ANTIMICROBIAL PACKAGING SYSTEM FOR OPTIMIZATION OF ELECTRON BEAM IRRADIATION OF FRESH PRODUCE

A Dissertation

by

JAEJOON HAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2006

Major Subject: Food Science and Technology

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Approved by:

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ABSTRACT

Antimicrobial Packaging System for Optimization of Electron Beam Irradiation of Fresh Produce. (August 2006)

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Chair of Advisory Committee: Dr. Elena Castell-Perez

This study evaluated the potential use of an antimicrobial packaging system in combination with electron beam irradiation to enhance quality of fresh produce. Irradiated romaine lettuce up to 3.2 kGy showed negligible ($p > 0.05$) changes in color, but texture and sensory attributes were less acceptable with increased dose.

We established the antimicrobial effectiveness of various active compounds incorporated into the low-density polyethylene (LDPE)/polyamide films to increase radiation sensitivity of surrogate bacteria (*Listeria innocua* and *Escherichia coli*). All films showed inhibition zones in an agar diffusion test. In the liquid culture test, the active compounds reduced the specific growth rate and decreased final cell concentration of strains. Films incorporated with active compounds increased the radiation sensitivity of the tested strains, demonstrating their potential to reduce the dose required to control microbial contamination using electron beam technology. The active compounds maintained their antimicrobial activity by exposure to ionizing radiation up to 3 kGy.

Antimicrobial activity of LDPE/polyamide films incorporated with trans-cinnamaldehyde was tested with fresh-cut romaine lettuce. Total aerobic plate counts (APC) and yeast and mold counts (YMC) were determined as a function of dose (0, 0.5, and 1.0 kGy) for 14 days of storage at 4°C. Irradiation exposure significantly lowered APCs of lettuce samples by 1-log CFU/g compared to the non-irradiated controls; however, it only slightly reduced YMCs. The effectiveness of using irradiation with antimicrobial films was enhanced with increased radiation dose and trans-cinnamaldehyde concentration.

Electron beam irradiation up to 20 kGy did not affect the tensile strength and toughness of the polymeric films. The film's flexibility and barrier properties were significantly improved by exposure to 20 kGy. The addition of an active compound did not affect the tensile strength and barrier properties of the films, but decreased the percent elongation-at-break and toughness, making them slightly more brittle.

Ionizing radiation affected the release kinetics of the antimicrobial agent from the packaging material into a model food system. Irradiated films exhibited slower release rates than non-irradiated film by 69%. In addition, release rate was lower at 4ºC by 62.6% than at 21-35ºC. The pH of the simulant solution affected release rate with pH 4 yielding higher rates than pH 7 and 10.

DEDICATION

To my wife, So-Yeon Kim

To my parents, Moo-Kyung Han and Sook-Ja Park

To my brother, Jae-Jin Han

To my sister, Hye-Jin Han

ACKNOWLEDGMENTS

I would like to express my deepest appreciation to my major professor, Dr. M. Elena Castell-Perez for the guidance, patience and knowledge she shared while serving as Chair of the Advisory Committee, as well as for her generosity and encouragement throughout my entire graduate program. She has been very generous in helping me to finish this dissertation and to have a vision for my future research.

I am also very grateful to Dr. Rosana G. Moreira, for her invaluable suggestions, encouragement and support during this study. I would like to thank to Dr. Luis Cisneros-Zeballos and Dr. Alejandro Castillo for giving me their advice and kindly allowing me to use their laboratory instruments. Thanks to them for serving as my Advisory Committee members. I am also indebted to Dr. Hung-Jue Sue for kindly allowing me to use the facilities of the Polymer Technology Center.

Profound thanks to Jong-Soon Kim, Paulo Da Silva, Maria Moreno and Carmen Gomes for sacrificing their time and generously providing help and resources vital to the completion of this study. I will always be grateful to their assistance and good friendship during my entire stay in graduate school.

Finally, I would like to thank to my parents and my wife, So-Yeon Kim. They have provided me with unconditional love.

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CHAPTER I

INTRODUCTION

In the United States, there has been a noticeable increase in the consumption of fresh fruit and vegetables in the last two decades, and more consumers are choosing the less labor-intensive ready-to-eat fresh produce. This trend originates in consumers' demand for premium product quality, convenience, fresh-like character, and health benefits. However, minimally processed produce is prone to lose its quality rapidly after processing due to undesirable biochemical reactions associated with wound response. In addition, these quality losses may lead to microbial contamination because wounded cells after minimal processing would release cell fluids in fresh-cut vegetables, which can be a suitable environment for microbial growth.

Leafy vegetables are overwhelmingly used as a popular raw product in salads, and grown in many countries in the world for commercial purposes. However, their shelf life is significantly decreased by minimally processing procedures. Elevated respiration and transpiration rates caused by wounded and senescing leaf tissues, water loss, and decay caused by microorganisms, contribute to the physiological processes that accelerate spoilage. Since the leafy vegetables are not subject to any lethal processing, they contain high levels of microorganisms. Therefore, it is the most serious concern in the fresh-produce industry that ready-to-eat fresh leafy produce such as iceberg, romaine

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This dissertation follows the style and format of the Journal of Food Science.

and butterhead lettuces, endive, spinach, mustard, kale, chicory, and escarole can be contaminated with food-borne pathogens and spoilage microorganisms (Sumner and Peter 1997).

Several preservation methods including antioxidant treatment, modified atmosphere packaging (MAP), refrigerated storage, washing with chlorinated water or ozone, and irradiation treatment have been applied to extend the shelf life and inhibit microbial spoilage of minimally processed fresh produce. The effectiveness of ionizing radiation (gamma rays or electron beam) to eliminate food-borne pathogens in fresh fruits and vegetables has been demonstrated for a variety of foods involving different treatment conditions and irradiation doses. However, there are still problems with the quality of the irradiated fresh produce, such as softening, browning and loss of nutritional factors, caused by the necessary high irradiation doses applied.

The bactericidal efficacy in a given dose of irradiation depends on the following: species of organism, number of organisms or spores originally present, the condition of the organisms, the physical state of the food, and extracellular environmental conditions such as pH, temperature, presence or absence of oxygen, and chemical composition of food during irradiation. Irradiation sensitivity of microorganisms varies among the species (Ingram and Roberta 1980). Thus, we may successfully reduce the undesirable changes in product quality induced by irradiation by increasing the sensitivity of the pathogens to a certain irradiation dose.

One approach is to investigate the synergistic effect of antimicrobial agents incorporated into film packaging materials combined with low-dose irradiation

treatments. These films can control microbial contamination by reducing the growth rate and maximum growth population, as well as extending the lag period of the target microorganism, and therefore should help to prolong the product shelf life and maintain its safety. Ideally, active films must reduce microbial growth of non-sterile foods or maintain the stability of pasteurized foods without post-contamination. If the packaging materials have self-sterilizing ability because of their own antimicrobial activity, they may eliminate the need for chemical sterilization. These novel polymeric materials may have great potential as a microbial hurdle against pathogenic bacteria and they could also be applied to medical supplies, containers and utensils.

The idea of incorporating antimicrobial agents in polymeric film packaging materials is not new and it has recently been developed into the concept of active food packaging systems. Although recent studies have reported the possibility of improved food preservation using these active packaging systems, the effect of a preservation technology – i.e. irradiation – on the effectiveness of the active system has not been thoroughly evaluated. Whether irradiation can be the controlling factor for antimicrobial release from the film into the food or not must be evaluated. Studies on these problems should provide vital information on the exact amount and release rate of the antimicrobial substances required to achieve a given effect (i.e., decontamination or pasteurization).

The main goal of this research was to establish the optimum conditions for a self-sterile active packaging system for potential applications in fresh-cut produce. The main hypothesis was that the use of antimicrobial packaging systems in combination with

low-dose electron beam irradiation (1-3 kGy) provides a synergistic preservative effect, and therefore one can ensure the microbial safety of minimally processed leafy vegetables with negligible quality loss.

The specific objectives are:

1. To quantify the effect of traditional electron beam treatment on the quality (objective and sensory) attributes of a leafy vegetable such as romaine lettuce (*Latuca sativa* var. *longifolia*).
2. To establish the antimicrobial effectiveness of various FDA approved synthetic and natural active compounds incorporated into plastic (low-density polyethylene) films. The antimicrobial activity of the active films against *Listeria innocua* ATCC 33090 and *Escherichia coli* ATCC 884 was evaluated in culture media using agar diffusion or liquid culture test method.
3. To determine the effectiveness of using the antimicrobial films with low-dose electron beam irradiation on the quality attribute and microbial safety of packaged fresh-cut romaine lettuce. The effectiveness of the active film systems with irradiation treatment as radiosensitizers was tested on a real food product (lettuce). In addition, the effect of irradiation on the film's mechanical and barrier properties was determined.
4. To quantify the effect of electron beam irradiation (0.1-20 kGy) on the release kinetics of the selected antimicrobial agent. The possibility of irradiation as a controlling factor for release of the antimicrobial agent in a model food system was evaluated.

CHAPTER II

LITERATURE REVIEW

2.1 Food-borne Pathogens in Minimally Processed Produce

The need for pathogen-free foods is more pronounced in ready-to-eat foods, because these foods are generally consumed raw or require minimal heat processing, which is usually not sufficient to destroy pathogens. The minimum processing required for fresh and fresh-cut produce, which omits any effective microbial elimination step, results in food products that can carry microorganisms naturally, some of which may be potentially hazardous to human health. There have been reports of some food-borne outbreaks where these foods have been implicated (Nguyen-the and Carlin 1994).

The number of food-borne bacteria recognized as human pathogens is increasing. The pathogenic bacteria of concern in fresh or minimally processed produce are *Aeromonas* spp., *Campylobacter* spp., *Clostridium botulinum, Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Staphylococcus aureus,* and *Yersinia enterocolitica*. Also, some parasites (*Cryptosporidium* spp., *Cyclospora* spp.) and viruses (Hepatitis A, Norwalk/Norwalk-like virus) are food-borne pathogens (Jay 1996; Sumner and Peter 1997).

Temperature control becomes critical for preventing bacterial reproduction on any cut produce item (Wiley 1994). Microorganisms often survive at refrigerated temperatures, even though these conditions reduce or eliminate the ability of the organisms to multiply. Exceptions are the psychrotrophic pathogens including non-

proteolytic *C. botulinum*, *L. monocytogenes*, *Y. enterocolitica*, and *A. hydrophila* (Sumner and Peter 1997).

Various enteric pathogens have been shown to multiply on the surface of minimally processed fruits and vegetables. Fresh-cut produce is injured through peeling, cutting, slicing, or shredding. During these operations, pathogenic microorganisms, if present, can transfer from the surface of the intact fruit or vegetable to the internal tissues. Tissue damage lowers the intrinsic resistance of the plant against invasion by microorganisms. Cellular leakage also provides a source of nutrients that can support extensive growth of microorganisms during subsequent storage. Injured cells and released cell fluids provide a nourishing environment for microbial growth (Seymour and Appleton 2001).

Most pathogens do not cause produce to spoil, even at relatively high populations. In the absence of spoilage, high populations of pathogens may be achieved and the item may be consumed because it is not perceived as spoiled. For this reason, specifications requiring very low microbial counts may, in some cases, compromise produce safety (FDA 2001a).

There is an important relationship between pathogenic and spoilage microorganisms on produce. Brackett (1992) suggested that reducing the native microbial populations by washing and sanitizing or by controlled atmosphere storage can allow pathogenic microorganisms to flourish on produce surfaces. This indicates that reduction in surface populations of microorganisms decreases competition for space and nutrients availability, thereby providing growth potential for pathogenic contaminants on

produce. Thus, an unspoiled product can be unsafe for consumption. For example, Berrang and others (1989) reported that they observed higher levels of food-borne pathogens in produce stored under controlled atmosphere for extended shelf life than in traditionally stored produce. The authors showed that it may not be desirable to prolong product shelf life if pathogens could grow before spoilage is detectable.

A variety of methods has been used to reduce populations of microorganisms on whole and minimally processed produces. Traditional methods of reducing microbial populations on produce involve physical and chemical treatments. Physical methods, which include temperature control (Wiley 1994) and the physical removal of microorganisms (Poulsen 1986), are commonly used. On the other hand, chemical treatments, such as chlorine, chlorine dioxide, organic acids, surfactants, and ozone, are common in practice in the fresh produce industry (Seymour and Appleton 2001). Usually, combined methods and hurdles are applied because most of the times a single method is not sufficient to reduce microbial populations.

2.2 Quality Changes in Minimally Processed Produce

Since 1996, the Food and Drug Administration (FDA) has responded to fourteen (14) outbreaks of food-borne illness for which fresh lettuce was the confirmed or suspected vehicle along with fresh tomatoes (FDA 2004). These outbreaks account for approximately 859 reported cases of illness. The outbreaks linked to lettuce were of U.S. and non-U.S. origin, and the causative agents were mainly *E. coli* O157:H7, *Cyclospora*, and Hepatitis A virus. Because fresh lettuce is commonly consumed in raw state without

processing to reduce or eliminate pathogens, the manner in which it is grown, harvested, sorted, packed, and distributed is crucial to ensure that the potential for microbial contamination is minimized, thereby reducing the risk of illness to consumers.

In general, minimal processing for lettuce includes trimming, coring, cutting, washing, spun-drying, and then packaging. These processes result in quality deterioration associated with water loss, softening, microbial contamination, increased respiration and ethylene production, and cut-surface browning (Rolle and Chism 1987). Enzymatic browning of processed lettuce is a major problem because visual quality is very important. Polyphenoloxidase is responsible for browning after tissue injury. This browning problem may be delayed as long as ascorbic acid is oxidized and quinines reduced back to the phenolic level (Walter and Purcell 1980), thus initial levels of ascorbic acid could influence enzymatic browning. However, there is no simple relationship between browning, phenolic content and/or polyphenoloxidase for fruits and vegetables (Matheis 1983). These reactions may be complex and therefore the interactions between phenolic substrates, cosubstrates, antioxidants, enzymatic activities and chemical polymerization which results in the browning reactions are not fully understood (Heimdal and others 1995).

Tissue injury caused by minimal processing induces losses in cellular membrane integrity that may lead to leakage of cellular components and disruption of enzyme and/or substrate compartmentation (Brecht 1995). Also, mechanical tissue damage elicits wound response reactions including increases in the activity of phenylalanine ammonia lyase, an enzyme which catalyses the synthesis of plant compounds collectively referred

as phenylpropanoid products (Ke and Saltveit 1989). The latter are oxidized to brown colored substances by the enzyme polyphenoloxidase leading to visible discoloration along the cut edge of the lettuce leaf (Lopez-Galvez and others 1996a). In addition, tissue respiration rates usually increase in proportion to the extent of tissue disruption (Kader 1992). Loaiza-Velarde and others (1997) have reported that warm water treatments may prove useful for quality improvement in minimally processed lettuce through inhibition of phenylalanine ammonia lyase activity.

2.3 Active Food Packaging Technology

The concept of antimicrobial polymeric materials was introduced and developed in the biomedical science field to protect sutures or implants from microbial contamination (Endo and others 1987; Desai and others 1992; Vigo, 1994; Rathinam and others 1996). Consequently, incorporation of chemical preservatives or antimicrobial agents into a food packaging material (film) should confer a way to enhance microbial safety (Han 2000). This observation is based on the principle that active agents can be effectively released from the packaging material to the foodstuffs. The key issue when designing such systems is to slowly deliver the active agent into the food, and to maintain adequate concentration of the agent in the food for efficient inhibition of microbial growth throughout the product shelf life.

Preservatives with antimicrobial properties play an important role in preventing spoilage and assuring safety of many foods. Many of these agents have been effectively 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, natamycin, glyceryl esters, propionic acid, acetic acid, benzoic acid, p-hydroxybenzoate alkyl esters, epoxides, antibiotics, various natural essential oils and others (Lindsay 1996; Appendini and Hotchkiss 2002). The antimicrobial agents used in food packaging materials have to be approved as safe by the FDA.

2.3.1 Novel Antimicrobial Agents

Researchers have been continually investigating the use of novel materials, such as plant-derived active compounds, to control pathogens or spoilage organisms in foods. Although different results are observed depending on test conditions, microorganisms and the source of the antimicrobial compound, some spices or essential oils always act very effectively in inhibiting microbial growth. Carvacrol and thymol are phenolic compounds present in the essential oil fraction of *Oreganum* and *Thymus* plants, respectively (Lagouri and others 1993; Juven and others 1994; Sivropoulou and others 1996). They are considered GRAS (General Recognized As Safe) food flavorings by FDA based on 21 CFR part 172.515 (CFR 2003a). Cinnamaldehyde (cinnamic aldehyde) is the main component in cassia oil as well as cinnamon bark oil, and is a GRAS for food use based on 21 CFR part 182.60 (CFR 2003b). Rosemary oleoresin is present in extracts of rosemary (*Rosmarinus officinalis* L.) leaves, and is a GRAS for food use based on 21 CFR part 182.20 (CFR 2003c). This compound has been recognized to possess significant antioxidant/antimicrobial properties due to the content of phenolic

diterpenes. The major phenolic diterpenes are carnosic acid and carnosol (Thorsen and Hildebrandt 2003).

Natural essential oils can be incorporated directly into polymers. Thermal polymer processing methods such as extrusion and injection molding are used for heat-stable antimicrobials like silver substituted zeolitres. For heat-sensitive antimicrobials such as enzymes and volatile compounds, solvent compounding may be a more suitable method into polymers. Meanwhile, antimicrobials (e.g. natural polyphenolics) that cannot tolerate the high temperatures used in polymer processing are often either coated onto the material after forming or added to cast films (Appendini and Hotchkiss 2002). The principle action of antimicrobial films is based on the release of antimicrobial agents, some of which could pose a safety risk to consumers if the release is not tightly controlled by the mechanisms within the packaging material (Ozdemir and Sadikoglu 1998). Thus, the active antimicrobial compounds have to be released at a controlled rate over prolonged period of time.

2.4 Irradiation Treatment of Minimally Processed Produce

Ionizing radiation from ^{60}Co, ^{137}Cs, or machine generated electron beams, either alone or in combination with other preservative treatments, is used to extend shelf life or enhance safety of produce (Diehl 1995; Thayer and others 1996). The emergence of pathogens in minimally processed produce has increased interest in the use of irradiation as a preservation technique (Monk and others 1995).

Recently, irradiation has been proposed for a variety of fresh and processed foods with different treatment conditions and irradiation doses for a certain application in food preservation and processing. It should be noted that produce treated by doses above the level of 1.0 kGy cannot use the term "fresh" (CFR 2002). Various studies have shown that ionizing radiation could effectively eliminate food-borne pathogens in fresh fruits and vegetables including lettuce (Langerak 1978; Hagenmaier and Baker 1997; Farkas and others 1997; Prakash and others 2000; Foley and others 2002; Fan and others 2003).

After irradiation treatment, more resistant pathogens are reduced in numbers, and the surviving flora is generally less resistant to other factors such as heat, pH change, salt concentration and antibiotics. Thus, a combination of irradiation with other food preservation methods can be used to achieve the inactivation of pathogens (Barbosa-Cánovas and others 1998).

Ionizing radiation is lethal for bacteria (Moseley and others 1990). For the vast majority of bacteria, the critical target for inactivation is the chromosome, which is a single and circular molecule of DNA containing several million base pairs. Most studies indicate that a primary cause of lethality is the damage to microbial DNA resulting in the loss of ability to reproduce, but damage to other sensitive and critical molecules (e.g., in membranes) may also promote inactivation (Ingram and Roberta 1980). The primary mechanism of microbial inhibition by ionizing radiation is the breakage of chemical bonds within the DNA molecules, or the alteration of membrane permeability and other cellular functions (Lopez-Gonzales and others 1999; Urbain 1986). This may facilitate

the contact between antimicrobial molecules and cell membranes, and increase their inhibitory effects.

Irradiation sensitivity of microorganisms differs with species and even with strain, although the range of resistance among strains of a single species is usually small enough to be negligible (Ingram and Roberts 1980). Gram-negative bacteria including common food spoilage organisms, and enteric species including pathogens, are generally more sensitive to irradiation than gram-positive bacteria. Irradiation resistance generally can be represented by the following sequence (Adams and Moss 1995):

$$\text{gram-negative} < \text{gram-positive} \approx \text{molds} < \text{spores} \approx \text{yeasts} < \text{virus}$$

Bacterial endospores are more resistant to the action of ionizing radiation than their corresponding vegetative cells. In general, the irradiation resistance of molds is equivalent to vegetative bacteria. Yeasts are distinctly more resistant to irradiation than molds, and as resistant as sporeforming bacteria. Viruses are even more irradiation resistant than bacteria, so that irradiation treatments that destroy bacteria will not reliably inactivate viruses (Ingram and Roberts 1980; Jay 1996).

The efficacy of irradiation is not only limited to the surface, but it can penetrate the product and eliminate microorganisms that are present in crevices and creases (Prakash and others 2000). Thus, irradiation should be an effective preservative treatment for leafy vegetables like lettuce, which have complex and non-uniform shapes where other chemical or physical treatments cannot be easily applied. However, irradiation can induce changes in texture or color in fruit and vegetable tissues (Somogyi and Romani 1964; Han and others 2004). The textural changes induced by irradiation are

still one of the main limiting factors for its use in fresh or minimally processed produce. Plant tissues can be softened with increasing doses of irradiation over critical thresholds. The effect of ionizing irradiation in fresh fruits and vegetables will depend on the produce, the dose level, and the process of irradiation. Han and others (2004) showed that electron beam irradiation (1.0, 1.5 and 3.2 kGy) of packaged fresh romaine lettuce hearts induced a 49% decrease in the firmness of leaves and 29% for the ribs, but the color was not affected when compared to non-irradiated samples stored up to 21 days at 4ºC.

Depending upon the irradiation treatment (dose and dose rate), produce can show some changes such as discoloration, loss of firmness (softening), changes in the respiration rate, thus inducing the physiological responses. Water content and cell turgidity may play an important role in tissue turgidity and firmness. Loss of cell turgidity and increased cell leakage may reduce firmness. However, tissues after low-dose irradiation can re-absorb fluid from intercellular space (Skou 1963), which may result in the partial recovery of firmness.

CHAPTER III

QUALITY OF PACKAGED ROMAINE LETTUCE HEARTS EXPOSED TO LOW-DOSE ELECTRON BEAM IRRADIATION*

3.1 Overview

We investigated the effects of low-dose electron beam irradiation (1.0, 1.5 and 3.2 kGy) on the quality of commercially prepackaged fresh romaine lettuce hearts. The impact of the irradiation treatment on the functionality of the package was also evaluated. Irradiated samples showed slight changes in color, but these changes were not significantly different ($p > 0.05$) from the non-irradiated (control) samples. Sample firmness decreased by 49.58% (leaves) and 29.13% (ribs), as the dose level increased. Sensory attributes such as overall quality, color, sogginess, and off-flavor were found less acceptable at the higher dose level. Irradiation affected the respiration rates inside the packages, with lower (10.38%) O_2 and higher (258%) CO_2 levels than the control. Irradiation at 1.5 and 3.2 kGy dose levels improved the oxygen barrier capability of the low-density polyethylene (LDPE) bags (7.67 and 4.48%, respectively). Water vapor permeability was unaffected at all the irradiation dose levels. The stiffness of LPDE films did not change due to irradiation treatment. Results from sensory evaluation of

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*Reprinted with permission from "Quality of packaged romaine lettuce hearts exposed to low-dose electron beam irradiation" by Han J, Gomes-Feitosa CL, Castell-Perez ME, Moreira RG, Silva PF, 2004. LWT–Food Science and Technology, 37(7), 705-715.

doi:10.1016/j.lwt.2004.02.007

produce overall quality suggest a potential fungicidal effect of low dose irradiation (1.0 kGy) of packaged romaine lettuce hearts without altering the overall quality of the produce as well as the LDPE packaging characteristics.

3.2 Introduction

Sales of minimally processed ready-to-eat fruits and vegetables have grown rapidly in the last decade and are expected to reach $19 billion by 2003 (Sloan 2000). Increasing markets for minimally processed fresh produces result from consumers' demand for premium product quality, convenience, and fresh-like character (Ohlsson 1994). Markets for such products could be expanded more if the products had longer storage life. For example, during conditioning, minimally processed fruits and vegetables, including romaine lettuce hearts, are subjected to stresses that make them more susceptible to physiological and microbial deterioration. Indeed, the shelf life of these products rarely exceeds 14 days, even under adequate refrigeration.

Several preservation methods including antioxidant treatment, modified atmosphere packaging (MAP), refrigerated storage, washing with chlorinated water or ozone, and irradiation have been applied to extend shelf life and inhibit microbial spoilage of minimally processed fresh produce (Wiley 1994; Ahvenainen 1996; Tapia de Daza and others 1996; Hoover 1997; Xu 1999; Prakash and others 2000; Fan and others 2003). Recently, ionizing radiation (gamma rays or electron beam) has been proposed for a variety of fresh and processed foods involving different treatment conditions and irradiation doses for application in food preservation and processing. Irradiation is an

effective non-thermal food processing technique to reduce and/or inhibit pathogenic and/or spoilage microorganisms (Morehouse 1998). Irradiation has been approved by the Food and Drug Administration (FDA 1998) for use on fruits and vegetables, including lettuce. The recommended 1.0-kGy dose is used for growth and maturation inhibition. This treatment has been shown to effectively reduce the number of food spoilage organisms and increasing the shelf-life of several fruits and vegetables (Yu and others 1996; Prakash and others 2000; Fan and others 2003). The efficacy of irradiation is not only limited to the surface, but it can penetrate the product and eliminate microorganisms that are present in crevices and creases (significant for vegetables like lettuce) (Prakash and others 2000); however, irradiation can cause the change of texture or color in fruit and vegetable tissues (Somogyi and Romani 1964; Bramlage and Lipton 1965; Howard and Buescher 1989).

Various studies have shown that ionizing irradiation could effectively eliminate food-borne pathogens in fresh fruits and vegetables. Langerak (1978) reported that radiation at 1-kGy resulted in reductions of bacterial populations while doubling the shelf-life of cut endive. Hagenmaier and Baker (1997) also found that commercially prepared fresh-cut lettuce irradiated at 0.19-kGy dose significantly reduced microbial populations for 8 days. They verified that it was feasible to combine chlorination with irradiation at 0.15-0.5 kGy to produce fresh-cut lettuce with reduced microbial population. Farkas and others (1997) showed that 1 kGy radiation reduced loads of spoilage bacteria and extended sensorial quality of precut peppers and carrots. Prakash and others (2000) found that cut romaine lettuce irradiated at 0.35- kGy decreased

aerobic counts by 1.5 logs and the difference was maintained through 22 days of storage at 4°C. They also reported that there was loss of firmness by 10% in irradiated cut romaine lettuce, but no changes occurred in sensorial attributes such as flavor, and appearance. Foley and others (2002) found that gamma ray irradiation at 0.55 kGy produced over a 5-log reduction in *E. coli* 0157:H7 in shredded iceberg lettuce without causing adverse effects on sensory attributes. Fresh-cut iceberg lettuce irradiated at 0.5 or 1 kGy after dipping in 5 or 47 °C water for 2 min had similar firmness as the controls, and even had better overall visual quality and less tissue browning than corresponding control samples after 14 and 21 days of storage (Fan and others 2003). Their study suggested that lettuce treated with warm water and irradiated at 0.5 or 1 kGy had the best sensory quality without significant loss in texture or nutritional qualities, and indicated that both cellular leakage and sogginess increased as radiation dose increased. The sogginess might be due to the leakage of fluid from cells and regarded as flaccidity or loss of turgidity.

Extensive research has been done on the effects of ionizing energy on foods. The accumulated data so far indicate that ionizing energy has some potential applications to fresh fruits and vegetables, but also has many limitations. Irradiation may induce undesirable changes in quality, such as softening, browning, and loss of nutritional factors. Thus, textural changes induced by irradiation are still one of the main limiting factors for its use in fresh produce. Plant tissues soften with increasing doses of irradiation over critical thresholds. The effect of ionizing irradiation in fresh fruits and vegetables will depend on the produce, the dose level, and the process of irradiation.

Depending upon the irradiation treatment, produce can show some changes such as discoloration, loss of firmness (softening) and, changes in the respiration rate, thus inducing the physiological responses. El Assi and others (1997) found that firmness loss in tomato pericarp tissues due to irradiation was apparent within 24 h following irradiation; however, by 7 days after treatment, firmness of pericarp treated at 0.7 kGy was comparable with that of pericarp from control fruit. Also, Fan and others (2003) reported that although firmness of cut-iceberg lettuce was lower in some irradiated samples at day 1, the difference disappeared as storage prolonged. Water content and cell turgidity may play an important role in tissue turgidity and firmness. Loss of cell turgidity and increased cell leakage may reduce firmness; however, tissues after low-dose irradiation can re-absorb fluid from intercellular space (Skou 1963), which may result in the partial recovery of firmness.

The action of ionizing radiation on polymers results in the following fundamental processes: crosslinking of the molecular chains, degradation of macromolecules, changes in the number and nature of the double bonds. These processes can take place simultaneously, controlled by the chemical nature of the polymer (Clegg and Collyer 1991). A polymer with the structure of $(-CH_2-CR_2-)_n$ will crosslink when at least one hydrogen exists at α-position (R = H). For example, crosslinking will occur in polyethylene (PE), polypropylene (PP), or polystyrene (PS) (Wilson 1974). Used in a wide range of products, PE is the most common and least expensive polymer material. PE, with a completely unbranched structure of $(-CH_2-)_n$, is the family name for such resins as low density polypropylene (LDPP) and high density polypropylene (HDPP).

LDPE is the largest volume single polymer used in food packaging, and it is currently the most commonly used commercial lettuce-packaging material with PP bags. It is a tough, slightly translucent material, and has good tensile strength, burst strength, impact resistance and tear strength. LDPE has an excellent barrier property to water vapor, but not a good barrier to gases. It has also excellent chemical resistance, particularly to acids and alkalis (Robertson 1993). According to Bradley (1984), high energy β-ray irradiation treatment by an electron beam accelerator can cause crosslinking between the chains on LDPE film and results in the evolution of hydrogen and a reduction in the crystallinity. Irradiation results in slight reduction of gas and water vapor transmission rates on LDPE film.

Most studies have been conducted using gamma rays as the irradiation source, but little information is available in the scientific literature on the effects of electron beam irradiation when used in fresh produce including romaine lettuce. Also, the effect of low energy irradiation on food packaging materials has not been studied extensively. The objective of this study was to evaluate the feasibility of using electron beam irradiation in a minimally processed food such as packaged romaine lettuce hearts. The specific objectives were (1) to determine the effect of low-dose irradiation on sensory and quality attributes of commercially packaged romaine hearts, and (2) to investigate the effect of irradiation on the packaging material.

3.3 Materials and Methods

3.3.1 Sample Preparation

A total of forty-eight (48) packaged romaine lettuce hearts (*Latuca sativa* var. *longifolia*) were purchased from a local grocery market. Average moisture content was 94.1% wet basis (AOAC 1990). In order to ensure highly reproducible results, the packages were obtained from the same brand, supplier and day of purchasing. The lettuce was already pre-treated by the suppliers by washing in chlorinated solution, spun-drying, and packaging in 50.8 μm thick perforated LDPE bags (16 perforations with 5-mm diameter each). Each bag (25-cm width, 30- cm length, 5-cm depth) contained approximately 510 g (3 romaine hearts). The packages were stored at 4°C overnight until further testing.

3.3.2 Irradiation Tests

Irradiation tests were carried out at the National Center for Electron Beam Food Research at Texas A&M University. This facility houses two vertically mounted opposing 10-MeV, 19-kW electron beam linear accelerators (LINAC). Irradiation experiments consisted of single beam exposure (bottom) at three different conveyor speeds (0.3, 0.2 and 0.1 m/s) to obtain doses of 1.0, 1.5 and 3.2 kGy, respectively. Packages were placed on a single layer at the middle of the conveyor. A dose of 1.0 kGy involves the absorption of 1.0 kJ of energy by each kilogram of matter through which the radiation passes. Irradiation dosage was measured by placing radiochromic film dosimeters (GEX Corporation, Centennial, Colo., U.S.A.) at four points on the exterior

at the top and bottom of the package (2 on each side). The blank dosimeter was used to estimate the dose absorbed by the dosimeter alone (no produce). Results indicated that dose was not uniformly distributed over the product. Doses at the top of the bags varied from 0.6 to 1.2 kGy (high conveyor speed); from 1.3 to 1.6 kGy (medium speed); and from 2.1 to 3.5 kGy (low speed). Differences in dose values are due to differences in product densities (723 ± 0.5 kg/m^3 for the leaves and 857 ± 0.5 kg/m^3 for the ribs).

After irradiation, samples were stored in a refrigerator (4°C) up to 21 days. Non-irradiated samples were considered as control. Quality and sensory attributes of samples were determined at 0, 7, 14 and 21-day intervals. The length of the experiment was based on the "best-if-used-by" date (shelf life) of commercially packaged romaine hearts, which is 14 days after packaging. The oxygen and water vapor permeabilities, and mechanical properties of the packaging material were also monitored.

3.3.3 Produce Evaluation

3.3.3.1 Color

A Labscan XE (16437) colorimeter (HunterLab, Inc., Reston, Va., U.S.A.) with the Universal Version 3.73 software (HunterLab, Inc., Reston, Va., U.S.A.) was used to assess changes in lettuce color using the CIELAB system. The measuring aperture diameter was 36 mm, and D65/10° was the illuminant/viewing geometry. The color meter was calibrated using the standard white and black plates. The reading was made separately for ribs and leaves. Three readings were made on each sample from each package and the mean values were used to determine the color coordinates L* (lightness),

a* (redness), and b* (yellowness). To evaluate the sample color on the 3-dimensional standard color space, sample hue angle and chroma (saturation) were calculated, where hue angle = $\tan^{-1}(b^*/a^*)$ and sample chroma = $(a^{*2} + b^{*2})^{1/2}$ (McGuire 1992).

3.3.3.2 Texture

Firmness of ribs and leaves was determined using a Kramer Shear Press with 5 blades (TA-91) attached to a TA-XT2 Texture Analyzer (Texture Technologies Corporation, Scardale, N.Y., U.S.A.). This method is commonly used to measure firmness of products, which require low force to be assayed (i.e., vegetables, bread, cake) (Bourne 1997). Twenty-g samples were cut to a length of ≈ 8 cm and placed into the sample holder (internal dimensions 82 × 63 × 89 mm) and a 5 flat-plate (1.5-mm thickness) plunger was forced through the lettuce. The probe was set at a height of 65 mm from the bottom of the 5 flat-plate plunger and moved downward at 1.0 mm/s. The maximum force (N) and work (J) until shear (cutting) were recorded by the Texture Expert software program, version 2.55 (Texture Technology corp., Scarsdale, NY). The work given by the area under the curve might be related to the process of mastication, i.e., that which occurs as the food is chewed and brought into a condition ready to be swallowed (Bourne 1997). Three measurements were performed for each sample. All experiments were conducted at room temperature (21˚C).

3.3.3.3 Sensory Evaluation

The influence of the combined effect of storage time and irradiation dose on the sensory characteristics of romaine lettuce hearts was evaluated (overall quality, color, sogginess and off-odor) for 0, 7, 14 and 21 days by 40 untrained, randomly chosen individuals. The samples (control and irradiated) were presented to each panelist at once for a total of 4 samples every week. For overall quality, the scale was 1-5 (five hedonic scale) as described by Carr (1999). On this hedonic scale, a score of 1 represented attributes most disliked and a score of 5 represented attributes most liked. For color, the samples were rated as 1-5 with 1 (no green) and 5 (green – fresh appearance). The sogginess was rated as 1 (crisp) to 5 (soggy), and for off-odor, the samples were rated as 1 (none) and 5 (the severest). The minimum score for commercial consumer acceptance was set at 3 for color and overall quality and 2 for sogginess and odor. These limits of acceptability were based on our "acceptance test" profile. For example, for "overall quality" a rating of 3 out of a 1-5 scale was defined as "fair" and thus considered as the minimum score for salability to consumers (Lopez-Galvez and others 1996b). The samples were placed on top of white paper plates identified by 3 digits and randomly placed in the trays. Samples were only evaluated by visual inspection.

3.3.3.4 Headspace Gases

Gases concentrations (O_2 and CO_2) inside the packages were measured during the entire duration of the experiment (21 days). The packages were stored under refrigeration at 4°C during the length of the study. Internal gases were withdrawn using

a 5-mL syringe having a side hole needle. The sampling holes were resealed by electrical tape, and packages were stored for future measurements. The withdrawn gases were immediately injected into a Model S-3A electrochemical O_2 analyzer (Applied Electrochemistry, Inc., Sunnyvale, Calif. U.S.A.) and a PIR-2000 infrared CO_2 gas analyzer (IRGA) (Horiba Instruments, Irvine, Calif. U.S.A.) to determine the levels of O_2 and CO_2, respectively. These instruments were abreast each other to obtain readings with only 1 injection (Saltveit and Strike 1989). Standard curves for CO_2 and O_2 concentrations were obtained, and the measured peaks fitted into a curve to obtain the corresponding gases concentration for each package (treated and control).

3.3.4 Packaging Material

3.3.4.1 Film Permeability

Oxygen and water vapor permeabilities of the packaging material were measured using oxygen and water diffusion systems, MAS 500 and MAS 1000 (MAS Technologies Inc, Zumbrota, Minn., U.S.A.), according to the ASTM F1770 Standard Method (ASTM 1997). The tests were performed under 65% relative humidity and 25°C temperature conditions. The material permeability coefficient (P, kg/s·m·Pa) was calculated from the following relationship,

$$P = \frac{F_e L}{p} \qquad (3.1)$$

where F_e is the film permeability flux in kg/m^2·s (given by the test machine), L is barrier thickness (50.8 μm), and p is test vapor pressure (1 atm).

3.3.5 Film Mechanical Properties

3.3.5.1 Stress Relaxation Measurements

On a stress relaxation method, an instantaneous strain is given to a strip of material and the stress required to maintain the deformation is recorded as a function of time. Important material properties can then be determined from this response (Steffe 1996). Stress relaxation tests were performed using a TA-XT2 Texture Analyzer (Texture Technologies Corporation, Scardale, N.Y., U.S.A.) with a bench-top movable system with one end attached to the Texture Analyzer platform and the other end attached to the Texture Analyzer arm. The distance between the two arms was set to 33 mm. Film strips (32 × 70 mm) of constant thickness (50.8 μm) were subject to tension at 3% strain (linear viscoelasticity region) for 300 s using tension mode, according to the method developed by Limanond and others (2002). The force (Newtons) and distance (% strain) values prior to the rupture of the sample were recorded using the Texture Expert software program, version 2.55 (Texture Technology Corp, Scarsdale, N.Y., U.S.A.) and later used to determine the changes on film stiffness and energy dissipated as a function of irradiation dose level. Tests were made in triplicate and conducted at room temperature.

The stiffness or ability of a material to withstand a tensile force is the ratio of homogeneous stress (σ) to the homogeneous strain (ε), which may be referred to as the

"modulus of elasticity" or "Young's modulus", *E* (Limanond and others 2002). This parameter is a good indicator of a material's strength. The higher the value corresponds to a stronger (firmer) material. The energy dissipated refers to the portion of strain energy released during periodic deformation, which will be transformed into heat. This parameter is an indicator of the material's ability to recover to its original shape after the tensile force is removed. The higher the value means the lesser the ability of a material to recover from deformation.

All the stress relaxation data was fitted into a 3-element generalized Maxwell model (Eq. 2) and transformed into creep compliance, stiffness and energy dissipated using MATLAB statistical software version 6.1.0.450 (The MathWorks, Inc., Natick, Mass., U.S.A.).

$$E = \frac{\sigma}{\gamma_o} = f(t) = E_e + E_1 e^{\left(-\frac{t}{\lambda_{rel}}\right)} \qquad (3.2)$$

where E_1 is the decay modulus; γ_o is the applied constant strain; λ_{rel} is the relaxation time in the model; E_e is the equilibrium modulus; and i$\sigma(t)$s the decaying stress at time t (Steffe 1996).

Data analysis was performed using the Statistical Analysis System (SAS) software, version 6.11 (SAS 1997). The effect of radiation dose, storage time as well as the interaction between these two factors was evaluated. 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 $p \leq 0.05$.

3.4 Results and Discussion

3.4.1 Lettuce Color

The effect of irradiation on the color of lettuce leaves and ribs is shown in Tables 3.1 and 3.2. Overall, the irradiation treatment induced the loss of pigments and accelerated the discoloration of the lettuce. The leaves of lettuce samples irradiated at 1.0 and 1.5 kGy showed the same level of color stability as the control (all color parameters) during the entire storage period. On the other hand, the samples treated with higher dose (3.2 kGy) showed some loss of color (changes in luminosity, hue, and chroma values) in comparison with the control samples.

The irradiated ribs showed more losses in color than the leaves regardless of dose level. The change in total color with time was mostly due to an increase in "a*" (relative redness-greenness) values, indicating a loss of green pigment, and an increase in "b*" (relative yellowness-blueness) values or increased yellow, as well as increased darkening (decreased "L*" value). There was no significant change in the control and irradiated samples throughout the entire storage period. The irradiated rib samples showed a significant ($p \leq 0.05$) decrease in the coordinate L* from the seventh day of experiment (Table 3.1). This result might be explained by the effect of phenolic oxidation. Loss of color quality is mainly from enzymatic browning due to wounding, and it is caused by the accumulation and oxidation of phenols by polyphenoloxidases

Table 3.1—Effect of irradiation treatment on the color characteristics (L value) of romaine lettuce hearts

	L*			
Storage Interval	**Control[1]**	**1.0 kGy**	**1.5 kGy**	**3.2 kGy**
Leaves				
Day 0	44.70^{ax} (6.55)[2]	44.11^{ax} (1.00)	45.98^{ax} (7.27)	44.68^{ax} (2.11)
Day 7	43.76^{ax} (3.99)	52.40^{bx} (5.17)	41.03^{ax} (0.95)	40.83^{axy} (1.05)
Day 14	35.97^{ax} (0.87)	45.79^{bx} (2.98)	47.45^{bx} (5.74)	36.59^{ay} (0.64)
Day 21	41.02^{ax} (1.89)	49.58^{abx} (2.24)	53.10^{bx} (4.35)	41.87^{axy} (5.46)
Ribs				
Day 0	57.34^{ax} (1.09)[2]	61.50^{ax} (2.64)	59.66^{ax} (4.07)	57.73^{ax} (4.47)
Day 7	52.44^{ay} (1.21)	49.73^{ay} (1.05)	48.95^{ay} (3.06)	50.19^{ay} (0.96)
Day 14	51.20^{ay} (1.58)	45.79^{bx} (2.98)	47.45^{bx} (5.74)	36.59^{ay} (0.64)
Day 21	50.57^{ay} (2.16)	47.96^{ay} (2.43)	51.70^{ay} (1.09)	47.23^{ay} (1.77)

[1] Control means samples not exposed to irradiation treatment
[2]Standard deviation
[a,b]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
[x,y]Means within a column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
Tests were conducted at room temperature (21^{o}C)

Table 3.2—Effect of irradiation treatment on the color characteristics (hue angle and chroma) of romaine lettuce hearts

	Hue Angle				Chroma			
Storage Interval	**Control[1]**	**1.0 kGy**	**1.5 kGy**	**3.2 kGy**	**Control[1]**	**1.0 kGy**	**1.5 kGy**	**3.2 kGy**
Leaves								
Day 0	116.15^{ax} (2.82)[2]	117.48^{abx} (2.36)	114.70^{abx} (3.38)	116.54^{ax} (0.64)	30.47^{ax} (4.90)	32.34^{ax} (4.52)	32.32^{ax} (0.51)	30.43^{ax} (2.51)
Day 7	116.98^{ax} (2.07)	113.40^{bx} (2.56)	114.28^{bx} (3.45)	109.83^{by} (1.64)	31.85^{abx} (1.43)	34.24^{ax} (2.94)	29.04^{by} (0.45)	30.32^{abx} (0.89)
Day 14	119.01^{ax} (0.82)	114.01^{bcx} (1.67)	113.87^{bx} (3.20)	109.52^{cy} (1.28)	26.20^{ax} (0.66)	32.55^{bx} (1.93)	33.47^{bx} (1.73)	28.07^{axy} (0.78)
Day 21	117.38^{ax} (0.84)	114.11^{ax} (0.51)	112.51^{ax} (2.26)	104.00^{az} (0.71)	30.39^{ax} (1.61)	33.78^{ax} (2.24)	33.91^{ax} (1.63)	25.69^{by} (1.85)
Ribs								
Day 0	110.98^{ax} (0.67)	105.63^{bx} (1.00)	108.35^{abx} (1.73)	109.98^{ax} (2.12)	20.67^{ax} (1.73)	19.00^{ax} (2.21)	19.24^{ax} (5.87)	21.77^{ax} (3.94)
Day 7	109.81^{ax} (0.43)	98.91^{by} (1.14)	101.71^{by} (3.05)	100.41^{by} (1.47)	23.88^{axy} (1.15)	26.80^{by} (1.15)	26.41^{by} (1.40)	29.89^{cy} (0.34)
Day 14	110.30^{ax} (0.49)	97.64^{bcyz} (1.76)	99.33^{byz} (1.68)	94.54^{cz} (2.23)	24.14^{axy} (3.42)	27.30^{aby} (1.36)	27.76^{aby} (0.67)	30.21^{by} (1.09)
Day 21	111.19^{ax} (0.95)	94.42^{bz} (2.72)	95.37^{bz} (1.68)	94.91^{bz} (1.23)	28.29^{ay} (0.98)	26.21^{ay} (1.83)	26.84^{ay} (1.42)	27.18^{ay} (1.01)

[1] Control means samples not exposed to irradiation treatment
[2]Standard deviation
[a,b]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
[x,y]Means within a column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
Tests were conducted at room temperature (21^{o}C)

and peroxidases to O-quinones that polymerize readily into dark pigments (Ke and Saltveit 1988). Since the package was a perforated bag, the O_2 concentration remained constant for all samples, which might have resulted in the enzymatic browning effect.

Irradiation does have an effect on the hue angle values ($\tan^{-1}(b^*/a^*)$) of the leaves. The hue angle describes the quality of the color value. All irradiated treatments showed a significant decrease in the hue angle value compared to the non-irradiated samples which means a change from the green to yellow spectrum. Only the high dose samples showed a significant decrease in hue angle by the end of the storage period. Again, the effect of treatments was more significant on the seventh day of storage (Table 3.2). No significant changes were observed for the control samples. The irradiated ribs showed a gradual decrease in hue angle values during the storage period while the non-irradiated samples showed no significant change (Table 3.2).

The chroma values of the leaves of the control sample remained constant for the entire storage period. In contrast, the leaves of samples irradiated at 1.0 kGy showed numerically higher chroma values than the control during the entire storage period. All the irradiated ribs had higher chroma values than the control at Day 7 and Day 14 but these values were lower at Day 21 (Table 3.2).

According to Bolin and Huxsoll (1991), chlorophyll breakdown in the cells would increase a* values. Darkening of the lettuce could be caused by phenolic oxidation and bacterial spoilage over time (King and Bolin 1989). This is in accordance with our results since the lettuce became more yellowish (due to the loss of green pigmentation) during the length of the study with results more accentuated for the ribs.

3.4.2 Lettuce Textural Characteristics

A decrease on the Kramer shear force value is a good indication of a softening effect on the fruit (Bourne 1997). There was significant difference among treatments since the first day of storage. Both the leaves and ribs of the irradiated samples were easier to cut (lower force values) than the control (Table 3.3). In general, the firmness of the leaves decreased by 15% at 1.0 kGy, 230% at 1.5 kGy and 50% at 3.2 kGy, and 27% at 1.5 kGy, and for the ribs, 29% at 3.2 kGy, respectively, at Day 0 (irradiation day). There was no significant difference in firmness of ribs between the control and those from samples irradiated at 1.0 kGy on Day 0. Higher irradiation dose yielded a produce softer than the control (lower shear force values). Storage time (up to 21 days) had no significant effect on firmness of all irradiated samples including the control.

Radiation-induced softening has been attributed to breakdown of cell wall constituents such as pectin, cellulose and hemicelluloses, and alteration of semi-permeable membranes, which result in structural weakening and loss of turgor in tissues (McArdle and Nehemias 1956; Kertesz and others 1964; Somogyi and Romani 1964; Howard and Buescher 1989; Yu and others 1996; Prakash and others 2000). These changes in cellular membranes might aggravate the loss of firmness. A loss of more than 5% water can cause texture breakdown in lettuce (Ryall and Lipton 1972), and besides high CO_2 levels can enhance tissue softening (Hamza and others 1996). This is in agreement with our observation that CO_2 levels in all irradiated samples were higher than control (Table 3.5); these samples were also softer than the control at Day 0.

Table 3.3—Effect of irradiation treatment on the texture characteristics of romaine lettuce hearts

	Kramer Shear Force (N)				Work (J)			
Storage Interval	**Control[1]**	**1.0 kGy**	**1.5 kGy**	**3.2 kGy**	**Control[1]**	**1.0 kGy**	**1.5 kGy**	**3.2 kGy**
Leaves								
Day 0	503.86^{ax}	427.26^{bx}	388.46^{bx}	254.07^{cx}	3.91^{ax}	3.50^{bxy}	3.02^{cx}	2.23^{dx}
	(43.28)[2]	(17.42)	(19.75)	(42.37)	(0.17)	(0.11)	(0.12)	(0.19)
Day 7	490.57^{ax}	392.17^{bx}	393.41^{bx}	342.85^{by}	4.02^{ax}	3.23^{by}	3.20^{bx}	2.70^{cxy}
	(28.45)	(37.93)	(34.25)	(6.23)	(0.34)	(0.08)	(0.17)	(0.06)
Day 14	520.68^{ax}	452.16^{bx}	365.49^{cx}	359.51^{cy}	3.92^{ax}	3.69^{bx}	3.20^{bx}	2.92^{by}
	(20.41)	(25.96)	(18.14)	(38.62)	(0.19)	(0.05)	(0.12)	(0.28)
Day 21	534.10^{ax}	428.01^{bx}	392.84^{bx}	290.35^{cxy}	4.29^{ax}	3.39^{bxy}	3.23^{bx}	2.55^{cxy}
	(20.21)	(50.58)	(32.92)	(28.96)	(0.01)	(0.22)	(0.02)	(0.26)
Ribs								
Day 0	508.23^{ax}	493.59^{ax}	372.48^{bx}	360.20^{bx}	4.67^{ax}	4.69^{ax}	3.80^{bx}	3.64^{bx}
	(44.47)	(21.89)	(29.67)	(38.90)	(0.36)	(0.20)	(0.14)	(0.37)
Day 7	479.25^{ax}	384.75^{byz}	367.80^{bx}	452.41^{ay}	4.65^{ax}	3.78^{bx}	3.75^{bx}	4.15^{abxy}
	(23.60)	(31.89)	(23.19)	(23.16)	(0.30)	(0.40)	(0.39)	(0.21)
Day 14	526.51^{ax}	355.35^{bz}	371.13^{bx}	492.56^{ay}	4.85^{ax}	3.82^{ax}	3.97^{ax}	4.51^{ay}
	(7.50)	(53.47)	(50.03)	(16.37)	(0.25)	(0.59)	(0.59)	(0.23)
Day 21	503.02^{ax}	438.93^{axy}	463.14^{ax}	449.22^{ay}	4.65^{ax}	4.53^{ax}	4.43^{ax}	4.04^{axy}
	(8.60)	(19.45)	(43.89)	(15.35)	(0.44)	(0.17)	(0.47)	(0.27)

[1] Control means samples not exposed to irradiation treatment
[2]Standard deviation
[a-d]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
[x,y]Means within a column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
Tests were conducted at room temperature (21^{o}C)

The irradiated leaves required less shear work than the non-irradiated samples until the end of the study (Day 21). Only the samples subjected to high dose showed a significant increase in work values for both leaves and ribs (Table 3.3) at all times. This finding suggests that the samples were harder to masticate. After the fourteenth day of storage, no significant changes in shear work values were found for all the ribs samples (control and irradiated). Although the values increased by Day 21, the difference is not significant ($p > 0.05$).

3.4.3 Sensory Attributes

The panelists were able to detect the differences among the treatments. The overall acceptability of the produce decreased as the dose level increased. Overall, the control sample rated better than the other samples. All sensory results are presented in Table 3.4.

3.4.3.1 Overall Quality

The control sample rated consistently higher than did the irradiated samples. The scores for the control and the low dose samples did not significantly change during the first 14 days of storage. As the irradiation dose increased, all the samples received lower scores. The samples exposed to high doses had the lowest scores at each storage time (1.13 at Day 21). After 21 days, the overall quality scores for samples exposed to low and medium doses were similar to the control (2.43 and 2.65 versus 2.38, respectively);

Table 3.4—Sensory attributes of romaine lettuce hearts irradiated at three different dose levels

Storage Interval	Control[1]	1.0 kGy	1.5 kGy	3.2 kGy
Overall quality				
Day 0	4.19^{ax}	3.67^{bx}	3.24^{cx}	1.67^{dx}
Day 7	4.42^{ax}	3.30^{bx}	2.88^{cxy}	1.16^{dy}
Day 14	4.26^{ax}	3.24^{bx}	2.59^{cy}	1.24^{dy}
Day 21	2.38^{ay}	2.43^{ay}	2.65^{ay}	1.13^{by}
Color				
Day 0	4.31^{ax}	3.93^{bx}	3.45^{cx}	2.81^{dx}
Day 7	4.55^{ax}	3.48^{by}	3.39^{bx}	1.91^{cy}
Day 14	4.33^{ax}	3.49^{by}	3.00^{cy}	2.13^{dy}
Day 21	3.20^{ay}	3.13^{ay}	2.93^{ay}	1.78^{by}
Sogginess				
Day 0	1.95^{ax}	2.29^{ax}	2.38^{ax}	3.40^{bx}
Day 7	1.70^{ax}	2.30^{bx}	2.64^{bx}	4.61^{cz}
Day 14	1.95^{ax}	2.08^{ax}	2.44^{ax}	3.87^{by}
Day 21	3.18^{ay}	2.78^{ay}	2.73^{ax}	4.93^{bz}
Odor				
Day 0	1.88^{ax}	2.00^{ax}	1.90^{ax}	2.36^{ax}
Day 7	1.48^{ax}	1.91^{ax}	2.03^{ax}	2.97^{by}
Day 14	1.46^{ax}	1.92^{abx}	2.23^{bxy}	3.18^{cy}
Day 21	2.93^{ay}	2.78^{ay}	2.68^{ay}	3.98^{bz}

[1] Control means samples not exposed to irradiation treatment

[a-c]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)

[x-z]Means within a column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)

Scores are averages of 40 randomly chosen panelists

however, all the scores were below the acceptability level of 3. The lower scores of the control samples were mainly due to the presence of molds by Day 21.

3.4.3.2 Color

Objective color measurements did not indicate obvious differences among the control and the irradiation treatments; however, the panelists gave higher scores to the control sample. As the irradiation dose increased, the scores for color decreased significantly ($p \leq 0.05$). However, the samples irradiated at low and medium doses received scores closer to the control sample after Day 21.

3.4.3.3 Sogginess

Control samples consistently received lower (less soggy) scores than the samples irradiated at low and medium doses at 0, 7, and 14 days; however, the changes were not significantly different. The low and medium dose samples received better scores (less soggy at $p \leq 0.05$) than the control after the 21-day study. The higher scores for the control sample at Day 21 were -- again -- due to the presence of molds on the surface of the produce, thus contributing to the soggier appearance.

3.4.3.4 Off-odor

After irradiation (at day 0), no significant differences in off-odor scores were observed. The sample exposed to a higher dose received higher off-odor scores as storage time proceeded. It also received significantly higher scores than the other

treatments and control at each storage interval. The control, low, and medium dose samples showed slight difference in off-odor scores during the 21-day storage studies.

3.4.4 Headspace Gases

The medium-dose treatment (1.5 kGy) yielded samples with significantly lower O_2 levels in the headspace (10%) than other treatments (Table 3.5). Samples irradiated at 1.5 and 3.2 kGy had significantly higher CO_2 levels than the control after irradiation at Day 0 with a 258.2% and 232.7% increase, respectively. The lower O_2 and higher CO_2 levels for the sample irradiated with 1.5 kGy indicated higher produce respiration rates than the control.

High CO_2 and low O_2 gas atmospheres could inhibit browning of fresh-cut lettuce (Gorny, 1997). The mechanism responsible for an increase in respiration due to irradiation is not well known. Gunes and others (2001) found that irradiation stimulated the catabolism of acetate to CO_2 in carrot tissues, and postulated that this explained the increased CO_2 evolution caused by irradiation. They also reported that irradiation at 2 kGy resulted in about 50% reduction in respiration rate of grated carrots. Moreover, an inhibitory effect of irradiation at 3 kGy on respiration rate was also reported for intact tomatoes. Such results seem to be in partial agreement with our study since the lettuce irradiated at 3.2 kGy showed significantly lower CO_2 and higher O_2 levels than the lettuce irradiated at 1.5-kGy doses. This could also be due to wound-induced responses of tissues. The high dose treatment could have wounded the tissues to a point that some of the lettuce cells died, thus the lower respiration rate. This aspect will be addressed in

Table 3.5—Package headspace content [O_2 and CO_2 concentration (%)] as a function of irradiation treatment

	O_2 (%)				CO_2 (%)			
Storage Interval	**Control[1]**	**1.0 kGy**	**1.5 kGy**	**3.2 kGy**	**Control[1]**	**1.0 kGy**	**1.5 kGy**	**3.2 kGy**
Day 0	21.48^{ax} $(0.06)^2$	20.86^{ax} (0.09)	19.25^{bx} (0.06)	20.49^{ax} (0.05)	0.55^{aw} (0.05)	0.72^{aw} (0.00)	1.97^{cw} (0.31)	1.28^{bx} (0.16)
Day 7	21.48^{ax} (0.06)	21.60^{ay} (0.00)	21.85^{ay} (0.03)	21.60^{ay} (0.05)	0.28^{ay} (0.01)	0.36^{bx} (0.01)	0.35^{bx} (0.01)	0.36^{by} (0.01)
Day 14	21.73^{ax} (0.06)	21.88^{ay} (0.03)	21.90^{ay} (0.04)	21.60^{ay} (0.00)	0.24^{ay} (0.00)	0.24^{ay} (0.01)	0.23^{bx} (0.00)	0.17^{cz} (0.00)
Day 21	21.23^{ax} (0.02)	21.29^{axy} (0.01)	21.42^{ay} (0.01)	21.18^{ay} (0.01)	0.48^{ax} (0.01)	0.20^{cz} (0.03)	0.24^{cx} (0.01)	0.30^{byz} (0.02)

[1] Control means samples not exposed to irradiation treatment
[2]Standard deviation
[a-c]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
[w-z]Means within a column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
Samples consisted of perforated LDPE bags (2 perforations with 5 mm diameter on each side)

a future study. The O_2 and CO_2 levels for all samples virtually reached the same levels in our study during the storage period because headspace gases might be exchanged with gases outside through the perforations. That is, CO_2 gas produced by respiration may be released without being accumulated inside, and also outside O_2 gas required for respiration was passing through the perforations.

3.4.5 Package Properties

3.4.5.1 Film Permeability

The LDPE bags irradiated at 1.5- and 3.2-kGy dose levels had significant reduction in oxygen permeability (cc·mil/m^2·day·atm) by 7.7% and 4.5%, respectively (Table 3.6). The improved oxygen barrier property of the films may be explained by the fact that generally, polymers undergo structural changes from irradiation such as crosslinking. The 1.0-kGy dose treatment was not sufficient to affect the structure of the packaging material. On the basis of these results, it can be assumed that the crosslinking processes induced by electron beam irradiation were enhanced at dose levels higher than 1.5 kGy. Although all the irradiation treatments caused a slight improvement on the film water vapor barrier properties, the effect was not significant in this study (Table 3.6).

3.4.5.2 Film Mechanical Properties

Table 3.7 presents the values of stiffness and energy dissipated for the LDPE films calculated using the method by Limanond and others (2002). In general, the higher dose treatment (3.2 kGy) caused an increase in stiffness of the polymer up to a

Table 3.6—Effect of irradiation treatment on the oxygen (cc·mil/m^2·day·atm) and water vapor permeability (g·mil/m^2·day·atm) of LDPE film (50.8 μm thickness)

Dose level	Oxygen permeability	Water vapor permeability
Control[1]	12.03^{x}	$1.326\times10^{-3\ x}$
	$(0.70)^{2}$	(3.88×10^{-4})
1.0 kGy	11.83^{x}	$1.045\times10^{-3\ x}$
	(0.46)	(3.19×10^{-5})
1.5kGy	11.11^{y}	$1.001\times10^{-3\ x}$
	(0.56)	(6.19×10^{-5})
3.2 kGy	11.49^{xy}	$1.225\times10^{-3\ x}$
	(0.59)	(2.75×10^{-4})

[1] Control means samples not exposed to irradiation treatment
[2]Standard deviation
[x,y]Means within a column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
Values at the end of study (Day 21)

Table 3.7—Effect of irradiation treatment on the mechanical properties of LDPE film (50.8 μm thickness)

	Control[1]	**1.0 kGy**	**1.5 kGy**	**3.2 kGy**
Stiffness (kPa)	$2.586\times10^{7\ ab}$ $(0.98\times10^{7})^{2}$	$2.283\times10^{7\ c}$ (1.04×10^{7})	$2.414\times10^{7\ bc}$ (0.78×10^{7})	$2.666\times10^{7\ a}$ (0.95×10^{7})
Energy dissipated (kJ/m^3)	$1.3126\times10^{-13\ a}$ (0.81×10^{-13})	$1.789\times10^{-13\ b}$ (1.79×10^{-13})	$1.452\times10^{-13\ ab}$ (0.79×10^{-13})	$1.088\times10^{-13\ a}$ (0.75×10^{-13})

[1] Control means samples not exposed to irradiation treatment
[2]Standard deviation
[a-c]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
Tests were conducted at room temperature (21°C)

value similar to the control. Consequently, the energy dissipated was also lower, as expected. Irradiation treatment with higher dose may have induced polymeric crosslinking, thus the stiffer plastic (more resistant to tear). This result is also in agreement with the improved oxygen barrier of the high-dose treated films. However, the changes in mechanical properties of irradiated LPDE films (exposed up to 3.2 kGy) were not significant.

3.5 Conclusions

This study shows that packaged romaine lettuce hearts can be irradiated using electron beam technology at low dose levels. The ribs and leaves of the lettuce samples had different responses to irradiation in terms of color and texture. The ribs seem to be more sensitive to irradiation than the leaves due to their higher density and the potential for higher dose absorption. Only the samples subjected to high doses showed a significant increase in work values (leaves and ribs). This means the samples could be more difficult to cut or chew. The higher dose treatment had a softening effect on the produce. Storage time (up to 21 days) had no significant effect on firmness of all samples, including the control. For the dose levels tested in this study, the sensory evaluation panelists better accepted the non-irradiated (control) samples. However, the low-dose irradiated sample (1.0 kGy) was found acceptable because the control sample had molds while the irradiated lettuce did not. The presence of molds in the control sample by the end of shelf-life (Day 21) suggests that low dose irradiation of lettuce may have some beneficial effect on the prevention of surface molds in the product. This

finding should be further investigated. The oxygen barrier capabilities of the packaging material improved with irradiation, while water vapor permeability and mechanical properties (stiffness) were unaffected by the treatments. Further studies will evaluate the effect of irradiation treatment on produce structure and its relationship with product quality and end of shelf-life.

CHAPTER IV

DEVELOPMENT OF ANTIMICROBIAL COATED LDPE/POLYAMIDE FILMS TO INCREASE RADIATION SENSITIVITY OF PATHOGEN SURROGATES

4.1 Overview

In this study, we established the antimicrobial effectiveness of various FDA-approved active compounds incorporated into low-density polyethylene (LDPE)/polyamide films to increase radiation sensitivity of selected food pathogen surrogate strains. We also evaluated the effects of electron beam irradiation (1-3 kGy) on the functional properties of the films. Sorbic acid, carvacrol, trans-cinnamaldehyde, thymol or rosemary oleoresin was added to a polyamide coating solution (1% of final solution weight). The coatings (≈ 3.03 ± 0.10 μm) were applied to one side of low-density polyethylene (LDPE) films and dried. Films were irradiated using a 10 MeV (18 kW) LINAC linear electron accelerator. We evaluated the antimicrobial effectiveness of the films against *Listeria innocua* ATCC 33090 and *Escherichia coli* ATCC 884 using agar diffusion and liquid culture tests. All films showed inhibition zones in agar diffusion test against both surrogate strains. In the liquid culture test, the active compounds significantly ($p \leq 0.05$) reduced the specific growth rate of *L. innocua*, and decreased final cell concentration of *L. innocua* and *E. coli* strains. Incorporation of active compounds in plastic films increased the radiation sensitivity of the tested strains, demonstrating the potential of this methodology to reduce required radiation dose to

control microbial contamination. Neither the presence of active compound nor irradiation dose affected the film's tensile strength and toughness, and films became more ductile (increased % elongation-at-break) and had improved moisture barrier capability. Film's oxygen permeability was not affected by either treatment. These results are encouraging for the future development of self-sterile active packaging materials for food safety applications.

4.2 Introduction

The emergence of pathogens in and on the food has increased the interest in the use of irradiation as a preservation technique (Diehl 1995; Monk and others 1995). The application of radiation treatment to fresh produce is a feasible way to extend shelf life and improve safety issues. After irradiation treatment, more resistant pathogens (like gram-positive *Listeria monocytogenes*) are reduced in numbers, and the surviving flora is generally less resistant to other factors such as heat, pH change, nutrients and antibiotics than untreated cells (Farkas 1990; Lacroix and Ouattara 2000). However, the use of ionizing radiation for decontamination purposes is limited by undesirable changes in produce quality. Depending upon the treatment (dose and dose rate), produce can show some changes such as discoloration, loss in texture or nutritional qualities (Somogyi and Romani 1964; Bramlage and Lipton 1965; Howard and Buescher 1989; Han and others 2004). An alternative is to increase the radiation sensitivity of the target pathogens in order to lower required radiation dose and successfully reduce these quality changes (Borsa and others 2004).

Most studies have been conducted using gamma rays as the irradiation source, but only a few studies were performed on the effect of electrons. The use of an electron accelerator offers certain advantages over using gamma radiation: (i) high efficiency for the direct deposition of energy, which results in high plant-product capacity; (ii) the efficient convertibility of electron power to X-ray, which means the capability of handling very thick products that gamma ray cannot penetrate; (iii) the easy variability of electron beam current and energy, which means a flexibility in the choice of surface and depth treatments; (iv) the monodirectional characteristic of the primary and secondary electrons, which permits a great flexibility in the food package design; (v) the ability to control and regulate, which means the capability of efficiently processing small, intricate or non-uniform shape; (vi) electron beams have superior dose rate to gamma rays (10^3-10^6 Gy/sec from electron beams and 1-100 Gy/min from ^{60}Co, respectively) (Koch and Eisenhower 1965; Cleland 1983).

One approach to lowering required dose is to use self-sterilizing packaging materials. For instance, 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 and Hotchkiss 2002; Cutter 2002). The concept of antimicrobial polymeric materials was introduced and developed in the biomedical science field to protect sutures or implants from microbial contamination (Endo and others 1987; Desai and others 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 effectively 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, natamycin, glyceryl esters, propionic acid, acetic acid, benzoic acid, p-hydroxybenzoate alkyl esters, epoxides, antibiotics, various natural essential oils and others (Lindsay 1996; Appendini and Hotchkiss 2002). The antimicrobial agents used in food packaging materials have to be approved as safe by the Food and Drug administration (FDA) in United States (CFR 2001).

Researchers have been continually investigating the use of novel materials, such as plant-derived active compounds, to control pathogens or spoilage organisms in foods. 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.

Listeria monocytogenes and *Escherichia coli* O157:H7 are common pathogenic bacteria associated with foods (Jay 1996; Sumner and Peter 1997), and their resistance to

a specific treatment can be mimicked using indicator or surrogate microorganisms. *Listeria innocua* ATCC 33090 and *Escherichia coli* ATCC 884 are commonly used non-pathogenic surrogate microorganisms for *L. monocytogenes* and *E. coli* O157:H7, respectively (FDA 2001b).

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 surrogates. Specific objectives included (1) testing the antimicrobial effectiveness of various FDA-approved compounds incorporated into polyamide coated low-density polyethylene (LDPE) films; (2) evaluating whether the antimicrobial film would aid in reducing the amount of dose required to inactivate the selected non-pathogenic surrogate bacteria when exposed to electron beam irradiation (1-3 kGy) and; (3) determining the effects of electron beam irradiation on the functional properties of the films.

4.3 Materials and Methods

4.3.1 Preparation of Films

Polyamide resin (Cognis Corporation, New Milford, Conn., USA) was dissolved in absolute alcohol with a 4:6 (w/w) ratio to prepare coating solution, and mixed using a magnetic stirrer for 12 hr. Polyamide was chosen as coating medium for incorporating active compounds on the surface of the low-density polyethylene (LDPE) film because it is approved for use in adhesives and coating components in food packaging materials (CFR 2003c). Next, various active compounds were added to the prepared polyamide solution at 1% of the final solution weight and mixed thoroughly for 2 min using a

vortex. These compounds consisted of sorbic acid, carvacrol, and trans-cinnamaldehyde (Aldrich, Milwaukee, Wis., U.S.A.), thymol (Sigma, St. Luis, Mo., U.S.A.) and rosemary oleoresin (Kalsec, Kalamazoo, Mich., U.S.A). The prepared coating medium was degassed by applying vacuum to remove dissolved air for 1 hr, and then applied manually on one side of LDPE film (50 μm thickness, Plastic Supplies Co., Fullerton, CA, U.S.A.) using a No.12 coating rod (RD Specialties Inc., Webster, N.Y., U.S.A.), and dried at 21°C for 12 hr in a laboratory fume hood. Final coating thickness (≈ 3.03 ± 0.10 μm) was measured by a comparator XL-750 (Brunswick Instrument, Niles, Ill., U.S.A.) after dried. Polyamide-coated LDPE film without addition of active compounds served as control.

4.3.2 Irradiation of Coated Films

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 a conveyor. Irradiation dosage was measured by placing a radiochromic film dosimeter (B3WIN Radiochromic Films, Gex Corp. Centennial, Colo., U.S.A.) at the top surface of the film. Irradiated and non-irradiated films were stored in a desiccator at room temperature until further testing.

4.3.3 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, Ill., U.S.A.). Original stock cultures were maintained in plastic vials (Protect^{TM} Bacterial System from Key Scientific, Round Rock, Tex., U.S.A.) 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, Tex., U.S.A.) at -80ºC. 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, Mich., U.S.A.), and maintained for 24 hr in an incubator (Equatherm, Curtin Matheson Scientific Inc., Houston, Tex., U.S.A.) at 37°C. To facilitate recovery, the working stock cultures were maintained on tryptic soy agar (TSA; Difco, Detroit, Mich., U.S.A.) slants at 4ºC in a refrigerator and grown in an incubator at 37°C for 24 hr until further testing.

4.3.4 Determination of Minimum Inhibitory Concentrations

The minimum inhibitory concentration (MIC) of tested active compounds against *L. innocua* ATCC 33090 was determined using the broth dilution method. Kim and others (1995) 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., U.S.A.) 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 and others 1995). Next, the mixture (antimicrobial solution) was sonicated for 10 min using a sonicator (Branson 220 Sonicator, Smithkline Company, Shelton, Conn., U.S.A.) 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 (0.0005-0.2%, w/v). Next, test tubes were incubated in an incubator at 37°C with agitation (200 rpm, Model G25, New Brunswick, Scientific Co. Inc., Edison, NJ, U.S.A.) for 36 hr. The culture was sampled (1.5 mL) every 3 hr up to 36 hr to obtain microbial growth profiles. The optical density (O.D.) of each culture sample was measured at λ = 600 nm using an UV-visible spectrophotometer (Spectronic 20D+, Milton Roy Company, Rochester, N.Y., U.S.A.), and the lowest concentration of active compound resulting in significant no growth was established as the MIC against *L. innocua* ATCC 33090.

4.3.5 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. In general, tests of antimicrobial activity are classified as diffusion, dilution or bioautographic methods (Rios and others 1988), but no standardized method has been developed for evaluating the antibacterial activity of possible preservatives against food-related microorganisms (Davidson and Parish 1989). 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 (Appendini and Hotchkiss 2002). Twenty (20) mL of melted TSA was poured into a Petri dish. Next, a square sample (12×12 mm) of plastic film was placed on the Petri dish with the coated side facing the TSA surface. Microorganisms were cultured in 5 mL of TSB for 24 hr in an incubator 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 hr in an incubator. The clear zone formed around the film square was recorded to the nearest millimeter as an indication of inhibition of the microbial species (Appendini and Hotchkiss 2002). A clear zone surrounding the film indicates antimicrobial diffusion from the film and subsequent growth inhibition (Fig. 4.1). All experiments were conducted in triplicate.

The inhibitory zone in agar diffusion test can be affected by the solubility and diffusion rate of the test compounds in agar medium, thus agar diffusion test does not accurately reflect the antimicrobial effectiveness of the test compounds (Kim and others 1995). The liquid culture test determines the antimicrobial activity of the test compounds

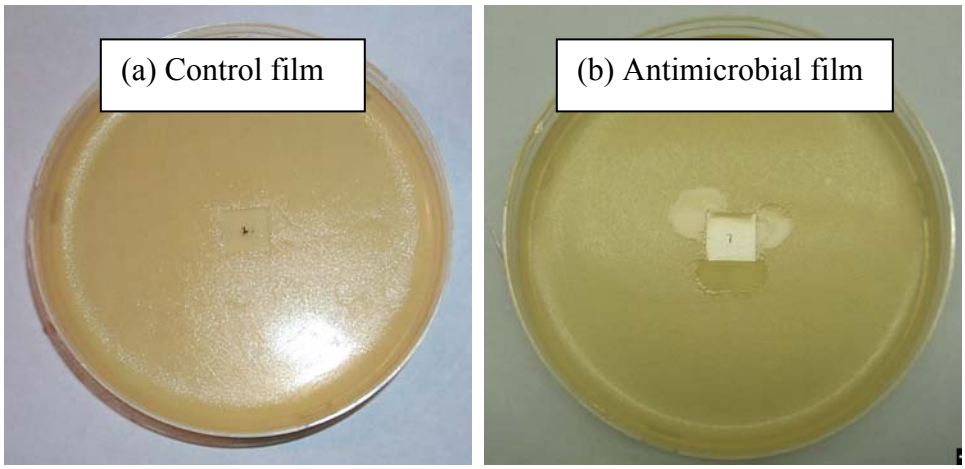

Figure 4.1—Effect of antimicrobial film on growth of *Listeria innocua* ATCC 33090 after 24 hr at 37°C by agar diffusion method. (a) Control film contains no active compound and (b) Antimicrobial film with trans-cinnamaldehyde.

by viable count and provides information on microbial growth kinetics, thus being more sensitive than the agar diffusion method (Mann and Markham 1998). For the liquid culture test, the films were cut into 30 × 50 mm rectangles using a sterile knife. Three film rectangles (45 cm^2 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. The culture was sampled (1.5 mL) periodically every 2 hr during the incubation period up to 36 hr to obtain microbial growth profiles. The optical density ($O.D._{600}$) of each culture sample was measured at λ = 600 nm using an UV-visible spectrophotometer (Spectronic 20D+) 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; (b) the specific cell growth rate (μ) during the exponential growth phase was calculated from:

$$\frac{dX(t)}{dt} = \mu X(t) \quad \text{and} \quad \mu = \frac{d \ln X(t)}{dt} \qquad (4.1)$$

where $X(t)$ is the cell concentration of inoculated microorganism in the medium ($O.D._{600}$), 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 ($O.D._{600}$) at stationary

phase using standard methods (Doran 1995; Chung and others 2003). All experiments were conducted in triplicate.

4.3.6 Properties of Films

The mechanical properties of the film strips (20 × 60 mm) were measured in accordance with the American Society of Testing Materials (ASTM) Standard Method D882-00 (ASTM 2000) using a TA-XT2 Texture Analyzer (Texture Technologies Corporation, Scardale, N.Y., U.S.A.) under large deformations (tension mode). 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. 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, Minn., U.S.A.), based on the ASTM F1770 Standard Method (ASTM 1997). The permeability coefficient (P, kg/s·m·Pa) was calculated as,

$$P = \frac{F_e L}{p} \qquad (4.2)$$

where F_e is the film permeability flux in kg/m^2·s, L is barrier (film) thickness, and p is test vapor pressure (1 atm). The testing conditions used were 65% relative humidity (RH) and 25°C temperature. All measurements were performed in quadruplicate.

Changes in color of the films were assessed using a Labscan XE (16437) colorimeter (HunterLab Inc., Reston, Va., U.S.A.) with the Universal Version 3.73 software (HunterLab Inc., Reston, Va., U.S.A) using the CIELAB system. The colorimeter was calibrated using the standard white and black plates. Hunter-lab color scale L^* (lightness), a^* (redness), and b^* (yellowness) values were determined. Total color difference (ΔE) was calculated as,

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (4.3)$$

The polyamide-coated LDPE film (control) containing no active compounds served as the reference. Film chroma (saturation) was calculated as,

$$Chroma = \sqrt{a^{*2} + b^{*2}} \qquad (4.4)$$

All measurements were repeated four times.

4.3.7 Statistical Analysis

Data analysis was performed using the Statistical Analysis System (SAS) software, version 8.1 (SAS Institute, Cary, N.C., 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 $p \leq 0.05$.

4.4 Results and Discussion

4.4.1 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. 4.2). The growth of *L. innocua* ATCC 33090 was inhibited by sorbic acid and rosemary oleoresin at 2,000 μg/mL (in 0.2%, w/v), which delayed the lag phase and lowered growth rate and final cell concentration of the microorganism. However, these two compounds (at concentration up to 2,000 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 2,000 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 2,000 μg/mL (in 0.2%, w/v) which resulted in significant ($p > 0.05$) no bacterial growth. The growth of *L. innocua* ATCC 33090 was inhibited by carvacrol at 500-2,000 μg/mL (in 0.05-0.2%, w/v), and the MIC of carvacrol was established as 2,000 μg/mL (in 0.2%, w/v) at which bacterial growth was inhibited completely ($p > 0.05$). Growth was completely inhibited by trans-cinnamaldehyde at the concentration range of 250-2,000 μg/mL (in 0.025-0.2%, w/v). Although test concentrations at 25-100 μg/mL (in 0.0025-0.01%, w/v) delayed the lag phase of the growth curve, they did not inhibit the microbial growth effectively which

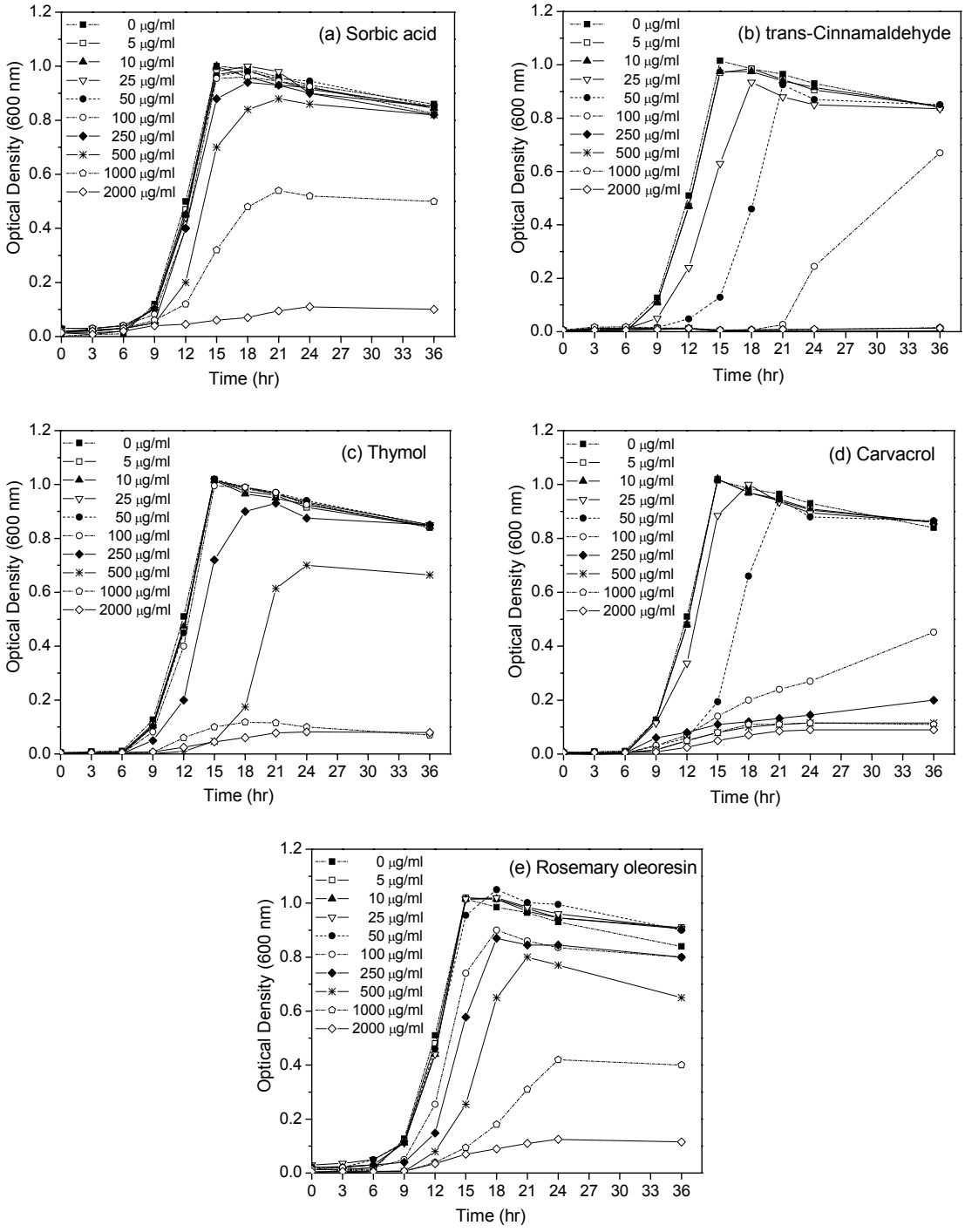

Figure 4.2—Growth of *L. innocua* ATCC 33090 in tryptic soy broth as a function of active compound concentration.

means there was a significant ($p \leq 0.05$) increase in bacterial population during the incubation period (36 hr). 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.

4.4.2 Agar Diffusion Test

All films with added active compound showed antimicrobial activity against both *L. innocua* ATCC 33090 and *E. coli* ATCC 884 (Table 4.1). 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 and 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).

4.4.3 Liquid Culture Test

Compared to the control (no active compounds 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. 4.3 and 4.4). *L. innocua* ATCC 33090 reached the stationary phase after 14 hr, and

Table 4.1—Mean zones of inhibition (mm) of antimicrobial films against 2 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[1]	0	0	0
	1	0	0
	3	0	0
Sorbic acid	0	5.33^{ax} (1.03)[2]	4.00^{ay} (0.84)
	1	5.00^{ax} (0.89)	4.08^{az} (0.80)
	3	5.17^{axy} (1.03)	3.83^{axy} (1.03)
Trans-cinnamaldehyde	0	5.92^{ax} (1.32)	4.92^{ay} (0.66)
	1	6.08^{ax} (0.80)	3.83^{ayz} (0.87)
	3	6.50^{ay} (1.26)	4.13^{ay} (0.76)
Thymol	0	6.08^{ax} (1.11)	3.58^{ay} (0.86)
	1	6.50^{ax} (1.05)	2.25^{bx} (0.27)
	3	4.67^{bx} (1.03)	3.17^{abxy} (1.12)
Carvacrol	0	5.25^{ax} (0.70)	2.08^{bx} (0.92)
	1	5.67^{ax} (0.87)	3.00^{axy} (0.32)
	3	4.75^{ax} (0.42)	3.75^{axy} (0.61)
Rosemary oleoresin	0	4.67^{ax} (0.41)	4.17^{ay} (0.93)
	1	5.00^{ax} (1.09)	4.25^{az} (1.08)
	3	5.83^{axy} (1.17)	2.50^{bxy} (0.55)

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

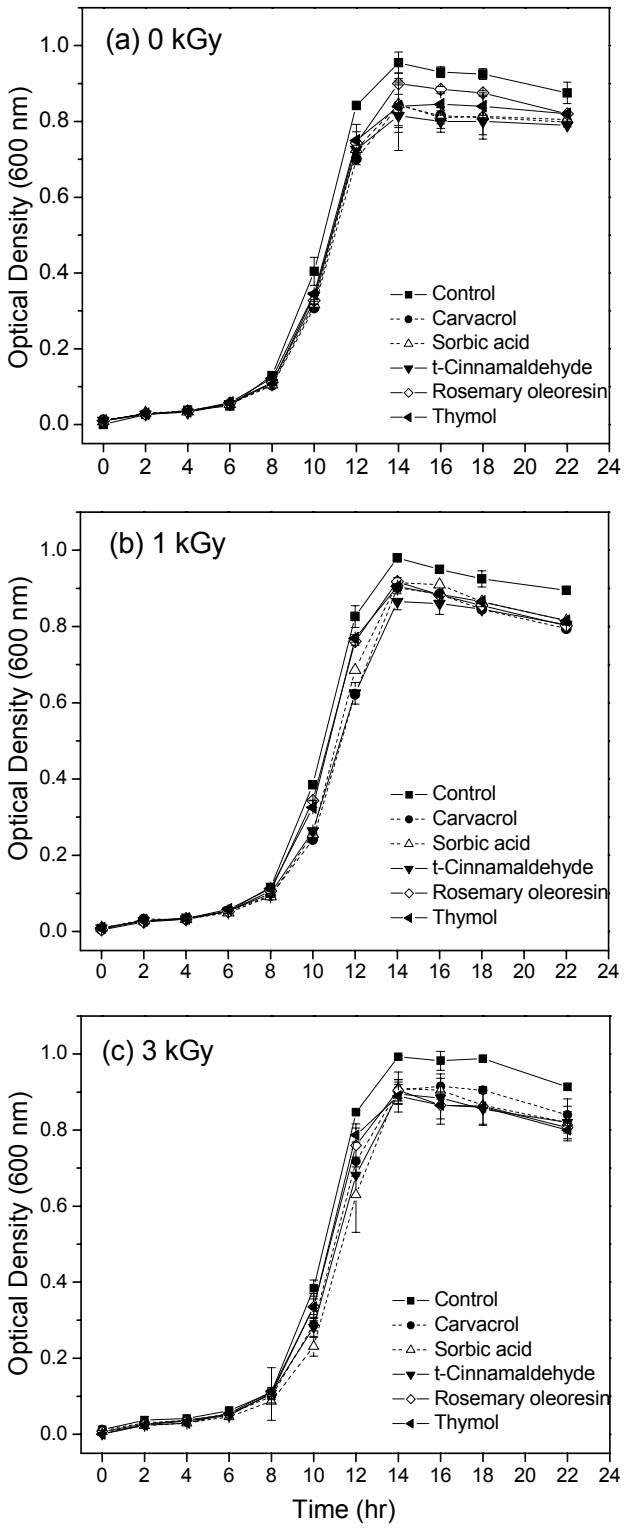

Figure 4.3—Growth of *Listeria innocua* ATCC 33090 in tryptic soy broth media in the presence of antimicrobial films which were irradiated at different doses.

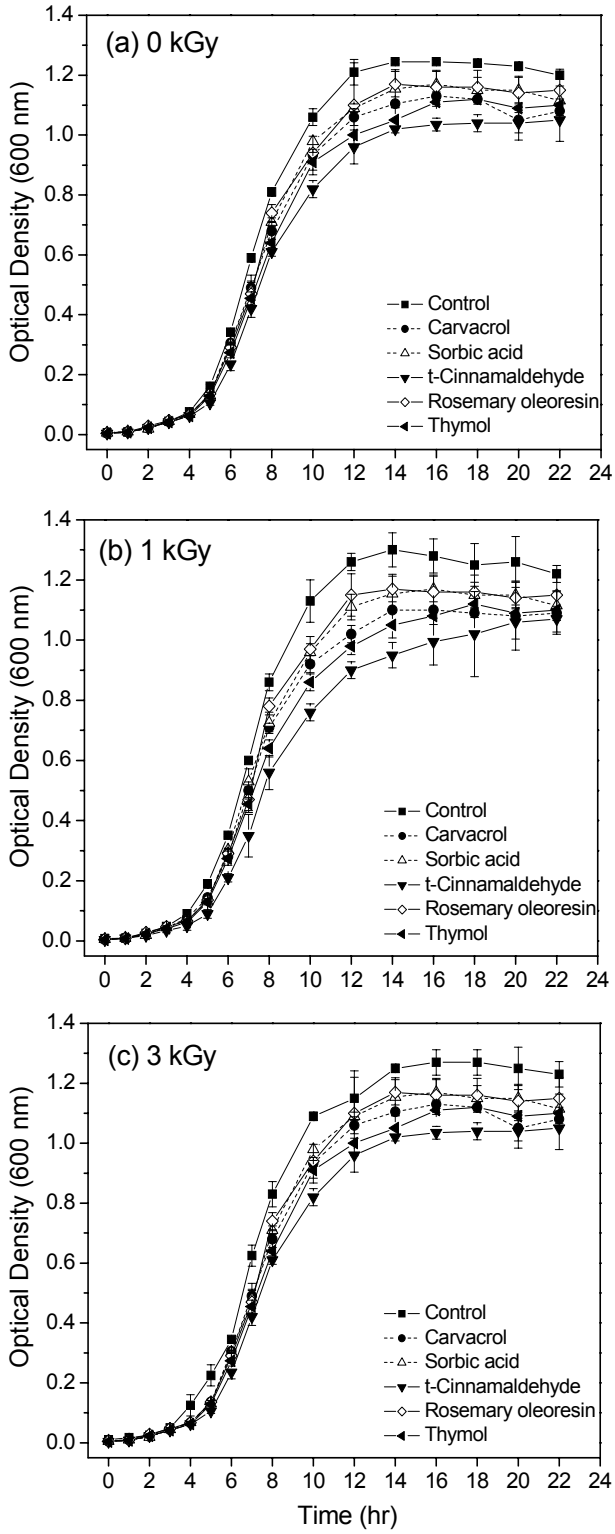

Figure 4.4—Growth of *Escherichia coli* ATCC 884 in tryptic soy broth media in the presence of antimicrobial films which were irradiated at different doses.

the culture media containing coated films significantly ($p \leq 0.05$) reduced microbial growth rates (μ) and final cell concentrations (C_f), when compared with the culture medium with control film (Fig. 4.3). The growth profiles of *E. coli* ATCC 884 were similar to the growth profiles of *L. innocua* ATCC 33090 (Fig. 4.4). *E. coli* ATCC 884 reached the stationary phase after 12 hr, and its final cell concentration was significantly ($p \leq 0.05$) reduced 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 4.2 and Fig. 4.3). 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 ($p \leq 0.05$) compared to the control film. Film with trans-cinnamaldehyde, which had the lowest MIC against *L. innocua* ATCC 33090 (Fig. 4.2), produced the highest reduction in final cell concentration (~ 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. 4.2 and Table 4.2).

Table 4.2—Effect of antimicrobial compound and dose level on the specific growth rates[2] (μ) and final cell concentrations (C_f) of *Listeria innocua* ATCC 33090 culture

Film treatment	Dose (kGy)	μ (hr^{-1})	% Reduction[3] of μ	C_f ($O.D._{600}$)	% Reduction[4] of C_f
Control film[1]	0	0.341^{ax}	-	0.96^{ax}	-
	1	0.349^{ax}	-	0.98^{ax}	-
	3	0.330^{axy}	-	0.99^{ax}	-
Sorbic acid	0	0.318^{ay}	6.74	0.85^{ayz}	11.52
	1	0.324^{axy}	7.16	0.92^{ay}	6.63
	3	0.335^{axy}	-1.43	0.91^{ay}	8.36
trans-Cinnamaldehyde	0	0.325^{axy}	4.70	0.82^{az}	14.66
	1	0.318^{ay}	8.88	0.87^{aby}	11.73
	3	0.328^{axy}	0.57	0.90^{by}	9.87
Thymol	0	0.323^{axy}	5.28	0.85^{ayz}	11.52
	1	0.327^{axy}	6.30	0.91^{by}	7.65
	3	0.344^{ax}	-4.01	0.90^{by}	10.37
Carvacrol	0	0.312^{ay}	8.50	0.84^{ayz}	11.83
	1	0.311^{ay}	10.80	0.90^{by}	7.86
	3	0.312^{ay}	5.16	0.92^{by}	7.85
Rosemary oleoresin	0	0.328^{axy}	3.81	0.90^{ay}	5.76
	1	0.341^{axy}	2.30	0.92^{ay}	6.33
	3	0.330^{axy}	0.00	0.91^{ay}	8.86

[1] Control means films not containing active compounds.
[2] Calculated μ from equation (4.1).
[3]% Reduction of μ = [1-(μ of active compound added film / μ of control film)] × 100; Films are at the same irradiation dose.
[4]% 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.
[a,b]Means within a same film treatment and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$).
[x-z]Means at same irradiation dose and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$).

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 4.2). However, films containing trans-cinnamaldehyde, thymol, and carvacrol were significantly ($p \leq 0.05$) 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 is affected by radiation exposure depending on the dose level or nature of the compound. In general, functional groups linked to the main chain of active compound molecules, such as hydrocarbons and hydroxyl groups in thymol and carvacrol, and carbonyl groups in trans-cinnamaldehyde are quite radiosensitive (Swallow 1960). Thus, the ionizing radiation energy may react with the radiosensitive bonds adjacent to functional groups, and change chemical structure of the active compounds, which can cause the loss of antimicrobial property. Meanwhile, films with sorbic acid and rosemary oleoresin had reduced ability to reduce in final cell concentration, but their values were not significantly ($p > 0.05$) changed by radiation exposure up to 3 kGy. However, in comparison with the control film, all irradiated films containing active compounds had significantly ($p \leq 0.05$) lower final cell concentration in TSB media after exposure up to 3 kGy than the control. In summary, radiation doses used in this study (1-3 kGy) did not affect the antimicrobial property of the active compounds.

The lag time and growth rate of *E. coli* ATCC 884 were not affected ($p > 0.05$) by the active compounds in films (Table 4.3). Film with added thymol did not affect the growth rate of the surrogate. Other tested compounds in film reduced the growth rate (μ)

Table 4.3—Effect of antimicrobial compound and dose level on the specific growth rates (μ) [2] and final cell concentrations (C_f) of *Escherichia coli* ATCC 884 culture

Film treatment	Dose (kGy)	μ (hr^{-1})	% Reduction[3] of μ	C_f ($O.D._{600}$)	% Reduction[4] of C_f
Control film[1]	0	0.571^{ax}	-	1.25^{ax}	-
	1	0.562^{ax}	-	1.30^{ax}	-
	3	0.586^{ay}	-	1.27^{ax}	-
Sorbic acid	0	0.556^{ax}	2.63	1.16^{ay}	7.20
	1	0.558^{ax}	0.71	1.19^{ay}	8.46
	3	0.556^{az}	5.12	1.18^{ay}	7.09
trans-Cinnamaldehyde	0	0.558^{ax}	2.28	1.04^{az}	16.80
	1	0.550^{ax}	2.14	1.07^{az}	17.69
	3	0.558^{az}	4.78	1.07^{az}	15.75
Thymol	0	0.571^{ax}	0.00	1.09^{ay}	12.80
	1	0.568^{ax}	-1.06	1.12^{ayz}	13.85
	3	0.571^{ayz}	2.56	1.13^{ay}	11.02
Carvacrol	0	0.564^{ax}	1.23	1.09^{ay}	12.80
	1	0.557^{ax}	0.89	1.13^{ayz}	13.08
	3	0.564^{ayz}	3.75	1.12^{ay}	11.81
Rosemary oleoresin	0	0.548^{ax}	4.03	1.15^{ay}	8.00
	1	0.551^{ax}	1.96	1.17^{ay}	10.00
	3	0.614^{bx}	-4.77	1.16^{ay}	8.66

[1] Control means films not containing active compounds.
[2] Calculated μ from equation (4.1).
[3]% Reduction of μ = [1-(μ of active compound added film / μ of control film)] × 100; Films are at the same irradiation dose.
[4]% 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.
[a,b]Means within a same film treatment and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$).
[x-z]Means at same irradiation dose and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$).

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 ($p \leq 0.05$) reduced by all films (by 7.2-16.8%) in TSB media (Table 4.3 and Fig. 4.4). 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.

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 (μ) or the final cell concentrations (C_f) 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.

4.4.4 Effect of Active Compound Incorporation and Radiation Dose on Film Properties

After radiation treatment, polymeric materials can undergo changes in mechanical and functional properties depending on the polymer types and absorbed radiation dose (Pentimalli and others 2000). During irradiation, crosslinking is the dominant reaction in polymer networks of LDPE and polyamide materials, which were used in this study (Wilson 1974; Hu and others 1999). However, the mechanical properties of the films were not affected ($p > 0.05$) by irradiation exposure at the dose levels (1-3 kGy) used in this study (Table 4.4).

Addition of active compounds in coating solution (1% of final solution weight) caused only slight changes ($p > 0.05$) in the tensile strength and toughness of the polyamide-coated LDPE films (Table 4.4). Meanwhile, the ability of the films to elongate increased significantly ($p \leq 0.05$) by 20.3-39.6% when incorporated with sorbic acid, carvacrol and rosemary oleoresin, making the films more flexible. The increased flexibility of films might be attributed to the added active compounds acting as a function of plasticizer (plasticizer effect) which is defined as a substance incorporated into a polymer material to increase its deformability (Sears and Darby 1982). Some hydrophobic compounds like vegetable oils, lecithin, and waxes were incorporated to polymeric films as plasticizers and they improved film flexibility (Kester and Fennema 1986; Lin and others 2004). In general, plasticizers are generally added into film-forming solutions to prevent film brittleness and cracks caused by intermolecular forces (Lieberman and Gilbert, 1973). Plasticizers are often added to modify the mechanical

Table 4.4—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[1]	0	17.62^{ax} (1.21)[2]	316.59^{ax} (70.18)	2.52^{ax} (0.43)
	1	19.05^{ay} (0.91)	455.09^{ax} (124.93)	3.69^{ay} (1.15)
	3	18.12^{axy} (0.79)	358.86^{ax} (76.40)	2.81^{ax} (1.17)
Sorbic acid	0	18.27^{ax} (0.90)	380.98^{axy} (78.20)	2.80^{ax} (0.45)
	1	18.55^{axy} (0.58)	355.88^{ax} (58.32)	3.27^{axy} (0.64)
	3	18.07^{axy} (0.56)	365.41^{ax} (35.84)	3.11^{ax} (0.34)
trans-Cinnamaldehyde	0	17.74^{abx} (0.26)	327.32^{ax} (14.37)	1.98^{ax} (0.72)
	1	16.96^{ax} (0.67)	313.99^{ax} (42.49)	2.21^{axy} (0.52)
	3	18.62^{bxyz} (0.65)	416.95^{bx} (27.10)	3.58^{bx} (0.27)
Thymol	0	17.34^{ax} (0.63)	317.80^{ax} (25.38)	2.53^{ax} (0.50)
	1	17.82^{axy} (0.57)	355.74^{ax} (56.90)	2.60^{axy} (0.73)
	3	19.51^{bz} (0.41)	436.56^{bx} (43.19)	3.41^{ax} (1.11)
Carvacrol	0	19.03^{ax} (0.88)	441.93^{ay} (44.80)	2.49^{ax} (0.97)
	1	17.83^{axy} (0.89)	356.61^{ax} (20.85)	1.81^{axy} (0.62)
	3	19.02^{ayz} (0.80)	400.86^{ax} (62.73)	2.15^{ax} (0.74)
Rosemary oleoresin	0	18.92^{ax} (0.55)	415.31^{axy} (56.03)	2.76^{ax} (1.20)
	1	18.43^{axy} (0.90)	348.31^{ax} (82.09)	3.02^{axy} (0.73)
	3	17.66^{ax} (0.40)	371.95^{ax} (48.21)	2.78^{ax} (1.00)

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

properties of films by deceasing intermolecular attractions between adjacent polymeric chains and increase film flexibility (Sothornvit and Krochta, 2001).

4.4.5 Film Barrier and Color Properties

Active compounds incorporation into films reduced ($p \leq 0.05$) the films water vapor permeability by 28.3-32.1%, but did not affect oxygen permeability (Table 4.5). This 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 ($p \leq 0.05$) water vapor permeability of control film (no compounds added) by 22.3% (Table 4.5). 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 agreement with the results on mechanical properties.

Irradiation treatment (dose) did not affect the color properties of the films (Table 4.6).

4.5 Conclusions

The antimicrobial activity of polyamide-coated LDPE films containing active compounds was clearly demonstrated against two commonly used surrogates. Antimicrobial films increased the radiation sensitivity of the tested microorganisms. Electron beam irradiation up to 3 kGy causes small or negligible changes on film functionality. Film flexibility and moisture barrier capability were improved by adding

Table 4.5—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 (g mil/m^2 day atm)	Oxygen permeability ($\times 10^3$cc mil/m^2 day atm)
Control film[1]	0	5.55^{ay} (0.22)[2]	11.39^{ax} (0.62)
	1	4.31^{bx} (0.45)	11.16^{ax} (0.44)
	3	4.51^{by} (0.25)	11.53^{axy} (0.62)
Sorbic acid	0	3.97^{ax} (0.23)	11.84^{ax} (0.55)
	1	3.96^{ax} (0.15)	11.31^{ax} (0.73)
	3	3.78^{ax} (0.11)	10.69^{ax} (0.49)
trans-Cinnamaldehyde	0	3.85^{ax} (0.12)	11.18^{ax} (0.44)
	1	3.92^{ax} (0.17)	11.22^{ax} (0.56)
	3	3.83^{ax} (0.15)	11.18^{axy} (0.48)
Thymol	0	3.83^{ax} (0.16)	11.46^{ax} (0.66)
	1	3.98^{ax} (0.11)	11.20^{ax} (0.67)
	3	3.81^{ax} (0.11)	11.05^{axy} (0.51)
Carvacrol	0	3.77^{ax} (0.09)	11.07^{ax} (0.86)
	1	3.96^{ax} (0.11)	11.77^{ax} (1.02)
	3	3.91^{ax} (0.15)	12.29^{ay} (0.94)
Rosemary oleoresin	0	3.98^{ax} (0.23)	11.91^{ax} (1.76)
	1	4.05^{ax} (0.12)	11.41^{ax} (0.73)
	3	4.43^{by} (0.21)	11.32^{axy} (0.54)

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

Table 4.6—Effect of electron beam irradiation treatment and antimicrobial coating on color characteristics of LDPE films

Film treatment	Irradiation dose (kGy)	*L* (lightness)	Δ*E*	Chroma
Control film[1]	0	8.50^{ax} (0.16)[2]	0.00^{aw}	4.15^{ax} (0.04)
	1	9.19^{axy} (0.94)	0.46^{bw} (0.10)	3.98^{ax} (0.24)
	3	8.36^{ax} (0.26)	0.29^{abw} (0.02)	4.11^{ax} (0.09)
Sorbic acid	0	15.90^{ay} (5.65)	2.40^{ax} (1.44)	3.35^{ax} (0.81)
	1	14.91^{az} (4.40)	2.38^{aw} (1.62)	3.58^{ax} (0.78)
	3	17.28^{ay} (0.20)	1.77^{awx} (0.53)	3.44^{ax} (0.86)
trans-	0	15.67^{ay} (1.67)	6.95^{ay} (0.61)	2.53^{ax} (0.43)
Cinnamaldehyde	1	13.21^{ayz} (1.93)	6.49^{ax} (1.85)	2.25^{ax} (0.36)
	3	11.55^{ax} (3.24)	6.02^{ay} (0.20)	3.24^{bx} (0.20)
Thymol	0	18.15^{ay} (2.08)	9.21^{az} (0.67)	2.68^{ax} (1.01)
	1	16.37^{az} (0.22)	8.83^{ax} (0.90)	2.43^{ax} (1.49)
	3	17.25^{ay} (0.45)	9.20^{az} (0.97)	3.11^{ax} (1.04)
Carvacrol	0	8.84^{ax} (1.05)	0.54^{aw} (0.11)	3.83^{ax} (0.10)
	1	7.86^{ax} (0.47)	0.59^{aw} (0.42)	3.82^{ax} (0.13)
	3	8.98^{ax} (1.14)	0.47^{aw} (0.24)	3.99^{ax} (0.10)
Rosemary	0	12.69^{axy} (2.69)	3.08^{ax} (0.97)	2.77^{ax} (0.96)
oleoresin	1	11.59^{axyz} (1.38)	2.87^{aw} (1.74)	2.91^{ax} (0.83)
	3	10.62^{ax} (1.26)	3.21^{ax} (1.66)	2.89^{ax} (0.86)

[1] Control means films not containing active compounds.
[2]Numbers in parenthesis are the standard deviation.
[a,b]Means within a same film treatment and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$).
[w-z]Means at same irradiation dose and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$).
Tests were conducted at room temperature (21^{o}C).

active compounds. Self-sterile active packaging materials combined with low dose (up to 3kGy) irradiation may be able to play an important role in ensuring the safety of food products in the near future. Further research is needed to assess the effectiveness of an antimicrobial packaging system on various types of foods such as fresh fruits and vegetables.

CHAPTER V

THE INFLUENCE OF ELECTRON BEAM IRRADIATION ON THE EFFECTIVENESS OF TRANS-CINNAMALDEHYDE-COATED LDPE/POLYAMIDE FILMS*

5.1 Overview

We have evaluated the effect of electron beam irradiation (up to 20 kGy) on the functional and barrier properties of trans-cinnamaldehyde coated low-density polyethylene (LDPE)/polyamide films. Irradiation did not affect the tensile strength and toughness of the films, but the 20 kGy treatment increased the % elongation-at-break significantly. The barrier properties were also enhanced (~18.8%) when the films were exposed to 20 kGy. Addition of trans-cinnamaldehyde with 3% of polyamide coating solution (w/w) did not affect the tensile strength and barrier properties of the films, but significantly decreased the % elongation-at-break and toughness. Films with 3% and 10% coating were used to wrap fresh-cut romaine lettuce samples to determine their antimicrobial activity. Total aerobic microbial counts and yeast and mold growth were determined as a function of dose (0, 0.5, and 1.0 kGy) for 14 days of storage at 4°C. Irradiation reduced the total aerobic plate counts (APC) and yeast and mold counts

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(YMC) as dose increased. The 0.5- and 1.0-kGy treatments decreased initial APCs by 1.2- and 1.5-logs, and no YMCs were observed in the 1.0-kGy treated samples at day 0. Irradiation exposure significantly lowered APCs of lettuce samples by almost 1-log CFU/g compared to the non-irradiated controls, though only slightly reduced YMCs. The effectiveness of using irradiation with antimicrobial films was enhanced with increased radiation dose and trans-cinnamaldehyde concentration (3 to 10%).

5.2 Introduction

Irradiation is an effective non-thermal food processing technique to reduce pathogenic and spoilage microorganisms, and it is becoming a well-established decontamination technology (Morehouse 1998). Irradiation can prevent subsequent microbial reinfection and insect exposure when it is applied to prepackaged foods (Riganakos and others 1999).

Various studies have shown that ionizing radiation could improve the microbial safety or quality attributes in prepackaged fresh fruits and vegetables (Langerak 1978; Hagenmaier and Baker 1997; Prakash and others 2000; Fan and others 2003; Han and others 2004). The efficacy of irradiation is not only limited to the surface, but it can penetrate the product and eliminate microorganisms that are present in crevices and creases (Prakash and others 2000). Thus, irradiation should be an effective preservative treatment for leafy vegetables like lettuce, which have complex and non-uniform shapes where other chemical or physical treatments cannot be easily applied. However, irradiation can induce changes in texture or color in fresh produces (Somogyi and

Romani 1964; Han and others 2004). Plant tissues can be softened with increasing doses of irradiation over critical thresholds. Han and others (2004) showed that electron beam irradiation (1.0, 1.5 and 3.2 kGy) of packaged fresh romaine lettuce hearts induced a 49% decrease in the firmness of leaves and 29% for the ribs, but the color was not affected when compared to non-irradiated samples.

After radiation treatment, polymeric materials used for food packaging can undergo changes in mechanical and functional properties depending on the polymer types and absorbed radiation dose (Pentimalli and others 2000). Most of the polymers listed in the US CFR 21 part 179.45 'Packaging Materials for Use During the Irradiation of Pre-packaged Foods' (CFR 2003d) were approved through petitions by the Atomic Energy Commission (up to10 kGy) and the US Army (up to 60 kGy) in the 1960s (Payne and others 1965; Killoran 1967; Sadler and others 2001).

The action of ionizing radiation on polymers results in the following fundamental processes: crosslinking of the molecular chains, degradation of macromolecules, changes in the number and nature of the double bonds. The tendency and range of changes in polymer properties is caused by whether crosslinking or degradation of the polymer dominates during the radiation process (Wood and Pikaev 1994). These processes can take place simultaneously, controlled by the chemical nature of the polymer (Clegg and Collyer 1991). A polymer with the structure of $(-CH_2-CR_2-)_n$ will crosslink when at least one hydrogen exists at α-position (R = H). For example, crosslinking will occur in polyethylene (PE), polypropylene (PP), polyamide (PA) or polystyrene (PS). On the other hand, a polymer will degrade by irradiation if it has no hydrogen at α-position ($R \neq$

H). For example, degradation will occur in polyisobutylene, polymethyl methacrylate or starch (Wilson 1974).

The idea of incorporating chemical preservatives or antimicrobial agents in polymeric film materials has recently been developed into the concept of active food packaging systems. There is limited benefit in direct application of antimicrobial agents onto food because the antimicrobial agents may be neutralized or diffused rapidly into the bulk of the food (Torres and others 1985; Siragusa and Dickson 1992). Antimicrobial packaging materials may contact the foodstuffs, deliver antimicrobial agents from the packaging material to the foodstuffs, and inhibit growth of microorganisms (bacteria, yeasts, molds) on the surface of the food (Vartiainen and others 2003). Consequently, incorporation of chemical preservatives or antimicrobial agents into a food packaging material (film) will give a way to enhance microbial safety (Han 2000).

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). Some essential oils always act very effectively in inhibiting microbial growth although different results are observed depending on test conditions, microorganisms and the source of the antimicrobial compound. A plant-derived trans-cinnamaldehyde (trans-3-phenyl-2-propenal), a GRAS for food use based on 21 CFR part 182.60 (CFR 2003b), occurs naturally in a wide range in essential oils of cinnamon, cassia, hyacinth, myrrh and Bulgarian rose (Furia and Bellanca 1975). It is a yellowish oily liquid with a cinnamon odor and a sweet taste. Its antimicrobial activity and stability against radiation exposure were shown in our previous study (Han and others 2005).

Various studies have also investigated its antimicrobial activity (Ouattara and others 2001; Weissinger and others 2001; Kwon and others 2003; Borsa and others 2004).

In general, natural antimicrobial compounds can be used advantageously in combination with other preservation systems in order to reduce extreme use of single treatment, which may cause adverse effects on the quality attributes of the food (Ouattara and others 2003). According to the food preservative hurdle concept, active films containing antimicrobial agents could be combined with irradiation treatment to increase the radiation sensitivity of the microorganisms thus enhancing the effectiveness of ionizing radiation as a decontamination technology (Ouattara and others 2001; Borsa and others 2004; Lacroix and others 2004).

The objectives of this study were to: (1) determine the effects of ionizing electron beam radiation (0-20 kGy) on the functional properties of antimicrobial films for food packaging applications, and (2) assess the effectiveness of using irradiated antimicrobial films (up to 1 kGy) for packaging of fresh produce such as fresh-cut romaine lettuce.

5.3 Materials and Methods

5.3.1 Film Surface Preparation

Low-density polyethylene (LDPE) film (49.50 ± 1.83 μm thickness, Plastic Supplies Co., Fullerton, Calif., U.S.A.) was used as the carrier surface for the antimicrobial agent. LDPE is commonly used in packaging applications involving direct contact with food. It is a thermoplastic polymer and chemically inert relatively, although some softening and swelling may occur due to absorption of the food components.

Polyamide was used as a binder medium for incorporating antimicrobial agents on the surface of the film because most fresh produce including romaine lettuce have high water content. Polyamide is approved for use in indirect food additives, such as adhesives and components of coating (CFR 2003c). An and others (2000) compared the solubility of several different coating binder media, and reported that coating with polyamide solution gave the LDPE film the highest stability in water.

The coating solution was prepared according to the procedure developed by Ha and others (2001). Polyamide resin (Cognis Corporation, New Milford, Conn., U.S.A.) was dissolved in absolute alcohol with a ratio of 4:6 (w/w), and mixed using a magnetic stirrer at medium speed for 12 hr. The antimicrobial agent, trans-cinnamaldehyde (Aldrich, Milwaukee, Wis., U.S.A.), was added to the prepared polyamide solution with 1, 2, 3, 5 or 10% of the final solution weight, and mixed thoroughly for 2 min using a vortex. The solution was degassed by applying vacuum to remove dissolved air. The solution was then applied manually on one side of the LDPE film using a No.12 coating rod (RD Specialties Inc., Webster, N.Y., U.S.A.), and dried at room temperature (21°C) for 12 hr. Control films consisted of only polyamide coating with no trans-cinnamaldehyde. Coating thickness ($\approx 3.03 \pm 0.10$ μm) was measured using a comparator XL-750 (Brunswick Instrument, Niles, Ill., U.S.A.). Films were stored in a desiccator at room temperature until further testing or analysis.

5.3.2 Irradiation of Coated Films

Irradiation tests were carried out using a 2-MeV Van de Graaff electron accelerator (High Voltage Engineering Corporation, Cambridge, Mass., U.S.A.) located in the Food Safety Engineering Lab at the Department of Biological and Agricultural Engineering, Texas A&M University. The dose rate was 0.005 kGy/s. Films were irradiated with doses ranging from 1 to 20 kGy. A dose of 1.0 kGy involves the absorption of 1.0 kJ of energy by each kilogram of matter through which the radiation passes. Dose was measured by placing radiochromic film dosimeters (GEX Corporation, Centennial, Colo., U.S.A.) at the surface of the film. Tests were carried out at room temperature (21°C).

5.3.3 Coated Film Properties

The mechanical properties of the film strips (20 × 60 mm) under large deformations (tension mode) were measured in accordance with ASTM method D882-00 (ASTM 2000) using a TA-XT2 Texture Analyzer. Tensile strength, percentage elongation-at-break, and toughness were calculated from the resulting stress–strain curve. Tests were repeated four times and conducted at room temperature (21°C).

Oxygen and water vapor permeabilities of the films were measured using oxygen and water diffusion systems, MAS 500 and MAS 1000 (MAS Technologies Inc., Zumbrota, Minn., U.S.A.), according to the ASTM F1770 Standard Method (ASTM 1997). The tests were performed four times under 65% relative humidity and 25°C temperature conditions. The permeability coefficient (P, kg/s·m·Pa) was calculated as,

$$P = \frac{F_e L}{p} \qquad (5.1)$$

where F_e is the film permeability flux in kg/m^2·s, L is barrier (film) thickness, and p is test vapor pressure (1 atm).

5.3.4 Antimicrobial Activity of Films in Broth Media

The minimum inhibitory concentration (MIC) of trans-cinnamaldehyde against *Listeria innocua* ATCC 33090 (National Center for Agricultural Utilization Research, Peoria, Ill., U.S.A.) was determined using the broth dilution method (Kim and others 1995). Trans-cinnamaldehyde was dissolved in 10 mL of sterile distilled water containing 0.1 g of Tween 20 (Sigma, St. Luis, Mo., U.S.A.). In each duplicate (50 mL test tube) having 19.6 mL of sterile tryptic soy broth (TSB), a 200 μL aliquot of bacterial suspension at 10^5 CFU/mL as well as the antimicrobial solution was added. The final concentration of the antimicrobial solution was adjusted to be 5, 10, 25, 50, 100, 250, 500, and 1000 μg/mL (in 0.0005-0.1%, w/v). Test tubes were then incubated at 37°C with agitation (200 rpm: model G25, New Brunswick, Scientific Co. Inc., Edison, N.J., U.S.A.) for 36 hr. A 1.5 mL aliquot was drawn from each test tube periodically every 3 hr during incubation for 36 hr, and turbidity at 600 nm was measured using an UV-visible spectrophotometer (Spectronic 20D+, Milton Roy Company, Rochester, N.Y., U.S.A.) to represent the cell concentrations of microorganisms in the media. The MIC

was determined as the lowest concentration of trans-cinnamaldehyde resulting in significant no growth of *L. innocua* ATCC 33090.

Next, trans-cinnamaldehyde coated films (1, 2, 3, 5 and 10% of the final coating solution weight) were cut into 30 × 50 mm rectangles using a sterile surgical knife. Three rectangles (45 cm^2 of total surface area) of film 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 the microbial culture, and then incubated at 37°C with agitation. The culture was sampled (1.5 mL) periodically every 2 hr during incubation for 36 hr to obtain microbial growth profiles. The optical density (O.D.) of each culture sample was measured at λ = 600 nm using an UV-visible spectrophotometer (Spectronic 20D+), and the lowest concentration resulting in significant no growth was established as the MIC of coated film against *L. innocua* ATCC 33090.

5.3.5 Effectiveness of Antimicrobial Films in a Food System

Packaged romaine lettuce hearts were obtained from the same brand, supplier and day of purchasing in order to ensure highly reproducible results. The produces were cut into ≈ 2 × 2 cm pieces using a sterile household chopper (Deluxe Chopper Model 106848, General Electric Company, Bentonville, Ark., U.S.A.). Twenty-five (25) gram of cut lettuce was transferred to sterile petri dishes (8.5 cm diameter, 1.5 cm height). The top surface was covered with the coated film, containing 0 (control), 3, or 10% trans-cinnamaldehyde, and treated with 0.0, 0.5, and 1.0 kGy radiation doses using a 10-MeV electron beam linear accelerator (LINAC) located at the National Center for Electron

Beam Food Research, Texas A&M University. The dose rate was 0.8 kGy/s. Irradiation experiments consisted of single beam exposure (top), and dosage was measured by placing radiochromic film dosimeters at four points on the exterior at the top and bottom of the sample (2 on each side). The blank dosimeter was used to estimate the dose absorbed by the dosimeter alone (no produce). After irradiation, samples were stored in a refrigerator (4°C) up to 14 days along with non-irradiated samples, and evaluated for color and microbial quality (aerobic plate count [APC] and yeast and mold count [YMC]).

At each storage interval (0, 4, 7, and 14 days), twenty-five (25) grams of lettuce samples were removed aseptically from the Petri dish and mixed with 225 mL of sterile 0.1% peptone water in a stomacher bag. The mixture was pummeled in a Laboratory Blender (Stomacher 400, Seward, London, U.K.) at high speed for 1 min. For APC, appropriate decimal dilutions of the homogenate were pipetted and spreaded on prepoured-dried tryptic soy agar (TSA) plates (Difco Laboratories, Detroit, Mich., U.S.A.). For YMC, same dilutions of the homogenate were pipetted and inoculated on Yeast and Mold Count Petri-film^{TM} plates (3M, St. Paul, Minn., U.S.A.). These plates were incubated at 25°C for 48 to 72 hr, and considered countable if the colonies were between 25 and 250. Results were expressed as $\log_{10}$ colony-forming unit (CFU)/g lettuce sample (Messer and others 1985). Each experiment was repeated twice.

A Labscan XE (16437) colorimeter (HunterLab, Inc., Reston, Va., U.S.A.) with the Universal Version 3.73 software (HunterLab, Inc., Reston, Va., U.S.A.) was used to assess changes in color of the lettuce using the CIELAB system. The measuring aperture

diameter was 36 mm, and D65/10° was the illuminant/viewing geometry. The color meter was calibrated using the standard white and black plates. Three readings were made on each sample from each package and the mean values were used to determine the color coordinates L^* (lightness), a^* (redness), and b^* (yellowness). To evaluate the sample color on the 3-dimensional standard color space, sample hue angle and chroma (saturation) were calculated, where hue angle = $\tan^{-1}(b^*/a^*)$ and sample chroma = $(a^{*2} + b^{*2})^{1/2}$ (McGuire 1992).

5.4 Results and Discussion

5.4.1 Film Properties

Tensile strength and toughness of the polyamide-coated LDPE films with trans-cinnamaldehyde did not change with irradiation treatment up to 20 kGy (Table 5.1). The ability of the films to elongate increased significantly ($p \leq 0.05$) when exposed to 20 kGy, making the films more flexible (i.e., less brittle).

When crosslinking is the major effect, tensile strength may increase to a maximum and then decrease, followed by a final increase at high crosslinking density when the polymer becomes brittle (Wilson 1974). In this study, radiation up to 20 kGy was not sufficient to affect tensile strength in this manner. Meanwhile, when the mechanical properties of control films and trans-cinnamaldehyde containing films were compared at the same irradiation dose levels, adding trans-cinnamaldehyde with 3% of the polyamide coating solution (w/w) did not affect the tensile strength (Table 5.1). However, this combination of ionizing radiation up to 5 kGy and coating solution

Table 5.1—Effect of irradiation treatment on the mechanical properties of LDPE/polyamide films

Film treatment	Irradiation dose (kGy)	Tensile strength (MPa)	Elongation at break (%)	Toughness (J)
Control film[1]	0	20.86^{ax} (0.54)[2]	460.38^{ax} (41.49)	4.04^{ax} (0.34)
	1	20.66^{ax} (0.11)	463.89^{ax} (26.73)	4.09^{ax} (0.27)
	3	20.53^{ax} (0.17)	454.45^{ax} (25.10)	3.93^{ax} (0.08)
	20	20.45^{ax} (0.95)	497.00^{bx} (43.86)	4.24^{ax} (0.62)
Film containing 3% Trans-cinnamaldehyde	0	20.58^{ax} (0.54)	414.97^{ay} (28.70)	3.49^{ay} (0.26)
	1	20.56^{ax} (0.56)	414.07^{ay} (20.93)	3.59^{ay} (0.21)
	3	20.30^{ax} (0.37)	433.30^{ax} (51.07)	3.52^{ax} (0.40)
	5	19.14^{b} (0.48)	435.34^{a} (56.06)	3.43^{a} (0.52)
	10	19.98^{b} (0.27)	505.97^{b} (7.92)	4.12^{ab} (0.05)
	15	20.03^{ab} (0.67)	454.00^{ab} (18.57)	3.81^{a} (0.20)
	20	20.63^{ax} (0.83)	510.84^{bx} (19.11)	4.43^{bx} (0.37)

[1] Control means films not containing trans-cinnamaldehyde
[2]Standard deviation
[a,b]Means within a same film treatment and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
[x,y]Means at same irradiation dose and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
Tests were conducted at room temperature (21^{o}C)

significantly ($p \leq 0.05$) decreased the elongation-at-break and toughness of the films, making them more brittle and easier to break under tension.

Film barrier properties improved after exposure to 3 kGy up to 20 kGy (Table 5.2). Water vapor permeability was significantly reduced in control and trans-cinnamaldehyde containing films (14.4% and 18.8%, respectively). Oxygen permeability decreased by 16.8% and 18.8% in control and trans-cinnamaldehyde containing films, respectively. These improved water vapor and oxygen barrier properties suggest that the LDPE/polyamide films undergo significant structural changes from exposure to irradiation, mainly crosslinking. During irradiation, crosslinking is the dominant reaction in polymer networks of LDPE and polyamide (Wilson 1974; Hu and others 1999), thus radiation-induced crosslinks caused the improved barrier property in our films. Although adding trans-cinnamaldehyde (3% of the polyamide coating solution, w/w) caused a slight reduction on the films permeabilities, its effect was not significant ($p > 0.05$) (Table 5.2).

5.4.2 Antimicrobial Activity

The growth of *L. innocua* ATCC 33090 was completely inhibited by trans-cinnamaldehyde at the concentration range of 250-1000 μg/mL (in 0.025-0.1%, w/v) (Fig. 5.1). Although test concentrations at 25-100 μg/mL (in 0.0025-0.01%, w/v) delayed the lag phase of the growth curve, they did not inhibit the microbial growth effectively. The MIC of trans-cinnamaldehyde against *L. innocua* ATCC 33090 was established as 250 μg/mL (in 0.025%, w/v) in this study. The MIC test of the films

Table 5.2—Effect of irradiation treatment on the water vapor and oxygen permeability of LDPE/polyamide films

Film treatment	Irradiation dose (kGy)	Water vapor permeability (g mil/m^2 day atm)	Oxygen permeability ($\times 10^3$cc mil/m^2 day atm)
Control[1]	0	5.40^{ax} (0.23)[2]	11.38^{ax} (0.82)
	1	5.17^{abx} (0.11)	10.73^{abx} (0.42)
	3	4.71^{bx} (0.17)	10.28^{bx} (0.13)
	20	4.62^{bx} (0.60)	9.47^{cx} (0.42)
Film containing 3% Trans-cinnamaldehyde	0	5.31^{ax} (0.13)	10.77^{ax} (0.68)
	1	5.02^{ax} (0.13)	10.68^{ax} (0.44)
	3	4.65^{bx} (0.51)	9.02^{bx} (1.35)
	5	4.52^{b} (0.13)	8.92^{b} (0.63)
	10	4.44^{b} (0.23)	9.25^{b} (0.72)
	15	4.42^{b} (0.04)	9.10^{b} (0.28)
	20	4.31^{bx} (0.16)	8.75^{by} (0.16)

[1] Control means films not containing trans-cinnamaldehyde
[2]Standard deviation
[a-c]Means within a same film treatment and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
[x,y]Means at same irradiation dose and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
Tests were conducted at 25^{o}C and 65% relative humidity

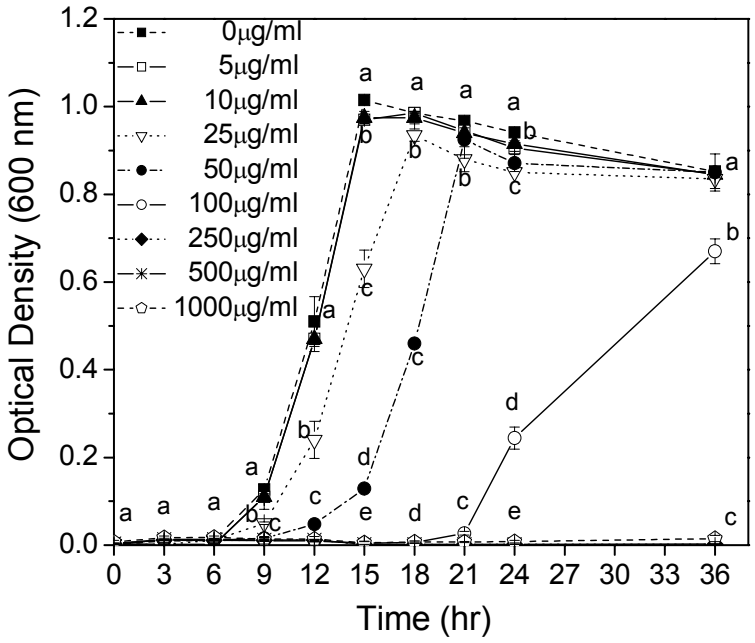

Figure 5.1—Growth of *L. innocua* ATCC 33090 in tryptic soy broth as a function of trans-cinnamaldehyde concentration. Means at a same time with the different letters are significantly different ($p \leq 0.05$)

containing trans-cinnamaldehyde against *L. innocua* ATCC 33090 was performed in same manner using the broth dilution method (Fig. 5.2). Results show that the microbial growth was suppressed as trans-cinnamaldehyde concentration in the film increased. The films containing trans-cinnamaldehyde at 3-10% significantly delayed lag phase, lowered cell growth rate, and decreased the final cell population at stationary phase. The MIC of trans-cinnamaldehyde containing film was established as 10% against *L. innocua* ATCC 33090 whose growth was effectively suppressed at this concentration level (Fig. 5.2).

5.4.3 Effect of Irradiation and Antimicrobial Films on Fresh Produce Quality

Tables 5.3 and 5.4 show the effects of applying low-dose (0.5 and 1.0 kGy) electron beam irradiation, using trans-cinnamaldehyde containing film (3 and 10% of the polyamide coating solution, w/w), and a combination of the two treatments, on the microbial populations of fresh- cut romaine lettuce stored at 4°C up to 14 days.

Right after irradiation (day 0), the initial aerobic microbial counts of the samples showed more than 1-log reduction compared to the initial aerobic microbial counts of non-irradiated samples (0 kGy) (Table 5.3). Furthermore, the 0.5- and 1.0-kGy treatments decreased initial counts of microbial populations of the samples covered with control film (0% trans-cinnamaldehyde) by 1.2- and 1.5-logs at day 0. Regardless of trans-cinnamaldehyde concentrations in films, the irradiation exposure significantly ($p \leq 0.05$) lowered APCs at each storage interval. This result clearly shows that irradiation

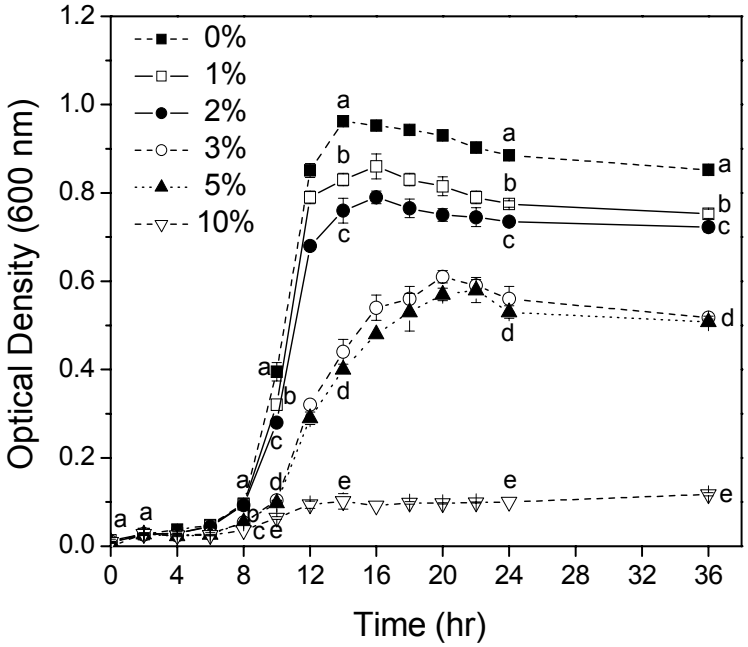

Figure 5.2—Growth of *L. innocua* ATCC 33090 in tryptic soy broth in the presence of films containing different trans-cinnamaldehyde concentrations. Means at a same time with the different letters are significantly different ($p \leq 0.05$)

Table 5.3—Change in populations of aerobic microorganisms (log_{10} CFU/g) on cut romaine lettuce exposed to irradiation and covered with trans-cinnamaldehyde (TC) coated film

Storage Interval	0 kGy + 0% TC[1]	0 kGy + 3% TC	0 kGy + 10% TC	0.5 kGy + 0% TC	0.5 kGy + 3% TC	0.5 kGy + 10% TC	1 kGy + 0% TC	1 kGy + 3% TC	1 kGy + 10% TC
Day 0	3.92^{bw} $(0.10)^{2}$	3.35^{cw} (0.14)	4.83^{aw} (0.12)	2.72^{dw} (0.09)	3.32^{cw} (0.10)	2.52^{ew} (0.10)	2.42^{fw} (0.09)	2.14^{gw} (0.12)	1.90^{hw} (0.07)
Day 4	9.53^{ax} (0.11)	8.02^{cx} (0.10)	6.92^{ex} (0.10)	8.61^{bx} (0.09)	7.00^{dx} (0.07)	6.44^{fx} (0.13)	6.79^{ex} (0.07)	6.07^{gx} (0.07)	6.03^{gx} (0.07)
Day 7	9.83^{ay} (0.11)	8.72^{by} (0.10)	8.12^{dy} (0.10)	8.63^{cx} (0.12)	7.01^{fx} (0.09)	7.01^{fy} (0.08)	7.85^{ey} (0.14)	6.63^{gy} (0.11)	6.20^{hy} (0.08)
Day 14	9.11^{az} (0.08)	9.01^{bz} (0.08)	8.12^{ey} (0.11)	8.33^{cx} (0.11)	7.41^{fy} (0.09)	7.02^{gy} (0.10)	8.21^{dz} (0.08)	7.06^{gz} (0.07)	6.21^{hy} (0.08)

[1] TC stands for trans-cinnamaldehyde
[2]Standard deviation
[a-h]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
[w-z]Means within a column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)

Table 5.4—Change in yeast and mold populations (log_{10} CFU/g) on cut romaine lettuce exposed to irradiation and covered with trans-cinnamaldehyde (TC) coated film

Storage Interval	0 kGy + 0% TC[1]	0 kGy + 3% TC	0 kGy + 10% TC	0.5 kGy + 0% TC	0.5 kGy + 3% TC	0.5 kGy + 10% TC	1 kGy + 0% TC	1 kGy + 3% TC	1 kGy + 10% TC
Day 0	1.30^{cw} $(0.07)^2$	1.82^{aw} (0.10)	1.82^{aw} (0.10)	1.00^{dw} (0.07)	1.60^{bw} (0.07)	1.00^{dw} (0.07)	0.00^{ew} (0.00)	0.00^{ew} (0.00)	0.00^{ew} (0.00)
Day 4	4.23^{ax} (0.11)	4.23^{ax} (0.11)	3.75^{bx} (0.14)	3.24^{cx} (0.13)	3.01^{cdx} (0.08)	2.11^{ex} (0.08)	2.93^{dx} (0.11)	1.65^{fx} (0.14)	1.30^{gx} (0.07)
Day 7	5.52^{ay} (0.09)	5.20^{by} (0.07)	4.91^{cy} (0.09)	5.21^{by} (0.09)	4.61^{dy} (0.08)	4.53^{dy} (0.12)	4.11^{ey} (0.08)	3.65^{fy} (0.14)	3.75^{fy} (0.14)
Day 14	5.83^{az} (0.11)	5.64^{az} (0.13)	5.60^{az} (0.07)	5.75^{az} (0.14)	5.11^{bz} (0.08)	5.02^{bcz} (0.09)	5.14^{bz} (0.12)	4.70^{dz} (0.08)	4.81^{cdz} (0.09)

[1] TC stands for trans-cinnamaldehyde
[2]Standard deviation
[a-g]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)
[w-z]Means within a column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$)

caused significant ($p \leq 0.05$) reduction of APCs depending on the dose intensity. These results were consistent throughout the 14-day storage period.

Irradiation treatment at 0.5- or 1.0-kGy did not completely inhibit the APCs, but reduced the APCs significantly ($p \leq 0.05$) by more than 0.7- and 0.9-log CFU/g at 0.5- and 1.0-kGy, respectively, compared to the non-irradiated samples (0 kGy), which were covered with control film (Table 5.3). However, aerobic microbial growth showed a fast rise during the initial storage interval (until day 4), regardless of treatment. This finding could be explained by the fact that during sample preparation, the lettuce underwent injury due to shredding and cutting, and the damaged tissues have lowered intrinsic resistance against invasion by microorganisms. Cellular leakage by sample preparation may also provide a source of nutrients that can support extensive growth of microorganisms during subsequent storage, thus causing the fresh-cut romaine lettuce to be easily affected by microbial growth.

No antimicrobial activity was observed in the samples covered by trans-cinnamaldehyde containing films at day 0, but their APCs were significantly ($p \leq 0.05$) lower than the control samples covered by the films without trans-cinnamaldehyde at each storage interval up to 14 days. After day 4, the films containing trans-cinnamaldehyde (3 or 10%) slowed down the microbial growth on the fresh-cut romaine lettuce samples throughout the 14-day storage period. This result could be due to a time-delayed release or delivery response of antimicrobial agents from the film surface to the inside air space or to the lettuce itself. In other words, the antimicrobial condition inside the package is not created instantaneously. Overall, the combination of irradiation

treatment and antimicrobial film wrapping resulted in a significant ($p \leq 0.05$) inhibition of total APCs, and the film's antimicrobial activity was enhanced with increased dose levels and trans-cinnamaldehyde concentrations.

Table 5.4 shows the effect of irradiation dose on the yeast and mold counts (YMCs) of fresh-cut romaine lettuce wrapped with the prepared films during the 14 days of storage at 4°C. Most observed colonies on the Petri-film^{TM} plates had the appearance of yeast. Generally, yeast and mold are more resistant to ionizing radiation than other gram-negative or gram-positive bacteria (Adams and Moss 1995). Yeast and mold growth was significantly ($p \leq 0.05$) inhibited by irradiation treatment, regardless of trans-cinnamaldehyde concentrations (0, 3 or 10%). Results show that the reduction of yeast and mold growth is dose-dependent. No yeast and mold growth was observed in the 1.0 kGy-treated samples at day 0. Meanwhile, the films, containing 3 or 10% trans-cinnamaldehyde, alone were not effective in reducing the YMCs, however their antimicrobial activity against yeast and mold improved significantly ($p \leq 0.05$) when the films were used in combination with irradiation treatment. The antimicrobial effect of 1.0 kGy radiation exposure was dominant, thus it might not be easy to differentiate among the different concentration levels of trans-cinnamaldehyde activity when treated with 1.0 kGy.

The fresh-cut romaine lettuce lost greenness and became darker during storage (Table 5.5). The color stability of lettuce did not improve when using the films containing trans-cinnamaldehyde (3 or 10%). However, the use of films improved the color stability when used in combination with irradiation treatment (Table 5.5). Hue

Table 5.5—Change in the color characteristics (L^*, hue angle, and chroma values) on cut romaine lettuce exposed to irradiation and covered with trans-cinnamaldehyde (TC) coated film

Color value	Storage interval	0 kGy + 0% TC[1]	0 kGy + 3% TC	0 kGy + 10% TC	0.5 kGy + 0% TC	0.5 kGy + 3% TC	0.5 kGy + 10% TC	1 kGy + 0% TC	1 kGy + 3% TC	1 kGy + 10% TC
L^*	Day 0	34.38^{ew}	33.52^{fw}	33.61^{fw}	38.18^{aw}	37.49^{bw}	35.37^{dw}	36.65^{cw}	34.90^{dw}	35.02^{dw}
		$(0.10)^2$	(0.07)	(0.17)	(0.07)	(0.13)	(0.26)	(0.67)	(0.09)	(0.13)
	Day 4	30.29^{bcx}	31.43^{abcx}	29.78^{cx}	32.55^{abx}	32.55^{abx}	31.06^{abcx}	32.68^{abx}	33.09^{ax}	32.44^{abx}
		(0.07)	(1.67)	(0.65)	(0.75)	(1.21)	(1.26)	(0.28)	(0.43)	(0.08)
	Day 7	25.68^{cz}	27.34^{by}	25.32^{dy}	28.18^{by}	27.07^{by}	30.51^{ax}	29.81^{ay}	30.17^{ay}	30.58^{ay}
		(0.53)	(0.16)	(0.69)	(0.62)	(0.26)	(0.19)	(0.95)	(0.91)	(0.43)
	Day 14	26.36^{cy}	25.49^{cdz}	26.88^{bcy}	27.01^{bcz}	25.57^{cz}	24.59^{dy}	27.16^{bcz}	31.26^{az}	28.12^{by}
		(0.12)	(0.33)	(0.51)	(0.56)	(0.55)	(0.39)	(1.24)	(1.02)	(0.26)
Hue angle	Day 0	107.95^{aw}	108.26^{ay}	108.252^{ax}	105.28^{bx}	105.32^{bx}	106.53^{abx}	105.66^{bw}	106.44^{bx}	106.72^{abx}
		$(0.37)^2$	(0.59)	(1.87)	(0.28)	(0.76)	(0.58)	(0.56)	(0.42)	(0.20)
	Day 4	209.77^{ax}	97.26^{bx}	91.69^{bw}	97.61^{bw}	101.49^{bw}	95.98^{bw}	101.03^{bw}	101.54^{bw}	99.18^{bw}
		(10.36)	(1.67)	(2.48)	(0.91)	(0.91)	(0.49)	(0.45)	(0.52)	(0.08)
	Day 7	261.31^{ax}	92.24^{bw}	264.02^{ay}	264.71^{ay}	265.92^{az}	95.13^{bw}	150.64^{bw}	266.25^{ay}	268.41^{az}
		(0.35)	(0.54)	(0.71)	(1.30)	(0.41)	(0.89)	(10.32)	(1.47)	(0.75)
	Day 14	268.02^{ax}	256.37^{cz}	261.61^{by}	265.17^{aby}	264.19^{aby}	253.37^{dy}	266.37^{ax}	267.42^{ay}	267.38^{ay}
		(1.82)	(1.01)	(1.59)	(2.77)	(0.58)	(2.88)	(1.48)	(0.83)	(0.41)
Chroma	Day 0	16.05^{bw}	15.02^{dw}	15.65^{cw}	16.86^{aw}	16.77^{aw}	15.81^{bcw}	16.14^{bw}	15.59^{cw}	15.57^{cw}
		$(0.14)^2$	(0.19)	(0.37)	(0.15)	(0.06)	(0.04)	(0.13)	(0.05)	(0.18)
	Day 4	12.43^{cx}	12.79^{bcx}	13.49^{abx}	13.48^{abx}	14.20^{ax}	12.88^{bcx}	13.77^{ax}	14.14^{ax}	13.73^{ax}
		(0.11)	(0.87)	(0.26)	(0.17)	(0.28)	(0.14)	(0.08)	(0.10)	(0.05)
	Day 7	10.88^{cy}	10.98^{cy}	11.93^{by}	11.79^{by}	11.28^{cy}	12.68^{ay}	12.65^{ay}	12.77^{ay}	12.95^{ay}
		(0.15)	(0.22)	(0.29)	(0.05)	(0.02)	(0.07)	(0.43)	(0.40)	(0.38)
	Day 14	11.04^{bcy}	10.81^{cy}	11.24^{bcz}	11.08^{bcz}	11.03^{bcy}	10.07^{dz}	11.13^{bcz}	13.16^{ay}	11.58^{bz}
		(0.23)	(0.21)	(0.20)	(0.08)	(0.25)	(0.13)	(0.24)	(0.43)	(0.04)

[1] TC stands for trans-cinnamaldehyde. [2]Standard deviation

[a-f]Means within a row, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$). [w-z]Means within a same color value and column, which are not followed by a common superscript letter, are significantly different ($p \leq 0.05$).

angle describes the quality of the color value. After exposed to irradiation at 0.5- or 1.0-kGy, the lettuce samples had significantly ($p \leq 0.05$) lower values of hue angle compared to the non-irradiated controls (Table 5.5). This result suggests that irradiation treatment may induce a color change from the green to yellow spectrum. No difference was observed between the irradiated and non-irradiated samples by the end of the storage period. All samples had significantly ($p \leq 0.05$) larger hue angle values after 14 storage days, possibly caused by increased reddish color in the lettuce. Chroma indicates the saturation or purity of the color. The chroma values of all samples decreased significantly ($p \leq 0.05$) throughout the 14 day storage regardless of irradiation dose level. The decrease in b^* value was fast and dominant during storage, in spite of the increased a^* value, thus decreasing chroma values. Irradiation treatment increased chroma values until day 7, but no differences were observed after 14 days.

5.5 Conclusions

This study demonstrates that electron beam irradiation (up to 20 kGy) causes small or negligible changes on the properties of polyamide-coated LDPE films.Water and oxygen barrier properties of the films improved with increasing radiation dose. Meanwhile, some mechanical characteristics of the films were affected when adding the antimicrobial agent, trans-cinnamaldehyde. However, this agent barely changed the film barrier properties.

This study shows the synergistic efficacy of low-dose irradiation (up to 1.0 kGy) and active films against aerobic microorganisms including mold and yeast growth in

fresh produce. The proposed methodology could prolong the shelf-life of ready-to-eat fresh produces, and furthermore, it may play an important role in ensuring the safety issues by controlling the postprocessing contamination in pre-packaged foods. Work on the effect of radiation treatment (up to 20 kGy) on the release rate of trans-cinnamaldehyde from the film into foodstuff is in progress.

CHAPTER VI

EFFECTS OF ELECTRON BEAM IRRADIATION ON RELEASE OF ANTIMICROBIAL AGENT FROM POLYAMIDE-COATED LDPE FILMS INTO FOOD SIMULANT SOLUTION

6.1 Overview

We investigated whether electron beam irradiation treatments up to 20 kGy affected the release kinetics of an antimicrobial agent from the packaging material into a model food system. As an antimicrobial agent, trans-cinnamaldehyde was incorporated into the polyamide-coated low-density polyethylene (LDPE) film. Irradiated LDPE/polyamide films exhibited up to 69% slower release rate of antimicrobial agent than non-irradiated control film. Release rate of trans-cinnamaldehyde was affected by storage temperature (4ºC to 35ºC) and pH (4 to 10) value of food simulant solutions (10% aqueous ethanol) as well as irradiation dose. Antimicrobial release rate decreased by 62.6% at refrigerated temperature (4ºC) compared to 21 and 35ºC conditions. The antimicrobial release rate in an acidic simulant solution (pH 4) was higher than in neutral (pH 7) and alkaline (pH 10) solutions by 101% and 345%, respectively. Trans-cinnamaldehyde was highly unstable to ionizing radiation in aqueous solution with a loss over 90% after exposure to 2 kGy. Fourier Transform Infrared (FTIR) analysis showed that exposure to ionizing radiation did not affect the structural conformation of the films and the trans-cinnamaldehyde up to 10 kGy, but it induced changes in the functional group of trans-cinnamaldehyde at dose as high as 20 kGy. Thus, release tests were

conducted with radiation resistant naphthalene to understand the effect of ionizing radiation on the release from the film into food simulant solution.

6.2 Introduction

Active food packaging is defined as a system that changes the condition of the packaging by interacting between the packaging material, the product, and the internal atmosphere in a positive way to extend shelf-life or improve safety or sensorial properties, which cannot be obtained otherwise (Vermeiren and others 1999). Active packaging confers additional functions by the interaction of the packaging material with the food to improve food quality, safety and convenience (Brody and others 2001). This concept involves some physical, chemical, or biological action for altering the relationships between the package, the product, and the package headspace to achieve certain desired outcome (Rooney 1995).

The concept of controlled release technology was introduced and developed in the drug industry, and has been applied and spreaded to other areas, such as the agrochemicals, fertilizers, veterinary drugs and food industries (Pothakamury and Barbosa-Cánovas 1995). Controlled release is a novel technology that can be used to increase the effectiveness of many ingredients, and it can be defined as a method by which one or more active agents or ingredients are made available at a desired site and time at a specific rate. Controlled release food packaging is therefore a type of active packaging, which contains active compounds attached on or embedded inside the food packaging materials, and the active compounds can be released at desirable and

controllable rates suitable for enhancing food safety and quality attributes during extended storage (LaCoste and others 2005). The main purpose of controlled release from the packaging is to compensate for the consumption or degradation of active compounds in food, thus maintaining adequate level of active compounds inside the package for a period of time.

There has been great interest in developing antimicrobial packaging materials, which slowly release antimicrobial agents to the surface of food and inhibit microbial contamination on food surface during storage. Food spoilage usually starts on food surfaces because of the presence and growth of spoilage or pathogenic microorganisms (Kim and others 2002). Thus, antimicrobial agents are often directly applied to foods using dipping, dusting or spraying to prevent surface contamination of food (Hotchkiss 1995). Direct application of antimicrobial agents on food surfaces has some limitations because the antimicrobial agents can be neutralized, evaporated, or diffused inadequately into the bulk of food (Torres and others 1985; Siragusa and Dickson 1992). In many cases, the antimicrobial agents are slowly released into the food surface and therefore remain at high concentration for extended period of time (Han 2000; Ouattara and others 2000). The gradual release of the antimicrobial agent from the packaging film to the food surface may have an advantage over direct application of antimicrobial agents like dipping or spraying. In these cases, antimicrobial activity may be rapidly lost due to inactivation of the antimicrobial agents by food components or dilution below active concentration due to migration into the bulk food matrix (Appendini and Hotchkiss

2002). Thus, slow and gradual release of antimicrobial agents is desirable to properly control microbial contamination in the food.

Recent food-borne microbial outbreaks result in a demand for innovative ways to prevent microbial growth in the foods while maintaining quality and safety (Appendini and Hotchkiss 2002). Films containing antimicrobial agents have been studied for use as a preservative method for improving food safety (Krochta and De Mulder-Johnston 1997). This is based on the principle that active compounds can be effectively released from the packaging material to the foodstuffs. The antimicrobial agent migrates to the surface of the packaging material and is then released to the food to inhibit microbial growth (Han 2000). The release rates and the amount of antimicrobial agent from the packaging material to food are very important to enhance microbial safety. The release of an antimicrobial agent over a period of time would allow for long-term effectiveness rather than an initial release, which could reduce the initial microbial load but have no effect on growth that could occur due to temperature abuse or extended shelf-life storage (Grower and others 2004).

Irradiation is often applied to prepackaged foods to prevent subsequent microbial reinfection and insect exposure, thus it can improve the microbial safety or quality attributes in prepackaged foods (Riganakos and others 1999). However, irradiation may induce undesirable changes in quality, such as softening, browning, and loss of nutritional factors (Somogyi and Romani 1964; Castell-Perez and others 2004; Han and others 2004; Moreno and others 2006). Thus, irradiation treatment is often combined

with other food preservative techniques such as antimicrobial packaging to lower required radiation dose.

One of the characteristic properties of irradiation is to cause ionization of the medium in which it is absorbed. Thus, the term 'ionizing radiation' is sometimes used (Swallow 1960). The chemical changes induced in food by ionizing radiation can be the result of direct or indirect action. In direct action, a sensitive target such as the DNA of a living organism is damaged directly by an ionizing particle or ray. In indirect action, on the other hand, changes to food are caused mostly by the products of water radiolysis that are transient in nature and disappear by reacting with each other or other food components (Simic 1983). The radiolysis of water is particularly important in food irradiation because water is a significant or major component in almost foods. When water is irradiated, a number of highly reactive entities like free radicals are formed (Stewart 2001). Therefore, when antimicrobial agents are combined with exposure to ionizing radiation as a mean to increase the radiation sensitivity of the microorganisms, the radiation sensitivity/stability of the antimicrobial agents should be considered not to lose their antimicrobial functionality.

The objectives of this study were (1) to determine the effects of electron beam irradiation (up to 20 kGy) on the radiation stability of the antimicrobial agent trans-cinnamaldehyde, (2) to investigate the effect of irradiation on the surface of trans-cinnamaldehyde incorporated low-density polyethylene (LDPE)/polyamide film, and (3) to evaluate temperature, pH, and irradiation dose, as controlling factors for release of the antimicrobial agent into model food systems.

6.3 Materials and Methods

6.3.1 Radiation Stability of Antimicrobial Agent

A plant-derived compound trans-cinnamaldehyde (trans-3-phenyl-2-propenal), a GRAS (Generally Recognized As Safe) additive for food use based on 21 CFR part 182.60 (CFR 2003b), occurs naturally in a wide range in essential oils of cinnamon, cassia, hyacinth, myrrh and Bulgarian rose (Furia and Bellanca 1975). It is a yellowish oily liquid with a cinnamon odor and a sweet taste. Various studies have investigated its antimicrobial activity (Ouattara and others 2001; Weissinger and others 2001; Kwon and others 2003; Borsa and others 2004; Han and others 2006).

A 0.25 mg of trans-cinnamaldehyde (Aldrich, Milwaukee, Wis., U.S.A.) was added to a 15 mL test tube having 10 mL of 10% aqueous ethanol mixture (v/v), and dissolved. Next, test tubes containing trans-cinnamaldehyde solution were irradiated using a 2-MeV Van de Graaff electron accelerator (High Voltage Engineering Corporation, Cambridge, Mass., U.S.A.) located in the Food Safety Engineering Lab at the Department of Biological and Agricultural Engineering, Texas A&M University. The dose rate was 0.005 kGy/s. Solutions containing trans-cinnamaldehyde were irradiated with doses ranging from 0.1 to 20 kGy at room temperature (21°C). After irradiation, the optical density (OD_{280}) of each solution was measured at $\lambda = 280$ nm using an UV-spectrophotometer (Spectronic 20D+, Milton Roy Company, Rochester, N.Y., U.S.A.) to determine the concentration of trans-cinnamaldehyde. In comparison with non-irradiated trans-cinnamaldehyde solution (control), the radiation stability of

trans-cinnamaldehyde was determined quantitatively. The tests were performed three times.

6.3.2 Preparation of Coated Film

Polyamide resin (Cognis Corporation, New Milford, Conn., U.S.A.) was dissolved in absolute alcohol with a ratio of 4:6 (w/w), and mixed using a magnetic stirrer at medium speed for 12 hr. The antimicrobial agent, trans-cinnamaldehyde, was added to the prepared polyamide solution with 10% of the final solution weight, and mixed thoroughly for 2 min using a vortex. This trans-cinnamaldehyde concentration (10%) is a minimum inhibitory concentration (MIC) against *Listeria innocua* ATCC 33090 (Han and others 2006). The coating solution was then applied manually on one side of the LDPE film using a No.12 coating rod (RD Specialties Inc., Webster, N.Y., U.S.A.), and dried at room temperature (21°C) for 12 hr in a laboratory fume hood. Control film was also prepared, which consisted of only polyamide coating with no trans-cinnamaldehyde added. Coating thickness (≈ 3.03 ± 0.10 μm) was measured using a comparator XL-750 (Brunswick Instrument, Niles, Ill., U.S.A.).

6.3.3 FTIR Analysis

The spectra of irradiated and non-irradiated trans-cinnamaldehyde coated LDPE/polyamide films were recorded by a Fourier Transform Infrared (FTIR) spectrometry (Nicolet Avatar 36 FT-IR, Madison, Wis., U.S.A.) at room temperature (21°C) at the Polymer Technology Center, Texas A&M University. The films were

placed directly onto the ZnSe ATR cell. The transmission infrared spectra of all film samples exhibited broad peaks in a range from 400 to 4000 cm^{-1}. For each spectrum, 64 consecutive scans at 2 cm^{-1} resolution were recorded in transmission mode. The spectra obtained were used to determine possible interactions caused by electron beam irradiation to the functional groups of trans-cinnamaldehyde and polyamide-coated LDPE film. The measurements were performed three times.

6.3.4 Release Test

Trans-cinnamaldehyde coated LDPE/polyamide film was cut into 47 × 65 mm strips and rolled into a cylindrical shape (coated surface inside) in 15 mm diameter and 65 mm high, and placed inside a test tube (15 mm diameter, 120 mm height) with 14 mL of 10% aqueous ethanol mixture (v/v, pH 7). This mixture simulates high water content foods like fruits and vegetables as specified by FDA (1993). Then, the test tubes containing the film sample and food simulant solution were stored at 4, 21 and 35°C for 120 hr at which the equilibrium cumulative amount of released trans-cinnamaldehyde reached. These temperatures simulate the refrigeration and ambient conditions to evaluate the effect of temperature on the release of the antimicrobial agent.

Next, the pH of 10% aqueous ethanol mixture (pH 7) was adjusted to pH 4 and pH 10 by adding 0.1 N hydrochloric acid (HCl) and 0.1 N sodium hydroxide (NaOH), respectively. The pH value of the food simulant solution was varied in order to simulate acidic or alkaline foods to determine the potential effect of food pH on the release of antimicrobial agent. Film samples were placed into a 15 mL test tube containing 14 mL

10% aqueous ethanol mixture at pH 4 or pH 10, and stored at 21°C for 120 hr at which the equilibrium cumulative amount of released trans-cinnamaldehyde reached.

During storage, the sample solution was withdrawn periodically from the test tube to determine the release of antimicrobial agent (trans-cinnamaldehyde) from the film into the food simulant solution as a function of time. The release of trans-cinnamaldehyde from the polyamide-coated LDPE film was measured using a spectrophotometer (Spectronic 20D+, Milton Roy Company, Rochester, N.Y., U.S.A.) at 280 nm. After completing the time interval sampling (120 hr), the amount of trans-cinnamaldehyde remaining in the film was extracted with 40 mL of absolute alcohol to determine the initial amount of trans-cinnamaldehyde in the coated film.

The release rate of trans-cinnamaldehyde was calculated using a first order relationship, one in which the rate of the reaction is proportional to the concentration of only one of the reacting substances (Benson 1960),

$$\frac{dC}{dt} = -kC \quad \text{and} \quad \ln\frac{C}{C_0} = -kt \qquad (6.1)$$

where C_0 is the initial concentration of antimicrobial agent in the food simulant medium (%), C is the concentration of agent in the food simulant medium at time t (%), k is the rate constant (h^{-1}), and t is time (h). The release rate of trans-cinnamaldehyde from the film into the food simulant medium was determined as a function of storage time. The tests were repeated three times.

Due to instability of trans-cinnamaldehyde at over 2 kGy, to understand the effect of high dose radiation (> 2 kGy) on the release of the antimicrobial agent from the film into the food simulant solution, radiation resistant naphthalene was added to the polyamide coating solution instead of trans-cinnamaldehyde. Naphthalene ($C_{10}H_8$) is an aromatic compound and consists of two fused benzene rings. It is known as one of the most stable compounds against irradiation (Swallow 1960; Sevast'yanov and others 1962).

A 3.5 mg of naphthalene (Aldrich, Milwaukee, Wis., U.S.A.) was added to a 15 mL test tube having 10 mL of 10% aqueous ethanol mixture (v/v), and dissolved. Next, test tubes containing naphthalene solution were irradiated using the 2-MeV Van de Graaff electron accelerator with doses from 0.1 to 20 kGy. After irradiation, the optical density (OD_{280}) of each solution was measured at $\lambda = 275$ nm using an UV-spectrophotometer (Spectronic 20D+, Milton Roy Company, Rochester, N.Y., U.S.A.) to determine the concentration of naphthalene. Non-irradiated naphthalene solution served as controls. The tests were performed three times.

Naphthalene was added to the polyamide solution (3% of the final solution weight), and mixed thoroughly for 2 min using a vortex (Han and others 2006). Then, the solution was applied onto the LDPE film using a No.12 coating rod in the same manner as for the trans-cinnamaldehyde incorporated film preparation described previously. Films were dried at room temperature (21°C) for 12 hr in a laboratory fume hood.

Naphthalene coated LDPE/polyamide film was cut and rolled into a cylindrical shape (coated surface inside) with 15 mm diameter and 65 mm height. Prepared cylindrical shaped film sample was put into a test tube (15 mm diameter, 120 mm height) with 14 mL of 10% aqueous ethanol mixture (v/v, pH 7). Next, the test tubes containing the film sample and food simulant solution were irradiated using same procedure and doses as described above to evaluate the effect of dose level on the release of the agent from the film. Irradiated and non-irradiated (control) samples were stored at room temperature (21°C) for 24 hr at which the cumulative amount of released naphthalene reached equilibrium. The release of naphthalene from the LDPE/polyamide film into the food simulant solution was determined using a spectrophotometer (Spectronic 20D+, Milton Roy Company, Rochester, N.Y., U.S.A.) at 275 nm. After completing the time interval sampling (24 hr), the amount of naphthalene remaining in the film was extracted with 40 mL of absolute alcohol to estimate the initial amount of naphthalene in the coated film. The measurements were performed three times.

The release rate of naphthalene as a function of irradiation dose was calculated from the kinetic curve based on equation (6.1).

6.3.5 Statistical Analysis

Data analysis was performed using the Statistical Analysis System (SAS) software, version 8.1 (SAS Institute, Cary, N.C., U.S.A.). 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 $p \leq 0.05$.

6.4 Results and Discussion

6.4.1 Radiation Stability of Trans-cinnamaldehyde

Trans-cinnamaldehyde concentration in the aqueous medium changed depending on absorbed radiation dose. Trans-cinnamaldehyde dissolved in 10% aqueous ethanol was not affected ($p > 0.05$) by irradiation exposure at the dose range 0.1-0.25 kGy, but it decreased significantly ($p \leq 0.05$) when irradiated above 0.5 kGy up to 20 kGy (Fig. 6.1). Trans-cinnamaldehyde concentration was reduced slightly by 3.9-15 % at the dose range 0.5-1 kGy. After exposure to 2-20 kGy, trans-cinnamaldehyde concentration was significantly ($p \leq 0.05$) reduced by 94-99%. Overall, trans-cinnamaldehyde dissolved in aqueous solution (10% ethanol, pH 7) was highly unstable against ionizing radiation, and the concentration of trans-cinnamaldehyde in aqueous solution decreased as the radiation dose increased. In addition, gaseous bubbles in solution were observed after radiation dose exceeded 2 kGy.

The loss of trans-cinnamaldehyde by exposure to over 2 kGy dose can be explained by direct and indirect reactions, based on radiation chemistry of organic compounds (Swallow 1960). First, the ionizing radiation energy may directly react with the radiosensitive bonds adjacent to carbonyl functional groups in trans-cinnamaldehyde, and change chemical structure of the active compounds, which can cause the loss of functionality. Carbonyl groups in trans-cinnamaldehyde will give carbon monoxide (CO) together with hydrogen (N_2) as main gaseous products from irradiation. While, water or aqueous solution is explicable in terms of the formation by the radiation of highly reactive free hydrogen atoms and hydroxyl radicals from the water (Swallow 1960). In

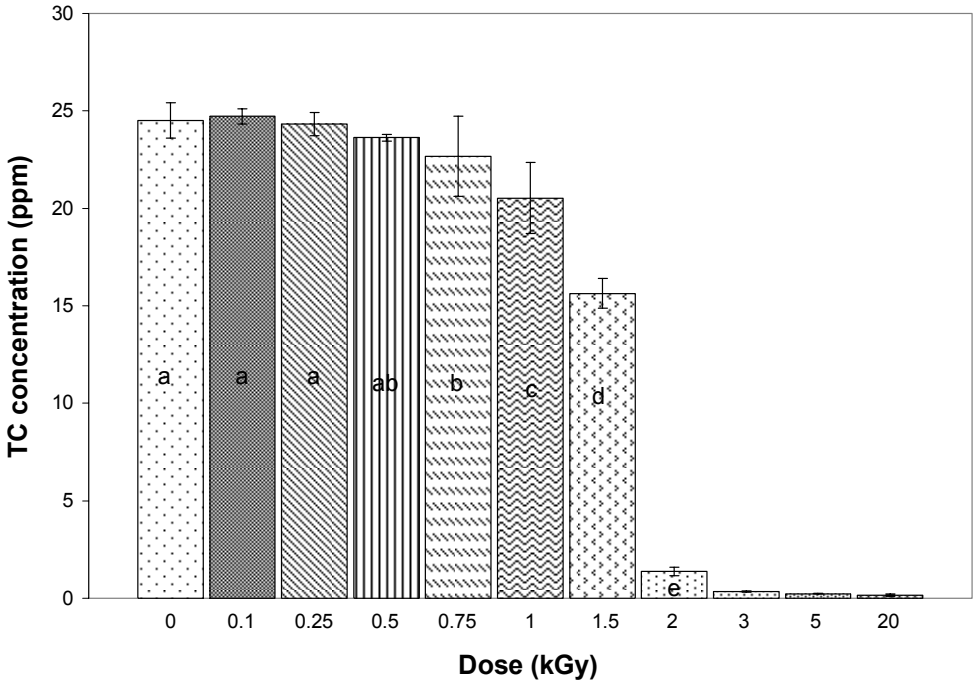

Figure 6.1—Radiation stability of trans-cinnamaldehyde (TC) in 10% aqueous ethanol solution (pH 7, 21ºC) after irradiation up to 20 kGy. Means with the different letters are significantly different ($p \leq 0.05$).

indirect action, the decomposition of trans-cinnamaldehyde may be caused by the highly reactive free radicals produced by water radiolysis, and free radicals quickly disappear by reacting with dissolved trans-cinnamaldehyde (solute) in aqueous solution.

6.4.2 FTIR Analysis

FTIR was used to determine the effect of irradiation on the structural changes of LDPE/polyamide film or incorporated trans-cinnamaldehyde. The transmission spectra of the LDPE/polyamide films incorporated with trans-cinnamaldehyde after exposure to different doses are shown in Fig. 6.2. All spectra showed similar patterns with the major peaks at 3300 cm^{-1}, 2850-2970 cm^{-1} broadened, and 1680 cm^{-1}. Absorption at these areas is from polyamide-coated LDPE film, which indicates stretching of the N-H bond in amide at 3300 cm^{-1}, the C-H bond at 2850-2970 cm^{-1}, and the C=O bond in amide at 1680 cm^{-1}. These similar patterns of spectra indicate that there was no major structural change caused by irradiation treatment up to 20 kGy in the LDPE/polyamide films. In addition, an absorption peak at 1725 cm^{-1} region is corresponding to the C=O stretching in aldehyde groups. This peak shows the presence of trans-cinnamaldehyde in the LDPE/polyamide films. After exposure to irradiation at the dose range 1-10 kGy, the peaks at 1725 cm^{-1} region were still sharp and of same height (spectra (b)-(f)). This implies that irradiation treatment up to 10 kGy did not react with trans-cinnamaldehyde incorporated into LDPE/polyamide film and did not affect carbonyl functional groups in trans-cinnamaldehyde. However, the peak at 1725 cm^{-1} almost disappeared after exposure to 20 kGy (spectrum (g)). This result may be attributed to the reaction between

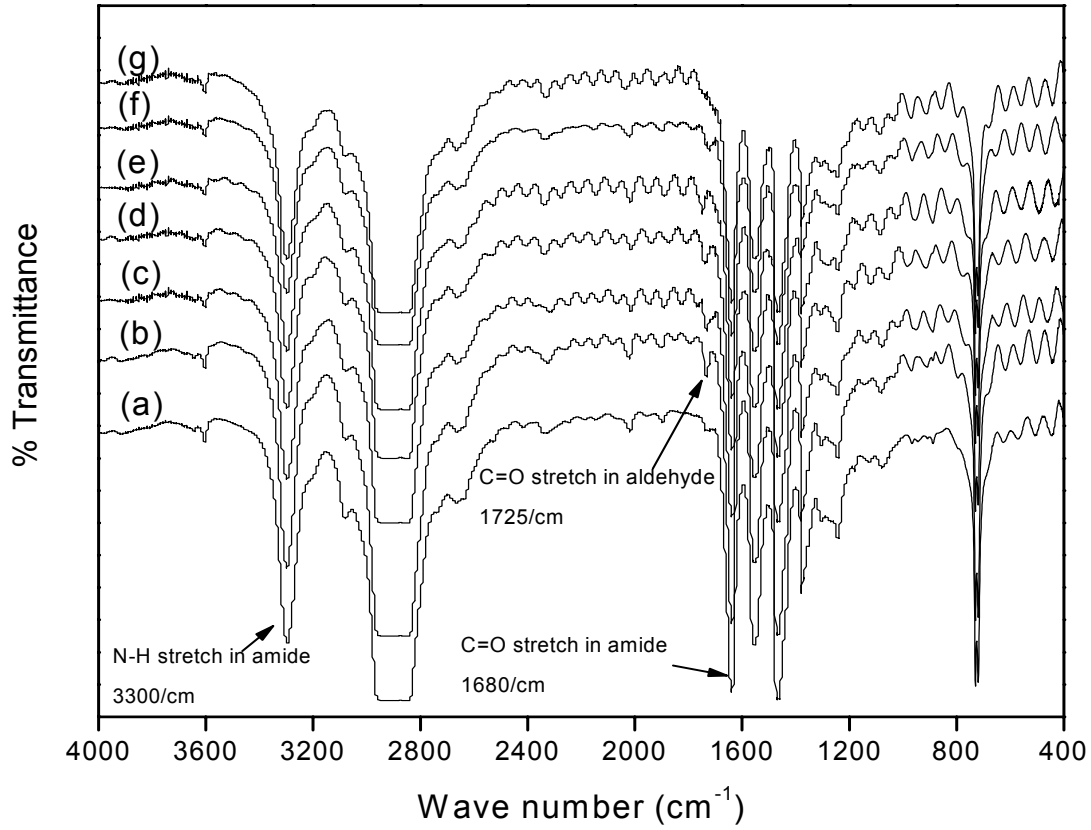

Figure 6.2—Fourier Transform Infrared (FTIR) spectra of trans-cinnamaldehyde (TC) coated LDPE/polyamide film after irradiation up to 20 kGy. (a) LDPE/polyamide; (b) LDPE/polyamide+TC, 0 kGy; (c) LDPE/polyamide+TC, 1 kGy; (d) LDPE/polyamide+TC, 3 kGy; (e) LDPE/polyamide+TC, 5 kGy; (f) LDPE/polyamide+TC, 10 kGy; (g) LDPE/polyamide+TC, 20 kGy. Tests were conducted at 21ºC.

ionizing radiation energy and carbonyl functional groups in trans-cinnamaldehyde at this dose level (20 kGy), which caused structural change in trans-cinnamaldehyde.

Trans-cinnamaldehyde was stable against ionizing radiation up to 10 kGy when incorporated in polymeric film (dry condition) (Fig. 6.2). This result suggests that trans-cinnamaldehyde incorporated film is not suitable for use in packaging of a liquid food in combination with irradiation treatment. However, it is suitable for treatment at doses between 0.1-0.25 kGy, good for most fresh food treatment. Its application to drier foods does have potential, however.

6.4.3 Release Kinetics of Trans-cinnamaldehyde

Figure 6.3 shows the cumulative amount of trans-cinnamaldehyde released from the polyamide-coated LDPE film into the food simulant medium (10% aqueous ethanol solution, pH 7) up to 120 hr. The release profiles of trans-cinnamaldehyde varied according to storage temperature. At 21°C, approximately 20% of trans-cinnamaldehyde was released from the film into the solution after 24 hr and this maximum cumulative amount (≈ 16 ppm, equilibrium value) was constant for 120 hr. The maximum cumulative amount of trans-cinnamaldehyde at 35°C was reached after 12 hr. Trans-cinnamaldehyde at 35°C was released at a significantly ($p \leq 0.05$) faster rate (18.1%) than at 21°C during the 120 hr storage period (Table 6.1). The release rate of trans-cinnamaldehyde at 4°C, a refrigerated temperature condition, was slower than at 20°C and 35°C by 55.8% and 62.6%, respectively. About 14% of trans-cinnamaldehyde was released after the 120 hr storage period at 4°C. As expected, release rate is a direct

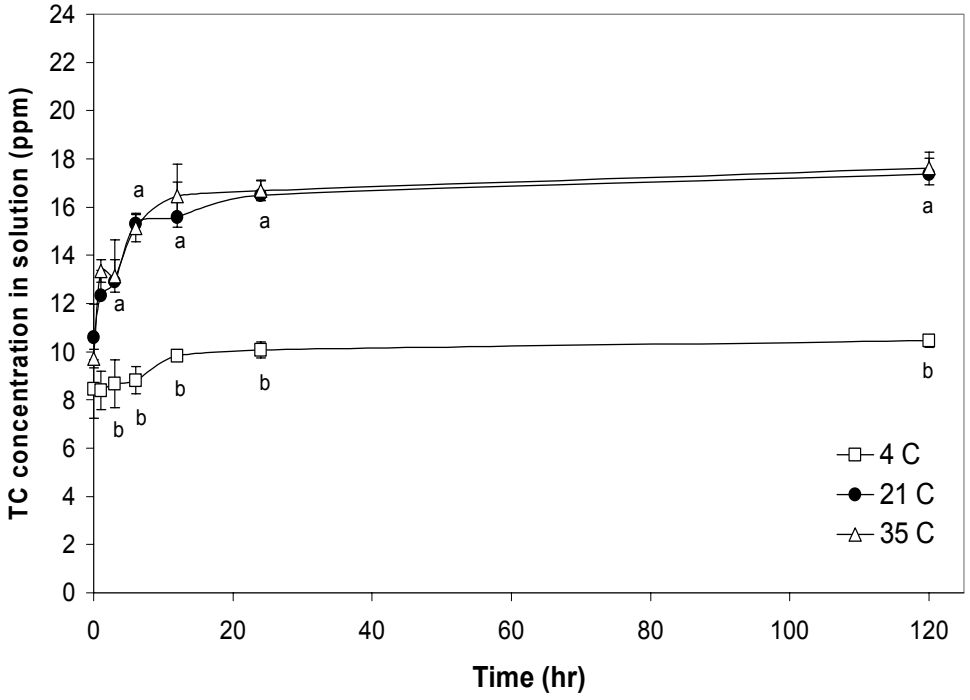

Figure 6.3—Release of trans-cinnamaldehyde (TC) from the LDPE/polyamide film into 10% aqueous ethanol solution (pH 7) as a function of storage temperature. Means at one time with different letters are significantly different ($p \leq 0.05$). Tests were conducted three times.

Table 6.1—Effect of storage temperature on the release rate of trans-cinnamaldehyde from the LDPE/polyamide film into food simulant medium (pH 7)

Storage temperature (ºC)	Release rate[1] (ppm/hr)
4	0.0129[a]
21	0.0292[ab]
35	0.0345[b]

[1] Equation (6.1).
[a,b] Means which are not followed by a common superscript letter are significantly different ($p \leq 0.05$). Values are means of three replications.

function of temperature. As temperature increased, the release rate of trans-cinnamaldehyde increased.

Release of trans-cinnamaldehyde was significantly ($p \leq 0.05$) affected by the pH of the contacting solution (Fig. 6.4). The cumulative amount of trans-cinnamaldehyde released into the food simulant medium (21ºC) significantly ($p \leq 0.05$) increased as the pH of the solution decreased. Release rate of trans-cinnamaldehyde at pH 4 was significantly ($p \leq 0.05$) faster than at pH 7 and pH 10 (Table 6.2). This increase can be explained by the structural change of polyamide molecules in acidic solution. Polyamide molecules degrade by hydrolysis (Yao and others 1987). Polyamide chain includes an amide group which can be hydrolyzed in water, leading to molecular chain scission. Results show a higher sensitivity of the amide bond in polyamide to acidic medium compared with neutral and alkaline media. Hydrolytic degradation of polyamide will cause release of trapped trans-cinnamaldehyde in the polyamide matrix degradation. Thus, higher degree of polyamide hydrolysis in acidic (pH 4) food simulant medium (21ºC) may result in higher release rate of trans-cinnamaldehyde than in neutral (pH 7) and alkaline (pH 10) media. This observation suggests that the pH of food should be considered when using the polyamide-based polymer materials for food packaging.

6.4.4 Effect of Dose on Release of Naphthalene from the Film

Naphthalene was very stable up to 5 kGy (Fig. 6.5). After exposure to 10 and 20 kGy, naphthalene concentration in aqueous solution was reduced by 19 and 36%, respectively.

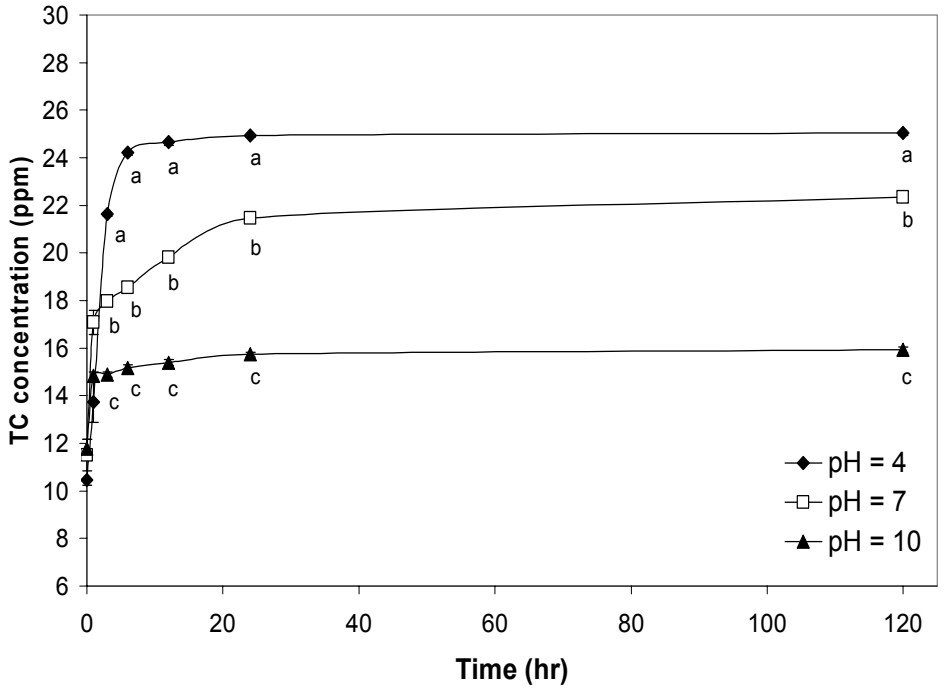

Figure 6.4—Release of trans-cinnamaldehyde (TC) from the LDPE/polyamide film into 10% aqueous ethanol solution (21ºC) as a function of pH. Means at one time with different letters are significantly different ($p \leq 0.05$). Tests were conducted three times.

Table 6.2—Effect of pH value of the food simulant medium on the release rate of trans-cinnamaldehyde from the LDPE/polyamide film (21ºC)

pH	Release rate[1] (ppm/hr)
4	0.0636[a]
7	0.0315[b]
10	0.0143[c]

[1] Equation (6.1).
[a-c] Means which are not followed by a common superscript letter are significantly different ($p \leq 0.05$). Values are means of three replications.

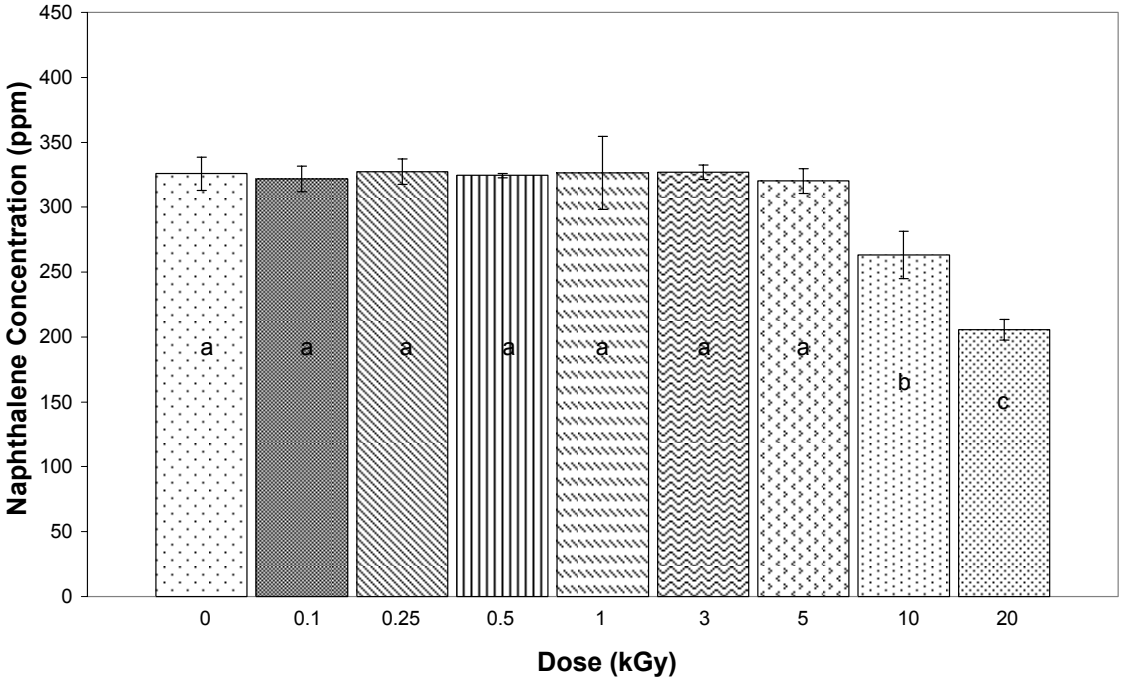

Figure 6.5—Radiation stability of naphthalene in 10% aqueous ethanol solution (pH 7, 21ºC) after irradiated up to 20 kGy. Means with the different letters are significantly different ($p \leq 0.05$). Tests were conducted three times.

The release pattern of naphthalene from the polyamide-coated LDPE film varied with irradiation dose (Fig. 6.6). Results show that irradiation treatment can function as a controlling factor for release of an active compound from the film. Table 6.3 shows the effect of different irradiation doses on release rate. The release rate was not affected ($p > 0.05$) by irradiation at 0.1 kGy, and the overall release pattern at this dose was the same as for non-irradiated control. Release rates at the irradiation doses in the range of 0.25-5 kGy declined as much as 33-69 %. Regardless of different rates of release at 0-5 kGy irradiation doses, final cumulative amounts of released compound were reached at the same ($p > 0.05$) level (≈ 1.5 ppm). This finding implies that slow and gradual controlled release of an active compound can be achieved by irradiation at these dose levels. After being released from the film into the aqueous simulant medium, the released naphthalene decomposed during the irradiation process at the dose range of 10-20 kGy, and decreased in final concentration after 24 hr.

This study provides information that supports the claim that exposure to electron beam dose (up to 5 kGy) results in slow and gradual release of the compound from the packaging film to the foodstuff.

6.5 Conclusions

Results indicate that the release kinetics of trans-cinnamaldehyde from the LDPE/polyamide film is affected by both the storage temperature and pH of contacting food simulant medium. Contact with acidic medium (pH 4) increased the release rate of trans-cinnamaldehyde from the LDPE/polyamide film by 102%, may be due to the

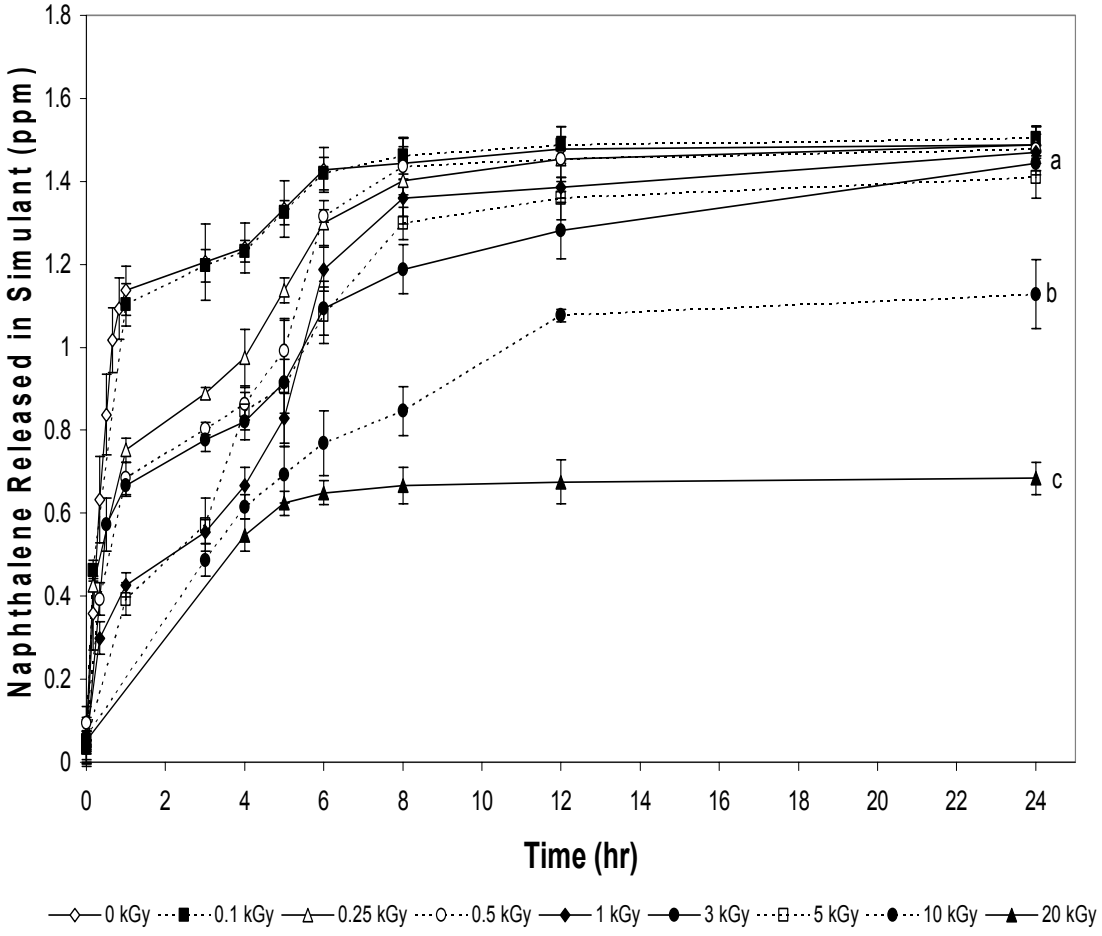

Figure 6.6—Release of naphthalene from the LDPE/polyamide film into 10% aqueous ethanol solution (pH 7, 21ºC) as a function of dose. Means at one time with different letters are significantly different ($p \leq 0.05$). Tests were conducted three times.

Table 6.3—Effect of irradiation dose on the release rate of naphthalene from the LDPE/polyamide film into food simulant medium (pH 7, 21ºC)

Irradiation dose (kGy)	Release rate[1] (ppm/hr)
0.0	0.2516[a]
0.1	0.2874[a]
0.25	0.1682[b]
0.5	0.1651[b]
1.0	0.0912[c]
3.0	0.0779[d]
5.0	0.0858[cd]
10.0	0.0790[d]
20.0	0.0639[e]

[1] Equation (6.1).
[a-e] Means which are not followed by a common superscript letter are significantly different ($p \leq 0.05$). Values are means of three replications.

increased polyamide degradation by hydrolysis. Trans-cinnamaldehyde was highly radiosensitive in aqueous medium, thus not being suitable for aqueous or liquid food packaging at dose over 2 kGy. Studies done with a radiation stable compound (naphthalene) showed that ionizing radiation induced the crosslinking in polymer networks of LDPE/polyamide film and caused slow and gradual release of the compound. Thus, irradiation served as a controlling factor for release of the active compound, and further studies on different food systems and dose levels should be done. This new understanding of irradiation effect on modes of antimicrobial release can aid in the design and development of reliable and effective active food packaging systems.

CHAPTER VII

CONCLUSIONS

1. Packaged romaine lettuce hearts can be irradiated using electron beam technology up to 1.0 kGy dose level.
2. The ribs and leaves of the lettuce samples had different responses to irradiation in terms of color and texture. The ribs were more sensitive to irradiation than the leaves due to their higher density and the potential for higher dose absorption.
3. The higher dose treatment (3.2 kGy) had a softening effect on the produce.
4. For the dose levels tested in this study (1-3.2 kGy), the sensory panelists better accepted the non-irradiated (control) samples. However, the low-dose irradiated sample (1.0 kGy) was found acceptable because the control sample had molds by the end of shelf-life (Day 21). Thus, it suggests that low dose irradiation of lettuce may have some beneficial effect on the prevention of surface molds in the product.
5. The antimicrobial activity of polyamide-coated LDPE films containing active compounds was clearly demonstrated. Antimicrobial films increased the radiation sensitivity of the tested microorganisms (*Listeria innocua* ATCC 33090 and *Escherichia coli* ATCC 884). Film flexibility and moisture barrier capability were improved when adding active compounds.
6. Electron beam irradiation (up to 20 kGy) causes small or negligible changes on the properties of polyamide-coated LDPE films. Water and oxygen barrier properties of the films improved with increasing radiation dose. Meanwhile, some mechanical

characteristics of the films were affected when adding the antimicrobial agent, trans-cinnamaldehyde. However, this agent barely changed the film barrier properties.

7. There was a synergistic efficacy of low-dose irradiation (up to 1.0 kGy) and active films against aerobic microorganisms including mold and yeast growth in fresh produce. The proposed methodology could prolong the shelf-life of ready-to-eat fresh produces, and furthermore, it may play an important role in ensuring the safety issues by controlling the postprocessing contamination in pre-packaged foods.
8. Release kinetics of trans-cinnamaldehyde from the LDPE/polyamide film varied depending on the storage temperature and pH of the contacting food simulant medium.
9. Release rate of trans-cinnamaldehyde increased with increased storage temperature and lower pH (acidic medium).
10. Trans-cinnamaldehyde was highly radiosensitive in aqueous medium, the packaging material containing trans-cinnamaldehyde in combination with irradiation treatment making it not suitable for aqueous or liquid food packaging.
11. Ionizing radiation induced the crosslinking in polymer networks of LDPE/polyamide film and caused slow and gradual release of naphthalene.
12. Irradiation served as a controlling factor for release of active compound.
13. The understanding of irradiation effect on modes of antimicrobial release can aid in the design and development of active food packaging systems.

14. Self-sterile active packaging materials combined with low dose irradiation (up to 2 kGy) will play an important role in ensuring the safety of produce by controlling the post-processing contamination in pre-packaged foods in the near future.

CHAPTER VIII

RECOMMENDATIONS FOR FURTHER STUDY

The followings are recommendations for future study.

1. Applying radiation protective treatment such as encapsulation to radiosensitive active compound to improve their radiation stability.
2. Incorporating active compound into different packaging materials such as biodegradable biopolymers which have different structural changes caused by ionizing radiation.
3. Determining the effectiveness of using antimicrobial packaging system with low-dose electron beam irradiation against food-borne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Clostridium botulinum,* etc.
4. Testing with real foods to understand the controlled release kinetics of active compound from the packaging material into various foodstuffs.

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