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Evaluating Kombucha and Fruit Juice Blends for a 5-Log Reduction of Acid-Adapted *Escherichia coli* O157

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Abstract

This study examined the survival of acid-adapted *E. coli* O157 in kombucha during fermentation and refrigerated kombucha mixed with fruit juices. Acidic and non-acidic kombucha mixes were fermented at 25˚C using a commercially available starter culture and inoculated with a 5-strain mixture of acid-adapted *E. coli* O157. There was >5-log reduction in the pathogen count for both starter mixes within 7 days of fermentation. For the kombucha-juice blends at refrigerated temperature, 14 ml of lemon, apple, orange, and mango juices were mixed with 186 ml kombucha separately. The treatments were inoculated with a 5-strain mixture of acid-adapted *E. coli* O157 and incubated at 5˚C for 14 days. >5-log reduction in the pathogen count was observed in lemon, control, and mango juice blend after 1, 3, and 14 days, respectively. The total reduction in pathogen count in the apple and orange juice blend after 14 days was 4.43 and 4.12 log CFU/ml, respectively. The inability of the kombucha fruit blend to cause a 5-log reduction of *E. coli* O157 suggests the need for following strict hygienic and good sanitation practices during blending and bottling for home fermenters and an approved HACCP plan for foodservice operators to ensure product safety.

Keywords

Kombucha, Fermentation, Kombucha-Fruit Blend, Refrigeration, Acid-Adapted *E coli* O157

1. Introduction

Kombucha is an acidic beverage made by brewing tea (most often black, green, or oolong tea) and sugar and fermenting 7 - 10 days with a Symbiotic Culture of Bacteria and Yeast (SCOBY). Kombucha started as a homemade beverage and

has rapidly entered the U.S. commercial market in recent years [1] [2]. Most of Kombucha's appeal is due to its perceived health benefits [2] [3]. Kombucha is generally unpasteurized since the live culture is considered a probiotic, and some manufacturers will use the live yeast to produce in-bottle carbonation.

If kombucha is made in a foodservice operation, it is considered a "Special Process," along with other fermented foods, by the FDA under the U.S. FDA model Food Code [4]. All Special processes under the U.S. FDA model Food Code require an approved food safety HACCP plan [5]. And, if the fermented kombucha is packaged together with fruit juice in a food establishment, it should be under a HACCP plan that demonstrates a 5-log reduction of *Escherichia coli* O157:H7 in the juice [6]. Similarly, if a food manufacturer produces fermented kombucha mixed with fruit juices, the fruit juice ingredient is required to receive a treatment that effects a 5-log reduction of *E. coli* O157:H7 following regulations in 21 CFR 120.7 (e) [7].

Brewer *et al.* reported >5 log reduction in *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) within 14 days of fermentation in four commercially available kombucha kits [8]. Several other studies have also demonstrated that the fermentation process of kombucha creates an environment in the product that is lethal to various pathogens such as *Salmonella*, *Staphylococcus aureus*, *Shigella sonnei, Listeria monocytogenes*, *Bacillus cereus,* and others [3] [9] [10] [11] [12] [13]. However, raw kombucha can be quite acidic, and it is often blended or mixed with fruit juice to meet consumer taste preferences. Adding unprocessed and low-acid fruit juice may introduce pathogens, change the pH, and dilute the kombucha acids leading to possible growth or survival of those pathogens. Nevertheless, some kombucha manufacturers, especially small processors, restaurants, and home fermenters, assume that adding unpasteurized fruit juice to raw kombucha will result in a 5-log reduction of *E. coli* O157 due to the antimicrobial effects of the organic acids and the microflora present. Therefore, this study was designed to determine if unpasteurized kombucha with added fruit juice achieves a 5-log reduction of *E. coli* O157.

2. Materials and Methods

2.1. Kombucha Fermentation

Kombucha was prepared using starter culture (Classic Scoby Kombucha Live Starter Culture; Fermentoholics LLC St. Petersberg, FL), black tea (Lipton Organic Black Tea; Unilever, Englewood Cliffs NJ), and sugar (Simple Truth Organic Cane Sugar; Kroger Co, Cincinnati, OH) and distilled water. 950 ml of distilled water were boiled in a steel pot and removed from heat, and then 12 tea bags each containing 2.5 g of black tea were placed in the pot with water and allowed to steep for 10 min and removed. Next, 406 g of cane sugar was added to the tea mixture and stirred until fully dissolved. The sweetened tea mixture was brought to room temperature and divided equally using a measuring cylinder into two sterile gallon-sized jars, and two treatments were prepared. The acidic

starter mix was prepared by adding the starter culture following the instructions provided with the starter culture, where the entire content of the starter culture pack was added to the jar. The pH reading was taken to ensure the pH was below 4.5. For the non-acidic starter mix, the liquid portion in the starter culture pack was discarded, and only the pellicle was used, which was washed with distilled water four times before addition into the jar. This was performed to obtain a pre-fermentation starter mix with a pH above 4.5. For each treatment, distilled water at room temperature was added to bring the final volume of the mixture to 3785 ml to prepare a gallon (U.S.) of the mixture. A coffee filter and rubber bands were used to secure the jars' mouths.

2.2. Kombucha Fruit Blend Preparation

For the preparation of the ready-to-drink Kombucha-fruit blend, four different fruit juices commonly used for preparing Kombucha-fruit blend were selected. The juices used were Lemon Juice (Santa Cruz Organic 100% Lemon Juice; Santa Cruz Natural Inc, Chico, CA), Orange Juice (Simply Orange Pulp Free Original Orange Juice; Simply Orange Juice Company, Apopka, FL), Apple Juice (Tree Top Fresh Pressed Three Apple Blend 100% Juice; Tree top INC, Selah, WA), and Mango Juice (Fresh juice prepared from whole fruits using a fruit juicer (Breville BJE510XL Juice Fountain Multi-Speed 900-Watt Juicer; Breville USA, Torrance, CA). The fruit juices and distilled water control were mixed with the kombucha previously fermented for 10 days (prepared following the instructions provided with the starter culture pack) at 93% (186 ml) kombucha and 7% (14 ml) distilled water or juice in 250 ml glass jar with screw caps to prepare five treatments of ready-to-drink Kombucha fruit blend. The treatments, treatment C (Kombucha with distilled water), treatment L (Kombucha with lemon juice), treatment M (Kombucha with mango juice), treatment A (Kombucha with apple juice), and treatment O (Kombucha with orange juice), were incubated at 25˚C for 24 h and transferred to 21˚C. After the next 24 h, the treatments were transferred again from 21˚C to 5˚C and stored at 5˚C for 15 days.

2.3. Inoculum Preparation

For inoculum, five strains of *E. coli* O157 of vegetable origins or related to vegetable outbreaks (H1730-human isolate from outbreak associated with letteuce, EC4042-clinical isolate from outbreak associated with spinach, EC4045-food-borne isolates from outbreak associated with spinach, EC4191-food-borne isolates from outbreak associated with spinach, EC4206-bovine isolate from outbreak associated with spinach) obtained from the culture collection of Dr. Donald Schaffner at Rutgers University and maintained at −80˚C in Dr. Taylor Oberg's laboratory were used. Working cultures for each strain were prepared by transferring 0.1 ml of thawed frozen stock into 10 ml of fresh tryptic soy broth (TSB) (TSB; Neogen Corp., Lansing, MI) and incubating at 37˚C for 24 h. Each strain was then transferred to TSB with 1% glucose for 24 h at 37˚C to induce

acid adaptation [14] [15]. The 5-strain mixture for the acid-adapted pathogen was prepared by combining 2 ml aliquots of each strain in a 15 ml conical centrifuge tube. Cells were pelleted by centrifugation (1509 × g for 15 min) and washed 3 times and resuspended in 10 ml of Butterfield Phosphate Buffer Solution (BPBS).

2.4. Sample Inoculation and Incubation

For the fermentation challenge, after the mixture of all the ingredients, the starter (Acidic and Non-acidic) mixes were inoculated with the 5-strain mixture of the acid-adapted *E. coli* O157 (10 ml/100ml) and incubated at 25˚C for 10 days for fermentation. For the Ready-to-drink refrigerated products challenge, the treatments prepared by mixing 93% Kombucha (fermented for 10 days) and 7% distilled water/juice were inoculated with the 5-strain mixture of acid-adapted *E. coli* O157 (10 ml/100ml). All treatments were incubated following commercial practice and were first incubated at 25˚C for 24 h and transferred to 21˚C for another 24 h followed by transfer to 5˚C for storage.

2.5. Microbial Analysis and pH Measurement

For the fermentation part of the study, the enumerations were first performed approximately after 30 min of inoculation. Subsequent enumerations were performed at 24 h intervals until the time point of no detectable growth. Similarly, for the kombucha fruit blend portion of the study, treatments were first enumerated approximately after 30 min of inoculation. After that, the treatments were enumerated at 24 h intervals for up to 10 days and after 14 days. For enumeration of *E. coli* O157, 1 ml of sample was pipetted into 9 ml of BPBS, and subsequent serial dilutions were performed. Each sample was then plated in duplicate on Sorbitol MacConkey Agar (Neogen Corp., Lansing, MI). Colonies were enumerated after 24 h of incubation at 37˚C. For pH measurements, approximately 10 ml of sample was taken at each enumeration point and measured using a double junction pH meter (pH Testr 30, Oakton Instruments, Vernon Hills, IL).

2.6. Data Analysis

The bacterial populations were interpreted as the log CFU (Colony Forming Unit) values per milliliter of the product. Three replications of the experiment were conducted for each stage of the experiment. In each replication of the two stages, the samples were inoculated and analyzed in duplicate for *E. coli* O157 counts at different data points. Data points are expressed as mean ± standard deviation. A two-way repeated measure design was used for the fermentation stage where the culture methods used were treatment between subjects. Similarly, a two-way repeated-measures design was used for the refrigerated storage stage where the fruit blend type was treatment between subjects. Analysis of variance (ANOVA) was used to analyze and compare the significance of the differences in mean values at an alpha value of 0.05 using R (version 4.0.4). The

Tukey's method was used for post-hoc analysis to determine the significant differences of mean values at an alpha = 0.05 over all comparisons.

3. Results and Discussions

3.1. Kombucha Fermentation

During the fermentation period, there was a decrease in the pH of the starter mix for both treatments (**Figure 1**). The initial pH of the acidic starter mix, prepared following the instructions provided with the starter culture pack, already had a lower initial pH of 3.27. The pH declined steadily from 3.27 to 3.15 after 4 days of fermentation. After 4 days, there was only a slight change in the pH with final pH of 3.10 after 10 days of fermentation. In the non-acidic starter mix, the initial pH was 6.05 as only washed pellicle from the starter culture pack was used. The pH decreased rapidly after 1 day of fermentation to 4.33, followed by a gradual and steady decrease to a final pH of 3.39 after 10 days. For both the acidic and non-acidic starter mixes, the final pH after 10 days of fermentation the final pH remained above 3, which was higher when compared to two other studies where the pH after 10 days was around 2.2 [16] [17]. However, the rapid initial decrease after 1 day of fermentation followed by a gradual decrease in pH for the non-acidic starter mix did follow a similar trend as observed in the study conducted by Teoh *et al.* [17].

The mean inoculum level added to the acidic starter mix was 9.13 log CFU/ml (**Figure 2**). *E. coli* O157 counts decreased significantly ($P < 0.05$) after 1 day of fermentation to 7.40 log CFU/ml. After 3 days, there were more than 6 log reductions in the pathogen count. The counts reached <1 CFU/ml of kombucha on 4 days, and no pathogen was detected following 4 days. Similarly, the mean inoculum level added to the non-acidic starter mix was 9.26 log CFU/ml. There was a significant decrease in *E. coli* O157 counts after 2 days of fermentation. More than five log reductions in the pathogen count were observed after 6 days, and the pathogens were undetectable after 8 days. A significant difference ($P < 0.05$) in the *E. coli* O157 counts between the acidic and non-acidic starter mixes was observed after 2 days of fermentation. In the acidic starter mix, *E. coli* O157 was inactivated in a shorter period than the non-acidic starter mix. The findings of this study are in agreeance with the study conducted by Brewer *et al.*, where they observed >5 log reduction in Shiga toxin-producing *Escherichia coli* (STEC) within 14 days of fermentation carried out using four commercially available kombucha kits [8]. The decrease in *E. coli* O157 count observed also agrees with a study conducted by Sreeramulu *et al.* where they found inhibition zones of increasing size in agar plates with 24 h old culture of *E. coli* when infused with kombucha of an increasing period of fermentation [3]. Similarly, other studies have also demonstrated the antimicrobial activity of kombucha against *E. coli,* but these studies mostly focused on the antimicrobial activity of kombucha after the end of the desired fermentation period [10] [11] [12] [18].

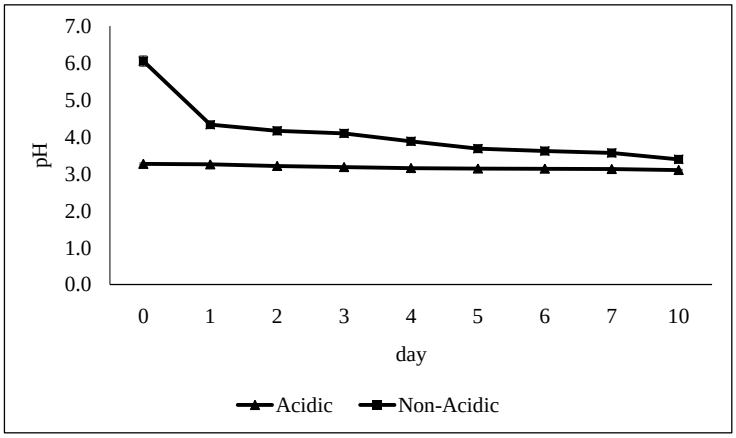

Figure 1. Change in pH during fermentation of Kombucha using acidic and non-acidic starter culture at 25˚C.

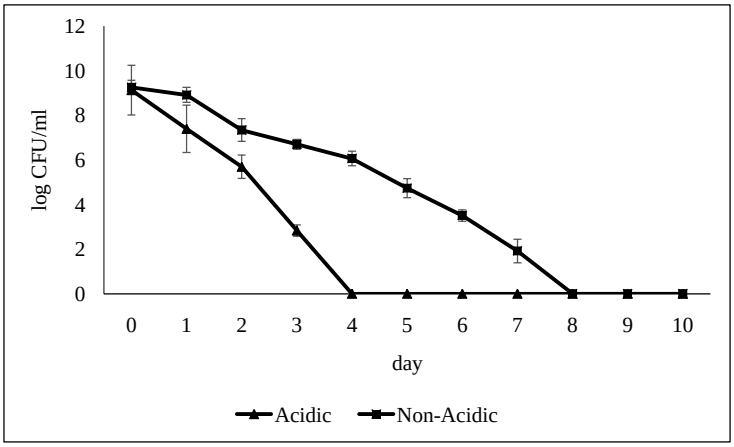

Figure 2. Survival of acid-adapted E. coli O157 during fermentation of Kombucha using acidic and non-acidic starter culture at 25˚C.

3.2. Kombucha Fruit Blend

The pH of the fruit juices were 2.40, 3.53, 3.61, and 3.84 for lemon, mango, apple and orange juice, respectively and the kombucha fermented for 10 days had a pH of 3.09 before adding the fruit juices. The pH of the treatments ranged from 2.68 to 3.49 after adding the fruit juices and distilled water (**Table 1**). The pH of all the treatments only decreased slightly throughout the study, ranging from 0.05 to 0.14. This change in pH is similar to the results observed in other studies relating to kombucha fermentation, where the rate of pH decrease was not prominent after a rapid decrease in pH for a few days of initial fermentation might be due to the complete utilization of all the sugar by microorganisms in the starter culture during the first few days of fermentation [3] [16] [17] [19] [20]. The addition of the fruit juices did not produce any noticeable decrease in pH, which might be due to the inactivation of the starter culture during storage at 5˚C. Inactivation of probiotics, especially the lactic acid bacteria, was also demonstrated in a study conducted to determine changes in probiotics during storage at 4˚C [21].

Table 1. pH of Kombucha fruit blend during incubation at 25˚C (for 24 h) followed by incubation at 21˚C (for 24 h) and storage at 5˚C[1].

	Treatments[2]				
Day	C	L	M	A	O
0	^{A} 3.18 ± 0.04 ^{a}	^{B} 2.68 ± 0.03 ^{ab}	^{A} 3.19 ± 0.03 ^{a}	^{C} 3.29 ± 0.04 ^{a}	^{D} 3.49 ± 0.03 ^{a}
1	^{A} 3.16 ± 0.03 ^{a}	^{B} 2.67 ± 0.02 ^{ab}	^{A} 3.20 ± 0.02 ^{a}	^{C} 3.28 ± 0.03 ^{ab}	^{D} 3.48 ± 0.02 ^{ab}
2	^{A} 3.16 ± 0.01 ^{a}	^{B} 2.67 ± 0.01 ^{ab}	^{A} 3.18 ± 0.03 ^{ab}	^{C} 3.26 ± 0.03 ^{abc}	^{D} 3.48 ± 0.03 ^{ab}
3	^{A} 3.14 ± 0.01 ^{a}	^{B} 2.67 ± 0.02 ^{ab}	^{A} 3.16 ± 0.01 ^{abc}	^{C} 3.26 ± 0.02 ^{abc}	^{D} 3.46 ± 0.01 ^{abc}
4	^{A} 3.14 ± 0.01 ^{a}	^{B} 2.69 ± 0.03 ^{a}	^{A} 3.16 ± 0.03 ^{abc}	^{C} 3.24 ± 0.01 ^{abc}	^{D} 3.45 ± 0.01 ^{abcd}
5	^{A} 3.15 ± 0.01 ^{a}	^{B} 2.68 ± 0.02 ^{ab}	^{A} 3.15 ± 0.01 ^{abc}	^{C} 3.24 ± 0.01 ^{abc}	^{D} 3.47 ± 0.02 ^{abc}
6	^{A} 3.13 ± 0.02 ^{a}	^{B} 2.67 ± 0.01 ^{ab}	^{A} 3.15 ± 0.02 ^{abc}	^{C} 3.23 ± 0.01 ^{bcd}	^{D} 3.45 ± 0.02 ^{abcd}
7	^{A} 3.14 ± 0.02 ^{a}	^{B} 2.66 ± 0.01 ^{ab}	^{A} 3.15 ± 0.01 ^{abc}	^{C} 3.24 ± 0.02 ^{bcd}	^{D} 3.44 ± 0.01 ^{bcd}
8	^{A} 3.14 ± 0.03 ^{a}	^{B} 2.67 ± 0.02 ^{ab}	^{A} 3.13 ± 0.01 ^{bcd}	^{C} 3.23 ± 0.01 ^{bcd}	^{D} 3.42 ± 0.01 ^{cd}
9	^{A} 3.12 ± 0.04 ^{ab}	^{B} 2.67 ± 0.01 ^{ab}	^{A} 3.12 ± 0.02 ^{cd}	^{C} 3.23 ± 0.01 ^{bcd}	^{D} 3.41 ± 0.02 ^{d}
10	^{A} 3.13 ± 0.03 ^{a}	^{B} 2.67 ± 0.02 ^{ab}	^{A} 3.09 ± 0.02 ^{de}	^{C} 3.21 ± 0.02 ^{cd}	^{D} 3.41 ± 0.01 ^{d}
14	^{A} 3.05 ± 0.04 ^{b}	^{B} 2.63 ± 0.02 ^{b}	^{A} 3.05 ± 0.03 ^{e}	^{C} 3.18 ± 0.01 ^{d}	^{D} 3.35 ± 0.03 ^{e}

[1]Data are presented as the mean values of 3 replications. [2]Treatments (93% Kombucha + 7% Fruit Blend): C = Kombucha + Distilled Water, L = Kombucha + Lemon Juice, M = Kombucha + Mango Juice, A = Kombucha + Apple Juice, O = Kombucha + Orange Juice. $^{A\text{ to }D}$ Means preceded by the same uppercase letters in the same row with each day of storage are not significantly different ($P \geq 0.05$). $^{a\text{ to }e}$ Means followed by the same lowercase letters in the same column within each treatment are not significantly different ($P \geq 0.05$).

The mean inoculum level for control, lemon blend, mango blend, apple blend, and orange blend were 9.38, 9.15, 8.96, 9.44, and 9.08 log CFU/ml, respectively (**Table 2**). A significant decrease ($P < 0.05$) was observed in the *E. coli* O157 count in all treatments after 1 day of incubation at 25˚C. *E. coli* O157 counts were undetectable (<1 CFU/ml) in lemon blend and control on 3 and 14 days, respectively. In contrast, the pathogen was detectable in mango, apple, and orange blend after the 14 days of observation. Five-log reduction in the pathogen count was observed in the lemon blend, control, and mango blend after 1, 3, and 14 days, respectively. For the apple and the orange blend, only about 4-log reductions were observed after 14 days of observations. During the duration of the study, the total decrease in *E. coli* O157 was the least for apple blend (4.43 log CFU/ml) followed by orange blend (4.12 log CFU/ml).

For the lemon blend, the rapid decrease of pathogen count can be attributed to the highly acidic nature of the treatment resulting from the naturally present citric acid in lemon juice. A similar rapid and greater than 5-log reduction in acid-adapted *E. coli* O157 was also observed in a study within 72 h in reconstituted single-strength lemon and lime juices stored at 22˚C [22]. In control, the decrease might be due to the lower pH originating from organic acids produced during fermentation and the absence of adequate nutrients for the survival of *E. coli* O157. Although, in the mango, apple, and orange blends, natural organic

Table 2. Survival (log CFU/ml) of acid-adapted *E. coli* O157 in different experimental treatments of Kombucha fruit blend during incubation at 25˚C (for 24 h) followed by incubation at 21˚C (for 24 h) and storage at 5˚C[1].

	Treatments[2]				
Day	C	L	M	A	O
0	^{A} 9.38 ± 0.4 ^{a}	^{A} 9.15 ± 0.1 ^{a}	^{A} 8.96 ± 0.2 ^{a}	^{A} 9.44 ± 0.6 ^{a}	^{A} 9.08 ± 0.1 ^{a}
1	^{A} 5.76 ± 0.2 ^{b}	^{B} 3.56 ± 0.1 ^{b}	^{A} 6.16 ± 0.3 ^{b}	^{C} 7.38 ± 0.3 ^{b}	^{C} 7.83 ± 0.2 ^{b}
2	^{A} 4.64 ± 0.5 ^{bc}	^{B} 1.71 ± 1.5 ^{c}	^{AC} 5.91 ± 0.3 ^{bc}	^{C} 6.72 ± 0.2 ^{bc}	^{C} 7.54 ± 0.4 ^{bc}
3	^{A} 4.22 ± 0.6 ^{c}	^{B} (UD, UD, UD) ^{d}	^{C} 5.75 ± 0.2 ^{bcd}	^{CD} 6.67 ± 0.1 ^{c}	^{D} 7.54 ± 0.7 ^{bc}
4	^{A} 3.94 ± 0.4 ^{cd}	^{B} (UD, UD, UD) ^{d}	^{C} 5.46 ± 0.3 ^{cde}	^{D} 6.59 ± 0.2 ^{c}	^{D} 7.02 ± 0.7 ^{bcd}
5	^{A} 3.93 ± 0.4 ^{cd}	^{B} (UD, UD, UD) ^{d}	^{C} 5.28 ± 0.1 ^{def}	^{D} 6.16 ± 0.1 ^{cd}	^{D} 6.9 ± 0.5 ^{bcd}
6	^{A} 3.64 ± 0.4 ^{cde}	^{B} (UD, UD, UD) ^{d}	^{C} 5.00 ± 0.1 ^{efg}	^{D} 6.10 ± 0.1 ^{cd}	^{E} 6.65 ± 0.2 ^{bcd}
7	^{A} 3.49 ± 0.3 ^{cde}	^{B} (UD, UD, UD) ^{d}	^{C} 4.86 ± 0.2 ^{efg}	^{D} 5.84 ± 0.2 ^{d}	^{D} 6.41 ± 0.3 ^{cd}
8	^{A} 2.99 ± 0.4 ^{de}	^{B} (UD, UD, UD) ^{d}	^{C} 4.73 ± 0.2 ^{fgh}	^{D} 5.86 ± 0.1 ^{d}	^{D} 6.33 ± 0.4 ^{d}
9	^{A} 2.96 ± 0.5 ^{de}	^{B} (UD, UD, UD) ^{d}	^{C} 4.58 ± 0.1 ^{gh}	^{D} 5.76 ± 0.1 ^{d}	^{D} 6.23 ± 0.3 ^{d}
10	^{A} 2.73 ± 0.3 ^{e}	^{B} (UD, UD, UD) ^{d}	^{C} 4.19 ± 0.2 ^{h}	^{D} 5.48 ± 0.2 ^{de}	^{D} 5.91 ± 0.2 ^{de}
14	^{A} (UD, UD, UD) ^{f}	^{A} (UD, UD, UD) ^{d}	^{B} 3.46 ± 0.3 ^{i}	^{C} 5.01 ± 0.1 ^{e}	^{C} 4.89 ± 0.3 ^{e}

[1]Data are presented as the mean values of 3 replications ± standard deviation. UD = undetectable (<1 CFU/ml). $^{A\ to\ E}$ Means preceded by the same uppercase letters in the same row with each day of storage are not significantly different ($P \geq 0.05$). $^{a\ to\ i}$ Means followed by the same lowercase letters in the same column within each treatment are not significantly different ($P \geq 0.05$). [2]Treatments (93% Kombucha + 7% Fruit Blend): C = Kombucha + Distilled water, L = Kombucha + Lemon Juice, M = Kombucha + Mango Juice, A = Kombucha + Apple Juice, O = Kombucha + Orange Juice.

acids like citric and malic acids known for antimicrobial activity are introduced [23] [24] [25]. The prolonged survival of *E. coli* O157 could be due to the lower concentration of the organic acids as well as the comparatively higher pH of the blends resulting from the higher initial pH of the juice itself. Another factor contributing to prolonged survival might be the interference from the added components. Although the pH of the treatments was highly acidic, the survival of *E. coli* O157 in four out of the five treatments for an extended period might be due to the high acid tolerance and acid adaptation of *E. coli* O157 and lower temperature of storage after 2 days as many studies have demonstrated that acid adaptation and refrigeration temperatures enhance the survival time of *E. coli* O157 in highly acidic food environments [26] [27] [28] [29].

4. Conclusions

The study's findings demonstrate that fermentation of kombucha using commercial cultures at room temperature for 10 days produces lethal effects against acid-adapted *E. coli* O157. Although the initial acidity of the kombucha starter kit will influence the duration to achieve lethality of the pathogen, fermentation, when carried out for the recommended duration of time using commercial cultures, the microbial safety of the final product is warranted, especially during home fermentation. However, when refrigerated at 5˚C, the survival period of

acid-adapted *E. coli* O157 in kombucha is enhanced. Also, with the addition of fruit juices in fermented kombucha under refrigeration which is primarily performed for the enrichment of flavor profile, the survival of acid-adapted *E. coli* O157 is prolonged. This is true even when the fruit juices added have low pH and are considered microbiologically safe.

Based on the study's findings, it is evident that 5-log reduction might not be achievable in fermented kombucha mixed with fruit juice(s) under refrigeration. Thus, for home fermenters and small retailers, pasteurized fruit juices are recommended as unpasteurized and fresh-squeezed juices might increase the levels of solids or particulate in the mixture resulting in interference of the antimicrobial activity of the kombucha fruit blend. For foodservice operators considering the addition of unpasteurized fruit juices after the fermentation stage, in addition to the above recommendations, an approved HACCP plan that demonstrates a 5-log reduction of *E. coli* O157 should be implemented, or use of unpasteurized fruit juices should be specified in the label with a warning. Also, for operators who seek to dilute the organic acids or raise the pH of kombucha, such as using higher pH fruit juices like banana or Asian pear, an abundance of caution and considerations in the HACCP plan are recommended as there is an expectation of greater possible survival of *E. coli* O157.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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