# Antifungal Poly(lactic acid) Films Containing Thymol and Carvone

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**Abstract.** The goal of this study was to develop antifungal poly(lactic acid) films for food packaging applications. The antifungal compounds, thymol and R-(-)-carvone were incorporated into poly(lactic acid) (PLA)-based polymer at 10, 15 and 20% by weight. Film converting process consists of three steps including melt blending, sheet extrusion and biaxial stretching. The incorporation of antifungal compounds into the polymer matrix resulted in decreased $T_g$ and $T_m$, increased gas permeabilility, reduced tensile strength and increased elongation at break of the antifungal PLA films.

## 1. Introduction

Biodegradable polymers from renewable resources have extensively been studied and developed as alternatives to petroleum-based materials in the past decade. Polylactide or poly(lactic acid) (PLA) is one of the most promising biopolymers for a wide range of packaging applications [1, 2]. PLA is biodegradability and biocompatibility. In addition, PLA exhibits good mechanical properties such as high strength and modulus, good thermal properties and good clarity.

New packaging materials with special functions have constantly been evolved. Development of packaging materials possessing antifungal properties are increasingly gaining attention. Antifungal material has found its way into a wide range of applications particularly in food packaging. Due to increased consumer's awareness of the potential health risk of synthetic substances, natural substances have gained greater prominence. Natural antimicrobials have been studied for their efficacy against various microorganisms involved in food spoilage.

Attempts have been made to incorporate natural antimicrobial compounds into polymeric materials to control or inhibit microbial growth. Poly(lactic acid)/poly(trimethylene carbonate) (PLA/PTMC) films incorporated with cinnamaldehyde (0, 3, 6, 9, and 12 wt%) were prepared by the solvent casting method [3]. LDPE/(clay/carvacrol) films were developed and exhibited superior antibacterial activity against *Escherichia coli* and *Listeria*

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*innocua*. The films also presented an excellent antifungal activity against *Alternaria alternata* [4].

Thymol, a principle component of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) essential oil, has received extensive interest as effective antimicrobial and antifungal agent [5, 6]. Thyme oil was incorporated into several films including soy edible film [7] and chitosan film [8]. Thymol showed the highest antifungal activity against anthracnose isolated from mango and avocado [9]. R-(-)-carvone, a major component of spearmint oil, showed high antifungal activity against *Colletotrichum gloeosporioides, Lasiodiplodia theobromae*, and an *Alternaria isolate* both *in vitro* and *in vivo* studies [10]. Therefore, the aim of this study was to develop the antifungal PLA film incorporated with thymol and R-(-)-carvone, which can be used to control fungal growth for food spoilage and postharvest decay. Thermal, physical and barrier properties of the films were also evaluated.

## 2.   Materials and Methods

**Materials.** The industrial biaxial grade PLA 4043D was purchased from NatureWork LLC. Two antifungal volatile compounds used in this study were 99% FCC grade thymol (solid phase, crystal) and 98% FCC grade R-(-)-carvone (oil phase), purchased from Sigma-Aldrich (St. Loius, USA) for incorporating into the films. Acetonitrile (HPLC grade) from Fisher Scientific (Waltham, USA) was used as a solvent for extraction.

**Film Preparation.** Thymol and R-(-)-carvone were incorporated with a PLA resin and converted into antifungal films by the three-step process using the laboratory scale machines. In the first step, PLA masterbatch with either 20 wt.% thymol or 20 wt.% R-(-)-carvone was prepared by a melt blending process to improve the dispersion and homogeneity of the antifungal compounds in the PLA polymer. This step was carried out using an internal mixer (Haake Rheomex OS, Germany) of 310 $cm^3$ with a fill factor of 0.7, and a rotor speed 40 rpm (0.06 G), at 180°C for 9 min. The temperature was controlled by a measuring drive Haake PolyLab OS, RheoDrive T, Germany). The 20 wt.% of thymol or R-(-)-carvone was introduced into the internal mixer once the polymer was in the melt state in order to avoid the losses of the compounds in the final materials. The solidified polymer blend was crushed into small pellets. Then, the masterbatches were casted into 400±50 μm sheets by premixing neat PLA pellets into the masterbatches to obtain 10, 15 and 20 wt.% of thymol (10T, 15T and 20T) and R-(-)-carvone (10C, 15C and 20C), respectively using single screw extruders (Thermo Scientific™ HAAKE Rheomex for the HAAKE PolyLab OS torque rheometer platform, Germany) connected with a 10cm sheet die. The barrel total length of the extruder was 120 cm with the 25:1 L/D ratio. Rotation rate of feeder was fixed at 80 rpm (0.22 G). The temperatures of the feeding zone, the compression zone and the metering zone and die were 100, 150, 155 and 165°C, respectively. Chill roll temperature was set at 25°C. Sheet without any active compound was fabricated as a control (PLA). The obtained sheet with 11–11.5 cm width then was cut into 9.5 × 9.5 $cm^2$. Finally, the sheets were converted into antifungal films by one-step simultaneous biaxial stretching mode. The stretching in two perpendicular directions was performed on a laboratory film stretcher KARO IV (Bruckner Maschinenbau GmbH, Germany) at the same rate of 100 % per second (pps) and to the same ratio at 3.5 × 3.5 drawing ratios at the drawing temperature 65°C which is close to the glass transition temperature of PLA, followed by annealing temperature at 65°C for 30 sec.

**Film Thickness.** The thickness of the resulting sheet and biaxial antifungal films was measured at 10 different points by thickness gauge (Mitutoyo, Japan). Average thickness was reported.

**Remaining Content.** The remaining contents of thymol and R-(-)-carvone incorporated into the PLA polymer resins after film processing were determined by thermogravimetric analysis (TGA). The TGA results give the indirect confirmation of the presence of additives

in the polymer matrix after processing. TGA data was obtained on a Mettler Toledo, TGA/SDTA 851e (Columbus, USA). Samples of approximately 10 mg from the sheets and 5 mg from the films were heated at 20°C min$^{-1}$ from 30–900°C, under a dry nitrogen gas flow rate of 60 mL min$^{-1}$. The data was calculated from % remaining of the additives in the films after processing to mg g$^{-1}$.

**Thermal Properties.** Glass transition temperature ($T_g$) and melting temperature ($T_m$) were determined using a Mettler Toledo DSC 822e differential scanning calorimeter (DSC) (Columbus, USA). under inert nitrogen atmosphere. Five milligrams of the films were introduced in aluminum pans (40 μL) and submitted to the following thermal program: heating from 40°C to 200°C at 10°C min$^{-1}$ (3 min hold), cooling at 10°C min$^{-1}$ to 40°C (3 min hold) and heating to 180°C at 10°C min$^{-1}$. The percentages of crystallinity for each material were calculated according to Eq. 1,

$$Crystallinity\ (\%)\ =\ \frac{\Delta H_m - \Delta H_c}{93.1} \times 100.$$

(1)

where $\Delta H_m$ (J g$^{-1}$) is the enthalpy of fusion and $\Delta H_c$ is the enthalpy of cold crystallization, and the theoretical enthalpy of fusion of 100% crystalline PLA is 93.1 J g$^{-1}$ [11].

**Mechanical Properties.** Tensile tests were carried out in both machine and transverse directions using an Instron 5965 (Instron, Grove City, USA) equipped with a 5 kN load cell. Tests were performed in rectangular probes (dimension: $100 \times 15$ mm$^2$), an initial grip separation was set at 50 mm and cross-head speed at 50 mm min$^{-1}$. Average tensile strength, elongation at yield, elongation at break and elastic modulus were calculated from the resulting stress-strain curves according to ASTM D882-09 Standard procedure (ASTM, 2009). Results were taken from the average of five measurements (±standard deviation).

**Barrier Properties.** The transmission rates of oxygen and carbon dioxide of all the films were measured using an Oxtran 2/21 and a Permatran C 4/41 (MOCON Inc., Minneapolis, MN, USA), respectively. Water vapor transmission rate was also measured using a Permatran-W Model 398 (MOCON Inc., Minneapolis, MN, USA). Permeability coefficients of oxygen (PO$_2$) and carbon dioxide (PCO$_2$) were calculated by multiplying the transmission rate with film thickness. The β value was calculated from PCO$_2$/ PO$_2$.

## 3.  Results and Discussions

**Antifungal Films.** All antifungal films and control (PLA) were homogeneous and transparent although R-(-)-carvone incorporated films were slightly yellow due to the color of the essential oil (Fig. 1). The resulting films demonstrated a good dispersion of thymol and R-(-)-carvone up to 20 wt.%. This was primarily attributed to a good polymer-filler interaction through hydrogen bonding between the carbonyl groups of PLA and the hydroxyl groups of the additives [12]. However, our preliminary study showed that thymol and R-(-)-carvone had limited solubility in the PLA polymer as 25–35 wt.% incorporation resulted in phase separation due to excess amount of thymol and R-(-)-carvone.



| PLA | 10T | 15T | 20T | 10C | 15C | 20C |

Fig. 1 Antifungal films after three-step processing.

**Remaining Content.** Remaining contents of antifungal compounds were analyzed by TGA and solvent extraction (Table 1). The results indicated that thermal processing had the effect on the amount of volatile compounds remaining in the films in all processing steps. Thermal processing enhanced partial evaporation or degradation of these natural volatile antifungal compounds [5, 13]. After blending with the initial concentration of each antifungal compound at 20 wt.% at the first step, the remaining contents of thymol and R-(-)-carvone was decreased to approximately 16.94 wt.% (169.4 mg $g^{-1}$) and 9.66 wt.% (96.6 mg $g^{-1}$), respectively. This could be due to the higher melting temperature of thymol at 50°C compared to 25°C of R-(-)-carvone. Furthermore, this could also be attributed to the lower vapor pressure of thymol (53.33 Pa at 25°C) as compared to R-(-)-carvone (79.99 Pa at 25°C). The higher the vapor pressure the more volatile the substance. In the second and third steps, the remaining content of thymol and R-(-)-carvone incorporated sheets and biaxial films were further decreased. For thymol incorporated antifungal films, the remaining amounts were 5.90 wt.% (59 mg $g^{-1}$), 8.14 wt.% (81.4 mg $g^{-1}$), and 8.33 wt.% (83.3 mg $g^{-1}$) for the formulations with expected concentration of 10, 15 and 20 wt.% respectively. For R-(-)-carvone incorporated antifungal films, the remaining contents were 3.67 wt.% (36.7 mg $g^{-1}$), 4.93 wt.% (49.3 mg $g^{-1}$), and 5.06 wt.% (50.6 mg $g^{-1}$) for the formulation with expected concentrations of 10, 15 and 20 wt.%, respectively. The results indicated that the final concentrations of the volatile compounds in the antifungal films were reduced to lower than 50% of initial concentration loaded. This was in agreement with the previous study of Ramos et al. (2012) [18] who prepared the thymol and carvacrol incorporated polypropylene films through the melt blending and compression molding processes and reported that the remaining concentrations were reduced from the initial loads of 4 wt.%, 6 wt.% and 8 wt.%, respectively to approximately 1 wt.%, 2 wt.% and 3.5 wt.%, respectively. The remaining content of the volatile compounds determined by the solvent extraction method showed the similar trends as the TGA results. The final amounts of thymol incorporated antifungal films were 54.5, 72.5 and 87.4 mg $g^{-1}$ of the film for formulation with expected concentration 10, 15 and 20 wt.% respectively. While the final amounts of R-(-)-carvone incorporated antifungal films were 36.5, 45.5 and 46.2 mg $g^{-1}$ of film for formulation with expected concentration 10, 15 and 20 wt.% respectively. Nevertheless, the antifungal films developed exhibited antifungal activity against postharvest disease as reported in another study of our group (data not shown).

TABLE 1. REMAINING CONTENTS OF THE MASTER BATCHES, SHEETS AND FILMS AFTER PROCESSING.

| Films | Approximately remaining contents [mg $g^{-1}$] | | | |
|---|---|---|---|---|
| | By TGA | | | By solvent extraction (Films) |
| | Masterbatches | Sheets | Films | |
| 10T | - | 70.6 | 59.0 | 54.5±0.5 |
| 15T | - | 108.6 | 81.4 | 72.5±0.2 |
| 20T | 169.4 | 128.3 | 83.3 | 87.4±0.1 |
| 10C | - | 71.5 | 36.7 | 36.5±0.2 |
| 15C | - | 86.3 | 49.3 | 45.5±0.6 |
| 20C | 96.6 | 88.1 | 50.6 | 46.2±0.3 |

**Thermal Properties**. As shown in Table 2, incorporation of thymol and R-(-)-carvone resulted in decreased $T_g$ and $T_m$ of the antifungal films developed. The results suggested that thymol and R-(-)-carvone acted as a plasticizer and rearranged the polymer chains layers [6]. PLA- thymol films had lower $T_m$ than PLA-carvone films. These results were related to the

degree of crystallinity of the antifungal films. The incorporation of thymol especially at 15 and 20 wt.% significantly reduced crystallinity of the antifungal films. This was consistent with the study of Wu et al. (2014) [6] who reported that as the amount of thymol increased, the $T_g$ value decreased from 46.7°C of PLA/PCL to 41.3°C of PLA/PCL/T12 composite film. A decrease in $T_g$ value was a good indication of the extent of the plasticizing effect provided by thymol. However, the incorporation of R-(-)-carvone at all concentrations resulted in an increase of about 5–7% crystallinity as compared to that of neat PLA. As the stretching takes place in the semi-solid state, high levels of orientation can be achieved depending on operating conditions [14]. The results suggested possible orientation and strain-induced crystallization in the R-(-)-carvone incorporated films. During stretching, polymer molecules are aligned, resulting in a decreased configurational entropy, which facilitates the incorporation into the crystalline phase, thereby increasing the crystallinity (strain-induced crystallization) of the film [15].

TABLE 2. GLASS TRANSITION TEMPERATURE ($T_G$), MELTING TEMPERATURE ($T_M$) AND DEGREE OF CRYSTALLINITY OF THE ANTIFUNGAL FILMS.

| Films | $T_g$ [°C] | $T_m$ [°C] | Crystallinity [%] |
|---|---|---|---|
| PLA | 57 | 150 | 27.6 |
| 10T | 52 | 142 | 25.6 |
| 15T | 50 | 143 | 16.4 |
| 20T | 56 | 140 | 13.6 |
| 10C | 54 | 144 | 32.3 |
| 15C | 54 | 145 | 32.5 |
| 20C | 54 | 144 | 34.9 |

**Barrier Properties**. The increasing antifungal contents affected the barrier properties of the antifungal films (Table 3). With increasing thymol content, both $PO_2$ and $PCO_2$ increased whereas increasing R-(-)-carvone content, resulted primarily in an increase of $PCO_2$ hence increasing the β value. This is due to the higher solubility of $CO_2$ in R-(-)-carvone than in thymol. The effect of antifungal contents on gas permeability of the films was more pronounced when increasing the concentration of thymol to 20 %. This could be because thymol acted as a plasticizer and rearranged the polymer chains layers [16] subsequently lower the degree of crystallinity and increased the free volume in the polymer matrix.

**Mechanical Properties.** Increasing thymol and R-(-)-carvone contents tended to reduce tensile strength but increase elongation at break of the antifungal films compared to the neat PLA film (Table 3). At 20% thymol, the film had lowest tensile strength but highest elongation at break at 145% and 116% in machine (MD) and transverse direction (TD), respectively. At 15% R-(-)-carvone, the film had the elongation at break at 103% and 122% in machine (MD) and transverse direction (TD), respectively. This was due to high plasticization effect of 20% thymol and 15% R-(-)-carvone in decreased brittleness and increased flexibility of the antifungal films [17]. As reported by Wu et al. (2014) [6], thymol acted as plasticizer, reducing the intermolecular forces of polymer chains, thus improving the flexibility and extensibility of the films. Moreover, the presence of thymol decreased the crystallinity of PLA phase. However, these additives also led to a decrease in oxygen barrier and thermal properties [18]. Increasing R-(-)-carvone concentration to 20% resulted in decreasing elongation at break of the PLA-carvone films which could be related to the higher crystallinity of the films as shown in Table 2.

TABLE 3. PERMEABILITY COEFFICIENTS OF OXYGEN ($PO_2$) AND CARBON DIOXIDE ($PCO_2$), B VALUES, YOUNG'S MODULUS, TENSILE STRENGTH AND % ELONGATION AT BREAK OF THE ANTIFUNGAL FILMS IN MACHINE DIRECTION (MD) AND TRANSVERSE DIRECTION (TD).

| Films | Thickness [μm] | $PO_2$ | $PCO_2$ | β | Young's modulus | | Tensile strength [mPa] | | Elongation [%] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | MD | TD | MD | TD | MD | TD |
| PLA | 36±8 | 21 | 65 | 3.1 | 3,434 | 2,509 | 89 | 103 | 40 | 54 |
| 10T | 30±11 | 17 | 41 | 2.4 | 2,941 | 2,916 | 61 | 64 | 49 | 48 |
| 15T | 29±12 | 24 | 61 | 2.5 | 2,667 | 2,090 | 50 | 33 | 43 | 43 |
| 20T | 45±14 | 50 | 120 | 2.4 | 901 | 956 | 17 | 18 | 145 | 116 |
| 10C | 28±12 | 20 | 59 | 3.0 | 2,553 | 2,678 | 51 | 36 | 62 | 59 |
| 15C | 33±6 | 18 | 61 | 3.4 | 2,538 | 2,151 | 50 | 45 | 103 | 122 |
| 20C | 33±7 | 22 | 82 | 3.7 | 2,733 | 2,760 | 58 | 63 | 33 | 40 |

## 4.  Summary

All antifungal films were homogeneous and transparent. Thermal processing resulted in the loss of antifungal compounds (thymol and R-(-)-carvone) remaining in the films at each processing step. The final concentrations of the volatile compounds in the antifungal films were reduced to lower than 50% of initial concentration loaded. Antifungal compounds especially thymol acted as a plasticizer, which lowered $T_g$ of the films. Incorporation of thymol and R-(-)-carvone resulted in increased gas permeabilility, reduced tensile strength and increased elongation at break of the antifungal films.

## 5.  Acknowledgements

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