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Presence and growth of microorganisms in Iowa apple cider

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GENERAL INTRODUCTION

E. coli O157:H7 was recognized as a human pathogen in 1982. Since the 1980 outbreak of *E. coli* O157:H7 associated with apple cider in Canada, it has been in the news over the last several years for other outbreaks related to apple cider. The illnesses caused by the pathogen include severe gastroenteritis (bloody diarrhea, vomiting, cramps) and Hemolytic Uremic Syndrome that is defined as a combination of thrombocytopenia (platelet count, $<150 \times 10^9$/L) microangiopathic hemolytic anemia and acute renal failure ($\geq$50% increase in serum creatinine level over baseline) (Steele et al., 1982, Besser et al., 1993). Apple cider was considered to be a highly acidic product and was therefore not considered to be conducive to the survival and growth of pathogens. But now research has demonstrated that *E.coli* O157:H7 possesses unusual tolerance to low pH and can thus survive in highly acidic products like apple cider (Semanchek et al., 1996).

In the United States two major outbreaks attributed to the organism in apple cider occurred in 1996 and 1999. In response to these outbreaks, the FDA has mandated a 5-log reduction in the populations of a target pathogen or the display of a warning label on unpasteurized products that warns consumers of the possible risk of acquiring food borne illness due to consumption. It also requires that cider producers should have a Hazard Analysis and Critical Control Point (HACCP) plan in place for their operations. Small and very small producers are not subject to the juice HACCP rule till January 21, 2003 and January 20, 2004 (FDA, CFR 21.2001), respectively. The National Advisory Committee on Microbiological Criteria (NACMCF) determined the 5-log reduction standard for foods by adding a 100-fold margin to the levels of *E.coli* O157:H7 that may typically be found in

juice. According to the FDA (2001) this performance standard has a built-in-safety factor that ensures additional consumer protection.

As a result of these regulations, cider-processing operations have begun to change their production practices. These changes include reducing the use of drop apples in cider production, addition of preservatives, and pasteurization of the final product. Thermal pasteurization of apple cider is a very effective way to ensure lower microbial loads in the final product. The cost involved in installing a small-scale pasteurization unit can lie anywhere between $10,000 and $20,000 (personal communication with producers). The high cost coupled with the reduction in the quality of cider (changes in color and flavor) upon pasteurization influence cider producers' decision on whether to continue to produce cider, with pasteurization, or to stop production. Since 1998, 10% of the certified apple cider producers in Iowa have stopped production (Iowa Fruit and Vegetable Growers Assoc., 1999). Other means of lowering *E.coli* O157:H7 levels in cider include ultraviolet light treatment, irradiation, high pressure, pulsed electric fields and the use of ozone.

Pasteurization of apple cider results in changes in its flavor compounds causing a decreased fruit-aroma score and an increase in cooked flavors (Poll, 1983). The principal flavor compounds produced by heating include furfural, hydroxymethylfurfural, benzaldehyde and 2,4-decadienal (Nursten and Woolfe, 1972). Changes in flavor compounds are also expected from the addition of preservatives and from both chemical changes and microbial growth and metabolism in cider during storage.

The purpose of this study was to survey the production practices of Iowa apple cider producers and to determine the microbial load present in the cider, on the apples used in cider production, and on the equipment used. HACCP plans were prepared on the basis of the

results obtained from a written survey and from in-person observations made during visits to the production sites. These plans were provided to the producers to help them improve their processes and thus ensure that their cider is safe for consumption. The second part of the study focused on the changes in microbial loads and flavor compounds during refrigerated storage of the cider samples. The flavor compounds that changed over time were identified with the help of gas chromatographic analysis and an electronic nose was used to determine if a relationship existed between microbial loads and the patterns of flavor compounds over storage time.

LITERATURE REVIEW

Apple Cider

Apple cider may be defined as the fresh pressed juice obtained from apples. Downing (1989) distinguished apple cider from apple juice on the basis of a darker color, less clarity and the presence of suspended solids. Traditionally cider production has been considered to be a by-product industry, and only those apples/ varieties were used for cider production that were considered to be surplus or drops were graded as culls. However, now attention is paid to the proper maturity and quality of apples used in cider production. The maturity of apples influences not only the quality of the finished juice but also affects the economy of plant operations, as the use of over-mature apples causes the pomace to adhere to the press cloths and makes cleaning very difficult, slowing down the whole operation (Moyer and Aitken, 1980). According to Childers (1983), the use of a particular variety to create the desired blend of flavors varies throughout the cider production season as different cultivars ripen at different rates. Processors also store apples in the cold (4°C) and use them as the season progresses.

Before use in cider production, the cider apples are subject to washing and/or brushing. This may be done either before apples are put in storage or right before pressing. Washing may be done by dumping the apples into water troughs containing water (sometimes chlorinated water may also be used) or strong water sprays may wash the apples as they move along a roller-type conveyor. The washed apples are then ground to a pulp suitable for juice extraction either by grinding or with the help of a hammer mill. Following grinding, juice is extracted from the pulp with the help of one of the following types of presses: hydraulic cider press, pneumatic fruit juice press, continuous screw type press,

continuous plate press, horizontal basket press or screening centrifuge (Moyer and Aitken, 1980).

The extracted juice is collected into trays and then pumped to holding tanks. Preservative addition may be done while the cider is kept in the holding tanks. The preservatives approved for addition to cider include potassium sorbate and sodium benzoate at $\leq 0.1\%$ concentrations. Once the preservative has been added, the cider may be allowed to sit from half an hour to overnight (depending on the processor) before the cider is pasteurized and/or bottled (personal observation).

Pasteurization can be done by using a variety of time-temperature combinations with times ranging from 1 sec to 11 sec and temperatures ranging from 73 to 84°C. Although pasteurization is very effective in eliminating *E. coli* O157:H7 contamination in apple cider, it is a very expensive option for roadside stand operators. As a result of the FDA regulations requiring a 5-log reduction in the final product, many small-scale cider processors have had to shut down their operations. Kozempel et al. (1998) calculated the cost of pasteurization for a medium-sized plant (producing 56 million L of cider/year) as 0.2 cents/L. But the production capacity of most orchards is less than this and thus installation of a pasteurization unit may prove to be much more costly.

Apples Used in Cider Production

The microbial load on the apples used for cider production can greatly affect the load in the final cider product. The apples can get contaminated from a variety of sources in the orchard, during harvesting and while processing. In the earlier outbreaks of *E. coli* O157:H7 in apple cider, the apples were proposed to be the main source of contamination. This was because prior to the outbreaks, the use of drop apples in cider production was a common

practice; it is possible that the apples picked from the ground could become contaminated from contact with animal fecal matter and soil. However, there is no direct evidence to link the use of drop apples to contamination of cider. Janiesievicz et al. (1998) suggested that the apples might be contaminated with *E. coli* O157: H7 from bird droppings and feces of domestic or feral animals. Flies and insects also can act as vectors. Fruit flies have the potential to transmit *E. coli* to apples because of the high frequency with which they are contaminated from a source and in turn are able to contaminate apple wounds (Janiesievicz et al., 1998). Kettle (1982) found that houseflies can contain up to 100 different pathogens and can transmit 65 of these. Ruminant animals like cattle, sheep and deer have also been identified as reservoirs of *E.coli* O157:H7 (Keene et al., 1997; Kudva et al., 1996; Zhao et al., 1995).

Riordan et al. (2001) collected fruit and environmental samples from 14 orchards throughout the U.S. to determine potential sources of *E. coli* O157:H7 and to characterize the microflora profile of the fruit and the orchard environment. No *E. coli* O157:H7 was found on any of the samples tested. Intact tree fruits had significantly lower counts of aerobic bacteria, coliforms, and yeasts and molds, with less microflora in the core of the fruit, than did dropped or damaged fruit. They identified the following environmental factors as critical to control to ensure a safe product: presence of fecal matter, application of manure, proximity to pasture lands and irrigation with non-potable water. The study confirmed that dropped and damaged fruit have increased microbial populations and are a potential source of *E.coli*. The authors suggested that these fruits should not be used for the production of unpasteurised juice or for the fresh or fresh-cut market.

Wounded /damaged apple tissue is a very good substrate for the growth of pathogens. Dingman (1999) tested the growth of *E. coli* O157:H7 in damaged apple tissue of various cultivars (McIntosh, Red Delicious, Macaun, Melrose and Golden Delicious) and found that it was present uniformly in the apples irrespective of the apple source. Higher pH and low Brix values in the damaged apple tissues enhanced bacterial growth. Fisher et al. (1998) looked at the growth and survival patterns of *Escherichia coli* O157:H7 in various cultivars of ground apples used in cider production. They reported that none of the cultivars showed a particular tendency to support the growth of *E.coli* but variations in apple pH during storage may negatively or positively affect the growth of *E. coli* at 25°C. They also associated increased apple pH with increased mold growth. Similarly, Zhao et al. (1993) observed that pathogens present on apples could contaminate the final product if the apples were improperly washed.

Buchanan et al. (1998) found that the outer core region of the apple had the highest concentration of pathogens among the various regions of the apple they tested. This cavity was difficult to reach while sanitizing/washing the apple and thus was a major problem area. They also found that the internalization of pathogens into apple tissues was dependent upon the temperature differentials between apples and wash water. Dipping of cold apples (4°C) in warm dye solution (21°C) did not cause any uptake of the dye whereas submergence of warm apples (22°C) in a cold solution (9°C) caused a considerable accumulation of the dye in the inner core regions of the apples.

Kenney et al. (2001) determined how bruising, washing and rubbing of apples affected the location of *E. coli* O157:H7 on the apple surface. They used transformed *E. coli* O157:H7 that produced a green fluorescent protein to inoculate undamaged and bruised

apples. The apples were then subjected to washing and rubbing treatments. Both found undamaged and bruised apples subject to the same wash and rub treatments did not differ in the number of cells recovered from their surface. Washing of both groups of apples decreased the number of cells on the surface compared to unwashed apples. No bacterial cells were found in the lenticels or wax platelets of the washed apples at a depth more than 6 μm. Bruising of apples by dropping them from a height allowed bacterial cells to be pushed deeper into the apple, thus protecting them from removal or contact with hydrophobic sanitizers. They also observed that cells that remained on the surface of the rubbed apples appeared to be sealed inside cracks and crevices in the waxy cutin platelets on the fruit surface. These organisms were thus protected from disinfection and may be released when apples were eaten or pressed for cider production. The authors suggested that the risk associated with raw apples or cider apples due to internalization of the pathogens in the wax platelets of the apples can be minimized by the use of sanitizers, which contain a surfactant, or solvent that aids in the removal of the protective natural wax on the apple surface.

Pathogens Found in Apple Cider and Related Outbreaks

Escherichia coli O157:H7 was first recognized as a pathogen in 1982 and is now known to be an important cause of bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome) in humans. *E.coli* belongs to the family *Enterobacteriacae*, members of which are identified as being small gram-negative rods, with 30-60% G-C (guanine-cytosine) content and the ability to ferment D-glucose (Janda et al., 1998). *E. coli* is a coliform as it can decompose lactose with acid and gas production (Kay et al., 1997). *E. coli* can be found in the intestinal tract of warm-blooded animals (including humans) and also as part of the bowel flora of birds (Janda et al., 1998).

At present five virotypes of *E. coli* are identified: enteropathogenic, enteroinvasive, enterotoxigenic, enteroaggregative, and enterohaemorrhagic. *E. coli* O157:H7 is an enterohaemorrhagic coliform (Kay et al., 1997). The principal virulence determinant of the pathogen is the production of cellular cytotoxins, which are similar to Shiga toxins obtained from *Shigella dysenteriae* (Neill, 1997). The infective dose for *E. coli* O157:H7 can be as few as 10 cells (Omaye, 2001). The main source of *E.coli* O157:H7 in the environment is proposed to be cattle and the main mode of infection is transmission from animal to animal or from animal to human (Phillips, 1999). A variety of food products including ground beef, raw milk, turkey sandwiches and apple cider have been demonstrated vehicles of *E. coli* O157:H7 (Doyle, 1991). *E. coli* O157:H7 can infect foods by different mechanisms: during processing of slaughtered animals at abattoirs or by excretion of biologic wastes by domestic or feral animals on agricultural lands (Sussman, 1997).

Apple cider has been implicated in a number of outbreaks involving *E. coli* O157:H7. In 1980, an outbreak of HUS in Canada was linked to the consumption of apple cider (Steele et al., 1982). Since then other outbreaks of HUS related to the presence of *E. coli* O157:H7 in apple cider have occurred in the United States. In the fall of 1991 there was an outbreak of *E.coli* O157:H7 in southeastern Massachusetts. The agent of infection in this case was identified to be fresh apple cider from a particular operation (Besser et al., 1993). A large part of the western United States and British Columbia, Canada, was in the news in 1996 because of a widespread outbreak of HUS linked to consumption of Odwalla apple juice; one death occurred in this outbreak (Cody et al., 1999).

In 1974, an outbreak of gastroenteritis caused by *Salmonella typhimurium* in non-sterile apple juice occurred in New Jersey. Goverd et al. (1979) reported the survival of

Salmonella spp. for up to 30 days in apple juice and also that the *Salmonella* survived well at pH as low as 3.68. But low temperatures (below 4°C) actually caused a decline in the population of *Salmonella typhimurium.* They also found that salmonellae were not able to survive well in the presence of ethanol; this and the changes in nutritional / physiological conditions caused during fermentation decreased the ability of the pathogen to grow in cider. Uljas and Ingham (1999) found that in frozen-thawed apple cider at pH 3.3, *S. typhimurium* DT104 decreased by 5 logs.

Another organism of concern that has been found to be present in apple cider is *Listeria monocytogenes.* Sado et al. (1998) detected this organism in unpasteurized apple juice. Because of the widespread occurrence of this pathogen in the environment, the chances of contamination are quite high.

Cummins (2001) identified some of the common microorganisms found in Iowa apple cider. Most bacteria found in the cider belonged to the *Enterobacteriaceae* family. Yeasts were also found to be prevalent on the apples and in unpasteurized cider. Passmore and Carr (1975) found six species of *Acetobacter* in their study on cider. *Zymomonas spp.* have also been found in apple cider but in very low numbers (Jay, 2000.)

Many types of yeasts and molds are also found in cider and on apples. The yeasts present in cider are generally capable of producing alcohol by the fermentation of sugars found in cider. The major yeasts present in the pressed apple juice and early stages of fermentation are *Hanseniaspora uvarum* and *Saccharomyces ludwigii, Metschnikowia pulcherrima and Dekkera spp* (Carr, 1984; Deak et al., 1996). Molds such as *Penicillium claviforme, P. expansum,* and *P.patulum,* some aspergilli and *Byssochlamys nivea* and *B.fulva* are capable of producing the mycotoxin patulin (Jay, 2000). Patulin is a known

carcinogen that can also cause liver damage (Klaassen, 1996). While the maximum acceptable level of patulin has been set at 50 ppb by the FDA (FDA, 2000), the levels of patulin in apple juice can be as high as 440 ppb and in cider up to 45 ppm. Thermal processing may produce only moderate reductions in patulin, which can survive pasteurization (WHO IARC, 1990).

Acid Tolerance of *E. coli*

E.coli is a pathogen that can adapt well to a variety of environmental conditions. Zhao et al. (2000) suggested that it does so by entering a viable but nonculturable (VNC) state in which cells remain metabolically active but do not undergo cellular division and produce colonies on conventional growth media. They studied the effect of pH (pH 4-7) on the entry of *E.coli* O157:H7 cells into VNC state (detected by emergence of two distinct morphological populations;typical rodshaped and coccoid shaped cells) and found that at pH 4 the cell population decreased to undetectable levels more rapidly than at pH 7. But the viable counts were similar for cells suspended in both the solutions.

Conner et al. (1994) studied the effect of various types of acids at varying pH levels and temperatures on the growth of *E. coli* O157:H7. Acetic and lactic acids were found to be the most effective for inhibition while tartaric acid was found to be the least inhibitory. Buchanan et al. (1992) also observed at pH 3.0, lactic acid was the most inhibitory organic acid. They found that of all the acids tested, hydrochloric acid was the least inhibitory one.

Marques et al. (2001) studied the acid resistance of three strains of *E. coli* O157:H7 inoculated in various fruit pulps whose pH ranged from 2.65 to 3.24. They found that all three strains survived for 4 days at all the pH's under refrigeration conditions. Their results indicate that acid resistance can persist for long periods during storage at 4°C and the acid

resistance systems remain active over prolonged storage periods. They also found that strains that had not been subject to acid shock treatments could rapidly develop the mechanisms needed to survive in low pH conditions. Miller et al. (1993) found that two strains of *E. coli* O157:H7 (ATCC 43889 and ATCC 43895) could survive in apple cider while the control strain *E. coli* B (FRIK124; Food research Institute Kaspar, culture collection isolate 124) showed rapid decrease in numbers and did not survive for long.

There are three acid resistance systems in *E. coli* O157:H7: an acid-induced oxidative system, a glutamate-dependent system, and an arginine-dependent system (Lin et al., 1996). All these systems are active during stationary-phase growth and once induced, they persist for at least 1 month at 4°C (Price et al., 2000). Price et al. (2000) suggested the involvement of a stationary-phase sigma factor S, encoded by rpoS. RpoS regulates the expression of stress response genes (Jyshiun et al., 1996). Price et al. (2000) also found that rpoS increases *E. coli* O157:H7 shedding in calves by inducing resistance in *E. coli* O157:H7 to gastro-intestinal stress.

Arnold et al. (1995) reported that the acid tolerance of *E. coli* O157:H7 was not dependent on prior exposure to a low pH; rather, entry into stationary phase or starvation of log-phase cells increased the acid tolerance of *E. coli* O157:H7 strains. There is a naturally increased resistance to various chemical and physical challenges in the stationary phase of cells. The proteins induced during starvation also protect the cell from environmental challenges.

Results from all these studies indicate that *E. coli* O157:H7 is capable of surviving in, and adapting to, the low pH of apple cider. Therefore it is necessary to include a processing

treatment in the production of cider to help reduce the risk of infection by this pathogen. One should not rely on the acidity of the product to make it safe for consumption.

Effect of Temperature on fate of *E. coli* O157:H7 in Apple Cider

Growth and survival of *E. coli* O157:H7 in apple cider is influenced by a number of factors such as pH, °Brix, temperature. Studies have shown that *E. coli* O157:H7 can survive in refrigerated cider. Zhao et al. (1993) reported that *E. coli* O157:H7 could survive for up to 31 days in apple cider when held at 8°C at pH 3.7. At 25°C, the cells could survive for only up to 2-3 days after inoculation. Dingman (1999) found that refrigeration at 1-4°C for 64 days caused a 1-log decrease in viable counts of *E.coli* O157:H7 while frozen cider (at–20°C) showed a drop of only 30%.

Corry (1976) found that high concentrations of sugars and solutes increased the heat resistance of microorganisms. Juven et al. (1978) reported that yeasts present in orange juice concentrates were more heat-resistant than the ones found in single-strength juice. Splittstoesser et al. (1995) studied the heat resistance of *E. coli* O157:H7 in apple juice concentrates and found that when the apple juice concentrates were diluted with water (1:3), the same heat resistance expected in single strength-juice was obtained. Addition of malic acid to the diluted concentrate reduced heat resistance but not to the extent expected. But an increase in °Brix values increased the heat resistance. They concluded that the high concentrations of solutes provided protection to the cells against heat while malic acid sensitized the cells and reduced heat resistance, but an unknown constituent of apple juice was having a greater effect on the heat resistance of *E. coli*.

Mazotta (2000) evaluated the heat resistance of stationary-phase and acid-adapted *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in apple juice. He found

that acid-adaptation of all three pathogens increased their heat resistance at 56, 60 and 62°C with acid -adapted *E. coli* O157:H7 showing the maximum heat resistance. But it was observed that *L. monocytogenes* had a higher z-value and extrapolation of the heat resistance to temperatures above 65°C indicated that *L. monocytogenes* was more heat-resistant at higher temperatures.

Mak et al. (2001) validated various time-temperature conditions employed for apple cider pasteurization in the states of Wisconsin and New York. Their results suggested that a time-temperature combination of 68.1°C for 14 s was successful in obtaining a 5-log reduction in the counts of two of the target organisms, i.e. *Salmonella* spp. and *E. coli* O157:H7. The third target microorganism, *Listeria* spp., did survive the treatment but died off within 24 h at 4°C. They concluded that *E. coli* O157:H7 is the most appropriate target organism for evaluating the efficiency of pasteurization treatments for cider.

Given the low heat resistance of *E. coli* O157:H7, heat treatment/pasteurization is a good method to ensure its removal from cider. As heat resistance of *E. coli* is affected by the other chemical and physical conditions of the cider, it is essential that any time-temperature combination that is validated for apple cider pasteurization should take into account these factors also.

Methods to Reduce *E. coli* O157:H7 Counts on Apples and in Cider

Beuchat (1992) reported only a 1-log reduction in microbial loads on fruits when washed with water alone. Use of chlorine in wash water can reduce microbial populations by another log . They reported that the use of flatbed washers instead of water dip does not help in decreasing the microbial loads even with the addition of antimicrobial agents because of the short exposure time and ineffective brushing.

The recommended levels of chlorine as a sanitizer on fruits and vegetables are 200 to 300 μg/ml. Other sanitizers used on apples include peroxyacetic acid, chlorine dioxide and a chlorine/phosphate buffer solution. Wisniewsky et al. (2000) reported that these sanitizers need to be used at high concentrations for at least 15 minutes to achieve the 5-log reduction. Sapers et al. (1999) conducted a study to determine the population reductions achieved on apples by the use of various sanitizers. They found that 0.5% hydrogen peroxide reduced the microbial populations on apples by 3 to 4 logs but the residual H_2O_2 levels on apples were high (1000 ppm). They also found that *E. coli* levels were reduced by 2 logs when the apple halves were treated with chlorine. Whole apples showed lower adherence of *E. coli* as compared to the halves.

Zook et al. (2000) examined the influence of sanitizer on the adaptive stress response in *E. coli* O157:H7. They exposed *E. coli* O157:H7 strains to a sublethal concentration of peroxyacetic acid (PAA) and found that cultures acutely exposed to the PAA developed tolerance to hydrogen peroxide (H_2O_2) but no thermal cross-resistance was observed. The use of acetic acid alone did not induce a significant peroxidative tolerance. This suggests that the H_2O_2 component of the PAA sanitizer (27.5% H_2O_2) is important for the induction of stress response to H_2O_2. These results need to be examined in applied scenarios such as fruit and vegetable sanitation to ensure that the use of sanitizers is not actually increasing the risk of contamination by *E. coli* O157:H7, rather than reducing it.

E. coli O157:H7 has been found to have no particular resistance to heat and pressure. Fleischman et al. (2000) studied the effect of hot water immersion (80-95°C for 30s) on the reduction of *E. coli* O157:H7 on whole apple surfaces. They found that this is an economical and effective method and reported reductions of up to 7 logs in *E. coli* O157:H7

populations on the apple surface. Use of other treatments such as sodium hypochlorite, acetic acid, and hydrogen peroxide with washing has resulted in a maximum decrease of 3 logs in the populations of pathogenic microorganisms. But this method was not found to be effective for internalized organisms.

Electrical pulses can cause microbial inactivation in liquid foods by causing permanent cell membrane breakdown (Ho et al., 1996). Iu et al. (2001) studied the reduction of *E. coli* O157:H7 in apple cider by pulsed electric fields and reported a greater than 5 log reduction in counts of *E. coli* O157:H7 cells in apple cider at 42°C with 10 electrical pulses at 80kV/cm. Combination of electrical pulse treatment with cinnamon or nisin increased cell inactivation to 6 to 8 logs. The inactivation effect was more pronounced on gram-negative bacteria than on gram-positive species and yeasts and molds.

Sage and Ingham (1998) reported that *E. coli* O157:H7 could be reduced in apple juice by freezing and thawing. Freezing helps in extending the shelf life of apple juice and also causes a 0.63-3.43 log in the reduction of *E. coli* O157:H7. Yamamoto and Harris (2000) found that the first cycle of freezing-thawing of frozen storage (24h) of apple juice resulted in 1.6-2.0 log reduction in the counts of the most sensitive strains of *E. coli* O157:H7; a slow decline in survival occurred after that. Injury and viability increased with each subsequent freeze/thaw cycle.

A number of methods are available which can be used to meet the 5-log reduction criteria for apple cider but the choice of a particular method is dependent upon a number of other factors such as the economics involved, feasibility, and effect on flavor characteristics of the cider. So far heat treatment seems to be the method that is being used most widely by producers, possibly because the flavor of pasteurized cider has found acceptability among

consumers who are familiar with the pasteurized flavor due to the widespread use of pasteurized milk.

Preservatives Used in Apple Cider

Preservatives are added to foods to prevent or delay spoilage. Sodium benzoate is mainly added to cider to inhibit yeast and mold growth while potassium sorbate can also prevent mold and yeast growth but is also effective against bacteria such as *Staphylococcus aureus, Vibrio parahemolyticus,* salmonellae and psychrotrophic spoilage bacteria (Miller and Kaspar, 1994). According to Turantas et al. (1999), yeasts, molds and bacteria are all inhibited effectively by sorbic acid while benzoic acid is more suitable for inhibition of yeasts and molds rather than bacteria. Sofos et al. (1985) studied the effect of sorbic acid on bacterial cells and spores and reported that sorbate acts on the bacteria by inhibiting spore germination, outgrowth, and vegetative cell division.

Brul et al. (1999) in their study on the modes of action of various preservatives proposed that bacterial growth is inhibited mainly by membrane disruption, inhibition of important metabolic reactions, upsetting the intracellular pH and accumulation of toxic anions. Induction of an energy-intensive stress response, which reduces the available energy pools for growth, is the principal mechanism by which yeast growth is inhibited.

Besser et al. (1993) reported that the addition of 0.1% sodium benzoate to cider stored at 8°C prevented the growth of *E. coli* O157:H7 with counts dropping to undetectable levels after 7 days. Potassium sorbate did not have much effect on the numbers of *E. coli* O157:H7 organisms.

Zhao et al. (1993) suggested the use of 0.1% sodium benzoate to increase the safety of apple cider by inhibition of *E. coli* O157:H7 growth and by suppression of yeasts and

molds. They found sodium benzoate to be more effective as a preservative than potassium sorbate at 8 and 25°C. A combination of 0.1 % sodium benzoate and 0.1% potassium sorbate was found to have the maximum inhibitory effect on *E. coli* O157:H7 populations. However, Miller and Kaspar (1994) did not see any significant effect of preservative on the survival of two strains of *E. coli* O157:H7. They suggested that this difference in results may be due to the presence of mold growth in the cider in previous studies (Zhao et al., 1993) that may have inhibited *E. coli* O157:H7, to variations among strains, or to other unidentified factors.

Splittstoesser et al. (1995) reported that both potassium sorbate and sodium benzoate reduced the heat resistance of *E. coli* O157:H7, with benzoate being about eight times more effective than sorbate. Dock et al. (2000) studied the combined effect of pH and preservatives on the heat resistance of *E. coli* O157:H7. They found that the addition of sorbate, benzoate and malic acid, individually and in combination, significantly reduced the heat resistance of *E. coli* O157:H7 in apple cider. The largest effect was seen with a combination of malic acid and benzoate, while sorbate had a lesser but still significant effect. The authors found higher thermal death times at higher temperatures (70°C) for cider containing benzoate as compared to cider without additives. This indicates that processors who add benzoate to cider before processing may be obtaining less than 5-log reductions of *E. coli* O157:H7 that would have occurred without the addition of benzoate.

It may be inferred from these studies that preservatives alone are not effective enough to reduce the populations of pathogenic microorganisms in apple cider. They are best utilized as agents that help in keeping down the microbial numbers during storage after the cider has received an initial kill treatment such as pasteurization or they may be effective when used in combination with some kind of heat treatment.

Production Practices

As a result of the numerous outbreaks related to apple cider, the FDA has issued regulations requiring the application of Hazard Analysis and Critical Control Point (HACCP) principles to juice production (FDA, 2001). The HACCP system was developed by the Pillsbury Company as a management system to prevent problems associated with the production of safe-to-consume food products. Good manufacturing practices (GMPs) and standard operating procedures (SOPs) are prerequisites for an appropriate HACCP plan (Stevenson et al., 1999). The GMPs and SOPs specific for cider production have been developed by some state agencies and in Canada (FDA, 1999).

The NACMCF recommended the use of *E. coli* O157:H7 or *Listeria monocytogenes* as the target microorganisms for studying microbiological control. The chosen indicator organism is useful as a verification tool for plant sanitation and for the HACCP plan. Lang et al. (1999) chose *E. coli* as the most useful indicator organism because of the fecal origin of *E. coli*, good survival characteristics, its association with apples and the relative ease of testing for the organism. The FDA plans to provide additional information in its HACCP Juice Hazards and Controls Guidance document to assist producers in identifying the pertinent microorganism for measuring the 5-log reduction (FDA, 21 CFR Part 120, 2001).

According to Senkel et al. (1999) the seven principles of HACCP are:

Principle 1: Conduct a hazard analysis.

Principle 2: Determine the critical control points (CCPs).

Principle 3: Establish critical limits.

Principle 4: Establish monitoring procedures.

Principle 5: Establish corrective actions.

Principle 6: Establish verification procedures.

Principle 7: Establish record-keeping and documentation procedures.

The HACCP principles require that a HACCP procedure be completed for each specific process, and hazard analysis, critical control points and control limits must be established for the identified hazards (Keller et al., 2002). Senkel et al. (1999) identified the following production steps as controls: pasteurization, exclusion of drop apples, chlorine soak or spray for apples, and temperature control of cider. With these steps identified as being critical to eliminate contamination, a producer can take precautions and appropriate measures in order to ensure the production of a safe final product.

Goverd et al. (1979) were the first to report on the incidence of foodborne pathogens in apple cider. They observed that the production practices followed by most of the plants varied and included practices such as the use of dropped apples. Sanitary conditions at the plants ranged from "primitive" to "well organised cleaning routines". The counts of *E. coli* obtained from these ciders varied from 0 to180 presumptive *E. coli*/100 ml by the most probable number (MPN) procedure.

The FDA conducted a survey of 237 cider manufacturers in 1997 (FDA, 1999) in 32 states. Emphasis was placed on the harvesting and processing practices and on the microbiological quality of the final product. Their findings indicated that 59% of the producers used only tree-picked apples. While 67% of the plants had good sanitation, 27% were marginal and 4% had poor sanitation.

Dingman (1999) noted that 64% of the mills in a survey of Connecticut cider producers used drop apples in addition to tree picked apples. *Escherichia coli* was found in

4% of the samples tested. Dingman observed an association between the time of year of cider production and the occurrence of *E. coli*. No *E. coli* was found in the final cider when the use of drop apples was maximum whereas when only tree-picked fruit was used, *E. coli* was found in the cider samples. Although guidelines have been issued by the FDA that discourage the use of drop apples in the production of cider, many orchards still use drop apples in their processes. It was suggested that other factors, in addition to the use of drop apples, including storage conditions, length of storage, and quality of fruit being used affect the contamination of cider.

Senkel et al. (1999) conducted a survey of Maryland cider producers to evaluate their production practices and to determine whether implementation of HACCP reduced the microbial contamination of the cider produced in the facilities. They found no change in the standard plate counts and total coliform counts after the implementation of improved practices. However, a highly significant decrease was seen in the number of bottled cider samples that contained *E. coli*. The authors suggested that the implementation of improved production practices as a part of the HACCP system could help reduce the risk of food borne illness in fresh apple cider by reducing bacterial levels and the likelihood of fecal contamination.

Senkel et al. (1999) found the occurrence of nonpathogenic *E. coli* on in-line apples and cider samples but not on the incoming apples. This implies that the microorganism could have been introduced during processing.

Keller et al. (2002) studied the efficacy of sanitation and cleaning methods in a small cider processing plant, under controlled conditions. They found that the total aerobic plate count and yeast and mold counts increased at subsequent steps throughout the trial. They

suggested that the higher levels of microorganisms found in the final cider as compared to incoming apples indicated substantial contamination from processing equipment. During processing of inoculated apples, a considerable microbial aerosol was observed. This is significant as the production of aerosols can account for the spread of contamination from the fruit to the entire facility. Some areas/equipment were observed to be difficult to clean and sanitize; the authors suggested that biofilms might have developed on these surfaces. When apples inoculated with *E. coli* K-12 were introduced, a population of this organism was established in the plant, which could not be removed by normal cleaning and sanitation. Therefore care should be taken to prevent the initial contamination of a facility.

It is very important to follow proper sanitation methods along with GMPs to ensure that the contamination of apples and cider from the equipment, plant environment and/or workers is minimized. Also proper handling of the product after pasteurization is needed to prevent post-process contamination. Although following a HACCP system alone may not ensure the production of a safe product, the implementation of GMPs and SOPs along with the application of a post pressing intervention step such as pasteurization can help in reducing the levels of contamination in the facility and the final product.

Electronic Nose

Electronic nose (e-nose) instrumentation contains multiple sensors that each measure one or more volatile components. Therefore, the e-nose assesses the mixture of volatiles comprising and emitted from the food under investigation (Spanier, et al, 1999).

The e-nose has, in recent years, been developed as an instrument being used primarily for quality control, which provides for a rapid, nondestructive and objective analysis. Bartlett et al. (1997) defined e- nose as "an array of chemical sensors, each of which

represents a group of olfactory receptors and produces a time-dependent electrical signal in response to the odor". This technology is now being used for a variety of applications such as the discrimination of coffee varieties (Gardner et al., 1992), determination of meat or fish freshness (Schweizer-Berberich, 1994), microbial classification of grains (Funazaki, 1995), discrimination among different types of wines (Di Natale et al., 1995), identification of different types of soft drinks (Tan et al, 1995). Shen et al. (2001) were able to determine a correlation between sensory evaluation and e-nose analyses for oxidized oils. Their results suggest that the electronic nose is capable of measuring changes in volatile compounds and can supplement sensory data.

Spanier et al. (1999) developed an e-nose method to distinguish differences in and keeping quality of whole, fresh-cut, and minimally processed Gala variety apple using a 32 sensor (AromaScan ^{TM} A32/50 S multisampler). They found that the method was able to differentiate between varieties of apples such as Granny Smith and Red Delicious and to aid in examining Gala apples during storage and fresh-cut storage. They reported that as the complexity of the samples increased, the AromaScan instrumentation could see differences among the samples. But they suggested that to train the nose to identify an unknown odor, large sample sizes would be needed to generate data libraries.

Garden and Craven (1996) used the e-nose to discriminate among six types of bacteria (*Clostriduim perfringens, Proteus, Haemophilus influenzae, Bacillus fragilis, Pseudomonas aeruginosa).* They also examined *E.coli* and *S.aureus.* The trained neural network was able to correctly classify 87% of the bacteria. Olsson et al. (1995) successfully used an e-nose to separate *Penicillium* species that produced various volatile metabolites. Gibson et al. (1997) obtained a 93.4 % classification rate for 12 different bacteria and a

96.3% classification rate for three similar yeast cultures. Hanson (1997) was able to detect pneumonia in patients by an e-nose analysis of the patients' breath.

The e-nose has also been tested for its application as a quality assessment tool for salmon fillets by Du et al. (2002). They reported that analysis by an e-nose can provide a viable approach to determine fish freshness and be useful as a quality control and inspection tool. They also suggested that proper training of neural networks with representative aromas could help in controlling seafood quality.

The e-nose can be used to develop a rapid and easy method for determining the shelf life and microbial levels of milk without any sample preparation (Korel and Balaban, 2002). These researchers were able to correlate the odor change of milk samples (detected with the help of the e-nose) inoculated with *Pseudomonas fluorescens* or *Bacillus coagulans* with microbial counts and sensory scores. The classification of odor changes in whole milk samples were 100% correct for both types of inoculations at all the storage temperatures except for a 96% classification at 12.8 C for *B.coagulans*. The classifications for reduced-fat milk samples were all 100% accurate.

The e-nose can be used as a quality control instrument to measure not only the characteristics/changes in aroma compounds but also the microbiological profile of a product. An e-nose analysis takes less time than conventional microbiological analysis, but is currently hampered by the lack of extensive data libraries. This technology still needs to be refined for use as a reliable tool for quality assessment.

Gas Chromatography

Headspace gas chromatography (GC) is one of the favored methods adopted for studying the properties and quality of food materials. This is because the sample

examined by the GC has, in theory, a similar composition to what is sniffed during sensory evaluation and is also the same as that present over the product during consumption (Poll and Flink, 1984). Poll (1988) used the GC to measure the effect of pulp holding time on the volatile components in apple juice. According to the aroma values based on aroma thresholds, hexanal, hexylacetate, ethyulbutanoate and ethyl-2-methyl-butanoate, and to a lesser degree butanol, trans-2-hexanal, butyl acetate and isopentylacetate were recognized as being the compounds most important for the aroma of the juice. The low aroma thresholds of esters and aldehydes make these compounds important contributors to the flavor of apple juice and other apple products.

Williams et al. (1977) studied the variation in flavor of Cox's orange Pippin apples with storage and found that there were almost 130 compounds that varied with storage. The most predominant ones were butanol, 2-hexenal, ethyl acetate, butyl acetate, pentyl acetate and hexyl acetate. When apples were naturally ripened, low-boiling esters increased up to a maximum in a few weeks post-harvest, depending on the temperature of holding. According to Williams et al. (1980), the volatile flavor compounds in apples and apple products depend on the variety, maturity, apple quality, and processing and storage conditions. Poll (1985) looked at the influence of apple ripeness and temperature of storage on the composition of apple juice flavor components and reported that juice made from unripe fruits showed a predominance of alcohols over esters and aldehydes. In samples stored for a long time, both esters and aldehydes decreased with the decrease in aldehydes being much greater. The GC examination of the juice showed that the volatile content in juices rose from "unripe" to "late picking," with a further increase for "ripe for eating;" for "longer storage", there was a slight decrease in volatile content. With the exception of butyl butyrate,

no esters were found in the "unripe" or "picking ripe" juices. Highest ester concentration was found in "ripe for eating" juices.

Cunningham et al. (1985) used the CHARM analytical procedure on several apple cultivars to describe their flavors. Charm is the ratio of the amount of an odor-active compound to its detection threshold in a gas chromatographic assay. They found that all the cultivars showed different odor activity and there was no single activity that was common to all of the cultivars. The most intense odor was caused by beta-damascenone, which is thought to be a necessary odor in apples. Hexyl butanoate and ethyl butanoate (fruity apple odor) and hexyl hexanoate (apple peel-like odor) were also identified. Ethyl-2 methyl butanoate seemed to be a minor contributor to odor at harvest but it was suggested that it might become more important as a result of post-harvest changes in apple volatile composition. The only alcohols detected by the charm analysis were hexanol and 3-(Z)-hexenol, which are lipid oxidation products formed when apples are damaged or crushed.

Dimick et al. (1981) in their review of apple flavor reported that a general description of apple flavor needs the presence of esters with a molecular weight between 100 and 130. The odor of apple juice and apple products made from crushed apples has a significant contribution of C-6 alcohols and aldehydes formed through lipid oxidation. They also found that trans-2-hexenal is not present in significant amounts in apples but is formed very rapidly upon crushing and that there is a good correlation between the odor of apple essences and the concentration of this compound. Poll and Flink (1984) found that increases in the amounts of alcohols present in cider were correlated with increased off-aroma in apple juice samples. Petro-Turza et al. (1986) studied apple aroma condensates and concluded that when butyl acetate, 3-methyl butyl acetate, hexyl acetate, hexanal and 2-hexenal increased in

concentrations, the desired apple aroma also increased. An increase in ethanol, hexanol and ethyl acetate concentrations decreased the desirable apple aroma.

Poll (1983) studied the fruit-aroma score of pasteurized apple juice and found that pasteurization resulted in a decreased content of esters and an increased cooked aroma accompanied with the formation of furfural and hydroxymethylfurfural. In this study, ethyl-2-methyl butyrate, hexyl acetate, hexanal, trans-2-hexenal and unsaturated C-6 alcohols were found to be the compounds necessary for fruit aroma in apple juice. Poll (1983) also found that valeraldehyde, amyl alcohol and trans-2-hexenal were degraded during storage. Nursten and Woolfe (1972) identified several Maillard reaction products including 5-methyl-2-furfural, benzaldehyde and 2,3-decadienal in cooked apple slices.

Mangas et al. (1996) reported good recoveries with adequate accuracy for alcohols, esters, lactones, phenols and fatty acids when solid phase extraction was used after determination of the trace aroma and flavor components with a GC-MS. The GC-MS method used in the study enabled the identification of a total of 36 cider aroma components, which included twelve alcohols, eight esters, two ketones, two phenols and twelve organic acids.

Vidrih et al. (1999) studied the synthesis of higher alcohols during cider formation. They found that higher alcohols found in cider generally have their origin in the fruit, with the exception of ethyl acetate, iso-amyl alcohol and 2-phenyl ethanol, which are metabolized during fermentation as a result of yeast activity. Yeasts produce 2-phenyl ethanol, which can hinder the growth of some bacteria.

Blanco-Gomis et al. (2002) characterized cider apples on the basis of the fatty acids found in them. Ten fatty acids were quantified with a GC, two of which (palmitic and stearic acid) constitute the major fatty acids in apple juice. Among unsaturated acids, oleic

was found to predominate over linoleic and palmitoleic acids while linolenic and arachidonic acids were present only in trace amounts.

Boylston et al. (2002) studied the effect of irradiation on the flavor and sensory characteristics of cider. Four esters (butyl acetate, 2-methyl butyl acetate, hexyl acetate and ethyl hexanoate) were found to decrease in pasteurized cider. They also reported an increase in 2-furfural and 5-hydroxymethylfurfural during pasteurization and irradiation, which correlated with the detection of cooked flavor by a sensory panel. No significant changes were reported in the contents of aliphatic alcohols and aldehydes as a result of either pasteurization or irradiation.

The GC has been used widely for the analysis of flavors of different products. The results obtained by the GC analysis of apples and apple juice/ cider have been able to identify certain compounds that are necessary for the characteristic flavors of these products. This is helpful in judging the quality of the apples or the cider and can be used to track changes caused by different treatments or during storage.

Objectives

The main objectives of this study were to audit the production facilities of the participating Iowa apple cider producers to help them produce safe apple cider and maintain clean and sanitary facilities. Based on the results obtained, the producers were provided with HACCP plans. Cider samples were also followed through storage to determine their shelf life and flavor changes. A relationship between coliform counts and flavor changes was also established.

A MICROBIOLOGICAL SURVEY OF THREE IOWA APPLE CIDER PRODUCERS

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(To be submitted to Dairy, Food and Environmental Sanitation)

Abstract

Apples and cider from three cider production facilities were subjected to standard enumeration methods for aerobic bacteria, yeasts, coliforms and *E. coli*. Microbial counts on apples ranged from 10^3 to10^7 per apple for aerobic bacteria, yeasts and molds. Washing apples with poor quality water increased microbial loads by 100 fold. Counts on apples were 100-1000 times higher in 2001 than in 2000. Counts for aerobes, yeasts and molds in raw cider ranged from 10^2 to 10^5 cfu/ml while in pasteurized cider counts were <10 cfu/ml. Coliform counts in raw cider ranged from 1 to 100 cfu/ml; in pasteurized cider coliforms were below the detection limit of 1 CFU/ml. *E.coli* were also below detection limits (10 cfu/ml) in both raw and pasteurized cider.

Introduction

In recent years, apple cider has been in the news for its association with outbreaks related to *Escherichia coli* O157:H7. The first of these outbreaks occurred in Canada in 1980 (Steele et al., 1982). This outbreak caused hemolytic uremic syndrome (HUS) and established the relationship between *E. coli* O157:H7 and HUS. Since then numerous outbreaks related to apple cider consumption have been reported in the United States. Hemorrhagic colitis was associated with apple cider for the first time in the US after a 1991 outbreak that occurred in Massachusetts (Besser et al., 1993). The most widespread and

publicized of these outbreaks was associated with Odwalla brand apple juice, which affected a large part of the western United States and British Columbia, Canada. There were a total of 45 cases including one death related to this outbreak (Cody et al., 1999). Cider has also been implicated in outbreaks of *Salmonella* and *Cryptosporidium* (CDC, 1997; Millard et al., 1994).

Apple cider was considered to be a microbiologically safe product because of its high acidity (pH 3.4-4.0) but the acid resistance of *E.coli* O157:H7 (Zhao et al., 1993; Semanchek et al., 1996) allows this organism to survive in cider. The fact that *E. coli* O157:H7 has a low infectious dose and that the raw cider is not subject to any treatment by the consumer before consumption makes the situation more alarming. These outbreaks and concerns led the FDA in 1998 to require processors to obtain a 5-log (100,000-fold) reduction in the population of a target pathogen or place a warning label on their product. The warning label reads as follows: "WARNING: This product has not been pasteurized and, therefore, may contain harmful bacteria which can cause serious illness in children, the elderly, and persons with weakened immune systems" (21 CFR Part 101 [Docket No. 97N-0524] RIN 0910-AA43). The final regulation on Hazard Analysis Critical Control Point (HACCP) was passed on January 19, 2001. This rule mandates the adoption of HACCP by all apple cider producers by January 20, 2004, (FDA, 21 CFR.2001). Juice produced by a processor not having the HACCP system that complies with Secs.120.6, 120.7, 120.8 of FDA 21 CFR (2001) will be considered to be adulterated.

The most commonly used method by producers to achieve the mandated 5-log reduction is thermal pasteurization. The states of New York and Wisconsin recommend a pasteurization time of 71.1°C for 6s for cider made from apple blends while a time-

temperature combination of 76.7°C for 2 s is recommended for cider made from red delicious apples (NY State Department of Agriculture and Markets, 1998; Wisconsin Department of Agriculture, Trade and Consumer Protection, 2000.). Mak et al. (2001) used six strains of *Escherichia coli* O157:H7 and acid-adapted *E. coli* O157:H7 (strains selected were linked to outbreaks or contained green fluorescent protein for differential enumeration) in pH and °Brix adjusted apple cider to validate a pasteurization treatment of 68.1°C for 14s for apple cider. Other methods that may achieve the desired reduction include ultraviolet light treatment, irradiation, high pressure, pulsed electric fields, and the use of ozone (Buchanan et al., 1998; Wright et al., 2000; Iu et al., 2001; Garcia-Graells et al., 1998). Cost of installing new processing equipment and flavor changes in cider due to processing are the main factors that influence a producer's decision to adopt one method over the other.

This study was undertaken to survey and audit the production practices of Iowa apple cider producers. The audit included comprehensive microbiological testing of the apples, cider and environmental and equipment samples on each site. After each production year HACCP plans were prepared based on the data obtained and on visual observations made, to help processors to lower counts in their product, maintain cleaner facilities and produce safe cider.

Materials and Methods

Iowa Cider Survey

A survey questionnaire of 45 questions, similar to the one used in the previous two years (Cummins, 2001) was compiled and given to the apple cider producers who participated in the study. Questions were asked regarding orchard practices, cider processing, and equipment maintenance and about the general practices followed from receiving apples up to sale of the finished cider. In the first year of the survey (2000-2001) only one producer was surveyed while in the second year (2001-2002), the survey was conducted with three processors, including the one from the first year. The questionnaires were returned anonymously and are attached as an appendix to this thesis.

Visits to the Cider Processing Operations

During the first year of the study, only one operation was visited. A total of seven visits were made to the plant on a monthly basis from October 2000 to April 2001; two visits were made in November and no visit was made in December. In the second year of the study, the original producer and two additional processing operations were visited once each month throughout the cider season .Two of the operations stopped pressing cider in December while the third operation continued until March. All the producers pasteurized their cider. The two additional producers had participated in a previous survey of cider production practices (Cummins, 2001).

Sample Collection

Apple samples: Apples were collected randomly from the grading table (just after they had been brought in from the orchard) before brushing, the refrigerated storage rooms

before washing, the wash tank and the conveyor belt of the cider press. Two apples were collected from each of the collection sites. The apples were stored at 7°C in sterile stomacher bags (Fisher Scientific Co., Itasca, IL), one apple per bag, until they were brought back to the laboratory.

Cider samples: Raw cider was collected immediately after it had been pressed, directly from the press plates, in sterilized glass bottles. Pasteurized cider with and without preservative (<0.1% potassium sorbate) was obtained from two producers; the third producer provided only pasteurized cider with preservative. In the case of pasteurized cider with preservative, preservative was added to the cider before pasteurization. The final cider was obtained immediately or within 24 hours of processing, in ½- or 1- gallon retail plastic containers.

Environmental samples: Apple wash water and chlorinated water used for cleaning hands during cider processing were collected in sterilized glass bottles. Conveyor belt, apple cider press, press plates, cider press chute, and randomly selected sites on the cider processing equipment and storage tanks were swabbed (10 x 10 cm^2 area) using sterile cotton swabs wetted with sterile 0.1% peptone (Difco Laboratories, Detroit, MI) diluent.

All samples were kept in a cooler at 7°C until they were brought to the laboratory within 2 hours of sample collection; the samples were stored at 7°C in the laboratory until they were analyzed within 4 to 24 hours of collection.

Sample Preparation

All dilutions were made with 0.1% peptone water. A 100-ml volume of diluent was added to each apple bag. The bags were shaken vigorously for 2 min and a 1-ml aliquot of

the diluent was taken for further dilution and plating. Cider and water samples were diluted directly. Swabs were added to 10 ml of diluent and shaken vigorously before dilution.

Enumeration of Microorganisms

Aerobic, mesophilic bacteria were enumerated by spread plating in duplicate onto Trypticase Soy Agar (TSA, Difco) according to standard methods (Gerhardt, 1994). Colonies were counted after incubation at 35°C for 48h. Yeasts and molds were counted on Potato Dextrose Agar (PDA, Difco), pH 3.5, after incubation at 25°C for 5 days. Coliforms were counted on Petrifilms according to the AOAC recommended method provided by the manufacturer (3M, St.Paul, MN). Pink gas-forming colonies after 24 h of incubation at 37°C were considered to be coliforms. Blue gas-forming colonies that developed after 48 hours of incubation at 37°C were counted as *E. coli*. Colonies without gas formation were not counted.

Statistical Analysis

Tukey's multiple comparison procedure and the nonparametric Wilcoxon rank sum tests (SAS analytical system, SAS Institute, Inc., Cary, N.C.) were performed to determine if any significant differences existed among the counts obtained over the two years for any of the cider producers.

Results and Discussion

Microbial Counts on Apples

Early in the season, all three producers used freshly harvested apples that had not been in storage for more than a week. Later in the season, apples stored for as long as three months were also used in cider processing. The apples were stored at 0-4°C in neatly stacked wooden or cardboard crates. Parish (1997) recommends refrigerated storage of apples as it can slow down microbial growth and also spoilage and rotting of apples. The storage areas for the producers surveyed were clean and were free of visible infestation by insects, birds, and small animals.

Figure 1 shows the overall microbial counts obtained at each sampling time on apples from the three producers surveyed for the 2000 and 2001 production years. Data from Producers B and C in 2000 are taken from a previous survey (Cummins, 2001). Apples sampled from the storeroom at various times during the cider season varied in counts by not more than 2 logs. Cummins (2001) also found that storage time did not significantly affect the microbial loads on apples. This may be due to the fact that the producers sorted the stored apples periodically and culled the rotten/bad apples. Aerobic bacteria and yeasts and molds were found in much higher numbers than coliforms throughout the season.

A nonparametric Wilcoxon rank test (Ott, 1993) was run on the data from all sampling times to determine if there were significant differences in counts throughout each production year for the individual producers; no significant differences were observed in any of the counts for the three producers. As the counts did not vary much among the sampling dates, the counts were averaged across sampling times for each producer for each production

Figure 1 a-f: Microbial counts on apples collected at several sampling times during the 2000 and 2001 cider seasons. Data for Producers B and C in 2000 are from Cummins (2001).

Figure 1a: Producer A (2000), 1b: Producer A (2001)

Figure 1c: Producer B (2000), 1d: Producer B (2001)

Figure 1e: Producer C (2000), 1f: Producer C (2001)

☐ = aerobic bacteria,

⁘ = yeasts and molds,

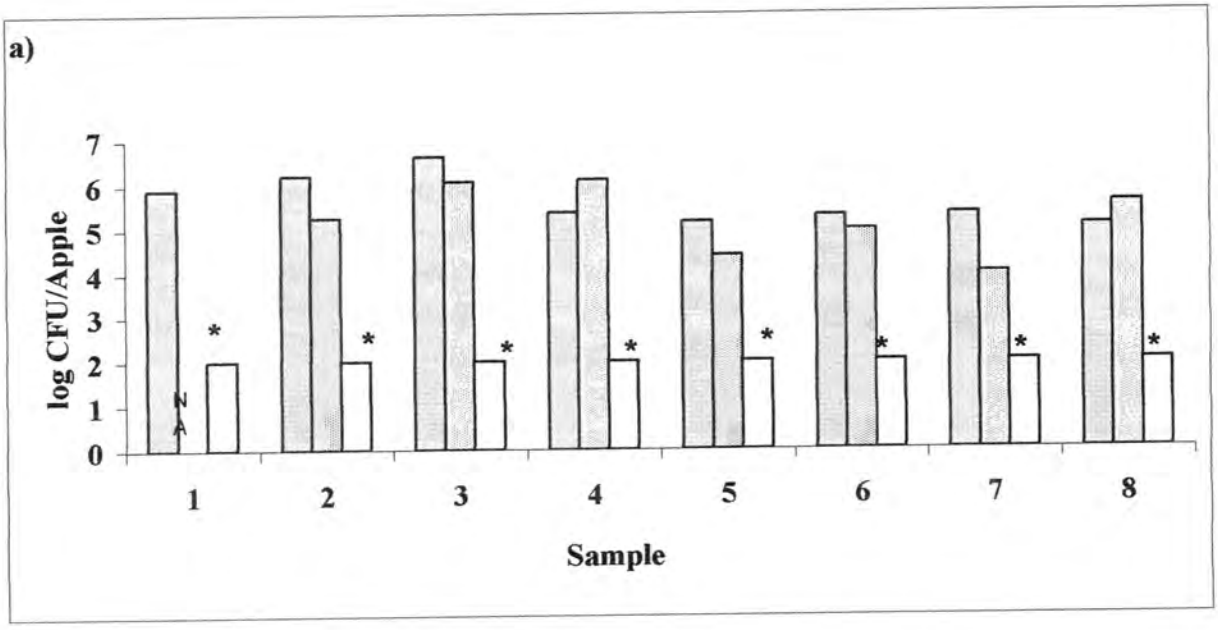
☐ = coliforms,

NA = counts not available

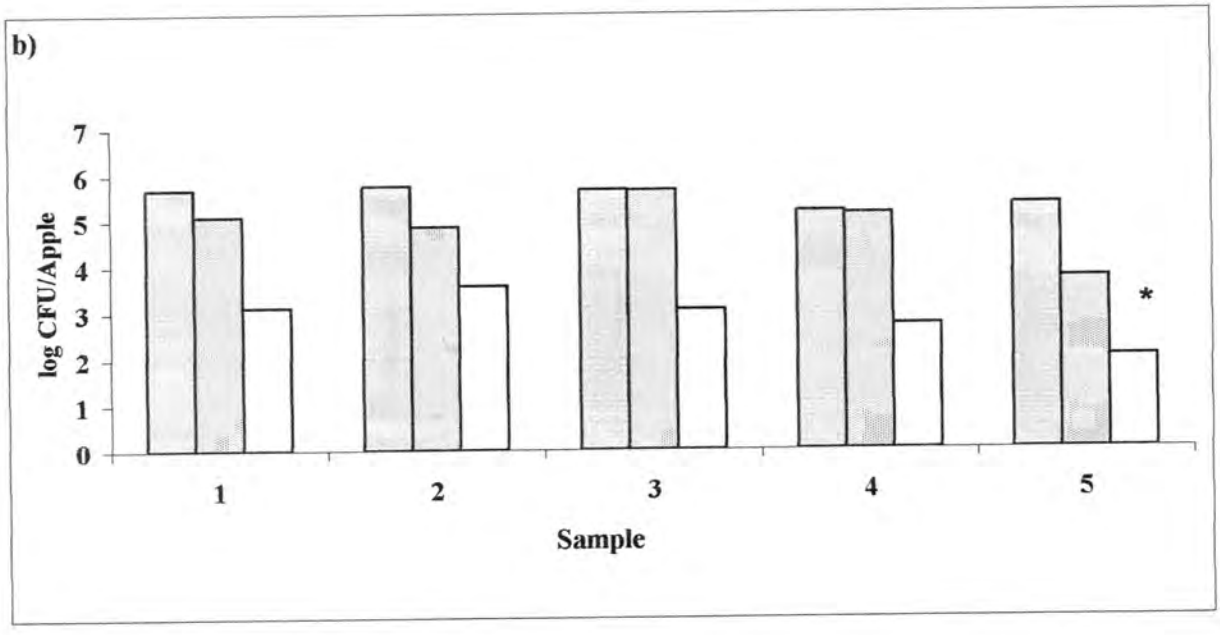
Limits of detection: 100 CFU/apple.

* = Counts were below the indicated limit of detection (100 CFU/apple)

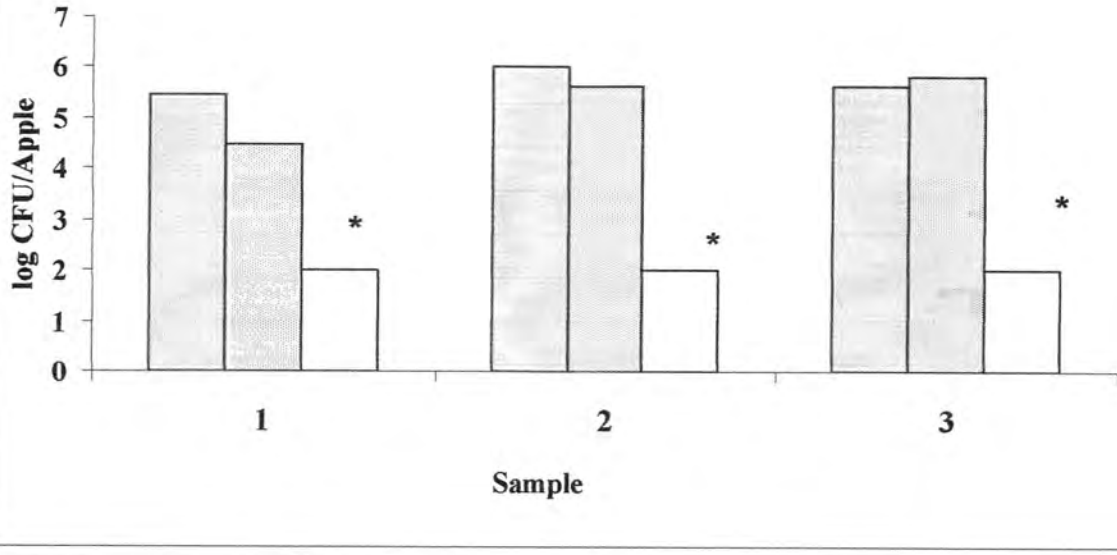
a)


log CFU/Apple
0
1
2
3
4
5
6
7
NA
*
1
2
3
4
5
6
7
8
Sample

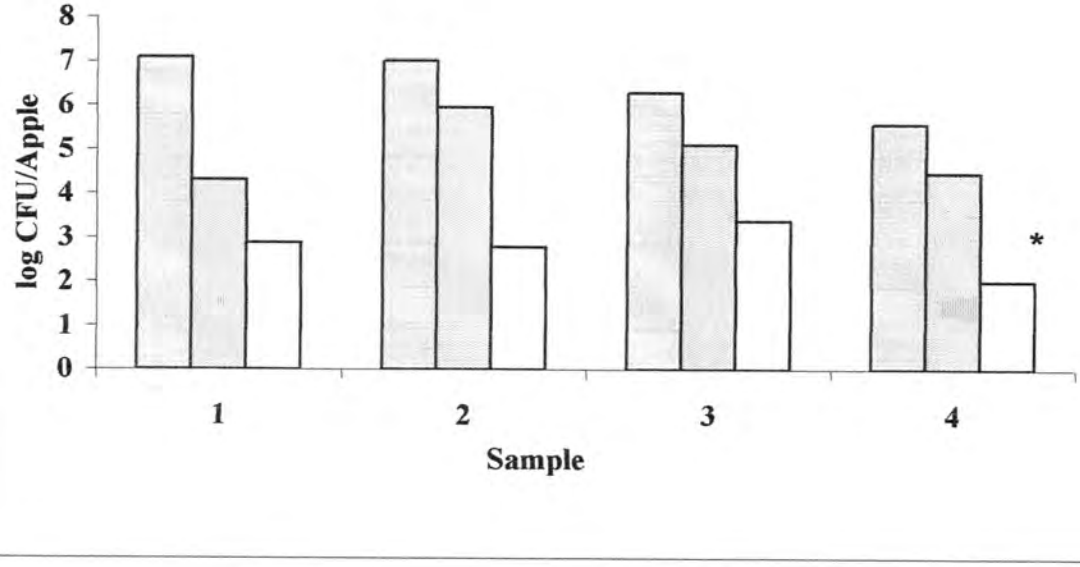
b)


log CFU/Apple
0
1
2
3
4
5
6
7
*
1
2
3
4
5
Sample

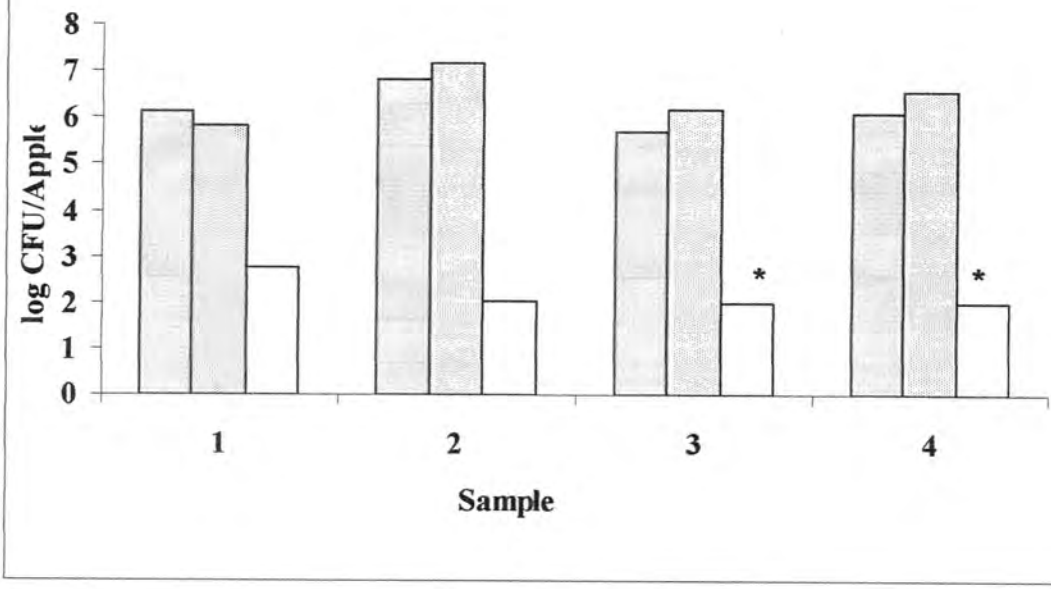
c)


log CFU/Apple
0
1
2
3
4
5
6
7
*
*
*
1
2
3
Sample

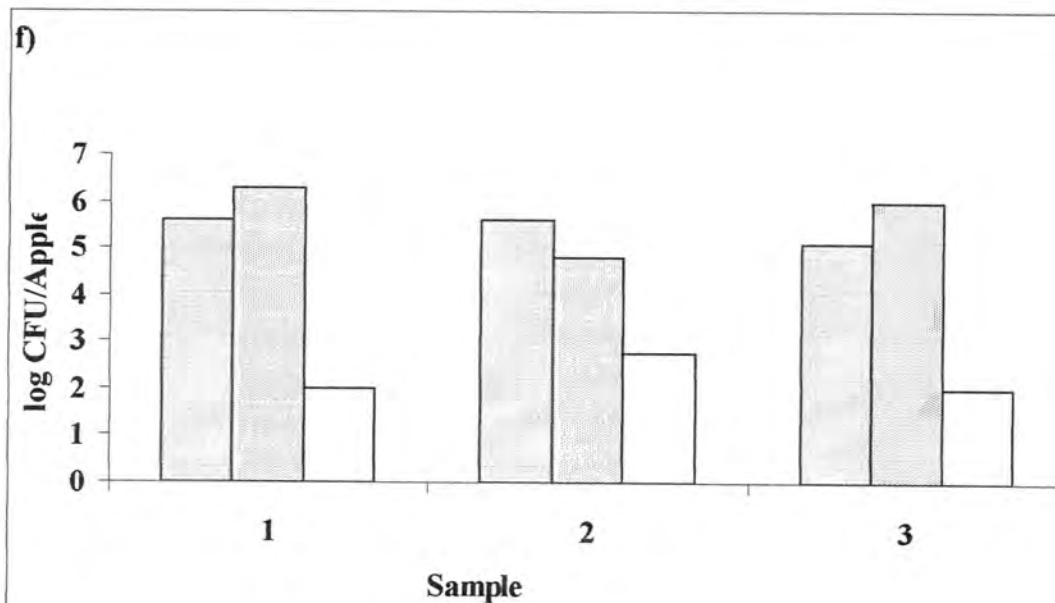
d)


log CFU/Apple
0
1
2
3
4
5
6
7
8
*
1
2
3
4
Sample

e)


log CFU/Apple
8
7
6
5
4
3
2
1
0
*
*
1
2
3
4
Sample

f)


log CFU/Apple
7
6
5
4
3
2
1
0
1
2
3
Sample

season. Figure 2 shows the average counts obtained on apples for the three producers for the two years.

The average aerobic bacteria counts on apples from Producer A varied by less than 1 log between the two years. In contrast, counts on apples from Producer B increased by one log and for Producer C decreased by one log between 2000 and 2001 (Fig. 2a). Highest average counts for 2000 were 2.4 x 10^5 CFU/apple (Producer C) and for 2001 were 6.0 x 10^6 CFU/apple (Producer B).

Average yeast and mold counts were similar over the two production years for Producers A and B while Producer C's counts decreased by one log in 2001 (Figure 2b). Highest average counts of yeasts and molds (over 10^7 CFU/apple) were observed on Producer C's apples in 2000. Yeasts predominated over molds in almost all cases; approximately 80% of the observed colonies were yeasts. Counts ranged from 10^5 - 10^7 CFU/apple in 2000, but were all around 10^5 CFU/apple in 2001. Deak et al. (1996) reported 10^2 to 10^6 yeasts and molds per apple and Riordan et al. (2001) reported 10^5 to 10^6 yeasts and molds per apple in previous surveys of US orchards. The results of the current study are in agreement with these previous studies.

Average coliform counts on apples from Producers A and B increased from below detection limits of 100 CFU/apple in 2000 to approximately 10^3 in 2001. Coliform counts on Producer C's apples did not change from 2000 to 2001 (Fig. 2c). The increase in counts for Producers A and B was seen consistently over the production season. Average coliform counts ranged from <100 to 1.0 x 10^3 CFU/apple over both production seasons; Cummins, who audited additional producers, reported a range of <100 to 10^6 coliforms/apple (Cummins et al., 2002). The 3M Petrifilms used for coliforms can distinguish between *E. coli* and other

Figure 2 a-c: Average microbial counts on apples from three producers (A, B and C) across the 2000 and 2001 production years.

a) Aerobic bacteria,

b) Yeasts and molds,

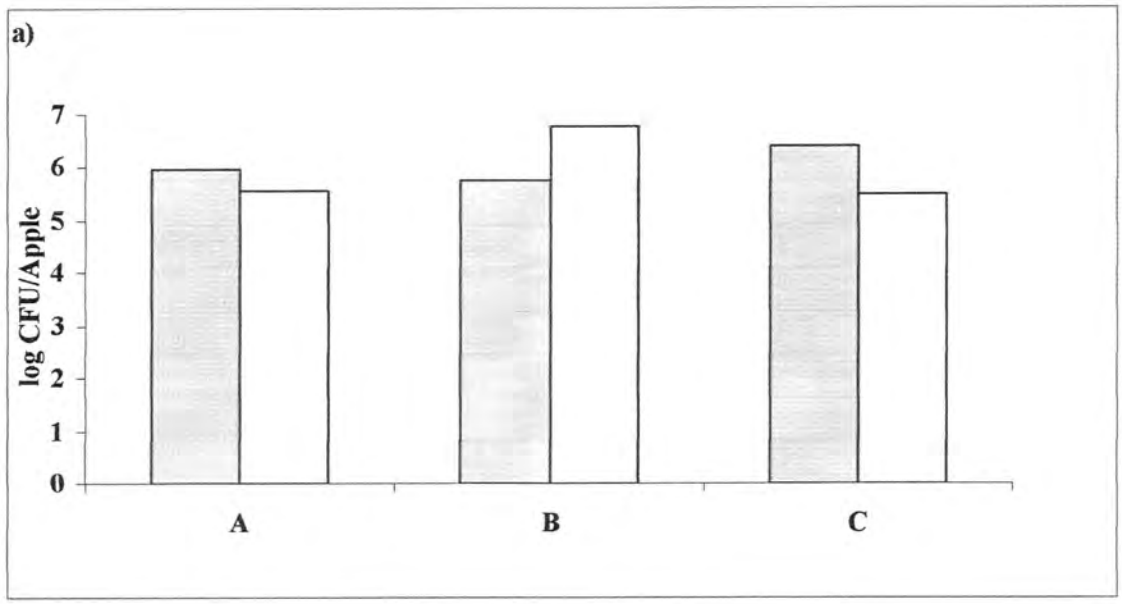
c) Coliforms,

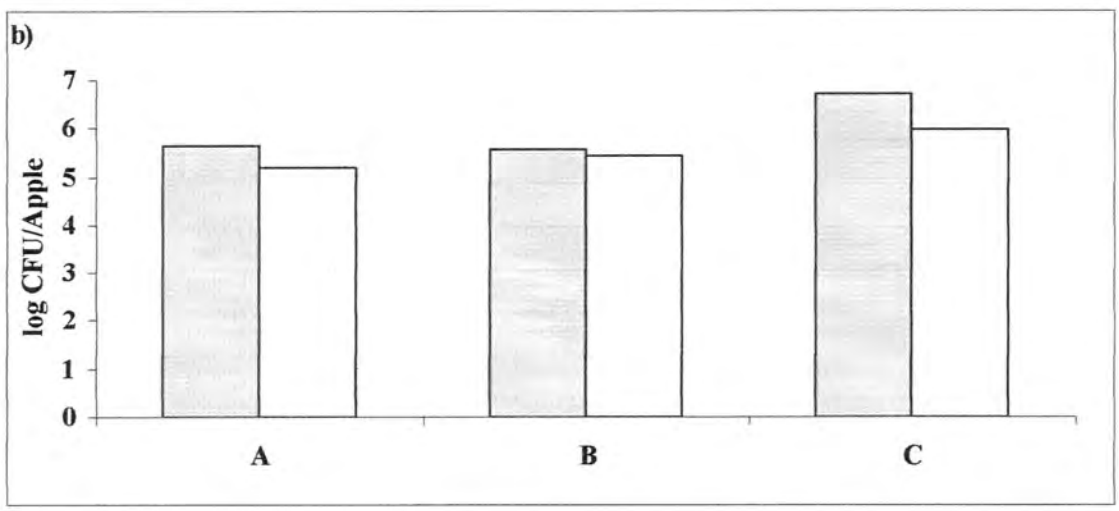
☐ = 2000,

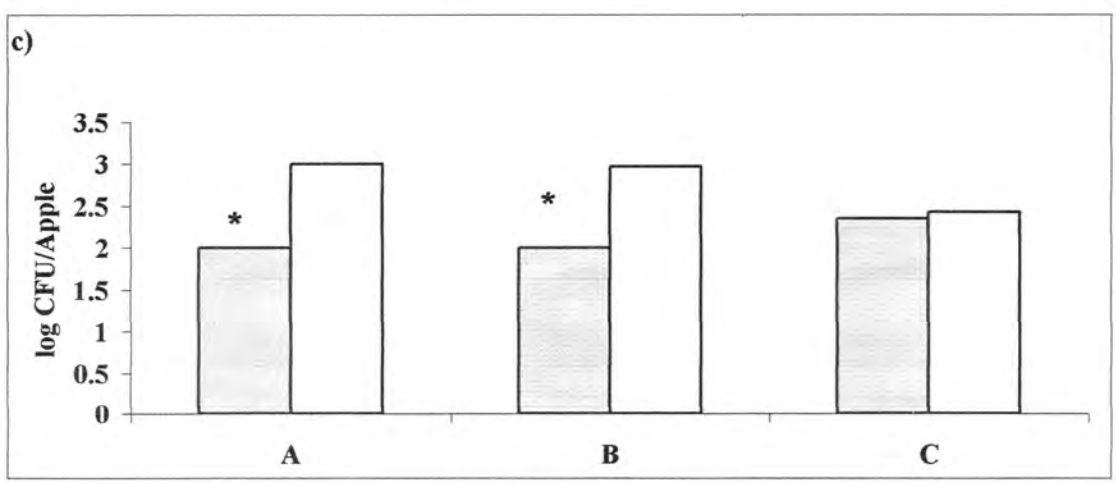
☐ = 2001,

Limits of detection: 100 CFU/apple.

* = Counts were below the indicated limit of detection (100 CFU/apple)


a)
log CFU/Apple
0
1
2
3
4
5
6
7
A
B
C


b)
log CFU/Apple
0
1
2
3
4
5
6
7
A
B
C


c)
log CFU/Apple
0
0.5
1
1.5
2
2.5
3
3.5
*
*
A
B
C

types of coliforms. No *E. coli* were detected in any of the apples tested in either year of the survey.

Microbial counts on apples may differ from year to year because of differences in a number of parameters such as weather, environmental conditions of the orchards, storage conditions, and source of the apples. In the case of Producer A it was noted on one occasion that apples obtained from another orchard had considerably higher aerobic bacteria and coliform loads than the producer's own apples. The counts on imported apples may be due to use of drops, increased handling and /or opportunities for microbial growth during transport.

Nguyen and Carlin (1994) observed that washing decreased microbial loads in various fruits and vegetables by at least 1 log. Wright et al. (2000) found that a wash step could reduce *E.coli* O157:H7 populations on apples by approximately 1.1 logs. All three producers washed apples before pressing. Producers A and B used a chlorinated wash. Producer A used a 10% sodium hypochlorite solution and Producer B added bleach (50 ppm) to the wash water. Producer C did not use a sanitizer in the washing/brushing step before pressing. Microbial counts on apples were reduced by about 1 log for Producers A and B (data not shown). However for Producer C, microbial loads on apples were higher after washing for each sampling time. Figure 3 shows counts on apples before and after washing averaged across all three sampling dates. Microbial counts increased by one to three logs after washing. Producer C's apple wash water was consistently found to contain about 10^3 aerobic bacteria/ml in 2001, while coliform and yeast and mold counts were below detection limits in these samples. Cummins (2002) found similar results in a previous survey of the facility in 2000.

Figure 3: Microbial counts on apples from Producer C in 2001 before and after washing.

a) Aerobic bacteria,

b) Yeasts and molds,

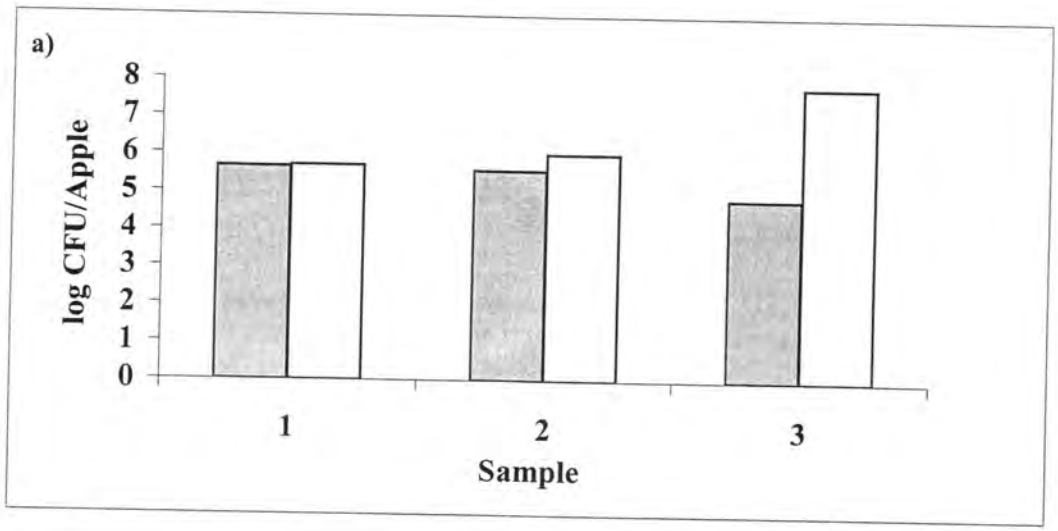
c) Coliforms,

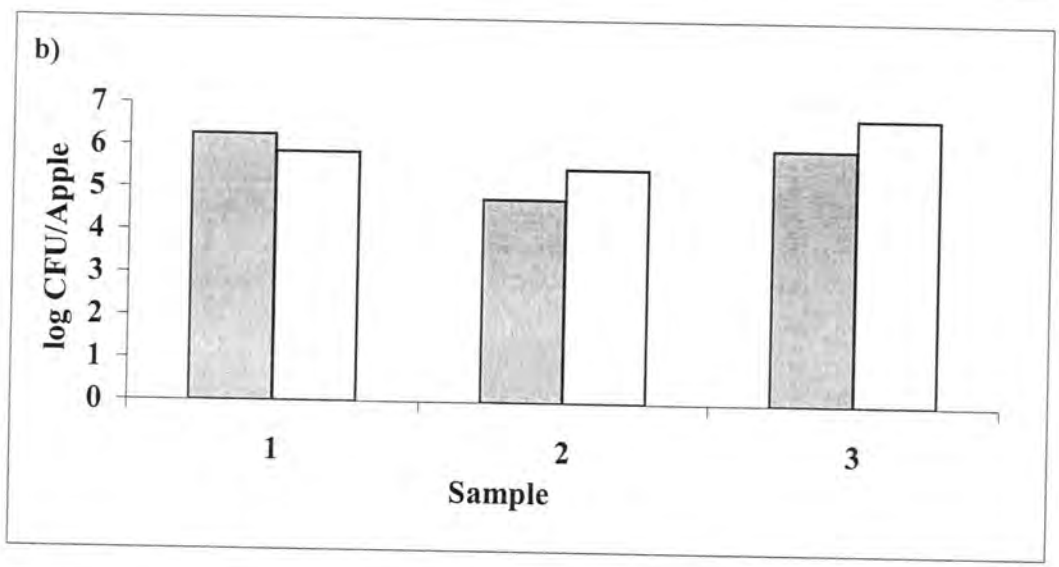
☐ = unwashed,

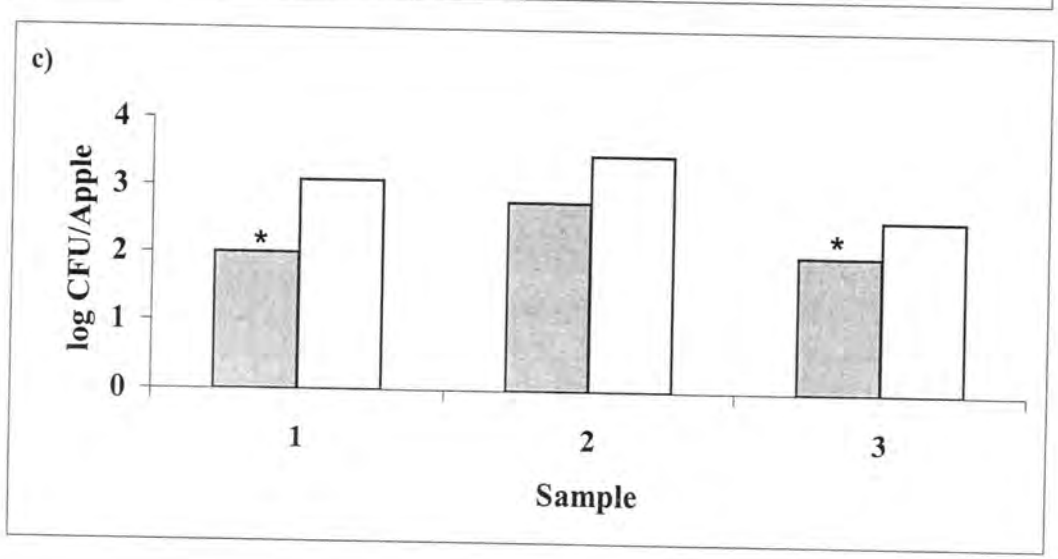
☐ = washed,

Limits of detection: 100 CFU/apple.

* = Counts were below the indicated limit of detection (100 CFU/apple)


a)
log CFU/Apple
0
1
2
3
4
5
6
7
8
1
2
3
Sample


b)
log CFU/Apple
0
1
2
3
4
5
6
7
1
2
3
Sample


c)
log CFU/Apple
0
1
2
3
4
*
*
1
2
3
Sample

Care should be taken with the apple wash water, as it is a potential source of contamination that can lead to higher post-washing counts on apples. It is recommended that the chlorine level be tested on a regular basis. Producer C chlorinated the well only once, before the start of the season in July 2001; chlorine levels in the well were not checked after that. Since high microbial counts were obtained on Producer C's apples even early in the season, it is possible that chlorination was not sufficient. Other reasons for the high counts in the water include inadequate cleaning and sanitizing of the washer brush or dirty or damaged water hoses.

According to Davidson et al. (1993), the antimicrobial activity of chlorine is dependent on several factors, such as pH, temperature, organic load and ionic concentration of the solution. Wright et al. (2000) recommended that producers should not rely only on chlorinated water washes to wash their apples and should use a sanitizer in their wash step to reduce the carryover of microorganisms to the subsequent processing steps. It is important to check the chlorine levels in water regularly with the help of chlorine dipsticks, and chlorinate the water at least twice during the cider season.

Producer A brushed apples as they were brought in from the orchard. Samples of apples before brushing and immediately after brushing were also tested, but no significant difference was seen in any microbial counts between these two stages. Brushing helps in the removal of soil, insects and other such material that may adhere to the apple surface, but may not be effective in removing microorganisms, especially if they have attached as biofilms to the apple surface.

Culling of rotten /bruised apples during storage and during processing was a practice followed by all three producers and should be helpful in lowering the counts in the final

product. This periodic removal of damaged fruit may also be the reason that counts on apples did not vary over longer storage times later in the season. The lower the microbial loads on the incoming apples, the lower should be the number of organisms present in the final cider if processing equipment is clean and good manufacturing practices are followed. Therefore, it is necessary to ensure that apples used in cider production are harvested, graded, stored and washed properly prior to use in cider manufacture.

Microbial Loads in Cider

During pressing, any organisms that are present on the apple (surface or inside) or on the equipment can be transferred to the apple cider. Pasteurization is a processing step that can kill *E. coli.* However, standard pasteurization conditions for apple cider have not been defined, and various time-temperature combinations may be used. Producers may also add up to 0.1% potassium sorbate to their cider as preservative.

Cider samples were obtained before and after pasteurization from all producers for both production years (2000 and 2001). Data from Producers B and C in 2000 are taken from a previous survey (Cummins, 2001). Pasteurized cider without preservative was obtained from Producer A, and on three occasions, raw cider with preservative was also obtained from this producer. Unpasteurized cider and pasteurized cider with preservative were obtained from Producer B. Producer C also provided samples of unpasteurized cider with preservative.

Table 1. Time and temperature conditions for pasteurization used by producers in this study

Producer	Temperature of Pasteurization		Time of Pasteurization (seconds)
	°C	°F	
A	71.1-73.9	160-165	2
B	71.7	161	11
C	72.8	163	2

Table 1 shows the time-temperature combinations for pasteurization used by the producers in this study. Although the severity and duration of treatments varied among the producers, the percent reductions in microbial loads between raw and pasteurized cider achieved by the processes were almost equal. Microbial counts in different types of cider obtained from the three producers across all sampling dates for the two production years are presented in Tables 2, 3, and 4. It can be seen from the counts for Producer A that the addition of preservative did not significantly affect the counts at time zero in pasteurized cider. However, on comparing unpasteurized to pasteurized cider, it is clear that pasteurization reduced the counts of microorganisms by 2 to 3 logs.

Table 5 shows the percent reductions achieved by pasteurization and addition of preservative in the different groups of microorganisms tested over the two years of this study. Averages of microbial loads were calculated for those sampling dates on which both raw and pasteurized with preservative cider samples were obtained. The formula used for calculating the percent reductions is as follows:

% Reduction ={(Average microbial load in raw cider)- (Average microbial load in pasteurized cider with preservative) / (Average microbial load in raw cider)} x 100

Maximum reduction (by 99%) in counts was achieved for yeasts and molds for all producers except for Producer A in 2001. Reduction in aerobic bacteria counts varied from 86% to 99% over the two years. Coliforms were reduced by 93% to 99%. For Producer A, the differences in percent reductions with and without the addition of preservatives did not differ by more than 2%; These results are similar to those obtained by Cummins (2001).

Table 2: Counts obtained on cider samples from Producer A

		Aerobes			**Coliforms**			**Yeasts & Molds**	
2000 Sample	Raw	Past./no Preserv.	Past.+ Preserv.	Raw	Past./no Preserv.	Past.+ Preserv.	Raw	Past./no Preserv.	Past.+ Preserv.
1	<10	<10	<10	<1	<1	<1	1500	<10	<10
2	2100	200	250	<1	<1	<1	1800	<10	<10
3	8200	700	1400	60	<1	<1	7700	4500	<10
4	2900	450	250	3	<1	<1	1300	<10	<10
5	2900	50	250	110	<1	<1	5400	<10	<10
6	7600	150	250	9	<1	<1	3000	<10	150

		Aerobes			**Coliforms**			**Yeasts & Molds**	
2001 Sample	Raw	Past./no Preserv.	Past.+ Preserv.	Raw	Past./no Preserv.	Past.+ Preserv.	Raw	Past./no Preserv.	Past.+ Preserv.
1	29000	<10	<10	25	<1	<1	25000	50	50
2	17000	200	<10	85	<1	<1	25000	100	150
3	39000	550	450	240	<1	<1	48000	1500	2500
4	28000	100	50	80	<1	<1	23000	<10	<10
5	30000	50	<10	25	<1	<1	8000	<10	<10

Limit of detection: 10 CFU/ml for aerobic bacteria and yeast and mold counts
CFU/ml for coliforms

Table 3: Counts obtained on cider samples from Producer B

	Aerobes		**Coliforms**		**Yeasts and Molds**	
2000 Sample	Raw	Past.+ Preserv.	Raw	Past.+ Preserv.	Raw	Past.+ Preserv.
1	230000	210	4000	<10	63000	25
2	110000	410	900	<10	7600	5
3	7000	520	60	<10	19000	<10

	Aerobes		**Coliforms**		**Yeasts and Molds**	
2001 Sample	Raw	Past.+ Preserv.	Raw	Past.+ Preserv.	Raw	Past.+ Preserv.
1	470000	500	6	<1	220000	<10
2	5300	250	45	<1	16000	100
3	620000	400	170	<1	19000	<10
4	25000	300	900	<1	15000	<10

Limit of detection: 10 CFU/ml for aerobic bacteria and yeast and mold counts
1CFU/ml for coliforms

Table 4: Counts obtained on cider samples from Producer C

	Aerobes			Coliforms			Yeasts & Molds		
2000 Sample	Raw	Raw + Preserv.	Past.+ Preserv.	Raw	Raw + Preserv.	Past.+ Preserv.	Raw	Raw + Preserv.	Past.+ Preserv.
1	5200	9200	410	150	55	<10	15000	24000	<10
2	6200	6500	150	180	55	<10	120000	120000	15
3	<1	8700	340	<1	20	<10	<1	63000	730
4	<1	4100	120	<1	90	<10	<1	46000	20

	Aerobes			Coliforms			Yeasts & Molds		
2001 Sample	Raw	Raw + Preserv.	Past.+ Preserv.	Raw	Raw + Preserv.	Past.+ Preserv.	Raw	Raw + Preserv.	Past.+ Preserv.
1	1300	4800	150	70	<1	<1	22000	12000	500
2	5700	3900	100	110	120	<1	180000	150000	100
3	6600	2800	50	70	50	<1	42000	60000	250

Limit of detection: 10 CFU/ml for aerobic bacteria and yeast and mold counts
1 CFU/ml for coliforms

Table 5: Average percent reductions in numbers of microorganisms achieved compared to those in raw cider, by pasteurization and addition of preservative during the 2000 and 2001 seasons

	2000			2001		
	Raw cider (CFU/ml)[1]	PWP Cider[2] (CFU/ml)	% Reduction	Raw Cider (CFU/ml)	PWP Cider (CFU/ml)	% Reduction
Aerobes:						
A	3800	500	86.84	28400	100	99.65
B[3]	115600	400	99.65	371600	400	99.89
C[3]	5700	400	93.42	4500	100	97.78
Coliforms:						
A	90	<1*	98.89	90	<1*	98.89
B	1700	10	99.41	300	<1*	99.67
C	200	10	93.94	80	<1*	98.75
Yeasts & Molds:						
A	4000	10	99.75	21300	500	97.65
B	52600	10	99.98	67500	300	99.56
C	67500	400	99.45	81300	300	99.65

1: Counts for raw and PWP cider are averages across sampling dates for which both samples were taken
2: Pasteurized with preservative cider
3: Data for 2000 for Producers B and C obtained from Cummins, 2001
*: Counts were below detection limits, so detection limit was used in calculations

The producers in the study indicated that 1-bushel of apples (120-130 apples) yields approximately 3.5 gallons of cider (personal communication with producers). Based on this information, the yield per apple can be assumed to be approximately 100 ml of cider. Figure 4 shows the microbial loads at various stages of processing from one processing day, for the three producers, with counts per apple reported as counts per ml after making adjustments based on the above mentioned assumption of volumes. Similar counts and trends were observed for all other sampling days. For Producers A and B an increase in counts (by at least 1 log) was seen in raw cider as compared to counts on apples. This may be due to contamination of the cider from the equipment used or from human sources. For Producer C poor quality wash water may have caused increased microbial loads on apples.

Average microbial counts in raw cider and pasteurized cider with preservative for the three producers, over both production seasons, are presented in Figure 5. A nonparametric Wilcoxon rank sum test (Ott, 1993) indicated that for Producer A, total aerobic bacteria and yeast and mold counts in raw cider differed significantly between 2000 and 2001, with 2001 counts being higher in both cases. This variation may have been caused by a variety of factors such as the incoming load on apples, the weather, surrounding environment and the overall cleanliness of the cider facility. No significant differences in counts between the two years were seen for other producers or types of organisms.

The average numbers of aerobic bacteria in raw cider ranged from 4.5×10^2 to 2.7×10^5 CFU/ml (Fig. 5a). As expected, pasteurization decreased the counts by 1 to 3 logs. Counts in pasteurized cider with preservative ranged from 1×10^2 to 3.9×10^2 CFU/ml (Fig. 5b). The range of counts for both types of cider is in accordance with those determined by

Figure 4: Average microbial counts at various stages of processing:

a) Producer A,

b) Producer B,

c) Producer C.

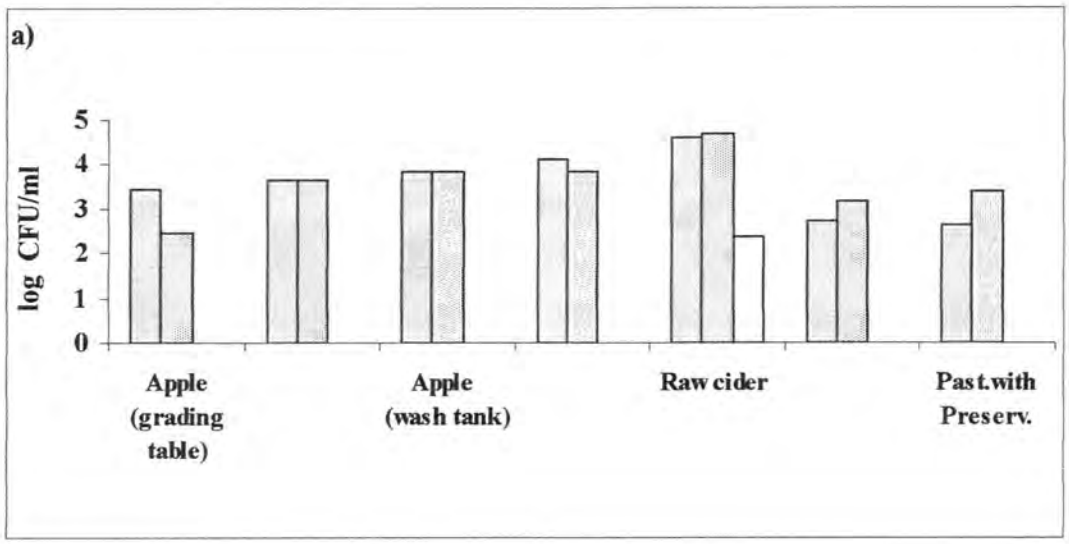
☐ = Aerobic bacteria,

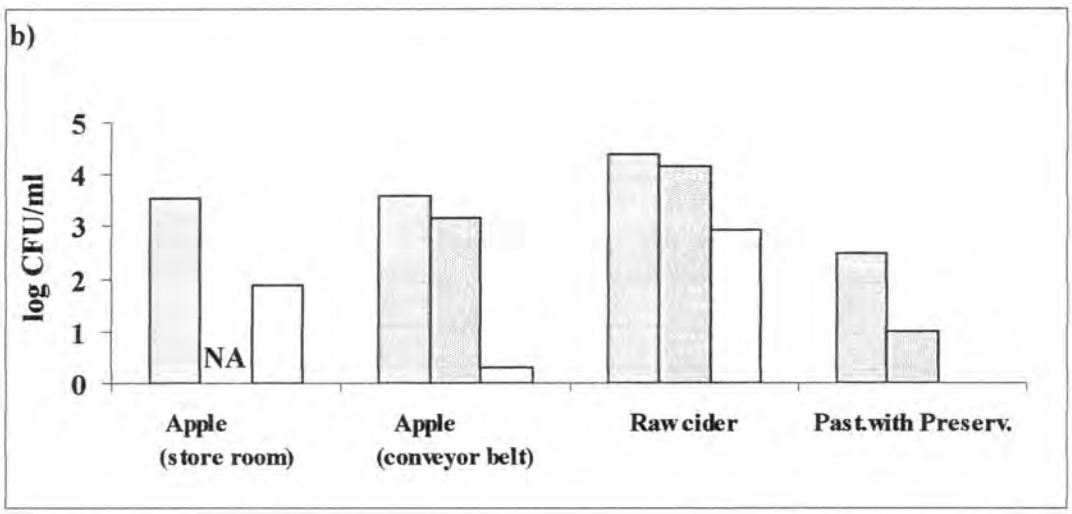
⊡ = Yeasts and molds,

☐ = Coliforms,

Limit of detection: 1 CFU/ml for aerobes, yeasts and molds and coliforms (for apples);

10 CFU/ml for aerobes and yeasts and molds and 1 CFU/ml for coliforms (for cider)


a)
log CFU/ml
0
1
2
3
4
5
Apple (grading table)
Apple (wash tank)
Raw cider
Past.with Preserv.


b)
log CFU/ml
0
1
2
3
4
5
NA
Apple (store room)
Apple (conveyor belt)
Raw cider
Past.with Preserv.

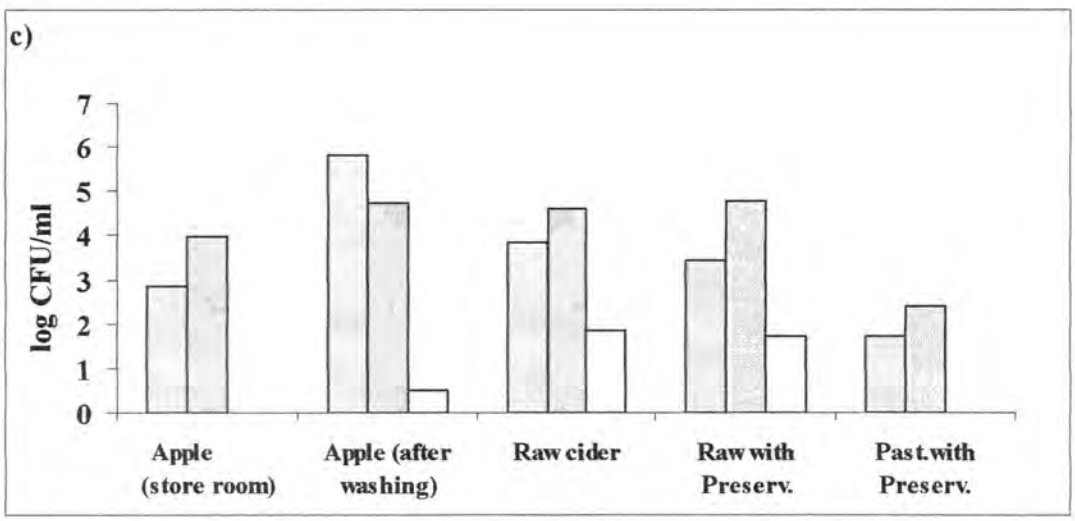

c)
log CFU/ml
0
1
2
3
4
5
6
7
Apple (store room)
Apple (after washing)
Raw cider
Raw with Preserv.
Past.with Preserv.

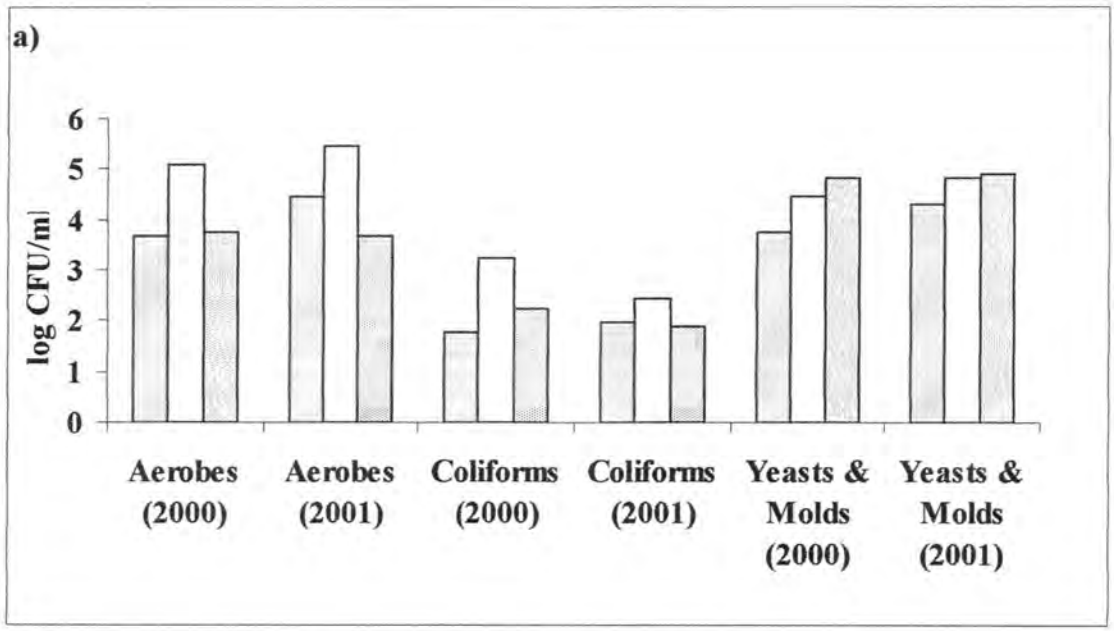
Figure 5: Average microbial counts for Producer A (□), B(□), and C (⊡) for the 2000 and 2001 production years

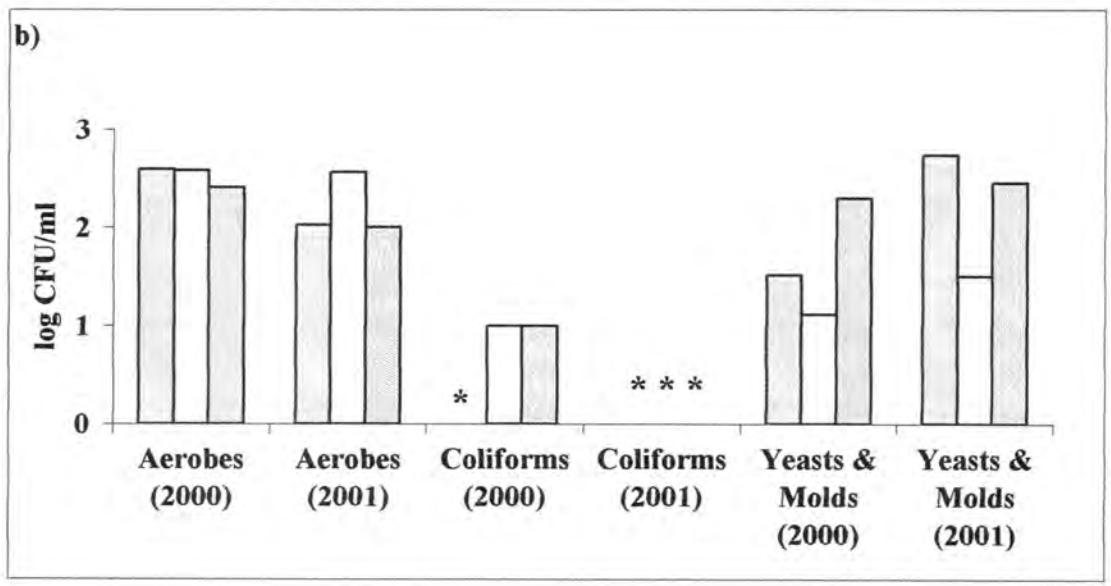
a) raw cider,

b) pasteurized cider

Limit of detection: 10 CFU/ml for aerobes and yeasts and molds and 1 CFU/ml for coliforms.

* = Counts were below the indicated limit of detection (10 CFU/ml or 1 CFU/ml)


a)
log CFU/ml
6
5
4
3
2
1
0
Aerobes (2000)
Aerobes (2001)
Coliforms (2000)
Coliforms (2001)
Yeasts & Molds (2000)
Yeasts & Molds (2001)


b)
log CFU/ml
3
2
1
0
*
* * *
Aerobes (2000)
Aerobes (2001)
Coliforms (2000)
Coliforms (2001)
Yeasts & Molds (2000)
Yeasts & Molds (2001)

Cummins et al. (2002). In contrast, Senkel et al. (1999) found 10^3 to 10^4 aerobic bacteria/ml in pasteurized cider in a survey of Maryland cider producers.

Coliform counts in raw cider made by Producers A and C were similar in 2000 and 2001 and were all in the range of 10 to100 CFU/ml (Fig. 5a). The average coliform count in Producer B's raw cider decreased from 1.6×10^3 to 3.5×10^2 CFU/ml between 2000 and 2001. Coliform levels in all producers' pasteurized cider were below the detection limit of 1 CFU/ml in 2001. Cummins et al. (2002) reported an average of <10 coliforms/ml in pasteurized cider from Producers B and C in 2000 (Fig.5b). Senkel et al. (1999) found much higher coliform levels: an average of 1.3×10^5 CFU/ml in unpasteurized cider and 3.2×10^3 CFU/ml in pasteurized cider. The 3M Petrifilms used for the coliform counts in this study can distinguish between *E. coli* and other coliforms; on the basis of the reactions observed on the Petrifilms, no *E. coli* were found in any of the samples tested.

Average yeast and mold counts in raw cider from Producer A increased by 1 log from 2000 to 2001. Counts averaged over all processing days for raw cider for the three producers ranged from 5.3×10^3 to 8×10^4 CFU/ml over both production seasons (Fig.5a) while those for pasteurized cider varied from 1.3×10^1 to 5.4×10^2 CFU/ml (Fig.5b). Pasteurization decreased the viable yeasts and molds by 3 logs from the numbers seen in the raw cider. The ranges for raw and pasteurized cider are similar to those obtained by Cummins et al. (2002). Figure 6 compares the counts obtained for unpasteurized cider and unpasteurized cider with preservative in three different samples obtained from Producers A and C. The addition of preservative did not significantly affect the microbial counts. While both producers used potassium sorbate at 0.1%, the samples obtained from Producer C had been

Figure 6: Comparisons of average microbial counts in unpasteurized cider with and without preservatives.

a) aerobic bacteria,

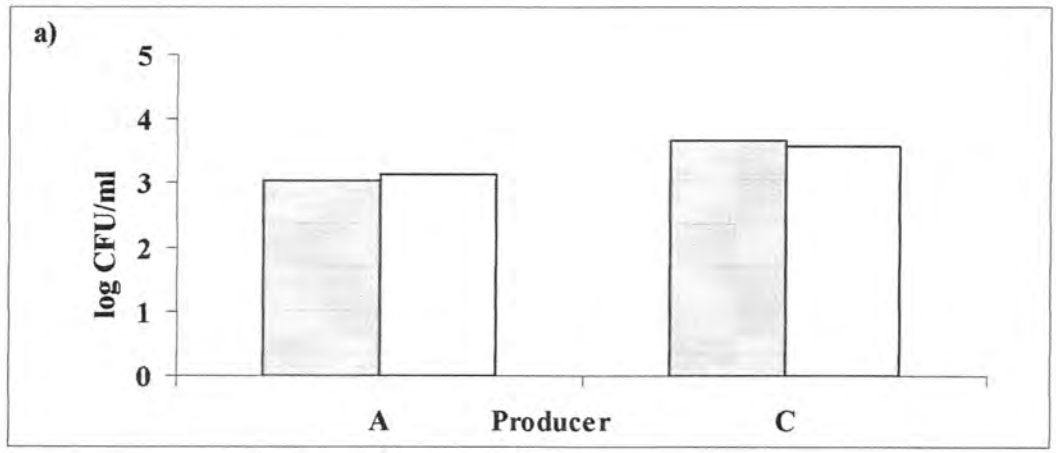
b) yeasts and molds.

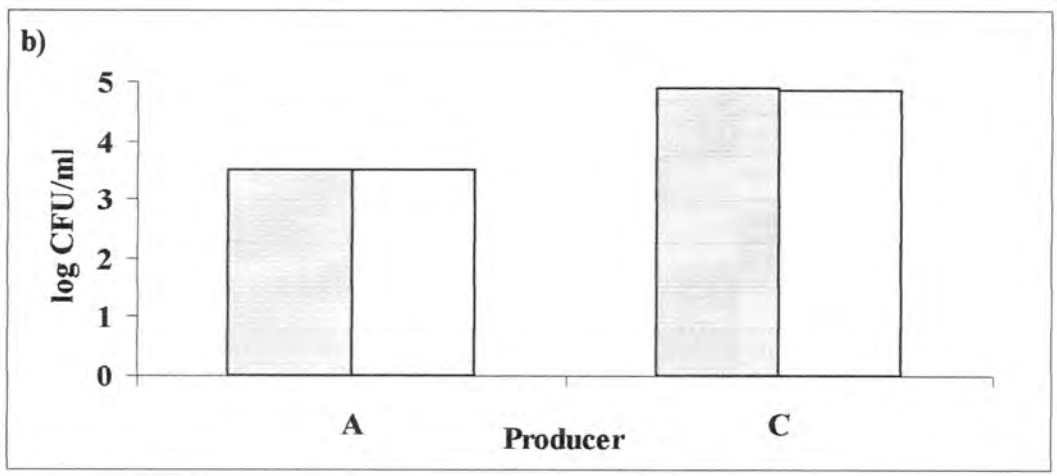
c) coliforms

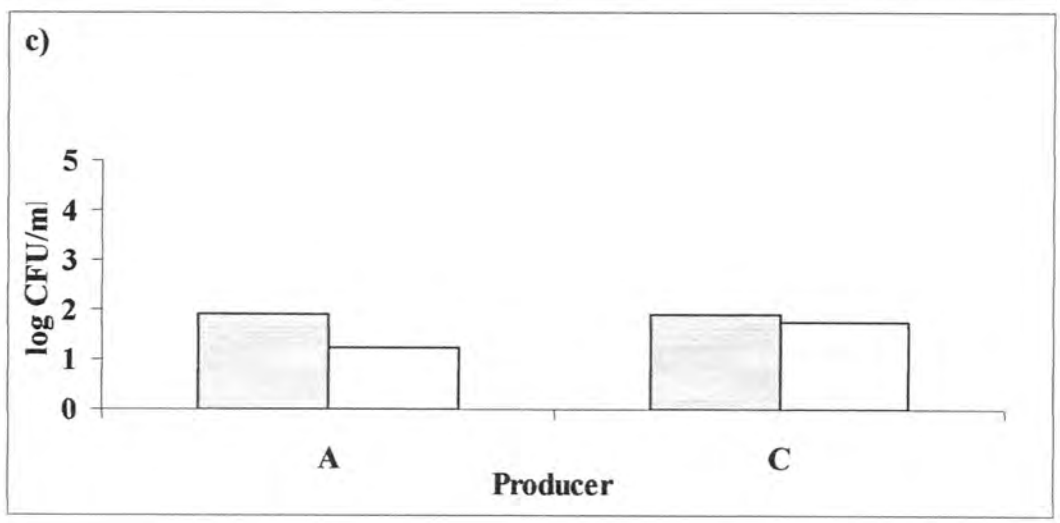
☐ = raw,

☐ = raw with preservatives.

Limit of detection: 10 CFU/ml for aerobes and yeasts and molds and 1 CFU/ml for coliforms.


a)
log CFU/ml
5
4
3
2
1
0
A
Producer
C


b)
log CFU/ml
5
4
3
2
1
0
A
Producer
C


c)
log CFU/ml
5
4
3
2
1
0
A
Producer
C

held for 24 hours after the addition of preservative while those obtained from Producer A had not been held for more than 2 hours. Zhao et al. (1993) found that potassium sorbate had minimal effects on the populations of *E. coli* O157:H7 in cider.

The results obtained for microbial counts in cider during this audit are in accordance with those obtained by Cummins et al. (2002) in a previous survey of apple cider produced in Iowa. No *E. coli* were found in any of the cider samples tested during the study. It was recommended to the producers that they have their pasteurization units inspected regularly by qualified personnel to verify that they are working properly. While potassium sorbate did not have a marked effect on the microbial counts in either raw or pasteurized cider, it was observed during a subsequent storage study conducted on the same samples that the addition of preservative reduced the growth of microorganisms in pasteurized cider over storage time (see next section of this thesis).

Producer C installed a new automatic bottling unit in the facility; this is a highly recommended practice since this reduces the risk of contamination of the pasteurized cider during bottling. Using only undamaged, tree-picked apples in making cider, following general good manufacturing practices, and ensuring that the pasteurization systems are working properly are important practices that help in keeping microbial counts low in the final cider.

Microbial Contaminants in the Environment and on Equipment

The equipment used in the manufacture of cider, as well as the surrounding environment, also play an important role in determining the microbial counts in the finished product. The cider press, conveyor belt for incoming apples, chute for disposing of pomace, bottler, and cider holding tanks were swabbed, and also samples of apple wash water and

unused lids were obtained during monthly visits to the cider producers in 2000 (Producer A only) and 2001 (all three producers). Producers B and C had been audited in a previous survey in 1999 and 2000 (Cummins, 2001), and data for 2000 for these producers are not presented here. All samples were collected while cider was being processed. Water samples were obtained from the supply line to the processing room. Sites sampled were not the same for all producers as each one's operation was set up differently. Occasionally, a piece of equipment was not accessible and could not be swabbed. Such a site is reported as not available (NA). Clean versus dirty samples were distinguished based on visual observations and numbers of organisms found during the audit. A piece of equipment was considered dirty if it had more than 100 organisms (of any type) per cm^2.

Average microbial counts per cm^2 of equipment surfaces or per ml of water, obtained over all the visits, are presented in Table 6. Raw data are reported in Appendix B of this thesis. Counts for all organisms on holding tanks, on bottler nozzles, and unused bottle caps were all <1 organism/cm^2 and these data are not presented in the table. These pieces of equipment and the bottle caps were considered clean. Some sites had high levels of microbial contamination and could be considered problem areas for specific producers. These problem areas were: chute for Producer A; cider press and chute for Producer B; conveyor belt and wash water for Producer C.

For Producers A and B, the well water used for washing apples had no detectable microbial contaminants (<10/ml). As apples were washed, microbial counts in the wash water rose considerably as organisms were transferred from the apples. The well water of Producer C contained high levels of aerobic bacteria and coliforms, and was likely responsible for the high microbial counts on washed apples for this producer, as noted previously.

Table 6: Average microbial loads on environmental and equipment samples

Microbial load at site sampled (CFU/ml or CFU/cm^2)	Producer and Year			
Aerobic Bacteria	**A (2000)**	**A (2001)**	**B (2001)**	**C (2001)**
Conveyor Belt	<10	142	<10	5
Chute	600	40	1500	NA
Cider Press	<10	300	2300	<10
Holding Tank	<10	<10	<10	NA
Wash Water (Before washing apples)	<10	<10	<10	7500
Wash Water (After washing apples)	5000	900	4000	NA
Coliforms				
Conveyor Belt	<1	5	<1	<1
Chute	6	<1	600	NA
Cider Press	<1	<1	60	2
Holding Tank	<1	<1	<1	NA
Wash Water (Before washing apples)	<1	<1	<1	200
Wash Water (After washing)	<1	80	<1	NA
Yeasts & Molds				
Conveyor Belt	<10	400	<10	600
Chute	400	40	<10	NA
Cider Press	<10	<10	2000	<10
Holding Tank	<10	<10	<10	NA
Wash Water (Before washing apples)	<10	<10	<10	10
Wash Water (After washing)	1300	60	10	NA

NA: Data not available.

For Producers A and B, the chute used for collection of pomace was contaminated with all three groups of microorganisms tested. However, only once for Producer A (in 2000) was an *E. coli* isolated from this piece of equipment. This producer used a canvas cloth as the funnel in the chute. Early in this study it was observed that the cloth was not cleaned before or after processing. The passage of large amounts of apple debris through this chute during processing could deposit large numbers of microorganisms on its surface. This was brought to the notice of the producer, who replaced the cloth with disposable, plastic funnels that were each used for only one processing batch. Microbial counts on Producer A's chute were considerably lower in 2001 compared to 2000.

Producer B used a steel funnel as a chute, which was cleaned after each use. However, high microbial counts were found here both in 2000, when the chute was sampled after it had been cleaned (Cummins, 2002), and in 2001, when it was swabbed while the equipment was in use. This chute should be cleaned more thoroughly after every batch process.

The cider presses for Producers A and B also harbored aerobic bacteria, yeasts and molds. As samples were taken during processing, this result is expected. However, Cummins (2002) also reported high aerobic bacteria and yeast and mold counts in 2000, when she sampled Producer B's equipment after cleaning. More thorough cleaning was recommended to Producer B. Producer A's press was tested twice after cleaning; counts for all organisms were below detection limits.

For Producer C the only piece of equipment that showed contamination was the conveyor belt, which had high levels of yeasts and molds. The belt for Producer A was also

contaminated with both aerobic bacteria and yeasts and molds. These counts likely can be attributed to the apples or wash water on the belt.

All employees at all three processing operations used gloves. Only Producer A had overalls for employees. Hairnets were not used at any of the sites; some employees sometimes wore baseball caps but this was not a regular practice. Employees of Producer A were required to wear gloves and use chlorinated, hot-water hand-dips periodically during processing and after touching unsanitary surfaces. Producers A and B conducted pressing and bottling operations in different rooms while Producer C carried out both operations in one room. All the producers had a clean-in-place system for treatment of the equipment after processing.

Following proper cleaning and sanitation procedures can solve a majority of the problems faced in equipment contamination. Producer B, whose equipment had high counts even after cleaning, should examine his cleaning procedures and should sanitize equipment both before and after use. The nature and concentration of the sanitizer should be checked for optimum effect in reducing microbial loads.

Producer C's most pressing problem is water quality, as evidenced by increased counts on apples after washing. In addition, the brush used for apple washing was not cleaned before or after use and could harbor high numbers of microorganisms. The hose used to bring water to the processing room should also be inspected and replaced, if necessary.

Comparisons of counts between 2000 and 2001 show that the problem areas identified by Cummins (2002) for Producers B and C in 2000 remained the same in 2001. The chute was a major problem area for Producer A in 2000, but in 2001 the producer solved this problem. The other problem areas for this producer were similar for both years. In 2001,

Producer A had problems with gnats and flies in the cider storage and bottling room. While measures (flytraps) were used to counter this problem, it is possible that insects could have been a reason for increased microbial counts in raw cider in this year. The conveyor belt for Producer A showed signs of flaking paint on its surface. This can be a physical/chemical hazard if any of the paint gets into the cider. Proper care of the equipment should be taken to safeguard against such hazards.

HACCP plans were developed for all the producers after both production seasons (see Appendix A of this thesis). These included generic lists of Standard Operating Procedures (SOP's) and Good Manufacturing Practices (GMP's). Each producer was also made aware of any problems immediately after discovery. Producers did attempt to follow these guidelines and took steps (e.g. providing gloves and overalls to employees, improving cleaning and sanitation processes) to improve their operations. Still, a number of deficiencies need to be rectified: hairnets should be provided to employees; all employees should regularly wash and sanitize hands during the process; eating should not be allowed in the process area; cider holding tanks should not be left uncovered. For clean and sanitary production of cider, it is important that good manufacturing practices be followed so that the risk of contamination of the final product is minimized.

Conclusions

The producers surveyed are following practices such as sanitation, use of preservatives, and pasteurization to help reduce the risk of microbial contamination of their cider. General hygiene of the employees and storage and processing conditions were satisfactory, with specific needed improvements in equipment sanitation and water chlorination noted. None of the producers used drop apples in their operations; most of the apples were from the producer's own orchards.

The microbial counts on apples were generally high and did not vary significantly between the two years of the study. No *E. coli* were detected on any of the apples tested. Proper handling of apples from harvesting to pressing is essential to help lower microbial loads in the cider. It was observed that equipment that came in contact with apples needed more sanitation and cleaning and the quality of wash water used also needed to be tested regularly to ensure that washing did not increase counts on the apples.

The only counts that differed significantly between the two production years were coliforms in raw cider samples obtained from two of the producers. Pasteurization decreased the counts to undetectable levels and the finished cider can be considered to be safe. The pasteurization time-temperature conditions used by the various producers varied but were equally effective in reducing microbial loads in cider. It is recommended that the producers should have their processes validated for the 5-log reduction of the target pathogen (*E. coli* O157:H7). Regular inspection, testing and calibration of the pasteurization equipment should also be done.

Good manufacturing practices should be followed throughout the process. Employee hygiene is a very important aspect of the operation as improper hygiene can result in

contamination of the final product. Care should be taken with post-pasteurization handling and storage of the cider to minimize the chance of contamination. When apples are obtained from other sources, the producer should ensure that their suppliers also follow sound orchard management practices. Incorporating a clause in the contract that specifically states that drop apples will not be accepted by the producer can help solve this problem.

No *E. coli* were found in any of the apple or cider samples tested. This does not mean that there are no *E. coli* on the apples and cider produced in Iowa, but it does indicate that the incidence of *E. coli* may be very low. Following GMP's, proper handling and storage of the raw material and the final product, proper pasteurization, and adequate equipment sanitization should help to keep Iowa apple cider safe.

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CHANGES IN MICROBIAL LOADS AND AROMA COMPOUNDS IN REFRIGERATED APPLE CIDER

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Abstract

Fresh cider samples from three different processors were stored under refrigerated conditions for a period of 2 to 8 weeks and analyzed at two-week intervals for changes in microbial loads and aroma compounds. Raw cider spoiled within two weeks while the pasteurized cider with preservative was shelf-stable for up to 8 weeks. Coliform levels in pasteurized cider with preservative remained below detection limits during storage. The aroma patterns obtained for the cider samples differed with time, producer, and treatment. Changes in aroma compounds measured by the electronic nose were significantly correlated (R^2=0.49) with coliform levels. Flavor compounds also showed changes in concentration with time, but no relationship could be detected between the aroma and microbial loads. Cider samples with added preservative maintained higher levels of some compounds during storage as compared to cider samples without preservative.

Introduction

Variety, maturity, apple quality, processing, and storage conditions all affect the volatile flavor compounds present in apples and apple products (Williams et al., 1980). The shelf life of apple cider is also determined by a number of factors including the microbial load, flavor changes, alcohol formation, and appearance.

Because of recent outbreaks of food-borne illness related to apple cider consumption, the FDA has mandated a 5-log reduction in the populations of a target pathogen. Although there are a number of methods such as irradiation, pulse electric field, ozone technology and ultra-violet light pasteurization, which can be employed to reach this target reduction, the most commonly used method has been pasteurization of raw cider. Pasteurization is able to reduce the numbers of microorganisms present in cider but it also produces changes in the flavor components of cider leading to the formation of a cooked flavor (Poll, 1983). Additionally, since apple cider is a very good medium for the growth of microorganisms, especially yeasts and molds, the microorganisms can recuperate during storage and start growing very rapidly, thus bringing about a number of changes in apple cider properties. In addition to pasteurization, preservatives (potassium sorbate or sodium benzoate at a maximum concentration of 0.1%) may also be added to the cider. The addition of preservatives helps to increase the shelf life of the product (Zhao et al., 1993; Besser et al., 1993). However, it is possible that this process may also alter the flavor composition of the product.

The present study was conducted in order to determine the changes produced in apple cider during storage. The quality of raw and pasteurized refrigerated cider was measured by determining its microbial load and aroma at two-week intervals with the help of standard microbiological techniques, headspace volatile flavor analysis and electronic nose techniques. The aim of the study was to determine a relationship between the microbial loads present in the cider at a given time and the changes in cider aroma.

Materials and Methods

Cider samples (raw, raw with preservative (RWP), pasteurized with preservative (PWP) and pasteurized without preservative (PNP)) were obtained from three Iowa apple cider producers over a period of two years. Details about the individual processors' methodology can be found in the previous section of this thesis. These samples were analyzed for changes in microbiological loads and aroma profiles during refrigerated storage (at 7°C) over 8 weeks. Each cider was sampled at 0, 2, 4, 6 and 8 weeks. Time 0 was specified as the week in which the samples were produced and obtained from the producers.

Enumeration of Microorganisms

Aerobic, mesophilic bacteria were enumerated by spread plating in duplicate on Trypticase Soy Agar (TSA Diffco) according to standard methods (Gerhardt, 1994). Colonies were counted after incubation at 35°C for 48 h.

Yeasts and molds were counted on Potato Dextrose Agar (PDA, Difco), pH 3.5, after incubation at 25°C for 5 days.

Coliforms were counted on Petrifilms according to the AOAC recommended method provided by the manufacturer (3M, St.Paul, MN). Pink gas-forming colonies after 24 h of incubation at 37° C were considered to be coliforms. Blue gas-forming colonies that developed after 48 h of incubation at 37°C were counted as *E. coli*. Colonies without gas formation were not counted.

Volatile Flavor Analysis

Solid-phase micro-extraction (SPME, Supelco, Inc., Bellefonte, PA) was used for the isolation of volatile flavor compounds. A 40-g sample of apple cider was transferred to a 100-ml headspace bottle and sealed with a Teflon septum. The sample was then held in a 37

to 40°C water bath, with stirring. The SPME fiber was exposed to the headspace over the cider for 45 min so that the volatiles were absorbed onto the SPME fiber, which was exposed to the headspace over the cider. A gas chromatograph equipped with a splitless injection port and flame ionization detector was used for the analysis of volatile flavor compounds (HP Model 6890, Hewlett-Packard, Inc., Wilmington, DE). The volatiles were thermally desorbed (225°C) for 3 min via the GC injection port onto a fused-silica capillary column (SPB-5, 30m x 0.25mm x 0.25 μm film thickness, Supelco Inc.). The column pressure was set at 18.0 psi with a helium flow rate of 1.9 mL/min. The oven was initially held at 30°C for 3 min and increased at a rate of 5°C/min to a final temperature of 200°C. The detector temperature was 220°C. The flow rates of detector gases were air, 400 mL/min; hydrogen, 30 mL/min; and nitrogen make-up gas, 23 mL/min. Volatile flavor compounds were identified using authentic standards (Sigma–Aldrich, Milwaukee, WI; AccuStandard, Inc., New Haven, CT) and confirmed with GC/MS analysis.

Electronic Nose Analysis

Cider samples (5 ml) were transferred into 500-ml taint-free plastic pouches with a special connector for attachment to the electronic nose (AromaScan$^{(R)}$, Mod. A32S. AromaScan, Inc Crewe, U.K.). The pouches were filled with air at 25°C and 15% relative humidity. The air used for the reference had 10% relative humidity. The apple cider samples were equilibrated at room temperature for 15-20 minutes before sampling. The headspace air was then pulled across all 32-polymer sensors. The sampling procedure for the electronic nose was: referencing (60 sec), sampling (120 sec), washing (60 sec) and referencing (60 sec). Referencing helps to eliminate the background noise, correct the baseline, and zero the sensors. Four replicates were used for the e-nose measurements for each treatment. Before

running the replicates, the sensors were flushed with air for 5 min. The air flush time between treatments was 10 min. Readings at 1min exposure of the sensors to the cider samples wereused for data analysis. Data were analyzed using readings from all 32 sensors. The AromaScan graphic program provided by the manufacturer was used to process the data.

Statistical Analysis

Tukey's multiple comparison procedure was performed on the microbial data using the SAS statistical analysis system (SAS Institute, Inc., Cary, N.C.) For the gas-chromatography data, analysis of variance and Fisher's least square difference tests ($P<0.05$) were conducted to determine the effects of processing treatment, time and their interactions on the content of volatile flavor compounds (SYSTAT, 1999). The gas-chromatography data from each producer was analyzed separately. The electronic nose data were analyzed using principal component analysis (AromaScan, Inc) and stepwise regression (SAS Institute, Inc., Cary, N.C.).

Results and Discussion

Microbial Changes During Storage

Differences in changes in microbial loads during storage due to differences in sampling times or treatments were analyzed by pooling together the data from all the producers. The data is presented in Appendix C of this thesis. The time of sampling did not cause any significant changes in total aerobic bacteria and coliform loads. But significant differences ($p<0.05$) were seen in the yeast and mold counts over the season; higher counts were reached more quickly during storage later in the season. This may be because the cider samples had a higher initial load. Comparisons of loads for the different treatments of cider show that counts of aerobes and yeasts and molds were similar in all the treatments. In the case of coliforms there was a significant difference ($p<0.05$) in the counts obtained for PNP and RWP ciders; counts during storage were higher in the RWP cider. Differences between any of the other treatments were insignificant.

Figures 1, 2 and 3 are representative of the storage study results obtained for Producers A, B and C, respectively, at one sampling time. The results obtained at other sampling times were similar. In the case of Producer A (Fig.1), the addition of preservative appeared to have a detrimental effect on the growth of microorganisms. PWP cider showed less rapid increases in aerobic bacteria (Fig.1a) and yeast and mold (Fig.1b) counts than did PNP cider. The range of counts obtained during storage for aerobic bacteria in pasteurized no preservative cider (also referred to as PNP cider in this paper) varied from 10^1 to10^6 CFU/ml while in the case of pasteurized with preservative cider (also referred to as PWP cider in this paper) the range was from 10^1 to10^3 CFU/ml. Yeast and mold counts did not show as much difference and ranged from 10^1 to 10^7 CFU/ml in PNP cider and from 10^1 to10^6 CFU/ml in

Figure 1: Shows changes in microbial loads during refrigerated storage of apple cider from Producer A.

Figure 1a: Aerobic bacteria,

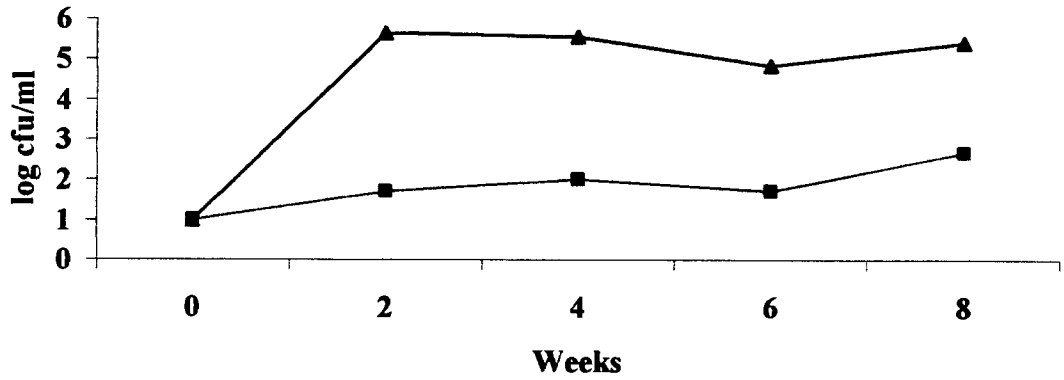
Figure 1b: Yeasts and molds,

Figure 1c: Coliforms,

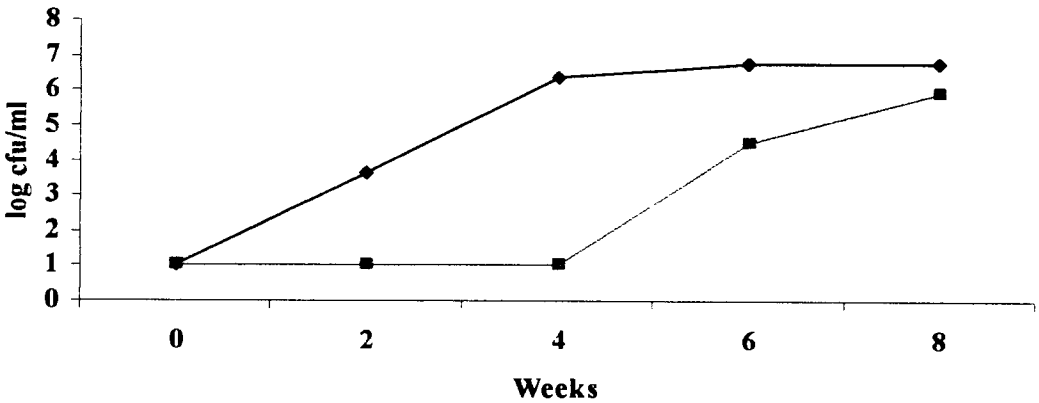
PNP (▲)

PWP (■)

a)


log cfu/ml
0
1
2
3
4
5
6
0
2
4
6
8
Weeks

b)


log cfu/ml
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1
2
3
4
5
6
7
8
0
2
4
6
8
Weeks

c)

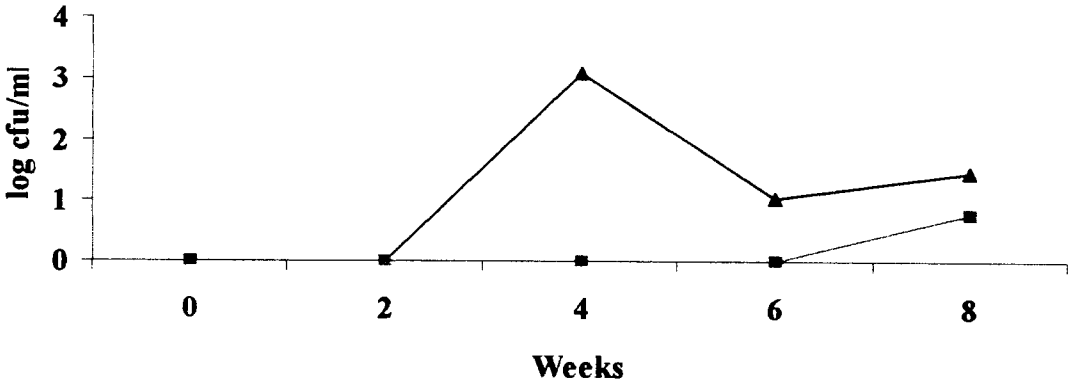

log cfu/ml
0
1
2
3
4
0
2
4
6
8
Weeks

Figure 2: Shows changes in microbial loads during refrigerated storage of apple cider from Producer B.

Figure 2a: Aerobic bacteria,

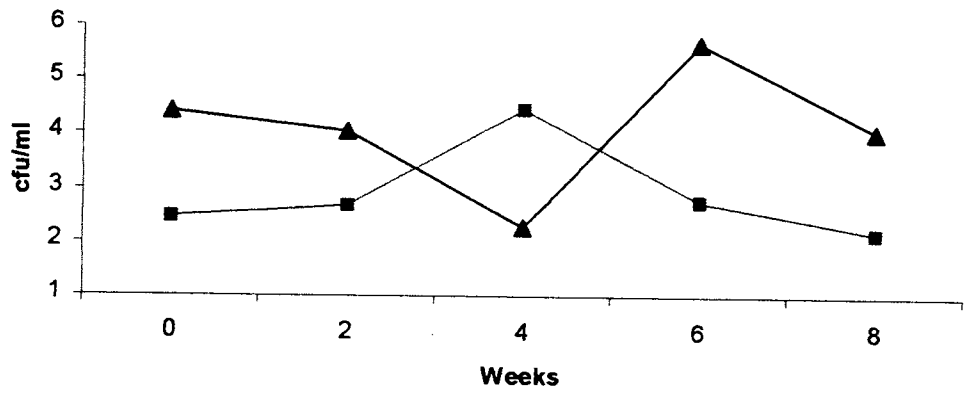
Figure 2b: Yeasts and molds,

Figure 2c: Coliforms,

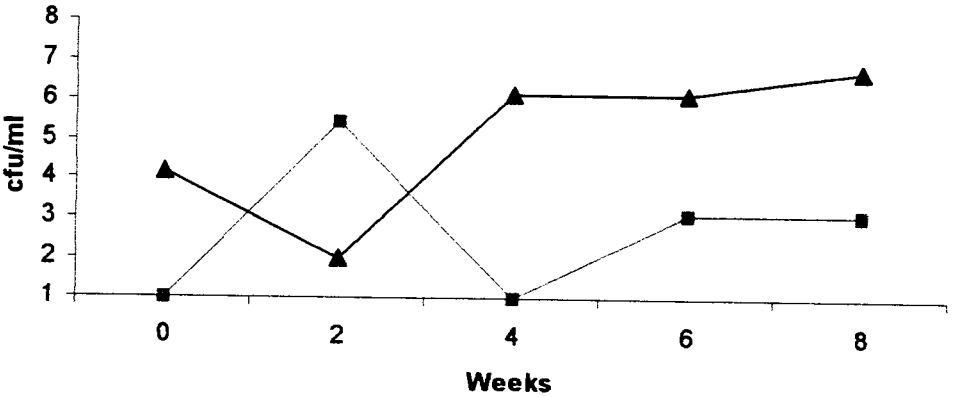
Raw (▲)

PWP (■)

a)


6
5
4
3
2
1
cfu/ml
0
2
4
6
8
Weeks

b)


8
7
6
5
4
3
2
1
cfu/ml
0
2
4
6
8
Weeks

c)

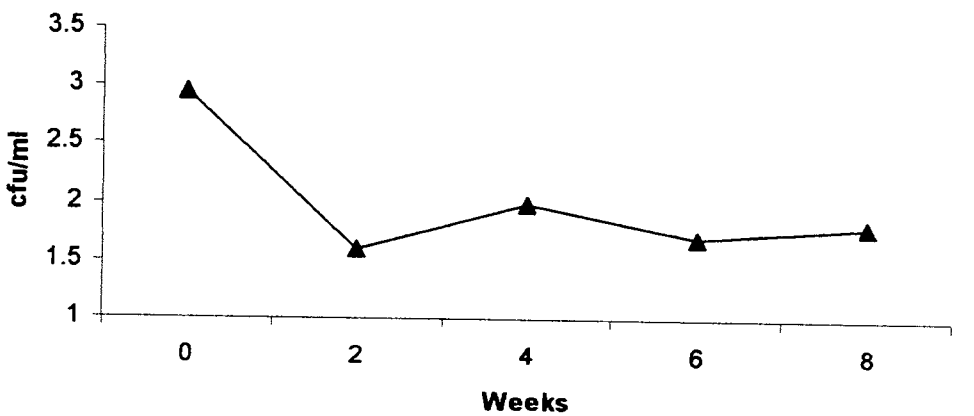

3.5
3
2.5
2
1.5
1
cfu/ml
0
2
4
6
8
Weeks

Figure 3: Shows changes in microbial loads during refrigerated storage of apple cider from Producer C.

Figure 3a: Aerobic bacteria,

Figure 3b: Yeasts and molds,

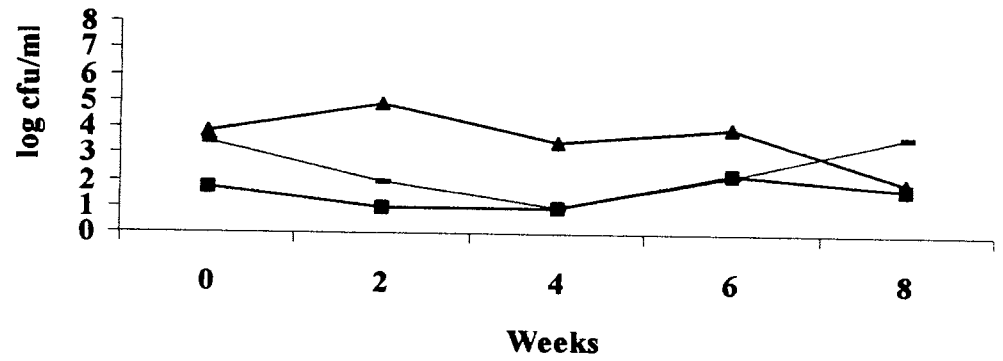
Figure 3c: Coliforms,

Raw (▲)

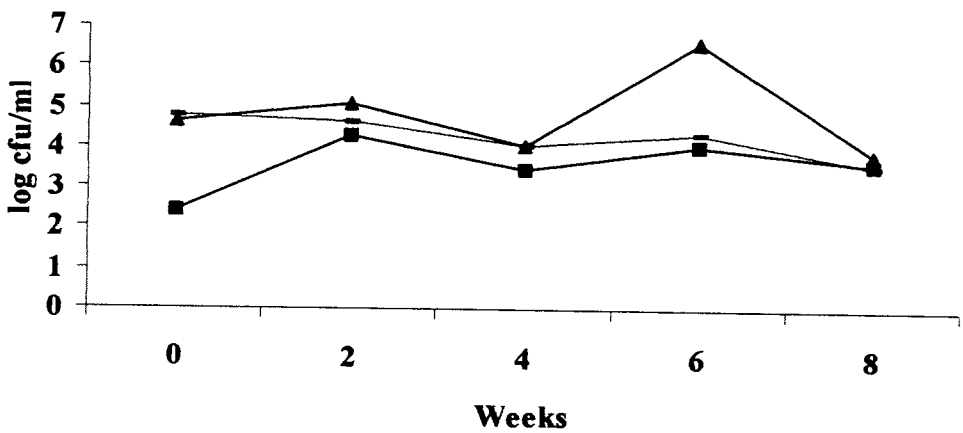
RWP (-)

PWP (■)

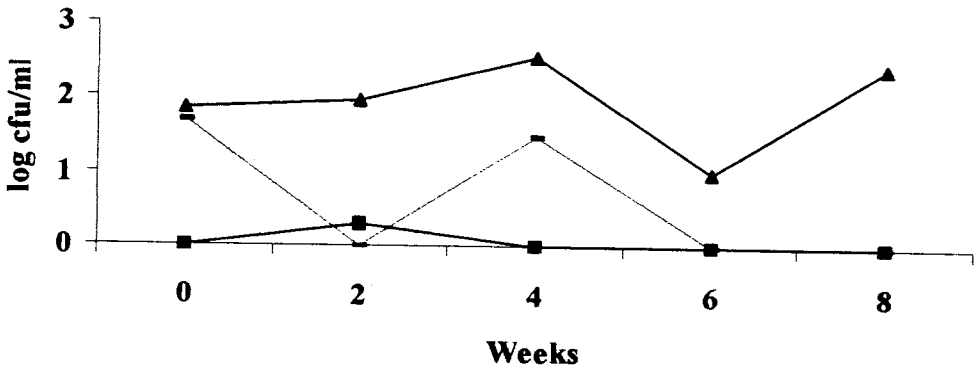
a)


log cfu/ml
8
7
6
5
4
3
2
1
0
0
2
4
6
8
Weeks

b)


log cfu/ml
7
6
5
4
3
2
1
0
0
2
4
6
8
Weeks

c)


log cfu/ml
3
2
1
0
0
2
4
6
8
Weeks

PWP. There was no growth of coliforms in PWP cider while PNP cider showed coliform counts up to 10^3 CFU/ml at various stages of storage (Fig. 1c).

Initial levels of microbes in raw cider from Producer B (Fig.2) were in the range of 10^4 to 10^6 CFU/ml of aerobic bacteria and yeasts and molds. Counts of aerobic bacteria reached10^6 CFU/ml during storage (Fig. 2a) while those for yeasts and molds rapidly increased to 10^6 CFU or higher (Fig. 2b). Coliforms were present in the range of 10^1 to10^3 CFU/ml and generally showed no change in numbers over storage or decreased over time (Fig. 2c). PWP cider had low initial microbial counts but during storage, these increased considerably. A trend was noted in the counts obtained for the PWP cider from this producer; if yeasts and mold counts were high during a week then the aerobic bacteria counts decreased during that week and vice versa. Cummins (2001) also noted similar trends in a storage study conducted on apple cider samples obtained from various producers in Iowa. It is possible that the yeasts and molds and aerobic bacteria compete with each other for nutrients and this may result in the fluctuations in counts. Counts were in the range of 10^2 to10^5 aerobic bacteria/ml and 10^1 to10^5 yeasts and molds/ml of PWP cider. No coliform growth was detected during the storage period.

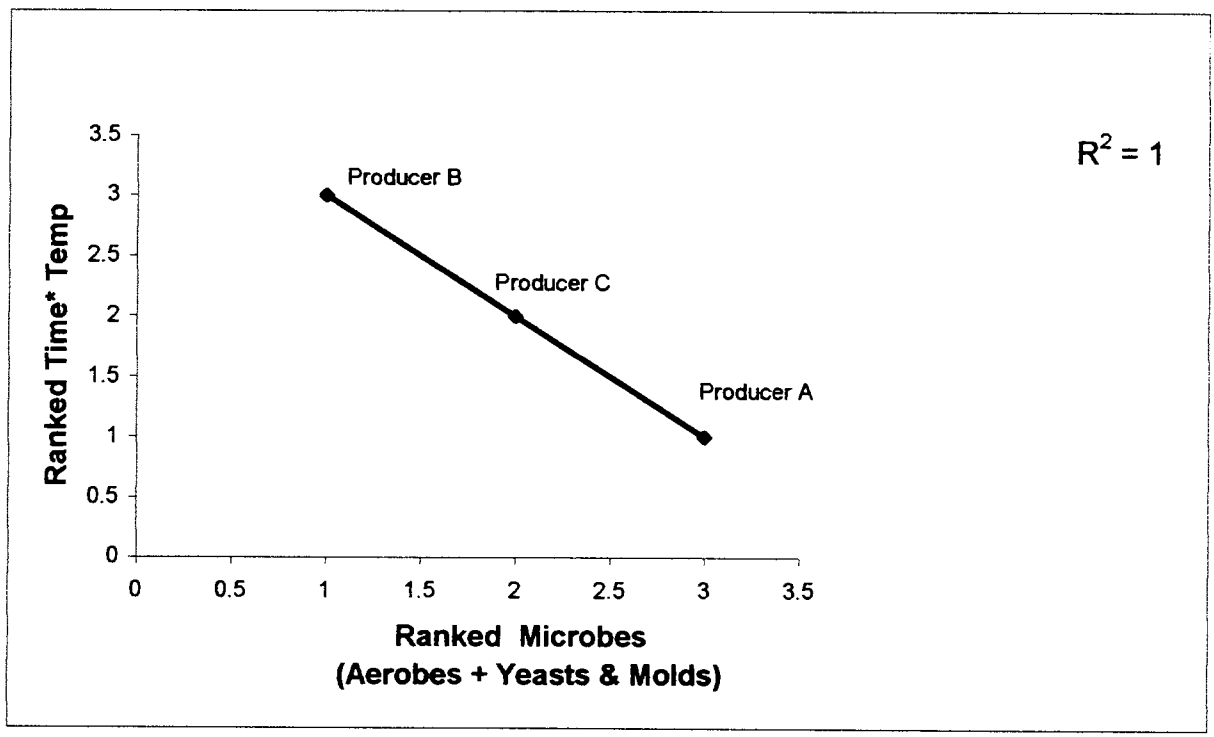
Producer C (Fig. 3) provided samples of raw, raw with preservative (also referred to as RWP in this paper) and PWP cider. There seemed to be no effect of preservative on the growth of microorganisms during storage in the raw cider; similar counts were obtained from both raw and RWP cider types. This suggests that the preservative alone may not be able to bring about the required reduction in cider; making the use of an additional initial kill step such as pasteurization necessary. It may be that the initial microbial counts in the raw cider are so high that the preservative is not as efficient in its ability to inhibit further microbial

growth, as it would be with a lower initial count. PWP cider had low initial counts but the maximum counts reached during storage were the same as those obtained in the case of unpasteurized ciders. The rate at which the maximum loads were reached was lower in PWP cider. Both types of raw cider had high (10^4 CFU/ml) yeast and mold counts initially and these increased to 10^6 CFU/ml by the second week of storage. PWP cider reached the 10^6 yeasts and molds /ml level by the eighth week (Fig. 3b). The growth of aerobic bacteria was slower as compared to yeast and mold growth in all the samples. The raw cider samples started out with 10^3 aerobic bacteria/ml and generally remained at the same level during most of the storage period with occasional 1-log fluctuations. Aerobic bacteria in PWP cider increased gradually during storage and reached the 10^4 CFU/ml level only in the eighth week of storage (Fig. 3a). Coliform levels were in the range of 10^1 to10^3 CFU/ml for the raw ciders and these levels did not change much during storage. Coliform growth in PWP cider was below detection limits (Fig. 3c).

It was observed that the PWP cider samples from Producer B spoiled at a slower rate as compared to the PWP cider from the other producers. As the three producers used different time-temperature combinations for pasteurizing, a rank test was conducted on microbial counts versus time of pasteurization x temperature of pasteurization (degree-second). An R of 1 was obtained by plotting the ranked values against each other (Fig. 4). The figure shows that at six weeks of storage, Producer B had the highest degree-second value and the lowest counts; Producer A had the lowest degree-second value and the highest counts. As Producer B pasteurized for the longest time (11 s), it is reasonable that the longer holding time caused the lower counts in the cider. Producers A and C both pasteurized for 2s

Figure 4: Plot of ranked time x temperature value against ranked microbial (total aerobes + total yeasts and molds) for each producer.

Lowest rank is the highest value for each parameter.



but Producer C pasteurized at a higher temperature than Producer A and this could explain the differences observed in the counts for these two producers. Therefore it can be assumed that, as expected, the time-temperature treatment of the cider will be reflected in the shelf life of the refrigerated cider even though time zero counts did not vary for the three producers.

A sample was considered to be deteriorated if it had microbial counts $>10^6$ CFU/ml. It was observed that the raw ciders spoiled very early during storage. Not only did the microbial counts increase greatly, but the aesthetic appearance, and odor of the cider also deteriorated within two weeks of storage. Pasteurized cider samples kept for a longer time; PNP cider deteriorated in appearance after six weeks and PWP cider had not deteriorated after eight weeks. These results are in accordance with those obtained by Cummins (2001) who estimated the shelf life of raw cider to be 2-3 weeks and that of pasteurized cider with preservative to be 10-12 weeks.

Considerable gas formation along with a strong alcoholic odor was observed in the unpasteurized ciders at two weeks and in the pasteurized cider without preservative at four weeks. Pasteurized cider with preservative did not show these effects even up to eight weeks. This suggests that the addition of preservative combined with pasteurization has a considerable effect on the inhibition of wild yeasts responsible for producing hard cider as was also noted by Deak et al., 1996.

The results obtained from the study indicate that the addition of preservative before pasteurization helps in increasing the shelf life of cider. Addition of preservatives alone may not be able to inhibit or kill the microbes present. Low initial counts also help in improving the efficacy of preservative and/or pasteurization treatments.

Electronic Nose Results

Differences were observed in the characteristic aroma patterns obtained on a Principal Component Analysis (PCA) plot of the cider samples at time zero (i.e. the week that cider was manufactured) across different sampling times for Producer A (Figure 5). The samples in the early and late season seem to be clustered together more tightly than mid-season samples that are more scattered. The addition of preservative did not change the aroma patterns; cider samples with preservative and without preservative being clustered in the same area of the plot. But there is a definite shift in the patterns with time. These differences may be due to factors such as the type of apples used, the length of time the apples were been in storage, and different time-temperature combinations used for pasteurization.

Figure 6 shows the aroma patterns obtained for PWP cider obtained from the three producers at approximately the same time in the season. The figure shows that all three producers have different aroma patterns for the ciders. The reasons for these differences may be due to the blends of apples used in making cider, the time for which apples had been in storage before cider production or the length of time for which the cider was held after addition of preservative. Different time-temperature combinations used for pasteurization, may have also affected the aroma composition of the final product.

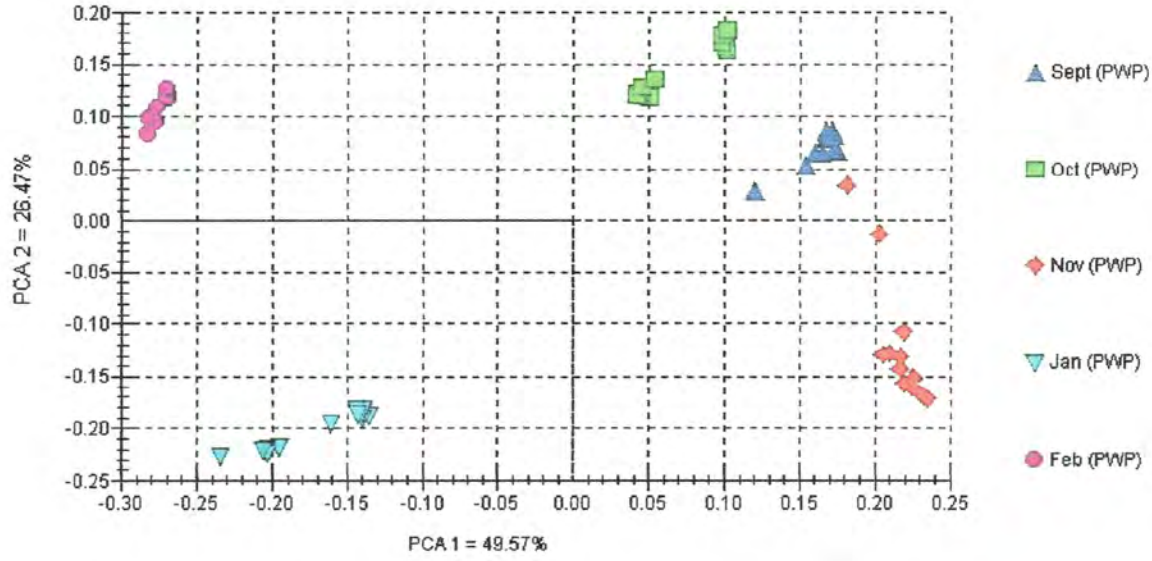
Figure 7 plots the raw, RWP and PWP cider obtained from Producer C. It can be seen that the time zero readings for the three treatments are similar. In week two the RWP and the PWP cider stayed the same as in week zero while the raw cider changed. The PWP cider changed relatively little throughout the storage period. Less microbial growth in this sample may have resulted in fewer aroma changes in the product.

Figure 5: Shows the aroma patterns obtained for cider samples from Producer A at different sampling times.

5a)PWP cider aroma patterns

5b)PNP cider aroma patterns

5a)



5b)

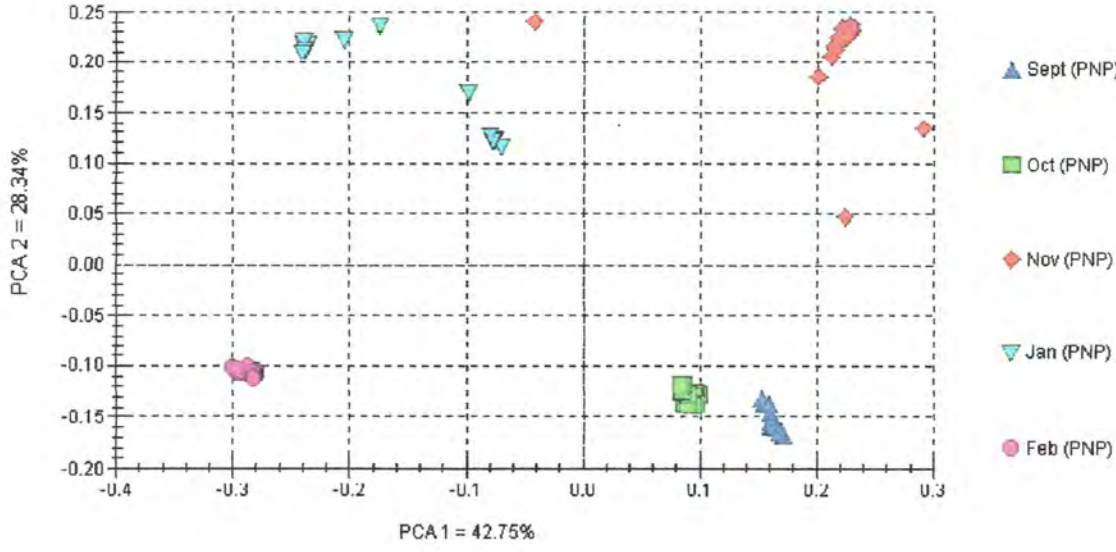


Figure 6: Shows the variation in aroma patterns in PWP cider from the three producers

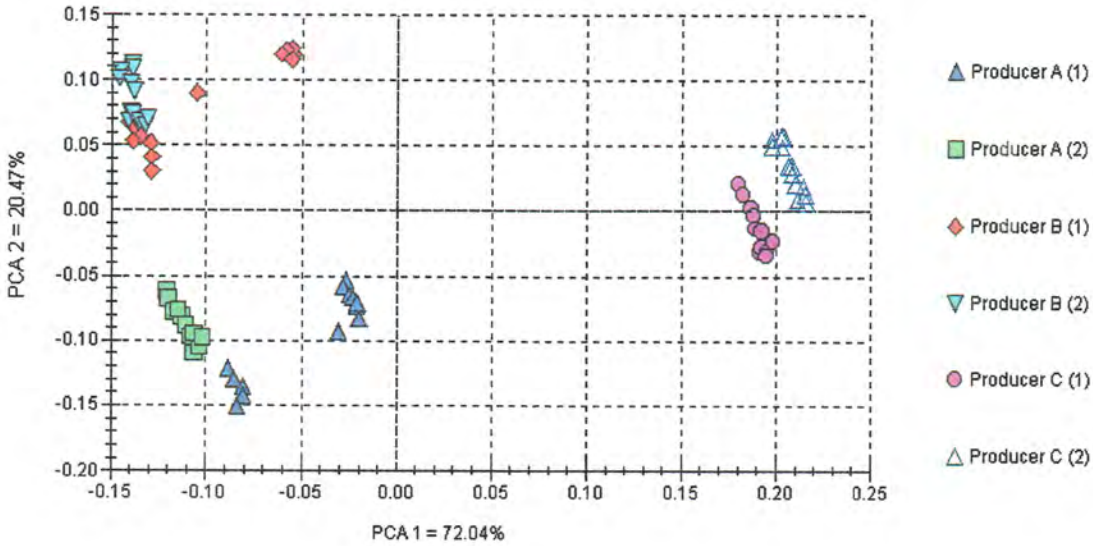
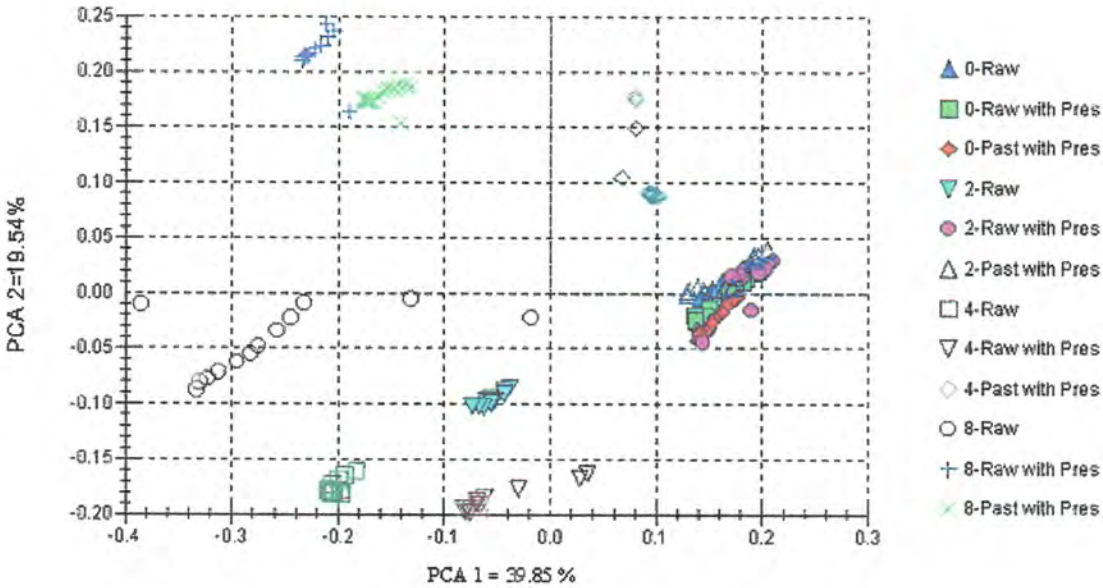


Figure 7: Shows the variation in aroma patterns in raw, RWP and PWP cider from Producer C



The average response for each of the 32 sensors was plotted against sensor number to identify particular sensors that could be used to predict changes in cider flavor compounds over time. Based on visual observations of data obtained from the analysis of raw, PNP and PWP ciders from all three producers (data not shown), sensors 5, 6, 18, 23, 24, 30 and 31 were found to vary in their response over time for a particular sample. But the responses recorded were not consistent and showed considerable variation over time.

It was hypothesized that changes in aroma compounds produced during storage could be related to changes in the corresponding microbial loads; thus, the aroma patterns observed at a particular time could be used as an indicator of the microbial load present in the cider and of the length of time the cider had been in storage. A stepwise regression test (SAS Institute) was conducted on the microbial and electronic nose data to test this hypothesis. Results obtained indicate that for aerobic bacteria counts, the sensors that were able to give the best prediction were 14, 17 and 24 (sensitive to amines, long chain alcohols, aromatic compounds, chlorinated hydrocarbons and esters) with a R-square value of 0.31. Sensor numbers 1 and 19 (detecting amines, alcohols, esters and carboxylic acids) gave an R-square value of 0.31 in the case of yeasts and molds. For coliforms sensors 24 and 32 (most sensitive to amines, alcohols, aromatic compounds and esters) were found to be the best predictive sensors with an R-square of 0.49. This indicates that the sensor response increased with increase in coliform levels and decreased when the coliform levels decreased. Therefore, it can be seen that except for a reasonable prediction of coliform levels, the electronic nose results could not be correlated to the microbial loads present in cider at a given time. The low prediction ability of the electronic nose sensors can be attributed to the large amount of variation found in cider samples with regard to producers, time of

processing, raw ingredients used (variety and maturity level of apples used), and processing conditions, all of which cause the cider to have varying initial microbial loads and populations and thus varying increase/ decrease in the numbers and types of microorganisms. Results obtained suggest that the electronic nose is sensitive to changes in coliform loads and it is possible to get a reasonable estimate of microbial loads in cider at a given time from the aroma scan data. To have a better predictive model, the variation will have to be reduced. But considering the fact that cider production practices vary from one producer to another and differences due to time and apples used cannot be eliminated, the utility of such a model may not be universal. The economics involved in purchasing an electronic nose unit may make this an unacceptable cost for the small-scale producers but for larger producers and commercial processing operations, this may prove to be a quick and reliable quality control technique.

Headspace Volatile Analysis

As noted in the electronic nose results, differences were observed in aroma compounds on samples from different producers, at different times with different treatments. Gas chromatographic analysis helped in the identification of some of the major compounds, which showed quantifiable changes during storage.

Comparing the peaks obtained in this study to those identified in a standard apple cider sample with the help of mass spectrophotometric analysis, 38 compounds were identified to be present in the various cider samples. Of these, 28 were esters, 4 were aldehydes, 3 were alcohols and 3 were classified as miscellaneous compounds. Among the compounds identified were ethyl-2-methyl butyrate, hexyl acetate, hexanal and unsaturated C-6 alcohols which have all been characterized as compounds necessary for fruit aroma in

apple juice (Poll, 1983). Ethyl-2-methyl butyrate is considered to be one of the most important aroma components of apple juice because of its low detection threshold. The only alcohols detected were 1-octanol, 1-octen-3-ol and hexanol. These are oxidation products, which are generally formed when the apples are crushed or damaged, or when the juice is exposed to air. Ethanol is an important alcohol present in fermented ciders but the equipment used for headspace volatile analysis in this study was not sensitive to the presence of ethanol in the samples and therefore was not detected at significant levels.

Figures 8(a) shows the changes in some representative ester compounds detected in stored cider obtained from Producer A averaged across two different times. Figure 8(b) shows the changes in esters in stored cider from Producer C. Producer B's cider was also analyzed but the data for this producer showed a lot of variation and it may be that the fiber used for this sample set was damaged. The data for all the esters can be found in the Appendix D of this thesis. The figures show that hexyl acetate decreased in all of the samples from both producers. For both of Producer A's samples, hexyl acetate initially decreased quite sharply after cider production followed by a gradual decrease later on. The decrease seen in the case of raw cider obtained from Producer C (Fig.8b) is very rapid and drastic as compared to the decrease in PWP and RWP ciders, which showed a similar pattern over time. The other esters shown in the graph include ethyl-2-methyl butyrate, butyl propionate, butyl acetate, and 2-phenylethyl acetate. All of these were found to be present in low concentrations and either decreased or remained at almost similar concentrations over time. The addition of preservative to cider seemed to have an effect on the changes in concentrations of the esters. Decrease of ester concentration over time was slower in cider with preservative (pasteurized as well as raw) as compared to cider without preservative.

Figure 8: Shows changes in representative ester compounds during refrigerated storage of cider samples

Figure 8 (a): Producer A (averaged across two different sampling times)

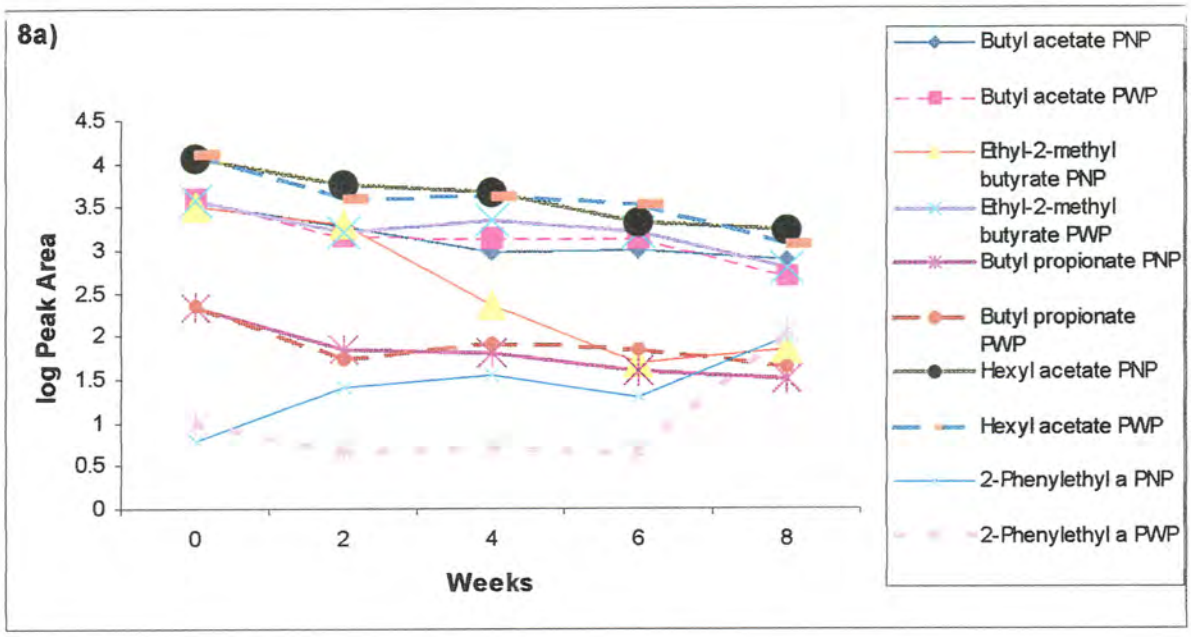
Figure 8 (b): Producer C

Raw: Unpasteurized cider

RWP: Unpasteurized cider with preservative

PNP: Pasteurized cider no preservative

PWP: Pasteurized cider with preservative


8a)
log Peak Area
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
Weeks
Butyl acetate PNP
Butyl acetate PWP
Ethyl-2-methyl butyrate PNP
Ethyl-2-methyl butyrate PWP
Butyl propionate PNP
Butyl propionate PWP
Hexyl acetate PNP
Hexyl acetate PWP
2-Phenylethyl a PNP
2-Phenylethyl a PWP

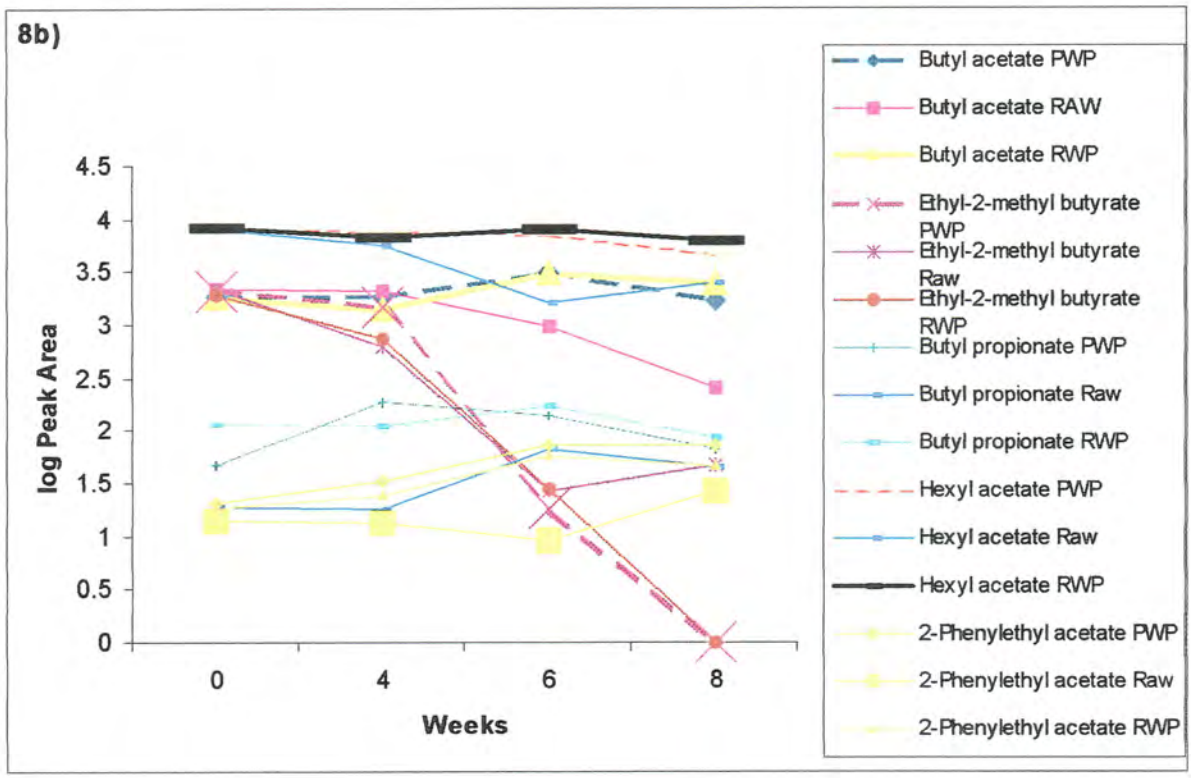

8b)
log Peak Area
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
4
6
8
Weeks
Butyl acetate PWP
Butyl acetate RAW
Butyl acetate RWP
Ethyl-2-methyl butyrate PWP
Ethyl-2-methyl butyrate Raw
Ethyl-2-methyl butyrate RWP
Butyl propionate PWP
Butyl propionate Raw
Butyl propionate RWP
Hexyl acetate PWP
Hexyl acetate Raw
Hexyl acetate RWP
2-Phenylethyl acetate PWP
2-Phenylethyl acetate Raw
2-Phenylethyl acetate RWP

Figure 9 shows the variation that occurred in hexanal concentrations in the stored cider samples. For Producer A (Figures 9a) hexanal was present in high concentrations in both the PWP and PNP ciders but the decrease seen over time is more rapid for the PNP cider. In the cider samples from Producer C (Figure 9b), hexanal was present at higher initial concentrations in the PWP and RWP ciders than in raw cider. With time, the concentrations in raw cider increased slightly with a subsequent decrease; whereas in the PWP and RWP ciders, there was a sharp decrease in hexanal concentrations from time zero onward. The other aldehydes detected included decanal and trans-2-octenal. The concentrations of these compounds were generally low and tended to drop off with time. Nonanal was detected during one of the sampling times for Producer A and its concentration showed a slight increase before decreasing to almost undetectable levels (data not shown)

The miscellaneous compounds detected in the cider samples included estragole, benzaldehyde and alpha-farnesene (Figure 10). Estragole was present at high concentrations in all the samples at time zero and underwent a sharp decrease in concentration in the raw cider from Producer C and the PNP and PWP ciders from Producer A. For the rest of the cases, there were decreases in concentration but these were more gradual. Estragole is an active volatile compound that contributes to the aroma of apples (Caccioni et al.,1997) and is a Generally Recognized As Safe (GRAS) flavoring component for food use (FDA 21 CFR Sec.182.20). No information could be obtained on the behavior of this compound during storage. The other two compounds were present at very low concentrations in the fresh cider and did not change in concentration over time.

Figure 9: Changes in aldehyde compounds during refrigerated storage of apple cider

Fig. 9a: Producer A (averaged across two different sampling times)

Fig. 9b: Producer C

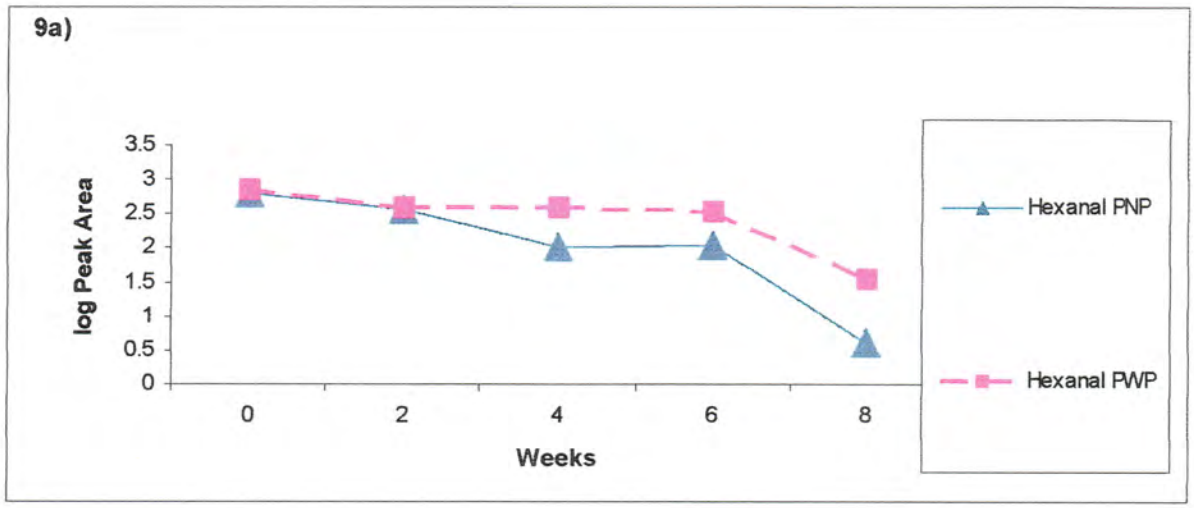
Raw: Unpasteurized cider

RWP: Unpasteurized cider with preservative

PNP : Pasteurized no preservative cider

PWP: Pasteurized with preservative cider

9a)


log Peak Area
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
Weeks
Hexanal PNP
Hexanal PWP

9b)

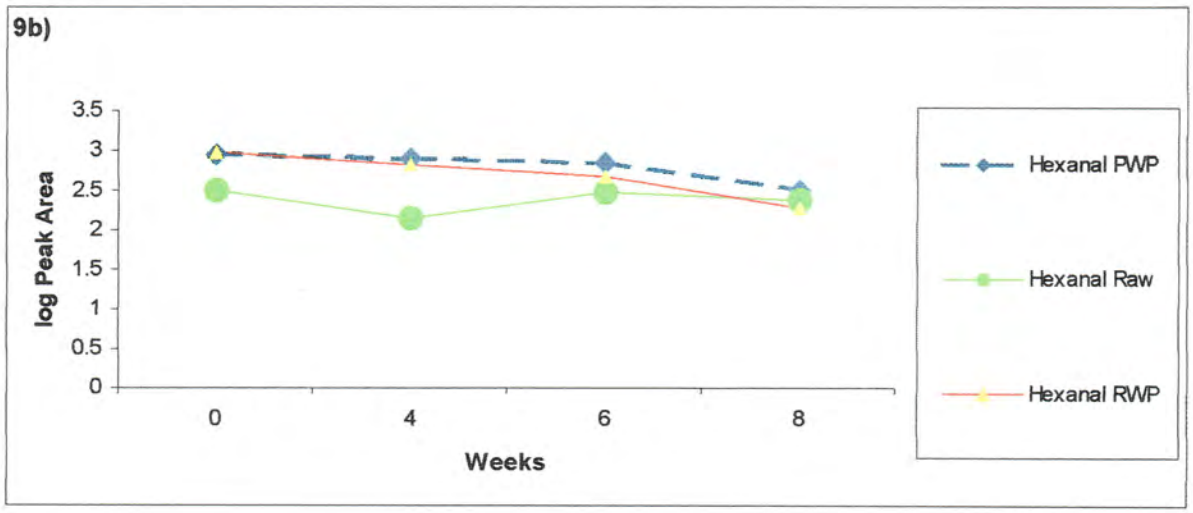

log Peak Area
3.5
3
2.5
2
1.5
1
0.5
0
0
4
6
8
Weeks
Hexanal PWP
Hexanal Raw
Hexanal RWP

Figure 10: Shows changes in estragole concentration during storage

Figure 10(a): Producer A (first sampling date)

Figure 10(b): Producer A (second sampling date)

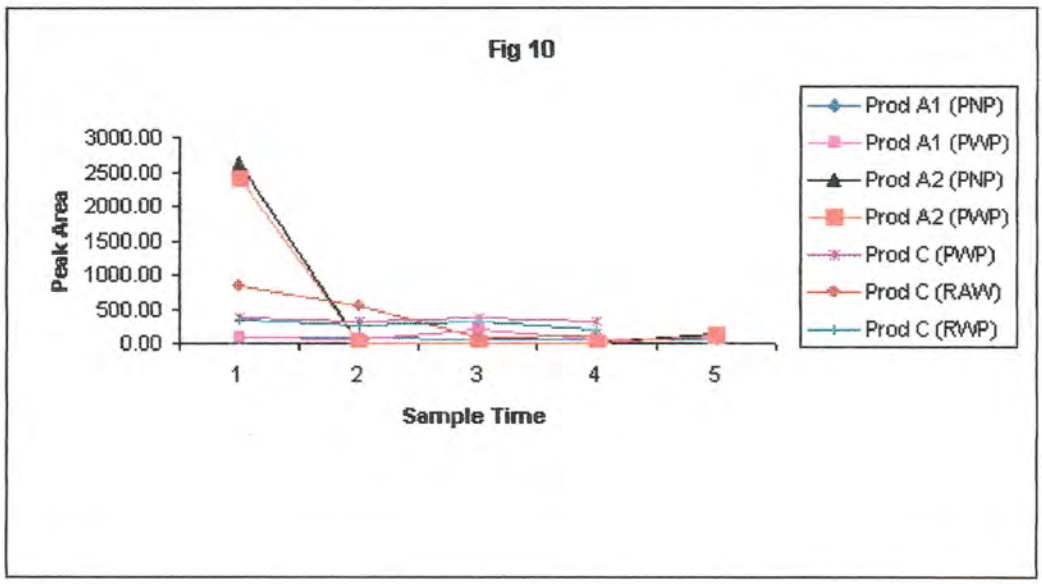
Figure 10(c): Producer C

Raw: Unpasteurized cider

RWP: Unpasteurized cider with preservative

PNP : Pasteurized no preservative cider

PWP: Pasteurized with preservative cider



Of the alcohols (data not shown), 1-octen-3-ol and octanol were found to be present in both Producer A and Producer C's samples but these were at low initial concentrations which further dropped to non-detectable levels for Producer A over storage, while for Producer C, they showed a slight increase in concentrations over time.

Poll (1983) reported that the ester content of stored apple juice samples decreases somewhat over time while aldehydes decrease much more markedly over time. Similar trends were noted in this study with both aldehydes and esters showing reductions in concentration. The aldehydes tended to drop off to nondetectable levels in almost all cases whereas only a few of the esters did so.

The changes in concentrations of the various compounds over storage were compared to the corresponding changes in aerobic bacteria, yeast and mold loads in the cider samples. Figures 11, 12 and 13 are representative of the relative changes seen in esters, aldehydes and other compounds, respectively, as compared to changes in microbial loads. Butyl acetate (Figure 11) concentrations in both the PNP and PWP ciders from Producer A did not seem to be affected by changes in aerobic bacteria or yeast and mold counts. Comparisons made for other esters from both Producers A and C also showed that on the whole ester concentrations during storage did not seem to be affected by microbial growth. Similarly it was observed no relationships existed between changes in aldehydes (hexanal-Figure 12) and other miscellaneous compounds (benzaldehyde-Figure 13) with microbial growth.

Figures 14 and 15 show the effect of treatment on the compounds, that did not show any change with time for cider from Producers A and C, respectively. From Figure 14 it can be seen that the addition of preservative increased the concentrations of all the compounds except for hexyl hexanoate, which was not affected by the treatment, and methyl-2-methyl

Figure 11: Shows changes in butyl acetate (representative ester) concentrations in cider samples from Producer A over storage as compared to changes in microbial loads

Figure 11 (a): Butyl acetate concentrations vs aerobic bacteria numbers in PNP cider

Figure 11 (b): Butyl acetate concentrations vs aerobic bacteria numbers in PWP cider

Figure 11 (c): Butyl acetate concentrations vs yeast and mold numbers in PNP cider

Figure 11 (d): Butyl acetate concentrations vs yeast and mold numbers in PNP cider

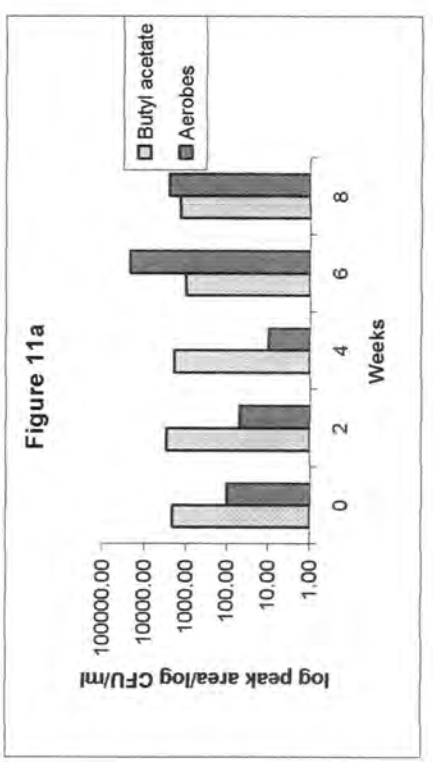


Figure 11a
Butyl acetate
Aerobes
log peak area/log CFU/ml
100000.00
10000.00
1000.00
100.00
10.00
1.00
0
2
4
6
8
Weeks

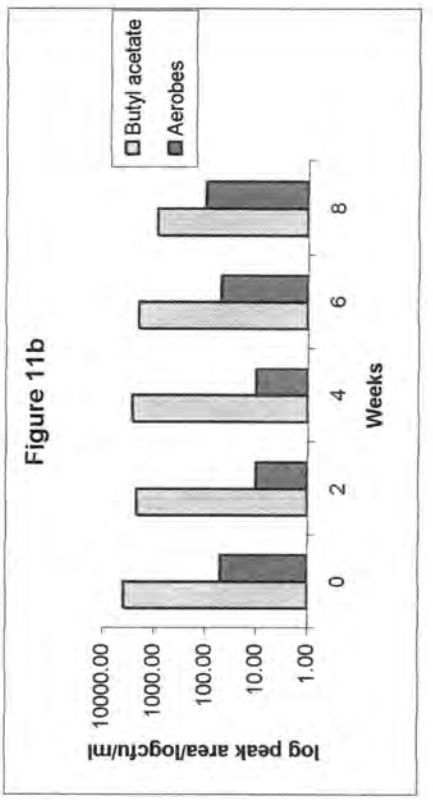


Figure 11b
Butyl acetate
Aerobes
log peak area/logcfu/ml
10000.00
1000.00
100.00
10.00
1.00
0
2
4
6
8
Weeks

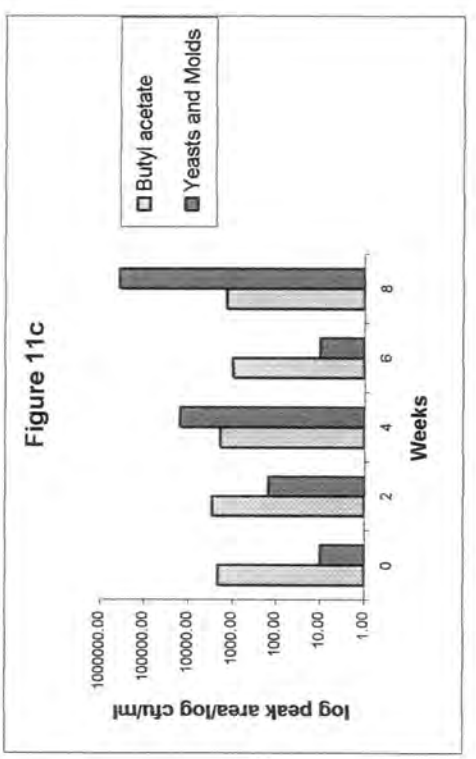


Figure 11c
Butyl acetate
Yeasts and Molds
log peak area/log cfu/ml
1000000.00
100000.00
10000.00
1000.00
100.00
10.00
1.00
0
2
4
6
8
Weeks

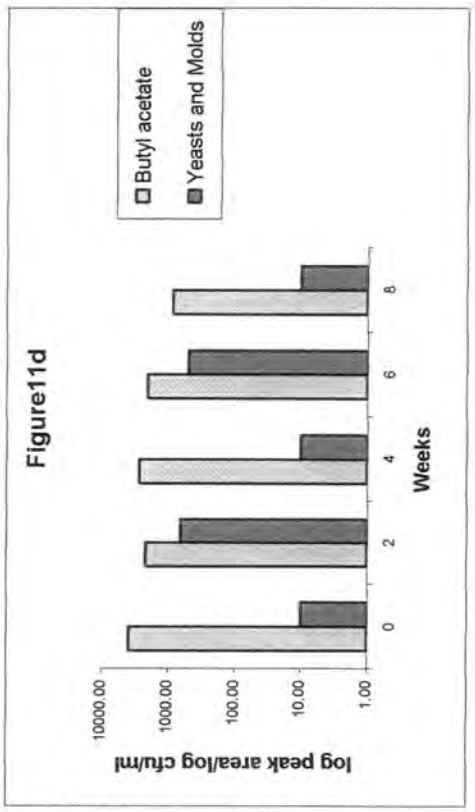


Figure11d
Butyl acetate
Yeasts and Molds
log peak area/log cfu/ml
10000.00
1000.00
100.00
10.00
1.00
0
2
4
6
8
Weeks

Figure 12: Shows changes in hexanal (representative aldehyde) concentrations in cider samples from Producer A over storage as compared to changes in microbial loads

Figure 12 (a): Hexanal concentrations vs aerobic bacteria numbers in PNP cider

Figure 12 (b): Hexanal concentrations vs aerobic bacteria numbers in PWP cider

Figure 12 (c): Hexanal concentrations vs yeast and mold numbers in PNP cider

Figure 12 (d): Hexanal concentrations vs yeast and mold numbers in PNP cider

Figure 12a

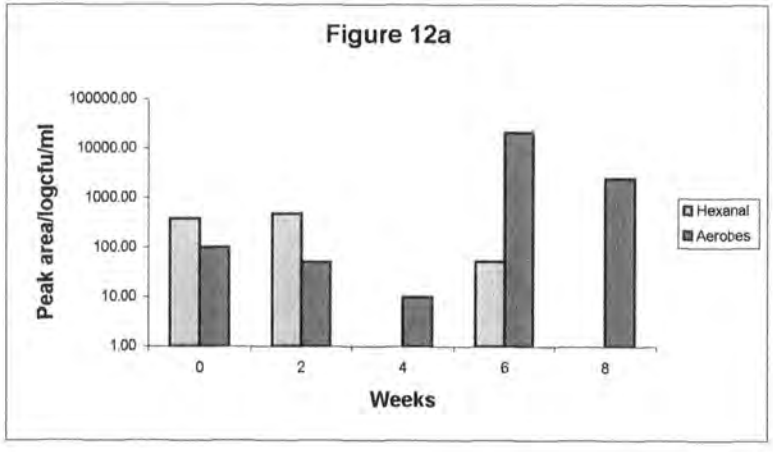


Figure12b

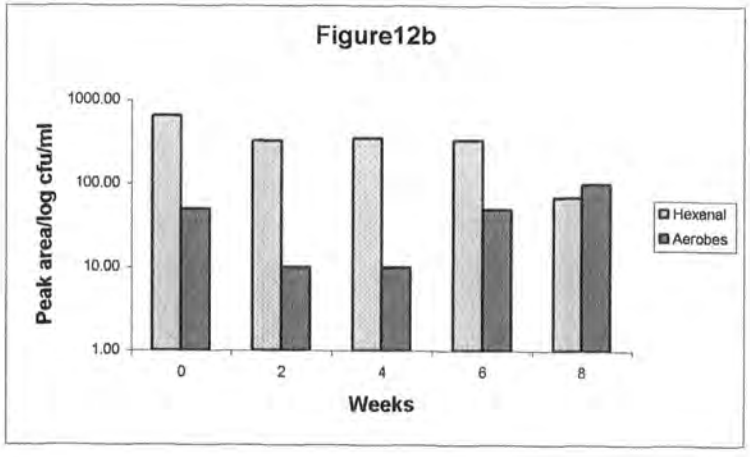


Figure 12c

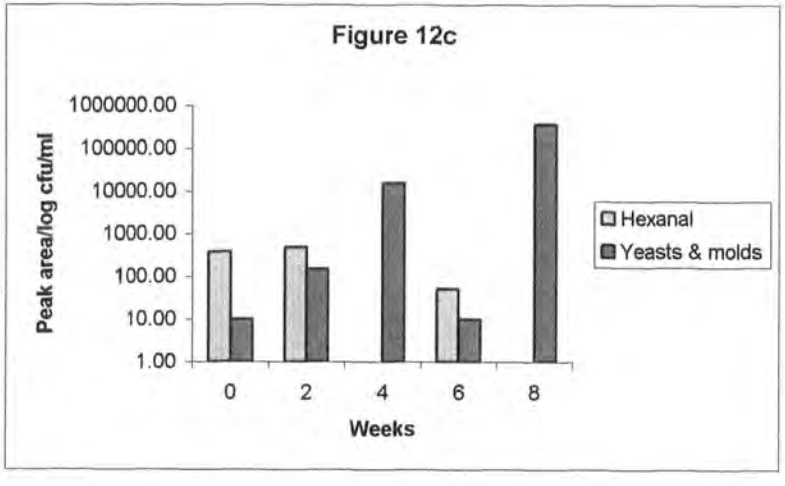


Figure 12d

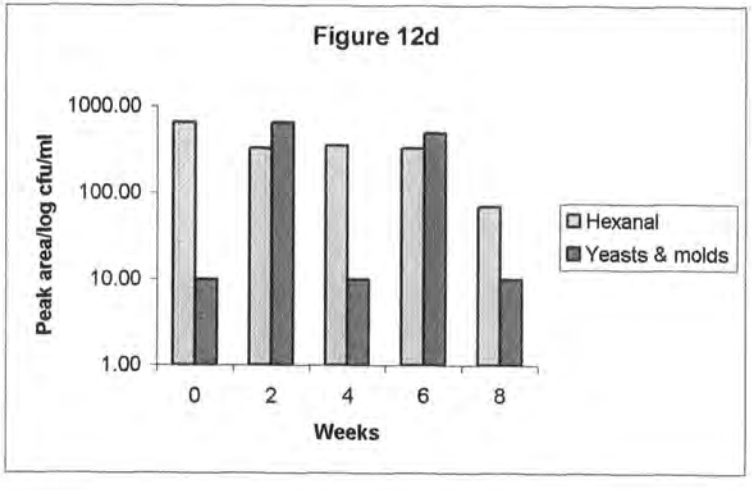


Figure 13: Shows changes in benzaldehyde (representative miscellaneous compound) concentrations in cider samples from Producer A over storage as compared to changes in microbial loads

Figure 13 (a): Benzaldehyde concentrations vs aerobic bacteria numbers in PNP cider

Figure 13 (b): Benzaldehyde concentrations vs aerobic bacteria numbers in PWP cider

Figure 13 (c): Benzaldehyde concentrations vs yeast and mold numbers in PNP cider

Figure 13 (d): Benzaldehyde concentrations vs yeast and mold numbers in PNP cider

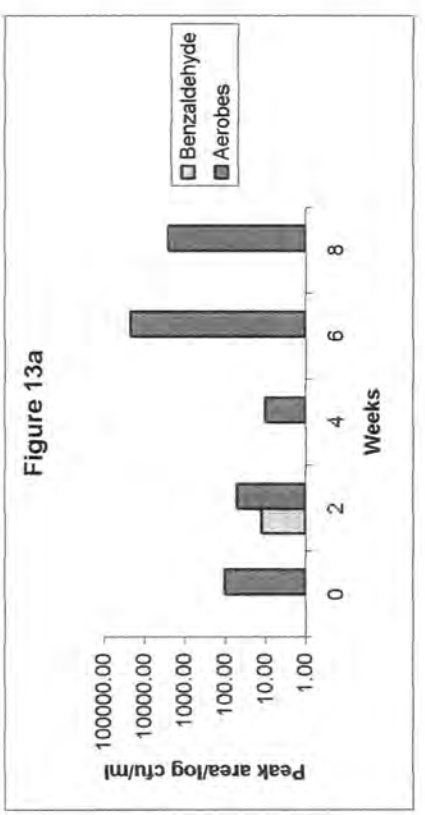


Figure 13a
Benzaldehyde
Aerobes
Peak area/log cfu/ml
100000.00
10000.00
1000.00
100.00
10.00
1.00
0
2
4
6
8
Weeks

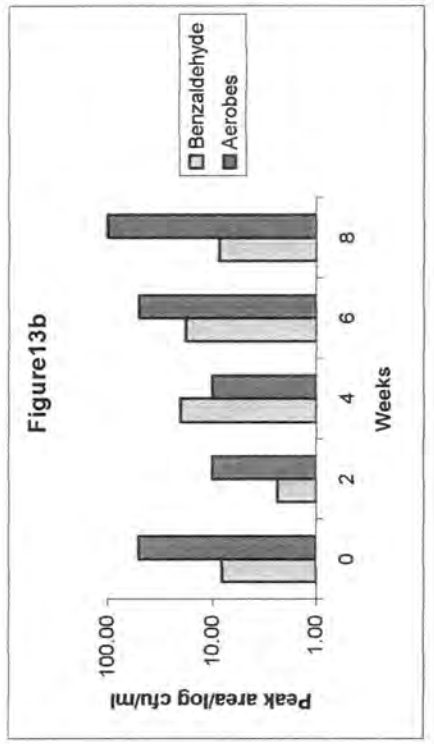


Figure13b
Benzaldehyde
Aerobes
Peak area/log cfu/ml
100.00
10.00
1.00
0
2
4
6
8
Weeks

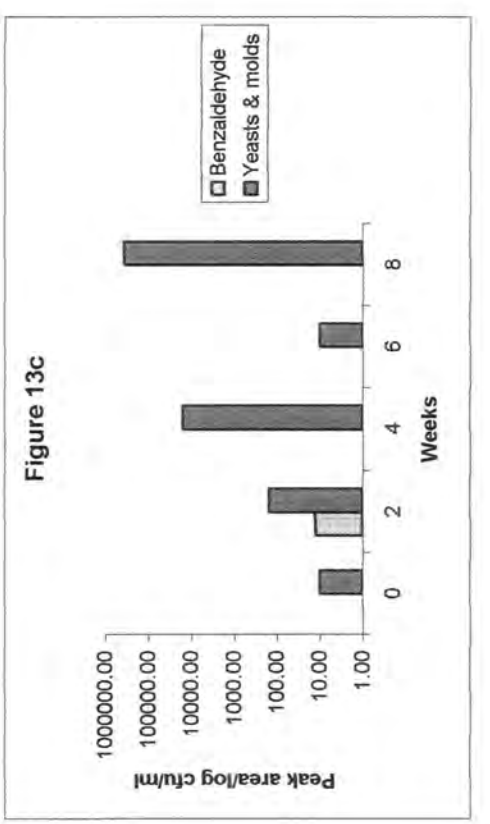


Figure 13c
Benzaldehyde
Yeasts & molds
Peak area/log cfu/ml
1000000.00
100000.00
10000.00
1000.00
100.00
10.00
1.00
0
2
4
6
8
Weeks

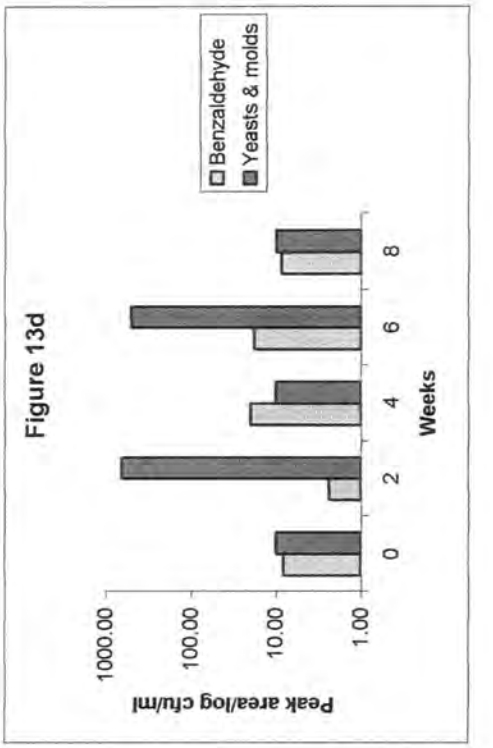


Figure 13d
Benzaldehyde
Yeasts & molds
Peak area/log cfu/ml
1000.00
100.00
10.00
1.00
0
2
4
6
8
Weeks

Figures 14 and 15: Effect of treatment on the flavor compounds in cider which did not show an interaction with time.

Figure 14: Shows this effect in Producer A's cider which was either PNP or PWP

Figure 15: Shows this effect in Porducer C's cider which was Raw, RWP and PWP

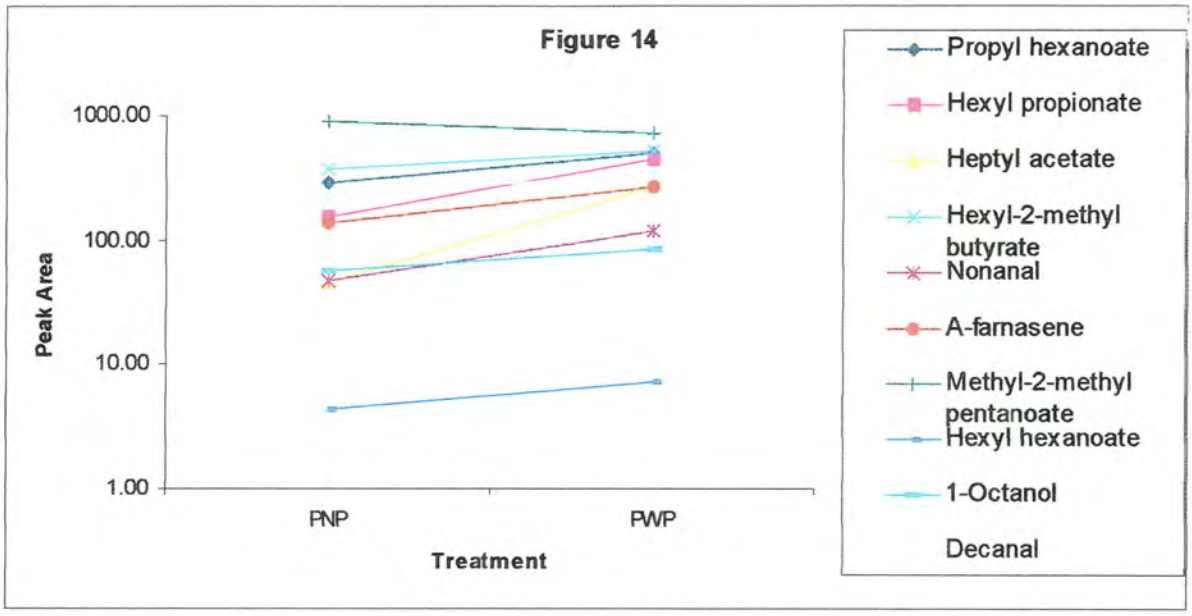


Figure 14
1000.00
100.00
10.00
1.00
Peak Area
PNP
PWP
Treatment
Propyl hexanoate
Hexyl propionate
Heptyl acetate
Hexyl-2-methyl butyrate
Nonanal
A-farnasene
Methyl-2-methyl pentanoate
Hexyl hexanoate
1-Octanol
Decanal

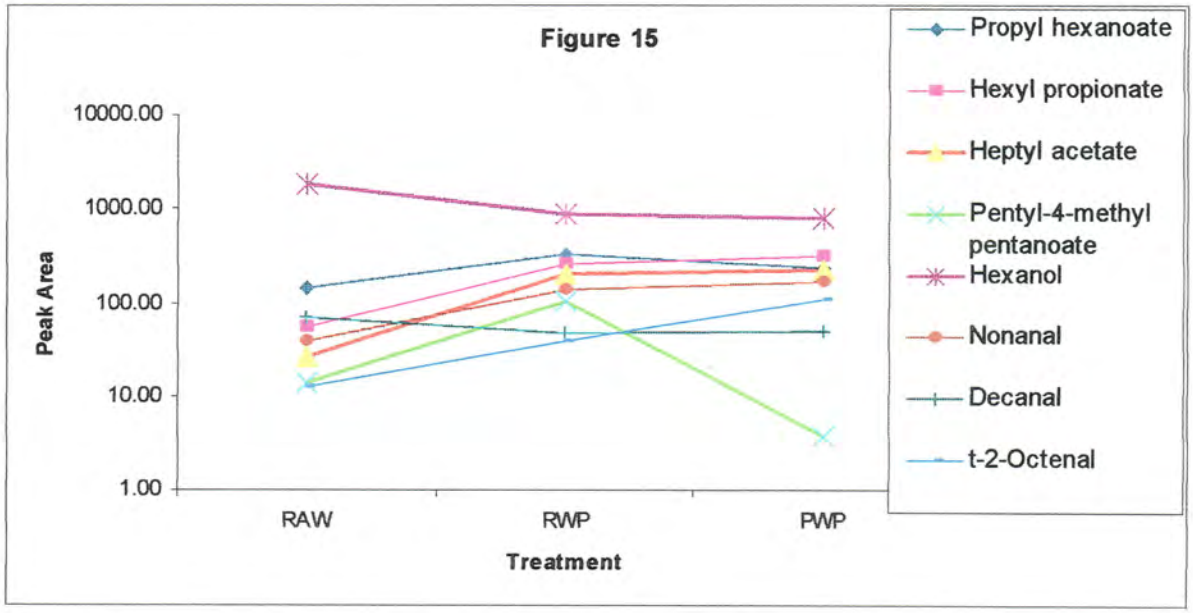


Figure 15
10000.00
1000.00
100.00
10.00
1.00
Peak Area
RAW
RWP
PWP
Treatment
Propyl hexanoate
Hexyl propionate
Heptyl acetate
Pentyl-4-methyl pentanoate
Hexanol
Nonanal
Decanal
t-2-Octenal

pentanoate, which showed a lower concentration in the PWP cider. For Producer C (Figure 15) it was seen that all compounds except hexanol increased in concentration upon addition of preservative. Also the differences between RWP and PWP ciders are not as large. Hexanol concentrations declined with each processing step; raw cider had more hexanol than pasteurized cider.

The addition of preservative to the cider affects the concentrations of the various compounds. It was observed that aldehydes were somehow protected from degradation over storage when preservative had been added to the cider whether it was pasteurized or not. Benzaldehyde also showed a similar pattern and was preserved longer in cider with preservative.

Nursten and Woolfe (1972) found several Maillard reaction products (furfural, benzaldehyde, 5-methyl-furfural) by boiling apple pieces to 100°C or more. Poll (1983) reported an increase in hydroxymethylfurfural concentration after storing apple juice (pasteurized at 90°C in a microwave oven) at 30°C for a year. In this study the only heat produced compound detected was benzaldehyde and that too was not present in very high concentrations. The reason for this may be that the heat treatment (boiling) used in the Nursten and Woolfe study and the storage time (1 year) and severe processing treatment used in Poll's study were more severe and longer as compared to those used in this study.

Conclusions

The results obtained from the storage study indicate that microorganisms are capable of increasing to quite high numbers during refrigerated storage. The counts in raw cider increased very rapidly and within two weeks of storage the product tended to give off a very strong off odor with visible sedimentation (i.e. visible separation of solids from the liquid portion of cider) and growth of a mold sheet on the surface. The pasteurized cider without preservative (PNP cider) kept for 4 to 6 weeks at which time it also showed sedimentation and mold growth. The pasteurized cider with preservative did not show such deterioration through 8 weeks of storage. This indicates that the shelf life of cider may be increased with the addition of preservatives to raw and pasteurized cider. Pasteurization, as expected, also helps in improving the shelf stability of cider as it reduces the majority of the microorganisms present in the raw cider. It was also observed that the pasteurization treatment of the cider is reflected in the refrigerated shelf life of the refrigerated cider; longer the holding time, slower the rate of growth of microorganisms during storage. A cider that starts out with a low microbial load will have a longer refrigerated shelf life.

The flavor analysis with the electronic nose showed differences in flavor patterns in cider obtained at different times, from different producers and subjected to different treatments. Addition of preservative to the cider helped keep the flavor patterns of the cider after two weeks almost to the patterns observed at time zero. This effect was seen in both unpasteurized and pasteurized ciders to which preservative was added. It may be due to more effective inhibition of microorganisms by the preservative or due to some effect that the preservative itself had on the flavor compounds. A reasonably good prediction ($R=0.70$) of coliform counts at various times during storage could be obtained from the response obtained

from sensors 1 and 19. A better predictive model would need more controlled conditions of cider processing and thus would be more useful for commercial juice/cider processors rather than small farm-based operations such as the ones that participated in the present survey.

Gas chromatographic analysis of the cider samples detected the presence of a number of typical cider compounds including esters and aldehydes. Most of these compounds were found to decrease during storage with almost all the aldehydes falling below detection limits quite rapidly. Although it was observed in the microbial storage study that yeasts and molds increased in the stored cider, and there was production of a strong alcoholic odor, only a few alcohols were detected by the GC. The GC analysis also showed that the addition of preservative helped in maintaining the levels of aldehydes and compounds such as benzaldehyde over storage. These compounds decreased considerably in the cider without preservatives whereas in cider with preservative these were generally found to remain constant with time. No relationship could be established between the microbial changes during storage and the changes observed in compound concentrations over time.

Overall, the study shows that addition of preservative to the cider alters the microbiological as well as the flavor composition of cider during storage. The mechanism by which it changes certain flavor responses still needs to be studied in greater detail. A predictive model for microbial loads based on flavor changes as detected by an electronic nose or the GC could be prove to be a very useful and time saving technique for quality control in the apple cider industry.

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OVERALL CONCLUSIONS

No *Escherichia coli* was found in any of the cider samples tested over both years of the survey. Although microbial loads in raw cider were high, pasteurization was effective in lowering the counts and also in keeping them low during storage. For the raw cider, coliform levels increased significantly for two of the producers in the second year. Reasons for this increase could be higher loads on apples either as they came in or while they were in storage, increased environmental contamination, or increased contamination from the equipment. The apples used for cider production had high counts and coliforms were also present on the apples as part of the natural microflora. But no *E. coli* were detected on the apples. Proper handling and storage of apples including periodic culling of rotten or damaged apples, washing and or sanitizing the apples prior to pressing, ensuring that drops are not included in the apples imported from other orchards are important points that the producers should keep in mind to reduce contamination.

The overall plant hygiene of the sites visited was found to be satisfactory although there were some manufacturing practices that needed improvement. Employee hygiene was an important focus area for all producers, as contamination by humans can occur at any point during cider manufacture. While bottling, special care should be taken, as any contamination at this time will be carried over to the consumers. To overcome the risk of contaminating the bottled product, it is advisable to have an automatic bottler installed in the facility.

Incorporation of HACCP plans in the plants was, to some degree, effective in improving the overall process safety of the plants. The producers accepted most of the specific suggestions regarding problem areas observed in the plant, and lower counts were observed on those areas. But these changes did not produce any significant drop in microbial

loads in the final cider. It is essential that the producers and their employees be given more thorough training in GMPs and the HACCP.

Pasteurization was equally effective for all three producers despite differences in the time-temperature combinations being used. It was seen that the pasteurization treatment had an effect on the microbial counts in stored cider with a longer shelf life being achieved by using a more severe treatment. It is necessary that the producers inspect, test and calibrate their pasteurizers regularly and also get them validated for meeting the 5-log reduction standard for the target pathogen. It is also important that producers ensure safe bottling, storage and handling practices of the final cider.

The storage study indicated that unpasteurized cider had a shelf life of 2 weeks while pasteurized cider could be stored for 4 to 8 weeks. Pasteurization not only decreased counts in the initial cider but also helped in maintaining low counts during storage. Addition of preservatives did not by itself result in decreased counts in the finished cider product but increased the shelf life of the pasteurized cider by at least 2 weeks.

Aroma analysis of the cider sample indicated that the composition of cider differed at different times during the season. This effect may be due to differences in the varieties, blends, maturity levels and storage length of the apples used. Differences were also observed in the pasteurized cider (with preservative) obtained from the different producers. Given the differences in pasteurization and processing techniques, these results are expected. Addition of preservative also produced an effect on the aroma and flavor patterns observed. The preservative helped in keeping concentrations of certain compounds such as aldehydes and benzaldehyde almost constant during storage.

No relationships could be established between the changes in flavor compounds and microbial loads. On the other hand, an R of 0.70 was obtained when changes in coliforms levels were correlated to changes in aroma compounds. The sensors giving this relationship were sensitive to changes in long-chain alcohols, aromatic compounds and esters. This could be helpful in quick determination of the coliforms levels in cider at a particular point in storage.

Based on this study, it can be concluded that the processors audited in the survey tried to produce as safe a product as possible. The microbial loads in the final cider were low with no *E. coli* being found in any of the samples tested. Pasteurization, addition of preservatives and following GMPs can help ensure that the final cider obtained is free from pathogens. These also help in lengthening the shelf life of cider by keeping microbial growth and spoilage under check.

The effect of preservative on microbial growth, aroma and flavor changes needs to be investigated in more detail as results from this study indicate that preservative addition affects all of these. Research to develop a predictive model for microbial quality of cider based on changes in aroma compounds is also recommended.

APPENDIX A:

(HACCP PLANS)

Survey Questionnaire

HARVESTING

1) Is manure fertilizer used in the orchard? YES NO

2) Is there a deer fence around the orchard? YES NO

3) Do you use drop apples in cider? YES NO

4) Are drop apples separated from tree picked apples? YES NO

5) Are apples from another supplier used? YES NO

If yes, are records kept documenting the source (drop vs tree-picked) of the supplier? YES NO

6) Do you provide hand wash stations and easily accessible toilets to field workers? YES NO

PROCESSING

7) Are outside windows or doors open during processing? YES NO

8) What is the water source used for processing? Well water Rural water Municipal water

9) Is your water source chlorinated? YES NO

10) Is your water source tested regularly for microbial counts? YES NO

Is your water source tested for chorine content? YES NO

11) Are apples stored inside a cooler? YES NO

What Temperature?_____

12) Are rotten apples discarded at any point during storage? YES NO

When_____

13) Are drop apples used in cider? YES NO

14) Are apples washed prior to processing? YES NO

15) Are brushes used on the apples? YES NO

16) Are apples sanitized prior to processing? YES NO

If yes, what is the sanitizer and concentration used?_____

17) Is an auger system used to dispose of pomace? YES NO

If yes, is the auger system enclosed? YES NO

If no, how is pomace removed? _____

18) Do you pasteurize? YES NO

If yes, what time and temperature do you use? _____

19) Is a preservative used? YES NO

What kind and concentration?_____

20) Is the cider filtered? YES NO

Through steel or mesh?_____

21) How long is cider allowed to settle before bottling?

1 Day 3 days 5 days 1 week more than 1 week

22) What temperature is cider held at during settling? _____

23) What type of bottling system is used? By hand Automatic

24) If bottling is done by hand, is the trough covered? YES NO

25) After bottling, approximately how long are bottles allowed to sit at room temperature before being transported to the cooler? _____

26) Are only new containers and caps used to bottle cider? YES NO

27) Is a date code or other method to identify lots used? YES NO

28) Is the "unpasteurized warning statement" used on the labels? YES NO

CLEANING/SANITATION

29) Is the processing equipment rinsed prior to startup? YES NO

30) Is processing equipment cleaned after each use? YES NO

31) Is a cleaner and/or sanitizer used on the equipment? YES NO

What kind and concentration?_____

32) Is a pest management system enforced? YES NO

Through what means?_____

EQUIPMENT

33) Are press cloths used that are specifically designed for cider production?

YES NO

Are the press cloths cleaned and/or sanitized after use? YES NO

34) What are the press racks made of? Food-grade plastic Wood Other

35) Are press racks made of properly maintained? YES NO

36) Are press racks and cloths stored off floors in a well-ventilated area?

YES NO

STANDARD OPERATING PROCEDURES

37) Has a written HACCP plan been developed? YES NO

If yes, are records maintained? YES NO

38) Are good manufacturing practices summarized and implemented?

YES NO

39) Have written standard operating procedures (SOPs) been developed? YES NO

40) Have you attended the Cider School? YES NO

41) Are you currently certified? YES NO

42) Have you been inspected yearly? YES NO

43) Where do you sell your cider?

On site Farmer's Market Retail Store Other

44) Has your cider sales volume increased or decreased over the past 2 years?

_____

45) Have you considered, or actually begun pasteurizing your cider in the past 2 years?

_____

Comments:

_____

_____

_____

_____

Good Manufacturing Practices

APPLICATION? YES	APPLICATION? NO	DESCRIPTION
		PERSONNEL
☐	☐	All employees wear hairnets in processing area
☐	☐	Jewelry in processing area?
☐	☐	Footwear/gloves required?
☐	☐	Smoking/eating in processing area?
		APPLES
☐	☐	Apples are free of visible filth and debris
☐	☐	Apples stored in clean, dry, area
☐	☐	Apples are cleaned and rinsed
☐	☐	Apples are sorted for bruises, cuts, and overall bad appearance
☐	☐	Are cores with rot/or worm damage used in cider
☐	☐	Rotten apples in contact with wholesome apples
☐	☐	Records kept for each lot of apples
		FACILITY
☐	☐	Good overall upkeep of orchard
☐	☐	Animal droppings on ground around orchard or facility
☐	☐	Good overall upkeep of facility
☐	☐	Proper usage of traps and bug lights (had fly strip hanging above pasteurizer)
☐	☐	Outside doors and windows properly covered
☐	☐	Proper disposal of waste/pomace (promptly removed and out of processing area)
☐	☐	Handwashing facility readily available
☐	☐	Toilets accessible and clean
		WAREHOUSE/STORAGE

☐ ☐ Boxes/pallets are stored away from the wall

☐ ☐ Boxes/pallets are raised above the ground

☐ ☐ Proper refrigeration/storage temperatures

☐ ☐ Proper storage of chemicals (chlorine based in dark, way from processing, etc)

☐ Animals prohibited from storage or processing area

☐ Are press racks and cloths stored off floors in well-ventilated area

HACCP

☐ ☐ Is a written HACCP plan used in facility

☐ ☐ Are records maintained for HACCP and/or GMP

☐ ☐ Have written SOP's been developed by the manufacturer

Are state GMP's and/or HACCP guidelines implemented

Pre-Operational Sanitization Standard Operating Procedures

1. Dry clean working area:
   - pick up and remove all large pieces of solid waste
   - put unused articles (packaging material, etc)away in the respective storage places

2. Pre-rinse all equipments and working surfaces with 120-140 ° F water.

3. Apply detergent to all places; reaching all framework bottoms, re-clean areas that have large buildup (funnel/chute bag). Allow detergent some reaction time (15-20 minutes) before rinsing .Do not let the detergent to dry on the surfaces.

4. Inspect for any missed areas.

5. Apply a pre-prepared sanitizer to all clean parts and surfaces.

6. Clean/rinse the floor.

7. Inspection by quality control manager and documentation.

Sanitization Standard Operating Procedures

SSOP#1

Detergent Preparation:

Frequency:

Before sanitizing equipment and working surfaces.

Person responsible:

QA Manager

Purpose:

To ensure that the detergent is food grade and used at the recommended levels.

Procedure:

Prepare the detergent before start of sanitization.

Carefully meter the amount of detergent and water to be added for the preparation of detergent mix. Ensure proper mixing of the two.

SSOP#2

Sanitizer Preparation:

Frequency:

Before sanitizing equipment and working surfaces.

Person responsible:

QA Manager

Purpose:

To ensure that the chlorine levels used for sanitization are below the recommended levels (200 ppm).

Procedure:

Prepare the chlorine mix before start of sanitization.

Carefully meter the amount of chlorine and water to be added for the preparation of sanitizer mix. Ensure proper mixing of the two.

Documentation for Sanitization Standard Operating Procedures

SSOP	Date	Time	Inspected by	Corrective Actions	Comments
Detergent preparation					
Sanitizer preparation					

Signature: ----------

Name & Title: ----------

SOP#1

Temperature Control of Coolers :

Frequency:

Daily

Person Responsible:

QA Manager

Purpose:

To ensure that the temperature of the coolers is 40 °F or below.

Procedure:

Monitor the coolers daily using a temperature sensor.

If the cooler temperature is over 40 °F for more than 2 hours, take action to reduce the temperature to an acceptable level.

Check temperature manually if the temperature sensor is not working.

SOP#2

Time and Temperature Control of Pasteurization:

Frequency:

Continuously while pasteurizing/bottling.

Person Responsible:

Pasteurizer Operator

Purpose:

To ensure that the correct time and temperature of pasteurization are achieved for pathogen destruction in cider.

Procedure:

Monitor the temperature of cider at exit of holding tube with the help of a temperature sensor.

Manually record temperature every 15 minutes as a comparison record.

If correct time-temperature treatment is not achieved, an alarm should go off and the cider should be diverted through a flow valve and repasteurized.

SOP#3

Preservative Addition:

Frequency:

Whenever preservative is to be added.

Person Responsible:

QA Manager

Purpose:

To ensure that the chemical limits for the level of preservative are not exceeded and that the preservative is uniformly dispersed in the cider.

Procedure:

Monitor and calibrate the weighing scales regularly.

After addition of preservative, disperse it thoroughly in the tank with the help of stirrers.

Documentation for Standard Operating Procedures

SOP	Date	Time	Inspected by	Corrective Actions	Comments
Temperature control of coolers					
Time-temperature control of pasteurization					
Preservative addition					

Signature: ----------

Name & Title: ----------

HACCP Plan No. 1 Product Category: 100% Apple Cider (Producer A)

Product Description

1. **Common name:**

100% Apple Cider

2. **How is it to be used?**

Consumed as purchased (ready-to-drink)

3. **Type of package?**

Plastic bottles (high density polyethylene)

4. **Length of shelf life, at what temperature?**

Approx. 40 days if not opened
Maximum acceptable storage temperature 10 C
Recommended 7 C

5. **Where will it be sold?**

Retail. Wholesale to Fareway

6. **Labeling instructions?**

Label should say "Keep Cold"
Ingredients, nutrition facts, net content, "Use by" / "Sell by" and Date of Production are Recommended
Warning label required for unpasteurised product.

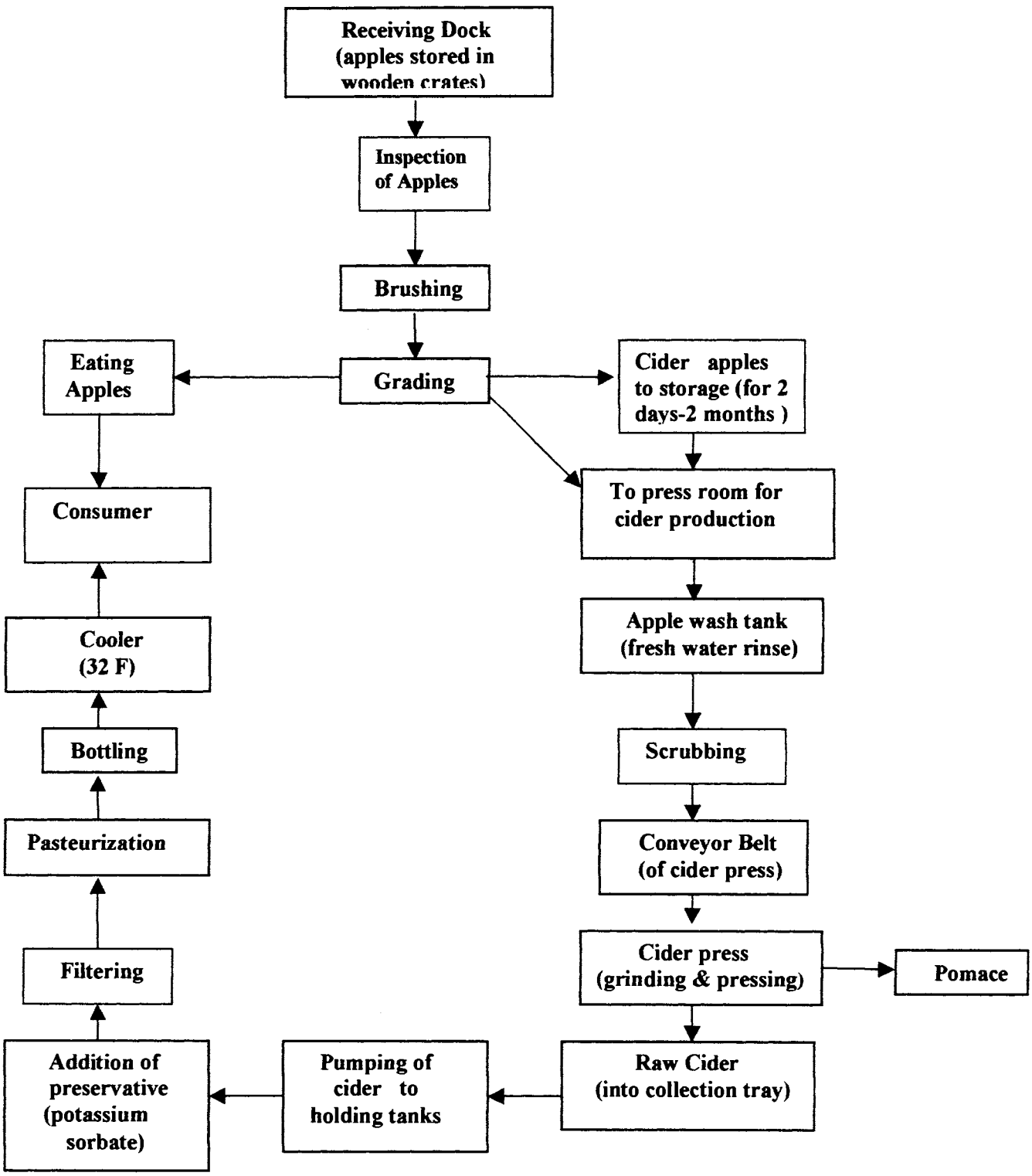
7. **Is special distribution control needed?**

Lot code or date of production needed for traceability.
Distribution and storage under acceptable refrigeration
(maximum recommended temperature 10 C)
Use of temperature monitoring devices recommended

Approved by: _____ **Date approved:** _____

List product ingredients

PRODUCER A: FLOW DIAGRAM FOR APPLE CIDER PRODUCTION



HACCP Plan No._____ Process Category_____

Product: 100% Pasteurized Apple Cider

Hazard Analysis and Identification of Critical Control Points

Processing Step		Potential Hazards Introduced	What control Measures Can be Applied to Prevent the Hazard?		Is the potential safety hazard significant & reasonably likely to occur?	CCP #
Raw ingredients (Apples)	B	Pathogens	-Inspection of apples (own & suppliers')	B	YES	1
Receiving and Inspection	P	Wood	-No drops -Grate separates large debris	P	NO	
	C	Pesticides	-Remove visibly spoiled & damaged apples	C	NO	
Storage of Apples	B	Pathogens Mold Growth Cross-contamination of good & apples	Storage inside cooler 32-42 F, no outside or uncovered storage Re-inspection weekly to Remove bad apples	B C	YES NO	2
	C	None				
	P	Rodents, Insect	Pest Control (GMP)	P	NO	
Brushing & Final Sorting	B	None	Good employee hygiene And GMP's	B	NO	
	C	None	Removal of visibly Spoiled and damaged apples	C	NO	
	P	None		P	NO	
Grinding and Pressing	B	Pathogens	Washing and sanitization of chute bag before start of operation	B	YES	3
	C	None		C	NO	
	P	Metal debris fr machine	Filter cider	P	NO	
Pumping cider to cooled settling ta	B	None	-Clean tubing and tank	B	NO	
	C	None		C	NO	
	P	None		P	NO	

Processing Step		Potential Hazards Introduced	What control measures can be applied to prevent the hazard?		Is the potential safety hazard significant & reasonably likely to occur?	CCP #
Add preservative (Pot. Sorbate)	B	None		B	NO	
	C	Chemical limits exceeded	-Good record keeping with SOP, monitoring & maintenance of	C	Yes	4
	P	None	weighing scales	P	NO	
Filtering (cheese cloth)	B	None	-Wash with sanitizer before use	B	NO	
	C	None	-Check filter daily for visible signs of damage	C	NO	
	P	None	-Replace as necessary	P	NO	
Pasteurization	B	Pathogen destruction	-Flash pasteurization at 161-165 F for 2 seconds	B	YES	5
	C	None		B	NO	
	P	None		P	NO	
Bottling	B	Pathogens	-Trough is covered or enclosed	B	YES	6
	C	Introduction of chemical hazard	-Capping performed in timely manner-containers not allowed to sit in open environment longer than 5 minutes	C	NO	
	P	Introduction of physical hazard	-Visual inspection of bottles for foreign materials -Checked for proper sealing (SOP)	P	YES	
Cooling	B	Improper cooling allowing pathog growth	-Bottles are sent to cooler immediately after sealing	B	NO	
	C	None	-Monitor cooler temperature (35-42 F)	C	NO	
	P	None		P	NO	

Approved by:_____ Date approved:_____

Critical Control Point (CCP)	Hazard(s) to be addressed in HACCP plan	Critical limits For each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 1 (B) Receiving and Inspection	Pathogenic Bacteria (prevention & destruction)	-No drops -No domestic manure in orchards -Removal of rotten or spoiled apples	Apple harvesting and shipments	Visual	Each harvest or shipment	Receiving Manager	No drops policy Reject drops
CCP 2 (B) (P) Storage of Apples	(B) Pathogens and mold growth	Storage inside Cooler (35-42 F)	Cooler temperature maintained	Temperature gauge-visual	Daily	Manager	Correct cooling problem, recalibrate and monitor hourly for next 6 hours. Move to other cooler if temperature has not been maintained within 6 hours.
	(P) Rodents and Insects	No outside or uncovered storage	Spoilt apples	Visual	Weekly	Manager	Discard any spoilt apples

Critical Control Point (CCP)	Hazard(s) to be addressed in HACCP plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 3 (B) Grinding and Pressing	Pathogenic bacteria (prevention & destruction)	Change chute bag after 4 processing operations Before use,make sure that bag is clean and does not have any residual debris from previous use. After use wash the bag with a sanitizer	Chute bag	Visual	After 4 processing operations Before each processing operation	Manager Operator	Change bag Regularly Keep an extra bag for use in case of presence of debris or evidence of improper sanitization.

Critical Control Point (CCP)	Hazard(s) to be addressed in HACCP plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 4 (B) Pasteurization	Pathogenic Bacteria (destruction)	Product target Time/temp : 163 F for 2 sec. Minimum temp. 161 F-alarm will sound	Temperature at exit of holding tube Temp. will also be recorded manually every 15 minutes as a Comparison Record	Temperature recorder at end of hold tube,low temperature manual divert valve	Continuous Recording	Pasteurizer Operator	Cider will be manually diverted if temp at end of holding tube is low and will be repasteurized -If divert valve doesn't work; production will be stopped and the portion of the cider in the holding tube and holding tank will be re-pasteurized.

Critical Control Point (CCP)	Hazard(s) To be Addressed In HACCP Plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 4 (B) Pasteurization (continued)							-Verification of temp. gauge & alarm system to ensure deviation does not occur again
			Seals on pumps and tubing and flow rate gauge	Visual check	At start up And once during processing	Pasteurizer Operator	-Questionable cider may be tested for pathogens before shipping -If seals are broken or gauge is not working properly;stop processing if necessary, recalibrate pump and/or reseal.

Critical Control Point (CCP)	Hazard(s) to be Addressed in the HACCP plan	Critical Limits For each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 5 (B) (P) Bottling	(B) Pathogens (P) environmental Debris	Cap within 5 minutes of filling	Length of time containers are exposed to open air environment	Monitored by timing device set at 5 min intervals	Every 5 minutes	Well trained employee	Dispose of bottled cider
		Proper seal achieved	Proper sealing of container	Visual	Every container	Operator	Reseal if necessary
		No visible debris	Physical contamination	Visual	Every container	Operator	Dispose of bottled cider
		Bottles sent to cooler in a timely manner	Length of time bottles are setting at room temperature	Time	Every 15 minutes	Operator	Dispose of bottled cider

Verification Activities	Record-Keeping Procedures
1. Maintenance calibrates divert valves & alarm system weekly 2. Verification of thermometers daily before start-up 3. Chute bag is changed at regular intervals and inspected before start of operation each time 4. Holding tube length and diameter are tested once per season with salt tracer test to validate the residence time 5.QA manager will review and initial records daily 6.QA checks pump flow rate gauge daily and enters data in pasteurization log	-Pasteurization log which includes temperature data -Calibration records for the thermometers, divert valves, alarm system, etc -QA flow verification log (pump flow rate info) -Corrective action logs

Verification:

Short term: # 1,2,3,5,6
Long term: # 4

Verification of overall process will also include microbial testing for coliforms / *E.coli* (samples will be taken from every batch)

HACCP Plan No. 1 Product Category: 100% Apple Cider (Producer B)

Product Description

1. **Common name:**

Pasteurized 100% Apple Cider

2. **How is it to be used?**

Consumed as purchased (ready-to-drink)

3. **Type of package?**

Plastic bottles (high density polyethylene)

4. **Length of shelf life, at what temperature?**

Approx. 40 days if not opened
Maximum acceptable storage temperature 40 ° F
Recommended < 38° F

5. **Where will it be sold?**

Retail

6. **Labeling instructions?**

Label should say "Keep Cold"
Ingredients, nutrition facts, net content, "Use by" / "Sell by" and Date of Production are Recommended
Warning label required for unpasteurized product.

7. **Is special distribution control needed?**

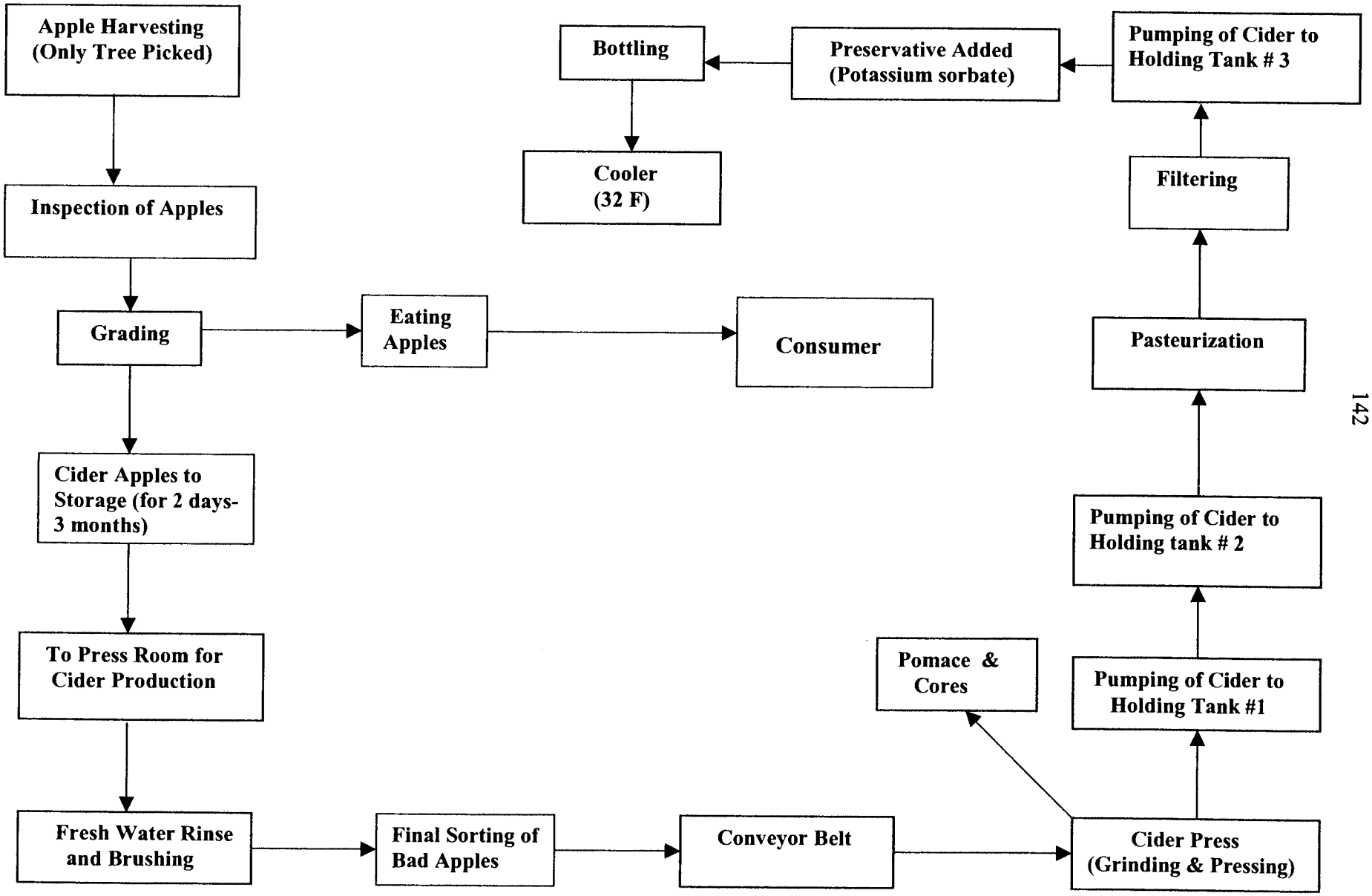
Lot code or date of production needed for traceability.
Distribution and storage under acceptable refrigeration (maximum recommended temperature 40° F)
Use of temperature monitoring devices recommended

Approved by: _____ **Date approved:** _____

List product ingredients

Apples. Potassium sorbate.

PRODUCER B: FLOW DIAGRAM FOR APPLE CIDER PRODUCTION



HACCP Plan No._____ Process Category_____

Product : Pasteurized 100% Apple Cider

Hazard Analysis and Identification of Critical Control Points

Processing Step		Potential hazards introduced	What control measures can be applied to prevent the hazard?		Is the potential safety hazard significant and reasonably likely to occur?	CCP #
Raw ingredients (Apples) Receiving & Inspection	B	Pathogens	-Inspection of apples (own & suppliers')	B	Yes	1
	P	Wood	-No drops			
			-Certified supplier audits	P	NO	
			-Grate separates large			
	C	Pesticides	debris			
			-Remove visibly spoiled & damaged apples	C	NO	
Fresh Water Rinse & Waxing	B	Pathogens, Parasites	-Potable water -Guaranteed testing by	B	NO	
	C	Metals, pesticides, Nitrites	municipal utilities -Food grade wax only	C	NO	
	P	None		P	NO	
Storage of Apples	B	Pathogens Mold Growth Cross-contamin tion from bad to good apples	-Storage inside cooler 35 to 40° F, no outside or uncovered storage -Re-inspection weekly to remove bad apples	B	Yes	2
				C	NO	
	C	None	-Pest Control (GMP)			
	P	Rodents, Insect		P	Yes	
Final Sorting	B	None	Good employee hygiene & GMP's	B	NO	
	C	None	Removal of visibly Spoiled and damaged	C	NO	
	P	None	apples	P	NO	
Grinding and Pressing	B	Pathogens from dirty equipment	-Washing & sanitization of cider press & before start of operation & after	B	Yes	3
	C	Cleaning residu	use (SOP)	C	NO	
	P	Metal debris fro machine	-Filter cider	P	NO	

Processing Step		**Potential hazards introduced**	**What control measures can be applied to prevent the hazard?**		**Is the potential safety hazard significant & reasonably likely to occur?**	**CCP#**
Pumping cider to press	B	-Contamination from equipment	-Clean tubing & press (SOP)	B	NO	
	C	-Cleaning residues	-Properly rinse equipment before & after use	C	NO	
	P	None		P	NO	
Pumping Cider to Holding tanks	B	-Contamination from equipment	- Ensure clean tubing (SOP)	B	NO	
	C	-Cleaning residues	-Properly rinse equipment before & after use	C	NO	
	P	None		P	NO	
Add preservative (Pot. Sorbate)	B	None		B	NO	
	C	Chemical limits exceeded	-Good record keeping with SOP, monitoring & maintenance of weighing scales	C	Yes	4
	P	None		P	NO	
Filtering	B	None	-Check filter daily for visible signs of damage	B	NO	
	C	None		C	NO	
	P	None	-Replace as necessary	P	NO	
Pasteurization	B	Pathogen destruction	-Flash pasteurization at 162° F for 11 seconds	B	YES	5
	C	None		B	NO	
	P	None		P	NO	
Bottling	B	Pathogens	-Bottling tube is clean & sanitized (SOP)	B	YES	6
	C	Introduction of chemical hazards	-Capping performed in timely manner-containers not allowed to sit in open environment longer than 5 minutes	C	NO	
	P	Introduction of physical hazards	-Visual inspection of bottles for foreign materials	P	YES	

Processing Step		**Potential Hazards Introduced**	**What control Measures Can be Applied to Prevent the Hazard?**		**Is the potential safety hazard significant and reasonably likely to o**	**CCP#**
Cooling	B	Improper cooling allowing pathogen growth	-Bottles are sent to cooler immediately after sealing	B	NO	
	C	None	-Monitor cooler temperature (35-40° F)	C	NO	
	P	None		P	NO	
Storage	B	Improper Refrigeration temp promotes bacterial growth	-Monitor storage and transportation temperature (35-40° F)	B	NO	
	C	None		C	NO	
	P	None		P	NO	

Approved by:_____ Date approved:_____

Critical Control Point (CCP)	Hazard(s) to be addressed in HACCP plan	Critical limits For each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 1 (B) Receiving and Inspection	Pathogenic Bacteria (prevention & destruction)	-No drops -No domestic manure in orchards -Removal of rotten or spoiled apples	Apple harvesting and shipments	Visual	Each harvest or shipment	Receiving Manager	No drops policy Reject drops
CCP 2 (B) (P) Storage of Apples	(B) Pathogens and mold growth	Storage inside Cooler (35-42 F)	Cooler temperatu maintaine	Temperatur gauge-visua	Daily	Manager	Correct cooling problem, recalibrate and monitor hourly for next 6 hours Move to other cooler if temperature has not been maintained with 6 hours.
	(P) Rodents an Insects	No outside or uncovered storage	Spoilt apples	Visual	Weekly	Manager	Discard any spoilt apples

Critical Control Point (CCP)	Hazard(s) to b addressed in HACCP plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 3 (B) Grinding and Pressing	Pathogenic bacteria (prevention & destruction)	Change chute bag after 4 processing operations Before use,make sure that bag is clean and does not have any residual debris from previous use. After use wash the bag with a sanitizer	Chute bag	Visual	After 4 processing operations Before each processing operation	Manager Operator	Change bag Regularly Keep an extra b for use in case o presence of debi or evidence of improper sanitization.
CCP 4 (C) Preservative Addition	Preservative concentration per bottle of cider	Preservative is fully dissolved before bottling	Dissolved potassium sorbate granules	Visual	Every contai	Operator	Pour cider back into tank and fil after preservativ is fully dissolve

Critical Control Point (CCP)	Hazard(s) to be addressed in HACCP plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 5 (B) Pasteurization	Pathogenic Bacteria (Destruction)	Product target Time/Temp: 162 ° F for 11 sec. Minimum temp. 161 F-alarm will sound	Temperature of cider at exit of holding tube Temp. will also be recorded manually every 15 minutes as a Comparison Record	-Temperature recorder at end of hold tube -Low temperature manual divert valve	Continuous Recording	Pasteurizer Operator	Cider will be manually diverted if temp at end of holding tube is low and will be re-pasteurized -If divert valve doesn't work; production will be stopped and the portion of the cider in the holding tube and holding tank will be re-pasteurized.

Critical Control Point (CCP)	Hazard(s) To be Addressed In HACCP Plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 5 (B) Pasteurization (continued)							-Verification of temp. gauge & alarm system to ensure deviation does not occur again
			Seals on pumps and tubing and flow rate gauge	Visual check	At start up & once during processing	Pasteurizer Operator	-Questionable cider may be tested for pathogens before shipping -If seals are broken or gauge is not working properly; stop processing if necessary, recalibrate pump and/or reseal.

Critical Control Point (CCP)	Hazard(s) to be Addressed in the HACCP plan	Critical Limits For each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 6 (B) (P) Bottling	(B) Pathogens	-Cap within 5 minutes of filling	-Length of time bottles are exposed to environment	Monitored by timing device set at 5 min intervals	Every 5 minutes and every container	Operator	Dispose of bottled cider
		-Proper seal achieved	-Proper sealing of container	Visual	Every container	Operator	Reseal if necessary
		-Bottles sent to cooler in a timely manner	-Length of time bottles are setting at room temp.	Time	Get estimate time for every pallet of bottled cider	Operator	Dispose of bottled cider if it has set at room temp for more than 1 hr
	(P) Environmental debris	-No visible debris	-Physical contamination	Visual	Every container	Operator	Dispose of bottled cider

Verification Activities	Record-Keeping Procedures
CCP#1: Pasteurization 1. Maintenance calibrates divert valves & alarm system weekly 2. Verification of thermometers daily before start-up 4. Holding tube length and diameter are tested once per season with salt tracer test to validate the residence time 5.QA manager will review and initial records daily 6.QA checks pump flow rate gauge daily and enters data in pasteurization log	-Pasteurization log which includes temperature data -Calibration records for the thermometers, divert valves, alarm system, etc -QA flow verification log (pump flow rate info) -Corrective action logs
CCP#2: Bottling 1. Ensure bottles are capped and sent to cooler in a timely manner. 2.QA manager reviews and initials records on a weekly basis.	-Log verifying bottling equipment and flavoring equipment was cleaned and sanitized before use -Bottles capped timely and sealing documetation -Log documenting discarded product due to biological and/or physical contamination -Corrective action logs

Verification:
Short term: # 1,2,3,5,6
Long term: # 4

Verification of overall process will also include microbial testing for coliforms / *E.coli* (samples will be taken from every batch)
Corrective actions will also be recorded and reviwed.

HACCP Plan No. 1 Product Category: 100% Apple Cider (Producer C)

Product Description

1. **Common name:**

Pasteurized 100% Apple Cider

2. **How is it to be used?**

Consumed as purchased (ready-to-drink)

3. **Type of package?**

Plastic bottles (high density polyethylene)

4. **Length of shelf life, at what temperature?**

Approx. 60 days if not opened
Maximum acceptable storage temperature 40 ° F
Recommended < 38° F

5. **Where will it be sold?**

Retail, Wholesale (In Iowa)

6. **Labeling instructions?**

Label should say "Keep Cold"
Ingredients, nutrition facts, net content, "Use by" / "Sell by" and Date of Production are Recommended
Warning label required for unpasteurized product.

7.**Is special distribution control needed?**

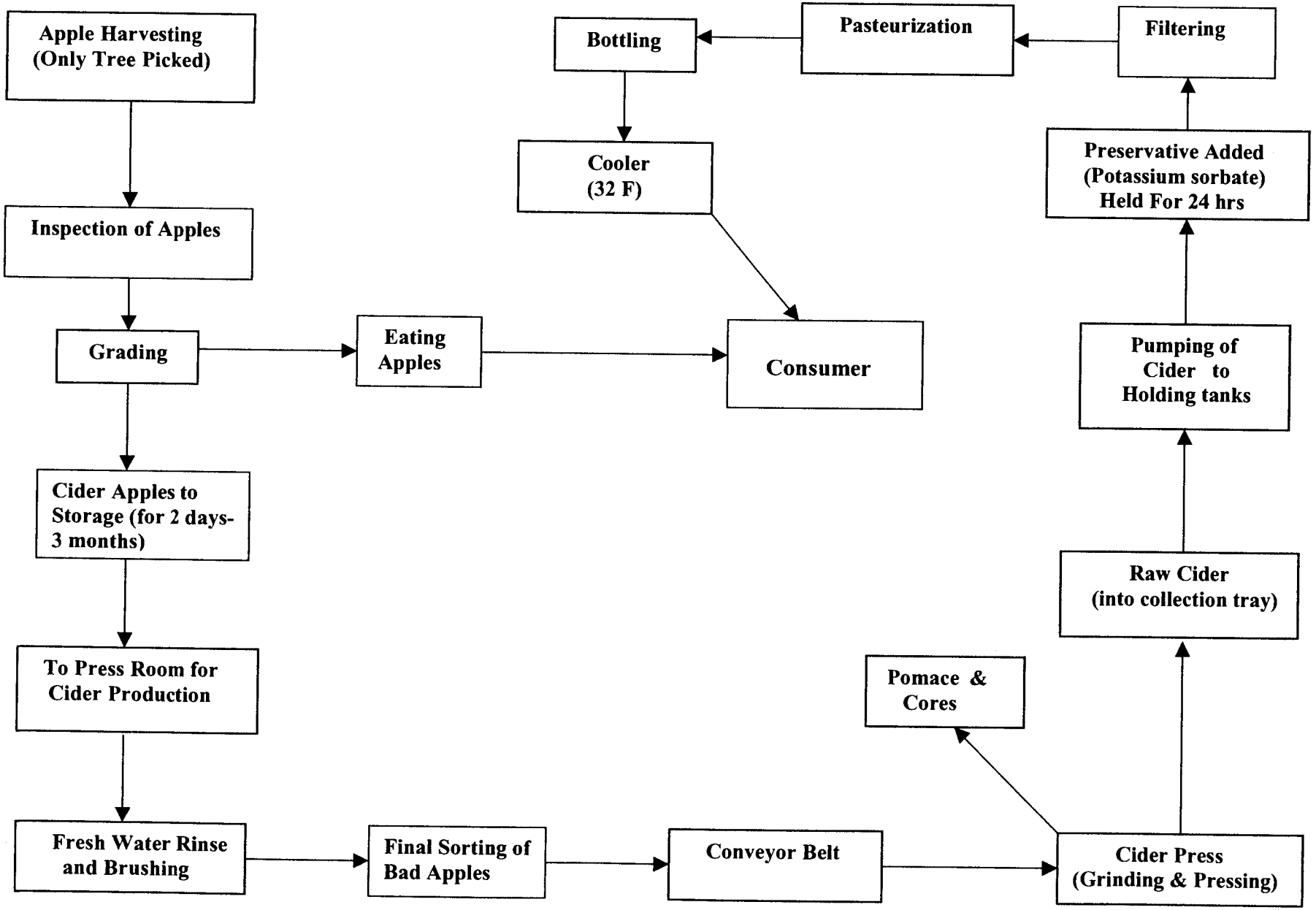
Lot code or date of production needed for traceability.
Distribution and storage under acceptable refrigeration (maximum recommended temperature 40° F)
Use of temperature monitoring devices recommended

Approved by: _____ **Date approved:** _____

List product ingredients

Apples. Potassium sorbate.

PRODUCER C: FLOW DIAGRAM FOR APPLE CIDER PRODUCTION



HACCP Plan No._____ Process Category_____

Product:100% Pasteurized Apple Cider

Hazard Analysis and Identification of Critical Control Points

Processing Step		**Potential hazards introduced**	**What control measures can be applied to prevent the hazard?**		**Is the potential safety hazard significant & likely to occur?**	**CC # **
Raw ingredients (Apples) Receiving and Inspection	B	Pathogens	-Inspection of apples (own & suppliers')	B	Yes	1
	P	Wood	-No drops		NO	
			-Certified supplier audits	P		
			-Grate separates large			
	C	Pesticides	debris		NO	
			-Remove visibly spoiled & damaged apples	C		
Storage of Apples	B	Pathogens Mold Growth Cross-contamina-	Storage inside cooler 35-40 F, no outside or uncovered storage	B	Yes	2
		tion from bad to good apples	Re-inspection weekly to Remove bad apples	C	NO	
	C	None				
	P	Rodents, Insects	Pest Control (GMP)	P	Yes	
Brushing & Final Sorting	B	None	Good employee hygiene And GMP's	B	NO	
	C	None	Removal of visibly Spoiled and damaged	C	NO	
	P	None	apples	P	NO	
Fresh water rinse and Brushing	B	Pathogens,parasit	-Potable water	B	NO	
	C	Metals,pesticides, Nitrites	-Guaranteed testing by municipal utilities	C	NO	
	P	None	-Sanitize brush before use	P	NO	
Grinding and Pressing	B	Pathogens from dirty equipment	Washing and sanitization of chute bag before start of operation	B	YES	3
	C	None		C	NO	
	P	Metal debris from machine	Filter cider		NO	
Pumping cider t settling tank	B	None	-Clean tubing and tank	B	NO	
	C	None	-Proper cooler temperature (35-40 F)	C	NO	
	P	None		P	NO	

Processing Step		**Potential hazards introduced**	**What control measures can be applied to prevent the hazard?**		**Is the potential safety hazard significant & likely to occur?**	**CCP #**
Add preservative (Pot. Sorbate)	B	None	-Good record keeping with SOP, monitoring & maintenance of weighing scales	B	NO	
	C	Chemical limits exceeded		C	Yes	4
	P	None		P	NO	
Filtering (25 & 50 mesh bags)	B	None	-Wash with sanitizer before use	B	NO	
	C	None	-Check filter daily for visible signs of damage	C	NO	
	P	None	-Replace as necessary	P	NO	
Pasteurization	B	Pathogen destruction	-Flash pasteurization at 163 F for 2 seconds	B	YES	5
	C	None		B	NO	
	P	None		P	NO	
Bottling	B	Pathogens	-Automatic bottler used -Capping performed in timely manner-containers not allowed to sit in open environment longer than 5 minutes	B	YES	6
	P	Introduction of physical hazard	-Visual inspection of bottles for foreign materials -Checked for proper sealing (SOP)	P	YES	

Processing Step		**Potential hazards introduced**	**What control measures can be applied to prevent the hazard?**		**Is the potential safety hazard significant & likely to occur?**	**CCP #**
Cooling	B	Improper cooli allowing pathog growth	-Bottles are sent to cooler immediately after sealing	B	NO	
	C	None	-Monitor cooler temperature (35-40 F)	C	NO	
	P	None		P	NO	
Storage	B	Improper refrig Temperature pr Bacterial growt	-Monitor storage and transportation temperature (35-40 F)	B	NO	
	C	None		C	NO	
	P	None		P	NO	

Approved by:_____ Date approved:_____

Critical Control Point (CCP)	Hazard(s) to be addressed in HACCP plan	Critical limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 1 (B) Receiving and Inspection	Pathogenic Bacteria (prevention & destruction)	-No drops -No domestic manure in orchards -Removal of rotten or spoiled apples	Apple harvesting and shipments	Visual	Each harvest or shipment	Receiving Manager	No drops policy Reject drops
CCP 2 (B) (P) Storage of Apples	(B) Pathogens and mold growth	Storage inside Cooler (35-42 F)	Cooler temperatu maintaine	Temperatur gauge-visua	Daily	Manager	Correct cooling problem, recalibrate and monitor hourly for next 6 hours Move to other cooler if temperature has not been maintained with 6 hours.
	(P) Rodents an Insects	No outside or uncovered storage	Spoilt apples	Visual	Weekly	Manager	Discard any spoilt apples

Critical Control Point (CCP)	Hazard(s) to b addressed in HACCP plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 3 (B) Grinding and Pressing	Pathogenic bacteria (prevention & destruction)	Change chute bag after 4 processing operations Before use,make sure that bag is clean and does not have any residual debris from previous use. After use wash the bag with a sanitizer	Chute bag	Visual	After 4 processing operations Before each processing operation	Manager Operator	Change bag Regularly Keep an extra b for use in case o presence of debr or evidence of improper sanitization.
CCP 4(C) Preservative addition	Preservative concentration per bottle of cider	Preservative is fully dissolved before bottling	Dissolved potassium sorbate granules	Visual	Every contai	Operator	Pour cider back into tank and fil after preservativ is fully dissolve

Critical Control Point (CCP)	Hazard(s) to be addressed in HACCP plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 5(B) Pasteurization	Pathogenic Bacteria (Destruction)	Product target Time/Temp: 162 ° F for 11 sec. Minimum temp. 161 F-alarm will sound	Temperature of cider at exit of holding tube Temp. will also be recorded manually every 15 minutes as a Comparison Record	-Temperature recorder at end of hold tube -Low temperature manual divert valve	Continuous Recording	Pasteurizer Operator	Cider will be manually diverted if temp at end of holding tube is low and will be re-pasteurized -If divert valve doesn't work; production will be stopped and the portion of the cider in the holding tube and holding tank will be re-pasteurized.

Critical Control Point (CCP)	Hazard(s) To be Addressed In HACCP Plan	Critical Limits for each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 4 (B) Pasteurization (continued)							-Verification of temp. gauge & alarm system to ensure deviation does not occur again -Questionable cider may be tested for pathogens before shipping -If seals are broken or gauge is not working properly; stop processing if necessary, recalibrate pump and/or reseal.
			Seals on pumps and tubing and flow rate gauge	Visual check	At start up & once during processing	Pasteurizer Operator	

Critical Control Point (CCP)	Hazard(s) to be Addressed in the HACCP plan	Critical Limits For each control measure	Monitoring				Corrective Action
			What	How	Frequency	Who	
CCP 6 (B) (P) Bottling	(B) Pathogens	-Ensure bottler tips are clean and sanitized	-Bottler is working properly and is free from contamination	Visual	Every 2 hours and before start-up	Operator	Dispose of bottled cider
		-Proper seal achieved	-Proper sealing of container	Visual	Every container	Operator	Reseal if necessary
		-Bottles sent to cooler in a timely manner	-Length of time bottles are setting at room temp.	Time	Get estimate time for every pallet of bottled cider	Operator	Dispose of bottled cider if it has set at room temp for more than 1 hr
	(P) Environmental debris	-No visible debris	-Physical contamination	Visual	Every container	Operator	Dispose of bottled cider

Verification Activities	Record-Keeping Procedures
CCP#1: Pasteurization 1. Maintenance calibrates divert valves & alarm system weekly 2. Verification of thermometers daily before start-up 4. Holding tube length and diameter are tested once per season with salt tracer test to validate the residence time 5.QA manager will review and initial records daily 6.QA checks pump flow rate gauge daily and enters data in pasteurization log	-Pasteurization log which includes temperature data -Calibration records for the thermometers, divert valves, alarm system, etc -QA flow verification log (pump flow rate info) -Corrective action logs
CCP#2: Bottling 1. Ensure bottles are capped and sent to cooler in a timely manner. 2.QA manager reviews and initials records on a weekly basis.	-Log verifying bottling equipment and flavoring equipment was cleaned and sanitized before use -Bottles capped timely and sealing documetation -Log documenting discarded product due to biological and/or physical contamination -Corrective action logs

Verification:
Short term: # 1,2,3,5,6
Long term: # 4

Verification of overall process will also include microbial testing for coliforms / *E.coli* (samples will be taken from every batch)
Corrective actions will also be recorded and reviwed.

APPENDIX B:

(RAW DATA)

Producer C 2001-2002 Results (CFU/Apple, CFU/ml or CFU/cm^2)

18-Oct			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple#1 from store	680000	<100	2800000
Apple #2 from store	130000	<100	920000
Apple (after washing)	510000	1200	710000
Raw cider	1300	70	22000
Cider (RWP)	4800	<1	12000
Cider (PWP)	150	<1	500
Apple wash water	7400	600	<10
Bottler nozzle	<10	<1	<10

15-Nov			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple (store room)	380000	600	60000
Apple (after washing)	1000000	3000	320000
Raw cider	5700	110	180000
Cider (RWP)	3900	120	150000
Cider (PWP)	100	<1	100
Water sample	3200	<1	<10

1-Dec			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple#1 from store	12000	<100	790000
Apple #2 from store	130000	<100	1200000
Apple#1after washing	3300000	1100	450000
Apple#1after washing	4500000	<100	12000000
Apple#1 conveyor belt	170000000	<100	4900000
Apple#1 conveyor belt	80000000	<100	4400000
Raw Cider	6600	70	42000
Cider (RWP)	2750	50	60000
Cider (PWP)	50	<1	250
Conveyor belt	>5600000	14	605000
Pressing roller	>5600000	11	1400000
Apple wash water	11850	1	<10

Producer B 2001-2002 Results (CFU/Apple, CFU/ml or CFU/cm^2)

13-Nov			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple#1- store room	1500000	4500	220000
Apple#2- store room	2200000	100	25000
Apple#1- conveyor belt	2300000	5000	110000
Apple#2- conveyor belt	970000	600	240000
Raw cider	620000	170	19000
Cider (PWP)	400	<1	<10
Conveyor Belt	<10	<1	<10
Chute	2900	1200	<10
Press Plate	5100	85	6400
Apple wash water	4700	<1	<10

14-Dec			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple#1- store room	330000	<100	5000
Apple#2- store room	380000	<100	5000
Apple#1- conveyor belt	330000	300	140000
Apple#2- conveyorbelt	480000	<100	160000
Raw cider	25000	900	15000
Cider (PWP)	300	<1	<10
Water Sample	<10	<1	<10

Producer B 2001-2002 Results (CFU/Apple, CFU/ml or CFU/cm^2)

18-Sep			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple #1- store room	14000000	100	35000
Apple #2- store room	9500000	1400	5000
Apple #1- conveyor belt	890000	200	<10000
Apple #2- conveyorbelt	730000	5000	40000
Raw cider	470000	6	220000
Cider (PWP)	500	<1	<10
Chute	NA	22	NA
Conveyor Belt	NA	10	NA
Press Plate	170000	80	54000
Apple wash water	<10	<1	<10

23-Oct			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple#1- store room	9800000	<100	1400000
Apple#2- store room	11000000	<100	410000
Apple#1- conveyor belt	4500000	<100	170000
Apple#2- conveyorbelt	5500000	800	60000
Raw Cider	5300	45	16000
Cider (PNP)	500	<1	<10
Cider (PWP)	250	<1	1000
Press Plate	<10	4	<10
Chute	<10	6	<10
Conveyor Belt	<10	<1	<10
Water Sample	<10	<1	<10

14-Jan			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple #1 from store room	180000	<100	190000
Apple #2 from store room	120000	100	80000
Apple #1 from conveyor belt	10000	<100	45000
Apple #2 from conveyor belt	15000	<100	25000
Raw Cider	27000	78	23000
Cider (PNP)	100	1	<10
Cider (PWP)	50	1	<10
Apple wash water	<10	<1	<10
Conveyor belt	<10	<1	<10
Chute	<10	<1	<10
Press plate	<10	<1	<10
Cider tank	<10	<1	<10

Producer A 2001-2002 Results (CFU/Apple, CFU/ml or CFU/cm^2)

27-Feb			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple #1 from store room	400000	500	5000
Apple #2 from store room	10000	<100	5000
Apple #1 from wash tank	15000	<100	10000
Apple #2 from wash tank	10000	<100	10000
Apple #1 from conveyor belt	830000	<100	10000
Apple #2 fromconveyor belt	15000	<100	5000
Raw Cider	30000	25	8000
Cider (PNP)	50	<1	<10
Cider (PWP)	<10	<1	<10
Apple wash water	<10	<1	<10
Conveyor belt	<10	<1	<10
Chute	<10	2	<10
Press plate	<10	1	<10
Cider tank	<10	<1	<10

Producer A 2001-2002 Results (CFU/Apple, CFU/ml or CFU/cm^2)

8-Nov			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple #1 from grading table	410000	<100	10000
Apple #2 from grading table	140000	<100	50000
Apple #1 from store room	750000	500	75000
Apple #2 from store room	160000	200	800000
Apple #1 from wash tank	180000	<100	690000
Apple #2 from wash tank	1200000	<100	NA
Apple #1 from conveyor belt	460000	<100	440000
Apple #2 from conveyor belt	2200000	<100	910000
Raw Cider	39000	230	48000
Cider (PNP)	550	<1	1500
Cider (PWP)	450	<1	2500
Apple wash water	63000	300	12000
Conveyor belt	4600	19	16000
Chute	<10	<1	<10
Press plate	<10	<1	<10
Cider tank	<10	1	<10

Producer A 2001-2002 Results (CFU/Apple, CFU/ml or CFU/cm^2)

11-Oct			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple #1 from grading table	140000	600	150000
Apple #2 from grading table	1100000	20000	130000
Apple #1 from store room	660000	2200	50000
Apple #2 from store room	470000	5000	95000
Apple #1 from wash tank	970000	17000	320000
Apple #2 from wash tank	1500000	500	180000
Apple #1 from conveyor belt	1700000	1500	130000
Apple #2 from conveyor belt	650000	500	190000
Raw Cider	17000	85	25000
Cider (PNP)	200	<1	100
Cider (PWP)	<10	<1	150
Conveyor belt	<10	<1	<10
Chute	4400	30	4700
Press plate	<10	<1	<10
Cider tank	<10	<1	<10
Apple wash water	380000	85	19000

Producer A 2001-2002 Results (CFU/Apple, CFU/ml or CFU/cm^2)

30-Aug			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple #1 from store	660000	2000	130000
Apple #2 from store	310000	500	120000
Apple #1 from wash tank	240000	33000	12000
Apple #2 from wash tank	>560000	13000	130000
Apple #1 from conveyor belt	250000	800	2500000
Apple #2 from conveyor belt	440000	1200	220000
Raw Cider	29000	25	2500
Cider (PNP)	<10	<1	50
Cider (PWP)	<10	<1	50
Wash Tank	5000	<1	<10
Conveyor Belt	2500	<1	<10
Pulp Bag	<10	<1	<10
Press Plate	<10	<1	<10
Cider Trough	8000	85	<10
Holding Tank(L)	<10	<1	<10
Holding Tank(R)	<10	<1	<10
Apple Wash Water	22000	<1	<10
Hand Dip Water	<10	<1	<10

3-Apr			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple store room	110000	<100	420000
Apple store room	120000	<100	310000
Apple wash tank	100000	<100	200000
Apple wash tank	5000	100	40000
Apple conveyor belt	20000	<100	80000
Apple conveyor belt	20000	<100	20000
Raw	7600	100	16000
Cider (PNP)	150	<1	<10
Cider (PWP)	250	<1	150
Apple wash water	<10	<1	<10
Hand dip (press rm)	<10	<1	<10
Pulp bag	<10	11	11000
Press plate	<10	1	<10
Holding tank	<10	<1	<10

12-Feb			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple store room	150000	<100	50000
Apple store room	250000	<100	140000
Apple wash tank	55000	<100	75000
Apple wash tank	25000	<100	5000
Apple conveyor belt	600000	<100	150000
Apple conveyor belt	120000	<100	180000
Raw	3000	110	5400
Cider (PNP)	450	<1	<10
Cider (PWP)	250	<1	<10
Apple wash water	<10	<1	<10
Hand dip (press room)	<10	<1	<10
Pulp bag	<10	<1	6300
Rope	<10	1	5000
Conveyor belt	<10	<1	<10
Press plate	<10	<1	<10
Holding tank	<10	<1	<10
Holding tank	<10	<1	<10
Bottling pipe	<10	<1	<10

Producer A 2000-2001 Results (CFU/Apple, CFU/ml or CFU/cm^2)

12-Mar			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple store room	400000	<100	1000
Apple store room	35000	<100	10000
Raw cider	2800	9	3000
Cider (PNP)	50	<1	<10
Cider (PWP)	250	<1	<10

Producer A 2000-2001 Results (CFU/Apple, CFU/ml or CFU/cm^2)

15-Jan			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple store room	150000	<100	25000
Apple conveyor belt	15000	<100	<10000
Apple wash tank	10000	<100	10000
Raw cider	8200	3	1300
Cider (PNP)	700	<1	4500
Cider (PWP)	1400	<1	<10
Holding tank	<10	<1	<10
Holding tank	<10	<1	<10
Pulp bag	<10	<1	6000
Press plate	<10	<1	<10
Rope	<10	<1	77000
Apple wash water	15000	<100	<10
Hand dip(press room)	<10	<1	<10

Producer A 2000-2001 Results (CFU/Apple, CFU/ml or CFU/cm^2)

16-Nov			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple before brushing	130000	<100	45000
Apple gradingtable	460000	<100	70000
Apple store room	7500000	<100	1800000
Apple store room	910000	<100	510000
Raw Cider	1300	250	6400
Cider (PNP)	200	<1	<10
Cider (PWP)	250	<1	<10
Bottling tube	<10	<1	<10
Holding tank	<10	<1	<10
Holding tank	<10	<1	<10
Trough	<10	6	<10
Press plate	<10	<1	<10
Hand dip (past room)	<10	<1	<10
Lid	<10	<1	<10
Pulp bag	1500	20	160000

14-Dec			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple store room	240000	<100	130000
Apple wash tank	10000	<100	10000
Apple conveyor belt	670000	<100	140000
Raw Cider	12000	55	8000
Apple wash water	<10	<1	<10
Cider press	<10	<1	<10
Holding Tank	<10	<1	<10
Pulp bag	1300	<1	12000
Hand dip(press room)	<10	<1	<10

2-Nov			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple Store room	610000	<100	210000
Apple after brushing	160000	<100	120000
Apple grading table	35000	<100	780000
Raw cider	2100	<1	1800
Cider (RWP)	2600	50	7000
Lid	<10	<1	<10
Press Plate	<10	<1	<10
Holding Tank	<10	<1	<10
Bottling Tube	<10	<1	<10
Bottle neck	<10	<1	<10
Dip(past room)	<10	<1	<10

Producer A 2000-2001 Results (CFU/Apple, CFU/ml or CFU/cm^2)

12-Oct			
	Aerobes	**Coliforms**	**Yeasts and Molds**
Apple #1 from store	840000	<100	NA
Apple #2 from store	750000	<100	NA
Apple #1 from wash tank	1700	<100	NA
Apple #1 from conveyor belt	470000	<100	NA
Apple #2 from conveyor belt	1400000	<100	NA
Apple before brushing	330000	<100	NA
Raw Cider	<10	<1	1500
Cider (PNP)	<10	<1	<10
Cider (PWP)	<10	<1	<10
Rope	<10	<1	3000
Water Sample	<10	<1	<10
Cleaning sponge	<10	<1	<10
Bottle neck	<10	<1	<10
Holding Tank(L)	<10	<1	<10
Holding Tank(R)	<10	<1	<10
Apple Wash Water	4500	<1	5000
Bottling pipe	<10	<1	<10
Press Plate	<10	<1	<10
Lid	<10	<1	
Hand Dip Water	<10	<1	<10
Hand Dip(Press room)	1800	<1	15000

APPENDIX C:

(MICROBIAL STORAGE DATA)

Producer A: Storage study results (2001)

Aug. 30			
	Week		
Aerobes		PNP	PWP
	0	<10	<10
	2	440000	50
	4	349500	100
	6	66500	50
	8	231750	450
Coliforms			
	0	<1	<1
	2	<1	<1
	4	1200	<1
	6	11	<1
	8	30	6
Y & M			
	0	<10	<10
	2	4150	<10
	4	2245000	<10
	6	5600000	30500
	8	5600000	800000

Oct. 11			
	Week		
Aerobes		PNP	PWP
	0	200	<10
	2	2385000	<10
	4	630000	1500
	6	<10	5000
	8	200	300
Coliforms			
	0	<1	<1
	2	45	<1
	4	480	<1
	6	20	<1
	8	1200	<1
Y & M			
	0	100	150
	2	1400000	134500
	4	3865000	150000
	6	5695000	95000
	8	5750000	12950

Nov.8				
	Week			
Aerobes		Raw	PNP	PWP
	0	39050	550	450
	2	320000	1300	100
	4	14575	800	<10
	6	560000	1550	<10
Coliforms				
	0	235	<1	<1
	2	165	65	<1
	4	100	30	<1
	6	800	600	<1
Y & M				
	0	47500	1450	2500
	2	6750000	9000000	20500
	4	2985000	1580000	316250
	6	3420000	1565000	315000

Jan.14			
	Week		
Aerobes		PNP	PWP
	0	100	50
	2	50	10
	4	<10	<10
	6	21875	50
	8	2500	100
Coliforms			
	0	<1	<1
	2	<1	<1
	4	<1	<1
	6	<1	<1
	8	<1	<1
Y & M			
	0	<10	<10
	2	150	650
	4	15425	<10
	6	<10	500
	8	371750	<10

Feb.27			
	Week		
Aerobes		PNP	PWP
	0	50	10
	2	10	10
	4	16300	100
	6	450	10
	8	40125	50
Coliforms			
	0	1	1
	2	1	1
	4	1	1
	6	1	1
	8	4	3
Y & M			
	0	10	10
	2	10	2500
	4	1620000	200
	6	1250000	174000
	8	1050000	2550

Producer B: Storage study results (2001)

Oct.23				
	Week			
		Raw	RWP	PWP
Aerobes	0	5250	500	250
	2	71000	53500	2300
	5	3750	150	250
Coliforms				
	0	45	<1	<1
	2	113	<1	<1
	5	250	<1	<1
Y & M				
	0	16200	5000	100
	2	5000000	1100000	890000
	5	3110000	1325000	109250

Nov.13			
	Week		
Aerobes		Raw	PWP
	0	620000	400
	2	213500	52000
	3	80500	
	4	905000	3700
	7	25000	50
	8	50500	215000
Coliforms			
	0	165	<1
	2	200	<1
	3	60	
	4	250	<1
	7	80	<1
	8	8	<1
Y & M			
	0	18700	<10
	2	560000	<10
	3	560000	
	4	560000	715000
	7	560000	468250
	8	560000	61500

Dec.12			
	Week		
		Raw	PWP
Aerobes	0	25250	300
	2	11375	500
	4	200	29150
	6	482250	600
	8	11000	150
Coliforms			
	0	900	<1
	2	40	<1
	4	100	<1
	6	50	<1
	8	65	<1
Y & M			
	0	14600	<10
	2	100	255500
	4	1405000	<10
	6	1390000	1250
	8	5600000	1250

Producer C: Storage study results (2001)

Oct.18				
	Week			
Aerobes		Raw	RWP	PWP
	0	1250	4750	50
	2	9050	7750	500
	5	1550	300	<10
	8	1450	800	15750
Coliforms				
	0	70	<1	<1
	2	75	15	<1
	5	21	<1	<1
	8	30	<1	2
Y & M				
	0	21875	11600	500
	2	5340000	3740000	22050
	5	7200000	151750	850000
	8	3710000	300000	1300000

Nov.29				
	Week			
		Raw	RWP	PWP
Aerobes	0	6600	2750	50
	2	78750	100	<10
	4	2800	<10	<10
	6	9850	150	200
	8	100	5000	50
Coliforms				
	0	70	50	<1
	2	90	<1	2
	4	360	30	<1
	6	10	<1	<1
	8	250	<1	<1
Y & M				
	0	42000	59500	250
	2	125000	44500	19250
	4	10800	11400	2800
	6	4010000	21250	11400
	8	6950	3700	4050

APPENDIX D

(GAS CHROMATOGRAPHY DATA)

Compounds affected by interaction between treatment of cider and time of storage (weeks): Producer A (Jan.)

Weeks Compounds		0		2		4		6		8	
Ethyl butyrate	PNP	469.79	bcx	599.81	cx	225.53	abx	95.94	ax	187.85	ax
	PWP	1069.83	by	571.09	ax	620.42	ay	597.90	ay	441.19	ax
Butyl acetate	PNP	2106.98	bcx	2859.54	cx	1852.78	abcx	985.79	ax	1357.45	abc
	PWP	3860.50	cy	2164.40	bx	2677.03	bx	2001.46	bx	858.50	ax
Ethyl-2-methyl	PNP	2456.64	bx	3109.67	by	130.28	ax	22.22	ax	101.66	ax
butyrate	PWP	4167.19	cy	2312.68	abx	2962.92	by	2314.97	aby	1197.76	ax
2-Methyl butyl	PNP	956.79	abx	1692.06	bx	1097.00	abx	666.38	ax	1070.21	abx
acetate	PWP	2098.33	cy	1033.02	abx	1688.38	bcx	1552.26	bcx	584.28	ax
Butyl propionate	PNP	67.22	abx	101.06	bx	69.24	abx	38.54	ax	54.00	ax
	PWP	150.95	cy	78.15	abx	108.64	bcx	91.11	aby	55.94	ax
Pentyl acetate	PNP	187.81	bx	263.03	bx	168.25	abx	65.13	ax	182.51	bx
	PWP	369.62	cy	198.33	abx	230.81	bcx	198.65	aby	115.31	ax
Isopropyl-2-	PNP	14.10	ax	31.26	ax	18.61	ax	12.23	ax	17.78	ax
methyl butyrate	PWP	56.64	ay	19.83	ax	36.64	abx	31.18	ax	18.16	ax
3-Methyl butyl	PNP	1.00		11.28		1.00		1.00		11.31	
propionate	PWP	6.14		1.00		9.96		8.74		12.70	
Butyl butyrate	PNP	410.03	abx	719.75	bx	372.28	ax	227.74	ax	320.27	ax
	PWP	926.27	by	424.62	ax	535.68	ax	435.94	ax	293.88	ax

Ethyl hexanoate	PNP	307.55	abx	666.76	bx	301.84	ax	139.35	ax	206.32	ax
	PWP	1222.10	cy	549.61	abx	817.74	by	680.74	by	310.96	ax
3-c-Hexen-1-yl	PNP	107.86	by	1.00	ax	107.28	by	61.94	by	77.76	bx
acetate	PWP	1.00	ax	48.09	abx	1.00	ax	1.00	ax	85.26	bx
Hexyl acetate	PNP	6152.70	bcx	8774.84	cx	4804.48	abx	2537.34	ax	3369.70	ax
	PWP	12273.70166	by	5401.46	ax	5351.55	ax	4004.79	ax	2060.40	ax
Butyl-2-Methyl	PNP	83.36	abx	156.12	bx	92.20	abx	47.68	ax	97.09	abx
butyrate	PWP	266.12	by	135.39	ax	167.19	ax	131.98	ax	112.34	ax
Hexyl butyrate	PNP	1725.12	bx	1777.41	bx	679.92	ax	385.80	ax	505.15	ax
	PWP	3222.34	by	1395.51	ax	1438.71	ax	995.30	ax	831.82	ax
2-Phenylethyl	PNP	6.13	ax	25.26	abx	35.07	by	20.04	abx	97.70	cx
acetate	PWP	9.87	ax	4.77	ax	5.14	ax	4.36	ax	113.36	bx
Hexanal	PNP	385.35	bx	492.40	bx	1.00	ax	51.70	ax	1.00	ax
	PWP	655.08	cy	329.05	bx	354.26	by	332.44	by	69.85	ax
Decanal	PNP	42.69	bx	83.02	cy	1.00	ax	19.16	abx	28.14	abx
	PWP	25.49	abx	23.57	ax	41.86	aby	28.12	abx	52.89	bx
t-2-Octenal	PNP	18.86	ax	41.15	bx	14.57	ax	7.54	ax	12.64	ax
	PWP	55.65	by	27.80	ax	41.35	ay	36.78	ay	35.97	ay
Benzaldehyde	PNP	1.00	ax	12.62	by	1.00	ax	1.00	ax	1.00	ax
	PWP	8.07	ax	2.38	ax	20.10	by	17.89	aby	8.65	ax
Estragole	PNP	86.59	ax	73.46	ax	58.91	ax	54.43	ax	63.96	ax
	PWP	95.56	ax	60.11	ax	187.25	by	62.15	ax	92.32	ax

Compounds affected by interaction between treatment of cider and time of storage (weeks): Producer A (Feb.)

Weeks / Compounds		0		2		4		6		8	
Ethyl butyrate	PNP	1671.18	dy	346.42	cx	307.80	bcx	172.05	abx	9.77	ax
	PWP	1459.10	cx	181.48	bx	287.22	bx	348.17	by	1.00	ax
Butyl acetate	PNP	5004.53	cy	930.80	bx	52.39	ax	999.68	bx	190.40	ax
	PWP	4029.05	cx	786.84	bx	71.72	ax	818.98	bx	146.51	ax
Ethyl-2-methyl butyrate	PNP	4018.75	cy	965.31	bx	327.35	ax	72.21	ax	39.16	ax
	PWP	3516.47	dx	923.04	cx	1498.25	by	883.30	cy	17.64	ax
2-Methyl butyl acetate	PNP	151.90	ax	1.00	ax	1.00	ax	1.00	ax	879.27	by
	PWP	1.00	ax	1.00	ax	16.49	ax	1.00	ax	572.27	bx
Butyl propionate	PNP	364.02	cy	36.73	abx	54.66	bx	39.31	abx	9.73	ax
	PWP	291.35	bx	27.00	ax	55.83	ax	48.91	ax	32.80	ax
Pentyl acetate	PNP	269.27	dy	51.15	abx	126.32	cx	67.35	bx	21.09	ax
	PWP	203.17	dx	61.38	bx	99.83	cx	93.56	bcx	18.04	ax
Isopropyl-2-methyl butyrate	PNP	5.01	ax	9.49	abx	15.06	cx	10.42	bx	5.56	ax
	PWP	4.11	ax	9.76	bx	11.62	bx	17.66	cy	1.00	ax
Butyl butyrate	PNP	2319.80	cy	152.17	abx	270.55	bx	163.14	bx	1.00	ax
	PWP	1680.51	cx	147.80	abx	217.91	bx	247.94	bx	1.00	ax
Ethyl hexanoate	PNP	2319.80	cy	354.33	abx	570.91	bx	144.84	ay	117.11	ax
	PWP	1680.51	cx	355.64	abx	607.13	bx	558.68	bx	68.47	ax

Hexyl acetate	PNP	16466.47	dy	2975.59	bx	4594.04	cx	1679.85	bx	67.13	ax
	PWP	12931.05	cx	2750.41	bx	3256.03	bx	2871.47	bx	287.45	ax
Propyl hexanoate	PNP	178.03	bx	81.45	ax	13.39	ax	46.48	ax	86.88	ax
	PWP	362.86	dy	181.14	cy	1.00	ax	466.80	by	82.51	ax
Hexyl propionate	PNP	65.47	bx	86.96	cx	13.76	ax	11.44	ax	23.16	ay
	PWP	176.03	cy	103.59	bx	111.56	by	107.15	by	1.00	ax
Heptyl acetate	PNP	49.62	ax	122.71	ax	54.31	ax	78.37	ax	110.79	ax
	PWP	324.66	dy	61.61	ax	209.99	by	149.61	abx	449.46	cy
Hexyl butyrate	PNP	2642.76	by	14.62	ax	17.00	ax	13.26	ax	1.00	ax
	PWP	2400.64	bx	16.14	ax	19.16	ax	16.07	ax	5.25	ax
Hexanal	PNP	900.36	dy	262.81	cx	208.16	cx	167.03	bx	7.45	ax
	PWP	803.62	dx	477.78	cx	456.61	cy	361.46	by	1.00	ax
Nonanal	PNP	8.51	abx	117.83	cx	33.51	bx	1.00	ax	23.16	abx
	PWP	113.68	by	273.77	dy	447.90	cy	466.80	cy	1.00	ax
1-Octen-3-ol	PNP	104.14	by	1.00	ax	1.00	ax	1.00	ax	3.96	ax
	PWP	82.59	bx	1.00	ax	1.00	ax	1.00	ax	1.00	ax
Estragole	PNP	2642.75	cy	3.34	ax	1.00	ax	1.00	ax	143.31	bx
	PWP	2400.63	cx	8.03	ax	1.00	ax	4.40	ax	101.08	by
A-farnasene	PNP	3.12	abx	11.18	bx	1.00	ax	8.88	abx	1.00	ax
	PWP	21.43	cy	4.88	abx	2.83	ax	2.56	ax	12.31	by
Benzaldehyde	PNP	16.88	bx	12.98	bx	1.00	ax	10.23	abx	1.00	ax
	PWP	13.83	abx	4.49	ax	15.37	by	16.07	bx	10.85	abx

Compounds affected by interaction between treatment of cider and time of storage (weeks) for Producer B:

Weeks / Compounds		0		4		6	
Butyl acetate	RAW	1.00	ax	1.00	ax	1.00	ax
	PWP	1.00	ax	3676.92	by	959.57	ax
Ethyl-2-methyl butyrate	RAW	2858.65	ax	7071.44	cy	4734.91	by
	PWP	2516.91	bx	45.48	ax	26.41	ax
2-Methyl butyl acetate	RAW	419.04	bx	1.00	ax	1.00	ax
	PWP	396.62	bx	552.10	cy	78.27	ax
Propyl butyrate	RAW	200.96	ax	409.19	by	315.51	by
	PWP	196.51	abx	231.08	bx	107.77	ax
Butyl propionate	RAW	255.65	ax	634.84	by	417.05	aby
	PWP	265.95	ax	167.88	ax	61.02	ax
Ethyl hexanoate	RAW	76.04	ax	275.61	cy	192.84	by
	PWP	81.43	bx	1.00	ax	1.00	ax
Butyl-2-Methyl butyrate	RAW	6814.48	ax	20595.28	by	12725.78	ay
	PWP	144.99	ax	213.18	ax	67.61	ax
Propyl hexanoate	RAW	574.48	bx	488.71	ay	511.66	aby
	PWP	516.00	cx	340.03	bx	116.87	ax
2-Phenylethyl acetate	RAW	13.58	ax	14.89	ax	8.58	ax
	PWP	10.49	ax	114.80	by	44.62	ax

Pentyl-4-methyl pentanoate	RAW	6.18	ax	1.00	ax	15.92	by
	PWP	6.82	ax	9.51	ay	4.63	ax
1-Octanol	RAW	1.00	ax	79.91	ax	1.00	ax
	PWP	1.00	ax	193.09	by	44.42	ax
t-2-Octenal	RAW	141.28	ax	408.94	by	44.61	aby
	PWP	28.52	ax	5.26	ax	3.02	ax
Nonanal	RAW	1.00	ax	1.00	ax	1.00	ax
	PWP	1.00	ax	32.05	by	9.18	ax
Hexanal	RAW	539.78	ax	1973.19	by	1128.41	ay
	PWP	372.46	ax	78.41	ax	22.93	ax
Decanal	RAW	84.65	ax	205.21	ax	324.60	ax
	PWP	60.68	ax	4193.23	by	5160.10	by
Estragole	RAW	1189.71	bx	1141.77	by	645.35	ay
	PWP	1255.91	bx	219.22	ax	1.00	ax

Compounds affected by interaction between treatment of cider and time of storage (weeks) for Producer C:

Week / Compound		0.00		2.00		6.00		8.00	
	PWP	**ND**	ax	42.79	abx	93.94	bx	98.83	bx
t-butyl acetate	Raw	22.02	ax	20.02	ax	111.83	bx	716.27	cy
	RWP	27.12	ax	24.71	ax	59.29	ax	78.55	ax
	PWP	7.15	ax	7.36	ax	6.20	ax	3.37	ax
2-Methyl propyl acetate	Raw	9.33	ax	9.21	ax	25.04	by	10.49	by
	RWP	10.44	ax	6.82	ax	10.24	ax	6.78	ax
Hexanal	PWP	899.90	bxy	809.03	by	697.01	by	314.40	ax
	RAW	682.61	bxy	267.14	ax	307.01	ax	243.61	ax
	RWP	921.93	dy	672.76	cy	470.63	bx	188.00	ax
1-Methyl propyl acetate	PWP	1811.46	abx	1862.05	ax	3030.88	by	1689.67	ay
Butyl acetate	RAW	2141.38	bx	2042.07	bx	956.65	ax	247.72	ax
	RWP	1834.87	abx	1402.72	ax	3010.26	by	2460.57	by
Ethyl-2-methyl butyrate	PWP	2061.74	cx	1481.60	by	17.86	ax	**ND**	ax
	Raw	2212.10	cx	619.68	bx	26.16	ax	46.97	ax
	RWP	1943.09	cx	726.61	bx	27.66	ax	**ND**	ax
Methyl-2-methyl pentanoate	PWP	1061.64	bcx	1167.73	cx	685.94	bx	159.39	ax
	RAW	915.30	ax	1032.20	ax	4698.22	bz	5594.84	cz
	RWP	1051.42	bcx	817.34	bx	1331.55	cy	571.95	aby
2-Methyl butyl acetate	PWP	273.79	ax	**ND**	ax	127.90	ax	191.46	ax
	Raw	**ND**	ax	113.09	abx	332.67	abx	452.25	bx
	RWP	248.40	ax	**ND**	ax	**ND**	ax	246.61	ax

Propyl butyrate	PWP	49.46	ax	72.35	axy	86.08	ax	81.51	ax
	Raw	77.39	ax	118.41	aby	240.45	cy	150.33	by
	RWP	50.27	ax	61.78	ax	99.93	ax	88.50	ax
Butyl propionate	PWP	47.65	ax	184.64	cz	134.45	by	64.95	ax
	Raw	19.20	ax	17.83	ax	65.59	bx	44.57	abx
	RWP	117.41	aby	109.94	ay	167.39	by	86.27	ax
Pentyl acetate	PWP	42.41	by	1.00	ax	1.00	ax	1.00	ax
	RAW	1.00	ax	1.00	ax	13.44	by	1.00	ax
	RWP	1.00	ax	1.00	ax	1.00	ax	17.79	by
	PWP	24.40	by	25.43	bz	1.00	ax	5.36	ax
Isopropyl-2-methyl butyrate	RAW	7.00	ax	12.40	aby	10.41	aby	15.47	by
	RWP	22.75	by	6.32	ax	1.00	ax	4.92	ax
	PWP	1.00	ax	1.00	ax	12.99	bx	10.72	bx
3-Methyl butyl propionate	RAW	1.00	ax	1.00	ax	16.84	bx	26.36	cy
	RWP	1.00	ax	1.00	ax	10.82	bx	5.59	abx
Butyl butyrate	PWP	233.87	ax	276.67	ax	667.76	bx	750.22	by
	RAW	267.08	ax	295.90	ax	525.50	bx	1172.35	cz
	RWP	268.45	ax	306.33	ax	522.52	bx	559.82	bx
Ethyl hexanoate	PWP	75.84	ax	77.62	ax	167.81	by	303.42	cy
	RAW	66.28	bx	56.68	bx	1.00	ax	1.00	ax
	RWP	75.97	ax	68.38	ax	236.94	bz	299.88	cy
Hexyl acetate	PWP	8139.66	bx	7482.16	bx	7099.46	by	4606.54	axy
Butyl-2-Methyl butyrate	Raw	7782.54	bx	5720.17	bx	1612.33	ax	2481.30	ax
	RWP	8313.56	ax	6809.75	ax	7993.32	ay	6124.62	ay

	PWP	102.66	ax	103.19	ax	227.64	by	307.52	cy
Butyl-2-Methyl butyrate	Raw	103.10	ax	144.43	ax	220.35	by	380.50	cz
	RWP	103.02	ax	106.72	ax	112.03	ax	148.04	ax
	PWP	12.19	ax	11.18	ax	6.88	ax	12.03	ax
Hexyl butyrate	RAW	10.30	bx	14.49	bx	1.00	ax	112.96	cy
	RWP	8.10	ax	12.36	ax	15.07	ay	11.41	ax
Octyl acetate	PWP	16.01	ax	32.40	ay	86.56	bz	69.00	by
	Raw	4.31	ax	7.67	ax	6.22	ax	45.12	bx
	RWP	16.10	ax	18.17	ay	62.30	by	49.38	bx
Hexyl-2-methyl butyrate	PWP	1.00	ax	721.33	cy	11.64	ax	273.59	bx
	RAW	1.00	ax	277.05	bx	4.40	ax	763.21	cy
	RWP	1.00	ax	248.93	bx	9.29	ax	222.05	bx
2-Phenylethyl acetate	PWP	20.60	ax	33.73	ay	70.40	by	71.01	bz
	Raw	14.09	abx	13.43	abx	9.12	ax	27.07	bx
	RWP	18.62	ax	24.29	ay	58.29	by	46.80	by
Decanal	PWP	16.40	ax	25.77	ax	82.88	by	52.84	cx
	RAW	47.51	ay	49.43	ay	51.67	ax	134.41	by
	RWP	12.43	ax	18.76	ax	91.82	cy	51.45	bx
Hexanal	PWP	899.90	cy	809.03	bcy	697.01	bz	314.40	ax
	Raw	316.91	bx	138.94	ax	295.76	by	231.42	abx
	RWP	921.93	dy	672.76	cy	470.63	bx	188.00	ax
t-2-Octenal	PWP	14.52	abx	19.58	bx	118.16	ay	228.57	cy
	Raw	11.31	ax	15.92	ax	9.83	ax	12.61	ax
	RWP	19.79	ax	31.05	ax	56.62	ay	40.64	ax

	PWP	16.80	ax	16.36	ax	46.19	bx	69.57	cy
1-Octen-3-ol	Raw	8.88	ax	11.99	ax	65.60	by	82.09	cy
	RWP	15.23	ax	14.30	ax	40.88	bx	43.24	bx
1-Octanol	PWP	31.87	abx	**ND**	ax	81.52	bx	40.80	abx
	Raw	40.97	ax	54.88	ax	82.59	ax	266.32	bx
	RWP	31.99	abx	23.85	ax	85.64	bx	54.97	abx
	PWP	379.59	ax	302.85	ax	372.97	ay	305.23	ay
Estragole	RAW	824.12	cy	545.27	by	90.62	ax	112.06	ax
	RWP	350.47	bx	258.65	abx	314.33	aby	214.00	axy
A-farnasene	PWP	**ND**	ax	4.04	ay	5.78	ay	**ND**	ax
	Raw	1.90	ax	3.39	ay	1.55	ax	3.82	ay
	RWP	**ND**	ax	**ND**	ax	2.88	ax	1.73	ax
Benzaldehyde	PWP	**ND**	ax	**ND**	ax	**ND**	ax	23.89	by
	Raw	**ND**	ax	12.40	by	10.41	by	15.46	bx
	RWP	**ND**	ax	9.55	ay	**ND**	ax	8.23	ax
Hexyl hexanoate									
	PWP	5.69	ax	5.97	ax	6.58	ax	16.09	by
	RAW	9.34	bx	2.63	ax	2.93	ax	3.10	ax
	RWP	6.03	ax	4.34	ax	4.31	ax	1.00	ax

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