

## Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in Cranberry, Lemon, and Lime Juice Concentrates

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### ABSTRACT

The production of thermally concentrated fruit juices uses temperatures high enough to achieve at least a 5-log reduction of pathogenic bacteria that can occur in raw juice. However, the transportation and storage of concentrates at low temperatures prior to final packaging is a common practice in the juice industry and introduces a potential risk for postconcentration contamination with pathogenic bacteria. The present study was undertaken to evaluate the likelihood of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* surviving in cranberry, lemon, and lime juice concentrates at or above temperatures commonly used for transportation or storage of these concentrates. This study demonstrates that cranberry, lemon, and lime juice concentrates possess intrinsic antimicrobial properties that will eliminate these bacterial pathogens in the event of postconcentration recontamination. Bacterial inactivation was demonstrated under all conditions; at least 5-log *Salmonella* inactivation was consistently demonstrated at  $-23^{\circ}\text{C}$  ( $-10^{\circ}\text{F}$ ), at least 5-log *E. coli* O157:H7 inactivation was consistently demonstrated at  $-11^{\circ}\text{C}$  ( $12^{\circ}\text{F}$ ), and at least 5-log *L. monocytogenes* inactivation was consistently demonstrated at  $0^{\circ}\text{C}$  ( $32^{\circ}\text{F}$ ).

Until recently, fruit juices were not recognized as vehicles of foodborne illness because of their low pH and high organic acid levels (18, 22). However, several outbreaks associated with unpasteurized fruit juices have been reported in the last decade, and as a consequence, issues surrounding the safety of juice products started to be addressed (1). The U.S. Food and Drug Administration (FDA) proposed a hazard analysis and critical control point (HACCP) regulation that includes a performance criterion to assure juice safety (7). The regulation, commonly known as juice HACCP, became effective January 2002 and requires juice processors to use methods to achieve a 5-log<sub>10</sub> reduction of an appropriate target organism in the juice process (8).

*Escherichia coli* O157:H7 and *Salmonella* serotype Typhimurium (3, 5, 6) have been involved in foodborne outbreaks transmitted by unpasteurized apple cider. *Salmonella* (12) has been linked to outbreaks transmitted by the consumption of unpasteurized orange juice. Although *Listeria monocytogenes* has not been implicated in foodborne outbreaks associated with juice, *L. monocytogenes* has been isolated from unpasteurized apple juice and an apple-raspberry blend (21). Furthermore, acid-adapted strains of *L. monocytogenes* can survive in acidic foods (10) and become potential hazards.

Fruit juices are thermally processed into concentrate by heating to temperatures far in excess of those necessary to

destroy pathogens reasonably likely to be associated with raw juice (15). Therefore, the presence of pathogens in juice concentrates would occur from postconcentration contamination, potentially during storage or transportation. In the event that postconcentration contamination with pathogens cannot otherwise be prevented, the FDA has determined that the requirement for a 5-log reduction would apply to concentrates as well as single-strength juice (8). However, juice concentrate is not typically repasteurized because of detrimental effects to product quality.

Questions have been raised about how long pathogenic bacteria such as *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* can survive in juice concentrates. According to National Food Processors Association member companies, transportation and storage of fruit juices and juice concentrates are usually at low temperatures, generally from  $-23$  to  $0^{\circ}\text{C}$ , to preserve the quality of the juice. Studies done by the National Food Processors Association have shown that *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* are able to survive in orange, apple, pineapple, and white grape concentrates and in banana puree at  $-23^{\circ}\text{C}$  for at least 12 weeks (20).

Cranberry, lemon, and lime concentrates have intrinsic characteristics (low pH values and high titratable acidities) that create a hostile environment for bacterial growth and survival. Cranberry, lemon, and lime concentrates also contain compounds described as having antimicrobial effects (4, 16, 23). The present study is focused on evaluating the likelihood of *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes* surviving in these concentrates at or above temperatures commonly used for transportation or storage of juice concentrates.

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TABLE 1. Properties of cranberry, lemon, and lime juice concentrates used in this study

Concentrate	pH	Titrateable acidity <sup>a</sup>	°Brix <sup>b</sup>
Cranberry	2.0–2.2	10.5–13.2	45–52.6
Lemon	1.8–2.0	30.0–36.0	48–55
Lime	2.2	32.8	50

<sup>a</sup> Units are g citric acid/g juice concentrate.

<sup>b</sup> Corrected at 23°C.

MATERIALS AND METHODS

**Juice concentrates.** The concentrates were obtained from National Food Processors Association member companies at °Brix consistent with levels used during transportation. The °Brix levels of the concentrates were determined by using a refractometer (Bausch and Lomb, Rochester, N.Y.). The pH was determined with an Orion 620 pH meter (Orion Research Inc., Boston, Mass.), and the titrateable acidity (wt/wt as citric acid) was calculated by NaOH titration to a pH 8.1 endpoint.

**Bacterial strains and culture conditions.** Composites of five strains were prepared for each pathogen used in this study. The *E. coli* O157:H7 composite included the following National Food Processors Association strains: N-4064, isolated from apple cider involved in a foodborne outbreak; N-4070, isolated from apple juice from a 1996 juice outbreak; N-4072, isolated from apple cider; N-4073, isolated from apple cider from a 1996 Connecticut outbreak; and N-4087, isolated from an outbreak involving salami. The *Salmonella* composite included the following juice-isolated serotypes, the first three originally obtained from M. E. Parish, University of Florida: N-4019 (*Salmonella* Rubislaw), N-4020 (*Salmonella* Gaminara), N-4021 (*Salmonella* Hartford), N-4088 (*Salmonella* Enteritidis) isolated from orange juice, and N-4089 (*Salmonella* Muenchen) isolated from orange juice from a 1999 orange juice outbreak. The *L. monocytogenes* composite included strains N-7003 (isolated from raw milk), N-7016 (isolated from meat), N-7175 (serotype 1/2b, isolated from a meat plant environment), ATCC 19113 (serotype 3, human isolate), and ATCC 7644 (human isolate).

Strains were transferred from tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants into individual tryptic soy broth (Difco) tubes and incubated at 35°C overnight. Cells were then transferred to tryptic soy broth adjusted to pH 5 with 1 N HCl and incubated at 35°C for an additional 24 h. Final acid adaptation was achieved by resuspension of the cells in cold 10 mM citrate buffer, pH 4, and holding overnight at 4°C. Cells were composited to obtain approximately equal numbers of each strain. Each strain and composite was enumerated by serial dilution in 0.1% peptone water and spread plating on tryptic soy agar. Plates were incubated at 35°C for 24 h.

**Inoculation protocol.** For all trials at –23°C; all trials investigating *E. coli* and *Salmonella* in cranberry, lemon, and lime juice concentrates at –11°C; and the first trial for *L. monocytogenes* in lemon juice concentrate at –11°C, the juice samples were dispensed in 10-g aliquots into sterile Whirl-Pak bags (Nasco, Fort Atkinson, Wisc.) and brought to or below the target temperature (–23 or –11°C) by holding in a temperature-controlled freezer overnight prior to inoculation. In the second trial for *L. monocytogenes* in lemon juice concentrate at –11°C, in both trials in lime and cranberry at –11°C, and in the trial for *L. monocytogenes* in cranberry concentrate at 0°C, the concentrate samples were dis-

TABLE 2. Inactivation of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* at –23°C

Inoculum (log CFU/10 g) AI <sup>c</sup>	Cranberry						Lemon						Lime						
	<i>E. coli</i>		<i>Salmonella</i>		<i>Listeria</i>		<i>E. coli</i>		<i>Salmonella</i>		<i>Listeria</i>		<i>E. coli</i>		<i>Salmonella</i>		<i>Listeria</i>		
	Trial: 1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
8.1	8.0	7.8	7.7	8.0	7.7	8.1	8.0	7.8	7.9	8.0	7.9	8.1	8.0	7.8	7.7	8.0	8.9	8.0	8.9
+	–	–	+	+	+	–	+	–	+	+	+	–	+	–	–	+	+	+	+
NT <sup>b</sup>	–	NT	–	NT	–	–	–	NT	–	–	–	NT	–	–	–	–	–	–	–
1 h	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 h	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24 h	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1 week	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 weeks	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

<sup>a</sup> Sampled within 15 min after inoculation (AI).

<sup>b</sup> Not tested.

TABLE 3. Inactivation of *E. coli* O157:H7 and *Salmonella* at  $-11^{\circ}\text{C}$ ; all samples in two trials were inoculated at 6.9 log CFU per 10 g of sample

Trial:	Cranberry				Lemon				Lime			
	<i>E. coli</i>		<i>Salmonella</i>		<i>E. coli</i>		<i>Salmonella</i>		<i>E. coli</i>		<i>Salmonella</i>	
	1	2	1	2	1	2	1	2	1	2	1	2
AI <sup>a</sup>	+	+	-	-	+	+	-	-	+	+	-	-
1 h	+	NT <sup>b</sup>	-	NT	-	-	-	-	+	NT	-	NT
6 h	-	-	-	-	-	-	-	-	-	-	-	-
24 h	-	-	-	-	-	-	-	-	-	-	-	-
1 week	-	-	-	-	-	-	-	-	-	-	-	-
2 weeks	-	-	-	-	-	-	-	-	-	-	-	-

<sup>a</sup> Within 15 min after inoculation (AI).

<sup>b</sup> Not tested.

pensed in 200-ml aliquots into sterile glass bottles and brought to the target temperatures prior to inoculation. All samples were inoculated with 0.1 to 0.85 ml of a composite, to achieve a minimum of 10<sup>6</sup> CFU of each strain per sample, and then quickly returned to the target temperature. For all experiments, Dickson SX 100 temperature recorders (Dickson, Addison, Ill.) were interspersed among the samples to monitor concentrate exposure temperatures. The samples were inoculated quickly to avoid higher temperatures in the concentrates. For all trials run at  $-11^{\circ}\text{C}$  or higher, samples were stomached or agitated briefly after inoculation to achieve an even distribution of the cells. At  $-23^{\circ}\text{C}$ , concentrates were solid, preventing mixing, so they were surface inoculated. Negative controls were prepared as above using sterile citrate buffer in place of the inoculum.

**Monitoring pathogen survival.** For trials of concentrates in 10-g bags, two samples were randomly selected after inoculation (within 15 min) and at designated times up to 2 weeks. At each sampling time, 90 ml of universal preenrichment broth (UPB, Difco) was added to the sample. For trials of concentrates stored in 200-ml bottles, a 10-ml sample was drawn from the bottle and added to 90 ml UPB. For all trials investigating *L. monocytogenes* inactivation in concentrates at  $-11$  and  $0^{\circ}\text{C}$ , decimal dilutions of the samples were prepared in UPB at each sampling time; this

approach was designed to estimate log reduction achieved in the event that survivors were detected after enrichment.

In all trials, the primary dilution of concentrate into UPB was neutralized to pH 7 by aseptic addition of a predetermined volume of 3 N NaOH prior to incubation or further dilution. This neutralization step was added to avoid any artifact of technique that would result in negative samples because of a low pH during enrichment. Samples were then incubated at  $35^{\circ}\text{C}$  for 72 h. After enrichment, samples were streaked onto selective plate media. UPB is recognized for its ability to recover sublethally injured bacteria (2, 12), so the cells recovered by enrichment would be expected to grow on selective media.

Sorbitol MacConkey (Difco) and EMB (Difco) agar plates were used for detection of *E. coli* O157:H7. Xylose-lysine-desoxycholate agar (Difco) plates were used for detection of *Salmonella*, and Palcam (Difco) plates were used for *L. monocytogenes*. All plates were incubated at  $35^{\circ}\text{C}$  for 24 h. Samples were scored as positive if typical colonies of the target organism were recovered on the selective agar plate. Atypical colonies were identified by Vitek 32 (BioMérieux, Hazelwood, Mich.).

**Detection limit.** The ability to detect low numbers of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* was verified in each experiment by adding UPB to three uninoculated samples, neu-

TABLE 4. Inactivation of *Listeria monocytogenes* in cranberry, lemon, and lime concentrates at  $-11^{\circ}\text{C}$

Concen- trate	Sample inactivation	Trial 1				Trial 2				
		Inoculum <sup>a</sup>	AI <sup>b</sup>	3 h	6 h	Inoculum	AI	3 h	6 h	
Lemon	Inoculum <sup>a</sup>	6.9	5.9	4.9	3.9	2.9	6.5	5.5	4.5	3.5
	AI <sup>b</sup>	2/2 <sup>c</sup>	2/2	2/2	2/2	2/2	1/2	2/2	0/2	0/2
	3 h	0/2	0/2	0/2	0/2	0/2	0/2	1/2	1/2	0/2
	6 h	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Lime	Inoculum	6.6	5.6	4.6	3.6	3.6	6.6	5.6	4.6	3.6
	AI	2/2	2/2	2/2	1/2	2/2	2/2	2/2	2/2	1/2
	3 h	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	6 h	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Cranberry	Inoculum	6.5	5.5	4.5	3.5	3.5	6.5	5.5	4.5	3.5
	AI	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
	3 h	0/2	0/2	0/2	0/2	0/2	0/2	1/2	0/2	0/2
	6 h	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5

<sup>a</sup> Inocula (log CFU/sample) calculated from enumeration of inoculum strains and decimal dilution prior to enrichment.

<sup>b</sup> Sampled within 15 min after inoculation.

<sup>c</sup> Number of positive samples per number of samples tested.

TABLE 5. Inactivation of *Listeria monocytogenes* in cranberry juice concentrate at 0°C

Inoculum <sup>a</sup>	Trial 1			Trial 2		
	7.0	6.0	5.0	7.0	6.0	5.0
AI <sup>b</sup>	4/4 <sup>c</sup>	4/4	4/4	4/4	4/4	4/4
6 h	4/10	1/10	0/10	0/10	0/10	0/10
24 h	0/4	0/4	0/4	0/4	0/4	0/4

<sup>a</sup> Inocula (log CFU/sample) calculated from enumeration of inoculum strains and decimal dilution prior to enrichment.

<sup>b</sup> Sampled within 15 min after inoculation (AI).

<sup>c</sup> Number of positive samples per number of samples tested.

tralizing with NaOH as described above, and inoculating with the appropriate dilutions of the composites to achieve 2, 1, and 0 log CFU/sample. Samples were incubated and then plated as described above. The sample inoculated with the lowest level of cells that demonstrated growth determined the limit of detection for the method.

## RESULTS AND DISCUSSION

Juice concentrates from different sources were used in our studies. The ranges of °Brix, pH, and titratable acidity are shown in Table 1.

For each trial, the cells were adapted to pH 4 before inoculation to trigger the acid tolerance response known to enhance cell survival in acidic foods (9, 11, 13, 14, 17). In this way, we tried to reproduce a worst-case scenario, in which the product is contaminated with acid-tolerant cells.

Temperature has been shown to influence bacterial pathogen survival in juice, with cells surviving longer at lower temperatures (19). Table 2 shows the inactivation of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* at -23°C, which could be considered a worst-case storage or transportation temperature. *Salmonella* was not detectable in any of the three concentrates held at -23°C within 15 min after inoculation.

As high as 8 log CFU *E. coli* O157:H7 and *L. monocytogenes* were generally unrecoverable within 15 min and 1 h after inoculation, respectively. However, positives were detected sporadically up to 2 weeks after inoculation. Positive controls performed with these trials confirmed a limit of detection of ≤40 CFU/sample.

In the trials at -23°C, the concentrates were solid at the time of inoculation and throughout storage. Therefore, in the positive samples, it is possible that the inoculum froze at the surface of the concentrate in locations of pure ice and did not come in contact with the concentrate and its antimicrobial properties during storage. However, the majority of samples exhibited >7-log CFU inactivation of pathogen, particularly for *Salmonella*, indicating that the antimicrobial properties of the concentrates can be effective at temperatures as low as -23°C.

The concentrates were all semiliquid at -11°C, so this temperature was selected for the next round of trials. Table 3 shows the inactivation of *E. coli* O157:H7 and *Salmonella* in cranberry, lemon, and lime concentrates at -11°C. *Salmonella* inoculated at levels as high as 6.9 log CFU was undetectable in concentrates 15 min after inoculation. *E.*

*coli* O157:H7 was undetectable in lemon concentrate by 1 h after inoculation and by 6 h after inoculation in cranberry and lime concentrates. Positive controls performed concurrently with these trials indicated that the limit of detection was ≤5 CFU/sample.

During early experiments at -11°C using similarly high inoculum levels, *L. monocytogenes* survivors were detected sporadically in cranberry, lemon, and lime concentrate samples more than 24 h after inoculation (data not shown). To quantify the level of inactivation of *L. monocytogenes* in these concentrates, samples were decimally diluted prior to enrichment. Results (Table 4) show a 5-log CFU reduction in lemon and lime concentrates by 6 h after inoculation in all cases. However, *L. monocytogenes* was recovered from one cranberry concentrate sample, even though *L. monocytogenes* were not recoverable from other samples with higher levels of inocula. The positive sample detected in cranberry concentrate after 6 h in trial 1 (Table 4) was the only positive among the 20 samples tested, including samples with 10 and 100 times higher levels of inocula. Positive controls run concurrently with these trials exhibited a limit of detection of 2 CFU/sample. This sporadic positive was not reproducible, and the reason for it is unknown. At -11°C, the cranberry concentrate was semiliquid; therefore, it is possible that some ice crystals remained, encapsulating and protecting the inoculum. Consequently, a trial was performed at a higher temperature, still consistent with commercial transportation conditions.

Table 5 shows the inactivation of *L. monocytogenes* in cranberry juice concentrate at 0°C. Samples taken 6 h after inoculation showed that a 5-log CFU reduction was already achieved, despite some positive samples at inoculum levels higher than 5 log CFU/sample. In all samples taken 24 h after inoculation, *Listeria* inocula as high as 7.0 log CFU were undetectable. The limit of detection in these trials was approximately 1 CFU/sample. Whether the increased storage temperature was entirely responsible for the complete inactivation of the inoculum is unknown and worthy of further research. However, under the conditions of this study, *L. monocytogenes* were consistently and rapidly inactivated in cranberry concentrate at 0°C.

## CONCLUSIONS

This study demonstrates that cranberry, lemon, and lime juice concentrates possess intrinsic antimicrobial properties, which will eliminate these bacterial pathogens in the event of postconcentration recontamination. The sporadic positives obtained in this study at -23 and -11°C might warrant further research, but at least a 5-log reduction of *Salmonella* was consistently demonstrated at -23 and -11°C, at least a 5-log reduction of *E. coli* O157:H7 was consistently demonstrated at -11°C, and at least a 5-log reduction of *L. monocytogenes* was consistently demonstrated in cranberry concentrate at 0°C. Consequently, cranberry, lemon, and lime juice concentrates, with properties consistent with the concentrates used in this study and held at the above temperatures or greater for more than a few hours, should not need to be repasteurized to control the potential presence of these pathogens.

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