiocyanate (AIT) release kinetics

Films were cut into $10 \text{ mm} \times 30 \text{ mm}$ pieces and placed in a petri dish (70 mm in diameter and 35 mm in height). Each dish contained 0.5 g of film sample. The dishes were then sealed with caps and incubated at 4, 10, and 20°C , respectively. After the set period of incubation dishes were withdrawn from the incubator and the film samples in the dishes were used for AIT analysis. The residual of AIT in films was determined by dissolving 0.2 g of PLA film samples in 50 ml methylene chloride, and then the absorbance of the resulting solution was recorded using a spectrophotometer at 245 nm. The readings were converted to the AIT concentrations referring to a predetermined standard curve. Triplicate experiments were conducted for each sample. The residual (R) of AIT in film after release was defined as shown below:

$$R = [1 - (C_i - C_t)/C_i] \times 100\%,$$

where C_i is initial concentration of AIT in film and C_t is concentration of AIT in film at time t .

2.8 Food preparation, package, film treatment and storage

Pak choi (*Brassica rapa* L. ssp. *chinensis*) was purchased from a local supermarket (Hangzhou, China), transported within 30 min to the laboratory and allowed to equilibrate at 6-8°C for 3 h. Damaged and yellow colored leaves were discarded. Pak choi samples were washed with running tap water to remove any dirt. Approximately 80 g of pak choi were placed in a PET box (125 × 165 × 64 mm) with a 4 × 8 cm² antimicrobial PLA film sample on the inner side of the box lid, as shown in Figure 6. Pak choi samples placed in PET boxes without film samples served as controls. All samples were stored at 4 and 10°C for 15 days. The changes in microbial populations, color and chlorophyll contents in samples were determined at 0, 3, 6, 9, 12, and 15 d, according to the methods described below.

2.8.1 Color determination

The color of pak choi leaves was determined using a handheld portable Chroma Meter (HYGROLAB CR-400, Konica Minolta, NJ, USA). Prior to use, black and white standard tiles were used to calibrate the instrument using those that came with the instrument. Ten measurements were performed for each treatment and storage time. L^* , a^* , and b^* values were recorded. The color changes during storage was represented as ΔE , and calculated using the following equations:

$$\Delta E = \sqrt{(a^* - a_0^*)^2 + (b^* - b_0^*)^2 + (L^* - L_0^*)^2}$$

where L_0^* , a_0^* , and b_0^* are the control values at day 0, and L^* , a^* , and b^* are the values for the treated samples at each storage time.

2.8.2 Chlorophyll measurement

Chlorophyll contents in pak choi leaves were estimated nondestructively using a portable chlorophyll meter (SPAD-520, Minolta Co. Ltd, Tokyo, Japan). Three leaves were randomly selected from each box and four readings per leaf were recorded. The results were reported as milligram per gram of fresh weight.

2.9 Microbiological analysis

The total aerobic bacterial load on pak choi samples was evaluated using the standard plate count method. Total plate count agar (PCA, Shanghai Dingguo Biotechnology Co. China) was used to determine total aerobic bacterial counts. A 25 g sample from each box was diluted in 225ml of sterile distilled water and homogenized using a sterilized kitchen blender homogenizer at 8,000 rpm for 2 min. Each sample was serially diluted and 1 mL of each dilution was transferred to a sterile petri dish. About 20 mL of sterile total plate count agar kept at temperature 40 - 45°C was poured into each petri dish, mixed well and allowed to set. Dishes were incubated at $36 \pm 1^\circ\text{C}$ for 48 h and then colony forming units (CFU) were counted and multiplied by the appropriate dilution factor to obtain total plate counts.

2.10. Statistical analysis

The experiments were triplicate and all data were expressed as means $\pm$ standard errors of triplicate measurements and analyzed by SAS software (SAS Institute, Cary, NC, USA). Least Square Differential method (LSD) was performed to analyze the significant differences

between control and treated samples. Significant differences were defined at $p < 0.05$.

3. Results and Discussion

3.1 Physical Properties of Films

3.1.1 Thermal Properties. The DSC thermograms of the PLA films are shown in Figure 1.

The glass transition and the crystalline status of test specimens are summarized in Table 1.

The glass transition temperature (T_g) and melting temperature (T_m) of PLA were found to be about 60 and 166°C, respectively. The addition of TBC reduced the T_g and T_m of the resultant films to 40.66 and 162.74 at 7.5% TBC, respectively. However, increasing the concentrations of TBC to 10% resulted in an increase in T_g , therefore, the formula with 7.5% TBC was used for incorporation of AIT. The inclusion of AIT further increased the T_g , and decreased the T_m . The peak of each sample broadened toward lower temperatures, and the onset of the transition also moved toward lower temperature ranges. Correspondingly, the T_g for each of the blends was also shifted to lower temperatures. The lowering of the T_g and T_m of the AM PLA films could be attributed to the presence of TBC (Table 1), which reduced the macromolecular interactions.

3.1.2 Mechanical Properties. The mechanical properties of PLA films are presented in Table

2. Increased TBC concentrations significantly reduced the tensile strength (TS) of the PLA films. The addition of AIT would not significantly influence the tensile strength (TBC+AIT vs 7.5%TBC). In contrast, increased TBC concentrations significantly increased the

elongation at break with a maximum value reached when TBC concentrations were at 7.5% (7.5%TBC). Similar to TS, the addition of AIT to 7.5%TBC had no significant effect on the elongation of the films (TBC+AIT vs 7.5%TBC). The effect of TBC concentrations on the elongation is corresponded to the effect on Tg, where the lowest Tg was found at 7.5% TBC (Table 1). Therefore the resultant AM PLA films had less strength, less stiffness and more flexibility, compared with pure PLA films. TBC is a plasticizer often used in plastic industries. The inclusion of TBC into PLA altered the brittle nature of PLA by increasing its flexibility, and thus the PLA/TBC blends were tougher than pure PLA specimens, which was expected because of the softening effect.

3.1.3 Permeability Characteristics. Table 2 also shows the water vapor, and O₂ and CO₂ transmission rates of PLA films. The water vapor transmission rate was significantly increased as TBC concentrations increased. The AM PLA film with 7.5% TBC and AIT had lower WVTR value than pure PLA film, although the difference was not significant ($p>0.05$). The increase in TBC concentrations resulted in lower oxygen and carbon dioxide transmission rates and addition of AIT further reduced oxygen permeability. The AM PLA films with TBC and AIT had significantly ($p<0.05$) lower O₂ and CO₂ transmission rates than the pure PLA films.

Based on the results of the thermal and mechanical properties and gas permeability characteristics, AM PLA film with 7.5% TBC and 5% AIT (TBC+AIT) was selected for further evaluation in comparison with pure PLA film.

3.1.4 SEM Morphology. The SEM images of pure PLA film and AM film (PLA+TBC+AIT) (cross section) are shown in Figure 2. A homogeneous, smooth morphology was observed for the blends of PLA+TBC+AIT (Figure 2A), indicating that three compounds were highly compatible. Micro pores could be clearly observed on the pure PLA film (Figure 2B). The pores were possibly created by the mixing and separation of air in the solution during stirring and solvent evaporation, respectively, resulting in the formation of a heterogeneous structure (Liu et al. 2009). The SEM images provide the evidence that AM films had lower gas transmission rates than the pure PLA films as presented in Table 2.

3.1.5 Fourier Transform Infrared Spectroscopy. Figure 3 showed the FTIR spectra of the pure PLA (A) and AM PLA films (B). The spectrum was classified into three regions, which correspond to the following peaks band assignment: --C=O ester carbonyl (1756 cm^{-1}), --CH-- deformation (1453 , and 1366 cm^{-1}), and --C--O-- stretch ($1000 \sim 1200\text{ cm}^{-1}$). The FTIR spectrum of PLA films is similar to PLA plastic cast disk from a 4% (w/v) solution in chloroform (FTIR). The IR spectral bands are not affected by the inclusion of TBC and AIT significantly. Two possible explanations for this are 1) the concentrations of TBC and AIT are low; 2) there is no specific chemical interaction between TBC/AIT and PLA, and they are therefore immiscible, suggesting that AIT was not chemically bonded to PLA, allowing for the release of AIT from the film.

3.1.6 UV–Vis Light Absorption. Figure 4 shows UV–vis absorption spectra of the films. An absorption peak for pure PLA film was centered at about 230 nm. The AM PLA film with

AIT and TBC shifted the peak to a slightly longer wavelength and centered at about 240 nm. The AM PLA film had a significantly higher UV absorption rate (3.5 X) than the pure PLA film.

3.2 AIT Release Kinetics

The release of AIT from the films were determined at 4, 10 and 20°C. These temperatures were representative of refrigeration temperature, temperature abuse, and room temperature, respectively. Figure 5 shows the release profiles of AIT up to 16 days. Avrami's equation was applied to demonstrate the release kinetics of AIT from the AM PLA film. This model is described by the following equation:

$$R = \exp [-(kt)^n],$$

where, R is the residual of AIT at time t ; k is the release rate constant and n is a parameter representing the release mechanism. The equation can be rewritten by double logarithm as follows:

$$\ln (-\ln R) = n \ln k + n \ln t.$$

A linear plot of $\ln (-\ln R)$ versus $\ln t$ from data in Figure 5 allows to determine the release rate constant k (intercept) and release parameter n (slope) that are listed in Table 3.

Our results clearly showed that the release kinetics of AIT depend on temperature, as the release rate constant k values increased as the temperature increased. As shown in Table 3, n values were found in the range from 0.64 to 0.90. The n value characterizes the release mechanism that describes the shape of a release time-course. Theoretically, the parameter $n = 0.54$ indicates a diffusive release and $n = 1$ a first-order release kinetics (Shiga et al 2001).

Hence, the release kinetics of AIT from the AM PLA film could be a combined mechanism of diffusion and first-order model. The obtained results were successfully fitted with the Avrami's equation, as the correlation coefficient R^2 for each test temperature exceeds 0.91.

3.3 Food Application of AM PLA Film

Pak choi was used as a model for fresh produce. A piece of AM PLA film was stuck on the inner side of the lid of the PET container before it was closed, as highlighted in Figure 6. Microbial growth and quality changes in pak choi were monitored during storage at 4 and 10°C. Table 4 summarizes the results of microbial growth and quality changes in pak choi during storage.

3.3.1 Microbial Growth. Populations of mesophilic aerobic bacteria of up to 8 log₁₀ cfu/g were commonly reported on fresh leafy vegetables, which including *Pseudomonas*, *Erwinia*, *Xanthomonas*, and *E. coli* (Barth et al. 2009; FAO 2008). Therefore, total plate count (TPC) method was used in the present study to determine the antimicrobial efficacy of the films. Bacterial populations increased during storage for all samples (Table 4). Samples with AM PLA film treatment had significantly ($p<0.05$) lower cell populations than controls regardless of storage temperature after 3 days. After storage for 15d, total plate counts in pak choi samples subject to film treatment at 4 and 10°C were 7.18 and 8.15 log CFU/g, and 9.58 and 10.34 log CFU/g in the controls at 4 and 10°C, respectively. Pak choi samples subject to film treatment had lower bacterial populations over the 15d storage period compared to the

controls, regardless of storage temperature. The AM PLA film was effective in inhibiting microbial growth at both temperatures.

3.3.2 Color Change. Color changes during storage occurred for all samples (Table 4). The total color difference ΔE was used to evaluate the degree of changes compared to original colors. In general, control samples stored at 10°C had the greatest color change during storage, and the rest had statistically similar color changes. After storage for 15d, the ΔE values in pak choi samples subject to film treatment at 4 and 10°C were 4.68 and 7.77, and 9.30 and 15.8 in the controls at 4 and 10°C, respectively. The AM PLA film improved the color stability of fresh pak choi during storage at both storage temperatures.

3.3.3 Chlorophyll Content Change. The chlorophyll contents in AM PLA film treated samples were well maintained for 9d regardless of storage temperature, after which they gradually decreased, while the chlorophyll contents in control samples started to decrease after 6 days storage, especially for those stored at 10°C (Table 4). After 15 days, the chlorophyll contents for all samples were not statistically different except for the control samples stored at 10°C (CK-10C). These results were similar to the total color difference ΔE . This is probably because the degradation of chlorophyll resulted in the yellowing of pak choi samples, leading to a greater total color change compared with the samples before storage. The b value (yellowness) for control samples stored at 10°C (CK-10C) was much higher than for the other samples (data not shown).

4. Discussion

Microbial contamination can occur at almost any point in the food production process before packaging. Conventional packaging does not stop the growth of pathogens and spoilage microorganisms on food after packaging and before consumption. Therefore, antimicrobial packaging provides the final hurdle to prevent microbial growth and food deterioration. In this study, the AM PLA film significantly inhibited the growth of total aerobic bacteria in pak choi over 15 days at 4 and 10°C compared to controls. The film treated samples also had the least color change and chlorophyll loss (Table 4). The typical pungent odor of AIT was not noticed on pak choi after storage. This may be due to the small size of the AM PLA films used in this study as well as the low AIT concentrations present in the films. These results indicate that it is necessary to subject vegetables to antimicrobial treatment to maintain their quality and extend their shelf-life, particularly when they are stored at high temperatures. Guo et al (2015) demonstrated that the antimicrobial effect of AIT-chitosan film is dependent on the concentration of AIT in film and the size of film used. The increase in film size allowed for greater concentrations of AIT to be incorporated into the films, resulting in the achievement of a greater antimicrobial effect. Their observations should apply in this study; if a larger sized film (> 4 x 6 cm) or AIT concentrations greater than 7% were used, a greater antimicrobial effect could be expected. Further studies are needed to verify this. The developed AM PLA films can be applied as a “patch” as shown in this study and placed inside any bags or containers used for food packaging. In addition, the film can be used as stand-alone packaging material for bags, boxes, or bottles. For stand-alone packaging materials, mechanical and gas permeability

properties are important for their applications. In this study, the resultant AM PLA films had less strength, less stiffness and more flexibility, compared with pure PLA films (Table 2) due to the plasticizing effect of TBC and AIT, which suggests that the AM PLA film is similar or superior to pure PLA film that is used as regular packaging material.

Food deterioration during storage can be microbiological and biochemical. Food lipids are food components that are very susceptible to oxidation processes. Oxidation reactions are one of the major causes of food deterioration during the manufacturing, storage, distribution and final preparation of foods (Johnson and Decker 2015). The oxidation of food products can not only reduce their shelf life, but also can be hazardous to human health. Lipid oxidation is caused by the presence of oxygen in the package and promoters such as UV light exposure. Therefore, oxygen and UV light barriers are needed for food, particularly those with high amounts of lipids.

The AM PLA films had similar water vapor but significantly ($p < 0.05$) lower O_2 and CO_2 transmission rates than the pure PLA films. In other word, the AM PLA film had better gas barrier than pure PLA film (Table 2). A decrease in O_2 permeability of packaging film would allow for the blockage of oxygen penetration into the food product, thus preventing food oxidation and maintaining product quality.

UV light is one of the oxidation promoters. The prolonged exposure of packaged food to daylight or artificial light during transportation, storage and display in retail stores could cause food oxidation. As shown in Figure 4, the AM PLA film had nearly three times higher UV absorbance than the pure PLA film and provides stronger UV protection for packaged foods. In addition, the AM film had a similar transparent appearance to the pure PLA film

(Figure 6). These optical properties of the AM PLA film are very beneficial for food applications when the films are used as stand-alone packaging.

Because a solvent-casting method was used for making films in the present study, evaporation of the solvent may cause the loss of AIT in the films. Therefore, the determination of release kinetics and residual of AIT is important. Avrami's equation has been used extensively for release kinetics description. This equation was used to correlate the release characteristics of essential oils from β -cyclodextrin (Wang et al. 2011; Yang et al. 2015), carbon dioxide from α -cyclodextrin (Neoh et al., 2006), and allyl isothiocyanate from α - and β - cyclodextrin (Li et al. 2007). Controlled release is important in research and development of encapsulation technology (Kytariolos et al. 2010). The results in this study showed that encapsulation of AIT in PLA film could efficiently control its release under defined temperature conditions (Figure 5). As presented in Table 3, the release rate constant k values increased with increasing temperature. Increased concentrations of AIT would be released from the films at higher temperatures than at lower temperatures. This would enhance the antimicrobial effect of AIT since the rate of microbial growth is faster at higher temperatures.

AIT or other volatile EOs can be encapsulated in other polymers similar to the method used in this study. However, the special characteristics of PLA, such as generally recognized as safe (GRAS) status, biodegradability and being a bio-resource put PLA in a unique position for food applications. Similarly, the food applications of PLA films can be extended to other ready-to-eat (RTE) foods, minimally processed foods, raw foods or semi-processed foods for which microbial contamination is a major concern and a longer shelf-life is required.

Nevertheless, further studies are needed for their applications. This is a preliminary study that provides a starting point, and further research will be required to evaluate these physiochemical and antimicrobial properties for the AM PLA films made with extrusion technology so that the antimicrobial packaging materials could be scaled up to a production scale.

5. Conclusions

Our preliminary study shows that the developed AM PLA films with AIT and TBC had similar or superior mechanical properties and gas permeability characteristics compared to pure PLA films. The AM PLA film treatment significantly inhibited bacterial growth in fresh pak choi at both 4 and 10°C for 15 days and maintained the quality with minimal color change and chlorophyll loss. The release behavior of AIT from PLA film was well simulated by Avrami's equation. The combination of biosourced/biodegradable food packaging materials and natural antimicrobials provides additional benefits for environmental protection and acceptance by consumers. The results from the present study provide the base for further studies. The AM PLA film has the potential to be utilized for extending the shelf life of a broad range of perishable food products.

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30, 179–187.

553 Table 1 Thermal Properties of PLA films

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Film sample	T_g (°C)	exotherm		endotherm	
		T_{cc} (°C)	ΔH_{cc} (J/g)	T_m (°C)	ΔH_m (J/g)
0% TBC	59.66	131.67	-7.35	168.44	29.35
2.5%TBC	51.56	123.33	-15.85	166.42	31.49
5%TBC	51.38	122.64	-11.91	165.08	29.64
7.5%TBC	40.66	113.33	-19.26	162.74	38.78
10%TBC	44.93	116.81	-13.38	162.74	26.08
7.5%TBC+5%AIT	50.06	126.53	-15.78	160.77	31.88

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Table 2 Mechanical and barrier properties of PLA films

	0% TBC	2.5% TBC	5% TBC	7.5% TBC	10% TBC	TBC+AIT*
TS	42.13±3.8a	32.60±1.4b	29.73±0.1bc	27.75±1.6c	22.25±0.8d	22.65±1.3c
EB	1.65±0.17d	38.50±7.10c	90.38±1.32b	109.3±17.35a	82.26±3.94b	113.3±7.93a
WV	177.7±4.5b	182.2±11.6b	204.6±9.3ab	206.1±13.8a	218.0±1.8a	166.6±8.3b
O ₂	386.8±14.6a	373.3±14.1a	366.5±22.5a	326.1±20.8b	309.8±26.5b	198.3±23.6c
CO ₂	3664±56a	1038±106b	860±122c	948±92bc	880±98c	459±57d

TS: tensile strength, MPa; EB: elongation at break, %; WV: water vapor permeability, $\text{g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$; O₂: oxygen permeability, $\text{ml} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$; CO₂: carbon dioxide permeability, $\text{ml} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. Data shows the means $\pm$ standard deviation. Different letters represented a significant difference within the same row ($p < 0.05$).

*The AM PLA film contained 7.5% TBC and 5% AIT.

567 Table 3 Release kinetic parameters of AIT from AM PLA film*

568

Temperature	<i>n</i>	<i>k</i>	Regression Eq.	R ²
4°C	0.6439	0.0571	y=0.6439x-1.8434	0.9390
10°C	0.9042	0.0960	y=0.9042x-2.1191	0.9478
20°C	0.7883	0.1947	y=0.7883x-1.2899	0.9179

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570 *AM PLA film contained 7.5% TBC and 5% AIT.

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Table 4 Microbial growth and quality changes in pak choi during storage at 10 and 4°C

Treatment		Storage time (day)					
		0	3	6	9	12	15
TPC	CK-10C	6.28±0.13a	6.40±0.17a	6.57±0.08b	7.41±0.01b	9.37±0.14a	10.34±0.02a
	FT-10C	6.28±0.13a	5.69±0.12b	5.55±0.11d	7.62±0.21ab	7.53±0.08c	8.15±0.04c
	CK-4C	6.28±0.13a	6.51±0.07a	6.98±0.08a	7.78±0.05a	8.40±0.06b	9.58±0.12b
	FT-4C	6.28±0.13a	5.53±0.14b	6.18±0.07c	6.89±0.12c	7.12±0.02d	7.18±0.06d
ΔE	CK-10C	0	11.20±4.7a	9.40±3.79a	16.48±4.81a	11.97±3.67a	15.80±5.18a
	FT-10C	0	3.51±1.20b	5.23±2.37ab	6.07±2.99b	6.11±2.74b	7.77±5.30bc
	CK-4C	0	4.28±2.60b	6.43±3.57ab	8.50±1.39b	8.14±4.41ab	9.30±2.93b
	FT-4C	0	3.77±1.48b	4.05±2.55b	6.50±4.15b	6.81±2.12b	4.68±1.48c
CHL	CK-10C	0.56±0.04a	0.51±0.11a	0.48±0.04a	0.41±0.01d	0.27±0.01c	0.23±0.01b
	FT-10C	0.56±0.04a	0.55±0.01a	0.52±0.02a	0.54±0.01b	0.36±0.01b	0.37±0.03a
	CK-4C	0.56±0.04a	0.51±0.04a	0.48±0.11a	0.47±0.02c	0.43±0.02a	0.38±0.01a
	FT-4C	0.56±0.04a	0.51±0.05a	0.57±0.09a	0.60±0.02a	0.47±0.03a	0.39±0.01a

TPC: total plate count, log CFU/g; CHL: chlorophyll, mg/g of fresh weight. CK-10C and CK-4C: control samples stored at 10 and 4°C; FT-10C and FT-4C: samples with film treatment and stored at 10 and 4°C. Data shows the means ± standard deviation. Different letters represent a significant difference within the same column and block ($p<0.05$).

Legends of Figures

Figure 1 DSC thermograms of PLA films: (from top to bottom) 0% TBC, 2.5% TBC, 5% TBC, 7.5% TBC, 10% TBC, 7.5% TBC+5% AIT.

Figure 2 SEM images of film cross section. A: AM PLA film; B: pure PLA film. AM PLA film contained 7.5% TBC and 5% AIT.

Figure 3 Fourier transform infrared spectra of pure PLA (A) and AM PLA films (B). AM PLA film contained 7.5% TBC and 5% AIT.

Figure 4 UV–Vis absorption spectra of pure PLA film and AM PLA film. AM PLA film contained 7.5% TBC and 5% AIT.

Figure 5 Release profiles of AIT from AM PLA films at three temperatures. Error bars represent the standard deviation of the means.

Figure 6 Picture of packaged Pakchoi with AM PLA film (4 x 8 cm) on the lid of PET box (highlighted).

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603

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606

A

B

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A

B

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