Food Sci. Biotechnol. Vol. 18, No. 1, pp. 162 ~ 166 (2009)

Food Science and Biotechnology

Effect of Glucose on *Listeria monocytogenes* Survival under Sequential Sublethal Stresses of Gamma Irradiation and NaCl

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Abstract This study evaluated glucose effect on *Listeria monocytogenes* survival under gamma irradiation and NaCl stress. *L. monocytogenes* in phosphate buffered saline (PBS) plus glucose (0-4%) was treated with gamma irradiation (0-0.5 kGy), and the samples were then exposed to NaCl (0-9%) in tryptic soy agar plus 0.6% yeast extract. D_{10} and t_{3D} values were determined, and a model for prediction of D_{10} values was developed. Cell counts of *L. monocytogenes* reduced as irradiation dose increased, and *L. monocytogenes* in PBS (no glucose) was more sensitive to irradiation and NaCl compared to those in PBS (2 or 4% glucose). D_{10} values were 0.07-0.1, 0.12-0.16, and 0.13-0.15 kGy for 0, 2, and 4% glucose, respectively. The t_{3D} values were 0.22-0.3 (0% glucose), 0.35-0.48 (2% glucose), and 0.40-0.44 (4% glucose). A model performance was acceptable. These results indicate that glucose in foods would increase the resistance of *L. monocytogenes* to gamma irradiation and NaCl stress.

Keywords: resistance, irradiation, *Listeria monocytogenes*, glucose, NaCl

Introduction

Listeria monocytogenes is a Gram-positive psychrotrophic pathogen causing sporadic cases of foodborne illness, and the pathogen is capable of surviving and growing at low pH as well as in high salt content (1). *L. monocytogenes* may easily be introduced and contaminate ready-to-eat (RTE) meat and poultry plants where it can survive and persist for very long periods of time, and cross-contaminate properly heat processed products during slicing and repackaging (2-4). This post-lethality treatment recontamination is a concern for products that allow growth of the pathogen and are not reheated before consumption (5). Thus, antimicrobial treatments have been used in RTE meat and poultry products to control the recontamination that may occur during post-lethality peeling, slicing, and packaging (6-14). However, these treatments are not sufficient to completely inhibit or destroy *L. monocytogenes* growth (15,16). Therefore, additional treatments should be applied to control *L. monocytogenes* in RTE meat and poultry products.

Irradiation has been in every part of our lives, and we encounter it every day in the natural circumstances (17). Of common types of irradiation, gamma irradiation is suggested as the best method for destroying pathogenic microorganisms in the final products after packaging because of its high permeability and lack of toxic compounds (18). In addition, use of gamma irradiation has positive effects in preventing decay by destroying microorganisms and by improving the food safety and quality of foods without compromising the nutritional or sensory quality (19). Thus, use of gamma irradiation technology has been gradually increasing worldwide (18). A study of Thayer and Rajkowski (20) showed that gamma irradiation effectively destroyed *Escherichia coli* O157:H7 from apple juice, *Toxoplasma gondii* and *Cyclospora cayetanensis* from raspberries, and *E. coli* O157:H7 and *Salmonella* from feeds and sprouts. Gamma irradiation (2 kGy) has also been shown to effectively destroy *L. monocytogenes* in processed meat and poultry products (21,22).

Glucose and NaCl are the compounds commonly found in various foods. In foods, glucose may contribute to resistance of foodborne pathogenic bacteria against various stresses (23-25). Moreover, NaCl may be related to survival of stress adapted cells of *L. monocytogenes* (1,23,24). Therefore, an objective of this study was to evaluate effects of glucose on resistance of *L. monocytogenes* under sequential stresses of gamma irradiation and NaCl, which stresses could be found during manufacturing of foods.

Materials and Methods

Preparation of inoculum and samples The 4-strain mixed inoculum of *L. monocytogenes* (KTCC3443, 3444, 3591, and 3587) was used in this study; the strains were obtained from the Korean Collection for Type Cultures (KTCC, Daejeon, Korea). Each strain was cultured in 10 mL of tryptic soy broth (Difco, Becton Dickinson and Company, Sparks, MD, USA) plus 0.6% yeast extract (Difco) (TSBYE) at 30°C for 24 hr. Portions (0.1 mL) of the cultures were then subcultured in 10 mL of TSBYE at 35°C for 24 hr. Stationary phase cells were harvested by centrifugation (523,224×g, 15 min, 4°C), washed, resuspended in phosphate buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.15 g of Na_2HPO_4, 8.0 g of NaCl, and 0.2 g of

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Received July 2, 2008; Revised August 25, 2008;
Accepted August 26, 2008

KCl in 1 L of distilled water), and diluted in 40 mL of PBS formulated with 0, 2, and 4%(w/v) of glucose to obtain an inoculum size of 7 log CFU/mL.

Gamma irradiation and microbial analysis The samples were then irradiated in a cobalt-60 irradiator (IR-221; MDS Nordion International Co., Ltd., Ottawa, ON, Canada) equipped with a 11.1 PBq strength at 15±0.5°C and operated at a dose rate of 10 kGy/hr. The test samples were irradiated at doses of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 kGy. Following irradiation, the samples were shipped in ice filled coolers for 2 hr and analyzed immediately after the samples arrived at the laboratory. The irradiated samples were serially diluted in 9 mL of sterile saline solution (0.85% NaCl), and plated on tryptic soy agar (Difco) plus yeast extract (Difco) supplemented with 0, 1.5, 3.0, 4.5, 6.0, 7.5, and 9%(w/v) NaCl. The plates were then incubated at 30°C for 48 hr, and colonies were recorded as number of CFU/mL.

Kinetics of irradiation decline Microbiological data were converted to log CFU/mL, and D_{10} values (the dose required to reduce 1 log CFU/mL of *L. monocytogenes*) for each of glucose and NaCl concentrations were determined by calculating the negative reciprocal of the slope after fitting survival data versus irradiation doses with the linear regression (26). The D_{10} values were then expressed using the following equation:

$$SQRT\ (D_{10})=a_1+a_2\text{glucose}+a_3\text{NaCl}+a_4\text{glucose}^2+a_5\text{NaCl}^2 \quad (1)$$

Where *SQRT* is the squared root and a_i (where 'i' represents any number from 1 to 5) are the coefficients to be estimated. In addition, the irradiation dose to achieve a 3-log inactivation (t_{3D}) was used to provide an integrated measure of the shoulder period and exponential phases (27). The t_{3D} value was calculated using the following equation:

$$t_{3D}=t_{\text{shoulder}}+3D \quad (2)$$

Where t_{shoulder} is the elapsed period until the cultures begin to die exponentially.

Statistical analysis The experiments were repeated twice with 2 samples in each replicate. A complete factorial design (irradiation doses [0, 0.1, 0.2, 0.3, 0.4, and 0.5 kGy] ×glucose [0, 2, 4%]×NaCl [0, 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0%]) was used in this study. Surviving cell counts for the irradiation dose, glucose, and NaCl concentration were analyzed by the mixed model procedure of SAS® version 9.1 (SAS Institute, Cary, NC, USA). All least squares mean comparisons in the 3-way interaction of irradiation dose, glucose, and NaCl concentration were performed with the pairwise *t*-test at $\alpha=0.05$.

Results and Discussion

***L. monocytogenes* survivors** The survivor of all gamma irradiated *L. monocytogenes* in PBS containing different concentrations of glucose are shown in Fig. 1. These results indicate that glucose in food may increase *L. monocytogenes* resistance to sequential sublethal stresses of irradiation and high concentration of NaCl (Fig. 1). The viability of *L. monocytogenes* were lower ($p<0.05$) in absence of glucose (0% glucose) than in presence of glucose (2 and 4%) after the pathogen was exposed to gamma irradiation (Fig. 1). In addition, the differences in surviving cell counts of *L. monocytogenes* between absence and presence of glucose became obvious as irradiation dose increased, and bacterial survivors of *L. monocytogenes* in PBS plus 2% glucose were lower ($p<0.05$) than those in PBS plus 4% glucose when the cells were challenged to high NaCl concentration (7.5%) (Fig. 1). The viability of *L. monocytogenes* exposed to 7.5% NaCl were lower ($p<0.05$) than those exposed to other concentrations (0 to 6%) of NaCl, while the number of *L. monocytogenes* challenged to 9% NaCl reduced below detection limit (0 log CFU/mL) for all glucose concentrations and irradiation doses; 9% NaCl was not then accounted for the calculation for D_{10} values and t_{3D} values. Other recent studies also showed that glucose would be related to *L. monocytogenes* resistance against stresses (28-30). A study of Sharma *et al.* (28) indicated that growth of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* in media formulated with 1% glucose resulted in cross protection to heat. Samelis *et al.* (29) also proved that growth of *L. monocytogenes* and *Salmonella* in TSBYE with 1% glucose improved their acid tolerance.

D_{10} and t_{3D} value To calculate irradiation doses required to reduce 1 log CFU/mL of *L. monocytogenes* for each of glucose and NaCl concentration, the negative reciprocals of the slopes of linear regression after fitting survival data of the pathogen were used as D_{10} values (26). From the fitting survival data to calculate D_{10} values, the coefficient of determination (R^2) were more than 0.926 except samples of 0% (R^2=0.599) and 2% (R^2=0.652) glucose for 7.5% NaCl (Table 1). D_{10} values ranged from 0.07 to 0.1 kGy, from 0.12 to 0.16 kGy, and from 0.13 to 0.15 kGy for 0, 2, and 4% glucose, respectively (Table 1). In our study, the calculated D_{10} values were lower than D_{10} values obtained from solid type foods (beef frankfurters, 0.52 kGy; cucumber, 0.34 kGy; blanched and seasoned spinach, 0.41 kGy) because bacteria in liquid type foods are easily sensitized to irradiation because of higher presence of free radicals (31). According to these results, it could be hypothesized that various ingredients presented in food structure may influence bacterial sensitivity to irradiation (31). Urbain (32) suggested that proteins in foods may play an important role as quenchers of the radicals formed by irradiation, leaving fewer ions to react with the organisms. Therefore, further studies need to be performed to evaluate effects of food ingredients such as fat, lean, starch, and proteins on resistance of foodborne pathogens to gamma irradiation. To provide an integrated measure of shoulder period and exponential phases of the inactivation curve, the equation ($t_{3D}=t_{\text{shoulder}}+3D$) was used (27). However, since no shoulder period was observed for all treatments, t_{shoulder} was not accounted for t_{3D} calculation. The t_{3D} values ranged from 0.22 to 0.3 kGy, from 0.35 to 0.48 kGy, and from 0.40 to 0.44 kGy for 0, 2, and 4% glucose, respectively (Table 1).

Model development To simulate predicted D_{10} values, the calculated D_{10} values were further expressed as a function of concentration of glucose and NaCl, and simulation to predict D_{10} value is presented in Fig. 2A, and the model used for the simulation was the following.

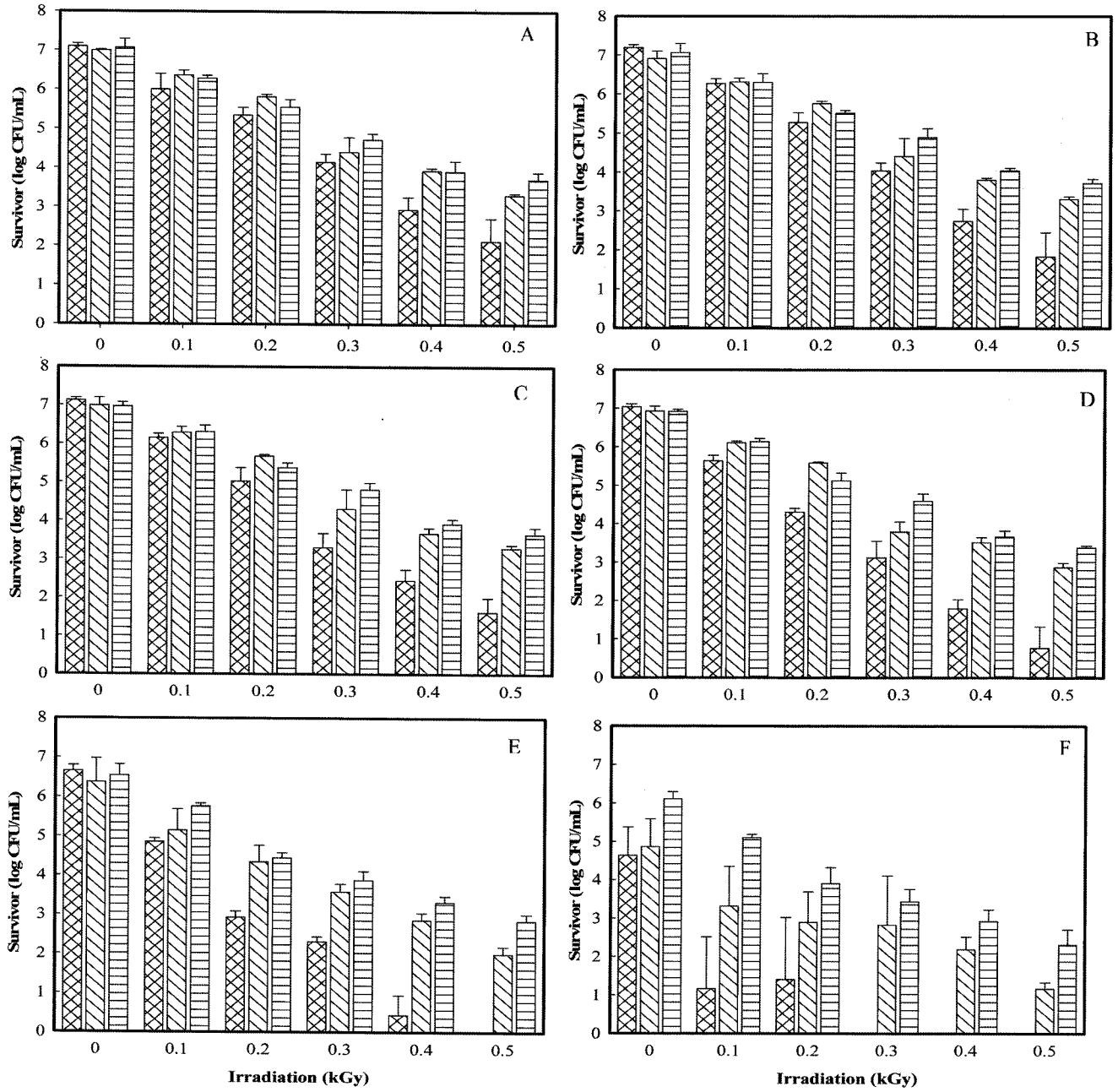

Fig. 1. Surviving cell counts (log CFU/mL) of *L. monocytogenes* recovered on tryptic soy agar plus 0.6% yeast extract containing various concentrations (A: 0%, B: 1.5%, C: 3.0%, D: 4.5%, E: 6.0%, F: 7.5%) of NaCl after gamma irradiation in PBS formulated with 0 (▩), 2 (▨), and 4% glucose (▤).

$$SQRT\ (D_{10}) = 0.300318 + 0.000712 \cdot \text{NaCl} + 0.039441 \cdot \text{Glucose} - 0.00553 \cdot \text{Glucose}^2$$

$$R^2 = 0.715$$

Standard error of fit=0.022

F-value=11.7318 (p=0.0004)

The regression statistics such as R^2 and standard error of fit showed that appropriate fitting. Moreover, normality of the developed model was also tested, and the model produces an approximately straight line (Fig. 2B). Thus, this means that the residuals produced by the model can be assumed to be normally distributed. This simulation could be useful in prediction of D_{10} values under different concentrations of glucose and NaCl.

In summary, presence of glucose in foods would increase resistance of *L. monocytogenes* to sequential stresses of gamma irradiation and NaCl. Therefore, this result suggest that irradiation dose to decrease *L. monocytogenes* populations should be determined according to levels of glucose or NaCl in foods, and the mathematical model developed in this study could be useful in finding appropriate irradiation doses to decrease *L. monocytogenes* populations in foods. Moreover, further studies need to be conducted to evaluate effects of food ingredients such as fat, lean, starch, and proteins on resistance of *L. monocytogenes* to gamma irradiation.

Table 1. D_{10} values (kGy) and t_{3D} values (kGy) of *L. monocytogenes* exposed to 0, 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0% of NaCl supplemented in tryptic soy agar plus 0.6% yeast extract after gamma irradiation (0, 0.1, 0.2, 0.3, 0.4, and 0.5 kGy) in PBS supplemented with 0, 2, and 4% of glucose

	Glucose (%)											
	0				2				4			
NaCl (%)	D_{10} (kGy)	R^2	Standard error	t_{3D} (kGy)	D_{10} (kGy)	R^2	Standard error	t_{3D} (kGy)	D_{10} (kGy)	R^2	Standard error	t_{3D} (kGy)
0.0	0.10	0.967	0.334	0.30	0.13	0.967	0.255	0.39	0.14	0.964	0.243	0.42
1.5	0.09	0.976	0.309	0.27	0.13	0.961	0.277	0.39	0.15	0.975	0.198	0.44
3.0	0.09	0.973	0.343	0.26	0.12	0.952	0.312	0.37	0.14	0.975	0.200	0.43
4.5	0.08	0.983	0.298	0.24	0.12	0.955	0.329	0.35	0.14	0.974	0.214	0.41
6.0	0.07	0.963	0.474	0.22	0.12	0.939	0.384	0.36	0.13	0.958	0.291	0.40
7.5	0.07	0.599	1.336	0.22	0.16	0.652	0.816	0.48	0.13	0.926	0.370	0.40
9.0[1)]	-	-	-	-	-	-	-	-	-	-	-	-

[1)] D_{10} values were not calculated because surviving cell counts of *L. monocytogenes* were below detection limits (0 log CFU/mL)

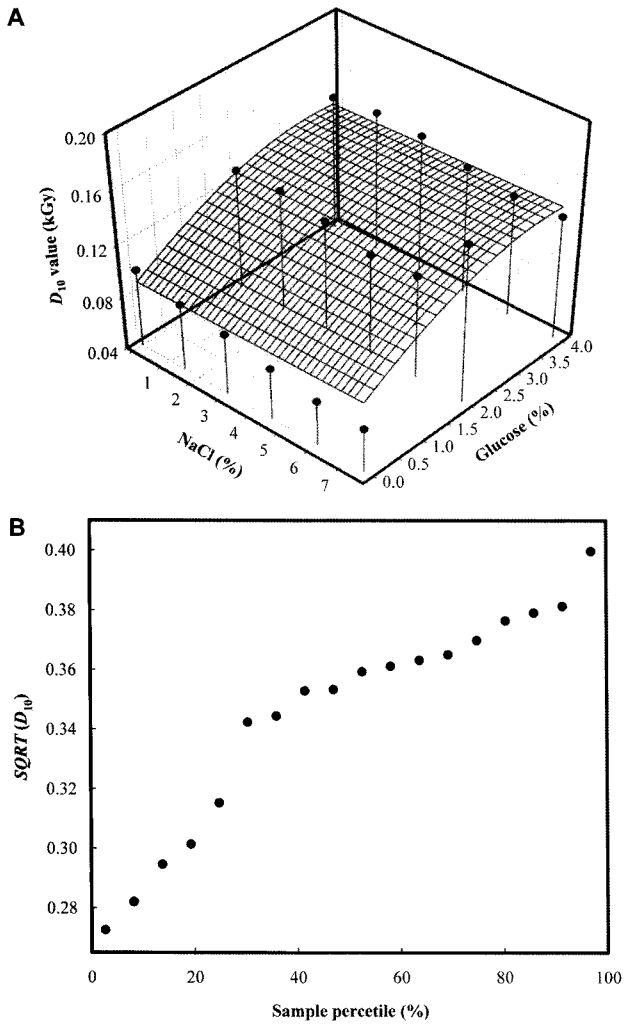

Fig. 2. Experimental data (●) and simulation (A) of D_{10} values (kGy) of *L. monocytogenes* as a function of glucose and NaCl concentration, and normal probability plot (B) of residuals vs. *SQRT* (D_{10}) for evaluation of normality of the model used for D_{10} value simulation.

Acknowledgments

This study was funded by a research grant from National Nuclear Technology Program of KOSEF, the Brain Korea 21 of Ministry of Education and the Environmental Biotechnology National Core Research Centre in Republic of Korea.

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