Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in Juice Concentrates

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MS 02-461: Received 13 December 2002/Accepted 4 April 2003

ABSTRACT

The survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* was studied in apple, orange, pineapple, and white grape juice concentrates and banana puree. Pouches of juice concentrate or puree were inoculated with pathogens at a level $10^3$ CFU/g and stored at $-23^\circ C$. Pathogen survival was monitored at 6 and 24 h, once a week for four consecutive weeks, and biweekly thereafter until 12 weeks. When pathogens were not detectable by direct plating, samples were enriched in universal preenrichment broth for 72 h and plated on selective media. Results showed that *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* were recoverable from all five concentrates through 12 weeks of storage at $-23^\circ C$.

Several incidents of foodborne disease have been associated with juices. *Escherichia coli* O157:H7 (4, 7, 8) and *Salmonella enterica* serotype Typhimurium (5) have been involved in foodborne outbreaks transmitted by unpasteurized apple cider. *S. enterica* serotypes Anatum, Enteritidis, Gaminara, Hartford, Muenchen, and Rubislaw have been linked to outbreaks transmitted by the consumption of unpasteurized orange juice (6, 9, 10, 17). It is known that *E. coli* O157:H7 can survive in single-strength apple juice (4, 7, 8) and fruit pulps (21), and *Salmonella* serovars have caused several outbreaks associated with unpasteurized orange juice (9, 22), but little is known about the survival of these pathogens in juice concentrates. To date, no outbreaks have been linked to juice concentrates.

The Food and Drug Administration has mandated the application of Hazard Analysis and Critical Control Point principles for the safe processing of fruit and vegetable juices. This regulation (10) became effective January 2002 for large juice processors and requires processors to include a 5-log pathogen reduction step prior to packaging for retail distribution. This pathogen reduction, often in the form of thermal processing, applies to juice concentrates under certain circumstances. Questions have been raised as to how long pathogens would survive in juice concentrates if a contamination occurred, e.g., during transportation of concentrates prior to final packaging. The goal of this study was to determine the survival of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in apple, orange, pineapple, and white grape juice concentrates and banana puree. For simplicity, banana puree is referred to as a juice concentrate in this paper.

MATERIALS AND METHODS

**Juice concentrates.** Concentrates at °Brix levels commonly produced by industry were obtained from Member companies of the National Food Processors Association. The °Brix levels of the concentrates were determined with a refractometer (Bausch and Lomb, Rochester, N.Y.), the pH with an Orion 620 pH meter (Orion Research Inc., Boston, Mass.), and the titratable acidity (% wt/ wt as citric acid) by NaOH titration to a pH 8.1 endpoint. Table 1 shows the characteristics of the juice concentrates used in the study.

**Test strains and inoculum preparation.** Five strains each of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* were acid adapted and handled as described previously (22). Each pathogen composite was prepared by combining 1 ml from each of the five strains in a sterile tube just prior to inoculation. Each strain and composite was enumerated by serial dilution in 0.1% peptone water and spread plating on tryptic soy agar and incubating at 35°C for 24 h.

**Inoculation protocol.** Each pathogen-concentrate combination was prepared and tested twice. Juice concentrates were dispensed into sterile plastic bags (Whirl-Pak, Nasco, Fort Atkinson, Wisc.) in 4.5- or 95-g aliquots and held at $-23^\circ C$ until inocula-

### TABLE 1. Characteristics of juice concentrates used in the experiments

| Juice concentrate | °Brix | pH | % titratable acidity
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Apple</td>
<td>54.9</td>
<td>3.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Banana</td>
<td>38.9</td>
<td>5.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Orange</td>
<td>63.4</td>
<td>3.7</td>
<td>3.8</td>
</tr>
<tr>
<td>Pineapple</td>
<td>59.8</td>
<td>3.6</td>
<td>3.2</td>
</tr>
<tr>
<td>White grape</td>
<td>66.2</td>
<td>3.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

a Corrected at 23°C.
b Calculated as g citric acid/g sample.
Each bag was inoculated at 10% (vol/vol) with a pathogen composite to achieve $10^3$ to $10^4$ CFU/g (i.e., $\geq 10^5$ CFU/100 g concentrate). Negative controls were prepared with sterile citrate buffer. Inoculated samples were stomached briefly. At $-23^\circ$C, concentrates were solid, which might have hindered homogeneous distribution of the cells. After inoculation, samples were quickly returned to $-23^\circ$C and kept at this temperature for the duration of the experiment.

**Monitoring of survival.** Each pathogen-concentrate combination was monitored for surviving cells by randomly drawing two 5-g samples for direct enumeration within 15 min after inoculation at 6 and 24 h, once a week for four consecutive weeks, and biweekly thereafter until 12 weeks. At 12 weeks, one 100-g sample was also tested. At each sampling point, the entire sample was tested to account for potential heterogeneous distribution of the inoculum.

Samples were serially diluted in universal preenrichment broth (UPB, Difco Laboratories, Detroit, Mich.) and spread plated in duplicate on tryptic soy agar and selective media. Sorbitol MacConkey (Difco) agar plates and EMB (Difco) were used for *E. coli* O157:H7; Palcam (Difco) plates were used for *L. monocytogenes*; and xylose-lysine-desoxycholate agar (Difco) plates were used for *Salmonella*. All plates were incubated at 35°C for...
FIGURE 2. Survival of E. coli O157:H7 inoculated at a lower level in juice concentrates and held at -23°C.

24 h. The initial UPB dilution was also incubated (35°C for 72 h) to detect pathogen survival if negative results were obtained from direct plating. After incubation, UPB enrichments were streaked on the selective agar appropriate to the pathogen inoculated in the sample.

Identification of isolates. Isolates were identified by growth on selective plating media. If the target organism was not recovered directly on selective media, presumptive isolates on tryptic soy agar were transferred to selective plating media and incubated at 35°C for 24 h. Gram staining and Vitek 32 (bioMérieux, Hazelwood, Mo.) were used for further confirmation of isolates if growth on selective plates was atypical.

RESULTS AND DISCUSSION

Our research was aimed at determining the potential for survival of E. coli O157:H7, L. monocytogenes, and Salmonella in different juice concentrates. Juices have not been implicated in any listeriosis outbreak. However, we included L. monocytogenes in our studies because it has been isolated from unpasteurized apple juice and because it can survive in acidic foods (13, 25).

Pathogens in citrate buffer were adapted to trigger the acid tolerance response that is known to confer enhanced cell survival to E. coli O157:H7, Listeria, and Salmonella in our studies because it has been isolated from unpasteurized apple juice and because it can survive in acidic foods (13, 25).

The temperature at which samples were stored (~23°C) was determined by surveying the National Food Processors Association’s Member companies and represents the lowest temperature typically used during transportation of juice concentrates.

Our choice of UPB as the enrichment broth was based on the ability of this medium to recover sublethally injured bacteria (3). A recent study reported that orange juice samples enriched with UPB yielded better recovery of inoculated Salmonella serovars when compared to samples enriched with lactose broth (15).

Enrichment of samples was needed for the recovery of L. monocytogenes in banana as soon as 6 h after inoculation and for the duration of the study. Enrichment was also needed for the recovery of Salmonella in orange and pineapple 12 weeks after inoculation and in white grape 10 weeks after inoculation and for the recovery of E. coli O157:H7 in pineapple and white grape concentrates 12 weeks after inoculation.

E. coli O157:H7 survived through 12 weeks at detectable levels in apple, banana, orange, pineapple, and white grape concentrates stored at -23°C (Fig. 1). Previous research with orange juice concentrate stored at -4°F has shown that E. coli can be detected at levels of 10^3 CFU/100 ml, 147 d after inoculating with 10^7 CFU/100 ml of juice (19). These results are not unexpected, considering other bacteria, such as Aerobacter spp., Klebsiella pneumoniae, and Streptococcus spp. have been isolated from frozen orange juice concentrate (12, 19, 24, 26).

In general, Salmonella did not survive in concentrates as well as E. coli O157:H7. However, Salmonella still survived for at least 12 weeks at detectable levels (Fig. 1). Reports suggest that S. enterica serovar Enteritidis can survive for 90 d in passion fruit nectar (pH 2.8 to 3.2) stored at -20°C (1, 2). However, other research has reported that, in orange juice stored at pH 3.8 and 0°C, S. enterica serovars Gaminara, Hartford, Rubislaw, and Typhimurium declined from 10^6 CFU/ml to below detection level (<0.2
most probable number/ml) at 24, 45, 39, and 29 d, respectively (23).

In all concentrates but banana, L. monocytogenes generally survived at higher levels than Salmonella or E. coli O157:H7 (Fig. 1). In banana concentrate, L. monocytogenes dropped quickly to levels that required enrichment of the samples for recovery and remained essentially at the same low level throughout the 12 weeks (Fig. 1b). The banana concentrate used in this study was not acidified, nor did it contain added preservatives.

Pathogen recoveries were highly variable between runs and between samples of the same run, as indicated by the bars in Figure 1. Some samples required enrichment for detection, whereas others remained at countable levels in duplicate samples. However, none of the pathogens were eliminated during storage in these juice concentrates under the conditions of the study.

To determine whether these juice concentrates might have a more lethal effect on lower levels of bacteria, an experiment was performed in which E. coli O157:H7 was inoculated at 1.95 log CFU/g of juice concentrate. Although E. coli O157:H7 showed a fast decline in some juice concentrates, survivors were still recoverable from all five concentrates 4 weeks after inoculation (Fig. 2).

Storage temperature could be critical for the survival of bacteria in juices and concentrates. Aea and Bushnell (2) found that passion fruit nectar at room temperature has a lethal effect for E. coli and Salmonella inoculated at levels of 10^3 to 10^5 CFU/ml of nectar. Two hours after inoculation, bacteria could not be detected in plating media or samples enriched in tetrahionate broth. Although bacteria were quickly inactivated at room temperature, passion fruit nectar held at −20°C allowed S. enterica serovar Enteritidis to survive at detectable levels for 90 d. Furthermore, bacteria could persist for 1 year or longer in nectar stored at −20°C (1). Although higher temperatures might enhance the lethality of juice concentrates to pathogens, the practicality of storage at such higher temperatures would need to be considered.

ACKNOWLEDGMENT

The authors thank Sandra Arze for her technical assistance.

REFERENCES