Active nanocomposite films based on low density polyethylene/organically modified layered double hydroxides/thyme oil to retain retail shelf life and quality of Hass avocados

Cite as: AIP Conference Proceedings **2289**, 020044 (2020); https://doi.org/10.1063/5.0029459
Published Online: 30 November 2020

Sreejarani Kesavan Pillai, Dharini Sivakumar, Suprakas Sinha Ray, Patience Obianom, Sharon Eggers, and Thobile Mhlabeni

Active Nanocomposite Films Based on Low Density Polyethylene/Organically Modified Layered Double Hydroxides/Thyme Oil to Retain Retail Shelf Life and Quality of Hass Avocados

Sreejarani Kesavan Pillai[1*], Dharini Sivakumar[2], Suprakas Sinha Ray[1,3], Patience Obianom[2], Sharon Eggers[1], Thobile Mhlabeni[1,3]

Centre for Nanostructures and Advanced Materials, DSI-CSIR Nanotechnology Innovation Centre, Council for Scientific and Industrial Research, Pretoria 0001, South Africa

[2]*Phytochemical Food Network Research Group, Department of Crop Sciences, Tshwane University of Technology, Private Bag X680, Pretoria West 0001, South Africa*

[3]*Department of Chemical Sciences, University of Johannesburg, Doornfontein 2028, Johannesburg, South Africa*

[*]Corresponding author:skpillai@csir.co.za, Phone: +27128413759

Abstract. In this study, the ability of an active film containing volatile bioactives in post-harvest disease control and preservation of quality in avocados is explored as a non-traditional treatment method. Antimicrobial transparent flexible trilayer low density polyethylene (LDPE) films containing organically modified layered double hydroxides (OLDH) and plant bioactive-thyme oil (TO) were made using single-step blown film extrusion. Antifungal effects of the packaging in comparison to commercial treatment and untreated control showed considerable reduction in anthracnose disease events in 'Hass' cultivar of avocados while improving the fruit quality. 2wt% OLDH loading improved the oxygen and moisture barrier properties while not affecting the transparency of the film. The results suggest that the synergistic effect of barrier and antimicrobial properties of the controlled volatile bioactive release of the nanocomposite film can be utilized as a prospective strategy to modify the headspace gas composition to combat anthracnose disease in avocados.

Introduction

Low-density polyethylene (LDPE) is the most extensively used commercial polymer in the food packaging sector owing to its low cost, easy processability, chemical inertness and desired physical properties (1). It is commonly used for the production of food packaging films, thanks to its relatively high mechanical properties such as impact and tensile strength (2,3). However, the performance of this polymer is often limited by its high permeability to degradative gases such as oxygen and water vapours which negatively affect the nutritional and organoleptic product qualities (4). A number of previous studies have been dedicated to the modification of polymer matrices with inorganic oxides such as SiO_x, and Al_2O_3 coating to improve barrier properties. However, the oxides have poor substrate adhesion, and due to the imperfections during deposition, the coating has a tendency to crack (1). Incorporation of nanosized fillers such as two dimensional inorganic clay nanostructures to develop high barrier films with good thermal and physical properties using traditional processing techniques since the material is environmentally friendly and cost-effective (5). Theoretically, once properly dispersed in the polymer matrix, clay layers can create a torturous and extensive diffusion pathway to the gas molecules which retard the transmission of the gas molecules through the matrix (6). However, organically modified clays are used as the pristine clays are incompatible with hydrophobic polymer matrices (7).

Layered double hydroxides (LDH), are synthetic clay minerals with two-dimensional layered structures having positive surface charge and diverse and replaceable anions in the interlayer space (8). Organically modified LDH (OLDH) fillers are largely explored as fire-retardant additives to PVC (9). Lately, OLDH based functional nanocomposite materials have been developed with improvement in mechanical and optical and barrier properties. Xu et. al. (10) as well as Jiao et al. (11) prepared LDPE films with improved heat retention properties using MgAl–CO_3 LDH and MgAl–(CO_3, SO_4) LDH as fillers. Wang et al. (12) also obtained LDPE composite films with exceptional infrared absorption in 1180–914 cm^{-1} region employing LDH particles intercalated with P-O based organic anion for agricultural film applications. Xie et al. (1) incorporated LDHs modified with lauryl phosphoric acid ester into LLDPE matrix by a solution casting technique, and the composite film showed enhancement in anti-drop, thermo-mechanical, optical, and barrier (water vapour) properties of LLDPE. The solution casting process is not ecologically benign owing to the use of organic solvents in the process, and furthermore, it is not suitable for the existing manufacturing processes, such as injection moulding and extrusion. Hence our aim was to prepare the active tri-layer films in a single step blown film extrusion process without the need for any intermediary steps.

Proceedings of PPS2019 Europe-Africa Regional Conference of the Polymer Processing Society
AIP Conf. Proc. 2289, 020044-1–020044-5; https://doi.org/10.1063/5.0029459
Published by AIP Publishing. 978-0-7354-4019-7/$30.00

Another aspect of the study was to extend the functionality of the film by adding an essential oil (EO) to yield active packaging materials with antimicrobial activity. In a recent study by our research group (13), effective antifungal packaging films were developed with LLDPE and thyme oil (TO) which is listed as GRAS compound and classified as minimum risk pesticide. The films containing 5 wt. % TO showed considerable inhibition activity against *Colletotrichum gloeosporioides* (*C. gloeosporioides*)-a fungus that leads to "anthracnose" disease in avocados in past-harvest storage conditions. The incorporation of EOs in the polymeric matrices decelerates the transmission rate of volatile active compounds, permitting superficial interaction of a larger concentration of antimicrobial molecules with the contaminated fruit surface, for a prolonged period (14). However, the incorporation of TO was found to have no effect on the gas transmission characteristics of the original films. Hence it was envisaged that the combination of antimicrobial TO molecules with proper dispersion of OLDHs in LDPE matrix could lead to novel antimicrobial packaging material with high barrier performance. According to our knowledge, no report was found on LDPE-OLDH-TO system at the time of conceptualization of the project, and so there was a motivation to explore it, owing to the success recorded in the previous study.

Experimental

Materials

LDPE pellets [film grade LT 033 with MFI: 0.33 g/10 min (2.16 kg, 230°C) and density 921 kg/m^3] were acquired from Sasol Polymers, South Africa. The TO additive which is free of any preservatives was attained from Vital Health Foods, South Africa. Nanosized Stearate modified Mg-Al-LDH used as OLDH was obtained from Hearty Chem, Korea. The post-harvest pathogen, *C. gloeosporioides* was isolated from diseased Hass avocado by Fruit and Vegetable Technology laboratory, Tshwane University of Technology. The isolate was preserved on malt extract agar at 25 °C and suspension of the spore was prepared as described by Bill et al. (14) for further experiments.

Methods

Preparation of active films

Tri-layer LDPE films of roughly 50–70 μm thickness were prepared using a single step blown film extrusion as reported by Kesavan Pillai et al. (13) with slight modifications. A blend of TO (5 wt %), OLDHs (2 and 5 wt %) and LDPE was used as the middle layer, whereas neat LDPE constituted the outer layers. The films were processed at 160°C with screw and roller speed of 60 rpm and 3.6 m/min respectively. To warrant proper dispersion of fillers and oil in the middle layer, TO, OLDHs and pulverized LDPE were mixed using a blender and kept for approximately 24 h in an air-light container. Trilayer film of neat LDPE prepared under similar processing conditions was used as a reference.

Characterization of active films

An UV-Vis spectrophotometer (Perkin-Elmer Lambda 750 S, USA) was used to characterize the visible light transmission properties of the LDPE and composite films in the 200– 800 nm range at room temperature. The film opacity was calculated by direct reading, of the absorbance of rectangular samples at 600 nm combined with the sample thickness (26) using the equation, Opacity (mm^{-1}) = absorbance @600 nm/sample thickness (mm). The water vapour, oxygen transmission rate films were analyzed using procedures described elsewhere (13). The headspace analysis was determined, according to Sellamuthu et al. (15).

Inoculation, packaging of the fruit and measurement of diseases development

Healthy 'Hass' avocado fruit, was purchased from Tzaneen, Limpopo at a mature commercial stage. For the inoculation process, triggered ripe fruit were chosen in accordance with the finger feel firmness score of 2 where 1= hard, 2 = semi hard (triggered ripe), 3= soft (16). The fruit surface was disinfected by immersing in 0.01% sodium hypochlorite for 5 min, and the fruit was air-dried at 25 ˚C prior to inoculation, packaging and storage. The sterilized fruit was uniformly wounded at the equatorial region on the pericarp, and 20 µL of *C. gloeosporioides* spore suspension (10^6 spores mL^{-1}) was used for inoculation before exposing to different post-harvest treatments. The fruit treated with commercially adopted prochloraz dip (1000 mg L^{-1}) treatment and sterile distilled water (designated as untreated) were used as controls for comparison. Three replicates bags of active films per packaging treatment each containing five fruit were used, and the experiments were repeated twice. The packaged fruit was held at 20 °C for 8 days before assessing the treated for disease development. Incidences of anthracnose and severity were determined on the 8th day after storage as described by Obianom and Sivakumar (17).

Effect of active films on the fruit quality

Assessment of fruit colour and firmness was carried out on ten fruit per treatment per analysis as described by Sellamuthu et al. (15) A group of five fruit per treatment were assessed for firmness at two equatorial points on the fruit by means of a penetrometer (Model DFM50 Ametek, Largo, Florida, USA). The fruit attained the soft ripe stage and the firmness was measured in kg (Standard ISO7619, International Organization for Standardization)]. After the firmness assessment, the fruit was cut up into two parts and the flesh colour was examined from two points with a Chroma Meter (CR0-2000, Minolta Camera Co. Ltd, Tokyo, Japan).

Results and discussion

Characterization of films

The transparency of packaging films can directly affect the appearance of packed fruit and hence influence consumer acceptance. Therefore, it is desired that the incorporated fillers and active compounds do not change (decrease) its transparency significantly. Table 1 shows the light transmittance of various films at 600 nm and the calculated opacity values.

TABLE 1 Light transmittance (%) and opacity values of LDPE and composite films with 5% TO and various concentrations of OLDH (2 and 5 wt %)

Packaging film Samples	$\%T_{600}$ (%)	A_{600} [2-log ($\%T_{600}$)]	Thickness (mm)	Opacity (mm^{-1})
LDPE	91.43 ±0.19	0.039±0.0009	0.067±0.006	0.59±0.043
LDPE-2 %OLDH-TO	90.96±0.15	0.041±0.0007	0.067±0.006	0.62±0.049
LDPE-5%OLDH-TO	90.80±0.14	0.042±0.0007	0.063±0.006	0.67±0.062

As can be observed, the opacity of neat LDPE is lower than the composite films indicating its higher transparency. The incorporation of TO and OLDH although increased the opacity values slightly (opacity increases with increase in the concentration of OLDH, but within the statistical limits), did not reduce the transparency of the LDPE film significantly which is apparent from transparency values above 90% for all the films between 400 and 800 nm.

WVPR and OPR of neat LDPE and composite films were considered to gauge their barrier performances, and the normalized values of the permeation rates are presented in Table 2.

TABLE 2 WVPR Data for neat LDPE and composite films measured at 37.8°C and 100% relative humidity

Packaging film Samples	WVTR g/[m^2-day]	WVPR g-mm/[m^2-day]	OTR cc/[m^2-day]	OPR cc-mm/[m^2-day]
LDPE	6.21±0.86	0.53±0.07	2.95±0.16	0.144±0.005
LDPE-2%OLDH-TO	7.64±0.05	0.47±0.04	0.54±0.02	0.033±0.002
LDPE-5%OLDH-TO	8.36±0.19	0.55±0.06	1.13±0.13	0.072±0.011

From table 2, it is clear that addition of 2% OLDH to the polymer matrix reduced the WVPR by 12%. However further increase in concentration of OLDH increased the permeation rate of water vapour molecules. More pronounced effect was observed for OPR. Compared to the neat LDPE, films containing 2 and 5 wt% of OLDH showed significant reduction in OPR where the values reduced by 76 and 50% respectively for 2 and 5 wt% OLDH based films. The enhancement in barrier property of the film at 2 wt% loading of OLDH can be attributed to the ordered arrangement of OLDH nanoparticles in LDPE matrix, which function as physical obstructions increasing the diffusion trail of the water molecules while they move through the film. The relative increase of WVPR and OPR at 5% OLDH concentration can be correlated to OLDH aggregation and consequently its reduced interaction with LDPE matrix. The modest improvement in WV barrier in comparison to a more pronounced oxygen barrier can be ascribed to the existence of hydroxyl groups in OLDH which can assist permeation of water molecules. Li et al. (18) prepared a series of composite films with poly (propylene carbonate) (PPC) and different amounts of OLDH through a melt-blending method. They observed best barrier properties for PPC film with 2% OLDH film where the coefficients of permeability for oxygen and water vapour were reduced respectively by 54% and 17%. Thus our observations are in line with the previous reports.

The cumulative concentration of thymol released to the headspace from LDPE- 2%OLDH-TO film was higher (90.09%) in comparison to and LDPE - 5% OLDH -TO film (79.67%). The results showed slower rate of release for TO from the film with higher concentration of OLDH. This could be due the increased preferential localization or adhesion of thymol to the organically modified clay phase due to its thermodynamic affinity thereby delaying its migration to the headspace from the film. LDPE being non-polar may not have any affinity for TO, thus indicating the potential of TO interacting with OLDH based on their chemical properties. These results hence indicate the scope of manipulation of the release rate of active molecules by varying the concentration of OLDH in the matrix. A similar slow release of thymol was observed by Moshe et al. (19) in their recent study for a system based on polypropylene/polyamide and montmorillonite nanoclays.

Antifungal effect of active films on diseases development in artificially infected 'Hass' avocado fruit

Figure 1a shows the effect of the prepared films on the percentage of anthracnose incidence and severity in inoculated Hass avocados. The results indicated a significant reduction of anthracnose incidence by less than 50% in fruit covered with LDPE- 2% OLDH -TO film in comparison to the untreated (100%) and commercial fungicide prochloraz (88%) treated counterpart. The film with 5 wt % of OLDH was found to be less effective, which is in line with the slower release of TO from the film and lower concentration of thymol released to the headspace. LDPE-2%OLDH-TO film prevented the growth of the pathogen effectively showing that 2 wt% concentration of OLDH is enough to achieve an adequate inhibition of the *C. gloeosporioides,* the causal agent of anthracnose. The TO was shown to provide residual protection against the *C. gloeosporioides* by inducing the defence mechanism and delaying the degradation of epictechin that acts the regulator for the maintenance of antifungal compound the dienes in avocados (14). Another factor that might contribute to the reduced anthracnose incidence and severity could be the high oxygen and moisture barrier properties of the film. The lowest oxygen and moisture transmission rate observed in the film with 2 wt% of OLDH could result in lower internal oxygen level in the packaging headspace as well as reduced moisture loss from the fruit which delays ripening, senescence and softening processes and hence ultimately helps to reduce the progression of latent infection. Jitareerat et al. (20) also observed a lower rate of anthracnose disease progression in chitosan-coated mangoes where chitosan acted as a barrier for oxygen which in turn delay the ripening through reducing respiration and ethylene production. Lunt et al. (21) reported that reduced moisture loss due to wax coating in avocados could contribute to the improvement of fruit quality by retarding fruit softening.

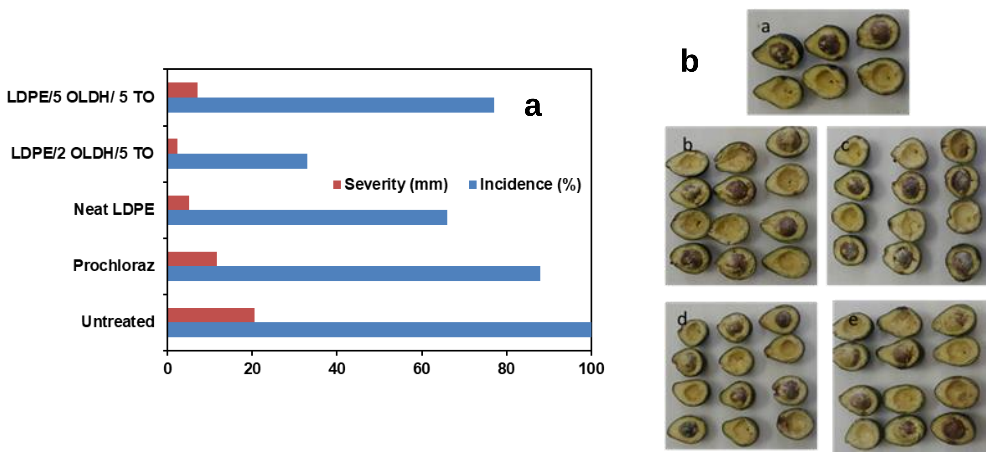

FIGURE 1 Effect of different treatments on a) the incidence and severity of anthracnose b) fruit quality of artificially infected 'Hass' avocado fruit after 8 days of storage.

Effect of active films on the fruit quality

LDPE film containing 2 wt% OLDH and TO was a very effective active packaging film which could modify the internal packaging atmosphere and successfully retard the development of anthracnose disease and more over the delay the ripening process improving the retail shelf life and fruit quality (Figure 1b). The fruit retained higher firmness in LDPE- 2% OLDH-TO (3.2 kg) treated to fruit, whereas the untreated control fruit (1.34 kg) showed the lowest fruit firmness. According to Perez et al. (22), there is an inverse relationship between the shelf-life of fresh produces and its rates of respiration and ethylene production. Hence by lowering the respiration rate by reducing the oxygen concentration within the limit can delay senescence which contributes to improved fruit quality (23). In this case, this could possibly be the result of high barrier property of the film containing 2 wt% OLDH, which reduces the headspace oxygen and in turn the respiration rate and ethylene production rate. The delayed ripening process at low oxygen levels, thus helped with the colour and firmness retention. Additionally, delay in decaying also contributed to the retention of fruit firmness (14).

Conclusions

The application of LDPE packaging films containing OLDH and TO, is an innovative technology that aids in the regulation of oxygen and moisture transport through the film and release of antimicrobials to the packaging headspace and hence is beneficial for the postharvest preservation stages of avocados. The LDPE- 2% OLDH-TO film as packaging for avocados clearly showed a synergistic effect of its antimicrobial and barrier properties in reducing the incidence of anthracnose, and preserving the quality of the fruit which shows its application prospects in retail shelf packaging. The use of such active packaging films should be commercially adopted for the use of horticultural crops as postharvest treatments to reduce food waste due to postharvest decay in the supply chain. Reduce the reliance on chemical fungicide based post-harvest treatments in avocados.

Acknowledgements

The authors sincerely thank the financial support (Project No: HGER20S) from Department of Science and Innovation and the Council for Scientific and Industrial Research South Africa. The funding from the National Research Foundation (grant 98352) for Phytochemical Food Network to Improve Nutritional Quality for Consumers is also acknowledged. The authors extend their appreciation to CeNAM characterization facility for the help in characterizing the samples.

References

1. J. Xie, H. Wang, Z. Wang, Q. Zhao, Y. Yang, G. I. N. Waterhouse, L. Hao, Z. Xiao, J. Xu, Nature Scientific Reports, 8, 52, 1 (2018).
2. P. H. D. L. Coelho, A. R. Morales, Polímeros, 23, 410 (2013).
3. V. R. Miranda, A. J. F Carvalho, Polímeros, 21, 353 (2011).
4. C. F. Ayuso1, A. A. Agüero, J. A. P. Hernández, A. B. Santoyo, E. G. Gómez, High Oxygen Barrier Polyethylene Films, Polymers & Polymer Composites, 25, 8, 571, 2017.
5. E. Avazverdi , M. Vandalvand, S. A. Javadinia, A. Shakeri, Progress in Rubber, Plastics & Recycling Technology, 32, 2, 99 (2016).
6. G. Laufer, C. Kirkland, A. A. Cain, J. C. Grunlan, Applied Materials and Interfaces, 4, 1643 (2012).
7. S. Sinha Ray, M. Okamoto, Progress in Polymer Science, 28, 1539 (2003).
8. Q. Wang, D. O'Hare, Recent Chemical Reviews, 112, 4124 (2012).
9. J. R. Rees, C. S. Burden, A. M. Fogg, Journal of Solid State Chemistry, 224, 36 (2015).
10. G. Xu, C. Guo, X. Duan, C. Jiang, Chinese Journal of Applied Chemistry, 16, 45 (1999).
11. Q. Z. Jiao, Y. Zhao, H. Xie, D. Evans, X. Duan, Chinese Journal of Applied Chemistry, 19, 1011 (2002).
12. L. J. Wang, L. R. Wang, Y. J. Feng, J. T. Feng, D. Q. Li, Applied Clay Science, 53, 592 (2011).
13. S. Kesavan Pillai, L. Maubane, S. Sinha Ray, V. Khumalo, M. Bill, D. Sivakumar, Journal of Applied Polymer Science, 133, 43045 (2016).
14. M. Bill, S. Kesavan Pillai, P. Tinyane, S. Sinha Ray, D. Sivakumar, Food Bioprocess and Technology, 11, 141
15. P. S. Sellamuthu, D. Sivakumar, P. Soundy, Journal of Food Safety, 33, 1, 86 (2013).
16. M. Bill, D. Sivakumar, M. Beukes, L. Korsten, Food chemistry, 194, 938 (2016).
17. C. Obianom, D. Sivakumar, Journal of Phytopathology, 166, 1, 1 (2018).
18. G. Li, W. Luo, M. Xiao, S. Wang, Y. Meng, Chinese Journal of Polymer Science 34, 1, 13 (2016).
19. I. Moshe, O. Weizman, M. Natan, G. Jacobi, E.Banin, A. Dotan, A. Ophir, Polymer for Advanced Technologies, 27, 1476 (2016).
20. P. Jitareerat , S. Paumchai , S. Kanlayanarat, S. Sangchote, New Zealand Journal of Crop and Horticultural Science, 35, 211 (2007).
21. R. E. Lunt, H. Smith, M. M. Darvas, South African Avocado Growers Association Yearbook, 4, 57 (1981).
22. K. Perez, J. Mercado, H. Soto-Valdez, Food Science and Technology International,10, 2, 73 (2004).