Combined effect of natural endogenous compound, modified atmosphere packaging and gamma irradiation on the microbial growth of ground beef

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***Abstract*-Selected Chinese cinnamon, Spanish oregano and mustard essential oils (EOs) were used in combination with irradiation in order to evaluate their efficiency to eliminate pathogenic bacteria, and extend the shelf life of medium-fat-content ground beef (23% fat). The shelf life was defined as the time when the growth level of the total bacteria count reached to 10^7 CFU/g. The shelf life of ground beef was determined for a period of 28 days at 4°C after treated with EOs. The concentrations of EOs were predetermined such that sensorial properties of cooked meat were maintained (0.025% for Spanish oregano or Chinese cinnamon, and 0.075% for mustard). Ground beef samples containing essential oils were then packaged under air or modified atmosphere, and irradiated at a dose of 1.5 kGy. Ground beef samples (10g) were taken during the storage period to evaluate the contents of total mesophilic aerobes, *Escherichia coli*, *Salmonella*, total coliforms, lactic acid bacteria, and *Pseudomonas*. The results showed that mustard EO was the most efficient to reduce the total mesophilic count and to eliminate pathogenic bacteria. Irradiation alone completely inhibited the growth of total mesophilic aerobic and pathogenic bacteria. The combined treatments of irradiation and EOs had better efficiency to reduce the lactic acid bacteria and *Pseudomonas*, especially in the presence of mustard and cinnamon EOs for lactic acid bacteria, and oregano and mustard for *Pseudomonas*, respectively. The best combined treatment to extend the shelf life of ground beef up to 28 days was the addition of EO, irradiation (1.5 kGy) and modified atmosphere packaging (MAP).**

***Index Terms*- Shelf life, microbial flora, essential oils, mustard, oregano, cinnamon, g-irradiation, beef, radiosensitization, modified atmosphere packaging.**

1. INTRODUCTION

Irradiation is a solution to eliminate bacterial contaminations, assure the safety, extend the shelf life and improve the overall quality of meat. This process has an enormous potential to extend the shelf life and to eliminate pathogenic bacteria such as *E. coli* and *Salmonella spp*. which are principally responsible for ground beef infection. The use of combined treatments in order to achieve an

increase of the bacterial sensitiveness and to limit the oxidation reactions represents an alternative. The use of essential oils (Eos) in combination with irradiation seems to have a great potential. The EO can act as flavoring compounds and at the same times increase the radiosensibility of *E. coli* and *Salmonella*. According to Chiasson *et al.* (2005), the addition of 0.5% of carvacrol (a compound founded in thyme EO) in ground beef could increase the radiosensibility of *E. coli* and *Salmonella* by more than 2 times. When irradiation treatment was combined with MAP (60% O_2; 30% CO_2; 10% N_2), the radiosensitivity of *Salmonella* increased by more than 10 times. However, the presence of carvacrol affected significantly the taste of the meat. The aim of this study was to evaluate the potential of the use of EOs without affecting the taste of ground beef in combination with irradiation under air or MAP to: (i) extend the shelf life; (ii) eliminate pathogenic bacteria (*E. coli, Salmonella,* and total coliforms); and (iii) reduce the total endogenous flora (total mesophilic aerobes, lactic acid bacteria and *Pseudomonas*).

II. MATERIALS AND METHODS

***A*. Meat handling and irradiation.** Ground beef containing 23% of fat was purchased at a local supermarket and brought to the Canadian Irradiation Centre (Laval, Québec, Canada) under refrigeration conditions (4 ± 1°C). The ground beef was vacuum-packaged and sterilized by irradiation at a dose of 25 kGy under frozen conditions (-80°C), using a IR-147 underwater calibrator (MDS Nordion, Kanata, Ontario, Canada) equipped with a ^{60}Co source having a dose rate of 17.698 kGy/h.

***B*. Preparation of bacterial cultures.** The natural bacterial floras were extracted from 200g of ground beef, which was homogenized in 200ml of sterile peptone water (0.1% wt/vol) and maintained at -80°C in tryptic soy broth (TSB; Becton-Dickinson and company, Sparks, MD USA) in presence of glycerol (10% vol/vol). Extracted bacterial cultures were used for meat inoculation. Before each experiment, stock cultures were propagated through two consecutive 24 h growth cycles in TSB at 35°C and washed twice in saline solution (0.85% wt/vol) to obtain a working culture containing approximately 10^9 CFU/ml.

***C*. Antimicrobial compounds.** Spanish oregano and Chinese cinnamon EOs were purchased from Robert et Fils (Montréal, Québec, Canada). Mustard essential oil was provided from Hilltech (Vankleek Hill, Ontario, Canada).

***D*. Meat preparation and inoculation procedures.** Sixteen **s**amples of medium-fat-content ground beef (450 g) were inoculated with a working bacterial culture of the natural flora from ground beef to obtain a final concentration of 10^7 CFU/g, and mixed for 1 min with a sterilized spoon. The EO compound was added to ground beef at a concentration of 0.025% or 0.075% (wt/wt) depending on the EO used, and mixed for another 1 min to achieve uniform dispersal of EO throughout the ground beef. Control samples were also prepared with only ground beef (no EO). Then, meat samples (25g each) were packaged in 0.5-mil metalized polyester/2-mil ethylene vinyl acetate (EVA) copolymer bags (205 ´ 355 mm, Winpack, St-Léonard, Québec, Canada). The packages were sealed under conventional air (78.1% N_2, 20.9% O_2, and trace amounts of other gases) for control or under modified atmosphere (60% O_2, 30% CO_2 and 10% N_2) for MAP, and stored at 4°C until irradiation treatment (approximately 15 h).

***E*. Bacterial radiosensitivity evaluation.** Inoculated ground beef samples were irradiated at the Canadian Irradiation Center under refrigerated conditions at 0 and 1.5 kGy. A UC-15A underwater calibrator (MDS Nordion, Kanata, Ontario, Canada) equipped with a ^{60}Co source having a dose rate of 17.698 kGy/h was used. Samples were analyzed immediately after irradiation and at each storage interval (3, 7, 14, 21 and 28 day).

***F*. Microbiological analysis.** Each sample was homogenized for 1 min in sterile peptone water (0.1% wt/vol) using a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From this mixture, serial dilutions were prepared, and pour-plated with tryptic soy agar (TSA; Becton Dickinson, Franklin Lakes, NJ, USA) for the determination of total mesophilic aerobes (TMA). Chromocult coliform agar (EMD Chemicals Inc., Gibbstown, NJ, USA) was used to determine *E. coli*, and *Salmonella*. Pseudomonas agar (EMD Chemicals Inc, Gibbstown, NJ, USA) was used for *Pseudomonas* determination. All bacterial enumerations were achieved after 24 h incubation at 35°C.

***G*. Statistical analysis.** All experiments were done in triplicate. For each replicate, three samples were analyzed for each treatment evaluated. Bacterial count results (log CFU per gram) were analyzed with the SPSS program (SPSS, Chicago, IL., USA) and mean comparison between each treatment was based on Duncan's multiple range tests ($P \leq 0.05$) as a function of storage time for each treatment and between treatments at each day of analysis.

III. RESULTS AND DISCUSSION

Total mesophilic aerobic bacteria. The results of total mesophilic aerobic bacteria (TMA) population during the storage of ground beef are shown in Table 1. The effect of irradiation alone reduced the level of TMA by 4.75 log (CFU/g). The irradiation in the presence of oregano or mustard EO was the most effective treatment, showing 4.24 and 4.72 log (CFU/g) reductions, respectively, as compared to the control. The combination of MAP and irradiation reduced the level of TMA below the level of detection (< 10 CFU/g) for 28 days. When mustard EO was added and packaged under modified atmosphere, this combined treatment extended shelf life to 3 days as compared to 1-day shelf life of the control packaged under air or modified atmosphere. When any of EO was combined with irradiation treatment and MAP, the shelf life of the ground beef was estimated more than 28 days.

Escherichia coli. The results of *E. coli* population in ground beef during the storage are presented in Table 2. When samples were irradiated regardless of EOs addition, the level of *E. coli* was stable and below the level of detection until the end of the experiment. MAP alone reduced the level of *E. coli* by 0.67 log (CFU/g). When mustard EO was added to the ground beef in MAP, this compound was able to stabilize the level of *E. coli* population to 3.72-3.98 log (CFU/g) for 14 days. The combination of MAP and irradiation, in presence or absence of EOs, reduced the level of *E. coli* population below the level of detection during 28 days of storage.

***Salmonella*.** The results *Salmonella* growth in ground beef during storage are presented in Table 3. Mustard and cinnamon reduced *Salmonella* growth by 3.26 and 2.93 log (CFU/g), respectively. Oregano was less effective than other EOs, showing a

reduction of *Salmonella* by 2.11 log (CFU/g). Irradiation alone at 1.5 kGy decreased the level of *Salmonella* population below the level of detection (< 10 CFU/g) until the end of the experiment. The MAP storage condition reduced the *Salmonella* growth by 1.72 log (CFU/g). When EOs were added to ground beef in combination with MAP, cinnamon, oregano and mustard EOs reduced the level of *Salmonella* by 3.09, 2.99 and 3.59 log (CFU/g), respectively, at day 1. Mustard EO reduced the level of *Salmonella* below the level of detection in combination with MAP up to 14 days. Regardless of conventional air or modified atmosphere condition, irradiation treatment even without EO reduced the level of *Salmonella* below the level of detection (< 10 CFU/g) up to 28 days.

***Pseudomonas*.** The level of *Pseudomonas* in ground beef during the storage is presented in Table 4. Results showed that *Pseudomonas* population was reduced by 1 log (CFU/g) at day 1 when mustard EO was added to ground beef. Irradiation alone reduced the level of *Pseudomonas* by 4.39 log (CFU/g). Addition of cinnamon, oregano, or mustard EO, in combination with irradiation, decreased *Pseudomonas* populations by 3.53, 4.85 and 6.08 log (CFU/g), respectively. MAP alone reduced the level of *Pseudomonas* by 1 log (CFU/g) as compared to the conventional air package. The combination of MAP and irradiation reduced the *Pseudomonas* growth by 6.54 log (CFU/g). The addition of cinnamon or oregano EO to ground beef in MAP had no significant ($P > 0.05$) effect on the *Pseudomonas* reduction. However, the combination of EO, MAP, and irradiation treatments reduced *Pseudomonas* population below the level of detection (< 10 CFU/g) for 28 days.

Our study showed that mustard and Chinese cinnamon EOs at a concentration of 0.075 and 0.025%, respectively, showed better antimicrobial activity than Spanish oregano EO at a concentration of 0.025%. The combination of EO, MAP, and irradiation can be used to maintain the safety of fresh or minimally processed ground beef. Irradiation inhibited foodborne pathogens, and subsequently extended the shelf life of ground beef. A complete inhibition of pathogenic bacteria was obtained with the treatment of low-dose radiation (1.5 kGy) regardless of EO addition. However, a combined treatment (irradiation with MAP) was necessary to reduce TMA below the level of detection. Bacterial counts indicated that the combination of irradiation and MAP played an important role in bacterial radiosensitization, producing a synergistic antimicrobial effect on the growth of bacteria in ground beef during the storage. The mustard EO showed the best efficiency to reduce the bacteria growth during the storage. A synergetic effect was observed when meat was stored in MAP. The addition of EOs alone did not have an antimicrobial activity against all tested bacteria, and might even promote microbial growth during the storage. In this study, mustard EO and MAP storage condition could significantly ($P \leq 0.05$) reduce the level of TMA, *E. coli*, total coliforms, LAB and *Pseudomonas*, and completely eliminate the growth of *Salmonella*. EOs are known as antimicrobial agents that could be used to control food spoilage and foodborne pathogenic bacteria. Lemay *et al.* (2002) studied the effect of mustard EO against a mixed culture of *Escherichia coli* and *Brochothrix thermosphacta*. When mustard EO was added to the nutrient media containing aerobic mesophilic bacteria, the levels of these bacteria were significantly ($P \leq 0.05$) lowered as compared to the control after 2 days of storage. Our results have demonstrated that mustard EO is also effective to reduce the bacteria growth during the storage of medium ground beef and a synergetic effect could be obtained when mustard EO is combined with MAP storage. *Samonella* and *E.coli* were stabilized and the presence of coliforms eliminated in

presence of mustard EO. De *et al.* (1999) have also reported that cinnamon had potential antimicrobial activities against *Escherichia coli* ATCC 10536. Karioti *et al.* (2006) showed that oregano EO had an antimicrobial activity and proved to be active against many microorganisms.

IV. CONCLUSION

In conclusion, our results indicated that irradiation completely inhibited the presence of *E. coli* and *Salmonella* in medium-fat-content ground beef up to 28 days. The g-irradiation (1.5 kGy) in presence of mustard EO (0.075 %) and MAP effectively eliminated the growth of TMA. Therefore, combination of Eos (at concentration which does not affect the taste), irradiation and MAP is needed to extend the shelf life and to assure the safety of ground beef during 28 days of storage at the concentration used.

V. ACKNOWLEDGMENTS

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REFERENCES

Anonymous. 1994. Foodborne pathogens, risk and consequences. Task force report no. 122. Council for Agricultural Science and Technology, Ames, IA.

Chiasson, F., J. Borsa, B. Ouattara & M. Lacroix. 2005. Combined effect of and packaging conditions on radiosensitivity of *Escherichia coli* and *Salmonella* Typhi in ground beef. *J. Food Prot. 68:2567-2570.*

De, M., K. De & A. B. Banerjee 1999. Antimicrobial screening of some Indian spices. *Phytother. Res.* 13:616-618.

Karioti, A., T. Vrahimi-Hadjilouca, D. Droushiotis, A. Rancic, D. Hadjipavlou-Litina & H. Skaltsa. 2006. Analysis of the essential oil of *Origanum dubium* growing wild in Cyprus. Investigation of its antioxidant capacity and antimicrobial activity. *Planta. Med*. 72:1330-1334.

Lemay, M. J., J. Choquette, P. J. Delaquis, G. Claude, N. Rodrique & L. Saucier. 2002. Antimicrobial effect of natural preservatives in cooked and acidified chicken meat model. *Int. J. Food Microbiol.* 78:217-226.

Table 1: Total mesophilic aerobic bacteria population in ground beef in presence of antimicrobial compounds for 28 days

Total mesophilic aerobic*				**AIR**		
Day	**1**	**3**	**7**	**14**	**21**	**28**
0 kGy Control	7.32±0.09 g, A*	>10^7 e, B	>10^7 f, B	>10^7 b, B	>10^7 b, B	>10^7 b, B
1.5 kGy Control	2.57±0.12 b, A	3.46±0.26 c, AB	4.26±0.13 c, B	>10^7 b, C	>10^7 b, C	>10^7 b, C
0 kGy Chinese cinnamon EO 0.025%	7.51±0.04 h, A	>10^7 e, B	>10^7 f, B	>10^7 b, B	>10^7 b, B	>10^7 b, B
1.5 kGy Chinese cinnamon EO 0.025%	3.41±0.18 d, B	2.13±0.18 b, A	5.60±0.16 e, C	>10^7 b, D	>10^7 b, D	>10^7 b, D
0 kGy Spanish oregano EO 0.025%	7.51±0.065 gh, B	6.98±0.25 d, A	>10^7 f, C	>10^7 b, C	>10^7 b, C	>10^7 b, C
1.5 kGy Spanish oregano EO 0.025%	3.08±0.20 c, A	3.18±0.23 c, A	4.93±0.1 d, B	>10^7 b, C	>10^7 b, C	>10^7 b, C
0 kGy Mustard EO 0.075%	7.35±0.12 g, A	>10^7 e, A	>10^7 f, B	>10^7 b, B	>10^7 b, B	>10^7 b, B
1.5 kGy Mustard EO 0.075%	2.60±0.23 b, A	2.36±0.21 b, A	3.81±0.17 b, B	>10^7 b, C	>10^7 b, C	>10^7 b, C
				MAP		
Day	**1**	**3**	**7**	**14**	**21**	**28**
0 kGy Control	6.25±0.11 e, A	>10^7 e, B	>10^7 f, B	>10^7 b, B	>10^7 b, B	>10^7 b, B
1.5 kGy Control	<10 a, A	<10 a, A	0.59±0.92 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Chinese cinnamon EO 0.025%	6.38±0.13 e, A	>10^7 e, B	>10^7 f, B	>10^7 b, B	>10^7 b, B	>10^7 b, B
1.5 kGy Chinese cinnamon EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Spanish oregano EO 0.025%	6.29±0.09 e, A	>10^7 e, B	>10^7 f, B	>10^7 b, B	>10^7 b, B	>10^7 b, B
1.5 kGy Spanish oregano EO 0.025%	2.68±0.39 b, A	<10 a, B	<10 a, B	<10 a, B	<10 a, B	<10 a, B
0 kGy Mustard EO 0.075%	5.63±0.08 e, A	7.12±0.13 d, B	>10^7 f, C	>10^7 b, C	>10^7 b, C	>10^7 b, C
1.5 kGy Mustard EO 0.075%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A

* Within each treatment and each column, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each row, means bearing the same capital letter are not significantly different ($P > 0.05$).

Table 2: *Escherichia coli* population in ground beef in presence of antimicrobial compounds for 28 days

Escherichia coli*			**AIR**			
Day	**1**	**3**	**7**	**14**	**21**	**28**
0 kGy Control	5.68±0.23 de, A*	>10^7 h, B	>10^7 e, B	>10^7 f, B	>10^7 b, B	>10^7 b, B
1.5 kGy Control	<10 a, A	2.08±0.08 b, B	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Chinese cinnamon EO 0.025%	5.82±0.08 e, A	>10^7 h, B	>10^7 e, B	>10^7 f, B	>10^7 b, B	>10^7 b, B
1.5 kGy Chinese cinnamon EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Spanish oregano EO 0.025%	6.10±0.2 f, A	>10^7 h, B	>10^7 e, B	>10^7 f, B	>10^7 b, B	>10^7 b, B
1.5 kGy Spanish oregano EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Mustard EO 0.075%	4.87±0.12 c, C	4.68±0.15 d, BC	4.45±0.27 c, AB	4.20±0.20 c, A	>10^7 b, D	>10^7 b, D
1.5 kGy Mustard EO 0.075%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
			MAP			
Day	**1**	**3**	**7**	**14**	**21**	**28**
0 kGy Control	5.01±0.11 c, A	6.10±0.04 f, 2¸B	>10^7 e, D	6.81±0.17 e, C	>10^7 b, D	>10^7 b, D
1.5 kGy Control	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Chinese cinnamon EO 0.025%	6.17±0.17 f, D	5.86±0.08 e, C	4.68±0.21 c, B	4.28±0.24 c, A	>10^7 b, E	>10^7 b, E
1.5 kGy Chinese cinnamon EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Spanish oregano EO 0.025%	5.55±0.12 d, A	6.43±0.11 g, C	6.11±0.27 d, B	6.17±0.13 d, B	>10^7 b, D	>10^7 b, D
1.5 kGy Spanish oregano EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Mustard EO 0.075%	3.98±0.13 b, B	3.99±0.21 c, B	3.58±0.10 b, A	3.72±0.28 b, A	>10^7 b, C	>10^7 b, C
1.5 kGy Mustard EO 0.075%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A

* Within each treatment and each column, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each row, means bearing the same capital letter are not significantly different ($P > 0.05$).

Table 3: *Salmonella* population in ground beef in presence of antimicrobial compounds for 28 days

Salmonella*	**AIR**					
Day	**1**	**3**	**7**	**14**	**21**	**28**
0 kGy Control	3.59±0.09 d, C*	3.38±0.09 d, B	3.92±0.05 f, D	3.27±0.10 e, A	>10^7 b, E	>10^7 b, E
1.5 kGy Control	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Chinese cinnamon EO 0.025%	0.66±0.61 b, A	0.63±0.99 ab, A	2.27±0.29 cd, A	4.08±0.37 f, C	>10^7 b, D	>10^7 b, D
1.5 kGy Chinese cinnamon EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Spanish oregano EO 0.025%	1.48±0.14 c, A	3.06±0.03 d, C	1.81±0.44 c, B	3.19±0.17 e, C	>10^7 b, D	>10^7 b, D
1.5 kGy Spanish oregano EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Mustard EO 0.075%	0.33±0.52 ab, A	0.74±0.61 ab, A	0.33±0.51 ab, A	0.17±0.41 a, A	>10^7 b, B	>10^7 b, B
1.5 kGy Mustard EO 0.075%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
	MAP					
Day	**1**	**3**	**7**	**14**	**21**	**28**
0 kGy Control	1.87±0.18 c, A	3.26±0.15 d, C	2.88±0.13 e, B	2.80±0.22 d, B	>10^7 b, D	>10^7 b, D
1.5 kGy Control	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Chinese cinnamon EO 0.025%	0.50±0.55 ab, A	1.94±0.10 c, C	0.68±0.75 b, AB	1.21±0.24 b, B	>10^7 b, D	>10^7 b, D
1.5 kGy Chinese cinnamon EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Spanish oregano EO 0.025%	0.60±0.55 b, A	2.31±0.03 c, B	2.62±0.22 de, B	2.39±0.16 c, B	>10^7 b, C	>10^7 b, C
1.5 kGy Spanish oregano EO 0.025%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A
0 kGy Mustard EO 0.075%	<10 a, A	0.38±0.6 ab, A	<10 a, A	<10 a, A	>10^7 b, A	>10^7 b, A
1.5 kGy Mustard EO 0.075%	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A	<10 a, A

* Within each treatment and each column, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each row, means bearing the same capital letter are not significantly different ($P > 0.05$).

Table 4: *Pseudomonas* population in ground beef in presence of antimicrobial compounds for 28 days

Pseudomonas*			**AIR**			
Day	**1**	**3**	**7**	**14**	**21**	**28**
0 kGy Control	7.31±0.14 f, A*					$>10^7$ b, B
1.5 kGy Control	2.92±0.34 c, C		3.13±0.15 c, C	2.61±0.08 c, B		<10 a, A
0 kGy Chinese cinnamon EO 0.025%	7.38±0.07 f, A		$>10^7$ e, B	$>10^7$ e, B		$>10^7$ b, B
1.5 kGy Chinese cinnamon EO 0.025%	3.78±0.20 d, D		1.14±0.60 b, B	2.50±0.29 c, C		<10 a, A
0 kGy Spanish oregano EO 0.025%	7.42±0.07 f, B	6.81±0.26 a, A	$>10^7$ e, B	$>10^7$ e, B		$>10^7$ b, B
1.5 kGy Spanish oregano EO 0.025%	2.46±0.65 c, C		1.39±0.24 b, B	1.81±0.31 b, C		<10 a, A
0 kGy Mustard EO 0.075%	6.12±0.07 e, A		6.10±0.17 d, A	6.47±0.29 d, A		$>10^7$ b, B
1.5 kGy Mustard EO 0.075%	1.23±1.25 b, B		1.28±0.64 b, B			<10 a, A
			MAP			
Day	**1**	**3**	**7**	**14**	**21**	**28**
0 kGy Control	6.38±0.17 e, A					$>10^7$ b, B
1.5 kGy Control	0.77±0.65 a, A		0.17±0.41 a, A	<10 a, A		<10 a, A
0 kGy Chinese cinnamon EO 0.025%	7.31±0.02 f, A					$>10^7$ b, B
1.5 kGy Chinese cinnamon EO 0.025%	<10 a, A		<10 a, A	<10 a, A		<10 a, A
0 kGy Spanish oregano EO 0.025%	7.19±0.03 f, A					$>10^7$ b, B
1.5 kGy Spanish oregano EO 0.025%	1.72±0.87 b, B		<10 a, A	<10 a, A		<10 a, A
0 kGy Mustard EO 0.075%	6.35±0.06 e, A					$>10^7$ b, B
1.5 kGy Mustard EO 0.075%	1.13±0.78 ab, B		<10 a, A	<10 a, A		<10 a, A

* Within each treatment and each column, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each row, means bearing the same capital letter are not significantly different ($P > 0.05$).