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Inactivation of *Escherichia coli* O157:H7 in Apple Juice as Affected by Cranberry Juice Concentration and Holding Temperature

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ABSTRACT

Cranberry juice concentration and holding temperature were evaluated for reducing *Escherichia coli* O157:H7 populations in pasteurized apple juice. Pasteurized, 100% cranberry (CJ) and apple juices were combined to yield mixtures containing 0 (control; pH 3.83), 10 (pH 3.55), 20 (pH 3.35), 30 (pH 3.20), 40 (pH 3.07), and 50% (pH 2.95) CJ. *E. coli* O157:H7 (5-strain mixture) was inoculated into the juice mixtures to obtain an initial population of approximately 7 log CFU/mL. Juices held at 4 and 25°C were sampled at 24-h intervals for up to six days, while juices held at 45°C were sampled at 0.5- or 1-h intervals for up to 8 h. Samples (in phosphate buffer, pH 6.4 – 6.63) were plated in duplicate on tryptic soy agar (TSA) and sorbitol MacConkey agar (SMAC). After six days storage at 4°C, *E. coli* O157:H7 populations were reduced < 1 log CFU/mL in 0 - 30% CJ mixtures, but were reduced by > 2 and 4 log CFU/mL in 40 and 50% CJ, respectively. In juices held at 25°C, *E. coli* O157:H7 populations were undetectable in 10% CJ after 120 h, in 20 and 30% CJ after 48 h, and in 40 and 50% CJ after 24 h. The population in 0% CJ was reduced by 5 log CFU/mL after 120 h. At 45°C, *E. coli* O157:H7 was reduced to non-detectable levels in 30, 40, and 50% CJ after 6, 4.5, and 4 hours, respectively. Reductions of about < 1, 2, and 6 log CFU/mL were observed in 0, 10, and 20% CJ, respectively. In all samples, substantial proportions of populations were sub-lethally injured during holding as indicated by poorer recover on SMAC in comparison to TSA; injury development was more pronounced at higher holding temperatures. At 45°C, 100% injury was observed in 20, 30, 40, and 50% CJ after holding for 7, 5.5, 4, and 3.5 h, respectively. When combined with temperatures of 25 or 45°C, with minimal holding time, concentrations of 30-50% pure CJ could serve to effectively reduce *E. coli* O157:H7 populations in juice.

INTRODUCTION

Recent outbreaks involving *Escherichia coli* O157:H7 in apple juice and cider has prompted research focused on developing practical, inexpensive, but effective methods for controlling contamination in unpasteurized juice. According to Rangel and others (2005), seven of the 183 *E. coli* O157:H7 outbreaks reported from 1982-2002 were from apple cider or apple juice. In response to these outbreaks associated with fruit juices, the U.S. Food and Drug Administration (FDA) issued regulations requiring juice processors to reduce the population of the pertinent microorganism by at least 5.0 log units (5D) by pasteurization or other treatments (FDA 2001). This reduction must be obtained in populations of the most resistant pathogen in their finished product compared to levels that may be present in untreated juice. Juice processors not achieving the population reduction are required to label each juice or cider container with a warning statement (FDA 1998).

Thermal pasteurization is considered the method of choice for achieving the specified pathogen reduction. However, due to cost of the pasteurization process to small processors (Kozempel and others 1998), anticipated changes in quality, and the consumer demand for non-thermally treated products, alternative methods are being investigated. Buchanan and others (1998) concluded that UV irradiation, which is FDA approved, would be sufficient to achieve a 5D inactivation at a dose of 1.8 kGy, and Quintero-Ramos and others (2004) agreed that the UV dose was very effective at inactivating *E. coli* O157:H7. Other processes that have been investigated include treatment with ozone (Williams and others 2004) and supercritical fluid processing (Mermelstein 1999).

Another method of pathogen inactivation in juices that is actively being explored is the addition of natural compounds with known antimicrobial activity. Some of these compounds

include essential oils such as carvacrol and p-cymene (Kisko and Roller 2005) and organic acids, such as benzoic and sorbic acids, and their salts (Koodie and Dhople 2001; Zhao and others 1993). Others have evaluated the indirect effects of organic acids on survival of *E. coli* O157:H7 in pineapple juice (Mutaku and others 2005), cranberry, lemon, and lime juice concentrates (Nogueira and others 2003), apple juice, and orange juice (Semanchek and Golden 1996; Williams and others 2004; Williams and others 2005). Specifically, the addition of cranberry juice to apple juice and cider has been investigated, likely due to the known antimicrobial properties of cranberries and their positive association with treatment of urinary tract infections (Raz and others 2004). Marwan and Nagel (1986) found that proanthocyanidins, flavonols, and benzoic acids in pure cranberry juice provided the majority of microbial inhibition. A "cran-cider process", which is the addition of cranberry juice at 15% (v/v) followed by warm hold (45 °C for 2 h) and freeze-thaw steps (-20 °C for 24 h, 5 °C for 24 h), was demonstrated to achieve the FDA-mandated pathogen reduction requirements (Ingham and others 2006).

The purpose of this investigation was to evaluate the effects of cranberry juice concentration and holding temperature on inactivation of *E. coli* O157:H7 to determine if these treatments would provide the regulatory 5D reduction.

MATERIALS AND METHODS

Preparation of inoculum

Five strains of *E. coli* O157:H7 [43888 (isolated from human feces), 43889 (feces of patient with hemolytic uremic syndrome, NC), 43890 (human feces, CA), 43894 (human feces from outbreak of hemorrhagic colitis, MI), 43895 (from raw hamburger meat implicated in a hemorrhagic colitis outbreak)], were used to inoculate juices. Test stains were cultured in tryptic

soy broth (TSB; Difco Becton Dickinson Microbiology Systems; Sparks, MD) for 24 h at 37°C. Cultures were transferred a minimum of three times at 24 h intervals before use. The five test strains were combined to yield a mixed culture containing equal proportions of each strain (25 mL total volume).

Preparation of juices

Pasteurized apple juice (100% apple juice from concentrate, no added sugar, and no preservatives) was purchased from a local supermarket. Pasteurized, 100% cranberry juice (CJ) (no added sugar, with added vitamin C) was purchased from a local health foods specialty store. Juices were stored at room temperature until opened, after which they were stored at 4 °C. Apple juice and CJ were combined in sterile 500 mL bottles to yield 250 mL of juice containing 0, 10, 20, 30, 40, and 50 % (v/v) CJ. The resultant pH was recorded for each mixture immediately after addition of CJ. Prepared juice mixtures were allowed to reach appropriate temperature (4, 25, or 45 °C) before inoculation.

Inoculation and sampling of juices

Juices were inoculated with 2.5 mL of a 24-h mixed culture (to yield about 7 log CFU/mL) and gently mixed to suspend cells. One milliliter samples were taken from each bottle at 24-h intervals for up to six days (144 h) for juices held at 4 and 25°C, while juices held at 45 °C were sampled at 0.5- or 1.0-h intervals for up to 8 h. Samples were serially diluted in 0.1 M phosphate buffer (PB; Becton Dickinson Microbiology Systems; Sparks, MD).

Enumeration of juice samples

Juice samples were surface plated on tryptic soy agar (TSA; Difco Becton Dickinson Microbiology Systems; Sparks, MD) and sorbitol MacConkey agar (SMAC; Oxoid Limited; Hampshire, England) in duplicate using a spiral plater (Don Whitley Scientific Limited;

Yorkshire, England). Plates were incubated for 48 h at 37 °C before enumeration of *E. coli* O157:H7 using a Protocol automatic plate counter (Synoptics Limited; Cambridge, UK).

Data analysis

All experiments were replicated three times. The statistical model consisted of a randomized block design, blocking on replication. Statistical analysis was conducted using the mixed models procedure (PROC MIXED) of SAS® 9.1 (SAS Institute Inc.; Cary, NC). Analysis of variance was used to determine statistical differences in survival of pathogens in juice.

RESULTS AND DISCUSSION

Initial pH of juice mixtures was 3.83, 3.55, 3.35, 3.20, 3.07, and 2.95 for 0, 10, 20, 30, 40, and 50 % CJ, respectively. Most samples were diluted in 0.1 M PB before plating, which sample pH to 6.4-6.63 before being plated. However, near the end of sampling, when *E. coli* O157:H7 populations were reduced to near the detection limit, it was necessary to surface plate directly from juice mixtures. We observed that plating samples at lower pH did not adversely affect recovery.

The effects of percent CJ, holding temperature, time, and all interactions were significant ($P<0.01$). Addition of CJ had a significant effect on lethality of *E. coli* O157:H7 ($P< 0.01$), which increased with percent CJ at all holding temperatures. Lethality was significantly greater ($P<0.01$) with increasing time and holding temperature. Ingham and others (2006) also found that the addition of CJ and subsequent holding temperature treatments significantly affected lethality of *E. coli* O157:H7 and *Salmonella* serovars. The most lethal treatment in this study was 50 % CJ held at 45 °C, where the combinations of low pH, CJ components, and elevated temperature provided the maximum synergistic effect.

In all samples, substantial proportions of populations were sub-lethally injured during holding as indicated by poorer recovery on SMAC as compared with recovery on TSA. Development of injury was more pronounced at higher holding temperatures, with 100% injury observed in 20, 30, 40, and 50% CJ after holding for 7, 5.5, 4, and 3.5 h, respectively at 45 °C. The development of injury, without death, is an important factor to consider, since injured organisms are typically more susceptible to additional, adverse treatments. As such, combinations of treatments that alone would not result in lethality could provide adequate reduction of *E. coli* O157:H7 in apple juice, as was observed in this study. Survival of *E. coli* O157:H7 held at 45 °C is illustrated in Figure 1. Populations were reduced to below detectable levels in 30, 40, and 50% CJ after 6, 4.5, and 4 h, respectively. A near 6-log reduction was observed in 20% CJ, while only a 1-2 log reduction occurred at 0 and 10 % CJ. Ingham and others (2006) reported similar results, stating that application of a warm hold (45 °C for 2 h) to 10 or 15 % CJ does not achieve the 5-log reduction target.

Figure 2 shows survival of *E. coli* O157:H7 held at 25 °C. Populations were undetectable in 20 and 30% CJ after 48 h, and in 40 and 50% CJ after 24 h. Mutaku and others (2005) found that, in pineapple juice (pH 3.57), a decline in *E. coli* O157:H7 populations occurred during ambient (20-25 °C) temperature storage but complete inhibition was not observed after 120 h. In the present study a decline in *E. coli* O157:H7 population in 0% CJ (pH 3.83) was observed, and complete inhibition in 10% CJ (pH 3.55) after 120 h storage at 25 °C. Uljas and Ingham (1999) found that when combined with freeze-thaw steps, holding pH 3.7 apple cider at 25 °C for 2 h provided the targeted 5-log reduction of *E. coli* O157:H7.

Marques and others (2001) demonstrated survival of *E. coli* O157:H7 during prolonged exposure to a pH range of 2.51-3.26, confirming that acid resistance systems remain active over

prolonged periods of cold storage. Similarly, we observed enhanced survival of *E. coli* O157:H7 held at 4 °C (Figure 3).. After 120 h of storage at 4 °C, populations were reduced < 1 log CFU/mL in 0-30% CJ, and 2- and 4-log reductions occurred in 40 and 50% CJ, respectively. Population reductions at 4 °C were much lower than at warmer holding temperatures, with none of the CJ mixtures providing a 5 log reduction at 4°C.

CONCLUSIONS

Results of this study demonstrate that the addition of CJ to apple juice, in combination with elevated holding temperature and time treatments, can provide the FDA mandated 5-log reduction in *E. coli* O157:H7. The purpose of this investigation was to evaluate and determine the most effective combination of added CJ and temperature. It was determined that when combined with warm hold temperatures of 25 or 45 °C, 30-50 % CJ could serve to effectively reduce *E. coli* O157:H7 populations in juice; 50% CJ would be the most effective concentration, but this would likely result in a product with that consumers find unacceptable due to undesirable sensory attributes of high CJ concentrations. Further study with individual antimicrobial components of CJ and their addition to juices is warranted, since the individual components could provide satisfactory inhibition without the undesirable sensory characteristics associated with CJ.

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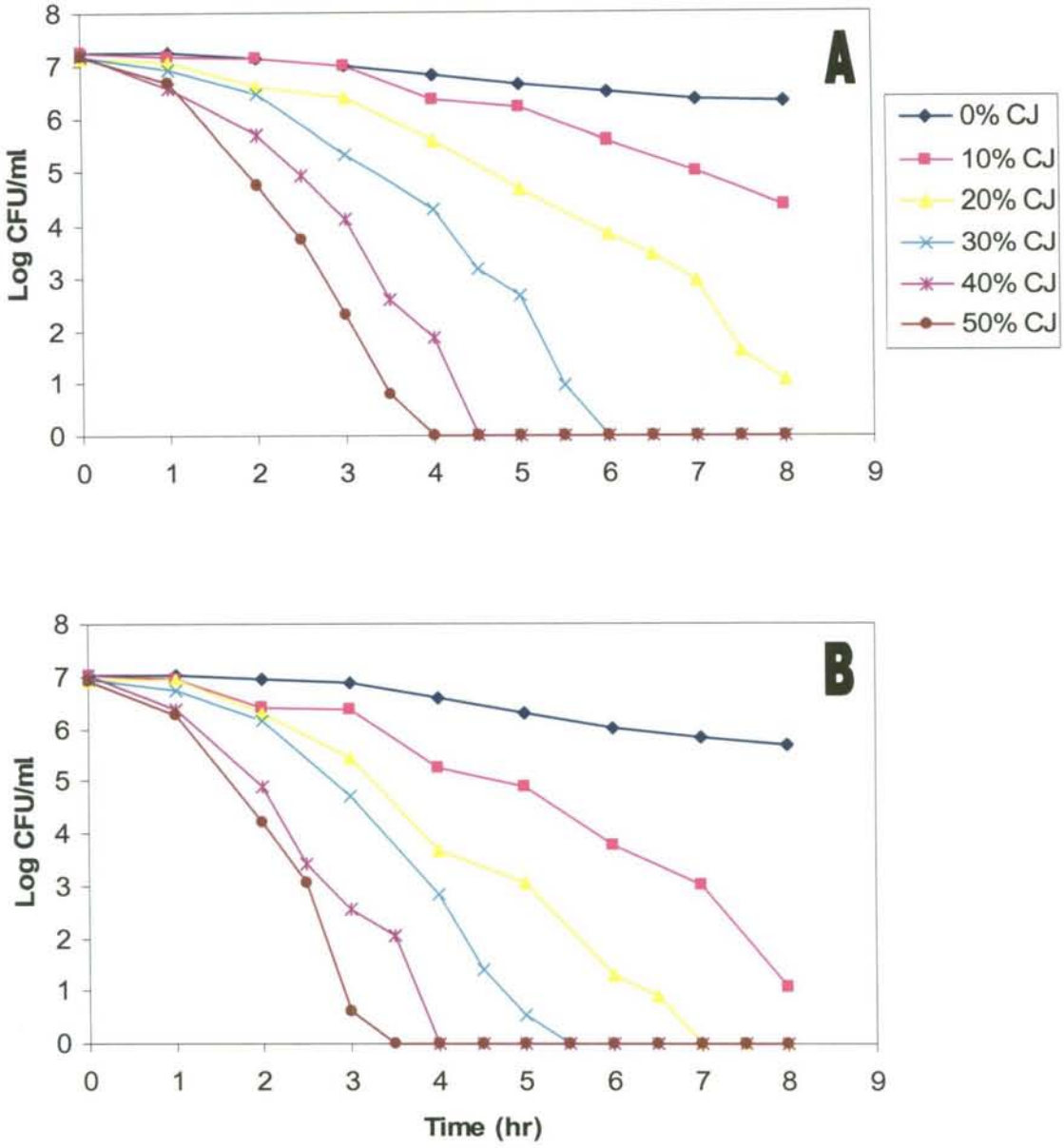

Figure 1. Survival of *E. coli* O157:H7 in apple juice containing 0-50 % cranberry juice (CJ) held at 45 °C and recovered on TSA (A) and SMAC (B). Data points represent means of three trials. Values shown as 0 Log CFU/mL represent no detection.

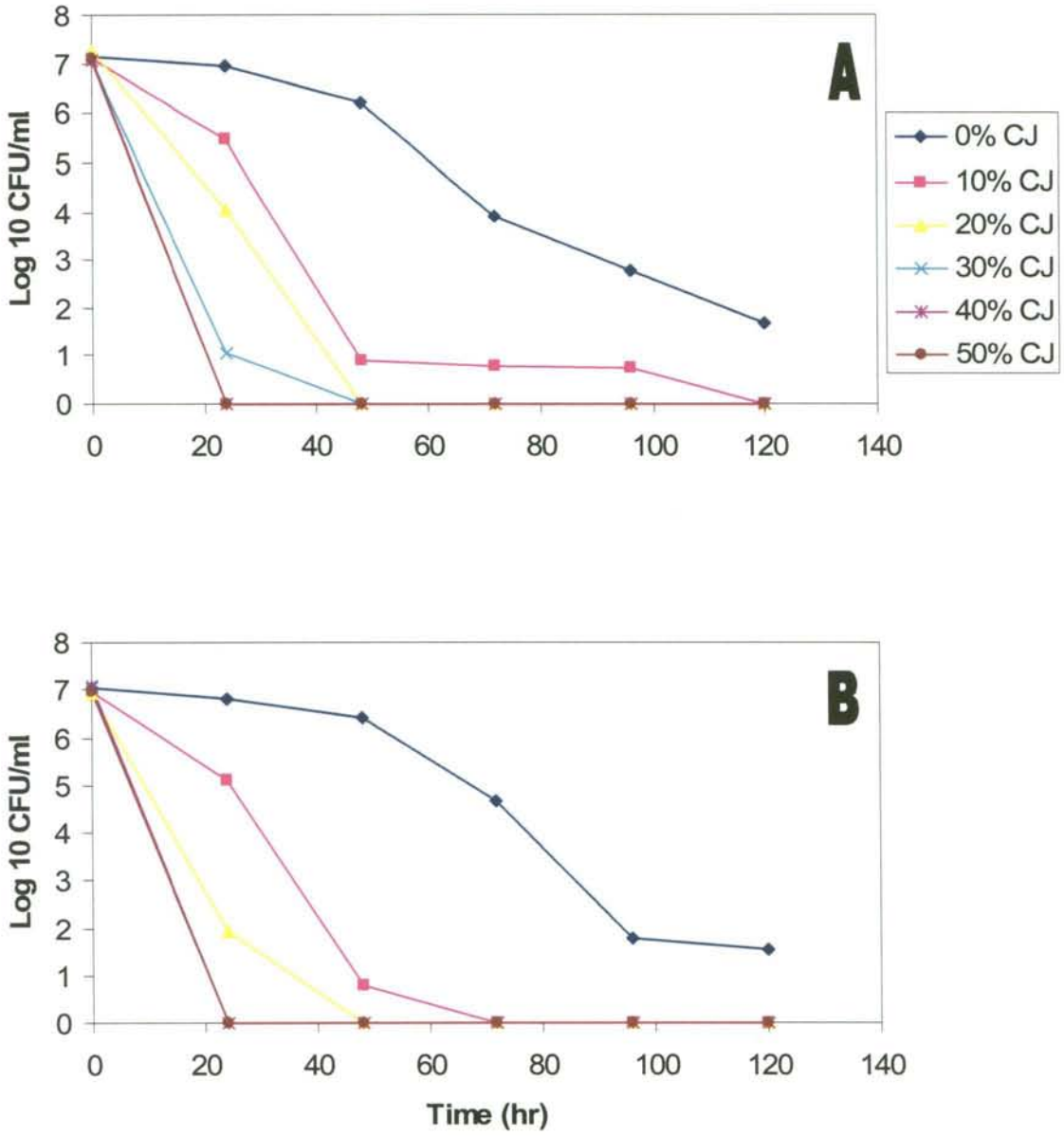

Figure 2. Survival of *E. coli* O157:H7 in apple juice containing 0-50 % cranberry juice (CJ) held at 25 °C and recovered on TSA (A) and SMAC (B). Data points represent means of three trials. Values shown as 0 Log CFU/mL represent no detection.

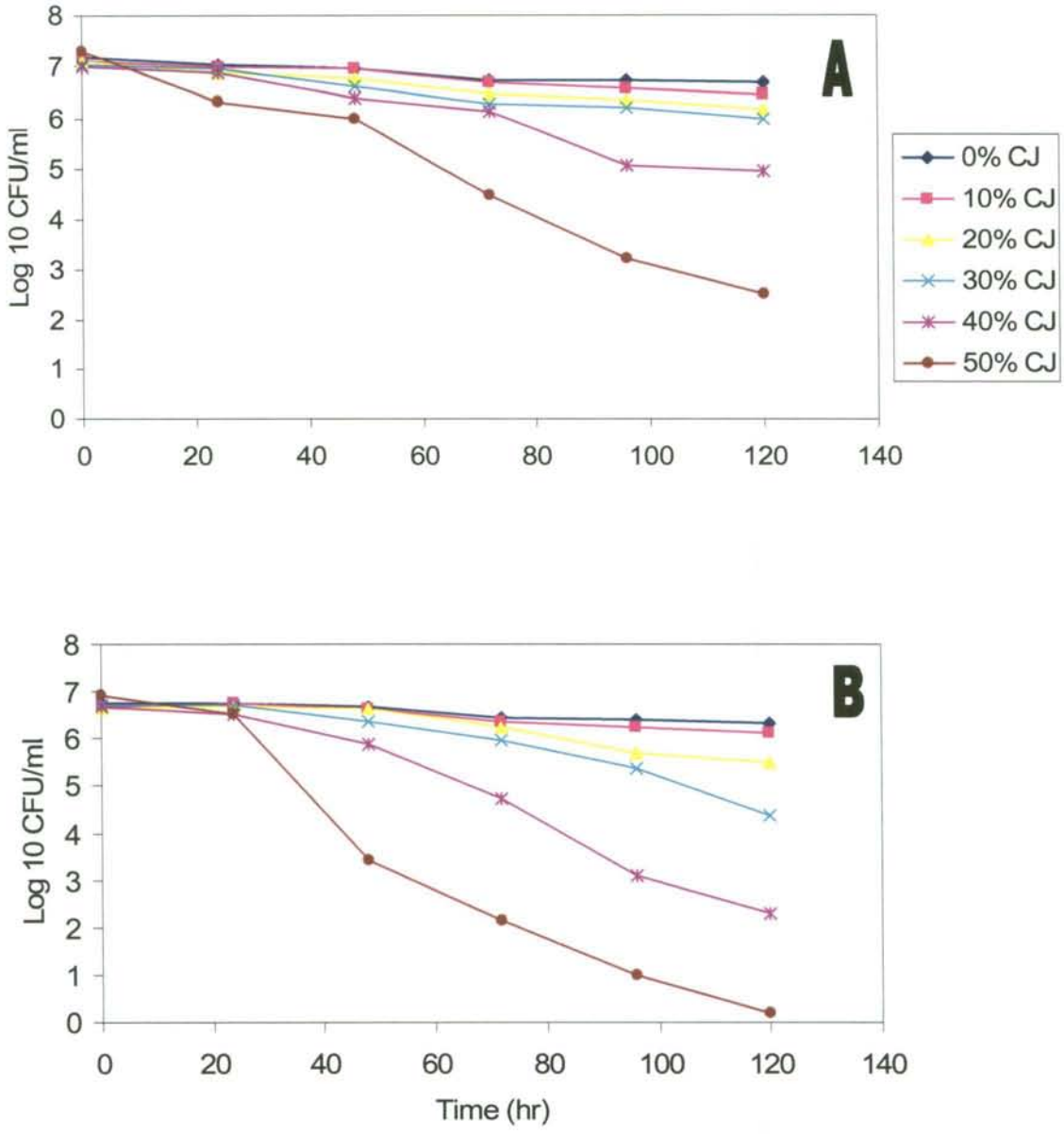

Figure 3. Survival of *E. coli* O157:H7 in apple juice containing 0-50 % cranberry juice (CJ) held at 4 °C and recovered on TSA (A) and SMAC (B). Data points represent means of three trials. Values shown as 0 Log CFU/mL represent no detection.