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Flavor characteristics of irradiated apple cider

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Flavor characteristics of irradiated apple cider

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This is to certify that the master's thesis of

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has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

I would like to dedicate this thesis to my parents,

Yohanes Hindarto and Tinawati Iskandar,

who support and love me unconditionally.

Without their support, I would not be the person I am today.

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Chapter 1. General Introduction

Introduction

Several outbreaks associated with *E. coli* O157:H7 focused attention to the need for an additional step is needed to increase the safety of apple cider (CDC 1996, 1997). The Food and Drug Administration (FDA) issued regulations for raw fruit juices such as warning label, HACCP plan and 5-$\log_{10}$ reduction (FDA 1998a, 1998b). Apple cider producers have to put a warning label on raw fruit juice, implement HACCP plan on their processing steps by 2004 and reduce 100,000 folds of microorganisms on their raw fruit juices by processing techniques. The FDA had not specified processing techniques used to achieve 5-$\log_{10}$ microbial reduction.

Pasteurization is commonly used by apple cider processors to meet the 5-$\log_{10}$ microbial reduction requirement. However, since pasteurization uses heat to reduce microorganisms, there are some changes in the characteristics of ciders (Fisher and Golden 1998). Irradiation is an alternative method that can be used to achieve a 5-$\log_{10}$ microbial reduction in apple cider. The effective dose of irradiation to achieve a 5-$\log_{10}$ reduction for *E.coli* O157:H7 in apple cider is between 1.8 to 2.47 kGy, according to Buchanan and others (1998) and Wang (2002).

Sensory evaluation panelists noted an undesirable flavor in irradiated cider such as "cardboard-like" flavor (Wang and others 2003) and sharp, strange bitter taste (Zegota 1991). Asselbergs and others (1958) reported there was an adverse effect of irradiation on acceptability of apple juice.

Consumer acceptability of irradiation as an alternative cider treatment or as an alternative processing treatment in general is an important issue. Even though irradiation can be used to achieve a 5-log reduction in apple cider, without consumer acceptability, an irradiation application of apple cider would be useless.

The overall objective of this research was to investigate an alternative processing technique (irradiation) for apple cider. The specific objectives of this study were to determine consumer preference for irradiated or pasteurized apple cider, to determine degree of liking of pasteurized apple cider with preservative and irradiated apple cider with preservative by consumers, to investigate flavor differences of apple ciders made by different processing treatments and with the presence of preservative by gas chromatography, and to evaluate the growth of coliforms, aerobic bacteria, yeasts and molds in raw, pasteurized and irradiated apple ciders and with and without preservative during 8-weeks of storage.

Thesis Organization

A general literature review chapter (Chapter 1) is followed by a chapter (Chapter 2) that addresses the first objective of this study: consumer evaluations and flavor characteristics of pasteurized and irradiated apple cider with potassium sorbate. The GC data in Chapter 2 may be included in a publication with additional data by Dr. Boylston. Chapter 3 describes continuing consumer evaluations, descriptive analysis tests and microbial analyses of raw, pasteurized and irradiated apple ciders. Chapter 4 explains the overall conclusions of Chapters 2 and 3.

Chapter 3 will be submitted to the Journal of Food Science as a research paper. All three authors (Fransiska Yulianti, Cheryll A. Reitmeier and Bonita A. Glatz) are from the

Department of Food Science and Human Nutrition, Iowa State University. All experimental methods and data collection were done by Fransiska Yulianti.

Table and Figures for Chapters 2 and 3 are given at the ends of these chapters. Data obtained in this study but not included in Chapters 2 and 3 are presented in the Appendix. References for Chapters 2 and 3 are given at the ends of these chapters. References for Chapters 1 are listed in Chapter 5. Acknowledgements are located at the end of the thesis (Chapter 6).

Literature Review

Apple cider

Cider production is important for apple growers to allow them to recapture and utilize the apples (*Malus pumila*) that do not meet USDA standards for grade (Friedrich 2001). High quality apples with no blemishes or bruises and with a good shape and size will be sold for retail sales. Lower quality apples are those with skin blemishes or abnormal shape or size (Somogyi and others 1996). These types of apples are used in apple cider production.

Although in many parts of the world, apple cider is a product of fermented apple juice, apple cider in the United States refers to "sweet cider" made with fresh apple juice (Somogyi and others 1996). In the United States, apple cider is a darker color, has less clarity and more suspended solids than apple juice (Downing 1989). Another definition is that apple cider is the product of freshly pressed juice of apples (Semanchek and Golden 1996).

Apple cider production begins with the selection of cultivars of apples (Childers 1983). Figure 1 (Cummins 2001) illustrates apple cider production beginning with apple

harvest to the consumption. Depending on the producer, different types of apples might be used to create desired apple cider flavors. Different cultivars are used depending on the time of making cider. In the early season (late August through mid-October), a producer might use harvested apples very quickly because of the demand for apple cider. In the late season, a producer uses apples that have been stored in the cold room (4°C) for apple cider production (Cummins 2001).

Apples may be washed and/or brushed prior to use, depending on the producer. Apples may be cleaned or dipped into sanitizing water before grinding. Chlorinated water might be used as a sanitizing agent for apples. Apples could be dipped and allowed to soak for a period of time. Rinsing with fresh water could also be applied during a series of rinsing and brushings on a moving belt. After washing and/or brushing, apples are ready to be ground into a pulp. The pulp and pomace are collected and wrapped on press cloth to be pressed. The pulp and pomace are stacked inside the press cloths between wooden boards. The pulp and pomace are pressed to extract the juice from the pulp. The extracted juice is pumped through a filter, such as cheese cloth, into a holding tank.

Preservatives such as potassium sorbate or sodium benzoate may be added up to 0.1% concentration to extend the shelf life of cider. Apple cider may be held for half an hour to overnight to allow precipitation of solids in a holding tank (21°C). Cider can be bottled or treated through pasteurization to kill harmful microorganisms. Various times and temperatures are used by each cider producer. Times of pasteurization could vary from 2 to 11 seconds and temperatures could vary from 160 to 175°F (Cummins 2001; Deol 2003).

Apple production

In the United States, apples are one of the most popular fruits consumed. Almost 230 million of bushels of apples are produced in the United States (USDA 2001). In 1998, 'Red Delicious' and 'Golden Delicious' were the leading cultivars of apples in the United States. 'Red Delicious' accounted for about 40% of apple production and 'Golden Delicious' accounted for about 15% (USDA 1998).

Apple trees bloom in the spring and apples are ready to be harvested in the fall. Apples are harvested during the fall season from August to October. To produce good quality apples, several steps are required to maintain apple trees. Three important steps necessary to maintain apple trees for optimal apple production are pruning, thinning, and pest management.

Pruning is an essential step in apple production. The purpose of pruning is to maintain the shape and structure of the tree so a strong framework will support fruit production. Pruning is usually done during the dormant or spring season. However, summer pruning is also done to control the growth of unwanted branches (Crassweller 2003). Apple trees, like many other trees, produce suckers in the spring. A sucker is an enlarged trunk growing on the side of the main branch at the bottom of the tree. Suckers can be detrimental to production if they are not removed because they compete with the tree for available water and nutrients. The suckers around the rootstock could grow to be as big as or bigger than the tree. A lopper is used for pruning suckers on apple trees. A lopper is a large scissor-like cutting device, strong enough to cut branches of the tree.

Thinning apple trees is an important step toward improving apple quality. Thinning is done to improve fruit size, to attain productivity, to improve quality and to avoid tree

breakage. Too many apples on the tree can cause the tree to use all its resources that year and decrease productivity the next season. Failure to thin early and adequately could affect tree growth, especially in dwarf apple trees. Thinning helps the tree to focus its resources on fewer fruit so fruit can be larger and have better quality. "To thin" means to provide 6 to 8 inches between fruit. Thinning should be done when fruits are smaller rather than larger, so less energy that has been put into apple growth is lost. Thinning is done using a chemical method, such as hormone-type chemical and pesticide, or a hand method using a clipper. A clipper is a scissor-like cutting device, strong enough to cut small branches of the tree.

Pest management is an important factor in apple production to prevent apples from insects and diseases. Pest management could be done by spraying trees about 7 to 10 times per season. The fewer apples with insect damage and diseases, the more high quality apples are produced. Integrated Pest Management (IPM) is a technique used to enhance sustainability of farms (Bessin and others 2003). Apple growers apply IPM procedures as needed based on orchard monitoring and predictive models of activities of certain insects and diseases rather than using a calendar schedule (Bessin and others 2003).

Before harvesting, a starch-iodine test is conducted to determine the maturity of the apple based on the starch to sugar ratio (Cowgill and others 2003). Apples are selected randomly from the lot of apple trees that will be harvested. The apples are sliced in half and an iodine solution is placed on the flesh of the apple and observed for a few minutes. The iodine solution can be made by dissolving 10 grams of iodine crystals and 25 grams of potassium iodide in 1 liter of water (Cowgill and others 2003). Dark blue color on the apple creates a different type and color of shadow depending on the amount of starch in the apple.

Iodine will react in immature apples with starch and will leave dark blue characteristic pattern. Iodine will not react in mature fruit because iodine does not react with sugar. The darker the shadow, the more starch is contained in the apple which means the apple is not ready to be harvested. The shadow is matched to the degree of ripeness chart to decide if the apples are ready to be harvested. The chart tells the maturity of the apple from score 1 (lowest score) to score 9 (highest score) to decide whether apples are ready to be harvested depending on the use of the apples. If apples are going to be stored in a cold room, apples with a lower maturity score will be harvested. If apples are going to be sold to the fresh market, apples with a high maturity score will be harvested (Chu 1988).

During harvest, apples are picked from the trees and put in canvas bags or lined buckets. Then, the apples are transferred to wooden boxes and loaded to the trucks. Fallen apples are separated out because of the possibility of contamination from animal fecal matter and soil. Apples might be contaminated with *E. coli* O157:H7 from bird droppings and feces of domestic or wild animals (Janiesievics and others 1998). If fallen apples are not separated, they could bring potential contamination to consumers of whole apples or apple products, such as apple cider.

After harvest, farmers usually separate and grade apples based on the quality of the apples. A high quality apple has no blemishes or bruises, has a good shape and large size. The appropriate size of a high quality apple will be determined by the buyer and the seller (USDA 1961). This high quality apple will be sold as a whole apple for retail sales. A lower quality apple is an apple that has skin blemishes or abnormal shape or size (Somogyi and others 1996). Most low quality apples are used for apple processing such as apple cider,

applesauce, sliced dried apples and canned products such as apple pie filling, whole baked apples, and spiced apple products (Somogyi and others 1996).

Good agricultural practices (GAP)

Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) have been introduced to farmers to improve the quality and the safety of apples during apple production. The Food and Drug Administration (FDA) announced the availability of a document "Guidance for Industry-Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" in October 1998 (Smith 1998). Good Agricultural Practices and Good Manufacturing Practices are used to minimize food safety hazards common to the growing, harvesting, packing and transport of unprocessed or minimally processed fruits and vegetables. Farmers need to be aware that foodborne illnesses, such as bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome), associated with apple cider potentially come from on-farm contamination and could be caused by *E. coli* O157:H7, *Salmonella sp.*, *Listeria*, and other pathogenic bacteria. Although it is impossible to produce microorganism-free products, it is possible to reduce potential contamination from the farm.

Potential contamination may be from soil, irrigation water, animal manure, inadequately composted manure, wild and domestic animals, inadequate field and plant worker hygiene, harvesting equipment, transport containers, unsanitary handling, transport vehicles and so forth (Rangarajan and others). GAP have been introduced to reduce *E.coli* O157:H7 contamination on apples and improve the safety of apple cider. Good Manufacturing Practices (GMPs) have also been established in packing and cider processing facilities to reduce the risk of contamination on apples and apple cider (Diehl 2000).

Sanitation Standard Operating Procedures (SSOPs) have been developed specifically for cider production by some state agencies and Canada (USDA 1999a).

E. coli

Escherichia coli is a gram-negative, rod-shaped bacteria in the family of *Enterobacteriaceae*. *E. coli* is a type of bacteria that lives in the intestines of animals and is an indicator of fecal contamination (Jay 2000; Padhye and Doyle 1992). Animal feces or manure from deer and birds present on a farm can be a potential hazard in apple cider. Apples that fall from trees and are collected may be contaminated with *E. coli*. For this reason, the FDA recommended that "drop apples" not be used for cider production.

Not all *E. coli* are harmful bacteria. *E. coli* has been classified based on serogroups (Bell and Kyriakides 1998). There are over 200 O serotypes of *E. coli* that have been recognized (Jay 2000). *E. coli* is grouped into six groups based on virulence properties, clinical syndromes, differences in epidemiology, and O:H serogroups: enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), enteropathogenic (EPEC), diffusely adherent (DAEC), and enterohemorrhagic (EHEC) (Bell and Kyriakides 1998; Buchanan and Doyle 1997; Gyles 1992; Padhye and Doyle 1992).

E. coli O157:H7 can cause serious illness and potentially can be fatal. *E. coli* O157:H7 contamination in food can cause bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome) in humans. In 1980, *E. coli* O157:H7 was first associated with apple cider after an outbreak in Canada (Steele and others 1982). In 1991 and 1996, *E. coli* O157:H7 was associated with apple cider in Massachusetts and the western

United States, respectively. In 1996, *E. coli* O157:H7 was associated with one individual's death after drinking unpasteurized apple cider (FDA 1996).

Regulation and HACCP

Since *E. coli* O157:H7 were associated with several apple cider outbreaks, it became important to improve the safety of apple cider for general consumption so there would not be any illnesses from drinking cider. The Food and Drug Administration established a regulation that required a warning label on fruit juices that have not been pasteurized. The warning statement says: "WARNING: This product has not been pasteurized and, therefore, may contain harmful bacteria that 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) (FDA 1998a). Children, the elderly and persons with weakened immune systems have higher risk of foodborne illnesses because their immune systems are not as healthy as normal healthy adults.

The FDA also passed a regulation for a 5-$\log_{10}$ reduction in microorganisms to be obtained in fruit juices (FDA 1998b). A guide for apple handlers and apple cider producers was released to minimize the risk of microbial contamination (FDA 1998b). The FDA also required that apple cider producers apply Hazard Analysis Critical Control Point (HACCP) program as a part of their mandatory preventive measurements by 2004 (FDA 2001).

A Hazard Analysis Critical Control Point program can assure quality and safety of apple cider when followed thoroughly by the apple cider producers. HACCP is a preventive program that involves seven principal that allow food industry to evaluate each of their processing step by step.

According to Jay (2000), the seven principles of HACCP are:

1. Conduct a hazard analysis.
2. Determine the critical control points (CCPs).
3. Establish critical limits.
4. Establish monitoring procedures.
5. Establish corrective actions.
6. Establish verification procedures.
7. Establish record-keeping and documentation procedures.

The HACCP principles require that a HACCP procedure be followed for each processing step and that hazard analysis, critical control points and control limits should be established for all potential hazards during processing. HACCP uses critical control points as a key to check the safety of product. For apple cider producers, the critical control points are exclusion of drop apples, a sanitation dip or spray for apples, temperature control of cider, and if pasteurization involved, the time and temperature of pasteurization (Senkel and others 1999).

Irradiation process

Other methods to eliminate *E. coli* (besides pasteurization) include high pressure, ozone treatment, ultraviolet light and irradiation (Sizer and Balasubramaniam 1999). The term irradiation comes from ionizing radiation. Irradiation is the process of exposing food to high levels of radiant energy to reduce or eliminate potentially dangerous microorganisms (USDA 1999b). Irradiation sources that can be used for food irradiation come from radionuclide or machine sources (Urbain 1986). Radionuclide sources permitted are cobalt-

60 and cesium-137, which emit gamma rays and machine sources which produce X-rays or electron beams. These high-energy beams have wavelengths that kill microorganisms that cause spoilage or food-born illnesses (Potter and Hotchkiss 1995). Cobalt-60, electron beams and X-rays are the most commonly used for food irradiation because of their cost, functionality and environmental characteristics (Satin 1993). Irradiation can be an alternative to chemical additives for preserving foods and can be applied to fresh fruits and vegetables for the purpose of controlling disease and deterioration (Monk and others 1995).

Irradiation does not make food sterile. Irradiation can reduce microorganisms, but not eliminate all organisms. The electron beam destroys the unwanted microorganisms, but it does not remain in the food. Irradiation can also be used for food preservation, microorganism control, sprouting control, ripening control and insect damage control (Potter and Hotchkiss 1995). Irradiation extends the shelf-life of food so it can be stored for longer period of time prior to cooking. Refrigerated ground beef (2.5 kGy) that has been through irradiation has two and a half times longer shelf-life compared to non-irradiated beef (Niemand and others 1981). The level of radiation controls the amount of energy used to destroy microorganisms. Each food has different energy level requirements needed to make it safe. The units of radiation that are commonly used are kiloGrays (kGy) or rads.

Irradiation has been used for a wide variety of foods such as fruits and vegetables, poultry, beef, wheat, potatoes, flour, spices, tea, pork, turkey and beef (Jay 2000). The USDA approved the irradiation of meat in December 1999 after the FDA determined that irradiation of raw meat was safe (USDA 1999b). Irradiated foods are required to have a radura symbol and the phrase "treated with radiation" or "treated by irradiation" on the packaging (Pauli and Tarantino 1995).

Recently, 40 countries have permitted irradiation as a treatment for one or more foods (Molins and others 2001). Irradiation of poultry has been approved in 12 countries, meat irradiation has been approved in 8 countries, and seafood irradiation has been approved in 13 countries (Molins and others 2001). In the United States, the Food and Drug Administration has approved food irradiation with specified doses permitted for specific foods (21 CFR 179.26). Fruit juice has been approved for irradiation by the FDA at 1 kGy (FDA 1998c)

Consumers acceptability of irradiation

Irradiation has been a method of food preservation since the 1950's but it is still a new and unfamiliar method of preservation to most people (Frenzen and others 2001). Only 53% of supermarket shoppers were aware of food irradiation in 1996 (Food Marketing Institute 1996). Food irradiation has been a controversial issue in the United States and other countries over the years because of the misconception consumers have about food irradiation. Over 60% of adults in a national survey were concerned that irradiated foods might be radioactive or capable of causing cancer or birth defects (American Meat Institute Foundation 1993). In a national survey, 69% of supermarket shoppers believed that irradiated foods were a health risk (Food Marketing Institute 1997). Food Marketing Institute conducts programs in research, education, industry relations, and public affairs to its member of food companies. Misconceptions about food irradiation have made the benefits of food irradiation less visible. The main problem is that the consumers need to be more informed about food irradiation (Olson 1998).

Frenzen and others (2001) conducted a telephone survey of 10,780 adults and 49.8% were willing to buy irradiated meat or poultry. Mailed questionnaires were sent to 918

consumers in the Atlanta, GA area and the results indicated that 72% of 918 respondents were aware of irradiation and 30% of respondents believed that irradiated food was radioactive (Resurreccion and others, 1995). Fox (2002) showed that counteracting negative information can positively affect consumers' willingness to buy irradiated foods. Marketing efforts should focus on women because women were less likely to be classified as proponents (those who always preferred irradiated products) of irradiation, especially with the presence of children under 18. Therefore, women need to be given more positive information before they will accept irradiated foods (Fox 2002).

Consumers need to be more informed about the irradiation process so they know that consuming irradiated foods is safe (Olson 1998). Although irradiation uses radioactive elements to produce high-energy beams, food does not contain any radioactive materials after it is processed. The energy kills microorganisms through the destruction of DNA but does not stay in the food (Jay 2000). By comparison, the human body goes through the same thing as irradiated food when they are X-rayed. The energy from X-rays does not stay in the body. After food is irradiated, the high-energy beams are gone too. Therefore, food irradiation does not make food radioactive. The World Health Organization approved irradiation as "unconditionally safe" up to 7 kGy (Jay 2000).

The other concerns that consumers have about food irradiation are that food can become toxic from chemical compounds formed during irradiation and that food loses nutritional content. The compounds that are formed in irradiated food are usually the same as ones formed by heat (Olson 1998). According to CDC (2003), there are no significant changes in the contents of amino acids, fatty acids or vitamins when food is irradiated at levels approved by the FDA. However, the vitamin thiamine is slightly reduced but not

enough to result in vitamin deficiency (CDC 2003). Liuzzo and others (1966) also reported that an irradiation dose of 2 to 6 kGy would partially destroy B vitamins.

The quality of foods could change by irradiation. Apple juice was reported to have a lighter color (bleaching effects) when irradiated (Asselbergs and others1958; Fan and Thayer 2002). Bhushan and Thomas (1998) reported that irradiation doses of 0.1 to 0.6 kGy did not cause any apparent effects on color, firmness, taste and aroma of whole apples. Boylston and others (2002) reported that ascorbic acid and carotenoid contents in papayas, rambutans and Kau oranges were not affected by irradiation at 0.75 kGy.

When the advantages of the irradiation process such as nutritional value, safety of workers from irradiation process and environmental safety from radioactive material are addressed, the majority of consumers will respond positively to purchase irradiated foods (Bruhn 1995). Communication to public about all factors that could be a concern regarding irradiation process will benefit consumer acceptability of irradiated foods.

Preservatives in cider

Preservatives, such as potassium sorbate and sodium benzoate, are important compounds in apple cider to extend the shelf-life. Sorbic acid and its salts, such as sodium and potassium, are widely used to inhibit yeasts and molds in fruit juices, bakery and dairy products (Jay 2000). Yeast and mold growth in apple cider can be inhibited by potassium sorbate (Miller and Kaspar 1994).

In aqueous solutions, sorbic acid was reported to undergo autooxidation, forming malonaldehyde and other carbonyls (Arya 1980). Hildegard and Sabalitschka (1965)

reported that an aqueous solution of sorbic acid underwent decomposition, forming acrolein, crotonaldehyde, and malonaldehyde.

Sorbic acid ($CH_3CH=CHCH=CHCOOH$) is a six-carbon compound that is effective for inhibition of yeasts and molds (Baroody and McLelland 1986). Potassium sorbate is very water soluble (CRC Handbook of Food Additives 1955) and its physical appearance of potassium sorbate is white and fluffy. Sorbic acid is generally recognized as safe (GRAS) by the FDA (2002a) described in 21CFR182.3089. The antimicrobial activity of sorbic acid increases as the pH decreases in food products (Li and others 1989). The maximum usage of sorbic acid in cider is 0.1% by the FDA described in 21CFR 150.141 (Sofos and Busta 1980; FDA 2002b).

According to Fischer and Golden (1998), pasteurization or irradiation did not decrease the yeasts and molds in cider. Because of this, preservative is added to decrease the yeasts and molds in cider. Zhao and others (1993) reported that sodium benzoate worked better than potassium sorbate. Potassium sorbate did not affect the survival of *E. coli* O157:H7 at 8°C and 25°C. *E. coli* O157:H7 survived 15 to 20 days at 8°C or 1 to 3 days at 25°C in apple cider with potassium sorbate (0.1%) compared to 2 to 10 days at 8°C or 1 to 2 days at 25°C in apple cider with sodium benzoate (0.1%) (Zhao and others 1993).

Dock and others (2000) reported that the D-values of *E. coli* O157:H7 could be reduced by the addition of sorbate, benzoate and malic acid, individually and in combination. Malic acid and benzoate were more effective in reducing *E. coli* O157:H7 compared to sorbate. As the temperature increased, D-values decreased with the addition of benzoate (0.1%) to cider (Dock and others 2000).

A combination of preservative and processing treatments is the best method to reduce pathogenic microorganisms in apple cider. Asselbergs and others (1958) reported that after irradiation at 621,000 rads (6.21 kGy) in the presence of 0.05% sodium sorbate and 0.75% ascorbic acid, apple juice can be kept at room temperature for 13 days without microbial growth. Comes and Beelman (2002) reported that the combination of fumaric acid (0.15%) and sodium benzoate (0.05%), followed by holding at 25°C for 6 h before 24 h refrigeration at 4°C achieved a 5-log reduction of *Escherichia coli* O157:H7 in apple cider.

There is anecdotal evidence that preservatives affect the flavor of apple cider but little current data. Sodium benzoate (0.1%) in cider had an undesirable flavor in cider (Fabian and others 1935; Tressler and Joslyn 1954). In feta cheese, the addition of sorbic acid was associated with the development of an off-flavor described as "plastic paint" or "kerosene" (Horwood and others 1981). Marth and others (1966) suggested that potassium sorbate could be degraded to pentadiene by *Penicillium sp.* molds which were isolated from sorbate-treated feta cheese.

Sorbic acid, when irradiated, was reported to undergo degradation in aqueous solution and the radiolytic degradation product(s) had higher antimicrobial activity than the parent compound (Ishizaki and others 1972). According to Thakur and others (1990), sorbic acid was more stable in alcohols and vegetable oils than in aqueous solutions. Sorbic acid was also found to degrade faster at a lower pH (Arya 1980).

Flavor isolation

Flavor isolation in food can be done by various methods depending on the volatility of flavor compounds in foods (Reineccius and Anandaraman 1984). Four methods used for

flavor isolation in food are headspace analysis, steam distillation, molecular distillation and solvent extraction. Headspace analysis can be done by direct injections, headspace concentration, cryogenic trapping and adsorbent traps. Steam distillation is a flavor isolation technique that uses volatility of flavor components and nonvolatility of most other food constituents. The stream strips volatiles from the food and the vapors co-condense in the condensate trap. Molecular distillation involves direct transfer of a volatile from food sample to a cold condenser. Solvent extraction is used to extract volatiles from food directly or to recover volatiles from dilute aqueous flavor distillates (Reineccius and Anandaraman 1984).

Headspace sampling with gas chromatography analysis (GC) is commonly used to evaluate volatile flavor compounds. Reineccius and Risch (2002) described the procedures for headspace flavor analysis. It starts by purging the volatiles from a liquid or food with an inert gas and collecting the volatiles onto an adsorbent matrix (fiber) or the volatiles are cryo-trapped by using liquid nitrogen or solid carbon dioxide in acetone. The volatiles are then thermally desorbed by injecting them into the GC port or the solvent extracts the volatiles from the adsorbent/cryo-concentrate.

The advantage of this method is that volatiles are highly enriched to enable chemical detection. There is minimal formation of thermally induced artifacts and the methods are easy, reproducible, and in some cases, automated. However, the disadvantage of this method is that different adsorbents or solvents could give selective recoveries. In headspace analysis, polar and high molecular compounds are often discriminated against non-polar ones. Another disadvantage of this method is that the moisture in a food system may limit the purge temperature and volume of sample that can be injected. This method can be time-and labor-intensive (Reineccius and Risch 2002).

Solid-phase microextraction (SPME) is a newer adsorption technique used for headspace gas chromatography (Yang and Peppard 1994). A SPME device is a fused silica fiber coated with polydimethylsiloxan or other materials depending on the polarity of the volatiles. The fiber is placed into a vial for adsorption of sample volatiles. For fruit juice analysis, SPME was introduced into the gas chromatography injector in splitless mode. It is important to keep the sample headspace as small as possible to obtain higher sensitivity (Yang and Peppard 1994). The advantage of SPME is that the method is very simple and easy to use because it does not need any solvent. It can be used for solid, liquid and gaseous samples. Chin and others (1996) showed that SPME-gas chromatography was a suitable method to identify cheese volatile profiles.

Apple compounds usually consist of alcohols, aldehydes and esters (Somogyi and others 1996). According to Poll (1983), ethyl-2-methyl butyrate, hexyl acetate, hexanal, trans-2-hexenal, and unsaturated C-6 alcohols were important compounds for fruit aroma of apple juice.

Table 1 – Flavor compounds found in apple juice/cider

Apple Compounds	Description
Hexanal	"Green apple" aroma
Hexyl acetate	Apple, floral aroma
Ethyl butyrate	Fruity, fragrant aroma
Ethyl-2-methyl butyrate	"Ripe apple" aroma
Trans-2-hexenal	"Green apple" aroma
Butyl acetate	Fruity, banana, pear aroma
Isopentylacetate	Fruity, banana, pear aroma

Source: Somogyi and others (1996) and Poll (1983)

Poll and Flink (1984) compared sensory responses with headspace gas chromatographic measurements and reported that an increase in off-aroma could be related to an increase in alcohol percentage in the headspace. The effect of irradiation on the flavor and sensory characteristics of apple cider was reported by Boylston and others (2003). Butyl acetate, 2-methyl butyl acetate, hexyl acetate and ethyl hexanoate decreased in pasteurized cider and 2-furfural and 5-hydroxymethylfurfural increased in pasteurized and irradiated apple cider compared to raw cider. A cooked flavor was noted by the sensory panel and was associated with the increase of 2-furfural and 5-hydroxymethylfurfural. Boylston and others (2003) reported that pasteurized and irradiated cider were not different in aliphatic alcohols and aldehydes compared to raw cider.

Blanco-Gomis and others (2001) evaluated the total fatty acids in apple cider by gas chromatographic analysis. The sources of fatty acids in cider could come from apples or the production of alcoholic fermentation. In this study, 12 fatty acids were identified and 4 (caproic, caprylic, capric, and palmitic) were major fatty acids in apple ciders. There were more saturated fatty acids than unsaturated fatty acids found in apple cider. This study is important for the identification of the fatty acid profile in cider which would be useful for recognizing the region of origin for the apples of ciders and for obtaining products with a high sensory quality, determined by the blend of apple cultivars.

A study of aroma compounds of 'Royal Gala' apple flavors was conducted using gas chromatography by Young and others (1996). 2-Methylbutyl acetate, butyl acetate, hexyl acetate, butanol, 2-methylbutanol and hexanol were major components in 'Royal Gala' apples. 2-methylbutyl acetate had the most effect on the sensory attributes of 'Royal Gala'

apples. Butanol was the most abundant component and had an impact on the aroma and flavor attributes of the apple flavor (Young and others 1996).

Sensory evaluation

Consumer acceptance of particular products can be measured using sensory evaluation. Human sensory data is the best model for how likely consumers are to perceive and react to food products in real life (Lawless and Heymann 1998). Acceptance and preference tests can be used for consumer tests. An acceptance test, such as a hedonic scaling, is a test that determines degree of liking for food products (Peryam and Girardot 1952). The most common hedonic scale is the 9-point hedonic scale, although 7-point and 5-point, are also used when it is felt that consumers will not express extreme reactions (Lawless and Heymann 1998). The scale is categorized by responses based on likes and dislikes.

In paired preference tests, consumers receive two coded samples and are asked to choose which sample they prefer. This test is designed to answer one question and consumers are responding to the product as a whole. The probability of this test is one chance in two (Lawless and Heymann 1998).

Comes and Beelman (2002) reported that consumers rated raw cider with preservative lower than pasteurized cider. At least 70 consumers were asked to evaluate raw, pasteurized and preservative-treated (raw) apple cider (0.15% fumaric acid, 0,05% sodium benzoate, held for 6 h at 25°C followed by 24 h at 4°C) on a 9-point hedonic scale. Sensory evaluations were conducted on early and late season ciders. Consumers rated preservative-treated ciders in the "like slightly" (6.45) category for the early season cider and in "neither like nor dislike" (5.62) category for the late season cider.

Acceptability of cider was also evaluated by Ingham and Schoeller (2002). Cider treated by multi-step intervention system (addition of 0.05% sodium benzoate and 0.05% potassium sorbate, warm hold at 35°C for 6 h, freezing and thawing) and cider treated by pasteurization (68.1°C for 14 s) was scored on 7-point hedonic scale. Consumers rated pasteurized cider higher (6.1) compared to the multi-step system cider (5.6).

Consumers had no preference of two apple ciders pasteurized using different temperature-time conditions (Mak and others 2001). One type of cider was pasteurized at 68.1°C for 14 s and the other at 71.1°C for 6 s. Unscreened panelists evaluated two ciders and asked "Which sample do you prefer the most?" on a 7-point hedonic scale. Mak and others (2001) reported that consumer acceptance of pasteurized cider was high.

Another technique of sensory evaluation is a descriptive analysis test which uses trained panelists to evaluate characteristics of products. Descriptive analysis describes objective measurement of important attributes in products. Panelists are trained to produce accurate and consistent data that would be reproducible. Training sessions are used for this test to teach panelists important attributes of the products. The objective of training is to teach panelists to use the same concept and to be able to communicate precisely with one another (Lawless and Heymann 1998). Descriptive analysis often uses a line-scale to measure the intensity of product attributes. A 15-cm line-scale is usually used for descriptive analysis with one end of the scale representing no intensity of the attribute and the other end of the scale representing strong intensity of the attribute.

Descriptive analysis tests were conducted by Wang and others (2003) in raw, pasteurized and irradiated ciders at 2 and 4 kGy. Panelists found an off-flavor called "cardboard-like" flavor in irradiated ciders. Other attributes used by their study were

sweetness, sourness, astringency, apple flavor and cooked apple flavor. Wang and others (2003) reported that sweetness, sourness, astringency, and cooked flavor were not different in all samples. Apple flavor was higher in raw cider compared to irradiated ciders at 2 and 4 kGy. "Cardboard-like" flavor was higher in irradiated ciders at 2 and 4 kGy compared to raw cider.

Zegota (1991) reported that undesirable flavors of fermented, moldy, musty juices or other strange odors and after-tastes were evaluated by trained panelists. Panelists noted a slight flavor of dried apple, slightly sharper in odor and sharp, strange bitter taste were noted in irradiated apple juice with 2.0 kGy dosage (Zegota 1991). This study was conducted to evaluate the sensory properties of irradiated apple juice concentrate. Dosages of 0.5, 1.0, 1.5 and 2 kGy were used to determine any differences in color, clarity, odor and taste.

Sensory evaluation has been used widely to measure consumers' acceptability and characteristics of food. The results obtained by sensory evaluation of apple juice and cider reported an off-flavor in irradiated apple juice/cider. Using consumer evaluations, consumer's acceptance of apple juice and cider were high. These results are helpful in looking at the quality and characteristics of ciders and as references for research in the future.

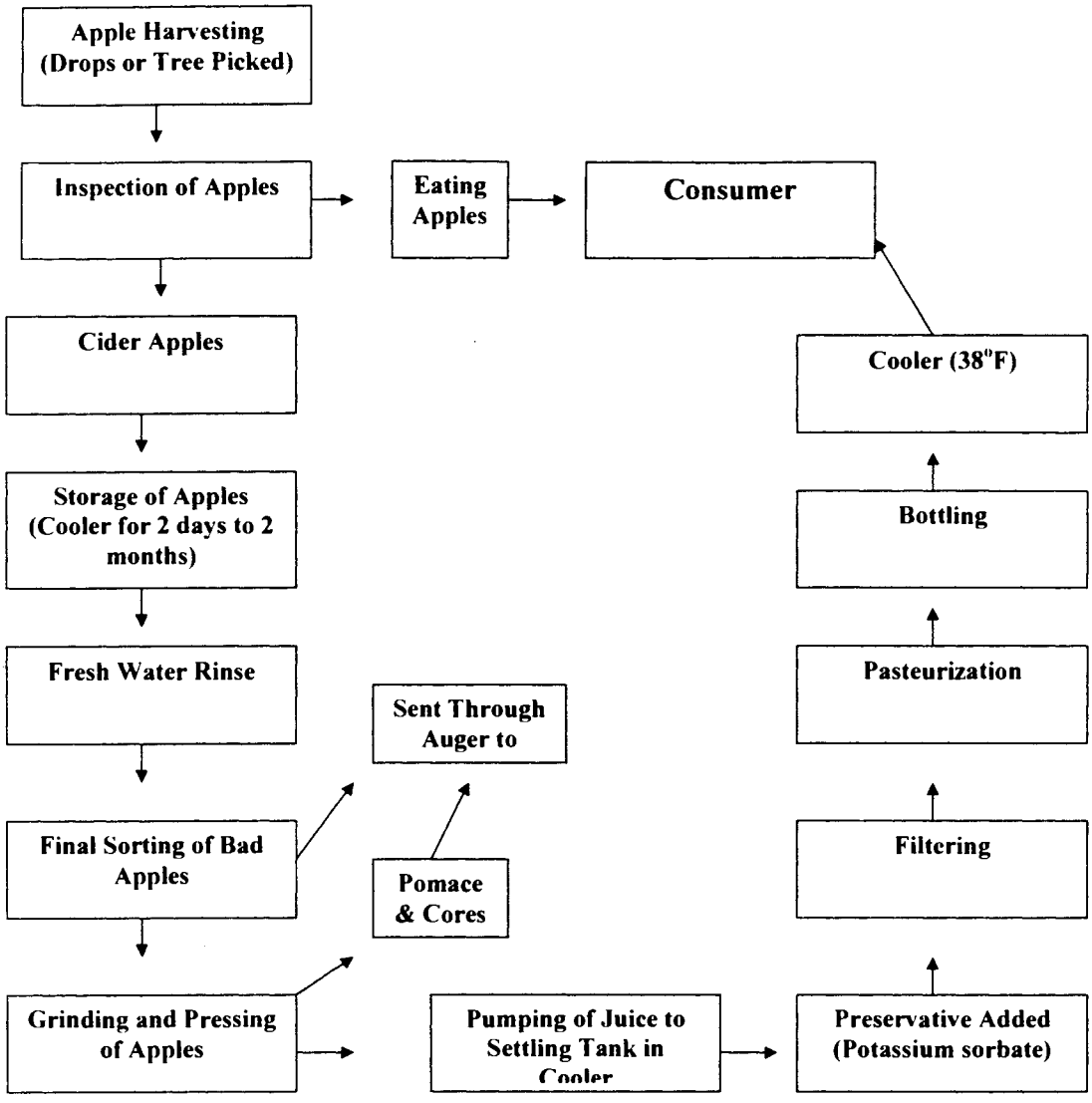

Figure 1 – Apple cider production (Cummins 2001)

Chapter 2. Sensory Evaluation and Flavor Characteristics of Pasteurized and Irradiated Apple Cider with Potassium Sorbate

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Abstract

The objectives of this research were to determine consumer preference for irradiated or pasteurized apple cider and to identify flavor compounds in apple cider by gas chromatography (GC).

Four consumer tests were conducted in Iowa. Consumers (n=599) were presented samples of irradiated apple cider with 0.05% potassium sorbate and pasteurized apple cider with 0.05% potassium sorbate. Color, pH, titratable acidity and soluble solids content were measured. A storage study was conducted to evaluate any changes in flavor compounds of apple cider over six weeks. Flavor compounds of apple cider were quantified using solid-phase microextraction (SPME) headspace analysis and gas chromatography (GC).

Consumers (n=199) at two locations had no preference for irradiated or pasteurized apple cider. Consumers at two different locations preferred irradiated cider (n=172 and 61) compared to pasteurized apple cider (n=128 and 39, respectively). The quality attributes of irradiated and pasteurized ciders were not different. Most flavor volatiles in pasteurized and irradiated ciders of consumer tests were not different. Higher concentrations of desirable apple compounds in irradiated cider may explain the preference for irradiated apple cider at 2 sites.

Irradiated apple cider was acceptable or more acceptable than pasteurized apple cider. No significant differences in the contents of key apple flavor compounds in irradiated and pasteurized ciders may result in a preference for either sample in consumer tests.

Introduction

Several outbreaks associated with *E. coli* O157:H7 in raw apple cider caused the Food and Drug Administration to examine the safety of apple cider (FDA 1996). The FDA issued a regulation for a warning label for fruit juices that are not pasteurized (FDA 1998a) and also issued a regulation for a 5-$\log_{10}$ reduction in microorganisms in fruit juices (FDA 1998b). A guide for apple producers and apple cider processors was released to aid processors and to minimize the risk of microbial contamination in cider (FDA 1998b). The FDA also required that apple cider producers apply a Hazard Analysis Critical Control Point (HACCP) program as a part of their mandatory preventive measurements by 2004 (FDA 2001). In 2003, the FDA provided guidelines for small and very small apple cider businesses to implement HACCP (FDA 2003).

Pasteurization is commonly used by apple cider processors to meet the 5-log microbial reduction requirement. However, pasteurization has an adverse effect on the color, flavor and viscosity of cider (Fisher and Golden 1998). Treatments with UV light, ozone and irradiation have also been used to reduce the microbial content in apple cider by 5-logs (Sizer and Balasubramaniam 1999).

Irradiation is an alternative method that can be used to achieve a 5-log microbial reduction in apple cider. According to Buchanan and others (1998), a dose of 1.8 kGy was needed for a 5-$\log_{10}$ reduction of *E. coli* O157:H7 in apple juice. Wang (2002) noted that the

effective dose of irradiation to achieve a 5-log reduction for *E.coli* O157:H7 in apple cider was between 1.70 to 2.47 kGy.

Irradiation has been a method of food preservation since the 1950's but it is still a new and unfamiliar method of preservation to many people (Frenzen and others 2001). Frenzen and others (2001) conducted a telephone survey of 10,780 adults and reported that 49.8% of respondents were willing to buy irradiated meat or poultry when asked if they would buy irradiated meat or poultry if it was available. Resurreccion and others (1995) indicated that 72% of consumers were aware of irradiation and 87.7% indicated that consumers had heard about irradiation but did not really know that much about the process. From this study, 30% of consumers also believed that irradiated food was radioactive.

Irradiated fruits such as mangoes, Hawaiian papayas, apples, and strawberries were evaluated in marketing studies by Bruhn (1995). Irradiated mangoes were sold successfully in Florida in 1986. In a 1-day trial sale, irradiated Hawaiian papayas outsold the identically nonirradiated counterparts by greater than ten to one in 1986. Irradiated apples were also favorably received in Missouri (Bruhn 1995). Irradiated strawberries, grapefruit and juice oranges continue to outsell their nonirradiated counterparts in a specialty produce store in Chicago, IL (Bruhn 1995).

Although pasteurization and irradiation can be used to reduce microbial content of bacteria such as *E. coli* O157:H7, these methods do not decrease yeast and mold contents in cider in significant amounts (Fischer and Golden 1998). Preservative is a necessary addition to apple cider because preservative can extend the shelf-life of cider by limiting yeast growth, which is the primary spoilage organism in apple cider (Jay 2000). Potassium sorbate

and sodium benzoate are common preservatives used in fruit juices including apple cider (Chipley 1993; Jay 2000).

A combination of preservative and processing treatments seems to be the best method to reduce both pathogenic and spoilage microorganisms. Asselbergs and others (1958) reported that after irradiation at 621,000 rep (6.21 kGy) in the presence of 0.05% sodium sorbate and 0.75% ascorbic acid, apple juice can be kept at room temperature for 13 days without microorganisms growth. Comes and Beelman (2002) reported that combination of fumaric acid (0.15%) and sodium benzoate (0.05%), followed by holding at 25 °C for 6 h before 24 h of refrigeration at 4 °C achieved 5-log reduction of *E. coli* O157:H7 in apple ciders.

The quality of apple cider is as important as the safety of apple cider. Apple quality, cultivar, maturity, and processing will affect the volatile flavor compounds in apples and apple products (William and others 1980). Apple compounds usually consist of alcohols, aldehydes and esters (Somogyi and others 1996). Pasteurized apple cider had lower ester content and more cooked aroma from the formation of furfural and hydroxymethylfurfural during heating (Poll 1983). Irradiation decreased the ester content of ciders and increased the content of alcohols and aldehydes compared to pasteurization (Boylston and others 2003)

Wang and others (2003) reported that sensory panelists detected an off-flavor in apple cider. A 'cardboard-like' flavor was in cider with preservative irradiated at 2 and 4 kGy (Wang and others 2003). It is unclear if the source of off-flavor in the apple cider was from the processing treatment or from the preservative. Zegota (1991) reported that trained panelists noted that irradiated juice had "an uncharacteristic after-taste".

Processing and preservative ensures the safety of apple cider for consumers. However, consumers may not be accustomed to the flavors caused by heat and preservatives. By evaluating the sensory quality of the cider, consumer acceptance can be measured.

The objectives of this research were to evaluate consumer preference for irradiated or pasteurized apple cider, to compare raw, pasteurized and irradiated ciders with and without potassium sorbate in different temperatures and to identify flavor compounds in raw, pasteurized and irradiated apple cider by gas chromatography (GC).

Preliminary tests (paired comparison with 64 consumers, paired comparison to determine effect of temperature, and paired preference with 197 consumers) were conducted to define the samples and parameters for the consumer evaluation.

Materials and Methods

Paired comparison test

Pasteurized and raw apple ciders with and without 0.05% potassium sorbate were purchased from local producers and frozen until 3 days before the sensory evaluation was conducted. Cider was pasteurized by the processor at 160 to 165 ^{o}F for 2 seconds. Cider (150 mL) was packaged in transparent polyethylene bags (Nasco, ID No. B736, 532 mL capacity) for irradiation. Raw cider was treated by electron beam irradiation at 2.0 kGy at Iowa State University Linear Accelerator Facility (Ames, IA). After irradiation, apple cider was refrigerated (4 ^{o}C) in the bags until the day of sensory panel (1 day).

Pasteurized cider, pasteurized cider with 0.05% potassium sorbate, irradiated cider, and irradiated cider with 0.05% potassium sorbate were paired in four different combinations. The first combination was pasteurized cider with 0.05% potassium sorbate

and irradiated cider with 0.05% potassium sorbate. The second combination was pasteurized and irradiated ciders. The third combination was irradiated cider with 0.05% potassium sorbate and irradiated cider. The fourth combination was pasteurized cider with 0.05% potassium sorbate and pasteurized cider.

Tests were conducted in the Center for Designing Foods to Improve Nutrition, Iowa State University. Samples of cider (~30 mL) (21°C) were poured into 3 oz. plastic cups labeled with three-digit random numbers. Ciders were served to the panelists at room temperature with water, expectorate cup and unsalted crackers. Panelists were given the choice to swallow or expectorate the samples.

Thirty-two panelists in 2 replicate sessions (64 panelists total) were asked "Are the samples the same or different?" Students, staff and faculty of the Food Science and Human Nutrition Department were volunteer panelists. Panelists tasted the samples in individual booths with red lighting to mask the color of ciders. Panelists circled the word "SAME" or "DIFFERENT" on the score sheet. Panelists were presented with four sets of paired samples in random order to avoid bias (Lawless and Heymann 1998).

Temperature-preservative study

Pasteurized apple cider with and without 0.05% potassium sorbate was purchased (3 days before sensory evaluation) from a supermarket in Ames, IA. The apple cider was pasteurized 3-5 days prior to purchase. Apple cider was stored at 5 °C after purchase. Irradiated and pasteurized apple ciders were purchased from the same processor but were not the same batch. Irradiated apple cider was prepared as described previously. The estimated average dose was 2.30 kGy. Irradiated cider was refrigerated (5 °C) for 3 days until sensory evaluation.

Samples were served at 7 °C, 21 °C and 55 °C. Samples were poured (~30 mL) into 3-oz. plastic cups labeled with 3-digit random numbers about 2 h before evaluation. Samples for evaluation at 7 °C were refrigerated. Samples for evaluation at 21 °C were placed on the counter and covered with paper towels. Samples for 55 °C (~ 30 mL) were poured into 50-mL glass beakers and refrigerated until evaluation. The cider temperature immediately after refrigeration was 7 °C. Samples were heated in a microwave oven to 55 °C (Amana Radarange, 800 watts). Each sample for each individual panelist was placed in the center of the microwave oven and heated for 25 seconds on high. Samples (55 °C) were poured into 3-oz. plastic cups and immediately served to the panelists.

Paired preference tests were conducted in the Center for Designing Foods to Improve Nutrition, Iowa State University. Thirty-two panelists evaluated pasteurized apple cider on 3 separate days (replications) and irradiated apple cider on 3 separate days (replications). Panelists evaluated samples in individual booths. The samples were presented in random order, with four possible serving sequences (AA, AB, BA, BB). Sample A was pasteurized or irradiated cider with preservative and sample B was pasteurized or irradiated cider without preservative. Blue light was used to mask the color differences between cider samples. Water, expectorate cup and unsalted crackers were available. Panelists were given the choice of either swallowing or expectorating the samples. Panelists were asked: "Are the samples same or different?" Panelists circled the word "SAME" or "DIFFERENT" on the score sheet. Panelists were presented with 3 sets of paired samples, with each set of samples presented at different temperatures, in random order to avoid bias (Lawless and Heymann 1998). Treats were given at the end of each panel session and a monetary award was given at the end of the last session.

Paired preference test

Pasteurized and raw apple ciders with and without 0.05% potassium sorbate were purchased from local producers one week before sensory evaluation. Cider (600 mL) was refrigerated until packaging in transparent polyethylene bags (Fisher, Cat No. 01-002-51, 1.8 L capacity). Cider was pasteurized by the processor at 165 ^{o}F for 2 seconds. Raw cider was irradiated at estimated average dose of 2.0 kGy with electron beam irradiation at the Linear Accelerator Facility, ISU, Ames, IA. After irradiation, apple cider was refrigerated in the bags until the day of sensory panel.

Three different combinations of paired samples were served to consumers. The first combination was pasteurized cider and irradiated cider. The second combination was pasteurized cider with 0.05% potassium sorbate and irradiated cider with 0.05% potassium sorbate. The third combination was irradiated cider with 0.05% potassium sorbate and irradiated cider.

Three combinations of samples were distributed randomly to avoid bias. Fifty-seven consumers evaluated the first combination of samples, 68 consumers evaluated the second combination of samples and 72 consumers evaluated the third combination of samples.

Tests were conducted in the lobby of LeBaron Hall, Iowa State University. Subjects (n=197) were volunteer participants, age 18 and above. Fluorescent light was used in the location of the test. Cider samples (~30 mL) were poured into 3 oz. plastic cups labeled with 3-digit random numbers 2 hours before the preference test to let the samples come to room temperature. The temperature of samples during serving was about 21 °C. Consumers were asked "Which sample do you prefer?" of the two samples presented to them. Participants circled the 3-digit random number of the sample they preferred on a score sheet.

Consumer tests

Larger scale consumer tests were conducted to determine consumers' preference for irradiated cider or pasteurized cider. Apple cider with 0.05% potassium sorbate was purchased from local producers during the fall season of 2001. Three different batches of cider were evaluated at 4 locations. Pasteurized cider (160 to 65 °F, 2 seconds) for consumer tests 1, 3 and 4 was purchased from the same producer. Pasteurized cider (161 °F, 11 seconds) for consumer test 2 was from a different producer. Consumer test 4 used the same cider as consumer test 3 but the test was conducted one week later. The time between processing and completion of each consumer test was one week. Irradiation procedures were the same as described previously. The estimated average dose over 3 irradiation processes was 2.23 kGy. After irradiation, cider was placed in 4-L plastic containers and refrigerated for 2 days until the day of the consumer test.

Subjects (n=599) were volunteer participants, age 18 and above. Participants (n=99) for consumer test 1 were consumers at a farmer's market in Ames, IA. Participants for consumer test 2 (n=300) and 3 (n=100) were consumers at two orchards in central Iowa. Participants for consumer test 4 (n=100) were students and faculty at Iowa State University. These locations were chosen because of participants' interest and familiarity with apple cider flavor.

Ciders were placed in a cooling container on the day of the consumer test and transferred to pitchers at the test location. Cider samples (~ 30 mL) were presented to panelists in alternate order to avoid order of presentation bias. Cider was poured into 3 oz. plastic cups. The temperature of samples during serving was about 10 °C. Consumers were

asked "Which sample do you prefer?" Participants circled the 3-digit random number on a scoresheet of the sample they preferred (Appendix A).

Physical and chemical analyses

Instrumental analyses were conducted on cider used for consumer tests. Raw ciders were analyzed with pasteurized and irradiated ciders as control in the analyses. The pH of apple cider was recorded using an analog pH meter (Model IQ240, Scientific Instruments, Inc., San Diego, CA), standardized with pH 4.0 and 7.0 buffers. Titratable acidity as malic acid was determined by measuring the amount of sodium hydroxide (0.1 N) needed to titrate 20 mL of apple cider to an endpoint of pH 7.0 and calculating the percent of malic acid in cider. The soluble solids content of apple cider was measured using a handheld refractometer (Model No. 18902, Extech, Japan).

Apple cider color (L, a, and b values) was measured with a HunterLab spectrocolorimeter (Model LS5100, Reston, VA) standardized with a white color tile (X=81.6, Y=86.68, Z=91.18). Cider (50 mL) was evaluated in a 6.4 cm x 3.7 cm glass cup covered with a box to exclude light. One-half inch port size was used, and D-65 was selected as the light source.

Volatile flavor analysis

Flavor analyses were conducted to relate consumer acceptability of pasteurized and irradiated ciders to the volatile flavor profile of ciders and to evaluate change of volatile flavor compounds in these ciders during a 6-week storage study. Ciders from consumer tests were stored and analyzed within a week to compare flavor compounds with consumer tests results. These ciders were stored for 6 weeks to determine the effects of storage and processing treatments on volatile flavor compounds. Raw, pasteurized and irradiated ciders

with 0.05% potassium sorbate (250 mL) were placed in glass bottles and stored at 5 ^{0}C for 6 weeks. Raw cider was used as a control in flavor analysis when comparing the processing effects of ciders.

Solid-phase microextraction (SPME, Supelco, INC., Bellefonte, PA) techniques were used for isolation of volatile flavor compounds. Apple cider (40 g) was transferred to a 100-mL headspace bottle and sealed with a teflon septum. Cider was placed into a water bath for 45 minutes at 37 °C for absorption of volatiles onto the SPME fiber. A gas chromatograph equipped with a splitless injection port and flame ionization detector was used for the analysis of volatile flavor compounds (Model 6890, Hewlett-Packard, Inc., Wilmington, DE). The volatiles were thermally desorbed (225 °C) for 3 minutes via the GC injection port onto a fused-silica capillary column (SPB-5, 30 m x 0.25 mm 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 minutes and increased at a rate of 5 °C/min. to a final temperature of 200 °C. The detector temperature was 220 °C. 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.

A gas chromatograph-mass spectrometer (Trio 1000, Fisons Instruments, Danvers, MA) with a quadrupole mass analyzer was used for the confirmation of the identity of the volatile compounds. The GC conditions were as for the chromatographic analysis. The conditions for the mass spectrometer were set as follow: source electron energy, 70 eV; source electron current, 150 μA; ion source temperature, 220 °C; interface temperature, 220

°C; source ion repeller, 3.4 V; electron multiplier voltage, 600 V; and scan range, 41-250 m/z. Mass spectra of the volatile flavor compounds were compared to the NBS Library and a flavor and fragrance database (*20*) for identification.

Statistical analysis

The paired comparison test results and temperature-preservative test were analyzed using Smith's Test; the exact probability was determined by calculated $Z = \frac{X - 0.5 - (np)}{\sqrt{npq}}$ (Lawless and Heymann 1999). Preference test and consumer tests results were analyzed by calculated $X = (z\sqrt{n} + n + 1)/2$ with X = number of correct responses; n = total number of responses; p = probability of correct decision by chance; q = 1 – p; z = 1.64 ($P < 0.05$) (Lawless and Heymann 1999).

Data from GC analyses were analyzed using SYSTAT (SYSTAT 1999) and SAS (SAS Institute Inc. 1996). Analysis of variance and Fisher's least square difference tests ($P < 0.05$) were conducted to determine the effects of the treatments on the content of volatile flavor compounds. Data from instrumental analyses were analyzed using SAS statistical program with analysis of variance and Fisher's least square difference tests ($P < 0.05$).

Results and Discussion

Paired comparison test

Panelists differentiated between pasteurized cider with preservative and irradiated cider with preservative, between irradiated ciders with and without preservative, and pasteurized ciders with and without preservative (Table 1). Panelists detected the presence

of potassium sorbate (0.05%) in pasteurized and irradiated cider. Panelists could not differentiate between pasteurized and irradiated ciders without preservative. Potassium sorbate caused a difference in flavor compared to ciders without potassium sorbate.

Temperature-preservative study

Panelists differentiated between pasteurized and irradiated apple cider with or without preservative at 4 °C and 21 °C (Table 2). Panelists did not differentiate between pasteurized and irradiated apple cider with or without preservative at 55 °C (Table 2). Serving temperature is important for the panelists to differentiate flavor characteristics. As expected, warm temperature affected the panelists' ability to differentiate flavor characteristics because the sense of taste is less acute at extreme temperature (Penfield and Campbell 1990). For optimum flavor perception, ciders should be served at 4 °C or 21 °C.

Paired preference test

Consumers preferred irradiated apple cider with 0.05% potassium sorbate compared to pasteurized apple cider with 0.05% potassium sorbate (Table 3). Consumers had no preference for pasteurized or irradiated ciders without potassium sorbate. Consumers had no preference for irradiated ciders with and without potassium sorbate (Table 3).

Three preliminary studies (paired comparison tests, temperature-preservative study, and paired preference tests) reported that consumers could detect the addition of preservative in apple cider. The most noticeable effects were in irradiated apple cider with preservative and pasteurized cider with preservative.

Consumer tests

Consumers had no preference for irradiated or pasteurized apple cider at 2 locations (Table 4). Consumers preferred irradiated apple cider to pasteurized apple cider at 2 other locations. Some consumers commented that the two samples had different flavors.

Although consumers had no preference for either cider at 2 locations and preferred irradiated apple cider at 2 locations, the number of consumers at one of the locations was larger than the three other locations. Consumers were forced to give their preference even though they may not have had any preference for either sample. The reason that "no preference" option was not given to consumers was because the difference between pasteurized and irradiated ciders was thought to be distinctive and that consumers would be able to choose easily between the two choices. After knowing the results from the consumer tests, "no preference" option may have resulted in a more accurate description of preference for some consumers. When consumers had the same preference for both samples, "no preference" option may have given better results for the consumer tests (Lawless and Heymann 1999).

Physical and chemical analyses

The quality attributes of the cider after pasteurization and irradiation were similar, as expected. Cider obtained for consumer test 2 contained more particulate matter than other ciders. It is unclear the reason soluble solids contents was higher in irradiated cider compared to pasteurized cider since the same batch of ciders was used. Irradiated cider had higher soluble solids content, darker color and less yellow color than pasteurized cider (Table 5). The lighter appearance of irradiated cider has been noted previously (Wang and others 2003; Asselbergs and others 1958; Fan and Thayer 2002). According to Fan and Thayer

(2002), irradiated juice darkened during storage. Chachin and Ogata (1969) also reported that irradiated apple juice became brown compared to non-irradiated apple juice. Variability in apple cultivar, pasteurization or irradiation methods, or amount of particulate matter may influence color measurement.

Flavor analyses related to consumer tests

Differences in the contents of key apple flavor compounds of irradiated and pasteurized ciders may contribute to the differences in consumer preference. In cider used for consumer test 1, few compounds were significantly different between processing treatments (Table 6). This may be the explanation for no preference between pasteurized and irradiated cider.

In consumer test 2, butyl butyrate, butyl-2-methyl butyrate and hexyl butyrate were higher in irradiated cider than in pasteurized cider (Table 7). Butyl butyrate is described as a powerful, fruity, fresh aroma (FlavorWorks, Flavometrics, version 2.0, Anaheim Hills, CA). Butyl-2-methyl butyrate is an important apple aroma in ciders. Hexyl butyrate is described as green, sweet, fruity, and fresh aroma. Propyl hexanoate was higher in pasteurized cider than in irradiated cider (Table 7). Propyl hexanoate contributes a winey and cheese aroma (FlavorWorks, Flavometrics, version 2.0, Anaheim Hills, CA). In consumer test 2, consumers preferred irradiated cider compared to pasteurized cider.

In consumer test 3, 2-methyl butyl acetate was detected in higher concentration in irradiated cider than in pasteurized cider (Table 8). 2-Methyl butyl acetate (a desirable apple flavor compound) is described as a fruity, banana, and pear aroma. Propyl hexanoate and hexyl butyrate were higher in pasteurized cider than in irradiated cider. In consumer test 3, consumers preferred irradiated cider compared to pasteurized cider.

In consumer test 4, there was more hexanol in irradiated cider compared to pasteurized cider (Table 9). Hexanol is described as an alcoholic, medicinal aroma. In this consumer test, consumers had no preference between pasteurized cider and irradiated cider.

No significant differences in the contents of key apple flavor compounds in irradiated and pasteurized ciders may result in a preference for either sample in consumer tests.

Flavor analyses for 6-week storage study

Esters contents were higher at the end of storage compared to the beginning of the storage which was not expected. Poll (1983) reported that ester and aldehyde contents decreased in apple juice stored for up to a year. The flavor compounds were highly variable and difficult to interpret. Better recovery of flavor compounds was found in weeks 4, 5, and 6 than in the first three weeks of the study. It was suspected that repeated use of the fiber may be responsible for poor recovery of the flavor compounds.

Solid Phase Micro-Extraction (SPME) is known to be a rapid and inexpensive aroma isolation technique (Budin 2002). However, Budin (2002) summarized that fibers have high variability and could cause problems for shelf-life studies. Thus, the same sampling fiber must be used to maintain accuracy of results. When fibers are used repeatedly over time for more than 150 injections, high variability can result (Budin 2002).

In future research, more accurate results could be obtained by strictly monitoring the use of fiber. Changing to a new fiber every week would be sufficient. A gas chromatography-olfactometry technique uses human senses to detect significant aromas and could be more sensitive than GC technique alone.

Conclusions

Irradiated cider was as acceptable as or more acceptable than pasteurized apple cider to consumers. The quality attributes of irradiated and pasteurized ciders were not different. Most flavor volatiles in pasteurized and irradiated ciders of consumer tests were not different.

Higher concentrations of desirable apple compounds in irradiated cider may explain consumer preference for irradiated apple cider at 2 sites. No significant differences in the contents of key apple flavor compounds in irradiated and pasteurized ciders may result in a preference for either sample in consumer tests.

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Table 1 – Paired comparison tests of pasteurized and irradiated apple ciders with and without 0.05% potassium sorbate[1]

Treatment[2]	Number of correct answers
Pasteurized + P vs. irradiated + P	43[a]
Pasteurized vs. irradiated	39[NS]
Irradiated + P vs. irradiated	41[a]
Pasteurized + P vs. pasteurized	43[a]

[1] 32 panelists x 2 replications = 64 responses.
[2] + P = with 0.05 % potassium sorbate.
[a] Significant at $p<0.05$, NS = Not Significant.

Table 2 – Paired comparison tests of pasteurized and irradiated apple ciders with and without 0.05% potassium sorbate

Temperature	Pasteurized[1]	Irradiated[2]
5 °C	46[a]	59[a]
21 °C	46[a]	57[a]
55 °C	39[NS]	52[NS]

[1] 24 panelists x 3 replications = 72 responses.
[2] 32 panelists x 3 replications = 96 responses.
[a] Significant at $p<0.05$ level, NS = Not Significant.

Table 3 – Paired preference tests of pasteurized and irradiated apple ciders with and without 0.05% potassium sorbate

Treatment[1]	No. of preference		Treatment	No. of preference	Total
Pasteurized	29	vs.	Irradiated	28	57[NS]
Pasteurized + P	27	vs.	Irradiated + P	41	68[a]
Irradiated + P	34	vs.	Irradiated	38	72[NS]

[1] + P = with 0.05 % potassium sorbate.
[a] Significant at $p<0.05$, NS = Not Significant.

Table 4 - Consumer preferences tests of irradiated or pasteurized apple ciders with 0.05% potassium sorbate

Consumer test	Location	No. of preference for pasteurized cider	No. of preference for irradiated cider	Total people surveyed
1	Farmers market	51	48	99 [NS]
2	Orchard	128	172	300[a]
3	Orchard	39	61	100[a]
4	University	41	59	100 [NS]

[a] Significant at $p<0.05$, NS = Not Significant.

Table 5 – pH, titratable acidity, soluble solids content and color of pasteurized and irradiated ciders with 0.05% potassium sorbate

		Titratable acidity	Soluble solids	Color		
Treatment	pH	(g / 100 mL)	(%)	L	a	b
Irradiated + P						
Consumer Test 1	3.52	0.42	12.20	14.96	0.08	4.62
Consumer Test 2	3.65	0.34	10.15	16.31	0.64	6.08
Consumer Test 3 + 4	3.64	0.33	12.95	13.50	-0.41	3.95
Mean	3.60[a]	0.37[a]	11.77[a]	14.99[b]	0.12[a]	4.90[b]
Pasteurized + P						
Consumer Test 1	3.47	0.44	12.55	13.87	-0.12	4.68
Consumer Test 2	3.66	0.41	10.30	19.20	0.10	6.29
Consumer Test 3 + 4	3.71	0.31	11.85	14.94	-0.07	5.40
Mean	3.61[a]	0.39[a]	11.57[b]	15.99[a]	-0.04[a]	5.45[a]

[a b] Means within the same column having the same superscript are not significantly different ($P<0.05$).

Table 6 – Effect of processing treatments on volatile flavor compounds of raw, pasteurized and irradiated ciders with 0.05% potassium sorbate in consumer test 1

	Treatments					
Compound	**Raw**		**Pasteurized**		**Irradiated**	
Esters						
Ethyl butyrate	31.20	a	31.84	a	38.62	a
Butyl acetate	27.46	a	57.27	a	71.42	a
Ethyl-2-methyl butyrate	412.61	a	471.22	a	446.14	a
Methyl-2-methyl pentanoate	20.63	a	19.80	a	21.27	a
2-Methyl butyl acetate	71.28	a	95.67	a	116.34	a
Hexyl acetate	171.71	a	284.03	a	255.68	a
Propyl hexanoate	1.00	a	640.96	a	587.72	a
Benzyl acetate	1.00	b	8.75	a	9.65	a
Hexyl butyrate	1.00	b	13.34	a	1.00	b
Aldehydes						
Hexanal	37.16	a	45.09	a	58.59	a
Alcohols						
Hexanol	78.14	a	77.61	a	91.66	a
Terpenes						
Alpha farnesene	61.91	a	172.23	a	92.86	a
Unknown						
RT 22.30	1.00	b	7.94	a	1.00	b
RT 24.49	12.97	a	17.93	a	17.03	a
RT 25.55	14.44	a	26.52	a	13.15	a
RT 27.04	3.68	a	7.05	a	1.00	a
RT 27.48	4.18	a	5.74	a	1.00	a

[a-c] Means within the same row having the same superscript are not significantly different ($P < 0.05$).

Table 7 – Effect of processing treatments on volatile flavor compounds of raw, pasteurized and irradiated ciders with 0.05% potassium sorbate in consumer test 2

	Treatments					
Compound	**Raw**		**Pasteurized**		**Irradiated**	
Esters						
Isopropyl acetate	8.66	a	7.33	a	9.80	a
Ethyl butyrate	82.44	a	57.35	a	65.95	a
Butyl acetate	90.55	a	94.14	a	146.09	a
Ethyl-2-methyl butyrate	692.25	a	775.47	a	817.39	a
Methyl-2-methyl pentanoate	22.81	a	29.88	a	31.71	a
2-Methyl butyl acetate	155.31	a	158.32	a	186.64	a
Pentyl acetate	18.15	a	17.13	a	23.11	a
Butyl butyrate	10.01	b	6.76	b	19.22	a
Ethyl hexanoate	29.46	a	17.43	a	24.02	a
Hexyl acetate	288.39	a	284.10	a	498.68	a
Butyl-2-methyl butyrate	1.00	b	1.00	b	11.59	a
Propyl hexanoate	627.49	a	662.51	a	267.32	b
Heptyl acetate (t)	81.44	a	63.97	a	88.84	a
Benzyl acetate	37.63	a	31.51	a	39.69	a
Hexyl butyrate	1.00	b	1.00	b	12.64	a
Alcohols						
Hexanol	94.63	a	118.35	a	125.06	a
Terpenes						
Alpha farnesene	1.00	a	7.75	a	1.00	a
Unknown						
RT 14.79	66.64	b	96.49	b	219.10	a
RT 22.30	1.00	b	1.00	b	6.59	a
RT 24.49	38.79	a	28.18	a	65.40	a
RT 27.04	13.49	a	9.66	a	20.42	a
RT 27.48	8.28	a	3.91	a	15.74	a
RT 27.68	13.43	a	10.20	a	10.40	a

[a-c] Means within the same row having the same superscript are not significantly different ($P < 0.05$)

Table 8 – Effect of processing treatments on volatile flavor compounds of raw, pasteurized and irradiated ciders with 0.05% potassium sorbate in consumer test 3

	Treatments					
Compound	**Raw**		**Pasteurized**		**Irradiated**	
Esters						
Isopropyl acetate	12.15	a	11.97	a	12.07	a
t-Butyl acetate	10.72	a	8.13	a	17.77	a
Ethyl butyrate	23.75	a	42.04	a	20.52	a
Butyl acetate	1.00	b	113.47	ab	188.86	a
Ethyl-2-methyl butyrate	803.61	a	694.17	a	774.39	a
Methyl-2-methyl pentanoate	46.12	a	32.68	a	45.19	a
2-Methyl butyl acetate	302.21	a	245.57	b	298.76	a
Pentyl acetate	22.84	a	21.33	a	27.58	a
Butyl butyrate	21.90	a	36.50	a	23.58	a
Ethyl hexanoate	19.44	a	25.36	a	32.24	a
Hexyl acetate	463.64	a	618.79	a	431.01	a
Butyl-2-methyl butyrate	12.18	a	19.20	a	17.53	a
Propyl hexanoate	677.32	a	692.69	a	251.10	b
Heptyl acetate (t)	107.99	a	106.40	a	98.45	a
Benzyl acetate	32.04	a	48.98	a	62.31	a
Hexyl butyrate	25.61	a	23.45	a	17.42	b
Aldehydes						
Hexanal	65.08	a	53.96	a	84.35	a
Alcohols						
Hexanol	233.70	a	221.55	a	240.25	a
t-2-Nonenol	4.47	a	16.05	a	8.33	a
Terpenes						
Alpha farnesene	137.73	a	168.77	a	111.52	a
Unknown						
RT 14.79	165.04	a	84.92	a	181.40	a
RT 22.30	15.02	a	11.74	b	9.47	b
RT 24.49	41.46	a	37.84	a	103.40	a
RT 25.55	12.45	a	22.52	a	10.31	a
RT 27.04	28.44	a	11.63	a	1.00	a
RT 27.48	9.73	a	9.07	a	18.18	a
RT 27.68	19.25	a	19.11	a	22.07	a

[a-c] Means within the same row having the same superscript are not significantly different ($P < 0.05$).

Table 9 – Effect of processing treatments on volatile flavor compounds of raw, pasteurized and irradiated ciders with 0.05% potassium sorbate in consumer test 4

	Treatments					
Compound	**Raw**		**Pasteurized**		**Irradiated**	
Esters						
Isopropyl acetate	17.13	a	15.85	a	17.30	a
t-Butyl acetate	17.23	a	14.19	a	13.38	a
Ethyl butyrate	29.41	a	48.50	a	32.34	a
Butyl acetate	239.68	a	140.06	a	276.48	a
Ethyl-2-methyl butyrate	1,111.05	a	888.00	b	995.51	ab
Methyl-2-methyl pentanoate	68.60	a	48.37	a	66.69	a
2-Methyl butyl acetate	401.66	a	318.78	a	407.40	a
Pentyl acetate	31.50	a	31.26	a	29.82	a
Butyl butyrate	37.23	a	42.21	a	34.93	a
Ethyl hexanoate	35.19	a	34.17	a	34.42	a
Hexyl acetate	752.85	a	644.50	a	599.26	a
Butyl-2-methyl butyrate	26.86	a	18.99	a	25.52	a
Propyl hexanoate	362.95	a	517.31	a	255.88	a
Heptyl acetate (t)	117.82	a	129.28	a	110.69	a
Benzyl acetate	55.70	a	55.94	a	48.89	a
Hexyl butyrate	44.53	a	29.35	b	22.92	b
Aldehydes						
Hexanal	110.14	a	91.27	a	79.63	a
Alcohols						
Hexanol	327.39	a	282.92	b	333.40	a
t-2-Nonenol	14.70	a	23.45	a	12.83	a
Terpenes						
Alpha farnesene	159.76	a	203.17	a	82.66	a
Unknown						
RT 14.79	320.77	a	278.65	a	269.59	a
RT 22.30	27.23	a	16.08	b	12.79	b
RT 24.49	26.07	a	30.25	a	58.91	a
RT 25.55	31.02	a	28.75	a	13.08	a
RT 27.04	20.49	a	15.80	a	7.81	a
RT 27.48	3.10	a	5.57	a	9.52	a
RT 27.68	23.09	a	16.21	a	20.03	a

[a-c] Means within the same row having the same superscript are not significantly different ($P < 0.05$).

Chapter 3. Consumer Tests, Sensory and Microbial Analyses of Irradiated Apple Cider

A paper will be submitted to the Journal of Food Science

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Abstract

Consumers (577) rated 'degree of liking' for irradiated and pasteurized apple cider with preservative on a 7-point hedonic scale (1=dislike very much to 7=like very much). Ten trained descriptive panelists evaluated the sensory characteristics of three kinds of apple cider: raw, pasteurized and irradiated (2 kGy). Microbial contents of raw, pasteurized and irradiated ciders during 8 weeks of storage were analyzed. Consumers rated pasteurized cider and irradiated cider the same at three locations and consumers rated pasteurized cider higher than irradiated cider at one location. At all locations, consumers rated both samples in the "like moderately" category. Irradiated and pasteurized ciders were not different in sourness, astringency, apple flavor or caramelized flavor. Trained panelists identified more "musty flavor" in irradiated apple cider than in raw or pasteurized ciders. Irradiated cider without potassium sorbate contained more yeasts than pasteurized and raw ciders by the end of the eight week study.

Introduction

Several outbreaks associated with *E. coli* O157:H7 has focused attention on the need for additional processing methods to improve the safety of apple cider (CDC 1996, 1997). The Food and Drug Administration issued additional regulations for raw fruit juices. A 5-

$\log_{10}$ reduction in microorganisms was required for fruit juices (FDA 1998). The FDA has not specified the processing methods to achieve a 5-$\log_{10}$ reduction. Pasteurization is often used by apple cider processors to meet the 5-log microbial reduction requirement.

Although pasteurization improves the safety of apple cider, the quality of pasteurized ciders is different than raw cider. Pasteurization has an adverse effect on the color, flavor and viscosity of cider (Fisher and Golden 1998). Irradiation is an alternative processing method that may improve food safety without the deleterious effects of heat. A 5-log microbial reduction in apple cider can be achieved by irradiation at 1.8 kGy to 2.47 kGy (Buchanan and others 1998; Wang 2002).

The hypothesis of this study was that irradiation would maintain the flavor characteristics in cider better than pasteurization because irradiation does not heat the food. However, sensory evaluation panelists noted that irradiated cider with preservative had more "cardboard-like" flavor compared to raw and pasteurized ciders (Wang and others 2003). A preliminary work has indicated that irradiated apple cider was acceptable or more acceptable than pasteurized apple cider to consumers in central Iowa (Yulianti 2003).

The objectives of this research were to determine degree of liking of pasteurized apple cider with preservative and irradiated apple cider with preservative by consumers in central Iowa, to investigate flavor differences of apple ciders treated by pasteurization, irradiation and preservative addition by sensory evaluation, and to evaluate the growth of coliforms, aerobic bacteria, yeasts and molds during 8 weeks storage of raw, pasteurized and irradiated apple ciders and with and without preservative.

Materials and Methods

Cider processing

Apple ciders were purchased from a local producer in central Iowa. Two types of ciders were purchased: pasteurized cider with and without preservative and raw cider with and without preservative. Ciders came from the same batch, were purchased, processed and stored for each consumer test. Cider used in consumer test 4 was the same cider as cider for consumer test 3 but the evaluation was conducted one week later.

Cider was pasteurized by the producer at 165 °F for 2 seconds. Cider (600 mL) was refrigerated at 5 °C until packaging in transparent polyethylene bags (Fisher, Cat No. 01-002-51, 1.8 L capacity). Raw cider was treated by an estimated average dose of 2.24 kGy with electron beam irradiation at the Linear Accelerator Facility, ISU, Ames, IA. The time between processing and completion of each consumer test was one week. For consumer tests, cider was transferred from bags to 4-L plastic containers after irradiation and refrigerated for 2 days.

The same procedures for processing were used for consumer and descriptive analysis evaluation, but separate samples were prepared for each replication. For descriptive analysis, six types of ciders were used: irradiated ciders with and without preservative, pasteurized ciders with and without preservative and raw ciders with and without preservative. Pasteurized cider on the first replication was pasteurized at 165 °F for 2 seconds; ciders for the second and third replications were pasteurized at 175 °F for 2 seconds. Ciders were purchased, processed and stored for each replicate of descriptive analysis. Ciders were served to panelists 3 to 5 hours after irradiation. The same samples from replications 2 and 3 of descriptive analysis were used for microbial study.

Consumer tests

Consumer evaluation of pasteurized and irradiated cider with 0.05% potassium sorbate was conducted at 4 locations in central Iowa. Ciders (10 °C) were placed in cooling containers on the day of the consumer test and transferred to pitchers for serving at the test location. Cider samples (~ 30 mL) were presented in alternate order to avoid order bias (Lawless and Heymann 1998). Cider was poured into 3 oz. plastic cups labeled with three digit random numbers. Consumers were asked to rate two samples based on degree of liking on a 7-point hedonic scale (1=dislike very much to 7=like very much) (Appendix B). The serving temperature was 10 °C.

Physical and chemical analyses

The pH of apple cider was recorded using an analog pH meter (Model IQ240, Scientific Instruments, Inc., San Diego, CA), standardized with 4.0 and 7.0 buffers. Titratable acidity as malic acid was determined by measuring the amount of sodium hydroxide (0.07 N) needed to titrate 20 ml of apple cider to an endpoint of pH 7.0. Soluble solids content of apple cider was measured using a handheld refractometer (Model No. 18902, Extech, Japan).

Apple cider color (L, a, and b values) was measured with a HunterLab colorimeter (Model LS5100, Reston, VA) standardized with a white color tile (X=81.6, Y=86.68, Z=91.18). Cider (50 mL) was evaluated in a 6.4 cm X 3.7 cm glass cup covered to exclude light. One-half inch port size was used, and D-65 was selected as the light source.

Descriptive analysis

Training. Ten panelists were trained about apple cider flavor characteristics in eleven 1-hour sessions. The panelists were selected based on their ability to differentiate between ciders with and without preservative and availability. At each session, panelists evaluated and compared apple cider characteristics using different type of apple ciders and standards. In the first session, each panelist responded to a questionnaire (Appendix C). The questionnaire gave panelists an opportunity to become comfortable as a group and to provide information for the panel leader. Panelists were asked to identify samples with five basic tastes: sweet, sour, bitter, salty and astringency and to rank the intensity of the basic tastes (Appendices D and E). In the second session, panelists did a scaling exercise to familiarize them with a line scale. Panelists were asked to identify basic tastes in cider. Standards were provided with basic tastes (Table 1). The standards used for the attributes sweetness, sourness and astringency were defined by Meilgaard and others (1991) and Lawless and Heymann (1998) (Table 2). Panelists agreed that 'Golden Delicious' apple described apple flavor. Artificial burnt sugar flavor was the standard for caramelized flavor (X-tra Touch®, Triple K MFG, Co. Inc., Shenandoah, IA). There was no standard used for the musty flavor. Seven brands of apple juice and three apple cider samples with a range of intensities of basic tastes and apple flavor were presented to panelists.

Five attributes of ciders were initially determined by the panelists: sweetness, sourness, astringency, apple flavor and cooked apple flavor. The panelists decided that caramelized flavor would better describe the cooked flavor of apple cider. Panelists noted that there was another attribute that needed to be added but could not agree on a term. Some descriptors were "musty flavor", "spoiled flavor", and "earthy flavor". Irradiated apple

ciders (2 and 4 kGy) with potassium sorbate had an "off-flavor". Panelists decided the off-flavor in the irradiated samples was best described as "musty flavor". Panelists agreed on the attributes sweetness, sourness, astringency, apple flavor, caramelized flavor and musty flavor (Appendix F).

Evaluation. Raw, pasteurized and irradiated ciders with and without preservative were evaluated. Three replications of descriptive analysis evaluation were completed. Blue light was used to mask the color differences among raw, pasteurized and irradiated ciders. Blue light was more effective than red, yellow, or green light to mask the color differences in cider. Ciders were evaluated at each session by all panelists. Samples (~45 mL) in 3 oz. plastic cups were numbered with three-digit random numbers. The temperature of samples during serving was 20 – 25 °C.

Panelists used a computerized 15-cm line scale program (Compusense five, version 3.8, Guelph, Ontario, Canada) and evaluated samples in individual booths in the Center for Designing Foods to Improve Nutrition, Iowa State University. The samples were presented in random order for each panelist. Panelists were instructed to taste the samples, hold the samples in his/her mouth for at least 10 seconds, to swirl it around the palate, then either to swallow the sample or to expectorate. Panelists were allowed to re-taste the samples and change ratings. The computer program linearly transformed panelists' ratings on the line scale to numbers between 0 to 15, with "0" corresponding to ratings for "None" and "15" to "Intense" for each attribute. Reference standards, water, expectorate cup and unsalted crackers were provided during sensory evaluation sessions.

The same panelists also evaluated "musty flavor" in 2 days (replications) for eight different samples: water, water with 0.05% potassium sorbate, irradiated water (2 kGy),

irradiated water with 0.05% potassium sorbate (2 kGy), apple cider, apple cider with 0.05% potassium sorbate, irradiated apple cider (2 kGy), and irradiated apple cider with 0.05% potassium sorbate (2 kGy). This test was conducted to determine if the musty flavor was coming from the irradiation process or the preservative.

Microbiological study

Ciders used in the microbiological study were the same samples used for descriptive analysis. Two replications were completed in an 8-week storage study. Raw, pasteurized and irradiated ciders with and without preservative were stored in sterilized bottles and stored at 7 °C. Two independent samplings were taken from each bottle, diluted and duplicated in 0.1% peptone water (Difco Laboratories, Detroit, MI).

Ciders were tested for total coliform count/*E.coli* count, aerobic bacteria and yeast and mold counts. The dilution used for the coliform/*E. coli* analysis was 10^{-1} dilution. Three initial dilutions (10^{-1}, 10^{-2}, 10^{-3}) were used for the bacteria, yeast and mold analyses. Dilutions were adjusted depending on the growth of microorganisms after storage.

Coliform and *E.coli* levels were determined by using Petrifilm *E.coli*/Coliform Count Plate (3M, St. Paul, MN). Incubation time and temperature followed AOAC Official Method 991.14 (3M, St. Paul, MN). Petrifilms were incubated for 24 h at 35 °C, counted, incubated for additional 24 h and observed for any changes. Coliforms were detected by the observation of red colonies with gas production after 24 h incubation. *E. coli* was detected by the observation of blue colonies with gas production after 48 h incubation.

Aerobic bacteria were determined by surface plating (0.1 mL) serial dilutions onto Trypticase Soy Agar (TSA, Difco) and incubating at 35 °C for 48 h. All colonies on the plate were counted as aerobic bacteria.

Yeasts and molds were determined by surface plating (0.1 mL) serial dilutions onto Peptose Dextrose Agar (PDA, Difco) and incubating at room temperature, (20 – 25 °C) for 5 days. Yeasts and molds were easily distinguished by colony morphology.

Statistical analysis

Analysis of variance (SAS Institute, Inc. 1996) was used (general linear model, PROC ANOVA) to test the effect of raw, pasteurized and irradiated ciders with and without preservative on consumer tests, sensory evaluation, pH, titratable acidity, soluble solids content and color. When a significant interaction between processing and preservative resulted, interaction means were reported. Main-effect means were reported when no significant interactions were found. When F-values were significant, mean differences were compared by using PDIFF as a procedure of mean separation.

Results and Discussion

Consumer tests

Consumers rated pasteurized cider and irradiated cider the same at three locations and consumers rated pasteurized cider higher than irradiated cider at one location (Table 2). At all locations, consumers rated both samples in "like moderately" category. One possibility that explained consumers' higher rating of pasteurized cider compared to irradiated cider was consumers' familiarity with pasteurized cider from that location (orchard). Ciders used for the consumer tests were produced at that orchard. Therefore, consumers who participated in the consumer tests were familiar with and liked pasteurized cider from that orchard. Many consumers commented that both samples tasted "good" but had different flavor.

Consumers had no preference for two apple ciders pasteurized using different temperature-time conditions (Mak and others 2001). One type of cider was pasteurized at 68.1 °C for 14 s and the other at 71.1 °C for 6 s. Unscreened panelists (n=192) evaluated two ciders and were asked "Which sample do you prefer the most?" on a 7-point hedonic scale. Mak and others (2001) reported that consumer acceptance of both pasteurized ciders were in the 'like moderately' category, cider pasteurized at 68.1 °C for 14 s was rated 6.0 and cider pasteurized at 71.1 °C for 6 s was rated 6.04.

An acceptability test of cider treated by multi-step intervention system (addition of 0.05% sodium benzoate and 0.05% potassium sorbate, warm hold at 35 °C for 6 h, freezing and thawing) and cider treated by pasteurization (68.1 °C for 14 s) was also conducted by Ingham and Schoeller (2002). Consumers rated pasteurized cider higher (6.1) compared to multi-step system cider (5.6) on a 7-point hedonic scale.

Consumer acceptance of pasteurized apple cider was high based on these studies. Consumers preferred pasteurized cider over irradiated or multi-step intervention system cider, although they did not differ much.

Physical and chemical analyses of consumer tests

There were no differences in pH and titratable acidity of irradiated and pasteurized ciders (Table 3). Pasteurized cider had higher soluble solids content than irradiated cider. Irradiated cider had higher "L" value, lower "a" value, and lower "b" value than pasteurized cider. Pasteurized cider was darker than irradiated cider due to the heat treatment from the pasteurization. Irradiated cider was reported to be lighter color than pasteurized cider (Asselbergs and others 1958; Fan and Thayer 2002b; Wang and others 2003). The bleaching effect of irradiation has been observed in strawberry juice likely caused by free radical

formation (Markakis and others 1959; Diehl 1982). Darker color in pasteurized cider could come from nonenzymatic and enzymatic browning reactions that occurred during processing (Carabasa-Giribet and Ibarz-Ribas 2000). The nonenzymatic browning reaction came from the heat treatment of pasteurization and the enzymatic browning reaction happened during the pressing of the pulp and pomace of apples. Zegota (1991) reported that brightening color was noted in irradiated concentrate apple juice (2.0 kGy).

Descriptive analysis

Main effect means were reported for processing treatment and preservative treatment for sweetness, sourness, astringency, apple flavor and caramelized flavor. An interaction between processing and preservative was found for musty flavor so interaction means are reported. Sweetness, sourness, astringency and caramelized flavor of raw, pasteurized and irradiated ciders were not different (Table 4). Astringency was a difficult attribute for the panelists because the astringency of the samples was similar. Raw cider had more intense apple flavor than irradiated cider, according to the sensory panelists. Sourness, astringency, apple flavor and caramelized flavor of ciders with and without preservative were not different (Table 5). Panelists perceived cider with preservative to be sweeter than cider without preservative.

For musty flavor, irradiated apple cider with preservative had more intense musty flavor compared to the other treatments (Table 6). There was a trend that the addition of preservative made the samples more intense in musty flavor compared to samples without preservative (Table 6). The musty flavor may have been the same attribute described as "cardboard-like" flavor by Wang and others (2003). The off-flavor produced in irradiated cider with preservative was a complex flavor. Different panelists may have described it in

different ways. Although the panelists were trained before evaluating the samples, the background and experience of panelists could influence their perception of the samples.

Undesirable flavors of fermented, moldy, musty juices or other strange odors and after-tastes were evaluated (Zegota 1991). This study was conducted to evaluate the sensory properties of irradiated apple juice concentrate. Panelists noted a slight flavor of dried apple, slightly sharper in odor and a sharp, strange bitter taste in apple juice irradiated with 2.0 kGy dose (Zegota 1991).

Physical and chemical analyses of descriptive analysis

Main effect means were reported when there was no interaction between processing and preservative treatment. The pH, soluble solids and yellow (b) color had no interaction (Table 7 and 8). Significant interactions were found for titratable acidity, L value and a value color measurements (Table 9). Raw cider was more acidic than pasteurized and irradiated ciders (Table 7). The soluble solids content of raw cider was higher than pasteurized cider. Raw cider had the most yellow (b) color compared to irradiated and pasteurized ciders. Irradiated cider had more yellow color compared to pasteurized cider.

Ciders with or without preservative did not differ in pH and soluble solids contents (Table 8). Apple cider with the addition of preservative had more yellow (b) color compared to cider without preservative.

There was interaction between processing and preservative treatments for titratable acidity, L value and a value (Table 9). Titratable acidity values were variable (Table 9), but raw cider with preservative and irradiated cider with preservative had more acid than other samples. Raw and irradiated ciders had lighter (L) color compared to other samples. Raw cider had the most intense red (a) color compared to all samples.

Musty flavor

Irradiated apple cider with preservative and irradiated water with preservative had the most intense musty flavor compared to all other samples (Table 10). The trend was that apple cider had more intense musty flavor than water. The preservative when irradiated produced a musty flavor. Irradiated apple cider had the same intensity of mustiness as raw cider, water, water with preservative and irradiated water.

Sorbic acid was reported to have developed an off-flavor described as "like plastic paint" or "kerosene" in commercial feta cheese (Horwood and others 1981). 1,3-Pentadiene, formed by decarboxylation of sorbic acid, was responsible for the development of off-flavor. Fan and Thayer (2002a) reported that malonaldehyde, formaldehyde and acetaldehyde were induced during irradiation in apple juice. Apple juice with sorbate addition had a higher acetaldehyde level than juice without sorbate addition (Fan and Thayer 2002a). Acetaldehyde was described as a pungent and irritating odor at high concentrations, however, at low concentrations acetaldehyde was described as a pleasant, fruity, sweet flavor (Fan and Thayer 2002a).

Comes and Beelman (2002) reported that consumers rated raw cider with preservative lower than pasteurized cider. At least 70 consumers were asked to evaluate raw, pasteurized and preservative-treated (raw) apple cider (0.15% fumaric acid, 0.05% sodium benzoate, held for 6 h at 25 °C followed by 24 h at 4 °C) on a 9-point hedonic scale. Sensory evaluations were conducted on early and late season ciders. Consumers rated preservative-treated ciders in "like slightly" (6.45) category for the early season cider and in "neither like nor dislike" (5.62) category for the late season cider.

Preservative can have an undesirable flavor in foods. Potassium sorbate was suspected to go through decarboxylation (Horwood and others 1981) or reacted with other compounds during irradiation, similar to changes in feta cheese (Horwood and others 1981), producing musty flavor. Therefore, the addition of preservative to cider should be done accurately, because too much preservative may produce an undesirable flavor.

Microbial storage study

Coliforms were found in raw ciders with and without preservative (especially at the beginning of storage) (Appendix G). No *E. coli* O157:H7 were found during storage. Not all coliform bacteria are harmful bacteria; therefore coliforms were not necessarily a concern. Coliforms in raw cider may come from apples that were not thoroughly cleaned or from dirty processing equipment. Coliforms may also come from the environment.

The number of coliforms decreased as storage continued. Refrigeration temperature may decrease the numbers of coliforms in the samples. There were greater numbers of coliforms found in raw cider without preservative, especially from week 3 increasing to week 5, and slowly reducing in week 6 and dying out in week 7 and 8. As shown in Appendix F, no coliforms were found in any samples with preservative. The decrease of coliforms over time was similar to the study conducted by Semanchek and Golden (1996). *E. coli* O157:H7 decreased from 6.4 log CFU/mL to an undetectable level in fermenting cider after 3 days at 20 ^{o}C or from 6.5 log CFU/mL to 2.9 log CFU/mL after 10 days at 20 ^{o}C.

No significant mold growth was observed during the 8-week storage. Because mold counts were so low, reported values were estimated. Mold may have come from the air. In most cases, the lowest possible detection level was ten. Because molds and yeasts were counted on the same plate, a higher detection level was used to count the number of yeast

colonies. The highest number of molds occurred in irradiated cider without preservative because there were two molds found on the plate with the lowest dilution of 10^{-3} which made the number large (Appendix J). In the entire storage study, mold counts were low and did not exceed the lowest detection level.

Two replications were treated separately in data analysis because the results were different. Yeast and aerobic counts did not exceed 1000 CFU/mL in raw cider with preservative (Figure 1). During the 8-week storage, yeast counts were relatively constant and aerobic bacteria decreased approximately 1 log. Yeast counts in replicate 2 were higher than yeast counts in replicate 1 in raw cider without preservative (Figure 2). Yeast counts increased a bit and aerobic bacteria were relatively constant or decreased a little. Yeast and aerobic counts started at 3-log CFU/mL. There was very little microbial growth during storage. The preservative may have slowed yeast growth. Deol (2003) showed that raw ciders spoiled within two weeks and had high microbial load (10^7 CFU/ml). The same apple cider processor was used by Deol (2003) and in this study; however the samples came from different seasons. Apples in different seasons may have different microbial loads.

No yeast growth was higher than the minimum detection level in pasteurized cider with preservative was found (Figure 3). Three samples showed growth of aerobic bacteria in weeks 5 and 6 in replicate 2 and aerobic bacteria in week 7 of replicate 1. There was no evidence of external contamination although results were not consistent throughout the storage study. In figure 4, there was a definite growth by yeasts and aerobic bacteria in replicate 1 but not in replicate 2. Yeast and aerobic counts in replicate 2 increased approximately 3-log per CFU/mL. In replicate 1, there was a possibility that colonies of aerobic bacteria were the yeast colonies that occurred in yeast counts. Since there was no

verification of the identity of the bacteria, this possibility could not be confirmed. In replication 2, both aerobic and yeast counts remained constant with no growth throughout the storage study.

No growth was found higher than the minimum detection level in irradiated cider with preservative for yeast and aerobic counts (Figure 5). Yeasts were slightly higher than the minimum detected level in the first week, but decreased in week 2 to the minimum detected level. There was significant growth of yeast in replicate 1 (Figure 6). Yeast counts increased approximately 4-log per CFU/mL, to levels higher than in raw ciders. In replicate 2, yeast counts increased approximately to 3.5-log per CFU/mL in week 5 and slowly decreased to 2-log per CFU/mL. Aerobic bacteria growth was very low and did not change much during the 8-week storage. There was very little competition from the aerobic bacteria in irradiated cider because of radiation-resistant yeasts.

There was no significant growth of yeast in pasteurized and irradiated cider with preservative. Potassium sorbate reduced yeast and aerobic bacteria growth. Irradiated cider without preservative had a higher number of yeasts compared to raw ciders and pasteurized cider. Radiation-resistant yeasts were also found by Wang (2002).

The microbial load in this study was not as high as the microbial study in a storage study conducted by Deol (2003). Deol noted that yeast and mold counts in pasteurized cider with preservative reached 10^6 CFU/mL by week 8. The yeast and mold counts were at the lowest detection level by week 8 of this study. These results were similar to results of Cummins (2001) who evaluated apple cider from various producers in Iowa.

Overall, microbial loads in all cider samples were low. This may be caused by different source of apples used for apple cider. Occasionally, producers use apples from

other orchards if they run out of their own apples. This may affect the microbial load of the cider since different orchards have different environmental factors that contribute microorganisms. A combination of preservative and processing treatment such as pasteurization and irradiation are effective in reduction of microbial load of yeasts and bacteria.

Conclusions

Consumers rated both pasteurized and irradiated ciders in the 'like moderately' category. There was no difference in the ratings at 3 locations. At one evaluation site, consumers rated pasteurized apple cider higher than irradiated apple cider because of familiarity with the product. Consumers did not dislike irradiated apple cider, but commented that irradiated apple cider had a different flavor than pasteurized apple cider.

For commercial applications, pasteurization is recommended rather than irradiation. Currently, pasteurization is more cost-effective and more efficient than irradiation. Irradiation would require transport of cider to a central location for processing. Pasteurization and irradiation have similar preservative effects, but pasteurized cider had less musty flavor than irradiated cider. The addition of potassium sorbate to apple cider effectively reduced yeast and aerobic bacteria counts, but contributed to off-flavors in irradiated cider.

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Table 1 - Reference standards used for descriptive analysis of apple cider

Attribute	Description	Reference standards
Sweetness	Taste of sugar	15 % (w/w) sucrose solution
Sourness	Taste stimulated by acids	0.20 % (w/w) citric acid
Astringency	Shrinking or puckering of the tongue surface	0.15 % (w/w) alum
Apple flavor	Fresh and ripe apple flavor	'Golden Delicious' fresh apple slice
Caramelized flavor	Caramel flavor	4 % (w/w) artificial burnt sugar flavor in water solution
Musty flavor	Earthy aroma	No standard

Table 2 - Consumer evaluations of pasteurized and irradiated ciders with 0.05% potassium sorbate

Location	Number of people	Degree of liking[1]:	
		Pasteurized + P [2]	Irradiated + P
Orchard	224	6.29[a]	6.30[a]
Orchard	136	6.44[a]	6.10[b]
Grocery Store	110	6.53[a]	6.42[a]
University	107	6.38[a]	6.14[a]

[1] 1=dislike very much, 7=like very much

[2] + P = 0.05% potassium sorbate

[a-b] Means within the same row having the same superscript are not significantly different ($P<0.05$)

Table 3 – Instrumental analyses of pasteurized and irradiated ciders with 0.05% potassium sorbate used in consumer evaluations

Treatment	pH	Titratable acidity (g/ 100 mL)	Soluble solids (%)	Color L	Color a	Color b
Irradiated + P [1]						
Consumer Test 1	3.6	0.43	14.2	11.26	-0.73	2.34
Consumer Test 2	3.5	0.28	13.1	11.12	-0.74	2.65
Consumer Test 3 + 4	3.7	0.33	12.1	14.19	-1.37	5.78
Mean	3.6[a]	0.34[a]	13.13[b]	12.26[a]	-0.94[b]	3.67[b]
Pasteurized + P						
Consumer Test 1	3.6	0.41	14.2	10.64	-0.50	2.98
Consumer Test 2	3.6	0.34	13.9	10.63	-0.34	3.01
Consumer Test 3 + 4	3.5	0.32	12.2	10.60	-0.96	8.28
Mean	3.57[a]	0.35[a]	13.45[a]	10.60[b]	-0.63[a]	4.74[a]
LSD[2]	0.10	0.03	0.28	1.14	0.17	0.93

[1] + P = with 0.05% potassium sorbate
[2] LSD = least significant difference
[a-b] Means within the same column having the same superscript are not significantly different ($P<0.05$)

Table 4 – Effect of processing treatments on sensory characteristics[1] of raw, pasteurized and irradiated apple ciders

Processing trt	Sweetness	Sourness	Astringency	Apple flavor	Caramelized flavor
Raw	10.89	9.92	10.23	10.27[a]	7.94
Pasteurized	10.65	10.04	10.27	9.66[ab]	8.30
Irradiated	10.41	9.92	10.48	8.96[b]	7.94
	NS	NS	NS		NS

[1] 0=none, 15=intense.
[a-b] Main effect means within the same column having the same superscript are not significantly different (P<0.05), NS = not significant.

Table 5 – Effect of preservative treatments of sensory characteristics[1] of raw, pasteurized and irradiated apple ciders

Preservative trt	Sweetness	Sourness	Astringency	Apple flavor	Caramelized flavor
With preservative	11.02[a]	10.18	10.64	9.55	8.26
No preservative	10.28[b]	9.85	10.02	9.71	8.12
		NS	NS	NS	NS

[1] 0=none, 15=intense.
[a-b] Means within the same column having the same superscript are not significantly different (P<0.05), NS = not significant.

Table 6 – Effect of processing and preservative treatments on musty flavor[1] of raw, pasteurized and irradiated apple ciders with and without 0.05% potassium sorbate

Interaction	Musty flavor
Raw	3.06[d]
Raw + P [2]	3.65[bcd]
Pasteurized	3.21[cd]
Pasteurized + P	4.73[bc]
Irradiated	5.02[b]
Irradiated + P	9.20[a]

[1] 0=none, 15=intense.
[2] + P = with 0.05% potassium sorbate.
[a-d] Means within the same column having the same superscript are not significantly different (P<0.05).

Table 7 – Effect of processing treatment on instrumental analyses of raw, pasteurized and irradiated ciders

			Color
Processing trt	**pH**	**Soluble solids (%)**	**b**
Raw	3.48[b]	12.67[a]	8.45[a]
Pasteurized	3.75[a]	12.40[b]	6.28[c]
Irradiated	3.80[a]	12.57[ab]	6.97[b]

[a-c] Means within the same column having the same superscript are not significantly different (P<0.05).

Table 8 – Effect of preservative treatments on instrumental analyses of raw, pasteurized and irradiated ciders

			Color
Preservative trt	**pH**	**Soluble solids (%)**	**b**
With preservative	3.68	12.47	8.18[a]
No preservative	3.68	12.62	6.28[b]
	NS	NS	

[a-b] Means within the same column having the same superscript are not significantly different (P<0.05), NS = Not significant.

Table 9 – Effect of processing and preservative treatments of instrumental analyses of raw, pasteurized and irradiated apple ciders with and without 0.05% potassium sorbate

	Titratable acidity	Color	
Interaction	**(g / 100 mL)**	**L**	**a**
Raw	0.27[b]	17.87[a]	1.23[a]
Raw + P[1]	0.29[a]	14.84[b]	- 0.17[b]
Pasteurized	0.26[b]	14.72[b]	- 0.65[c]
Pasteurized + P	0.26[b]	14.03[b]	- 0.76[c]
Irradiated	0.26[b]	18.06[a]	0.02[b]
Irradiated + P	0.28[a]	14.97[b]	- 0.66[c]

[a-c] Means within the same column having the same superscript are not significantly different (P<0.05), NS = Not significant.
[1] + P = with 0.05% potassium sorbate.

Table 10 – Sensory evaluation of musty flavor in apple cider and water with and without 0.05% potassium sorbate

Treatments	Musty flavor
Apple Cider	3.68^{cd}
Irradiated Cider	4.04^{cd}
Apple Cider + P [1]	5.68^{bc}
Irradiated Cider + P	9.72^{a}
Water	3.64^{cd}
Irradiated Water	2.32^{d}
Water + P	3.54^{cd}
Irradiated Water + P	7.92^{ab}

[a-d] Means within the same row having the same superscript are not significantly different ($P<0.05$).
[1] + P = with 0.05% potassium sorbate.

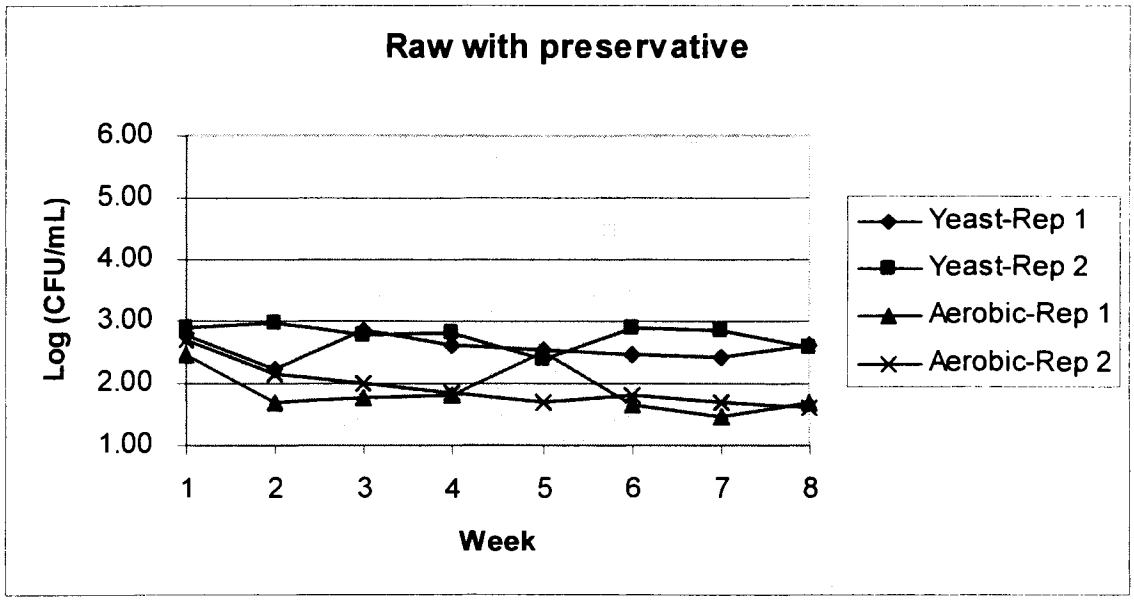

Figure 1 - Yeasts and aerobics counts during 8-weeks storage of raw cider with 0.05% potassium sorbate

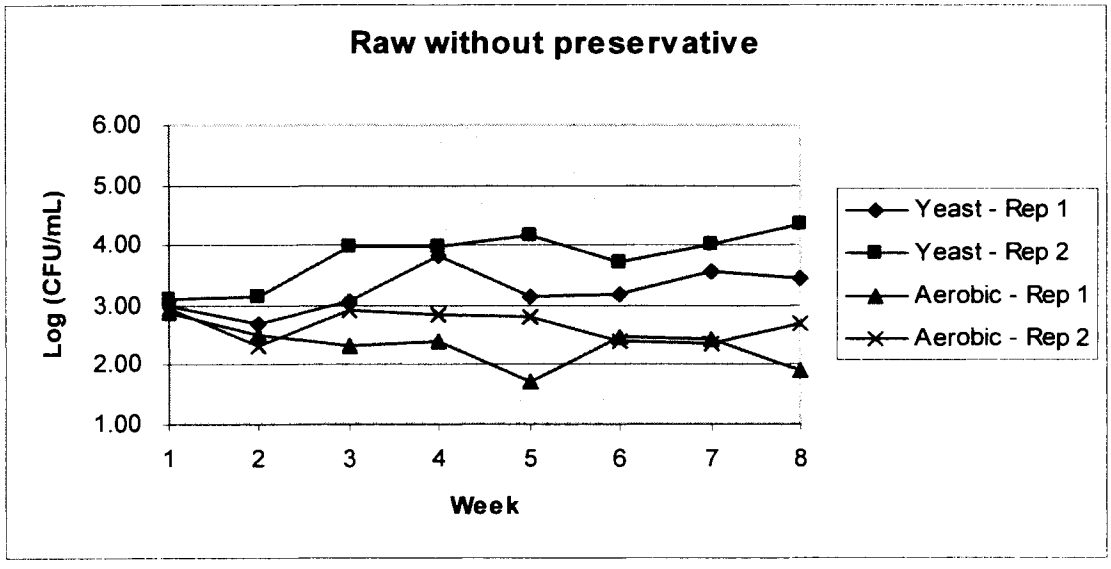

Figure 2 - Yeasts and aerobics counts during 8-weeks storage of raw cider without 0.05% potassium sorbate

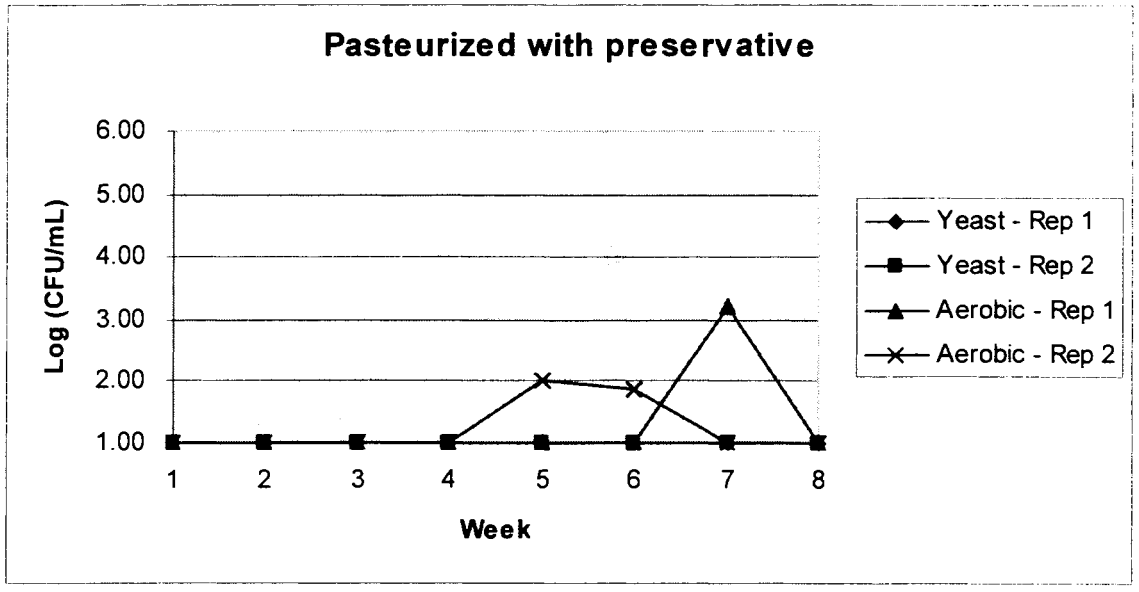

Figure 3 - Yeasts and aerobics counts during 8-weeks storage of pasteurized cider with 0.05% potassium sorbate

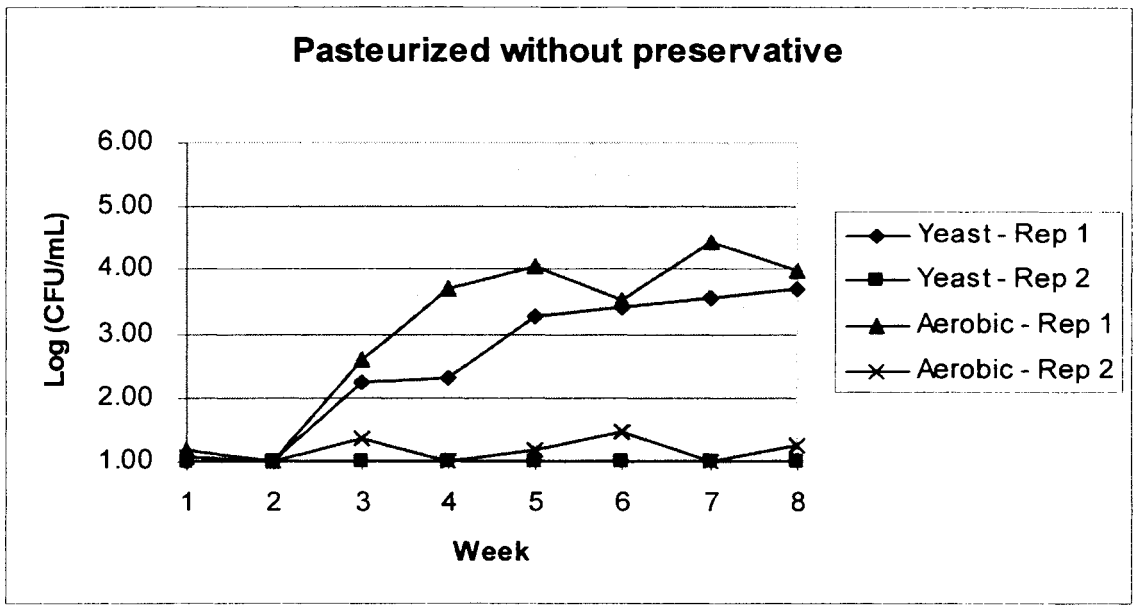

Figure 4 - Yeasts and aerobics counts during 8-weeks storage of pasteurized cider with 0.05% potassium sorbate

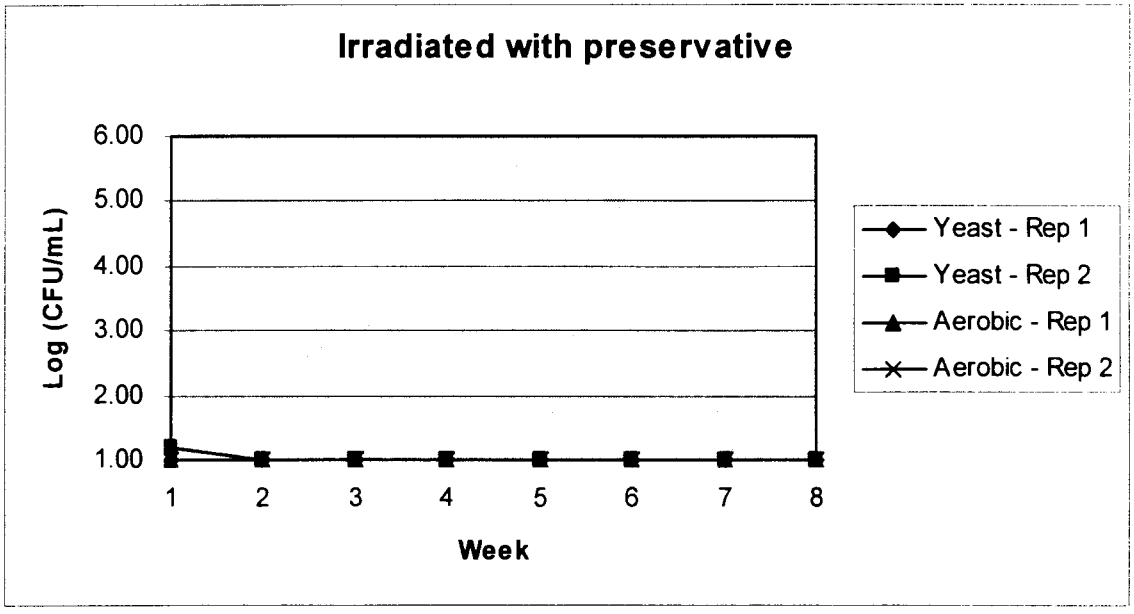

Figure 5 - Yeasts and aerobics counts during 8-weeks storage of irradiated cider with 0.05% potassium sorbate

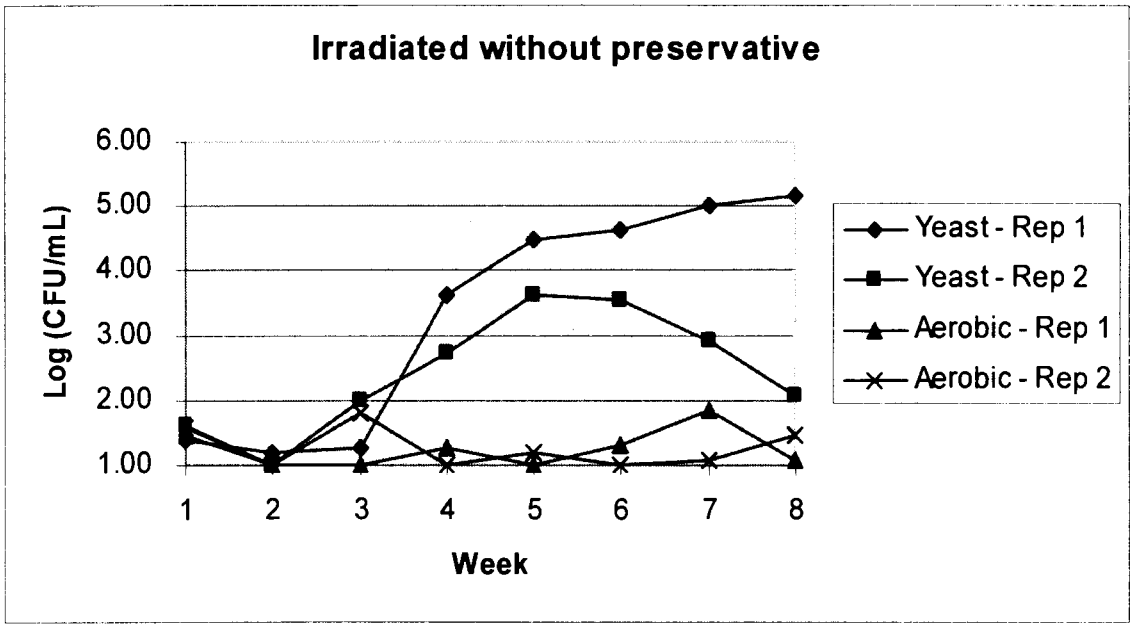

Figure 6 - Yeasts and aerobics counts during 8-weeks storage of irradiated cider without 0.05% potassium sorbate

Chapter 4. General Conclusions

Pasteurized and irradiated ciders were similar in flavor characteristics according to trained panelists. Without the addition of preservative, sensory attributes of both pasteurized and irradiated ciders were the same. An off-flavor was detected in irradiated apple cider with preservative. Irradiated apple cider with preservative had more intense musty flavor than raw or pasteurized ciders. The cause of the musty flavor was irradiated potassium sorbate.

Consumers did not detect a musty flavor in ciders and rated both ciders similarly. In the first study, irradiated cider was as acceptable as or more acceptable than pasteurized apple cider. However, in the second study, consumers at three sites rated "degree of liking" for pasteurized apple cider the same as irradiated apple cider. The absence of key apple flavor compounds in both ciders may have contributed to consumers' lack of preference at 2 locations.

The preservative effectively reduced yeasts and aerobic bacteria growth during storage. Therefore, if preservative is added to cider, it should be measured carefully so consumers will not detect any musty flavor.

For commercial applications, pasteurization is recommended rather than irradiation. Currently, pasteurization is more cost-effective and more efficient than irradiation. Irradiation would require transport of cider to a central location for processing. Pasteurization and irradiation have similar preservative effects, but pasteurized cider had less musty flavor than irradiated cider. The addition of potassium sorbate to apple cider effectively reduced yeast and aerobic bacteria counts, but contributed to off-flavors in irradiated cider.

Appendix A – Scorecard for Preference Test

APPLE CIDER EVALUATION

DATE: September 29, 2001

CONSENT:

YOU MUST BE OF **18 YEARS AGE** TO PARTICIPATE IN THIS EVALUATION. There are two samples of apple cider that are pasteurized or irradiated and do contain a preservative. The cider was purchased from a local producer. Cider was pasteurized by a local processor or irradiated at the Linear Accelerator Facility, ISU. The cider has been refrigerated and handled in a sanitary manner.

This evaluation will require about 5 minutes. Fransiska Yulanti and Dr. Cheryll Reitmeier, Department of Food Science and Human Nutrition, ISU, will be available throughout the evaluation to answer questions. There is no risk associated with the consumption of apple cider that has been pasteurized, irradiated or has preservatives. Responses to the sensory evaluation are anonymous.

INSTRUCTIONS:

The two samples are coded with 3-digit random numbers. Taste each of the coded samples and answer the question: Which sample do you prefer? Please make an independent choice and do not consult with others about your answer. Circle the coded number of the sample you prefer.

Thank you.

WHICH SAMPLE DO YOU PREFER? 322 683

How often do you consumer apple cider during the season? Check the appropriate space below.

_____ I consume apple cider more than once a week.

_____ I consume apple cider 2-4 times a month.

_____ I consume apple cider once a month.

_____ I consume apple cider less than once a month.

Appendix B – Scoresheet of Consumer Test

APPLE CIDER EVALUATION

DATE: October 12, 2002

CONSENT:

YOU MUST BE OF 18 YEARS AGE TO PARTICIPATE IN THIS EVALUATION. There are two samples of apple cider that are pasteurized and irradiated and do contain preservative. The cider was purchased from a local producer. Cider was pasteurized by a local processor or irradiated at the Linear Accelerator Facility, Iowa State University. The cider has been refrigerated and handled in sanitary manner.

This evaluation will require about 5 minutes. Fransiska Yulianti and Dr. Cheryll Reitmeier, Department of Food Science and Human Nutrition, Iowa State University, will be available throughout the evaluation to answer questions. There is no risk associated with the consumption of apple cider that has been pasteurized, irradiated or has preservative. Responses to the sensory evaluation are anonymous.

INSTRUCTIONS:

The two samples are coded with 3-digit random numbers. Taste each of the coded samples and check the box that best describes your overall opinion of each sample (If you have any questions, please ask the server now.)

Sample No. **557**	Sample No. **674**
☐ Like very much	☐ Like very much
☐ Like moderately	☐ Like moderately
☐ Like slightly	☐ Like slightly
☐ Neither like nor dislike	☐ Neither like nor dislike
☐ Dislike slightly	☐ Dislike slightly
☐ Dislike moderately	☐ Dislike moderately
☐ Dislike very much	☐ Dislike very much

Comments:

THANK YOU

Appendix C – Questionnaire

Name: _____
Address: _____
Phone: _____

HEALTH:

1. Do you have any of the following?

Dentures	_____
Diabetes	_____
Oral or gum disease	_____
Hypoglycemia	_____
Food allergies	_____
Hypertension	_____

2. Do you take any medications which affect your senses, especially taste and smell?

_____

FOOD HABITS:

1. What is (are) your favorite foods? _____
2. What is (are) your least favorite foods? _____
3. How often do you eat out in a month? _____
4. What foods do you not like to eat? _____
5. Is your ability to distinguish smell and tastes:

	SMELL	TASTE
Better than average	_____	_____
Average	_____	_____
Worse than average	_____	_____

FLAVOR QUIZ:

1. How would you describe the difference between flavor and aroma?

_____

2. How would you describe the difference between flavor and texture?

_____

3. Describe some of the noticeable flavors in cola

_____

Appendix D – Scorecard for Basic Tastes Recognition Test

Name: _____

Date: _____

Taste the samples and indicate whether they are sweet, sour, salty or bitter. Please rinse between samples. You do not need to swallow the samples.

Samples	Sweet	Sour	Salty	Bitter	Astringency
325	_____	_____	_____	_____	_____
553	_____	_____	_____	_____	_____
932	_____	_____	_____	_____	_____
797	_____	_____	_____	_____	_____
286	_____	_____	_____	_____	_____

Appendix E - Scorecard for Intensity Ranking

Name: _____

Date: _____

Taste the samples and rank them from the least intense to the most intense for each of the four basic tastes.

	Code
Least salty	_____
	_____
Most salty	_____

	Code
Least sweet	_____
	_____
Most sweet	_____

	Code
Least sour	_____
	_____
Most sour	_____

	Code
Least bitter	_____
	_____
Most bitter	_____

	Code
Least astringency	_____
	_____
Most astringency	_____

Appendix F – Scoresheet for Sensory Evaluation

APPLE CIDER EVALUATION

DATE: November 7, 2002
NAME: _____

INSTRUCTIONS:

Please rinse your mouth with water before starting and between sets of samples. Eat a piece of cracker between samples, if you desire.
Please place a perpendicular mark on each line below.

Sweetness

None Intense

Sourness

None Intense

Astringency

None Intense

Apple Flavor

None Intense

Caramelized Flavor

None Intense

Musty Flavor

None Intense

Comments:

Thank you.

Appendix G

Coliforms counts in apple cider per 8 weeks storage period (cfu/ml) – Rep 1

Treatment[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Raw + P	*	*	*	*	*	*	*	*
Raw - P	*	*	1.7×10^2 est	4.5×10^1 est	2.8×10^1 est	*	*	*
Pasteurized + P	*	*	*	*	*	*	*	*
Pasteurized - P	*	*	*	*	*	1.2×10^1 est	*	*
Irradiated + P	*	*	*	*	*	*	*	*
Irradiated - P	*	*	*	*	*	*	*	*

[a] + P = with 0.05% potassium sorbate, - P = without potassium sorbate
* Means counts < 10 CFU/mL

Coliforms counts in apple cider per 8 weeks storage period (cfu/ml) – Rep 2

Treatment[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Raw + P	5.0×10^1 est	*	*	*	*	*	*	*
Raw - P	*	*	9.2×10^1 est	1.5×10^3	1.6×10^3	3.2×10^2	*	*
Pasteurized + P	*	*	*	*	*	*	*	*
Pasteurized - P	*	*	*	*	*	*	*	*
Irradiated + P	*	*	*	*	*	*	*	*
Irradiated - P	*	*	*	*	*	*	*	*

[a] + P = with 0.05% potassium sorbate, - P = without potassium sorbate
* Means counts < 10 CFU/mL

Appendix H

Aerobic counts in apple cider per 8 weeks storage period (cfu/ml) – Rep 1

Treatment[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Raw + P	2.9×10^2	5.0×10^1 est	6.0×10^1 est	6.5×10^1 est	3.2×10^2	4.5×10^1 est	2.8×10^1 est	4.8×10^1 est
Raw - P	7.6×10^2	3.3×10^2	2.0×10^2 est	2.4×10^2 est	5.0×10^1 est	2.9×10^2	2.6×10^2	8.2×10^1 est
Pasteurized + P	*	*	*	*	*	*	1.6×10^3	*
Pasteurized - P	1.5×10^1 est	*	3.8×10^2	5.0×10^3	1.1×10^4	3.2×10^3	2.8×10^4	9.4×10^3
Irradiated + P	*	*	*	*	*	*	*	*
Irradiated - P	3.0×10^1 est	*	*	1.8×10^1 est	*	2.0×10^1 est	7.0×10^1 est	1.2×10^1 est

[a] + P = with 0.05% potassium sorbate, - P = without potassium sorbate
* Means counts < 10 CFU/mL

Aerobic counts in apple cider per 8 weeks storage period (cfu/ml) – Rep 2

Treatment[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Raw + P	4.8×10^2	1.4×10^2 est	9.8×10^1 est	7.0×10^1 est	5.0×10^1 est	6.2×10^1 est	4.8×10^1 est	4.2×10^1 est
Raw - P	9.0×10^2	2.0×10^2 est	8.4×10^2	6.9×10^2	6.6×10^2	2.5×10^2	2.2×10^2 est	5.0×10^2
Pasteurized + P	*	*	*	*	1.0×10^2 est	7.5×10^1 est	*	1.0×10^1 est
Pasteurized - P	1.2×10^1 est	*	2.2×10^1 est	*	1.5×10^1 est	3.0×10^1 est	*	1.8×10^1 est
Irradiated + P	*	*	*	*	*	1×10^1 est	*	*
Irradiated - P	3.8×10^1 est	*	6.2×10^1 est	*	1.5×10^1 est	*	1.2×10^1 est	2.8×10^1 est

[a] + P = with 0.05% potassium sorbate, - P = without potassium sorbate
* Means counts < 10 CFU/mL

Appendix I

Yeast counts in apple cider per 8 weeks storage period (cfu/ml) – Rep 1

Treatment[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Raw + P	6.0×10^2	1.7×10^2 est	7.2×10^2	4.0×10^2	3.4×10^2	2.8×10^2	2.6×10^2	4.0×10^2
Raw - P	1.0×10^3	4.8×10^2	1.2×10^3	6.8×10^3	1.4×10^3	1.5×10^3	3.6×10^3	2.9×10^3
Pasteurized + P	*	*	*	*	*	*	*	*
Pasteurized - P	*	*	1.7×10^2 est	2.0×10^2 est	1.8×10^3	2.5×10^3	3.6×10^3	5.0×10^3
Irradiated + P	*	*	*	*	*	*	*	*
Irradiated - P	2.5×10^1 est	1.5×10^1 est	1.8×10^1 est	4.0×10^3	2.8×10^4	4.2×10^4	1.0×10^5	1.4×10^5

[a] + P = with 0.05% potassium sorbate, - P = without potassium sorbate
* Means counts < 10 CFU/mL

Yeast counts in apple cider per 8 weeks storage period (cfu/ml) – Rep 2

Treatment[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Raw + P	7.6×10^2	9.2×10^2	6.0×10^2	6.3×10^2	2.4×10^2 est	7.4×10^2	7.2×10^2	3.9×10^2
Raw - P	1.3×10^3	1.4×10^3	9.0×10^3	9.2×10^3	1.4×10^4	5.0×10^3	1.0×10^4	2.2×10^4
Pasteurized + P	*	*	*	*	*	*	*	*
Pasteurized - P	*	*	*	*	*	*	*	*
Irradiated + P	1.5×10^1 est	*	*	*	*	*	*	*
Irradiated - P	4.0×10^1 est	*	1.0×10^2 est	5.6×10^2	4.2×10^3	3.4×10^3	8.5×10^2 est	1.2×10^2 est

[a] + P = with 0.05% potassium sorbate, - P = without potassium sorbate
* Means counts < 10 CFU/mL

Appendix J

Mold counts in apple cider per 8 weeks storage period (cfu/ml) – Rep 1

Treatment[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Raw + P	8.0×10^1 est	*	*	2.2×10^1 est	1.5×10^1 est	*	*	1.2×10^1 est
Raw - P	1.1×10^3	9.8×10^2	4.1×10^2	1.8×10^2 est	8.5×10^1 est	8.5×10^1 est	2.2×10^1 est	2.5×10^1 est
Pasteurized + P	*	*	*	*	*	*	*	*
Pasteurized - P	*	*	*	*	*	*	*	2.5×10^1 est
Irradiated + P	*	*	*	*	*	*	*	*
Irradiated - P	1.2×10^1 est	*	*	1.1×10^2 est	*	*	*	2.0×10^3 est

[a] + P = with 0.05% potassium sorbate, - P = without potassium sorbate
* Means counts < 10 CFU/mL

Mold counts in apple cider per 8 weeks storage period (cfu/ml) – Rep 2

Treatment[1]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Raw + P	2.8×10^1 est	*	*	*	*	*	*	*
Raw - P	9.8×10^1 est	2.8×10^1 est	*	*	*	*	*	*
Pasteurized + P	*	*	*	*	*	*	*	*
Pasteurized - P	*	*	*	*	*	*	*	*
Irradiated + P	*	*	*	*	*	*	*	*
Irradiated - P	*	*	*	*	*	*	*	*

[a] + P = with 0.05% potassium sorbate, - P = without potassium sorbate
* Means counts < 10 CFU/mL

Appendix H: Microbiological study (raw data)

MICRO RESULTS Week 1

11/2/2002		**11/3//02**			**11/6//02**			**11/6/2002**		
Coliform		**TSA (AEROBIC)**			**YEAST**			**MOLD**		
	10 E-1	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3
1A	0	19	4	2	10	4	2	10	1	1
1A	0	29	4	1	62	5	1	14	1	1
1B	0	36	4	5	60	4	0	6	0	2
1B	0	33	2	1	110	7	1	2	1	1
2A	0	71	7	3	55	8	3	65	18	3
2A	0	94	7	1	180	8	2	168	13	1
2B	0	77	4	2	76	7	1	96	32	2
2B	0	65	10	0	88	11	2	125	17	4
3A	0	0	0	1	0	0	0	1	0	1
3A	0	1	0	1	0	0	0	1	0	1
3B	0	1	0	0	0	0	0	0	3	3
3B	1	1	0	4	2	0	1	1	1	1
4A	0	0	0	74	0	0	0	2	0	2
4A	0	1	0	0	0	0	0	0	0	2
4B	0	4	0	0	1	0	0	0	0	1
4B	0	1	1	1	0	0	0	0	0	0
5A	0	1	0	0	0	0	0	0	0	0
5A	0	0	0	0	0	0	0	0	0	1
5B	0	0	0	0	0	0	0	0	1	1
5B	0	0	1	0	0	0	0	1	1	1
6A	0	3	0	0	2	0	0	1	1	1
6A	0	3	0	0	2	0	0	1	1	1
6B	0	4	0	0	4	1	0	2	1	1
6B	0	2	0	0	2	0	0	1	0	0

Legend: Samples 1-6 Irradiation Date October 31, 2002

1= raw with preservative
2= raw without preservative
3=pasteurized with preservative
4=pasteurized without preservative
5=irradiated with preservative
6=irradiated without preservative

A=Dilution blank 1
B=Dilution blank 2

MICRO RESULTS WEEK 2

11/9/2002			11/10/2002			11/13/2002			11/13/2002		
Coliform			**TSA (AEROBIC)**			**YEAST**			**MOLD**		
	10 E-1	10 E-2	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3
1A	0	0	7	1	0	17	3	0	1	0	0
1A	0	0	4	1	0	28	3	0	2	0	0
1B	0	0	6	1	2	11	4	1	0	0	1
1B	0	0	3	1	1	13	3	0	0	0	0
2A	1	0	41	7	0	46	11	0	90	13	1
2A	0	0	42	7	0	51	12	0	110	18	1
2B	0	0	27	1	0	45	8	0	69	14	1
2B	0	0	23	6	0	51	6	0	126	13	2
3A	0		0	0	0	0	0	1	0	0	0
3A	0		0	0	0	0	1	0	0	0	0
3B	0		1	0	0	0	0	0	0	0	0
3B	0		0	0	0	0	0	0	0	0	0
4A	0		0	0	0	0	0	0	0	0	0
4A	0		0	0	0	0	0	0	0	0	0
4B	0		1	0	0	0	0	0	0	0	0
4B	0		2	0	0	0	0	0	0	0	0
5A	0		0	0		0	0	0	0	0	0
5A	0		0	0		0	0	0	2	0	0
5B	0		0	0		0	0	0	0	0	0
5B	0		0	0		0	0	0	0	0	0
6A	0		1	0		0	0	0	0	0	0
6A	0		0	0		3	0	0	0	0	0
6B	0		0	0		2	0	0	1	0	0
6B	0		0	0		1	0	0	0	0	1
7A	2	0	33	7	0	67	8	1	1	0	0
7A	10	1	64	8	0	111	10	5	3	1	0
7B	3	0	42	2	2	60	7	0	3	0	0
7B	5	0	52	4	0	65	13	1	4	1	1
8A	1	0	72	9	0	152	21	5	9	0	0
8A	0	0	100	8	1	143	17	2	13	5	1
8B	0	0	95	13	1	125	9	2	4	3	0

8B	2	0	93	5	0	110	22	2	13	5	0
9A	0		0	0	0	0	0	0	1	0	0
9A	0		0	0	0	0	0	0	1	0	1
9B	0		1	0	0	0	0	0	0	0	2
9B	0		0	0	0	0	0	0	0	0	0
10A	0		0	0	0	1	0	0	0	0	0
10A	0		2	0	0	0	0	0	0	0	0
10B	0		1	0	0	0	0	0	0	0	0
10B	0		2	0	0	0	0	0	0	0	0
11A	0		0	0		0	0	0	1	1	1
11A	0		0	0		3	1	0	0	0	0
11B	1		0	0		1	0	3	1	0	0
11B	0		2	0		2	0	0	0	0	0
12A	0		0	0		4	1	0	1	0	0
12A	0		7	0		5	0	0	0	0	0
12B	0		6	0		5	1	0	1	0	0
12B	0		2	0		2	0	0	0	0	0

Legend:

Samples 1-6 Irradiation Date October 31, 2002

1= raw with preservative
2= raw without preservative
3=pasteurized with preservative
4=pasteurized without preservative
5=irradiated with preservative
6=irradiated without preservative

A=Dilution blank 1
B=Dilution blank 2

Samples 7-12 Irradiation Date November 7, 2002

7=raw with preservative
8=raw without preservative
9=pasteurized with preservative
10=pasteurized without preservative
11=irradiated with preservative
12=irradiated without preservative

MICRO RESULTS WEEK 3

11/16/2002	11/17/2002			11/20/2002			11/20/2002		
Coliform	**TSA (AEROBIC)**			**YEAST**			**MOLD**		
	10 E-1	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3
1A	0	6	1	1	85	6	0	0	0	0
1A	0	7	3	0	70	5	0	1	1	0
1B	0	8	0	0	63	4	0	0	1	0
1B	0	3	0	0	69	4	0	1	0	0
2A	23	19	1	0	153	17	1	37	8	4
2A	13	22	0	0	133	16	0	57	10	2
2B	11	15	1	0	70	17	1	21	5	0
2B	21	22	2	0	133	11	1	49	10	1
3A	0	1	0	0	0	0	0	0	0	0
3A	0	0	0	0	0	0	0	0	0	2
3B	0	0	0	1	0	0	0	0	0	0
3B	0	0	0	0	0	0	0	0	0	0
4A	0	37	3	0	18	0	0	0	0	0
4A	0	30	7	1	17	0	0	1	0	0
4B	0	34	1	0	19	0	0	0	0	0
4B	0	52	2	0	14	0	0	0	0	0
5A	0	0	0	0	0	0	0	0	0	0
5A	0	0	0	0	0	0	0	0	0	0
5B	0	0	0	0	0	0	0	0	0	0
5B	0	0	1	0	0	0	0	0	0	0
6A	0	0	0	0	1	0	0	0	0	1
6A	0	0	0	0	0	0	0	0	0	0
6B	0	0	0	0	3	0	0	0	0	0
6B	0	0	0	0	3	0	0	0	0	0
7A	0	17	1	0	98	3	0	0	0	0
7A	0	10	2	1	66	2	1	0	2	0
7B	0	15	2	1	90	8	2	2	0	0
7B	1	16	2	1	114	12	0	0	0	0
8A	0	19	5	0	155	14	1	2	1	0
8A	0	21	2	0	114	12	1	4	1	0
8B	1	29	5	1	160	14	1	1	0	0

8B	0	14	1	0	121	8	1	4	1	0
9A	0	0	0	0	0	0	0	0	0	0
9A	0	0	0	0	0	0	0	0	0	0
9B	0	0	0	0	0	0	0	0	0	0
9B	0	0	0	0	0	0	0	0	0	0
10A	0	0	0	0	0	0	0	0	1	0
10A	0	0	0	0	0	0	0	0	0	0
10B	0	0	0	0	0	0	0	0	0	1
10B	0	0	0	0	0	0	0	0	1	0
11A	0	0	0	0	0	0	0	0	0	0
11A	0	1	0	0	1	0	0	0	1	0
11B	0	0	0	0	0	0	0	0	0	0
11B	0	0	0	0	1	0	0	0	0	0
12A	0	1	0	1	1	0	0	0	0	0
12A	0	0	0	0	0	0	0	0	0	1
12B	0	0	0	0	2	0	0	0	0	0
12B	0	0	0	0	0	0	0	0	0	0

Legend:

Samples 1-6 Irradiation Date October 31, 2002

1= raw with preservative
2= raw without preservative
3=pasteurized with preservative
4=pasteurized without preservative
5=irradiated with preservative
6=irradiated without preservative

A=Dilution blank 1
B=Dilution blank 2

Samples 7-12 Irradiation Date November 7, 2002

7=raw with preservative
8=raw without preservative
9=pasteurized with preservative
10=pasteurized without preservative
11=irradiated with preservative
12=irradiated without preservative

MICRO RESULTS WEEK 4

11/16/2002		11/17/2002				11/20/2002			11/20/2002		
Coliform		**TSA (AEROBIC)**				**YEAST**			**MOLD**		
	10 E-1		10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3
1A	0		7	0	0	55	2	0	2	0	0
1A	0		6	1	0	52	8	0	3	0	0
1B	0		5	1	1	13	5	0	0	0	0
1B	0		8	1	1	43	6	0	4	1	0
2A	3		33	16	1	TMTC	77	5	35	66	3
2A	4		23	8	4	TMTC	88	4	35	5	0
2B	4		22	10	0	TMTC	35	5	0	21	2
2B	7		19	15	0	TMTC	72	14	0	0	3
3A	0		0	1	0	0	0	0	0	0	0
3A	0		1	0	0	0	0	0	0	0	0
3B	0		1	0	0	0	0	0	0	0	0
3B	0		0	0	0	0	0	0	0	0	0
4A	0	TMTC		41	0	10	0	0	0	0	1
4A	0	TMTC		35	0	14	10	0	0	0	0
4B	2	TMTC		49	5	36	12	1	0	0	0
4B	0	TMTC		74	9	18	0	0	0	0	17
5A	0		1	0	0	0	0	0	0	0	0
5A	0		0	0	1	0	0	0	0	0	0
5B	0		0	0	0	0	0	0	0	0	0
5B	0		0	0	0	0	0	0	0	0	0
6A	0		2	0	0	90	45	4	6	0	0
6A	0		0	0	0	TMTC	53	3	19	4	0
6B	0		2	0	0	114	29	10	10	1	0
6B	0		3	1	0	TMTC	36	13	10	2	1
7A	0		8	2	3	82	3	0	0	0	0
7A	1		9	2	1	40	4	1	0	2	0
7B	1		8	2	0	53	6	1	1	0	0
7B	0		14	2	1	68	3	0	2	0	1
8A	8		69	58	2	TMTC	52	10	0	0	0
8A	5		64	51	0	TMTC	105	10	0	8	1
8B	13		96	81	0	TMTC	87	20	1	0	0

8B	11	105	57	1	TMTC		114	10	0	2	1
9A	0	0	0	0		0	0	0	0	0	0
9A	0	0	0	0		0	0	0	0	0	0
9B	0	0	0	0		0	0	0	0	0	0
9B	0	0	1	0		0	0	0	0	0	0
10A	0	6	1	0		0	0	0	0	0	0
10A	0	0	0	0		0	0	0	0	0	0
10B	0	1	0	0		0	0	0	0	0	0
10B	0	2	1	1		0	0	0	0	0	0
11A	0	0	0	1		0	0	0	0	0	0
11A	0	0	0	0		1	0	0	0	0	0
11B	0	0	0	0		1	0	0	0	0	0
11B	0	0	0	0		0	0	0	0	0	0
12A	0	8	0	0		9	0	0	1	0	0
12A	0	7	0	0		11	1	0	1	0	0
12B	0	6	0	0		8	1	0	0	0	0
12B	0	4	1	0		12	1	0	0	0	0

Legend:

Samples 1-6 Irradiation Date October 31, 2002

1= raw with preservative
2= raw without preservative
3=pasteurized with preservative
4=pasteurized without preservative
5=irradiated with preservative
6=irradiated without preservative

A=Dilution blank 1
B=Dilution blank 2

Samples 7-12 Irradiation Date November 7, 2002

7=raw with preservative
8=raw without preservative
9=pasteurized with preservative
10=pasteurized without preservative
11=irradiated with preservative
12=irradiated without preservative

MICRO RESULTS WEEK 5

11/16/2002		11/17/2002			11/20/2002			11/20/2002		
Coliform		**TSA (AEROBIC)**			**YEAST**			**MOLD**		
	10 E-1	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3
1A	0	50	3	1	35	10	0	3	1	0
1A	0	30	2	0	45	3	0	0	0	0
1B	0	28	4	1	26	1	0	3	0	0
1B	0	23	4	0	32	1	0	0	1	0
2A	4	8	1	0	80	17	2	5	0	0
2A	2	5	1	0	155	16	3	13	0	0
2B	5	5	1	0	168	30	4	8	1	1
2B	0	2	0	0	154	24	2	8	1	0
3A	0	1	1	0	0	0	0	1	0	0
3A	0	0	0	0	0	0	0	0	0	0
3B	0	1	0	1	0	0	0	0	0	0
3B	0	1	0	0	0	0	0	0	0	0
4A	0	TMTC	87	24	TMTC	16	1	0	0	0
4A	0	TMTC	99	28	TMTC	8	3	0	0	0
4B	0	TMTC	121	34	176	5	4	0	0	0
4B	0	TMTC	138	29	TMTC	29	6	0	0	0
5A	0	0	0	1	0	0	0	0	0	0
5A	0	0	0	1	0	0	0	0	0	0
5B	0	0	0	0	0	0	0	0	0	0
5B	0	0	0	0	0	0	0	0	0	0
6A	0	0	0	0	TMTC	250	39	0	0	0
6A	0	0	0	0	TMTC	156	13	0	0	0
6B	0	0	0	0	TMTC	230	38	0	0	0
6B	0	0	0	0	TMTC	193	22	0	0	0
7A	0	10	4	0	54	4	0	0	0	0
7A	0	4	1	0	63	7	1	0	0	0
7B	0	8	4	0	62	1	0	0	0	0
7B	0	6	5	0	73	1	0	0	0	0
8A	204	105	21	1	TMTC	79	12	0	1	0
8A	154	72	12	4	TMTC	100	10	0	0	1
8B	131	48	7	1	TMTC	107	8	0	0	0

8B	111	51	4	1	TMTC		82	10	0	0	0
9A	0	0	0	0		0	0	0	0	0	0
9A	0	0	0	0		1	0	0	0	0	0
9B	0	0	0	0		0	0	0	0	0	0
9B	0	0	0	0		0	0	0	0	0	0
10A	0	1	1	1		0	0	0	0	0	0
10A	0	0	0	0		0	0	1	0	0	0
10B	0	0	0	0		0	0	1	0	0	0
10B	0	0	0	0		0	0	0	0	0	0
11A	0	0	0	0		0	0	0	0	0	0
11A	0	0	0	0		0	0	0	0	0	0
11B	0	0	1	0		1	0	0	0	1	0
11B	0	0	0	0		0	0	0	0	0	0
12A	0	1	0	0		50	4	0	0	0	0
12A	0	0	0	0		42	6	0	1	0	0
12B	0	0	1	0		78	2	0	0	0	0
12B	0	0	0	0		57	6	1	0	0	0

Legend:

Samples 1-6 Irradiation Date October 31, 2002

1= raw with preservative
2= raw without preservative
3=pasteurized with preservative
4=pasteurized without preservative
5=irradiated with preservative
6=irradiated without preservative

A=Dilution blank 1
B=Dilution blank 2

Samples 7-12 Irradiation Date November 7, 2002

7=raw with preservative
8=raw without preservative
9=pasteurized with preservative
10=pasteurized without preservative
11=irradiated with preservative
12=irradiated without preservative

MICRO RESULTS WEEK 6

12/7/2002			12/8/2002			12/11/2002			12/11/2002		
Coliform			**TSA (AEROBIC)**			**YEAST**			**MOLD**		
	10 E-1	10 E-2	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3
1A	0		5	2	0	31	6	0	0	0	0
1A	0		3	0	0	14	8	2	0	0	0
1B	0		6	0	0	42	2	0	0	0	0
1B	0		4	0	0	26	2	0	0	2	0
2A	1		27	1	1	TMTC	17	1	0	0	1
2A	0		32	0	0	240	13	1	10	2	1
2B	0		36	0	1	TMTC	9	1	0	2	0
2B	0		21	0	0	TMTC	22	2	24	4	0
3A	0		0	0	0	0	0	0	0	0	0
3A	0		0	0	0	0	0	0	0	0	0
3B	0		2	1	0	0	0	0	0	0	0
3B	0		0	0	0	0	0	0	0	0	1
4A	5		TMTC	40	6	276	2	0	0	0	0
4A	0		TMTC	34	9	238	2	0	0	0	0
4B	0		TMTC	28	13	TMTC	3	0	0	0	0
4B	0		TMTC	29	6	TMTC	5	0	0	0	0
5A	0		0	0	0	0	0	0	0	0	0
5A	0		0	0	0	1	0	0	0	0	0
5B	0		0	1	1	0	0	0	0	0	0
5B	0		0	0	0	0	0	0	0	0	0
6A	0		0	0	0	TMTC	TMTC	28	0	4	0
6A	0		5	0	0	TMTC	227	40	0	0	0
6B	0		1	0	0	TMTC	250	53	0	0	0
6B	0		2	0	0	TMTC	TMTC	50	0	0	1
7A	0		4	3	0	27	1	0	0	0	0
7A	0		6	6	0	22	0	0	0	0	0
7B	0		5	0	1	4	0	0	0	0	0
7B	0		5	0	1	45	2	0	0	1	0
8A	194	17	73	15	1	TMTC	149	18	0	3	0
8A	166	15	85	5	2	TMTC	171	15	0	6	0
8B	141	4	40	16	2	TMTC	113	15	0	0	0

8B	174	8	64	1	0	TMTC		108	17	0	2	0
9A	0		16	0	0		0	0	0	0	2	0
9A	0		1	0	0		0	0	0	0	0	1
9B	0		23	1	0		0	0	0	0	0	0
9B	0		0	0	0		0	0	0	0	1	0
10A	0		1	0	0		0	0	0	1	0	0
10A	0		4	0	0		0	0	0	0	0	0
10B	0		1	0	0		0	0	0	0	0	0
10B	0		0	0	0		0	0	0	1	0	0
11A	0		1	0	1		0	0	0	0	0	0
11A	0		0	0	1		0	0	0	0	0	0
11B	0		0	0	0		0	0	0	0	1	0
11B	0		0	0	0		0	0	0	0	0	0
12A	0		0	0	0	TMTC		43	2	0	1	0
12A	0		0	0	0	TMTC		43	0	0	1	0
12B	0		4	2	0	TMTC		42	4	0	1	0
12B	0		2	0	0	TMTC		42	9	0	0	0

Legend:

Samples 1-6 Irradiation Date October 31, 2002

1= raw with preservative
2= raw without preservative
3=pasteurized with preservative
4=pasteurized without preservative
5=irradiated with preservative
6=irradiated without preservative

A=Dilution blank 1
B=Dilution blank 2

Samples 7-12 Irradiation Date November 7, 2002

7=raw with preservative
8=raw without preservative
9=pasteurized with preservative
10=pasteurized without preservative
11=irradiated with preservative
12=irradiated without preservative

MICRO RESULTS WEEK 7

12/14/2002			12/15/2002			12/18/2002				12/18/2002			
Coliform			TSA (AEROBIC)			YEAST				MOLD			
	10 E-1	10 E-2	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-4	10 E-1	10 E-2	10 E-3	10 E-4
1A	0		2	2	0	26	1	0		1	0	0	
1A	0		8	1	0	22	0	0		0	0	0	
1B	0		1	0	0	23	0	0		0	0	0	
1B	0		0	0	0	34	0	0		1	0	0	
2A	0		29	1	0	248	31	8		4	0	0	
2A	0		20	1	0	289	25	3		0	0	1	
2B	1		34	3	0	187	51	3		5	0	0	
2B	0		24	0	0	TMTC	35	8		0	3	1	
3A	0		268	8	1	0	1	spr		0	0	spr	
3A	0		220	13	3	0	0	0		0	0	0	
3B	0		27	7	1	1	0	0		0	1	0	
3B	0		124	4	2	0	0	0		0	0	0	
4A	0		TMTC	176	29	TMTC	43	10		0	0	0	
4A	0		TMTC	139	25	TMTC	32	7		0	0	0	
4B	0		TMTC	101	31	TMTC	30	8		0	0	0	
4B	0		TMTC	89	30	TMTC	42	3		0	0	0	
5A	0		1	1	0	0	0	0		0	0	0	
5A	0		2	0	0	0	0	0		0	0	0	
5B	0		0	2	1	0	0	0		0	0	0	
5B	0		0	5	0	0	0	0		0	0	0	
6A	1		10	0	1		TMTC	72	14		0	0	0
6A	0		12	0	0		TMTC	112	15		0	0	0
6B	0		3	0	0		TMTC	108	12		0	0	0
6B	0		3	1	0		TMTC	128	18		0	3	0
7A	0		7	39	0	70	7	1		0	0	0	
7A	0		8	2	0	62	6	0		0	0	0	
7B	0		6	1	0	82	4	0		0	0	0	
7B	0		4	0	0	83	8	0		0	0	0	
8A	26	1	30	33	4	TMTC	50	12		0	0	0	
8A	44	2	23	32	4	TMTC	42	7		0	0	0	
8B	32	2	22	3	0	TMTC	51	6		0	0	0	
8B	28	1	25	6	0	TMTC	57	4		0	0	0	

9A	0	3	0	1	0	0	0		0	0	0	
9A	0	6	2	0	0	0	0		0	0	0	
9B	0	20	1	0	0	0	1		0	0	0	
9B	0	1	2	0	0	0	0		0	0	0	
10A	0	6	3	3	0	0	0		0	0	0	
10A	0	4	2	0	0	0	0		0	0	0	
10B	0	0	0	0	0	0	0		0	0	0	
10B	0	2	0	0	0	0	0		0	0	0	
11A	0	1	0	0	0	0	0		0	0	0	
11A	0	0	0	0	0	0	0		0	0	0	
11B	0	2	0	0	0	0	0		0	0	0	
11B	0	1	0	0	0	0	0		0	0	0	
12A	0	1	0	0		39	12	1		0	0	0
12A	0	0	0	0		38	7	0		0	0	0
12B	0	0	0	0		27	5	0		0	0	0
12B	0	0	0	0		35	12	0		0	0	0

Legend:

Samples 1-6 Irradiation Date October 31, 2002

1= raw with preservative
2= raw without preservative
3=pasteurized with preservative
4=pasteurized without preservative
5=irradiated with preservative
6=irradiated without preservative

A=Dilution blank 1
B=Dilution blank 2

Samples 7-12 Irradiation Date November 7, 2002

7=raw with preservative
8=raw without preservative
9=pasteurized with preservative
10=pasteurized without preservative
11=irradiated with preservative
12=irradiated without preservative

MICRO RESULTS WEEK 8

12/21/2002			12/22/2002			12/27/2002					12/27/2002			
Coliform			TSA (AEROBIC)			YEAST					MOLD			
	10 E-1	10 E-2	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-4	10 E-5	10 E-1	10 E-2	10 E-3	10 E-4
1A	0		2	4	1	38	5	0			2	0	0	
1A	0		6	1	0	42	0	0			1	0	0	
1B	0		6	1	0	33	1	0			0	0	0	
1B	0		5	1	0	46	4	0			2	0	0	
2A	0		7	1	4		34	5	0			0	0	0
2A	0		10	0	0		37	5	0			1	0	0
2B	0		11	3	0		23	1	1			0	0	0
2B	0		5	1	0		22	0	0			0	0	0
3A	0		0	5	0	0	0	0			0	0	0	
3A	0		0	0	0	0	0	0			0	0	0	
3B	0		0	0	1	0	0	0			0	0	0	
3B	0		0	0	0	0	0	0			0	0	0	
4A	0		TMTC	91	18		23	2	1			0	0	0
4A	0		TMTC	72	21		34	2	2			0	0	0
4B	1		TMTC	104	44		85	11	1			0	0	0
4B	0		TMTC	109	32		60	2	0			1	0	0
5A	0		0	0	0	0	2	0			0	0	0	
5A	0		0	0	0	0	0	0			0	0	0	
5B	0		0	1	2	0	0	0			0	0	0	
5B	0		0	4	0	0	0	0			0	0	0	
6A	0		1	0	0			153	14	1			4	0
6A	0		1	0	0			191	17	4			4	0
6B	0		2	0	0			125	15	1			0	0
6B	0		1	0	0			123	6	1			0	0
7A	0		7	36(spr)	0	80	3	0			1	0	0	
7A	0		2	0	0	77	7	0			0	0	0	
7B	0		4	2	0	77	3	1			0	0	0	
7B	0		6	0	0	55	3	0			0	0	0	
8A	1	0	18	4	0		105	15	2			0	0	0
8A	0	0	15	2	0		101	24	1			0	0	0
8B	0	0	24	4	0		103	23	1			0	0	0

8B	0	0	32	2	0		115	20	0			0	0	0
9A	0		0	0	0	0	0	0			0	0	0	
9A	0		0	0	0	0	0	0			0	0	0	
9B	0		0	0	0	0	0	0			0	0	0	
9B	0		0	0	0	0	0	0			0	0	0	
10A	0		0	1	0	0	1	0			0	0	0	
10A	0		0	0	0	0	0	0			0	0	0	
10B	0		1	0	0	0	0	0			0	1	2	
10B	0		2	0	0	0	0	0			0	0	0	
11A	0		0	1	0	0	0	0			0	0	1	
11A	0		0	0	0	0	0	0			0	0	0	
11B	0		0	0	1	0	0	0			0	0	1	
11B	0		0	0	0	0	0	0			0	0	0	
12A	0		2	0	0		12	1	0			0	0	0
12A	0		0	0	0		4	5	0			0	0	0
12B	0		3	1	0		12	3	0			0	0	0
12B	0		0	0	0		6	2	0			0	0	0

Legend:

Samples 1-6 Irradiation Date October 31, 2002

1= raw with preservative
2= raw without preservative
3=pasteurized with preservative
4=pasteurized without preservative
5=irradiated with preservative
6=irradiated without preservative

A=Dilution blank 1
B=Dilution blank 2

Samples 7-12 Irradiation Date November 7, 2002

7=raw with preservative
8=raw without preservative
9=pasteurized with preservative
10=pasteurized without preservative
11=irradiated with preservative
12=irradiated without preservative

MOLD
10 E-5

0
0
0
0

MICRO RESULTS WEEK 9

12/28/2002			12/29/2002			1/1/2003				1/1/2003			
Coliform			**TSA (AEROBIC)**			**YEAST**				**MOLD**			
	10 E-1	10 E-2	10 E-1	10 E-2	10 E-3	10 E-1	10 E-2	10 E-3	10 E-4	10 E-1	10 E-2	10 E-3	10 E-4
7A	0		5	0	0	31	4	1		0	2	0	
7A	0		4	0	0	41	1	0		0	0	0	
7B	0		4	0	0	43	0	0		0	0	0	
7B	0		4	0	0	42	3	0		0	0	0	
8A	1	0	54	5	1		170	18	4		0	1	0
8A	0	0	41	8	0		182	29	0		0	0	0
8B	0	0	57	8	1		189	24	5		1	0	0
8B	0	0	46	10	0		199	32	2		0	0	0
9A	0		1	0	0	1	0	0		0	0	0	
9A	0		1	0	0	0	0	0		0	0	0	
9B	0		1	0	0	0	0	0		0	0	0	
9B	0		1	0	0	0	0	0		0	0	0	
10A	0		3	0	0	0	0	0		0	0	0	
10A	0		1	0	0	0	0	0		0	0	0	
10B	0		3	0	2	0	0	0		0	0	0	
10B	0		0	0	0	0	0	0		0	0	0	
11A	0		0	0	0	0	0	0		1	0	0	
11A	0		0	0	0	0	0	0		0	0	0	
11B	0		0	0	0	0	0	0		0	0	0	
11B	0		0	0	0	0	0	0		0	0	0	
12A	0		1	1	0	8	2	0		0	0	0	
12A	0		4	0	0	13	1	0		0	0	0	
12B	0		3	0	0	17	2	0		0	0	0	
12B	0		3	0	0	13	1	0		0	0	0	

Legend: Samples 7-12 Irradiation Date November 7, 2002

7=raw with preservative

8=raw without preservative

9=pasteurized with preservative

10=pasteurized without preservative

11=irradiated with preservative

12=irradiated without preservative

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