# Combined Effect of Ascorbic Acid and Gamma Irradiation on Microbial and Sensorial Characteristics of Beef Patties during Refrigerated Storage

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The present study was undertaken to evaluate the effect of ascorbic acid concentrations (0.03 to 0.5%) and irradiation doses (0.5 to 4 kGy) on microbial growth, color coordinates (L*, a*, and b*), and sensory characteristics (taste and odor) of beef patties during storage at 4 ± 1 °C. Ascorbic acid was also compared to citric acid at a similar pH value in order to differentiate the effects of ascorbic acid from those of pH reduction. Results showed significant reduction ($p \leq 0.05$) of aerobic plate counts (APCs) and total coliforms, and a significant interaction ($p \leq 0.05$) between ascorbic acid and irradiation dose was observed. The irradiation treatment had detrimental effects on redness, yellowness, and hue angle values of meat. However, incorporation of ascorbic acid into the meat before irradiation resulted in significant ($p \leq 0.05$) stabilization of color parameters. The color improvement obtained with ascorbic acid was not related to the pH reduction. Also, no significant detrimental effect on taste or odor was found in irradiated samples containing ascorbic acid.

**Keywords:** *Beef patties; irradiation; ascorbic acid; sensorial evaluation*

## INTRODUCTION

Visual appearance is one of the major criteria used by consumers to assess the quality and the palatability of meat and meat products (*1* and *2*). Meat color, of ground beef in particular, is often used by consumers to determine whether it is safe to eat. Extensive research has been conducted to reduce or prevent color change in meat and meat products during processing and storage (*1*, *3*, *4*). Studies have included influence of formulation (*3*) and use of various packaging conditions (vacuum, modified atmosphere, and controlled atmosphere) (*5*).

Irradiation as a method of meat preservation has excellent potential to improve meat safety and extend shelf life (*6* and *7*). In 1981, the use of irradiation was approved by the FAO/IAEA/WHO joint committee on the wholesomeness of irradiated food. Since then, significant progress has been made in this respect by using irradiation doses lower than 10 kGy to control the growth on meat and meat products of pathogenic and spoilage bacteria such as *Listeria monocytogenes* and *Salmonella typhimurium* (*8*), *Escherichia coli* O157:H7 and *Yersinia enterocolitica* (*9*). However, a number of studies reported that the generation of free radicals during food processing or storage can react with myoglobin or hemoglobin, resulting in undesirable color (*10* and *11*). As oxygen from air comes in contact with the exposed meat surfaces it binds to the myoglobin molecule to form oxymyoglobin. This pigment gives fresh meat its bright red color. Upon irradiation, the free binding site (FBS) reacts instead with free radicals such as hydroxyl (•OH) and sulfuryl (•SH) radicals to form metmyoglobin and sulfmyoglobin, respectively (Figure 1). Metmyoglobin is associated with the brown color of meat and sulfmyoglobin is responsible for green color (*12*).

Food antioxidant additives possess scavenging properties for free radicals (*13*). Decker and Xu (*14*) recently reviewed the potential of endogenous and exogenous antioxidant compounds that can be added to muscle food to minimize rancidity and discoloration. That review included α-tocopherol, carotenoids, phenolic compounds from plant extracts, ascorbate, and ascorbic acid. Schaefer et al. (*15*) found that intravenous infusions of ascorbic acid immediately before harvest led to greater stability of oxymyoglobin in skeletal muscle and less discoloration. Furthermore, ascorbic acid, like many other edible organic acids, has been reported to inhibit the growth of many meatborne pathogenic and spoilage bacteria (*16*−*18*). The objective of this study was to evaluate the effect of ascorbic acid incorporation before irradiation treatment on bacterial growth, color coordinates L*, a*, and b*, and sensory characteristics of ground beef. Color measurements were also performed on samples contain-

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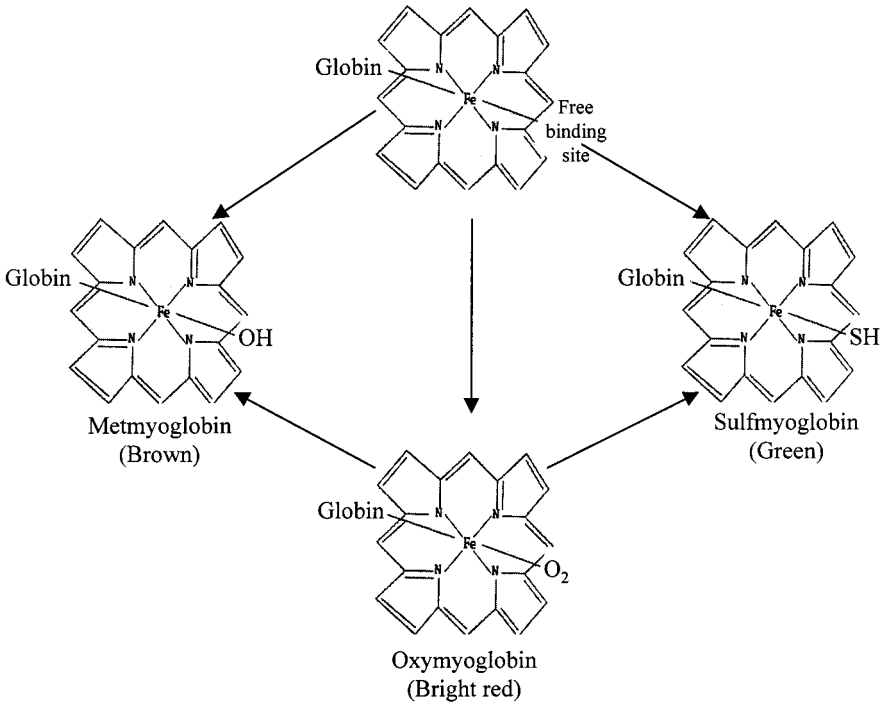


**Figure 1.** Simplified structure of myoglobin showing the free binding site involved in various reactions with free radicals.

ing citric acid at the same pH. This experiment was done in order to differentiate the effect of ascorbic acid from that of the pH reduction.

## MATERIALS AND METHODS

**Samples Preparation.** Fresh lean beef patties containing 23% fat were purchased at a local grocery store (IGA, Laval, Québec, Canada) and transported to the Canadian Irradiation Center (CIC) under refrigerated conditions in an ice-filled thermal container. The beef patties were divided into five separate batches of 0.5 kg each, and ascorbic acid (Sigma Chemicals, St. Louis, MO) was added to final concentrations of 0 (control), 0.03, 0.10, 0.30, and 0.50% (w/w). The total amount of ascorbic acid was first dissolved in 7.5 mL of sterile distilled water. The ascorbic acid solution was then incorporated into the ground beef and mixed in a Kenwood food blender (Kenwood Small Appliance Ltd, New Lane, UK). For microbiological analysis, patties weighing 10 g were prepared and packed individually into oxygen-permeable WIN68 bags (Winpak Technologies Inc., St-Léonard, Québec, Canada). For color and sensory analysis, patties were prepared in the same manner, but samples weighed 30 ± 3 g. Samples were irradiated at a total dose of 0.0, 0.5, 1.0, 2.0, 3.0, and 4.0 kGy in a $^{60}$Co underwater calibrator unit (UC-15; MDS Nordion, Kanata, Ontario, Canada), with a mean dose rate of 18.83 kGy/h. The irradiator was certified by the National Institute of Standards and Technology (Gaithersburg, MD), and dose rate was established using a correction for decay of source. Beef samples were evaluated for bacterial, color, and sensory parameters immediately after the irradiation treatment (day 1), and on days 3, 5, or 7 during storage at 4 ± 1 °C.

Comparison with samples containing citric acid instead of ascorbic acid at the same pH was performed to discriminate the pH effect from other functions of the molecule. The pH of the meat was adjusted with citric acid (Sigma Chemicals, St. Louis, MO) to the same pH of meat containing ascorbic acid (0.5%), using a model 420A Orion pH meter (Orion Research Inc., Boston, MA). Beef patties without acids served as negative control. At day 1, the pH of beef patties containing 0.5% ascorbic acid was 5.1, compared to 5.9 for control samples. At day 1, samples were irradiated at a mean dose of 2 or 4 kGy as described above and were stored at 4 ± 1 °C for 7 days. Color coordinates L*, a*, and b*, and hue angle values were determined at 1, 3, 5, and 7 days.

**Microbiology.** Ten grams of each sample was homogenized using a Lab-blender 400 stomacher (Laboratory Equipment, London, UK) in 90 mL of sterile peptone water (Bacto-peptone, Difco Laboratories, Detroit, MI). Further serial dilutions were prepared and spread-plated on a sterile Petri plate for numeration of total aerobes (APCs) using Plate Count Agar (PCA, Difco Laboratories, Detroit, MI); plates were incubated at 35 ± 1 °C for 48 h. For the numeration of total coliforms, 0.1 or 1 mL of appropriate dilutions were poured-plated with melted violet red bile agar (VRBA, Difco Laboratories, Detroit, MI); plates were incubated at 35 ± 1 °C for 24 h.

**Color Analysis.** Color measurements were performed on unwrapped patties using a Colormet (Instrumar Ltd, Newfoundland, Canada) using the CIELAB system. Measurements were taken three times on each sample 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 was calculated, where hue angle = $\tan^{-1}$ (b/a). Low hue values in meat samples are indicative of more red color and less yellow color.

**Sensory Analysis.** The influence of the combined effect of ascorbic acid and irradiation on the sensory characteristics of beef patties was evaluated. Control samples (without ascorbic acid) and samples containing ascorbic acid (0.5%, w/w) were irradiated at 0, 1, 2, 3, and 4 kGy. Afterward, samples were assessed by a panel of 30 members for the evaluation of odor and taste. Two samples corresponding to two different treatments (unirradiated and irradiated) were evaluated individually by each panelist at each session. Samples for taste anlysis were cooked to an internal temperature of 75 °C for 30 min using a model 71A electric cooker (General Electric, Mississauga, ON) set at 425 °F. Samples were served warm. The overall procedure was performed according to the nine points hedonic scale described by Larmond (*19*). On this hedonic scale, a score of 1 represented attributes most disliked and a score of 9 represented attributes most liked. The experiment was replicated two times.

**Statistical Analysis.** The experiment on APCs and total coliforms was a 2 × 5 × 5 factorial design with 2 patty treatments (0 and 0.5% ascorbic acid), 5 irradiation dose levels (0, 1, 2, 3, and 4 kGy), and 5 storage times (1, 3, 5, 7, and 10 days). The experiment was replicated three times and duplicate measurements were taken on each sample. The effects of ascorbic acid and irradiation on color parameters was a 3 × 3

**Table 1. Summarized Results of Variance Analysis Showing the Significance of Simple Effects and Combined Effects of Ascorbic Acid, Irradiation Dose, and Storage Time on APCs and *Enterobacteriaceae***

| | | P (F > Fcal) | |
|---|---|---|---|
| | DF | APCs | *Enterobacteriaceae* |
| ascorbic acid | 1 | < 0.001 | < 0.001 |
| irradiation | 4 | < 0.001 | < 0.001 |
| time | 4 | < 0.001 | < 0.001 |
| ascorbic × irradiation | 4 | 0.018 | < 0.001 |
| ascoribic × time | 4 | 0.214 | < 0.001 |
| irradiation × time | 16 | < 0.001 | < 0.001 |
| ascorbic × irradiation × time | 16 | 0.084 | < 0.001 |

× 4 factorial design with 3 patty treatments (control, ascorbic acid, and citric acid), 3 irradiation dose levels (0, 2, and 4 kGy), and 4 storage times (1, 3, 5, and 7 days). The experiment was replicated two times and triplicate measurements were taken for each sample. To evaluate the difference between different irradiation doses and ascorbic acid concentrations, analysis of variance (ANOVA) was applied using the GLM procedure of the SAS statistical package (SAS Institute, Cary, NC). The Duncan multiple-range test was used to determine significant differences between treatments. Student's *t* test was used to differentiate samples containing ascorbic acid from those containing citric acid. Differences between means were considered significant when $p \leq 0.05$.

## RESULTS

**Microbiology.** Results of variance analysis showed significant ($p \leq 0.05$) simple effects of ascorbic acid, irradiation, or storage time on APCs and total coliforms (Table 1). All the combined effects were also significant except ascorbic acid × time and ascorbic acid × irradiation × time on total APCs. All the irradiation doses resulted in significant ($p \leq 0.05$) reductions of total APCs in both samples without or with ascorbic acid (Table 2). Irradiation at a level of 1 kGy after 1 day storage produced a 1.78 log unit reduction of APCs in samples without ascorbic acid and a reduction of 3.77 log units in samples containing ascorbic acid. For irradiation doses ≥2 kGy, bacterial growth was below the detection level after 1 day of storage. Lag periods before the initiation of bacterial growth were 5 and 7 days for samples irradiated at 3 and 4 kGy, respectively. In the presence of ascorbic acid, lag periods were 5 days for samples irradiated at 2 kGy and 7 days for those irradiated at 3 and 4 kGy. A synergistic effect was observed between irradiation and the presence of ascorbic acid for samples treated at doses of 2 and 3 kGy.

Total coliforms count in unirradiated samples was 4.30 log CFU/g on day 1 and reached 6.39 log CFU/g after 10 days of storage (Figure 2). The addition of ascorbic acid reduced total coliform counts by 2 log units, and this effect was maintained for 3 days. The combination of ascorbic acid and irradiation at 1 kGy resulted in a colony count below detection level during 7 days. In the absence of ascorbic acid, the same irradiation dose (1 kGy) resulted in a colony count below detection level for only 5 days. Our results also showed that a dose of 2 kGy was sufficient to eliminate the growth of total coliforms during all the experiment period (results not shown).

**Color Analysis.** The effect of irradiation treatment and ascorbic acid addition on the color coordinates L*, a*, and b* of beef patties was also evaluated. Results are shown in Table 3.

*Lightness (L*).* In samples without ascorbic acid and in samples containing 0.03 and 0.3% of ascorbic acid, the lightness of beef patties was significantly reduced ($p \leq 0.05$) by irradiation treatment at doses ≥2kGy. At 0.5% of ascorbic acid, no significant difference was observed between control and irradiated samples, regardless of the dose of irradiation. Moreover, addition of ascorbic acid at 0.5% increased significantly ($p \leq 0.05$) the L* value in unirradiated samples.

*Redness (a*).* The redness (a* values) also decreased with increase of irradiation dose (Table 3). Addition of ascorbic acid at a level of 0.5% also decreased significantly the a* values. The a* value of unirradiated samples without ascorbic acid was 15.38 as compared to 13.22 for that of unirradiated samples containing 0.5% of ascorbic acid. When 0.5% of ascorbic acid was added to the samples, irradiation treatment at 4 kGy reduced the a* to 10.22. However, this value (10.22) was not significantly different ($p > 0.05$) from the value obtained for samples without ascorbic acid and irradiated at the same dose (11.07). Results also showed that addition of 0.1% of ascorbic acid increased significantly the redness of irradiated beef patties. After addition of 0.1% ascorbic acid, a* value increased from 11.30 to 15.35 for samples irradiated at 1 kGy and from 11.07 to 12.83 samples irradiated at 4 kGy.

*Yellowness (b*).* Similarly to the color coordinates L* and a*, the yellowness (b*) decreased with irradiation and increased when ascorbic acid was added to the samples at concentrations ≤0.3% (w/w). In unirradiated beef patties, b* values increased from 20.18 to 21.85 when ascorbic acid concentration increased from 0 to 0.3%. Incorporation of ascorbic acid before irradiation also produced a significant ($p \leq 0.05$) increase of meat yellowness, regardless of the irradiation dose. A maximum b* value was obtained by addition of ≤0.3% of ascorbic acid. The values observed in unirradiated samples containing ascorbic acid were around 22. Nevertheless, the values between 20 and 21 could be maintained in irradiated samples by the addition of ascorbic acid.

*Discriminating Intrinsic Ascorbic Acid Effect from pH Effect.* Results showing the comparative effects of ascorbic acid and citric acid treatment on the color coordinates (L*, a*, and b*) of beef patties are shown in Figure 3. In general, incorporation of ascorbic acid did not affect significantly the lightness of beef patties when irradiated at 2 or 4 kGy. No significant difference ($p > 0.05$) was observed between samples without acid and those containing ascorbic or citric acids during storage of irradiated samples. Regardless of the dose of irradiation, the L* values remained stable (approximately 45) until day 7. However, for unirradiated samples L* values were stable until day 5 of storage and decreased quickly to approximately 40 on day 7.

For unirradiated samples, the addition of 0.5% ascorbic acid increased the a* value only during the first 3 days of storage. At day 7 of storage, no significant difference ($p > 0.05$) was observed between samples without acid and those containing ascorbic or citric acid. Results were quite different for irradiated samples. Incorporation of ascorbic acid permitted stabilization of the a* values of beef patties during irradiation and storage under refrigeration. At day 1, the a* value recorded for fresh unirradiated beef patties without ascorbic acid was 17.20, and it decreased to 14.80 after 7 days of storage. During the same storage period, a* values of samples containing ascorbic acid remained at 16.47 and 15.60 after irradiation at 2 and 4 kGy,

**Table 2. Aerobic Plate Counts of Irradiated Beef Patties Containing 0.5% (w/w) of Ascorbic Acid at 4 °C[a,b]**

| | | irradiation doses (kGy) | | | | |
|---|---|---|---|---|---|---|
| time (d) | treatment | 0 | 1 | 2 | 3 | 4 |
| 1 | control | $5.53 \pm 0.13_a$ | $3.75 \pm 0.09_b$ | $0.65 \pm 0.12_c$ | $Bdl_d$[c] | $Bdl_d$ |
| | ascorbic acid | $4.16 \pm 0.08_a$* | $1.76 \pm 0.28_b$* | $Bdl_c$* | $Bdl_c$ | $Bdl_c$ |
| 3 | control | $5.71 \pm 0.36_a$ | $3.79 \pm 0.49_b$ | $2.58 \pm 0.38_c$ | $Bdl_d$ | $Bdl_d$ |
| | ascorbic acid | $4.97 \pm 0.14_a$* | $1.86 \pm 0.10_b$* | $Bdl_c$* | $Bdl_c$ | $Bdl_c$ |
| 5 | control | $5.98 \pm 0.30_a$ | $5.10 \pm 0.20_b$ | $4.23 \pm 0.18_c$ | $2.32 \pm 0.30_d$ | $Bdl_e$ |
| | ascorbic acid | $6.05 \pm 0.28_a$ | $3.68 \pm 0.32_b$* | $3.50 \pm 0.05_c$* | $Bdl_d$* | $Bdl_d$ |
| 7 | control | $9.39 \pm 0.40_a$ | $4.63 \pm 0.30_b$ | $4.61 \pm 0.20_b$ | $4.23 \pm 0.22_b$ | $2.99 \pm 0.14_c$ |
| | ascorbic acid | $8.49 \pm 0.27_a$ | $4.60 \pm 0.04_b$ | $4.77 \pm 0.37_b$ | $3.82 \pm 0.11_c$ | $1.11 \pm 0.10_d$ |
| 10 | control | ND[d] | $5.14 \pm 0.25_a$ | $4.83 \pm 0.10_a$ | $4.94 \pm 0.30_a$ | $4.37 \pm 0.23_a$ |
| | ascorbic acid | ND | $4.96 \pm 0.32_a$ | $4.99 \pm 0.29_a$ | $4.73 \pm 0.28_a$ | $3.91 \pm 0.38_a$ |

[a] In the same line, means with identical letters are not significantly different ($p > 0.05$), $n = 6$. [b] At different storage times and irradiation doses, APCs means of ascorbic acid treated samples followed by asterisks are significantly lower ($p \leq 0.05$) than the corresponding control samples. [c] Bdl = below detection level (10 bacterial cells/g). [d] ND = not determined.

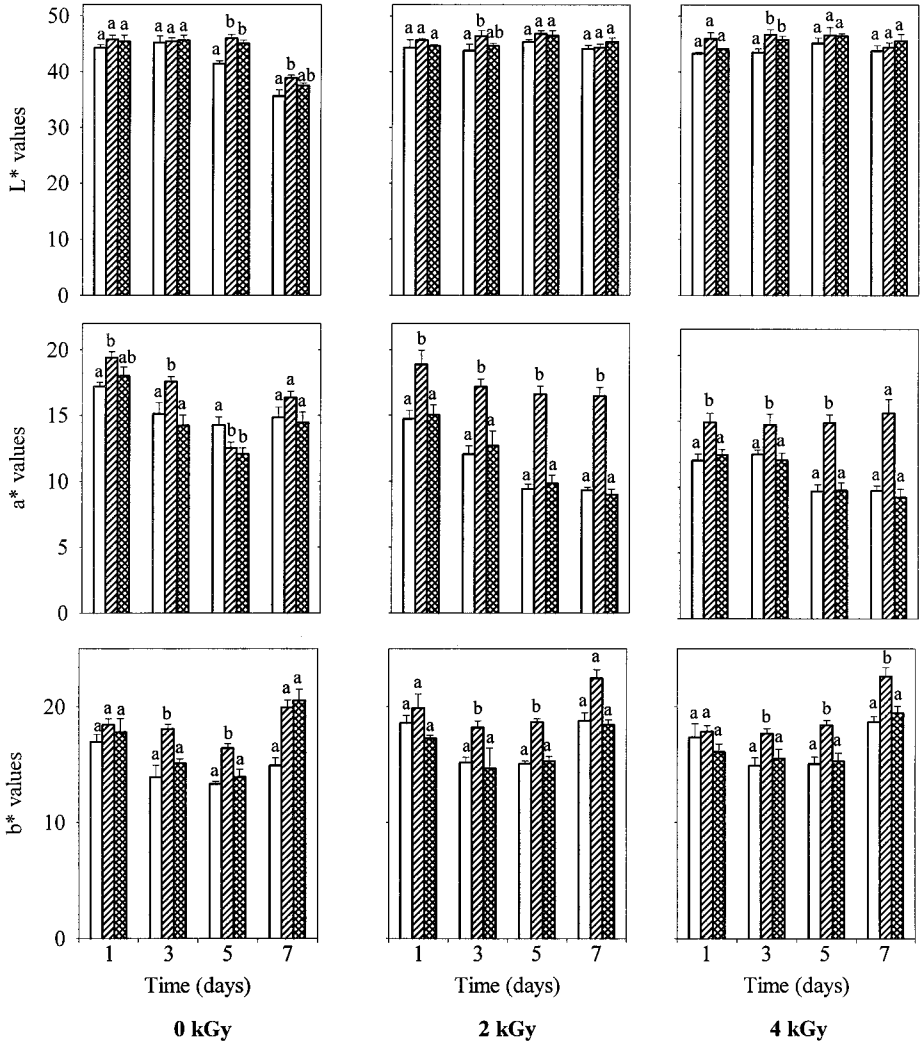


**Figure 2.** Effect of citric and ascorbic acids on the color coordinates L*, a*, and b* of beef patties. Meat samples were unirradiated (0 kGy) or irradiated at 2 or 4 kGy. Open bar, control (without acid); //// bar, ascorbic acid; xxx bar, citric acid. Means with different superscripts are significantly different ($p \leq 0.05$).

respectively. In contrast, a* values of both samples without ascorbic acid and those containing citric acid decreased continuously to reach values as low as 9.00 at the end of the storage. Similarly to redness, yellowness of beef patties was also retained with addition of ascorbic acid. Over all the experimental period, b* values of samples containing ascorbic acid were significantly higher ($p \leq 0.05$) than those of samples without acid and samples containing citric acid.

Figure 4 shows hue angle values of samples treated at 0, 2, and 4 kGy during storage. This figure demonstrated that hue angle increased, and thus the redness decreased, during storage. At 0 kGy, and after 7 days of storage, hue angle values of samples with ascorbic acid (72.30) were significantly ($p \leq 0.05$) higher than those of samples without acid (53.32) or containing citric acid (54.91). However, when samples were irradiated at 2 or 4 kGy, and after 3 d of storage, hue angle values of samples containing ascorbic acid were significantly lower and more red than values of samples without acid or with citric acid. At day 7, the hue angle values of samples containing ascorbic acid (53.72−54.91) were

**Table 3. Influence of Irradiation and Ascorbic Acid Concentration on the Color Coordinates L*, a*, and b* in Beef Patties[a,b]**

| | | ascorbic acid concentration | | | | |
|---|---|---|---|---|---|---|
| | kGy | 0 | 0.03 | 0.1 | 0.3 | 0.5 |
| L* | 0.0 | $45.02 \pm 1.51_{a}^{(1)}$ | $46.25 \pm 0.86_{bc}^{(1)}$ | $45.93 \pm 0.77_{ab}^{(1,2)}$ | $46.32 \pm 0.44_{bc}^{(1,2)}$ | $47.42 \pm 1.10_{c}^{(1)}$ |
| | 0.5 | $45.62 \pm 1.28_{a}^{(1)}$ | $46.25 \pm 1.41_{a}^{(1)}$ | $45.93 \pm 1.52_{a}^{(1)}$ | $46.23 \pm 0.43_{a}^{(1)}$ | $45.75 \pm 2.30_{a}^{(2)}$ |
| | 1.0 | $44.50 \pm 0.45_{a}^{(1,2)}$ | $45.43 \pm 0.67_{a}^{(2)}$ | $44.65 \pm 1.00_{a}^{(2)}$ | $46.88 \pm 0.63_{b}^{(2)}$ | $46.73 \pm 1.04_{b}^{(1,2)}$ |
| | 2.0 | $44.31 \pm 1.44_{a}^{(3)}$ | $44.27 \pm 0.64_{b}^{(3)}$ | $44.53 \pm 0.37_{b}^{(2,3)}$ | $45.27 \pm 0.27_{c}^{(3)}$ | $45.67 \pm 0.27_{c}^{(2)}$ |
| | 3.0 | $43.27 \pm 1.39_{a}^{(2)}$ | $43.52 \pm 0.66_{a}^{(4)}$ | $45.02 \pm 1.47_{b}^{(1,2,3)}$ | $44.80 \pm 0.42_{b}^{(3)}$ | $46.00 \pm 1.26_{c}^{(1,2)}$ |
| | 4.0 | $43.25 \pm 2.31_{a}^{(4)}$ | $44.32 \pm 0.39_{b}^{(3,5)}$ | $43.33 \pm 1.13_{c}^{(3)}$ | $43.25 \pm 0.77_{c}^{(4)}$ | $45.88 \pm 1.13_{d}^{(1,2)}$ |
| a* | 0.0 | $15.38 \pm 0.96_{a}^{(1)}$ | $15.20 \pm 0.76_{a}^{(1)}$ | $16.05 \pm 0.97_{a}^{(1)}$ | $15.42 \pm 0.38_{a}^{(1)}$ | $13.22 \pm 0.73_{b}^{(1)}$ |
| | 0.5 | $11.92 \pm 0.93_{ac}^{(2,3)}$ | $15.12 \pm 0.57_{b}^{(1)}$ | $15.97 \pm 1.61_{b}^{(1)}$ | $13.93 \pm 0.38_{ac}^{(2)}$ | $12.97 \pm 2.39_{c}^{(1)}$ |
| | 1.0 | $11.30 \pm 0.63_{a}^{(2)}$ | $13.95 \pm 0.75_{b}^{(2)}$ | $15.35 \pm 1.19_{c}^{(1,2)}$ | $12.10 \pm 1.04_{a}^{(3)}$ | $11.90 \pm 0.85_{a}^{(1,2,3)}$ |
| | 2.0 | $12.35 \pm 0.14_{a}^{(3)}$ | $13.77 \pm 0.45_{b}^{(2,3)}$ | $14.43 \pm 0.25_{c}^{(1)}$ | $12.90 \pm 0.38_{d}^{(4)}$ | $10.88 \pm 0.51_{e}^{(2,3,4)}$ |
| | 3.0 | $11.23 \pm 1.36_{a}^{(2)}$ | $13.22 \pm 0.71_{b}^{(3)}$ | $12.42 \pm 0.18_{c}^{(3)}$ | $11.80 \pm 0.32_{a}^{(3)}$ | $9.82 \pm 1.16_{d}^{(3,4)}$ |
| | 4.0 | $11.07 \pm 0.46_{a}^{(2)}$ | $11.08 \pm 0.31_{a}^{(3)}$ | $12.83 \pm 1.68_{b}^{(3)}$ | $11.65 \pm 0.20_{c}^{(3)}$ | $10.22 \pm 1.82_{a}^{(4)}$ |
| b* | 0.0 | $20.18 \pm 1.35_{a}^{(1)}$ | $22.15 \pm 0.65_{b}^{(1)}$ | $21.85 \pm 0.39_{b}^{(1)}$ | $21.85 \pm 0.33_{b}^{(1)}$ | $19.73 \pm 1.00_{a}^{(1)}$ |
| | 0.5 | $19.78 \pm 0.98_{a}^{(1)}$ | $21.75 \pm 0.60_{b}^{(1,2)}$ | $21.67 \pm 1.21_{b}^{(1)}$ | $21.07 \pm 0.73_{b}^{(2,5)}$ | $19.93 \pm 0.96_{a}^{(1)}$ |
| | 1.0 | $18.63 \pm 0.82_{a}^{(2)}$ | $21.02 \pm 0.87_{bc}^{(2,4)}$ | $21.18 \pm 0.98_{b}^{(1,2)}$ | $20.43 \pm 0.74_{bc}^{(3,5)}$ | $20.00 \pm 1.19_{c}^{(1)}$ |
| | 2.0 | $17.42 \pm 0.40_{a}^{(3)}$ | $19.85 \pm 1.38_{b}^{(3,4)}$ | $21.00 \pm 0.23_{c}^{(1,2)}$ | $20.35 \pm 0.45_{bc}^{(3,4,5)}$ | $20.78 \pm 0.56_{c}^{(1)}$ |
| | 3.0 | $18.37 \pm 1.48_{a}^{(2,3)}$ | $19.17 \pm 1.47_{ab}^{(3,4)}$ | $20.18 \pm 1.19_{b}^{(2)}$ | $19.80 \pm 0.25_{b}^{(4)}$ | $19.78 \pm 0.75_{b}^{(1)}$ |
| | 4.0 | $18.07 \pm 0.34_{a}^{(2,3)}$ | $20.25 \pm 0.69_{b}^{(4)}$ | $19.90 \pm 1.27_{b}^{(2)}$ | $20.67 \pm 0.44_{b}^{(5)}$ | $19.55 \pm 2.88_{ab}^{(1)}$ |

[a] In the same line, means with identical letters are not significantly different ($p > 0.05$). [b] In the same column, means with identical numbers are not significantly different ($p > 0.05$). $n = 6$.

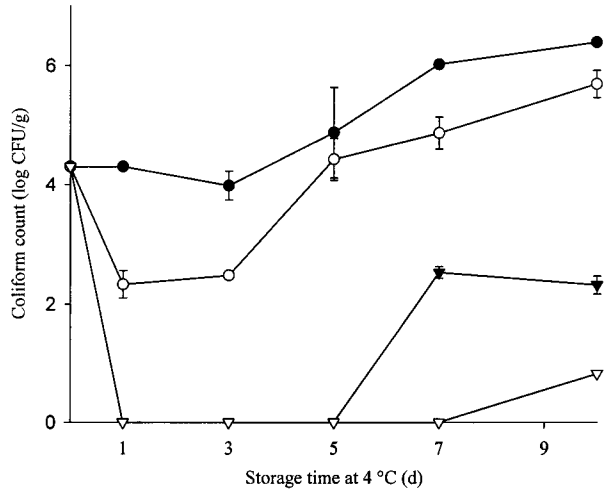


**Figure 3.** Synergistic effect of ascorbic acid and irradiation (1 kGy) on the growth of total coliforms in beef patties. ● Unirradiated without acid; ○ unirradiated with acid; ▼ irradiated without acid; ▽ irradiated with acid.

significantly lower than those of samples without acid (63.61−62.49) or with citric acid (63.99−64.65).

**Sensory Analysis.** Table 4 shows the results of sensory evaluation. The effects of irradiation and addition of ascorbic acid at a level of 0.5% (w/w) on the odor and the taste are presented. Regardless of the irradiation dose used in the experiment, no significant difference ($p > 0.05$) was found between samples without ascorbic acid and those containing ascorbic acid. All the results ranged from 5 (not good, nor bad) to 6 (moderately good). There was no evidence of detrimental effect of irradiation treatment (dose ≤ 4 kGy) and addition of ascorbic acid on sensory characteristic of beef patties. In many cases, however, odor and taste scores of samples containing ascorbic acid appeared slightly lower than those for samples without acid.

## DISCUSSION

The reduction of APCs and total coliforms by the addition of ascorbic acid in beef patties is consistent with previous reports on the antimicrobial properties of

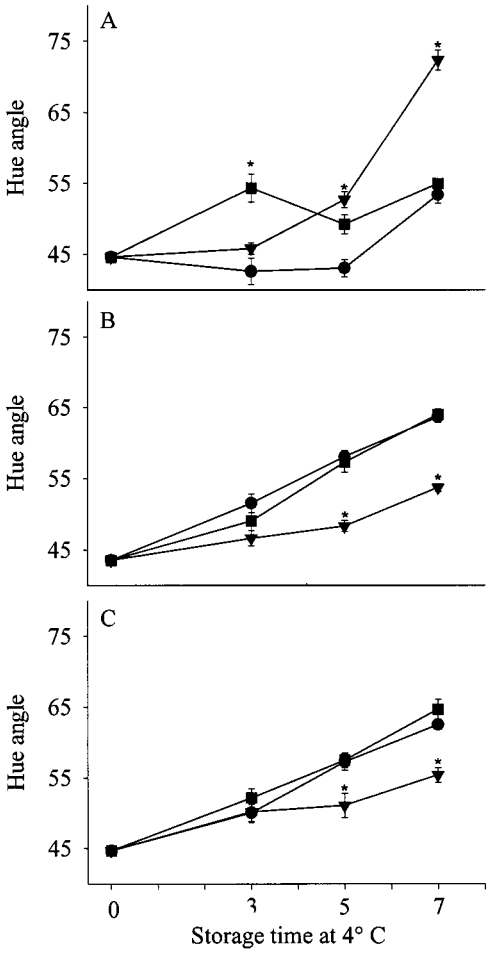


**Figure 4.** Effect of citric and ascorbic acids on the hue angles of beef patties unirradiated (A) or irradiated at 2 (B) or 4 (C) kGy. ● Control (without acid); ▼ ascorbic acid; ■ citric acid.

organic acids against meat spoilage and pathogenic bacteria (*16*−*18*, *20*). The antimicrobial action of ascorbic acid may be due mainly to the reduction of the pH of the beef patties (0.8 unit reduction). The reduction of the pH of ground beef (0.8 unit reduction) may have increased the proportion of uncharged molecules and at least facilitated the contact with bacterial cells. Also,

**Table 4. Sensory Evaluation Scores of Irradiated Ground Beef with or without Ascorbic Acid (0.5% w/w, final concentration)[a]**

| | odor | | taste | |
|---|---|---|---|---|
| kGy | with acid | without acid | with acid | without acid |
| 0 | 5.13 ± 1.36 | 6.24 ± 1.55 | 4.97 ± 1.22 | 6.30 ± 1.40 |
| 1 | 5.37 ± 1.30 | 6.27 ± 1.01 | 5.80 ± 1.10 | 6.17 ± 1.34 |
| 2 | 5.50 ± 1.25 | 6.17 ± 1.37 | 5.60 ± 1.16 | 6.33 ± 0.99 |
| 3 | 5.77 ± 1.30 | 6.03 ± 1.33 | 5.93 ± 1.51 | 5.93 ± 1.34 |
| 4 | 5.77 ± 1.10 | 6.57 ± 1.22 | 6.07 ± 1.51 | 6.47 ± 1.57 |

[a] No significant difference was found between control samples (without ascorbic acid) and samples containing 0.5% ascorbic acid. $n = 60$.

on the basis of the scanvenging properties of ascorbic acid (*4* and *13*), the inhibitory effect could be attributed to its ability to bind to critical compounds such as metal ions, sulfhydryl, and amino groups of proteins. These compounds are generally associated with transport of nutrient and membrane functions in bacteria.

Results showed that the combination of ascorbic acid and gamma irradiation resulted in enhanced antibacterial effects compared to those of control samples, with significant ($p \leq 0.05$) interaction on both APCs and total coliforms. This result confirms those obtained by Farkas and Andrassy (*21*) which indicated that a combination of irradiation and pH reduction by ascorbic acid reduced microbial growth in meat products even at abused temperatures. Lee et al., (*22*) also observed a synergistic effect of irradiation and naturally occurring antioxidants such as ascorbyl palmitate, α-tocopherol, and $\beta$-carotene on the microbial stability of beef patties. The results presented in this study are also in agreement with the literature showing greatest radiation sensitivity of gram$^-$ bacteria compared to gram$^+$ bacteria. Irradiation doses required to obtain complete inhibition of bacterial growth for 7 d were 2 kGy for total coliforms and 3 kGy for APCs. Similarly, Lefebvre (*23*) reported $D_{10}$ values of 0.035 kGy for *Pseudomonas fluorescens* and 1.827 kGy for *Staphylococcus sp*.

Irradiation as a method of meat preservation is known to possess excellent potential to improve meat safety and to extend shelf life (*24*). However, irradiation may generate ionizing substances which can react with sensitive pigments such as myoglobin and modify meat color. Zhao and Sebranek (*2*) reported significant decreases in L* and b* values of pork chops irriadiated at 1 kGy.

The use of ascorbic acid to protect meat color during industrial processing is well documented (*1*, *25*−*27*). From these studies, it appears that ascorbic acid can increase color stability of both intact cuts of beef and beef patties. Recently, Lee et al. (*4*) incorporated ascorbic acid in ground beef to a final concentration of 0.1% (w/v) and found significant inhibition of metmyoglobin formation and resulting brown color development on the surface. Our results of color analysis are consistent with these previous findings because the color coordinates a* (redness) and b* (yellowness) we recorded were significantly ($p \leq 0.05$) higher when ascorbic acid was added to the formulations. Also, in this study, a significant reduction ($p \leq 0.05$) of Hue angle values found in meat samples containing ascorbic acid is indicative of more red color, as previously reported by Berry (*3*). Many reasons have been suggested to explain this effect, particularly the ability of ascorbic acid to act with naturally occurring tocopherols in restricting formation of peroxides and to act as an oxygen scavenger (*1*, *13*, *28*).

As ascorbic acid has a great affinity of free radicals, it could be used as an alternative means to prevent pigment oxidation. As indicated by Mitsumoto et al. (*13*), ascorbic acid functions as an antioxidant with some substrates by scavenging oxygen and inhibiting radical formation at double bonds. Based on that hypothesis, Zhao and Sebranek (*2*) dipped pork meat in a solution of sodium ascorbate (550 ppm) prior to irradiation and showed that color parameters were stabilized. Similar results were obtained in our study relative to the effect of ascorbic acid on color parameters, where a* and b* values were stabilized during storage of irradiated ground beef under refrigeration conditions. Furthermore, our results indicated that the effect of ascorbic acid on color stabilization was not due to a pH reduction, because no red color stabilization was observed with citric acid at a similar pH.

Also, scores for taste and odor were not significantly ($p \leq 0.05$) affected by addition of ascorbic acid. Results obtained in the present study for odor and taste did not show a significant ($p > 0.05$) difference between samples containing ascorbic acid and control samples. An addition of ascorbic acid at a concentration of 0.5% (w/w) seems to be adequate to maintain the color stability during storage.

## CONCLUSION

The present study demonstrated that incorporation of ascorbic acid in beef patties before irradiation treatment stabilized the color parameters during storage. Redness (a*) and yellowness (b*) were significantly higher ($p \leq 0.05$) when ascorbic acid was added to meat samples and the effect was not due to a reduction of pH. A concentration of 0.5% of ascorbic acid was needed to reduce by 1 log the APCs in unirradiated samples. A synergistic effect was also found between addition of ascorbic acid and irradiation on microbial growth. Also, it appears that ascorbic acid can be used in concentrations up to 0.5% (w/w) without detrimental effects on sensory characteristics (taste and odor). The use of ascorbic acid in combination with irradiation increased the shelf life of ground meat without any detrimental effect on the color.

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## LITERATURE CITED

(1) Demos, B. P.; Gerrard, R. W.; Mandigo, X.; Tan, J. Mechanically recovered neck bone lean and ascorbic acid improve color stability of ground beef patties. *J. Food Sci.* **1996**, *61*, 656−659.

(2) Zhao, Y.; Sebranek, J. G. Physicochemical and sensory qualities of fresh pork chops as affected by phosphate, ascorbate or sorbate dipping prior to irradiation. *J. Food Sci.* **1996**, *61*, 1281−1284.

(3) Berry, B. W. Color of cooked beef patties as influenced by formulation and final internal temperature. *Food Res. Int.* **1997**, *30*, 473−478.

(4) Lee, B. J.; Hendricks, D. G.; Cornforth, D. P. A comparison of carnosine and ascorbic acid on color and lipid stability in a ground beef pattie model system. *Meat Sci.* **1999**, *51*, 245−253.

(5) Renerre, M.; Labadie, J. Fresh meat packaging and meat quality. Review Paper. *Proc. Int. Congr. Meat Sci. and Technol.* **1993**, *39*, 361−387.
(6) Dogbevi, M. K.; Vachon, C; Lacroix, M. Physicochemical and microbiological changes in irradiated fresh pork loins. *Meat Sci.* **1999**, *51*, 349−354.
(7) Giroux, M.; Lacroix, M. Nutritional adequacy of irradiated meat − a review. *Food Res. Int.* **1998**, *31*, 257−264.
(8) Fu, A.-H.; Sebranek, J. G.; Murano, E. A. Survival of *Listeria monocytogenes*, *Yersinia enterocolitica* and *Escherichia coli* O157:H7 and quality changes after irradiation of beef steaks and ground beef. *J. Food Sci.* **1995**, *60*, 972−977.
(9) Fu, A.-H.; Sebranek, J. G.; Murano, E. A. Survival of *Listeria monocytogenes* and *Salmonella typhimurium* and quality attributes of cooked pork shops and cured ham after irradiation. *J. Food Sci.* **1995**, *60*, 1001−1005, 1008.
(10) Kamarei, A. R.; Karel, M.; Wierbicki, E. Spectral studies on the role of ionizing radiation in color changes of rappertized beef. *J. Food Sci.* **1979**, *44*, 25−31.
(11) Satterlee, L. D.; Duane Brown, W.; Lycometros, C. Stability and characteristics of the pigment produced by gamma irradiation of metmyoglobin. *J. Food Sci.* **1972**, *37*, 213−217.
(12) Judge, M. D. Principles of Meat Science, 2nd ed. Kendall/Hunt: Dubuque, IA, 1989.
(13) Mitsumoto, M.; Cassen, R. G.; Scheafer, D. M.; Arnold, R. N.; Scheller, K. K. Improvement of color and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatments. *J. Food Sci.* **1991**, *56*, 1489−1492.
(14) Decker, E. A.; Xu, Z. Minimizing rancidity in muscle foods. *Food Technol.* **1998**, *52*, 54−59.
(15) Schaefer, D. M.; Liu, Q.; Yin, M. C. Supranutritional administration of vitamins E and C improves oxidative stability of beef. *J. Nutr.* **1995**, *125*, 1792−1798.
(16) Ahamad, N.; Marth, E. H. Behavior of *Listeria monocytogenes* at 7, 13, 21, and 35 °C in tryptose broth acidified with acetic, citric, or lactic acids. *J. Food Prot.* **1989**, *52*, 688−695.
(17) Dickson, J. S.; Anderson, M. E. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. *J. Food Prot.* **1992**, *55*, 133−140.
(18) Ouattara, B.; Simard, R. E.; Holley, R. A.; Piette, G. J.-P.; Bégin, A. Inhibitory effect of organic acids upon meat spoilage bacteria. *J. Food Prot.* **1997**, *60*, 246−253.
(19) Larmond, E. Laboratory methods for sensory evaluation of foods. Research Brand of Agriculture Canada Publication No. 1637. Ottawa, ON, 1977.
(20) Anderson, M. E.; Huff, H. E.; Naumann, H. D.; Marshall, R. T. Counts of six types of bacteria on lamb carcasses dipped or sprayed with acetic acid at 25 °C or 55 °C and stored vacuum packaged at 0 °C. *J. Food Prot.* **1988**, *51*, 874−877.
(21) Farkas, J.; Andrassy, E. Interaction of ionizing radiation and acidulants on the growth of the microflora of a vacuum-packed chilled meat product. *Int. J. Food. Microbiol.* **1993**, *19*, 145−152.
(22) Lee, J.-W.; Yook, H.-S.; Kim, S.-A.; Lee, K.-H.; Byun, M.-W. Effects of antioxidants and gamma irradiation on the shelf life of beef patties. *J. Food Prot.* **1999**, *62*, 619−624.
(23) Lefebvre, N. Éssai d'amélioration de la salubrité et de la période de conservation de du bœuf haché à l'aide de rayonnements ionisants. M.Sc. Thesis; Institut Armand-Frappier, Université du Québec, Laval, PQ, 1990.
(24) Dempster, J. F. Radiation preservation of meat and meat products. *Meat Sci.* **1985**, *12*, 61−89.
(25) Counsell, J. N. Meat processing with ascorbic acid. *Process Biochem.* **1971**, *12*, 25−28.
(26) Karasz, A. B.; Maxstaddt, J. J.; Reher, J.; Decocco, F. Preservatives and artificial sweeteners. Rapid screening procedure for the determination of preservatives in ground beef: sulfites, benzoates, sorbates, and ascorbates. *J. AOAC* **1975**, *59*, 766−769.
(27) Kulshrestha, S. A.; Rhee, K. S. Precooked reduced-fat beef patties chemical and sensory quality as affected by sodium ascorbate, lactate and phosphate. *J. Food Sci.* **1996**, *61*, 1052−1057.
(28) Lawrie, R. A. Meat Science. 5th ed.; Pergamon Press: Oxford, 1979.

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