PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 42, No. 4, p. 284–291
https://doi.org/10.4315/FPT-21-031

2900 100th Street, Suite 309, Des Moines, IA 50322-3855, USA

Sujan Acharya and Brian A. Nummer*

Dept. of Nutrition, Dietetics and Food Science, Utah State University, 8700 Old Main Hill, Logan, UT 84322-8700, USA

Survival of *Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes* in Ethanol and Juice Mixtures at Ambient Temperature

ABSTRACT

Some bars and restaurants have begun displaying alcoholic fruit and vegetable cocktail mixes at ambient temperature. Operators often believe that ethanol makes this practice safe, and conversely, some regulators question the safety. It has been reported that ethanol may inhibit the growth of some bacteria at concentrations starting at 8–10% and may be biocidal at concentrations ≥ 30%. In this study, *Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes* did not grow in banana puree, pear puree, orange juice, and apple juice cocktail mixtures prepared with ethanol concentrations from 10 to 50% at 25°C. Inoculated pathogens were not detected in juice plus ethanol at 40 or 50% after 48 h. Juices with ethanol concentrations of 10–30% exhibited different log reductions over time for each of the pathogens introduced. This study has implications for how regulators assess the risk of ethanol juice mixtures held at ambient temperature under the U.S. Food and Drug Administration (FDA) Food Code. Operators that desire to display ethanol and fruit juice mixtures at room temperature need to ensure a pH < 4.2 or a combination of pH and water activity values based on *Table B* of the FDA Food Code, or they must keep the product under refrigeration at a temperature ≤ 41°F.

INTRODUCTION

Bars and restaurants are always looking for new ways to attract customers by creating unique products *(7, 12)*, including new and innovative cocktail drinks *(10)*. Some operators have begun displaying these alcoholic cocktails, often with fruit and vegetable inclusions, in glass display dispensers at ambient temperature. The establishments often believe that the alcohol will kill microorganisms. However, regulators have questioned this practice.

The U.S. Food and Drug Administration (FDA) Food Code cites that foods with water activity (a_w) > 0.88 and a pH ≥ 4.2 must be assessed for control of both vegetative foodborne illness organisms and spores (*Table B*, Chapter 1, Temperature Control for Safety [TCS] Foods) *(13)*. Foods below pH 4.2 are deemed non-TCS foods, permitting storage at any temperature. Foods with pH ≥ 4.2 require further assessment based on a_w or may require a laboratory product assessment. Foods deemed to be

*Author for correspondence: Phone: +1 435.797.2116; E-mail: brian.nummer@usu.edu

TCS could only be displayed at ambient temperature for up to 4 h and would require a discard after 4 h *(13)*. The FDA Food Code has no references directly in it to evaluate the percentage of ethanol as a food preservative or antimicrobial with the exception of providing for the option of performing a product assessment under the TCS definition in Chapter 1 *(13)*.

Ethanol is the type of alcohol in beer, wine, and liquor and has been used for many years as a disinfectant *(3, 6)*. De Villiers states that 15–17.5% ethanol based on free water (a_w) is an effective preservative for oral pharmaceutical preparations *(4)*. Kalathenos and Russell, when reviewing ethanol as a food preservative, stated that solutions containing <30% (vol/vol) were rarely biocidal; however, bacterial growth could be inhibited starting at 8–11% (vol/vol) *(5)*. Waite and Daeschel reported that log reductions of *Staphylococcus aureus* and *Escherichia coli* O157:H7 occurred in 20 min in wine with 12.1–14.7% ethanol at pH 2.74–3.72 *(14)*. Other studies have established that ethanol at a 5% concentration produces a strong bacteriostatic effect on the growth of pathogens like *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* *(2, 8, 11)*.

This study was undertaken to evaluate the fate of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* inoculated into alcoholic cocktail drinks prepared with ethanol concentrations of 10, 20, 30, 40, and 50% in various fruit juices stored at ambient temperature. The data can be used by regulators to ascertain the relative risk of storing these beverages at ambient temperature for more than 4 h.

MATERIALS AND METHODS

Cocktail preparation

Ethanol concentrations of 10, 20, 30, 40, and 50% (vol/vol) were prepared using 190 proof ethyl alcohol and distilled water. For each concentration, 1,000 ml of ethanol solution was prepared. The 100-ml cocktail mixtures were prepared at each concentration by mixing 50 ml of ethanol solution and 50 ml of fruit juice or puree using banana puree, Asian pear puree, orange juice (Pulp Free Original Orange Juice, Simply Orange Juice Company, Apopka, FL), and apple juice (Fresh Pressed Three Apple Blend 100% Juice, Tree Top Inc., Selah, WA). A control for each concentration was also prepared by mixing 50 ml of ethanol and 50 ml of distilled water. The treatments for cocktails for each ethanol concentration were control (ethanol and distilled water), pear (ethanol and Asian pear puree), banana (ethanol and banana puree), orange (ethanol and orange juice), and apple (ethanol and apple juice).

Inoculum preparation

Five strains of *E. coli* O157 of vegetable origin or related to vegetable outbreaks (H1730, EC4042, EC4045, EC4191, and EC4206) were used; these were obtained from the culture collection of Dr. Donald Schaffner at Rutgers University and maintained at −80°C in Dr. Taylor Oberg's laboratory. Similarly, five strains of *Salmonella*, Thompson FSIS 120 (chicken isolate), Enteritidis H3502 (clinical isolate, phage type 4), Enteritidis H3527 (clinical isolate, phage type 13a), Typhimurium H3380 (clinical isolate, phage type DT104), and Heidelberg F5038BG1 (ham isolate) were used; these were also obtained from Schaffner's culture collection at Rutgers University and maintained at −80°C in Oberg's laboratory. For *L. monocytogenes*, five strains—J1-177 (serotype 1/2b, human isolate), C1-056 (serotype 1/2a, human isolate), N3-013 (serotype 4b, food isolate), R2-499 (serotype 1/2a, sliced turkey isolate), and N1-227 (serotype 4b, food isolate)—were obtained from Oberg's culture collection at Utah State University. Cultures for each pathogen strain were prepared from frozen stock maintained at −80°C by transferring 0.1 ml of thawed frozen stock into 10 ml of fresh tryptic soy broth (TSB; Neogen Corp., Lansing, MI) and incubating at 37°C for 24 h. Individual strains were then grown in TSB for 24 h at 37°C before inoculation. The five-strain mixture for each pathogen was prepared by combining 2-ml aliquots of each strain in a 15-ml conical centrifuge tube. Cells were pelleted by centrifugation (1,509 × g for 15 min) and resuspended in 10 ml of Butterfield phosphate buffer solution (BPBS) 3 times.

Sample inoculation and incubation

The treatments prepared from the five varieties of cocktail mixtures at 10, 20, 30, 40, and 50% ethanol concentrations were transferred to sterile glass containers, loosely capped with sterile caps, and stored at 25°C. The five-strain mixture of each pathogen was then inoculated (10/100 ml) in the treatments separately. All inoculated treatments were transferred to 25°C for incubation for up to 48 h.

Microbial analysis, pH, and a_w measurement

Inoculated pathogens were first enumerated after approximately 30 min of inoculation into the treatments. Subsequent enumerations were performed at 3, 6, 9, and 12 h, followed by enumeration at 12-h intervals for up to 48 h. For enumeration, 1 ml of the sample was pipetted into 9 ml of BPBS, and subsequent serial dilutions were performed. The samples were then plated in duplicates. The treatments inoculated with *E. coli* O157 were plated in Sorbitol–MacConkey agar (Neogen), and colonies were enumerated after 24 h of incubation at 37°C. For treatments inoculated with *Salmonella*, *Salmonella Shigella* agar (Neogen) was used to enumerate colonies after 24 h of incubation at 37°C. Similarly, for treatments inoculated with *L. monocytogenes*, PALCAM agar base (Neogen) containing PALCAM supplement (Neogen) was used to enumerate colonies after 48 h of incubation at 37°C. For pH measurement, approximately 10 ml of the sample was taken and the pH was measured using a double-junction pH meter (pHTestr 30, Oakton Instruments, Vernon Hills, IL). Likewise, a_w was measured by pipetting 3 ml of the sample into the sample cups using a LabSwift portable water activity meter (Novasina AG, Lachen, Switzerland).

TABLE 1. pH and a_w of treatments of ethanol–fruit cocktail mixtures prepared with 10, 20, 30, 40, and 50% ethanol concentrations

Treatments	Ethanol % (vol/vol)									
	10		20		30		40		50	
	pH	a_w	pH	a_w	pH	a_w	pH	a_w	pH	a_w
Control	6.68	0.976	6.76	0.967	6.84	0.959	6.87	0.951	6.94	0.944
Banana	4.95	0.965	5.03	0.956	5.04	0.947	5.17	0.936	5.21	0.927
Pear	4.84	0.970	4.87	0.963	4.99	0.955	5.04	0.948	5.11	0.941
Orange	4.12	0.972	4.17	0.963	4.23	0.955	4.28	0.947	4.32	0.938
Apple	3.82	0.969	3.89	0.962	3.96	0.953	4.01	0.945	4.07	0.938

Data analysis

The bacterial populations were enumerated as the log CFU values per milliliter of product. For each pathogen, three replications of the experiment were conducted. In each replication, the samples were inoculated and analyzed in duplicate for *E. coli* O157, *Salmonella*, and *L. monocytogenes* counts at different data points. Data points were expressed as mean ± standard deviation of the three replications.

RESULTS

pH and a_w

The pH levels of the different juice mixes and control ranged from 3.82 to 6.94 *(Table 1)*. The highest pH among the cocktails was in control, and apple juice cocktail had the lowest pH. The pH of the mixtures increased with increasing concentrations of ethanol. Most treatments, fruit juices, and ethanol mixtures were pH ≥ 4.2 and would require refrigeration as TCS foods under the FDA Food Code *(13)*. The a_w ranged from 0.938 to 0.969 for all mixtures, where control had the highest and banana puree cocktail had the lowest a_w. The a_w of the mixtures decreased with increasing concentrations of ethanol, supporting that ethanol acts as a hydrogen-bonding, polar humectant *(5)*.

Control (ethanol plus distilled water)

The initial inocula of *E. coli* O157 in control were 6.98, 7.11, 6.80, 6.79, and 6.37 log CFU/ml in 10, 20, 30, 40, and 50% ethanol concentrations, respectively. There was a reduction in *E. coli* O157 counts for all concentrations *(Figure 1)*. *E. coli* O157 survived for 48 h of incubation in the lower three concentrations evaluated. The total reductions were 0.41, 1.36, and 1.72 log CFU/ml for 10, 20, and 30% ethanol concentrations after 48 h of incubation. In the cocktails with 40 and 50% ethanol concentrations, *E. coli* O157 was not detected (<1 CFU/ml) after 48 and 24 h of incubation. In control inoculated with *Salmonella*, the mean inoculum levels were 6.84, 6.55, 6.12, 6.16, and 6.13 log CFU/ml in 10, 20, 30, 40, and 50% ethanol concentrations, respectively. The counts reached <1 CFU/ml in 30, 40, and 50% concentrations after 24, 12, and 6 h, and no *Salmonella* was detected for up to 48 h, whereas survival was observed for 48 h with a decrease of 0.67 log CFU/ml in a 10% concentration and 2.9 log CFU/ml in a 20% concentration *(Figure 1)*. In *L. monocytogenes*-inoculated control, mean inoculum levels were 7.89, 7.08, 7.22, 6.94, and 6.97 log CFU/ml in 10, 20, 30, 40, and 50% ethanol concentrations, respectively. At a 10% concentration, *L. monocytogenes* survived the 48 h of incubation with a total reduction of 2.13 log CFU/ml. In 20, 30, and 40% concentrations, *L. monocytogenes* was not detected after 6 h, and it was not detected after 3 h in a 50% concentration for up to 48 h of incubation *(Figure 1)*.

Banana puree cocktail

The banana puree cocktails with 10, 20, 30, 40, and 50% ethanol had initial inocula of 8.94, 7.97, 7.86, 7.59, and 7.45 log CFU/ml of *E. coli* O157, respectively. In the two lower concentrations evaluated, *E. coli* O157 survived for 48 h with a total reduction of 2.05 log CFU/ml in 10% ethanol and 3.72 log CFU/ml in 20% ethanol. The counts for *E. coli* O157 reached an undetectable level after 12, 9, and 3 h in 30, 40, and 50% concentrations, respectively, and were undetected for up to 48 h of incubation *(Figure 2)*. For *Salmonella*, the mean inoculum levels were 7.63, 6.79, 6.49, 6.36, and 6.35 log CFU/ml in 10, 20, 30, 40, and 50% ethanol concentrations, respectively. *Salmonella* survived for 48 h in the 10 and 20% concentrations, with reductions of 1.04 and 1.49 log CFU/ml. The counts reached an undetectable level after 9, 3, and 3 h for 30, 40, and 50% concentrations and remained the same until 48 h of incubation *(Figure 2)*. In the cocktails with 10, 20, 30, 40, and 50% ethanol inoculated with *L. monocytogenes*, the levels of inoculum were 8.62, 7.87, 5.56, 5.49, and 5.65 log CFU/ml, respectively. *L. monocytogenes* survived for 48 h in 10 and 20% concentrations, with reductions of 1.58 and 3.73 log CFU/ml, respectively, whereas in 30, 40, and 50% concentrations, the pathogen was undetectable after 9, 6, and 3 h, respectively *(Figure 2)*.

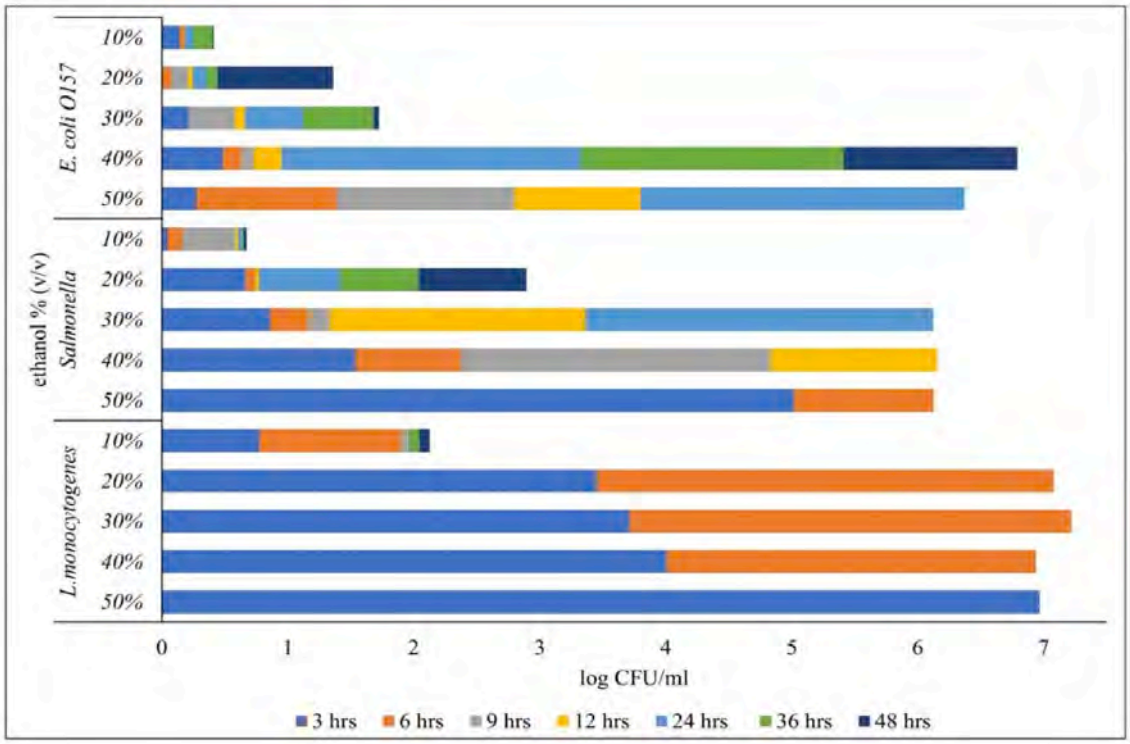

Figure 1. Reduction (log CFU/ml) of *E. coli* O157, *Salmonella*, and *L. monocytogenes* counts in control (ethanol + distilled water) after each time period at different ethanol concentrations during storage at 25°C.

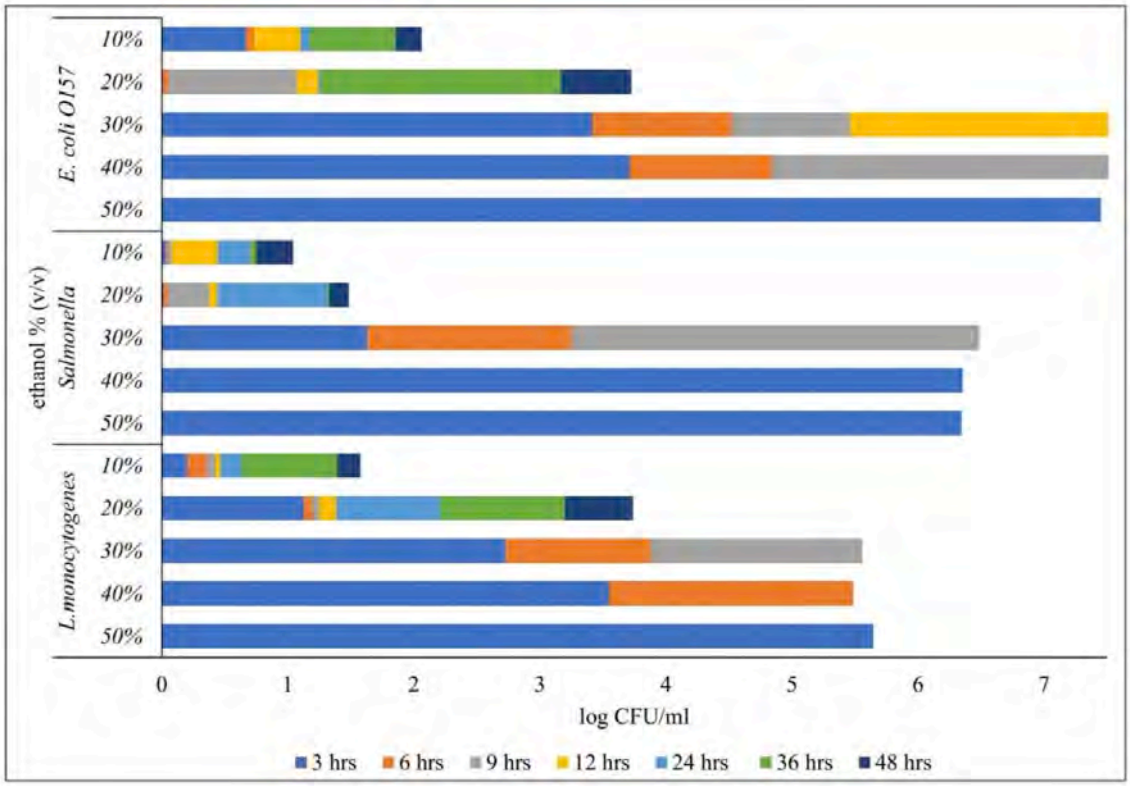

Figure 2. Reduction (log CFU/ml) of *E. coli* O157, *Salmonella*, and *L. monocytogenes* counts in banana puree cocktail after each time period at different ethanol concentrations during storage at 25°C.

Asian pear puree cocktail

The initial mean inocula for *E. coli* O157 in Asian pear puree cocktails with 10, 20, 30, 40, and 50% ethanol were 8.29, 8.59, 8.11, 8.20, and 8.00 log CFU/ml, respectively. *E. coli* O157 was detected after 48 h in the lower three concentrations, with total reductions of 1.26, 1.66, and 3.99 log CFU/ml in 10, 20, and 30% concentrations, respectively. In 40 and 50% concentrations, the pathogen was not detected after 48 and 36 h, respectively *(Figure 3)*. For *Salmonella*-inoculated cocktails, the inoculum levels in 10, 20, 30, 40, and 50% concentrations were 7.91, 7.75, 7.69, 7.31, and 7.21 log CFU/ml, respectively. *Salmonella* counts were undetectable for 40 and 50% concentrations after 9 and 6 h, whereas for the lower three concentrations, the pathogen was still detected after 48 h *(Figure 3)*. The total reductions for 10, 20, and 30% concentrations were 1.11, 3.27, and 4.86 log CFU/ml after 48 h. In the case of the Asian pear puree cocktails inoculated with *L. monocytogenes*, the levels of inoculum for 10, 20, 30, 40, and 50% ethanol were 7.28, 7.29, 7.05, 7.02, and 7.00 log CFU/ml, respectively.

L. monocytogenes was detected after 48 h with total reductions of 0.76 and 3.14 log CFU/ml for 10 and 20% concentrations, respectively. For 30, 40, and 50% concentrations, the pathogen reached an undetectable level after 36, 9, and 6 h, respectively *(Figure 3)*.

Orange juice cocktail

In the orange juice cocktails with 10, 20, 30, 40, and 50% ethanol, the initial inoculum levels of *E. coli* O157 were 7.94, 7.57, 7.11, 6.82, and 6.79 log CFU/ml, respectively. In 10 and 20% concentrations, *E. coli* O157 survived for 48 h with reductions of 0.98 and 2.42 log CFU/ml, respectively. *E. coli* O157 counts were at an undetectable level after 6 h and for the remainder of the 48 h for 30 and 40% concentrations, whereas no pathogen was detected after 3 h in a 50% concentration *(Figure 4)*. For *Salmonella*-inoculated cocktails, the inoculum levels in 10, 20, 30, 40, and 50% concentrations were 6.92, 6.76, 6.18, 6.09, and 6.05 log CFU/ml, respectively. In both 10 and 20% concentrations, *Salmonella* survived for 48 h with total reductions of 1.81 and 2.64 log CFU/ml, respectively. *Salmonella* counts reached an undetectable level at 9 h in a 30% concentration, whereas in 40 and 50% concentrations, the pathogens were undetected after 3 h *(Figure 4)*. With *L. monocytogenes*-inoculated cocktails, the inoculum levels were 8.12, 7.81, 7.62 log CFU/ml in 10, 20, 30, 40, and 50% concentrations, respectively. In 10, 20, and 30% concentrations, there were total reductions of 2.02, 2.30, and 3.88 log CFU/ml, respectively, after 48 h. In 40 and 50% concentrations, the pathogen was undetectable after 12 and 3 h, respectively *(Figure 4)*.

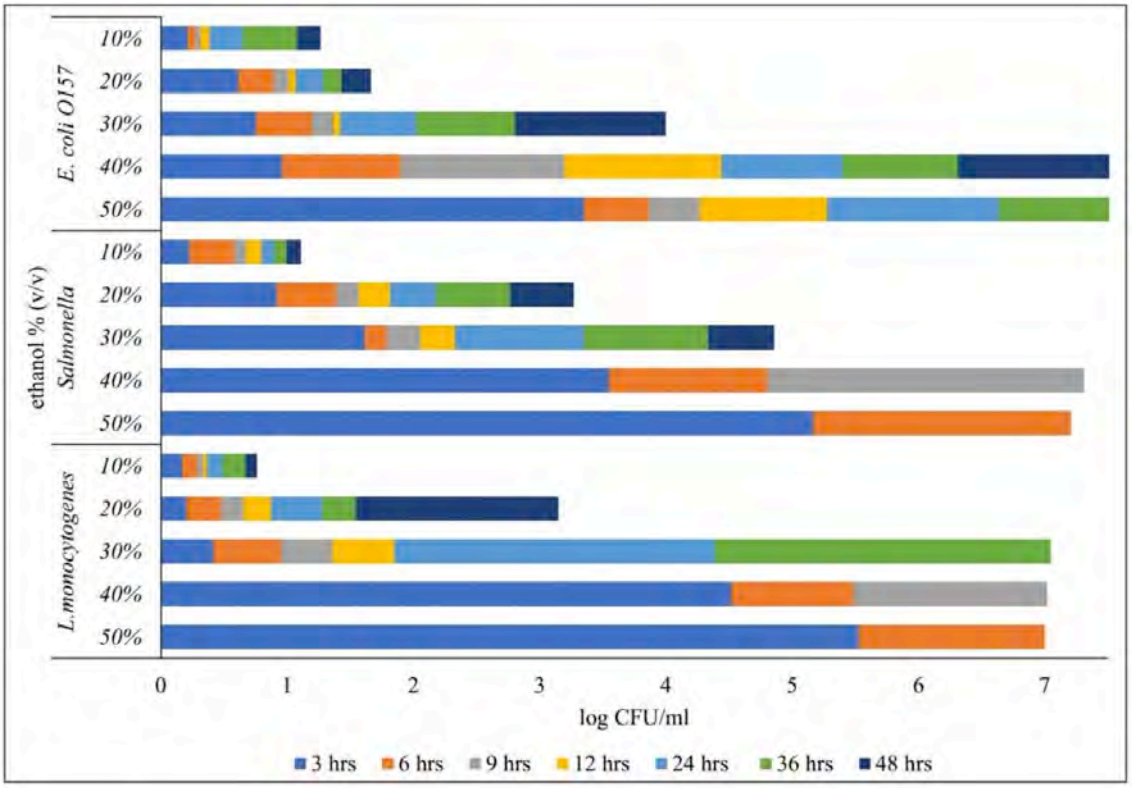

Figure 3. Reduction (log CFU/ml) of *E. coli* O157, *Salmonella*, and *L. monocytogenes* counts in Asian pear puree cocktail after each time period at different ethanol concentrations during storage at 25°C.

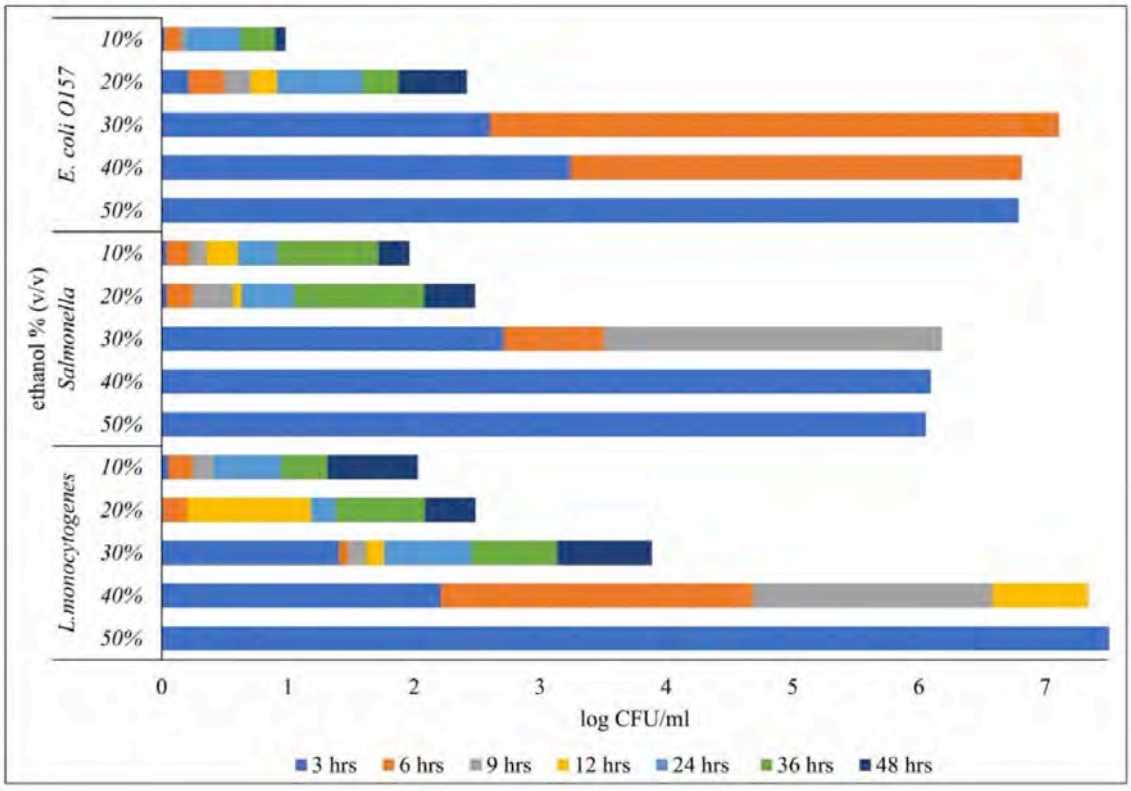

Figure 4. Reduction (log CFU/ml) of *E. coli* O157, *Salmonella*, and *L. monocytogenes* counts in orange juice cocktail after each time period at different ethanol concentrations during storage at 25°C.

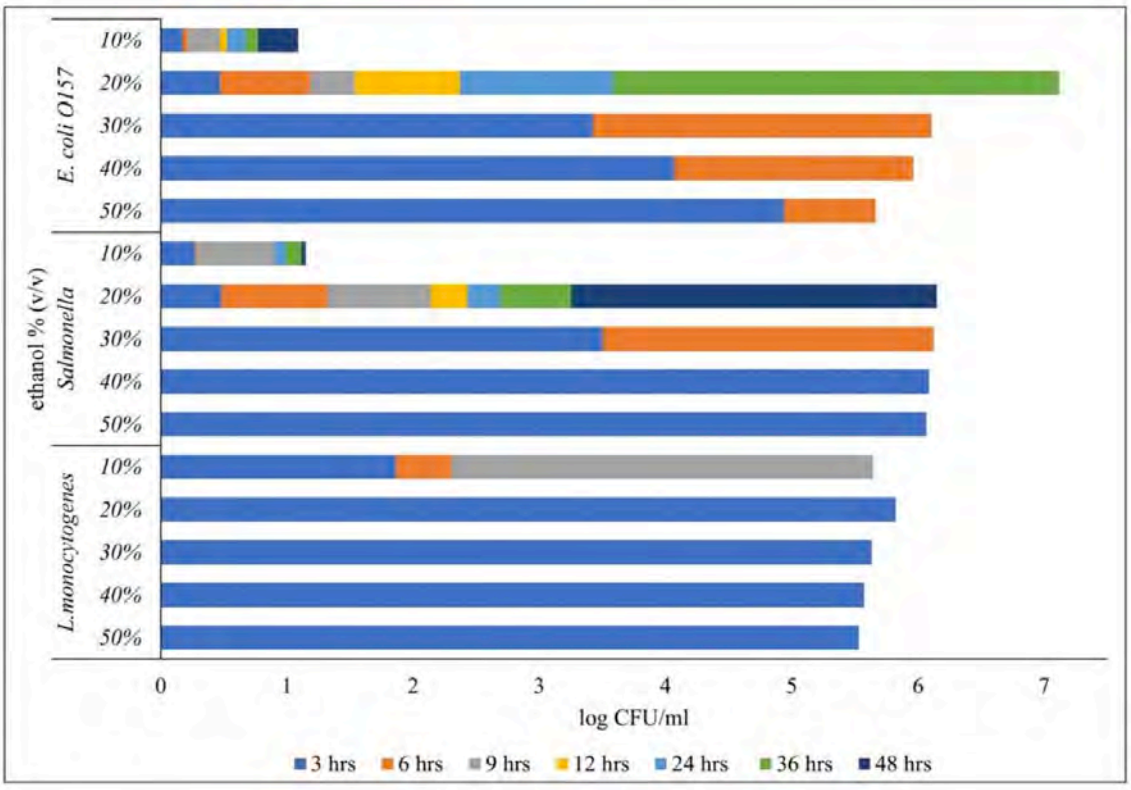

Figure 5. Reduction (log CFU/ml) of *E. coli* O157, *Salmonella*, and *L. monocytogenes* counts in apple juice cocktail after each time period at different ethanol concentrations during storage at 25°C.

Apple juice cocktail

The initial mean inocula for *E. coli* O157 in apple juice cocktails with 10, 20, 30, 40, and 50% ethanol were 7.73, 7.12, 6.11, 5.96, and 5.66 log CFU/ml, respectively. In a 10% concentration, *E. coli* O157 survived for 48 h with a reduction of 1.08 log CFU/ml, whereas in a 20% concentration, the pathogen was not detected after 36 h. In the higher three concentrations, the *E. coli* O157 counts reached an undetectable level after 6 h of incubation *(Figure 5)*. *Salmonella*-inoculated cocktails had initial inocula of 6.81, 6.15, 6.12, 6.09, and 6.07 log CFU/ml in 10, 20, 30, 40, and 50% concentrations, respectively. In a 10% concentration, *Salmonella* survived for 48 h with a decrease of 1.15 log CFU/ml. In a 20% concentration, the *Salmonella* counts were undetectable after 36 h. For the higher three concentrations, the pathogen was undetectable after 6 h in 30% and 3 h in 40 and 50% concentrations *(Figure 5)*. For *L. monocytogenes*, the inoculum levels were 5.65, 5.82, 5.63, 5.57, and 5.53 log CFU/ml of the cocktails with 10, 20, 30, 40, and 50% ethanol. *L. monocytogenes* was not detected after 9 h for a 10% concentration, whereas for the higher four concentrations, the pathogen was not detected after 3 h of incubation *(Figure 5)*.

DISCUSSION

There were reductions in pathogen counts for all cocktail drinks and control with concentrations of ethanol from 10 to 50%. This supports statements by Kalathenos and Russell that bacterial growth could be inhibited with ethanol starting at 8–11% (vol/vol) *(5)*. Inoculated pathogens were detected for all 10% ethanol cocktail mixtures after 48 h except for *L. monocytogenes* in apple juice cocktail, where the counts were undetectable after 9 h. For fruit juice mixtures with a 20% ethanol concentration, *L. monocytogenes* reached an undetectable level in control after 6 h but was detected for 48 h in Asian pear puree, banana puree, and orange juice. *E. coli* O157 and *Salmonella* were detected after 48 h in control, Asian pear puree, banana puree, and orange juice.

For the fruit juices with a 30% ethanol concentration, significant reductions of all pathogens were observed after 3 h. The counts for all three pathogens in banana puree and apple juice cocktails reached an undetectable level within 48 h. However, at the same time, *E. coli* O157 and *Salmonella* were detected after 48 h in Asian pear puree cocktail. In addition, for control and orange juice cocktail, *E. coli* O157 and *L. monocytogenes*, respectively, were detected after 48 h. Waite and Daeschel also observed increased log reductions of *S. aureus* and *E. coli* O157:H7 when the ethanol concentration in wine was increased from 12.1 to 14.7% *(14)*.

For the fruit juices with 40 and 50% ethanol concentrations, there was a significant decrease in the pathogen counts after 3 h of incubation in all cocktail mixtures, and the counts of all three pathogens evaluated reached an undetectable level within 48 h. These data agree with statements by Kalathenos and Russell that solutions containing >30% (vol/vol) ethanol could be biocidal *(5)*.

Among the three pathogens evaluated, *L. monocytogenes* was found to be the most sensitive to ethanol, and the counts decreased rapidly in almost all treatments and more prominently in the lower three concentrations. However, *E. coli* O157 survived longer in the presence of ethanol and had the least log reduction compared with *L. monocytogenes* and *Salmonella*. For all treatments tested, increasing ethanol concentration with lower pH demonstrated increased pathogen reduction. At higher ethanol concentrations, the effect of pH was somewhat masked because the rate of decrease in the pathogens counts was similar, but the reduction for lower pH was still comparatively quicker than that for higher pH. This aligns with the concept that pH and ethanol act as hurdles for pathogens and together can exert synergistic action and that the effect is increased with increasing concentration of alcohol and decreasing pH *(1, 9)*.

CONCLUSION

Based on the pH and a_w of the cocktail mixtures evaluated and ethanol increasing the pH value of the juices, many cocktail drinks prepared in bars and restaurants are likely to be categorized as TCS (pH > 4.2). This study indicates that a cocktail made with 10–50% ethanol and fruit juice would likely show no growth of selected pathogens, suggesting these beverages are low risk. From a pathogen reduction standpoint, ethanol at a 40–50% concentration mixed with fruit juice resulted in significant lethality of all three pathogens studied (>6 log) within 48 h at 25°C.

Operators who are under the FDA Food Code and who wish to prepare and display these types of ethanol and fruit juice mixtures at room temperature need to ensure that the pH of the beverage is <4.2 or have a combination of pH and a_w values based on *Table B* of the FDA Food Code. If the beverage cannot be modified into the non-TCS category, then the beverage should only be displayed and dispensed for 4 h and then discarded afterward or kept under refrigeration at a temperature ≤ 41°F until served. In addition, operators should use caution when adding low-acid foods (whole, pieces, purees, or juices) to the cocktail mixture such that the equilibrium pH increases above pH 4.6.

REFERENCES

1. Barker, C., and S. F. Park. 2001. Sensitization of *Listeria monocytogenes* to low pH, organic acids, and osmotic stress by ethanol. *Appl. Environ. Microbiol.* 67:1594–1600.
2. Brewer, R., M. R. Adams, and S. F. Park. 2002. Enhanced inactivation of *Listeria monocytogenes* by nisin in the presence of ethanol. *Lett. Appl. Microbiol.* 34:18–21.
3. Centers for Disease Control and Prevention. 2021. Alcohol use basics: Frequently asked questions. Available at: https://www.cdc.gov/alcohol/faqs.htm. Accessed 10 October 2021.

4. de Villiers, M. 2009. Antimicrobial preservatives, p. 203–215. *In* J. E. Thompson (ed.). A practical guide to contemporary pharmacy practice, 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.
5. Kalathenos, P., and N. J. Russell. 2003. Ethanol as a food preservative, p. 196–217. *In* N. J. Russell and G. W. Gould (eds.), Food preservatives. Springer US, Boston, MA.
6. Kampf, G. 2018. Efficacy of ethanol against viruses in hand disinfection. *J. Hosp. Infect.* 98:331–338.
7. Lee, C., R. Hallak, and S. R. Sardeshmukh. 2016. Drivers of success in independent restaurants: A study of the Australian restaurant sector. *J. Hosp. Tour. Manag.* 29:99–111.
8. Menz, G., P. Aldred, and F. Vriesekoop. 2011. Growth and survival of foodborne pathogens in beer. *J. Food Prot.* 74:1670–1675.
9. Møretrø, T., and M. A. Daeschel. 2006. Wine is bactericidal to foodborne pathogens. *J. Food Sci.* 69:M251–M257.
10. Ocejo, R. E. 2010. What'll it be? Cocktail bartenders and the redefinition of service in the creative economy. *City Cult. Soc.* 1:179–184.
11. Oh, D. H., and D. L. Marshall. 1993. Antimicrobial activity of ethanol, glycerol monolaurate or lactic acid against *Listeria monocytogenes*. *Int. J. Food Microbiol.* 20:239–246.
12. Ottenbacher, M., and R. J. Harrington. 2007. The innovation development process of Michelin-starred chefs. *Int. J. Contemp. Hosp. Manag.* 19:444–460.
13. U.S. Food and Drug Administration. 2017. FDA Food Code 2017. U.S. Food and Drug Administration, Washington, D.C.
14. Waite, J. G., and M. A. Daeschel. 2007. Contribution of wine components to inactivation of foodborne pathogens. *J. Food Sci.* 72:M286–M291.