Jurnal Teknologi

Full paper

Potential of Antimicrobial Film Containing Thymol with pH Indicator to Increase Biosafety of Packed Food

Nozieana Khairuddin[a], Ida Idayu Muhamad[a*]

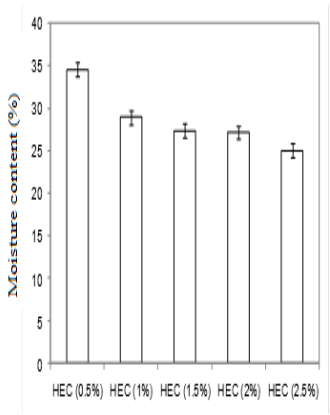
[a]*Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

*Corresponding author: idayu@cheme.utm.my

Article history

Received :19 November 2012
Received in revised form :
8 January 2013
Accepted :18 Januaryr 2013

Graphical abstract



Abstract

Antimicrobial (AM) packaging incorporated with pH indicator is a promising form of smart food packaging with great potential; economically, environmentally and increase biosafety of packed food. An AM Smart Packaging is made by incorporating suitable AM agents into food package matrices and applying a bio switch concept to inhibit the spoilage and the pathogenic microorganisms for the safety of food. The value-added pH indicator system will be used to monitor the condition of packaged foods to give information about the chemical-microbiological quality of the packaged food during transport and storage. The main objective of this study was to develop the formulation of AM starch-based film in which the active compound, thymol was incorporated into the polymeric material. A solution casting method was used in the film preparation and thymol was incorporated prior to casting. The physical and chemical characterizations of the prepared film gave valuable information on moisture content and chemical composition of the films. The AM film had a relatively smoother, cleaner and more compact surfaces compared to the control film. The resultant film with reduction in moisture content was used to indicate the relationship between thymol and water molecules in the diffusion mechanism throughout the film matrices. FTIR analysis implied the consistency of the chemical composition and structure of the AM film compared to the control film indicating that the addition of thymol into the film did not affect or alter the carbonyl functional groups and stability of the film.

Keywords: Antimicrobial wheat-based packaging; thymol; Fourier Transform Infra-red (FTIR); moisture content

Abstrak

Pembungkusan antimikrob (AM) yang digabungkan dengan penunjuk pH menjanjikan pembungkusan makanan pintar yang berpotensi besar; menjimatkan, mesra alam dan meningkatkan biokeselamatan makanan yang dibungkus. AM Smart Packaging diperbuat dengan menggabungkan agen AM yang sesuai ke dalam matriks pembungkus makanan dan menggabungkan konsep suis bio untuk menghalang kerosakan dan mikroorganisma patogen untuk keselamatan makanan. Sistem penunjuk pH yang tambah nilai akan digunakan untuk memantau keadaan makanan yang dibungkus dan memberikan maklumat tentang kualiti kimia-mikrobiologi makanan yang dibungkus semasa penghantaran dan penyimpanan. Objektif utama kajian ini adalah untuk membangunkan formulasi filem AM berasaskan kanji dimana kompoun aktifnya, thymol digabungkan ke dalam bahan polimer. Kaedah penuangan larutan telah digunakan dalam pembuatan filem dan thymol telah dimasukkan terlebih dahulu sebelum penuangan. Pencirian fizikal dan kimia filem yang disediakan memberikan maklumat yang penting terhadap kandungan lembapan dan komposisi bahan kimia filem. Filem AM mengandungi permukaan yang lebih lembut, bersih dan lebih padat berbanding filem kawalan. Filem yang dihasilkan dengan pengurangan dalam kandungan lembapan menunjukkan hubungan antara thymol dan molekul air dalam mekanisma resapan pada seluruh matriks filem. Analisis FTIR menunjukkan konsistensi komposisi kimia dan struktur pada filem AM berbanding dengan filem kawalan yang menunjukkan penambahan thymol ke dalam filem tidak mempengaruhi mahupun mengubah fungsi kumpulan karbonil dan kestabilan filem.

Kata kunci: Pembungkus antimikrob berasaskan gandum; thymol; Fourier Transform Infrared (FTIR); kandungan lembapan

1.0 INTRODUCTION

Nowadays, food quality is a major issue and the introduction of active packaging particularly antimicrobial (AM) food packaging could play a role in food security assurance. In recent years, the demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products, globalization of food trade, and distribution from centralized processing pose major challenges for food safety and quality.[1-2] Currently, food-borne microbial outbreaks are driving a search for innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness and safety. Active packaging interacts with the product or the headspace between the package and the food system, to obtain a desired outcome. Likewise, AM food packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food or packaging materials itself.[3]

The ability of starch to interact with many additives or components of the food is also an advantage. As a result of this interaction, different properties of either the additives or the starch might be affected. Han (2002) reported that edible films could be used as carrier for AM substances to increase shelf life of food product as well as carrier for flavours and colouring agents.[4-5] Famá *et al*. reported that starch can interact with AMs such as sorbic, benzoic and p-benzoic acids and the nature of this interaction depends on the type of starch as well as on the concentration and the chemical characteristic of the preservative [6]. Several researches also reported the use of edible films as a way of supporting and slowly releasing AMs in food product such as for slow release of lysozyme[7] and for slow release of propylparaben.[8]

Previous researches have reported that thymolposses both fungistatic and antibacterial activity against a broad range of microorganism.[9-13] The mode of action of thymol is by the alteration of the membrane fatty acid composition for pathogenic or spoilage microorganisms.[14]

The main objective of this study is to develop the formulation of AM wheat-based film in which the active compound, thymol, is incorporated into the polymeric material.

2.0 EXPERIMENTAL

2.1 Preparation of AM Starch-based Film

The films were prepared by slightly modifying the method described by Gennadios *et al.* and Mitchell.[15-16] A 0.5 g thymol was dissolved in 20 ml of absolute ethanol. Then 0.01 g of bromothymol blue and methyl red was added to the solution. The solution was then filtered using filter paper. The filtrate was added to the 80 ml distilled water containing 4 g of HEC and 5 g of wheat starch. After the solution was completely dissolved, 5 ml glycerin (HmbG Chemicals) was added as plasticizer and the mixture was heated slowly to a mild boiling. Films were casted into square plate (20 x 20 cm). The casting plate was placed for 24 hours in an oven (Memmert) set at 60°C.The same step was repeated for the preparation of 1 %, 1.5 %, 2 %, and 2.5 % of thymol.

2.2 Moisture Content

The determination of moisture content in this study followed method from Finkenstadt *et al*.[17]. A Moisture Determination Balance FD-620 was used to determine the moisture content (MC) of the starch products by gravimetric method using equation 1.

$$MC = \frac{Mf - Mi}{Mi} \times 100 \quad (1)$$

where M_i is the initial weight of the sample and M_f is the final weight after drying

Determination of moisture content was performed on 3 replicates and the average is reported.

2.3 Fourier Transform Infra-red (FTIR) Analysis

Fourier-transform infrared (FTIR) spectra were recorded on a Perkin-Elmer Spectrum One FT-IR Spectrometer. FTIR analysis was performed with a resolution of 4 cm-1 in the range of 4000 – 400 cm-1 and was averaged over 16 scans.

2.4 Zone Inhibition Assay

The agar diffusion test was carried out using the method described previously [9, 10]. The strain selection represented typical spoilage organism groups commonly occurring in various kinds of food products. The strains were as follows: (1) *Escherichia coli*, a conventional hygiene indicator organism, a Gram-negative rod belong to the same family of Enterobacteriaceae as for example *Salmonella* sp. (2) *Bacillus subtilis*, a Gram-positive rod capable of forming heat-resistant spores. The control and AM starch-based films were cut into circular discs (6 mm in diameter) and sterilized using UV light for 15 min. The cut pieces were aseptically placed on nutrient agar plates seeded with 0.1 mL of bacterial solution. The plates were incubated at 37°C for 24 h. After the incubation process, the diameters of the clear zones that formed around the film samples were measured using a Verniercalliper and reported as the zone of inhibition. Duplicate agar plates were prepared for each type of film and control film.

The calculation of an AM index (AMI) was defined in equation 2:

$$AMI = (d1 - d2)/d1 \quad (2)$$

where d1 is the diameter of clear zone and d2 is the diameter of circular film.

3.0 RESULTS AND DISCUSSION

3.1 Moisture Content

Figure 1 show the moisture content (%) for different concentration of AM film after 24 hours of film development. The result shows a decrease of moisture content with the increase of AM concentration after 24 hours of the samples incorporated with thymol. This indicates that the ability of AM film to absorb and retain moisture is reduced after 24 hour development. Previous study suggested that the increase in the crystalline phase of a semi-crystalline material is highly linked with the decrease in its moisture content.[18] Perhaps, the changes caused by the increase in crystalline fraction with the addition of antimicrobial agent. Therefore, it is shown that the percentage of moisture content decrease in the film with the addition of AM agent. This is in agreement with previous study reported by Fama' *et al*. [6]

The reduction in moisture content of AM film indicates the relationship between thymol and water molecules in the diffusion mechanism throughout the film matrices. This behaviour of AM film towards moisture implies the suitability of application and control aspects that are needed in practice.

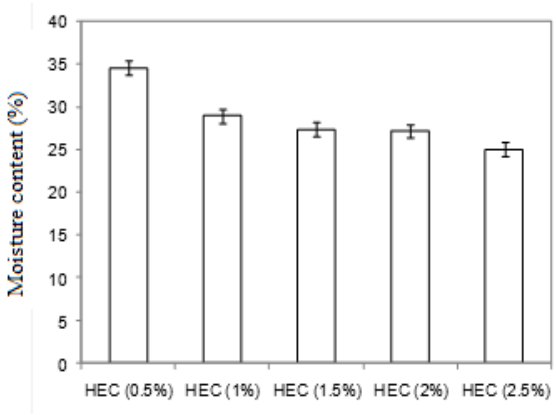


Figure 1 Moisture of antimicrobial film containing different concentration of thymol

3.2 Fourier Transform Infra-red (FTIR) Analysis

FTIR was used to determine the effect of incorporation of substance on the structural changes of film.[19] The infra red spectra of control film and AM films are shown in Figure 2. For the spectra of control film, the strong and broad absorption peak at 3381.60 cm-1 was assigned to the characteristic absorption peak of the stretching vibration of -OH. The bands at 1155.68 cm-1 and 1112.68 cm-1 were attributed to the stretching vibration of C-O in C-O-H groups and the band at 1042.32 cm-1 was attributed to the stretching vibration of C-O in C-O-C groups. The bands detected on the starch-based film were in agreement with the previous study of starch-based film.[20-21]

Interestingly, the result implies the consistency of the chemical composition and structure of the AM film compared to the control film. This clearly indicates that the addition of thymol into the starch-based film did not affect or alter the carbonyl functional group of the starch-based film. This indicates there is no chemical interaction occurring between thymol and starch matrix..

Figure 2 FTIR spectra of (a) Control film and (b) Antimicrobial film containing thymol

3.3 Inhibition of *Escherichia coli* and *Bacillus subtilis* on Agar Plate Test

Antibacterial activity of AM film against two pathogenic bacteria was expressed in terms of zone inhibition. The agar diffusion test simulates wrapping of foods and therefore can be used to estimate how much the antimicrobial agent migrates from the film to the food when the film contacts contaminated surfaces.[3,19]

Table 1. Analysis of the zone of inhibition data in agar plate test for *E.coli* and *B. subtilis* at 37ºC in the presence of HEC-wheat starch-based film incorporated with thymol

	AMI zone of inhibition				
	0.5 %	**1.0 %**	**1.5 %**	**2.0 %**	**2.5 %**
Escherichia coli	0.772 ± 0.01	0.769 ± 0.01	0.763 ± 0.03	0.719 ± 0.02	0.772 ± 0.02
Bacillus subtilis	0.771 ± 0.03	0.762 ± 0.02	0.801 ± 0.01	0.751 ± 0.01	0.740 ± 0.03

All samples were examined for possible inhibition zones after incubation at 37ºC for 24 hours. Table 1 lists calculated inhibition area for each plate test. The control films showed no inhibition area and colonies were formed all over the plate (Figure 3).

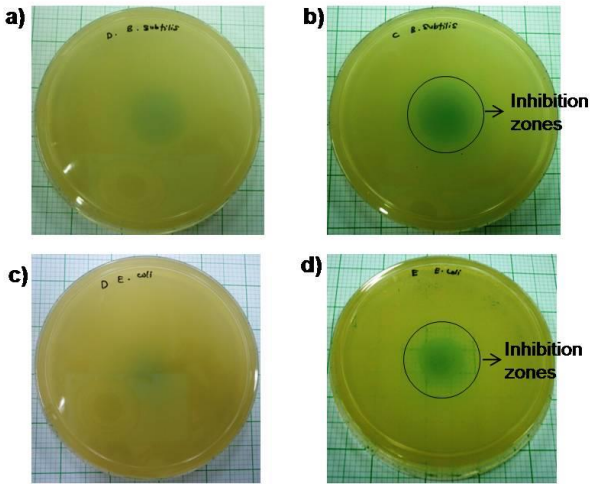


Figure 3 Inhibition of (a,b) *Bacillus subtilis* and (c,d) *Escherichia coli* on solid media by HEC-wheat starch-based film incorporated with thymol after incubation for 24 hours at 37ºC with (a,c) no AM agent and (b,d) with AM agent

■4.0 CONCLUSION

The resultant film with reduction in moisture content indicated the relationship between thymol and water molecules in the diffusion mechanism throughout the film matrices. FTIR analysis implied the consistency of the chemical composition and structure of the AM film compared to the control film indicating that the addition of thymol into the film did not affect

or alter the carbonyl functional groups and stability of the film. This study had established the AM effectiveness of thymol incorporated into the film against *E. coli* and *B. subtilis* in the microbial contamination study which was clearly observed as clear zone formation in the agar plate test.

Acknowledgements

The authors would like to thank the Ministry of Science Technology and Innovation (MOSTI) Malaysia, the Ministry of Higher Education (MOHE) and Research Management Centre, UTM for financial support of this project.

References

[1] Vermeiran, L., Devlieghere, F., van Beest, M., de Kruijf, N., and Debevere, J. 1999. Developments in the Active Packaging of Foods. *Trend in Food Science and Technology*. 10: 77–86.

[2] Suppakul, P., Miltz, J., Sonneveld, K. and Bigger, S. W. 2003. Antimicrobial Properties of Basil and Its Possible Application in Food Packaging. *Journal of Agricultural and Food Chemistry*. 51: 3197–3207.

[3] Appendini, P. and Hotchkiss, J. H. 2002. Review of Antimicrobial Packaging Food Packaging. *J. Innovative Food Science & Emerging Technologies*. 3: 113–126.

[4] Han, J. H. 2002. Protein-based Edible Films and Coatings Carrying Antimicrobial Agents. In: A. Gennadios (Ed). Protein-based Films and Coatings. Boca Raton, Florida: CRC Press LC. 485–499.

[5] Talja, R. A., Helén, H., Roos, Y. H., and Jouppila, K. 2007. Effect of Various Polyols and Polyol Contents on Physical and Mechanical Properties of Potato Starch-based Films. *Carbohydrate Polymers*. 67: 288–295.

[6] Fama', L., Flores, S., K., Gerchenson, L., & Goyanes, S. 2006. Physical Characterization of Cassava Starch Biofilm with Special Reference to Dynamic Mechanical Properties at Low Temperatures. *Carbohydrate Polymers*. 66: 8–15.

[7] Buonocore, G. G., Del Nobile, M. A., Panizza, A., Corbo, M. R., and Nicolais, L. 2003. A General Approach to Describe the Antimicrobial Agent Release From Highly Swellable Films Intended for Food Packaging Applications. *Journal of Controlled Release*. 90: 97–107.

[8] Chung D., Papadakis S. E. and Yam K. L. 2002. Evaluation of a Polymer Coating Containing Triclosan as the Antimicrobial Layer for Packaging Materials. *International Journal of Food Science and Technology*. 38: 165–169.

[9] Kuorwel, K. K., Cran, M. J., Sonneveld, K., Miltz, J. and Bigger, S. W. 2011. Antimicrobial Activity of Natural Agents against Saccharomyces cerevisiae. Packaging Technology and Science. 24: 299–307.

[10] Kuorwel, K. K., Cran, M. J., Sonneveld, K., Miltz, J.and Bigger, S. W. 2011. Antimicrobial Activity of Natural Agents against Staphylococcus Aureus. *Journal of Food Science.* 76(8): 531–537.

[11] Lim, G-O., Jang, S-A., and Song K. B. 2010. Physical and Antimicrobial Properties of Gelidiumcorneum/Nano-Clay Composite Film Containing Grapefruit Seed Extract or Thymol. *Journal of Food Engineering*. 98: 415–420.

[12] Del Nobile, M. A., Conte, A., Incoronato, A. L., and Panza, O. 2008. Antimicrobial Efficacy and Release Kinetics of Thymol from Zein Films. *Journal of Food Engineering*. 89: 57–63.

[13] Dusan, F., Marion, S., Katarina, D., Dobroslava, B. 2006. Essentials Oils-Their Antimicrobial Activity Against Escherichia coli and Effect on Intestinal Cell Viability. *Toxicology in Vitro.* 20: 1435–1445.

[14] Di Pasqua, R., Hoskin, N., Betts, G., and Mauriello, G. 2006. Changes in Membrane Fatty Acids Composition of Microbial Cells Induced by Addcition of Thymol, Carvacrol, Limonene, Cinnamaldehyde and Eugenol in the Growing Media. *Journal of Agricultural and Food Chemistry*. 54: 2745–2749.

[15] Gennadios, A. and Testin, R. F., 1993. Temperature Effect on Oxygen Permeability of Edible Protein-Based Films. *Journal of Food Science.* 58: 212–219.

[16] Mitchell, R. L., Murphy1, C. F. 1972. Heat Sealable Hydroxyethyl Cellulose Film And Process for Preparing same. US 3652316.

[17] Finkenstadt, V. L., and Willet, J. L. 2004. A Direct-Current Resistance Technique for Determining Moisture Content in Native Starches and Starch-Based Plasticized Materials. *Carbohydrate Polymers*. 55: 149–154.

[18] Chang, P., Chea, P. B., Seow, C. C. 2000. Plasticizing-Antiplasticizing Effects of Water on Physical Properties of Tapioca Starch Films in the Glassy State. *Journal of Food Science*. 65(3): 445–451.

[19] Han Jaejoon. 2006. Antimicrobial Packaging System for Optimization of Electron Beam Irradiation of Fresh Produce. Ph.D. Thesis. Texas A&M University.

[20] Soares, R. M. D., Lima, A. M. F., Oliveira, R. V. B., Pires, A. T. N., and Soldi, V. 2005. Thermal Degradation of Biodegradable Edible Films Based on Xanthan and Starches from Different Sources. *Polymer Degradation and Stability*. 90: 449–454.

[21] Xiong, H., Tang, S., Tang, H., and Zou, P. 2008. The Structure and Properties of a Starch-based Biodegradable Film. *Carbohydrate Polymers.* 71: 263–268.