Short Communication

Inactivation of Escherichia coli O157:H7 in apple juice and apple cider by trans-cinnamaldehyde

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This study investigated the antimicrobial effect of low concentrations of trans-cinnamaldehyde (TC) on Escherichia coli O157:H7 in apple juice and apple cider. A five-strain mixture of E. coli O157:H7 was inoculated into apple juice or cider at ~6.0 log CFU/ml followed by the addition of TC (0%v/v, 0.025%v/v, 0.075%v/v and 0.125%v/v). The inoculated apple juice samples were incubated at 23 °C and 4 °C for 21 days, whereas the cider samples were stored only at 4 °C. The pH of apple juice and cider, and E. coli O157:H7 counts were determined on days 0, 1, 3, 5, 7, 14 and 21. TC was effective (P<0.05) in inactivating E. coli O157:H7 in apple juice and apple cider. At 23 °C, 0.125 and 0.075%v/v TC completely inactivated E. coli O157:H7 in apple juice (negative by enrichment) on days 1 and 3, respectively. At 4 °C, 0.125 and 0.075%v/v TC decreased the pathogen counts in the juice and cider to undetectable levels on days 3 and 5, respectively. Results indicate that low concentrations of TC could be used as an effective antimicrobial to inactivate E. coli O157:H7 in apple juice and apple cider.

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1. Introduction

Enterohemorrhagic Escherichia coli O157:H7 has emerged into a significant food-borne pathogen in the United States. Although the majority of E. coli O157:H7 outbreaks have been associated with the consumption of undercooked ground beef and raw milk (Armstrong et al., 1996; Hancock et al., 1997; Mao et al., 2001), a variety of acidic foods such as apple juice and apple cider, traditionally considered of low risk, have also been implicated in disease outbreaks (Steele et al., 1982; Anonymous, 1996; 1998; Cody et al., 1999; Rhee et al., 2003). E. coli O157:H7 is an acid tolerant pathogen (Glass et al., 1992; Jordan et al., 1999; Mao et al., 2001) and can survive the low pH of apple juice and cider for more than one to two-weeks of refrigeration (Roering et al., 1999). E. coli O157:H7 may contaminate apple juice and cider by using “drop” or “windfall” apples tainted with the pathogen from animal fecal materials in the soil of orchards (McEllan and Splittstoesser, 1994) or contamination during the manufacturing process (Gower et al., 1979; Viedma et al., 2008). Application of cattle manure as a fertilizer in apple orchards and inadequate washing of apples before processing can also potentially contribute to contamination of the juice or cider (Hancock et al., 1997; Buchanan et al., 1999). The National Advisory Committee on Microbiological Criteria for Foods, of the United States Department of Agriculture (USDA) recommends that manufacture of fruit juices should include effective treatments that can result in a cumulative 5-log reduction in E. coli O157:H7 levels (Anonymous, 1997).

Plant-derived essential oils represent a group of natural antimicrobials that have been traditionally used to preserve foods as well as enhance food flavor. Trans-cinnamaldehyde (TC) is an aldehyde present as a major component of bark extract of cinnamon (Cinnamomum verum) (Holley and Patel, 2005). TC is classified as a GRAS (generally regarded as safe) molecule by the United States Food and Drug Administration and is approved for use in foods (21 CFR 182.60) (Adams et al., 2004). Although TC has been reported to possess an antimicrobial property against food-borne pathogens (Bilgrami et al., 1992; Burt, 2004; Holley and Patel, 2005), its use for improving the safety of apple juice and cider needs to be validated. Moreover, cinnamon is a common flavor used in apple products. Thus this study was undertaken to determine the efficacy of low concentrations of TC for inactivating E. coli O157:H7 in apple juice and apple cider during storage at refrigeration (4 °C) and room temperature (23 °C).

2. Materials and Methods

2.1. Bacterial culture and media

Five different strains of E. coli O157:H7 from our culture collection were used for this study. The strains used included E6 (milk isolate), E10 (meat isolate), E22 (calf feces isolate), E7927 (apple cider isolate) and E1-O (apple juice isolate). All bacteriological media used in the study were purchased from Difco (Sparks, MD). Each strain of the pathogen was cultured separately in 10 ml of sterile Tryptic soy broth.
without dextrose (TSB) at 37 °C for 24 h with agitation (150 rpm). After incubation, the cultures were sedimented by centrifugation (4 °C, 8000×g for 10 min), washed twice, and resuspended in 10 ml of sterile phosphate buffered saline (PBS, pH 7.3). The bacterial population in each culture was determined by plating 0.1-ml portions of appropriately diluted culture on duplicate Tryptic soy agar plates (TSA), with incubation at 37 °C for 24 h. Equal volumes containing approximately equal populations from each of the five strains were combined, and 200 μl of the appropriately diluted suspension was used as the inoculum (~7 log CFU). The bacterial count of the five-strain mixture of the pathogen was also confirmed by plating 0.1-ml portions of appropriate dilutions on TSA plates.

2.2. Trans-cinnamaldehyde

TC (catalog # 239968) was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). TC concentrations used for this study included 0.025, 0.075, and 0.125% v/v.

2.3. Sample preparation, inoculation and storage

Fresh pasteurized apple juice and apple cider free of any preservatives were purchased from a local grocery store. The apple juice and cider were tested for the presence of E. coli O157:H7 by directly plating on Sorbitol MacConkey agar (SMA) supplemented with 0.1% 4-methylumbelliferyl-β-D-glucuronide (MUG, Oxoid, Ogdensburg, N. Y.), or inoculating 1 ml of juice or cider in 10 ml of TSB and incubating at 37 °C for 24 h. The enrichment was done in TSB because selective broths can potentially inhibit the recovery of acid-stressed and TC injured E. coli O157:H7. Following incubation, the enriched culture was streaked on SMA + MUG plates.

A volume of 200 μl of the appropriately diluted five-strain mixture of the E. coli O157:H7 was separately added to 20-ml aliquots of the apple juice or apple cider to obtain an inoculation level of approximately 6.0 log CFU/ml, followed by addition of one of the three concentrations of TC. A volume of 5 μl, 15 μl, and 25 μl of TC was added to 20 ml of apple juice to obtain 0.025, 0.075, and 0.125% v/v concentrations respectively. Inoculated apple juice or apple cider samples without any added TC (0%v/v) served as controls. The inoculated samples of apple juice were incubated at 4 °C and 23 °C, whereas the apple cider samples were stored only at 4 °C.

2.4. Microbiological analysis

The population of E. coli O157:H7 in apple juice and cider was enumerated on days 0, 1, 3, 5, 7, 14 and 21 by plating directly or after serial dilutions (1:10 in PBS) on duplicate TSA and SMA plates. Representative colonies (~3–4 colonies on each plate) were confirmed as E. coli O157:H7 by streaking on SMA + MUG plates and using the E. coli O157 latex agglutination test kit (Oxoid). When E. coli O157:H7 was not detected by direct plating, samples were tested for surviving cells by enriching 1 ml of the juice and cider samples in 100 ml of TSB for 24 h at 37 °C, followed by streaking on SMA + MUG plates. Three samples for each treatment and control were included at each of the specified temperatures, and the entire study was replicated thrice.

2.5. pH measurement

The pH of each treatment and control apple juice and cider was determined using an Accumet pH meter (Fisher) on days 0, 1, 3, 5, 7, 14 and 21.

2.6. Statistical analysis

For each treatment, the data from independent replicate trials were pooled and were analyzed using the general linear model of Statistical Analysis software (the SAS system for Windows, version 8, SAS Institute, Cary, N. C.). The model included the treatment concentrations, storage temperature and time as the major effects. Least significant difference test was used to determine significant differences (P<0.05) due to TC concentrations and storage temperature on E. coli O157:H7 counts.

3. Results

Direct plating of the uninoculated apple juice and cider on day 0 did not yield any yeast, mold, E. coli O157:H7 or inherent bacteria on the respective media plates. Since selective enumeration media such as SMA can potentially inhibit the recovery of acid-stressed and TC injured E. coli O157:H7, the population of surviving E. coli O157:H7 in apple juice and cider was enumerated on both TSA and SMA. The counts of E. coli O157:H7 recovered on SMA were slightly lower (P<0.05) than those obtained on the non-selective medium (TSA). Therefore, E. coli O157:H7 counts from the TSA plates were used for statistical analysis and discussion.

The mean pH of the apple juice and apple cider was 3.80±0.02 and 3.60±0.02, respectively. Addition of TC did not result in any significant change in the juice or cider pH (data not shown).

The effect of TC on E. coli O157:H7 in apple juice stored at 23 °C is shown in Fig. 1. The average population of E. coli O157:H7 in the
treatment and control samples at 0 h was \( \sim 5.5 \log \text{CFU/ml} \). TC concentrations at 0.125%v/v and 0.075%v/v completely inactivated \( E. \ coli \) O157:H7 (enrichment negative) on days 1 and 3 of storage, respectively, whereas 0.025%v/v TC reduced the pathogen to undetectable levels on day 5. However, \( E. \ coli \) O157:H7 population in the control samples decreased gradually and the juice samples tested negative for the pathogen only on day 14 of storage.

The fate of \( E. \ coli \) O157:H7 in control and TC-supplemented apple juice and apple cider stored at 4 °C is presented in Figs. 2 and 3 respectively. \( E. \ coli \) O157:H7 population in the control juice and cider samples declined by \( \sim 5 \) and 2.5 log CFU/ml respectively, over the 21-day storage period. However, the pathogen populations in the treated juice and cider were reduced to undetectable levels on days 3, 5 and 14 by 0.125, 0.075 and 0.025%v/v TC, respectively.

4. Discussion

\( E. \ coli \) O157:H7 populations in apple juice and cider can be inactivated by heat treatment such as pasteurization, however, potential concerns with alteration in composition and flavor properties of thermally processed fruit juices exist (Buchanan et al., 1998). Moreover, commonly used preservatives in cider such as potassium sorbate and sodium benzoate have been shown to exert only minimal lethal effect on \( E. \ coli \) O157:H7 (Ulijas and Ingham, 1999). These aforementioned problems along with a consumer demand for minimally processed foods could potentially be used as an effective antimicrobial to inactivate \( E. \ coli \) O157:H7 at room temperature compared to those at refrigeration temperature (Yuste and Fung, 2004). Similarly, \( E. \ coli \) O157:H7 survived at a greater rate at 4 °C compared to room temperature in the control samples, which could also be attributed to the lower metabolic, growth and death rates of \( E. \ coli \) O157:H7 at room temperature compared to 23 °C (Fig. 1). However, the same concentration of TC brought about significant magnitude of reduction in bacterial counts only by the end of the second week of storage at 4 °C (Fig. 2). Similar differences in pathogen inactivation at these temperatures were also observed with the higher TC concentrations, although not as prominent as that observed with 0.025%v/v TC. A similar finding was previously reported by Yuste and Fung (2004), who also reported a higher rate of inactivation of \( E. \ coli \) O157:H7 and \( S. \ aureus \) in apple juice by a combination of nisin and cinnamon at 20 °C compared to 5 °C. The enhanced antibacterial effect of TC at 23 °C could be attributed to the higher metabolic, growth and death rates of \( E. \ coli \) O157:H7 at room temperature compared to 4 °C.

For example, at 23 °C, 0.125, 0.075 and 0.025%v/v TC completely inactivated \( E. \ coli \) O157:H7 (negative by enrichment) on days 1, 3, and 5 of storage, respectively (Fig. 1). Similarly, at 4 °C, complete killing of \( E. \ coli \) O157:H7 (enrichment negative) was observed in juice and cider containing 0.125 and 0.075%v/v TC on days 3 and 5 respectively, whereas samples containing 0.025%v/v TC tested positive for the pathogen until day 14 of refrigerated storage (Fig. 2).

Temperature was another factor that exerted a significant effect (\( P<0.05 \)) on the antibacterial activity of TC on \( E. \ coli \) O157:H7 in apple juice. For example, 0.025%v/v TC decreased \( E. \ coli \) O157:H7 counts to undetectable levels by 5 days of storage at 23 °C (Fig. 1). However, the same concentration of TC brought about significant magnitude of reduction in bacterial counts only by the end of the second week of storage at 4 °C (Fig. 2). Similar differences in pathogen inactivation at these temperatures were also observed with the higher TC concentrations, although not as prominent as that observed with 0.025%v/v TC. A similar finding was previously reported by Yuste and Fung (2004), who also reported a higher rate of inactivation of \( E. \ coli \) O157:H7 and \( S. \ aureus \) in apple juice by a combination of nisin and cinnamon at 20 °C compared to 5 °C. The enhanced antibacterial effect of TC at 23 °C could be attributed to the higher metabolic, growth and death rates of \( E. \ coli \) O157:H7 at room temperature compared to those at refrigeration temperature (Yuste and Fung, 2004).

The results of this study indicate that TC at low concentrations could potentially be used as an effective antimicrobial to inactivate \( E. \ coli \) O157:H7 in apple juice and cider. Although addition of TC to apple juice and cider did not result in any change in the pH and appearance of the juice, sensory studies to determine the organoleptic properties of apple juice containing TC are necessary.

References


Cody, S.H., Glynn, M.K., Farrar, J.A., Cairns, K.L., Grif...