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Use of gamma-irradiation technology in combination with edible coating to produce shelf-stable foods

B. Ouattara[a,b], S.F. Sabato[c], M. Lacroix[a,b,*]

[a] *Canadian Irradiation Center (CIC), 535 Cartier Boulevard West, Laval, Que., Canada H7V 3S8*

[b] *INRS-Institut Armand-Frappier, Research Center in Microbiology and Biotechnology, 531, des Prairies blvd, Laval, Que., Canada H7V 1B7*

[c] *Departemento de Aplicaçoes Nucleares, IPEN-CNEN/SP, Travessa R., 400, 055508-900, Saõ Paulo, SP, Brazil*

Abstract

This research was undertaken to determine the effectiveness of low-dose gamma-irradiation combined with edible coatings to produce shelf-stable foods. Three types of commercially distributed food products were investigated: precooked shrimps, ready to cook pizzas, and fresh strawberries. Samples were coated with various formulations of protein-based solutions and irradiated at total doses between 0 and 3 kGy. Samples were stored at 4°C and evaluated periodically for microbial growth. Sensorial analysis was also performed using a nine-point hedonic scale to evaluate the organoleptic characteristics (odor, taste and appearance). The results showed significant ($p \leqslant 0.05$) combined effect of gamma-irradiation and coating on microbial growth (APCs and *Pseudomonas putida*). The shelf-life extension periods ranged from 3 to 10 days for shrimps and from 7 to 20 days for pizzas, compared to uncoated/unirradiated products. No significant ($p > 0.05$) detrimental effect of gamma-irradiation on sensorial characteristics (odor, taste, appearance) was observed. In strawberries, coating with irradiated protein solutions resulted in significant reduction of the percentage of mold contamination.

Keywords: Gamma irradiation; Edible coating; Shelf life

1. Introduction

The current interest in "minimally processed foods" has attracted the attention for combination of mild treatments to improve food safety and shelf-life extension. Food irradiation offers several substantial technical benefits in food-processing technology including microbial decontamination and pathogen elimination (Lacroix and Ouattara, 2000). 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 (Aureli et al., 1992; Eloff, 1999; Mahrour et al., 1998; 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 food (Siragusa and Dickson, 1992; Torres et al., 1985). Currently, a new concept is being 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 a longer storage time (Gennadios et al., 1997; Ming et al., 1997; Siragusa and Dikson, 1992). According to the microbiological hurdle concept (Leistner, 1992), active films or coating containing antimicrobial agents can also be combined with low-dose gamma irradiation to obtain a synergistic inhibitory effect (Farkas, 1990; Lacroix and Ouattara, 2000) in order to produce shelf-stable foods. The present study was undertaken to evaluate the combined effect of gamma irradiation and

*Corresponding author. Tel.: +1-450-6875010x4489; fax: +1-450-687-5792.

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

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edible coating on the shelf-life extension of pre-cooked shrimp, ready-to-eat shrimps and fresh strawberries.

2. Material and methods

2.1. Sample preparation

2.1.1. Shrimp

The base-coating solution was prepared from a mixture of soy-protein isolate (Dupont Campbell Protein Technologies, St-Louis, MO, USA) and whey-protein isolate (Food Research and Development Centre, St-Hyacinthe, Quebec, Canada). Chilled peeled shrimp (*Penaeus* spp.) samples were purchased at a local grocery store (IGA, Laval, Quebec, Canada) and randomly assigned into four treatment lots consisting of one control lot (uncoated) and three lots treated with the following coating solutions: base and base + essential oils, final concentration of 0.9% (vol/wt) (EO09) or 1.8% (vol/wt) (EO18). Uncoated and coated shrimps were divided into two groups. One group was irradiated at a total dose of 3 kGy and at a dose rate of 31.24 kGy/h, using a ^{60}Co source UC-15A (MDS-Nordion International Inc., Kanata, Ontario, Canada). The second group serves as an unirradiated control. All the plates were stored at 4°C and duplicate samples were taken at 1, 3, 6, 9, 1, and 21 days for aerobic plate count (APC) determination. Day 1 corresponded to the day of irradiation. In a separate experiment, the effect of gamma-irradiation and coating was evaluated on artificially contaminated shrimp with *Pseudomonas putida* isolated from refrigerated beef at the Food Research and Development Center (St-Hyacinthe, Quebec, Canada) at a level of approximately 10^5 colony forming units (CFU)/ml of *P. putida*.

2.1.2. Ready to eat pizzas

A protein-based coating solution Longevita® (Bio-Envelop Technologies Inc.) was used in this experiment. All dressed refrigerated pizzas were purchased from Sorrento Inc. (Chicoutimi, Quebec) and used within 24 h after manufacturing. Pizza samples were first assigned to two treatment groups (uncoated and coated with Longevita®). For each group, three subgroups were constituted and irradiated at 0, 1 and 2 kGy. Both shrimps and pizzas were stored at 4°C for 21 days, and duplicate samples were taken periodically for APCs.

2.1.3. Strawberries

Two types of coating solutions were prepared following a procedure previously developed in our laboratories (Brault et al., 1997): (i) a base-coating solution made from a mixture of calcium caseinate and whey protein isolate (1/1) with glycerol and (ii) the base solution plus a mixture of polysaccharides (PLS) (0.2%, w/w). All the solutions were irradiated at 32 kGy in a ^{60}Co irradiator (Gammacell-200, MDS Nordion, Kanata, Ontario, Canada) at the Canadian Irradiation Center (Laval, Quebec, Canada). "Kent" strawberries were used for the analysis. Samples were randomly assigned to three groups: (i) uncoated control, (ii) coated with the base solution, and (iii) coated with protein solution containing PLS. Samples were stored in a large refrigerator at 4 ± 1°C and mold growth (%) was noted until 100% contamination was observed.

2.2. Microbial analysis

Samples were homogenized for 2 min in 90 ml of sterile peptone water (0.1%) using a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From these mixtures, serial dilutions were prepared and appropriate ones were spread on sterile petri plates containing Plate Count Agar (Difco Laboratories, Detroit, MI, USA) and incubated at 35 ± 1°C, for 24 h, for the numeration of total APCs. The numeration of *P. putida* was done on brain infusion agar (BHA, Difco Laboratories, Detroit, MI, USA), 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 bacteria/g for shrimp (Ayres, 1960) and 10^6 bacteria/g for pizzas.

2.3. 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 3 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 was performed by 11 trained panelists (students and employees of INRS-Institut Armand-Frappier, Laval, Quebec, 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.

2.4. 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 Inc. Chicago, IL, USA). The least-squares significant difference (LSD) test was used at each sampling time for

point-by-point determination of the influence of coating. Difference between unirradiated and irradiated samples was determined using the Student t-test. Differences between means were considered significant when $p \leqslant 0.05$.

3. Results

3.1. Shrimp

3.1.1. 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 ($p \leqslant 0.05$) 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 base solution, bacterial count decreased significantly ($p \leqslant 0.05$) compared to uncoated controls. The patterns of bacterial growth in irradiated samples were quite different from those observed in unirradiated samples. The irradiation process resulted in a significant ($p \leqslant 0.05$) increase of lag periods before initiation of bacterial growth. For both uncoated and coated samples, no viable colony-forming unit was detected during the first 7 days of storage. In general, combination of gamma irradiation with coating resulted in a more inhibitory effect against bacterial growth. In irradiated samples, regardless of the type of coating, total APCs in coated samples were significantly ($p \leqslant 0.05$) lower than uncoated control samples. Based on the onset of shrimp spoilage established at 10^7 bacteria/g, the shelf-life periods of unirradiated and irradiated shrimps were estimated. 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 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.

3.1.2. Pseudomonas putida

Data related to the growth of *P. putida* in unirradiated and irradiated shrimp are illustrated in Fig. 2. Bacterial growth in unirradiated shrimp increased significantly to reach maximum values of 10.76–12.24 CFU/g after 21 days. Although total counts of *P. putida* were lower in EO09 and EO18 treatment, no significant ($p > 0.05$) effect of coating was found during the first 7 days of storage. At the end of the experimental period (21 days), only the EO18 coating solution showed a significant ($p \leqslant 0.05$) reduction in the growth of *P. putida*. When shrimps 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

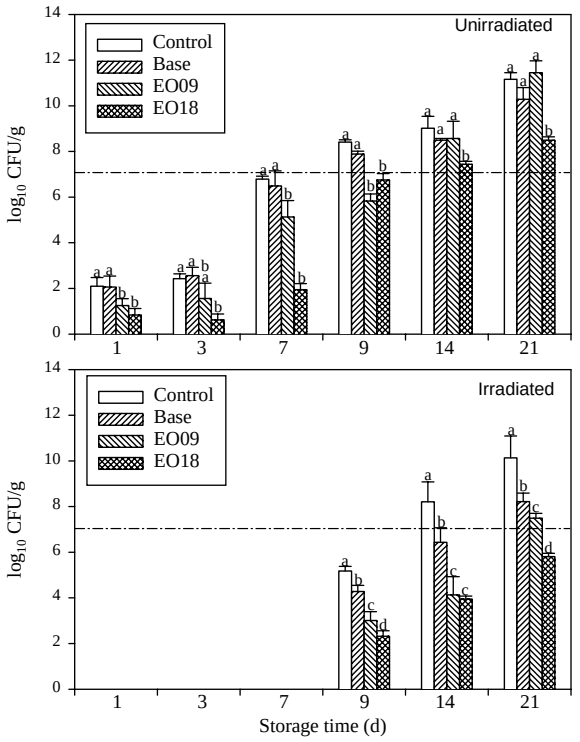

Fig. 1. Changes in total bacterial counts (APCs) on unirradiated and irradiated shrimp during storage at 4°C.

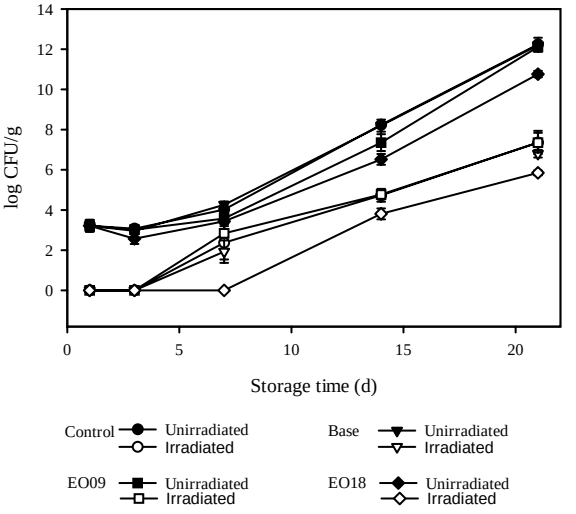

Fig. 2. Effect of gamma irradiation and antimicrobial coating on the growth of *P. putida* during storage at 4°C.

observed after 3 days for control, base and EO09, and after 7 days for EO18. Total APCs for both control and coated samples remained significantly lower ($p \leqslant 0.05$) in irradiated samples compared to unirradiated ones during the entire storage period (21 days). Bacterial counts in samples coated with EO18 solutions were significantly lower ($p \leqslant 0.05$) than in all the other samples during the entire experimental period (21 days). No significant ($p > 0.05$) antibacterial effect was observed for the base and EO09 solutions.

3.1.3. Sensorial evaluation

Table 1 shows the results of variance analysis relative to sensorial evaluation of the shrimp. None of the sensorial 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 ($p \leqslant 0.05$), the acceptability of odor and taste. There was no significant combined effect of gamma irradiation and coating on 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. Appearance of shrimp 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 and from 6.45 to 6.73 for irradiated ones. For odor and taste, no significant difference ($p > 0.05$) was observed between uncoated control samples and samples coated with the base solution, or with 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 ($p \leqslant 0.05$). 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 EO18 solution. In both the cases (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 irradiated samples.

Table 1
Summarized results of variance analysis showing main and interaction effects of gamma irradiation and coating on the sensorial characteristics of shrimp

		$P\ (F > F_{cal})$		
	df	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

3.2. Ready-to-eat pizzas

The effect of irradiation alone and irradiation combined with antimicrobial coating on the shelf life of pizza samples is presented in Fig. 3. The level of contamination before irradiation and coating treatments was 4.3 log CFU microorganism/g. Gamma irradiation alone produced 2–3.5 log unit reduction of APCs depending on the dose (Fig. 3A). Furthermore, growth rates during storage were significantly reduced ($p \leqslant 0.05$). Shelf-life periods obtained were 3 days for unirradiated samples compared to 12 and 14 days for samples irradiated at 1 and 2 kGy, respectively. Combining irradiation with antimicrobial coating resulted in a synergistic inhibitory effect (Fig. 3B). Indeed, the shelf-life periods were extended to 21 for coated samples irradiated at 1 kGy and to more than 21 days for those irradiated at 2 kGy.

3.3. Strawberries

Coating with irradiated protein solution resulted in significant reduction of mold growth on fresh strawberries. Visible contamination was observed at day 3 for uncoated control samples, at day 9 for samples coated with the base solution, and at day 15 for samples coated

Table 2
Effect of coating and gamma-irradiation on the organoleptic properties of shrimp after 3 days of storage[a,b]

	Sensorial parameters					
	Appearance		Odor		Taste	
	Unirradiated	Irradiated	Unirradiated	Irradiated	Unirradiated	Irradiated
Control	6.56 ± 2.30a	6.45 ± 1.37a	7.20 ± 1.93a	7.22 ± 97a	7.30 ± 1.34a	7.70 ± 1.49a
Base	6.40 ± 2.07a	6.55 ± 1.57a	6.89 ± 1.45a	6.55 ± 1.75ab	6.78 ± 1.20ab	6.82 ± 1.99a
EO09	6.70 ± 2.16a	6.73 ± 1.49a	6.25 ± 1.49ab	4.50 ± 1.66ab	4.56 ± 1.46ab	5.00 ± 2.24ab
EO18	6.40 ± 2.22a	6.64 ± 1.57a	4.86 ± 1.86b	4.14 ± 1.57b	4.17 ± 1.67b	4.38 ± 1.92b

[a] Means within a column bearing the same letter are not significantly different ($p > 0.05$) as determined by the least significant difference test.

[b] No significant difference ($p > 0.05$) was found between irradiated and unirradiated samples as determined by the Student t-test.

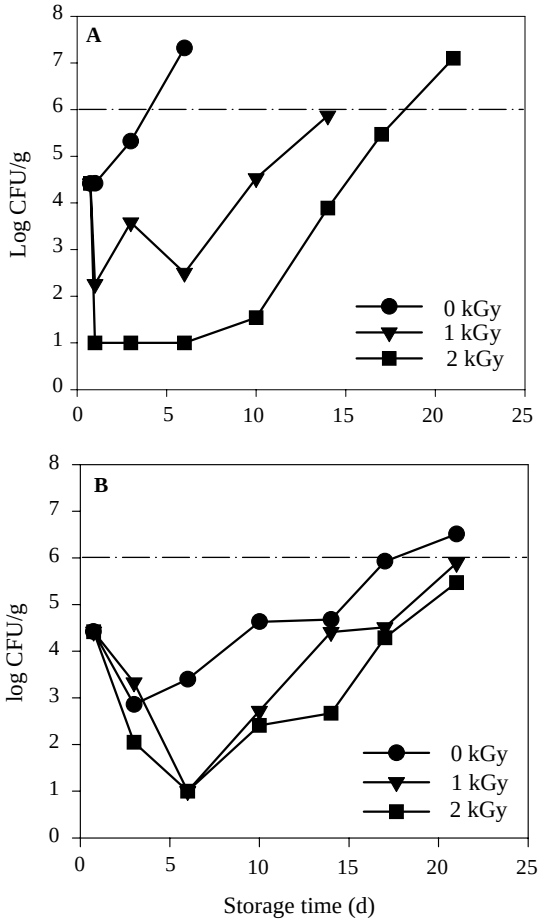

Fig. 3. Shelf-life extension of ready-to-eat pizzas as affected by gamma irradiation and edible coating during storage at 4°C.

with the base solution + PLS. The level of contamination reached 100% at day 18 for uncoated controls, at day 25 for samples coated with the base solution and day 35 for samples coated with the base solution + PLS (Fig. 4).

4. Discussion and conclusions

Our results showed a significant synergistic effect of gamma irradiation and antimicrobial coating in reducing the growth of bacterial in-peeled shrimp and refrigerated pizzas. This effect was characterized by longer lag period, lower growth rates, and therefore, significant shelf-life extension in irradiated samples. 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 are

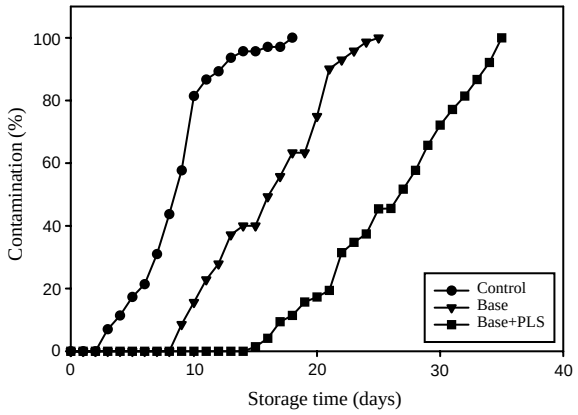

Fig. 4. Effect of coating fresh strawberries with irradiated protein solutions on mold growth during storage at 4°C.

untreated ones (Farkas, 1990; Lacroix and Ouattara, 2000). These observations are also supported by the report of Mahrour et al. (1998), who combined marinating in natural plant extracts with gamma irradiation, and obtained significant reduction of irradiation dose required to control pathogenic *Salmonella* on fresh poultry. Immobilizing antimicrobials into coating solutions is a very advantageous technology for food preservation. The resulting biofilms 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 sufaces (Hotchkiss, 1995; Torres et al., 1985). From the present study, changes in appearance, odor and taste, as affected by gamma irradiation, were not detectable by the sensorial evaluation panelist. These results agreed with the reports 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 effect on biochemical and nutritional characteristics.

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