

Irradiation Inactivation of Four *Salmonella* Serotypes in Orange Juices with Various Turbidities[†]

BRENDAN A. NIEMIRA,* CHRISTOPHER H. SOMMERS, AND GLENN BOYD

Food Safety Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

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ABSTRACT

Reconstituted orange juice inoculated with *Salmonella* Anatum, *Salmonella* Infantis, *Salmonella* Newport, or *Salmonella* Stanley was treated with gamma radiation at 2°C. To determine the relationship between juice antioxidant power and D_{90} (dose required to achieve 90% mortality), juice solids were removed prior to inoculation by centrifugation and/or filtration to create juice preparations of varying turbidity. In unadulterated orange juice, *Salmonella* Anatum ($D_{90} = 0.71$ kGy) was significantly more resistant than the other species tested. *Salmonella* Newport ($D_{90} = 0.48$ kGy) and *Salmonella* Infantis ($D_{90} = 0.35$ kGy) were significantly different, while *Salmonella* Stanley ($D_{90} = 0.38$ kGy) was intermediate between the two. Neither the resistance of each isolate nor the pattern of relative resistance among isolates was altered in reduced turbidity juice preparations. Although total antioxidant power was associated with the level of juice solids resuspended in phosphate buffer, antioxidant power was not significantly associated with turbidity in the juice preparations or with D_{90} of any species. The variable resistance to irradiation of the *Salmonella* isolates suggests this as a more significant factor than turbidity or antioxidant power in designing antimicrobial juice irradiation protocols.

Freshly squeezed, nonpasteurized orange juice is a highly desirable commodity (2, 3). However, it has also been implicated as the food source in several recent outbreaks of salmonellosis (4, 9, 10). The National Advisory Committee on Microbiological Criteria for Foods has recommended that all fruit juices receive treatments sufficient to produce a cumulative 5-log reduction of bacteria associated with foodborne illness, such as *Escherichia coli* O157:H7 and *Salmonella* (8). A variety of nonthermal technologies have been proposed to achieve this level of reduction in fruit and vegetable juices while preserving heat-sensitive flavor and aroma components (13). Irradiation is one potential alternative to thermal pasteurization.

In a study of apple juice inoculated with *E. coli* O157:H7, the amount of gamma radiation necessary to reduce the bacterial population by 90% (D_{90}) was dependent on the bacterial strain examined and the level of suspended solids in the apple juice (3). The suggested mechanism for the effect of juice turbidity on D_{90} is the contribution of antioxidant (oxygen radical scavenging) potential of the suspended solids, which may serve to absorb the oxygen radicals produced during the irradiation process, thereby protecting the suspended bacteria and increasing D_{90} (3). Pathogenic bacteria exhibit increased D_{90} in solutions amended with chemical antioxidants such as formate or polyethylene glycol (7). However, the effect of naturally occurring antioxidant power associated with suspended solids on the ra-

diation sensitivity of bacteria in irradiated food media such as orange juice has not been studied. The objectives of this study were to determine (i) the efficacy of gamma radiation for inactivation of *Salmonella* in regular and reduced-turbidity orange juice and (ii) the antioxidant power of reduced-turbidity orange juice and resuspended orange juice solids.

MATERIALS AND METHODS

Microorganisms. Four *Salmonella* isolates were obtained from the Centers for Disease Control (Atlanta, Ga.). The cultures were outbreak strains originally isolated from seeds used to produce edible sprouts: *Salmonella* Anatum F4317, *Salmonella* Infantis F4319, *Salmonella* Newport H1275, *Salmonella* Stanley H0558. Stock cultures were maintained in tryptic soy broth (Difco, Detroit, Mich.) at 2°C and transferred bimonthly.

Inoculation. Working cultures were grown by inoculation of 10 ml sterile tryptic soy broth with 0.1 ml of stock culture and incubation at 37°C for 18 h without agitation. The concentration of the working culture was approximately 10^8 CFU/ml as determined by dilution and pour plating using tryptic soy agar (Difco). Aliquots of 0.4 ml working culture per 10 ml juice preparation to be inoculated were centrifuged at $5,000 \times g$ for 10 min to pelletize cells. The tryptic soy broth supernatant was discarded, and the pelletized cells were resuspended with a small aliquot of the juice. This suspension was then added to the juice. The final bacterial concentration in inoculated juice preparation was approximately 10^7 CFU/ml.

Gamma irradiation. The inoculated juice preparations were treated with 0.0 (control), 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, or 2.5 kGy and held on ice during transport to and from the irradiator. Time between inoculation and irradiation was typically

* Author for correspondence. Tel: 215-836-3784; Fax: 215-233-6445; E-mail: bniemira@arserrc.gov.

[†] Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE 1. Characterization of regular, centrifuged, and filtered orange juice and resistance of *Salmonella* isolates to gamma radiation

	Orange juice preparation ^a			
	Regular	Centrifuged	Filtered	
pH ^b	3.86 A	3.92 A	3.92 A	
Turbidity ^{b,c}	1.30 A	1.24 A	0.33 B	
Antioxidant power ^b	339.73 A	300.26 A	289.30 A	
<i>Salmonella</i> D _γ ^d				
<i>Salmonella</i> Anatum ^e	0.71 A	0.70 A	0.66 A	NSD ^f
<i>Salmonella</i> Infantis ^e	0.35 B	0.37 B	0.35 B	NSD
<i>Salmonella</i> Newport ^e	0.48 C	0.50 C	0.47 C	NSD
<i>Salmonella</i> Stanley ^e	0.38 B,C	0.42 B,C	0.38 B,C	NSD

^a Regular, reconstituted frozen concentrated orange juice; centrifuged, bulk solids removed, 2,500 × g, 20 min; filtered, centrifuged, filtered through VWR 454 and Whatman GF/B filter papers to remove all particles larger than 1 μm.

^b Mean of five samples; numbers in a given row with different letters are significantly different ($P < 0.05$, analysis of variance).

^c Absorbance at 560 nm.

^d D_γ, the dose of gamma radiation (kGy) required to reduce bacterial population by 90%.

^e Numbers in a given column with different letters are significantly different ($P < 0.05$, analysis of covariance).

^f NSD, no significant difference in D_γ for the isolate in the different juice preparations ($P < 0.05$, analysis of covariance).

less than 1 h. The tubes were arranged in a circular plastic rack within the chamber using the same geometry each time. A single juice preparation was examined per irradiation, with one *Salmonella* isolate per tube per dose. Each isolate and preparation combination was irradiated three times in separate trials. The three juice preparations (described below) were evaluated sequentially.

The samples were irradiated using a Lockheed-Georgia cesium-137 self-contained gamma radiation source, with a dose rate of 0.101 kGy/min. The sample temperature (2°C) was maintained during irradiation by injection of gas coming from liquid nitrogen into the sample chamber. Following irradiation, noninoculated juice samples were examined for gross changes in product quality related to visual appearance and aroma. A subsequent simultaneous evaluation of all three juice preparations using *Salmonella* Newport confirmed the consistency of the irradiation procedure.

Alanine pellets (lot IVR4, Bruker Instruments, Rjeomstettem, Germany) were used for dosimetry. The pellets were stored in a desiccator at 51% relative humidity until used. One pellet per dose was weighed and loaded into a Nalgene Cryoware 1.2-ml cryogenic vial (Thomas Scientific, Swedesboro, NJ). The vial was placed inside a 16- by 125-mm glass tube for irradiation. After irradiation, the vials were returned to the desiccator and held until being read. The pellets were read on a Bruker EMS 104 EPR analyzer and compared with a previously determined standard curve. Actual dose was typically within 5% of the nominal dose.

Calculation of D_γ. Pour plating with tryptic soy agar was used to determine the surviving bacterial population in the irradiated juice preparations. Serial dilution was with 9.0-ml blanks of Butterfield's phosphate buffer. Three pour plates per dilution were incubated for 24 h at 37°C and counted with an automatic plate counter. The data for each isolate were normalized against the control and plotted as the log₁₀ reduction using the nominal doses. The slopes of the individual survivor curves were calculated with linear regression using a computer graphics program (SigmaPlot 5.0, SPSS Inc., Chicago, Ill.). D_γ was calculated by taking the negative reciprocal of the survivor curve slope (QuattroPro, Corel Corp., Ottawa, Ontario, Canada). The significance of differences between slopes was determined with analysis of covariance (SAS, SAS Institute, Inc., Cary, N.C.) using data pooled from the three trials.

Orange juice preparations. To minimize experimental variation, reconstituted frozen concentrated orange juice was used as a model system for fresh (not from concentrate) orange juice. The frozen concentrated orange juice was obtained from local markets. The same manufacture lot was used throughout the study and stored at -70°C until used. Frozen concentrated orange juice was aseptically reconstituted with 1,000 ml distilled water per 355 ml frozen concentrate. A magnetic stirrer was used to dissolve completely the frozen concentrated orange juice and keep all solids in homogeneous suspension. Pour plating with tryptic soy agar showed no native microflora in the reconstituted juice, to the limit of detection (10¹ CFU/ml).

Three juice preparations were tested. Regular juice consisted of unadulterated reconstituted juice. Centrifuged juice was prepared by bulk centrifugation of the reconstituted juice at 2,500 × g for 20 min to remove the pulp and large solids. Filtered juice was prepared by centrifugation of the juice as described, followed by successive vacuum filtration through VWR 454 and Whatman GF/B filter papers to remove all particles larger than 1 μm. Non-inoculated samples of each juice preparation were characterized for pH, turbidity (absorbance at 560 nm), and antioxidant power (Table 1).

Antioxidant measurement. The antioxidant strength of each juice preparation was measured using the ferric reducing-antioxidant power (FRAP) assay, a colorimetric assay that measures the total antioxidant power of a solution by development of blue pigmentation (1). Each juice preparation was diluted 1:10. Samples (100 μl) were placed in spectrophotometer cuvettes (five per juice preparation), and 3 ml of fresh FRAP reagent solution was added, fully mixing the solutions. The reaction was allowed to proceed for 6 min at room temperature to allow full development of the pigmentation. The absorbance of the reacted solution was read at 593 nm, and the value converted to FRAP μM equivalent using a previously determined standard curve (1,000 μM ascorbic acid = 2,000 μM FRAP), accounting for previous dilution of the original juice preparation. The study was performed twice. The solids removed from the juice by centrifugation were resuspended in Butterfield's phosphate buffer or in filtered juice at various concentrations ranging from 0 to 10 g per 100 ml liquid. The amended fluids were then analyzed with the FRAP assay as described.

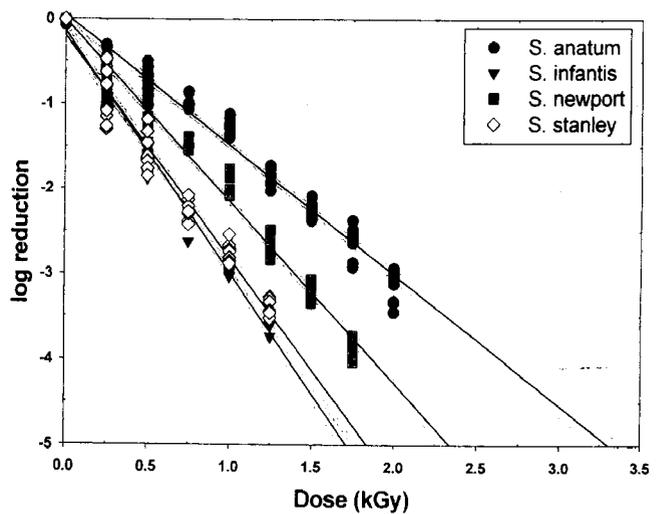
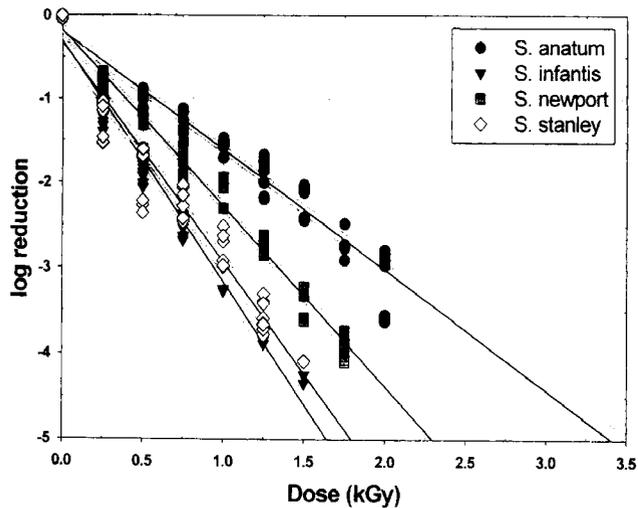


FIGURE 1. Survival of *Salmonella Anatum* CDC-F4317, *Salmonella Infantis* CDC-F4319, *Salmonella Newport* CDC-H1275, and *Salmonella Stanley* CDC-H0558 in orange juice following gamma irradiation.

FIGURE 3. Survival of *Salmonella Anatum* CDC-F4317, *Salmonella Infantis* CDC-F4319, *Salmonella Newport* CDC-H1275, and *Salmonella Stanley* CDC-H0558 in centrifuged and filtered orange juice following gamma irradiation.

RESULTS

Gamma radiation effectively reduced the level of *Salmonella* in all three juice preparations examined (Figs. 1 through 3). At the highest doses used (2.25 and 2.50 kGy), populations of bacteria in irradiated samples fell below detectable levels. As the r^2 values of the linear survivor curves were consistently above 0.95, inactivation kinetics were considered to be first order, and D_{γ} were calculated from these linear regressions. Standard error of D_{γ} among the replications for each isolate or juice preparation was low, less than 4% of the mean, indicating low variation between replications. In regular (unadulterated) orange juice, the D_{γ} of the more resistant species, *Salmonella Anatum* and *Salmonella Newport*, differed significantly (analysis of covariance, $P < 0.05$) from each other and from the less resistant species, *Salmonella Stanley* and *Salmonella Infantis*, that did not differ from each other (Table 1). D_{γ}

in centrifuged and filtered juice preparations followed a similar pattern (Table 1). The turbidity of the juice preparation did not significantly (analysis of covariance, $P < 0.05$) influence the D_{γ} obtained for any of the isolates tested (Table 1). There were no evident changes in the visual appearance or aroma of any noninoculated juice preparation at any radiation dose.

Variation in antioxidant power among the juice preparations did not rise to the level of significance (analysis of variance, $P < 0.05$) (Table 1). The antioxidant power of both phosphate buffer and filtered juice increased by 2.5 to 3.5 μM FRAP per mg amended juice solids (Fig. 4). The correlation coefficient was high for amended phosphate buffer (0.99) and relatively low for amended filtered juice (0.69). The contribution of amended solids to the total an-

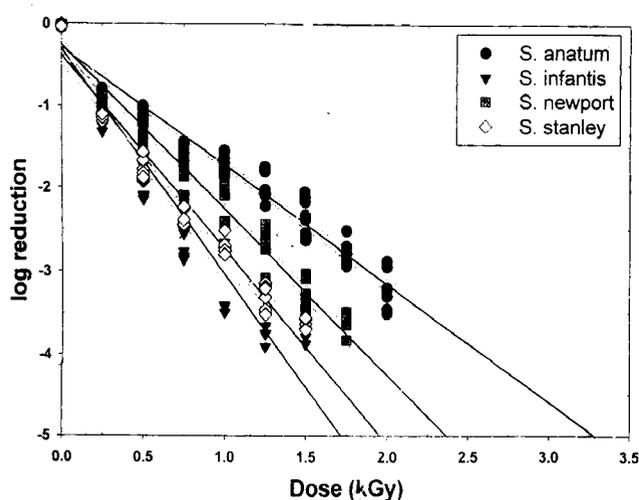


FIGURE 2. Survival of *Salmonella Anatum* CDC-F4317, *Salmonella Infantis* CDC-F4319, *Salmonella Newport* CDC-H1275, and *Salmonella Stanley* CDC-H0558 in centrifuged orange juice following gamma irradiation.

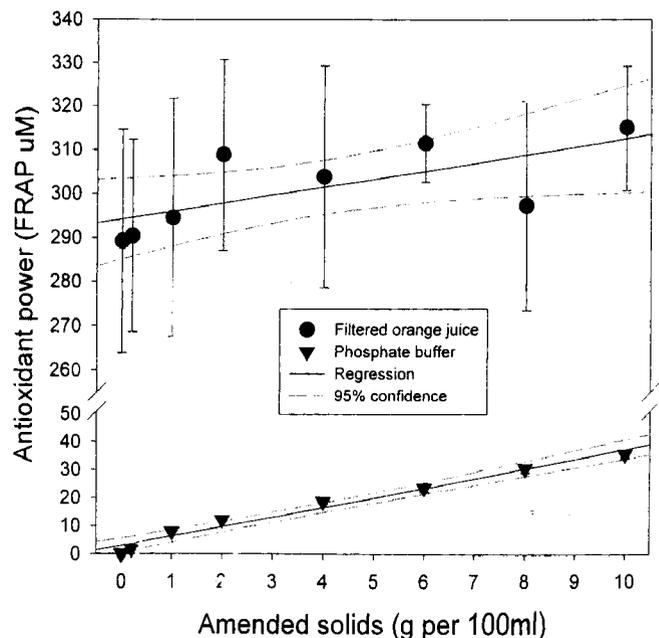


FIGURE 4. Antioxidant power of phosphate buffer and filtered orange juice amended with juice solids.

antioxidant power of the filtered juice was relatively low, even at the highest amendment level.

DISCUSSION

Irradiation was effective in reducing the populations of *Salmonella* spp. in reconstituted orange juice. The different isolates differed significantly in sensitivity to irradiation. Achieving the recommended 5-log reduction (8) of the least resistant isolate, *Salmonella* Infantis ($D_{10} = 0.35$ kGy), would require 1.75 kGy. By contrast, the most resistant isolate tested, *Salmonella* Anatum ($D_{10} = 0.71$ kGy), would require 3.55 kGy for the same reduction. The difference in resistance to irradiation among the *Salmonella* species examined suggests that the most conservative recommendation is warranted, i.e., that based on the most resistant species tested.

In these studies, variation in juice turbidity was not associated with altered D_{10} of any isolate. Studies of pathogenic isolates of *E. coli* suspended in apple juices of naturally varying turbidity showed a relationship between the level of suspended solids and D_{10} (3). The authors of that work cited the polyphenolic nature of the suspended solids, conjecturing that the suspended solids acted as antioxidants, enhancing scavenging of biocidal hydroxyl radicals produced during the irradiation. Total dissolved phenol concentration of orange juice is associated with antioxidant power (11). In this study, while juice solids suspended in phosphate buffer increased the total antioxidant power of the solution, the contribution of the solids was only a minor component of the total antioxidant power of the orange juice. Turbidity varies more widely in commercial apple juices than in commercial orange and citrus juices (3). The range of turbidity observed in commercial citrus juices is less than that of the three juice preparations examined herein (unpublished data). As reduced turbidity was not associated with decreased D_{10} for any of the isolates examined, juice turbidity is not expected to be a major factor in determination of effective dose rates for the elimination of bacteria in orange juice.

Irradiation of fruit and fruit juices was recently reviewed (15). A variety of fruit juices, including orange juice, were irradiated by Fetter et al. (6) to a maximum of 5 kGy with no impact on flavor. More recently, orange juice irradiated to doses as high as 5 kGy at 2°C demonstrated little reduction in product color or concentration of key aroma and flavor compounds (5). Irradiation of citrus fruits and juices is known to oxidize a portion of the total ascorbic acid (vitamin C) to the dehydro form (12). However, both of these forms of the vitamin are biologically active, suggesting minimal nutritional impact (14). Therefore, temperature-controlled application of the radiation doses re-

quired for a 5-log reduction of the most resistant isolate examined would have little impact on the organoleptic or nutritional quality of orange juice. The efficacy of temperature-controlled irradiation of orange juice to reduce bacterial contamination suggests this technique as an important area of further research.

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REFERENCES

1. Benzie, I. F. F., and J. J. Strain. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* 299:15–27.
2. Braddock, R. J. 1999. Single strength juices and concentrates, chapter 5, p. 53–64. In R. J. Braddock (ed.), *Handbook of citrus by-products and processing technology*. John Wiley & Sons, Inc., New York.
3. Buchanan, R. L., S. G. Edelson, K. Snipes, and G. Boyd. 1998. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. *Appl. Environ. Microbiol.* 64:4533–4535.
4. Cook, K. A., T. E. Dobbs, G. Hlady, J. G. Wells, T. J. Barrett, N. D. Pouhr, G. A. Lancette, D. W. Bodager, B. L. Toth, C. A. Genese, A. K. Highsmith, K. E. Pilot, L. Finelli, and D. L. Swerdlow. 1998. Outbreak of *Salmonella* serotype Hartford infections associated with unpasteurized orange juice. *JAMA* 280:1504–1509.
5. Fan, X. 2000. Personal communication.
6. Fetter, F., G. Stehlik, J. Kovacs, and S. Weiss. 1969. Das flavourverhalten einiger gamma-bestrahlter fruchtsaeften. *Mitteilungen: -rube, -wein, -obstbau und fruechteverwertung.* 19:140–151.
7. Kim, A. Y., and D. W. Thayer. 1995. Radiation-induced cell lethality of *Salmonella typhimurium* ATCC 14028: cooperative effect of hydroxyl radical and oxygen. *Radiat. Res.* 144:36–42.
8. National Advisory Committee on Microbiological Criteria for Food. 1997. Recommendations for controlling the transmission of pathogenic microorganisms in juices. Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C.
9. Parish, M. E. 1997. Public health and non-pasteurized fruit juices. *CRC Crit. Rev. Microbiol.* 23:109–119.
10. Parish, M. E. 1998. Coliforms, *Escherichia coli* and *Salmonella* serovars associated with a citrus processing facility in a salmonellosis outbreak. *J. Food Prot.* 61:280–284.
11. Rapisarda, P., A. Tomaino, R. Lo Cascio, F. Bonina, A. De Pasquale, and A. Saija. 1999. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food Chem.* 47:4718–4723.
12. Romani, R. J., J. Van Kooy, L. Lim, and B. Bowers. 1963. Radiation physiology of fruit—ascorbic acid, sulfhydryl and soluble nitrogen content of irradiated citrus. *Radiat. Bot.* 3:58.
13. Sizer, C. E., and V. M. Balasubramaniam. 1999. New intervention processes for minimally processed juices. *Food Technol.* 53:64–67.
14. Thayer, D. W. 1994. Wholesomeness of irradiated foods. *Food Technol.* 48:132–136.
15. Thayer, D. W., and K. T. Rajkowski. 1999. Developments in irradiation of fresh fruits and vegetables. *Food Technol.* 53:62–65.