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# Effects of *Allium sativum* essence oil as antimicrobial agent for food packaging plastic film

Suet-Yen Sung [a], Lee Tin Sin [a,*], Tiam-Ting Tee [a], Soo-Tueen Bee [a,*], A.R. Rahmat [b]

[a] Department of Chemical Engineering, Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Jalan Genting Kelang, 53300 Setapak, Kuala Lumpur, Malaysia
[b] Department of Polymer Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia

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ABSTRACT

This research is focused on the development of antimicrobial (AM) plastic packaging by using blown film extrusion machine. Plastic films incorporated with 0, 2, 4, 6 and 8% *Allium sativum* essence oil (AEO) (% w/w) were tested for AM activities against beef related bacteria namely *Listeria monocytogenes*, *Escherichia coli* and *Brochothrix thermosphacta*. Mechanical properties, thermal properties and microstructure of films were investigated to justify the effects of AEO on the film physicomechanical functionality. The retraction zone in agar disk diffusion result shows that low-density-polyethylene/ethylene-vinyl-acetate (LDPE/EVA) co-polymer film with 8% AEO significantly reduce the concentration of bacteria with inhibition strength of *L. monocytogenes* > *B. thermosphacta* > *E. coli*. For challenge test, the AM films sufficiently reduced the growth rate of *L. monocytogenes* on cooked beef at 4 °C. High amount of AEO only slightly weakens the mechanical properties whereby film crystallinity increases significantly when small amount of AEO incorporated.

*Industrial relevance:* The current innovation is applied for the meat industry where a prolong storage of meat is required. *A. sativum* extract is a natural essential oil which will not cause health risk when in contact with food when applied in the packaging.



## 1. Introduction

It is commonly known that the spoilage of foods is mainly caused by microbial contamination. Most of the food preservation methods such as fermentation, drying, thermal processing, freezing, refrigeration and modified atmosphere are effective but having their own limitation especially when applied on fresh meat which required minimal changes of meat texture (Quintavalla & Vicini, 2002). Since microbial contamination always occurs primarily on food surface, many studies suggested the addition of antimicrobial (AM) agents onto the food surfaces to suppress the growth of microbial to prolong the shelf life of food. However, the direct application of AM agents onto foods is not effective to inhibit microorganisms due to rapid diffusion of AM agent into food and denaturization of the active substances by food constituent. In order to maintain the meat quality, variety alternative packaging systems have been developed to preserve foods as well as ensuring the safety of foods. In fact, the latest development of food packaging is about utilization of specialty films which containing bio-active ingredients to suppress the reactivity of degrading agents (Gutierrez, Sanchez, Batlle, & Nerin, 2009). In this context, AM agents are normally added as the active agent into the packaging (Rodriguez, Sepulveda, Bruna, Guarda, & Galotto, 2012). AM packaging film is defined as packaging containing AM agent where this agent could embed inside polymer chains by offering a slow and continuously migration to form antimicrobial layer on the food surface. In other words, the AM agent could maintain in high concentration over a long time of application (Quintavalla & Vicini, 2002).

Generally, there are various existing chemical components of plant-origin possessing antimicrobial effects which include saponin and flavonoids, thiosulfinates, glucosinolates and saponins. Spices such as cumin, cinnamon and cloves exhibited the greatest AM effects against bacteria such as *Escherichia coli*, *Enterococcus faecalis*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, and *Candida albicans* by using the method of disk-diffusion (Agaoglu, Dostbil, & Alemdar, 2007). Besides, Pattaratanawadee, Thongson, Mahakarnchanakul, and Wanchaitanawong (2006) concluded that *Zingiber officinale*, *Alpinia galanga*, *Curcuma longa*, and *Boesenbergia pandurata* extracts can fight against Gram-positive and Gram-negative pathogenic bacteria especially at the range of 0.2–0.4% v/v and 8–10% v/v for finger root and all of the spices respectively (Pattaratanawadee et al., 2006). Generally, some essential oils are made up mainly of terpenoids and sesquiterpenes with different groups of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones also possessing the AM effects (Fisher & Phillips, 2006). For instance, Sudjana et al. (2009) found that olive leaves (*Olea europaea*) exhibited AM effects against *Campylobacter jejuni*, *Helicobacter*

* Corresponding author. Tel.: +60 3 4107 9802; fax: +60 3 4107 9803.
*E-mail addresses:* direct.tinsin@gmail.com (L.T. Sin), direct.beest@gmail.com (S.-T. Bee).

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*pylori* and *S. aureus*. Rahman and Kang (2009) have shown that ethanolic leaf extract of *Lonicera japonica* Thunb has AM effects against some food-borne pathogens. In this study, *Allium sativum* essence oil (AEO) was utilized as the AM agent to be added into the packaging film. *A. sativum* or commonly known as garlic is well recognized of its antibacterial functionality for supplement nutrients of mankind. They are natural and complex compounds normally formed by aromatic plants as secondary metabolites. They benefit humans because of the properties of antibacterial, antifungal and antioxidant to build up body immunity (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). The AM properties of garlic were first studied at year 1844. In later time, Cavallito, Bailey, and Buck (1944) found that allyl sulfides are the major constituent of crushed garlic and allicin is responsible for the antibacterial activities. Other than the AM benefit, garlic has been reported to reduce blood lipids, modulate cardiovascular, enhance immune functions, and having antioxidant and anticancer properties. Up to date, there are only a few published papers about utilizing AEO as AM agent in food packaging system. Most of the studies were focused on edible film, and as far as we know, researches done on AEO incorporated-plastic film produced by blown film extrusion technique are lacking. The reason may be due to allicin that is very unstable and easily decompose under high processing temperature and pressure. Nevertheless, the AM activities may not loss since allicin tends to transform to diallyl sulfide (DAS), diallyl disulfide (DAD), diallyl trisulfide (DAT) and ajoene, which is relatively more stable under high temperature. These compounds also possess comparable AM effect with allicin (Corzo-Martinez, Corzo, & Villamiel, 2007; Tansey & Appleton, 1975). Overall, this study was focused on the effectiveness of AEO-incorporated plastic films to inhibit beef related pathogenic and spoilage bacteria in-vitro. The mechanical and thermal properties of films were assessed to determine the compatibility between AEO and polyethylene compound.

## 2. Experimental

### 2.1. Materials

Raw material for film production, low-density-polyethylene (LDPE), was purchased from Titan Petchem (M) Sdn. Bhd., Malaysia. Ethylene-vinyl-acetate (EVA) copolymer with the grade of UE629 (10% vinyl acetate content) was supplied by USI Corporation, Taiwan. EVA was added with LDPE as the compatibilizer of deodorized *A. sativum* essence oil (AEO) and LDPE matrix. On the other hand, the culture medium, namely Tryptone Soya (agar, CM0131B and broth, CM0129B) and saline peptone powder (CM0733B), was purchased from Oxoid Microbiology, United Kingdom. The AM agent, i.e. deodorized *A. sativum* essence oil was obtained from Xiamen Forever Green Source Biochem Tech. Co., Ltd., China.

### 2.2. Antimicrobial film preparation

The AM plastic films were prepared from pre-mixed 90% low-density-polyethylene (LDPE) pellet and 10% ethylene-vinyl-acetate (EVA) copolymer powder. EVA copolymer pellet was grounded into powder by using ball mill grinder, then blended with AEO (0, 2, 4, 6, 8% w/w respectively) thoroughly in drum tumbler and continued with the addition of LDPE pellet. The masterbatch was manufactured into films by blown film extrusion machine (Tai King Machinery, Taiwan). The temperature in the extruder was set to 170 °C in all heating zones.

### 2.3. Bacterial cultures

Bacterial cultures used in this study were typical beef contaminants including Gram-negative bacteria, *E. coli* (ATCC 10536) and Gram-positive bacteria, *Listeria monocytogenes* (ATCC 13932) and *Brochothrix thermosphacta* (ATCC 11509). *L. monocytogenes* and *E. coli* are pathogenic bacteria and *B. thermosphacta* is beef spoilage bacteria. They were purchased from American Type Culture Collection (ATCC), US. The bacteria cultures were maintained according to the steps describe in manual obtained from supplier. For ceasing bacteria activities, the bacteria strains were stored at −80 °C in Tryptone Soya Broth (TSB) that contained 20% glycerol. For experiment purpose, the bacteria were regularly subcultured on Tryptone Soya Agar (TSA) and stored at 4 °C. In the preparation for antibacterial test, one colony of bacteria was transferred from TSA into 50 ml Tryptone Soya Broth (TSB) and incubated in incubator shaker at 37 °C, 200 rpm for 18 h. Serial dilution was conducted to obtain required concentration of bacteria.

### 2.4. Agar disk diffusion

The AM activities were carried out by agar disk diffusion test. The plastic films were cut into 26 mm diameter disks and placed on TSA plates with approximately $10^6$ cfu/ml of tested bacteria. The plates were incubated for 24 h at 37 °C for *L. monocytogenes* and *E. coli*, and 25 °C for *B. thermosphacta*. After 24 h, the growth of bacteria surrounding and underneath the film was observed and the area of inhibitory/retraction zone was measured. The test was done in triplicate.

### 2.5. Challenge test on cooked beef

In order to further verify the AM activity of the films in a more real situation, the films were tested on cooked beef. The cooked beef was cut into smaller size that weighted 5 g per piece and exposed to UV light for 15 min in prior to test. The meats were then inoculated with 0.1 ml of *E. coli*, *L. monocytogenes and B. thermosphacta* respectively with concentration of $10^6$–$10^7$ cfu/ml. The inoculums spread evenly to obtain bacteria concentration at around $10^5$ cfu/g. The beef was then wrapped tightly with plastic films containing 0, 2, 4, 6 and 8% w/w of AEO respectively and stored immediately at 4 °C for 15 days as normal retail display temperature (Torstveit & Magnussen, 1998). The numbers of bacteria counts were examined immediately after inoculation and periodically after 3, 6, 9 and 15 days of inoculation by using serial dilution method. On the sampling days, two packages for each formulation were opened and the bacteria are extracted by 50 ml saline peptone water with homogenizer (BagMixer). 0.1 ml homogenate was pipette into centrifuge tube and serially diluted with 0.9 ml saline peptone water. 0.1 ml of each diluted homogenate was then transferred onto TSA plate and incubated at 37 °C (*E. coli* and *L. monocytogenes*) or 25 °C (*B. thermosphacta*) in incubator chamber. After 24 h, the number of colonies formed was calculated and expressed as cfu/g.

### 2.6. Tensile test

Tensile properties of the plastic films were determined according to ASTM D882-10 by using Universal Testing Machine Instron 5567. Prior to the test, the film was conditioned to room temperature for 48 h. Each film with different formulations was cut in machine direction (MD) for five sheets with dimension of 100 mm length and 12.7 mm width. The specimen was placed in grips of machine with initial grip distance of 50 mm and the rate of grip separation used was 500 mm/min.

### 2.7. Differential scanning calorimetry (DSC)

The melting behavior of plastic films was determined by using Mettler Toledo DSC823 DSC according to ASTM D3418-08. Samples were cut into approximately 5 mg and sealed by aluminum pan. Another empty pan was used as reference. The DSC machine was set