

**ANTIBACTERIAL ACTIVITY OF HYDROGEN PEROXIDE AGAINST
ESCHERICHIA COLI O157:H7 AND *SALMONELLA* SPP. IN FRUIT JUICES,
BOTH ALONE AND IN COMBINATION WITH ORGANIC ACIDS**

by

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ABSTRACT

The antibacterial efficacy of hydrogen peroxide treatments in four fruit juices was determined. Preservative free apple cider, white grape, and purple grape juice were inoculated with ~ 6.4 log CFU/ml of a five strain, acid adapted, nalidixic acid resistant *E. coli* O157:H7 cocktail. Orange juice was inoculated with a comparable *Salmonella* spp. cocktail. In the first study, 0.017% and 0.012% H₂O₂ was added in combination with 0.1% and 0.3% of the dominant organic acid (OA) to 4°C and 25°C juices, with samples taken each day for 21 days. H₂O₂ was a significant factor in all juices (p < 0.05) except white grape (lack of data), and both 0.017% H₂O₂ treatments reduced counts in apple cider, orange juice, and white grape to undetectable numbers within 48 hrs as cultured on tryptone soy agar + 0.05% nalidixic acid (TSAN). Treatments in purple grape juice were less effective overall, and more dependent on OA concentration (p < 0.001) than H₂O₂.

There were instances where bacterial survival in apple cider, purple grape, and orange juice continued for 21 days after treatment, and sometimes outlasted the control. These occurrences were dependent on temperature (25°C) and H₂O₂ (0.012%), but not on OA. However, OA concentration was a significant factor (p < 0.05) overall in apple cider and purple grape juice, but not in orange juice.

In the second study, 0.015% and 0.03% H₂O₂ was added to 10, 25, and 40°C apple cider and orange juice inoculated with 6.4 log CFU/ml *E. coli* O157:H7 and *Salmonella* spp. respectively. Only 0.03% H₂O₂ was effective in reducing counts to undetectable numbers in both juices. However, both temperature and H₂O₂ were significant factors (p < 0.0001) in bacterial destruction, with 0.03% H₂O₂ at 40°C giving

undetectable numbers at ≤ 3 and ≤ 6 hours in orange juice and apple cider respectively.

It has been demonstrated that at $\sim \geq 0.017\%$, H_2O_2 can provide a 5 log reduction of these pathogens in fruit juice. Increasing temperature and organic acid concentration can improve its rate of effectiveness in certain juices. However, sensory concerns may negate its use in some products.

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INTRODUCTION

Fresh, unpasteurized fruit juices hold a favorable appeal to many consumers due to their distinct flavor characteristics and perceived nutritional superiority. Producers of unpasteurized juice have traditionally relied upon a juice's inherent acidity to render their product microbiologically safe. However, documented outbreaks of *Salmonella* and *Escherichia coli* O157:H7 associated with unpasteurized juices have dispelled this belief. As early as 1922 and 1944, outbreaks of typhoid fever have been linked to sweet cider and orange juice consumption (Parish, 1997). In 1980, before recognition of *E. coli* O157:H7 as a human pathogen, an outbreak of hemolytic uremic syndrome (HUS), likely from *E. coli*, was reported in apple cider (Parish, 1997). Sixty six cases of *E. coli* O157:H7 infection, including the death of a child resulted from a 1996 outbreak of *E. coli* O157:H7 in unpasteurized apple cider (CDC, 1997). More recently, between 1999 and 2000, over 500 confirmed cases of *Salmonella* infection resulted from contaminated orange juice (66 FR 6138).

Contamination could arise from fecal contact, but also other sources. Fecal contamination from the use of dropped, unwashed apples has been implicated as the source of *E. coli* in some apple cider outbreaks (Besser et al., 1993; CDC, 1996). However, vectors such as birds could potentially deposit this pathogen on tree-bound fruit (Wallace et al., 1997). In a 1995 outbreak of Salmonellosis from unpasteurized orange juice, *Salmonella* spp. were isolated from amphibians around the processing facility (Parish, 1997).

As further support of the need for concern, researchers have demonstrated the ability of both *E. coli* and *Salmonella* to survive for periods of 18 days or more in fruit juice (Fisher and Golden, 1998; Linton et al., 1999). In addition, prior exposure to sub-lethal pH (~ 5.0) incurs adaptive mechanisms which greatly enhance their ability to withstand acidic conditions (Ryu et al., 1999; Brudzinski and Harrison, 1998). For *E. coli*, such adaptation could potentially be induced while inhabiting the bovine digestive system, thereby increasing resistance to juice acidity, as well as stomach acidity upon ingestion (Lin et al., 1996).

In response to these outbreaks and studies, the FDA has taken regulatory action

dealing with the reduction of pathogens in fresh fruit juice. The final ruling on January 19, 2001 required the use of Hazard Analysis Critical Control Point (HACCP) principles for juice processing, with procedures capable of attaining a 5 log reduction in the pertinent pathogen. In the case of apple cider and orange juice, this would be *E. coli* O157:H7 and *Salmonella* spp. respectively, but *E. coli* is recommended for those juices with no specific product/pathogen association. The compliance period is three tiered based on processor size, with small processors having 36 months to comply (66 FR 6138). During this time, non-pasteurized juice producers will be looking for economically viable methods of juice processing.

Given the breadth of evidence supporting potential internalization of pathogens into fruit (Zhuang et al., 1995; Buchanan et al., 1999; Merker et al., 1999), simple wash procedures may not provide an adequate solution to the problem. A pathogen reduction step for the expressed juice is necessary. Pasteurization is not mandated as part of the HACCP plan, thus leaving room for non-thermal approaches. Though pasteurization offers a reliable method for pathogen reduction, economic and sensory concerns make it undesirable for many processors. Pasteurization costs increase greatly at lower production scales, while flavor and aroma profiles are affected (Kozempel et al., 1998; Su and Wiley, 1998). A possible alternative may be the use of chemical applications such as hydrogen peroxide and organic acids.

The bactericidal efficacy of hydrogen peroxide has been demonstrated in both water and food systems (Yoshpe-Purer and Eylan, 1968; Liao and Sapers; 2000; Forney et al., 1991), with gram negative organisms having the most susceptibility (Davidson and Branen, 1993; Lillard and Thomson, 1983). It has been effective in extending the shelf life of cantaloupe, mushrooms, bell peppers, grapes, and raisins (Sapers and Simmons, 1998; Forney et al., 1991). This antimicrobial action stems from its ability to form reactive oxygen species such as the hydroxyl radical and singlet oxygen, which can damage DNA and membrane constituents (Juven and Pierson, 1996). In addition, its effectiveness appears to improve at both higher temperatures and more acidic pH (Toledo et al., 1973; Baldy, 1983). An important characteristic of this molecule is that it rapidly degrades into oxygen and water upon contacting organic material, thus having no long

term residual activity. However, any residual peroxide could be removed by adding catalase (Davidson and Branen, 1993). Hydrogen peroxide has GRAS (Generally Regarded as Safe) status, and is currently allowed as an antimicrobial in starch processing and in milk for cheese manufacturing (21 CFR 184.1366).

As natural components of fruits, organic acids such as malic, citric, and tartaric acid lower the pH and help maintain the proper sugar/acid balance in fruit juices (Nagy et al., 1993). In broth systems, their bacteriostatic and bacteriocidal effects have been evaluated for both *E. coli* O157:H7 and *Salmonella* (Chung and Goepfert, 1970; Conner and Katrola, 1995; Ryu et al., 1999), with pH and degree of dissociation being major factors in a particular acids efficacy. Undissociated acids are more cell permeable, and upon entering the cell can dissociate and lower intracellular pH (Lück and Jager, 1997). Thus, organic acids can affect both the intercellular and extracellular pH. As a natural method of lowering juice pH, increasing the organic acid concentration of juices may improve the antimicrobial efficacy of hydrogen peroxide treatments.

Sensory changes as a result of hydrogen peroxide and organic acid additions are an important consideration. By contributing a sour or acidic taste, organic acids are only practical in concentrations that do not adversely upset the sugar/acid ratio. Fruit juices are sensitive to oxygen in terms of stability, appearance, and flavor. In fact, the color of apple juice is almost solely derived from oxidative reactions with phenolic constituents (Nagy et al., 1993). The use of hydrogen peroxide for extending the shelf life of strawberries and raspberries was negated due to anthocyanin bleaching (Sapers and Simmons, 1998). Thus, hydrogen peroxide, as both an oxidative molecule and liberator of oxygen upon degradation, may adversely affect sensory qualities of fruit juice.

The purpose of this study was to examine the use of hydrogen peroxide as an antimicrobial agent for *E. coli* O157:H7 in apple cider, purple grape, and white grape juice, and *Salmonella* spp. in orange juice. Furthermore, the role of temperature and organic acid concentration in the effectiveness of these treatments was investigated. Changes in the sensory characteristics of these juices as a result of such treatments were also evaluated.

LITERATURE REVIEW

A. U.S. fruit and juice industry

In 1999, total U.S. commercial production of apples, grapes, and oranges were reported at roughly 5.3, 6.2, and 13.1 million tons respectively (USDA, 2001). For apples and grapes, production was valued at 1.5 and 3 billion dollars (U.S. Apple Association, 2001; USDA, 2001). Though consumed mostly as fresh fruit (56%), 23% of the total 1999 U.S. apple crop went towards the production of apple juice and apple cider, equaling approximately 1.12 million tons (U.S. Apple Association, 2001; USDA, 2001).

Florida is the second largest producer of oranges in the world, with Brazil as the largest (FDOC, 2000). The Florida Department of Citrus reports that more than 90% of Florida oranges are used in the production of orange juice (99% pasteurized), contributing a substantial portion of the 1998 total of 1.2 billion gallons (FDOC, 2000; USDA, 2000). Over 85% of the 1998 grape crop went towards the production of wines, juices, jams, and jellies (USDA, 2000).

Unpasteurized apple cider, orange, and grape juice are available, but make up only a small portion of the juice processed from these fruits. In 1998, approximately 98% of juice produced in the U.S. underwent pasteurization. However, the remaining 2% equaled approximately 40 million gallons of unpasteurized product (Meskin, 1998). Whether due to their perceived superior flavor, aroma, or nutrient content, unpasteurized juices have occupied a distinct niche in the fruit juice market.

B. Outbreaks

Numerous outbreaks of *E. coli* and *Salmonella* infection from consumption of fresh juices, namely apple cider and orange juice, have been documented. In 1922 and 1944, outbreaks of typhoid fever from both sweet cider and orange juice were reported (Parish, 1997). A 1980 outbreak of hemolytic uremic syndrome (HUS), likely due to *E. coli* O157:H7, was associated with apple cider (Steele et al., 1982). Unpasteurized orange juice served in a Florida theme park caused an outbreak of salmonellosis in 1995 (Cook et al., 1998). Apple cider, from unwashed apples, was implicated in an outbreak of

diarrhea and HUS from *E. coli* O157:H7 in 1991. During this investigation, *E. coli* O157:H7 was reported to have survived 20 days in refrigerated cider (Besser et al., 1993). Two apple cider related outbreaks of HUS from *E. coli* O157:H7 were reported in 1996, one involving a death (CDC, 1997). In 1999, 423 confirmed cases of *Salmonella muenchen* infection were traced to consumption of unpasteurized orange juice, and 8 cases of *E. coli* O157:H7 infection were due to apple cider consumption. Most recently, a 2000 outbreak in orange juice involved 88 cases of *S. enteritidis* infection (66 FR 6138).

As is obvious from the above information, the incidence of reported outbreaks has increased throughout this past century. Such a trend is likely due to an increase in our ability to isolate and identify these organisms, as well as an increased level of efficiency and communication between health agencies such as the Centers for Disease Control and Prevention (CDC).

C. Organisms of concern

I. *Escherichia coli* O157:H7

Escherichia coli is a gram-negative, rod shaped, facultative anaerobe (Ray, 1996). As a part of the normal intestinal flora of humans and warm-blooded animals, it is a good indicator of fecal contamination. Only a few *E. coli* strains are pathogenic to humans. Pathogenic strains can cause infections of the urinary tract, lungs (pneumonia), blood (bacteremia), and intestines. Intestinal infections are caused by five types of *E. coli*: enterotoxigenic (ETEC), enteroaggregative (EAaggEC), enteropathogenic (EPEC), enteroinvasive (EIEC), and enterohemorrhagic (EHEC) or verotoxin producing (VTEC). The EHEC strains produce two different cytotoxins that acts like the Shiga toxin produced by *Shigella dysenteriae*. These toxins cause both hemorrhagic colitis (inflammation of the large intestine) and hemolytic-uremic syndrome. Hemorrhagic colitis is characterized by abdominal cramps, diarrhea, and bloody discharge. Hemolytic-uremic syndrome causes anemia, kidney damage, and possibly kidney failure (Sussman, 1997).

E. coli O157:H7 is the most widespread of the VTEC type. The letter and number

designations refer to the antigenic type: O (lipopolysaccharide) antigen number 157 and H (flagellar protein) antigen number 7 (Sussman, 1997). Unlike most other *E. coli* serotypes, *E. coli* O157:H7 does not ferment sorbitol. Sorbitol positive O157:H7 mutants have been isolated, but they are not pathogenic (Fratamico, 1993). O157:H7 strains are also negative for B-glucuronidase. Such characteristics allow for isolation via the use of agars selective for sorbitol and B-glucuronidase negative bacteria.

Of particular interest to this study is the acid tolerance associated with *E. coli* O157:H7. Though the acid pH limit for growth is approximately 4.5 (Slonczewski et al., 1987), *E. coli* O157:H7 can tolerate much more acidic conditions for extended periods of time. Factors such as acid adaptation, bacterial strain, growth phase, food type, and storage temperature all play a role in the degree of acid tolerance.

The survival of *E. coli* O157:H7 in acidic fruits and fruit juices, most notably apple products, is well documented. A survival time of 31 days was seen in apple cider (pH 3.7) held at 8°C (Zhao et al., 1993). Fisher and Golden (1998) showed that *E. coli* O157:H7 can survive in crushed apples up to 18 days, with possible growth at 25°C. Cells survived better when placed in apple juice compared to TSA acidified with organic acids, possibly indicating the presence of protective juice constituents (Uljas and Ingham, 1998). In orange juice, survival of greater than 25 days at pH 3.9 and 13 days at pH 3.4 has been demonstrated (Linton et al., 1999). *E. coli* O157:H7 has been shown to survive in other low pH foods, such as yogurt (Hudson et al., 1997), pepperoni (Riordan et al., 1998), ketchup (Tsai and Ingham, 1997), and mayonnaise (Zhao and Doyle, 1994).

Numerous researchers have shown that exposing *E. coli* cultures to sublethal pH enhances survival under subsequent low pH conditions (Ryu et al., 1999; Brudzinski and Harrison, 1998; Leyer et al., 1995; Goodson and Rowbury, 1988). Brudzinski and Harrison (1998) reported an almost 1000-fold increased survival of acid adapted cultures when exposed to pH 4.0. In apple cider (pH 3.4), acid adapted cells were detected up to 81 hours (Leyer et al., 1995). Acid adapted cultures survived at least 48 hours on TSA acidified with organic acids (citric pH 3.9, malic pH 3.4) (Ryu et al., 1995). As a consequence, one hypothesis suggests that the bovine intestine could induce this adaptation. Upon human consumption, the organisms would be more likely to survive

the stomach's protective acidity and cause intestinal infection (Lin et al., 1996).

E. coli O157:H7 outbreak strains survived better than other low-pH food isolates during mineral and organic acid exposure (McKellar and Knight, 1999). Conner and Kotrola (1995) acidified TSB with organic acids to pHs of ≥ 4.0 and witnessed *E. coli* O157:H7 survival for up to 56 days. In one extreme case, *E. coli* O157:H7 strain ATCC 43895 maintained viability for 5 hours at pH 3.0 and 2.5, with survival highest in late stationary phase (Benjamin and Datta, 1995). As previously stated, this has clinical significance since the organism must survive extreme acidity in the stomach before colonization of the intestine.

A number of possible mechanisms exist for the acid tolerance response. Heyde and Portalier (1990) reported the induction of multiple acid shock proteins upon a drop in pH. Lin et al. (1996) showed that multiple acid resistant systems contribute to tolerance under various acid stresses (extremely low pH or moderate with concentrated volatile fatty acids). *lacZ* fusions indicated two separate gene expression systems, dependent on either internal or external pH variance (Slonczewski et al., 1987). Thus, the acid tolerance response is strongly correlated to the production of specific proteins.

In contrast to the above findings, chloramphenicol had an insignificant effect on acid tolerance in *E. coli*, indicating that *de novo* protein synthesis is not required for survival under acidic conditions (Benjamin and Datta, 1995). The researchers suggest that proton pump efficiency could play a major role in internal pH homeostasis. Regardless of the mechanism, *E. coli* O157:H7 has demonstrated its ability to survive acid stress.

Another important aspect of *E. coli* physiology is its response to oxidative stresses such as hydrogen peroxide exposure. Interestingly, *E. coli* exhibits a bimodal pattern of hydrogen peroxide mediated killing. Mode one killing involves actively metabolizing cells which show maximal death rates at ~ 2.5 mM hydrogen peroxide, but as the concentration increases to 10 mM the death rate decreases to half-maximal. This mode of killing appears due to DNA damage (Imlay et al., 1988). Mode two killing occurs in metabolically inactive cells, follows a multiple-order dose response curve up to much higher H₂O₂ concentrations (~ 50 mM), but the method of cellular damage is unknown

(Imlay and Linn, 1987).

At least two transcriptional systems have been identified in *E. coli* to help cope with oxidative stress, as well as develop resistance to further H₂O₂ challenge. The OxyR protein is activated upon H₂O₂ exposure, and initiates the transcription of numerous genes, most notably *katG*, which codes for production of the enzyme catalase (White, 2000). Catalase is an extremely efficient enzyme that converts hydrogen peroxide to water and oxygen. Imlay and Linn (1987) found that after H₂O₂ exposure, *E. coli* induced a 10-fold increase in catalase production. A similar response was seen by Demple and Halbrook (1983), but to a much smaller degree.

Due to the high diffusivity of H₂O₂, it is doubtful that catalase production alone would protect a cell from such stress (Ma and Eaton, 1992). Catalase production has been found to incur H₂O₂ resistance in at least one study (Imlay and Linn, 1987), but this is in contradiction to a number of others. In fact, heat shocked *E. coli* O157:H7, with virtually no catalase activity, were found to survive H₂O₂ exposure better than unshocked *E. coli* with much higher catalase production (Murano and Pierson, 1992). In another study, catalase production did not protect individual cells or dilute suspensions, though it did exert a strong protection on high density or colonial *E. coli*. Findings such as the latter have bolstered the idea that multicellular organisms evolved due to competition under oxidatively stressful conditions (Ma and Eaton, 1992).

H₂O₂ also induces the SOS response, which acts to repair damaged DNA. Increased levels of DNA repair have been implicated in both the survival and induced resistance of *E. coli* to hydrogen peroxide. Similar to the acid tolerance response, exposure to sublethal levels of H₂O₂ can render *E. coli* more resistant to H₂O₂ mediated killing (Imlay and Linn, 1987; Demple and Halbrook, 1983). In addition, oxidative stress has been shown to incur resistance to a number of other environmental toxicants such as certain antibiotics, organic solvents, and heavy metals (White, 2000).

II. *Salmonella*

Salmonellae are facultatively anaerobic, gram negative, straight rods, consisting of over 2,300 serovars (Holt et al., 1996). They occur in the intestinal tracts of warm and

cold blooded animals including birds, reptiles, farm animals, humans, and occasionally insects. Foods such as eggs, poultry, meat, and meat products are the most common vehicles of salmonellosis for humans. However, outbreaks associated with fruits and vegetables have been reported (Jay, 1996).

Salmonellae are the most reported cause of foodborne gastroenteritis outbreaks in the United States (Bean and Griffin, 1990). An infective dose of 10^7 - 10^9 cells/g is usually needed to cause illness, but much lower numbers have been reported. Upon ingestion, Salmonellae can cause gastroenteritis, septicemia, enteric fever, and most severely, typhoid fever. Typhoid fever is characterized by high fever (up to 104°F), headache, stomach pains, and the highest mortality of all Salmonella associated illnesses (CDC, 2000).

Near neutral pH is optimal for the growth of Salmonellae, but growth and survival are possible at much lower pH. In a 1970 study, using a number of acidulants, the minimum pH for growth was reported as 4.05 (Chung and Goepfert, 1970). A more recent study found that *Salmonella* spp. could grow at pH levels as low as 3.8 in HCl acidified tryptone-yeast extract-glucose medium at 30°C (Ferreira and Lund, 1987). However, minimum growth pH was inversely proportional to incubation temperature, with growth inhibited below pH 4.4 at 10°C. Foster (1991) demonstrated that *S. typhimurium* could survive extremely low pH (3.0) if first adapted at less severe conditions (pH 5.5 to 6.0).

Growth and survival of Salmonellae on and within fruits and vegetables of various acidity has been demonstrated by a number of investigators. Cantaloupe, watermelon, and honeydew melons (pH 5.90-6.67) support substantial growth of *Salmonella* spp. at 23°C, and survival at 5°C (Golden et al., 1993). On both the surface and interior of tomatoes, *S. montevideo* grew significantly at 20 and 30°C, but was inhibited at or below 10°C (Zhuang et al., 1995). In tomatoes of internal pH 3.99 to 4.37, three *Salmonella* serovars were shown to grow prolifically at 22 and 30°C, but remained static at 7°C (Asplund and Nurmi, 1991).

Survival of Salmonellae below minimum growth pH has been demonstrated in orange juice (Parish et al., 1997). In this study, four *Salmonella* serovars were acid

adapted in pH 5.0 orange serum prior to inoculation of orange juice adjusted to pH 3.5, 4.1, 4.8, and 4.4 with citric acid or 2N NaOH. Results (reported as lag time and death rate) showed that the time necessary to reduce populations from 10^6 CFU/ml to undetectable levels increased as the pH increased, with a minimum of 27 days (pH 3.5). Thus, *Salmonella* could survive long enough in orange juice to cause illness if present in sufficient numbers.

The mechanism of acid resistance is not precisely known for Salmonellae. Similar to *E. coli*, exposure to acid results in the production of numerous acid shock proteins (Foster and Hall, 1990; Foster, 1991). However, unlike *E. coli*, maintenance of pH homeostasis was found sensitive to protein synthesis inhibitors (Foster and Hall, 1991). Habituation to sub-lethal pH employs resistance to further decreases in pH (i.e. acid adaptation) (Foster and Hall, 1991), as well as other stresses such as heat and salt (Leyer and Johnson, 1993).

Hydrogen peroxide exposure induces the production of at least 30 proteins in *Salmonella typhimurium*, many of which overlap those expressed during other forms of stress (Morgan et al., 1986). Increases in *katG* (catalase) expression (Morgan et al., 1986) and a corresponding 4-5 fold increase in catalase production (Christman et al., 1985) has been observed. Proteins produced after heat shock, as well as nalidixic acid and ethanol treatments, overlap with many of those produced during hydrogen peroxide exposure (Morgan et al., 1986). As in *E. coli*, exposure to sub-lethal levels can incur resistance to levels that were previously lethal (Christman et al., 1986).

Culture conditions and isolate type can play a role in the H₂O₂ resistance of Salmonellae. Human stool isolates have been found to have much higher resistance to 10 mM H₂O₂ exposure than those isolated from chicken carcasses. In addition, growth to the stationary phase (compared to log-phase cells) greatly increased resistance in the human stool isolates (Humphrey et al., 1995).

D. Internalization and Sources of Contamination

Human pathogens are carried by an array of animate vectors. Animals such as deer and cattle are reservoirs for *E. coli* O157:H7 (Jay, 1996). Birds and insects have

also been implicated as carriers of this pathogen (Wallace et al., 1997; Janisiewicz et al., 1999). *Salmonella* is harbored by a number of domestic animals as well as humans (Jay, 1996). Investigations into a 1995 outbreak of Salmonellosis in orange juice isolated the pathogen from frogs and toads around the processing facility (Parish, 1997).

Pathogen contamination may occur via contact with the feces of these vectors. Windfallen or dropped fruit, having a greater likelihood of fecal contamination, has been implicated as a pathogen source in recent *E. coli* O157:H7 outbreaks from cider (Besser et al., 1993; CDC, 1996). Under such circumstances, typical brushing and washing techniques may remove surface fecal contamination, but these techniques become less effective if pathogens are internalized.

Though the mechanisms by which pathogens enter fruit are still questionable, internalization can occur. External injuries such as cuts and abrasions would offer easy attachment and access. Liao and Sapers (2000) found that apple disks with no skin retained 13 – 19% more *Salmonella* Chester than disks with skin, indicating easier attachment to abraded or wounded fruit. Natural structures such as the stem, stem scar, and calyx are also potential sites for internalization. Of those *Salmonella* Chester attached to apples after artificial inoculation, 94% were located on the stem or calyx region (Liao and Sapers, 2000).

Wash water quality and temperature may also play a role. Zhuang et al. (1995) found that tomatoes at 25°C dipped in a 10°C cell suspension of *Salmonella montevideo* internalized a significant number of the pathogen. Similar uptake of *E. coli* was witnessed in apples dipped in cold peptone water (Buchanan et al., 1999). Studies by the FDA using dyed water indicate that microorganisms could potentially be internalized simply through the skin of undamaged fruit, when contacting aqueous suspensions of lower temperature (Merker et al., 1999). A warm fruit, cool water interface creates a slight vacuum due to the decreased partial vapor pressure at the fruit surface, thus potentially sucking bacteria beneath the skin, calyx, or stem scar. Employing such a mechanism, it is reasonable to assume that warm fruit, still on the tree, could internalize pathogens from bird feces, if subjected to a cool rain.

Bacterial soft rot in fruit and vegetables has also been associated with increased

levels of *Salmonella* contamination. Wells and Butterfield (1997) reported that *Salmonella* contamination was present in at least 18 – 20% of soft rotted samples, compared to 9-10% in healthy samples. In addition, vegetable disks inoculated with *Erwinia carotovora* (soft rot bacterium) and *Salmonella typhimurium* supported 10 times the *S. typhimurium* levels of vegetable disks inoculated with *S. typhimurium* alone.

E. Selective vs Nonselective Media

A host of research has shown that in terms of culturing stressed or injured cells, nonselective media such as tryptone soy agar (TSA) outperforms selective media. TSA with and without a Violet Red Bile Agar (VRBA) overlay was found to recover more acid injured *E. coli* cells than a number of selective medias including Sorbitol McConkey Agar (SMA), *E. coli* Petrifilm, Eosin Methylene Blue Agar (EMBA), or VRBA alone (Silk and Donnelly, 1997; Silk et al., 1997). Amezcuita and Brashears (2000) saw better recovery of lactic acid stressed *E. coli* O157:H7 on TSA than on selective or recovery media, but *Salmonella* recovery was equivalent on all medias used.

Recovery from fruit juice has shown similar preference for nonselective media. In orange, cranberry, white grape, and red grape juices, TSA and Rainbow Agar provided equal recovery of *E. coli* O157:H7, but better recovery than SMA, MEMB, and Hemorrhagic colitis agar (HC). In apple cider, TSA outperformed all other agars tested (Lakins et al., 2000). Similarly, Semanek and Golden (1996) found that in both fermenting and nonfermenting apple cider, TSA provided better recovery of *E. coli* O157:H7 compared to SMA.

F. Juice Regulatory History

On August 28, 1997, in response to juice associated illnesses and a recommendation from the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), the FDA issued a notice of intent to develop a juice HACCP (Hazard Analysis Critical Control Point) program, warning label statement, and an educational program for fruit and juice beverage processors. In July of 1998, the FDA published the warning statement rule (63 FR 37030), which required the labeling of juices not

processed to a level necessary for a 5-log reduction (i.e. pasteurization) in the most persistent pathogen. Technical workshops for the citrus and apple cider industries were conducted to provide assistance with sanitation and processing techniques necessary to achieve the 5-log reduction.

On April 24, 1998, FDA proposed a required implementation of HACCP for juice processors, with an initial comment deadline of July 8, 1998. Included in the plan was a requirement for processors to achieve a 5-log reduction of the pertinent pathogen. However, the proposal gives no definition as to the method(s) that must be used to achieve this reduction. The comment period for the HACCP proposal was extended and reopened a number of times up to a deadline of January 24, 2000 (64 FR 65669).

January 19, 2001 marked the final ruling titled “Hazard Analysis Critical Control Point (HACCP); Procedures for the Safe and Sanitary Processing and Importing of Juice” (66 FR 6138). The ruling requires the implementation of HACCP principles in the production of juice or juice products. Within the HACCP plan, procedures capable of obtaining a 5-log reduction in the most pertinent pathogen must be included. The FDA states that historical association will determine a particular products pertinent pathogen, though the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) recommends *E. coli* O157:H7 as a benchmark. Pasteurization is not mandated, thus leaving room for alternative methodologies such as UV, ozone, chemical treatments, or wash treatments in combination with these technologies. If surface treatments are part of the 5-log reduction protocol, microbiological testing of the final product must be included in the HACCP plan. Such end product testing is not required for processes achieving the full 5-log reduction in the expressed juice.

The time frame for implementation of these regulations is three tiered, based on size and sales of the processor. Processors with more than 500 employees are given 12 months for implementation. Those with between 100 and 500 employees have 24 months to comply. Very small processors, defined as those with less than \$500,000 annual sales, greater than \$500,000 annual sales but less than \$50,000 from food, or less than 100 full time employees or less than 100,000 units sold per year, have 36 months before necessary compliance. During this interim period, small processors will likely be looking at non-

thermal options in order to avoid the high capital costs of pasteurization equipment.

G. Juice composition

Fruit juice is mainly the liquid expressed from fruit cell vacuoles, but also includes insoluble particles and bits of fruit tissue. Though primarily water, this organic medley contains sugars such as glucose, fructose, and sucrose, organic acids (malic, citric, and tartaric), fats, proteins, various volatile compounds, and vitamins (Nagy et al., 1993).

Taste and flavor qualities are formed by the sugars, organic acids, and aroma compounds present in juice. Sugars and organic acids make up the bulk of the soluble solids fraction, and a proper balance between the concentrations of both are important in the palatability of the juice. Thus, organic acid additions are only reasonable within the scope of maintaining an acceptable sugar/acid ratio. Aroma arises from a number of volatile compounds whose composition is essential to juice quality yet very sensitive to processing techniques. Aroma profile modification is of great importance when considering processing methods for juice manufacturing (Nagy et al., 1993).

Enzymes released during juice expression cause a host of chemical changes, some of which may detract from the appearance and stability of a juice product. In the case of apple juice under aerobic conditions, polyphenol oxidase catalyzes the polymerization of phenolic constituents which leads to brown coloration (melanins) (Fennema, 1996). In addition to being the sole production of color, these oxidative polymerizations can change the flavor and aroma of the juice. Thus, addition of an oxidant such as hydrogen peroxide would likely have a noted effect on the color, flavor, and aroma of apple juice. In orange juice, pectin methylesterase converts pectin to pectic acid, with the end result being cloud loss and juice separation (Fennema, 1996; Nagy et al., 1993). Such changes are obvious in unpasteurized orange juice, which has had no heat treatment to inactivate pectin methylesterase.

As mentioned, the characteristic brown color of apple juice is a result of oxidative reactions after juice extraction. However, orange and grape juice color is derived from pigments initially present in the fruit. Orange juice derives its color from carotenoids

such as β -cryptoxanthin, antheraxathin, α -carotene, β -carotene, and leutin within the juice vesicles. These color components are rather stable to processing, but vary in intensity according to the fruit's growing season (Nagy et al., 1993). In purple grape juice, color comes from anthocyanins and phenolic components mostly in the skin of the grape, but must be extracted into the initially clear juice. For wine production, fermentation aids in color extraction, but in juice production, heat ($\sim 60^{\circ}\text{C}$) is used to extract color components from the skins (Baldy, 1998; Nagy et al., 1993).

H. Pasteurization

Pasteurization is regarded as the most reliable method for pathogen reduction in juices. Typical time/temperature applications for apple cider and orange juice pasteurization are 88°C for 5 seconds and $95 - 90^{\circ}\text{C}$ for 15 - 60 seconds respectively (Kozempel et al., 1998; Nagy et al., 1993). Such processing accomplishes not only microbial destruction, but also inactivation of enzymes. In fact, pasteurization time/temperature requirements for juices are actually set for enzyme inactivation, with sufficient microbial destruction resulting as an added benefit (Nagy et al., 1993).

Although its efficacy is unchallenged, pasteurization has economic and sensory constraints. Kozempel et al. (1998) demonstrated that pasteurization costs are reasonable for medium sized (107 L/min) processors, but costs increase dramatically as the production rate and days of processing decrease. Therefore, small, seasonal processors might be unable to afford the costs for such equipment and utilities. In addition to the question of economics, sensory characteristics such as flavor and aroma are believed adversely affected by heat treatment. High-end pasteurization temperatures ($\sim 85^{\circ}\text{C}$) have been shown to reduce a number of flavor compounds in apple juice (Su and Wiley, 1998), as well as cause negative flavor changes in orange juice (Nagy et al., 1993). Similar temperatures were shown to reduce all volatile fractions of mango juice (El-Nemr et al., 1988). Such findings are important when considering market acceptance of a pasteurized product. Nonthermal alternative such as UV, ozone, and chemical preservatives may prove useful for processors considering the market appeal and costs of pasteurizing their juice.

I. Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is an antiseptic (compared to a preservative) since it quickly acts to kill microorganisms and has no long-term or preserving effect (Lück and Jager, 1997). This short-lived action is due to hydrogen peroxide's rapid decomposition to oxygen and water upon contact with organic material. The antimicrobial action of hydrogen peroxide is not due to its oxidative properties as a molecule, but primarily in the production of other powerful oxidants such as singlet oxygen, superoxide radicals, and the hydroxyl radical (Davidson and Branen, 1993). These reactive oxygen species cause irreversible damage to a host of cell components such as enzymes, membrane constituents, and DNA. In fact, aqueous solutions of H_2O_2 alone will not cause protein, lipid, or nucleic acid modification without the presence of radical formation catalysts (Juven and Pierson, 1996). H_2O_2 is naturally produced by enzymatic systems, and is notably utilized by phagocytes in the destruction of bacteria within the phagolysosome (Brock et al., 1997).

Of those radicals mentioned above, hydroxyl radical ($\text{HO}\cdot$) production likely plays the largest role in the toxicity of hydrogen peroxide (Imlay and Linn, 1988). When produced adjacent to DNA, hydroxyl radicals are unique in that they can "both add to DNA bases and abstract H-atoms from the DNA helix" (Cadenas, 1989). Hydroxyl radicals may also damage cell membranes. In a study of model membrane systems, Anzai et al. (1991) found that hydroxyl radicals (generated from hydrogen peroxide and $\text{Cu}(\text{en})_2$) increased lipid peroxidation as well as the ion permeability of model membrane systems, though via independent mechanisms. Furthermore, after 17 minutes of hydroxyl radical exposure, complete membrane breakdown was observed.

Production of $\text{HO}\cdot$ from H_2O_2 has been reported to occur in a number of ways. A commonly cited example is the Fenton reaction whereby a reducing agent such as the superoxide radical reduces Fe^{3+} to Fe^{2+} , which then reacts with H_2O_2 to produce hydroxyl anions, hydroxyl radicals, and Fe^{3+} (Juven and Pierson, 1996). Accordingly, growing *Staphylococcus aureus* cells in broths of increasing iron concentrations was found to increase killing by H_2O_2 , whereas addition of $\text{HO}\cdot$ scavengers had a protective effect against such killing (Repine et al., 1981). In a contrasting study, the ferryl radical,

not the hydroxyl radical, was indicated as the DNA damaging species in *E. coli* (Imlay et al., 1988).

A host of research related to the activity of hydrogen peroxide on various bacteria, molds, and yeast has been performed. Its activity appears greatest against anaerobic and gram-negative bacteria (Davidson and Branen, 1993). Lillard and Thomson (1983) found that concentrations of 5,300 – 12,000 ppm in poultry chiller water reduced *E. coli* populations 97 to > 99.9%. In addition, the Enterobacteriaceae were found to be more sensitive than other organisms tested. *E. coli* showed a D-value of 0.57 minutes when exposed to 3% H₂O₂, compared to 2.35 minutes for *Staphylococcus aureus*, 8.55 for *Aspergillus niger*, and 18.3 for *Candida parapsilosis* (Turner, 1983). The significant sporicidal activity of H₂O₂ on *Bacillus subtilis* spores was witnessed in both the liquid phase (Baldry, 1983) and the vapor-phase (Klapes and Vesley, 1990). As a water disinfectant, hydrogen peroxide had only a moderate immediate effect on *E. coli*, but substantial immediate effect on *S. typhi* (Yoshpe-Purer and Eylan, 1968).

Hydrogen peroxide has been used as an antimicrobial agent since the early 1800's, and is well known for its use as a topical skin application in 3% concentrations (Davidson and Branen, 1993). In foods, hydrogen peroxide was used as a disinfectant in milk as early as 1904 (Lück and Jager, 1997). Hydrogen peroxide has GRAS status (21 CFR 184.1366), and is approved by the FDA for packaging and surface sterilization in the food industry (21 CFR 178.1005).

Allowed uses of hydrogen peroxide as a direct additive to foods are limited. For antimicrobial purposes, H₂O₂ is allowed for treating milk used in cheese manufacturing, thermophile free starch production, and the preparation of modified whey, at levels of 0.05, 0.15, and 0.4% respectively. It is used as an oxidizing and reducing agent in wine, dried eggs, and corn syrup, and as a bleaching agent in tripe, beef feet, instant tea, colored cheese whey, and certain emulsifiers. Residual peroxide must be removed by an appropriate means, typically by addition of catalase (21 CFR 184.1366).

The use of hydrogen peroxide to extend the shelf life of minimally processed fruits and vegetables was reviewed by Sapers and Simmons (1998). Applied as a vapor, 60 minutes of hydrogen peroxide exposure improved the shelf life of whole cantaloupe,

and drastically reduced the mold count on raisins. Similarly, in two varieties of grape, 10 minute applications of 40°C vapor phase H₂O₂ was found to significantly reduce *Botrytis cinera* spores and enhance shelf life, without affecting grape color (Forney et al., 1991). Vapor phase treatments of mushrooms showed excessive browning, but 30 second wash treatments with 5% H₂O₂ and subsequent erythorbate dip (browning inhibitor) gave acceptable bacterial control without compromising color. On apple disks, 6% hydrogen peroxide gave a greater reduction of *Salmonella* Chester than trisodium phosphate, calcium hypochlorite, or sodium hypochlorite (Liao and Sapers, 2000). While products such as zucchini and bell peppers show promise for use of H₂O₂ in controlling soft rot, others like strawberries and raspberries show great sensitivity to anthocyanin bleaching at bactericidal peroxide levels (Sapers and Simmons, 1998).

There is a substantiated correlation between temperature and the antimicrobial efficacy of hydrogen peroxide. Toledo et al. (1973) found a notable increase in the sporicidal activity of H₂O₂ as the temperature increased above room temperature. In liquid whole egg, 1% H₂O₂ was more effective (~ 1 log) at decreasing survival of *Salmonella typhimurium* in 24 hours at 20°C in comparison to the same peroxide concentration at 5°C (Ünlütürk and Turantas, 1986). Kuchma (1998) noted a synergistic effect (killing of *E. coli* K-12 and *P. aeruginosa* 102) between microwave heating and low H₂O₂ concentrations, with maximum lethality at 50°C and 0.08% H₂O₂.

Though little if any work related to H₂O₂ use in juice has been done, an important observation is that the efficacy of H₂O₂ appears to increase with decreasing pH (Davidson, 1993). 150 μ mol hydrogen peroxide was found to be bacteriostatic towards *Pseudomonas aeruginosa* at pH 5.0, yet 1.5 mmol were required at pH 8.0. In addition, a 3% solution of hydrogen peroxide was sporicidal against *Bacillus subtilis* in 3 hours at pH 5.0, but needed 6 hours to achieve the same effect at pH 6.5 and 8.0 (Baldy, 1983). Thus, hydrogen peroxide might be more effective in combination with acidulants such as organic acids.

J. Organic acids

Organic acids occur throughout nature and are used extensively in food systems.

In addition to their use as microbial inhibitors, they can serve as defoaming agents and emulsifiers, aid in setting of pectin gels, and have a strong effect on the taste of a food (Fennema, 1996). With a characteristically sour taste, organic acids have an important role in the flavor of fruits and their juices by balancing the sugar/acid ratio (Arthey and Ashurst, 1996).

The inhibitory effect of organic acids depends on the undissociated form, as well as its ability to donate hydrogen ions in an aqueous system (Uljas and Ingham, 1998). The degree of dissociation for a particular acid is related to its dissociation constant and the acidity of the product. Dissociation constants indicate the pH at which there is a 50/50 distribution of undissociated and dissociated forms. At lower pHs, more undissociated acid is present (Bruice, 1995). In this form, the cell membrane is more permeable to the acid, allowing it to enter the cell. Upon entering the cytoplasm, the acid dissociates, thus lowering the internal pH of the cell and disrupting cellular functions (i.e. enzyme stability) (Lück and Jager, 1997). In addition to affecting enzymes, excess protons in the cytoplasm upset the membrane potential necessary for energy production and transport across the cell membrane (White, 1999). Thus, organic acids can act on a cell by affecting both the external and internal pH.

In both culture media and food system, the varying bacteriostatic and bactericidal effects of organic acids have been demonstrated. Chung and Goepfert (1970) showed that various organic acids are bacteriostatic to *Salmonella* spp. at different pH levels. In our lab, apple cider and orange juice acidified with between 0 and 3% malic and citric acid respectively, were analyzed for the survival of *Listeria monocytogenes*. Untreated apple cider (unknown pH) reduced *L. monocytogenes* to undetectable levels within 48 hours at 4°C, but orange juice with 3% citric acid (unknown pH) took at least 4 days to have the same effect, and significant numbers survived at least 10 days in untreated orange juice. For *E. coli* O157:H7, inactivation in acidified tryptic soy broth (TSB) and agar was demonstrated for citric, malic, and tartaric acids (Conner and Katrola, 1995; Ryu et al., 1999). In one study, survival of *E. coli* O157:H7 was greater in acidified apple juice compared to acidified TSB, suggesting a protective effect of juice constituents. However, contrary to other studies, acidified apple juice enhanced survival

compared to untreated apple juice at 4°C, suggesting a protective effect from the acid under refrigeration (Uljas and Ingham, 1998).

Citric acid [HOOC-CH₂-COH(COOH)-CH₂-COOH] is one of the more widely used food acidulants. It is a common constituent of fruits, namely citrus fruits, and imparts a pleasant sour taste. Citric acid is commonly employed as an acidulant in canned vegetables and dairy products (Davidson and Branen, 1993). In skim milk, citric acid was the most potent inhibitor of *S. typhimurium* compared to lactic acid and HCl (Subramanian and Marth, 1968). Fischer et al. (1985) reported a 0.75% solution of citric acid to sufficiently reduce *S. typhimurium*, *Yersinia enterocolitica*, *E. coli*, and *Staphylococcus aureus* on hard-boiled eggs. Under good manufacturing practices, citric acid is approved as a GRAS substance (21 CFR 184.1033).

Malic acid (HOOC-CHOH-CH₂-COOH), along with citric acid, comprise the main organic acids in fruits (Arthey and Ashurst, 1996). In apples, malic acid is the predominant organic acid (Nagy et al., 1993). It is used for its flavoring and acidification properties in beverages, jams, jellies, and sherbets (Davidson and Branen, 1993). Malic acid has GRAS status (21 CFR 184.1069).

Unlike most other fruits, the main organic acid in grapes is tartaric acid (HOOC-CHOH-CHOH-COOH) (Nagy et al., 1993). Tartaric acid is useful in supporting grape-like flavors. As an antimicrobial agent, tartaric acid is believed to act only by lowering the pH of the product (Davidson and Branen, 1993).

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METHODS AND MATERIALS

I. Effect of hydrogen peroxide and organic acid treatments in apple cider, white grape, purple grape, and orange juice.

Bacteria and culture conditions. Five *E. coli* O157:H7 strains; 994 (salami), H1730 (lettuce), E0019 (beef), F4546 (alfalfa sprout), Cider, and five *Salmonella* serotypes; Montivideo (tomato), Baildon (lettuce/tomato), Agona (alfalfa sprout), Michigan (cantaloupe), and Gaminara (orange juice) were obtained from Larry R. Beuchat (University of Georgia). All strains except *E. coli* O157:H7 Cider, 994, and *Salmonella* Gaminara were isolated from stools of patients suffering from illness due to consumption of the specific food. Nalidixic acid resistance was induced by successive transfers of each strain into tryptic soy broth (TSB) with increasing concentrations of nalidixic acid (ICN Biomedicals Inc., Aurora, OH), to a final concentration of 0.05 g/L. Strains were stored at 4°C on tryptone soy agar (TSA) (Difco Laboratories, Detroit, MI) slants and grown in (TSB) (Difco Laboratories) at 35°C. After two successive 24 hour transfers into TSB, a third transfer was made into pH 5.0 TSB (adjusted with 1 N HCl) in order to induce acid adaptation. After 18 hours incubation, five strain cocktails of *E. coli* O157:H7 and *Salmonella* were made by combining the acid adapted cultures of each strain in equal volumes. The cocktails were centrifuged and washed twice with 0.1% peptone (Difco Laboratories) before adding to the juice. One ml of this suspension was added to 98 ml of the respective juice, giving an approximate inoculum of 2.5×10^6 CFU/ml (6.4 log CFU/ml).

Juice preparation. Apple cider was obtained unpasteurized from a local processor, stored frozen, and thawed at 4°C before use. Orange juice (Kroger Original Premium Pasteurized Orange Juice) (Kroger, Cincinnati, OH) was obtained from a local supermarket and stored frozen until use. Purple grape juice (Welch's 100% Grape Juice) (Welch's, Concord, MA) and white grape juice (Santa Cruz Organic White Grape Juice) (Santa Cruz, Chico, CA) were stored at room temperature prior to use. All juices were preservative free. To remove background microflora, the apple cider and orange juice

was brought to 65°C and quickly cooled before use. Juice pH and sugar content was measured using a pH meter (Fisher Scientific) and a Brix hydrometer (Fisher Scientific). Titratable acidity was measured by titrating with NaOH as described by Zoecklein et al. (1995).

Hydrogen peroxide and organic acid treatments. Two levels of hydrogen peroxide (Fisher Scientific, Fair Lawn, NJ); 0.017% and 0.012% (wt/vol), and two levels of organic acid (Presque Isle Wine Cellars, North East, PA); 0.3% and 0.1% (wt/vol), were combined for a total of four possible treatment combinations. Prior experimentation determined that hydrogen peroxide levels below 0.012% were mostly ineffective. Organic acid levels were determined from preliminary experiments indicating that 0.1% additions alone had no apparent antimicrobial effect in any juice compared to controls, while > 0.3% were impractical in terms of taste modification. The organic acids used corresponded to the dominant organic acid of the particular juice; citric, malic, and tartaric for orange juice, apple cider, and grape juices respectively. Untreated juice served as a control. All treatments and controls were stored at 4°C and 25°C.

Treatment and sampling protocol. Organic acids (dry, crystalline) were added to dry 125 ml bottles and autoclaved. Ninety eight ml portions of juice were added to each bottle and stored overnight at the respective temperatures until inoculation the following day. One ml of the five strain acid adapted *E. coli* O157:H7 cocktail was added to the apple cider, white grape, and purple grape juices to give an initial level of approximately 6.4 log CFU/ml. One ml of the five strain acid adapted *Salmonella* cocktail was added to the orange juice preparations to give an initial level of approximately 6.4 log CFU/ml. Within 20 minutes of inoculation, 1 ml of diluted 30% (wt/vol) hydrogen peroxide was added to give a final concentration of either 0.017% or 0.012% (wt/vol) in the juice. The actual concentration of the hydrogen peroxide stock solution was found to be 29.5% using the potassium permanganate test (E. Merck, 2000). One ml juice samples were taken every 24 hours for 21 days, serially diluted in 0.1% peptone, and pour plated using TSA with 0.05 g/L nalidixic acid (TSAN). Plates were incubated for 48 hours at 35°C.

Colonies were enumerated using a Quebec™ colony counter. Isolate identification was periodically tested using API 20 E strips (BioMérieux, France) and the *E. coli* O157:H7 Prolex Latex Agglutination System (Pro-Lab Diagnostics, Canada).

Statistical analysis and design. Reported values are averages from three independent trials converted to logarithmic units. Juices were analyzed separately by the general linear model procedure using SAS statistical software (SAS Institute Inc., Cary, N.C.). A 4 way analysis of variance was performed to test for interaction between treatment, temperature, and time. A 3 way analysis of variance was performed on days 1, 3, and 7 to determine significant differences ($\alpha = 0.05$) in the means, treatment contrasts, and test for hydrogen peroxide/organic acid interaction.

II. Effect of hydrogen peroxide in apple cider and orange juice at different temperatures.

Bacteria and juices were maintained and prepared in the same manner as in experiment I, except that only apple cider and orange juice were used in this section.

Hydrogen peroxide treatments. Hydrogen peroxide was added at 0.015% and 0.03% to inoculated apple cider and orange juice held at 10°C, 25°C, and 40°C. Untreated juice at each temperature served as a control.

Treatment and sampling protocol. Ninety eight ml portions of juice were added to sterile 125 ml bottles and stored overnight at the respective temperatures until inoculation the following day. One ml of the five strain acid adapted *E. coli* O157:H7 cocktail was added to the apple cider to give an initial level of approximately 6.4 log CFU/ml. One ml of the five strain acid adapted *Salmonella* cocktail was added to the orange juice to give an initial level of approximately 6.4 log CFU/ml. Following inoculation, 1 ml of diluted 30% (wt/vol) hydrogen peroxide was added to give final concentrations of 0.015% or 0.03% in the juice. Five ml samples were taken at 1, 3, 6, 12, and 24 hours post treatment and added to test tubes containing 0.1 ml fungal catalase (ICN Biomedicals

Inc.) (1000 EU/ml) (1 EU decomposes 1 μ mol hydrogen peroxide per minute at pH 7.0) in order to stop the hydrogen peroxide activity. The catalase treated juice was then serially diluted in 0.1% peptone (Difco Laboratories) and pour plated with TSAN. Plates were incubated for 48 hours at 35°C. Colonies were enumerated and identified as in experiment I.

Residual hydrogen peroxide measurement. A number of methods were tested in an attempt to measure residual hydrogen peroxide concentration. Both the vanadium oxide test and EM Science Quant test strips gave false positives. However, the degree to which gas was liberated after catalase addition to H₂O₂ treated juice did serve as a qualitative measure of residual peroxide.

Statistical analysis and design. Reported values are averages from three independent trials converted to logarithmic units. Juices were analyzed separately by the general linear model procedure using SAS statistical software (SAS Institute Inc., Cary, N.C.). A 3 way analysis of variance was performed to determine significant differences ($\alpha = 0.05$) in the means.

III. Sensory evaluation. To establish flavor quality standards, taste evaluations were performed on apple cider and orange juice with added H₂O₂ and organic acids (OA) (malic and citric respectively) using the In/Out method (Munoz et al., 1992). Twenty five untrained panelists from the Department of Food Science and Technology tasted 5 juice samples (control, juice with 0.1% H₂O₂, 0.03% H₂O₂, 0.3% OA, or 0.1% OA added) and rated the samples as either “In” or “Out” of personal specification, according to their individual perception of flavor quality. If deemed “Out”, taste descriptors were requested. A minimum of 65% agreement was required to consider a particular H₂O₂ or OA treatment as “In” specification.

Unpasteurized apple cider and orange juice, and pasteurized purple grape and white grape juices were used for describing changes in color and appearance as a result of H₂O₂ additions.

RESULTS

I. Effect of hydrogen peroxide and organic acid treatments in apple cider, white grape, purple grape, and orange juice.

Tables 2, 3, 4, and 5 show the numbers of culturable *E. coli* O157:H7 (apple cider, purple grape, white grape) and *Salmonella* spp. (orange juice) at 24, 48, and 96 hours after hydrogen peroxide/organic acid (H₂O₂/OA) treatments. In most instances, H₂O₂/OA treatments reduced *E. coli* O157:H7 and *Salmonella* spp from approximately 6.4 log CFU/ml to an undetectable number within 24 – 48 hours. Notably, H₂O₂/OA treatments were less effective in reducing *E. coli* O157:H7 in grape juice than in apple cider or white grape juice. In contrast to the general efficacy of these treatments, in all juices except white grape juice, there were instances where *E. coli* O157:H7 and *Salmonella* spp. showed prolonged survival after treatment, and in some cases survived better than the controls. These occurrences were not consistent between trials, but were H₂O₂ concentration and temperature specific. Such findings, as well as unique observations are described for each individual juice.

Apple cider. The effect of malic acid additions on apple cider pH, titratable acidity, and sugar content can be seen in Table 1. Culturable *E. coli* O157:H7 cells were reduced to undetectable numbers (<1 CFU/ml) within 48 hours for most treatment combinations. An analysis of day 1 (24 hours after treatment application) showed that averaged over both temperatures, H₂O₂ and malic acid concentration were significant factors in microbial reduction ($p < 0.05$). Thus, increasing the concentration of either factor significantly decreased the number of culturable *E. coli* O157:H7 cells. No interaction was found between H₂O₂ and malic acid concentration. Though day 1 counts were generally lower at 25°C, temperature did not exhibit a statistically significant effect on the efficacy of these treatments in apple cider. However, two important observations were noted related to temperature. First, at both malic acid concentrations, 0.017% hydrogen peroxide reduced culturable *E. coli* O157:H7 cells to undetectable levels within 24 hours at 25°C, whereas at 4°C, between 1 and 2 log CFU/ml were cultured on TSAN.

Second, at 25°C and the lowest treatment level (0.012% H₂O₂ + 0.1% malic acid), bacterial survival was witnessed for the duration of the experiment, but never at 4°C. As is depicted in Table 2, following a rapid decrease in culturable organisms 24 hours post treatment, a large number of cells eventually regained their ability to grow in TSAN (~ 4.5 logs by day 4). This occurred in only one of the three experimental trials, but it was observed that in this trial, bacterial numbers were higher in the treated juice compared to the control from days 12 to 21 (data not shown).

Orange juice. The effect of citric acid additions on orange juice pH, titratable acidity, and sugar content can be seen in Table 1. Reduction of *Salmonella* spp. in orange juice was dependent on both H₂O₂ concentration ($p < 0.001$) and temperature ($p < 0.05$). Citric acid concentration did not significantly affect bacterial populations ($p > 0.05$). Treatments with 0.017% H₂O₂ at 25°C reduced *Salmonella* spp. to undetectable levels within 24 hours, but not at other treatment/temperature combinations. Temperature was a significant factor, but its role changed over time (i.e. an analysis of day x temperature indicated significant interaction ($p < 0.05$)). Thus, on day 1, increasing the temperature from 4 to 25°C was found to significantly decrease culturable Salmonellae across all treatments ($p < 0.05$), but by day 3, survival was greater at 25°C. As is depicted in Table 3, after an initial sharp decrease in organisms cultured on TSAN, treatments of 0.012% H₂O₂ with both 0.1% and 0.3% added citric acid at 25°C demonstrated a quick upswing in culturable Salmonellae from days 2 to 4. Orange juice (25°C) with 0.012% H₂O₂ + 0.1% citric acid treatments eventually supported higher *Salmonella* numbers than the controls (data not shown).

Purple grape juice. The effect of tartaric acid additions on purple grape juice pH, titratable acidity, and sugar content can be seen in Table 1. The initial efficacy of H₂O₂/OA treatments was much less pronounced in purple grape juice. One day after application, *E. coli* O157:H7 reduction was no more than ~ 1 log across all treatments and temperatures, compared to the controls (Table 4). No significant differences were observed between the various factor levels on day 1. By day 3, both H₂O₂ and tartaric

acid levels were significant factors in bacterial reduction, and it appears that in this juice, increasing the tartaric acid level affected survival ($p < 0.001$) more so than increasing H_2O_2 concentration ($p < 0.01$). *E. coli* O157:H7 numbers had dropped to undetectable levels at day 3 for both $H_2O_2 + 0.3\%$ tartaric acid treatments at $25^\circ C$, but not with 0.1% tartaric acid. Furthermore, by comparing $0.017\% H_2O_2 + 0.1\%$ tartaric acid and $0.012\% H_2O_2 + 0.3\%$ tartaric acid at $4^\circ C$ in Figure 1, it can be seen that increasing H_2O_2 from 0.012% to 0.017% decreased survival from 15 days to 12 days whereas increasing tartaric level from 0.1% to 0.3% decreased survival to 10 days. By day 7, estimates of low vs. high hydrogen peroxide and low vs. high tartaric acid level contrasts were 1.9 and 2.9 logs respectively. Except for one case, survival was greatly enhanced in $4^\circ C$ treatments ($p < 0.001$), with organisms detected up to 15 days compared to ≤ 4 days at $25^\circ C$. As was described for *E. coli* O157:H7 in apple cider and *Salmonella* spp. in orange juice, enhanced survival (compared to the control) was witnessed in the lowest treatment combination at $25^\circ C$ in one trial of this experiment (data not shown). In this one trial, viable colonies were higher after day 16 compared to the control, and continued survival (> 2.4 logs) was seen until day 21.

White grape juice. The effect of tartaric acid additions on white grape juice pH, titratable acidity, and sugar content can be seen in Table 1. The highest reductions in *E. coli* O157:H7 from H_2O_2/OA application were seen in white grape juice. In most cases, culturable organisms were undetectable within 24 hours, and never after 48 hours (Table 5). The lowest H_2O_2 level treatments at $4^\circ C$ consistently had counts of between 1 and 20 CFU/ml at day 1, regardless of organic acid level. However, no significant differences were seen between factors due to lack of data at day 1.

II. Effect of hydrogen peroxide in apple cider and orange juice at different temperatures

As a continuation of experiment 1, this aspect of the study was designed to examine hydrogen peroxide's bactericidal efficacy over 24 hours, at temperatures illustrating moderate heating or cooling around ambient temperature. Tables 6 and 7

show the H₂O₂/time/temperature dependent reduction of *Salmonella* spp. and *E. coli* O157:H7 in orange juice and apple cider respectively, as cultured on TSAN. H₂O₂ treatments significantly reduced levels of these pathogens in their respective juices compared to untreated controls ($p < 0.0001$).

Reductions of *E. coli* O157:H7 in apple cider from 6.4 log CFU/ml to undetectable levels was seen in all treatments except the lowest H₂O₂/temperature combination, with reduction rates dependent on temperature and H₂O₂ concentration ($p < 0.0001$). There was temperature x H₂O₂ interaction ($p < 0.0001$), such that increasing the temperature from 25 – 40°C was more effective than increasing 10 – 25°C. This is obvious in contrasting mean counts at hours 3 and 6. Furthermore, for this pathogen/juice combination, increasing temperature appears to be a more important factor in hydrogen peroxide's efficacy than increasing H₂O₂ concentration. For example, 6 hours after treatment, increasing temperature from 10°C to 40°C at 0.015% H₂O₂ was more effective (2 logs) than maintaining 10°C and increasing H₂O₂ concentration to 0.03%.

Results for *Salmonella* in orange juice were similar in regards to those of *E. coli* O157:H7 in apple cider except for one case. In one of the three trials, *Salmonella* spp. survived the 0.015% at 40°C treatment, and was detected for the following week during storage at 25°C. Thus, only 0.03% H₂O₂ treatments were universally effective. Increasing temperature significantly increased the efficacy of 0.03% H₂O₂ treatments ($p < 0.0001$), with ≤ 3 hours at 40°C required for undetectable levels of *Salmonella*.

Gas produced as a result of catalase addition to H₂O₂ treated juice samples provided a qualitative measure of residual peroxide concentration. No gas bubbles were produced in untreated juice. At 0.015% H₂O₂, the degree of gas production (foaming) in treated juices appeared dependent on both time and temperature. Gas liberation was obvious in 10 and 25°C samples (both juices) for the duration of the experiment, but noticeably decreased in volume from 12 to 24 hours, indicating a decrease in H₂O₂ concentration. Samples at 40°C had very little gas production at 12, and none at 24 hours.

III. Sensory

At levels used in this study, hydrogen peroxide caused noticeable color changes in apple cider, orange juice, and purple grape juice, but not in white grape juice. Unpasteurized apple cider darkened considerably within minutes of hydrogen peroxide whereas unpasteurized orange juice lightened slightly. In orange juice, the lightening was not detrimental and seemed to enhance the appearance. For both unpasteurized orange juice and apple cider, a portion of the orange pulp and apple particulates commonly formed a floating layer above the rest of the juice. Purple grape juice (pasteurized) lost most of its characteristic color and looked diluted quickly after treatment. This was excessive and likely not desirable.

Taste panels on hydrogen peroxide and organic acid additions to apple cider and orange juice demonstrated that on the basis of taste alone, hydrogen peroxide levels used in this study ($\leq 0.03\%$) were within panelists perception of flavor quality. Panelists that perceived hydrogen peroxide treated samples as out of personal specification provided descriptors such as “bitter”, “astringent”, and a generally unappealing aftertaste. Adding 0.1% of the dominant organic acid to apple cider and orange juice were within specification, but 0.3% additions were considered unqualified for consumption for most individuals.

DISCUSSION

Results using TSAN indicate that at levels of 0.017% or above, H_2O_2 is an effective antimicrobial for *E. coli* O157:H7 and *Salmonella* spp. in various fruit juices, though the death rate varied between juice type and temperature. Below this concentration, both organisms were found to survive such treatments, and in some cases become more robust, outlasting those cells in untreated juice. This behavior was inconsistent between trials, but occurred at 25°C and at H_2O_2 concentrations of 0.012% and 0.015%.

Increases in the dominant organic acid (OA) concentration significantly lowered juice pH, but had variable results in terms of increasing treatment effectiveness. In

reducing *E. coli* O157:H7, OA was a mildly significant treatment factor in apple cider, but very significant in purple grape juice. Continued survival of this organism in either product was stopped by increasing the OA level of H₂O₂/OA treatments from 0.1% to 0.3%. Thus, it is reasonable to assume that if applying H₂O₂ to these juices, increasing OA concentrations will increase the efficacy. For *Salmonella* spp. in orange juice, citric acid additions were not found to significantly affect treatment efficacy, nor was it a consistent factor in halting continued survival.

As described earlier, in those cases where continued survival occurred, the common scenario was a quick drop in organisms, followed by a gradual increase in bacterial counts. This curve is likely due to a large number of viable but non-culturable (VBNC) or sub-lethally injured cells, which gradually regained their ability to form colonies in TSAN. Given the breadth of research not reporting growth of these organisms in juice, and the fact that levels never went above the initial inoculum, increased counts from bacterial growth is unlikely. TSA has been demonstrated to improve the recovery of sub-lethally injured cells from juice compared to selective media (Lakins et al., 2000; Semanchek and Golden, 1996), but initially, numerous cells in this study escaped TSA's recovery capabilities. Similarly, Wolfson and Sumner (1994) found that 2.5mM H₂O₂ in TSB reduced *S. typhimurium* to undetectable levels on plate count agar, but that samples confirmed positive using DNA hybridization kits. Consequently, these counts should be viewed as numbers of cultivable organisms, not total viable organisms.

A number of explanations exist for the temperature/ H₂O₂ dependent enhancement of survival (compared to controls) seen in this study. H₂O₂ decomposes to water and oxygen when contacting organic material, and this rate of decomposition should increase with increasing temperature and organic load. Thus, bacterial exposure to H₂O₂ is shortened at increasing juice temperatures. In addition, it has been demonstrated that catalase positive cells such as *E. coli* and *Salmonella* protect each other in high density or colonial units, but may be less hardy as individual cells during H₂O₂ exposure (Ma and Eaton, 1992). Exposure to sub-lethal concentrations of H₂O₂ induces the production of multiple stress response proteins in these organisms, some of which overlap those

induced from acid, heat, and other stresses (Morgan et al., 1986; White, 2000). As a result, mild H₂O₂ exposure can induce resistance to further oxidative stress, as well as certain antibiotics, organic solvents, and heavy metals (White, 2000). Combining these three statements, survivors due to colonial protection or shortened exposure times could be subjected to sub-lethal doses which incur adaptive mechanisms, making the cell more robust and apt to survive in adverse environments such as fruit juice.

The overall role of temperature in this study was highly variable. At H₂O₂ concentrations of 0.017% and 0.03%, increases in temperature increased bacterial reduction rate, but at 0.012% and 0.015%, the inverse was sometimes true. As mentioned, increases in temperature would likely increase H₂O₂ degradation in the juice. Thus, when dealing with very low H₂O₂ concentrations, lower temperatures may extend H₂O₂ contact times and increase efficacy. Also, low temperatures such as 4°C may provide a sufficient hurdle to keep a cell from repairing itself after oxidative injury. However, overall it is reasonable to assume that if sufficient H₂O₂ concentrations are used, increasing application temperature will improve the microbial reduction rate.

In purple grape juice, it was seen that H₂O₂/OA treatments were initially less effective in pathogen reduction compared to the other juices tested, and that tartaric acid concentration was more important than H₂O₂ concentration. A reasonable explanation for this is that antioxidant constituents such as flavonoids, anthocyanins, or other phenolic compounds degraded the H₂O₂ before exerting much bactericidal effect, leaving tartaric acid concentration as the main antimicrobial factor. Color components such as anthocyanins are present in higher concentrations in purple grape skins compared to white grape skins, possibly explaining the discrepancy between results in white grape and purple grape juice. In support, Wang et al. (1996) found that purple grape juice contained 3 times the total antioxidative capacity of orange juice and apple juice. Also, this capacity was only minimally due to ascorbic acid, which was exogenously added. These observations, and the reduction in color from H₂O₂ make purple grape juice and unlikely candidate for H₂O₂ treatments.

Changes in the appearance of these juices as a result of hydrogen peroxide treatment were not unexpected. The brown coloration of apple juice is due to oxidative

polymerization of phenolic constituents via polyphenol oxidase activity. In fact, apple juice would be relatively clear without oxygen exposure (Nagy et al., 1993; Fennema, 1996). Thus, as an oxidative molecule and liberator of oxygen upon decomposition, hydrogen peroxide would enhance the rate of phenolic polymerization and melanin formation, resulting in a dark brown color. In purple grape juice, the loss of color was likely due to decomposition or bleaching of anthocyanins. Similar anthocyanin degradation has been demonstrated in sour cherry juice, as well as in whole strawberries and raspberries after hydrogen peroxide exposure (Sapers and Simmons, 1998; Özkan et al., 2000). The brightening effect seen in orange juice probably stemmed from hydrogen peroxide induced carotenoid oxidation. Analogous to the must cap formed during red wine fermentation, the orange pulp and apple particulate layer formed after H₂O₂ addition was likely a result of oxygen liberated upon hydrogen peroxide's degradation, which attached to the particulates and increased their buoyancy.

CONCLUSIONS

Hydrogen peroxide shows promise as an antimicrobial agent for *Salmonella* spp. in orange juice, and *E. coli* O157:H7 in apple cider and white grape juice, though sensory concerns may negate its potential use in apple cider and purple grape juice. Effective levels ($\sim \geq 0.017\%$) were below those allowed for use in other food products (21 CFR 184.1366). Hydrogen peroxide concentrations $\leq 0.015\%$ may initiate protective mechanisms within the cell that allow for enhanced survival in all juices except white grape juice. These occurrences were temperature dependent (25°C) but sporadic between trials. Increasing temperature improved the effectiveness of $\geq 0.017\%$ hydrogen peroxide treatments, but not $\leq 0.015\%$ treatments. Based on our results, increasing the dominant organic acid concentration may increase efficacy of hydrogen peroxide treatments in apple cider, purple grape, and white grape juice, but not in orange juice.

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Table 1. pH, titratable acidity, and sugar content of apple cider, orange, white grape, and purple grape juices with and without added (wt/vol) organic acids (OA)^a

	pH ^b		
	Control	0.1% OA	0.3% OA
apple cider	3.8	3.6	3.3
orange juice	3.8	3.7	3.6
purple grape	3.1	3.0	2.8
white grape	3.3	3.2	3.1

	Titratable acidity (g/L) ^c		
	Control	0.1% OA	0.3% OA
apple cider	4.0	5.6	8.5
orange juice	10.0	12.1	14.8
purple grape	10.7	12.3	15.1
white grape	5.1	6.6	9.5

	Sugar content (°Brix) ^d		
	Control	0.1% OA	0.3% OA
apple cider	11.4	11.4	11.4
orange juice	11.3	11.4	11.5
purple grape	15.6	15.1	15.4
white grape	15.3	15.4	15.4

^aOrganic acids used were the dominant organic acid in the particular juice. Malic, citric, and tartaric in apple cider, orange juice, and grape juices respectively.

^bpH \pm 0.05

^c \pm 0.3 (g/L)

^d \pm 0.2 (°Brix)

Table 2. Effect of hydrogen peroxide, malic acid (MA), and temperature on *E. coli* O157:H7 in apple cider after 24, 48, and 96 hour incubation, as cultured on TSAN. (n = 3)

Treatment	Culturable organisms (log CFU/ml)					
	24 hrs		48 hrs		96 hrs	
	4°C	25°C	4°C	25°C	4°C	25°C
Control	6.24	6.09	6.23	6.15	6.07	6.71
0.012% H ₂ O ₂ + 0.1%MA ^a	2.51	0.84	0.3	1.34	ND	4.52
0.012% H ₂ O ₂ + 0.3%MA	1.57	0.85	1.18	ND	ND	ND
0.017% H ₂ O ₂ + 0.1%MA	1.72	ND ^b	ND	ND	ND	ND
0.017% H ₂ O ₂ + 0.3%MA	1.07	ND	ND	ND	ND	ND

^aPercent malic acid added (wt/vol)

^bBelow the limit of detection for this assay (< 1 CFU/ml)

Table 3. Effect of hydrogen peroxide, citric acid (CA), and temperature on *Salmonella* spp. in orange juice after 24, 48, and 96 hour incubation, as cultured on TSAN. (n = 3)

Treatment	Culturable organisms (log CFU/ml) after					
	24 hrs		48 hrs		96 hrs	
	4°C	25°C	4°C	25°C	4°C	25°C
Control	6.21	6.07	5.95	6.07	5.78	6.51
0.012% H ₂ O ₂ + 0.1%CA ^a	1.57	2.68	ND	0.94	ND	5.93
0.012% H ₂ O ₂ + 0.3%CA	1.44	1.83	ND	0.22	ND	4.22
0.017% H ₂ O ₂ + 0.1%CA	0.82	ND ^b	ND	ND	ND	ND
0.017% H ₂ O ₂ + 0.3%CA	0.43	ND	ND	ND	ND	ND

^aPercent citric acid added (wt/vol)

^bBelow the limit of detection for this assay (< 1 CFU/ml)

Table 4. Effect of hydrogen peroxide, tartaric acid (TA), and temperature on *E. coli* O157:H7 in purple grape juice after 24, 48, and 96 hour incubation, as cultured on TSAN. (n = 3)

Treatment	Culturable organisms (log CFU/ml) after					
	24 hrs		48 hrs		96 hrs	
	4°C	25°C	4°C	25°C	4°C	25°C
Control	5.82	5.82	5.69	5.76	5.29	5.12
0.012% H ₂ O ₂ + 0.1%TA ^a	5.43	5.46	5.4	4.01	4.59	4.03
0.012% H ₂ O ₂ + 0.3%TA	5.13	5.22	4.4	2.86	3.97	ND ^b
0.017% H ₂ O ₂ + 0.1%TA	5.23	5.21	5	2.72	4.49	ND
0.017% H ₂ O ₂ + 0.3%TA	4.26	4.72	2.9	0.3	1.66	ND

^aPercent tartaric acid added (wt/vol)

^bBelow the limit of detection for this assay (< 1 CFU/ml)

Table 5. Effect of hydrogen peroxide, tartaric acid (TA), and temperature on *E. coli* O157:H7 in white grape juice after 24 and 48 hour incubation, as cultured on TSAN. (n = 3)

Treatment	Culturable organisms (log CFU/ml) after			
	24 hrs		48 hrs	
	4°C	25°C	4°C	25°C
Control	6.02	5.79	5.79	5.76
0.012% H ₂ O ₂ + 0.1%TA ^a	1.12	ND ^b	ND	ND
0.012% H ₂ O ₂ + 0.3%TA	1.14	ND	ND	ND
0.017% H ₂ O ₂ + 0.1%TA	0.52	ND	ND	ND
0.017% H ₂ O ₂ + 0.3%TA	ND	0.3	ND	ND

^aPercent tartaric acid added (wt/vol)

^bBelow the limit of detection for this assay (< 1 CFU/ml)

Table 6. Effect of hydrogen peroxide at different temperatures on numbers of *E. coli* O157:H7 in apple cider, as cultured on TSAN. (n = 3)

Temp (°C)	H ₂ O ₂ %	Culturable organisms (log CFU/ml) after				
		1 hr	3 hr	6 hr	12 hr	24 hr
10	0	6.62	6.67	6.65	6.67	6.59
25	0	6.66	6.75	6.61	6.59	6.45
40	0	6.43	6.61	6.45	6.2	5.15
10	0.015	6.4	5.25	4.43	2.84	0.78
25	0.015	6.3	5.38	3.81	2.08	ND
40	0.015	5.63	3.08	0.48	ND	ND
10	0.03	5.77	4.11	2.48	0.3	ND
25	0.03	5.72	3.53	1.99	ND	ND
40	0.03	4.08	1.15	ND ^a	ND	ND

^aBelow the limit of detection for this assay (< 1 CFU/ml)

Table 7. Effect of hydrogen peroxide at different temperatures on numbers of *Salmonella* spp. in orange juice, as cultured on TSAN. (n = 3)

Temp (°C)	H ₂ O ₂ %	Culturable organisms (log CFU/ml) after				
		1 hr	3 hr	6 hr	12 hr	24 hr
10	0	6.41	6.28	6.32	6.35	6.25
25	0	6.36	6.27	6.01	5.86	6.12
40	0	5.66	5.53	5.33	4.97	5.47
10	0.015	6.04	4.57	3.82	2.74	ND
25	0.015	5.36	4.4	3.57	1.58	ND
40	0.015	3.36	1.9	0.3	0.3	1.28
10	0.03	4.75	3.18	0.3	0.3	ND
25	0.03	4.4	1.98	ND	ND	ND
40	0.03	1.36	ND ^a	ND	ND	ND

^aBelow the limit of detection for this assay (< 1 CFU/ml)

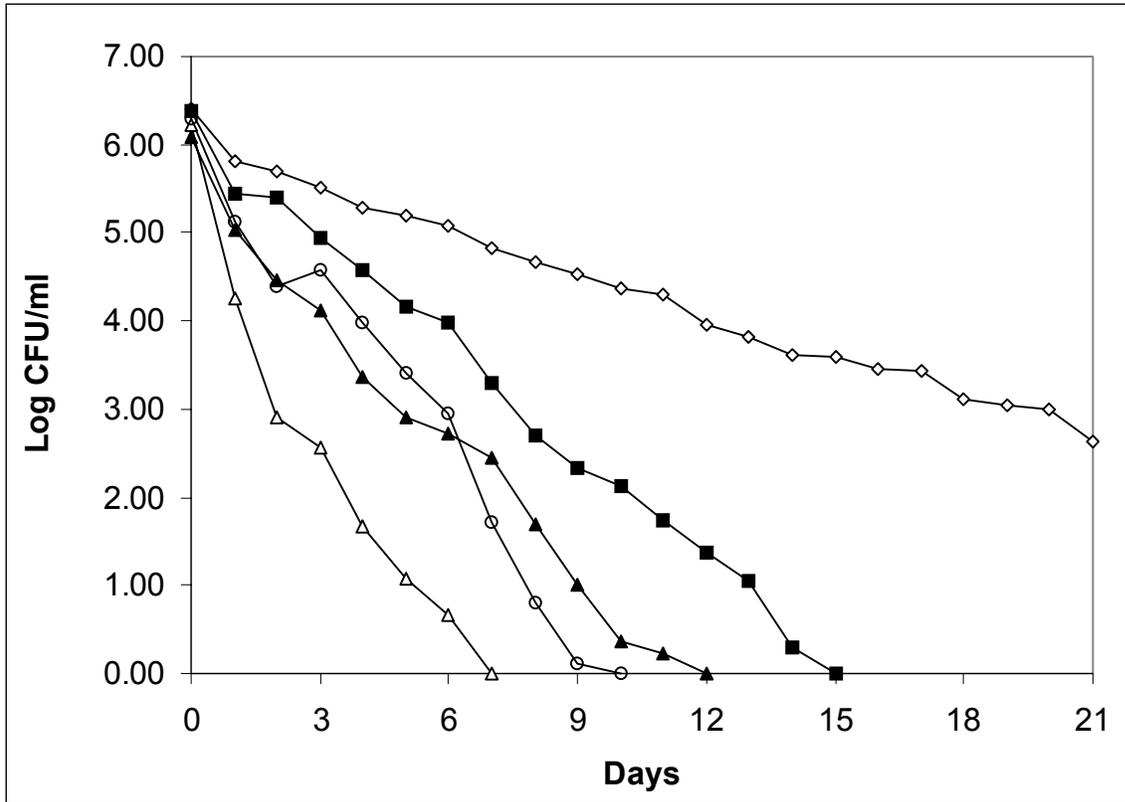


Figure 1. Effect of H₂O₂ and tartaric acid (TA) on the survival of *E. coli* O157:H7 in 4°C purple grape juice as enumerated on TSAN. Grape juice control (◇); 0.017% HP + 0.3% TA (Δ); 0.017% HP + 0.1% TA (▲); 0.012% HP + 0.3% TA (○); 0.012% HP + 0.1% TA (■).

APPENDIX

Evaluation of Organic Acid Added to Apple Juice

Name: _____
Number: _____
Date: 4/27/01

Instructions:

Rinse your mouth with water before beginning. Expectorate the water into the container provided. You will review 5 samples. For each sample please indicate your acceptance for the taste by circling "In" or rejection for the taste by circling "Out". Only evaluate the taste (not the color or odor). Rinse your mouth with water between samples and expectorate all samples and the water into the cup provided.

Thank you for your participation! It is much appreciated!

Comments

Sample #: In Out

VITAE

John Schurman was born and raised in Richmond, Virginia. He attained his Bachelor's degree in Food Science and Technology in December of 1999 from Virginia Polytechnic Institute and State University. During the summer of 1998, he worked as a research intern for the Food Science and Technology Department. His Master's program in Food Science and Technology at Virginia Tech began in August of 1999. While at Virginia Tech, he was a member of the Institute of Food Technologists, and played trumpet in various musical ensembles including the Virginia Tech Jazz Orchestra, Olim Jazz Quintet, and Trumpet Ensemble.