Food Microbiology 72 (2018) 199–205

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

# Survival of foodborne pathogens on commercially packed table grapes under simulated refrigerated transit conditions

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ARTICLE INFO

*Article history:*
Received 1 June 2017
Received in revised form
22 November 2017
Accepted 6 December 2017
Available online 9 December 2017

*Keywords:*
*Listeria monocytogenes*
*Escherichia coli* O157:H7
*Salmonella enterica*
Sulfur dioxide-generating pads
Polyethylene box liners
Table grapes
Refrigerated transit temperature
Packaging

ABSTRACT

We examined the survival of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enterica* Thompson inoculated on commercially packed table grapes under simulated refrigerated transit conditions (1.1 ± 0.5 °C; 90% RH). Grapes were placed in perforated polyethylene cluster bags, within a commercial expanded polystyrene box equipped with either a $SO_2$-generating pad; a perforated polyethylene box liner; a $SO_2$-generating pad and a box liner; or none of them. *L. monocytogenes* was most sensitive to $SO_2$-generating pad. $SO_2$-generating pad or $SO_2$-generating pad with box liner inactivated this pathogen completely on day 12 following the inoculation. *S. enterica* Thompson displayed a similar cold sensitivity as *L. monocytogenes*, but was more resistant to $SO_2$-generating pad than *L. monocytogenes*. While $SO_2$-generating pad eliminated *S. enterica* Thompson on day 20, a combination of box liner with $SO_2$-generating pad inactivated this pathogen completely on day 13. *E. coli* O157:H7 had the highest tolerance to transit temperature and to $SO_2$-generating pad; $SO_2$-generating pad inactivated this pathogen completely on Day 20. Our data suggest that use of $SO_2$-generating pad combined with box liner is effective in reducing foodborne pathogens *L. monocytogenes* and *S. enterica* Thompson, while the use of $SO_2$-generating pad alone was more effective on *E. coli* O157:H7.

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## 1. Introduction

Table grapes are a non-climacteric fruit that is rich in phytonutrients and antioxidants (Morre and Morre, 2006; Pezzuto, 2008). In California, most table grapes are packaged in the field before storage. Stem browning due to desiccation and gray mold infections caused by the fungus *Botrytis cinerea* are the main factors that reduce table grape postharvest quality. Sulfur dioxide ($SO_2$) fumigation is an effective way to control fungal infections and to prevent decay during storage (Palou et al., 2002; Smilanick and Henson, 1992). Standard industry practice for harvested California table grapes is to perform initial $SO_2$ fumigation during pre-cooling of the grapes, followed by weekly fumigations during cold storage. To preserve quality of postharvest table grapes during long-distance export marketing or long retail handling in which $SO_2$ fumigation cannot be applied, the use of in-package $SO_2$-generating pads, which was first developed in California in the later 1960's, is a common practice by table grape industries worldwide (Gentry and Nelson, 1968; Nelson, 1983; Nelson and Ahmedullah, 1972, 1973, 1976). The $SO_2$-generating pads contain sodium metabisulfite, which reacts with moisture in the grape container, releasing gaseous $SO_2$ constantly and slowly. The perforated polyethylene liners help maintain a high humidity within the package, thus preventing water loss from table grapes. The use of the slow-release $SO_2$-generating pad in combination with a perforated polyethylene box liner was shown to reduce water loss and prevent grey mold infection without enhancing $SO_2$ phytotoxicity under California conditions (Crisosto et al., 1994). Although the effect of $SO_2$-generating pads on controlling grey mold and pests have been examined extensively (Lichter et al., 2008; Yokoyama et al., 2001), little information is available on the antimicrobial activity of $SO_2$-generating pad on foodborne pathogens.

We previously reported the fate of three common bacterial foodborne pathogens, *Listeria monocytogenes, E. coli* O157:H7, and *Salmonella enterica* Thompson inoculated on freshly harvested table grapes following the initial and weekly $SO_2$ fumigations under standard cold storage condition currently used by California table

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https://doi.org/10.1016/j.fm.2017.12.004
0740-0020/Published by Elsevier Ltd.

grape industry (Carter et al., 2015). *L. monocytogenes* was highly sensitive to $SO_2$ fumigation, whereas *E. coli* O157:H7 has the highest tolerance to $SO_2$ fumigation. Consequently, multiple weekly fumigations were required for elimination of *E. coli* O157:H7 from the artificially surface-inoculated table grapes. $SO_2$-generating pads are commonly used during long distance transportation of table grapes, during which the temperature is often maintained between 0 and 1.7 °C and the $SO_2$ concentration within the perforated liner is about 10 ppm generally (Crisosto et al., 1994; Lichter et al., 2008). To gain insight into the antimicrobial effect of $SO_2$ generated from an in-package $SO_2$-generating pad under the refrigerated transit condition, in this study we investigated the fate of these three pathogens inoculated on commercially packed table grapes stored at a refrigerated transit temperature (1.1 ± 0.5 °C) with 90% relative humidity. To reveal the impact of box liner on the bactericidal effect of $SO_2$-generating pad, the four packaging combinations included: (A) no box liner and no $SO_2$-generating pad; (B) box liner only; (C) $SO_2$-generating pad only; and (D) both box liner and $SO_2$-generating pad.

## 2. Materials and methods

### 2.1. Bacterial strains

A representative strain of *Escherichia coli* O157:H7, *Salmonella enterica* serovar Thompson, and *Listeria monocytogenes* was selected based on isolation source and virulence properties. *E. coli* O157:H7 strain H1827 is a clinical isolate linked to a lettuce-associated outbreak in 1996 in the US (Hilborn et al., 1999). *S. enterica* Thompson strain 99A2342 is a clinical isolate associated with an outbreak linked to cilantro in California (Campbell et al., 2001). *L. monocytogenes* strain 91E01275-9 serotype 1/2b was isolated from a garlic surveillance sample in California. A spontaneous rifampicin resistant mutant of *E. coli* O157:H7 strain H1827, *S. enterica* Thompson strain 99A2342, and *L. monocytogenes* strain 91E01275-9 were described previously (Brandl, 2008; Brandl and Amundson, 2008; Brandl and Mandrell, 2002; Carter et al., 2015) and used in this study.

### 2.2. Table grapes and packaging conditions

California table grapes were harvested approximately 1 day prior to inoculation and shipped overnight, packed in vented plastic bags that were placed inside a commercial expanded polystyrene box under one of the four packaging combinations: (A) without a box liner or a $SO_2$-generating pad; (B) with a box liner; (C) with a $SO_2$-generating pad; and (D) with a box liner and a $SO_2$-generating pad. The Experiment 1 was conducted with early season green grapes and the Experiment 2 was conducted with mid to late season red grapes. The $SO_2$-generating pads used in this study were slow-release (UVASYS, Grapetek (Pty) Ltd, Cape Town, South Africa), containing approximately 7 g of sodium metabisulfite. A macro-perforated box liner with 1.05% vented area (hole diameter, 4.75 mm) was used in Experiment 1, while a micro-perforated box liner with 3.5% vented area (hole diameter, 1.00 mm) were used in Experiment 2. The grapes were stored at 4 °C prior to inoculation.

### 2.3. Bacterial inoculation and in vivo survival test

Single colonies of each pathogen were inoculated into LB broth (Lennox) supplemented with rifampicin at a final concentration of 25 μg/ml and grown at 28 °C overnight on a shaker (150 rpm). Cells were collected by centrifugation at 8000 g for 3 min and washed twice in KP buffer (10 mM $K_2PO_4$, pH 7.0). On the day of the experiment, grapes were moved to a biosafety cabinet and air-dried for 30 min. Only firm and intact grapes were used for inoculation. Grape berries were placed with pedicel side down in each well of a 24-well culture plate (Corning No. 3473). A five micro liter cell suspension containing a concentration of $10^6$ cells/ml was spot-inoculated on the surface of grapes. The absolute inoculum concentration was determined by counting colony forming units (CFU) of 10-fold serial dilutions of the cell suspension. At least six biological replicates were prepared for each pathogen under each test condition (six inoculated berries per pathogen for each sampling day). Inoculated grapes were put back to their original packing boxes and stored in a cold room that was maintained at 1.1 ± 0.5 °C with 90% RH for one month. The inoculated grapes were periodically retrieved from the cold room and placed in a 50 ml conical tube containing three ml KP buffer. Cells were released from grapes to the KP solution as described previously (Carter et al., 2012, 2015). The viable cells were recovered by plating cell suspensions onto rifampicin-containing LB agar plates (50 μg/ml). To increase the detection limit, a 40-fold concentrated cell suspension was made by centrifugation of a 2 ml grape washing buffer and re-suspending the cell pellet in a 50 μl KP buffer. The resulted cell suspension was plated onto rifampicin-containing LB agar plates. Percent survival was calculated by comparing viable pathogen cells retrieved from each grape on each sampling day to that in the corresponding initial inoculum. For samples that yield zero CFUs, an enrichment procedure was used to detect any viable pathogen cells. Briefly, 1.5 ml of the grape wash solution was mixed with an equal volume of 2 x tryptic soy broth (TSB) and incubated at 37 °C over-night. The culture was then plated on rifampicin-containing LB agar plates to detect the presence of the inoculated pathogen.

### 2.4. Statistical analysis

Statistical analysis was performed with GraphPad Prism 7.0 (GraphPad Software Inc.). An unpaired *t*-test was performed for a two-group comparison, and the Two-way Analysis of Variance (ANOVA) followed by Tukey's test was performed for multiple comparisons.

## 3. Results

### 3.1. Fate of Listeria monocytogenes on packed table grapes

The survival of *L. monocytogenes* on packed table grapes under the four simulated refrigerated transit conditions were examined periodically for 23 (Experiment 1) or 22 days (Experiment 2) following the inoculation (Fig. 1). Two-way ANOVA test revealed that both storage time and packaging combination played a significant role in the survival of *L. monocytogenes* on table grapes (*in vivo* survival) (Table 1). The population of *L. monocytogenes* decreased rapidly on the grapes stored without a box liner or a $SO_2$-generating pad (Fig. 1, Packaging combination A). The viable pathogen cells retrieved from the grapes on and after two days of incubation were significantly fewer than the initial inoculum. The survival of *L. monocytogenes* was on average 0.66% and 0.48% on day 12 and day 13 in Experiment 1 and 2, respectively. No viable L. *monocytogenes* cells were detected on any grapes on day 23 in Experiment 1; whereas, in Experiment 2, five out of six grapes were negative for this pathogen on day 22.

The box liner did not alter the cold sensitivity of *L. monocytogenes* under the condition examined. There was no significant difference in *in vivo* survival of *L. monocytogenes* between the grapes stored without a box liner or a $SO_2$-generating pad and the grapes stored with a box liner only on any of sampling days (Fig. 1, Packaging combination B). The response of *L. monocytogenes* to the in-package $SO_2$-generating pad was