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Combined Effect of Gamma Irradiation, Ascorbic Acid, and Edible Coating on the Improvement of Microbial and Biochemical Characteristics of Ground Beef

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ABSTRACT

This study was conducted to evaluate the combined effect of gamma irradiation and the incorporation of natural antimicrobial compounds in cross-linked films on the microbiological and biochemical characteristics of ground beef. Medium-fat (23% fat) ground beef patties were divided into three separate treatment groups: (i) control samples without additives, (ii) ground beef samples containing 0.5% (wt/wt) ascorbic acid, and (iii) ground beef samples containing 0.5% ascorbic acid and coated with a protein-based cross-linked film containing immobilized spice powders. Meat samples were irradiated at doses of 0, 1, 2, and 3 kGy and stored at 4 ± 2°C. Microbial growth (based on total aerobic plate counts [APCs] and total coliforms) was evaluated, as were the content of thiobarbituric acid–reactive substances (TBARS) and that of free sulfydryl groups. At the end of the storage period, *Enterobacteriaceae,* presumptive *Staphylococcus aureus,* presumptive *Pseudomonas* spp., *Brochothrix thermosphacta,* and lactic acid bacteria were enumerated. Regardless of the treatment group, irradiation significantly ($P \leq 0.05$) reduced the APCs. Irradiation doses of 1, 2, and 3 kGy produced immediate APC reductions of 2, 3, and 4 log units, respectively. An APC level of 6 log CFU/g was reached after 4, 7, and 10 days for samples irradiated at 1, 2, and 3 kGy, respectively. Lactic acid bacteria and *B. thermosphacta* were more resistant to irradiation than were *Enterobacteriaceae* and *Pseudomonas.* The TBARS and free sulfydryl contents were stabilized during postirradiation storage for samples containing ascorbic acid and coated with the protein-based cross-linked film containing immobilized spice powders.

Irradiation as a method of meat preservation has excellent potential to improve the safety and extend the shelf life of meat *(4, 12, 13).* In 1981, the use of irradiation was approved by the Food and Agriculture Organization/International Atomic Energy Agency/World Health Organization joint committee on the wholesomeness of irradiated food. Since then, significant progress has been made in this respect through the use of irradiation doses of <10 kGy to control the growth of pathogenic and spoilage bacteria such as *Listeria monocytogenes* and *Salmonella* Typhimurium *(9)* and *Escherichia coli* O157:H7 and *Yersinia enterocolitica (10)* on meat and meat products. The Food and Drug Administration's approval of the use of meat irradiation in 1997 *(8)* has made consumers more confident and attracted the interest of industries concerned with food quality. Currently, more than 40 countries have approved the use of irradiation for specific foods, and more than 34 countries are irradiating one or more food products for commercial purposes *(23).*

New trends in food irradiation technology include the development of combined treatments involving heat, headspace gas, antimicrobial agents, etc., to reduce the irradiation doses required to kill pathogenic bacteria and/or reduce overall microbial loads *(14).* Mahrour et al. *(24)* reduced the irradiation dose required for the complete elimination of *Salmonella* on fresh poultry by combining gamma irradiation with marination in natural plant extract. Similarly, Farkas and Andrassy *(7)* reported that the combination of gamma irradiation at 2 kGy with ascorbic acid or gluconodelta-lactone produced significant reductions in aerobic viable cell counts and *Enterobacteriaceae* in vacuum-packaged, chilled meat products. A recent study conducted in our laboratories *(13)* showed a significant ($P \leq 0.05$) additional antimicrobial effect between irradiation at 1 to 4 kGy and the addition of ascorbic acid.

Edible coating or biodegradable packaging is a new technology that has been introduced into food processing to obtain products with longer shelf lives. Several applications for meat, poultry, and seafoods have been reviewed by Gennadios et al. *(11)* with particular emphasis on lipid oxidation reduction, weight loss, moisture loss, microbial load, and volatile flavor loss. The coating can serve as a carrier of antimicrobial compounds for the maintenance of high concentrations of preservatives on food surfaces. For example, Ouattara et al. *(26)* incorporated selected antimicrobial compounds, including organic acids, fatty acids, and essential oils, and obtained a controlled diffusion of active

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compounds and subsequent inhibition of bacterial growth on processed meats. Immobilization of organic acids in a calcium alginate gel has also been used to control *L. monocytogenes* on beef tissue *(31)*.

Studies conducted in our laboratories *(1, 29, 36)* have shown heat and gamma irradiation to result in cross-linking and to improve physical and functional properties of edible and cross-linked films via the production of cross-links between protein molecules. On the basis of the report of Papadokostaki et al. *(28)* on the relationship between polymer structure and the transport of active molecules through the network, it can be hypothesized that structure modification induced by gamma irradiation will increase the capacity of edible and cross-linked films to retain immobilized antimicrobial compounds. The application of coating films or solutions made from such polymers on solid or semisolid foods will increase antimicrobial efficacy by maintaining high concentrations of active molecules on food surfaces, where most microbial growth occurs. Coating with cross-linked films containing antimicrobial compounds may also allow a controlled release of the active molecules into the bulk of the food.

The present study was undertaken to evaluate the combined effect of gamma irradiation and the application of cross-linked film coatings containing spice powders on microbial growth. The types of microorganisms and some biochemical characteristics (the content of thiobarbituric acid–reactive substances [TBARS] and that of free sulfydryl [−SH]) of ground beef were also evaluated at the end of the storage period.

MATERIALS AND METHODS

Preparation of cross-linked film coatings. Calcium caseinate (91.8% [wt/wt] protein) was provided by New Zealand Milk Product Inc. (Santa Rosa, Calif.). Whey protein isolate was produced by ultrafiltration and diafiltration at the Food Research and Development Centre (St.-Hyacinthe, Quebec) and was transported to the Canadian Irradiation Center (Laval, Quebec) under refrigeration conditions (4 ± 2°C). The whey protein isolate solution was freeze-dried (Model 12 research freeze-dryer, Virtis Company Gardiner, New York, N.Y.) and dried at 100°C for 3 h in a model 019 vacuum oven (Precision Scientific Inc., Chicago, Ill.). Calcium caseinate and whey protein isolate were mixed at a ratio of 1:1 (wt/wt) and solubilized in distilled water containing 2.5% (wt/wt) glycerol and 0.25% (wt/wt) low-viscosity carboxymethyl cellulose (Sigma Chemical Co., St. Louis, Mo.), for a total protein concentration of 5% (wt/wt, dry weight basis) in the coating solution. Spice powders (thyme, rosemary, and sage) were mixed together at a ratio of 1:1:1 (dry weight basis) and added to the protein solution at a final concentration of 3% (wt/wt). The protein-based solution was subjected to thermal treatment (90°C for 30 min) followed by gamma irradiation treatment at a dose of 32 kGy with a ^{60}Co source UC-15A (MDS-Nordion International Inc., Kanata, Ontario) at 36.639 kGy/h *(36)*. Film-forming solutions were cast onto sterile petri plates (8.5 cm in diameter) and dried overnight at 20 ± 1°C in a climatic chamber with a relative humidity of 45 to 50%.

Sample preparation. Fresh medium-fat (23% fat) ground beef was purchased at a local grocery store (IGA, Laval, Quebec) and transported to the Canadian Irradiation Center under refrigerated conditions in an ice-filled cooler container. The ground beef was divided into three separate groups: (i) control samples with no additives, (ii) samples containing ascorbic acid at a concentration of 0.5% (wt/wt), and (iii) samples containing ascorbic acid at a concentration of 0.5% (wt/wt) and coated with a cross-linked film containing immobilized spice powders. Sterile petri plates (8.5 cm in diameter) containing the cross-linked films were filled to the brim with ground beef. Coating films were applied to the entire sample surface and kept in close contact with the meat with the lid of the petri plate. Meat samples from each group were irradiated at 0, 1, 2, and 3 kGy with a ^{60}Co source UC-15A at 36.6 kGy/h. Samples were stored at 4 ± 2°C and tested periodically (on days 1, 4, 7, and 10) for microbial growth. At the end of the storage period (determined on the basis of the limit of microbiological acceptability), different groups of microorganisms and biochemical characteristics (TBARS and −SH contents) were evaluated.

Microbial analysis. Each sample of ground beef was weighed aseptically (ca. 10 ± 0.2 g) and homogenized for 2 min in 90 ml of sterile peptone water (0.1%; Difco Laboratories, Detroit, Mich.) with a Lab-blender 400 stomacher (Seward Medical, London, UK). From the resulting homogenate, serial dilutions were prepared, and the appropriate dilutions were spread plated onto sterile petri plates containing plate count agar (Difco), which were incubated at 35 ± 1°C for 48 h. The shelf life of the product was estimated on the basis of a limit of acceptability of 10^6 bacteria per g. At the end of the storage period, each sample was tested for total *Enterobacteriaceæ* on violet red bile glucose agar (Difco), and plates were incubated at 35°C for 24 h. Lactic acid bacteria, *Brochothrix thermosphacta,* and presumptive *Pseudomonas* spp. were enumerated on deMan Rogosa Sharpe agar, streptomycin thallous acetate actidione agar, and cephaloridin fucidin cetrimide agar, respectively, with incubation at 25°C for 48 h *(30)*. Presumptive *Staphylococcus aureus* was enumerated on Baird-Parker agar enriched with egg yolk tellurite emulsion (Difco) with incubation at 37°C for 48 h.

Lipid oxidation. Lipid oxidation potential was evaluated by the determination of TBARS. A 10-g portion of each sample was blended with 50 ml of distilled, deionized water and 10 ml of trichloroacetic acid (final concentration, 15%). The homogenate was filtered through Whatman 0.45-μm filter paper (Whatman International Ltd., Maidstone, UK). To 8 ml of the filtrate, 2 ml of 0.06 M thiobarbituric acid was added. The solution was mixed and placed into an 80°C water bath for 90 min and then cooled in an ice bath. Absorbance was read at 520 nm on a DMS 200 spectrophotometer (Varian Techtron Pty. Ltd., Mulgrade, Australia), and the concentration of malonaldehyde was calculated on the basis of a standard curve obtained with serial dilutions of a solution of 1,1,3,3-tetraethoxypropane (Sigma) *(17, 21)*. The TBARS values were expressed as micrograms of malonaldehyde per gram of meat. Analyses were carried out on day 0 and at the end of the storage period (determined on the basis of the limit of microbiological acceptability [10^6 CFU/g]).

Sulfydryl content. The total concentrations of free sulfydryl groups (−SH) were determined as follows. First, meat samples (15 g) were homogenized and proteins were extracted in a 10 mM sodium phosphate buffer (pH 7.0) containing 60 mM sodium chloride, 1 mM magnesium chloride, and 0.02% sodium azide *(4)*. From the total protein solution, the −SH concentration was determined by using Ellman's reagent *(22)*. Briefly, protein extracts were mixed with 0.1 ml 5,5′-dithiobis-2-nitrobenzoic acid (Sigma) for 15 min at room temperature, and absorbance was measured at

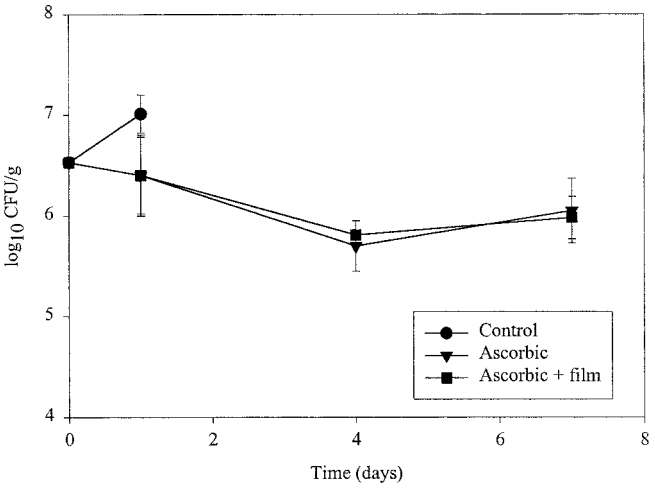

FIGURE 1. *Change in total APCs for unirradiated ground beef containing ascorbic acid and for unirradiated ground beef containing ascorbic acid combined with an edible coating during storage at 4°C.*

412 nm with a DMS 200 spectrophotometer. Analyses were carried out on day 0 and at the end of the storage period (determined on the basis of the limit of microbiological acceptability [10^6 CFU/g]).

Statistical analysis. Experiments for microbiological and biochemical analysis were a 3 × 4 × 5 factorial design with 3 ground beef treatments (control, ascorbic acid, and ascorbic acid plus cross-linked film coating), 4 irradiation dose levels (0, 1, 2, and 3 kGy), and 5 storage periods (0, 1, 4, 7, and 10 days). The experiments were replicated three times, and duplicate measurements were taken for each sample. Analysis of variance was carried out with the GLM procedure of the SAS statistical package (SAS Institute, Cary, N.C.) and the least significant difference test was use to differentiate treatments. Differences between means were considered significant when $P \leq 0.05$.

RESULTS

Microbiology. Changes in total bacterial counts (APCs) for unirradiated ground beef samples during refrigerated storage are presented in Figure 1. Total APCs for control samples increased by 0.5 log units (from 6.53 to 7.01 log CFU/g) within 1 day of storage. Samples containing ascorbic acid and those containing ascorbic acid combined with cross-linked film coating showed a 1-log decrease in bacterial growth (from 6.53 to 5.70 log CFU/g) during the first 4 days, followed by a slight increase from day 4 to day 7. APC values were 6.53, 5.70, and 6.05 log CFU/g on days 1, 4, and 7, respectively, for samples containing ascorbic acid. APC values for samples containing ascorbic acid combined with cross-linked film coating were 6.53, 5.81, and 5.98 log CFU/g on days 1, 4, and 7, respectively. However, no significant ($P > 0.05$) difference was observed between samples containing ascorbic acid alone and those containing ascorbic acid combined with cross-linked film coating.

Figure 2 shows the effects of gamma irradiation at 1, 2, and 3 kGy on total APCs during refrigerated storage. The effect of irradiation on bacterial destruction was immediate, and the magnitude of the APC reduction was proportional to the irradiation dose. On day 1, total counts were reduced by 2, 3, and 4 log units for ground beef samples irradiated at 1, 2, and 3 kGy, respectively. Shelf life extension periods estimated on the basis of a limit of 6 log CFU/g for APCs were 4, 7, and 10 days for samples irradiated at 1, 2, and 3 kGy, respectively. The incorporation

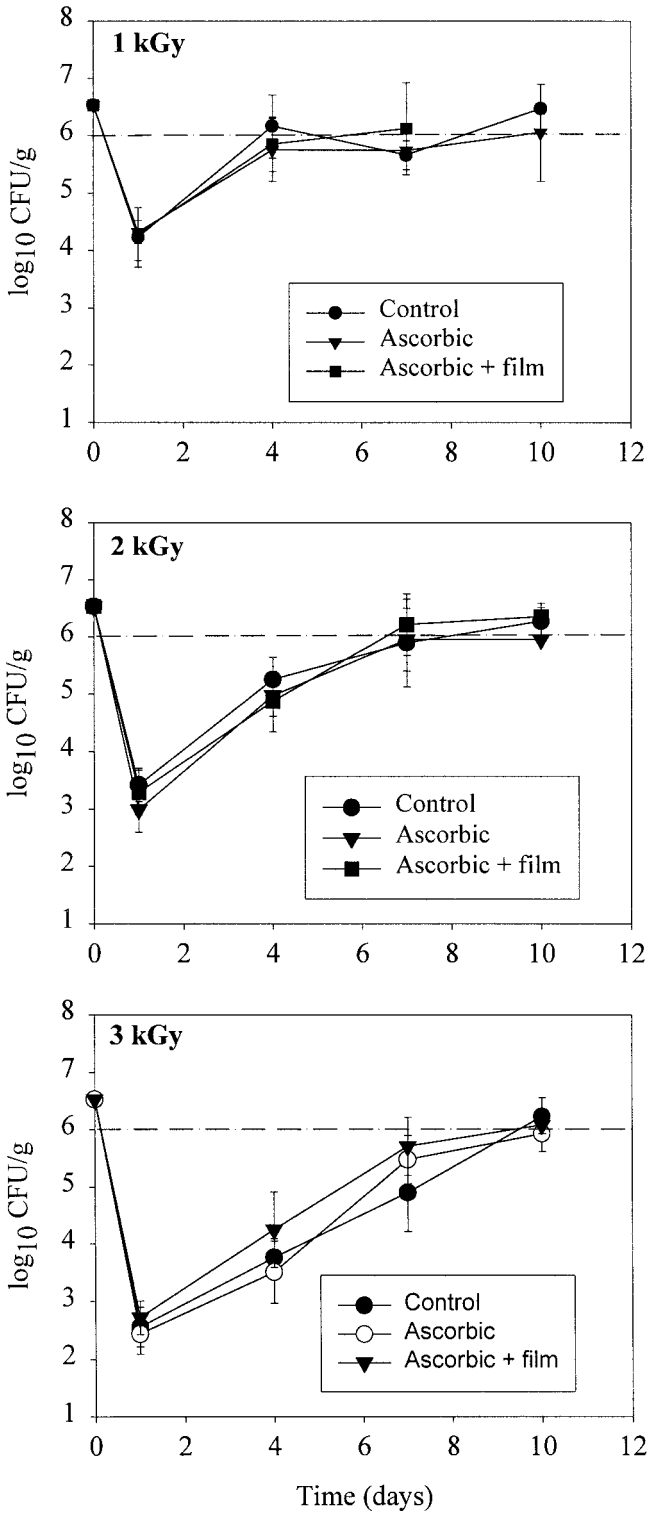

FIGURE 2. *Combined effects of ascorbic acid, edible coating, and gamma irradiation at 1, 2, and 3 kGy on total APCs for ground beef during storage at 4°C. Dash-dotted lines represent a limit of acceptability of 6 log CFU/g.*

TABLE 1. *Counts of total coliforms,* Enterobacteriaceae, Pseudomonas *spp., presumptive lactic acid bacteria, presumptive* Staphylococcus aureus, *and* Brochothrix thermosphacta[a]

Organism(s)	Irradiation dose (kGy)	Control	Ascorbic acid	Ascorbic acid + film
Total coliforms	0	4.75 ± 1.63 Ab	4.74 ± 1.53 Ab	4.64 ± 1.61 Ab
	1	2.33 ± 1.20 Aa	1.67 ± 0.68 Aa	1.73 ± 0.74 Aa
	2	1.31 ± 0.96 Aa	1.03 ± 0.11 Aa	1.06 ± 0.03 Aa
	3	1.04 ± 0.43 Aa	1.03 ± 0.02 Aa	1.04 ± 0.03 Aa
Enterobacteriaceae	0	4.46 ± 1.58 Ac	4.63 ± 1.50 Ab	4.59 ± 1.86 Ab
	1	2.53 ± 1.23 Ab	1.88 ± 0.53 Aa	1.96 ± 0.92 Aa
	2	1.01 ± 0.01 Aa	1.08 ± 0.25 Aa	1.06 ± 0.03 Aa
	3	1.04 ± 0.04 Aa	1.03 ± 0.02 Aa	1.04 ± 0.03 Aa
Pseudomonas spp.	0	7.87 ± 0.58 Ac	7.78 ± 0.50 Ac	8.06 ± 1.24 Ac
	1	3.69 ± 0.94 Ab	4.14 ± 0.74 Ab	4.20 ± 1.22 Ab
	2	2.59 ± 0.95 Aa	2.28 ± 0.47 Aa	2.31 ± 0.48 Aa
	3	2.29 ± 0.44 Aa	2.25 ± 0.44 Aa	2.29 ± 0.39 Aa
Lactic acid bacteria	0	8.16 ± 0.33 Ab	7.80 ± 0.26 Ab	8.06 ± 0.61 Ab
	1	7.38 ± 0.42 ABa	7.15 ± 0.57 Aa	7.76 ± 0.28 Ab
	2	7.28 ± 0.28 Aa	7.14 ± 0.22 Aa	7.70 ± 0.18 Ab
	3	7.09 ± 0.45 Aa	7.10 ± 0.49 Aa	6.90 ± 0.46 Aa
S. aureus	0	6.32 ± 0.55 Ac	6.02 ± 0.57 Ac	6.35 ± 0.84 Ac
	1	3.55 ± 0.77 Ab	3.35 ± 0.54 Ab	3.48 ± 0.99 Ab
	2	3.06 ± 0.79 Aab	2.42 ± 0.50 Aa	2.31 ± 0.48 Aa
	3	2.29 ± 0.44 Aa	2.28 ± 0.44 Aa	2.29 ± 0.50 Aa
B. thermosphacta	0	7.10 ± 0.19 Bc	6.53 ± 0.29 Ac	6.87 ± 0.67 Ac
	1	6.49 ± 0.52 Abc	6.47 ± 0.26 Ac	6.10 ± 0.53 Ac
	2	5.79 ± 0.82 Bb	4.67 ± 1.28 Ab	5.22 ± 0.93 Ab
	3	3.66 ± 1.41 Aa	3.15 ± 1.51 Aa	2.55 ± 0.6 Aa

[a] Means with the same small capital letter in the same row are not significantly different ($P > 0.05$); means with the same lowercase letter in the same column are not significantly different ($P > 0.05$).

of ascorbic acid into ground beef and the combination of ascorbic acid with the cross-linked film coating containing spice powders did not improve the inhibitory effect of gamma irradiation significantly ($P > 0.05$).

At the end of the experiment, various microorganisms and groups of microorganisms were enumerated for unirradiated and irradiated ground beef samples (Table 1). Growth levels of total coliforms, *Enterobacteriaceae,* and *Pseudomonas* spp. were significantly ($P \leq 0.05$) reduced by gamma irradiation treatment. For all of the treatment groups, irradiation significantly reduced total coliform growth. Values obtained for samples irradiated at 1 kGy were 1.67 to 2.33 log CFU/g, compared with 4.64 to 4.75 log CFU/g for unirradiated samples. Higher irradiation doses (2 and 3 kGy) produced additional bacterial inhibition, with total counts of 1.03 to 1.31 log CFU/g at 2 kGy and 1.03 to 1.04 log CFU/g at 3 kGy. All of the irradiation doses tested kept the total counts of coliforms below the maximum level (3 log CFU) recommended by the Canadian Food Inspection Agency *(2)* for raw meat. Similar sensitivity behavior was observed for *Enterobacteriaceae,* with final bacterial concentrations of 1.88 to 2.53 log CFU/g at 1 kGy, 1.01 to 1.08 log CFU/g at 2 kGy, and 1.03 to 1.08 log CFU/g at 3 kGy, corresponding to average bacterial reductions of 2.43, 3.51, and 3.52 log units, respectively. The population of *Pseudomonas* spp. was reduced from a value of 7.78 to 8.06 log CFU/g to a value of 3.69 to 4.20 log CFU/g after irradiation at 1 kGy. Irradiation at 2 and 3 kGy resulted in *Pseudomonas* counts of 2.28 to 2.59 log CFU/g and 2.25 to 2.29 log CFU/g, respectively. No significant difference ($P > 0.05$) was observed between control samples, samples with ascorbic acid, and samples with ascorbic acid plus cross-linked film coating for all treatment groups. Presumptive *S. aureus* also exhibited significant sensitivity, with counts of 3.35 to 3.55 log CFU/g at 1 kGy, 2.31 to 3.06 log CFU/g at 2 kGy, and 2.28 to 2.29 log CFU/g at 3 kGy, while bacterial counts for unirradiated samples were 6.02 to 6.35 log CFU/g. Total counts of presumptive *S. aureus* in samples irradiated at 2 and 3 kGy were below the maximum limit of acceptability of 3 log CFU *(2). B. thermosphacta* counts were significantly ($P \leq 0.05$) reduced when samples were irradiated at 2 and 3 kGy, with bacterial counts of 4.67 to 5.79 log CFU/g and 2.55 to 3.66 log CFU/g, respectively, compared with 6.53 to 7.10 log CFU/g for unirradiated samples. No significant difference ($P > 0.05$) was observed between the three treatment groups (control samples, samples with ascorbic acid, and samples with ascorbic acid plus cross-linked film coating) for any of the microorganisms analyzed. The greatest resistance and/or rate of recovery during storage was obtained with cells enumerated on deMan Rogosa Sharpe agar, which is designed for presumptive lactic acid bacteria.

TABLE 2. *Concentrations of TBARS and −SH in ground beef samples containing ascorbic acid (A) or ascorbic acid plus cross-linked film coating (A+F) irradiated at 2 kGy*

Time	TBARS content (μg/g)[a]			−SH content (μM/g)[a]		
	Control	A	A+F	Control	A	A+F
Initial	5.28 ± 0.42	5.28 ± 0.42	5.28 ± 0.42	5.68 ± 1.47	5.68 ± 1.47	5.68 ± 1.47
After irradiation[b]	7.34 ± 0.79 A	6.95 ± 1.93 A	10.26 ± 4.72 B	5.62 ± 1.12 A	7.03 ± 0.67 AB	7.54 ± 0.65 B
End of storage[c]	21.08 ± 0.79 A	21.32 ± 4.49 A	14.01 ± 1.70 B	7.45 ± 1.86 A	10.28 ± 2.36 B	8.16 ± 0.43 AB

[a] Means with different letters in the same row are significantly different ($P \leq 0.05$).
[b] Samples were analyzed for TBARS and −SH contents immediately after irradiation.
[c] The end of the storage period corresponded to the limit of microbiological acceptability (10^6 log CFU/g) (day 8 for all samples).

Only a 1-log reduction in total counts was observed even at the highest irradiation dose used (3 kGy).

Biochemistry. Concentrations of TBARS and −SH radicals were determined for unirradiated ground beef samples and for samples irradiated at 2 kGy. Comparisons were also made between control samples (without additives), samples containing ascorbic acid, and samples containing ascorbic acid combined with cross-linked film coating on day 0 and at the end of the storage period, as determined on the basis of the limit of microbiological acceptability. The initial concentrations of TBARS and −SH in ground beef were 5.28 μg/g and 5.68 μM/g, respectively. The limit of microbiological acceptability of unirradiated samples was reached after only 1 day, with concentrations of 8.10 μg/g for TBARS and 7.39 μM/g for −SH groups (results not shown).

Immediately after irradiation at 2 kGy, the TBARS contents increased to 7.34 μg/g for control samples, to 6.95 μg/g for samples containing ascorbic acid, and to 10.26 μg/g for samples containing ascorbic acid combined with cross-linked film coating (Table 2). Further increases in TBARS concentrations were observed during postirradiation storage for controls and for samples containing ascorbic acid. However, the TBARS values remained stable for samples containing ascorbic acid combined with cross-linked film coating. Values obtained were 10.26 ± 4.77 μg/g at the beginning of the experiment and 14.01 ± 1.70 μg/g at the end of the experiment. At the end of the storage period, TBARS values were significantly ($P \leq 0.05$) higher for control samples and samples containing ascorbic acid (21.08 and 21.32 μg/g, respectively) than for samples containing ascorbic acid combined with cross-linked film coating (14.01 μg/g). For control samples, no significant effect ($P > 0.05$) of gamma irradiation on −SH content was observed (5.68 and 5.62 μM/g before and after irradiation, respectively) immediately after treatment. Samples containing ascorbic acid or ascorbic acid plus cross-linked film coating showed a slight increase in −SH content immediately after irradiation at 2 kGy. The values obtained were 7.03 μM/g for samples containing ascorbic acid and 7.54 μM/g for those containing ascorbic acid combined with cross-linked film coating. At the end of the storage period (day 8), −SH contents remained stable for control samples and for samples containing ascorbic acid plus cross-linked film coating (7.45 and 8.16 μM/g, respectively). In contrast, theses values increased significantly ($P \leq 0.05$) for samples containing only ascorbic acid, reaching 10.28 μM/g at the end of the storage period.

DISCUSSION

The results of the present study indicate that the incorporation of ascorbic acid, alone or in combination with cross-linked film coating, into ground beef had an appreciable effect in stabilizing microbial growth (Fig. 1). Compared with control samples, for which a significant APC increase was observed, samples treated with ascorbic acid alone or in combination with cross-linked film coating showed stabilization of microbial growth after 4 days of storage (Fig. 1). This finding is consistent with those of several previous studies on the antimicrobial properties of organic acids with regard to meat spoilage and pathogenic bacteria *(3, 27).* A previous study conducted in our laboratories *(13)* also demonstrated that a reduction in bacterial growth was strongly related to a reduction in pH resulting from the incorporation of ascorbic acid into ground beef. Ascorbic acid may also act through a scavenging effect by binding to some critical compounds, such as metal ions, sulfydryl, and amino groups of proteins *(18).* These compounds are generally associated with membrane functions and the transport of nutrients in bacteria *(13).*

Gamma irradiation treatment of ground beef produced an immediate, dose-dependent inhibition of bacterial growth (Fig. 2). Hence, the immediate reduction in the microbial populations was expected, since irradiation treatment is known to have excellent potential to improve the safety and extend the shelf life of meat *(13).*

Irradiation treatment causes several chemical changes in the bacterial cell components, including (i) effects on synthetic processes, particularly the synthesis of DNA and RNA; (ii) alteration of the cell membrane, which affects the transfer of critical nutritional compounds; and (iii) effects on energy metabolism through the reduction of phosphorylation *(35).* However, the irradiation doses used in the present study (1, 2, and 3 kGy) corresponded to a radurization treatment *(35)* and therefore could not bring about the complete destruction of microorganisms. Hence, total APCs in ground beef increased progressively after 4 days of storage.

The results of this study are also in agreement with the literature reporting greater radiation sensitivity for gram-negative bacteria than for gram-positive bacteria *(13).* The

growth levels of total coliforms, *Enterobacteriaceae,* and *Pseudomonas* spp. were more compromised than those of lactic acid bacteria and presumptive *B. thermosphacta.* Similarly, Lefebvre et al. *(19)* reported D_{10} values of 0.035 kGy for *Pseudomonas fluorescens* (gram negative) and 1.827 kGy for *S. aureus* (gram positive). Thayer and Boyd *(34)* reported significant inhibition of the growth of *Salmonella* Typhimurium, *Pseudomonas aeruginosa,* and *Serratia marcescens* with irradiation doses of <5 kGy. However, in our study, the effect of irradiation treatment for *S. aureus* was similar that for the gram-negative bacteria, i.e., total coliforms, *Pseudomonas* spp., and *Enterobacteriaceae.*

Food preservation by combined methods generally results in enhanced antibacterial effects and therefore extended shelf life *(20).* Farkas and Andrassy *(7)* showed that irradiation combined with pH reduction brought about by ascorbic acid reduced microbial growth in meat products even at abused temperatures. Lee et al. *(18)* also observed a synergistic effect of irradiation and naturally occurring antioxidants such as ascorbyl palmitate, α-tocopherol, and β-carotene on the microbial stability of beef patties. Our results, however, failed to confirm the effect of irradiation in combination with ascorbic acid and in combination with ascorbic acid plus a cross-linked film coating containing spices. The discrepancy is probably related to factors such as the gram-negative/gram-positive ratio of the indigenous microflora of meat samples. A predominance of gram-positive bacteria, particularly lactic acid bacteria, may reduce the antimicrobial effectiveness of ascorbic acid because of the resistance of these bacteria to irradiation treatment under acidic conditions *(19, 26).* The lack of a synergistic antimicrobial effect may result in active compounds being present at low concentrations in the spice powders. McGimpsey et al. *(25)* and Sivropoulou et al. *(32)* reported seasonal and intraspecific variations of the compositions of many spices and essential oils. Incorporation of essential oils or pure active compounds into the cross-linked coating film formulation would probably result in an increased antimicrobial effect. Other competition processes in the ground beef samples may also have influenced the overall efficiencies of the treatments. These processes include the kinetics of diffusion of the active molecules from the surface to the interior of the product, the interaction with ground beef constituents, and the level of initial contamination of the product. In a previous study in which the initial level of contamination was 5.5 log CFU APC/g, a reduction in bacterial counts of up to 1 log was obtained with 0.5% ascorbic acid, and additional reductions resulted after this treatment was combined with 1 to 4 kGy irradiation *(13).* The absence of an additive interaction effect for the combination irradiation treatment with ascorbic acid and spice powders could also be related to a protective action of these compounds against the free radicals generated by gamma irradiation due to their antioxidant properties. This hypothesis is consistent with the report of Stecchini et al. *(33),* who found that the toxicity of peroxy radicals was counteracted by the incorporation of carnosine. We also evaluated the scavenging properties of ascorbic acid by the DPD (*N,N*-diethyl-*p*-phenylenediamine) colorimetric method described by Dumoulin et al. *(6).* Ascorbic acid concentrations of 0.033, 0.075, and 0.15% resulted in 55, 98, and 100% free radical scavenging capacity, respectively (unpublished data).

The results of this study also show that gamma irradiation increased lipid oxidation in ground beef samples. This finding is consistent with the report of Lambert et al. *(16),* who observed rapid fat oxidation in pork samples irradiated at 0.25 to 1 kGy under O_2-permeable conditions. The lipid oxidation was attributed to the combination of free radicals with O_2 to form hydroperoxides. Although −SH groups are known to be very sensitive to gamma irradiation *(5),* the irradiation dose used in the present experiment (2 kGy) was not high enough to produce a significant reduction. The increase in −SH content in the samples containing ascorbic acid can be attributed to the reduction of the pH of ground beef resulting from the incorporation of this compound. Li-Chan *(22)* also reported a significant effect of pH on the −SH content of whey protein concentrate during heating, with higher values occurring at lower pHs (6.7 μM/g at pH 4.6, 5.8 μM/g at pH 7.0, and 3.4 μM/g at pH 8.0). In our study, the incorporation of ascorbic acid reduced the pH of ground beef from 6.06 to 5.56, but the combination of ascorbic acid with cross-linked film coating containing spice powders may have protected the meat samples against lipid oxidation and production of −SH radicals during postirradiation storage. These results are probably related to the presence of some active compounds with scavenging properties in the mixture of spice powders incorporated into the cross-linked film coating formulation, as previously reported *(17, 37).* This hypothesis is also in agreement with the previous report of Lacroix et al. *(15),* who found that the addition of powdered rosemary or thyme decreased the lipid radiolysis induced by gamma irradiation by 52.5 to 80.5%.

The present study dealt with the use of combined treatments involving gamma irradiation, natural spice compounds, and a cross-linked film coating to improve the quality of ground beef. The results obtained indicate that a large part of the antimicrobial effect was due to gamma irradiation. Incorporation of ascorbic acid both alone and in combination with the cross-linked film coating into ground beef resulted in a significant reduction in bacterial growth for unirradiated samples. However, these treatments in combination did not increase the antimicrobial effectiveness of gamma irradiation significantly. As expected, *Enterobacteriaceae* and other gram-negative bacteria (total coliforms and *Pseudomonas* spp.) exhibited greater sensitivity to the irradiation treatment than did the gram-positive bacteria tested (*B. thermosphacta, S. aureus,* and lactic acid bacteria). Lactic acid bacteria appear to be the most resistant organism tested, followed by *B. thermosphacta,* while presumptive *S. aureus* showed a sensitivity comparable to those of the gram-negative bacteria, i.e., *Enterobacteriaceae,* total coliforms, and *Pseudomonas* spp. Ascorbic acid and a cross-linked film coating containing spice powders reduced lipid oxidation and −SH radical production during postirradiation storage.

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