Fate of Bacterial Pathogens and Indicator Organisms in Liquid Sweeteners

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ABSTRACT

The survival of pathogenic and indicator microorganisms in liquid sweeteners was studied. Seven sweeteners—liquid sucrose, 42% high-fructose corn syrup (HFCS), 55% HFCS, 25 DE (dextrose equivalent) corn syrup (CS), 36 DE CS, 63 DE CS, 50% medium invert sucrose, and 65% high-maltose corn syrup (HMCS) were inoculated with Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and coliforms at a level of 10⁵ cells per g. The inoculated products were stored both at or near their normal holding temperatures (32 to 46°C) and at 26.7°C (the lower limit during transportation). In most of the products the number of microorganisms fell below the detection limit in less than 3 days when the sweeteners were stored at their normal holding temperatures. However, in liquid sucrose S. aureus survived up to 2 weeks. When the products were stored at 26.7°C, the reduction in the number of microorganisms occurred at a slower rate. At 26.7°C the fastest rates of reduction were observed in 42 and 55% HFCS and in 50% medium invert sucrose. In these products the number of bacteria fell below the detection limit in 3 to 6 days. The slowest rate of the reduction was observed in the liquid sucrose, in which S. aureus survived up to 1 month. These results indicate that incidental contamination of liquid sweeteners with microbial pathogens will not present a public health or regulatory hazard.

Over the past several decades, a great deal has been learned about the spoilage of liquid sweeteners and how it can be controlled. The physical and chemical attributes, e.g., low water activity and low pH, of liquid sweeteners dictate the organisms of concern. The minimum water activity and pH requirements for most of the spoilage and pathogenic bacteria are 0.9 and 4.0, respectively (1). In contrast to bacteria, xerophilic molds and osmophilic yeasts are able to grow at lower water activity and pH values and are therefore more likely to cause spoilage problems in liquid sweeteners (4). They are able to grow in products with water activity values as low as 0.6 and pH values as low as 1.5 to 2.0 (1). Thus, some liquid sweeteners with water activity values between 0.6 to 0.8 may support the growth of osmophilic yeasts and xerophilic molds, but not pathogenic microorganisms of public health concern; organisms such as Salmonella spp., Staphylococcus aureus, Listeria monocytogenes, or Pseudomonas spp.

Nevertheless, in recent years, corn sweetener customers have requested product testing to demonstrate the absence of specific pathogens. Finished product testing is not a realistic way to assure the absence of any organism in a shipment of product. An effective way to provide such assurance is to have in place a hazard analysis critical control point (HACCP) system designed to eliminate the hazard of potential pathogen contamination. HACCP systems are based on prevention of hazards through process controls and product design. There are usually critical control points (CCPs) in the process that assure the destruction of bacterial pathogens, should any be present. Even with HACCP systems in place, there still remains a remote possibility that contamination of the finished sweeteners by pathogens could occur during loadout, after the final critical control point. The incidental contamination of corn sweeteners with microbial pathogens has been a concern for the corn milling industry. Work done previously by Marceau (3) showed that when Salmonella spp. and Escherichia coli were inoculated into liquid sweeteners, there was complete kill of these pathogens at the end of 2 weeks. However, an all-encompassing evaluation of liquid sweeteners and pathogens has not been made; therefore there is not a thorough understanding of the safety and stability of these products. The objectives of the present study were to determine the fate of microbial pathogens and indicator microorganisms in liquid sweeteners and to validate the safety of liquid sweeteners.

MATERIALS AND METHODS

Properties of liquid sweeteners. The physical and chemical properties of each product were determined according to the following procedures:

Sulfur dioxide measurement, titratable acidity, and percent solids were determined following the method of the Corn Refiners Association (2).

Water activity (a_w) of each sample was measured both at normal holding temperature and at 26.7°C using an Aqua Lab water activity instrument model CX2 (Decagon, Pullman, WA).

For pH determination each product was diluted 1:1 with warm distilled water. The pH was determined by immersing an electrode from a standardized pH meter into the sample. The sample was...
RESULTS AND DISCUSSION

The reduction in numbers of Salmonella spp., E. coli, coliforms, P. aeruginosa, L. monocytogenes, and S. aureus in 42% HFCS fell below the detection limit (<10 CFU/g) from an initial level of approximately $10^4$ to $10^5$ cells per g within 3 days regardless of the storage temperature (Fig. 1). The complete reduction of these pathogens in the 55% HFCS also occurred within 3 days when the product was held at 38°C and at 26.7°C, with the exception of E. coli at 26.7°C, which fell below the detection limit in 5 days (Fig. 2). When the 25 DE CS was held at or near its normal storage temperature (Fig. 3A), the microorganisms fell below the detectable limit within 3 days. The pathogens and indicator organisms were able to survive slightly longer in the 25 DE CS at the lower temperature. Populations fell below the detectable limit in 4 days for Pseudomonas, within 7 days for S. aureus, within 11 days for Salmonella spp., L. monocytogenes, and E. coli, and in 15 days for the coliform strains (Fig 3). Figure 4 shows the fate of the microorganisms in 36 DE CS. At the product's normal storage temperature, the microorganisms died off within 3 days (Fig. 4A). At 26.7°C (Fig. 4B) die-off was more gradual: Salmonella spp. and P. aeruginosa died off in 7 days while the remainder of the organisms fell below the detectable limit within 11 days. As was observed for the other products, the microorganisms all died off within 3 days in the 63 DE CS when held at the upper temperature (Fig. 5A). When the 63 DE CS was held at the lower temperature (Fig. 5B), the reduction of the microbial populations was slower. L. monocytogenes and P. aeruginosa died off in 11 days, the E. coli and Salmonella spp. in 15 days, and the S. aureus and coliforms in 19 days. Figure 6 illustrates the

**FIGURE 1. Fate of microbial pathogens in 42% HFCS stored at: (A) 32°C. (B) 26.7°C. Symbols: ●, Salmonella; ○, E. coli; □, coliform; *, Pseudomonas; ●, L. monocytogenes; ○, S. aureus.
die-off of the organisms in the 50% medium invert sucrose. When the product was stored at 46°C (Fig. 6A), all of the organisms fell below 10 CFU/g within 4 days, with the exception of S. aureus, which fell below the detectable limit in 15 days. At 26.7°C (Fig. 6B) L. monocytogenes, S. aureus, and P. aeruginosa reached levels below 10 CFU/g within 3 days, Salmonella spp. within 4 days, and E. coli and coliforms within 6 days. Generally, the organisms were able to survive for a longer time in the liquid sucrose at both temperatures. At 38°C (Fig. 7A) P. aeruginosa, Salmonella spp., and E. coli fell below the detectable limit in 3 days and L. monocytogenes in 6 days. S. aureus was able to survive for a longer period, falling below the detectable limit in 15 days.
Enrichment of the products was performed after the number of microbial pathogens fell below the detection limit. None of the microbial pathogens were recovered from any of the enriched samples, except those from 50% MIS and liquid sucrose in which S. aureus was detected. For these products the enrichment step was repeated after 15 days of storage, and S. aureus was not recovered.

Chemical properties of the liquid sweeteners used in this study were determined (Table 1). The water activity values ranged from 0.664 for 55% HFCS to 0.851 for liquid sucrose, and the pH values ranged from 3.74 for the 25 DE CS to 5.20 for the 63 DE CS. Pathogenic and indicator microorganisms used in this study generally have minimum requirements of water activity values of 0.9 and pH values of 4.0 for their survival and growth, except S. aureus, which is able to survive and grow at water activity values of 0.86. Considering that liquid sucrose has a water activity of 0.851 and pH of 4.2, it is not surprising to find that S. aureus could survive in the liquid sucrose for a longer time than the other bacteria.

Our results are in agreement with those of Marceau (3). In his work, HMCS, high-conversion CS, regular-conversion CS, extra-low-DE CS, low-DE CS, and liquid dextrose were inoculated with Salmonella spp. at a level of $6 \times 10^6$ cells per g. Marceau (3) reported that three of the products (extra-low-DE CS, low-DE CS, and liquid dextrose) were negative for Salmonella spp. after 24 h of storage at 38°C, and three (HMCS, high-conversion CS, and regular-conversion CS) were negative after 48 h.

The inoculation level used in this research ($10^4$ to $10^5$ cells per g) is not typical of the level of contamination which might occur, but rather represents a worst case and also helps to generate a death curve to demonstrate the anticipated die-off of the bacteria in each product. Typical contamination, if it occurs, may actually be less than 1 cell per g of product. Thus, under normal commercial conditions, complete die-off may occur within hours rather than days.

Overall the fastest reductions occurred in 42% HFCS, in 55% HFCS, and in 50% MIS, in which the levels of all microorganisms fell below the detection limit (<10 CFU/g) from an initial inoculation level of approximately $10^4$ to $10^5$.

### Table 1. Properties of liquid sweeteners used

<table>
<thead>
<tr>
<th>Sample(^a)</th>
<th>% solids</th>
<th>Water activity ($a_w$)</th>
<th>Titratable acidity, meq</th>
<th>SO₂ ppm</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>42% HFCS</td>
<td>71.00</td>
<td>0.755</td>
<td>0.00</td>
<td>0.96</td>
<td>3.88</td>
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<tr>
<td>55% HFCS</td>
<td>77.00</td>
<td>0.664</td>
<td>0.00</td>
<td>0.08</td>
<td>4.41</td>
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<tr>
<td>25 DE CS</td>
<td>78.20</td>
<td>0.738</td>
<td>Negl.(^b)</td>
<td>20.32</td>
<td>3.74</td>
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<tr>
<td>36 DE CS</td>
<td>80.40</td>
<td>0.700</td>
<td>0.00</td>
<td>23.50</td>
<td>4.97</td>
</tr>
<tr>
<td>63 DE CS</td>
<td>80.90</td>
<td>0.586</td>
<td>0.00</td>
<td>32.20</td>
<td>5.20</td>
</tr>
<tr>
<td>65% HMCS</td>
<td>81.00</td>
<td>0.693</td>
<td>0.00</td>
<td>0.05</td>
<td>4.95</td>
</tr>
<tr>
<td>50% MIS</td>
<td>77.30</td>
<td>0.683</td>
<td>Negl.(^b)</td>
<td>0.48</td>
<td>5.03</td>
</tr>
<tr>
<td>Liquid sucrose</td>
<td>66.90</td>
<td>0.851</td>
<td>Negl.(^b)</td>
<td>0.16</td>
<td>4.16</td>
</tr>
</tbody>
</table>

\(^a\) HFCS = high-fructose corn syrup; DE = dextrose equivalent; CS = corn syrup; HMCS = high-maltose corn syrup; MIS = medium invert sugar.

\(^b\) Negl. = negligible amount.
cells per g within 3 to 6 days. In addition to pH and $a_w$, the rapid death of the microorganisms can be attributed to osmotic pressure. The high-fructose corn syrups and the invert sucrose syrup possess higher amounts of monosaccharides than the other sweeteners, and thus a higher osmotic pressure, even though the total solids are lower, making them less susceptible to bacterial growth (5). In general, all the microorganisms could survive for a longer period of time when the products were stored at 26.7°C instead of their normal holding temperatures. For products kept at 26.7°C, the complete death of microorganisms occurred within 3 to 4 weeks. It was also observed that the rate of death is faster in liquid corn sweeteners than in liquid sucrose sweeteners.

This research shows that all of the bacterial pathogens and indicator organisms tested not only are incapable of growth but also die off to undetectable levels, whether the sweeteners are held at their normal commercial temperature or at a lower temperature more favorable to the microorganisms. Hence, incidental contamination of these syrups with microbial pathogens will not present a public health or regulatory hazard.

The results of this research can be used to support the hazard analysis process of those companies who develop HACCP plans for products that contain liquid sweeteners. Because the liquid sweeteners cause the destruction of vegetative pathogens, these ingredients can be considered to be free of microbiological hazards.

ACKNOWLEDGMENTS

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REFERENCES