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Control of bacteria growth on ready-to-eat beef loaves by antimicrobial plastic packaging incorporated with garlic oil

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ABSTRACT

This study was designated to ascertain the effectiveness of low density polyethylene (LDPE) based film incorporated with garlic oil for inhibition of food pathogen on ready-to-eat beef loaves. The blown film extrusion method was employed to produce film samples added with garlic oil in 2, 4, 6 and 8% w/w as well as sample with 0% w/w which served as control throughout the study. Besides, several analyses were also conducted to determine the water vapour barrier properties, thermal stability and bonding interaction of the plastic packaging as influenced by the incorporation of garlic oil. The outcomes of challenge test showed that regardless of the garlic oil amount (2–8% w/w), the antimicrobial plastic packaging was able to reduce the number of *Listeria monocytogenes* on beef loaves after 3, 6, 9 and 15 days of storage at 4 °C. However, there were insignificant effects on both *Escherichia coli* and *Brochothrix thermosphacta*. For water vapour barrier properties, films with higher amount of garlic oil proved to have weaker barrier properties. There was lack of significant difference in the thermal stability for all samples when tested with thermogravimetry analyser. Also, the infrared analysis indicated garlic oil does not change the polymer structure.

1. Introduction

Cooked meat for retail sale is often subjected to microbial contamination. Among the microorganisms, *L. monocytogenes* and *Escherichia coli* were the most common bacteria that found in beef products that contributed to food-borne diseases. Consuming foods contaminated by *L. monocytogenes* would lead to fatal diseases such as meningitis and pneumonia which often occurs in the new born, pregnant women and elder folks. *L. monocytogenes* has been involved in food-borne illness outbreak at 1998 and 1999 which caused 21 deaths among 100 reported cases. In fact in year 2002, 10 deaths occurred in United States related to consumption of contaminated meat (Ye, Neetoo, & Chen, 2008). As reported by the government of United States in year 2000, the annual economic loss associated with *L. monocytogenes* was $2.3 billion. *E. coli* O157:H7 is also a facultative anaerobe foodborne illness bacterial that particularly problematic for the beef industry. Consuming foods contaminated by *E. coli* O157:H7 could cause hemorrhagic colitis disease resultant in bloody diarrhea, severe cramping and occasional vomiting that last for 2–9 days (Feng, 2000). For more serious case, it could cause fatal as reported by Mead, Slutsker, and Dietz (1999) where 52 people out of total 1843 were death.

In order to prevent the growth of pathogenic and spoilage microorganisms on Ready-to-Eat (RTE) meat products, antimicrobial (AM) packaging has been developed. AM packaging is defined as packaging containing AM agents and this packaging is more effective compared to direct adding of AM agents into foods. This is because direct adding of AM agent can cause rapid diffusion of the agent into foods; consequently, the active substances denaturized by food constituents rapidly. Whereas, AM packaging offers slow and continuous migration of AM agent from packaging material to food surfaces which enables AM agent to maintain at high concentration over a long period (Quintavalla & Vicini, 2002). Example of the AM agents used commercially in packaging systems included silver substituted zeolite, chlorine dioxide, ethanol, sulfur dioxide, triclosan, and horseradish extract.

In this study, garlic essential oil was used as the AM agent to be incorporated into plastic packaging film by using blown film extruder. Garlic or *Allium sativum* in scientific name, has been used widely as medicine many years ago. It is used traditionally as food

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preservative to inhibit the growth of pathogens and spoilage microorganisms where a wide range of microorganisms included bacteria, mold, fungi, parasites and viruses can be inhibited. 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 related to garlic oil used as AM agent in food packaging system. Most of the studies were focus on edible film, and as per known, no research has studied garlic oil incorporated-plastic film produced by blown film extrusion technique. The reason may be due to the AM agent in garlic, known as allicin, is very unstable and easily decompose when process under high temperature and pressure. However, the antimicrobial activities may not loss since the breakdown products of allicin, i.e., diallyl sulphide (DAS), diallyl disulphide (DAD), diallyl trisulphide (DAT) and ajoene have reported effective against certain type of microorganisms (Corzo-Martinez, Corzo, & Villamiel, 2007; Tansey & Appleton, 1975). Therefore, this study was conducted to verify the AM effectiveness of blown film extruded AM film when used in actual beef packaging conditions. Besides, several analyses were also carried out to determine the water barrier properties, thermal stability and bonding interaction of the plastic packaging as affected by the incorporation of garlic oil.

2. Experimental

2.1. Materials

Deodorized garlic essential oil which contained more than 35% of allicin was purchased from Xiamen Forever Green Source Biochem Tech. Co. Ltd. For the packaging film, LDPE with 2.0 g/10 min of melt flow index was purchased from Titan Group whereby ethylene vinyl acetate copolymer (EVA) with the grade of UE629 (10% vinyl acetate content) was supplied by USI Corporation, Taiwan. Tryptone Soya Agar (CM0131B) (TSA), Tryptone Soya Broth (CM0129B) (TSB), and saline peptone powder (CM0733B) were from Oxoid, United Kingdom which were supplied by Choice Care Sdn. Bhd., Malaysia. Microbe strain used were typical beef contaminants included gram-negative bacteria, *E. coli* (ATCC 10536) and gram-positive bacteria, *L. monocytogenes* (ATCC 13932) and *B. 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), United States.

2.2. Bacteria culture maintenance

The bacteria cultures were maintained according to the steps described in manual obtained from supplier. For ceasing bacteria activities, the bacteria strains were stored at −80 °C in Tryptone Soy Broth (TSB) that contained 20% glycerol. For experiment purpose, the bacteria were regularly subculture on Tryptone Soya Agar (TSA) and stored at 4 °C. In the preparation for challenge 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.

2.3. Film preparation

LDPE film were added with various amount of garlic oil ranging from 0, 2, 4 6 and 8 weight percent (% w/w). Firstly, a masterbatch preparation was prepared in such a way that the EVA was grinded to powder form using ball mill grinding machine and 2% w/w of garlic oil was added. The mixture was then mixed thoroughly by using tumbler. LDPE pellets were added and mixed to get 5 batches. The process was repeated with 4, 6 and 8% w/w of garlic oil respectively. The films were produced by blown film extruder. The temperature was set at 170 °C throughout all the zones from barrel to die, and the target film thickness was 40–50 μm. After extrusion, the films were wrapped with aluminium foil to prevent loss of AM agent by evaporation. Summary of film formulation is listed in Table 1.

2.4. Challenge test

The antimicrobial activity of the films was tested on Ready-to-Eat (RTE) beef loaves. Once purchased from local retailer, the meats were steamed to cooked and cut into loaf shape that weighted 5 g per piece. In order to sterilize the beef loaves, every single side of the meat surfaces was exposed to UV light for 15 min prior to test. The meat were then randomly divided into 3 sets for different bacteria inoculations, and each set divided into five lots for different packaging formulation, i.e., garlic oil in 0, 2, 4, 6 and 8% w/w respectively. For inoculation of bacteria on beef, 0.1 ml of each bacteria strain (*E. coli*, *L. monocytogenes and B. thermosphacta*) with concentration of 10^6–10^7 cfu/ml was transferred onto top and bottom surfaces of meat and spread evenly to obtain bacteria concentration of approximately 10^5 cfu/g. The samples were left for 5 min to allow the inoculums to soak and attached to the meats before wrapped with plastic films containing 0, 2, 4, 6 and 8% w/w of garlic oil respectively. The meats were tightly contacted with the films and the three open sides of films were sealed and stored immediately at 4 °C for 15 days to mimic the normal retail display temperature (Torstveit & Magnussen, 1998). The counts of bacteria were determined by using serial dilution method immediately. All these were done after inoculation and periodically at the end of 3, 6, 9 and 15 days of inoculation. For determination the number of bacteria growth on the sampling days, two packages for each formulation were opened. The bacteria on beef were extracted by adding 50 ml saline peptone water into each sample and homogenized with laboratory blender (Bagmixer) for 2 min. 0.1 ml homogenate was pipette transferred into centrifuge tube and serially diluted with 0.9 ml saline peptone water. 0.1 ml of each diluted homogenate were then transferred onto TSA plate and incubated at 37 °C (*E. coli and L. monocytogenes*) and 25 °C (*B. thermosphacta*) in incubator chamber. After 24 h, the number of colonies formed was calculated and expressed as cfu/g.

2.5. Water vapour barrier properties

The water vapour barrier properties of the film samples were determined in term of water vapour transmission rate (WVTR) at 23 °C and 60% relative humidity (RH) according to the ASTM E96/E96M-10 (ASTM, 2010). The circular test cup was filled with silica gel (desiccant) activated at 200 °C (0%RH) and then sealed by the test films. It was covered with a lid having 60 mm of opening (film tested surface area) which allowed vapour to pass through. The cups were placed inside a control chamber which was maintained

Table 1
Summary of film formulations.

Weight of LDPE (kg)	Weight of EVA (kg)	Weight of garlic oil (kg)	Weight percent of garlic oil (% w/w)
3.6	0.4	0.00	0 (Control)
3.6	0.4	0.08	2
3.6	0.4	0.16	4
3.6	0.4	0.24	6
3.6	0.4	0.32	8