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# Combined effect of antimicrobial coating and gamma irradiation on shelf life extension of pre-cooked shrimp (*Penaeus* spp.)

B. Ouattara<sup>a</sup>, S.F. Sabato<sup>b</sup>, M. Lacroix<sup>a,\*</sup>

<sup>a</sup> Canadian Irradiation Center (CIC), Research Center in Microbiology and Biotechnology, INRS-Institut Armand-Frappier, 531, des Prairies Boulevard, Laval, Quebec, Canada H7V 1B7

<sup>b</sup> Departemento de Aplicaçoes Nucleares, IPEN-CNEN / SP, Travessa R. 400, 05508-900, São Paulo, SP, Brazil

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## Abstract

The present study was conducted to evaluate the combined effect of low-dose gamma irradiation and antimicrobial coating on the shelf life of pre-cooked shrimp (*Penaeus* spp.). Antimicrobial coatings were obtained by incorporating various concentrations of thyme oil and *trans*-cinnamaldehyde in coating formulations prepared from soy or whey protein isolates. Coated shrimps were stored at  $4 \pm 1^{\circ}$ C under aerobic conditions and were periodically evaluated for aerobic plate counts (APCs) and *Pseudomonas putida*. Sensory evaluations were performed for appearance, odor, and taste using a hedonic test. Results showed that gamma irradiation and coating treatments had synergistic effects ( $p \le 0.05$ ) in reducing the APCs and *P. putida* with at least a 12-day extension of shelf life. Without irradiation, the inhibitory effects of the coating solutions were closely related to the concentration of thyme oil and *trans*-cinnamaldehyde. No detrimental effects of gamma irradiation on organoleptic parameters (appearance, odor, and taste) were observed. However, incorporation of thyme oil and *trans*-cinnamaldehyde reduced the acceptability scores for taste and odor. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Antimicrobial coating; Gamma irradiation; Shrimp; Shelf life

## 1. Introduction

Shrimp is a perishable product. Its shelf life and wholesomeness during refrigerated storage and shipping is greatly influenced by both enzymatic and microbiological changes (Al-Dagal and Bazaraa, 1999; Benner et al., 1994). During the last several years, reliable methods have been developed to extend the shelf life of shrimp and to avoid health hazards for consumers (Al-Dagal and Bazaraa, 1999). Such preservation methods include cold storage in ice (Shamshad et al., 1990), modified ice storage (Harrison and Heinsz, 1989), and cook-chill processes (Venugopal, 1993). Recently, a biopreservation method involving *Bifidobacterium breve* has been successfully used to extend the shelf life of whole and peeled shrimp (Al-Dagal and Bazaraa, 1999).

The use of chemical antimicrobial compounds to preserved shrimp has been mainly focused on organic acids and their salts (Benner et al., 1994).

<sup>&</sup>lt;sup>\*</sup> Corresponding author. Tel.: +1-450-687-5010 ext. 4489; fax: +1-450-687-5792.

*E-mail address:* monique.lacroix@inrs-iaf.uquebec.ca (M. Lacroix).

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Microbial inhibition characteristics of organic acids in shrimp were found to be similar to those reported in fresh meat and other seafoods (Dickson and Anderson, 1992). Through these investigations, the mechanisms of microbial inhibition by organic acids have been elucidated and related to the undissociated forms of the acids (Freeze et al., 1973). Due to the increasing demand for natural food additives, more extensive efforts are currently being made in research for alternative antimicrobial compounds based on plant extracts. For example, essential oils and their active components have been used against many foodborne pathogenic bacteria including Escherichia coli (Eloff, 1999). Salmonella typhimurium and Staphylococcus aureus (Juven et al., 1994: Mahrour et al., 1998). Listeria monocytogenes (Aureli et al., 1992), and Vibrio (Koga et al., 1999). The inhibitory effects of essential oils against meat spoilage bacteria including Brochothrix thermosphacta, Carnobacterium piscicola, Pseudomonas fluorescens, Serratia liquefaciens, have also been investigated (Ouattara et al., 1997).

However, direct application of antimicrobial substances onto food has often been found to have limited benefits because the substances were neutralized or diffused rapidly into the bulk of the food (Siragusa and Dickson, 1992; Torres et al., 1985). Currently, a new concept is been developed in which antimicrobial compounds can be incorporated into packaging films or coatings in order to maintain high concentrations of preservatives on the surface of foods for longer storage time (Gennadios et al., 1997). Due to environmental concerns, edible coatings prepared from proteins, polysaccharides and lipids are generally used as carriers for various antimicrobials. Significant progress has already been made in this respect by fixing bacteriocins on cellulose casting (Ming et al., 1997), or by immobilizing organic acids on a calcium alginate gel (Siragusa and Dickson, 1992) to control L. monocytogenes. At the present time, little is known about the efficiency of such technology for the control of microbial growth and extension of shelf life of shrimp.

The recent approval of meat irradiation by the Food and Drug Administration (Food and Drug Administration, 1997) has made consumers more confident and attracted the interest of industries concerned with food quality and safety. It is well known that

the application of this process can virtually eliminate all pathogens from food. The recent review of Lacroix and Ouattara (2000) indicates that more than 26 countries throughout the world are using irradiation on a commercial scale. According to the microbiological hurdle concept (Leistner, 1992), active films or coating containing antimicrobial agents can be combined with low doses of gamma irradiation to obtain a synergistic inhibitory effect (Farkas, 1990; Lacroix and Ouattara, 2000). The study presented here was conducted to determine the effect of incorporation of thyme oil and *trans*-cinnamaldehyde in a protein-based coating, on the microbial growth in pre-cooked shrimp and its shelf life extension.

# 2. Material and methods

#### 2.1. Preparation of coating solutions

Soy protein isolate (SPI) containing 90% protein (moisture-free basis) was purchased from Dupont Campbell Protein Technologies (St. Louis, MO, USA). Whey protein isolate (WPI; 87% wt/wt) was produced by ultrafiltration and diafiltration at the Food Research and Development Centre (St. Hyacinthe, OC, Canada) and transported to the Canadian Irradiation Center (Laval, OC, Canada) under refrigerated conditions  $(4 + 2^{\circ}C)$ . The WPI solution was lyophilized (Model 12 Research freeze dryer, The Virtis Gardiner, New York, USA) and dried at 100°C for 3 h in a model 019 vacuum oven (Precision Scientific, Chicago, IL, USA), prior to incorporation in the film-forming solution. The total protein concentration in the lyophilized WPI and SPI powder were determined using a Leco FP-428 combustion oven apparatus (Leco, St. Joseph, MI, USA). SPI and WPI were mixed in a ratio of 1/1 (wt/wt) in distilled water containing 0.5% (wt/wt) of polyvinyl alcohol (PVA) (Sigma, St. Louis, MO, USA). The total protein concentration in the solution was 5% (wt/wt, dry weight basis). The pH of the mixture was adjusted to 8.5 with 1M Na<sub>2</sub>CO<sub>3</sub>. Glycerol and low viscosity carboxymethyl cellulose were added at the concentration of 2.5% and 0.25% (wt/wt), respectively, and the solutions sterilized by autoclaving (120°C; 15 min). Antimicrobial coating solutions were obtained by incorporating trans-cinnamaldehyde (Sigma) or thyme oil from *Thymus* saturoïdes (Robert and Fils, Montréal, QC, Canada). Three formulations of coating solution were prepared: (i) base solution containing SPI, WPI, PVA, and Glycerol, (ii) EO-0.9 containing de Base solution plus L- $\alpha$ -phosphatidylcholine (20%, wt/wt, Sigma) (0.5%), thyme oil (0.75%), and *trans*-cinnamaldehyde (0.15%), (iii) EO-1.8 containing the base solution plus L- $\alpha$ -phosphatidylcholine (0.5%), thyme oil (1.50%), and *trans*-cinnamaldehyde (0.30%).

# 2.2. Shrimp samples

Pre-cooked frozen peeled shrimp (*Penaeus* spp.) samples were purchased at a local grocery store (IGA, Laval, QC, Canada) and transported to the Canadian Irradiation Center in a thermal container. Upon arrival (within 20 min of purchase), samples were defrosted overnight at  $4 \pm 1$ °C prior to application of the coating solutions.

## 2.3. Treatment of shrimps

Shrimp samples were randomly assigned into four treatment lots consisting of one control lot (uncoated) and three lots treated with the following coating solutions: base coating and base coating + essential oils, final concentration of 0.9% (vol/wt) (EO09) or 1.8% (vol/wt) (EO18). For each coated lot, approximately 200 shrimp  $(140 \pm 5 \text{ g})$  were immersed for 5 min in 500 ml of the coating solution with gentle swirling using a sterile glass rod to ensure complete contact of the shrimp with the coating solution. Shrimp were removed and allowed to drain for 5 min on a pre-sterilized metal net under a biological containment hood. After draining of the excess coating solution, samples were placed into sterile Petri plates (8.1 cm i.d.) (approximately 15 shrimp/plate). Plates containing either uncoated or coated shrimp were divided into two groups. One group was irradiated at the Canadian Irradiation Center (Laval, OC, Canada) at a total dose of 3 kGy and at a dose rate of 31.24 kGy/h, using a <sup>60</sup>Co source UC-15A (MDS-Nordion International, Kanata, ON, Canada). Amber perspex 3042s (Atomic Energy Research Establishment, Harwell, OXF, UK) was used to validate the dose distribution throughout the samples. The irradiator was also certified by the National Institute of Standards and Technology and the (Gaithersburg, MD, USA), and the dose rate was established using a correction for decay of source. The second group served as an unirradiated control. All the plates were stored at  $4^{\circ}$ C and duplicate samples were taken at 1, 3, 6, 9, 14, and 21 days for aerobic plate count (APC) determination. Day 1 corresponded to the day of irradiation.

In a separate experiment, the effects of gamma irradiation and coating were evaluated on shrimp artificially contaminated with *P. putida* isolated from refrigerated beef at the Food Research and Development Center. Samples were prepared following the procedure described above, but shrimp were first dipped in BHI broth containing approximately  $10^5$  colony forming units (CFU)/ml of *P. putida*. The mean level of contamination obtained at Day 1 was approximately 2  $\log_{10}$  bacterial cells/g of shrimp before irradiation.

# 2.4. Microbial analysis

Each shrimp sample was weighed (ca.  $10 \pm 2$  g) and homogenized for 2 min in 90 ml of sterile peptone water (0.1%) using a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From this mixture, serial dilutions were prepared and appropriate ones were spread-plated on sterile petri plates containing Plate Count Agar (Difco Laboratories, Detroit, MI, USA) and incubated at  $35 + 1^{\circ}C$ for 24 h for the enumeration of aerobic plate counts (APCs). The enumeration of *P. putida* was done on brain infusion agar (BHA, Difco Laboratories), following the same procedure. Experiments were done in duplicate and three samples were analyzed at each sampling time. The limit of acceptability was calculated based on the onset of shrimp spoilage which was considered to be  $10^7$  to  $10^8$  bacteria/g (Avres. 1960).

# 2.5. Sensorial evaluation

The sensorial evaluation was performed only on uninoculated samples. In order to minimize variations of the organoleptic properties due to difference in microbial growth, all the treatments were evaluated after three days of storage. The sensory testing was done at the Canadian Irradiation Center (CIC). The sensory lab was equipped with individual partitioned booths and sensorial analysis were performed by 11 trained panelist (students and employees of INRS-Institut Armand-Frappier, Laval, OC, Canada), using a nine-point hedonic scale ranging from 1 (most disliked) to 9 (most liked) (Larmond, 1977). Odor and taste were evaluated under a red light to mask any difference of color. A second nine-point hedonic scale test was carried out under a normal light to evaluate the degree of acceptability based on appearance. Samples were heated at 50°C in a water bath, and presented with unsalted Premium crackers and drinking water on a polystyrene tray. Four different samples were simultaneously presented in bowls coded with a three-digit random number. The samples were presented in a randomized complete block design. Consumers were asked to eat a bite of cracker and rinse palate with water between samples to minimize any residual effect, and to evaluate the samples from left to right.

## 2.6. Statistical analysis

Data were subjected to an analysis of main effects and interaction effects of type of coating and irradiation using the ANOVA procedure of SPSS (SPSS, Chicago, IL, USA). The least square significant difference (LSD) test was used at each sampling time for point-by-point determination of the influence of coating. Differences between unirradiated and irradiated samples were determined using the Student's *t* test. Differences between means were considered significant when  $p \le 0.05$ .

# 3. Results

#### 3.1. Aerobic plate counts

Counts of bacterial population in unirradiated samples are shown in Fig. 1. In both control (uncoated) samples and samples coated with various solutions, APCs increased significantly ( $p \le 0.05$ ) during the 21 days of storage. No significant difference (p > 0.05) was found between uncoated samples and samples coated with the base solution. In contrast, when essential oils were incorporated in the

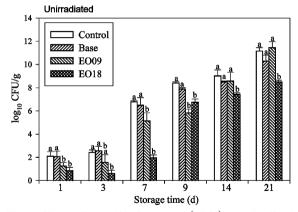


Fig. 1. Changes in aerobic plate counts (APCs) on unirradiated shrimp during storage at  $4^{\circ}$ C. EO09 and EO18: coating solutions containing 0.9% and 1.8% (v/w) of essential oils.

base solution, bacterial counts decreased significantly (p < 0.05) compared to uncoated controls. The antibacterial effectiveness of the coating solution containing 0.9% essential oils (EO09) was significantly higher than uncoated samples until day 9. but the difference tended to disappear after 9 days of storage. At day 14 and 21, no significant difference (p > 0.05) was observed between the EO09 solution and uncoated samples. Bacterial counts in samples coated with EO18 solution remained significantly  $(p \le 0.05)$  lower than bacterial counts in uncoated samples at day 21. The patterns of bacterial growth in irradiated samples were quite different from those observed in unirradiated samples (Fig. 2). The irradiation process resulted in a significant ( $p \le 0.05$ ) increase in the phase lag before the initiation of bacterial growth. For both uncoated and coated samples, no viable colony forming units were detected during the first 7 days of storage. In general, the combination of gamma irradiation with the coating resulted in a greater inhibition of bacterial growth. In irradiated samples, regardless of the type of coating, total APCs in coated samples were significantly (p  $\leq 0.05$ ) lower than uncoated control samples. Based on the onset of shrimp spoilage established at  $10^7$ bacteria/g, the shelf life of unirradiated and irradiated shrimp was estimated (Fig. 3). Data indicated that without irradiation, the limit of acceptability was reached after 7 days for uncoated, 8 days for samples coated with the base solution, and 12 days for samples coated with EO09 and EO18. With gamma

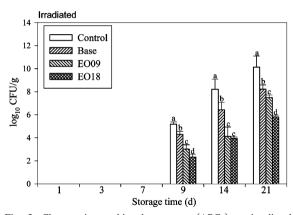


Fig. 2. Changes in aerobic plate counts (APCs) on irradiated shrimp during storage at 4°C. EO09 and EO18: coating solutions containing 0.9% and 1.8% (v/w) of essential oils.

irradiation, the shelf life was 12 days for uncoated samples, 17 days for samples coated with the base coating solution, 20 days for samples coated with EO09, and more than 21 days for samples coated with EO18.

#### 3.2. Growth of P. putida

Data related to the growth of *P. putida* in unirradiated and irradiated shrimp are illustrated in Fig. 4.

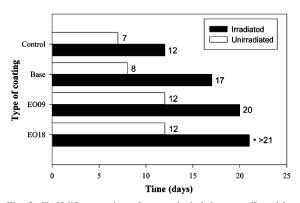


Fig. 3. Shelf life extension of pre-cooked shrimp as affected by gamma irradiation and antimicrobial coating during storage at 4°C. Results are expressed in terms of the time to reach the onset of shrimp spoilage  $(10^7 \text{ bacteria/g})$ . (\*) The onset of spoilage was not reached at the end of the storage period (21 days). EO09 and EO18: coating solutions containing 0.9% and 1.8% (v/w) of essential oils.

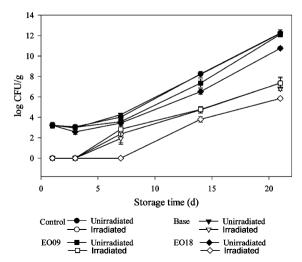


Fig. 4. Effect of gamma irradiation and antimicrobial coating on the growth of *P. putida* during storage at 4°C. EO09 and EO18: coating solutions containing 0.9% and 1.8% (v/w) of essential oils.

Bacterial growth in unirradiated shrimp increased significantly to reach maximum values of 10.76 to 12.24 CFU/g after 21 days. Although the counts of P. putida were lower on the EO09 and EO18 coated shrimp, no significant (p > 0.05) effects of coating were found during the first 7 days of storage. At the end of the experimental period (21 days), only the EO18 coated shrimp showed a significant ( $p \le 0.05$ ) reduction of the growth of *P. putida*. When shrimp were subjected to gamma irradiation, complete inhibition of P. putida occured during the first 3 days for all the samples. The initiation of bacterial growth was observed after 3 days for the control, and base and EO09, treated sample and after 7 days for the EO18 coated samples. Total APCs for both control and coated samples remained significantly lower (p < 0.05) in irradiated samples as compared to the unirradiated ones during the entire storage period (21 days). Bacterial counts in samples coated with EO18 solutions were significantly lower ( $p \le 0.05$ ) than all the other samples during the whole experimental period (21 days). No significant (p > 0.05) antibacterial effects were observed with the base and EO09 solutions.

#### 3.3. Sensory evaluation

Table 1 shows the results of variance analysis relative to sensory evaluation of shrimp. None of the

#### Table 1

Summarized results of variance analysis showing main effects and interaction effect of gamma irradiation and coating on the sensory characteristics of shrimp after 3 days of storage

	$\mathrm{DF}^{\mathrm{a}}$	$p (F_{\text{critical}} > F_{\text{calculated}})^{\text{b}}$		
		Appearance	Odor	Taste
Irradiation	1	0.851	0.099	0.489
Coating	3	0.975	0.001	0.001
Irradiation × coating	3	0.972	0.416	0.865

<sup>a</sup>DF: Degree of freedom.

<sup>b</sup>Level of significance of the F test. Probability that the critical F value is greater than or equal to the calculated value of F.

organoleptic parameters (appearance, odor and taste) was significantly affected by gamma irradiation (p > 0.05). Coating did not affect the appearance of shrimps, but reduced significantly ( $p \le 0.05$ ) acceptability for odor and taste. There was no significant combined effect of gamma irradiation and coating on the appearance, odor, or taste. Results of comparison of means for significant differences between types of coatings for unirradiated and irradiated samples are summarized in Table 2. Shrimp appearance was not significantly (p > 0.05) affected by coating. The mean values on the hedonic scale ranged from 6.40 to 6.70 for unirradiated samples, to 6.45 to 6.73 for irradiated ones. For odor and taste, no significant differences (p > 0.05) were observed between uncoated control samples and samples coated with the base solution, or with the EO09 solution (0.9%)essential oils). When essential oils were added to the base solution at a level of 1.8% (EO18), odor and taste acceptability of shrimp decreased significantly ( $p \le 0.05$ ). In unirradiated samples, acceptability values for odor decreased from 6.89 for the base solution to 6.25 for EO09 and 4.86 for EO18. For taste, values were 6.78 for the base solution, 4.56 for EO09 solution, and 4.17 for the EO18 solution. For both odor and taste, the acceptability values were significantly lower only for the coating solution containing 1.8% (vol/wt) essential oils. A similar significant decrease of acceptability values was also observed in the irradiated samples.

# 4. Discussion

The results generated from this study showed that edible active food packaging films or coatings can be developed by incorporating natural compounds with antimicrobial properties against spoilage bacteria. Protein-based coating containing trans-cinnamaldehvde and thyme oil were found to reduce bacterial growth on pre-cooked shrimp. This observation is consistent with the results obtained by several other workers using various film-forming polymers (proteins, polysaccharides, lipids) and antimicrobial compounds (organic acids, essential oils, lysosyme, nisin). For example, Padgett et al. (1998) successfully incorporated food grade nisin and lysosyme into biodegradable soy protein isolate and corn zein. The resulting films demonstrated significant antibacterial effects against Lactobacillus plantarum. Similar applications of this technology have been reported for cellulosic casing containing pediocin

Table 2

Effect of coating and gamma irradiation on the organoleptic properties of shrimp after 3 days of storage<sup>1,2</sup>

	Sensorial parameters							
	Appearance		Odor		Taste			
	Unirradiated	Irradiated	Unirradiated	Irradiated	Unirradiated	Irradiated		
Control	$6.56 \pm 2.30^{\mathrm{a}}$	$6.45 \pm 1.37^{\mathrm{a}}$	$7.20 \pm 1.93^{\mathrm{a}}$	$7.22\pm97^{a}$	$7.30 \pm 1.34^{\mathrm{a}}$	$7.70 \pm 1.49^{a}$		
Base	$6.40 \pm 2.07^{a}$	$6.55 \pm 1.57^{a}$	$6.89 \pm 1.45^{a}$	$6.55 \pm 1.75^{ab}$	$6.78 \pm 1.20^{ab}$	$6.82 \pm 1.99^{a}$		
EO09	$6.70 \pm 2.16^{a}$	$6.73 \pm 1.49^{a}$	$6.25 \pm 1.49^{ab}$	$4.50\pm1.66^{ab}$	$4.56 \pm 1.46^{ab}$	$5.00 \pm 2.24^{ab}$		
EO18	$6.40\pm2.22^{a}$	$6.64 \pm 1.57^{\text{a}}$	$4.86 \pm 1.86^{\rm b}$	$4.14 \pm 1.57^{\mathrm{b}}$	$4.17 \pm 1.67^{\mathrm{b}}$	$4.38 \pm 1.92^{\mathrm{b}}$		

EO09 and EO18: coating solutions containing 0.9% and 1.8% (v/w) of essential oils.

<sup>1</sup>Means within a column bearing the same letter are not significantly different (p > 0.05) as determined by the Least Significant Difference test.

<sup>2</sup>No significant difference (p > 0.05) was found between irradiated and unirradiated samples as determined by the Student t test.

and nisin (Ming et al., 1997), polyvinyl chloride films and agar coating containing nisin (Padgett et al., 1995), protein films containing lauric acid (Dawson et al., 1997), and chitosan-based edible films formulated with selected organic acids, fatty acids or essential oils (Ouattara et al., 2000a,b).

Immobilizing antimicrobials into film-forming solutions is a very advantageous technology for food preservation. The resulting bio-active films or coatings provide more inhibitory effects against spoilage and pathogenic bacteria by lowering the diffusion processes and maintaining high concentrations of the active molecules on the food surface (Hotchkiss, 1995: Torres et al., 1985). Siragusa and Dickson (1992) immobilized various organic acids into a calcium alginate gel and obtained a greater reduction of the growth of L. monocytogenes on lean beef muscle, than when the organic solutions were directly applied by dipping. Similarly, a chitosan-based bio-active film containing acetic acid, propionic acid, trans-cinnamaldehyde, or lauric acid has been found to produce significant extension ( $p \le 0.05$ ) of the shelf life of cooked meat products by inhibiting the growth of total Enterobacteriaceae and S. liquefaciens (Ouattara et al., 2000b). From the present study, it appears that without irradiation, the inhibitory effect of the coating applied on pre-cooked peeled shrimp was closely related to the concentration of essential oils added to the solutions. With irradiation, the inhibitory effect was greatly improved due to an additive interaction effect. The antibacterial effect found with coating solutions containing essential oils was to be expected, since preliminary studies done in our laboratories showed that thyme oil and *trans*-cinnamaldehyde possessed significant ( $p \le 0.05$ ) antibacterial effects (result not shown). Furthermore, thyme oils contain high concentrations of thymol. a well-known antimicrobial molecule (Aureli et al., 1992). Also, several previous studies identified *trans*-cinnamaldehyde and thymol as the major antibacterial constituents of cinnamon and thyme oil, respectively (Ouattara et al., 1997; Sivropoulou et al., 1996).

The mechanism by which microorganisms are inhibited by thymol or other phenolic compounds involves a sensitization of the phospholipid bilayer of the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents (Juven et al., 1994; Kim et al., 1995), or impairment of bacterial enzyme systems (Wendakoon and Sakaguchi, 1995). *Trans*-cinnamaldehyde acts by inhibiting the amino acid decarboxylase in target bacteria (Wendakoon and Sakaguchi, 1995). The greater resistance of *P. putida* to *trans*-cinnamaldehyde and thyme oil incorporated in the protein-based coating can be explained by the difference in susceptibility between gram-positive and gram-negative bacteria (Chanegriba et al., 1994). This resistance is generally attributed to the cell wall lipopolysaccharides of gram-negative bacteria, which may prevent active compounds from reaching the cytoplasmic membrane (Russel, 1991).

Our results showed a significant additive interaction effect of gamma irradiation and antimicrobial coating in reducing the growth of bacterial in precooked peeled shrimp. This effect was characterized by longer lag periods, lower growth rates, with a resulting significant shelf life extension in irradiated samples. The primary mechanism of microbial inhibition by ionising radiation is the breakage of chemical bonds within the DNA molecules, or alteration of membrane permeability and other cellular functions (Lopez-Gonzales et al., 1999; Urbain, 1986). This may facilitate the contact between antimicrobial molecules and cell membranes, and increase their inhibitory effects. Several previous reports on the combination of gamma irradiation and other treatments suggested that microorganisms which survive radiation treatment, will probably be more sensitive to environmental conditions (temperature, pH, nutrients, inhibitors, etc.) than untreated cells (Farkas, 1990; Lacroix and Ouattara, 2000). These observations are also supported by Mahrour et al. (1998) who combined marinating in natural plant extracts with gamma irradiation, and obtained a significant reduction in the irradiation dose required to control Salmonella spp. on fresh poultry. Also, incorporation of ascorbyl palmitate (200 ppm) in ground beef prior to irradiation at 1.5 kGy resulted in an additional 3-log units reduction in total aerobic and lactic acid bacteria counts (Giroux et al., 2000; Lee et al., 1999). In the current literature, no chemical interaction has been reported between protein molecules and thymol or trans-cinnamaldehyde. However, it can be hypothesized that the incorporation of active compounds into a polymeric matrix will reduce their

diffusion, and lead to a higher concentration of the compounds at the surfaces of food products for longer periods. This is consistent with published data on the diffusion, characteristics of active compounds incorporated into polymer solutions and films (Ouattara et al., 2000a; Redl et al., 1996).

A significant additive interaction effect of gamma irradiation and coating was also observed with the base solution (without essential oils). A combination of 3 kGy irradiation of shrimp and coating with the base solution, resulted in a 5-day extension of shelf life as compared to irradiation alone, and an additional 9-day extension of shelf life as compared to coating alone. Coating acts as an additional parameter by increasing microbial susceptibility through modifications of some environmental factors such as oxygen availability at the surface of products (Cuq et al., 1995).

From the present study, changes in appearance, odor and taste as affected by gamma irradiation was not detectable by the sensory evaluation panel. These results agreed with those of Giroux and Lacroix (1998) and Kanatt et al. (1998) who found that low dose irradiation can be use to extend the shelf life of food products, without any detrimental effects on biochemical and nutritional characteristics. The irradiation dose used in the present study (3 kGy) was not high enough to induce production of unacceptable odors or flavors from lipid and protein components of shrimp. The lower scores obtained in sensory evaluation tests for odor and taste can be related to the intrinsic sensory characteristics of thyme oil and *trans*-cinnamaldehyde.

## 5. Conclusion

The present study dealt with the control of bacterial growth of pre-cooked shrimp using gamma irradiation combined with edible antimicrobial coatings. This technology showed significant potential for inhibiting aerobic bacteria including *P. putida*, and, as a result, the microbial shelf life was extended by 5 days with gamma irradiation, and more than 11 days with gamma irradiation combined with a proteinbased coating containing thyme oil and *trans*-cinnamaldehyde. A synergistic effect was also observed between irradiation and coating with the base solution (without essential oils). The appearance of shrimp was not affected by the treatment as well as odor and taste for essential oil concentrations up to 0.9%. However, incorporation of 1.8% essentials oils in the coating solutions significantly ( $p \le 0.05$ ) decreased the acceptability of the products.

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# References

- Al-Dagal, M.M., Bazaraa, W.A., 1999. Extension of shelf life of whole and peeled shrimp with organic acid salts and bifidobacteria. J. Food Prot. 62, 51–56.
- Aureli, P., Costantini, A., Zolea, S., 1992. Antibacterial activity of some plant essential oils against *Listeria monocytogenes*. J. Food Prot. 55, 344–348.
- Ayres, J.C., 1960. The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry, and eggs. J. Appl. Bacteriol. 23, 471–486.
- Benner, R.A., Miget, R., Finne, G., Acuff, G.R., 1994. Lactic acid/melanosis inhibitors to improve shelf life of brown shrimp (*Penaeus aztecus*). J. Food Sci. 59, 242–250.
- Chanegriba, N., Sabaou, N., Baaliouamer, A., Meklati, B.Y., 1994. Activité antibactérienne et antifungique de l'huile essentielle. Riv. Ital. EPPOS 12, 5–12.
- Cuq, B., Gontard, N., Guilbert, S., 1995. Edible films and coatings as active layers. In: Roony, M.L. (Ed.), Active Food Packaging. Blackie Academic & Professional, Bishopbriggs, Glasgow, pp. 111–142.
- Dawson, P.L., Han, I.Y., Padgett, T.R., 1997. Effect of lauric acid on nisin activity in edible protein packaging films. Poult. Sci. 76, 74 (Suppl.).
- Dickson, J.S., Anderson, M.E., 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. J. Food Prot. 55, 133–140.
- Eloff, J.N., 1999. It is possible to use herbarium specimens to screen for antibacterial components in some plants. J. Ethnopharmacol. 67, 355–360.
- Farkas, J., 1990. Combination of irradiation with mild heat treatment. Food Control 1, 223–229.
- Food and Drug Administration, 1997. Irradiation in the production, processing and handling of food. Fed. Regist. 62, 64107– 64121.

- Freeze, E., Sheu, C.W., Galliers, E., 1973. Function of lipophilic acids as antimicrobial food additives. Nature 241, 321–325.
- Gennadios, A., Kurth, L.B., 1997. Application of edible coatings on meats, poultry and seafoods: a review. Lebensm.-Wiss. Technol. 30, 337–350.
- Giroux, M., Lacroix, M., 1998. Nutritional adequacy of irradiated meat. A review. Food Res. Int. 31, 337–350.
- Giroux, M., Ouattara, B., Yefsah, R., Smoragiewicz, W., Saucier, L., Lacroix, M., 2000. Combined effect of ascorbic acid and gamma irradiation on microbiological and sensorial characteristics of ground beef during refrigerated storage. J. Agric. Food Chem. (submitted for publication).
- Harrison, M.A., Heinsz, L.J., 1989. Shelf life extension of raw brown shrimp (*Penaeus aztecus*) with potassium sorbate in ice and dip. J. Food Qual. 12, 243–248.
- Hotchkiss, J.H., 1995. Safety consideration in active packaging. In: Roony, M.L. (Ed.), Active Food Packaging. Blackie Academic & Professional, Bishopbriggs, Glasgow, pp. 238–253.
- Juven, B.J., Kanner, J., Sched, F., Weisslowicz, H., 1994. Factors that interact with the antibacterial action of thyme oil and its active constituents. J. Appl. Bacteriol. 76, 626–631.
- Kanatt, S.R., Paul, P., D'Souza, S.F., Thomas, P., 1998. Lipid peroxidation in chicken meat during chilled storage as affected by antioxidant combined with low-dose gamma irradiation. J. Food Sci. 63, 198–200.
- Kim, J., Marshall, M.R., Wei, C., 1995. Antibacterial activity of some essential oils components against five food borne pathogens. J. Agric. Food Chem. 34, 2839–2845.
- Koga, T., Hirota, N., Takumi, K., 1999. Bactericidal activities of essential oils of basil and sage against a range of bacteria and the effect of these essential oils on *Vibrio parahaemolyticus*. Microbiol. Res. 154, 267–273.
- Lacroix, M., Ouattara, B., 2000. Combined industrial processes with irradiation to assure innocuity and preservation of food products: a review. Food Res. Int. 33, 719–724.
- Larmond, E., 1977. Laboratory Methods for Sensory Evaluation of Foods. Research Branch of Agriculture Canada publ. 1637, Ottawa, Ontario.
- Lee, J.-W., Yook, H.-S., Kim, S.-A., Lee, K.-H., Byun, M.-W., 1999. Effects of antioxidants and gamma irradiation on the shelf life of beef patties. J. Food Prot. 62.
- Leistner, L., 1992. Food preservation by combined methods. Food Res. Int. 25, 151–158.
- Lopez-Gonzales, V., Murano, P.S., Brennan, R.E., Murano, E.A., 1999. Influence of various commercial packaging conditions on survival of *Escherichia coli* O157:H7 to irradiation by electron beam versus gamma rays. J. Food Prot. 62, 10–15.
- Mahrour, A., Lacroix, M., Nketsa-Tabiri, J., Calderon, N., Gagnon, M., 1998. Antimicrobial properties of natural substances in irradiated fresh poultry. Radiat. Phys. Chem. 52, 81–84.

- Ming, X., Weber, G.H., Ayres, J.W, Sandine, W.E., 1997. Bacteriocins applied to food packaging materials to inhibit *Listeria monocytogenes* on meats. J. Food Sci. 62, 413–415.
- Ouattara, B., Simard, R.E., Holley, R.A., Piette, J.-P.G., Bégin, A., 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. 37, 155–162.
- Ouattara, B., Simard, R.E., Piette, J.-P.G., Bégin, A., Holley, R.A., 2000a. Diffusion of acetic and propionic acids from chitosan-based antimicrobial films. J. Food Sci. 65, 668–673.
- Ouattara, B., Simard, R.E., Piette, J.-P.G., Bégin, A., Holley, R.A., 2000b. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. Int. J. Food Microbiol. (In press).
- Padgett, T., Han, I.Y., Dawson, P.L., 1995. Incorporation of lysosyme into biodegradable packaging packaging films. Poult. Sci. 74, 175 (Suppl.).
- Padgett, T., Han, I.Y., Dawson, P.L., 1998. Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. J. Food Prot. 61, 1330–1335.
- Redl, A., Gontard, N., Guilbert, S., 1996. Determination of sorbic acid diffusivity in edible wheat gluten and lipid films. J. Food Sci. 61, 116–120.
- Russel, A.D., 1991. Mechanisms of bacterial resistance to non-antibiotics: food additives and food pharmaceutical preservatives. J. Appl. Bacteriol. 71, 191–201.
- Shamshad, S.I., Nisa, K., Riaz, M., Zuberi, R., Qadri, R.B., 1990. Shelf life of shrimp (*Penaeus merguiensis*) stored at different temperatures. J. Food Prot. 55, 1201–1205.
- Siragusa, G.A., Dickson, J.S., 1992. Inhibition of *Listeria mono-cytogenes* on beef tissue by application of organic acids immobilized in a calcium alginate gel. J. Food Sci. 57, 293– 296.
- Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T., Arsenakis, M., 1996. Antibacterial and cytotoxic activities of *Oreganum* essential oils. J. Agric. Food Chem. 44, 1202–1205.
- Torres, J.A., Motoki, M., Takano, M., 1985. Microbial stabilization of intermediate moisture food surfaces: I. Control of surface preservative concentration. J. Food Process. Preserv. 9, 75–92.
- Urbain, W.M., 1986. Biological effects of ionizing radiation. In: Schweigert, B.S. (Ed.), Food Irradiation. Academic Press, Orlando, FL, pp. 83–117.
- Venugopal, V., 1993. Cook-chill process to extend refrigerated shelf life of peeled and deveined shrimp and white pomfret. Int. J. Food Sci. Technol. 28, 273–278.
- Wendakoon, C.N., Sakaguchi, M., 1995. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J. Food Prot. 58, 280–283.