Elimination of *Escherichia coli* O 157 : H7 and *Listeria monocytogenes* from raw beef sausage by γ-irradiation

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The effectiveness of low γ-irradiation doses in the destruction of *Escherichia coli* O 157 : H7 and *Listeria monocytogenes* in raw beef sausages was investigated. Raw samples of fresh manufactured beef sausage were subjected to γ-irradiation at doses of 0, 1, 2, and 3 kGy. Then samples were cold-stored (4 ± 1°C) for 12 days and the effects of irradiation and storage on the counts of these harmful bacteria were studied. Moreover, the effects of irradiation and storage on the percentages of free fatty acids (FFAs) in lipids, on the *p*-anisidine values of lipids, solubility of sarcoplasmic and myofibrilar proteins, and water-holding capacity (WHC) were also determined. The results showed that γ-irradiation at 1 and 2 kGy significantly reduced the counts of *E. coli* O 157 : H7 and *L. monocytogenes*, while 3 kGy dose effectively eliminated these bacteria by more than 4 log and 3 log units, respectively, and could keep their counts below the detection level during storage. γ-Irradiation had no significant effects on the percentages of FFAs in lipids, solubility of sarcoplasmic and myofibrilar proteins, and WHC of samples, while it significantly increased the *p*-anisidine value of lipids. During storage, significant increases in the percentages of FFAs and *p*-anisidine values were observed for lipids of irradiated and nonirradiated sausages, while the solubility of sarcoplasmic and myofibrilar proteins showed no significant changes. Moreover, samples of irradiated and nonirradiated sausages showed significant decreases in their WHC during the first 6 days of storage at 4 ± 1°C, then showed no significant changes. Finally, γ-irradiation at a dose of 3 kGy appeared to be sufficient to improve the microbiological safety of raw beef sausages without adverse effects on their chemical properties.

Keywords: *Escherichia coli* O 157 : H7 / Irradiation / *Listeria monocytogenes* / Sausage

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1 Introduction

Enterohemorrhagic *Escherichia coli* O157 : H7 and *Listeria monocytogenes* are pathogenic bacteria that have emerged as foodborne pathogens of major public health concern [1 – 3], causing food poisoning and disease outbreaks around the world [3 – 6]. Sausage is one of the meat products that are gaining popularity because of convenience, variety, economy, and nutritional value. It takes little time in preparation, with some sausage being ready to serve, and others needing only to be warmed [7]. However, sausage may act as a main source of food poisoning and has been reported as a vehicle transmitting *E. coli* O157 : H7 and *L. monocytogenes* in many cases of food-borne diseases [8, 9].

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Abbreviations: FFA, free fatty acid; **WHC**, water-holding capacity

Irradiation as a method of meat preservation has an excellent potential to improve meat safety and shelf-life [10, 11]. The approval of meat irradiation by FDA in 1997 has attracted the attention of industry and consumers concerned with improving food safety. It is well-established that the application of this process can virtually eliminate most vegetative pathogens from fresh meats [12 – 15]. Its use within a Hazard Analysis Critical Control Points System in meat processing plants could positively impact the safety of these products [3, 15]. Therefore, the objective of the present work was to examine the incidence of *E. coli* O157 : H7 and *L. monocytogenes* in raw beef sausage and their elimination by low doses of γ-irradiation.

2 Materials and methods

2.1 Experimental design

This study was divided into two stages. In the first stage, we determined the incidence of *E. coli* O 157 : H7 and *L. mono-*

cytogenes in raw beef sausage. Sixty samples of well-sealed prepackaged raw sausages (400 g in a polyethylene pouch) were randomly collected from three different meat product markets (in El-Sharkia Governorate, Egypt) which distributes meat products of the most famous meat processing plants in Egypt. Samples were transported to the laboratory in an ice-chest and the microbiological analyses were performed immediately on arrival at the laboratory after 1.5 h of delivery. Analyses were conducted using triplicate separated subsamples for each package of sausages. The bacteriological survey revealed that 8.3% of the samples (5 samples) contained *E. coli* O 157 : H7, while 35% of the samples (21 samples) contained *L. monocytogenes*, meanwhile 6.7% of the samples (4 samples) contained *E. coli* O 157 : H7 in addition to *L. monocytogenes*. Therefore, due to low probability for the incidence of *E. coli* O 157 : H7 in samples compared to *L. monocytogenes*, the second stage of this work was designed to study the effects of irradiation on the elimination of this pathogen through inoculation studies, while the study was carried out on *L. monocytogenes* that may be naturally present in samples due to their relatively higher incidence in sausage samples.

2.2 Beef sausages

Raw beef sausages were purchased freshly after manufacturing from a meat processing company (10^{th} of Ramadan City, Egypt) and used in the second stage of the present study. The main formula consisted of frozen beef meat (flank (65%) and forequarters (10%)), dextrose (0.695%), sodium caseinates (0.794%), soya (0.993%), ecolin (0.894%), dried garlic (0.198%), tripolyphosphate (0.396%), sodium chloride (1.142%), water and ice (19.148%), nitrite and nitrate (0.01%), and spices (0.730%). The obtained sausages were ground and well-mixed under aseptic conditions using a Braun Hand Mixer (model #MR5550MBC), then divided into three portions. The first and second portions were subdivided into appropriate samples (~100 g) in polyethylene pouches for chemical determinations and determinations of *L. monocytogenes*, respectively. The pouches were sealed by heat. The third portion of the ground sausage was used for inoculation with *E. coli* O 157 : H7.

2.3 Inoculation of *E. coli* O 157 : H7

Inoculation of *E. coli* O 157 : H7 in ground sausage was done as described by López-González *et al.* [15]. Confirmed cells of *E. coli* O 157 : H7, which were isolated from sausage samples in the first stage of our study and confirmed using the dry-spot *E. coli* O 157 : H7 latex test (Oxoid), were incubated in tripticase soy broth (TSB; Difco Laboratories, Detroit, MI, USA) for 24 h at 35°C, followed by storage at 4 ± 1°C. When needed, 24 h before purchasing the samples, cultures were grown in 10 mL TSB at 35°C for 18 h. Then, 0.1 mL of the culture was transferred to 10 mL TSB and grown at 35°C to early stationary phase (8 h), at which time the approximate bacterial population in the culture reached 10^8 cells per mL (as indicated by the direct microscopic count using a hemocytometer before inoculation, while the confirmed count reached 1.04×10^8 cfu/mL as indicated by the plate count on Sorbitol MacConky agar (Oxoid). One mL of the culture was transferred to 100 mL TSB and the diluted culture was added to the ground sausage at 10 mL per each 500 g. The inoculated sample was mixed at slow speed (139 rpm) in a meat mixer (Hobart, N-50 with stainless steel beater), followed by grinding and further mixing by the hand mixer to assure uniform distribution of the inoculum. The inoculated samples were also subdivided into appropriate samples (~100 g) in polyethylene pouches and sealed by heat. Then all pouches of the three portions under investigation were transferred to irradiation treatments.

2.4 Irradiation and storage

Samples of the three sausage portions under investigation were exposed to γ-irradiation at doses of 0, 1, 2, and 3 kGy using a Cobalt-60 source, providing a dose rate of 9.23 kGy/h (National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt). Irradiation was carried out at room temperature and dosimetry was conducted with ferrous sulfate/cupric sulfate dosimeters. Then all samples were stored at 4 ± 1°C for 12 days and periodically analyzed at 3-day intervals. Analysis of all samples was stopped when decomposition of the control nonirradiated samples became apparent (after 12 days).

2.5 Bacteriological analyses

For bacteriological determinations (in all stages of this study), pouches were aseptically opened. The initial 1/10 dilution as well as the other appropriate serial dilutions were prepared in 0.1% peptone water diluent. Then the colony forming units of *E. coli* O 157 : H7 were enumerated by surface plating on Sorbitol MacConkey agar and incubation at 35°C for 24 h, while representative colonies were confirmed as *E. coli* O157 : H7 using dry-spot *E. coli* O 157 : H7 latex test [16]. *Listeria monocytogenes* was counted (after enrichment using selective enrichment medium prepared from buffered Listeria enrichment broth base and Listeria selective supplement, Oxoid) on Listeria selective medium (prepared from Listeria-selective agar base CM856 and Literia-selective supplement SR140, Oxford formulation) after 24–48 h of incubation at 35°C [16]. Cultures were examined after 24 and 48 h incubation and typi-

cal colonies were confirmed through biochemical tests according to Bille and Doyle [17].

2.6 Chemical analyses

2.6.1 Lipid analysis

Lipids were extracted with chloroform/methanol (2 : 1) according to the method of Folch *et al.* [18] and the FFAs were determined in the extracted lipids according to the official methods of AOCS [19], while the lipid oxidation potential was evaluated by the determination of the *para*-anisidine value as described by Egan *et al.* [20].

2.6.2 Protein solubility and water-holding capacity

Sarcoplasmic proteins were determined according to the method described by Diaz *et al.*, [21] while myofibrilar proteins were determined as described by Warner *et al.* [22]. Water-holding capacity (WHC) was determined as described by Rocha-Garza and Zayas [23].

2.7 Statistical analysis

All analyses were performed using triplicate samples (pouches) per treatment. The results were statistically analyzed by randomized complete block design (two factors) using a microcomputer program for design, management, and analysis of agronomic research experiments (MSTAT-C), while the differences between the means ($p < 0.05$) were determined by the least significant difference [24]. Values of bacteriological determinations were converted from decimal numbers to integer numbers before statistical analysis.

3 Results and discussion

3.1 Bacteriological properties

3.1.1 Incidence of *E. coli* O 157 : H7 and *L. monocytogenes* in fresh raw beef sausage

In the bacteriological survey conducted on samples of fresh raw beef sausage, collected in the first stage of the present study, the results revealed that the counts of *E. coli* in the positive samples were in the range of 5.7×10^1 to 3.6×10^2 cfu/g, while the counts of *L. monocytogenes* ranged from 1.25×10^2 to 1.0×10^3 cfu/g (Table 1). It was stated that cattle appeared to be the primary origin for serotype O 157 : H7, however, it was also found in deer, sheep, and other animals [25]. Moreover, this pathogen has the ability to survive up to 9 months in frozen storage at −20°C, can tolerate up to 8% salt, and survive any acidity developed in fermented sausage [9, 26]. In addition, even if only a small portion of isolated *E. coli* can be harmful for humans, the infectious dose of this enterohemorrhagic *E. coli* O 157 : H7 was estimated to be very low being in the range of 10 – 100 cells [25, 27]. Meat products, including sausage, have also been identified as products which promote growth and can serve as a source of *L. monocytogenes* [8, 28]. This organism can also survive freezing [29] and may become established within the processing environment [30], while surviving cleaning and disinfection routines [31].

Table 1. Bacteriological survey for the incidence of *E. coli* O 157 : H7 and *L. monocytogenes* in raw fresh beef sausage

Percentage of positive samples[a)]	Mean count (cfu/g)			
	E. coli O 157 : H7		*L. monocytogenes*	
	Lower observed count	Higher observed count	Lower observed count	Higher observed count
8.3%	7×10^1	3.6×10^2	ND	ND
35.0%	ND	ND	1.3×10^2	1.05×10^3
6.7%	5.7×10^1	1.8×10^2	1.25×10^2	7.3×10^2

a) Number of the analyzed samples = 60; ND, not detected

3.1.2 Effects of irradiation and refrigerated storage on *E. coli* O 157 : H7

Samples of raw beef sausage inoculated with *E. coli* O 157 : H7 had an initial count of 2.11×10^4 cfu/g. Subjecting raw beef sausage, inoculated with *E. coli*, to γ-irradiation at doses of 1 and 2 kGy significantly reduced ($p < 0.05$) the initial count of this pathogen in the samples (Table 2). However, refrigerated storage (4 ± 1°C) significantly increased ($p < 0.05$) the counts of *E. coli* O 157 : H7 that survived the 1 kGy dose as well as the counts in control nonirradiated samples. In addition, gradual but insignificant increases in the counts of *E. coli* O 157 : H7 were observed during cold storage of samples irradiated at a 2 kGy dose, then the count significantly increased on day 12 of cold storage. Irradiation at a dose of 3 kGy effectively eliminated these bacteria by more that 4 log cycles; *E. coli* O 157 : H7 was not detected during storage of samples. It has been reported that low-dose irradiation was an effective means of eliminating *E. coli* O 157 : H7 [12]. Moreover, the relatively low irradiation dose of 1.5 kGy was sufficient to give a 10 000 fold reduction in the numbers of *E. coli* O 157 : H7 at 5°C [3] and a dose of 2 – 3 kGy can be sufficient to decontaminate raw meats of this pathogen [25].

3.1.3 Effects of irradiation and refrigerated storage on *L. monocytogenes*

The initial count of *L. monocytogenes* naturally present in raw beef sausage was found to be 1.78×10^3 cfu/g. Application of γ-irradiation at doses of 1 and 2 kGy induced significant reduction ($p < 0.05$) in the counts of *L. monocytogenes*

Table 2. Colony-forming units of *E. coli* O 157 : H7 in raw beef sausage as affected by γ-irradiation and cold storage (4 ± 1°C)

Storage (days)	Count (mean ± SD cfu/g)/Irradiation dose (kGy)			
	0.0	1.0	2.0	3.0
0	h $2.11 \times 10^4 \pm 1.6 \times 10^2$	j $1.47 \times 10^4 \pm 1.45 \times 10^2$	n $7.44 \times 10^3 \pm 0.9 \times 10^2$	ND
3	f $2.70 \times 10^4 \pm 2.0 \times 10^2$	i $1.95 \times 10^4 \pm 1.7 \times 10^2$	m $7.94 \times 10^3 \pm 0.85 \times 10^2$	ND
6	e $3.27 \times 10^4 \pm 2.61 \times 10^2$	g $2.71 \times 10^4 \pm 2.21 \times 10^2$	lm $8.30 \times 10^3 \pm 1.0 \times 10^2$	ND
9	c $4.06 \times 10^4 \pm 2.58 \times 10^2$	d $3.68 \times 10^4 \pm 2.1 \times 10^2$	l $8.81 \times 10^3 \pm 1.01 \times 10^2$	ND
12	a $4.75 \times 10^4 \pm 2.7 \times 10^2$	b $4.24 \times 10^4 \pm 2.5 \times 10^2$	k $9.33 \times 10^3 \pm 0.92 \times 10^2$	ND

LSD at $P < 0.05 = 4.356 \times 10^2$
Means with different letter differ significantly ($P < 0.05$)
ND, not detected

Table 3. Colony-forming units of *L. monocytogenes* in raw beef sausage as affected by γ-irradiation and cold storage (4 ± 1°C)

Storage (days)	Count (mean ± SD cfu/g)/Irradiation dose (kGy)			
	0.0	1.0	2.0	3.0
0	f $1.78 \times 10^3 \pm 1.9 \times 10^2$	g $1.32 \times 10^3 \pm 0.8 \times 10^2$	i $7.22 \times 10^2 \pm 0.77 \times 10^2$	ND
3	e $2.29 \times 10^3 \pm 1.48 \times 10^2$	f $1.80 \times 10^3 \pm 0.66 \times 10^2$	hi $7.78 \times 10^2 \pm 0.84 \times 10^2$	ND
6	d $3.04 \times 10^3 \pm 0.87 \times 10^2$	e $2.29 \times 10^3 \pm 1.05 \times 10^2$	hi $8.18 \times 10^2 \pm 0.47 \times 10^2$	ND
9	c $3.92 \times 10^3 \pm 1.06 \times 10^2$	d $3.04 \times 10^3 \pm 1.64 \times 10^2$	h $9.30 \times 10^2 + 0.6 \times 10^2$	ND
12	a $5.09 \times 10^3 \pm 1.05 \times 10^2$	b $4.08 \times 10^3 \pm 1.21 \times 10^2$	g $1.20 \times 10^3 \pm 0.56 \times 10^2$	ND

LSD value ($P < 0.05$) = 1.555×10^2
Means with different letter differ significantly ($P < 0.05$)
ND, not detected

in sausage (Table 3). During storage at 4 ± 1°C, further significant increases ($p < 0.05$) were observed in the counts of *L. monocytogenes* for both nonirradiated samples and those which received a 1 kGy irradiation dose, being at higher rates for control samples. Sausage samples irradiated at 2 kGy showed a gradual but insignificant slight increases in the viable counts of *L. monocytogenes* during storage for 9 days at 4 ± 1°C, then a significant increase ($p < 0.05$) in the count was observed on day 12 of storage, but the rate of increase was much lower than that in the control and with 1 kGy irradiated samples. A more than 3 log reduction in the count of *L. monocytogenes* was achieved by irradiation of sausages at a dose of 3 kGy, and this dose was sufficient to keep the count of this pathogen below the enumeration level during storage of samples at 4 ± 1°C. This is in agreement with our previous study [32]. It has been reported that a dose of 1.5 kGy should reduce the viable population of *L. monocytogenes* by at least 10^2 cfu, while a dose of 2.5 kGy should inactivate at least 10^3 cfu of this pathogen [33] and this is in agreement with the observed results. Therefore, irradiation at a dose of 3 kGy appeared to be sufficient to improve the microbiological safety of raw sausage through elimination of *E. coli* O 157 : H7 and *L. monocytogenes* present in samples.

3.2 Chemical properties

3.2.1 FFAs

Table 4 indicates that irradiation of sausage at the dose levels used was insufficient to significantly alter ($p < 0.05$) the liberation of FFAs from lipids. However, refrigerated storage (4 ± 1°C) has more effects on the significant development ($p < 0.05$) of lipolysis and liberation of FFAs which may be attributed to the activity of both meat and microbial

Table 4. FFAs of lipids separated from irradiated and non-irradiated raw beef sausage during cold storage (4 ± 1°C)

Storage (days)	FFAs% (mean ± SD)/irradiation dose (kGy)			
	0	1	2	3
0	0.817 ± 0.03^{i}	0.800 ± 0.03^{i}	0.813 ± 0.04^{i}	0.827 ± 0.03^{i}
3	0.900 ± 0.02^{h}	0.890 ± 0.03^{h}	0.883 ± 0.02^{h}	0.883 ± 0.02^{h}
6	1.297 ± 0.03^{d}	0.983 ± 0.03^{g}	0.913 ± 0.02^{h}	0.900 ± 0.02^{h}
9	1.733 ± 0.04^{b}	1.233 ± 0.04^{e}	1.080 ± 0.04^{f}	0.977 ± 0.02^{g}
12	2.047 ± 0.06^{a}	1.563 ± 0.07^{c}	1.267 ± 0.04^{de}	1.083 ± 0.05^{f}

LSD value ($p < 0.05$) = 0.05227
Means with different letter differ significantly ($p < 0.05$)

lipases. These lipolytic enzymes are not fully inactivated even by doses on the order of 50 kGy [34]. The rate of lipolysis during storage of sausages decreased with increasing the applied dose, which may indicate the reduction of lipolytic microorganisms due to irradiation treatments.

3.2.2 Oxidation of lipids

The influence of irradiation and refrigerated storage on the oxidation of sausage lipids was evaluated by the determination of *p*-anisidine value (Table 5). As shown, lipids separated from sausages subjected to the increasing doses of irradiation had a significantly higher ($p < 0.05$) *p*-anisidine value than lipids of nonirradiated sausages. Moreover, subsequent significant increases ($p < 0.05$) in *p*-anisidine values for lipids of sausages were also observed during storage of both irradiated and non irradiated samples, but with higher rates for the irradiated ones. Ouattara *et al.* [35] also reported the oxidation of lipids in ground beef due to irradiation and storage. The higher surface-to-volume ratio of ground beef could have led to greater contact with air, making it more susceptible to oxidation [36].

3.2.3 Solubility of sarcoplasmic and myofibrilar proteins

The results in Tables 6 and 7 show that sarcoplasmic and myofibrilar proteins in fresh nonirradiated sausages amounted to 9.58 and 17.33 g/100 g dry matter, respectively. These values represented 19.4 and 35% of the total protein (referred to dry matter), respectively, and may reflect the possible denaturation of beef proteins as indicated by their relatively reduced solubility [22]. It was reported that sarcoplasmic and myofibrilar proteins represent 33.37 and 53.55% of the total proteins (referred to dry matter), respectively, in mammalian muscles before degradative changes [37]. The lower solubility of sausage proteins may be due to the utilization of frozen meat in the preparation of the product in addition to curing. Gracey [38] mentioned that muscle plasma proteins become insoluble and do not regain their solubility when the meat was frozen and thawed, while Astiasaran *et al.* [39] found that curing of

Table 5. *p*-Anisidine values of lipids separated from irradiated and nonirradiated raw beef sausage during cold storage (4 ± 1°C)

Storage (days)	Values (mean ± SD)/irradiation dose (kGy)			
	0	1	2	3
0	7.757 ± 0.11^{m}	8.437 ± 0.43^{l}	9.580 ± 0.14^{k}	11.140 ± 0.22^{h}
3	8.687 ± 0.02^{l}	9.790 ± 0.55^{k}	10.100 ± 0.12^{j}	12.790 ± 0.07^{e}
6	9.737 ± 0.07^{k}	10.160 ± 0.05^{j}	11.600 ± 0.12^{g}	14.100 ± 0.21^{c}
9	10.650 ± 0.20^{i}	11.300 ± 0.07^{h}	12.960 ± 0.13^{de}	15.120 ± 0.23^{b}
12	12.180 ± 0.18^{f}	13.100 ± 0.25^{d}	14.020 ± 0.24^{c}	15.920 ± 0.18^{a}

LSD value ($p < 0.05$) = 0.2863
Means with different letter differ significantly ($p < 0.05$)

Table 6. Effects of γ-irradiation and cold storage of raw beef sausage (4 ± 1°C) on solubility of sarcoplasmic proteins

Storage (days)	Amount (mean ± SD g/100 g dry matter)/irradiation dose (kGy)			
	0	1	2	3
0	9.587 ± 0.54^{a}	9.623 ± 0.57^{a}	9.587 ± 0.54^{a}	9.627 ± 0.64^{a}
3	9.240 ± 0.62^{a}	9.237 ± 0.53^{a}	9.247 ± 0.48^{a}	9.243 ± 0.57^{a}
6	9.073 ± 0.40^{a}	9.110 ± 0.33^{a}	9.067 ± 0.40^{a}	9.077 ± 0.41^{a}
9	8.990 ± 0.36^{a}	9.033 ± 0.55^{a}	9.020 ± 0.55^{a}	8.953 ± 0.38^{a}
12	8.900 ± 0.23^{a}	8.953 ± 0.19^{a}	8.987 ± 0.25^{a}	8.987 ± 0.22^{a}

LSD value ($p < 0.05$) = 0.05227
Means with the same letter (a) do not differ significantly ($p < 0.05$)

Table 7. Effects of γ-irradiation and cold storage of raw beef sausage (4 ± 1°C) on solubility of myofibrilar proteins

Storage (days)	Amount (mean ± SD g/100 g dry matter)/irradiation dose (kGy)			
	0	1	2	3
0	17.33 ± 0.34^{a}	17.39 ± 0.43^{a}	17.31 ± 0.34^{a}	17.32 ± 0.44^{a}
3	17.33 ± 0.39^{a}	17.40 ± 0.38^{a}	17.37 ± 0.41^{a}	17.32 ± 0.39^{a}
6	17.31 ± 0.42^{a}	17.31 ± 0.44^{a}	17.36 ± 0.35^{a}	17.39 ± 0.34^{a}
9	17.30 ± 0.43^{a}	17.30 ± 0.31^{a}	17.21 ± 0.40^{a}	17.29 ± 0.41^{a}
12	17.33 ± 0.40^{a}	17.33 ± 0.30^{a}	17.32 ± 0.33^{a}	17.29 ± 0.34^{a}

LSD value ($p < 0.05$) = 0.6337
Means with the same letter (a) do not differ significantly ($p < 0.05$)

sausage reduced the extractability of these proteins. With regards to the effects of irradiation and cold storage of sausage, the presented data clearly illustrate that neither γ-irradiation nor refrigeration (4 ± 1°C) could significantly alter ($p < 0.05$) the solubility of sarcoplasmic and myofibrilar proteins. Similar findings were observed by Emam [40].

3.2.4 WHC

The ability of meat and meat products to retain moisture before, during, and after processing or cooking plays a criti-

Table 8. WHC of raw beef sausage as affected by γ-irradiation and cold storage at (4 ± 1°C)

Storage (days)	WHC % (mean ± SD)/irradiation dose (kGy)			
	0	1	2	3
0	13.300 ± 0.50[a]	13.300 ± 0.64[a]	13.300 ± 0.56[a]	13.280 ± 0.46[a]
3	12.230 ± 0.36[b]	12.260 ± 0.33[b]	12.260 ± 0.30[b]	12.280 ± 0.45[b]
6	11.170 ± 0.07[c]	11.150 ± 0.09[c]	11.140 ± 0.15[c]	11.130 ± 0.10[c]
9	10.060 ± 0.07[d]	10.050 ± 0.08[d]	10.020 ± 0.06[d]	10.040 ± 0.06[d]
12	9.713 ± 0.03[d]	9.690 ± 0.04[d]	9.663 ± 0.07[d]	9.647 ± 0.07[d]

LSD value ($p < 0.05$) = 0.4116
Means with different letter differ significantly ($p < 0.05$)

cal role in palatability and consumer acceptance of the product [41]. Irradiation of sausage at the different doses used in the present work showed no significant effects ($p < 0.05$) on the WHC of the samples (Table 8). However, samples of irradiated and nonirradiated sausages showed significant decreases ($p < 0.05$) in their WHC during the first six days of subsequent refrigerated storage (Table 8). This loss of WHC may probably be due to the observed protein denaturation as a result of freezing of meat utilized in the production of sausages. Edwards [42] illustrated that freezing of muscle tissue resulted in protein denaturation and loss of WHC.

In conclusion, irradiation of raw beef sausage at a dose of 3 kGy effectively reduced the counts of *E. coli* O 157 : H7 and *L. monocytogenes* by more than 4 log and 3 log units, respectively. Thus, a 3 kGy dose of γ-irradiation appeared to be sufficient to improve the microbiological safety of raw beef sausages during 12 days of refrigerated storage (4 ± 1°C) without adverse effects on their chemical properties.

4 References

[1] Tarr, P. I., Besser, T. E., Hancock, D. D., Keene, W. E., Goldoft, M., Verotoxigenic *Escherichia coli* infections: US overview. *J. Food Prot.* 1997, *60*, 1466 – 1471.

[2] Huffman, R. D., Current an future technologies for decontamination of carcasses and fresh meat. *Meat Sci.* 2002, *62*, 285 – 294.

[3] Satin, M., Use of irradiation for microbial decontamination of meat: situation and prospectives. *Meat Sci.* 2002, *62*, 277 – 283.

[4] Mermelstein, N. H., Controlling *Escherichia coli* O 157 : H7 in meat. *Food Tech.* 1993, *47*, 90 – 91.

[5] Anonymous, *Annual epidemiological report*, Swedish Institute for Infectious Disease Control, 2000.

[6] Donnelly, C. W., *Listeria monocytogenes*, in: Labbé, R. G., García, S. (Eds). *Guide to Foodborne Pathogens*, Wiley-Interscience, New York 2001, pp. 99 – 132.

[7] Pearson, A. M., Gillett, T. A. (Eds.), *Processed Meats*, Chapman & Hall, New York 1996.

[8] Anonymous, *Microbiological Monitoring of Ready-to-Eat Products, 1993 – 1996*, US Department of Agriculture, Food Safety and Inspection Service, Washington, DC 1997.

[9] Todd, E. C. D., Epidemiology and globalization of foodborne disease, in: Labbí, R. G., García, S. (Eds.), *Guide to Foodborne Pathogens*, Wiley-Interscience, New York 2001, pp. 1 – 22.

[10] Dogbevi, M. K., Vachon, C., Lacroix, M., Physicochemical and microbiological changes in irradiated fresh pork loins. *Meat Sci.* 1999, *51*, 349 – 354.

[11] Giroux, M., Lacroix, M., Nutritional adequacy of irradiated meat: a review, *Food Res. Int.* 1998, *31*, 257 – 264.

[12] Clavero, M. R. S., Monk, J. D., Beuchat, L. R., Doyle, M. P., Brackett, R. E., Inactivation of *Escherichia coli* O 157 : H7, Salmonella, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Appl. Environ. Microb.* 1994, *60*, 2069 – 2075.

[13] Fu, A. H., Sebranek, J. H., Murano, E. A., Survival of *Listeria monocytogenes*, *Yersinia enterocolitica* and *Escherichia coli* O 157 : H7 and quality changes after irradiation of beef steaks and ground beef. *J. Food Sci.* 1995, *60*, 972 – 977.

[14] Andrews, L. S., Ahmedna, M., Grodner, R. M., Liuzzo, J. A., *et al*., Food Preservation using ionizing radiation. *Rev. Environ. Contam. Toxicol.* 1998, *154*, 1 – 53.

[15] López-González, V., Murano, P. S., Brennan, R. E., Murano, E. A., Influence of various commercial packaging conditions on survival of *Escherichia coli* O 157 : H7 to irradiation by electron beam versus gamma rays. *J. Food Prot.* 1999, *62*, 10 – 15.

[16] *Oxoid, The Oxoid Manual*, 8th ed. Oxoid Ltd., Hampshire, UK 1998.

[17] Bille, J., Doyle, M. P., Listeria and Erysipelothrix, in: Balows, A., Hauster, W. J. Jnr., Herrman, K. L., Isenberg H. D., Shadomy H. J. (Eds.), *Manual of Clinical Microbiology*, 5th ed. American Society for Microbiology, Washington, DC 1991, pp. 287 – 295.

[18] Folch, Y., Lees, M., Sloena-Stanley, G. H., A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 1957, *226*, 497 – 509.

[19] AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed., Champaign, IL 1998.

[20] Egan, H., Kirk, R. S., Sawyer, R. (Eds.), *Pearson's Chemical Analysis of Foods*, Churchill Livingstone, London 1981.

[21] Diaz, O., Fernández, M., García de Fernando, G., de la Hoz, L., Ordóñez, J. A., Effect of the addition of pronase E on the proteolysis in dry fermented sausage. *Meat Sci.* 1993, *34*, 205 – 216.

[22] Warner, R. D., Kuffman, R. G., Greaser, M. L., Muscle protein changes post mortem in relation to pork quality traits. *Meat Sci.* 1997, *45*, 339 – 352.

[23] Rocha-Garza, A. E., Zayas, J. F., Quality of broiled beef patties supplemented with wheat germ protein flour. *J. Food Sci.* 1996, *61*, 418 – 421.

[24] Nissen, O., *MSTAT-C*, A microcomputer programme for the design, management and analysis of agronomic research experiments, version 1, Department of Agricultural Econmics, Michigan University, 1993.

[25] Feng, P., *Escherichia coli*. in: Labbé, R. G. , García, S. (Eds.), *Guide to Foodborne Pathogens*, Wiley-Interscience, New York 2001, p. 143 – 162.

[26] Faith, N. G., Shere, J. A., Brosch, R., Arnold, K. W., *et al.*, Prevalence and clonal nature of *Escherichia coli* O 157 : H7 on dairy farms in wisconsin. *Appl. Environ. Microb.* 1996, *62*, 1519 – 1525.

[27] Caya, F., Fairbrother, J. M., Lessard, L., Quessy, S., Characterization of the risk to human health of pathogenic *Escherichia coli* isolated from chicken carcasses. *J. Food Prot.* 1999, *62*, 741 – 746.

[28] Glass, K. A., Doyle, M. P., Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environ. Microb.* 1989, *55*, 1565 – 1569.

[29] Palumbo, S. A., Williams, A. C., Resistance of *Listeria monocytogenes* to freezing in foods. *Food Microb.* 1991, *8*, 63 – 68.

[30] Boerlin, P., Piffaretti, J. C., Typing of human, animal, food and environmental isolates of *Listeria monocytogenes* by multilocus enzyme electrophoresis. *Appl. Environ. Microb.* 1991, *57*, 1624 – 1629.

[31] Borch, E., Arinder, P., Bacteriological safety issues in red meat and ready-to-eat meat products, as well as control measures. *Meat Sci.* 2002, *62*, 381 – 390.

[32] Badr, H. M., Use of irradiation to control foodborne pathogens and extend the refrigerated market life of rabbit meat. *Meat Sci.* 2004, *67*, 541 – 548.

[33] Thayer, D. W., Use of irradiation to kill enteric pathogens on meat and poultry. *J. Food Safety* 1995, *15*, 181 – 192.

[34] Lefebvre, N., Thibault, C., Charbonneau, R., Piette, J.-P. G., Improvement of shelf-life and wholesomeness of ground beef by irradiation. 2. Chemical analysis and sensory evaluation. *Meat Sci.* 1994, *36*, 371 – 380.

[35] Ouattara, B., Giroux, M., Yefsah, R., Smoragiewicz, W., *et al.*, Microbiological and biochemical characteristics of ground beef as affected by gamma irradiation, food additives and edible coating film. *Rad. Phys. Chem.* 2002, *63*, 299 – 304.

[36] Rodriguez-Estrada, M. T., Penazzi, G., Caboni, M. F., Bertacco, G., Lercker, G., Effect of different cooking methods on some lipid and protein components of hamburgers. *Meat Sci.* 1997, *45*, 365 – 375.

[37] Adams, M. R., Moss, M. O. (Eds.), *Food Microbiology,* The Royal Society of Chemistry, Cambridge 1995.

[38] Gracey, J. F. (Ed.), *Meat Hygiene*, English Language Society/ Bailliére Tindall, London 1991.

[39] Astiasaran, I., Villanueva, R., Bello, J., Analysis of proteolysis and protein insolubility during the manufacture of some varieties of dry sausage. *Meat Sci.* 1990, *28*, 111 – 117.

[40] Emam, O. A. M., Effects of irradiation on some foodstuffs and their products. *PhD Thesis*, Faculty of Agriculture, El-Mansoura University, Egypt 1990.

[41] Xiong, Y. L., Structure – Function relationships of muscle proteins, in: Damodaran, S., Paraf, A. (Eds.), *Food Proteins and their Applications*, Marcel Dekker Inc., New York 1997, pp. 341 – 392.

[42] Edwards, M., Changes in cell structure, in: Beckett, S. T. (Ed.), *Physico-chemical Aspects of Food Processing*, 1st ed., Chapman & Hall, London 1995, pp. 212 – 233.

