

# Fat content and ascorbic acid infusion influence microbial and physicochemical qualities of electron beam irradiated beef patties

Peter Y.Y. Wong, A. Nilmini Wijewickreme<sup>1</sup>, David D. Kitts<sup>\*</sup>

Food, Nutrition and Health, Faculty of Agricultural Sciences, The University of British Columbia, 6640 N.W. Marine Drive, Vancouver, BC, Canada V6T 1Z4

Received 8 October 2003; received in revised form 6 February 2004; accepted 6 February 2004

## Abstract

The effects of fat content and post-slaughter ascorbic acid (AA) infusion on microbial and physicochemical qualities of beef patties processed by electron beam irradiation were investigated in a 4 °C storage trial. Beef muscles from AA-infused or control animals were ground and mixed with tallow to achieve a final fat content of 4%, 17% and 30%, respectively. Beef patties were irradiated at 5 and 10 kGy with a linear electron beam accelerator. Non-irradiated and non-infused ground beef patties served as a control. The addition of fat significantly ( $p < 0.05$ ) increased aerobic, total coliform, *E. coli*, and psychrotrophic bacteria counts in beef patties during storage. Irradiation at both dosages exerted a pasteurization effect on psychrotrophic bacteria for up to 7 days of storage. No viable aerobic, total coliform, or *E. coli* bacteria were detected in any irradiated beef patties during storage. Physicochemical changes caused by lipid oxidation and surface discoloration of beef patties were significantly ( $p < 0.05$ ) increased by both the addition of fat and irradiation processing. Beef patties made from AA-infused animals did not alter bacterial counts. Instead, post-slaughter infusion of AA exerted a pro-oxidant effect in the beef patties that led to a significant ( $p < 0.05$ ) increase in lipid oxidation and surface discoloration of stored patties.

© 2004 Elsevier Ltd. All rights reserved.

**Keywords:** Electron beam irradiation; Fat content; Ascorbic acid; Psychrotrophic bacteria; Lipid oxidation; Surface discoloration

## 1. Introduction

*Escherichia coli* O157:H7 contamination of beef poses both a food safety and an economic concern for the beef industry. During the past decade, the emergence of *E. coli* O157:H7 in beef products has cost the beef industry an estimated \$242.7 billion dollars in lost sales, resulting in a decrease in beef prices and increased expenditure on food safety procedures. Moreover, an increase in recall costs was also recorded (Kay, 2003). Therefore, there is continuing interest in the study of innovative food safety measures to reduce and eradicate this pathogen from beef.

Irradiation is widely accepted as an effective method for reducing microbial population in meats, thereby improving food safety and extending shelf-life of fresh meats. Roberts and Weese (1998) reported a 1 and 3 log reduction in aerobic plate counts of gamma-irradiated ground beef at 1 and 3 kGy dosage, respectively. In general, low dosages of irradiation, between 2 and 3 kGy, can eliminate up to 2 log cycles of the pathogenic bacteria (e.g., *E. coli* O157:H7 or *Salmonella* spp.) and reduce the number of spoilage bacteria (e.g., pseudomonads or lactic acid bacteria) in ground beef (Radomycki, Murano, Olson, & Murano, 1994). However, the refrigerated shelf-life of irradiated beef was decreased by the addition of fat aimed to create commercial grade of lean (<17%), medium (<23%), and regular (<30%) ground beef (Berry, Wells, Cross, Emswiler, & Muse, 1980). This was attributed to the presence of aerobic bacteria in various anatomical fat trimmings.

Numerous studies have indicated that irradiation-induced lipid oxidation in fresh meats can severely limit

<sup>\*</sup> Corresponding author. Tel.: +1-604-822-5560; fax: +1-604-822-3959.

E-mail address: [ddkitts@interchange.ubc.ca](mailto:ddkitts@interchange.ubc.ca) (D.D. Kitts).

<sup>1</sup> Present address: Cantest Ltd. 4606 Canada Way, Burnaby, BC, Canada V5G 1K5.

the product shelf-life. The generation of hydroxyl radicals in meats from the radiolysis of water in meat muscle cells is responsible for initiating lipid oxidation. In particular, the oxidation of phospholipids in muscle cell membranes contributes to 90% of lipid oxidation in meats (Pikul, Leszczynski, & Kummerow, 1984). However, recent studies by Ahn, Lutz, and Sim (1996, 1998, 1999) and Jo, Lee, and Ahn (1999a, 1999b) have shown that added fat, in the form of triglycerides, will increase lipid oxidation of pork patties and sausages. Therefore, the oxidative stability of fresh meats may be affected by both the phospholipids and triglyceride content and quality, and this can be further exacerbated by irradiation pasteurization treatment (Ahn et al., 1998).

Other studies have shown that the blending of ascorbic acid (AA) into ground beef, at concentrations up to 0.1%, retarded lipid oxidation by 58% in non-irradiated patties (Lee, Hendricks, & Cornforth, 1999) and by 79% in irradiated patties (Nam, Min, Park, Lee, & Ahn, 2003). Increasing AA concentration in meats, up to 0.5%, reduced lipid oxidation by 73% in non-irradiated ground beef (Mitsumoto et al., 1991a). However, topical application of antioxidants, such as dipping beefsteaks in 10% AA solution, only reduced lipid oxidation by 56% (Harbers, Harrison, & Kropf, 1981; Mitsumoto et al., 1991a, 1991b). The lower efficiency of topical application of antioxidant was believed to be due to the inadequate penetration of the antioxidant into the muscle matrix to inhibit oxidation. In contrast, the endogenous administration of AA to cattle decreased surface discoloration caused by the oxidation of oxymyoglobin (Hood, 1975; Wheeler, Koobmaraie, & Shackelford, 1996). More recently, AA infusion to steers was shown to make no improvement in lipid stability in the resulting beef steaks and ground beef (Katsanidis et al., 2003). The effect of deposited AA on lipid oxidation of beef muscles, however, has not been thoroughly investigated.

In general, little information exists on the effects of altering fat content, or infusing AA to animals at slaughter, on the quality of subsequent ground beef products processed by irradiation. The objectives of this study were to determine the microbial and physicochemical properties of electron beam-irradiated ground beef patties formulated with different fat contents (Experiment 1) or derived from cattle infused with ascorbic acid at slaughter (Experiment 2).

## 2. Materials and method

### 2.1. Experiment 1: fat level

#### 2.1.1. Ground beef patties

Ground beef patties were produced from a pool of *semimembranosus*, *semitendinosus*, *biceps femori* and *glu-*

*teus medius* muscles derived from three Hereford x Angus non-pregnant beef cows raised in Agriculture and Agri-Food Canada Research Station in Lacombe, Alberta. All muscles were first trimmed of visible fat and connective tissues, and ground in a Hobart Grinder Model 84142 (Don Mills, ON) using a 3/16 in. plate at 4 °C. Ground beef samples were randomly removed for crude fat analysis by the method of Folch, Lee, and Stanley (1957) and were found to contain, on average, 4% crude fat. Extra lean ground beef (approximately 4% fat) was made by grinding trimmed beef muscles only, whereas lean (approximately 17% fat) and regular (approximately 30% fat) ground beef were made by grinding trimmed beef muscles with added beef tallow to the desired crude fat content. The resulting ground beefs were converted into patties of 150 g in weight, with dimensions of 10.5 cm (diameter) × 1.5 cm (thickness), using a hand-held patty maker. All patties were stored in a ZipLoc™ polyethylene bag and frozen at –30 °C prior to irradiation.

#### 2.1.2. Electron beam irradiation

Individually packaged frozen patties were arranged in a single layer to be irradiated at ambient temperature on one side using a 60 kW high capacity high energy (10 MeV) linear electron beam accelerator (Iotron Technologies Inc.; Port Coquitlam, BC) at 5 and 10 kGy. Irradiation dosages were verified by a dosimeter placed on the conveyor belt along with the samples. Non-irradiated samples (i.e., control) were kept at room temperature during the irradiation process. All ground beef patties were returned to storage at 4 °C in individual ZipLoc™ bags for a period of 14 d.

#### 2.1.3. Bacterial analyses

Aerobic plate count, total coliform, *E. coli* and psychrotrophic count were determined from homogenized beef patties serially diluted in 0.1% peptone. 3M Petrifilm Aerobic Count Plate and *E. coli*/Coliform Count Plate (St Paul, MN) were used for the enumeration of aerobic bacteria, *E. coli* and total coliforms after 48 h of incubation at 35 °C. Psychrotrophic bacteria were also enumerated on 3M Petrifilm Aerobic Count Plate after 5 d at 20 °C.

#### 2.1.4. Physicochemical analyses

**2.1.4.1. pH.** The pH values of ground beef patties were determined from a clarified beef homogenate in distilled deionized water (1:10 dilution), at ambient temperature, using an Accumet pH Meter (Fisher Scientific, NJ).

**2.1.4.2. Lipid oxidation.** Lipid oxidation in ground beef patties was assessed by quantifying the production of malondialdehyde (MDA) according to the procedure described in detail by Buege and Aust (1978).

**2.1.4.3. Surface discoloration.** Surface discoloration of ground beef patties was determined by the tristimulus colour system involving the *L*, *a*, *b* colour coordinates. Prior to analysis, ground beef patties was allowed to “bloom” for approximately 1 h at 4 °C and the surface of each patty was scanned twice with a Hunterlab Scan 6000 model spectrophotometer (Hunterlab Associated Laboratories Inc., VA). Positive *a* value from the Hunterlab scan was reported as surface redness.

## 2.2. Experiment 2: L-AA infusion

### 2.2.1. Ascorbic acid (AA) infusion and ground beef patties

Three Hereford x Angus non-pregnant beef cows raised at the Agriculture and Agri-Food Canada Research Station in Lacombe, Alberta, were used for the infusion study. Prior to infusion, all cows were stunned, shackled by both hind legs, raised and exsanguinated according to commercial practices. Afterwards, the abdominal cavity was opened, contents removed, and the carcass was arbitrarily divided into a control half and an infusion half. The femoral vein on the infusion half of each carcass was severed to limit circulation of the infused 17.5 l of 10,100 and 500 mM sodium ascorbate solutions into the respective cow, via the femoral artery at 37 °C under 15 psi pressure. An equal volume of saline solution was infused into the control half under the same conditions to serve as a within-animal control.

Ground beef patties were produced from these animals using a pool of *semimembranosus*, *semitendinosus*, *biceps femori* and *gluteus medius* muscles obtained from the respective sides of each carcass (i.e., control and infused) in the manner described in Experiment 1.

### 2.2.2. Electron beam irradiation

All patties (control and treatment) were irradiated at 5 and 10 kGy with a linear electron beam accelerator as described in Experiment 1.

### 2.2.3. Bacterial analyses

Enumeration of aerobic bacteria, *E. coli*, total coliform, and psychrotrophic bacteria, in all patties, were conducted on 3M Petrifilm Aerobic Count Plate and Total coliform and *E. coli* Count plate under the conditions described in Experiment 1.

### 2.2.4. Physicochemical analyses

pH, lipid oxidation and surface discoloration of all patties were determined as described in Experiment 1.

### 2.2.5. Statistical analyses

A three factorially arranged, 3 (levels of fat) × 3 (dosages of irradiation) × 5 (days of storage) randomized complete block design, with 3 replications serving as a block, was used to evaluate the bacterial and

physicochemical qualities of treated ground beef patties in Experiment 1. A three factorially arranged, 4 (levels of infusion) × 3 (dosages of irradiation) × 5 (days of storage) randomized complete block design, with 3 replications serving as a block, was used to evaluate the bacterial and physicochemical qualities of treated ground beef patties in Experiment 2. Since infusion was not found to be statistically significant ( $p > 0.05$ ) by ANOVA, all three levels of infused materials were pooled together as a single treatment and ANOVA was re-done with 2 levels of infusion (i.e., control and infused). All data are reported as means ± SEM. Treatment effects of fat, infusion, irradiation, storage time and all interactions were analyzed by a three-way ANOVA and significant differences between mean values were determined by the Tukey's test, using the Minitab Statistical Software version 12.0 (MiniTab Inc., PA).

## 3. Results and discussion

### 3.1. Experiment 1

#### 3.1.1. Bacterial analyses

The shelf-life of fresh ground beef is greatly influenced by the level of bacterial contamination in the lean meat and fat tissue used in processing. Beef trimmings, such as diaphragm meat, head meat, and chuck meat, are known to harbour high levels of aerobic and psychrotrophic bacteria (Berry & Chen, 1976; Duitschaver, Bullock, & Arrott, 1977; Field, Smith, Deane, Thomas, & Kotula, 1977). Since the ground beef patties used in this study were made from the same pool of minced muscles, detectable differences in bacterial counts in patties were attributed to the introduction of fatty tissues.

Adipose tissue can support the growth of psychrotrophic bacteria such as *Flavobacterium* (Berry, Smith, & Carpenter, 1973). Although the bacterial contamination in the subcutaneous fat used in this study was not determined, the addition of fat (i.e., 17 and 30% fat patties) did result in a significantly ( $p < 0.05$ ) higher initial load of aerobic, total coliform, *E. coli*, and psychrotrophic bacteria in ground beef patties (Fig. 1). This suggested that the subcutaneous fat used was contaminated with aerobic, total coliform and *E. coli* bacteria. Similar findings have been reported by Berry et al. (1980) and Chestnut, Emswiler, Kotula, and Young (1977), where the observed increase in bacterial counts of high fat ground beef was attributed to the addition of bacterially contaminated fatty tissues. Furthermore, adipose tissues from different anatomical locations may harbour different levels of bacteria and this resulted in a non-uniform microbial quality in ground beef made with different sources of fatty tissues (Berry et al., 1980). Unfortunately, the location of subcutaneous fat used in

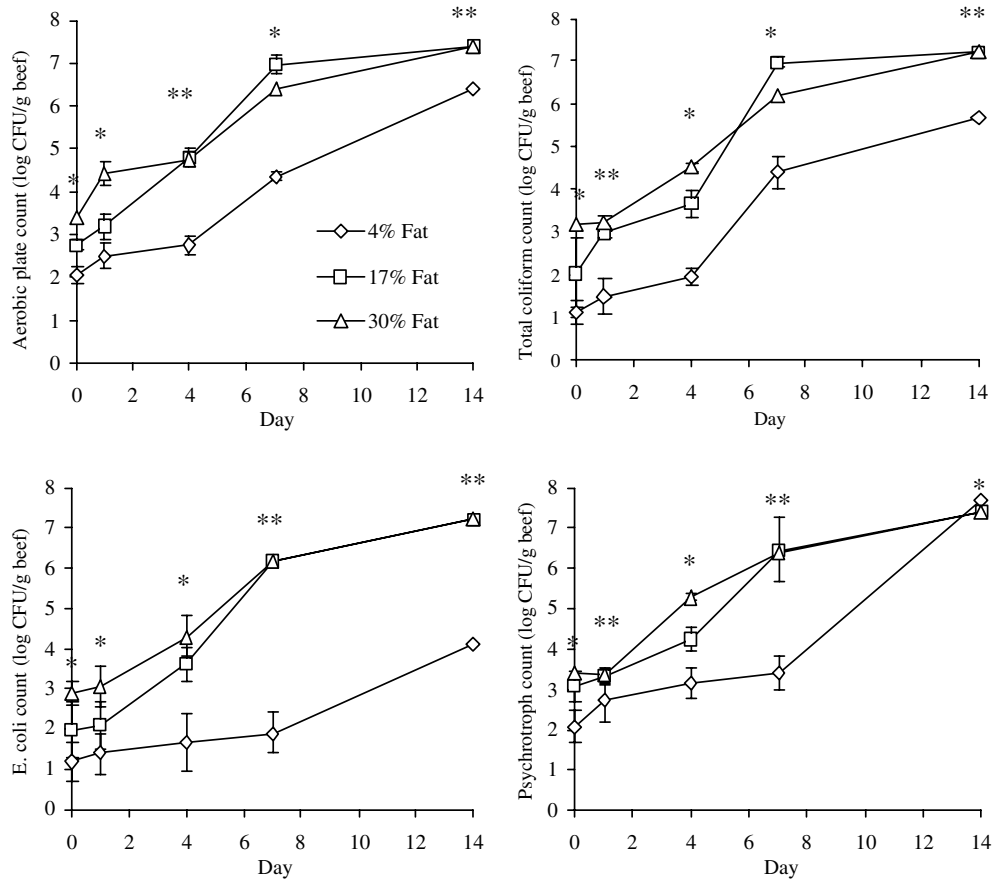


Fig. 1. Effects of fat content on viable bacterial count of non-irradiated ground beef patties during storage. \* All data are significantly ( $p < 0.05$ ) different from each other. \*\* 4% fat data are significantly ( $p < 0.05$ ) different from 17% and 30% fat data.

this study was not determined. Nevertheless, we conclude that subcutaneous fat is a major source of bacterial contamination that may influence the microbiological quality of the resultant ground beef and can reduce shelf-life of these products.

Irradiation pasteurization is an effective tool for eradicating pathogenic and reducing spoilage bacteria in fresh meats. Low dosages of irradiation, between 2 and 3 kGy, reduce pathogenic bacteria by 2 log cycles and drastically lower the level of spoilage bacteria in ground beef (Radomyski et al., 1994). The higher dosages of 5 and 10 kGy used in electron beam irradiation immediately eradicated the viable aerobic, coliform, and *E. coli* bacteria in all ground beef patties (Data not shown). No viable bacteria were detected during the 14 day storage at 4 °C. The 5 kGy dosage, however, did not eradicate the psychrotrophic bacteria in 30% fat patties and a viable count was detected starting on day 7 of storage (Fig. 2). A higher dosage of 10 kGy was effective in delaying the growth of psychrotrophic bacteria up to 14 days storage in 30% fat patties. These results suggest that 5 and 10 kGy of irradiation only provided a sub-lethal antibacterial effect on psychrotrophic bacteria in ground beef. The greater the bacterial contamination in

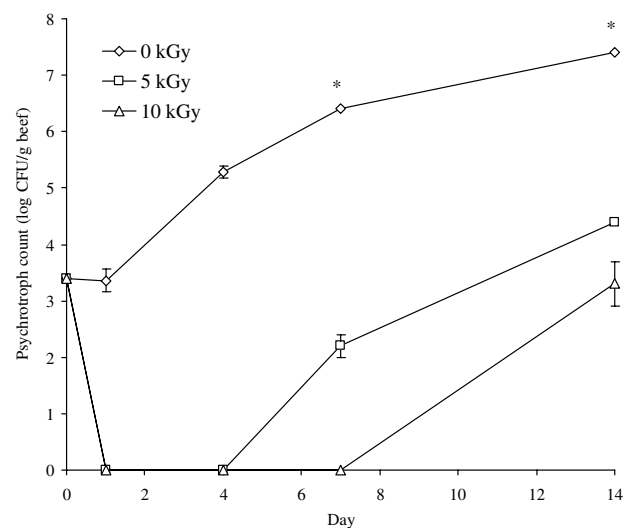


Fig. 2. Effects of irradiation on viable psychrotrophic count of 30% fat ground beef patties during storage. \* All data are significantly ( $p < 0.05$ ) different from each other.

30% fat patties, the greater the reduced efficiency of irradiation observed herein. We suspect that the ability of injured psychrotrophic bacteria to repair and grow

during favourable storage conditions of 4 °C did not occur for aerobic bacteria, *E. coli* and coliforms. Ray (1979, 1986) reported that sub-lethal irradiation treatment of food products may only injure microorganisms and cellular repair is often observed during post-irradiation storage. However, injured microorganisms often exhibited more exacting nutritional and physical requirements, thereby requiring a longer lag phase from growth, and they thus exhibit partially different physiological and taxonomical characteristics.

### 3.1.2. pH

pH, a reliable indicator of food stability is associated with microbial and chemical reactions that cause food deterioration. The optimal pH for bacterial growth is near 7.0, with a range between 4.0 and 9.0 (Jay, 1996). A pH analysis of all ground beef patties showed pH values to vary with the range 5.3–5.6, which agrees with the reported range of 5.4–5.5 for beef tissue (Lawrie, 1985). During storage, beef patty pH increased, especially in the non-irradiated patties and irradiated patties with 4%

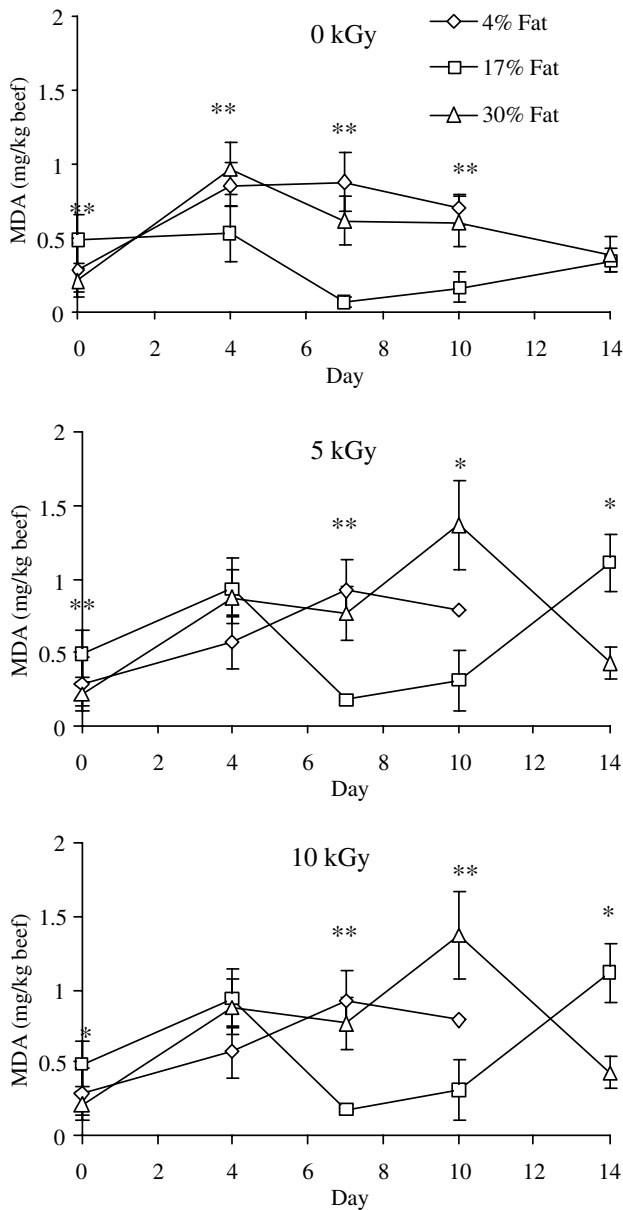


Fig. 3. Effects of fat content on lipid oxidation of irradiated ground beef patties during storage. \* All data are significantly ( $p < 0.05$ ) different from each other. \*\* 17% fat data is significantly ( $p < 0.05$ ) different from 4% and 30% fat data.

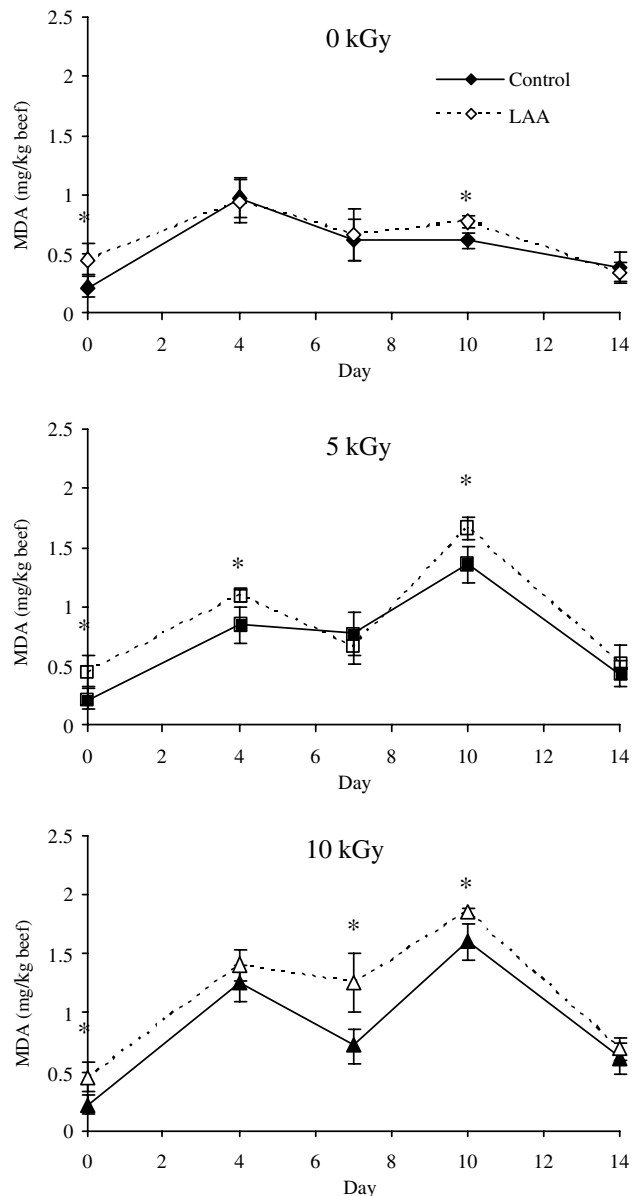


Fig. 4. Effects of L-ascorbic acid (LAA) infusion on lipid oxidation of irradiated ground beef patties during storage. Control represents saline-infused samples. \* All data are significantly ( $p < 0.05$ ) different from each other.

fat (Table 2). This increase in pH of non-irradiated patties has been attributed to the production of alkaline metabolites, such as ammonia and amines which are derived from the proteolysis of urea and amino acids in beef by viable bacteria, not eradicated by electron beam irradiation (An-Hung, Sebranek, & Murano, 1995; Lefebvre, Thibault, & Charbonneau, 1992).

### 3.1.3. Lipid oxidation

Lipid oxidation represents a major source of both nutritional and organoleptic quality losses in ground beef. Typically, the fatty acid content and composition in meats will influence the extent of oxidative deterioration. In meats, the oxidation of phospholipids in muscle cell membrane accounts for 90% of the lipid oxidation (Pikul et al., 1984). As such, the addition of tallow (46% saturated fatty acids) to adjust the lean-to-fat ratio in ground beef should not drastically increase the lipid oxidation in the resulting ground beef. This was evident from our MDA results in ground beef patties where the addition of tallow to increase the fat content from 4% to 17% did not increase the susceptibility to lipid oxidation (Fig. 3). Shivas et al. (1984) also reported no increase in MDA concentration in ground beef patties containing 20% and 25% fat. However, a further increase of fat content of beef patties used in this study to 30% resulted in a significant ( $p < 0.05$ ) increase in beef patty lipid oxidation during storage. Recent studies by Ahn et al. (1996, 1998, 1995) and Jo et al. (1999a, 1999b) have also shown that adding lard to ground pork increased lipid oxidation.

Irradiation-induced oxidative damages in meat involve either direct ionizing of food molecules, or the indirect oxidation of food molecules by hydroxyl radicals that are generated during radiolysis of water. Moreover, the extent of lipid oxidation in irradiated meats is dependent on the irradiation dosage applied (Lefebvre et al., 1992). In this study, increasing electron beam irradiation dosage only enhanced the production of MDA in beef patties having the higher fat content of 30%. This effect confirmed a similar result observed in our former study (Poon, Wong, Dubeski, Durance, & Kitts, 2003). Engeseth and Gray (1994) reported that the fat content of meat does not influence the initial level of lipid oxidation, since the initiation of lipid oxidation occurs in the muscle membrane. However, Jo et al. (1999a, 1999b) reported an increase in lipid oxidation between 4% and 15% fat ground pork treated with 4.5 kGy of electron beam irradiation. Ahn, Kawamoto, Wolfe, and Sim (1995) also demonstrated earlier, that total fat content and composition of fatty acids in the lipid of meat were very important in determining the development of lipid oxidation of aerobically packaged meat during storage. The increase in lipid oxidation of irradiated high fat ground beef patties observed herein, was likely due to the greater development of lipid oxidation supported by the larger quantity of added fat.

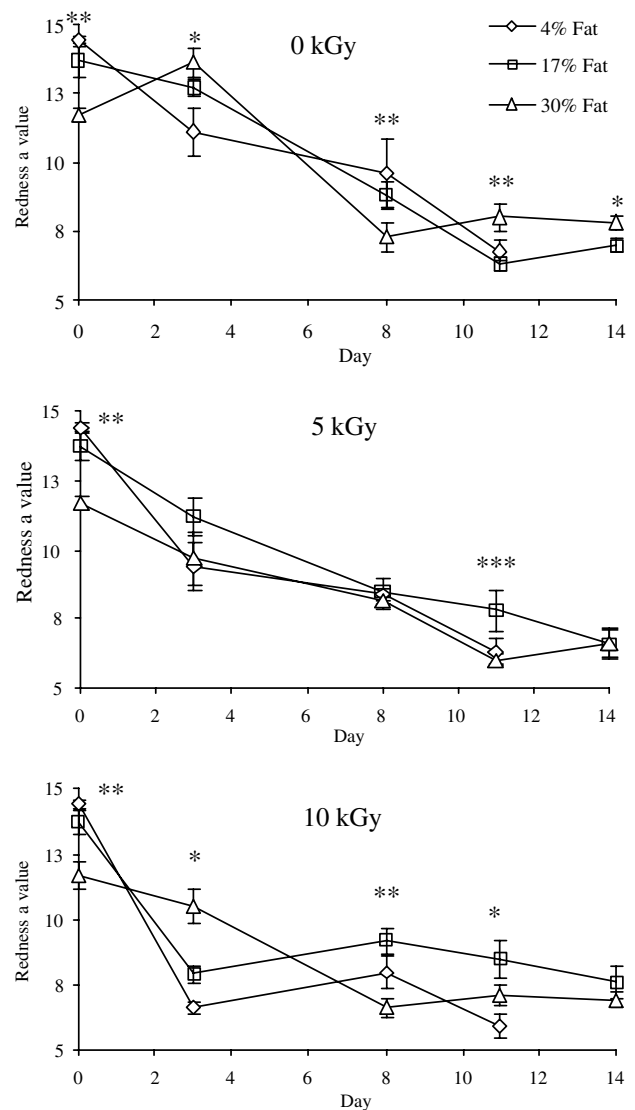


Fig. 5. Hunterlab a value for surface redness irradiated ground beef patties having various fat contents during storage. \* All data are significantly ( $p < 0.05$ ) different. \*\* 30% fat data is significantly ( $p < 0.05$ ) different from 4% and 17% fat data. \*\*\* 17% fat mean value is significantly ( $p < 0.05$ ) different from 4% and 30% fat data.

### 3.1.4. Surface discoloration

Surface redness of beef is the primary determinant of quality as evaluated by consumers. The oxidation of the red pigment, oxymyoglobin (oxyMb), to the brown pigment, metmyoglobin (metMb), reduces the surface redness of beef. Furthermore, the addition of fat in ground beef formulation also reduces the surface redness of beef. These changes often result in a lighter coloured ground beef, similar to the 17% and 30% fat ground beef patties used in this study; where a higher initial  $L$  value for lightness (data not shown) and a lower initial  $a$  value for redness were observed (Fig. 5). Increasing fat content, in ground beef (Berry et al., 1980) and ground pork (Jo et al., 1999a, 1999b), increased the

degree of lightness and decreased the degree of redness. Increasing fat content, in this study, to 30% decreased surface redness. It has been suggested that lipid oxidation and beef discoloration are interrelated (Ladikos & Lougovois, 1990; Schaefer, Liu, Faustman, & Yin, 1995). The severe surface discoloration observed in high fat ground beef patties (i.e., 30%) in this study might be explained by the increased oxidation of oxyMb, attributed to the greater potential of peroxy radicals derived from increased fat content.

Irradiation promotes the surface discoloration of beef by initiating hydroxyl radical-induced oxidation of oxyMb to metMb. Increasing irradiation dosages will also lead to a rapid decrease in surface redness on ground beef, as observed in this study (Fig. 5) and our former study with ground beef patties (Poon et al., 2003). The addition of fat, to 30%, further exacerbated

the surface discoloration on ground beef patties. Jo et al. (1999a, 1999b) found similar results and reported that irradiation accelerated lipid oxidation and this resulted in the more rapid conversion of red colour to brown colour in pork. Thus, the irradiation pasteurization of beef patties may induce beef discoloration by directly ionizing the ferrous ion in oxyMb, as a result of free radicals produced from irradiation-induced accelerated lipid oxidation.

### 3.2. Experiment 2

#### 3.2.1. Bacterial analyses

The presence of antioxidant in irradiated meat was hypothesized to lower the oxidation effect of irradiation by scavenging hydroxyl radicals generated. We report here that the pasteurization effect of irradiation was not

Table 1  
Effects of AA infusion and irradiation on viable bacterial count of 30% fat ground beef patties

Parameter	Treatment	Dose (kGy)	Days of storage				
			0	1	4	7	14
Aerobic <sup>d</sup>	Control	0	3.4 ± 0.2	4.4 ± 0.3	4.8 ± 0.1	>6.4 ± 0.0 <sup>a</sup>	>7.4 ± 0.0 <sup>b</sup>
	AA	0	3.4 ± 0.2	3.5 ± 0.5	4.7 ± 0.2	>6.4 ± 0.0 <sup>a</sup>	>7.4 ± 0.0 <sup>b</sup>
Coliform <sup>d</sup>	Control	0	3.2 ± 0.3	3.3 ± 0.2	4.5 ± 0.3	>6.4 ± 0.0 <sup>a</sup>	>7.4 ± 0.0 <sup>b</sup>
	AA	0	3.2 ± 0.3	3.2 ± 0.3	4.4 ± 0.1	>6.4 ± 0.0 <sup>a</sup>	>7.4 ± 0.0 <sup>b</sup>
<i>E. coli</i> <sup>d</sup>	Control	0	2.9 ± 0.3	3.5 ± 0.5	4.3 ± 0.4	>6.4 ± 0.0 <sup>a</sup>	>7.4 ± 0.0 <sup>b</sup>
	AA	0	2.9 ± 0.4	3.0 ± 0.3	4.3 ± 0.2	>6.4 ± 0.0 <sup>a</sup>	>7.4 ± 0.0 <sup>b</sup>
Psychrotroph	Control	0	3.4 ± 0.1	3.5 ± 0.3	5.3 ± 0.1	>6.4 ± 0.0 <sup>a</sup>	>7.4 ± 0.0 <sup>b</sup>
		5	3.4 ± 0.2	ND <sup>c</sup>	ND	2.2 ± 0.2	4.4 ± 0.3
		10	3.4 ± 0.2	ND	ND	ND	3.3 ± 0.4
	AA	0	3.4 ± 0.1	3.4 ± 0.2	5.2 ± 0.2	>6.4 ± 0.0 <sup>a</sup>	>7.4 ± 0.0 <sup>b</sup>
		5	3.4 ± 0.1	ND	ND	2.3 ± 0.1	4.4 ± 0.1
		10	3.4 ± 0.1	ND	ND	ND	3.4 ± 0.3

Values represent mean ± SEM ( $n = 18$ ). ND denotes none detected.

<sup>a</sup> TNTC at 1:10,000 dilution.

<sup>b</sup> TNTC at 1:100,000 dilution.

<sup>c</sup> Less than 25 at 1:10 dilution.

<sup>d</sup> No viable bacteria detected in 5 and 10 kGy irradiated processed ground beef patties.

Table 2  
pH values of irradiated and AA-infused ground beef patties containing various fat content during storage

Treatment	Fat (%)	Dose (kGy)	Days of storage		
			0	7	14
Control	4	0	5.38 ± 0.02 <sup>ax</sup>	N/A	6.68 ± 0.19 <sup>ay</sup>
		5	5.38 ± 0.02 <sup>ax</sup>	N/A	6.80 ± 0.25 <sup>ay</sup>
		10	5.38 ± 0.02 <sup>ax</sup>	N/A	6.27 ± 0.22 <sup>by</sup>
Control	17	0	5.56 ± 0.03 <sup>bx</sup>	5.55 ± 0.01 <sup>ax</sup>	6.47 ± 0.20 <sup>aby</sup>
		5	5.56 ± 0.03 <sup>bx</sup>	5.54 ± 0.03 <sup>ax</sup>	5.59 ± 0.02 <sup>cx</sup>
		10	5.56 ± 0.03 <sup>bx</sup>	5.61 ± 0.01 <sup>by</sup>	5.61 ± 0.02 <sup>cy</sup>
Control	30	0	5.31 ± 0.06 <sup>ax</sup>	5.58 ± 0.04 <sup>ay</sup>	6.71 ± 0.05 <sup>az</sup>
		5	5.31 ± 0.06 <sup>ax</sup>	5.65 ± 0.03 <sup>by</sup>	5.63 ± 0.01 <sup>by</sup>
		10	5.31 ± 0.06 <sup>ax</sup>	5.69 ± 0.04 <sup>by</sup>	5.58 ± 0.02 <sup>cz</sup>
AA	30	0	5.05 ± 0.16 <sup>bz</sup>	5.48 ± 0.01 <sup>by</sup>	6.36 ± 0.07 <sup>bx</sup>
		5	5.05 ± 0.16 <sup>bz</sup>	5.61 ± 0.02 <sup>ax</sup>	5.58 ± 0.02 <sup>dx</sup>
		10	5.05 ± 0.16 <sup>bz</sup>	5.61 ± 0.02 <sup>ax</sup>	5.56 ± 0.01 <sup>dy</sup>

Values represent mean ± SEM ( $n = 18$ ). N/A denotes not available. abc Means in a column with different letters are significantly different ( $p \leq 0.05$ ). xyz Means in a row with different letters are significantly different ( $p \leq 0.05$ ).

in fact reduced by the prior infusion of antioxidant, since all bacterial counts observed were not significantly affected ( $p > 0.05$ ) by AA infusion (Table 1). This result confirms earlier reports by Sanchez-Escalante, Dienane, Torrescano, Beltran, and Roncales (2003) and Shivas et al. (1984), that the water-soluble antioxidant AA is not effective in reducing microbial growth in ground beef. Cabedo, Sofos, and Smith (1998) and Chan et al. (1995) also found that fat-soluble antioxidants, such as  $\alpha$ -tocopherol did not affect the irradiation-induced reduction of pathogenic and spoilage bacterial in ground beef. Quattara, Giroux, Smoragiewicz, Saucier, and Lacroix (2002) and Giroux et al. (2001), however, both reported an antibacterial effect of 0.5% ascorbic acid in gamma-irradiated ground beef and attributed the antibacterial effect mainly to the reduction of meat pH by the presence of ascorbic acid. A pH analysis of all ground beef patties used in this study did not reveal a drastic reduction in meat pH as a result of AA infusion (Table 2). This may have been the difference between the antibacterial effect of AA in beef proposed by Quattara et al. (2002) and Giroux et al. (2001), was but not observed in this study.

### 3.2.2. Lipid oxidation

The addition of low levels of AA to meats has been linked to an overall increase in oxidative stability by directly decreasing lipid oxidation (Giroux et al., 2001; Lee et al., 1999; Nam et al., 2003; Quattara et al., 2002; Shivas et al., 1984) and regenerating endogenous  $\alpha$ -tocopherol (Nam et al., 2003; Schaefer et al., 1995). Hood (1975) and Wheeler et al. (1996) have shown a deposition of AA into meat muscle following injection of live cattle or post-mortem beef muscle. Contrary to the former studies, our results indicated that the infusion of high levels of AA into beef muscle produced a significant increase ( $p < 0.05$ ) in the lipid oxidation of ground beef patties (Fig. 4). Previous studies, conducted with high levels of AA infusion, have reported increased lipid oxidation in ground beef (Katsanidis et al., 2003; Morrissey, Sheely, Galvin, Kerry, & Buckley, 1998; Sanchez-Escalante et al., 2003). Since rapid oxidation of AA to ascorbyl radical anion occurs rapidly (Wong & Kitts, 2001), it can be postulated that the post-mortem state of high levels of ascorbyl radicals may lead to increased lipid oxidation (Mahoney & Graf, 1986; Wong & Kitts, 2001). Moreover, high levels of AA will promote oxidation by reducing ferric ion to ferrous ion, enabling the ferrous ion to participate in the Fenton reaction or lipid oxidation (Mahoney & Graf, 1986).

### 3.2.3. Surface discoloration

The reducing property of AA, to regenerate oxyMb from metMb (Schaefer et al., 1995) will prevent surface discoloration of ground beef (Hood, 1975; Lee et al., 1999; Nam & Ahn, 2003; Shivas et al., 1984; Wheeler

et al., 1996). Moreover, the regeneration of  $\alpha$ -tocopherol by AA, which in turn inhibits the oxidation of oxyMb to metMb by lipid free radical, can further reduce surface discoloration in beef (Schaefer et al., 1995). However, the application of AA at a level greater than 500 ppm, to meats stored aerobically will result in the decomposition of AA into hydrogen peroxide that can lead to surface discoloration (Ladikos & Lougovois, 1990). The pro-oxidant effect of AA can also be attributed to its rapid oxidation to ascorbate radical anion that oxidizes oxyMb to metMb. Thus, the infusion of AA, at 10–500

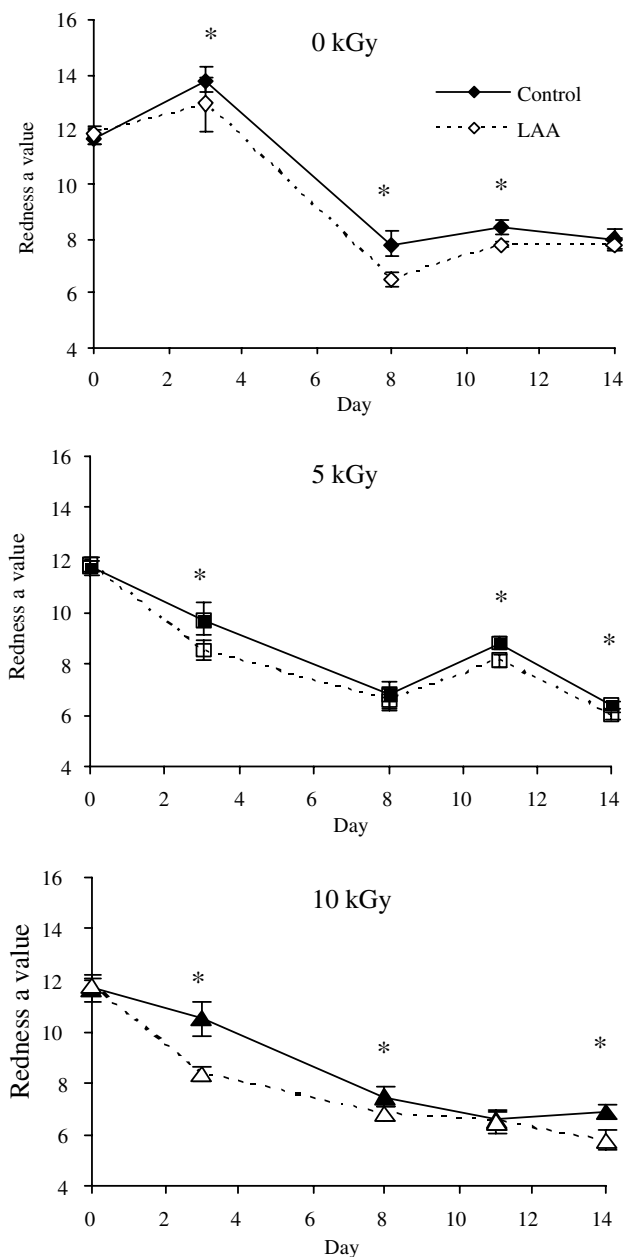


Fig. 6. Hunterlab a value for surface redness of AA-infused and irradiated ground beef patties during storage. \* All data are significantly ( $p < 0.05$ ) different from each other.



mM, can be attributed to the significant decrease ( $p < 0.05$ ) in surface redness obtained in those meat products (Fig. 6). Sanchez-Escalante et al. (2003) also report a loss of surface redness in ground beef patties containing AA. Our finding, that the combination of irradiation and AA resulted in a synergistic pro-oxidant effect on the oxyMb, explains why redness values in the irradiated and infused ground beef patties were lower than controls. This finding contradicts the protective effect of AA in irradiated ground beef patties reported by Giroux et al. (2001); however, these workers employed lower dosages of irradiation (0.5 and 4 kGy) and infused lower levels of AA.

#### 4. Conclusion

The addition of fat to beef patties affected the microbial and chemical deterioration of the ground beef during storage. Cold pasteurization of the contaminated ground beef patties with electron beam irradiation at 5 and 10 kGy reduced ( $p < 0.05$ ) viable psychrotrophic bacteria only up to 7 days storage at 4 °C. Irradiation at the same dosage effectively eradicated aerobic, coliform and *E. coli* bacteria in the patties. Irradiation-induced lipid oxidation was exacerbated by the presence of fat and the prior post-slaughter infusing of AA to animals that contributed to the beef muscle. We conclude that the electron beam irradiation represents an effective pasteurization measure for ground beef of low fat content. Post-slaughter AA infusion of carcasses will not control the chemical deteriorations induced by irradiation, but instead may reduce shelf-life with a pro-oxidant effect.

#### Acknowledgements

Authors wish to thank Drs. Paula Dubeski and Alan Schaefer for their assistance at Lacombe Research Station. This study was supported by a research grant from BC Cattleman's Association and Alberta Agricultural Research Institute (AARI) to author D.D.K. and University of British Columbia UGF award to author P.Y.Y.W.

#### References

- Ahn, D. U., Lutz, S., & Sim, J. S. (1996). Effect of dietary  $\alpha$ -linolenic acids on the fatty acid composition, storage stability and sensory characteristics of pork loin. *Meat Science*, *43*, 291–299.
- Ahn, D. U., Kawamoto, C., Wolfe, E. H., & Sim, J. S. (1995). Dietary alpha-linolenic acid and mixed tocopherols and packaging influence lipid stability in broiler chicken breast and leg muscle tissue. *Journal of Food Science*, *60*, 1013–1018.
- Ahn, D. U., Olson, D. G., Jo, C., Chen, C., & Lee, J. I. (1998). Effect of muscle type, packaging, and irradiation on lipid oxidation, volatile production, and color in raw pork patties. *Meat Science*, *49*, 27–39.
- Ahn, D. U., Olson, D. G., Jo, C., Love, J., & Jin, S. K. (1999). Volatiles production and lipid oxidation in irradiated cooked sausage as related to packaging and storage. *Journal of Food Science*, *64*, 226–229.
- An-Hung, F., Sebranek, J. G., & Murano, E. A. (1995). Survival of *Listeria monocytogenes*, *Yersinia enterocolitica* and *Escherichia coli* O157:H7 and quality changes after irradiation of beef steaks and ground beef. *Journal of Food Science*, *60*, 972–977.
- Berry, B. W., Wells, L. H., Cross, H. R., Emswiler, B. S., & Muse, H. D. (1980). Shelf-life characteristics and aerobic plate counts of ground beef as influenced by fat level and fat source. *Journal of Food Protection*, *43*, 713–716.
- Berry, B. W., & Chen, A. T. (1976). Bacterial shelf life and consumer acceptance characteristics of chopped beef. *Journal of Milk and Food Technology*, *39*, 405–407.
- Berry, B. W., Smith, G. C., & Carpenter, Z. L. (1973). Growth of two genera of psychrotrophs on beef adipose tissue. *Journal of Food Protection*, *36*, 1074–1075.
- Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Method in Enzymology*, *52*, 302–310.
- Cabedo, L., Sofos, J. N., & Smith, G. C. (1998). Bacterial growth in ground beef patties made with meat from animals fed diets without or with supplemental vitamin E. *Journal of Food Protection*, *61*, 36–40.
- Chan, W. K. M., Hakkarainen, K., Faustman, C., Schaefer, D. M., Scheller, K. K., & Liu, Q. (1995). Color stability and microbial growth relationships in beef as affected by endogenous  $\alpha$ -tocopherol. *Journal of Food Science*, *60*, 966–971.
- Chestnut, C. M., Emswiler, B. S., Kotula, A. W., & Young, E. P. (1977). Bacteriological quality of ingredients used in ground beef manufacture. *Journal of Animal Science*, *44*, 213–217.
- Duitschaver, C. L., Bullock, D. H., & Arrott, D. R. (1977). Bacteriological evaluation of retail ground beef, frozen beef patties and cooked hamburger. *Journal of Food Protection*, *40*, 378–381.
- Engeseth, N. J., & Gray, J. I. (1994). Cholesterol oxidation in muscle tissue. *Meat Science*, *36*, 309–320.
- Field, R. A., Smith, F. C., Deane, D. D., Thomas, G. M., & Kotula, A. W. (1977). Sources of variation at the retail level in bacteriological conditions of ground beef. *Journal of Food Protection*, *40*, 385–388.
- Folch, J., Lee, M., & Stanley, G. H. S. (1957). A simple method of the isolation and purification of total lipid from animal tissues. *Journal of Biological Chemistry*, *226*, 242–250.
- Giroux, M., Ouattara, B., Yefsah, R., Smoragiewicz, W., Saucier, L., & Lacroix, M. (2001). Combined effect of ascorbic acid and gamma irradiation on microbial and sensorial characteristics of beef patties during refrigerated storage. *Journal of Agriculture and Food Chemistry*, *49*, 919–925.
- Harbers, C., Harrison, D., & Kropf, D. (1981). Ascorbic acid effects on bovine muscle pigments in the presence of radiant energy. *Journal of Food Science*, *46*, 7–12.
- Hood, D. E. (1975). Pre-slaughter injection of sodium ascorbate as a method of inhibiting metmyoglobin formation in fresh beef. *Journal of the Science of Food and Agriculture*, *26*, 85–90.
- Jay, J. M. (1996). *Modern food microbiology* (fifth ed.). New York: Chapman&Hall.
- Jo, C., Lee, J., & Ahn, D. U. (1999a). Lipid oxidation, color changes and volatiles production in irradiated pork sausage with different fat content and packaging during storage. *Meat Science*, *51*, 355–361.
- Jo, C., Ahn, D. U., & Lee, J. (1999b). Lipid and cholesterol oxidation, color changes, and volatile production in irradiated raw pork batters with different fat content. *Journal of Food Quality*, *22*, 641–651.

- Kay, S. (2003). \$2.7 Billion the cost of *E. coli* O157:H7. *Meat and Poultry*, 49, 26–34.
- Katsanidis, E., Meyer, D. C., Addis, P. B., Yancey, E. J., Dikeman, M. E., Tsiamyrtzis, P., & Pullen, M. (2003). Vascular infusion as a means to improve the antioxidant-prooxidant balance of beef. *Journal of Food Science*, 68(4), 1149–1154.
- Lawrie, R. A. (1985). *Meat science* (fourth ed.). Oxford: Pergamon Press.
- Ladikos, D., & Lougovois, V. (1990). Lipid oxidation in muscle foods: A review. *Food Chemistry*, 35, 295–314.
- Lee, B. J., Hendricks, D. G., & Cornforth, D. P. (1999). A comparison of carnosine and ascorbic acid on colour and lipid stability in a ground beef patties model systems. *Meat Science*, 51, 245–253.
- Lefebvre, N., Thibault, C., & Charbonneau, R. (1992). Improvement of shelf life and wholesomeness of ground beef by irradiation. Microbial aspects. *Meat Science*, 32, 203–213.
- Mahoney, J. R., & Graf, E. (1986). Role of alpha tocopherol, ascorbic acid, citric acid and EDTA as oxidant in model systems. *Journal of Food Science*, 51, 1293–1296.
- Mitsumoto, M., Faustman, C., Cassens, R. G., Arnold, R. N., Schaefer, D. M., & Scheller, K. K. (1991a). Vitamin E and C improve pigment and lipid stability in ground beef. *Journal of Food Science*, 56, 194–197.
- Mitsumoto, M., Cassens, R. G., Schaefer, D. M., Arnold, R. N., & Scheller, K. K. (1991b). Improvement of color and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatment. *Journal of Food Science*, 56, 1489–1492.
- Morrissey, P. A., Sheely, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, 49S, S73–S86.
- Nam, K. C., & Ahn, D. U. (2003). Effects of ascorbic acid and antioxidants on the color of irradiated ground beef. *Journal of Food Science*, 68, 1686–1690.
- Nam, K. C., Min, B. R., Park, K. S., Lee, S. C., & Ahn, D. U. (2003). Effects of ascorbic acid and antioxidants on the lipid oxidation and volatiles of irradiated ground beef. *Journal of Food Science*, 68(5), 1680–1685.
- Quattara, B., Giroux, M., Smoragiewicz, W., Saucier, L., & Lacroix, M. (2002). Combined effect of gamma irradiation, ascorbic acid and edible coating on the improvement of microbial and biochemical characteristics of ground beef. *Journal of Food Protection*, 65, 981–987.
- Pikul, J., Leszczynski, D. E., & Kummerow, F. A. (1984). Relative role of phospholipids, triacylglycerols and cholesterol esters on malonaldehyde formation in fat extracted from chicken meat. *Journal of Food Protection*, 49, 704–708.
- Poon, P. W. B., Wong, P. Y. Y., Dubeski, P., Durance, T. D., & Kitts, D. D. (2003). Application of electron-beam irradiation pasteurization of ground beef, from steers fed vitamin E fortified diets: Microbial and chemical effects. *Journal of the Science of Food and Agriculture*, 83, 542–549.
- Radomski, T., Murano, E. A., Olson, D. G., & Murano, P. S. (1994). Elimination of pathogens of significance in food by low-dose irradiation: A review. *Journal of Food Protection*, 57, 73–86.
- Ray, B. (1979). Methods to detect stressed microorganisms. *Journal of Food Protection*, 42, 346–355.
- Ray, B. (1986). Impact of bacterial injury and repair in food microbiology: Its past, present and future. *Journal of Food Protection*, 49, 651–655.
- Roberts, T. W., & Weese, J. O. (1998). Shelf life of ground beef patties treated by gamma radiation. *Journal of Food Protection*, 61, 1387–1389.
- Sanchez-Escalante, A., Dienane, D., Torrescano, G., Beltran, J. A., & Roncales, P. (2003). Antioxidant action of borage, rosemary, oregano, and ascorbic acid in beef patties packaged in modified atmosphere. *Journal of Food Science*, 68, 339–344.
- Schaefer, D. M., Liu, Q., Faustman, C., & Yin, M. C. (1995). Supranutritional administration of vitamin E and C improves oxidative stability of beef. *Journal of Nutrition*, 125S, 1792S–1798S.
- Shivas, S. D., Kropf, D. H., Hunt, M. C., Kastner, C. L., Kendall, J. L. A., & Dayton, A. D. (1984). Effects of ascorbic acid on display life of ground beef. *Journal of Food Protection*, 47, 11–15.
- Wheeler, T. L., Koobmaraie, M., & Shackelford, S. D. (1996). Effect of vitamin E concentration and co-injection with calcium chloride on beef retail display color. *Journal of Animal Science*, 74, 1846–1853.
- Wong, P. Y. Y., & Kitts, D. D. (2001). Factors influencing ultraviolet and electron beam irradiation-induced free radical damage to ascorbic acid. *Food Chemistry*, 74, 75–84.