Effectiveness of Acidic Calcium Sulfate as an Inhibitor of *Escherichia coli* 0157:H7 in Apple Cider

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ABSTRACT

Acidic calcium sulfate (trade name pHresh 5.0) produced by pHresh Technologies was examined for antimicrobial effects against *Escherichia coli* 0157:H7 in apple cider (*Malus domestica* cv Red Delicious). The cider was initially determined to be free of *E. coli* 0157:H7 by PCR using the Qualicon BAX System. Triplicate samples of pasteurized and non-pasteurized apple cider were then adjusted to pH values of 3.0, 3.2 and 3.5 using acidic calcium sulfate. Once the pH values were adjusted, the cider samples were inoculated with a five strain cocktail of *E. coli* 0157:H7. Untreated cider was also inoculated to serve as a control. The non-pasteurized apple cider was sampled from days 0 to 5 at 12 hour increments for the first 2 days and then every 24 hours until day 5. Pasteurized cider was sampled from days 0 to 9 at 12 hour increments for the first 3 days and then every 48 hours for the next 6 days. Both types of cider were plated on Sorbitol MacConkey agar to enumerate the *E. coli* 0157:H7. Acidic calcium sulfate was shown to inhibit *E. coli* O157:H7 in both non-pasteurized and pasteurized apple cider by reducing their levels by at least 3 logs over the sampling period. This experiment demonstrates that acidic calcium sulfate could potentially provide another hurdle against the growth of *E. coli* O157:H7 in apple cider due to its ability to lower the pH without negatively affecting sensory attributes.

INTRODUCTION

Due to the low infectious dose and severity of illnesses caused by *E. coli* 0157:H7, it is of high importance to control this organism in the food system. There have been several outbreaks of food poisoning caused by *E. coli* 0157:H7 in apple cider (1). Even with the low pH of cider, *E. coli* 0157:H7 has still been able to survive and cause infection (2).

Acidic calcium sulfate has previously been shown to prevent the outgrowth of *Listeria monocytogenes*, *Salmonella* serotype Enteritidis, and *Staphylococcus aureus* (3). Due to the antimicrobial properties of this acidulant, the main objective of this experiment was to determine the ability of acidic calcium sulfate to control *E. coli* 0157:H7 in apple cider.

pHresh INFORMATION

- Liquid acidifier with little or no acidic taste, colorless, and odorless
- Prevents enzymatic browning of fruits and vegetables
- Wide range of product applications including, sauces, dressings, bakery items, beverages, or any ready to eat foods
- Allows preservation of high moisture products due to its antimicrobial properties
- FDA approved with generally recognized as safe status

MATERIALS AND METHODS

Apple Cider: Freshly pressed, unfiltered red delicious apple cider was obtained from a local Nebraska producer (Diagram 1). The cider was frozen until the time of the experiment. Before the experiment, half of the cider was pasteurized and the remaining portion was left un-pasteurized.

***E. coli* 0157:H7 Inoculum:** A five strain cocktail of *E. coli* 0157:H7 was prepared using streptomycin and acid resistant strains along with one that was isolated in human feces from an outbreak. The steps to prepare the inoculum are outlined in the Diagram 2.

Cider Treatments: Flasks sanitized with potassium metabisulfite were filled with 1.5L of cider. Three flasks were treated with pHresh 5.0 and adjusted to a pH value of 3.0, 3.2 or 3.5 and one was left untreated to serve as the control. To adjust the cider to pH 3.0 it took 7.0mL of pHresh, pH 3.2 required 5.7mL and 3.5 required 4.0mL. Malic acid was also used to adjust one flask to pH 3.0 which required 25g of the acid.

Inoculation and Enumeration: Each cider treatment was inoculated with 3mL of the *E. coli* 0157:H7 cocktail which resulted in a final count of 10^7/ml. Survival of the *E. coli* 0157:H7 was monitored by enumeration using the standard plate count method on Sorbitol MacConkey agar.

Informal Sensory Panel: Two informal sensory panels were conducted on the same day with six panelists in the morning and seven in the afternoon. Panelists evaluated cider samples that contained either acidic calcium sulfate or malic acid at pH 3.0 or 3.5. Panelists were asked to fill out a form for each sample and assign a number value representing their evaluation on a scale ranging from 1 to 10 with 10 being highly preferred.

Diagram 1: Process Overview

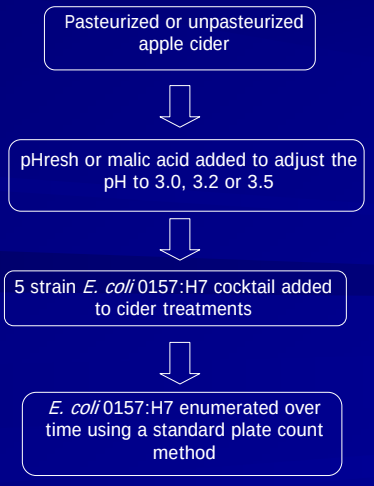
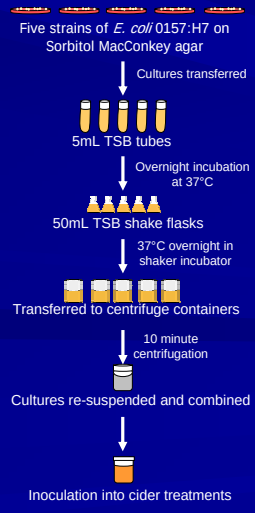


Diagram 2: Inoculum Preparation



RESULTS

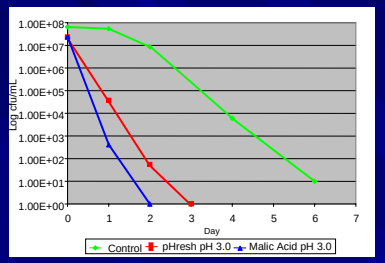


Figure 1. Survival of *Escherichia coli* 0157:H7 Over Time in non-pasteurized Apple Cider

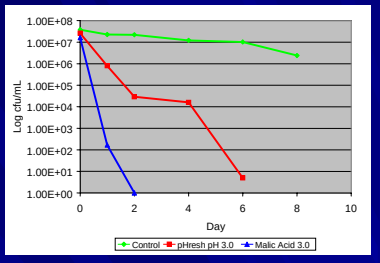


Figure 2. Survival of *Escherichia coli* 0157:H7 Over Time in Pasteurized Apple Cider

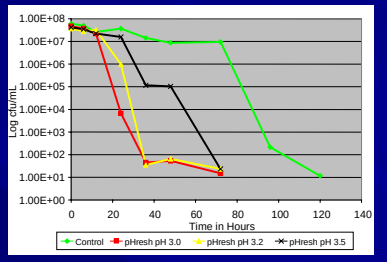


Figure 3. Survival of *Escherichia coli* 0157:H7 Over Time in non-pasteurized Apple Cider

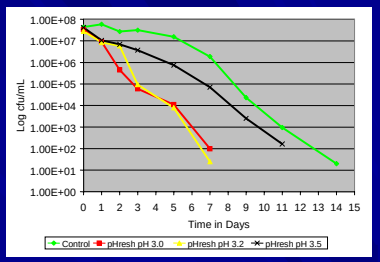


Figure 4. Survival of *Escherichia coli* 0157:H7 Over Time in Pasteurized Apple Cider

	Overall Acceptability (AM)	Overall Acceptability (PM)
Control	5.3	4.2
pHresh pH 3.5	3.8	3.7
pHresh pH 3.0	5.8	6.0
Malic Acid pH 3.5	3.7	3.6
Malic Acid pH 3.0	1.7	1.3

Table 1. Informal Sensory Panel Overall Acceptability Ratings of Cider Samples

CONCLUSIONS

Acidic calcium sulfate and malic acid were most effective in the unpasteurized juice samples. This may be attributed to thermal pasteurization reducing some of the natural antimicrobials present in the juice by precipitating and denaturing proteins, complexing tannins and minerals, and removing anthocyanin derivative compounds.

The difference in reduction times caused by the two acidulants may be attributed to their titratable acidities. Malic acid is a stronger acid and it created a higher titratable acidity causing the faster reduction time.

Adjusting apple cider samples to low pH values using acidic calcium sulfate caused significant reductions in the amount of *E. coli* 0157:H7, however, it should be used in combination with pasteurization to ensure five log reduction levels are met.

Malic acid was able to cause a reduction in the amount of *E. coli* 0157:H7, however, the addition of malic acid would not be practical due to its bitter taste and unacceptability to consumers.

REFERENCES

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