Pakistan J. Zool., vol. 48(5), pp. 1373-1379, 2016.

Inhibition of *Escherichia coli* 0157:H7 Growth by Gamma Radiation Improves the Hygienic Quality of Chilled Fresh Beef Meat

Wafaa Sayed Mohamed*[1] and Afaf Mohamed Ezz El-Deen[2]
[1] *Radiation Health Research Department, Vet. Res. Unit, The National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority. O. Box: 29 Nasr City, Cairo, Egypt*
[2]*Department of Bacteriology and Food Hygiene, Animal Health Research Institute, Giza Laboratory, Egypt.*

ABSTRACT

The focus of this study was to evaluate the effect of γ radiation and chilling on the hygienic quality of fresh beef meat, fatty acids content and survival of *Escherichia coli* as food poisoning microorganisms. Sixty fresh beef samples were hygienically examined, surveyed for *E. coli O157: H7*; eight samples were positive (13.3%). Sensory examination revealed that there were no significant changes between γ radiated (at 3 kGy) and non-irradiated groups in color, odor and texture. γ radiation caused slight significant effect on the chemical analysis after irradiation; there was no significant changes in pH values it slightly increased the amount of total volatile basic nitrogen (TVN), thiobarbituric acid reactive substances (TBA) and peroxide value. Fatty acids of irradiated chilled samples were slightly affected as compared with control samples; the irradiated samples had higher saturated fatty acid content (C16:0, C18:0) and lower unsaturated fatty acid content (C18:1, C18:2) than the non-irradiated sample. We recommend exposing meat to 3 kGy of γ radiation to prolong its chilling life hygienically.

Article Information
Received 18 May 2015
Revised 15 January 2016
Accepted 29 January 2016
Available online 1 August 2016

Authors' Contribution
WSM designed the research, performed chemical analysis and described sensory characters. AMEE performed microbiological and statistical analysis. Both authors interpreted the data and finalized the manuscript.

Key words
***Escherichia coli O157: H7*, gamma radiation, beef meat chilling, fatty acids, sensory, chemical.**

INTRODUCTION

The increased microbial load in food causes food-borne infections, their destruction leads to a significant increase in the acceptable shelf life of food (Osés *et al.*, 2012). Data indicating trends in food-borne infectious intestinal disease is limited to few industrialized countries (CDC, 2013). The causes of diarrhoea include viruses, bacteria and parasites; bacterial pathogens contribute in 60% of the food borne illnesses that lead to hospitalization and account for two thirds of the estimated number of food borne pathogens related deaths via beef consumption (Al-Gallas *et al*., 2007).

Escherichia coli are bacterial species that normally live in the intestines of people and other warm-blooded animals. Diarrheagenic *E. coli* is the main causative pathogens that result in endemic and epidemic diarrhoea (Ganguly *et al.*, 2012). Most *E. coli* are harmless and actually are important for healthy human intestinal tract produce vitamin K and help to prevent colonization by disease-causing bacteria (Kim *et al*., 2012). *E. coli* can contaminate the farm, water catchments and food processing environment via faeces then contaminate foods. *E. coli O157:H7* is a potentially deadly bacterium that can cause dehydration, bloody diarrhoea and abdominal cramps (3–4 days) after exposure to the organism (FSIS, 2015). The presence of *E. coli* in meat is an indicator of direct or indirect contamination and lack of hygiene in handling and production operation of meat, inadequate storage and post process contamination (Sofos, 2014). The extent of bacterial contamination increase during the grinding process of meat because of lack in temperature controls (Müller *et al*., 2012).

Typical Gram-negative spoilage organisms are very sensitive to irradiation. Combinations of irradiation and low storage temperature can produce hygienic meat (O′ Bryan *et al.*, 2008). The alteration in lipid composition as a result of irradiation depends on the dose of irradiation, storage temperature and packaging conditions (Özden *et al.*, 2007). The composition of beef is about 18% lipids and its fatty acids content is divided into 46% saturated, 51% mono-unsaturated and 3% poly-unsaturated. Some of the fatty acids found in meat play important roles in metabolism. Recent interest in trans fatty acids was sparked off by epidemiological evidence linking trans fatty acids to higher plasma total cholesterol and low-density-lipoprotein cholesterol and increased the incidence of coronary heart disease (Park *et al*., 2010). Taste, appearance, texture, and microbiological safety are required to be preserved within a foodstuff for the longest period of time (Zhanga, 2010).

* Corresponding author: wsmas1960@yahoo.com.
0030-9923/2016/0005-1373 $ 8.00/0

This work aimed to estimate the effect of gamma irradiation of fresh beef on the survival of *E. coli O157: H7*, its sensory attributes, chemical composition and fatty acid content.

MATERIALS AND METHODS

Sampling

Sixty fresh beef samples were collected from different grocery shops in Cairo and Giza governorate (Egypt). The samples were aseptically transferred to the laboratory in an icebox and were kept at 4±1°C.

Irradiation process

The irradiation process was carried out using the Russian Medical Sterilizing CM-20 γ cell located at the National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The source was giving a dose rate of 2.4 kilo Gray kGy/hour at the time of experiment. Dose levels of 0, 3 and 7 kGy were used. The samples were kept cold during irradiation.

Microbiological survey

Out of each sample, 25 g were inoculated into 250 ml peptone water (enrichment broth), incubated at 37°C for 24 h and then, cultivated onto nutrient agar, eosin methylene bile agar (Kim and Bhunia, 2008) and MacConkey agar media and incubated for 24 h at 37°C. Also, 1 ml of the inoculated peptone water was transferred to 9 ml selenit "F" broth (pre-enrichment fluid media, Oxoid) and incubated at 37°C for 12-18 h. An inoculum was then cultured onto Salmonella - Shigella (S.S.) agar medium. After overnight incubation of MacConkey agar, a part of single typical well isolated lactose fermenting colonies was tested for sorbitol fermentation by cultivating onto sorbitol MacConkey agar (S.M.A.) (Oxoid), and incubated at 37°C over night. From sorbitol MacConkey agar, colorless or pale colonies were considered as non-fermenters of sorbitol and pink colonies as sorbitol fermenters. Non-sorbitol fermenter colonies on S.M.A. and non-lactose fermenter colonies with or without black centre on S-S media were examined culturally, morphologically, biochemically, as well as serologically. Non-sorbitol fermenting isolates were serotyped using *E. coli* antisera (polyvalent 3 and monovalent 0157, H7) according to Collins and Lyne (1976). Antisera were obtained from Denka Seiken Laboratory, Tokyo. Japan to serology unit, Animal Health Research Institute, Dokki, Giza.

Polymerase chain reaction (Stacy-Phipps et al*., 1995)*

For identification of the strain *E. coli O157:H7*, genomic DNA was isolated from the cultures by chromosomal DNA extraction kit (Gen Jet Genomic DNA purification kit K0271, Fermentas), and used for amplification of a 350- bp segment of the shiga like toxin I gene (*sItI*) using a pair of primers specific to *sItI* gene (Trochimchuka *et al*., 2003). The sequence of the forwarded and reverse primer was
5'- ACC TCA CTG ACG CAG TCT GTG G-3'
(nucleotide position 508) and
5'- TCT GCC GGA CAC ATA GAA AA-3',
respectively.

The PCR reaction mixture (total volume of 50 µl) was 25 µl Dream green PCR Reddy Mix (Dream Taq Green PCR Master Mix (2X) Fermentas Company, cat., No.K1080, USA.), 5 µl target DNA, 2 µl of each primers (containing 10 p mole/µl) and the mixture was completed by sterile distilled water to 50 µl PCR amplification conditions for yeast isolates were 5 min initial step followed by 35 cycles each of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and a final extension step at 72°C for 7 min using Gradient Thermal cycler (1000 S Thermal cycler Bio-Rad USA)

Bacteriological examination

Aerobic bacterial count was carried out on standard plate count agar at 35°C for 48 h according to APHA (1992). Ten grams of each sample were homogenized with 90 ml of 1% sterile peptone water for 1 minute using stomacher (Lab-blender 400Seward. Serial No. 30469 type BA 7021, London), to provide 10^{-1} dilution, then tenfold decimal serial dilution up to 78 were prepared. Aerobic bacterial count was carried out on a standard plate count agar at 35°C for 48 h according to FDA (2001a). Yeast and mold counts were performed on Sabouraud dextrose agar medium (Oxoid) supplemented with chloramphenicol (0.05mg/ml) and incubated at 25°C for 5 days as described by Koneman *et al*. (1994).

Chemical evaluation

The pH was determined by blending the samples with distilled water (1:10) and the pH values of the suspension was evaluated by pH meter directly as the mean value of three measurements (El- Sheshetawy *et al*., 2009).

Total volatile basic nitrogen (TVN) was determined using standard methods (AOAC, 2010). Peroxide values were determined according to Shantha and Decker (1994).

Thiobarbituric acid (TBA) detection of malonaldehyde (a common lipid peroxidation product) was performed in triplicates according to Tarladgis *et al*. (1960) using trichloroacetic acid 7.5% freshly prepared 0.02M TBA solution and the absorbance of the developed red color was measured at wave length 538nm

malonaldehyde formed during lipid oxidation in fresh meat samples were measured and reported in units of mg MDA/kg sample.

Lipid analysis

After irradiation the fatty acids of beef minced meat were extracted according to Scholfield *et al.* (1963). Then fatty acid analysis was conducted using Hp 6890 gas chromatograph instrument equipped with: HP5-5% phenyl methyl siloxane 25 ml; i.d.0.32µm; 0.17 µm film thickness. Oven temperature was 170°C for 1 min, then raised with a rate of 5°C/min to 190°C, hold for 1 min, then raised with a rate of 2°C/min to 210°C hold for 1 min, then raised with a rate of 5°C/min to 240°C hold for 10 min. Carrier gas: Nitrogen; Flow rate, 1.3 ml/min; Detector, FID, 275°C; Inj. temp, 260°C.

Sensory examination

The samples were evaluated physically before and after irradiation for colour, odour and texture according to O′ Bryan *et al.* (2008).

Statistical analysis

The experiment was conducted 3 times and the results were presented as means, standard deviation and simple error (Draper and Smith, 1998). All statistical procedures were computed using the Microsoft Excel 2007 in order to compare the mean values of the investigated parameters.

RESULTS AND DISCUSSION

E. coli O157:H7 *count*

E. coli O157:H7 was isolated from 8 out of 60 examined samples (13.3%) and the count of the organism was followed during 7 days of refrigeration at 4±1˚C (Fig. 1).

E.coli 0157: H7 count increased till it reached log 4.5 in control (non-irradiated) stored samples. It was noticed that the counts of the organism increased as the storage period increased in control and γ radiated samples but to less extent. Li *et al.* (2015) reported that irradiation at 7 kGy controlled *E. coli* in refrigerated and frozen meat and at 3 kGy *E. coli* were reduced by 5 log CFU/g. It was noticed that the counts of the organism increased as the storage period increased (Fratamico and Bagi, 2007). Irradiation at 1 kGy of beef carcasses lowered the hazard represented by *E. coli* and reduced the viability of two groups of non-*O157 E. coli* mixtures by ≤ 4.5 and ≤ 3.9 log CFU/g (Kundua *et al.*, 2014). Yoona *et al.* (2013) found that total bacterial and *E. coli O157:H7* populations remained unchanged during chilling storage.

The identification of the bacteria was confirmed by PCR amplification of *slt1* gene which is characteristics of *E. coli O157:H7* (Fig. 2).

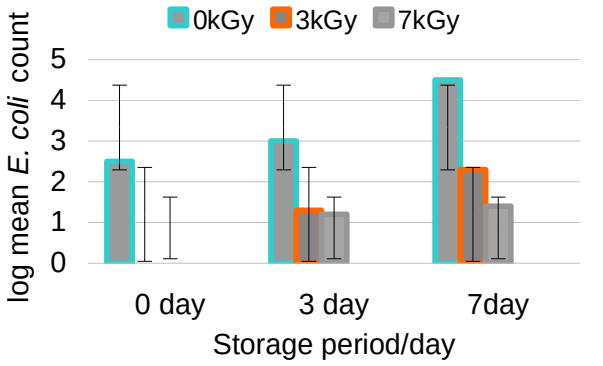

Fig. 1. The effect of irradiation and storage at 4±1ºC for 7 days on log mean *E.coli O157:H7* count.

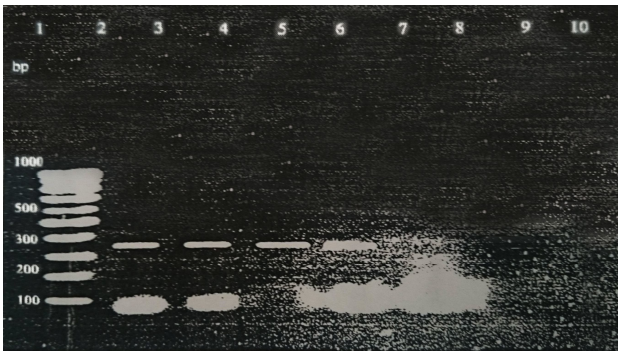

Fig. 2. The **e**lectrophoretic pattern of amplified *E.coli O157:H7* PCR product by using pair primers specific to *s1t1* gene with amplification of 350-bp. Lane 1, 100 bp DNA ladder. Lane 2, amplicon of the reference strain *E. coli O157:H7.* Lanes 3-5, positive samples. Lane 6, negative control reaction.

Similar results were recorded by Beutin *et al.* (2007) who stated that the combination of serotyping and stx genotyping was found useful for identification and for assignment of food- borne pathogens.

Hygienic quality of fresh beef

Microbiological evaluation

Table I shows that the initial total bacterial count of beef meat during chilling storage at 4±1°C for 7 days was higher than γ radiated samples.

Beef meat control (non -irradiated) samples were rejected at the end of storage period according to EOS (2005) which stated that the meat product is rejected if

the total bacterial count exceeds 10^6 CFU/g. It was clear from the results in Table I that γ radiation at 3 and 7 KGy reduced the bacterial counts as they decreased immediately after irradiation at 3 kGy to non-detectable counts and post irradiation storage at 4±1ºC. The total mold and yeast count on day 7 in all samples irradiated at 3 and 7 kGy was lower than the count in non-irradiated samples on day 0.

Table I.- Changes of mean total bacterial count (TBC) and total yeast & mold count (TY&MC) of beef meat stored at 4±1°C after γ radiation.

	Storage period/ day		
	Day 0	**Day 3**	**Day 7**
TBC (CFU/g)			
0	5.3×10^5	$4.1x10^6$	$7.8x10^7$(R)
3	<10	$1.4x10^3$	$3.4x10^4$
7	<10	<10	$3.4x10^2$
TY&MC			
0	$9.1x10^2$	$5x10^4$	$9.6x10^7$(R)
3	<10	<10	$5.4x10^3$
7	<10	<10	$1.9x10^2$

R: Rejected

Sommers (2012) showed that microbial resistance to radiation usually decreases in the order: bacterial spores followed by yeast and molds. Gram-positive bacteria were less sensitive than Gram-negative bacteria. Peter *et al*. (2005) reported that doses 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. The study revealed that irradiation (3 and 7 kGy) and storage at low temperature had a reduction effect on microbial loads of beef meat samples. These findings indicated that food spoilage microorganisms are generally very susceptible to γ radiation and came in parallel with Yazdi and Jouki (2012).

Chemical evaluation

Table II shows that γ radiation at 0, 3, 7 kGy did not cause significant changes in pH values but there was an increase in TBA and peroxide values after 3 and 7 kGy of irradiation.

The pH value is considered to be an important factor, because of its influence on many characteristics, including shelf life, color, water holding capacity and texture of meat and products. According to the results in Table II there was a slight increase of pH values during refrigeration storage especially in non-irradiated samples. These results are consistent with Kim *et al*. (2012). Lipid oxidation in meat is one of the major degradative change responsible for loss of meat quality. It results in the formation of warmed off flavor, destruction of essential fatty acids and loss of vitamins. TBA values were considered as an index of lipid rancidity. γ radiation induced the oxidation of lipids and production of lipid oxides. Subsequent decomposition of the unstable lipid oxides produce malonaldehyde (Yoon, 2003). The effect of various levels of γ irradiation on TVN contents which is an indicator for bacterial decomposition that occurred and protein breakdown during storage of fresh beef (Table II). The results showed that irradiation caused slight increase in TVN as irradiation dose increased. Storage significantly increased the TVN for non-irradiated samples, these results come in accordance with Brewer (2009). This slight increase may be due to effect of proteolytic enzymes on protein with formation of products from protein breakdown as carbonyl group, ammonia, hydrogen peroxide and organic peroxide (Park *et al*., 2010).

Table II.- Effect of γ radiation on deterioration criteria of fresh beef meat during chilling storage period (4±1ºC).

Dose (kGy)	Storage period/day		
	Day 0	**Day 3**	**Day 7**
pH			
0	5.87 ± 0.26	5.70 ± 0.22	5.70 ± 0.24
3	5.77 ± 0.36	5.70 ± 0.10	5.77 ± 0.15
7	5.77 ± 0.23	5.67 ± 0.26	5.67 ± 0.26
Total volatile basic nitrogen (TVN: mg/100gm)			
0	12.30±0.36	13.37±0.15	18.8±0.26
3	12.20±0.28	13.36±0.36	14.10±0.33
7	12.20±0.25	13.82±0.23	14.00±0.29
Thiobarbituric acid (TBA: mg MD/kg meat)			
0	0.18±0.31	0.27±0.32	1.05±0.32
3	0.20±0.28	0.30±0.21	0.52±0.22
7	0.19±0.22	0.35±0.41	0.56±0.12
Peroxide value (mEq/O_2/Kg)			
0	2.90 ± 0.21	3.90 ± 0.28	8.0 ± 0.42
3	3.10 ± 0.11	3.41± 0.12	4.80 ± 0.12
7	3.21± 0.11	3.31± 0.22	5.20 ± 0.12

(Results are expressed as Means ± S.E).

Peroxide value and TBA measurements were chosen as representative of primary and secondary lipid oxidation. Table II also revealed that there was an increase in the TBA and peroxide values after 3 and 7 kGy of γ radiation; they increased with the storage time at refrigeration temperature but within the permissible limits (0.90 mg MD/kg meat) approved by the ESS-1972/2005.

In comparison with non-irradiated samples, similar results were also reported by Kumar *et al.* (2013).

Lipid analysis

The fatty acids composition after different irradiation doses is presented in Table III. Most chemical changes in irradiated meat are associated with free radical reactions, and the resultant sulphur compounds could be controlled by using appropriate packaging methods and additive combinations which can control color, off-odor and lipid oxidation in irradiated raw meat during storage. This change may be due to a decrease in salt soluble protein and water soluble protein (Mahboob *et al.*, 2015).

Table III.- Fatty acid composition (%) of γ radiated chilled beef meat.

Irrad. dose	C14.0	C16.0	C18.0	C18.1	C18.2	Trans f. a.
Control	2.32	21.2	1.59	16.5	38.2	20.19
3 kGy	1.27	19.3	1.99	12.1	25.7	39.64
7 kGy	0.84	7.31	2.37	4.8	9.4	75.28

Table III showed that fatty acid C16 (palmitic acid) usually is the major saturated fatty acid, oleic (C18:1) and linoleic (C18:2) are the major unsaturated fatty acids. There is a decrease in linoleic acid. There is an increase of unknown acids (trans fatty acids) related to the increase in irradiation dose which occur due to a change in molecular structure of fatty acids, breaking down double bonds, forming free radicals and trans fatty acids. Fan and Kays (2008) found that C18:1 trans was the dominant fatty acid; when expressed as the percentage of total sample weight it increased from 3.99 % for control beef meat to 4.05 % for 5 kGy irradiated samples. Kim *et al.* (2011) stated that samples irradiated at 4 kGy had a higher saturated fatty acid content (C14:0, C16:0, C18:0) and significantly lower unsaturated fatty acid content (C16:1, C18:1, C18:3).

Sensory evaluation

It was found that the color, odor and texture were not changed and remained acceptable during the storage period. Also there were no differences between γ radiated and non- irradiated minced meat samples (Table IV).

The final evaluation was performed in individual sensory booths, under red lights to prevent color differences from influencing the panellists. All the samples were visually examined for abnormal color, odor and texture. Karada and Günefi (2008) reported that irradiation doses up to 3 kGy did not cause any change in the sensory attributes but sensory changes became a problem after long storage periods or higher irradiation doses. Park *et al.* (2010) stated that the use of irradiation up to 10 kGy on patties was useful in reducing bacterial populations with no adverse effect on most of sensory characteristics.

Table IV. Sensory evaluation of irradiated and non-irradiated beef meat during chilling storage (4±1°C) period.

Dose (kGy)	Storage period/day		
	Day 0	Day 3	Day 7
Color			
0	4.9±0.02	4.2±0.31	3.1±0.15
3	4.81±0.10	4.71±0.1	4.2±0.30
7	4.85±0.11	4.7±0.24	4.5±0.11
Odor			
0	4.5±0.11	4.5±0.23	1.7±0.30
3	4.72±0.02	4.6±0.01	3.6±0.14
7	4.74±0.13	4.61±0.04	3.8±0.54
Texture			
0	4.89±0.01	4.3±0.60	1.8±0.22
3	4.9±0.02	4.5±0.14	3.2±0.14
7	4.85±0.10	4.6±0.03	3.2±0.16

(Results are expressed as Means ± S.E)

Meat color is perceived by consumers as indicative of freshness in that they discriminate against meat that has turned brown in color (O'Bryan *et al.*, 2008). Irradiation of fresh beef showed no significant change in color and all samples were accepted by the panellists. Off odor appear as irradiation dose increase. This could be overcome by decreasing the temperature of the product during irradiation (Ahn and Lee, 2009).

CONCLUSION

The application of γ radiation 3 and 7 kGy to chilled fresh beef meat induced significant reduction in total aerobic bacterial count and total mold and yeast counts. Doses of 3 and7 kGy eradicated *E. coli O157:H7*. γ radiation showed slight significant effect on the chemical analysis but were still within the permissible limits approved by the ESS-1972/2005 and had no adverse effect on its quality. The irradiated samples had higher saturated fatty acid. The increase of trans fatty acids in the irradiated beef is one of the important factors that can be considered in the irradiation process.

ACKNOWLEDGMENTS

Authors would like to thank Radiation Health Department, Veterinary Research Unit. The National

Centre for Radiation Research and Technology (NCRRT)–Atomic Energy Authority. Animal Health Research Institute, Giza Lab.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Al-Gallas, N., Bahri, O., Bouratbeen, A., Haasen, A.B. and Aissa, R.B., 2007. Aetiology of acute diarrhoea in children and adults in Tunis, Tunisia, with emphasis on diarrheagenic Escherichia coli: Prevalence, phenotyping, and molecular epidemiology. *Am. J. trop. Med. Hyg.*, **77**: 571- 582.

AOAC, 2010. Association of Official Analytical Chemists. In: *Official methods of analysis* (15th Ed), Arlington, Kennesh Helrich (Ed.), Virginia 2220, Washington, DC USA, pp. 1452-1473.

APHA (American Public Health Association), 1992. *Compendium of methods for the microbiological examination of foods*. Third Ed. APHA Technical Committee on Microbiological Methods for Food. Washington. DC. USA.

Beutin, L., Miko, A., Krause, G., Pries, K., Haby, S., Steege, K. and Albrechet, N., 2007. Identification of human pathogenic strains of Shiga toxin producing *E. coli* from food and a combination of serotyping and molecular typing of Shiga toxin gene. *Appl. environ. Microbiol.*, **73** :4769-4775.

Brewer, M.S., 2009. Irradiation effects on meat flavour: A review. *Meat Sci.*, **81**:1-14.

Centre for Disease Control and Prevention (CDC), 2013. Multistate outbreak of Shiga toxin-producing *Escherichia coli O157:H7* infections linked to ready-to-eat salads. Found on the CDC *E. Coli* Web Page.

Collins, C. H. and Lyne, R. M., 1976. Estimating bacterial numbers. In: *Microbiological methods*, 4th Ed., Butterworth, London, pp. 194-210.

Draper, N.R. and Smith, H., 1998. *Applied regression analysis*. 3rd ed., John Wiley, New York, pp. 131-153.

El-Sheshetawy, H. E., Yasin, N. M. N. and Abo-El-Fetoh, S. M., 2009. Tarhana dough as a source of probiotics in processing of functional fermented meat sausage (as a home-made trial). *Annl. agric. Sci*., **54**: 339-348.

Egyptian Organization for Standardization and Quality Control, 2005. Egyptian standard specification (EOS). No. 1694 for minced beef.

Fan, X. and Kays, S.E., 2008. Formation of trans fatty acids in ground beef and frankfurters due to irradiation. *J. Fd. Sci*., **74**: C79–C84.

FDA, 2001a. BAM, Aerobic plate count. In: *Bacteriological analytical manual* (eds. L. Maturin and J.T. Peeler), (8thEd). Research/Laboratory Methods/ucm063346.htm. http://www.fda.gov/Food/FoodScience.

Fratamico, P. M. and Bagi, L. K., 2007. Comparison of methods for detection and isolation of cold- and freeze-stressed *Escherichia coli O157:H7* in raw ground beef. *J. Fd. Prot*., **70**: 1663 -1669.

FSIS, 2015. The U.S. Department of Agriculture's Food Safety and Inspection Service, Washington Firm Recalls. Boneless Beef Trim Product Due To Possible *E. coli O157:H7* Contamination. *On line.*

Ganguly, S., Mukhopadhayay, S.K. and Biswas, S., 2012. Potential threat to human health from foodborne illness having serious implications on public health - a review. *Int. J. Clin. Biol. Sci*., **1**: 65-68.

Karada, A. and Günefi, G., 2008. The effects of gamma irradiation on the quality of ready-to-cook meatballs. *Turk. J. Vet. Anim. Sci*., **32**: 269-274.

Kim, H. and Bhunia, A.K., 2008. SEL, a Selective Enrichment Broth for Simultaneous Growth of *Salmonella enterica*, *Escherichia coli O157:H7*, and *Listeria monocytogenes*. *Appl. environ. Microbiol.*, **74**: 4853-4866.

Kim, I.S., Yang, M., Jo, C., Ahn, D.U. and Kang, S.N., 2011. Effect of gamma-irradiation on trans fatty acid, free amino acid and sensory evaluation of dry-fermented sausage. *Korean J. Fd. Sci. Anim. Res.*, **4**:580-587.

Kim, I.S., Jo, C., Lee, K.H., Ahn, D.U. and Kang, S.N., 2012. Effect of low-level gamma irradiation on the characteristics of fermented pork sausage during storage. *Rad. Phys. Chem*., **81**: 466-472.

Koneman, E., Allen, S., Janda, M., Schreckenberger, P. and Winn, W., 1994. *Diagnostic microbiology*. 6th Ed. JB Lippincott, Philadelphia.

Kumar, R.J., Rajamanickam, G., Lakshmana. R., Kathiravan, J.H., Nataraju, T.S. and Nadanasabapathi, S., 2013. Effect of gamma irradiation and retort processing on microbial, chemical and sensory quality of ready-to-eat (RTE) chicken pulav. *Int. Fd. Res. J*., **20**: 1579-1584.

Kundua, D., Gillb, A., Luia, C., Goswamia, N. and Holleya, R., 2014. Use of low dose e-beam irradiation to reduce *E. coli O157:H7*, non-*O157 (VTEC) E. coli* and *Salmonella* viability on meat surfaces. *Meat Sci*., **96**: 413–418.

Li, S., Kundu, D. and Holley, R.A., 2015. Use of lactic acid with electron beam irradiation for control of *Escherichia coli O157:H7*, non-*O157 VTEC E. coli*, and *Salmonella* serovars on fresh and frozen beef. *Fd. Microbiol.*, **46**: 34–39.

Mahboob, S., Al-Ghanim, K. A., Sultana, S., Alkahem AlBalawi, H. F., Sultana, T., Ashraf, A., Al-Misned, F. and Ahmed, Z., 2015. Assessment of meat quality and dressing losses in wild and farmed *Cyprinus carpio*. *Pakistan J. Zool*., **46**: 1753-1759

Muller, K., Aabo, S., Birk, T., Mordhorst, H., Bjarnadottir, B. and Agerso, Y., 2012. Survival and growth of epidemically successful and nonsuccessful *Salmonella enterica* clones after freezing and dehydration. *J. Fd. Prot*., **75**: 456-464.

O′ Bryan, C., Crandall, P., Ricke, S. and Olson, D., 2008. Impact of irradiation on the safety and quality of poultry and meat products: a review. *Crit. Rev. Fd. Sci. Nutr.*, **48**: 442- 457.

Osés, S.M., Luning, P.A., Jacxsens, L., Santillana, S., Jaime, I. and Rovira, J., 2012. Microbial performance of food safety management systems implemented in the lamb production chain. *J. Fd. Prot*., **75**: 95- 103.

Özden, Ö., Inugur, M. and Erkan, N., 2007. Effect of different dose gamma radiation and refrigeration on the chemical and sensory properties and microbiological status of aqua cultured sea bass (*Dicentrarchus labrax*). *Rad. Phys. Chem.*, **76**: 1169–1178.

Park, J.G., Yoon, Y., Han, I.J., Song, B.S., Kim, J.H., Hwang, H. J., Han, S.B. and Lee, J.W., 2010. Effects of gamma irradiation and electron beam irradiation on quality, sensory, and bacterial populations in beef sausage patties. *Meat Sci.*, **85:** 368-372.

Peter, Y.Y., Wijewickreme, N. and Kitts, D., 2005. Fat content and ascorbic acid infusion influence microbial and physicochemical qualities of electron beam irradiated beef patties. *Fd. Chem*., **89**: 93–102.

Scholfield, C.R., Jones, R.O., Butterfield and Dutton, H.J., 1963. Analysis of triglycerides. *Anal. Chem*., **35**:1588-1591.

Shantha, N.C. and Decker, E.A., 1994. Rapid, sensitive iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J. Analyt. Office. Agric. Chem. Int.*, **77**:422-425.

Sofos, J.N., 2014. Safety of food and beverages: meat and meat products encyclopaedia. *Fd. Safe*., **3**:268-279.

Sommers, C.H., 2012. Microbial decontamination of food by irradiation. In: *Microbial decontamination in the food industry*. Wiley-Blackwell 2nd ed. Chapter 11, pp. 322-343.

Stacy-Phipps, S., Mecca, J.J. and Weiss, J.B., 1995. Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *E. coli* DNA during course of infection. *J. clin. Microbiol*., **33**: 1054- 1059.

Tarladgis, B.G., Watts, B. M., Younathan, M.T. and Dugan, L.R., 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil. Chem. Soc*., **37**:44- 48.

Trochimchuka, T., Fotheringhama, J., Toppb, E., Schrafta, H. and Leunga, K.T., 2003. A comparison of DNA extraction and purification method to detect *E. coli O157:H7* in cattle manure. *J. Microbiol. Meth.*, **54**:165-175.

Zhanga, W., Xiaoa, B,S., Samaraweeraa, H., Eun Joo Leea, E.J., Dong, U. and Hna, U., 2010. Improving functional value of meat products. *Meat Sci.*, **86**: 15–31

Yazdi, F.T. and Jouki, M., 2012. Gamma irradiation effects on microbial decontamination of ostrich meat. *Sci. J. Microbiol*., **1**: 24-28.

Yoon, K.S., 2003. Effect of gamma irradiation on the texture and microstructure of chicken breast meat. *Meat Sci*., **63**: 273-277.

Yoona, Y., Georgnarasa, I., Mukherjeea, I., Belka, K.E., Scangaa, J.A., Smitha, G.C. and Sofosa, J.N., 2013. Effects of cooking methods and chemical tenderizers on survival of *E. coli O157:H7* in ground beef patties. *Meat Sci*., **95**: 317–322.