Available online at www.sciencedirect.com
ScienceDirect
LWT 40 (2007) 1545–1554
LWT
www.elsevier.com/locate/lwt

The influence of electron beam irradiation of antimicrobial-coated LDPE/polyamide films on antimicrobial activity and film properties

J. Han, M.E. Castell-Perez*, R.G. Moreira

Department of Biological and Agricultural Engineering, Texas A&M University, College Station, TX 77843-2117, USA

Received 26 July 2006; received in revised form 8 November 2006; accepted 16 November 2006

Abstract

We evaluated the effects of ionizing radiation (1–3 kGy) and incorporation of antimicrobials on the functional properties of low-density polyethylene (LDPE)/polyamide films. We established the antimicrobial effectiveness of several coatings of FDA-approved antimicrobial compounds including sorbic acid, carvacrol, trans-cinnamaldehyde, thymol and rosemary oleoresin using selected food pathogen surrogates. The antimicrobial coatings were applied to one side of the LDPE films and dried. Films were irradiated using a 10 MeV linear electron beam accelerator at room temperature. All films showed inhibition zones in an agar diffusion test against *Listeria innocua* ATCC 33090 and *Escherichia coli* ATCC 884. In the liquid culture test, the antimicrobials significantly ($p \leqslant 0.05$) reduced the specific growth rate of *L. innocua* by 3.8–8.5%, and decreased final cell concentration of both strains by 5.7–14.6% and 7.2–16.8%, respectively. All active compounds retained the antimicrobial activity when exposed to 1–3 kGy. Neither the presence of active compound nor dose affected the film's tensile strength and toughness. Additionally, films became more ductile and had improved moisture barrier functionality. Film's oxygen permeability was not affected by either treatment. Results are an initial step toward the development of self-sterile active packaging materials for use in combination with irradiation treatment of foods.

Keywords: Electron beam irradiation; Active compound; Packaging; *Listeria*, *Escherichia coli*

1. Introduction

The application of radiation treatment to fresh produce is a feasible way to extend shelf life and improve safety issues. However, the use of ionizing radiation for decontamination purposes is limited by undesirable changes in produce quality. Depending upon the dose and dose rate used in a particular treatment, discoloration as well as loss in texture and nutritional qualities may occur (Somogyi & Romani, 1964; Bramlage & Lipton, 1965; Howard & Buescher, 1989; Han, Gomes-Feitosa, Castell-Perez, Moreira, & Silva, 2004). An alternative is to increase the radiation sensitivity of the target pathogens in order to lower required radiation dose and successfully reduce product quality change (Borsa, Lacroix, Ouattara, & Chiasson, 2004).

Most radiation studies are conducted using gamma rays as the irradiation source and only a few were performed with electron beam, a more effective (direct deposition of energy) and easier to control technology with superior dose rate to gamma rays—10^3–10^6 Gy/s from electron beams and 1–100 Gy/min from ^{60}Co, respectively (Cleland, 1983). In addition, after radiation treatment, polymeric materials can undergo changes in mechanical and functional properties depending on the polymer types and absorbed radiation dose (Pentimalli et al., 2000). Therefore, this potential effect must be evaluated.

One approach to lowering required dose is to use antimicrobial packaging materials. These novel materials can control microbial contamination by reducing the growth rate and maximum growth population, as well as extending the lag period of the target microorganism, contributing to prolong the product shelf life and maintain its safety (Appendini & Hotchkiss, 2002; Cutter, 2002). The concept of antimicrobial polymeric materials was introduced and developed in the biomedical science field to

*Corresponding author. Tel.: +1 979 862 7645; fax: +1 979 845 3932.
E-mail address: ecastell@tamu.edu (M.E. Castell-Perez).

0023-6438/$30.00
doi:10.1016/j.lwt.2006.11.012

protect sutures or implants from microbial contamination (Endo, Tani, & Kodama, 1987; Desai, Hossainy, & Hubbell, 1992; Vigo, 1994). Likely incorporation of chemical preservatives or antimicrobial agents into a food packaging material (film) demonstrated to enhance microbial safety (Han, 2000). This observation is based on the principle that active compounds can be effectively released from the packaging material to the foodstuffs.

Preservatives with antimicrobial activity play an important role in preventing spoilage and assuring safety of various foods. Many of these agents have been successfully incorporated directly into packaging materials to confer antimicrobial property. Examples include sulfites and sulfur dioxide, nitrite and nitrate salts, sorbic acid and its sodium and potassium salts, glyceryl esters, propionic acid, acetic acid, epoxides, antibiotics, various natural essential oils and others (Lindsay, 1996; Appendini & Hotchkiss, 2002).

The interest in the development and usage of natural antimicrobial agents as additives in packaging materials has increased markedly due to their potential safety advantages (Roller, 2003). Although different results are observed depending on test conditions (such as strain type, growth condition of microorganisms, and the source and composition of the antimicrobial compound), some GRAS (generally recognized as safe) spices or essential oils always act very effectively in inhibiting microbial growth.

There are various ways to incorporate antimicrobial agents into polymers. Thermal polymer processing methods such as extrusion and injection molding are used for heat-stable antimicrobial agents like silver substituted zeolitres. Meanwhile, for heat-sensitive antimicrobials such as enzymes and volatile compounds, solvent compounding may be a more suitable method into polymers. Antimicrobial compounds that cannot tolerate the high temperatures used in polymer processing are often either coated onto the film material after forming or are added to cast films (Appendini & Hotchkiss, 2002), thus natural essential oils should be incorporated directly into polymeric materials.

Listeria monocytogenes and *Escherichia coli* O157:H7 are common pathogenic bacteria associated with foods (Jay, 1996; Sumner & Peter, 1997), and their resistance to a specific treatment can be mimicked using indicator or surrogate microorganisms. *Listeria innocua* ATCC 33090 and *E. coli* ATCC 884 are commonly used non-pathogenic surrogate microorganisms for *L. monocytogenes* and *E. coli* O157:H7, respectively (FDA, 2001). Our main goal was to determine the feasibility of increasing pathogen sensitivity to electron beam irradiation using antimicrobial films by studying their effect on the selected surrogates. Specific objectives included: (1) testing the antimicrobial effectiveness of various FDA-approved compounds incorporated into low-density polyethylene (LDPE)/polyamide films; (2) establishing whether ionizing radiation (1–3 kGy) would influence the antimicrobial effectiveness of the applied coatings and; (3) determining the effects of electron beam irradiation on the functional properties of the films.

2. Materials and methods

2.1. Preparation of films

Polyamide was chosen as coating medium for incorporating active compounds on the surface of the LDPE film because it is approved by the FDA for use in adhesives and coating components in food packaging materials (CFR, 2003). Polyamide resin (Cognis Corporation, New Milford, CT, USA) was dissolved in absolute alcohol with a 4:6 (w/w) ratio, and mixed using a magnetic stirrer for 12 h at medium speed. Next, various active compounds were added to the prepared polyamide solution (100 mg antimicrobial/10 g polyamide solution) and mixed thoroughly for 2 min using a vortex. These compounds consisted of sorbic acid, carvacrol, and trans-cinnamaldehyde (Aldrich, Milwaukee, WI, USA), thymol (Sigma, St. Luis, MO, USA) and rosemary oleoresin (Kalsec, Kalamazoo, MI, USA). The prepared coating medium was then degassed by applying vacuum to remove dissolved air for 1 h, and applied manually on one side of LDPE film (20 cm width, 25 cm height, 50 μm thickness, Plastic Supplies Co., Fullerton, CA, USA) using a No. 12 coating rod (RD Specialties Inc., Webster, NY, USA), and dried at 21 °C for 12 h in a laboratory fume hood. Final coating thickness averaged $\approx 3.03 \pm 0.10$ μm and it was measured by a comparator XL-750 (Brunswick Instrument, Niles, IL, USA) after drying. Polyamide-coated LDPE film without addition of active compounds served as control.

2.2. Film irradiation

Irradiation tests were carried out using a 10-MeV (18 kW) electron beam linear accelerator (LINAC) located at the National Center for Electron Beam Food Research, Texas A&M University. The double beam fixture (top and bottom) configuration at two different conveyor speeds (0.3 and 0.1 m/s) was used to obtain doses of 1.0 and 3.0 kGy, respectively. Films (≈ 53 μm thickness) were placed on a single layer at the middle of cardboard boxes, and then placed on top of the conveyor. Irradiation dosage was measured by placing a radiochromic film dosimeter (B3WIN Radiochromic Films, Gex Corp. Centennial, CO, USA) at the top surface of the film. Tests were carried out at room temperature (21 °C). Irradiated and non-irradiated films (controls) were stored in a desiccator at room temperature until further testing.

2.3. Bacteria culture and media

L. innocua ATCC 33090 and *E. coli* ATCC 884 strains were obtained from the National Center for Agricultural Utilization Research (Peoria, IL, USA). Original stock cultures were maintained in plastic vials (Protect™ Bacterial System from Key Scientific, Round Rock, TX, USA) containing glycerol as cryopreservative solution and porous plastic beads, which were chemically sterilized.

The vials were stored in a Harris freezer (Scimetric Inc., Missouri City, TX, USA) at −80 °C. For recovery of culture, one plastic bead containing the desired culture was aseptically transferred into a tube containing 10 mL of tryptic soy broth (TSB; Difco, Detroit, MI, USA), and maintained for 24 h in an incubator at 37 °C. To facilitate recovery, the working stock cultures were maintained on tryptic soy agar (TSA; Difco, Detroit, MI, USA) slants at 4 °C in a refrigerator and grown in an incubator at 37 °C for 24 h until further testing.

2.4. Determination of minimum inhibitory concentrations (MICs)

The MIC of the active compounds against *L. innocua* ATCC 33090 was determined using the broth dilution method (Kim, Marshall, & Wei, 1995). The same authors reported that a gram-positive *L. monocytogenes* is more resistant to selected essential oil constituents than a gram-negative *E. coli*. Thus, in this study, the MICs of the active compounds were determined regarding their antimicrobial activity against the more resistant *L. innocua* ATCC 33090.

Each compound was dissolved in a mixture of 9 mL of sterile distilled water containing 0.1 g of Tween 20 (in 1%, w/v; Sigma, St. Luis, MO, USA) and 1 mL of ethanol. Tween 20 was added to increase the solubility of the hydrophobic compounds in aqueous solvent and improve the penetration of the compounds into bacterial cell wall and membrane (Kim et al., 1995). Next, the mixture (antimicrobial solution) was sonicated for 10 min using a sonicator (Branson 220 Sonicator, Smithkline Company, Shelton, CT, USA) to increase the solubility of the test compound in solvent. A 200 μL aliquot of bacterial suspension at 10^5 CFU/mL and the prepared antimicrobial solution was added to a 50 mL test tube having 19.6 mL of sterile TSB. The final concentration of antimicrobial solution was adjusted to be 5, 10, 25, 50, 100, 250, 500, 1000, and 2000 μg/mL. Next, test tubes were incubated in an incubator at 37 °C with agitation (200 rpm, Model G25, New Brunswick, Scientific Co. Inc., Edison, NJ, USA) for 36 h. The culture was sampled (1.5 mL) every 3 h up to 36 h to obtain microbial growth profiles. The optical density (OD) of each culture sample was measured at $\lambda = 600$ nm using an UV–visible spectrophotometer (Spectronic 20D+, Milton Roy Company, Rochester, NY, USA), and the lowest concentration of active compound resulting in significant no growth was established as the relative MIC against *L. innocua* ATCC 33090.

2.5. Antimicrobial activity

The antimicrobial activity of the coated films against *L. innocua* ATCC 33090 and *E. coli* ATCC 884 strains were evaluated using both agar diffusion and liquid culture test methods. For the agar diffusion method, 20 mL of melted TSA was poured into a Petri dish. A square sample (12 × 12 mm) of plastic film was placed on the Petri dish with the coated side facing the surface of a TSA plate. Microorganisms were grown in 5 mL of TSB for 24 h in an incubator (Model G25, New Brunswick, Scientific Co. Inc., Edison, NJ, USA) at 37 °C, and 0.2 mL of *L. innocua* ATCC 33090 (cell concentration of 1.9×10^9 CFU/mL) or *E. coli* ATCC 884 (cell concentration of 3.1×10^8 CFU/mL) culture was spreaded on the agar plate. The plate with the TSA medium was incubated at a constant temperature (37 °C) for 24 h. The clear zone formed around the film square was recorded to the nearest millimeter as an indication of inhibition of the microbial species (Appendini & Hotchkiss, 2002). A clear zone surrounding the film indicates antimicrobial diffusion from the film and subsequent growth inhibition. All experiments were conducted in triplicate.

For the liquid culture test, the films were cut into 30 × 50 mm rectangles using a sterile knife. Three film rectangles (45 cm^2 of total surface area) were immersed in 40 mL TSB containing 0.4 g of Tween 20 in a 50 mL glass test tube and inoculated with 0.4 mL of *L. innocua* ATCC 33090 (cell concentration of 10^5 CFU/mL) or *E. coli* ATCC 884 (cell concentration of 10^5 CFU/mL) culture, and then incubated at 37 °C with agitation (200 rpm, Model G25, New Brunswick, Scientific Co. Inc., Edison, NJ, USA). The culture was sampled (1.5 mL) periodically every 2 h during the incubation period to obtain microbial growth profiles. The optical density (OD_{600}) of each culture sample was measured at $\lambda = 600$ nm using an UV–visible spectrophotometer (Spectronic 20D+, Milton Roy Company, Rochester, NY, USA) to represent the cell concentrations of microorganisms in the media. The microbial growth kinetic parameters were estimated as follows: (a) the lag time (t_{lag}) was estimated from the duration of the lag phase, and (b) the specific cell growth rate (μ) during the exponential growth phase was calculated from:

$$\frac{dX(t)}{dt} = \mu X(t) \quad \text{and} \quad \mu = \frac{d}{dt}(\ln X(t)), \tag{1}$$

where $X(t)$ is the cell concentration of inoculated microorganism in the medium (OD_{600}), μ is the specific growth rate of microorganism (h^{-1}), and t is time (h). The final cell concentration (C_f) was estimated from the optical density (OD_{600}) at stationary phase using standard methods (Dorran, 1995; Chung, Papadakis, & Yam, 2003). All experiments were conducted in triplicate.

2.6. Properties of films

We evaluated the potential effect of irradiation on the mechanical properties of the film using standard methods (ASTM, 2000). Film strips (20 × 60 mm) were subjected to large deformations (under tension mode) using a TA-XT2 Texture Analyzer (Texture Technologies Corporation, Scardale, NY) in accordance to ASTM method D882-00 (ASTM, 2000). The value of the load cell was 25 kg, and the cross head speed was 30 mm/min. Tensile strength, percentage elongation-at-break, and toughness

were determined from the stress–strain data obtained from the force–deformation curves. The area under the stress–strain curve represents the films' toughness. All tests were conducted four times at room temperature (21 °C).

Barrier properties—oxygen and water vapor permeability—were measured using the MAS 500 and MAS 1000, oxygen and water diffusion systems (MAS Technologies Inc., Zumbrota, MN, USA), based on the ASTM F1770 Standard Method (ASTM, 1997). The permeability coefficient (P, kg/s m Pa) was calculated as

$$P = \frac{F_e L}{p} \quad (2)$$

with F_e as the film permeability flux in kg/m^2 s, L as the barrier (film) thickness, and p as the water vapor or oxygen partial pressure difference on each side of the film. The testing conditions used were 65% relative humidity (RH) and 25 °C temperature. All measurements were performed in quadruplicate.

2.7. Statistical analysis

Data analysis was performed using the Statistical Analysis System (SAS) software, version 8.1 (SAS Institute, Cary, NC, USA). The General Linear Models Procedure was used for analysis of variance, with main effect means separated by the Student–Newman–Keuls test. Significance was defined at $p \leqslant 0.05$.

3. Results and discussion

3.1. Growth inhibition effectiveness of active compounds

The different test compounds showed various degrees of growth inhibition against *L. innocua* ATCC 33090 using the broth dilution method (Fig. 1). The growth of *L. innocua* ATCC 33090 was inhibited by sorbic acid and rosemary oleoresin at 2000 μg/mL, which delayed the lag phase and lowered growth rate and final cell concentration of the microorganism. However, these two compounds (at concentration up to 2000 μg/mL) did not completely inhibit the microbial growth of the surrogate. Sorbic acid and rosemary oleoresin were least effective in terms of inhibiting growth, and their MICs could well be over 2000 μg/mL, to inhibit the growth of *L. innocua* ATCC 33090. Thymol inhibited bacterial growth at 1000–2000 μg/mL (in 0.1–0.2%, w/v) effectively, and its MIC was established as 2000 μg/mL which resulted in significant ($p > 0.05$) no bacterial growth. The growth of *L. innocua* ATCC 33090 was inhibited by carvacrol at 500–2000 μg/mL (in 0.05–0.2%, w/v), and the MIC of carvacrol was established as 2000 μg/mL at which bacterial growth was inhibited completely ($p > 0.05$). Growth was completely inhibited by trans-cinnamaldehyde at the concentration range of 250–2000 μg/mL. Although test concentrations at 25–100 μg/mL delayed the lag phase of the growth curve, they did not inhibit the microbial growth effectively which means there was a significant ($p \leqslant 0.05$) increase in bacterial population during the incubation period (36 h). The MIC of trans-cinnamaldehyde against *L. innocua* ATCC 33090 was established as 250 μg/mL (in 0.025%, w/v) in this study. Trans-cinnamaldehyde had the strongest inhibitory activity against *L. innocua* ATCC 33090, followed by carvacrol and thymol, while sorbic acid and rosemary oleoresin were the least inhibitory compounds with higher MICs.

3.2. Agar diffusion test

All films coated with active compound showed antimicrobial activity against both *L. innocua* ATCC 33090 and *E. coli* ATCC 884 (Table 1). Inhibition zones surrounding the film square were formed, ranging from 4.6 to 6.5 mm against *L. innocua* ATCC 33090, and from 2.1 to 4.9 mm against *E. coli* ATCC 884, respectively, which indicates that antimicrobial sensitivity of microorganisms varies with strains (Adams & Moss, 1995). All active compounds were equally effective in terms of their antimicrobial ability, regardless of type of compound or radiation dose (1–3 kGy).

3.3. Liquid culture test

Compared to the control (no antimicrobials added), active films effectively inhibited both microbial growth of *L. innocua* ATCC 33090 and *E. coli* ATCC 884 in TSB, and their growth inhibition ability varied depending on the type of compounds (Figs. 2 and 3). *L. innocua* ATCC 33090 reached the stationary phase after 14 h, and the culture media containing coated films significantly ($p \leqslant 0.05$) reduced microbial growth rates (μ) and final cell concentrations (C_f), up to 8.5% and 14.6%, respectively, when compared with the culture medium with control film (Fig. 2). The growth profiles of *E. coli* ATCC 884 were similar to the growth profiles of *L. innocua* ATCC 33090 (Fig. 3). *E. coli* ATCC 884 reached the stationary phase after 12 h, and its final cell concentration was significantly reduced ($p \leqslant 0.05$) up to 16.8% by the films containing active compounds.

The type of active compound used affected the growth rate (μ) during the exponential growth phase and final cell concentration (C_f) at the stationary growth phase of *L. innocua* ATCC 33090 in TSB (Table 2 and Fig. 2). None of the tested active compounds caused a change in the lag time (data not shown), though the compounds reduced the growth rate by 3.8–8.5% and the final cell concentration by 5.7–14.6%, respectively, compared to the control film. Films with carvacrol and sorbic acid were the most effective in reducing growth rates (by 6.7–8.5%).

Films containing active compounds reduced the final cell concentrations significantly ($p \leqslant 0.05$) compared to the control film. Film with trans-cinnamaldehyde, which had the lowest MIC against *L. innocua* ATCC 33090 (Fig. 1), produced the highest reduction in final cell concentration

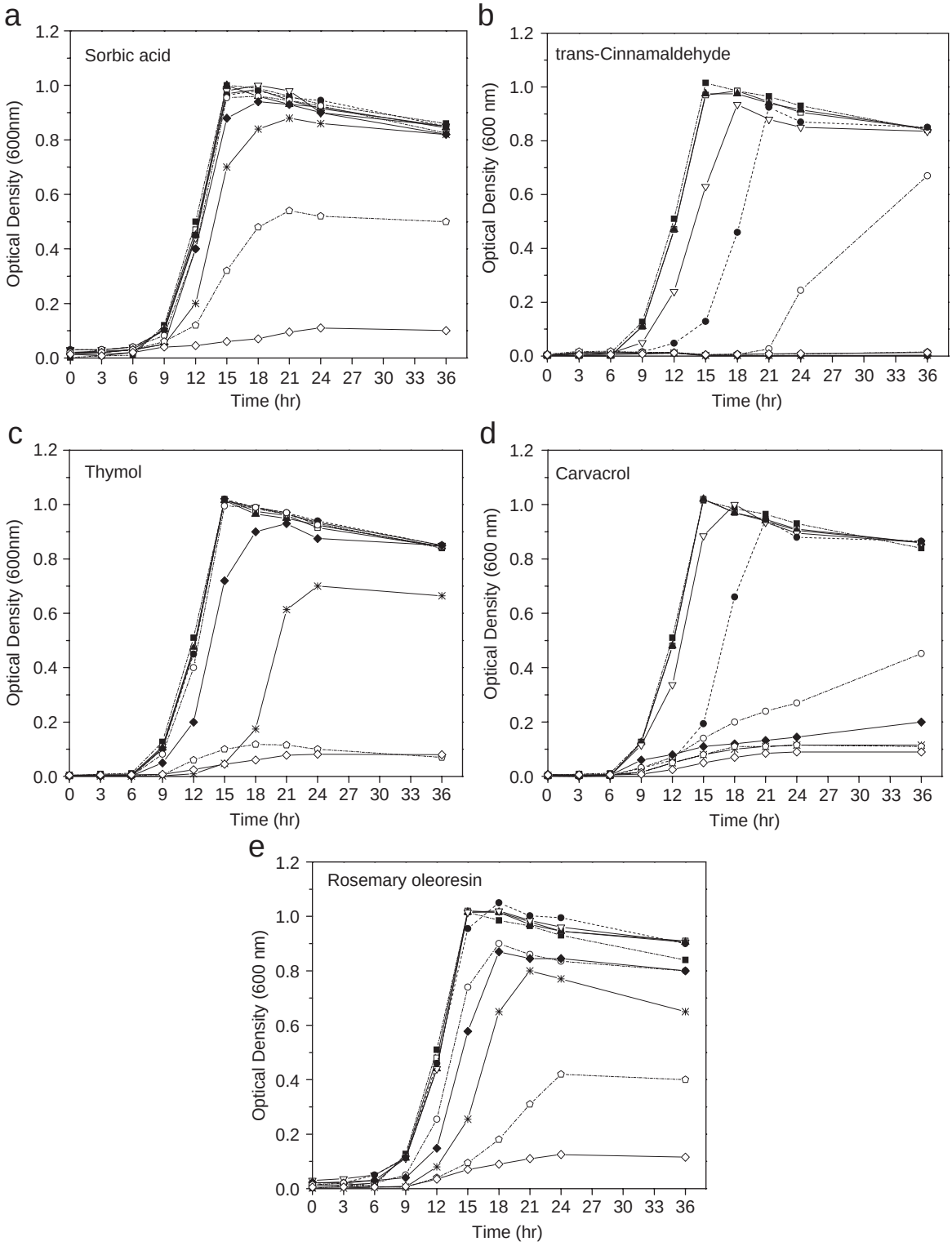

Fig. 1. Growth of *L. innocua* ATCC 33090 in tryptic soy broth as a function of active compound concentration. Key: ■, 0 μg/mL; □, 5 μg/mL; ▲, 10 μg/mL; ∇, 25 μg/mL; ●, 50 μg/mL; ○, 100 μg/mL; ◆, 250 μg/mL; *, 500 μg/mL; ⌂, 1000 μg/mL; ◇, 2000 μg/mL.

(~14.6%). Films containing sorbic acid, thymol and carvacrol had similar ability to reduce the final population of the microorganism. The film with rosemary oleoresin, which had the highest MIC against *L. innocua* ATCC 33090, showed the least bactericidal activity with only 3.8% reduction in growth rate and 5.7% reduction in final cell concentration, respectively (Fig. 1 and Table 2).

Radiation exposure up to 3 kGy did not change ($p > 0.05$) the films' ability to reduce growth rates (μ) of *L. innocua* ATCC 33090 (Table 2). However, films containing

Table 1
Mean zones of inhibition (mm) of antimicrobial films against two different non-pathogenic surrogate microorganisms measured by the agar diffusion test

Film treatment	Dose (kGy)	*Listeria innocua* ATCC 33090	*Escherichia coli* ATCC 884
Control film[a]	0	0	0
	1	0	0
	3	0	0
Sorbic acid	0	5.33ax (1.03)[b]	4.00ay (0.84)
	1	5.00ax (0.89)	4.08az (0.80)
	3	5.17axy (1.03)	3.83axy (1.03)
Trans-cinnamaldehyde	0	5.92ax (1.32)	4.92ay (0.66)
	1	6.08ax (0.80)	3.83ayz (0.87)
	3	6.50ay (1.26)	4.13ay (0.76)
Thymol	0	6.08ax (1.11)	3.58ay (0.86)
	1	6.50ax (1.05)	2.25bx (0.27)
	3	4.67bx (1.03)	3.17abxy (1.12)
Carvacrol	0	5.25ax (0.70)	2.08bx (0.92)
	1	5.67ax (0.87)	3.00axy (0.32)
	3	4.75ax (0.42)	3.75axy (0.61)
Rosemary oleoresin	0	4.67ax (0.41)	4.17ay (0.93)
	1	5.00ax (1.09)	4.25az (1.08)
	3	5.83axy (1.17)	2.50bxy (0.55)

a–c—Means within a same film treatment and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
x,y—Means at same irradiation dose and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
[a]Control means films without added active compounds.
[b]Numbers in parenthesis are the standard deviation.

trans-cinnamaldehyde, thymol, and carvacrol were significantly ($p \leqslant 0.05$) affected by irradiation since their bactericidal (reduced final cell concentration (C_f)) declined by 9.5–9.7% when exposed to 3 kGy dose. This result implies that the antimicrobial activity of active compounds may be affected by radiation exposure depending on the dose level or nature of the compound. The mechanism responsible for this effect needs yet to be understood.

The lag time and growth rate of *E. coli* ATCC 884 were not affected ($p > 0.05$) by the active compounds in films (Table 3). Film with added thymol did not affect the growth rate (μ) of the surrogate. Other tested compounds in film reduced the growth rate (μ) only by 1.2–4.0%, which is not significantly ($p > 0.05$) different from the control film. However, the final cell concentrations (C_f) were significantly ($p \leqslant 0.05$) reduced by all films (by 7.2–16.8%) in TSB media (Table 3 and Fig. 3). Films with trans-cinnamaldehyde were the most effective in reducing the final cell concentration of *E. coli* ATCC 884 (by 16.8%) as well as *L. innocua* ATCC 33090 (by 14.6%), followed by thymol, carvacrol, rosemary oleoresin and sorbic acid. Regardless of radiation dose, the active films were equally effective in reducing the final cell concentrations of *E. coli* ATCC 884, compared to the non-irradiated films.

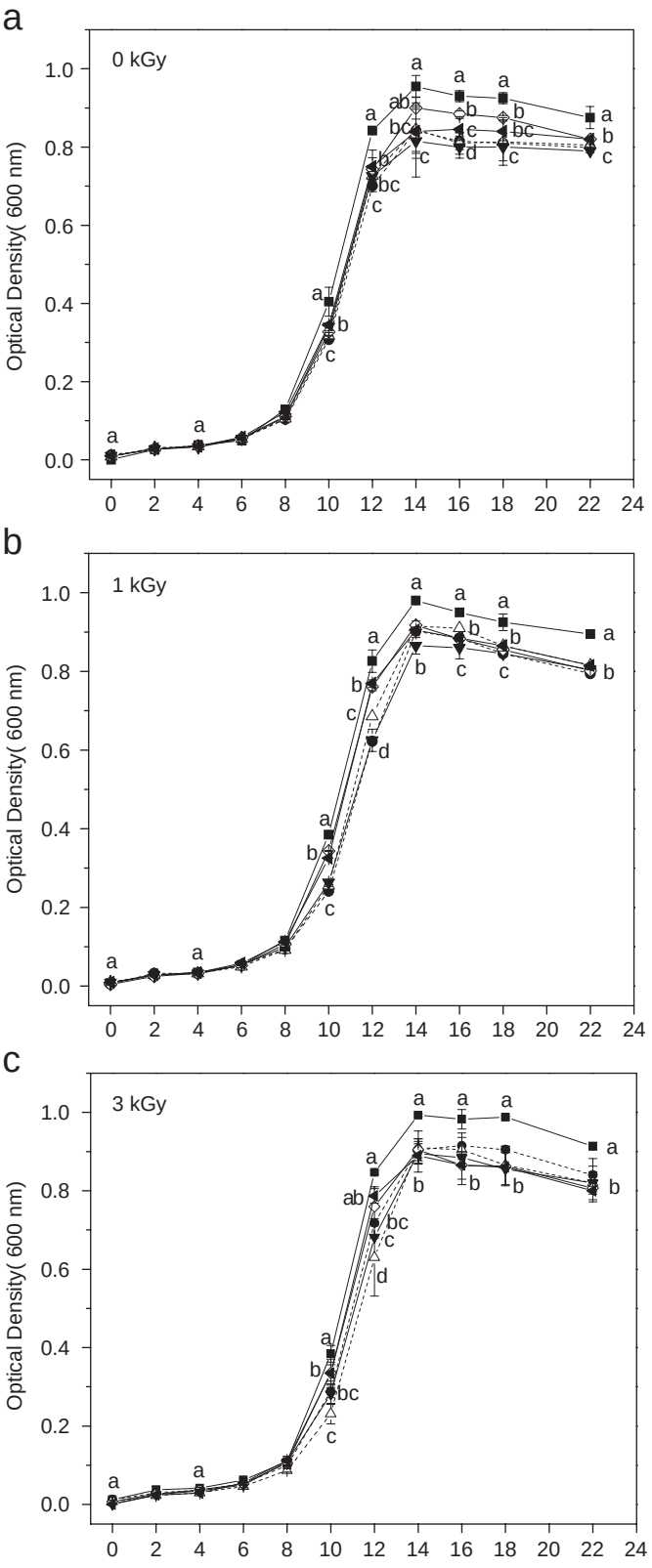

Fig. 2. Growth of *Listeria innocua* ATCC 33090 in tryptic soy broth media in the presence of previously irradiated (1–3 kGy) antimicrobial films. Zero kGy indicates non-irradiated film. Concentration of active compound in film was 100 mg active compound/10 g polyamide solution. Key: ■, control; ●, carvacrol; Δ, sorbic acid; ▼, trans-cinnamaldehyde; ◇, rosemary oleoresin; ◀, thymol. a–d: means at each time interval with different letters are significantly different ($p \leqslant 0.05$).

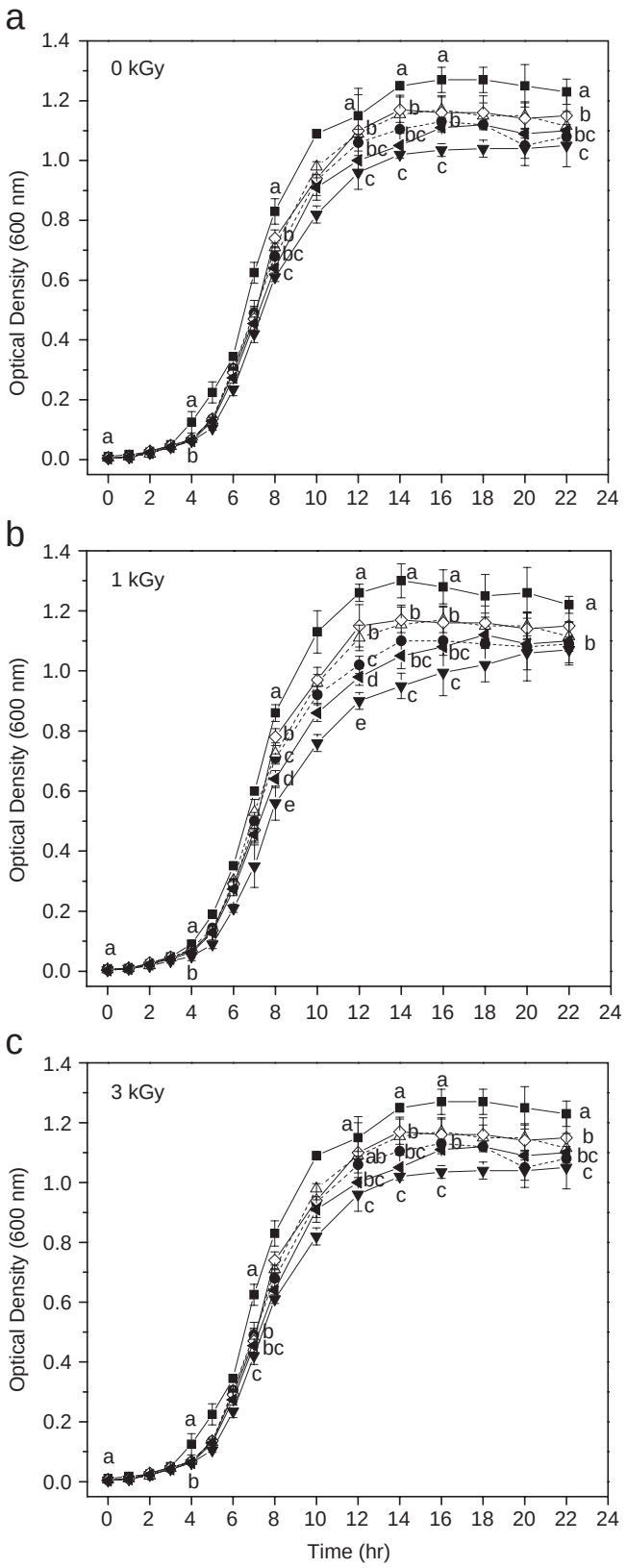

Fig. 3. Growth of *E. coli* ATCC 884 in tryptic soy broth media in the presence of previously irradiated (1–3 kGy) antimicrobial films. Zero kGy indicates non-irradiated film. Concentration of active compound in film was 100 mg active compound/10 g polyamide solution. Key: ■, control; ●, carvacrol; Δ, sorbic acid; ▼, trans-cinnamaldehyde; ◇, rosemary oleoresin; ◀, thymol. a–e: means at each time interval with different letters are significantly different ($p \leqslant 0.05$).

Results show that the MIC values of tested compounds are positively correlated with their bactericidal activity when these compounds are incorporated into LDPE/polyamide films. For instance, active compounds with low MICs were more effective in reducing the growth rates or the final cell concentrations of *L. innocua* ATCC 33090 and *E. coli* ATCC 884 in TSB when applied at the same concentration level. Trans-cinnamaldehyde showed the lowest MIC and it was the most inhibitory compound against *L. innocua* ATCC 33090 and *E. coli* ATCC 884.

All active compounds used in this study were effective in reducing the final bacterial population of *L. innocua* and *E. coli* strains, and maintained their bactericidal ability even after exposure to ionizing radiation up to 3 kGy. These results suggest that selected active compounds incorporated into polyamide-coated LDPE films could help increase the radiation sensitivity of pathogenic microorganisms, thus reducing required radiation doses that could cause detrimental food quality changes. Recent studies using trans-cinnamaldehyde demonstrate this (Han, Castell-Perez, & Moreira, 2006).

3.4. Effect of active compound incorporation and radiation dose on film properties

Irradiation exposure at the dose levels (1–3 kGy) used in this study did not affect ($p > 0.05$) the mechanical properties of the films (Table 4). Addition of active compounds in coating solution (100 mg active compound/10 g polyamide solution) caused only slight changes ($p > 0.05$) in the tensile strength and toughness of the polyamide-coated LDPE films (Table 4). Meanwhile, the films became significantly ($p \leqslant 0.05$) more ductile (increased elongation) by 20.3–39.6% when incorporated with sorbic acid, carvacrol and rosemary oleoresin. The increased flexibility of films might be attributed to the plasticizer effect of the added compounds (Sears & Darby, 1982) by decreasing intermolecular attractions between adjacent polymeric chains and increasing film flexibility (Lieberman & Gilbert, 1973; Sothornvit & Krochta, 2001; Lin, Lee, & Wang, 2004). Studies on the microstructure of these films are currently in progress.

3.5. Film barrier and color properties

Active compounds incorporation into films reduced ($p \leqslant 0.05$) the films water vapor permeability by 28.3–32.1%, but did not affect oxygen permeability (Table 5). This result may be due to the hydrophobic attributes of the active compounds, which may decrease the solubility of water in the polyamide-coated LDPE film.

Irradiation treatment did affect ($p \leqslant 0.05$) water vapor permeability of control film (no compounds added) by 22.3% (Table 5). However, barrier properties of films with active compounds were not affected by exposure to irradiation, which means the films did not undergo structural changes at these dose ranges (1–3 kGy). This result is in

Table 2
Effect of antimicrobial compound and dose level on the specific growth rates[a] (μ) and final cell concentrations (C_f) of *Listeria innocua* ATCC 33090 culture

Film treatment	Dose (kGy)	μ (h^{-1})	% Reduction[b] of μ	C_f (OD_{600})	% Reduction[c] of C_f
Control film[d]	0	0.341ax	–	0.96ax	–
	1	0.349ax	–	0.98ax	–
	3	0.330axy	–	0.99ax	–
Sorbic acid	0	0.318ay	6.74	0.85ayz	11.52
	1	0.324axy	7.16	0.92ay	6.63
	3	0.335axy	−1.43	0.91ay	8.36
Trans-cinnamaldehyde	0	0.325axy	4.70	0.82az	14.66
	1	0.318ay	8.88	0.87aby	11.73
	3	0.328axy	0.57	0.90by	9.87
Thymol	0	0.323axy	5.28	0.85ayz	11.52
	1	0.327axy	6.30	0.91by	7.65
	3	0.344ax	−4.01	0.90by	10.37
Carvacrol	0	0.312ay	8.50	0.84ayz	11.83
	1	0.311ay	10.80	0.90by	7.86
	3	0.312ay	5.16	0.92by	7.85
Rosemary oleoresin	0	0.328axy	3.81	0.90ay	5.76
	1	0.341axy	2.30	0.92ay	6.33
	3	0.330axy	0.00	0.91ay	8.86

a,b—Means within a same film treatment and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
x–z—Means at same irradiation dose and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
[a]Calculated μ from Eq. (1).
[b]% Reduction of $\mu = [1 - (\mu$ of active compound added film/μ of control film)] × 100; films are at the same irradiation dose.
[c]% Reduction of $C_f = [1 - (C_f$ of active compound added film/C_f of control film)] × 100; films are at the same irradiation dose.
[d]Control means films not containing active compounds.

Table 3
Effect of antimicrobial compound and dose level on the specific growth rates (μ)[a] and final cell concentrations (C_f) of *Escherichia coli* ATCC 884 culture

Film treatment	Dose (kGy)	μ (h^{-1})	% Reduction[b] of μ	C_f (OD_{600})	% Reduction[c] of C_f
Control film[d]	0	0.571ax	–	1.25ax	–
	1	0.562ax	–	1.30ax	–
	3	0.586ay	–	1.27ax	–
Sorbic acid	0	0.556ax	2.63	1.16ay	7.20
	1	0.558ax	0.71	1.19ay	8.46
	3	0.556az	5.12	1.18ay	7.09
Trans-cinnamaldehyde	0	0.558ax	2.28	1.04az	16.80
	1	0.550ax	2.14	1.07az	17.69
	3	0.558az	4.78	1.07az	15.75
Thymol	0	0.571ax	0.00	1.09ay	12.80
	1	0.568ax	−1.06	1.12ayz	13.85
	3	0.571ayz	2.56	1.13ay	11.02
Carvacrol	0	0.564ax	1.23	1.09ay	12.80
	1	0.557ax	0.89	1.13ayz	13.08
	3	0.564ayz	3.75	1.12ay	11.81
Rosemary oleoresin	0	0.548ax	4.03	1.15ay	8.00
	1	0.551ax	1.96	1.17ay	10.00
	3	0.614bx	−4.77	1.16ay	8.66

a,b—Means within a same film treatment and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
x–z—Means at same irradiation dose and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
[a]Calculated μ from Eq. (1).
[b]% Reduction of $\mu = [1 - (\mu$ of active compound added film/μ of control film)] × 100; films are at the same irradiation dose.
[c]% Reduction of $C_f = [1 - (C_f$ of active compound added film/C_f of control film)] × 100; films are at the same irradiation dose.
[d]Control means films not containing active compounds.

Table 4
Effect of electron beam irradiation treatment (dose) and antimicrobial coating on mechanical properties of LDPE/polyamide films

Film treatment	Dose (kGy)	Tensile strength (MPa)	Elongation-at-break (%)	Toughness (J)
Control film[a]	0	17.62ax (1.21)[b]	316.59ax (70.18)	2.52ax (0.43)
	1	19.05ay (0.91)	455.09ax (124.93)	3.69ay (1.15)
	3	18.12axy (0.79)	358.86ax (76.40)	2.81ax (1.17)
Sorbic acid	0	18.27ax (0.90)	380.98axy (78.20)	2.80ax (0.45)
	1	18.55axy (0.58)	355.88ax (58.32)	3.27axy (0.64)
	3	18.07axy (0.56)	365.41ax (35.84)	3.11ax (0.34)
Trans-cinnamaldehyde	0	17.74abx (0.26)	327.32ax (14.37)	1.98ax (0.72)
	1	16.96ax (0.67)	313.99ax (42.49)	2.21axy (0.52)
	3	18.62bxyz (0.65)	416.95bx (27.10)	3.58bx (0.27)
Thymol	0	17.34ax (0.63)	317.80ax (25.38)	2.53ax (0.50)
	1	17.82axy (0.57)	355.74ax (56.90)	2.60axy (0.73)
	3	19.51bz (0.41)	436.56bx (43.19)	3.41ax (1.11)
Carvacrol	0	19.03ax (0.88)	441.93ay (44.80)	2.49ax (0.97)
	1	17.83axy (0.89)	356.61ax (20.85)	1.81axy (0.62)
	3	19.02ayz (0.80)	400.86ax (62.73)	2.15ax (0.74)
Rosemary oleoresin	0	18.92ax (0.55)	415.31axy (56.03)	2.76ax (1.20)
	1	18.43axy (0.90)	348.31ax (82.09)	3.02axy (0.73)
	3	17.66ax (0.40)	371.95ax (48.21)	2.78ax (1.00)

a,b—Means within a same film treatment and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
x–z—Means at same irradiation dose and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
Tests were conducted at room temperature (21 °C).
[a]Control means films not containing active compounds.
[b]Numbers in parenthesis are the standard deviation.

Table 5
Effect of electron beam irradiation treatment (dose) and antimicrobial coating on water vapor and oxygen permeability of LDPE/polyamide films

Film treatment	Dose (kGy)	Water vapor permeability ($\times 10^2$ g mm/m^2 d atm)	Oxygen permeability ($\times 10^4$ mL mm/m^2 d atm)
Control film[a]	0	14.10ay (0.56)[b]	2.89ax (0.16)
	1	10.95bx (1.14)	2.83ax (0.11)
	3	11.46by (0.64)	2.93axy (0.16)
Sorbic acid	0	10.08ax (0.58)	3.01ax (0.14)
	1	10.06ax (0.38)	2.87ax (0.19)
	3	9.60ax (0.28)	2.71ax (0.12)
Trans-cinnamaldehyde	0	9.78ax (0.30)	2.84ax (0.11)
	1	9.96ax (0.43)	2.85ax (0.14)
	3	9.72ax (0.38)	2.84axy (0.12)
Thymol	0	9.73ax (0.41)	2.91ax (0.17)
	1	10.11ax (0.28)	2.84ax (0.17)
	3	9.68ax (0.28)	2.81axy (0.13)
Carvacrol	0	9.58ax (0.23)	2.81ax (0.22)
	1	10.06ax (0.28)	2.99ax (0.26)
	3	9.93ax (0.38)	3.12ay (0.24)
Rosemary oleoresin	0	10.11ax (0.58)	3.02ax (0.45)
	1	10.29ax (0.30)	2.90ax (0.19)
	3	11.25by (0.53)	2.88axy (0.14)

a,b—Means within a same film treatment and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
x,y—Means at same irradiation dose and column, which are not followed by a common letter, are significantly different ($p \leqslant 0.05$).
Tests were conducted at 25°C and 65% relative humidity.
[a]Control means films not containing active compounds.
[b]Numbers in parenthesis are the standard deviation.

agreement with our results on mechanical properties. Irradiation treatment (dose) did not affect the color properties (lightness, total color difference, chroma) of the films.

4. Conclusions

The antimicrobial activity of polyamide-coated LDPE films containing active compounds was clearly demonstrated against two commonly used surrogates with significant reduction of microbial growth rates and final cell populations of the tested microorganisms. Electron beam irradiation up to 3 kGy causes small or negligible changes on film functionality. An interesting finding was that film flexibility and moisture barrier capability improved with the addition of active compounds due to their hydrophobic nature. Further research is needed to assess the effectiveness of an antimicrobial packaging system on various types of foods such as fresh fruits and vegetables and planning of irradiation treatments for decontamination of fresh produce.

Acknowledgments

We thank J. Kim, Biological and Agricultural Engineering Department, and J. Maxim, National Center for Electron Beam Food Research, Texas A&M University, for their assistance with the irradiation experiments. We also thank Dr. Alejandro Castillo, Department of Animal Science, for his assistance with the microbiological analyses. This research was funded in part by a USDA/NRI Grant No. 2002-35201-12441 and a USDA/CSREES Grant No. 2002-51110-01968.

References

Adams, M. R., & Moss, M. O. (1995). *Food microbiology*. Cambridge, United Kingdom: The Royal Society of Chemistry.

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science and Emerging Technologies*, *3*(2), 113–126.

ASTM. (1997). Standard test method for solubility, diffusivity, and permeability of flexible barrier materials to water vapor. F1770-97. In *Annual book of ASTM standards* (pp. 1394–1440). Philadelphia: American Society for Testing and Materials.

ASTM. (2000). Standard test method for tensile properties of thin plastic sheeting. D882-00. In *Annual book of ASTM standards* (pp. 165–173). Philadelphia: American Society for Testing and Materials.

Borsa, J., Lacroix, M., Ouattara, B., & Chiasson, F. (2004). Radiosensitization: Enhancing the radiation inactivation of foodborne bacteria. *Radiation Physics and Chemistry*, *71*, 135–139.

Bramlage, W. J., Lipton, W. J. (1965). *Gamma radiation of vegetables to extend market life*. Marketing Research Report No. 703. Washington, DC: Agricultural Research Service, U.S. Department of Agriculture.

CFR (Code of Federal Regulations) (2003). Title 21. Indirect food additives (adhesives and components of coatings): Resinous and polymeric coatings for polyolefin films. Part 175.320 (pp. 184–187).

Chung, D., Papadakis, S. E., & Yam, K. L. (2003). Evaluation of a polymer coating containing triclosan as the antimicrobial layer for packaging materials. *International Journal of Food Science and Technology*, *38*, 165–169.

Cleland, M. R. (1983). Radiation processing: Basic concepts and practical aspects. *Journal of Industrial Irradiation Technology*, *1*(3), 191–218.

Cutter, C. N. (2002). Microbial control by packaging: A review. *Critical Reviews in Food Science and Nutrition*, *42*(2), 151–161.

Desai, N. P., Hossainy, F. A., & Hubbell, J. A. (1992). Surface-immobilized polyethylene oxide for bacterial repellence. *Biomaterials*, *13*(7), 417–420.

Dorran, P. M. (1995). *Bioprocess engineering principles*. San Diego, CA: Academic Press, Inc.

Endo, Y., Tani, T., & Kodama, M. (1987). Antimicrobial activity of tertiary amine covalently bonded to a polystyrene fiber. *Applied and Environmental Microbiology*, *53*(9), 2050–2055.

FDA. (2001). *Food and Drug Administration. Evaluation and definition of potentially hazardous foods*. Center for Food Safety and Applied Nutrition. 〈http://www.cfsan.fda.gov/~comm/ift4-toc.html〉.

Han, J., Castell-Perez, M. E., & Moreira, R. G. (2006). The influence of electron beam irradiation on the effectiveness of trans-cinnamaldehyde coated LDPE/polyamide films. *Journal of Food Science*, *71*(5), E245–E251.

Han, J., Gomes-Feitosa, C. L., Castell-Perez, M. E., Moreira, R. G., & Silva, P. F. (2004). Quality of packaged romaine lettuce hearts exposed to low-dose electron beam irradiation. *Lebensmittel Wissenschaft und Technologie*, *37*(7), 705–715.

Han, J. H. (2000). Antimicrobial food packaging. *Food Technology*, *54*(3), 56–65.

Howard, L. R., & Buescher, R. W. (1989). Cell wall characteristics of gamma-radiated refrigerated cucumber pickles. *Journal of Food Science*, *54*, 1266–1268.

Jay, J. M. (1996). *Modern food microbiology* (5th ed.). New York, NY: Chapman & Hall.

Kim, J., Marshall, M. R., & Wei, C. (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry*, *43*, 2839–2845.

Lieberman, E. R., & Gilbert, S. G. (1973). Gas permeation of collagen films as affected by cross-linkage, moisture, and plasticizer content. *Journal of Polymer Science*, *41*, 33–43.

Lin, W. J., Lee, H. K., & Wang, D. M. (2004). The influence of plasticizers on the release of theophylline from microporous-controlled tablets. *Journal of Controlled Release*, *99*, 415–421.

Lindsay, R. C. (1996). Food additives. In 3rd ed.O. R. Fennema (Ed.), *Food chemistry*, pp. 780–782). New York, NY: Marcel Dekker, Inc.

Pentimalli, M., Capitani, D., Ferrando, A., Ferri, D., Ragni, P., & Segre, A. L. (2000). Gamma irradiation of food packaging materials: An NMR study. *Polymer*, *41*, 2871–2881.

Roller, S. (2003). *Natural antimicrobials for the minimal processing of foods*. Boca Raton, FL: CRC Press.

Sears, J. K., & Darby, J. R. (1982). *The technology of plasticizers*. New York, NY: Wiley-Interscience.

Somogyi, L. P., & Romani, R. J. (1964). Irradiation induced textural changes in fruits and its relation to pectin metabolism. *Journal of Food Science*, *29*, 366–371.

Sothornvit, R., & Krochta, J. M. (2001). Plasticizer effect on mechanical properties of β-lactoglobulin films. *Journal of Food Engineering*, *50*, 149–155.

Sumner, S. S., & Peter, D. L. (1997). Microbiology of vegetables. In D. S. Smith, J. N. Cash, W. K. Nip, & Y. H. Hui (Eds.), *Processing vegetables: Science and technology* (pp. 87–106). Lancaster, PA: Technomic Publishing Co, Inc.

Vigo, T. L. (1994). Advances in antimicrobial polymers and materials. In C. Gebelein, & C. Carraher (Eds.), *Biotechnology and bioactive polymers* (pp. 225–237). New York, NY: Plenum Press.