



RESEARCH FOCUS

Back-sweetened Wine and Apple Cider Inhibit Foodborne Pathogens

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Gibney lab working on yeast at Stocking Hall, Ithaca, NY. From left to right: Alisha G. Lewis, Glycine Zhujun Jiang, Anqi Chen.

KEY CONCEPTS

- Wine and hard apple cider are bactericidal due to their low pH and alcohol content.
- Foodborne pathogens can be introduced into back-sweetened alcoholic beverages, posing a health risk to the consumers.
- Pathogen die-off times need to be established in the back-sweetened wine and hard apple cider.
- Model back-sweetened wines were made from white grape juice and apple juice in this study.
- We measured survival of *E. coli*, *Salmonella enterica*, and *Listeria monocytogenes* in the model back-sweetened wines under different conditions for 96 hours.
- Holding times to achieve 5-log reduction of *E. coli* ranged from 6-24 hours (at juice pH =3) ,12-72 hours (at Juice pH=4), and 72-96 h (at juice pH=5).
- Higher alcohol content and lower pH reduced the amount of time needed to achieve a 5-log reduction in all pathogens.

Wine and alcoholic apple cider are commonly back-sweetened with unpasteurized juice, which could be contaminated with foodborne pathogens. Currently, there are few additional control measures taken by the industry to ensure the safety of back-sweetened alcoholic beverages. In this study, we evaluated the survival of three common foodborne pathogens – *E. coli*, *Salmonella enterica* and *Listeria monocytogenes* in model back-sweetened wines and alcoholic apple ciders. We identified safe holding times to ensure 5-log reduction of pertinent pathogens, which could inform federal guidelines on the safety of back-sweetened alcoholic beverages.

Table 1. Time to reach at least 5-log reduction^a for three foodborne pathogens under different combinations of pH and ethanol content in modified white grape and apple juice.

Juice type ^b	pH	Ethanol content	Time to reach at least 5-log reduction (hours)		
			<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>
Grape	3	0%	96	48	72
Grape	3	7%	24	6	24
Grape	3	10%	12	6	6
Grape	3	12%	6	2	6
Grape	3	14%	2	2	2
Grape	4	10%	72	72	72
Grape	4	12%	36	24	36
Grape	4	14%	12	12	24
Grape	5	12%	96	72	96
Grape	5	14%	72	48	48
Apple	3.2	0%	72	72	72
Apple	3.2	3.2%	48	36	48
Apple	3.2	5%	36	36	36
Apple	3.2	7%	24	12	36
Apple	3.2	8.5%	12	6	12
Apple	3.7	7%	\ ^c	96	\ ^c
Apple	3.7	8.5%	96	72	96

^a The log reduction of pathogen load at these time points is significantly larger than 5 ($p < 0.05$) according to student's t-test at 95% confidence level.

^b Modified conditions that are not mentioned here did not achieve at least 5-log reduction compared to time 0 in 96 hours.

^c "\^c" denotes that no time points within 96 h achieved at least 5-log reduction compared to time 0.

ter meets the desire of the consumers, and is also allowed within the scope of 27 C.F.R. § 24.332 (2020).

Three foodborne pathogens can contaminate back-sweetened wine and apple cider

Unpasteurized juices are considered fresh, natural, and nutritious. They are popular choices for back-sweetening, especially for small cidermaking operations that lack pasteurization equipment. However, back-sweetening with unpasteurized juices presents health risks from potential foodborne pathogens. The foodborne pathogen that is commonly associated with fruit juices is *Escherichia coli* O157:H7, which could be introduced through raw fruit that comes into contact with fecal material, or contaminated equipment during processing. *E. coli* O157:H7 can withstand low pH conditions of apple juice and grape juice, and pathogenic strains

Back-sweetening is used in the production of sweet wine and apple cider

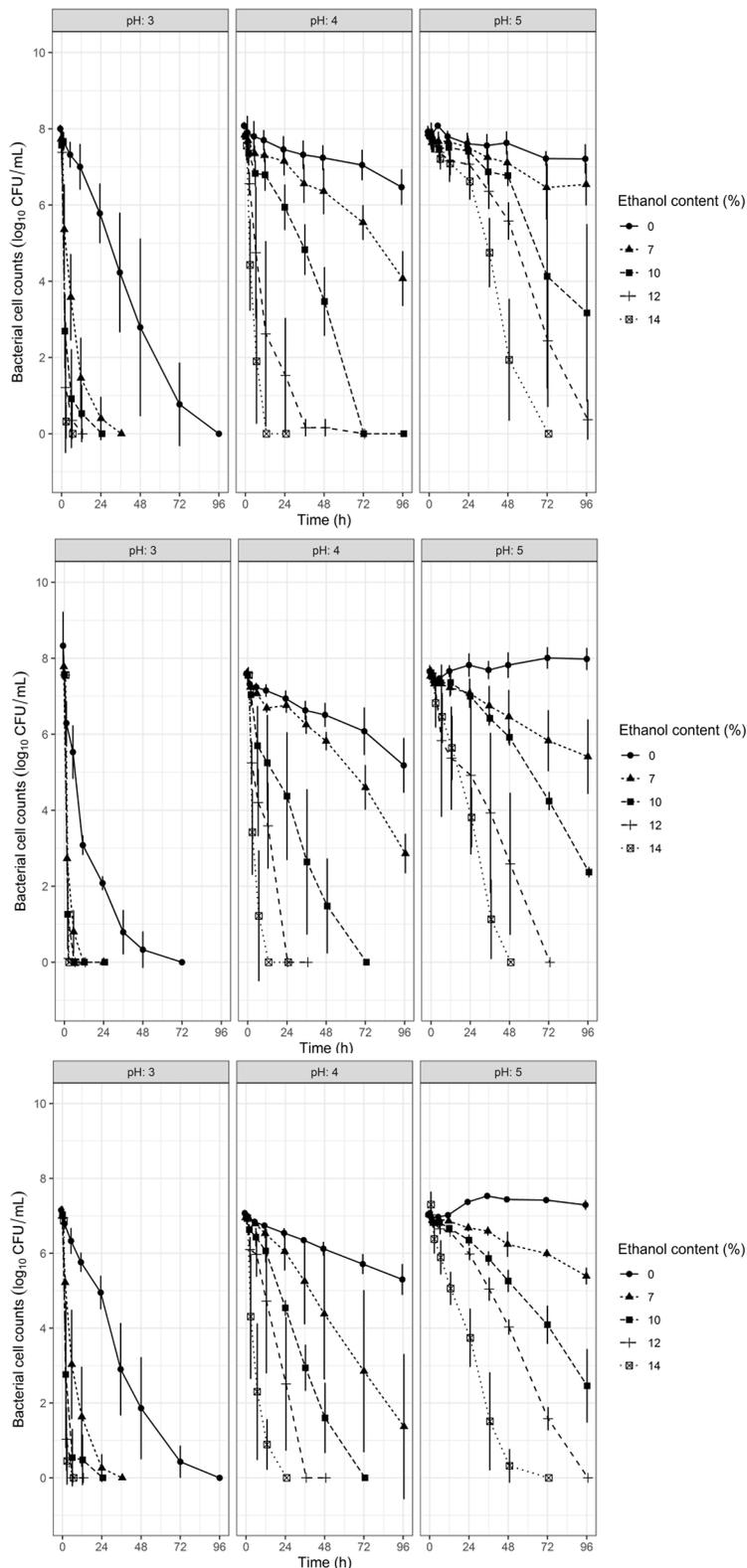
There are two general methods for producing wine with residual sugar: 1. Arrest the fermentation before all of the sugar has been converted to alcohol and carbon dioxide; or 2. Add a sweetener such as sugar or unfermented juice (MacNeil, 2015) after the fermentation has completed. This latter method is commonly referred to as "back-sweetening". According to the code of federal regulations (Title 27, section 24.179), sugar, juice or concentrated juices from the same kind of fruits may be added after fermentation to sweeten wine, however the juice used to sweeten the wine must comply with FDA Juice HACCP regulations (TTB 2020).

Similar procedures are also common in another popular alcoholic beverage – apple cider. Apple cider in this article refers to the alcoholic, fermented apple juice, commonly termed hard cider in the US market. Hard cider production is relatively small in the US compared to other alcoholic beverages, but has grown significantly over the last few years (Miles et al., 2020). Back-sweetening apple cider with sugar or juices can create a product that bet-

like Shiga toxin-producing *E. coli* can cause diarrhea, abdominal cramps and other gastrointestinal symptoms (Centers for Disease Control and Prevention, 2017). Several outbreaks of food-borne illness resulting from consumption of unpasteurized or improperly pasteurized apple juice and unfermented apple cider have been reported (Cody et al., 1999, Vojdani et al., 2008).

Two additional pathogens of concern that can survive in juices are *Salmonella enterica* and *Listeria monocytogenes*. *S. enterica* can be introduced to the juice through fecal and water contamination. The symptoms of *Salmonella* infection include diarrhea, fever and stomach cramps. Most infections are self-limiting, but severe illness can occur, especially in immunocompromised groups (Centers for Disease Control and Prevention, 2020b). Outbreaks associated with orange juice and apple cider contaminated with *S. enterica* serovar Typhimurium have also been documented (Centers for Disease Control and Prevention, 1975; Jain et al., 2009; Mihajlovic et al., 2013).

Listeria (caused by *L. monocytogenes*) is known to be cold-tolerant and acid-tolerant. Although no previous outbreaks in juice products were associated with *L. mono-*



Figures 1-3. Top: The survival curve of *E. coli* O157:H7 in modified white grape juice in 96 hours. Bacterial cell counts were recorded at time 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h. Error bars indicate standard deviation, $n = 3$. Middle: The survival curve of *Salmonella enterica* in modified white grape juice in 96 hours. Bacterial cell counts were recorded at time 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h. Error bars indicate standard deviation, $n = 3$. Bottom: The survival curve of *Listeria monocytogenes* in modified white grape juice in 96 hours. Bacterial cell counts were recorded at time 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h. Error bars indicate standard deviation, $n = 3$.

cytogenes, it is possible that fruit juices could be contaminated via infected soil or the processing environment. *L. monocytogenes* causes listeriosis with a variety of symptoms including fever, headache, and muscle aches. Pregnant women, older adults, and people with weakened immunity are more susceptible to *L. monocytogenes* foodborne illness (Centers for Disease Control and Prevention, 2020a). All three pathogens could be introduced into finished wine or apple cider through the use of unpasteurized juices, especially by commercial and home brewers who do not follow Good Manufacturing Practices during the back-sweetening process. Foodborne pathogens in these back-sweetened beverages could present a serious health risk to consumers.

Sufficient kill of foodborne pathogens needs to be validated for back-sweetening

Currently, few or no additional measures are taken by industry to address the safety issue of back-sweetening with unpasteurized juices. The justification for lack of control measures is that, in general, a combination of low pH and the high level of ethanol in fermented products will inhibit the growth of foodborne pathogens (Mørsetrø & Daeschel, 2004).

However, because of variations in alcohol and pH of wines and apple ciders in the market, the bacterial pathogens in the unpasteurized juice likely vary in their inactivation rates. Holding time between back-sweetening and consumption of both wines and apple ciders should be validated to reduce pathogens to ensure a safe finished product. According to the Juice Hazard Analysis and Critical Control Points (HACCP) regulations, a 5-log reduction (5 orders of magnitude) of pertinent microorganisms in juice products is required (U.S. Food and Drug Administration, 2004). The Juice HACCP regulation applies to alcoholic beverages back-sweetened with unpasteurized juices. Extended holding time to produce a reliable 5-log reduction on pertinent foodborne pathogens should be included in the federal guidelines for the wine and cider industry.

Experiments were performed with juice models and pathogen cocktails

To determine the safe holding time for back-sweetened products, we modified grape and apple juice to use as models for wine and hard cider. We adjusted the juices to desirable pH (3, 4, 5 for grape juice and 3.2, 3.7, 4.2 for apple juice) and filtered through 0.22 μm filters. We added undenatured ethanol to reach desirable alcohol content (0%, 7%, 10%, 12%, 14% for grape juice and 0%, 3.2%, 5%, 7%, 8.5% for apple juice). A five-strain cocktail for each pathogen (*E. coli*, *S. enterica* and *L. monocytogenes*).

genes) was mixed using overnight culture. We separately inoculated pathogen cocktails into modified juices, with an initial inoculum of 7-9 log/mL. We plated the samples onto tryptic soy agar at 0 h, 2 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h, 96 h. Plates were incubated at 37 °C for 48 hours and plate counts were recorded. We performed three biological replicates to ensure reproducibility of our experiments.

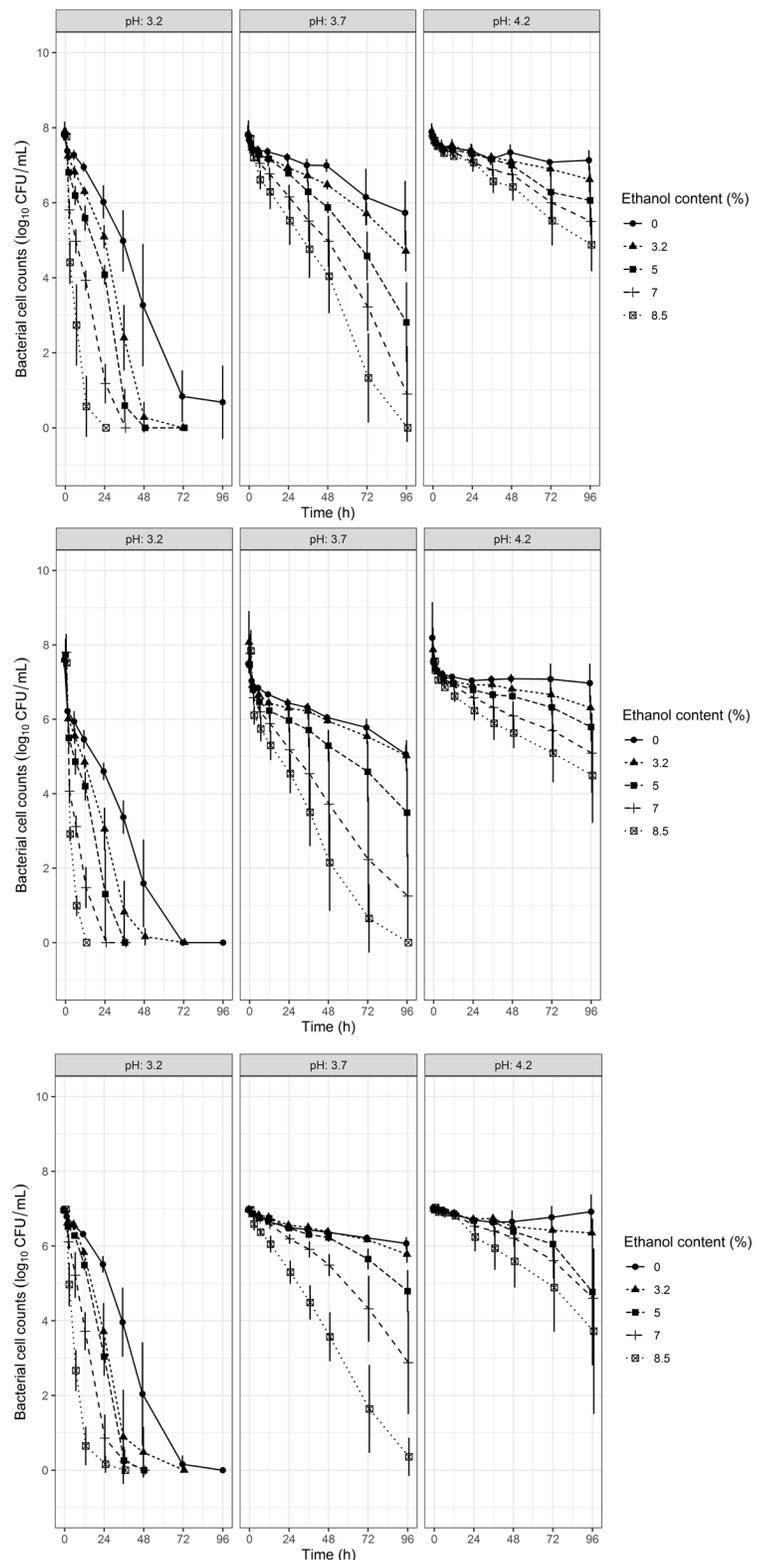
Survival curves of pathogens in back-sweetening models revealed necessary holding times

Survival curves of *E. coli*, *S. enterica*, and *L. monocytogenes* in modified white grape and apple juice are shown in **Figure 1-6**. Conditions achieving a 5-log reduction or greater of each tested pathogen compared to time 0 are summarized in **Table 1**, based on one-sample student's t test ($p < 0.05$). In apple juices with pH 4.2 and ethanol content between 0% to 8.5%, we found that the pathogen reduction was not significantly larger than 5-log in 96 hours and was not included in **Table 1**.

In this study, we recorded the reduction of pathogens in modified juice models up to 96 hours, with 9 time points of sampling. In general, pathogen load reduction was faster in conditions with lower pH and higher ethanol content. Survival of *E. coli* O157:H7 in 0% ethanol decreased as pH decreased, with a 1-2 log reduction in 96 hours at pH 4 and 5, and a faster reduction at pH 3 with an 8-log reduction in 96 hours (**Figure 1**). As ethanol content increased, at all pH levels, a faster reduction of pathogen load and a shorter time required for 5-log reduction can be observed.

However, the bactericidal effect of ethanol was less when the pH was higher. At higher pH values, longer holding time was required to meet the 5-log reduction threshold. Five-log or greater reduction of *E. coli* O157:H7 was achieved under the following conditions: pH 3: 7% ethanol and 24 hour hold time, 10% ethanol and 12 hour hold time, 12% ethanol and 6 hour hold time; pH 4: 10% ethanol and 72 hour hold time, 12% ethanol and 36 hour hold time, 14% ethanol and 12 hour hold time; pH 5: 12% ethanol and 96 hour hold time, 14% ethanol and 72 hour hold time.

Similar conclusions can be drawn for *S. enterica* and *L. monocytogenes* in modified white grape juice (**Figure 2 and 3**). Under pH 5 and 0% ethanol, slight increase (less than 1 log) in 96 hours for *S. enterica* and *L. monocytogenes* was observed. The nutrients in the modified white grape juice can support the growth of *S. enterica* and *L. monocytogenes* when no ethanol was present and pH was permissive for pathogen growth. Comparatively, under 0% ethanol, less than 2-log reduction of *S. enterica* and *L. monocytogenes* in pH 4 was achieved in 96 hours. While in pH



Figures 4-6. Top: The survival curve of *E. coli* O157:H7 in modified apple juice in 96 hours. Bacterial cell counts were recorded at time 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h. Error bars indicate standard deviation, $n = 3$. Middle: The survival curve of *Salmonella enterica* in modified apple juice in 96 hours. Bacterial cell counts were recorded at time 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h. Error bars indicate standard deviation, $n = 3$. Bottom: The survival curve of *Listeria monocytogenes* in modified apple juice in 96 hours. Bacterial cell counts were recorded at time 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h. Error bars indicate standard deviation, $n = 3$.

3, more than 5-log reduction of *S. enterica* and *L. monocytogenes* was achieved in 48 hours and 72 hours, respectively. For both *S. enterica* and *L. monocytogenes*, higher ethanol content resulted in a faster reduction for both pathogens, and a lower pH can further contribute to the bactericidal effect, resulting in a shorter time required to reach 5-log reduction (Table 1).

Apple Juice

As for apple juice models, the bactericidal effects of low pH and ethanol can be further illustrated. Similar to the trends observed in Figure 1 to 3, a higher concentration of ethanol and lower pH resulted in a faster reduction of all three pathogens in apple juice models. In our study, considering that apple cider generally has a lower alcohol content than wine, we modified apple juice models with up to 8.5% ethanol. The bactericidal effects of modified apple juice models were not as effective as grape juice models, which had higher concentrations of ethanol (up to 14% v/v). The influence of pH is also clearly demonstrated. While even the juice with 0% ethanol saw acceptable reduction at pH 3.2, the ethanol content of 8.5% was not sufficient to achieve the targeted 5-log reduction in pH 4.2 for all three pathogens in our apple juice models.

Take-home message: hold your back-sweetened product for the necessary time to ensure its safety

Alcoholic beverages with an alcohol content above 5% are not commonly associated with foodborne pathogens. However, for back-sweetened wine and hard cider, foodborne pathogens could be introduced through unpasteurized grape juice and apple juice. Consuming these beverages without holding for the necessary time could potentially cause foodborne illness, especially for the elderly and immune-compromised patients.

Currently, back-sweetened alcoholic beverages are regulated under FDA Juice HACCP regulations. However, wine and cider producers are not provided with clear guidelines on the safety of juice addition and safe holding time. In our study, we summarized the conditions under which a 5-log pathogen reduction was achieved in the juice models. To ensure that back-sweetened alcoholic beverages are safe to consume, manufacturers need to follow the holding times suggested for the appropriate conditions. For example, for a back-sweetened white wine with pH 4 and ethanol concentration of 12% v/v, holding the product for 36 hours at ambient temperature after back-sweetening is needed to ensure that all three pathogens are reduced at least 5-log. For a back-sweetened apple cider with pH 3.7 and ethanol concentration of 8.5% v/v, the safe holding time is 96 hours. Similar estimations can be made based on our results summarized in Table 1.

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Questions?

If you have questions about this work, please contact Patrick Gibney at pag235@cornell.edu.

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Glycine Zhujun Jiang is a PhD candidate in the Department of Food Science at Cornell University, working with Dr. Patrick Gibney in wine microbiology. She was a winemaker in France and is now doing research on understanding the impact of yeast on wine aromas and flavors with an emphasis on microbial terroir.



Zirui Ray Xiong is a PhD candidate in the Department of Food Science and Technology at Cornell University. His research is focused on using metagenomic methods to study the microbiome of food, and the application of bacterial metabolites for food safety and quality.



Anqi Chen is a Ph.D. candidate in the Gibney Lab at the Department of Food Science. Her research focuses on understanding cellular stress response and sugar metabolism by studying yeast as a model organism.



Christine D. Sislak is a technician in the Gibney Lab working primarily on industry questions regarding beer yeast physiology and genetics. The detection and understanding of diastatic activity in beer yeast is a main focus of her current research.



*Alisha G. Lewis is a Food Science PhD candidate in the Gibney lab. Her work focuses on the role of the electron transport chain in the regulation of starvation survival in *Saccharomyces cerevisiae*.*



Dr. Randy Worobo is a Professor of Food Microbiology in the Department of Food Science. Alternative approaches to enhance the safety and quality of food is the primary objective of his research. Short-term research includes the investigation of new technologies and combinations with existing food processing technologies to enhance the safety and quality. Long-term research projects include the chemical and genetic characterization of antimicrobial peptides (bacteriocins) produced by bacteria.



Dr. Patrick Gibney is an assistant professor of wine microbiology in the Department of Food Science. His lab is interested in a variety of research topics that span eukaryotic cell biology to wine microbiology. His research topics include understanding fundamental aspects of biology to collaborating with the fermented beverage industry on more applied projects.



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