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RESEARCH NOTE

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Effect of Irradiation on the Microbial Content of Ready-to-Use Cooked Carrot

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Abstract The aim of this study was to investigate the effect of irradiation treatment on the inactivation of pathogens in ready-to-use cooked carrot. The pathogens tested were *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria inocua*. Following the inoculation of these organisms into cooked carrot (about 10^6-10^8 CFU/g), the growth of each was inhibited due to irradiation for 24 hr of storage at 20°C. *S. typhimurium* and *E. coli* inoculated into cooked carrot were not detected following irradiation with 3 kGy. *S. aureus* and *L. inocua* inoculated into the cooked carrot decreased by 5 logs (CFU/g) following 2 kGy irradiation. The range of D_{10} values was from 0.30-0.50. The Hunter color, L*-, a*-, and b*-values, and the hardness of the cooked carrot were not effected by irradiation treatment. The sensory score of irradiated cooked carrot was not statistically different from that of non-irradiated samples (p>0.05). These results indicate that low dose irradiation can enhance the microbial safety and extend the shelf-life of ready-to-eat foods such as cooked carrot.

Keywords: carrot, ready-to-use, pathogen, irradiation, microbiological safety

Introduction

Gamma irradiation technology can significantly delay food spoilage caused by microorganisms, thus improving the safety and shelf-stability of food products without compromising their nutritional or sensory qualities (1). Recently, the US Centers for Disease Control (CDC) and Prevention estimated that if half of the ground beef, pork, poultry, and processed luncheon meats in the US were irradiated, there would be over 880,000 fewer cases of foodborne illness, 8,500 fewer hospitalizations, 6,660 fewer catastrophic illness, and 352 lives saved every year (2).

The application of a gamma irradiation up to a dose level of 10 kGy can eliminate or greatly reduce the numbers of microorganisms associated with food spoilage and/or illness in food products without compromising their nutritional or sensory qualities (1).

Cooked carrot is a common ready-to-use food in Korea which can be used in traditional prepared foods such as *kimbap* (steamed rice rolled by dried laver) or *bibimbap* (mixed rice with vegetables). However, various bacterial pathogens have been reported to survive and grow on vegetables and fruits. Carrots showed average mold and yeast counts of 5.3×10^4 and a detected range was between less than 100 to 2.1×10^5 CFU/g, respectively (3). Also, pathogens such as *Listeria inocua* are a food safety concern with regard to minimally processed vegetables sold in a ready-to-use or ready-to-eat form (4). Ionizing irradiation has recently been used to eliminate *Escherichia coli* O157:H7 from apple juice, Toxoplasma gondii and or Cyclospora cayetanensis from raspberries, and *E. coli* O157:H7 and salmonellae from seeds and sprouts.

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The objective of the present study was to demonstrate the effect of irradiation on pathogens of public health significance, including *Staphylococcus aureus*, *L. inocua*, *Salmonella typhimurium* and *E. coli*, in ready-to-use cooked carrot, and thereby ensuring the safety and quality of such ready-to-use foods.

Materials and Methods

Sample preparation Carrots were purchased from a local store and sliced into 1×1 cm portions. The sliced carrot pieces were fried for 2 min at 180°C. Ten g samples were packed into oxygen-impermeable nylon bags (2 mL O_2/m^2/24 hr at 0°C, 0.09 mm thickness; Sunkyung Co., Ltd., Seoul, Korea). The packed samples were then exposed to an irradiation dose of 30 kGy except for the sample for texture and sensory analysis (point source, AECL, IR-79; MDS Nordion International Co., Ltd., Ottawa, ON, Canada) at 12±0.5°C to achieve complete inactivation of the indigenous microflora.

Strains and culture conditions Four pathogens, *Salmonella typhimurium* (KCTC 1925), *Escherichia coli* (KCTC 1682), *Staphylococcus aureus* (KCTC 1916), and *Listeria inocua* (KCTC 3586) were used in this study and obtained from the Korean collection for type cultures (KCTC, Daejeon, Korea). *E. coli*, and *S. aureus* were grown in tryptic soy broth (Difco Laboratories, Sparks, MD, USA). *L. inocua* and *S. typhimurium* were grown in brain heart infusion broth and nutrient broth (Difco), respectively. The incubation temperature for *E. coli*, *S. aureus*, *S. typhimurium* and *L. inocua* was 37°C. The bacterial cultures were grown for 24 hr in sterilized medium following inoculation with 1 colony from an agar slant. 0.1 mL of this culture was then transferred to new medium and grown for 18 hr. The cultures were pelleted at

2,795×g for 10 min at 4°C in a refrigerated centrifuge (Vs-5500; Vision Scientific, Co., Seoul, Korea). The bacterial pellets were washed twice with sterile 0.1% peptone water. The pellet was then suspended in sterile peptone water to a cell density of about 10^8-10^9 CFU/mL.

Inoculation of test organisms Cooked carrot samples were inoculated with a cell suspension of each test organism. Two hundred μL of test culture suspension (10^8-10^9 CFU/mL) was aseptically spread onto cooked carrot samples (10 g). Each sample was kept at a sterile workstation for 1 min to allow for absorption of the inoculum.

Irradiation Cooked carrot samples inoculated with each test organism was irradiated in a cobalt-60 irradiator (point source AECL, IR-79; MDS Nordion International Co.) at the Korea Atomic Energy Research Institute (KAERI, Daejeon, Korea). The source strength was approximately 100 kCi with a dose rate of 10 kGy/hr at 12±0.5°C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free-radical signal was measured by using a Bruker EMS 104 EPR Analyzer. The dosimeters were calibrated against an international standard set by the International Atomic Energy Agency (Vienna, Austria). The applied doses in this study were 1, 2, and 3 kGy. After irradiation, the samples were transferred to a laboratory and microbiological analysis was performed during storage at room temperature (20°C).

Microbiological analysis Ten g samples of inoculated cooked carrot were aseptically homogenized for 2 min in a sterile stomacher bag containing 90 mL of sterile 0.1% peptone water by using a bag mixer® (Model 400; Interscience Co., France). Plates for the enumeration of *E. coli, S. typhimurium, S. aureus*, and *L. inocua* were prepared with tryptic soy agar (Difco). Plates were incubated at the 37°C for 48 hr and colony forming units (CFU) per gram were counted following dilution to 30-300 CFU per plate. D_{10} values (the dose required to inactivate 90% of a population) for each of the organisms tested were determined by calculating the reciprocal of the slope of the population. Experiments with each bacterial culture were conducted, independently, twice.

Color measurement Carrot samples were prepared, cooked, and packed by the methods described above. After irradiation, carrot color was measured with a Hunter color difference meter (Spectrophotometer CM-3500d; Minolta Co., Ltd., Osaka, Japan). The numerical value of the color was expressed as Hunter L*-, a*-, and b*-values. The Hunter values were monitored by a computerized system using spectra magic software (version 2.11; Minolta Cyberchrom Inc., Osaka, Japan).

Texture profile analysis The texture analysis of each sample was performed using a texture analyzer (Model TA-XT 2i; Stable Micro Systems Ltd., Surrey, UK). Hardness was measured by the method of Lee *et al.* (5) except for the modification of using the 2 mm cylinder probe, and processing the data with the texture expert software system (V. 1.22).

Sensory evaluation Sensory analysis of cooked carrot samples was determined as described by Jo *et al.* (6). Each cooked carrot sample was served to 10 panelists to evaluate its sensory qualities. This was assessed using a 9-point hedonic scale which addressed carrot appearance, odor, texture assessment, overall acceptability, and irradiation off-odor. Approximately 20 g of cooked carrot was analyzed at a time and water was provided for panelists to wash their oral cavities after each test.

Statistical analysis Each set of microbial data represents the mean of 2 different experiments in which 2 measurements were taken for each experiment. The texture and sensory evaluation tests included 10 replications. One-way analyses of variance were performed by using SAS software (version7.0; SAS Inst., Cary, NC, USA) along with Duncan's post hoc tests to compare the differences among the mean values.

Results and Discussion

The effects of irradiation on 4 pathogens, *S. typhimurium, E. coli, S. aureus*, and *L. inocua*, inoculated into ready-to-use cooked carrot are shown in Table 1. *Salmonella* spp. have been associated with outbreaks of disease due to their ingestion with vegetables and fruits. The number of *S. typhimurium* inoculated into cooked carrot was 6.56 $\log_{10}$ CFU/g. However, following 3 kGy of irradiation, there was no detectable live *S. typhimurium* in the cooked carrot (Table 1). Several authors have already suggested the use of irradiation to improve the microbiological safety of vegetables (7, 8). In addition, Jo *et al.* (9) reported that *S. typhimurium* inoculated into fried egg and ham showed 4.6 and 5.7 $\log_{10}$ CFU/g, respectively following irradiation at 1 kGy, whereas irradiation at 3 kGy reduced the CFU/g to undetectable levels.

E. coli showed a growth pattern similar to *S. typhimurium* (Table 1). *E. coli* inoculated into cooked carrot was undetectable after irradiation at 3 kGy. Jo *et al.* (10) reported that irradiation at 2 kGy decreased *E. coli* levels in the prepared imitation crab leg to below 2 log CFU/g. Bharathi *et al.* (11) reported that with an initial inoculum of 4.3 log CFU/g, the storage of washed carrot at 4°C and below resulted in a maximum viable population of 6.4 log CFU/g within 10 days. The author confirmed that the microbial safety of minimally processed vegetables depends largely upon the initial population of microbial contaminant, followed by the storage period and temperature.

Aycicek *et al.* (12) reported that out of 512 Russian salad, vegetable salad, and meatball samples, 48 (9.4%) contained coagulase-positive *S. aureus* ranging form 2.2 to 4.3 log CFU/g. *S. aureus* was not detected in inoculated cooked carrot samples following 3 kGy of irradiation regardless of the storage period (Table 1). Irradiation at 1 kGy decreased the *S. aureus* levels in cooked carrot to below 4 $\log_{10}$ CFU/g. Jo *et al.* (9) reported that irradiation at 3 kGy resulted in approximately 1 log CFU/g of viable cells in marinated beef rib at 4°C, but no viable cells were detected after 2 weeks of storage.

Table 1. Effect of irradiation on the growth (log_{10} CFU/g) of *Salmonella typhimurium* (KCTC 1925), *Escherichia coli* (KCTC 1682), *Staphylococcus aureus* (KCTC 1916), and *Listeria innocua* (KCTC 3586) in cooked carrot during storage at 20°C[1)]

Pathogens	Irradiation dose (kGy)	Viable cell counts (log_{10} CFU/g)		
		0 hr	8 hr	24 hr
Salmonella typhimurium	0	6.56±0.31	6.82±0.06	7.29±0.36
	1	4.19±0.03	4.30±0.01	4.39±0.12
	2	1.90±0.08	2.42±0.06	2.98±0.09
	3	ND[2)]	ND	ND
Escherichia coli	0	8.16±0.06	8.23±0.02	8.58±0.03
	1	4.96±0.04	4.54±0.02	4.70±0.01
	2	1.48±0.67	1.85±0.53	1.87±0.12
	3	ND	ND	ND
Staphylococcus aureus	0	6.99±0.04	7.14±0.09	7.57±0.13
	1	2.63±0.04	4.15±0.21	4.39±0.12
	2	1.15±0.21	1.39±0.12	1.45±0.21
	3	ND	ND	ND
Listeria inocua	0	8.18±0.12	8.59±0.05	8.84±0.07
	1	4.00±0.08	5.98±0.20	6.93±0.08
	2	3.78±0.06	3.85±0.12	4.01±0.10
	3	2.15±0.21	2.87±0.24	3.20±0.04

[1)]Mean±SD (n=2).
[2)]Viable cells were below the detection limit (<10^1 CFU/mL).

Table 2. Radio-sensitivity (D_{10}-value) of the pathogens inoculated into cooked carrot

Pathogens	D_{10} value
Salmonella typhimurium KCTC 1925	0.43
Escherichia coli KCTC 1682	0.30
Staphylococcus aureus KCTC 1916	0.34
Listeria inocua KCTC 3586	0.50

Table 3. Hunter color value changes for cooked carrot following irradiation[1)]

Irradiation (kGy)	L*	a*	b*
0	57.35±1.297	6.95±2.741	8.98±2.046
1	57.20±1.340	5.86±1.684	8.34±1.685
2	57.80±1.389	6.83±1.915	9.14±1.652
3	57.48±1.741	6.75±3.360	8.79±2.504

[1)]Mean±SD (n = 10).

Table 4. Hardness of cooked carrot following irradiation[1)]

Irradiation (kGy)	Hardness
0	8.4±0.84
1	8.0±0.37
2	8.2±1.59
3	8.5±0.55

[1)]Mean±SD (n=10).

Inoculated *L. inocua* in ready-to-use cooked carrot was relatively resistant to irradiation (Table 1). There was a 4-log reduction following 1 kGy of irradiation at 0 hr. Similarly, *L. monocytogenes* inoculated into pre-cut carrots and bell peppers, stored at 1-16°C, was reduced due to 1 kGy of gamma irradiation (13). An effective method for killing *Listeria* spp. in foods would reduce the food-borne outbreaks of listeriosis, and decrease economic losses in the food industry.

Table 2 shows the calculated D_{10} values following irradiation of ready-to-use cooked carrot samples inoculated with each pathogen. The D_{10} values ranged from 0.30-0.50. Yu *et al.* (14) reported that 1.98 mg of gallic acid equivalent per carrot seed oil was detected. Chaibi *et al.* (15) reported that savage carrot essential oil (400 ppm) inhibited the growth of *C. botulinum* 62A spores and the differences among essential oil antimicrobial activities were ascribed to differences in their active fractions (phenolic, acid, aldehyde, ketone, oxide, hydrocarbons). Thayer *et al.* (16) determined that the irradiated D value of a mixture of *Salmonella* serovars, which included isolates associated with outbreaks of salmonellosis caused by the consumption of alfalfa sprouts, was 0.97±0.03 kGy for sterile alfalfa seed. Chung *et al.* (17) reported that the D_{10} value of pathogens inoculated into *kimbap*, which is a popular ready-to-eat food in Korea, were 0.31-0.44 kGy.

To investigate changes in quality of cooked carrot due to irradiation, color, hardness, and sensory analyses were performed. The Hunter color value and hardness of cooked carrot were not affected by irradiation at any of the doses tested ($p>0.05$). Byun *et al.* (18) reported that the color characteristics of cooked soybean showed no significant differences between irradiated samples and the non-irradiated control. Yim and Sohn (19) confirmed that the Hunter color values of carrot purees decreased significantly after sterilization at 115, 120, and 125°C.

The value of the sensory score, including appearance, odor, texture, and overall acceptability, showed a decreasing trend following irradiation, however this was not statistically significant ($p>0.05$). Zang *et al.* (20) reported that by the 8th day of storage at 4°C, the sensory quality of fresh-cut lettuce irradiated with 1.0 kGy appeared to be the best, which could be explained by the fact that the non-irradiated and 0.5 kGy-irradiation treated samples were not effective in controlling the microbial population. Chaudry *et al.* (21) reported that changes in the firmness of minimally processed carrots exposed to different doses of a gamma radiation were not significant ($p<0.05$) and their sensory characteristics were also acceptable. Treatment of a fermented vegetable with 20 kGy of gamma irradiation did not bring about any genotoxic effects under the described experimental conditions (22).

These results indicate that the irradiation of cooked carrot and perhaps ready-to-use vegetables in general, can minimize the risk of pathogenic bacteria without any adverse effects on food quality. Furthermore, microbial

Table 5. Sensory evaluation of cooked carrot following irradiation[1)]

Irradiation (kGy)	Appearance	Odor	Texture	Overall acceptability	Irradiation odor
0	6.5±1.81	5.2±1.67	5.8±2.23	6.4±2.11	3.0±2.0
1	5.2±2.23	4.7±2.15	5.7±2.05	5.0±2.05	2.9±1.51
2	5.1±1.58	4.6±1.50	5.6±1.81	4.8±1.54	4.2±1.47
3	5.7±1.35	4.7±1.01	4.9±1.81	4.7±1.74	3.2±1.47

[1)]Sensory panelists (n=10) were each provided with test sample and a sensory score sheet. A 9-point hedonic scale was used ranging from 'like very much' (9) to 'extremely dislike' (1).

hurdle effects such as good manufacturing practices, hazard analysis critical control point (HACCP) or pre-pasteurization methods such as a heating may ensure the safety of prepared foods in combination with irradiation.

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