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Pasteurization of Apple Cider with UV Irradiation

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By

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A THESIS

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An Abstract of the Thesis Presented
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In a period of increasing concern about food safety, food poisoning outbreaks where unpasterurized apple cider or apple juice was found contaminated with *Escherichia coli* 0157:H7 reinforces the need for using the best technologies in apple cider production. Most apple cider is sold as an unpasteurized raw product. Because of their acidity, it was believed that juice products do not usually contain microorganisms such as *E. coli* O157:H7, *Salmonella,* and *Crytosporidium.* Yet all of these foodborne pathogens are capable of being transmitted in unpasteurized juices. It is known that these pathogens can survive for several weeks in a variety of acidic juices. Although heat pasteurization is probably the best method to eliminate these pathogens, it is not the most desirable method as it changes sensory properties and also is very costly for small to mid-sized apple cider processors.

Pasteurization of apple cider with Ultraviolet Irradiation (UV) is a potential alternative to heat pasteurization. Germicidal UV irradiation is effective in inactivating microorganisms without producing undesirable by-products and changing sensory properties.

Unpasteurized raw apple cider from a small local processor was purchased for this study. The effects of physical parameters, exposure time and dosage on the UV treatment efficacy were examined as well as the effects of the UV light on apple cider quality. UV light with principal energy at a wavelength of 254.7 nm, was effective in reducing bacteria (*E .coli,* ATCC 25922) inoculated apple cider. The UV dosage absorbed by the apple cider was mathematically calculated. A radiation dose of 8,777 $\mu W\text{-}s/cm^2$ reduced bacteria an average of 2.20 logs and in multiple passes, the FDA mandated 5-log reduction was achieved.

Sensory analysis showed there was no significant difference between the UV treated and non-treated cider. Experiments with UV treated apple cider indicated a significant ($p < 0.01$) extension of product shelf life through inhibition of yeast and mold growth.

The extension of the researched performed is applicable to other fruit juice processing operations.

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1. INTRODUCTION

1.1 Apples

Apples are a very important crop in the state of Maine and North America in general. In Maine, the total production of apples for the year 1999 was 32.6 million kg, this was slightly less than one percent of national production (MDA 2000). Total utilization of apples was 27.6 million kg. Utilized price per pound is $0.20, (20 cents) which leads to an income of $1,335,000 (MDA 2000). Maine orchards are largely geared to MacIntosh production. The apple harvesting season in Maine starts in late August and usually lasts through October.

Eighty four percent of the apple is water and the remaining 16 % is total solids. This 16% contains nitrogen, fatty materials, minerals, carbohydrates, astringents, color compounds, enzymes, volatiles, vitamin A, C and flavonoids (Smock and Neubert 1950). Maine apples usually contain less than 1% fat, have no cholesterol or sodium, are low in calories and have most of the essential vitamins and minerals (MDA 2000).

Apples are an excellent source of fiber. A medium sized apple has about 5 g of fiber - 25% of the recommended daily intake of fiber (20 g). Apples contain both soluble and insoluble fiber, the majority of fiber that apples contain is a soluble fiber called pectin, which is a form of fiber that has cholesterol reduction properties (Smock and Neubert 1950).

The amount of sugars and acids in fresh apples can vary. Maturity, production regions and varieties of apples, as well as the weather conditions throughout the year

create differences in apple composition. There are numerous flavor compounds in apples, they are complex and volatile. Many of these flavor compounds are lost in making a clarified, preserved juice from freshly squeezed apples. Pectins are responsible for the viscosity of cider and the tannins for the astringency. Apple cider contains small amounts of ethanol in addition to acetaldehyde (Jay 2000).

Apples contain two primary enzymes: polyphenol oxidase and peroxidase. These two enzymes are responsible for the oxidized flavor in apple cider. If they are not inactivated quickly enough, they accelerate the oxidation of tannins and natural flavors. Oxidized flavor and color change in unpasteurized cider is due to these oxidized compounds. These flavors may be the reason why some consumers prefer unpasteurized cider to the pasteurized product (Pearson 1976). Throughout this thesis cider is the juice from freshly squeezed apples separated from the pumice but not filtered.

1.2 Cider and *Esherichia coli* (*E. coli*)

Although some apple cider is heat pasteurized to ensure microbiological safety, unpasteuzied cider is still the choice of many consumers even though it has been established as a vector for foodborne illnesses. Increased incidences of foodborne illnesses associated with apple cider are generating interest in cider safety. Enterohemorrhagic *Esherichia coli* (*E. coli*) was first recognized as a human pathogen in 1982 (Griffin *et al.* 1991). *E. coli* is an important and common human pathogen which causes diarrhea, bloody diarrhea (hemorrhagic colitis) and also the post diarrheal disorder, hemolytic uremic syndrome (HUS) which is life threatening

(Karmali *et al.* 1989, Riley *et al.* 1983). In past years, many food-associated outbreaks were caused by enterohemorrhagic *E. coli.* It was not until 1991 that *E. coli* was associated with hemorrhagic colitis when an outbreak was epidemiologically linked to fresh apple cider in Massachusetts (Besser *et al.* 1992). Until this outbreak most of the cases involving this pathogen were associated with raw milk, undercooked ground beef or person to person contact (Griffin *et al.* 1991). Following the initial outbreak associated with fresh apple cider, two other outbreaks occurred in Connecticut in 1996. In both of these outbreaks, *E. coli* 0157:H7 was linked to the consumption of apple cider (CDC 1997).

The US Food and Drug Administration (FDA) considers foods with pH $\leq$ 4.6 to be high-acid foods, which are not microbiologically hazardous (FDA 1977). Due to its low pH (< 4.0), apple cider was thought not to support the survival of *E. coli* 0157:H7. However, Miller and Kapsar (1994) reported that there was no change in the population of *E. coli* 0157:H7 after 24 hours in apple cider or in low pH adjusted Trypticase Soy Broth (TSB); TSB was adjusted to a pH of 3.0 with hydrochloric acid. Several other studies had shown that *E. coli* 0157:H7 survives in apple cider (pH 3.7) stored at 8 °C for up to 31 days. Typically apple cider has a pH of 3.5 to 4.0, and it is considered a highly acidic food. As stated earlier, food studies and outbreaks suggest that *E. coli* 0157:H7 possesses unusual tolerance to low pH. Survival of *E. coli* 0157:H7 was shown in other acidic foods such as mayonnaise-based sauces and also in reduced-calorie and real mayonnaise stored at 5 °C (Erickson *et al.* 1995). It has been reported that refrigeration enhances the survival of *E. coli* 0157:H7 in some acidic foods. Zhao *et al.* (1993) reported that *E. coli* 0157:H7 cells die faster in apple

cider stored at 8 °C versus 25 °C. It was also found that *E. coli* 0157:H7 survived longer in ketchup stored at 5 °C than 23 °C (Tsai *et al.* 1997). Outbreaks and studies such as these, along with developing regulatory requirements, have resulted in the need for processors to ensure the destruction of this pathogen in their food products.

Other outbreaks associated with different pathogens in cider have been reported as well. During 1974, apple cider was implicated in a large outbreak of salmonellosis in New Jersey, which caused 296 people to become ill (CDC 1975). Research has shown that some serotypes of *Salmonella* could also survive at a pH of 4.0 for up to 30 days at 4 °C (Goverd *et al.* 1979).

Cryptosporidium spp. outbreaks have also been linked to the consumption of apple cider. One of the outbreaks occurred in Maine in 1993 and 160 people became ill. The second outbreak occurred during 1996 in New York (CDC 1997).

As *E. coli* 0157:H7 is the most common foodborne illnesses linked to apple cider, it is the target organism in the study performed at the University of Maine and therefore will be discussed in more detail.

1.3 How *E. coli* gets into the cider

The source of *E. coli* in fresh apple cider has generally been attributed to contact of the apples with animal feces prior to cider processing. Typically, this might occur in the orchard if farm animals or wild animals were grazing and apples dropped to the ground and contacted the feces of these animals. If these apples were later used in apple cider processing, *E. coli* could be introduced into the cider. Coliforms and

generic *E. coli* are among the organisms that may be found on produce, however the presence of *E. coli* does not necessarily indicate that pathogens (such as *E. coli* 0157:H7) are present (NFPA 2000). The International Commission on Microbiological Specifications for Foods (ICMSF) does not recommend using microbiological criteria in acceptance and rejection of raw vegetables.

If proper procedures (Good Manufacturing Practices) are not followed, microorganisms found on apples might be in the finished product, apple cider, as well (Besser *et al.* 1993). Incidences where improper handling procedures resulted in contamination of cider with *E. coli* 0157:H7 and other pathogens such as *Salmonella typhimurium* were reported (Goverd *et al.* 1979). There has not been a study showing that *E. coli* 0157:H7 was found in apple cider made from non-drop apples (Riordan *et al.* 2001, Kenney *et al.* 2001).

1.4 Ways to eliminate *E. coli* from apple cider

Heat Pasteurization: *E. coli* 0157:H7 is completely destroyed in properly pasteurized products similar to milk, during high temperature short time pasteurization (HTST). The FDA mandates pasteurizing apple cider at 72° C for 10 seconds (FDA 1998). However, pasteurization is not typically applied to fresh apple cider. Even though pasteurization can destroy all the human pathogens and achieve a high food safety level, it changes the sensory qualities of the apple cider and makes it undesirable for consumption. Raw, unpasteurized apple cider is still the choice of consumers due to its flavors and textures.

Chemicals: Use of chemicals has been examined as a method to eliminate *E. coli* from cider. It was shown that a combination of sodium sorbate and mild heat greatly increased the shelf-life of fresh apple cider (Robinson and Hills 1958). Other studies performed by Besser *et al.* (1993) and Zhao *et al.* (1993) reported that sodium benzoate decreases the survival time of *E. coli* 0157:H7 in apple cider.

New Technologies: Several new technologies are available to accomplish a microbiological reduction in juices. Ozonation is being examined (Dock 1999), minimal thermal processes, Pulsed Electric Field, batch and continuous high-pressure processing systems and Ultraviolet (UV) irradiation have been offered commercially (Sizer and Balasubramaniam 1999). In Table 1.1 a comparison of some of these processes are made.

Table 1.1. Comparison of nonthermal juice processes*

Process	Temperature	Enzyme Inactivation	Equipment Costs[1]
Pulsed Electric Field	Ambient (slight increase due to process)	None	$$$$$
UV light	Ambient	None	$
Minimal thermal process	70° C for 6 sec	Minimum	$$
Batch High Pressure	Ambient plus compression heating	Selective inactivation	$$$$$
Continuous high pressure	Ambient plus compression heating	Selective inactivation	$$$$$

*Adapted from (Sizer and Balasubramaniam 1999).
[1]Compared to the estimated cost of thermal pasteurization equipment, which ranges from $20,000 to $30,000 ($$$$$), UV irradiation equipment has a lower capital cost of $10,000 to $15,000 ($) (Kozempel *et al.* 1998, Majchrowicz, 1999, Brown 2001).

For this research UV irradiation is the treatment of interest. One of the reasons for choosing UV irraditaion was its cost efficiency as the project is mainly geared towards the small local producers in Maine (see Table 1.1).

1.5 Food irradiation

Microbiological hazards still exist even though the United States (US) food supply has achieved a high level of safety. Irradiation of foods for the purpose of killing spoilage microorganisms and pathogens has been recognized as a preservation technique for several decades. It is one of the solutions to eliminate foodborne illnesses and their consequences. It was estimated that microbial pathogens cause as many as 76 million cases of foodborne disease, including 5,200 deaths annually

(Economic Research Service, ERS 2000). Recent outbreaks of illness and death caused by *E. coli* O157:H7 have focused attention on this emerging pathogen. ERS estimates that, each year in the United States, foodborne *E. coli* O157:H7 disease costs $659.1 million to society. ERS for the first time also estimated the cost due to other strains of *E. coli* that produce Shiga toxins (STEC). These strains of *E. coli* are known as *E. coli* non-O157:H7 STEC. Foodborne *E. coli* non-O157 STEC disease costs $329.7 million for a combined (both *E. coli* O157:H7 and *E. coli* non-O157:H7 STEC) total cost of $988.8 million (See Table 1.2).

Table 1.2. Estimated annual costs due to foodborne *E. coli* O157:H7 and foodborne *E. coli*, non-O157 STEC, August 2000*

	E. coli O157:H7 Cases	*E. coli* non-O157:H7 STEC Cases	*E. coli* O157:H7 Costs	*E. coli* non-O157:H7 STEC Costs
	Number	**Number**	**Million $**	**Million $**
Acute [1]				
Medical:				
No medical care	35,632	17,816	Not estimated	Not estimated
Physician visit	24,983	12,492	6.9	3.4
Hospitalized and survived, HC	921	460	8.2	4.1
Hospitalized and survived, HUS	869	435	24.7	12.4
Hospitalized and died, HC	0	0	0	0
Hospitalized and died, HUS	52	26	1.5	0.8
Subtotal	62,458	31,229	41.3	20.7
Productivity loss/premature deaths:				
No medical care	35,632	17,816	9.8	4.9
Physician visit	24,983	12,492	13.7	6.8
Hospitalized and survived, HC	921	460	1.8	0.9
Hospitalized and survived, HUS	869	435	3.8	1.9
Hospitalized and died, HC	0	0	0	0
Hospitalized and died, HUS [2]	52	26	447.5	223.8
Subtotal	62,458	31,229	476.6	238.3
Subtotal—Acute	**62,458**	**31,229**	**517.9**	**259**
Chronic [3]				
Medical:				
No medical care	0	0	0	0
Physician visit	0	0	0	0
Hospitalized, HUS	46	23	36.5	18.3
Subtotal	46	23	36.5	18.3
Productivity loss/premature deaths:				
No medical care	0	0	0	0
Physician visit	0	0	0	0
Hospitalized, HUS[3]	46	23	104.7	52.4
Subtotal	46	23	104.7	52.4
Subtotal—Chronic	**46**	**23**	**141.2**	**70.7**
Total	62,458	31,229	659.1	329.7

*Adapted from Economic Research Service, USDA, Oct. 20, 2000.

[1]Data from the Centers for Disease Control and Prevention (Mead *et al.* 1999) except for physician visits and hospitalized case subcategories, which were estimated by ERS. These estimated cases and costs are for the secondary complications, hemolytic uremic syndrome (HUS), and hemorrhagic colitis (HC), during the first year of the illness.

[2]Cost calculations are based on the labor market approach for valuing the cost of premature deaths.

[3]Note that all components of the chronic section are for the subset of acute illness cases that go on to develop chronic HUS cases and incur costs beyond the first year.

1.5.1 What is Irradiation?

Irradiation exposes food to radiant energy. Radiation is the transport of energy by electromagnetic waves or atomic particles. This might either be an ionizing (Gamma Ray, X Ray) energy source or a non-ionizing one (Ultraviolet radiation (except the high energy end of the UV-spectrum), visible light, infrared radiation, microwaves and radio waves). Ionizing radiation has shorter wavelengths and is capable of converting atoms and molecules to ions by removing electrons. This type of irradiation creates ions in the irradiated material such as free radicals. A substance produced from irradiation is called the radiolytic product. Radiation with less energy than that required to produce ions in the irradiated material is called non-ionizing radiation. UV light is a non-ionizing energy source (Swallow *et al.* 1991, Diehl 1995).

Irradiance (or Intensity) is the radiant energy reaching the defined surface and is measured in microwatts per centimeter squared ($\mu W/cm^2$) or milliwatts per centimeter squared (mW/cm^2). Two types of units are used for radiation, units of activity and units of exposure (dosage). Units of activity quantify the amount of radiation emitted by a given radiation source. Units of exposure quantify the amount of radiation absorbed or deposited in a specific material by a radiation source. A Gray is the International System of Unit (SI) of absorbed radiation. Irradiation dose is the amount of kiloGray used to irradiate a product. One joule of energy is absorbed per kilogram of matter being irradiated; 1,000 Gray (Gy) = 1 kiloGray (1 kGy).

1.5.2 Approval of food irradiation in the US

Food irradiation is the food processing technology, which took the longest for its approval, 40 years (AMA 1993). The FDA treats food irradiation as a food additive, which is one reason for lengthy approval. Research has been comprehensive and has included wholesomeness, toxicological, and microbiological evaluation. Worldwide, 38 countries permit irradiation of food. More than 12.7 billion kg of food are irradiated annually in Europe (Loaharanu *et al.* 1994).

1.5.3 FDA regulatory aspects of food irradiation

Radiation doses allowed by the FDA are the most restrictive of all countries in which irradiation is allowed (AMA 1993). It has been shown that low doses of irradiation permit microorganisms to survive but does not replace proper food handling (Diehl 1995). Thus, the handling of foods processed by irradiation should be the same as all other foods. Food irradiation cannot enhance the quality of a food that is not fresh. Irradiation does cause changes in food, all of which have been found to be benign. The studies that were done in the last 40 years showed no toxic effects from the irradiated foods (Thayer 1994). Additionally, human volunteers consuming up to 100% of their diets as irradiated food have shown no ill effect (Diehl 1995). A food that has been irradiated must be labeled and the packaging that is used to hold the food must be tested and a regulation should be issued for its use (Pauli and Tarantino 1994).

World Health Organization (WHO) policy statement released in 1992 was also accepted by the American Medical Association. The statement is as follows:

"Irradiated food produced under established Good Manufacturing Practices is to be considered safe and nutritionally adequate because: i) the process of irradiation will not introduce changes in the composition of the food which, from a toxicological point of view, would impose an adverse effect on human health; ii) the process of irradiation will not introduce changes in the microflora of the food which would increase the microbiological risk to the consumer; iii) the process of irradiation will not introduce nutrient losses in the composition of the food, which, from a nutritional point of view, would impose an adverse effect on the nutritional status of individuals or populations" (AMA 1993).

FDA also dealt with UV irradiation under the indirect food additives category, which took some time to be approved. It is mandatory that all irradiated foods in the United States be labeled with a Radura (Figure 1.1), the international symbol for irradiation and the words "treated by irradiation" or "treated with radiation." (Stevenson 1994).

Figure 1.1. International symbol for irradiation (Radura)

1.5.4 Nutritional adequacy of irradiated food

Nutritional studies related to irradiated foods have been directed towards the problems of potential destruction of nutrients or if there is a change in nutrient bioavailabilty or not (Swallow 1991, Diehl 1995, AMA 1993, International Atomic Energy Agency 1991). It has been demonstrated that the macronutrients undergo minimal changes due to irradiation. Studies where wheat, potatoes, chicken, rice and fish were subjected to low to medium doses of irradiation illustrated this (Thomas 1977, 1981, 1983).

1.5.5 Consumer/Producer issues

Irradiated foods are not widely used due to consumer issues, despite their advantages and regulatory approval. The concept of wholesomeness has been used for food irradiation. It is a specifically defined term and it includes concepts of microbiological and toxicological safety and nutritional adequacy (Skala *et al.* 1986). In a 1995-96 study, after seeing a 10 minute video describing irradiation, interest in buying irradiated foods among California and Indiana consumers increased from 57% to 82% (Center for Consumer Research 1996). If accurate information is given to the public by health specialists regarding irradiation, consumers will be more willing to purchase irradiated food. Even if some consumers are familiar with food irradiation, many have little knowledge of the process and its advantages.

1.5.6 UV light as food irradiation source

Pasteurization of apple cider with UV irradiation appears to be a potential alternative to heat pasteurization. Germicidal UV irradiation does not produce undesirable by-products and is also effective in inactivating microorganisms (Morris *et al.* 1972, Yip *et al.* 1972). Light with wavelengths shorter than 400 nm are in the UV range. There are three types of UV light; UVA, UVB, and UVC (Figure 1.2). The light, which is used for the inactivation of bacteria and viruses, is the UVC light with the wavelength range of 220-300 nm, which has germicidal properties similar to gamma radiation. UV irradiation germicidal properties are due to the DNA absorption of the UV light which causes cross linking between the neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand (Miller *et al.* 1999). Due to this, the DNA transcription and replication is blocked, which compromises cellular functions and leads to cell death (Bachman *et al.* 1975, Miller *et al.* 1999). UV light is a physical rather than a chemical process, which makes this system ideal due to ease of maintenance, low operating cost, and operator safety.

Typical UV units consist of eight UV lamps, two sensors to detect the UV exposure and concentric tubes where fluid flows as a thin film. Total energy coming from the lamps can be expressed in terms of power, Watts (W). As the light beam passes from source to the UV lamps, then to the apple cider, there are some reflection, refraction and absorption processes. The efficiency of UV light in the destruction of microorganisms and also properties of UV irradiation is discussed in detail in the following sections concerning UV light.

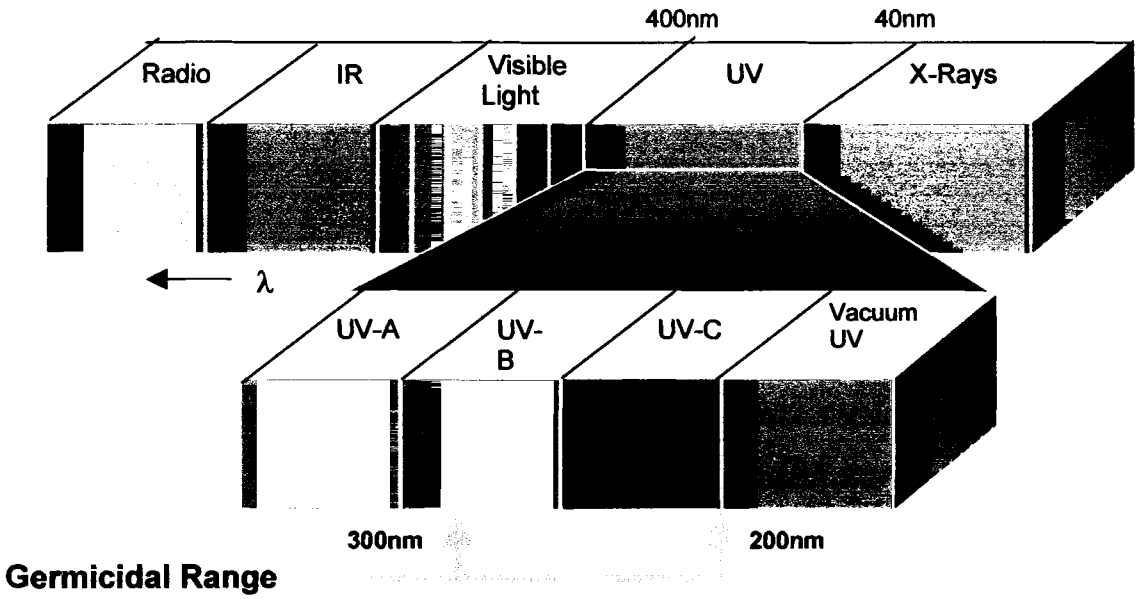

Figure 1.2. Electromagnetic spectrum illustrating breakdown of UV light types. Adapted from (Malley 2000)

In the past, few investigations were performed concerning the use of UV to reduce bacterial numbers in foods. Some of the investigations include reducing microorganisms by 99% in flowing maple sap using UV (Kissinger *et al.* 1966) and extension of shelf life of fresh Mackerel by seven days over the control (Huang *et al.* 1982). Harrington and Hills (1968) employed specifically designed UV lamps for reducing the bacterial population of fresh cider. It was also found that a significant increase in the caselife of beef might be obtained by exposure to UV light (Reagan *et al.* 1973). Another study demonstrated that bacteria counts on a smooth surface of fresh fish can be reduced by 2 log cycles using UV light (Stermer *et al.* 1987). In a

study performed by Zhao *et al.* (1994), the possibility of UV light being an alternative to the use of chemical preservatives, such as potassium sorbate and sodium benzoate, was discussed as these only minimally affect the survival of *E. coli* 0157:H7 in fresh apple cider.

As mentioned earlier, UV treatment of foods is a physical rather than a chemical process. This is an advantage as there might be residuals in chemical processes (Qualls *et al.* 1983). Treating foods with UV light offers advantages to consumers, retailers, and food manufacturers such as improved microbiological quality, replacement of chemical treatments, and extended shelf life. This results in the reduced use or elimination of chemical treatments. The disadvantage may be the poor penetration property of UV light. One significant disadvantage of using UV light in treating apple cider is that the presence of small amounts of particulates in a liquid can greatly reduce the penetration of UV (Bachman *et al.* 1975, Shama *et al.* 1996). Due to the high turbidity (which decreases the clarity of the fluid) of apple cider and low penetration capability of UV light, apple cider should be exposed to UV irradiation as a thin film (FPE 2000).

1.5.7 FDA regulatory aspects of UV irradiation

It was mandated by the FDA that a warning label should be placed on any juice that has not been processed to prevent, reduce, or eliminate pathogenic microorganisms that may be present, so juices not receiving 5-log reduction would be required to have a warning statement (FDA 1998). The term "log" is short for

logarithm. A logarithm is a power of ten. Each log reduction is a reduction of 90%. So, a one log reduction is a 90% reduction, a two log reduction is 99%, and a five log reduction is 99.999%. Recently the FDA has recommended that fruit and vegetable processors should implement Hazard Analysis Critical and Control Point programs (FDA 2001). FDA has approved the use of UV irradiation of juices, to reduce contamination from pathogens and microorganisms (FDA, 2000). The FDA considers the treatment with UV irradiation a way to achieve "considerable reduction" in the level of pathogens and microorganisms.

1.6 Summary and Objectives

Previous research clearly shows that *E. coli* O157:H7 has been implicated in numerous foodborne illness outbreaks from contaminated, raw apple cider. *Cryptosporidium parvum*, a protozoan parasite, and *Salmonella* have also been implicated as causative agents in foodborne illness outbreaks from apple cider as well. Increasing concern in the safety of food products related to these outbreaks makes processors seek low cost and reliable methods to achieve the required safety in apple cider. UV irradiation is one promising non-thermal processes alternative to heat pasteurization. It achieves the FDA required 5-log reduction, yet does not impair the sensory and nutritional qualities of apple cider and is much cheaper.

The objectives of this study were:

1. To examine the efficacy of a low-cost UV treatment process to achieve the FDA-required 5-log reduction in *E. coli* colonies in unpasteruized apple cider,

2. To evaluate microbiological and physical characteristics of UV treated apple cider for its food safety and extended refrigerated storage period,
3. To observe the effects of color, soluble solids, turbidity, viscosity and pH on the UV treatment efficiency. Critical factors having an affect on the percentage of microbial kill were determined,
4. To accurately define the UV dose required inactivating *E. coli*, and
5. Consumer acceptability of irradiated apple cider was investigated.

2. MATERIALS AND METHODS

The following section will describe the raw materials used (2.1), preparation of microbiological media (2.2), growing the *E. coli* (ATCC 25922) culture (2.3), use of spectrophotometer to determine the culture strength (2.4), analysis on apple cider including physical and microbiological (2.5), experimental design for testing (2.7), calculations (flow rate, characteristics and energy) regarding the UV machine used in this research (2.8), sensory analysis (2.9), shelf-life study (2.10) and statistical analyses (2.11).

2.1 Raw materials

Apple cider used in this project was purchased from a small local producer, the Apple Farm (Skowhegan, ME). The Apple Farm implements Hazard Analysis Critical Control Point (HACCP) procedures and uses only handpicked apples in apple cider production. According to state of Maine regulations, apple cider produced in Maine must contain at least 50% MacIntosh apples. The remaining apples used in cider production are Cortland, Golden Delicious, Macoun and Mutsu, depending on the availability of the species at the time of pressing. The first step in making cider is to wash the apples with chlorinated water. The next step is to crush them. A conveyor carries the apples up to the crusher. Crushed apples are then dropped into a wooden square frame for pressing, after the apples are pressed the cider runs freely to the juice collector. Finally, the cider is bottled and is ready to sell (This is shown is Figure 2.1).

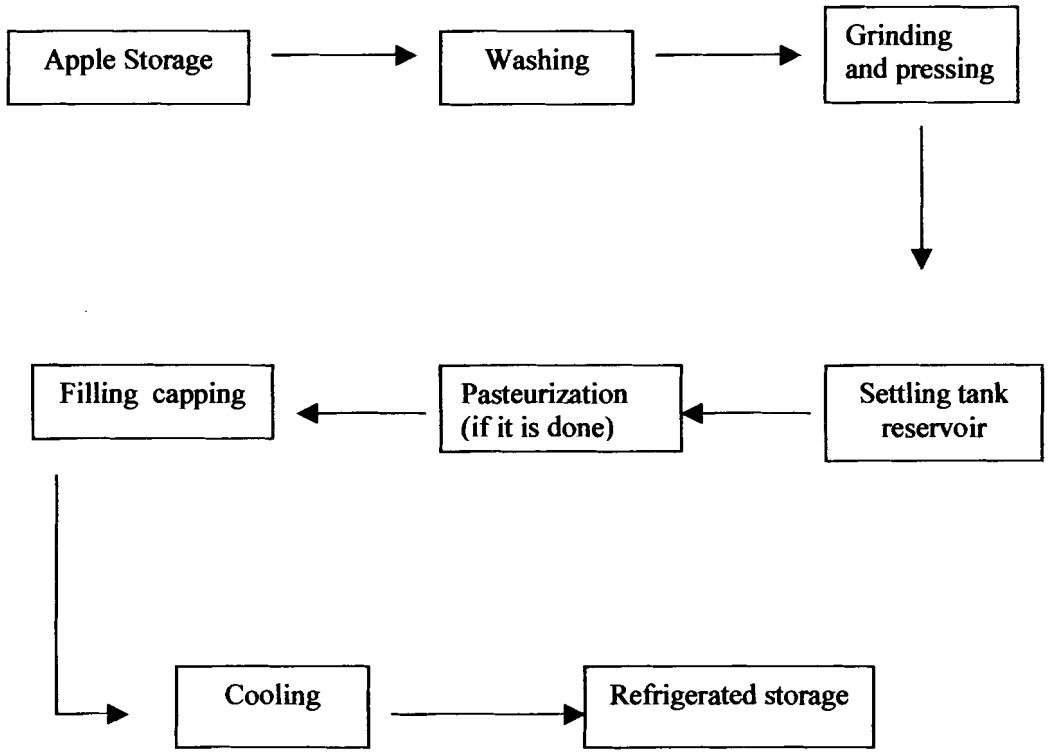

Figure 2.1. Flow diagram of apple cider production

By the use of this technique, apple cider is pressed with a force of 907.2 kg. If the cider is pasteurized, it is done before bottling at 71-72 °C for 10 seconds. For our research purposes, raw unpasteurized apple cider was purchased and used. The cider was purchased in gallon jugs and was transported to the Biological Engineering Laboratory (BEL) at the University of Maine (UMaine) in coolers filled with ice to limit the growth of the microorganisms already present in raw cider and to prevent contamination. Ice maintains low temperatures not to favor the growth of microorganisms. The apple cider was kept at 4 °C in a standard laboratory refrigerator

and was used within the next two weeks for experiments. Daily temperature checks were performed to ensure maintaining a temperature of 4 °C.

E. coli (ATCC 25922) was obtained from the American Type Culture Collection (ATCC), (Manassas VA, USA). Worobo (1999) and Duffy *et al.* (2000) showed that this strain of *E. coli* (ATCC 25922) is acid resistant and behaves the same way as *E. coli* 0157:H7 and therefore can be used as a surrogate organism of *E. coli* 0157:H7 for experimental purposes. The strain was delivered as a freeze-dried sample and then resuspended in TSB (Trypticase Soy Broth, Difco Chemical Beckton Dickinson, Sparks, MD). After resuspension, the mixture was transferred into 100 ml of TSB and was placed in a shaker at 35 °C overnight (Model 132000, Boekel Industries Inc. Feasterville, PA). Ten ml of the *E. coli* suspension was kept as a frozen culture in the event something went wrong with sampling. One ml of the mixture was transferred onto each of three Trypticase Soy Agar (TSA) plates and incubated. From these three parent plates; triplicates of TSA were made with the streaking technique and incubated at 35 °C to keep the bacteria growing. The plates were streaked in order to isolate the *E. coli* colonies. Prior to each test, a fresh *E. coli* sample was grown and isolated. An isolated colony was transferred into 100 ml of TSB to be grown overnight in the incubator shaker (Series 25, Incubator Shaker, New Brunswick Scientific, Edison, NJ) at 35 °C.

The culture was restreaked on TSA plates every three to four days to keep it viable. In addition to this, once a month, a colony was transferred to a selective medium such as Sorbitol MacConkey Agar (SMAC) or MacConkey Agar (MAC) to verify that the colonies growing on TSA plates were *E. coli* colonies. Microbiological

media used in this study included TSA (Trypticase Soy Agar), TSAP (Trypticase Soy Agar supplemented with Pyruvic acid, (Sigma Chemical Company, St Louis, MO), MAC (MacConkey), Special Yeast and Mold (YM) medium (Difco Laboratories, Detroit, MI) and SMAC (Sorbitol MacConkey). SMAC was used to test if there was *E. coli* 0157:H7 present in the apple cider when purchased. While other *E. coli* strains like ATCC 25922 form pink colonies on SMAC, *E. coli* 0157:H7 forms clear colonies because it cannot ferment sorbitol present in SMAC. This media was mainly used to check the background microflora of cider used in the sensory testing (discussed later).

Chloramphenicol (Fisher Scientific, Fair Lawn, New Jersey) was added to the yeast and mold media. This is an antibiotic that prevents the growth of bacteria on the medium and also facilitates the growth of yeast and molds. Normally, with yeasts and molds it takes at least four days before the colonies can be counted but the use of chloramphenicol reduces this to two days. This antibiotic also has the advantage of being autoclave stable, which is more convenient as it can be added to the media before autoclaving. Bactopeptone (Difco Laboratories, Detroit, MI) was used as a dilution medium. This is a protein used to prevent the lysis of cells, which can occur with the use of distilled water only. Distilled water used for the experiments was obtained from Hitchner Hall and carried to the BEL a day prior to testing. Five-gallon carboys were used to store the distilled water at room temperature in the BEL.

2.2 Preparation of microbiological media

The media mentioned previously in section 2.1, were prepared and spread plating technique was used. When preparing media, a water bath (Isotemp 120, Water Bath, Fisher Scientific, Fair Lawn, NJ) and heated stir plates (Fisher Stirring Hot plate Model: 11-502-49SH, Fisher Scientific, Fair Lawn, NJ) were employed. The stir plates were used to mix the media thoroughly before autoclaving and the water bath was used after autoclaving to keep the media at the right temperature. Media were made in advance and stored in the laboratory refrigerator at 4 °C. Prior to testing, the media were allowed to come to room temperature. McCarthy *et al.* (1998) determined a 3-log difference in *E. coli* counts between the TSA and SMAC plates, so for stressed organisms detection SMAC should not be used, instead in our experiments TSAP was used. This is an enrichment media for determining if sublethal cells are present.

2.3 Growing the *E. coli* culture

The *E. coli* culture (ATCC 25922) was kept on TSA at 4 °C. A day before the test, one loop of *E. coli* from the TSA plate was transferred into a flask, containing 100 ml of TSB. This was then placed into the incubator shaker rotating at 250 rpm for 18-22 hours at 37 °C.

Dilution tubes containing nine ml of 0.1 % Bactopeptone were prepared in advance. When dilutions are made, apple cider should not be kept in bactopetone for more than twenty minutes because it is a nutrient rich medium and the colonies might increase in number, therefore, dilutions were plated immediately.

2.4 Spectrophotometer

On the test day, a spectrophotometer (Spectronic 1001, Split Beam Spectrophotometer, Miltron Roy, Boston, MA) was used to measure the strength of the culture grown a day prior to testing. The spectrophotometer was turned on at least 30 minutes before the testing in order to warm up; it was blanked, standardized and readings were taken at 600 nm. After the *E. coli* were taken out of the shaker, a dilution of *E. coli* and TSB was made before taking the spectrophotometer reading. According to the spectrophotometer protocol, the absorbance reading of the culture should be 0.04 or higher. The measured reading is converted to CFU/ml. The factor used in the conversion of spectrophotometer reading into microorganisms per milliliter is $2.77*10^8$, a multiplying factor found by the use of a standard curve. A standard curve in the BEL was used to verify the multiplying factor (calculations not shown). After the equation of the line is found the factor is calculated; and using the factor, the absorbance value is converted into the CFU/ml. The amount of *E. coli* that would be inoculated to achieve the target concentration was calculated. This was done by equation (1) shown below:

$$C_1*V_1=C_2*V_2 \quad (1)$$

Where C_1 is the strength (concentration) of the culture, C_2 is the target microbial concentration of apple cider, V_1 is the required volume of the culture and V_2 is the volume of apple cider that will be tested.

2.5 Analyses on apple cider

2.5.1 Physical analysis

The physical properties of cider that were examined included color, turbidity, and viscosity. Temperature, pH, brix and density were also recorded. The analyses were performed on both the UV irradiated and non irradiated (control) apple cider to determine the effects of UV on the physical parameters of the cider. The analyses performed are explained below in detail.

2.5.1.1 Color

Color of the apple cider was analyzed using the Hunter Lab Scan I I Spectrocolorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Hunter "L", "a", and "b" values were measured. The optical aperture that was selected was 4.45 cm (the largest size available). The D_{65} illuminant was used for color analysis, 60 ml of sample was collected. In each treatment, the reflectance measurement was obtained from the average of three readings.

2.5.1.2 Turbidity

Turbidity of the cider was analyzed using a turbiditimeter (NTU Turbidity, Hach Model 2100A, Hach Company, Loveland, CO), consisting of a nephelometer with a light source illuminating the sample. The intensity of light was scattered at 90 degrees (perpendicular) to the path of incident light. Turbidity was indicated with the photoelectric detectors through a readout device. The method compared the intensity

of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The measurement of NTU does not give the sizes of the particles, nor does it indicate the amount of particles present. It is a qualitative, rather than quantitative way of measuring turbidity. For turbidity testing, 40 ml of the control and irradiated apple cider were collected separately and analyzed on the test day. First the turbiditimeter was adjusted using a 100 nephelometric turbidity units (NTU) standard. Due to the high turbidity of apple cider, a dilution was prepared for accuracy. Therefore a 50% dilution of apple cider and distilled water was prepared and the turbidimeter reading was multiplied by two in order to calculate the final turbidity of the apple cider (Greenberg *et al.* 1992). The procedure used for turbidities above 40 NTU was followed for this testing, as the turbidity of apple cider is generally higher than 40 NTU. Equation 2 was used for the calculation of turbidity;

$$\text{Nephelometric turbidity units (NTU)} = \frac{A^{*}(B+C)}{C} \qquad (2)$$

Where "A" is the NTU found in the diluted sample, "B" is the volume of dilution water (ml) and "C" is the cider volume used for dilution (ml). Using equation 2 the final turbidity value of the diluted apple cider was determined.

2.5.1.3 Viscosity

Five hundred ml of the control and UV irradiated apple cider were tested for viscosity, using a Brookfield viscometer (Model DV-III Programmable Rheometer, Brookfield Engineering Laboratories, Stoughton, MA). The apple cider was tested

with spindle number one and the rpm was adjusted according to torque percentage. The reading (in Centipoise, cP) was obtained. As temperature affects viscosity, temperature readings were recorded for each corresponding viscometer reading. Viscosity values were then mathematically adjusted according to the temperature - viscosity relationship shown in Figure 2.2. The range of temperature readings were between 5 and 15 °C. The viscosity values in literature are calculated at room temperature, and therefore different than those obtained here. The experimental temperature range examined is lower than room temperature and therefore cannot accurately predict what the values would be at room temperature. The equation defines the relationship between temperature and viscosity for the measured range of values and any of the predictions made outside of this range will not be accurate.

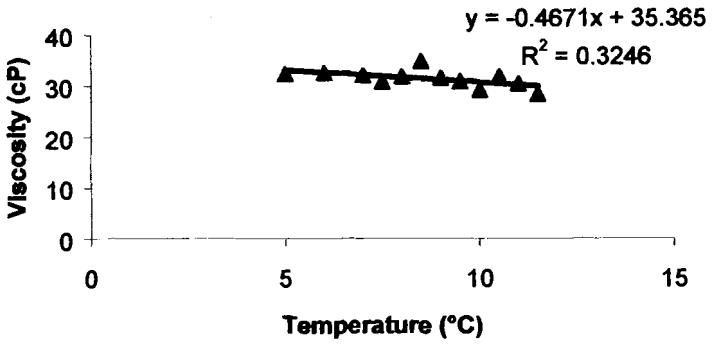

Figure 2.2. The relationship between the temperature and viscosity for this study

2.5.1.4 Temperature and pH

Before and after each test, temperature and pH values of the apple cider samples (20 ml) were recorded using a digital pH meter (Chemcadet Model 5986-60, Singapore) which was calibrated by using standard solutions of pH 4.0 and 7.0.

2.5.1.5 Brix and soluble solids values

To obtain a brix value, apple cider was centrifuged in an Eppendorf Model 5415C centrifuge (Brinkmann Instruments, Westbury, NY) at setting 10 (10,000 rpm) for 2 minutes. Soluble solids are calculated using the brix value measured by a refractometer (Model 9001 0-30% Solids (Brix) Tester, Spectronic Instruments Inc., Rochester NY).

2.5.2 Microbiological analysis

For microbiological analysis, one ml of sample is serially diluted in 9 ml of 0.1% Bactopeptone. Appropriate serial dilutions were then spread plated in triplicate using a sterile glass hockey stick and incubated (35 °C) for 24 hours. The colonies were counted manually using a standard plate count method to determine the colony forming units per ml (CFU/ml) (Vanderzant *et al.* 1992). For background microflora, fresh non-inoculated apple cider was plated on TSA to determine total bacterial populations. SMAC and MAC were used to selectively enumerate any possible *E. coli* 0157:H7 and other strains of *E. coli*. Yeast and Mold media (YM) supplemented with 0.01% Chloramphenicol (Sigma, Chemical Company, St. Louis, MO) was used for the enumeration of yeasts and molds.

In order to test the log reduction in inoculated *E. coli* (ATCC 25922), plate counts were taken before and after UV treatment. Again, appropriate dilutions were plated in triplicate after one ml of test sample (whether UV irradiated or unirradiated apple cider) was serially diluted in 0.1% Bactopeptone. The reason to plate a sample

from the non- irradiated cider is to determine if the calculated concentration of *E. coli* was the same as the target concentration calculated by using the multiplying factor (discussed previously in Section 2.4). TSA supplemented with pyruvic acid (TSAP) was used as a recovery medium. This is a nutrient enriched medium and it is used to determine if there are any sublethlally damaged cells. Colonies on plates were counted after incubation and the incubation specifications for each media is shown below, in Table 2.1.

Between the replications, distilled water for rinsing was run through the UV machine at setting 2 (6.30 L/min). The rinse water was also plated on SMAC and MAC plates to determine if there was cross-contamination.

Table 2.1. Time/Temperature combinations for incubation of media

Media	Temperature	Time	Plate Position
MAC	35°C	24 hours	Inverted
SMAC	35°C	24 hours	Inverted
TSA	35°C	24 hours	Inverted
TSAP	35°C	24 hours	Inverted
YM*	24°C(Room T)	48 hours	Lid up/non-inverted

* Yeast and Mold plates should be covered with aluminum foil, as they are photosensitive

2.6 Experimental design

Four separate tests were performed to determine the effectiveness of UV light in killing the most pertinent microorganisms in apple cider. Each test consisted of three replications. The study was performed as shown in Table 2.2. "Pass" refers to a pass through the UV machine; therefore "double pass" would indicate a sample going two passes (cycles) through the UV machine.

Table 2.2. An example of an experimental set up for single test (Study # 1, 05 February 2001)

Replication 1	Replication 2	Replication 3
Apple cider control	Apple cider control	Apple cider control
Single pass	Single pass	Single pass
Double pass	Double pass	Double pass
Triple pass	Triple pass	Triple pass
Four passes	Four passes	Four passes

As stated before, four separate studies were performed, each study was performed on different dates (February 5, March 28, April 2 and July 29, 2001). Each study is a combination of three replications. In Table 2.2 the experimental design of one test day is shown. The way each replication was performed is given below.

First, fresh, non-inoculated apple cider was plated on SMAC for background microorganisms. After plating the fresh apple cider, *E. coli* was inoculated into the apple cider to achieve the target concentration. To confirm that the target concentration was achieved, *E. coli* inoculated apple cider was plated. These were labeled as "before UV samples". At the same time, samples for physical analysis were taken from the same cider before it was exposed to UV irradiation. Following this, *E. coli* inoculated apple cider was exposed to UV light for 2.03 (single), 4.06 (double), 6.09 (triple) and 8.12 (four passes) seconds, respectively, at a flow rate of 6.30 L/min. Treatment 1 refers to apple cider given one UV treatment (single pass) by pumping it once through the UV chamber which has eight lamps at a flolw rate of 6.30 L/min. Double pass is referred to as Treatment 2 and so on to Treatment 4. Throughout the results and discussion these different UV irradiation levels (machine passes) will be referred to as Treatment 1, 2, 3 and 4. The apple cider was inoculated

to 10^5, 10^6 and 10^7cells/ml for initial target concentration. This was done in order to see if initial cell concentration would make a difference on the log reduction effects of UV irradiation. Experimental procedure for a single test day is shown below in Figure 2.3, steps for one repetition of one experiment is explained here.

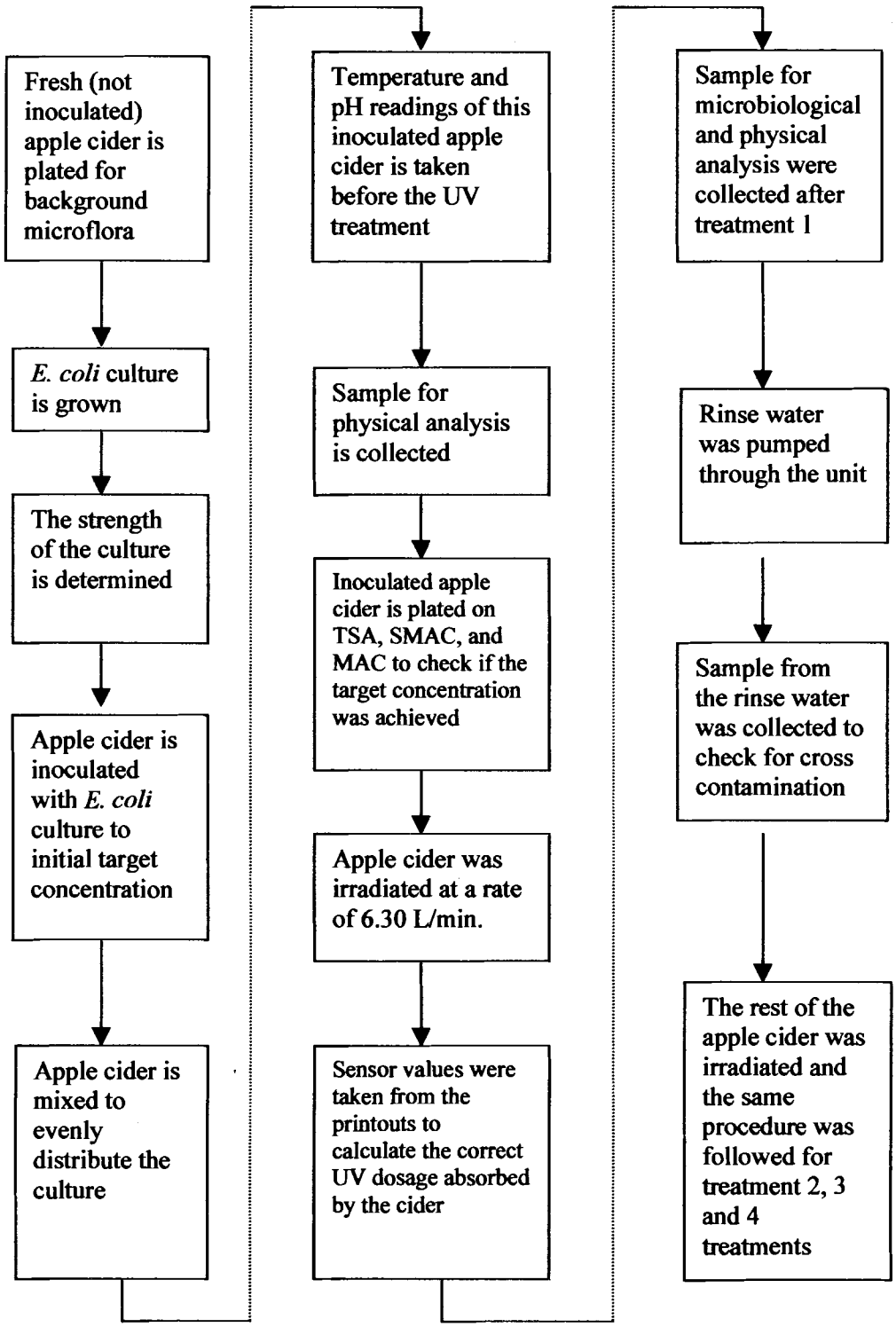

Figure 2.3. UV irradiation scheme for apple cider experiment

2.7 Design of the UV machine

The UV machine (FPE 1500®, FPE Inc., Macedon, NY) used consists of two compartments as shown in Figure 2.4. One section is a compartment where electronic controls are located and the other is the tube where fluid flows. The fluid tube has three components, these are the two concentric tubes (outer stainless steel and inner quartz) and the sensors.

The apple cider being treated flows between the stainless steel tube and the quartz tube (see Figures 2.5 and 2.6). The gap between these two tubes is 0.762 mm, which allows for a thin film of apple cider to be exposed to UV light. Eight UV lamps are placed around the inside diameter of the quartz tube. A fan is located at the top of the unit to cool the lamps. The source of UV irradiation for this experiment is a low-pressure mercury arc lamps, which is calibrated by the use of sensors. There are two sensors located in the mid-stream of the fluids (Figure 2.7). Power density can be determined using the sensor values. Once the coefficient of absorption of cider is determined and combined with the sensor readings UV dosage can be calculated. As mentioned earlier, machine was operated at 6.30 L/min for the apple cider testing.

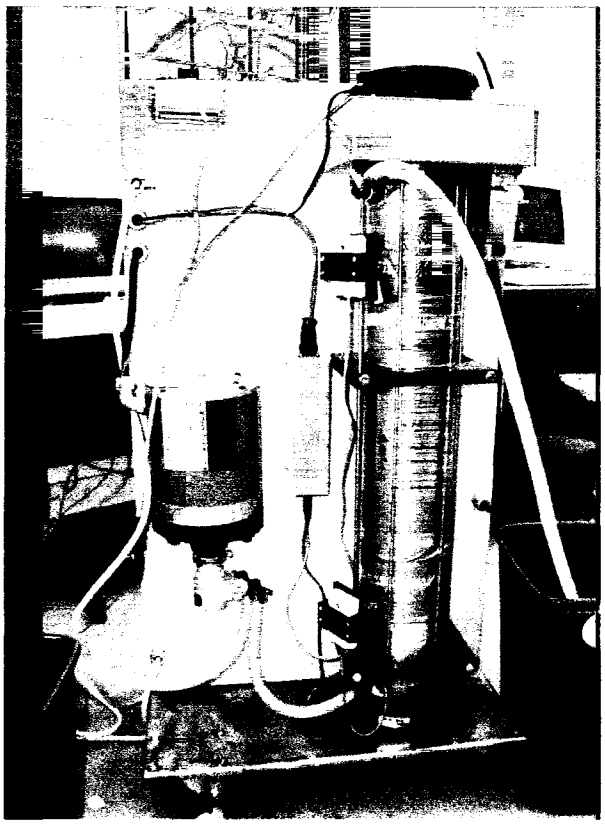

Figure 2.4. Picture of the UV machine (FPE 1500®, FPE Inc., Macedon, NY)

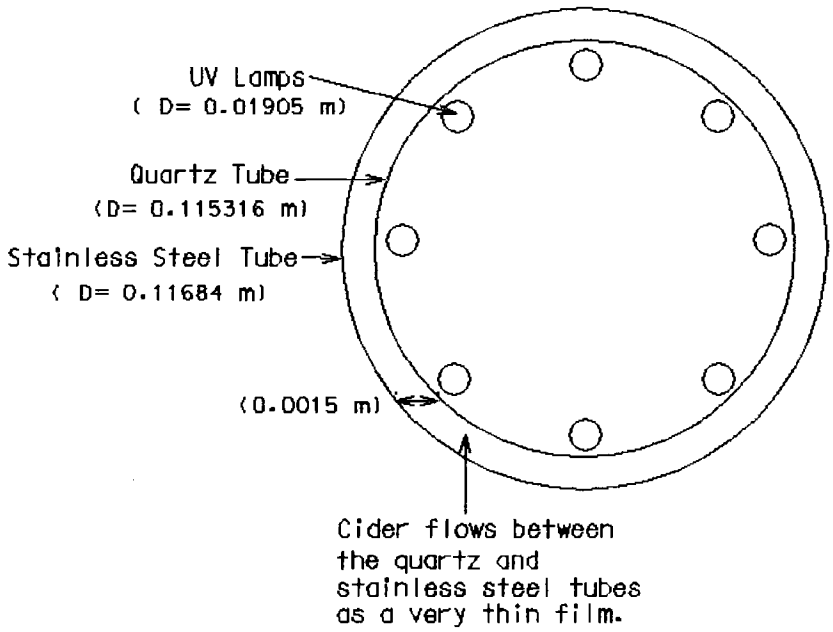

Figure 2.5. The top view of the UV machine (2 dimensional, not to scale)

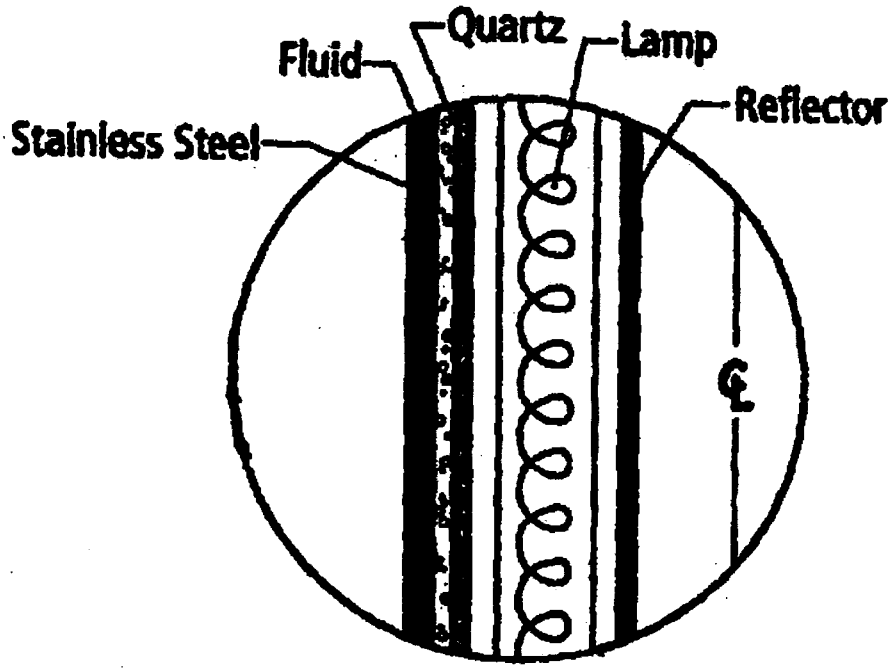

Figure 2.6. Fluid chamber

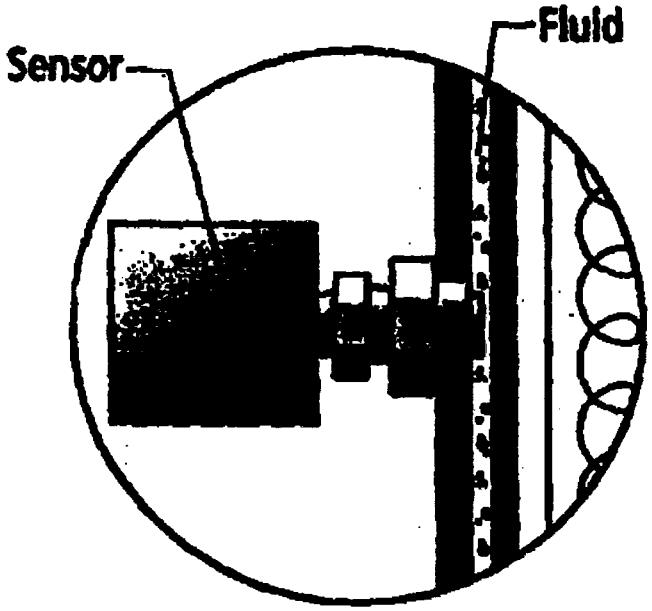

Figure 2.7. Instream UV detector

2.8 Calculations regarding UV machine

2.8.1 Flow rate calculation

Reynolds number was calculated to determine the flow characteristics of the apple cider flowing through the UV machine. By weighing a known volume of cider and computing its mass, density was calculated. There are two types of flow, turbulent and laminar. Turbulent flow is obtained at higher liquid velocities, whereas laminar, or streamline flow occurs at slower liquid velocities. A Reynolds number of 2100 or above is considered as turbulent flow, whereas 400 or below is the laminar flow region; between these two, 400 and 2100, transitional flow occurs. The flow type is an important factor as UV light has a very poor penetration through the apple cider, so creating turbulence in the fluid improves the efficiency of UV light as it provides mixing and all particles in apple cider are exposed to UV light (Harrington and Hills 1968, Murakami *et al.* 2001). Reynolds number is defined as

$$N_{Re} = \frac{\text{Inertial forces}}{\text{Viscous forces}} = \frac{\rho D v}{\mu} \qquad (3)$$

Where ρ is the density of apple cider in kg/m^3, D is the diameter of the gap through which apple cider flows (m), v is the velocity (m/s) and μ is the viscosity of apple cider (Pa*s).

Reynolds number calculations are shown below:

1. $D = D_2 - D_1 = 0.11684 - 0.115316 = 0.001524$ m
2. $v = 1.5136$ m/s (Calculated using the flow rate and volume of apple cider used per pass)
3. $\rho = 1013.7$ kg/m^3 (Calculated in the BEL lab)
4. $\mu = 0.03$ Pa*s at 10 °C

The calculated average viscosity of the apple cider at 10 °C is 0.03 Pa*s. Viscosity will decrease with an increase in temperature. In the literature (Singh and Heldman 1993) the viscosity value for an apple juice of 20° Brix at 27 °C is given as 0.0021 Pa*s and the measured average viscosity in our experiments is 0.03 Pa*s, this value is for the range of 8-13 °C and any estimation made out of this range will not be as accurate, therefore the values calculated in the BEL of UMaine were used. These parameters gives a Re number (N_{Re}) of approximately 78 indicating laminar flow type at the location where the cider is exposed to UV light in a thin film. Other flow types might exist prior to the inlet to and after the outlet from the thin film area where UV exposure occurs.

2.8.2 Calculation of the exposure time

To determine how long the apple cider was exposed to UV irradiation, the following calculations were made. Apple cider flows between the stainless steel and quartz tube as a very thin film. The outer diameter of the stainless steel tube is 0.11684 m and the inner diameter, which is the diameter of the quartz tube, is 0.1153 m. The amount of apple cider that flows through the machine is the difference in volumes of these two tubes: 812.6 l – 791.6 l = 210 ml at machine setting 2, the measured flow rate is 6.30 l/min therefore exposure time is calculated as 0.0338 min (2.03 s). This is used as exposure time for a single pass through (Treatment 1) the UV machine. Further passes through the UV machine are multiplies of this exposure time.

2.8.3 UV dosage absorbed by the apple cider during the exposure time

To calculate the dosage, time of exposure and intensity at the surface of the fluid should be known and the possible energy losses must be taken under consideration. Then using the equation 4, the dose absorbed by the apple cider is calculated.

DOSE= Intensity (micro Watts) * Time (seconds) / Area (cm^2) (4)

2.8.4 Calculation of the energy losses

Energy loss as heat

Energy loss, as heat, occurs when the temperature in the UV chamber is not equal to the optimal operating temperature for the lamps. The lamps provide a given output of power, 13.8 W at their optimal operating temperature. The temperature of the laboratory where the experiments carried was approximately 18 °C, then the temperature of the UV lamps is approximately 29 °C (84 °F). Looking at the temperature chart (Figure 2.7, Phillips Lighting 1992) for the UV lamps, it shows that at 29 °C the lamp efficiency is 70%, illustrating a 30% loss of energy. When heat losses are considered the output of each lamp reduces to 9.66 W.

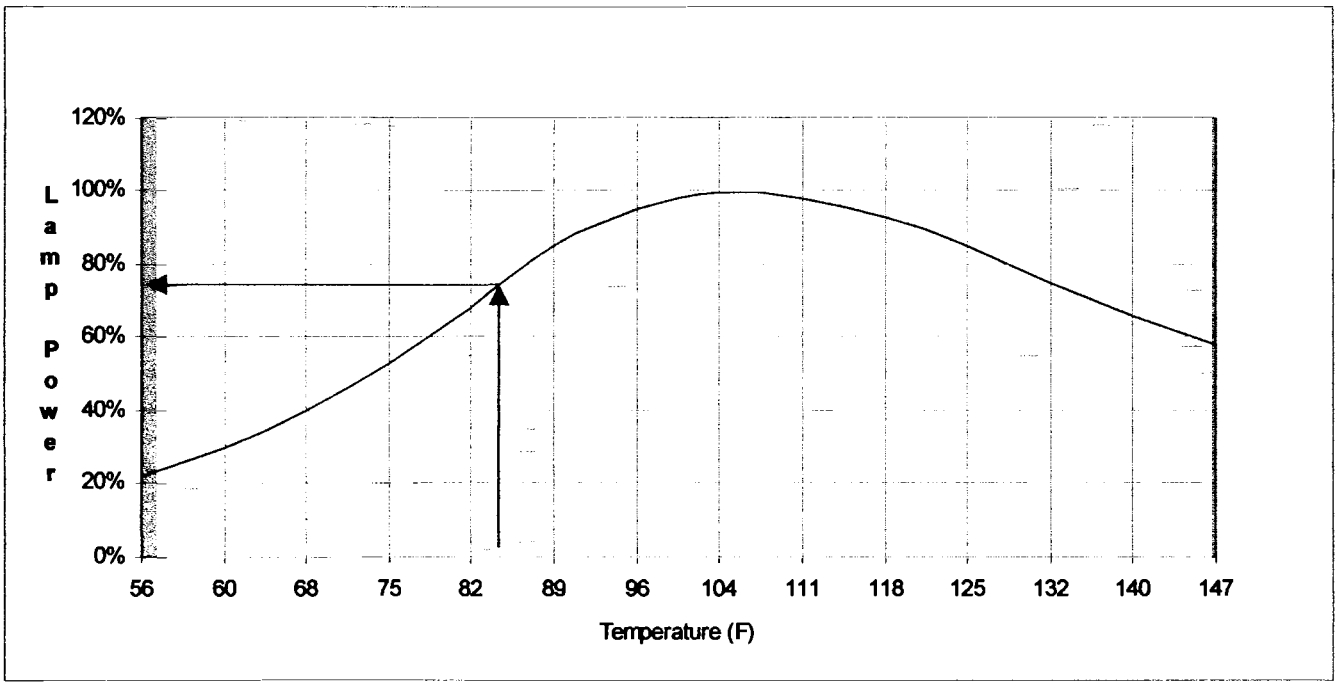

Figure 2.8. Lamp output versus temperature of the lamp (adapted from Phillips lighting 1992)

Energy loss due to the transmissivity of the quartz tube

The UV transmissivity of the quartz tube is 80% at 254.7 nm (Figure 2.8). Therefore 20% of energy is lost in passing the UV light through the quartz tube.

Power calculation

To calculate the dosage, first the power should be calculated. There are eight UV lamps in UV unit, which gives a total power of 110.4 W (13.8*8), without the energy losses. To correctly determine the dosage reaching the microorganism, all the energy losses should be considered. When the energy loss as heat is considered (30%) the power value is reduced to 77.28 W and also when the transmissivity of the UV tube is taken under consideration there is an additional loss of 20%, so the power calculated and delivered is 61.824 W, which is a reduction of 56%.

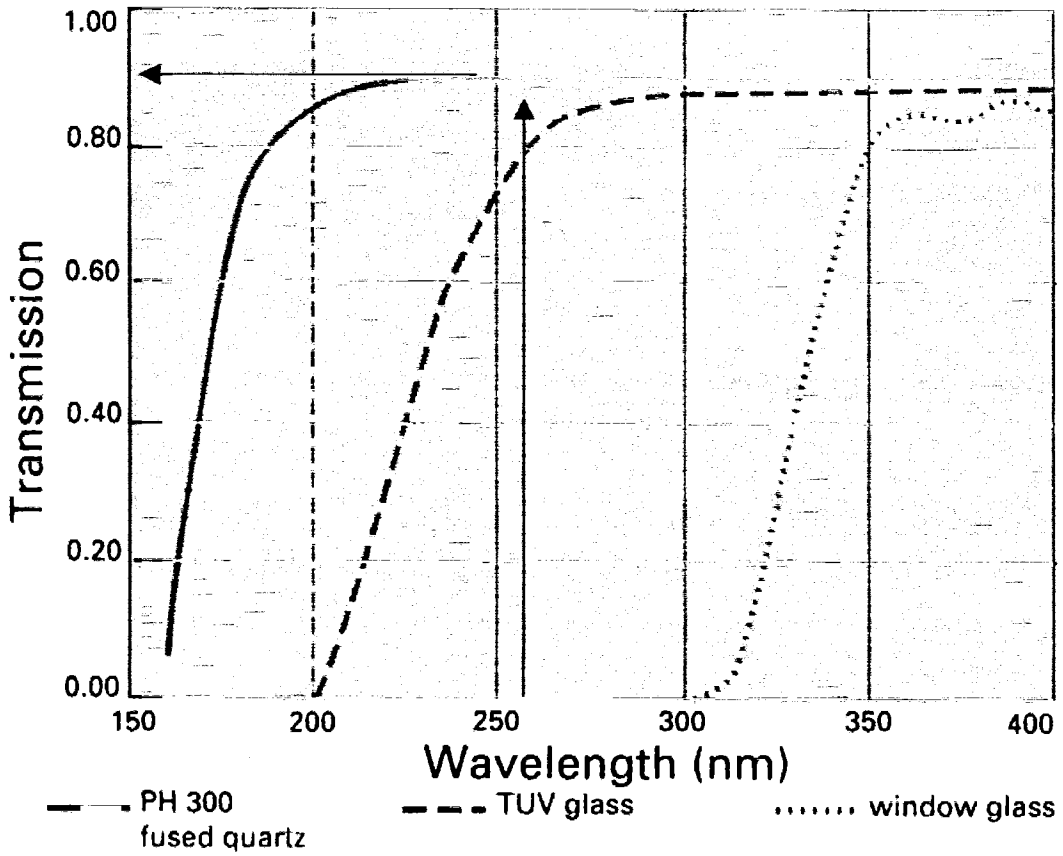

Figure 2.9. Special transmission of glasses (adapted from Phillips lighting 1992)

Power density calculation

Power density determines how much energy reaches the known surface. There are two ways of determining power density, one is calculating it mathematically and the other is measuring it in the laboratory.

Mathematical calculation (calculated power): First the area of the quartz tube is calculated as 2775.5 cm^2. The power density is then computed as 61.824 W / 2775.5 cm^2 = 22,289 $\mu W\text{-}s/cm^2$.

Calculation in the laboratory (measured power): Power density was also measured through experiments in the lab. This was performed by running distilled water through the UV system as it is assumed that UV light intensity is the same for every depth of clear liquids. The power measured was 3,200 μW-s/cm^2. Phillips Lighting (1992) states that the effective penetration depth for a 90% kill may vary from 3 m for distilled water down to 12 cm for normal drinking water. The sensors of the UV machine are located at a depth of 0.4572 mm, so it is assumed that all of the generated power reaches that depth. This means that the power at the sensor depth can be assumed to be the same as the power at the surface. However, if the liquid is turbid, penetration depth can go down below 0.5 mm. Absorptive liquids decrease the germicidal intensity exponentially with the penetration depth x. This relationship is shown by equation 5. This indicates that the power density read by the sensors may not be the same as the one at the surface of the absorptive liquid. When the distilled water was used to measure the average power, a difference between the measured and calculated power was determined and this difference is computed as a sensor replacement factor, which is 6.97 (22, 289/3,200 μW-s/cm^2).

$$E = E_o * e^{-\alpha x} \qquad (5)$$

E_o = Incident Intensity
E = Intensity at depth x
α = Absorption coefficient

Power was calculated for each treatment of every test. This was performed by calculating the power at the sensor level using the sensor output, and then multiplying this power with the sensor replacement factor to find the power at the surface. This was done for every replication and then the resulting power was averaged. The

resulting average power multiplied by the exposure time gives the dosage (μW-s/cm^2). The resulting average exposure is 8,777μW-s/cm^2.

2.9 Sensory analysis

Sensory evaluation is the scientific discipline used to measure, analyze and interpret reactions to those characteristics of materials as they are perceived by the senses of sight, smell, taste, touch and sound (Meiglard *et al.* 1999). The objective of apple cider sensory evaluation was to determine if UV changes the flavor of apple cider.

There are many types of tests that are used to evaluate sensory perceptions of a product. Difference testing is one of the methods used in sensory evaluation. These differences can include ingredients, processing, or differences in packaging. The Triangle Difference Test was used for sensory evaluations of UV treated and non-treated apple cider. This test was approved by the Human Subjects Committee of the University of Maine (See Appendix B.1). The test was conducted at the University of Maine's Consumer Testing Center in the Department of Food Science and Nutrition, Holmes Hall. An example of the ballot that was used is shown in Appendix B.3.

For this analysis twenty-three UMaine staff and students who regularly drink apple cider, were recruited using flyers and the campus computer conference system. Panelists received three coded samples. They were told that two of the samples were the same and that one was different. Panelists were then asked to identify the different sample.

2.9.1 Preparation for the sensory testing

Before the sensory analysis, the UV machine was thoroughly cleaned with distilled water. Each wash cycle was one gallon of distilled water with 0.85% active chlorine concentration. The first four rinse cycles were a gallon each whereas the last rinse cycle was 5 gallons of distilled water only. Five wash and rinse cycles were run through the machine and a sample from the last rinse cycle was plated to determine if there were any microorganisms in the rinse water.

After the five wash and rinse cycles were completed an additional five rinse cycles was run through. Five gallons of distilled water were used in each of the last five cycles for complete rinsing at a rate of 6.30 L/min. Once the machine was thoroughly cleaned, one and a half gallons (5.7 L) of cider for sensory testing was pumped through the UV machine. The sensory study was performed using double pass (Treatment 2, 4.06 s exposure) cider.

2.9.2 Sensory materials and experimental design

For the apple cider sensory testing 3 oz. (88.7 ml) matt (non-clear) cups (No.44, Solo, IL) cups were used because any kind of light effect may alter the decision of panelists leading to biased results. Apple cider was kept in the refrigerator before serving and the temperature was recorded to assure 4 °C. Each individual was randomly given each of the three samples. The test for proportions was used where the $p = 1/3$.

2.10 Shelf-life study and experimental design

The purpose of doing the shelf life study for apple cider was to determine the amount of time that the UV treated product can remain available to the consumer. Thus, it was determined which property/properties of the product would be the first cause of either non-palatability or potential hazards. To obtain useful information, control (non-irradiated) apple cider was also stored under the same conditions. To simulate actual storage conditions in an end-product market refrigeration system, apple cider was stored at 4 °C. As part of the ongoing shelf-life study, stored jugs were periodically inspected to detect leakage or any abnormal conditions (due to microbial activity, processing or storage conditions). The shelf-life study was performed for 27 days. During this study, samples were also surveyed for incidences of the human pathogen, *E. coli* 0157:H7.

The shelf-life study was performed after the microbiological experiments. Three separate apple cider samples were treated with UV irradiation once (Treatment 1), twice (Treatment 2), three times (Treatment 3) and four times (Treatment 4). A control was also stored under the same conditions as the irradiated samples. Triplicates of the treated sample and a control were stored at 4 °C and the temperature of the refrigerator was recorded daily. There was no temperature abuse (unexpected increase or decrease in the refrigeration temperature) during the study. Turbidity, viscosity, pH and color were measured once each week to determine any fluctuation in these parameters between treatments and these were also compared to the control.

Every five days, samples of the apple cider were plated on Yeast and Mold media supplemented with chloramphenicol. One ml of sample was serially diluted in 9 ml of 0.1% bactopeptone, and then appropriate serial dilutions were plated in triplicate on Yeast and Mold plates. These plates were stored in the dark at room temperature for two days, when colonies were counted. Yeast and mold numbers were also employed to determine fermentation of apple cider.

2.11 Statistical analysis

Data were analyzed using the SYTAT® version 9 (SPSS Science, Chicago, IL). Different analyses were used depending on the data. Log survival values for the *E. coli* were used to determine the log reduction in the bacteria. The following were investigated.

2.11.1 The effect of UV irradiation on the physical properties of cider – a repeated measures analysis of variance (ANOVA) was used to determine the differences among treatments and F test was used to determine the level of significance (α=0.05)

2.11.2 The effect of physical parameters on the log reduction in the E. coli population – Multiple regression analysis was used to determine the effects of physical properties on the log reduction.

2.11.3 Statistical significance of injured cells during irradiation – ANOVA was used to compare the differences in bacterial counts on TSAP and SMAC media.

2.11.4.1 Yeast and Mold counts during the shelf-life study - an analysis of variance was used to analyze the data and the Yeast and Mold counts were transformed to log scale. Tukey's (HSD)10% test was used for pair wise comparison.

2.11.4.2 Change in the physical parameters of UV treated cider versus the control during the shelf-life period - ANOVA with the repeated measures analysis was used for the evaluation of these data.

2.11.5 Sensory analysis to statistically determine the acceptability of UV treated apple cider - z test for proportions were used at p= 1/3.

3. RESULTS AND DISCUSSION

The section below gives the results and discussion of apple cider experiments including physical (3.1.1), microbiological (log reduction) (3.1.2), sensory analysis (3.1.3) as well as shelf life results (3.1.4).

3.1 Analysis on apple cider

3.1.1 Physical analysis

The effect of UV irradiation on the physical properties of apple cider was examined. Physical properties analyzed included color, turbidity, viscosity, pH and temperature. These properties of the cider are an indication of its quality; therefore the effect of UV treatment was analyzed to see if there was a change in quality.

When the statistical analyses were performed to see the effect of UV irradiation on the apple cider properties, changes in some of the physical properties were observed. The effect of UV irradiation on each physical property of apple cider is individually reported and discussed below and the mean value for each property of apple cider is shown in Table 3.1. The results will be given then a summary/discussion section will follow.

Table 3.1. Mean values for the change in physical parameters of apple cider after each UV irradiation cycle (at the level of α=0.05 and n=36)

	UV TREATMENT (Number of passes)				
Parameter	**Control**	**1#**	**2**	**3**	**4**
Color (Hunter L)	20.94^{a*}	20.53^{b}	20.24^{c}	20.15^{c}	19.87^{c}
Turbidity (NTU)	92.02^{d}	90.44^{e}	89.24^{f}	89.62^{f}	89.69^{f}
Viscosity (Pa*s)	0.031^{h}	0.031^{h}	0.030^{h}	0.030^{h}	0.029^{i}
pH	3.23^{j}	3.35^{k}	3.34^{k}	3.33^{k}	3.37^{l}
Temperature (°C)	11.5^{m}	16.0^{n}	18.5^{p}	20.5^{r}	21.5^{s}

#= UV treatment indicative of number of passes through UV machine

• The same superscripts indicate no significant difference between the two values at the level of α=0.05. When the superscript is different than the previous treatment in adjacent columns within the same row, this indicates that there is a significant treatment differences between the two at the level of $p < 0.05$. (For example looking at color control is different than treatment 1 as the superscript of the control is a and the treatment 1 is b).

3.1.1.1 Results of quality tests

The results of the physical analyses are given in this section and are discussed in section 3.1.1.2.

Color: Hunter tristimulus color values showed the irradiated samples maintained significantly lower "L"(lightness/darkness) values than did the control samples. A lower L-value indicates a darker sample. The color change is significant ($p < 0.05$) between the control and Treatment 1 and also significant between Treatment 1 and 2. It is not significant between 2 and 3 or between 3 and 4. The L-values were

considered because this value shows if the UV treatment is affecting any of the enzymatic and non-enzymatic systems that could cause the cider to get darker.

Turbidity: Results of turbidity analysis indicated significant differences between some of the treatments. The mean values for the turbidity of apple cider are shown in Table 3.1. Irradiating the cider once (Treatment 1) created significant differences ($p < 0.05$) from control as is difference between Treatment 1 and 2. Further UV treatments did not cause any significant changes in cider turbidity. Irradiation tends to slightly decrease the turbidity of cider. Even though there is a slight increase in turbidity between treatment 3 and 4, this was found to be statistically not significant.

Viscosity: Viscosity of apple cider was significantly different ($p< 0.05$) between the last two treatments (3 and 4), see Table 3.1. Increasing irradiation (dosage) caused a decrease in viscosity. It was demonstrated that the cider began to get less viscous when the dosage received was 26,331 μ W-s/cm^2 (treatment 3) or more.

Temperature: Statistical analysis illustrated significant temperature differences ($p <0.001$) between each of the treatments shown in Table 3.1 The calculated temperature increase is 4.5°C for the first treatment. It is 2.5, 1.8, and 1.1 °C respectively, for each cycle after (Table3.1).

The effect of UV irradiation on apple cider physical properties is shown in Figures 3.1-3.5. The numbers 1-5 on y-axis indicates treatments, 1 being the control and 5 being treatment 4.

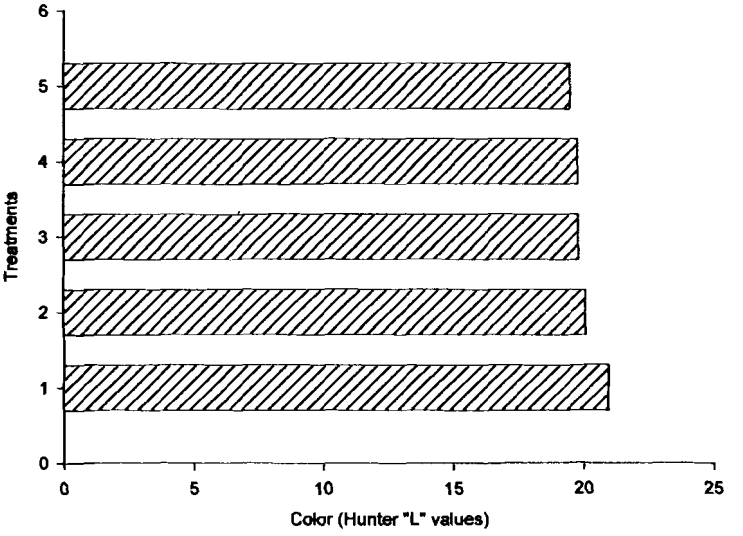

Figure 3.1. The Hunter L-values decrease as the exposure to UV light increases

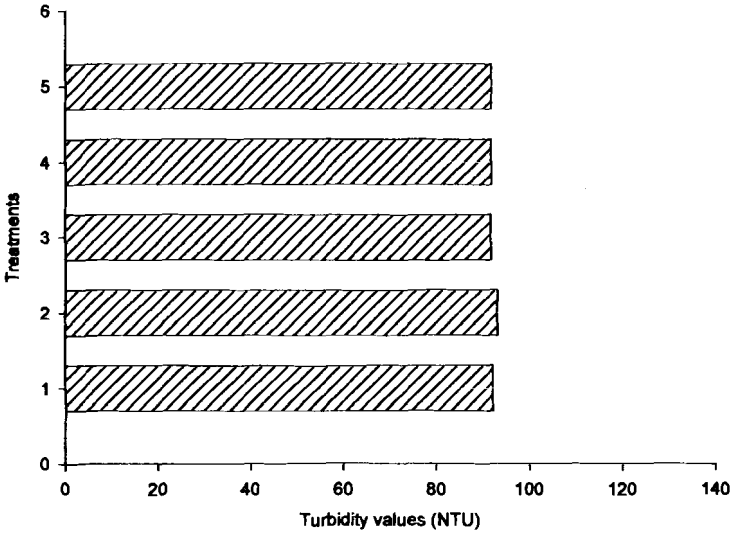

Figure 3.2. Turbidity as the exposure to UV light increases

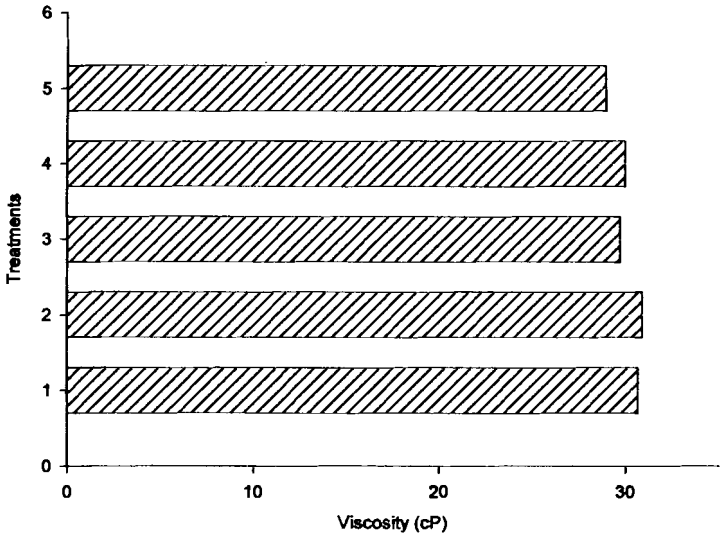

Figure 3.3. Viscosity as the exposure to UV light increases

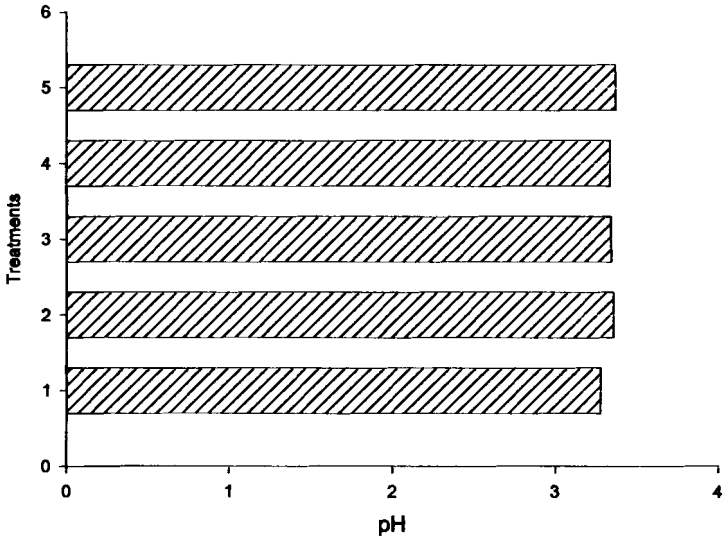

Figure 3.4. pH values as the exposure to UV light increases

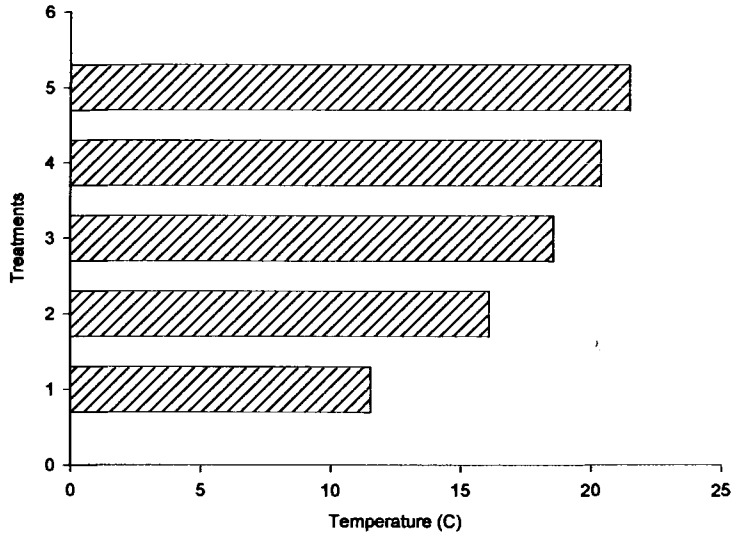

Figure 3.5. Temperature as the exposure to UV light increases

3.1.1.2 Summary and discussions

Change in some of the characteristics of apple cider due to UV light exposure was determined. It was shown that apple cider gets darker (Hunter "L" value decreases) when the exposure time to UV increases (Figure 3.1). This was found to be significant ($p < 0.05$) between the treatments shown in Table 3.1. There are not many studies, which observed the effect of UV light on the color change of apple cider. The darkening can be the result of browning reactions. The possible browning reactions, which can take place in apple cider during UV irradiation, are either enzymatic browning (polymerization of some reaction products of the enzyme *polyphenol oxidase*) or non-enzymatic, Maillard browning (Amino groups and reducing sugars at high temperatures, Fraser 1997). Enzymatic browning would be more prevalent in cider where compartmentilization between substrates and enzymes are destroyed during pressing.

In these tests there is an increase in temperature and it may be that this increase in temperature is also the contributing factor to the maillard reactions. When observed it can be seen that a change in color is reported for treatment 1 and 2 only. The increase in temperature is more in these two cycles compared to the last two irradiation cycles. The increase in temperature for treatment 1 is 4.5°C and it is 2.5 °C for treatment 2. These are higher temperature increase compared to the increase in treatment 3 (1.8 °C), and treatment 4 (1.1 °C). The increase in temperature is not very high, so it is speculated that the enzymatic browning is the major reason for the browning reactions.

Turbidity significantly ($p < 0.05$) decreases from the control to treatment one and again from treatment 1 to treatment 2 (Figure 3.2). The turbidity tends to follow a different trend and increase between treatments 2 and 3 and 3 and 4, but this increase was found not to be statistically significant (Table 3.1). A possible reason may be that when apple cider is first exposed to UV light its affect on the cider is the greatest and then with further treatments less change occurs. There are not any studies in the literature, which observed the effect of UV irradiation on turbidity of fluids. One possible reason why turbidity is first decreasing might be the result of the decreased yeast and mold counts in apple cider due to UV irradiation. Yeast and molds contribute to the turbidity of apple cider and if these are decreased in number then the turbidity is speculated to show a decrease.

The change in viscosity of apple cider was found to be significant only between treatments 3 and 4. During the irradiation cycle viscosity increased and decreased but generally remained around the 0.030 and 0.031 Pa*s range, which

made the change not statistically significant. When the last irradiation dosage was applied the cider tended to become less viscous. This could be due to the fact that viscosity is temperature dependent and the relationship between them is non-linear, once it reaches a certain temperature then viscosity changes. The last irradiation cycle was significantly ($p < 0.01$) higher in temperature compared to the other cycles and this may make the viscosity lower, confirming the well-established fact that viscosity is inversely proportional to temperature.

The change in pH of apple cider was found to be significant between the control and treatment 1, as well as treatments 3 and 4. This might be due the H^+ ions that are created in apple cider once exposed to UV light.

The temperature of apple cider increases with increasing dosage of irradiation and this was found to be statistically significant between each treatment. A reason for a significant temperature change might be experimental error during testing. The refrigerated apple cider was taken out and then after inoculation it was immediately irradiated; if the temperature of cider was brought to room temperature before irradiation, there might not have been as much difference in the temperature. Considering the first law of thermodynamics, energy is not lost it is converted from one form to another. As there is a heat exchange between the apple cider and the ambient temperature of the UV machine, if apple cider is cold, the temperature increase will be higher to come to the equilibrium with the machine temperature. If the apple cider was warmer to begin with, not much of an increase in temperature would be observed. However, this procedure was used for each experiment therefore the error was consistent throughout the experiments.

Even though statistical differences in the quality of irradiated apple cider were detected, the consumer panel could not distinguish between the treated and non-treated apple cider. One possible reason for this might be that the triangle test which was performed to see the differences between treated versus non-treated apple cider, is an overall difference test, which means that the physical properties of apple cider were not individually investigated by consumers. But if the differences between the physical characteristics of apple cider were greatly different then the panelists would be able to recognize it. Thus, even if statistical differences were detected, the degree of the differences in color might be below the level that can be detected by a panelist.

3.1.2 Microbiological analysis

During this study, the effect of UV exposure time, dosage and physical properties on the log reduction rate in a bacterial population was analyzed. Raw, unpasteurized apple cider was plated for *E. coli* 0157:H7 and no organisms were detected in any of the cider purchased for UV testing. The reason apple cider was plated for background microflora was to see if the initial concentration of microorganisms would have an effect on log reduction. The following section will discuss the effect of exposure time and dosage as well as the effect of physical properties of apple cider, on the log reduction in the *E. coli* cells. Recovery of injured cells will also be discussed.

3.1.2.1 Effect of exposure time and dosage on the log reduction

The evaluation of log reduction was made by comparing the initial and final plate counts from each pass through the UV machine. Log reduction is recorded in cumulative numbers. Between each pass from the UV machine (between each treatments) the wash and rinse water was plated to see whether there was a contamination or not. No contamination in wash and rinse water was found between any of the treatments. The exposure dosage is calculated using the sensor values. An average sensor value of 140 μW-s/cm^2 was obtained when the apple cider was given only one UV treatment. When the calculations were made, these sensor values give an average exposure dosage of 8,777μW-s/cm^2 per single pass through the UV unit, accounting for all the energy losses. A single pass through the UV machine takes 2.03 seconds. Increasing the time of exposure to UV irradiation increases the dosage absorbed by apple cider. The dosage absorbed by apple cider was calculated for each pass through the UV machine separately. An average of 2.20 log reduction was calculated for a dosage of 8,777 μ W-s/cm^2. Table 3.2 represents the cumulative log reduction in the population of *E. coli* with the increasing exposure time. In this table the log reduction achieved for each treatment through the UV machine is noted in parenthesis.

Table 3.2. Log reductions with increasing exposure time

Treatment	Exposure t(sec)	Calculated dosage (μW-s/cm^2)	Initial pretreatment concentration		
			5	6	7
			Log reduction*	Log reduction**	Log reduction***
1	2.03	8,777	1 (1)$^+$	1 (1)	2 (2)
2	4.06	17,554	2 (1)	3 (2)	4 (2)
3	6.09	26,331	3 (1)	4 (1)	4
4	8.12	35,108	5# (2)	6# (2)	7# (3)

Log reduction meets FDA mandated 5-log
*The log reduction of *E. coli* in apple cider when initial level of this bacteria was 5 log (CFU/ml).
** The log reduction of *E. coli* in apple cider when the initial level of this bacteria was 6 (log CFU/ml).
*** The log reduction of *E. coli* in apple cider when the initial level of this bacteria was 7 (log CFU/ml).
$^{(+)}$ Log reduction of bacteria is shown for each individual treatment
The log reduction numbers shown are rounded to the nearest whole number.

The literature states that an exposure of 7,000 μW-s/cm^2 is required to achieve a 3 log reduction in *E. coli.* (Phillips Lighting 1992). The average value obtained in this study (8,777 μ W-s/cm^2, taking energy losses into account) is close to the reference value, yet requires a higher exposure dosage for less of a log reduction. This was expected, because all the UV dosage emitted by the lamps might not be uniformly distributed in the apple cider. Another reason is that all of the emitted irradiation might not be reaching the bacteria due to the suspended particles and also the high absorbance of apple cider. The dosage value calculated in the reference (7,000 μW-s/cm^2) is done assuming that the energy is distributed in the liquid uniformly. This leads to the conclusion that, the calculated exposure dosage being higher and yielding less reduction is reasonable. The reason for this is that as energy

is not absorbed uniformly by the apple cider and that there is 56% energy loss, higher exposure dosages are required for less log reduction. One additional reason which supports the idea of higher dosage for lower reduction might be that the flow of apple cider in the UV tube is not turbulent, which means that all the surfaces of the apple cider are not exposed to UV light, as there is not enough mixing in the light exposure chamber (Murakami *et al.* 2001).

Three different initial *E. coli* concentrations were analyzed and log reduction was recorded in cumulative numbers. For each of these initial concentrations, it was observed that the last irradiation cycle is the one when the greatest reduction is achieved. Although they did not follow a certain trend, the reduction for the last irradiation cycle was the one to achieve the FDA mandated 5-log reduction in microorganisms. Looking at Table 3.2, it can be seen that greater reduction can be achieved with the same dosage of irradiation. The increased kill rate obtained in the last irradiation cycle may be interpreted as the result of higher number of injured organisms existing in the apple cider after the exposure to UV light more than once. Even though statistical results showed that there was not a significant difference in counts between the enrichment media and selective media (no injured cells), it can still be postulated that the greater reduction can be due to injured cells, because the counts on selective media were higher than the counts on the enrichment media. Even though this was not statistically significant this does not mean that there are not any cells that might have been damaged. When the organisms are partially injured it is more likely that they will be inactivated with additional UV treatment. When the population of *E. coli* was 5, 6, and 7-log CFU/ml, all (plates having counts less than

30 microorganisms were counted as zero plates) of the *E. coli* were eliminated (were none detectable as they were below the countable limit) in 4 passes through the UV machine.

These experiments demonstrated that UV treatment significantly ($p < 0.05$) reduced the populations of ATCC 25922 in apple cider inoculated to 5, 6 and 7 log of colonies. A maximum of 7 log reduction was obtained with multiple passes. Experiments demonstrate that UV radiation is effective in eliminating bacteria from apple cider with multiple passes, supporting the results of other researchers (Harington and Hills 1968, Worobo *et al.* 2000, Wright *et al.* 2000). A study performed by Wright *et al.* (2000) did not achieve 5-log reduction of *E. coli* in apple cider, even though the exposure dosage calculated was higher than the one found here at UMaine. Dosages obtained by Wright *et al.* (2000) ranged from 9,402 to 61,500 μ W-s/cm^2 and the mean log reduction for all the treated samples was 3.81 log CFU/ml. This leads one to speculate on the importance of machine design and energy losses in the effectiveness of delivering UV irradiation to the cider. In the present study the dosage absorbed by the microorganisms was calculated and several energy losses were considered, illustrating the importance of machine design.

Figure 3.6 shows the survival of *E. coli* on SMAC plates when treated with UV light. On x-axis treatments are shown and 0 is the control and 4 is the treatment 4. The effect of increasing the dosage is shown. When the dosage was increased the survival number of *E. coli* is greatly reduced and eliminated (were below the countable limits) in the last irradiation cycle, which exposes apple cider to a total

dosage of 35,108 μW-s/cm^2. Table 3.3 gives the calculated dosages for every pass of each apple cider with three different initial pretreatment concentrations.

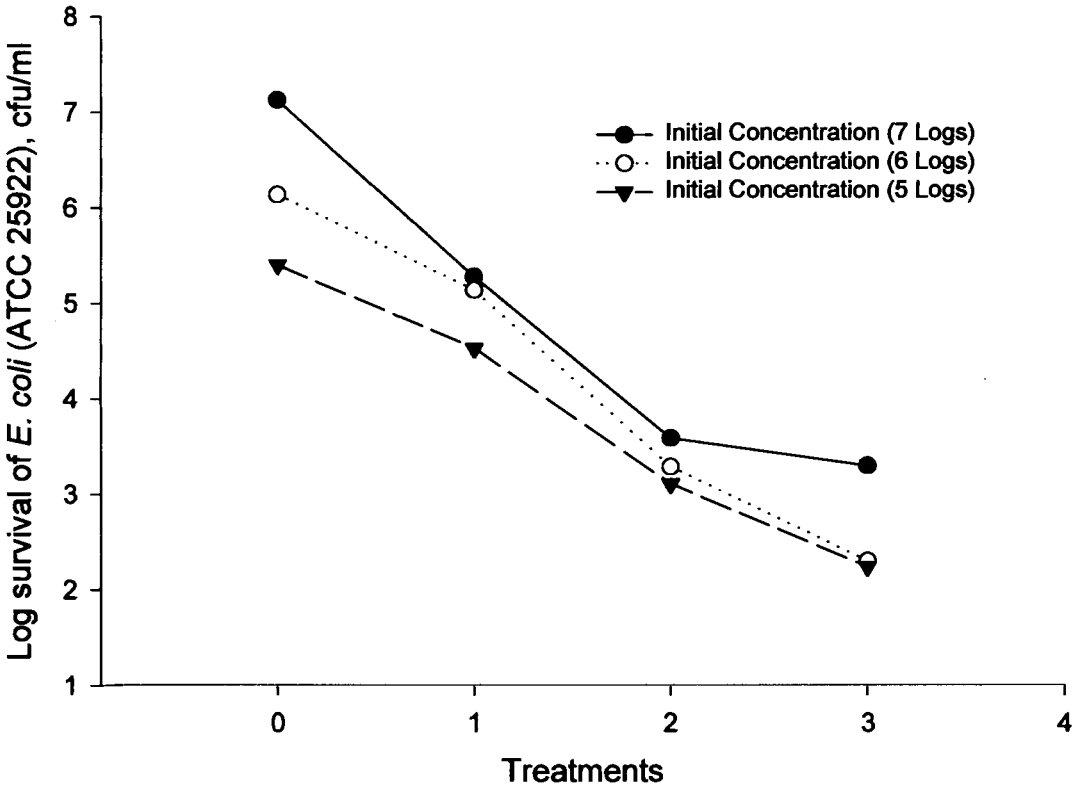

Figure 3.6. Log survival on SMAC plates when the starting concentration was 5, 6 and 7 logs (0 = control and 4 = treatment 4)

Table 3.3. Calculated UV dosages for every pass of each apple cider (which has 3 different initial pretreatment concentration of 5, 6 and 7)

Treatment	Dosage (μ W-s/cm^2)	*E. coli* Survival (log scale)	Dosage (μ W-s/cm^2)	*E. coli* Survival (log scale)	Dosage (μ W-s/cm^2)	*E. coli* Survival (log scale)
Initial Concentration		7.1		6.1		5.4
1	7652.3	5.3	7630.0	5.1	7282.9	4.5
2	7861.5	3.6	7314.4	3.3	7289.1	3.1
3	8272.2	3.3	7303.2	2.3	7501.9	2.2
4	8497.5	-	7495.2	-	7186.2	-

3.1.2.2 Effect of physical properties on the log reduction

Statistical analyses (Multiple Linear Regression) were performed to determine the effect of each physical property of apple cider on the log reduction of *E. coli* cells. Regression analysis to illustrate the effect of physical properties was performed for both SMAC and MAC. Both media followed the same pattern therefore only the results for SMAC are presented in this study. A strong correlation between color and turbidity (R^2=0.979) was found in this study, therefore only color was used in this regression. Table 3.4 shows the results of regression analysis. From the p - values in the table ($p < 0.05$), it is evident that both viscosity and color have an association with log reduction of *E. coli* population in apple cider. Only an association can be determined because the results shown could be due to actual effects of UV treatment. Other parameters, such as pH and temperature, have no effect on the reduction of *E. coli* in apple cider. Residual analysis was performed to examine the suitability of the regression model. Although the regression coefficient is low, the uniform distribution of the residuals around zero indicates a good model fit (Figure 3.7).

Table 3.4. Multiple regression models relating the physical properties to the bacterial log reduction on SMAC agar

Effect	p values
CONSTANT	0.056
Viscosity (Pa*s)	0.046
Color (Hunter L Values)	0.002

p value indicates the significance of the test (α=0.05)
$y=26.139-0.935x_1+0.372x_2$
y = is the log reduction in the population of *E. coli* in apple cider
x_1=viscosity of apple cider
x_2=color of the apple cider

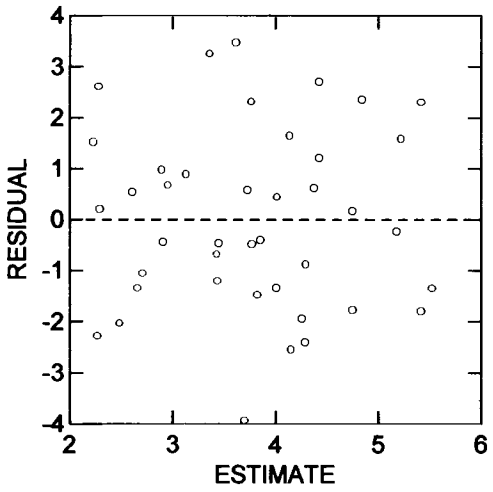

Figure 3.7. Plot of residuals against predicted values for the multiple regression model

The results show that turbidity, color and viscosity of apple cider are associated with the log reduction of bacteria in apple cider. Other parameters such as pH and temperature had no effect or association in the reduction of the *E. coli* population. When turbidity is high the log reduction was lower and previous studies showed that the particulates and organic matter are associated with low transmissivity

of UV light (Shama *et al.* 1996). A reason for this relationship might also be due to the fact that the change in turbidity is a result of UV treatment. As the cider gets less turbid (as UV treatment occurs) more bacterial log reduction is achieved. So we can say that they are associated. In the past, very few studies were performed relating the effect of color, viscosity or turbidity to log reduction, therefore there are not many studies to compare results. Effect of other parameters on the log reduction such as the effect of flow rate, the background microflora and total solids were examined by different researchers (Wright *et al.* 2000, Murakami *et al.* 2001). The results of other researchers showed that increasing the flow rate increases the log reduction, so it is speculated that creating turbulence will help us achieve a higher reduction in less treatments.

3.1.3 Recovery of injured *E. coli* cells

Additional testing was performed that would allow for the detection and enumeration of the number and extent of sublethally damaged microorganisms. This means that they can be reactivated again, when the right nutrients are provided. Such microorganisms can pose a human health hazard, and precautions must be taken to prevent this reactivation. These microorganisms are called "stressed" or "sublethal" microorganisms and are demonstrated by the counts between selective and non-selective media. Even though the recovery on TSAP (enrichment media) was greater than colony counts on SMAC and MAC (shown in Figures through 3.8-3.10), the difference in counts was shown not to be statistically significant. These tests also demonstrate that there are some injured but not completely dead cells. This might be

due to the fact that UV light has poor penetration and shadowing effects. Because of high turbidity of cider and suspended particles (Qualls *et al.* 1983), some of the light might not directly reach the bacteria. For the destruction of bacteria to be effective with UV light, it should reach the bacteria directly. The high concentration of suspended particles in apple cider tends to scatter the UV light rather than absorb it. This decreases the efficiency of cell destruction. The typical penetration of UV light in juices is on the order of 1 mm depth for absorption of 90% of the light (Sizer *et al.* 1999). This can be improved by creating a turbulent flow pattern so all surfaces of the juice are exposed to UV light as a thin film. The machine design provides laminar flow where the apple cider is exposed to UV light. The fact that irradiation inactivated all the *E. coli* colonies in apple cider with multiple passes makes one assume that there are some turbulence in the flow. One reason turbulence might occur in the flow is due to the sudden diameter change at the inlet and outlet to the thin film treatment area. In other words, when cider first enters the inlet tube, the diameter of the inlet hose is much smaller than the diameter of the quartz tube, so when the apple cider flows from the inlet hose into the gap between quartz and stainless steel tubes turbulence might occur. As turbulence brings mixing into the system, more surfaces of apple cider are exposed to the UV light providing more effective inactivation.

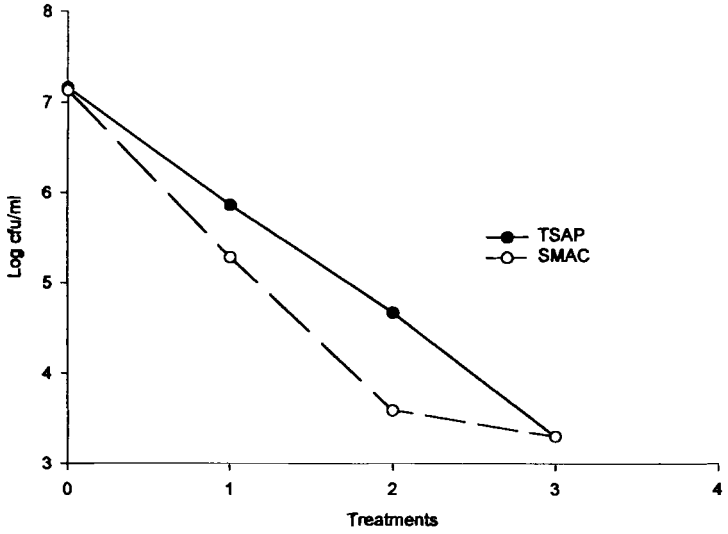

Figure 3.8. Evaluation of the survival of *E. coli* O157 when plated on TSAP and SMAC (initial concentration was ~7 logs)

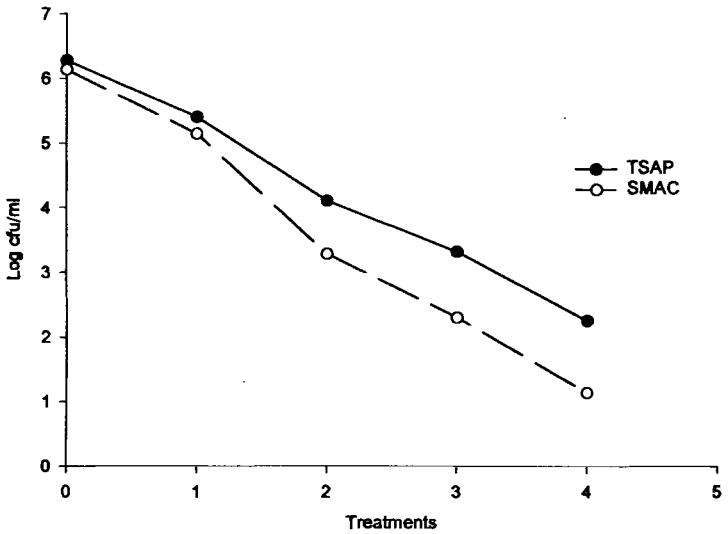

Figure 3.9. Evaluation of the survival of *E. coli* O157 when plated on TSAP and SMAC (initial concentration was ~6 logs)

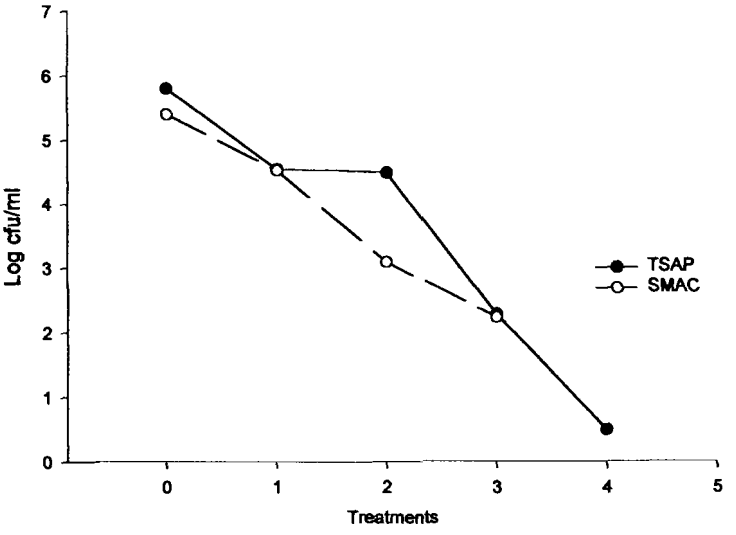

Figure 3.10. Evaluation of the survival of *E. coli* O157 when plated on TSAP and SMAC (initial concentration was ~5 logs)

Figures 3.8-3.10 demonstrates a higher survival numbers of *E. coli* on TSAP versus SMAC but this was found to be statistically insignificant.

3.1.4 Summary and discussions

Previous work on the survival of *E. coli* 0157:H7 in apple cider exposed to UV irradiation was carried out by other researchers (Harrington and Hills 1968, Worobo *et al.* 2000, Wright *et al.* 2000). This is the first time it has been studied in the state of Maine using Maine apples. Also this was the first attempt to mathematically calculate the UV dosage reaching the bacteria, taking into consideration all the energy losses (56%) in the system. Significant reductions ($p < 0.05$) in the *E. coli* population after UV treatment of laboratory-inoculated apple cider was shown in this study. Present work suggests that the turbidity and color as

well the viscosity of *E. coli* inoculated apple cider are associated with log reduction achieved. These results agree with research by Qualls *et al.* (1983) who has shown that UV light has poor penetration properties, which are further reduced with increasing turbidity and apple cider turbidity is high (~ 80 NTU).

The present study shows a strong correlation between color and turbidity of apple cider ($R^2 = 0.979$); the darker the color the more turbid the fluid. One problem with high turbidity is the effectiveness of UV light is reduced because particles can prevent the light from reaching the bacteria cells.

An average of 2 log reduction was obtained in the *E. coli* (ATCC 25922) population of apple cider per pass through the UV machine. Ultimately an FDA mandated 5-log reduction was achieved in this study by multiple treatments (passes). The pretreatment initial concentration of *E. coli* (5, 6, 7 log CFU/ml) seemed not to have an effect on the resulting log reduction.

3.1.5 Sensory analysis

The results of a taste panel are shown below in Table 3.5. The results of the triangle difference test shows that the panelists could not tell the difference between the samples. Results suggest that out of 25 people only 4 of them identified the different sample. This difference is not significant which means that UV light does not affect the sensory qualities of apple cider. These results agree with previous studies (Harrington and Hills 1968). In their research, the results of a standard 9-point hedonic test, showed no significant difference between the UV irradiated samples and the control. In the Harrington and Hills (1968) study UV treated apple cider was irradiated for 120 seconds. Treatment 4 in our study (the cider which has

been exposed to the highest amount of UV light) is irradiated for only 8.12 seconds. Comparing this with Harrington and Hills (1968) study, we can speculate that a hedonic test would lead to no significant differences between the UV treated and control apple cider.

Table 3.5. Summary of trials with successes, p-values and significance of cider Triangle Test

Attribute	Probability	Trials	Successes	p-value	Significance
Overall	0.333	21.0	4.0	0.954	Non-significant

3.1.6 Shelf-life study of UV-irradiated apple cider

To evaluate shelf-life data, two separate statistical analyses were performed. The categories analyzed were physical properties and the yeast and mold counts over the 27 day shelf-life period. The yeast and mold counts determine the length of the shelf –life period whereas the physical properties of apple cider are observed for its quality.

The ANOVA results showed no significant ($p > 0.05$) differences in physical properties between the control and UV irradiated samples meaning there were no treatment differences in physical properties (turbidity, color, viscosity, pH) of apple cider and the control. Since there were no within treatment differences an average value for each treatment was used to examine between week changes in properties Table 3.6 gives the results of shelf-life study.

Table 3.6. Significance values for physical properties of apple cider[#]

Physical Parameter	Difference between week 1&2 values	Difference between week 2&3 values
Color (Hunter L values)	+2.850 *	+0.020 *n.s.*
Turbidity (NTU)	+0.209 *n.s.*	+1.225 *n.s.*
Viscosity (Pa*s)	-0.0013*	-0.0002*n.s.*
pH	-0.034*n.s.*	+0.052*n.s*

[#] The table shows changes between average values for week to week comparisons. Actual mean values are given in Table A.2 in Appendix A.

*= Significant ($p < 0.05$)
n.s.= Not significant ($p > 0.05$)

3.1.7. Results and discussions of quality analysis

Color: Hunter "L" values were recorded over the storage period and a significant ($p < 0.05$) color change was observed in the cider between the first and second weeks. Yet the change was not significant between weeks two and three. The color of the cider got lighter over the three weeks. One reason for can be that the increase in yeasts and molds. Yeasts and molds use some of the compounds that cause dark color, as their growth requirements, so it is postulated that the change in color can be related to increases in yeasts and molds.

Turbidity: There was no significant change in turbidity for the shelf-life period. As color turbidity are highly correlated a change in turbidity was expected, as color got lighter over the weeks. The reason no change was observed in the turbidity

might also be due to the yeasts and molds are using the end product of browning reactions and balancing the turbidity of the cider.

Viscosity: A significant ($p < 0.05$) viscosity change was observed in the apple cider between the values of the first and second week. The viscosity change was not significant for the third week of the study. One reason can be as yeasts and molds increase in number they produce gas, by using available substrate (end products of browning reactions). When substrate is limited there is less gas production and growth, which might lead to a less viscous apple cider.

pH: The quality tests showed no significant differences in pH of apple cider samples processed with UV light throughout the shelf-life study.

Yeast and mold counts: As yeast and mold counts are expected to increase exponentially over storage time, the log transformation of the counts for each week was taken and the log mean values of yeast and mold counts over each week are shown in Table 3.7. A negative correlation was observed between the yeast and mold counts and exposure time. Fermentation was first detected in the control and treatments one and two on the 16^{th} day of storage, but they were not actively fermenting [Actively fermenting is an observational (by author) quality of the apple cider made throughout the shelf-life study]. The increase in deterioration of cider quality, starting on day 16, may be attributed to the elevated yeast and mold counts, which might have accelerated the fermentation in the stored products. Treatment four first showed signs of fermentation on the 27^{th} day of the shelf-life study.

Table 3.7. The mean values of yeast and mold counts over the storage period stored at 4° C (log scale) for 27 days

Treatment	Week 1	Week 2	Week 3
	YM* counts (Log scale)	YM counts (Log scale)	YM counts (Log scale)
Control	9.0	11.3	12.4
Treatment 1	7.8	9.8	11.7
Treatment 2	7.5	9.2	12.1
Treatment 3	6.9	8.8	10.1
Treatment 4	5.5	8.4	10.1

* = Yeasts and molds

Figure 3.10 shows the same log pattern of growth over the three weeks but in the third week treatment two has higher counts than treatment one. The higher counts obtained in some of the jugs analyzed in the third week, were probably due to experimental error and/or the existence of more injured cells in treatment two compared to the other treatments which became active in cider and resulted in higher counts compared to the treatment one. Statistical analysis performed for the yeast and molds indicated significant differences between UV treatments within each week ($p < 0.001$). This means that within the same week different treatments had different yeast and mold counts (For example, Treatment 1 had different counts than treatment 4). Therefore treatments within each week were analyzed separately with Tukey's LSD (10%) and yielded the following results:

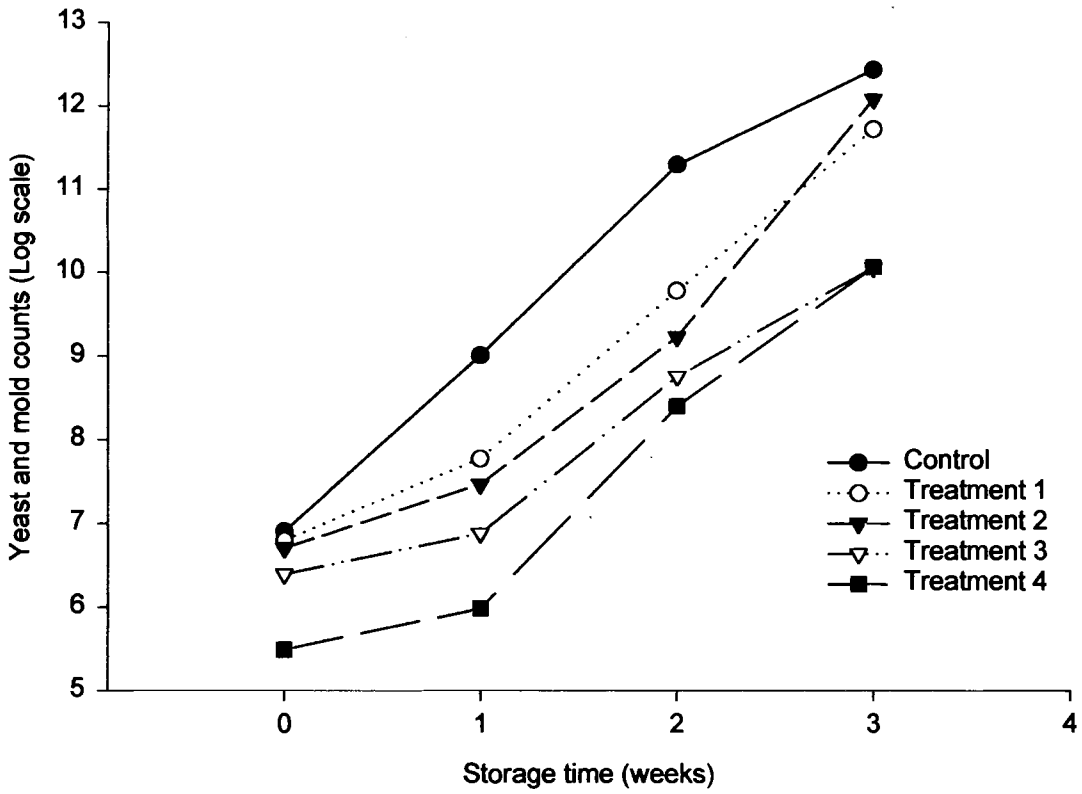

Fig 3.11. Yeast and mold counts during the shelf-life period (week 0 indicates the initial yeast and mold counts)

Week 1: The analysis of each treatment within a week showed that during the first week of the shelf-life study only treatment four was significantly different from the control apple cider and treatment one ($p < 0.1$). There were no other differences observed between the rest of the treatments during this week.

Week 2: In the second week of the storage study increases in yeast and mold numbers can be seen from Table 3.8 and Figure 3.10. In this week the control is significantly different ($p < 0.1$) from all other treatments. Treatment two is different from treatment one and four, treatment three is different from treatment one, and treatment

four is different from treatment one as well. The counts for the differences for each treatment can be seen in the Fig. 3.10.

Week 3: During the last week of the shelf-life study, treatment one and two were not significantly different from the control, but these three are significantly different ($p < 0.1$) from treatments three and four.

Summary and discussions

Benefits of UV irradiation on the shelf-life were demonstrated in this study. First the physical properties were examined and the tests showed no significant differences in the pH and turbidity of apple cider samples treated with UV light throughout their shelf-life. Significant differences in color and viscosity were observed.

It was shown that refrigerated storage of apple cider was extended when yeast and mold counts were reduced. In the last (third) week of shelf-life study, control, treatment 1 and 2 were not significantly different from each other but these three were significantly different from treatment 3 and 4. This shows that UV irradiation extended the shelf-life of the refrigerated cider over the control.

The results generally agree with those of Harrison and Hills (1968) but they stored the apple cider at 2.2 °C, which was lower than the storage temperature (4° C) in this study. The reason cider was stored at 4° C in this study was it would be closer to the actual storage condition of apple cider in commercial markets. A slightly higher storage temperature compared to that of Harrison and Hills (1968), resulted in a

shorter shelf-life for the irradiated apple cider. In their study apple cider was irradiated for up to 54 seconds, whereas in this study the highest exposure time was 8.12 seconds (treatment 4). Less irradiaton time compared to the Harrington and Hills also results in a shorter shelf-life. The irradiated apple cider (8.12 seconds) from our study was still not fermenting until day 27 of storage and in their study the 54 seconds irradiated apple cider was good up to 35 days. It is speculated that consumers will use the apple cider in a week or two and assuming that the cider will not stay in the commercial market more than 10 days, shelf life determined in our study and also in Harrington and Hills (1968) is above the average time apple cider will be kept refrigerated.

Considering the big difference in the irradiation time, there is not a very big difference between the days of refrigerated storage when the study is compared to that of Harrington and Hills (1968). We can still conclude that UV light extended the shelf-life of apple cider by more than seven days over the control. During the last week of shelf life study, treatment 3 and 4 resulted in a fewer yeast and mold counts in apple cider, compared to the control apple cider.

4. CONCLUSIONS AND FUTURE WORK

The FPE 1500® UV machine was successful in addressing the concerns related to apple cider safety. Through the experiments at UMaine, we found that the FDA mandated 5-log reduction is possible with UV treatment of apple cider. The following are concluded from this study.

1. FDA required 5-log reduction has been achieved in multiple passes through the UV machine for approximately 8.12 seconds.
2. The log reduction achieved is associated with turbidity, color and viscosity of apple cider.
3. It was shown that apple cider gets darker when the exposure time to UV light increases and turbidity tends to decrease as UV exposure time increases.
4. Significant temperature increase was observed in apple cider when irradiated.
5. Increase in pH (less acidic) and decrease in viscosity was observed.
6. Sensory analysis showed no difference between the UV treated and non-treated apple cider using a triangle difference test.
7. UV irradiated and cold-stored (4 °C) apple cider had at least seven days longer shelf-life than the untreated control.

The results from these laboratory experiments using the UV machine show that the ability to kill bacteria is associated with the turbidity, color and viscosity of apple

cider as well as the exposure time to UV light. Viscosity values started to get significantly altered when the exposure time was 8.12 seconds. The change in pH of apple cider was found to be significant between control and treatment 1 and between treatments 3 and 4.

Initially, there was a concern that irradiating apple cider may lead to changes in the sensory qualities of apple cider. This concern was alleviated through the triangle difference test and it was shown that UV irradiation can be used to treat apple cider. The effectiveness of UV light at reducing initial bacterial counts and prolonging the refrigerated shelf life of apple cider was demonstrated.

In summary these findings reiterate the efficacy of UV irradiation as a pasteurization technique. It should be kept in mind that food irradiation is not a substitute for proper sanitation, however, when combined with the Good Manufacturing Practices, it can reduce the risk of foodborne illnesses caused by pathogenic microorganisms by a significant amount.

We can still conclude that UV treatment is effective in reducing the *E. coli* and other microorganisms in apple cider and preserving the qualities where the resulting changes caused by the UV treatment are not significant.

FUTURE WORK: There are a few suggestions that can be made to make the UV unit more efficient in killing microorganisms. The calculated flow rate here indicated laminar flow and other research (Murakami *et al.* 2001) has shown that creating a turbulent flow or near turbulent, improves the log reduction with UV treatment. It has not been shown previously that turbulence can be achieved in the current design

of this UV unit. A number of modifications to the design of the chamber need to be explored. One of the possible approaches can be placing a mesh-like corrugated metal between the quartz and the stainless steel tube. This will increase mixing and provide turbulence to achieve more uniform exposure of the apple cider to UV light.

To increase the effectiveness of UV light and achieve a 5-log reduction in fewer cycles, different approaches can be considered. Apple cider might be exposed to ultrasound before it is exposed to UV light. Oliver and Cosgrove (1975) found that if they dispersed the sample by ultrasonication, the fluid would be more sensitive to UV light. This way bacteria are not protected from UV light (Qualls *et al.* 1983). Ultrasonication might be investigated as an additional process step prior to UV treatment to help increase the efficiency of the UV light. However, it should be investigated if ultrasonication changes the sensory properties of apple cider.

Pretreatment filtration is not recommended, as filtrated apple cider is considered to behave like apple juice. Other researchers have found positive results using filtration (Worobo *et al.* 2000), however, filtering the apple cider causes changes in texture and organoleptic properties that might be undesirable to the consumer.

Another interesting aspect to pursue would be to add sensory testing to correlate the sensory attributes of the UV irradiated apple cider at each stage of the shelf-life study. Further testing should be performed to test the individual parameters of apple cider and stored cider. Sensory testing using hedonic scale can be performed to examine the perceived effect of individual parameters of apple cider.

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Appendices

Appendix A: Results of Statistical Analysis

Table A.1. Pearson correlation matrix showing the correlation between the physical parameters of apple cider

	Color	Turbidity	Viscosity	pH
Color	1.000[+]	------	------	------
Turbidity	0.979	1.000	------	------
Viscosity	0.501	0.440	1.000	------
pH	-0.130	-0.125	-0.064	1.000

[+] The higher the number is the higher the correlation between the two physical parameter shown.
– Indicates inverse relationship between the two variables.

Table A.2. Mean values of the physical parameters for apple cider for 27 days stored at 4 ° C

Physical property	**Storage Period**			
	Week 0	Week 1	Week 2	Week 3
Color (Hunter L values)	21.4	21.540	24.390	24.410
Turbidity (NTU)	84.298	84.320	84.525	85.750
Viscosity (Pa*s)	0.0312	0.031	0.030	0.030
pH	3.55	3.364	3.330	3.382

Table A.3. Matrix of pair wise comparison probabilities for the treatments in week 1 (Tukey HSD Multiple Comparisons). **WEEK 1**

	1	2	3	4	5
1	1.00	------	------	------	------
2	0.98	1.00	------	------	------
3	0.60	0.86	1.00	------	------
4	0.06	0.11	0.33	1.00	------
5	0.62	0.44	0.20	0.03	1.00

1= Treatment one 2= Treatment two 3= Treatment three
4= Treatment four 5= Control

The matrix above shows the p-values between the treatments. The p-value indicates whether or not there is a difference between the two treatments in the same week. This allows for the determination of how more irradiation can be better or not. The table shows the treatment differences in only week 1 of the shelf – life study.

Table A.4. Matrix of pair wise comparison probabilities for the treatments in week 2 (Tukey HSD Multiple Comparisons).**WEEK 2**

	1	2	3	4	5
1	1.00	------	------	------	-----
2	0.07	1.	------	------	------
3	0.00	0.13	1.00	------	------
4	0.00	0.02	0.39	1.00	------
5	0.00	0.00	0.00	0.00	1.00

1= Treatment one 2= Treatment two 3= Treatment three
4= Treatment four 5= Control

The matrix above shows the p-values between the treatments. The p-value indicates whether or not there is a difference between the two treatments in the same week. This allows for the determination of how more irradiation can be better or not. The table shows the treatment differences in only week 2 of the shelf – life study.

Table A.5. Matrix of pair wise comparison probabilities for the treatments in week 3 (Tukey HSD Multiple Comparisons). **WEEK 3**

	1	2	3	4	5
1	1.00	------	------	------	------
2	0.94	1.00	------	------	------
3	0.06	0.02	1.00	------	------
4	0.09	0.04	1.00	1.00	------
5	0.83	0.98	0.06	0.07	1.00

1= Treatment one 2= Treatment two 3= Treatment three
4= Treatment four 5= Control

The matrix above shows the p-values between the treatments. The p-value indicates whether or not there is a difference between the two treatments in the same week. This allows for the determination of how more irradiation can be better or not. The table shows the treatment differences in only week 3 of the shelf – life study.

Table A.6. Univariate F Tests showing the significance between the treatments on the physical parameters of apple cider as a result of UV irradiation

	Turbidity	Color	Viscosity	Temperature	pH
Treatments#	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
Control-1	0.012*	0.043*	0.127 *n.s*	0.000*	0.009*
1-2	0.007*	0.005*	0.140 *n.s*	0.000*	0.700 *n.s*
2-3	0.497 *n.s*	0.434 *n.s*	0.090 *n.s*	0.000*	0.667 *n.s*
3-4	0.926 *n.s*	0.069 *n.s*	0.019*	0.000*	0.048*

=Treatments which are; Control = No UV treatment, Treatment 1= Single Pass through the UV machine, Treatment 2= Double Pass, Treatment 3= Triple Pass, Treatment 4 = 4 passes
* = Significant differences between treatments for each cycle ($p < 0.05$)
n.s. = Non significant differences

Appendix B: Sensory Materials

Triangle Difference Test Between UV Treated Apple Cider and Non Treated Apple Cider

Objectives: The objective of this study is to determine whether UV irradiation causes a detectable taste difference in apple cider. UV treatment may be an alternative method to high temperature pasteurization. Current high temperature pasteurization techniques causes change in sensory properties of apple cider and cider makers have been resistant to pasteurization because they do not want an inferior product. Because of this we are looking for alternative pasteurization methods that will produce safe and appealing cider.

Sensory Evaluation: The purpose of this sensory test is to determine if untrained panelists (consumers) can detect a difference between apple cider pumped through the UV unit and untreated (not passed through the machine, CONTROL) non pasteurized apple cider. Two separate triangle tests will be conducted. In the first one a sample pumped through the UV unit with no exposure will be compared to control and in the second one, sample exposed to UV treatment will be compared to control. Panelists will evaluate the fresh apple cider for taste difference using the triangle test ballot (see the attached form).

Personnel: The personnel who will have contact with the consumer sensory panel will be Nazife Canitez, a graduate student from Bio-Resource Eng department who has currently taken the Sensory Course and Dr. Mary Ellen Camire, Ph.D, from Food Science and Human Nutrition Department who has over 16 years' of experience in consumer testing.

Figure B.1. Application for the approval of sensory testing

Subject Recruitment: The subject population, to be recruited for participation in the sensory panel, will be members of the University of Maine community who have consumed cider in the past twelve months. The recruitment announcements will also state that persons who are allergic to apples or may be pregnant, nursing, have HIV, hepatitis or are otherwise immunocompromised should not participate in the project. The ages of the participants will be approximately between ages of 18-45. These subjects will be recruited by oral and written communication via posted announcements on campus.

Informed Consent: Panelists will have to sign an informed consent form before they participate in the sensory project.

Risks to subjects: Recruitment announcements will state that unpasteurized cider will be tested and that a small risk for food-borne illness is possible. *E.coli 0157* has been associated with outbreaks of illness from drinking unpasteurized cider. Cider will be bought from a local producer (North Star Orchards) located in Madison, ME. They use stored apples and they press apple cider every week. Cider samples will be analyzed prior to sensory testing for this pathogen to make sure that unpasteurized samples are not contaminated. Each bottle of cider that will be used for consumer testing will be analyzed by plating on EMB plates. Otherwise the risks involved are minimal since only small volumes of cider will be consumed.

Benefits: The results of this sensory test will be very beneficial to this project. It will allow us to determine whether the processing methods affects sensory quality of apple cider.

Figure B.1. Continued

INFORMED CONSENT FORM

Date: September 14, 2000

Nazife Canitez is authorized to include me in the following research study: Consumer acceptance of Ultra Violet light (UV) treated apple cider. I have been asked to participate in a difference test to evaluate apple cider. The purpose of this study is to determine if UV irradiation causes a detectable difference in apple cider.

It has been explained to me that the reason for my inclusion in this study is because I am at least 18 years old and I have consumed apple cider in the past year. As a participant, I will evaluate three samples of 2 ounces of unpasteurized apple cider in 4 ounce cups. I will evaluate a total of 6 ounces but I know that I don't have to drink all 6 ounces. Participation may take 15-20 minutes.

The study described above may involve the following risks: possible microbial contamination. The samples will be tested for the *E. coli* pathogen prior to sensory analyses to make sure that samples are not contaminated. ***I understand that I will not be allowed in this study if I have any allergies to apple cider or If I am pregnant, nursing, have HIV, hepatitis or are otherwise immunocompromised.***

The PI of this study is Nazife Canitez. Nazife Canitez will be available to address any concerns I may have about this project. Nazife can be contacted at 581-2724 or on the University's First Class e-mail system. If the study design or use of information is to be changed, I will be so informed and my consent reobtained. While there is no direct benefit to me for participating other than the small incentive such as a candy bar I will receive at the end of the test, the results of this study will help determine whether processing methods affect sensory quality of apple cider

I have the right to refuse to participate in, or to withdraw from, this research at any time. This test is anonymous, so I should not write my name on thew ballot.

If I have further questions, comments, or concerns about the study or the informed consent process, I may contact any of the following:

1.Mary Ellen Camire (Faculty Sponsor) at 581 1627
2. Michael Dougherty (Scientific Technician) at 581 3581
3. Nazife Canitez (PI) at 581-2724.

Figure B.2. Informed Consent form submitted to Human Subjects Committee

TRIANGLE TEST

ATTENTION! DO NOT WRITE YOUR NAMES ON THE BALLOT

Panelist No. _____ Date:_____

Type of Sample:_____

Instructions

Taste the samples on the tray from left to right. Two of the samples are identical; one is different. Select the **odd** sample and indicate by placing an X under the code of the sample.

715	488	937

Figure. B.3. An example of the sensory ballot using in the triangle testing

UNIVERSITY OF MAINE

Office of Research and Sponsored Programs
Protection of Human Subjects Review Board

5717 Corbett Hall
Orono, ME 04469-5717
207/581-1498
FAX 207/581-1446

MEMORANDUM

TO: Mary Ellen Camire
Nazife Canitez

FROM: Gayle Anderson
Assistant to the IRB

SUBJECT: "Triangle Difference Test Between UV Treated Apple Cider and Non-Pasteurized Apple Cider," #2000-05-05

DATE: May 12, 2000

Dr. Michael Robbins, Chair of the Protection of Human Subjects Review Board, conducted an expedited review of the above referenced project. He judged your application exempt from further review under category 6 of the regulations with the following modifications:

1) It seems that there is no reason that the triangle test couldn't be anonymous. You state under "Confidentiality" that panelist names will not be pertinent information. Unless you have a reason for collecting names on the ballot, please revise the form to remove the name or any type of coding that would link responses with names.

2) Informed Consent Form:

   a) Delete the "I hereby" and the "I understands" throughout the form. Those phrases are discouraged because they tend to make the form read like a legal document. The purpose of an informed consent form is to provide potential subjects with the information necessary to make an "informed" decision whether to participate, and it should be written in an easy-to-read manner.
   b) Include the purpose of the study.
   c) Explain the procedures, so that potential participants know what they will be asked to do.
   d) Include a time estimate of how long it may take to participate.

Figure B.4. Approval of Human Subjects Committee

e) Include your name and phone number as a contact (describing that you are the faculty sponsor on the project). (Are you the PI? The application lists you as PI, but the informed consent does not.)
f) Under the risk that you currently list, explain that samples will be tested for the E. coli pathogen prior to testing to make sure samples are not contaminated.
g) For a benefit statement, use a statement such as, "while there is no direct benefit to me for participating, the results of the study will help determine whether the processing methods affect sensory quality of apple cider."
h) In the fifth paragraph, delete "without penalty or loss of benefits to which I am entitled," since there are neither penalties nor benefits.
i) Delete the sixth paragraph unless you have a reason for not conducting this as an anonymous study. Instead state that the study is anonymous and they should not write their name on the ballot.
j) Delete the second to last paragraph.
k) Delete the signature line, as it is not required for projects exempt from further review.

3) Forward a copy of the ad that will be used to recruit subjects.

Please forward the revised informed consent form and testing ballot (or explanation why you need the names) to me at 424 Corbett Hall. If you have any questions, please contact me at 1-1498. On behalf of the Board, thank you for your cooperation, and we wish you success with your project.

Figure B.4. Continued

Biography of the Author

Nazife Canitez was born in Lefkosa, Cyprus, on November 26, 1977. She was raised in Lefkosa and she graduated from Turk Maarif College in 1994. She attended the Ege University in Izmir, Turkey, the same year. She graduated with a Bachelor of Science degree in Food Engineering. In 1998, she worked in Horozoglu Chocolate Factory in Lefkosa, Cyprus, as a Food Scientist.

She enrolled in the University of Maine, Orono, Maine in September 1999 and served as a graduate research assistant in the Department of Bio-Resource Engineering. She is a candidate for the Master of Science degree in Bio-Resource Engineering from The University of Maine in May, 2002.