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Influence of modified atmosphere and varying time in storage on the irradiation sensitivity of Salmonella on sliced roma tomatoes

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HIGHLIGHTS

- *Salmonella* radiation D_{10} increased on sliced tomatoes by MAP and time in refrigerated storage.
- Reduced oxygen caused higher D_{10} values, up to 0.335 kGy for 100% N_2.
- Refrigerated holding may alter the efficacy of irradiation if reduced oxygen MAP is used.

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ABSTRACT

Salmonella contamination of tomatoes is a recurrent food safety concern. Irradiation inactivates pathogens on fresh and fresh cut produce. However, the interaction of time in refrigerated storage and modified atmosphere packaging (MAP) may influence the response of pathogens to irradiation. Roma tomatoes were sliced and inoculated with a cocktail of outbreak strains of *Salmonella*. The inoculated tomatoes were packaged under one of four atmospheres: air, 10/90 O_2/N_2, 5/95 O_2/N_2 or 100% N_2. The packages were kept in refrigerated storage (10 °C) for various times after inoculation, to simulate the potential time delay between packaging and irradiation treatment. Tomatoes were irradiated immediately (0 h), or after 24 or 48 h in storage. The surviving populations were recovered and enumerated. Irradiation effectively reduced *Salmonella* at all times. Estimated D_{10} value (the dose necessary for 1 log reduction) varied significantly among the combinations of time and MAP, ranging from 0.165–0.335 kGy. Tomatoes packaged in air, irradiated at 0 h, had a D_{10} of 0.165 kGy; all other combinations showed significantly higher D_{10}. Reduced oxygen generally resulted in higher D_{10} values, with the highest D_{10} of 0.335 kGy obtained for 100% N_2, 0 h. Time in storage pre-irradiation tended to increase D_{10} for air and 5/95 O_2/N_2, but not for 10/90 O_2/N_2 or 100% N_2. These results suggest that time required for refrigerated holding of processed Roma tomatoes or shipment to an off-site irradiation service provider may alter the efficacy of irradiation if reduced oxygen MAP is used.

1. Introduction

Fresh produce is an identified vector for foodborne illness outbreaks (Gombas et al., 2003; Horby et al., 2003; Sivapalasingam et al., 2004). Sorting, cutting, blending, and other handling procedures for fresh and fresh-cut produce are known opportunities for contamination to occur (Doyle and Erickson, 2008). In 2004, a *Salmonella* outbreak associated with pre-sliced Roma tomatoes, sold in tray packs for use in deli-counter convenience foods, sickened more than 500 people in PA, MD, OH, WV and VA, resulting in more than 120 hospitalizations (Anon, 2004). Food safety interventions are recognized as a valuable tool for the various stages of processing and distribution (Doyle and Erickson, 2008).

Irradiation effectively reduces pathogens on a variety of fresh and fresh-cut fruits and vegetables, including intact and cut tomatoes (Fan et al., 2008; Niemira, 2007; Prakash et al., 2002; Todoriki et al., 2009). In 2008, the US FDA approved irradiation for iceberg lettuce and spinach as a food safety process (FDA (US Food and Drug Administration), 2008). Irradiation reduced *Salmonella* on diced tomatoes up to 5 logs, depending on dose (Prakash et al., 2007a). Optimization of best practices for using irradiation includes consideration of the point at which to irradiate in the supply chain – off-site at an irradiation service provider or in-line using in-house equipment. Transshipment to a third-party irradiation service provider entails time in transit, during which the product is held in an unbroken cold chain. In-line irradiation is a terminal, post-packaging

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process applied immediately after handling and packaging. Time in refrigerated storage between a contamination event and irradiation reduced the antimicrobial efficacy of irradiation for lettuce and spinach leaves contaminated with *Escherichia coli* O157:H7 (Niemira and Cooke 2012), but had no significant effect on tomato slices contaminated with *Salmonella* (Niemira, 2011).

Modified atmosphere packaging (MAP) is also known to influence pathogen response to irradiation. Cell membranes are sensitive to oxidative attack by oxygen free radicals, which can lead to undesirable sensory effects. Irradiating under reduced MAP can reduce these effects, but the same changes in the availability of oxygen radicals can also alter the efficiency of the treatment (Niemira and Deschenes, 2005). Reduced-oxygen, enhanced-CO_2 packaging led to a suppression of *Listeria monocytogenes* on irradiated endive, compared with regrowth in refrigerated storage for samples packaged in air (Niemira et al., 2004). Strawberries packaged under 7% oxygen and 20% CO_2 and irradiated at 1 kGy had reduced decay incidence and suppression of spoilage organisms (Brecht et al., 1992). Total aerobic counts under MAP were reduced by ~3 logs on iceberg lettuce (Hagenmaier and Baker, 1997) after a dose 0.19 kGy. Romaine lettuce treated with 0.35 kGy under MAP showed a reduction of total aerobic counts of ~1.5 logs (Prakash et al., 2000). In that study, the shelf life was extended by 4–8 days. Hagenmaier and Baker (1998) showed that MAP plus 0.45 kGy reduced total aerobic plate counts by ~2 logs on shredded carrots. This suppression persisted throughout a 9 day storage evaluation. Oxygen is consumed in sealed packages of sliced and chopped vegetables in sealed packages as it is consumed during respiration, with the levels sensitive to the gas permeability of the packaging material (Fan and Sokorai, 2002).

The objective of this study was to evaluate the efficacy of irradiation to remove *Salmonella* from sliced Roma tomatoes, and to determine the combined impacts of (a) reduced oxygen MAP and (b) time in refrigerated storage between a contamination event and irradiation processing.

2. Materials and methods

2.1. Microorganisms

All isolates utilized in this study were from the USDA-ARS-ERRC culture collection. Three human outbreak strains of *Salmonella* were chosen. The isolates were maintained in tryptic soy broth (TSB, Difco, Sparks, MD): *S.* Anatum F4317, *S.* Stanley H0558 and *S.* Enteritidis PT30. Fresh cultures of each isolate were grown overnight in TSB at 37 °C. The cell concentration of these individual cultures was approximately 9.0 $\log_{10}$ CFU/mL, as determined by serial dilution and plate count on TSA incubated overnight at 37 °C. Selective media has historically been problematic for irradiation studies, since it can prevent the growth on sublethally injured cells, while strains rendered antibiotic resistant can have an altered sensitivity to irradiation (Niemira 2005; Niemira and Lonczynski, 2006). For these reasons, inoculation materials were prepared so as to have a pathogen concentration which would effectively overwhelm native microflora by several orders of magnitude, so as to ensure plate counts would represent *Salmonella*. Aliquots of 15 mL were drawn from each of the three fresh cultures and combined (combined volume=45 ml) and mixed with 405 mL of sterile buffered peptone water (BPW) in a sterile container to make a 450 mL inoculation bath. The final concentration of the 1:10 dilution was approximately 8.0 $\log_{10}$ CFU/ml.

2.2. Inoculation and storage

Ripe Roma tomatoes (*Lycopersicon esculentum* cv. Roma) were obtained from local markets and held at 10 °C until the experiments. The exterior of the tomatoes were wiped with a sterile Kimwipe and a 70% ethanol solution. The tomatoes were allowed to air dry. Slices of tomato (1 cm thickness) were made using a sterile knife. The slices were transferred into the *Salmonella* inoculum bath and submerged for 1 min. The slices were withdrawn using tongs and allowed to drip away excess inoculum. The slices were allowed to dry in the airflow of a biosafety cabinet for 15 min before placement into No. 400 Stomacher bags (Tekmar, Inc., Cincinnati, OH), approximately 20 g per bag. Exact weight of tomato material per bag was recorded. The sample bags packaged in air were heat-sealed and placed into refrigerated storage. Sample bags to receive MAP were placed into a Multivac A300/16 gas packaging system (MULTIVAC, Kansas City, MO) and flushed with bottled mixtures of 10/90 O_2/N_2 or 5/95 O_2/N_2 or with 100% N_2 from house supply lines. The bags were flushed twice and heat-sealed, then transferred to a refrigerator at 10 °C for 0 h (i.e. processed immediately), 24 h or 48 h before processing. One sample was used as an untreated control for each storage time, with all treatments and controls done in triplicate in separate experiments.

2.3. Irradiation

At each storage time, separate samples were treated with 0.0 (control) 0.25, 0.50, 0.75, 1.0 or 1.5 kGy as previously described (*11*). In all cases, the irradiation was conducted at 4 °C. Temperature control was maintained during irradiation by injection of gas coming from liquid nitrogen into the sample chamber. The samples were irradiated using a Lockheed-Georgia (Marietta, GA) cesium-137 self-contained gamma radiation source, with a dose rate of 4.42 kGy/h. The dose rate was established using alanine transfer dosimeters from the National Institutes of Standards and Technology (Gaithersburg, MD). Alanine pellets (Bruker, Inc., Billerica, MA) were used for dosimetry. The pellets were read on a Bruker E-SCAN EPR analyzer (Bruker, Inc., Billerica, MA) and compared with a previously determined standard curve. Actual dose was typically within 5% of the nominal dose.

2.4. Sampling

Sterile BPW was added to each of the bags, with the precise amount adjusted based on the weight of the tomato slices, making a 5:1 ratio of total solution:tomato by weight. The bags were stomached on high (260 rpm) for 2 min. An aliquot (0.1 mL) of the fluid was drawn and serially diluted using sterile BPW. Dilutions appropriate for each treatment were plated onto aerobic count petrifilm (3 M, Forest City, IA) and incubated overnight at 37 °C. The minimum detection limit of the lowest dilution used was 5 CFU/mL. Three plates per dilution were counted on a digital cell counter.

2.5. Statistical analysis

The estimated radiation D_{10} for the *Salmonella* cocktail was calculated for each storage time and MAP combination, using the entire data set, based on the negative reciprocal of the slope for the linear regression line. The significance of differences among the D_{10} values were determined with analysis of covariance (ANCOVA) (Excel, Microsoft Corp. Redmond, WA).

3. Results and discussion

Irradiation effectively reduced the population of *Salmonella* on tomato slices at all storage times and MAP regimes tested, with reduction directly related to dose (Figs. 1–4).