

Research Note

Citrus Juice Composition Does Not Influence Radiation Sensitivity of *Salmonella* Enteritidis[†]

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ABSTRACT

Food substrate chemistry is known to influence radiation sensitivity of pathogenic bacteria. The sensitivity of a citrus juice outbreak strain of *Salmonella* Enteritidis to gamma radiation was determined in five commercial orange juice formulations. The juices differed in pH (3.87 to 4.13), calcium concentration (2.1 versus 36.9 mM), juice composition (orange versus orange-tangerine blend), and antioxidant power (11,751 to 12,826 μ M ferric reducing-antioxidant power units). The D_{γ} (dose required to achieve 90% destruction) varied only slightly (0.35 to 0.37 kGy), with no significant ($P < 0.05$) differences among any of the suspending juices. These results indicate that *Salmonella* Enteritidis sensitivity to gamma radiation is not strongly influenced by the composition of formulated commercial orange juices.

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, 10, 11). 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* spp. (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 (12). Ionizing radiation is one potential alternative to thermal pasteurization.

The radiation sensitivity of foodborne pathogenic bacteria varies among species and among isolates of a given species (14, 17). The sensitivity may also vary with changes in the composition of the food product in which the suspended bacteria are irradiated (13). The extent to which formulated juice products may influence D_{γ} , the amount of gamma radiation necessary to effect a 90% reduction in population of suspended bacteria, has not been extensively studied. The objective of this study was to determine the effect of orange and mixed citrus juice formulation on the radiation sensitivity of a *Salmonella* Enteritidis strain associated with a foodborne illness outbreak.

MATERIALS AND METHODS

Microorganism. A culture of *Salmonella* Enteritidis isolated from salmonellosis patients who consumed contaminated, unpas-

teurized orange juice was obtained from the Wyoming Department of Health, Cheyenne, Wyo. 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, or 1.25 kGy and held on ice during transport to and from the irradiator. Time between inoculation and irradiation was typically less than 1 h. The tubes were arranged in a circular plastic rack within the chamber using the same geometry each time. The inoculated juices were irradiated with one tube per dose, 10 ml juice per tube (16-ml capacity). The study was repeated three times in separate trials.

The samples were irradiated using a Lockheed-Georgia (Marietta, Calif.) ^{137}Cs self-contained gamma radiation source, with a dose rate of 0.101 kGy/min. The sample temperature (2°C) was maintained during irradiation with the gas phase of liquid nitrogen injected into the sample chamber. Following irradiation, noninoculated juice samples were examined for gross changes in product quality, i.e., readily apparent changes in juice visual appearance and aroma.

Alanine pellets (lot IVR4; Bruker, Billerica, Mass.) 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 (Fischer

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† 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. Characteristics of reconstituted commercial frozen concentration citrus juices

Brand	Juice type	Pulp ^a	Acidity	Calcium	Comments
A	Orange	Not adjusted	Not adjusted	Not adjusted	
B	Orange	Pulp added	Not adjusted	Not adjusted	
C	Orange	Not adjusted	Reduced	Not adjusted	"Reduced acid"
D	Orange	Not adjusted	Not adjusted	Calcium added ^b	"Calcium enriched"
E	Orange, tangerine	Pulp reduced	Not adjusted	Calcium added ^b	"Pulp free, calcium enriched"

^a Descriptions of composition are reported from product label.

^b Calcium added as tricalcium phosphate and calcium lactate.

Scientific, Pittsburgh, Pa.). 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 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 (ARI, Newtown, Conn.). 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 \log_{10} 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 Inc., Ottawa, Ontario, Canada). The significance of differences between slopes was determined with analysis of covariance (ANCOVA; SAS version 6.12; SAS Institute, Inc., Cary, N.C.) using data pooled from the three trials. Correlation analysis among physical parameters and D_{γ} was performed with spreadsheet software (QuattroPro).

Orange juice brands. To minimize experimental variation, reconstituted frozen concentrated orange juice was used as a model system for fresh (not from concentrate) orange juice. Five commercial brands of frozen concentrated orange juice were obtained from local markets that differed in pulp content, pH, calcium amendment, and fruit juice composition (Table 1). 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 the frozen concentrated orange juice and to 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 (10^1 CFU/ml). Noninoculated samples of each juice preparation were characterized for pH ($n = 4$), turbidity (absorbance at 566 nm, $n = 6$), and antioxidant power ($n = 6$) (Table 2). The assays were performed in three trials, and the pooled data sets for each parameter for each juice were analyzed using analysis of variance (ANOVA; SigmaStat 2.0; SPSS).

Antioxidant measurement. The antioxidant strength of each juice brand 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 μ l) were placed in spectrophotometer cuvettes (six per juice brand), 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 was converted to the FRAP μ M equivalent using a previously determined standard curve (1,000 μ M ascorbic acid = 2,000 μ M FRAP).

RESULTS

The juices varied significantly (ANOVA, $P < 0.05$) in pH, ranging from 3.87 to 4.13, and in antioxidant power, ranging from 11,751 to 12,826 μ M FRAP (Table 2). The calcium concentration of calcium-enriched juices was 17-fold greater than that of nonaugmented juice, as reported in the product composition information. The juices did not vary in turbidity (Table 2).

TABLE 2. Analysis of reconstituted commercial frozen concentrated citrus juices

Brand	Turbidity (A_{556}) ^{a,b}	pH ^a	Calcium (mM) ^c	Antioxidant power (FRAP μ M) ^a	D_{γ} (kGy) ^d
A	1.295 A	3.87 A	2.1	12,214 AB	0.365 A
B	1.351 A	3.90 B	2.1	12,153 AB	0.370 A
C	1.310 A	4.04 C	2.1	11,751 B	0.368 A
D	1.339 A	4.13 D	36.9	12,739 A	0.365 A
E	1.336 A	4.13 D	36.9	12,826 A	0.351 A

^a Numbers followed by different letters are significantly different (ANOVA, $P < 0.05$).

^b Sample size: turbidity, $n = 6$; pH, $n = 4$; antioxidant power, $n = 6$.

^c Concentration of calcium as reported on commercial package label.

^d Numbers followed by different letters are significantly different (ANCOVA, $P < 0.05$).

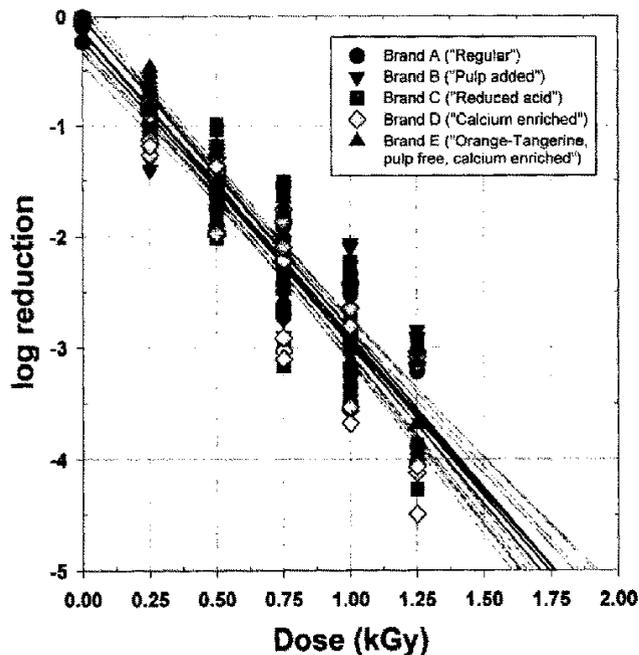


FIGURE 1. Reduction of *Salmonella Enteritidis* population in irradiated citrus juices. Lines indicate linear regression, with 95% confidence intervals.

The suspending juice type did not lead to significant differences in either the survival in nonirradiated controls (data not shown) or in the radiation sensitivity of *Salmonella Enteritidis* (Fig. 1). D_{γ} ranged from 0.35 to 0.37 kGy. There was no correlation between D_{γ} and any of the physical parameters measured, including antioxidant power, and the physical parameters did not significantly correlate with each other. Irradiated noninoculated juice did not exhibit any gross (i.e., readily apparent) changes in visual appearance or aroma.

DISCUSSION

The radiation sensitivity of pathogenic bacteria is often, although not always, influenced by the chemistry of the suspending medium, and the extent of the influence is species-dependent (15, 16). Pathogenic bacteria exhibit increased D_{γ} in solutions amended with chemical antioxidants such as formate or polyethylene glycol (6). Antioxidants can absorb the oxygen radicals produced during the irradiation process, thereby protecting the suspended bacteria and increasing D_{γ} (3). Natural variation in food composition has been shown to result in variation in D_{γ} . *Salmonella* suspended in pork showed greater radiation sensitivity than the same culture suspended in beef, lamb, or turkey meat (15), although subsequent studies with the same *Salmonella* cultures suspended in bison, ostrich, alligator, and caiman meat did not indicate substrate-dependent radiation sensitivity (16). Similarly, for bacteria suspended in formulated meat products, the nature of the specific formulation examined, including meat type or additives containing salt, fat, sugars, spices, or preservatives, may or may not influence radiation sensitivity (5, 7, 13, 15–17).

The effect of substrate composition on radiation sen-

sitivity has not been as well studied in plant products. The radiation sensitivity of *E. coli* O157:H7 suspended in apple juice varied with the level of suspended solids in several commercial brands of apple juice (3). In that study, D_{γ} ranged from 0.26 to 0.35 kGy in juices ranging in turbidity from very clear ($A_{550} \sim 0.1$) to very turbid ($A_{550} \sim 2.0$). The suggested mechanism for the effect of juice turbidity on radiation sensitivity was the contribution of antioxidant potential of the suspended solids. Studies of artificially manipulated levels of orange juice solids indicated no significant effect on antioxidant power or radiation sensitivity of four suspended *Salmonella* isolates (9). In the results presented herein, significant variation in commodity mixture (orange versus orange-tangerine), calcium amendment, antioxidant power, and pH did not correlate with any significant variation in the radiation sensitivity of an outbreak strain of *Salmonella* Enteritidis. The different responses of irradiated *Salmonella* and *E. coli* cultures to changes in juice composition highlight the complex influence of food chemistry on radiation sensitivity of suspended bacteria. It is expected that additional studies of varied bacteria-commodity combinations will continue to improve our understanding of radiation pasteurization of formulated food products.

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