Journal of Food Protection, Vol. 77, No. 2, 2014, Pages 339–344
doi:10.4315/0362-028X.JFP-13-133

Research Note

Inactivation of *Alicyclobacillus acidoterrestris* Spores in Apple and Orange Juice Concentrates by Gamma Irradiation

SU-YEON LEE,† SANG-HYUN PARK,† AND DONG-HYUN KANG*

Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

MS 13-133: Received 4 April 2013/Accepted 8 August 2013

ABSTRACT

The objective of this study was to evaluate the effect of different concentrations of reconstituted apple and orange juice on reduction of *Alicyclobacillus acidoterrestris* spores by gamma irradiation. Spores of *A. acidoterrestris* were inoculated into three concentrations of apple (18, 36, and 72°Brix) and orange (11, 33, and 66°Brix) juice and subjected to five radiation doses (1, 3, 5, 7, and 10 kGy). No significant reductions ($P > 0.05$) in spores were observed after the 1-kGy treatment for all apple and orange concentrations. Spores in 18, 36, and 72°Brix apple juice concentrates subjected to 10 kGy were reduced to 4.34, 3.9, and 3.84 log CFU/ml, respectively. Similar results were observed for orange juice. When 10 kGy was applied to 11°Brix orange juice, populations of spores were reduced by 5 log CFU/ml. The reduction of spores in 33 and 66°Brix orange juice concentrates exposed to 10-kGy gamma irradiation was 4.54 and 3.85 log CFU/ml, respectively. Juice concentration did not affect ($P > 0.05$) the number of surviving *A. acidoterrestris* spores from the same kGy treatment. Gamma irradiation treatment did not change the pH or water activity of the juice ($P > 0.05$).

Alicyclobacillus acidoterrestris is a gram-positive, thermoacidophilic, aerobic, nonpathogenic, and spore-forming bacterium *(7)*. *A. acidoterrestris* was first documented by Cerny et al. *(8)* as a spoilage bacterium of apple juice in Germany in 1982, and spoilage incidents related to *Alicyclobacillus* spp. have been reported in various fruit juices *(17, 24),* fruit juice blends *(18),* and carbonated fruit juice drinks *(16)*. This bacterium produces a medicinal off-flavor compound (guaiacol) and other taint chemicals in fruit and vegetable juices *(19, 32)*. *A. acidoterrestris* also causes spoilage of fruit juice concentrates and other fruit juice products. Spoilage of commercial juice concentrates by *A. acidoterrestris* vegetative cells from germinated spores have been reported *(2, 6)*. The incidence of spoilage attributed to *A. acidoterrestris* may increase with increased international trade of fruit concentrates *(17)*.

Shelf-stable fruit juice is generally pasteurized at 95 to 98°C for 30 s, and common fruit juice spoilage microorganisms can be eliminated under these conditions *(12)*. However, spores produced by *A. acidoterrestris* are highly heat resistant, although *D*- and *z*-values are variable depending on the strains and growth conditions *(4, 12, 37)*. Ceviz et al. *(9)* reported *D*-values for *A. acidoterrestris* spores in apple and orange juice (10°Brix, pH 4.0) of 27.8 and 20.8 min at 95°C. Therefore, it is difficult to inactivate *A. acidoterrestris* spores using thermal pasteurization without adversely affecting the sensory properties of commercial products.

Gamma irradiation can effectively control or eliminate pathogenic microorganisms in fruit and vegetable juices *(26, 36)*. This treatment is particularly suitable for destroying bacterial spores in ingredients of low moisture content *(15)*. In 1981, a joint committee consisting of the U.S. Food and Drug Administration, the International Atomic Energy Agency, and the World Health Organization approved the use of irradiation for food processing and stated that foods treated in this way are safe and do not suffer from toxicity or nutritional degradation when the average radiation dose is less than 10 kGy *(20)*. Gamma irradiation technology can be used to prevent spoilage by inactivating microorganisms and to enhance the safety and shelf stability of food products *(39)*.

Although gamma irradiation has been applied to various fruit juices to control foodborne pathogenic bacteria *(14, 30)* and bacterial spores *(28),* the effects of this treatment on thermoacidophilic spores suspended in juice concentrates apparently have not been studied. The main objective of this study was to investigate the effect of gamma irradiation on *A. acidoterrestris* spores in apple and orange juices of various concentrations and to evaluate changes in pH, water activity, and color of juice concentrates.

MATERIALS AND METHODS

Bacterial strains and media. *A. acidoterrestris* type strain ATCC 49025 was obtained from the Korea Culture Center of

* Author for correspondence. Tel: +82-2-880-4927; Fax: +82-2-883-4928; E-mail: kang7820@snu.ac.kr.
† Equal contribution.

Microorganisms (Seoul). The strain was cultured using *Bacillus acidocaldarius* medium (BAM). BAM was first described by Darland and Brock *(11)* and made according to the formula modified by Silva et al. *(34)*. The inoculated plates were incubated for 3 days at 45°C and then stored at 4°C as stock cultures.

Preparation of spore suspension and inoculation. Cells grown at 45°C for 3 days on BAM were spread on acidified potato dextrose agar (PDA; pH 3.5; Difco, BD, Sparks, MD) in petri dishes and incubated at 43°C for 7 days until 80 to 90% sporulation was reached as determined by microscopic examination. Spores were harvested by depositing 1 to 2 ml of sterile deionized water onto the surface of acidified PDA culture plates and gently rubbing with a sterile cotton swab. Pooled spore suspensions of 15 plates were centrifuged at 4,000 × *g* for 20 min (4°C). The supernatant fluid was decanted, and the pellet was resuspended with sterile distilled water. The centrifugation and washing process was performed three times. The final pellets were resuspended in sterile water corresponding to approximately 10^6 to 10^7 spores per ml, combined, and stored in 2-ml cryogenic vials (Corning Inc., Corning, NY) at −20°C until used in subsequent experiments.

Unpasteurized apple and orange juice concentrates (all previously screened to ensure they were free of *A. acidoterrestris* spores) were supplied by a commercial producer (Edentown F&B, Incheon, Korea). Apple concentrates (72°Brix) were diluted to two different sugar concentrations (36 and 18°Brix) with sterile deionized water. Orange concentrates (66°Brix) were diluted to 33 and 11°Brix. A digital refractometer (PR-101, ATAGO Co., Tokyo, Japan) was used to measure °Brix. Individual 50-ml conical centrifuge tubes containing 20 ml of samples were inoculated with 0.3 ml of spore suspensions. The final spore level was ca. 10^5 spores per ml.

Gamma irradiation treatments. Inoculated fruit juice samples in conical centrifuge tubes were irradiated in a cobalt-60 gamma irradiator (point source AECL, IR-79, MDS Nordion International Co., Ltd., Ottawa, Ontario, Canada) at the Advanced Radiation Technology Institute (Jeongeup, Korea). The source strength was approximately 100 kCi with a dose rate of 10 kGy/h. The applied doses in this study were 0.0 (control), 1.0, 3.0, 5.0, 7.0, and 10 kGy. Nonirradiated controls were also maintained with the same storage, transport, and handling conditions as the irradiated samples. For enumeration of surviving spore populations, 1 ml of each juice concentration was taken from conical centrifuge tubes, 10-fold serial dilutions were prepared in buffered peptone water (Difco, BD), and 0.1 ml of sample or diluents was spread plated onto acidified PDA. When low spore populations were anticipated, 250 μl of sample was plated onto four plates of acidified PDA and incubated at 43°C for 2 days. All experiments were performed three times.

Measurement of physical properties. Triplicate measurements of sample pH and water activity were performed at room temperature with a pH meter (Seven multi 8603, Mettler Toledo, Greifensee, Switzerland) and a water activity meter (AQUA Lab 4TE, Decagon Inc., Pullman, WA), respectively, before and after gamma irradiation.

Color measurement. Hunter color values for *L* (lightness), *a* (redness), and *b* (yellowness) were measured with a colorimeter (CR400, Minolta Co., Osaka, Japan) to evaluate color changes of gamma-irradiated samples. Each bottle was shaken to suspend solid particles, and 10 ml of each sample was placed in the bottom half of a glass petri dish. The measuring head of the colorimeter was placed directly into the sample. All measurements were carried out in triplicate.

Scanning electron microscopy. The method used for scanning electron microscopy was similar to that described by Bae et al. *(1)*. Treated spores were rinsed three times with 0.1 M phosphate-buffered saline (pH 7.3) and collected by centrifugation at 4,000 × *g* for 10 min. The pellet was fixed in modified Karnovsky's fixative (2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer) at 4°C for 2 to 4 h. After primary fixation, spores were centrifuged and washed three times with 0.05 M sodium cacodylate buffer. Spores were then fixed in 1% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.2) at 4°C for 2 h and washed twice briefly with distilled water. Spores were dehydrated in a graded series of 30, 50, 70, 80, 90, and 100% ethanol and dried twice with 100% hexamethyldisilazane for 15 min. Dried samples were mounted on metal stubs and sputter coated with gold. Images were obtained with a Field-Emission scanning electron microscope (SUPRA 55VP, Carl Zeiss, Jena, Germany).

Statistical analysis. All experiments were conducted three times. Data were analyzed with an analysis of variance and Duncan's multiple range test (SAS Institute, Cary, NC). Differences in the results of the processing treatments were considered significant at $P < 0.05$.

RESULTS

Effects of gamma irradiation on *A. acidoterrestris* spores. As radiation doses increased from 1 to 10 kGy, surviving populations of *A. acidoterrestris* spores decreased in proportion. The levels of surviving spores were significantly reduced ($P < 0.05$) by 4.34, 3.90, and 3.84 log CFU/ml in 18, 36, and 72°Brix apple juice concentrations, respectively, when treated with 10 kGy of gamma radiation (Fig. 1). Treatment with 1 kGy of radiation produced no significant reductions ($P > 0.05$) in any of the apple juice preparations, and there were no significant differences ($P > 0.05$) in surviving populations of *A. acidoterrestris* spores in the various apple juice concentrations subjected to the same kGy treatment. When 10 kGy was applied, populations of *A. acidoterrestris* spores were significantly reduced ($P < 0.05$) by 5.00, 4.54, and 3.85 log CFU/ml in 11, 33, and 66°Brix orange juice concentrations, respectively (Fig. 2). Similar to apple juice, 1-kGy treatment produced no significant reduction ($P > 0.05$) in the number of *A. acidoterrestris* spores, and there were no significant differences ($P > 0.05$) between orange juice concentrations in populations of *A. acidoterrestris* spores subjected to the same kGy treatment.

Physical properties of apple juice and orange juice treated by gamma irradiation. After gamma irradiation treatment at each dose, there were no significant changes ($P > 0.05$) in water activity and pH of apple and orange juice concentrates (data not shown).

Color change of apple juice and orange juice after gamma irradiation. The Hunter color value changes of apple and orange juice concentrates after gamma irradiation are shown in Tables 1 and 2. The *L* value increased with an

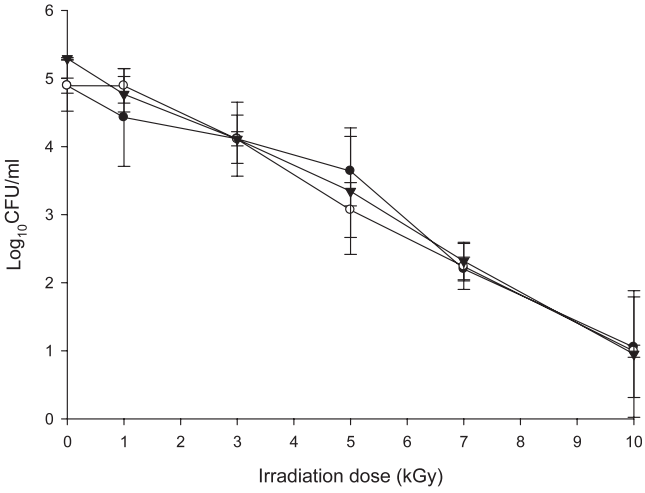

FIGURE 1. *Survival of* A. acidoterrestris *spores in apple juice concentrates (72°Brix) and diluted juice concentrates (36 and 18°Brix) treated with gamma irradiation (1, 3, 5, 7, and 10 kGy).* ●, *72°Brix;* ○, *36°Brix;* ▼, *18°Brix.*

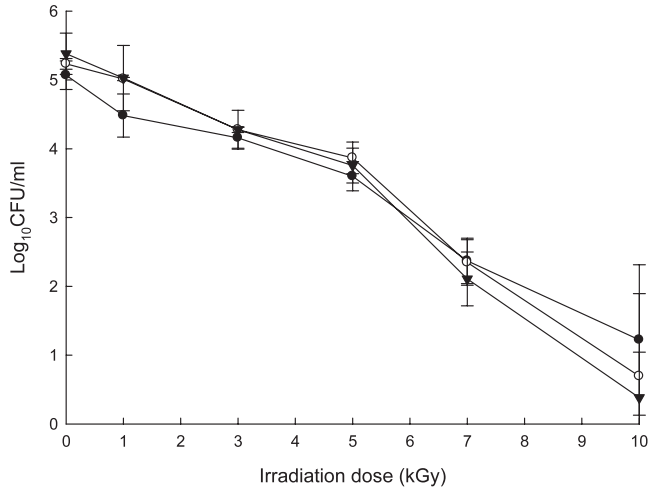

FIGURE 2. *Survival of* A. acidoterrestris *spores in orange juice concentrates (66°Brix) or diluted juice concentrates (33 and 11°Brix) treated with gamma irradiation (1, 3, 5, 7, and 10 kGy).* ●, *66°Brix;* ○, *33°Brix;* ▼, *11°Brix.*

increase in radiation dose in both apple and orange juice concentrates. The general *a* value of apple and orange juice concentrates significantly decreased ($P < 0.05$) with increasing radiation dose. The *b* value generally increased in apple and orange juice concentrates except in the 18°Brix apple juice concentrates.

Microstructural alteration of *A. acidoterrestris* spores after gamma irradiation. The structural and morphological changes of *A. acidoterrestris* spores after exposure to gamma irradiation were examined. Scanning electron microscopy was employed to examine spores irradiated at 7 and 10 kGy in 72°Brix apple juice concentrate (Fig. 3). The scanning electron micrographs revealed that treated spores were crushed and exhibited a high degree of hollowness on the spore surface, whereas the untreated spores were short, planiform elliptical rods.

DISCUSSION

The presence of *Alicyclobacillus* in fruit juice products poses a serious problem for the juice industry because *Alicyclobacillus* spores can survive normal pasteurization procedures and produce spoilage without apparent changes to packaging or juice clarity *(10, 22, 25, 35, 38).* Many researchers have studied the inactivation of *A. acidoterrestris* spores. Murakami et al. *(27)* reported that *D*-values at 90°C in citrate and phosphate buffers (20 mM concentration)

TABLE 1. *Color values of gamma-irradiated apple juice concentrate*

Sugar concn (°Brix)	Radiation dose (kGy)	Parameter[a]		
		L	*a*	*b*
18	0	21.52 ± 0.14 A	1.02. ± 0.83 A	9.75 ± 0.16 A
	1	21.98 ± 0.65 AB	0.06 ± 0.20 B	9.14 ± 0.37 A
	3	22.20 ± 0.62 AB	−0.35 ± 0.07 B	5.40 ± 1.13 B
	5	22.34 ± 0.47 AB	−0.50 ± 0.08 B	4.70 ± 0.10 BC
	7	22.43 ± 0.13 B	−0.53 ± 0.09 B	4.34 ± 0.15 C
	10	22.30 ± 0.45 AB	−0.53 ± 1.04 B	4.55 ± 0.26 BC
36	0	21.76 ± 0.26 A	2.98 ± 0.08 A	10.15 ± 0.19 AB
	1	22.34 ± 0.17 A	2.32 ± 0.10 B	9.59 ± 0.92 A
	3	22.28 ± 0.64 A	1.25 ± 0.16 C	9.84 ± 0.15 A
	5	23.16 ± 0.59 B	0.76 ± 0.21 D	10.82 ± 0.31 BC
	7	24.74 ± 0.31 C	0.56 ± 0.12 D	11.00 ± 0.15 C
	10	24.59 ± 0.31 C	−0.57 ± 0.13 E	11.13 ± 0.01 C
72	0	14.01 ± 0.54 A	7.30 ± 0.02 A	7.98 ± 0.15 A
	1	14.02 ± 0.31 A	7.64 ± 0.40 AB	8.05 ± 0.15 A
	3	15.65 ± 0.42 B	8.95 ± 0.08 C	9.92 ± 0.08 B
	5	16.97 ± 0.23 C	8.58 ± 0.35 C	10.19 ± 0.08 B
	7	17.29 ± 0.58 C	8.25 ± 0.17 BC	11.48 ± 0.17 C
	10	20.97 ± 0.79 D	8.58 ± 0.97 C	13.32 ± 0.42 D

[a] Values are means ± standard deviations for color parameters of *L* (lightness), *a* (redness), *b* (yellowness). Within a column, means followed by different letters are significantly different ($P < 0.05$).

TABLE 2. *Color values of gamma-irradiated orange juice concentrate*

Sugar concn (°Brix)	Radiation dose (kGy)	Parameter[a]		
		L	*a*	*b*
11	0	45.03 ± 0.35 A	−5.10 ± 0.16 A	16.48 ± 1.08 A
	1	45.07 ± 0.47 A	−5.17 ± 0.25 A	16.31 ± 0.99 A
	3	45.06 ± 0.04 A	−5.33 ± 0.11 A	15.55 ± 0.35 A
	5	45.02 ± 0.96 A	−5.44 ± 0.33 A	15.49 ± 0.57 A
	7	44.97 ± 0.61 A	−5.62 ± 0.95 A	15.17 ± 0.35 A
	10	44.53 ± 3.82 A	−5.70 ± 1.04 A	16.02 ± 0.18 A
33	0	43.89 ± 0.31 A	1.27 ± 1.87 A	24.06 ± 1.40 A
	1	46.34 ± 0.30 B	1.03 ± 1.03 B	24.44 ± 1.70 AB
	3	48.97 ± 0.98 C	−2.70 ± 0.58 BC	26.65 ± 0.91 BC
	5	47.25 ± 1.39 BC	−3.89 ± 1.23 C	26.43 ± 1.40 BC
	7	48.93 ± 1.10 C	−4.34 ± 0.52 C	28.25 ± 0.67 C
	10	49.06 ± 1.84 C	−4.78 ± 1.02 C	27.30 ± 0.61 C
66	0	33.95 ± 0.33 A	10.91 ± 0.42 A	20.83 ± 0.65 A
	1	36.09 ± 1.18 B	9.54 ± 0.74 A	21.94 ± 1.01 A
	3	37.07 ± 0.37 B	10.32 ± 0.80 A	24.27 ± 1.02 B
	5	37.84 ± 0.96 B	9.91 ± 1.02 A	25.12 ± 0.99 BC
	7	40.22 ± 1.36 C	7.51 ± 0.62 B	25.88 ± 0.45 BC
	10	41.45 ± 1.79 C	6.98 ± 0.67 B	26.59 ± 0.99 C

[a] Values are means ± standard deviations for color parameters of *L* (lightness), *a* (redness), *b* (yellowness). Within a column, means followed by different letters are significantly different ($P < 0.05$).

were 13.6 and 12.9 min, respectively. Baysal and Icier *(3)* investigated the effectiveness of ohmic heating for inactivating spores of *A. acidoterrestris*. *D*-values at 70, 80, and 90°C were 58.48, 12.24, and 5.97 min, respectively, at 30 V/cm. Chemical and physical methods have also been used to control heat-resistant *A. acidoterrestris* spores. Treatment with 40 ppm of chlorine dioxide reduced *A. acidoterrestris* spores in aqueous suspension and on apple surfaces by more than 4 log units after 5 min *(23)*. Orr and Beuchat *(31)* reported that about 2.2-, 0.4-, and 0.1-log reductions in *A. acidoterrestris* spores were achieved when spores were treated for 10 min with 200 ppm of chlorine, 500 ppm of acidified sodium chlorite, or 0.2% hydrogen peroxide solution, respectively. Several researchers have reported that the efficacy of high pressure against spores is enhanced when combined with heat treatment. Lee et al. *(21)* reported that the level of *A. acidoterrestris* spores in apple juice was significantly reduced by a combined treatment of high pressure and high temperature. In apple juice (17.5°Brix), the combination of high pressure and high heat resulted in spore reductions of more than five log units at 71 and 90°C.

In the present study, treatment with gamma irradiation produced significant reductions of *A. acidoterrestris* spores in apple and orange juice concentrates. Nakauma et al. *(28)* reported that *D*-values (radiation doses) for electron-beam– and gamma-irradiated *A. acidoterrestris* spores on filter paper were 1.02 and 1.10 kGy, respectively. This confirmed that electron-beam and gamma irradiation are effective for reducing *A. acidoterrestris* spores in dry materials.

Juice concentrations with high soluble sugar concentrations have low water activity, which affects the resistance of microorganism to inactivation methods *(33)*. Lee et al. *(21)* reported that high-pressure treatment (621 Mpa) at 71 and 90°C did not inactivate *A. acidoterrestris* spores in apple juice concentrate. Silva et al. *(34)* investigated the thermal inactivation of *A. acidoterrestris* spores in fruit concentrates with various concentrations of soluble solids. The *D*-value of *A. acidoterrestris* spores at 91°C in 58°Brix black current concentrates was 24.1 min, whereas that in 26.1°Brix light black current concentrates was 3.84 min. In this study, juice concentrates and their dilutions were used. No significant differences ($P > 0.05$) were observed between juice concentrations in the surviving populations of *A. acidoterrestris* spores for the same kGy treatment. These results indicate that gamma irradiation could effectively inactivate *A. acidoterrestris* spores regardless of the concentration of the juice.

The water content of spores is lower than that of vegetative cells *(29)*. The low water content of spores may influence the formation of radicals, which play an important role in inactivation *(5)*. Mechanisms of bacterial inactivation of ionizing radiation involve absorption of photon energy by a target molecule, which results in damage to the target *(5)*. Photon energy also is absorbed by a nearby molecule such as water, resulting in the formation of highly reactive radicals, which in turn react with target molecules in the microorganism *(5)*. Ionizing radiation also could affect the structure of spores. Fiester et al. *(13)* reported that ionizing radiation decreased inner spore membrane and coat integrity in *Bacillus atrophaeus,* which could cause spillage of cytoplasmic contents. The results of the present study are in accordance with the results of Fiester et al. *(13)*. The scanning electron micrographs (Fig. 3) revealed a dose-dependent increase in damage to the spore structure. Visible holes and areas of hollowness on spore surfaces increased with increasing doses of gamma radiation.

These results confirm that gamma irradiation is effective for inactivating *A. acidoterrestris* spores in apple

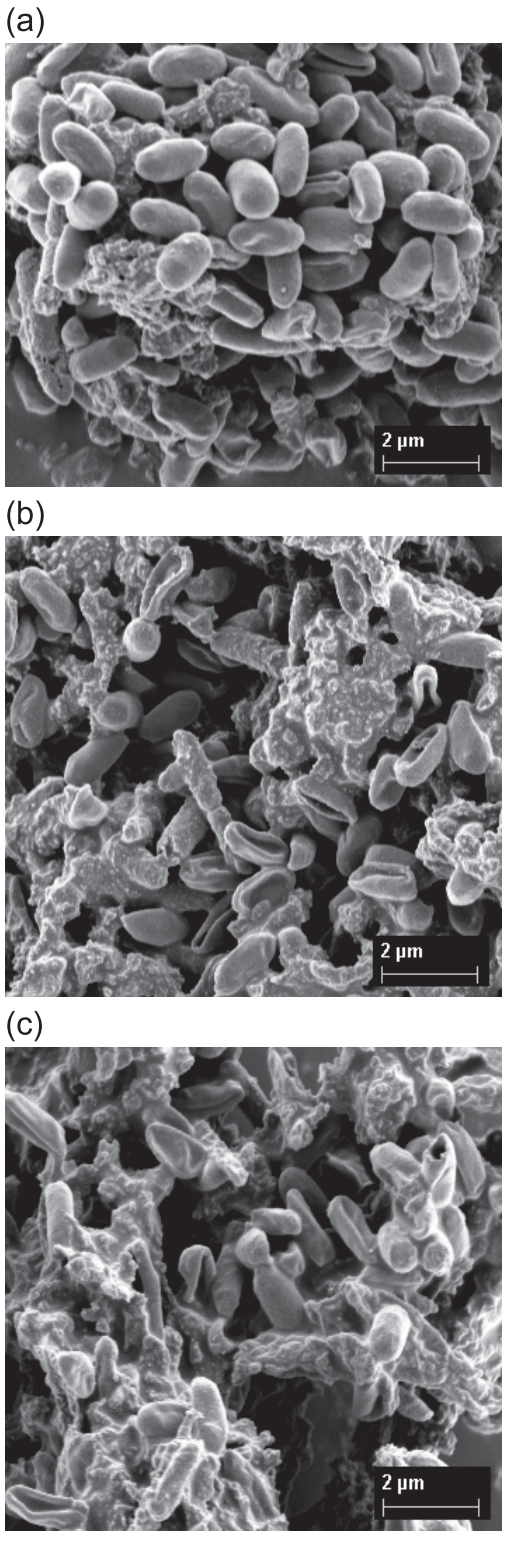

FIGURE 3. *Scanning electron micrographs of* A. acidoterrestris *spores in apple juice concentrate (72°Brix). Untreated control spores (a) and spores treated with gamma irradiation at 7 kGy (b) and 10 kGy (c).*

and orange juice concentrates. The effects of inactivation are dependent on the radiation dose. Gamma irradiation could be applied by the fruit juice industry instead of conventional methods used to control spores of *A. acidoterrestris*. Further investigation of combination techniques with gamma irradiation is needed to enhance the effectiveness of gamma irradiation.

ACKNOWLEDGMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (0635-20120004). This research was also supported by the Agriculture Research Center program of the Ministry for Food, Agriculture, Forestry and Fisheries, South Korea.

REFERENCES

1. Bae, Y. Y., H. J. Lee, S. A. Kim, and M. S. Rhee. 2009. Inactivation of *Alicyclobacillus acidoterrestris* spores in apple juice by supercritical carbon dioxide. *Int. J. Food Microbiol.* 136:95–100.
2. Bahceci, K., V. Gokmen, and J. Acar. 2005. Occurrence of *Alicyclobacillus acidoterrestris* on apple and in apple juice concentrates and effects of process technology on *A. acidoterrestris* spores in apple juice. *Fruit Process.* 15:328–331.
3. Baysal, A. H., and F. Icier. 2010. Inactivation kinetics of *Alicyclobacillus acidoterrestris* spores in orange juice by ohmic heating: effects of voltage gradient and temperature on inactivation. *J. Food Prot.* 73:299–304.
4. Bevilacqua, A., M. Sinigaglia, and M. R. Corbo. 2008. *Alicyclobacillus acidoterrestris*: new methods for inhibiting spore germination. *Int. J. Food Microbiol.* 125:103–110.
5. Blatchley, E. R., A. Meeusen, A. Aronson, and L. Brewster. 2005. Inactivation of *Bacillus* spores by ultraviolet or gamma radiation. *J. Environ. Eng.* 131:1245–1252.
6. Borlinghaus, A., and R. Engel. 1997. *Alicyclobacillus* incidence in commercial apple juice concentrate (AJC) supplies—method development and validation. *Fruit Process.* 7:262–266.
7. Casas, J., M. T. Valverde, A. Marín-Iniesta, and L. Calvo. 2012. Inactivation of *Alicyclobacillus acidoterrestris* spores by high pressure CO_2 in apple cream. *Int. J. Food Microbiol.* 156:18–24.
8. Cerny, G., W. Hennlich, and K. Poralla. 1984. Spoilage of fruit juice by bacilli: isolation and characterization of the spoiling microorganism. *Z. Lebensm-Unters. -Forsch.* 179:224–227.
9. Ceviz, G., Y. Tulek, and A. H. Con. 2009. Thermal resistance of *Alicyclobacillus acidoterrestris* spores in different heating media. *Int. J. Food Sci. Technol.* 44:1770–1777.
10. Danyluk, M. D., L. M. Friedrich, C. Jouquand, R. Goodrich-Schneider, M. E. Parish, and R. Rouseff. 2010. Prevalence, concentration, spoilage, and mitigation of *Alicyclobacillus* spp. in tropical and subtropical fruit juice concentrates. *Food Microbiol.* 28: 472–477.
11. Darland, G., and T. D. Brock. 1971. *Bacillus acidocaldarius* sp. nov., an acidophilic thermophilic spore-forming bacterium. *J. Gen. Microbiol.* 67:9–15.
12. Eiroa, M. N. U., V. C. A. Junqueira, and F. L. Schmidt. 1999. *Alicyclobacillus* in orange juice: occurrence and heat resistance of spores. *J. Food Prot.* 62:883–886.
13. Fiester, S. E., S. L. Helfinstine, J. C. Redfearn, R. M. Uribe, and C. J. Woolverton. 2012. Electron beam irradiation dose dependently damages the *Bacillus* spore coat and spore membrane. *Int. J. Microbiol.* 2012:1–9. doi:10.1155/2012/57959.
14. Foley, D. M., K. Pickett, J. Varon, J. Lee, D. B. Min, R. Caporaso, and A. Prakash. 2002. Pasteurization of fresh orange juice using gamma irradiation: microbiological, flavor, and sensory analyses. *J. Food Sci.* 67:1495–1501.
15. Food and Agriculture Organization. 1961. Report of the European meeting on the microbiology of irradiated foods, Paris, 20–23 April, 1960. Food and Agriculture Organization, Rome.
16. Gouws, P. A., L. Gie, A. Pretorius, and N. Dhansay. 2005. Isolation and identification of *Alicyclobacillus acidocaldarius* by 16S rDNA from mango juice and concentrate. *Int. J. Food Sci. Technol.* 40:789–792.
17. Jensen, N. 2000. *Alicyclobacillus* in Australia. *Food Aust.* 52:282–285.

18. Jensen, N., and F. B. Whitfield. 2003. Role of *Alicyclobacillus acidoterrestris* in the development of a disinfectant taint in shelf-stable fruit juice. *Lett. Appl. Microbiol.* 36:9–14.
19. Komitopoulou, E., I. S. Boziaris, E. A. Davies, J. Delves-Broughton, and M. R. Adams. 1999. *Alicyclobacillus acidoterrestris* in fruit juices and its control by nisin. *Int. J. Food Sci. Technol.* 34:81–85.
20. Lacroix, M., and B. Ouattara. 2000. Combined industrial processes with irradiation to assure innocuity and preservation of food products. *Food Res. Int.* 33:719–724.
21. Lee, S.-Y., H.-J. Chung, and D.-H. Kang. 2006. Combined treatment of high pressure and heat on killing spores of *Alicyclobacillus acidoterrestris* in apple juice concentrate. *J. Food Prot.* 69:1056–1060.
22. Lee, S.-Y., R. H. Dougherty, and D.-H. Kang. 2002. Inhibitory effects of high pressure and heat on *Alicyclobacillus acidoterrestris* spores in apple juice. *Appl. Environ. Microbiol.* 68:4158–4161.
23. Lee, S.-Y., P. M. Gray, R. H. Dougherty, and D.-H. Kang. 2004. The use of chlorine dioxide to control *Alicyclobacillus acidoterrestris* spores in aqueous suspension and on apples. *Int. J. Food Microbiol.* 92:121–127.
24. Matsubara, H., K. Goto, T. Matsumura, K. Mochida, M. Iwaki, M. Niwa, and K. Yamasato. 2002. *Alicyclobacillus acidiphilus* sp. nov., a novel thermo-acidophilic, ω-alicyclic fatty acid–containing bacterium isolated from acidic beverages. *Int. J. Syst. Evol. Microbiol.* 52: 1681–1685.
25. McIntyre, S., J. Y. Ikawa, N. Parkinson, J. Hagludn, and J. Lee. 1995. Characteristics of an acidophillic *Bacillus* strain isolated from shelf-stable juices. *J. Food Prot.* 58:319–321.
26. Molins, R. A., Y. Motarjemi, and F. K. Kaferstein. 2001. Irradiation: a critical control point in ensuring the microbiological safety of raw foods. *Food Control* 12:347–356.
27. Murakami, M., H. Tedzuka, and K. Yamazaki. 1998. Thermal resistance of *Alicyclobacillus acidoterrestris* spores in different buffers and pH. *Food Microbiol.* 15:577–582.
28. Nakauma, M., K. Saito, T. Katayama, M. Tada, and S. Todoriki. 2004. Radiation-heat synergism for inactivation of *Alicyclobacillus acidoterrestris* spores in citrus juice. *J. Food Prot.* 67:2538–2543.
29. Nicholson, W. L., N. Munakata, G. Horneck, H. J. Melosh, and P. Setlow. 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol. Biol. Rev.* 64:548–572.
30. Niemira, B. A., C. H. Sommers, and G. Boyd. 2001. Irradiation inactivation of four *Salmonella* serotypes in orange juices with various turbidities. *J. Food Prot.* 64:614–617.
31. Orr, R. V., and L. R. Beuchat. 2000. Efficacy of disinfectants in killing spores of *Alicyclobacillus acidoterrestris* and performance of media for supporting colony development by survivors. *J. Food Prot.* 63:1117–1122.
32. Orr, R. V., R. L. Shewfelt, C. J. Huang, S. Tefera, and L. R. Beuchat. 2000. Detection of guaiacol produced by *Alicyclobacillus acidoterrestris* in apple juice by sensory and chromatographic analyses, and comparison with spore and vegetative cell populations. *J. Food Prot.* 63:1517–1522.
33. Palou, E., A. Lopez-Malo, G. V. Barbosa-Canovas, J. Welti-Chames, and B. G. Swanson. 1997. Combined effect of high hydrostatic pressure and water activity on *Zygosaccharomyces balii* inhibition. *Lett. Appl. Microbiol.* 24:417–420.
34. Silva, F. M., P. Gibbs, M. C. Vieira, and C. L. M. Silva. 1999. Thermal inactivation of *Alicyclobacillus acidoterrestris* spores under different temperature, soluble solids and pH conditions for the design of fruit processes. *Int. J. Food Microbiol.* 51:95–103.
35. Smit, Y., M. Cameron, P. Venter, and R. C Witthuhn. 2011. *Alicyclobacillus* spoilage and isolation—a review. *Food Microbiol.* 28:331–349.
36. Song, H. P., D. H. Kim, C. Jo, C. H. Lee, K. S. Kim, and M. W. Byun. 2006. Effect of gamma irradiation on the microbiological quality and antioxidant activity of fresh vegetable juice. *Food Microbiol.* 23:372–378.
37. Walker, M., and C. A. Phillips. 2008. *Alicyclobacillus acidoterrestris*: an increasing threat to the fruit juice industry? *Int. J. Food Sci. Technol.* 43:250–260.
38. Walls, I., and R. Chuyate. 1998. *Alicyclobacillus*—historical perspective and preliminary characterization study. *Dairy Food Environ. Sanit.* 18:499–503.
39. World Health Organization. 1999. High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. WHO technical report series 890. World Health Organization, Geneva.