DEVELOPMENT AND EVALUATION OF LOW-DENSITY POLYETHYLENE-BASED ANTIMICROBIAL FOOD PACKAGING FILMS CONTAINING ESSENTIAL OILS FOR JACKFRUIT

Nor Hanis Aifaa Yusoff
Horticulture Research Centre
MARDI Headquaters, 43400 Serdang Selangor
Email: aifa@mardi.gov.my

Nurzam Ezdiani
Food Science Research Centre
MARDI Headquaters, 43400 Serdang Selangor
Email: ezdiani@mardi.gov.my

Siti Aisyah Abdullah
Horticulture Research Centre
MARDI Headquaters, 43400 Serdang Selangor
Email: ctaisyah@mardi.gov.my

Nur Adliza Baharom
Horticulture Research Centre
MARDI Headquaters, 43400 Serdang Selangor
Email: nuradliza@mardi.gov.my

ABSTRACT

Antimicrobial films (LDPE) containing essential oils as antifungal agent (Cinnamon, Clove and Lemongrass) were developed. These essential oils were incorporated into a low-density polyethylene (LDPE) substrate using the techniques extrusion film blowing. Different film formulations containing different types and concentration of essential oils were investigated for their potential to enhance the retention and to control the release of the essential oils from the films. The physical and mechanical properties of the films were evaluated in order to assess the effect that the addition of essential oil has on the ultimate properties of the film. Films were tested for activity in-vitro enabling the optimum concentrations for essential oil activity to be obtained and the effect of essential oil incorporated into films on the fungal inhibition related to the postharvest disease of Jackfruit such as Lasiodiplodia sp. The effects of incorporated essential oil in LDPE based films were also studied on postharvest storage life of Jackfruit. Having identified the best combination of essential oil and ldpe in laboratory media, the films were then used to package Jackfruit in order to assess the usefulness of essential oil films in the enhancement of Jackfruit shelf life and control postharvest disease. The essential oil incorporated in LDPE films had a positive effect on the inhibition of Lasiodiplodia sp during in vitro condition. The fungal inhibition by essential oil films was affected by the type and concentration of the essential oil. However, essential oil incorporated in LDPE film did not improved the microbiological preservation of the fruit, and also did not maintained quality parameters of the fruit at stable levels for longer times.

Key words: *Lasiodiplodia* sp, essential oil, active packaging, shelf life.

INTRODUCTION

One of the most common problems in the jackfruit trade is post-harvest fruit rot. Although the condition rarely occurs before maturity, it often develops at the onset of ripening and can cause significant losses at the wholesaler and retailer level. Causal agents of fruit rot in jackfruit is *Lasiodiplodia* sp. Propiconazole (250 ppm) fungicide were recommended to control disease until 2 weeks of storage. However, increased concerns about fungicides has demanded free fungicide produce.Food packaging has been traditionally defined as a passive barrier that delays environment effects on food products (Lopez-Rubio et al. 2004). However, trends in current research involve the development of packaging materials that can positively interact with the environment and food, playing an active role in preservation. To date, active packaging is a novel food biopreservation technique for extending the shelf life of food products (Barros Velazquez 2011).

Antimicrobial packaging, an innovative concept, can be defined as a kind of active packaging in which the package, the product, and the environment interact to reduce, inhibit, or retard the growth rate of microorganisms (Suppakul et al. 2008). In these technologies, researchers are developing food packaging materials such as synthetic films (Suppakul et al. 2006, 2008; López et al. 2007) and edible films (Campos et al. 2010) with antimicrobial properties. Polymers are effective vehicles for the active substance (Lopez-Rubio et al. 2004), giving the possibility to incorporate different antimicrobial additives. A first example is low-density polyethylene (LDPE), which may incorporate imazalil (Vartiainen et al. 2003), organic acids (Dobias et al. 2000), nisin (Scannell et al. 2000) or food preservatives (Devlieghere et al. 2000b). A second example is polyethylene (PE), which may be coated with an antimicrobial peptide (Miltz et al. 2006). A third example is hydroxypropylmethylcellulose, which may incorporate nisin by cross-linking (Sebti et al. 2003).

Although many studies have demonstrated the antimicrobial effect of essential oils and their active compounds against a broad spectrum of pathogenic bacteria in food (Carson et al. 2006), there are very few publications that discuss their incorporation as additives in polymeric plastic films (Suppakul et al. 2006, 2008). Given that the US Food and Drug Administration categorizes natural extracts such as essential oils (EOs) and their constituents as generally recognized as safe, packaging manufacturers and demanding consumers consider the incorporation of these natural extracts in plastic films an appealing way of avoiding microbial food spoilage (Nerín et al. 2008). The current study aims to develop antimicrobial LDPE films using two incorporation methods, to determine the antimicrobial activity of these films against *Lasiodiplodia* sp. and to assess the effect of incorporation of selected essential oils on mechanical and barrier properties in the active films.

MATERIALS AND METHODS

MATERIALS

Essential oils of Cinnamon, Clove and Lemongrass were used as the antimicrobial agents. The EOs were obtained from Soul Brand, Segambut Kuala Lumpur and were selected for their high antimicrobial activity and wide availability. The polymer used in these studies was LDPE; attractive characteristics of this polymer include ease of processing, chemically inertness, and low cost. This material is commonly used to pack jackfruit.

Development of Antimicrobial Films

Extrusion

The EOs were preblended with polymer resin LDPE into a master batch (Brabender Instruments, Inc., South Hackensack, NJ, USA). Two hundred fifty grams of resin was added to the mixer with 40 ml of EO at 110 °C and 50 rpm for 30 min. The preblended oil resin was then ground in a knife mill to produce 2-mm fragments and finally incorporated with virgin resin pellets in a single screw extruder (Kilion Extruders Inc.) with an L/D ratio screw of 24:1 and an operating speed of 30 rpm. The temperature profile from the fed zone to the die was 120/125/135/145/175/175 °C. LDPE films incorporating 1, 5, 10 and 15% (w/w) of each EOs and control films without EOs were produced. The antimicrobial films developed were immediately wrapped in aluminum foil to minimize the loss of the antimicrobial agent by evaporation and were then stored at room temperature for up to 1 week prior to testing. An average of 10 measurements were taken at different points on the film sample using a digital micrometer (Digimatic Outside Micrometer, Mitutoyo, Japan) to measure the thickness of the sample..

Tensile Properties of the Films

A standard method D882-02 (ASTM 2002) was used to measure the tensile properties of the films. Films were cut into strips with a test dimension of 169×19 mm according to the standard method D638-08 (ASTM 2008). All films were conditioned for 48 h at 23±2 °C and 50±2% RH before testing. The strips were mounted and clamped with pneumatic grips on a universal testing machine (United Calibration Corp. and United Testing Systems, Inc., California, USA) with a 100-N load cell. The initial gauge length was set to 100 mm, and films were stretched using a crosshead speed of 10 mm/min. The parameters of tensile strength and elongation at break were determined. Measurements were performed on five replicates.

Determination of Antifungal Effect of Active Films

The antifungal activities of active films were determined quantitatively by Poison Food Medium Method. The zone of inhibition on solid media was used to determine the antimicrobial effects of active films against *Lasiodiplodia* sp. Agar disc with mycelia (5mm diameter) were cut from the periphery of actively growing regions of the 7 day old pure cultures using sterile cork borer and aseptically inoculated at the center of petri plates. Then, each antimicrobial film (2×2 cm) was placed over the surface of the agar plate medium. The plates were incubated at 37 °C for 24 h in an appropriate incubation chamber. The antimicrobial activity was observed as a zone of inhibition of the targeted microorganisms around the active film, and the diameter of the zone was measured with a digital micrometer (Digimatic Outside Micrometer, Mitutoyo). All tests were performed in triplicate. LDPE films without essential oils were included as controls.

Effect of LDPE Film Containing Essential Oil on Shelf Life Jackfruit

The fruits of jackfruit (J33), were harvested at commercial maturity, rinsed with tap water and dried with ambien temperature. Afterwards they were packed in LDPE film containing lemongrass eseential oils. The control treatment consisted only of washing fruits and fruit dipping in propiconazole (250 ppm) fungicides. Then fruits were packed in corrugated fiber box and were stored at 12 °C and 90% relative humidity for 3 weeks. Upon at removal each week, fruits were stored ambient until ripe and fruits were analyzed for physical and biochemical changes.

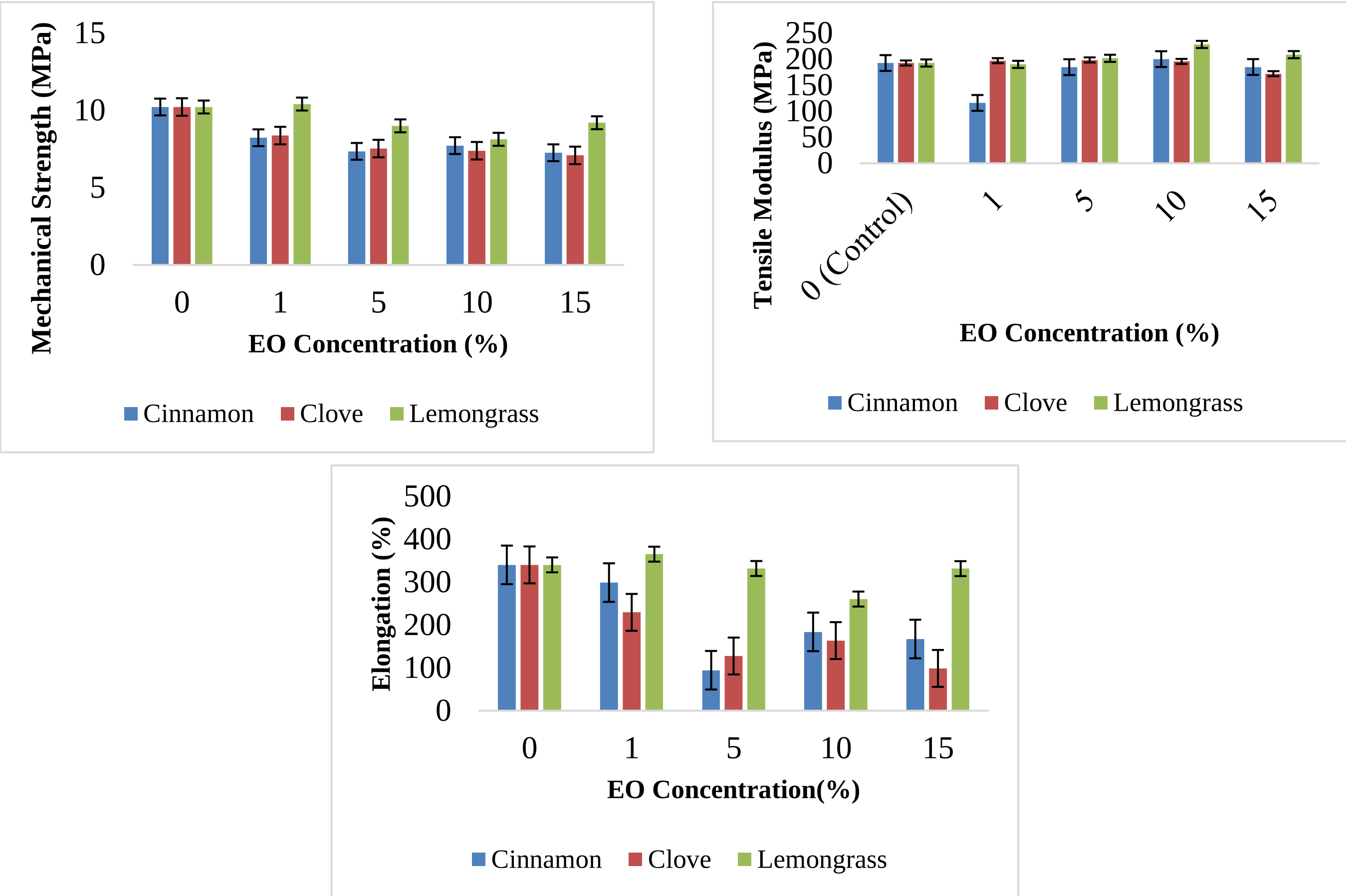
Statistical analyses of the treatment responses were conducted using Analysis of Variance (ANOVA) and Least Significance Difference (LSD) to determine whether the comparison between different treatments and different storage durations showed significant differences ($p < 0.05$). The main effect means are presented in the tables and figures. Experimental data are presented as means ± standard deviation of the determinations for each sample. For comparison of more than two means, the mean separation was done by Duncan Multiple Range Test (SAS Inst. 1985).

RESULTS AND DISCUSSION

Mechanical Properties of Antimicrobial LDPE Films

The activation of LDPE films with EOs was performed using extrusion methods. Tensile and mechanical strength and elongation and thickness were measured in order to study the effect of EO incorporation on the mechanical properties of films. LDPE films with thickness of 0.065-0.180 mm using the extrusion method. Mechanical properties of films with and without EOs are shown in Figure 1. The active films developed using the extrusion method showed a significant reduction in mechanical strength and in percentage of elongation. However, not showed significantly different effect in tensile strength. These results suggest that incorporating EOs in LDPE films using the extrusion method produces a plasticizing effect on the films.

Figure 1: Effect of the incorporation of essential oils on mechanical properties of LDPE films



Antimicrobial Activity Assay of Active Films

Figure 2 shows the antimicrobial activity of LDPE active films against *Lasiodiplodia* sp. As expected, in all cases, the films without essential oils did not show antimicrobial activity against *Lasiodiplodia* sp. LDPE film containing essential oils of Cinnamon and Clove showed lowest inhibition activities on mycelial growth even at higher concentration (Figure 2).

The LDPE active films developed using the extrusion method incorporating 5% and 10% (w/w) of Lemongrass EO showed antifungal activity against *Lasiodiplodia* sp (Fig. 3). Other authors also reported the effectiveness of LDPE films incorporating essential oils (propolis, clove, basil, oregano, and cinnamon) at concentrations from 0.5% to 5% (w/w) (Suppakul et al. 2008) with different activation procedures than those presented in this study. These results demonstrate the powerful utility of LDPE films as antimicrobial packaging materials when they are formulated with EOs.

The results suggest that the extrusion method allowed a better incorporation of the active compounds on the polymer. Figure 2 and 3 also shows pictures representing the zones of inhibition of antimicrobial films incorporated with EO against tested *Lasiodiplodia* sp. There were also differences in the antifungal activity as a function of the EO incorporated; the films containing lemongrass were found to be more effective than those containing Cinnamon and Clove, as illustrated in Fig. 2. The interactions of EOs have crucial effects on the antimicrobial activity of active films. Raweewon (2008) observed strong antiungal properties of lemongrass essential oil when directly applied against *Colletotrichum gloeosporiodes*. These authors found that the inhibitory effect of lemongrass was due to the high concentration of phenolic compounds such as citral. Citral acts as a fungicidal agent because it is able to form a charge transfer complex with an electron donor of fungal cells, resulting in fungal death (Kurita et al., 1981).

Figure 2: Effect of ldpe films containing essential oil at different concentration on the growth of Lasiodiplodia sp. (TRT 1) Cinnamon 1%, (TRT 2) Cinnamon 5%, (TRT 3) Cinnamon 10%. (TRT 4) Cinnamon 15% , (TRT 5) Clove 1%, (TRT 6) Clove 5%, (TRT 7) Clove 10%.and (TRT 8) Clove 15%.

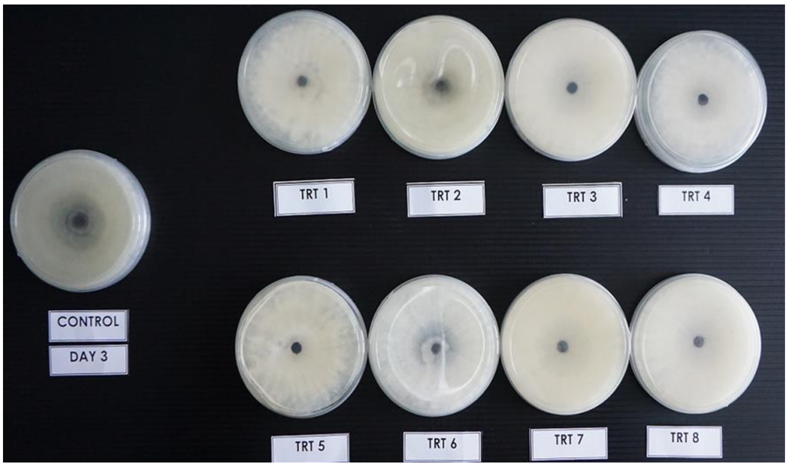
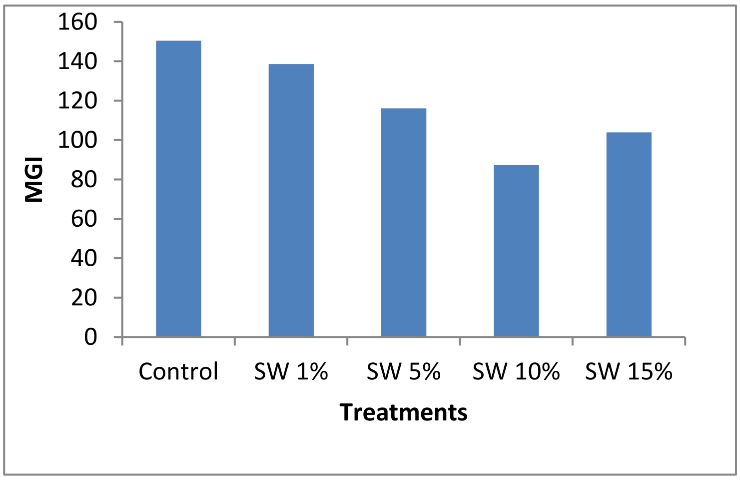
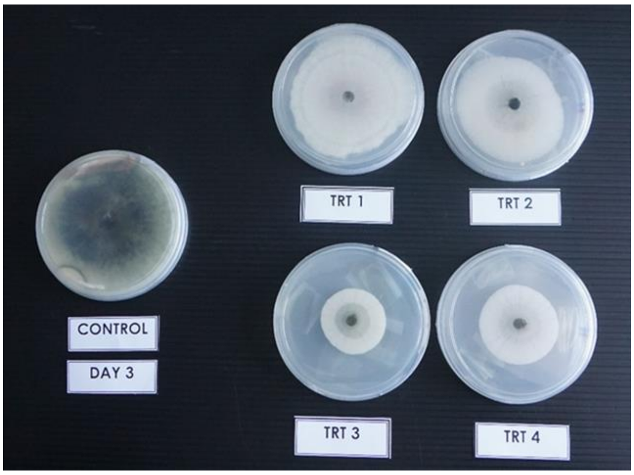


Figure 3: Antimicrobial activity of LDPE films incorporating Lemongrass (SW) essential oils against Lasiodiplodia. (TRT 1) Lemongrass 1%, (TRT 2) Lemongrass 5%, (TRT 3) Lemongrass 10% and (TRT 4) Lemongrass 15%.





LDPE Film Containing Essential Oil on Shelf Life Jackfruit

There was no change in shelf life of jackfruit with ldpe film containing lemongrass essential oils. There were also no significantly different in weight loss, skin colour of skin and pulp and total soluble solids content, titratable acidity and vitamin C with different treatments.

CONCLUSIONS

The direct addition of lemongrass EOs into LDPE polymer matrices using extrusion methods was possible. The antifungal films developed showed antifungal activity against postharvest disease of Jackfruit during in vitro only. Antifungal films incorporating 10% (w/w) of the EOs developed using the extrusion method showed a greater inhibitory. The incorporation of essential oils into LDPE films changed some characteristics of the packaging material slightly, such as the mechanical and barrier properties. However, this film cannot give positive effect on postharvest shelf life of Jackfruit during storage duration. Further research is needed to confirm this finding.

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