IMPACT OF THERMAL AND NON-THERMAL PROCESSING TECHNIQUES ON APPLE AND GRAPEFRUIT JUICE QUALITY

By

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To my daughter Suha and my husband, Niyas

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Increased consumer demand for fresh-like products with minimum flavor and nutritional losses has paved the way for alternatives to heat processing. The impact of non-thermal techniques, including pulsed electric field (PEF), radio frequency electric field (RFEF) or ultraviolet irradiation (UV) and thermal pasteurization on quality of fruit juices after 4 weeks of storage at 4ºC were studied in this project. All treatments were optimized to achieve equivalent five log reduction in *E. coli* K12 levels.

The effect of PEF, UV and thermal treatments on apple cider quality was investigated based on microbial, color, physical properties, volatile and sensory analysis. PEF and thermally processed cider maintained good microbial quality during 4 weeks of storage, but UV treated cider fermented after 2 weeks. Thermal and UV pasteurized cider color faded significantly ($p<0.05$) during storage. CIE L* (lightness) and b* (yellow) values increased compared to PEF cider. Significant differences ($p<0.05$) in the level of key cider odorants were observed between treated apple ciders after 4 weeks of storage. Thermal samples lost 30% and PEF cider lost less than 2% of the total ester and aldehydes during storage. In UV cider, hexanal and 2-(E)-hexenal were completely lost after 4 weeks of storage. Microbial spoilage in UV cider was

chemically confirmed by the detection of the microbial metabolite 1,3 -pentadiene. Triangle sensory analysis indicated a significant difference ($p<0.05$) in aroma between treatments after 4 weeks storage. PEF treated cider was preferred over thermally treated cider by 91% of the sensory panel. PEF treated apple cider had a longer shelf life than UV treated cider and a better aroma and color than the thermally processed sample.

The effect of PEF, RFEF, UV and thermal techniques on the quality of red grapefruit juice were investigated based on microbial, color, physical properties, vitamin C content, non-enzymatic browning, pectin esterase inactivation, aroma volatiles and sensory analysis. Thermal treatment was more effective compared to non-thermal treatment in terms of microbial stability after 4 weeks storage. However, RFEF treatment ensured microbial safety of grapefruit juice for 3 weeks of storage with significantly ($p<0.05$) higher Vitamin C content and equivalent PME inactivation compared to thermally treated juice. PEF and UV treatments maintained good microbial quality for 1 week with significantly ($p<0.05$) higher Vitamin C content, as well as better color preservation compared to thermal. Thermal, as well as non-thermal, pasteurization affected the aroma profile of fresh grapefruit juice considerably. The effects of treatments on juice aroma were highlighted by loss in desirable fruity and citrus odorants, along with an increase in undesirable cooked/catty odorants. Aroma of treated juice differed significantly ($p<0.05$) from fresh juice by sensory evaluation. Non-thermally treated juice had a shorter shelf life but higher vitamin C content and superior physical qualities compared to thermally treated juice.

CHAPTER 1
INTRODUCTION

Public perception of juices as a healthy natural source of nutrients and heightened public interest in health issues have led to increased fruit juice consumption worldwide. However, in recent years many outbreaks of foodborne illness have been reported after consumption of unpasteurized juice due to the presence of microorganisms like *Salmonella sp*., *Escherichia coli* O157:H7 and *Cryptosporidium parvum* (Besser and others). The 1999 Salmonella outbreak in the United States was caused by consumption of unpasteurized orange juice. Under the federal Juice HACCP rule published in 2001, juice processors must implement treatments to reduce populations of "pertinent" microorganism by 5 log cycles. The "pertinent" microorganism is defined as the most resistant microorganism of public health significance that is likely to occur in the juice in question. At present, *E. coli* O157:H7 and *Cryptosporidium parvum* are accepted as the pertinent organisms for apple juice and *Salmonella* are the accepted pertinent organisms in citrus juice.

Thermal processing is the most commonly used technique to reduce spoilage and pathogenic microorganisms in fruit juices (Choi and Nielsen 2005). Unfortunately, thermal pasteurization can produce undesirable quality changes like loss of color and flavor in addition to reducing the nutritional quality of juice (Rouseff and others 2001; Vikram and others 2005). Recently, non-thermal processing alternatives like high pressure, dense phase CO_2, pulse electric fields, oscillating magnetic fields, pulsed high intensity light and ultraviolet irradiation have been examined for their efficacy in extending shelf life and enhancing juice, pulp or cider microbial safety while minimizing

quality and nutritional losses (Cserhalmi and others 2006; Tandon and others 2003; Donahue and others 2004).

Even though there are many non-thermal processing techniques available for the pasteurization of juices, a lot more research is required to make them commercially successful. Particularly, studies about the effects of non-thermal techniques on quality and consumer acceptability compared to the effects of thermal pasteurization are required. Most often the conditions used for thermal processing are severe in terms of temperature and time of exposure to heat. Thus, shelf life studies comparing the effects of non-thermal to thermal techniques on acceptability of juice using conditions that do not achieve the same reduction of a concerned microorganism would result in incorrect conclusions (Rivas and others 2006; Aguillar-Rosas and others 2007; Yeom and others 2000b) Although a large amount of microbial information concerning thermal and non-thermal processed fruit juice can be found in the literature, little information exists on the effects of these processes on the flavor of juice. A major motivation for non-thermal processing technologies is a minimal change to organoleptic properties. Therefore an in-depth analysis of the effect of the above processes on the flavor profile of juice and its relation to sensory quality is necessary.

A recent study by Sentandreu and others (2006) compared the sensory acceptability of citrus juices (Valencia, Clementine and Ortanique) pasteurized by pulsed electric field (PEF) and heat treatment. Each treatment was optimized to achieve comparable 90% pectin methyl esterase (PME) inactivation. A simple ranking test revealed no significant difference in sensory acceptability between PEF (25kV/cm for 330 μs) and heat (85 ºC for 10s) treated juices.

It is hypothesized that processing of juices by thermal and non-thermal techniques at equivalent microbial inactivation conditions would have comparable effects on juice quality. The main objectives of this study were to:

a) determine optimal conditions for apple cider and red grapefruit juice pasteurization by thermal and non-thermal (PEF, RFEF & UV) techniques
b) compare the impact of thermal and non-thermal pasteurization techniques on quality of apple cider and red grapefruit juice
c) study the change in quality of apple cider and red grapefruit juice during 4 weeks storage at 4ºC

CHAPTER 2
LITERATURE REVIEW

Flavor Perception

Flavor is one of the major influences on consumer purchases and consumption. It is an amalgamation of aromatic character, gustatory stimuli and trigeminal sensations. The aromatic character is imparted by a complex mixture of volatiles, whereas taste and trigeminal components are mostly non-volatile, hydrophobic molecules soluble in saliva (Laing and Jinks 1996). Aroma is sensed in the nasal cavity and encompasses most of what is considered flavor. Odorants first interact with olfactory sensory neurons located in the olfactory epithelium lining the nasal cavity. Conformational changes in the receptor, due to binding of odorant, results in a cascade of biochemical changes leading to transmission of signals to the olfactory bulb. These signals are further relayed to other brain areas where the information is processed to given an integrated flavor profile (Hatt 2000; Lancet and others 1993). A major breakthrough with regards to odorant and receptor specificity came from the Nobel-Prize winning work of Buck and Axel (Buck and Axel 1991). The three major points they presented were: a) each odor receptor can recognize multiple odorants, b) each odorant can be detected by multiple receptors, and c) the olfactory system uses a combinatorial receptor coding scheme to encode odor identity. The receptor specificity for certain molecular groups is evident in the case of the rat 17 odor receptor. This receptor binds to seven to nine carbon aliphatic aldehydes with maximum affinity to octanal and lesser interaction with molecule-like heptanal (Krautwurst and others 1998). However, with substitution of the aldehyde group with any other functional group, this affinity is completely lost (Araneda and others 2000). The functional group of compounds appears to be significant for recognition of odorants.

On the other hand, each odorant can be recognized by multiple receptors and thus stimulate several receptors at same time. It is predicated that those odor receptors which interact with the same odorant would have a similar protein sequence. For example, highly related odor receptors S6, S50 and S79 interact with odorant nonanedioic acid (Malnic and others 1999). Each odorant thus activates a unique combination of olfactory cells and send a unique pattern of neuronal activity to the olfactory bulb. The signal is then relayed to brain to encode the odor identity. Humans have about 350 different odor receptors; hence, the olfactory system is well adapted to recognize an endless list of odorants and to discriminate them (Zozulya and others 2001).

Gustatory stimuli (or taste) is the perception of sweet, sour, salty, bitter, and umami sensations in the mouth. Umami is the characteristic taste imparted by glutamate and 5'-nucleotides like inosine and guanine. Taste cells are clustered in taste buds on the tongue, palate and pharynx. The tastants interact either with ion channels (salty, sour) or specific G-protein coupled receptors (bitter, sweet, umami) on the taste cells and transducts the signal to brain. Generally, chemicals that elicit sour and salty tastes are ionic in nature and are associated with high concentrations of H^+ and Na^+, respectively (Lindemann 1996). Ions enter the cell via selective membrane channels resulting in depolarization of cell. This causes the release of neurotransmitters between taste cells and taste neurons. Bitter, sweet and umami taste components bind to G-protein coupled receptors similar to olfactory receptors, although the chemical recognition is not as discriminating as it is in the case of the olfactory system (Zhang and others 2003). Bitter receptors belong exclusively to a class known as the T2R

family (Mueller and others 2005; Chandrashekar and others 2000). Bitter tasting compounds have an important role in survival, as they signal the presence of potentially dangerous components. In nature, numerous and biologically diverse bitter compounds exist, such as alkaloids, flavonoids, glycosides, steroids, phenols, N-heterocyclic compounds, and urea and related compounds. In comparison to the wide range of bitter compounds, there are far fewer T2Rs (~ 25) identified in mammals. Studies using functional expression of T2R transcripts in taste receptor cells have shown that each bitter sensing taste cell co-expresses the majority of T2R genes (Adler and others 2000). Meyerhof and others (2010) suggested that T2Rs are broadly tuned to perceive a large variety of bitter molecules. When 25 hTAS2Rs (human bitter taste receptors) were challenged with 104 bitter chemicals, they found that 3 of these hTAS2Rs were able to detect ~50% of the bitter compounds. However, the ability of bitter receptors to discriminate between the hTAS2Rs was limited (Adler et al. 2000). Thus, quite often many bitter compounds taste the same. Both sweet and umami receptors belong to the family of receptors called T1R (Nelson and others 2001; Nelson and others 2002). Heterodimers of these receptors, namely T1R1-T1R3, T1R2-T1R3 or T1R3, function as receptors for sweet tastes. These receptors expressed differentially among the taste cells enable binding of a wide range of structurally dissimilar molecules which are perceived as sweet, like sweet sugars, sweet proteins and artificial sweeteners (Nelson et al. 2001). Heterodimer T1R1-T1R3 acts as umami taste receptors and binds to monosodium L-glutamate and other L-amino acids (Nelson et al. 2002). Because this type of heterodimer is common to both umami and sweet tastes, there may be a relationship between these two taste modalities. Compared to the olfactory system, the

taste system is incapable of detailed chemical analysis due to the small number of taste receptors and ion channels.

Another system found in olfactory epithelium is the trigeminal nerve receptors. The trigeminal nerve extends to second set of nerve endings in areas around the mouth, eyes and nasal cavity. They are responsible for tactile sensations, pressure, pain and temperature. Some food components like capsaicin, menthol, flavonoids and alkaloids are responsible for trigeminal sensations like pungency, cooling and astringency. The trigeminal system influences the perception of taste and smell. A study by Prescott and others (1993) showed that capsaicin inhibits the perceived sweetness of sucrose. In another study, the burning sensation produced by capsaicin was influenced by the presence of sodium chloride but not by the presence of sucrose (Prescott and Stevenson 1995). More studies of the role of trigeminal system in flavor perception are required.

The perception of aroma is also influenced by whether the odorant is perceived via the orthonasal or retronasal path. Orthonasal refers to when the aroma is taken directly through the nose (Linforth and others 2002). Retronasal refers to the aroma volatiles produced after the food is swallowed and air from the lungs is exhaled through the nasal cavity. In an orthonasal case, the release of aroma compounds from foods depends on the partition coefficient between air phase and food matrix. In retronasal olfaction, aroma release depends on the partition coefficient between the water phase (saliva) and the food matrix. The eating process is a dynamic process, and the release of volatiles from food depends on the different physical and chemical conditions in the mouth like pH, temperature, mastication and enzymes. The rate of release of odorants

is also affected by the degree of interactions between aroma compounds and both macro (carbohydrates, proteins and lipids) and micro (acids, salt) food constituents. The retronasal impact is thus a combination of olfaction and gustatory perception. Retronasal aroma would most likely leave a lasting impression on the consumer.

Fruit Flavors

Fruit and fruit juice consumption have increased recent past years due to their perceived health benefits. Because flavor is one of the major influences of consumer purchases and consumption, a significant amount of research in last 30 years has been conducted in area of fruit flavors. The focus of research has been on characterizing the key volatile components that deliver the characteristic flavor unique to particular fruits.

Apple Juice/Cider Aroma

Apple cider (sometimes called soft cider) is the name used in the United States for an unfiltered, unsweetened, non-alcoholic beverage produced from apples. It is opaque due to the fine apple particles in suspension and may be tarter than conventional filtered apple juice depending on varietal characteristics of the apples used. In European countries, cider refers to an alcoholic beverage made from the fermented juice of apples.

Volatile compounds in fresh apples have been a subject of interest to many investigators. Over 300 volatile compounds have been detected in apples comprising of alcohols, aldehydes, carboxylic esters, ketones and ethers (Dimick and Hoskin 1983). Esters are present in highest amounts (78-92%) followed by alcohols (6-16%). Ester volatiles in apples give a fruity odor and are classified as ethyl esters, butyric esters, propanoic esters and hexanoic esters. The alcohols mostly are ethyl, butyl and hexyl alcohols.

The volatile composition and content in apple varies according to variety, maturity and storage conditions. Most of the changes in flavor and aroma characteristics develop after harvest. The aroma volatiles in whole, intact fruits are mostly formed via beta-oxidation or the lipoxygenase pathway from fatty acids, which are the major precursors of volatiles (Dimick and Hoskin 1983). The straight chain esters are synthesized via beta-oxidation of fatty acids to give acetic, butanoic and hexanoic acids. These acids are then reduced to corresponding alcohols and esters using Acyl CoA enzyme (Paillard and Rouri 1984a). As the fruit ripens, the cell wall and membranes becomes more permeable and the lipoxygenase enzyme comes in contact with fatty acids like linoleic and linolenic acid, converting them into C6 and C9 aldehydes (Dever and others 1992). These aldehydes are responsible for the green notes in apple aroma. The C6 and C9 aldehydes formed in homogenized fruit reach their maximum concentration within the first hour after homogenization.

Varietal differences affect volatile profile of apples. Granny Smith and Nico varieties are characterized by a high concentration of ethyl butyrate and hexanol, whereas Cox's Orange Pippin and Jonathan apples are characterized by high levels of hexanal and 2-E-hexenal (Dixon and Hewett 2000; Schamp and others 1988). In addition to the ripening and varietal effect, storage time and environment also impacts apple flavor (Aaby and others 2002; Plotto and others 1999). Apples are mostly held in controlled atmospheres during storage to delay ripening and reduce respiration. The reduced oxygen environment induces acetaldehyde and ethanol accumulation and decreases ester and aldehyde levels. Exposure of apples to low temperatures for more than 3 months decreases volatile concentration (Streif and Bangerth 1988).

One of the earliest studies on sensory contribution of apple volatiles to aroma was by Flath and others (1967b). They performed GC sniffing of Delicious apple essence. In total, 56 compounds were identified by mass spectrometry. Compounds with apple characteristics were listed as ethyl 2-methyl butyrate, hexanal and 2-E-hexenal. A study by Durr and others (1981) on 48 samples of commercial apple essence showed that the concentration of 2-E-hexenal and butyl acetate had a correlation of 0.78 and 0.72, respectively, to the odor intensity of apple essence. Good apple essence was characterized by high levels of C6 aldehydes and esters and low alcohol levels.

In Red Delicious apples, ethyl butyrate, ethyl 2-methyl butyrate, propyl 2-methyl butyrate, hexyl acetate, ethyl hexanaote and 1,3,5 (E,Z)-undecatriene were identified as key odorants by GCO analysis (Cunningham and others 1986). In Gala apples stored in a regular atmosphere, the key contributors to aroma were butyl acetate, hexyl acetate, butyl 2-methyl butyrate, hexyl 2-methyl butyrate and hexyl propanoate (Plotto and others 2000). Its profile changed in a controlled atmosphere by a decrease in esters hexyl acetate and butyl acetate.

Fuhrmann and others (2002) compared GCO data of three apple cultivars, Cox Orange, Elstar and Royal Gala, to determine the key contributing odorants. They studied the odor profile in the headspace of whole fruit as well as homogenized fruit. The key ester volatiles in whole fruit for different apple varieties were ethyl 2-methyl butyrate, ethyl butyrate (Elstar), ethyl 2-methyl propanoate, ethyl butyrate, 2-methyl butanol (Cox) and methyl 2-methyl butyrate, ethyl 2-methyl butyrate, and propyl 2-methyl butyrate (Royal Gala). In homogenized fruit, the FD profile changed compared to whole fruit. High FD values for compounds hexanal, cis-3-hexenal and hexyl acetate in

Cox and Elstar varieties were noted. The increase in levels of aldehydes was due to oxidation of linoleic acid and linolenic acid by liopxygenase released from crushed cells. Other volalites noted with high FD values were β-damascenone and p-allyl anisole. Beta-damascenone has a rosy, fragrant odor, which adds intensity to the fruity odor of an apple. Para-allyl anisole with musk herbaceous odor, along with its isomer anethole, imparts spicy notes to an apple's aroma.

Mehanigic and others (2006) characterized odor volatiles of three apple cultivars (Fuji, Golden Delicious and Braeburn) at different maturity stages. They found 15 odorants common to all varieties, the important ones being ethyl butyrate, ethy-2-methyl butyrate, butyl acetate, hexyl acetate and hexanal. The overall quantity of odorants increased during maturation in Fuji and Golden Delicious apples with more alcohols and esters (butyric & hexanoic) and less aldehydes. The data from various literature reports on different varieties of apples indicate esters like ethyl butyrate **(4)**, ethyl-2-methyl butyrate **(5)**, butyl acetate **(6)** and hexyl acetate **(7)** (Figure 2-1) as key odorants of apple aroma.

Grapefruit Juice Aroma

Grapefruit juice has a unique flavor due to its sweet-tart and bitter taste combined with a characteristic aroma. Quantitative and qualitative studies on volatile constituents of grapefruit juice, essence and oils have been carried out by many groups (Buettner and Schieberle 1999; Lin and others 2002a; Coleman and others 1972). Moshonas and others (1971) reported a total of 32 compounds in grapefruit essence extracted from canned grapefruit juice . Based on the relative amounts by GC-FID, they reported major volatiles as ethanol, acetal, ethyl acetate, ethyl butyrate, isoamyl alcohol, cis and tran-linalool oxide, linalool, octanol, alpha-terpineol, ethyl 3- hydroxyhexanoate and terpinen-

4-ol as major components. In contrast to essence, fresh juice had high ethanol content followed by volatiles limonene, beta-caryophyllene, ocimene and myrcene. Other compounds found in lower quantities were linalool, citral, α-terpeniol, β-phelladrene, copaene and nootkatone (Shaw and others 2000; Nunez and others 1985).

Pino and others (1986) studied the relationship between sensory response and 32 compounds present in grapefruit juice. Some of the important aroma compounds identified were limonene, acetaldehyde, decanal, nootkatone, ethyl acetate, methyl butyrate and ethyl butyrate. They determined the odor threshold value of the compounds in water and quantified the amount of each compound in juice. The relative flavor contribution of each compound was calculated based on the ratio of amount in juice to odor threshold in water. Ethyl acetate and acetaldehyde had the highest relative threshold values and nootkatone the lowest. The authors performed a sensory analysis wherein panelists scored the odor of juice with four different dilutions of compounds in increasing order. These sensory scores were then related to quantities of compounds from GC data using linear regression analysis. Nootkatone, limonene, decanal, ethyl butyrate and methyl butyrate showed positive correlation, which indicates that they contribute significantly to good grapefruit juice flavor. However, an increase in concentration of α-terpineol, cis and trans epoxydihydro linalool had a negative correlation with sensory scores. In another study by Jella and others (1998), a positive correlation in grapefruit juice acceptability with beta-caryophyllene levels and negative correlation with myrcene and linalool levels were reported.

Two compounds, nootkatone **(1)** and p-1-menthene-8-thiol **(2)** (Figure 2-1), have been identified and proposed as character impact components of grapefruit juice aroma

(Macleod and Buigues 1964; Demole and others 1982). The flavor threshold of nootkatone is at 1ppm in water and 6ppm in reconstituted grapefruit juice (Berry and others 1967). Nootkatone at levels more than 6-7ppm impart an unpleasant bitterness to juice. The importance of nootkatone to grapefruit juice flavor is debatable, as Shaw and others (1981) found that addition of nootkatone to grapefruit juice had very little impact on juice flavor. They suggested the presence of other aroma components in juice that are significant to grapefruit flavor. Sulfur compound 1-p-menthene-8-thiol is a character impact odorant in grapefruit juice. Demole and others (1982) isolated 1-p-menthene-8-thiol (1-PMT) and its bicyclic epimer 2, 8-epithio-cis-p-menthane, from canned grapefruit juice. They reported 1-PMT as the most potent odorant in nature with a very low odor threshold at 0.1×10^{-9} g/L. It occurs at 200 fold higher concentration in juice, implying its significance to grapefruit flavor. The threshold of the1-PMT isomer was about 10^5 greater than the thiol, indicating lesser contribution to grapefruit flavor. To investigate other important odorants in grapefruit juice, further studies by the same authors led to the identification of 15 related sesquiterpenes ketones in grapefruit juice (Demole and Enggist 1983). One of these ketones, 8,9-didehydronootkatone, was a more intense (lower threshold) grapefruit aroma component than nootkatone.

Most of the odor active volatiles important to grapefruit aroma are present in very low levels and often not detected by mass spectrometry or FID. Numerous GC-olfactometry studies have been performed to identify the character impact volatiles in grapefruit juice. A recent study by Buettner and others (1999) detected 37 odor-active volatiles in fresh grapefruit juice. Although acetaldehyde and limonene were the most abundant compounds present in grapefruit juice, they had a minor impact on juice

flavor. The authors reported high FD values for: ethyl butyrate, 1-p-menthene-8-thiol, (Z)-3-hexenal, 4, 5-epoxy-(E)-2-decenal, 4-mercapto-4-methyl pentan-2-one (4-MMP) **(3)**, 1-hepten-3-one and wine lactone. The trace component 4-MMP had the highest odor activity value. The same authors conducted further studies by quantitation, reconstitution and omission methods to determine the contribution of above odorants (Buettner and Schieberle 2001). . Sensory evaluation of the reconstituted grapefruit aroma model using 20 odor active components in water showed the sulfurous grapefruit-like odor quality due to 4-MMP and 1-p-menthene -8-thiol to be important. Single compound omission of 4-MMP from aroma models indicated that it had a higher impact on grapefruit odor than 1-p-menthene-8-thiol. Other grapefruit juice sulfur compounds reported in other studies included: 4-mercapto 4-methyl pentanol, 3-mercapo hexanol and 3-mercapto hexyl acetate (Lin and others 2002b). Hydrogen sulfide was also reported as a potential contributor to grapefruit juice aroma as it is present at levels above its odor threshold in juice (Shaw and others 1980). Grapefruit juice has 2-4X more sulfur components compared to orange juice (Rouseff and others 2003).

Volatile Extraction

The first step when characterizing odor active chemicals in a complex mixture is to separate them from nonvolatile components. This is accomplished through a variety of techniques such as solvent extraction, headspace concentration or distillation. The results can vary significantly in terms of final composition and flavor-component mixture, depending on the method used for volatile collection and concentration of aromas. For example, low impact methods of flavor isolation like hydrodistillation and solvent extraction are preferred for concentrating aromas from fruits and blossoms, as they are

less invasive and use mild temperatures. However, these methods might not be suitable for reaction flavors that have already been subjected to heat treatment.

The extraction methods used in the past for apple volatiles include: vacuum hydro distillation, dynamic and static headspace and liquid extraction (Komthong and others 2006a; Matich and others 1996; Mehinagic and others 2003). Young and others (2004) found that butanol was detected at the highest levels in Royal Gala apples by vacuum hydrodistillation, while butyl acetate was detected at the highest levels using headspace methods. Matich and others (1996) compared solid phase micro extraction (SPME) and solid phase extraction (SPE) method for Granny Smith apple volatiles. SPME provided a greater adsorption of high molecular components but required long period of time for equilibration and exposure times.

Various methods like solvent extraction, vacuum distillation, purge and trap, steam distillation extraction (SDE) and SPME have been used for extraction of grapefruit juice volatiles (Nunez and others 1984; Cadwallader and Xu 1994; Yoo and others 2004). SDE method was good for extraction of medium- and low-volatile components, but the high temperature used during the procedure combined with the presence of oxygen could alter composition of volatiles (Nunez et al. 1985). Purge and trap extraction eliminates some artifact formation. However, the method showed a few drawbacks when used for grapefruit juice volatile analysis. Compounds having lower vapor pressure, like oxygenated mono and sesquiterpenes, could not be effectively collected and therefore could not be detected. Moreover, sulfur compounds significant for grapefruit juice aroma were also not detected using the above extraction method (Cadwallader and Xu 1994). Solvent extraction is reported to be a better method of

volatile extraction for grapefruit juice, but it is cumbersome and loss of volatiles may occur during solvent evaporation (Buettner and Schieberle 1999; Jella and Rouseff 1997). Reports on SPME extraction of grapefruit volatiles are limited (Yoo et al. 2004).

Static headspace analysis by solid phase micro extraction (SPME) is a simple, rapid and solventless technique for extraction of volatiles (Kataoka and others 2000). Equilibrium is established among the analyte concentrations above the sample headspace and the polymer coating on the fused silica fiber. The amount of analyte adsorbed by the fiber depends on the thickness of polymer coating, the type of fiber coating and the distribution constant for the analyte. In addition to fiber, the transfer of analyte from food matrix to SPME fiber also depends on the matrix composition, time and temperature of extraction. Some of the fiber coatings used are polydimethylsiloxane (PDMS), divinylbenzene (DVB), carbowax (CW), carboxen and polyacrylate. Most often a combination of these coatings is used for broad-range volatile extraction.

Gas Chromatography Olfactometry

Even though food aroma is a complex mixture of volatiles, only a fraction of these volatiles are odor active. Most of the potent odorants are not often detected by instrumental techniques due to very low odor thresholds. Human olfaction has superior sensitivity and selectivity to odor compounds compared to many instrumental systems. The hyphenation of olfactometry to gas chromatography technique is an effective tool for the discrimination of relevant food flavor components, enabling the differentiation of a multitude of volatiles into odor active and non-odor active categories. In GCO, assessors judge the olfactory impressions elicited by the volatile compounds immediately after elution from a GC column in order to associate odor activity with the

eluting compounds. GC/O methods are classified into three categories: dilution methods, time- intensity methods and frequency of detection methods.

Time intensity methods are dynamic and odor intensity is recorded with the time during peak elution. “Osme” is a time-intensity approach for evaluating the significance of odor compounds in the GC effluent. Trained subjects sniffing the GC effluent mixed with humidified air directly record the odor intensity and duration time of each odor-active compound while describing its odor quality. The plot of the retention time/index versus odor intensity provides an easily interpretable representation of the compound’s odor significance in the flavor extract, called an “Osmegram” (Bazemore and others 1999). Higher peaks suggest greater sensory importance of the compounds. Time–intensity olfactometry has been used to identify key odorants in various fruits like apples, oranges, grapefruits, blueberries and cashew apples (Plotto et al. 2000; Lin and Rouseff 2001; Rouseff et al. 2001; Su and Chien 2010; Garruti and others 2003).

Processing

Fresh fruit juices can undergo quality degradation due to microbiological and enzymatic activities, as well as chemical reactions. In recent years, there has been increased concern about the safety of unpasteurized fruit juices due to outbreaks of *Escherichia coli* O157:H7, *Salmonella spp.* and *Cryptosporidium parvum* (Besser and others 1993a; Parish 1997). On January 19, 2001, the FDA published a final rule in the Federal Register that requires processors of juice to develop and implement Hazard Analysis and Critical Control Point (HACCP) systems for their processing operations (66 FR 6138). HACCP is a systemic preventive process applied in the food industry to address food safety by control and analysis of physical, biological and chemical hazards

at various processing stages. Under this rule, juice processors must comply with two requirements:

(1) subpart A of the rule requires use of HACCP principles and systems in their operations, and

(2) subpart B of the rule requires that processors implement treatment(s) to reduce a theoretical population of “pertinent” microorganisms in the juice by 99.999% or 5-log cycles.

The “pertinent” microorganism is defined as the most resistant microorganism of public health significance that is likely to occur in the juice. At the present time, *Salmonella* is generally recognized as the pertinent organism for citrus juices and *E. coli* O157:H7 and *Cryptosporidium parvum* for apple juice (FDA 2001).

Thermal Pasteurization

Thermal treatment has been widely used to inactivate spoilage, pathogenic microorganisms and enzymes to extend shelf life of juice products. Initially, pasteurization of fruit juices was done at low temperatures for long periods of time (63 ºC for 30 min). But extended heat treatment lowered the sensory and nutritional qualities of juices (Moshonas and Shaw 2000). Current pasteurization methods in practice are high temperatures, short time (HTST) and ultra high temperature (UHT).

UHT process is performed at 135-150 ºC for a few seconds (2 - 45 s). There are 2 methods: direct heating by steam injection and indirect heat transfer by heat exchanger. The product is aseptically packaged after UHT processing in order to obtain a product with a shelf life of 1 to 2 years at ambient temperatures. UHT destroys all pathogenic microorganisms, as well as spores. It is commonly used for milk pasteurization (David

and others 1996). However, UHT causes considerable change to the quality of milk, as it renders a heated flavor.

HTST, or flash pasteurization, is the most common method used for fruit pasteurization (David et al. 1996). Flash pasteurization is used for perishable beverages like milk, fruit and vegetable juices, and beer. It is done prior to pouring the beverages into containers in order to kill spoilage microorganisms in an effort to make the products safer and to extend their shelf life. The liquid moves in a controlled, continuous flow through a heat plate exchanger system subjected to temperatures of 72 - 74 °C for about 15 to 30 s. It increases the shelf life by 2-3 weeks under refrigerated conditions. Flash pasteurization destroys all pathogenic microorganisms but does not inactivate spores. The time/temperature requirement for pasteurization of juice depends on the initial microbial load, pH of the product and if enzyme inactivation is required.

Citrus juice thermal processing

In addition to microbial inactivation, citrus juice processing requires pectin esterase (PE) inactivation, as this enzyme leads to cloud loss in stored citrus juices. Normally, temperatures above 71 ºC for few seconds are enough to kill pathogens and spoilage bacteria in orange juice (Kale and Adsule 1995). However, treatment of juice at 90 ºC for 0.8min to 1min is required for 99% inactivation of PE activity (Wicker 2004). The food industry has different time and temperature parameters for enzymatic inactivation and destruction of microbial population in citrus juices by heat (Table 2-1) (Crupi and Rispoli 2002).

Apple juice thermal processing

Flash pasteurization of apple juice involves heating the juice to 71 ºC for 6s or heating cider to an equivalent time /temperature combination (AFDO 2001). However,

the cost of flash pasteurization for small cider producers is unaffordable, and therefore options like the hot fill technique are advantageous when the whole packaged product is pasteurized. This reduces the cost of sterilizing the container. Hot filling is conducted by passing the final product through a heat exchanger and raising the temperature such that the temperature of the juice filling container reaches a recommended temperature of 88 to 95 ºC. The juice is usually held for at least 3 min before cooling. The hot fill technique is adequate for acidic beverages like apple juice, cranberry juice and grape juice. Usually a shelf life extension from 9 to 12 months in glass bottles can be achieved by this method (McLellan and Padilla-Zakour 2004).

Effect of Thermal Pasteurization on Juice Flavor

Conventional juice pasteurization by heat causes significant loss of fresh flavor, color and nutritional content (Braddock 1999). There are few studies regarding the effect of thermal processing on the volatile composition of apple juice. A decrease in ester concentrations due to thermal pasteurization has been found in all these reports (Kato and others 2003; Su and Wiley 1998; Aguillar-Rosas et al. 2007). Su and others (1998) reported a decrease in apple juice aroma components iso-butyl acetate, ethyl butyrate, ethyl 2-methyl butyrate and hexanal after pasteurization at 85 ºC for 10 min. Apple juice processed by HTST at 90 ºC for 30 s resulted in more than 50% loss in hexanal, ethyl acetate, ethyl butyrate, methyl butyrate and acetic acid concentrations (Aguillar-Rosas et al. 2007). Kato and others (2003) correlated the change in apple juice volatiles pasteurized at various time –temperatures (2-320s; 80-120 C) to sensory data by principal component analysis. They found that the change in volatile concentrations were very complicated with respect to different thermal processing conditions, as the odor attributes of processed apple juice were not predictable. Principal component

analysis indicated that treatment temperature affected the volatile concentration more than treatment time.

Citrus juice flavors are also sensitive to thermal pasteurization. Shaw and others (2000) found a decrease in volatile constituents in heat pasteurized orange juice during a 9 week storage study. Tatum and others (1975) reported occurrence of alpha-terpineol, 4-vinyl guaiacol and Furaneol® in thermally treated canned orange juice stored at elevated temperatures. PVG was found to be the most detrimental to orange juice flavor, as it gave a rotten flavor to the juice. Furanones like Furaneol® are formed from Maillard reactions between amino acids and sugars present in juice and impart a caramel, cooked odor quality to juice (Perez-Cacho and Rouseff 2008). The acid catalyzed degradation of limonene under acidic conditions results in formation of compounds like α-terpineol, cineole, carvone and carveol (Marcotte and others 1998). Alpha-terpienol is considered to be a marker for thermally abused citrus juices.

In contrast to orange juice, there are few reports on the thermally-induced changes in grapefruit juice aroma (Lin et al. 2002b; Lin and others 2001; Lin et al. 2002a). Shaw and others (1982) compared the headspace levels of sulfur compounds in fresh and commercially heat processed grapefruit juice. They reported higher levels of hydrogen sulfide (tentatively identified) in fresh juice compared to processed juice, whereas dimethyl sulfide was detected only in processed juice. In a more recent study, GCO analysis of thermally processed grapefruit juice showed a loss of 6 odor active odorants and formation of meaty, cooked odorants 2-methyl 3-furnathiol and 2-acetyl 2-thiazoline (Lin and Rouseff 2001). These compounds negatively affect the flavor of juice. A sulfur volatile quantitation using sulfur chemiluminescence detector showed that pasteurized

grapefruit juice had higher levels of sulfur compounds compared to untreated juice (Lin et al. 2002a). Sulfur containing the amino acids methionine, glutathione and cysteine were proposed as the possible precursors for these sulfur volatile compounds.

A comparison of single strength and grapefruit juice reconstituted from concentrate without volatile restoration showed more than 90% loss of volatile components in the reconstituted juice (Lin et al. 2002b). Olfactometric analysis showed a drastic loss in fresh/citrusy and sulfur/grapefruit characteristic notes. The loss in fresh/ citrusy notes was attributed to a loss of limonene, octanal, and nonanal, whereas the loss in 1, 10-dihrydronootkatone, 3-mercaptohexyl acetate, 3-mercapto hexanol and 4-mercapto 4-methyl pentanol contributed to a decrease in sulfur/grapefruit odor. Thermal instability, as well evaporation during heating, contributed to flavor loss. In addition to flavor loss, thermal processing also causes degradation of vitamin C. Saguy and others (1978) found 7-12% loss of Vitamin C content in grapefruit juice pasteurized at 95 ºC for 1min.

Non-Thermal Processing Methods

Consumer demand for safer and minimally processed food has resulted in investigation of non-thermal processing alternatives. The main objective of non-thermal preservation methods is to minimize degradation of food quality by limiting heat damage to foods. In addition to preservation of food quality, non-thermal process should also achieve equivalent microbial safety level as thermal processes.

Juices are graded in United States based on standards such as Brix, Brix/acid ratio, color and flavor (AMS 1983). Therefore, it is important to study the impact of non-thermal processing on these quality parameters to determine if they maintain the

Agricultural Marketing Standards (AMS). Citrus juices are consumed for their nutritional benefits (Vitamin C) as well as for their flavor. However, ascorbic acid is heat and oxygen sensitive and undergoes degradation during processing and storage. Thus, the effect of non-thermal processing on ascorbic acid content is an important parameter to study.

Enzymes present in juice cause deterioration in the quality of juice by modification of pectin, degradation of ascorbic acid and browning. They change the flavor, color and texture of juice. In citrus juices, it is equally important to maintain the cloud stability equally as flavor and color. About 5% of the cloud is pectin, and it has a dominating effect on the behavior of clouds. Pectin methyl esterase enzyme present in juice demethylates the pectin molecule, causing aggregation of pectin molecules. These aggregates settle out of the juice, resulting in juice clarification or cloud loss (Guiavarc'h and others 2005). Thermal pasteurization is the most common method used for PME inactivation. The efficiency of non-thermal processing to inactivate PME is a crucial factor in terms of commercial viability.

Some of the non-thermal techniques extensively studied for juice pasteurization are high pressure (HPP), dense phase CO_2, pulse electric fields (PEF), oscillating magnetic fields, pulsed high intensity light and ultraviolet irradiation (UV) (Elez-Martinez and others 2006; Boff and others 2003; Sampedro and others 2009; Donahue et al. 2004; Koutchma 2008). Many reports have been published in the last decade studying the efficacy of non-thermal techniques to extend shelf life and enhance the safety of fresh juice while minimizing changes to organoleptic and nutritional qualities of the juice (Grahl and Markl 1996; Tandon et al. 2003).

Regulatory approval is important for the commercialization of non-thermal technologies. Some of the FDA approved technologies for juice processing are PEF, UV and HPP. However, it has to be kept in mind that no single technology can be practically applied to all products and, within technologies, pathogen reduction varies among products treated.

Pulsed electric field

PEF processing involves treating liquid foods with high voltage pulses in the range of 20- 80 kV for microseconds. A high electric field is generated between two electrodes (treatment chamber), which results in a large electrical flux flow through the product. A schematic diagram of a typical bench top PEF unit is given in Figure 2-2 (Jia and others 1999). The apparatus consists of a high voltage power supply, a pulse generator, treatment chambers and a water bath for temperature control. PEF process achieves both microbial and enzymatic inactivation while minimizing heating of foods and associated detrimental changes to sensory and physical properties of foods. Though there are several theories on microbial inactivation, the most accepted is by electroporation. The high voltage pulses break cell membranes of vegetative microorganisms, thus altering the membrane permeability. This results in protein inactivation and leakage of cellular contents and eventually disruption of microbial cells (Sale and Hamilton 1967). The factors that affect the efficiency of microbial inactivation by PEF are product composition, microbial characteristics and treatment parameters.

Product Parameters. Only pumpable food products can be treated by PEF. The critical parameters pertaining to food products are electrical conductivity, density, viscosity, pH and water activity (Cserhalmi et al. 2006). The food products should have low electrical conductivity, as conductivity increases the difference in conductivity

between medium and microbial cytoplasm resulting in a weakening of membrane structure (VegaMercado and others 1996). An enhanced efficiency in microbial inactivation in acidic environment was also reported by the same authors.

Microbial Characteristics. Generally, gram positive bacteria are more resistant to PEF than gram negative bacteria (Hulsheger and others 1983). Yeasts and mold show higher sensitivity to PEF than bacteria due most likely to their larger cell size (Qin and others 1996). Wouters and others (2001) showed that *Lactobacillus* species in different sizes or shapes had different membrane permeability to PEF. In addition to the type of microorganism, the growth stage also changes the sensitivity to PEF. Bacteria and yeasts at their logarithmic stage are more sensitive than those at the stationary or lag growth stage (Pothakamury and others 1996). PEF processing is more efficient at vegetative cell inactivation compared to spores. Raso and others (1998) compared the effect of PEF treatment (32 -36.5 kV/cm) on vegetative cells and *Zygosaccharomyces baili* ascospores. PEF treatment decreased the vegetative cell population by 4.5-5 log cycles and ascoproes by 3.5-4 log cycles.

Treatment Parameters. The critical process factors for PEF treatment are electric field intensity, treatment time, pulse wave shape, pulse length, number of pulses and temperature. Microbial inactivation efficiency increases with an increase in electric field strength above the critical electric field (Qin and others 1998). The critical electric field is the intensity below which no microbial inactivation occurs. Hulseger and others (1983) found a linear relationship between electric field strength and *E. coli* inactivation.

Treatment time is another important parameter that affects the efficiency of PEF treatment. It is the product of number of pulses and pulse duration. Therefore, an

increase in any of the above two parameters results in increased microbial inactivation. However, longer pulse width also increases food temperature. Peleg and others (1995) showed that the rate of microbial inactivation by PEF at a constant electric field strength increased with an increase in treatment time.

The electric field pulse can be applied in the form of exponential decaying, square wave and oscillatory pulses. Zhang and others (1994) compared the effect of square wave, exponentially decaying and charge reverse pulses on the shelf life of orange juice. Square wave pulses yielded juice with longest shelf life. PEF treatment of juice can lead to the formation of a shielding layer on electrodes in treatment chambers due to migration of charged molecules to surface of electrode. This layer reduces the efficiency of PEF treatment. Bipolar pulses prevent the formation of shielding layer, as the polarity reversal causes a corresponding change in direction of charged molecules (Evrendilek and Zhang 2005). Therefore, bipolar pulses are used with square wave or exponential pulses to increase efficiency of PEF processing.

Moderate temperatures (40-60 ºC) exhibit synergistic effects with PEF on the inactivation of organism. At moderate temperatures the membrane fluidity of cell is altered, increasing sensitivity to PEF treatment. Heinz and others (Heinz and others 2003) found that *E. coli* inactivation increased from 1 to 6.5 log cycles with a temperature increase from 32 ºC to 55 ºC in inoculated apple juice. However, since application of electric field strength also increases the temperature of product, a proper post treatment cooling is necessary.

Microbial stability studies in PEF treated juices. Microbial stability in PEF treated juices have been studied in various fruit juices like apple juice, apple cider,

orange juice, cranberry juice, tomato juice, and mango juice, as well as in orange milk beverages (Ayhan and others 2001; Charles-Rodriguez and others 2007; Jin and Zhang 1999; Yeom and others 2004; Min and Zhang 2003). There are no reports to date on microbial stability in PEF treated grapefruit juice.

The commercial-scale processing of orange juice by PEF treatment at 40 kV/cm for 97 μs reduced the total aerobic count and yeast and mold count by 6 log cycles (Min and others 2003a). Commercially processed PEF juice had a shelf life of 196 days at 4 ºC. Aseptically packaged PEF treated orange juice (29.5 kV/cm, 60 μs) had a shelf life of 7 months at 4 ºC (Qiu and others 1998).

In apple juice, PEF treatment (34 kV/cm, 166 μs) using a bench scale system resulted in a 4.5 log reduction in inoculated *E. coli* O157:H7 levels (Evrendilek and others 2000b). The same juice treated using a pilot scale unit at 35 kV/cm for 94 μs and combination of heat treatment at 60 ºC for 30 s gave a shelf life of more than 67 days at both 4 ºC and 22 ºC.

Lately, the synergistic effect of PEF with antimicrobials to achieve improved microbial safety has been a subject of interest. Liang and others (2002) reported a 5.9 log reduction in inoculated *Salmonella* levels in fresh orange juice at 90 kV/cm, 50 pulses and temperature at 55 ºC. Addition of antimicrobials nisin and lysozyme increased the inactivation by 1.37 logs. Interestingly, they also reported that PEF inactivation was significantly more extensive in pasteurized juices than fresh juice, probably due to differences in their composition. This is an important parameter to look into when comparing the effect of varying PEF parameters on microbial inactivation in juices. Similar studies of apple juice also showed an increase in microbial inactivation

by 1-2 log cycles using electric field strength in range 27-33 kV/cm in combination with nisin and lysozyme (Liang and others 2006).

Though numerous studies on microbial stability and shelf life of products are present in the literature, there are fewer studies on the effect of PEF technology on different microorganisms. McDonald and others (2000) achieved more than 5 log reductions of *Leuconostoc mesenteroides*, *E. coli* and *Listeria innocua* in orange juice at 30 kV/cm and 50 kV/cm electric field strength using varying pulses. Inactivation of spores was more difficult, as a maximum of 2.5 log reductions of *S. cerevisiae ascospores* was achievable at 50 kV/cm, 50 ºC.

Garcia and others (2005) found that *E. coli* populations exhibited greater sensitivity to subsequent holding of apple juice at refrigerated conditions than immediately after PEF treatment (40kV/cm, 80 pulses). An increase in inactivation from 0.5 log to 5 log was noted after 3 days of storage under refrigeration. This phenomenon was attributed to increased sensitivity of injured cells to the acidic conditions of apple juice.

In summary, due to the numerous critical process factors and different experimental conditions used, definite conclusions about the critical process parameter effect on specific pathogen reductions are difficult to establish. Research that provides conclusive data on the effect of critical PEF factors on microbial inactivation is required for commercialization of the PEF process.

Effect of PEF processing on juice quality. The effect of PEF treatment on aroma, nutrition, color, sensory and other physiochemical parameters of orange, apple, cranberry and tomato juices are present in literature (Jin and Zhang 1999; Biasioli and others 2003; Aguillar-Rosas et al. 2007; Ayhan and others 2002).

In apple juice, PEF extended the shelf life of fresh juice for up to 56 days with no change in sensory or physiochemical properties compared to freshly squeezed juice (Qin and others 1995). Evrendilek and others (2000b) found no significant change in sensory acceptance of PEF treated (35kV/cm for 94 μs) apple juice compared to fresh juice by paired preference test. Aguillar-Rosas and others (2007) reported a significantly higher loss in eight select volatiles (acetic acid, hexanal, butyl hexanoate, ethyl acetate, ethyl butyrate, methyl butyrate, hexyl acetate, and 1-hexanal) in HTST treated apple juice (90 ºC, 30 s) compared to PEF treated juice (35kV/cm, 4μs bipolar pulse).

The effect of PEF treatment on orange juice has been more extensively studied compared to other beverages. Jia and others (1999) noted that the loss of volatile compounds in PEF (30kV/cm for 240 μs or 480 μs) and heat processed (90 ^{0}C for 1min) orange juice were greatly influenced by compound type and processing methods. Loss in volatiles α-pinene, limonene and ethyl butyrate were common in both treatments. However, decanal and octanal were lost only in heat treated samples. The flavor loss by PEF process was mainly due to the vacuum degassing system rather than PEF application.

Yeom and others (2000b) investigated the effects of PEF treatment on microorganisms and pectin methyl esterase activity. They compared vitamin C, volatile and other attributes of PEF treated juice (35kV/cm, 59μs) to heat pasteurized (94.6^{0}C for 30s) orange juice. PEF processing prevented growth of microorganisms at 4, 22 and 37^{0}C for 112 days and inactivated 88% of PME activity. PEF treated juice also retained greater amounts of vitamin C and aroma compounds (pinene, myrcene, octanal, limonene and decanal) compared to heat-treated samples.

In contrast, Ayhan and others (2002) reported a significant increase in hydrocarbons d-limonene, alpha-pinene, myrcene and valencene for PEF treated single strength orange juice (35kV/cm for 50 µs) compared to untreated juice. They proposed that the hydrophobic flavor compounds present inside pulp of orange juice become more available during PEF application.

Because most of the previous PEF studies were performed in laboratories or pilot plant scales, Min and others (2003a) studied the effect of commercial scale PEF processing (40kV/cm for 97 µs) on microbial stability, ascorbic acid content, flavor compounds and other sensory attributes compared to thermally processed (90 ºC for 90 s) and freshly squeezed orange juice. PEF and thermally processed juice had similar microbial shelf lives at 4 ºC for 196 days. PEF processed juice retained more ascorbic acid, flavor and color compared to thermally treated juice. Preference sensory analysis indicated significantly higher sensory attributes of texture, flavor and overall acceptability for PEF treated juice compared to thermally processed juice.

Only one reference in the literature on PEF processed grapefruit juice could be found (Cserhalmi et al. 2006). The authors investigated the effect of PEF technology on physical properties, color, non-enzymatic browning and flavor components. They reported no significant changes in any of the quality parameters post processing compared to fresh, untreated juice (Cserhalmi et al. 2006). The aroma analysis in the study was limited to eight select volatiles (terpinen-4-ol, ethyl octanoate, decanal, carvone, geraniol, geranyl acetate, geranyl acetone, and nootkatone) in grapefruit juice.

Inactivation of enzymes by PEF. Enzyme conformational change is suggested as the possible mechanism of enzyme inactivation by PEF (Castro AJ 2001). The

authors found that alkaline phospatase molecules tend to associate and aggregate due to polarization created by electric charges.

Yeom and others (2002) reported an 83.2 % inactivation of PME in fresh orange juice at 35 kV/cm for 180 ms at 30 ºC. However, a 90% PME inactivation was achieved at 25 kV/cm when the temperature was increased to 50 ºC, indicating a synergistic effect of heat and PEF treatment on PME inactivation. In apple juice, PEF reduced the PPO activity by 50-70% at treatment condition 38.5kV/cm, 300 pps and 50 ºC (Sanchez-Vega and others 2009). Min and others (2003b) reported 88.1% inactivation in lipoxygenase in tomato juice treated by PEF at 30 kV/cm for 50 μs at 50 ºC.

Radio frequency electric field

Radio frequency electric fields (RFEF) processing is a relatively new pasteurization technique and is not FDA approved yet. The key equipment components of the process include a radio frequency power supply and a treatment chamber that is capable of applying high electric fields to liquid foods (Figure 2-3) (Geveke and others 2007). The process is similar to the pulsed electric fields process, except that the power supply is continuous using an AC generator rather than pulses, thus reducing the capital cost.

Ukuku and others (2008) worked on *E. coli* inoculated apple juice to understand the mechanism of RFEF on microbial inactivation. They demonstrated a 4 log reduction in inoculated apple juice at 15kV/ cm entirely due to the non-thermal effect of RFEF and not due to temperature increase during treatment. Membrane damage in bacterial cell wall leading to leakage of intracellular material was cited as a mechanism of microbial inactivation.

The process parameters affecting RFEF efficiency are electric field strength, frequency, treatment time, output temperature and number of treatment cycles. Radio frequencies of 15 and 20 kHz were more effective in inactivation of *E. coli* than 30-70 kHz (Geveke and Brunkhorst 2004). Increasing electric field strength (20 – 30 kV/cm), treatment time (140 – 420 μs) and outlet temperature (50 – 60 ºC) increased inactivation of *E. coli* in apple cider (Geveke and others 2008). An increase in treatment cycles also increased inactivation of inoculated *E. coli* K12 at conditions 18kV/cm, 170 μs and 50 ºC outlet temperature. No studies on the effect of RFEF on physiochemical properties, flavor or color of apple juice are reported in the literature.

In orange juice, an electric field strength of 20 kV/cm and outlet temperature of 65 ºC gave a 3.9 log reduction in *E. coli K12* population with no significant non-enzymatic browning and loss in ascorbic acid content (Geveke et al. 2007). Because RFEF is a relatively new pasteurization technique, not much information is available on its effect of the quality of juices.

Ultraviolet radiation

UV light in the wavelength 220 -300nm exhibits germicidal properties and is used for inactivation of bacteria and viruses. UV light absorbed by the DNA of microorganism causes cross-linking between neighboring pyrimidine nucleoside bases (thymidine and cytosine) in the same DNA strand and prevents cell replication. UV light has low penetration in juices and other beverages due to the presence of color compounds, organic solutes and suspended particles. Therefore, UV irradiation is limited to microbial inactivation of food surfaces, packaging material or processing plants. In recent years, due to many developments in design of treatment chambers by utilization of turbulent flow to form continuous renewed surface, microbial inactivation in juices and other

beverages is enabled. In 2000, the FDA approved UV irradiation as an alternative to thermal treatment for fresh juices. UV light is now commercially used for water disinfection and apple cider pasteurization. A picture of UV processing unit used by Geveke (2008) for pasteurization of liquid egg whites is given in Figure 2-4. The unit consists of a bi-pin base, a 30 W germicidal UV bulb, UV transparent tubing and silicon rubber tape. The lamp generated 90% of its energy at 254nm. The product flows through tubing, and the time of exposure to UV light is calculated based on the flow rate. The effectiveness of microbial inactivation by UV process depends on the UV light source, the product composition and microbial characteristics.

UV light sources. The different light sources used for UV are mercury lamps, excimer lamps, microwave lamps and broadband pulsed lamps (Koutchma 2009). The correct UV source enhances microbial inactivation by increasing UV penetration in liquid, as well as by employing higher UV intensity from pulsed sources. Mercury lamps are generally used due to their low cost and effective microbial inactivation. Based on the vapor pressure of mercury when lamps are operating, they are categorized as: low pressure lamps, low pressure high-output lamps and medium pressure lamps. Low pressure mercury lamps are generally used in food processing and are approved by the FDA. In pulsed lamps, alternating current is stored in a capacitor and energy is discharged in the form of intense emission of light within microseconds. This technology has been applied to food surface treatment in fresh produce and meats, but it has not yet been established in the field of liquid foods.

Product composition. The physical, chemical and optical properties of juices impact the effectiveness of microbial inactivation by UV. The variation in UV absorption

between different juices is due to the difference in their pH, viscosity, soluble solid and suspended solid content. Clear juices are better candidates for UV treatment compared to opaque juices, as the presence of dissolved solutes in juice causes strong UV attenuation effects (Koutchma and others 2007). Carrot and pineapple juices (opaque juices) had higher UV absorptivity than semi-transparent watermelon and apple juice. Ascorbic acid present in juices also has strong absorbance between 220 -300 nm even at very low concentrations. Tran and others (2004a) found a 17% loss in vitamin C content in UV treated orange juice. It is important to study the ascorbic acid content and its destruction during UV treatment in terms of UV dose delivery. Suspended solids present in juice not only attenuate UV dose but also provide a site for aggregation of bacteria to particle surfaces. Suspended solids also cause scattering in light such that microbes far away from the UV light are not effectively inactivated (Murakami and others 2006).

Microbial characteristics. The information on the effect of UV on different pathogens is very limited. Factors like microbial species, strain, growth media, and initial microbial load, as well as stage of growth, affect the efficiency of microbial inactivation by UV. Generally, gram positive bacteria are more resistant to UV than gram negative bacteria due to difference in cell wall thickness and composition. Bacterial spores, like *Bacillus subtilis,* are very resistant to UV requiring 36mJ/cm ^{2} for 1-log reduction in water (Chang and others 1985). Mold spores are considered to be more UV resistant than yeast and lactic bacteria. They are the major spoilage microorganisms in juices.

Microbial inactivation studies in UV treated juices. UV light inactivates microorganisms by causing cross-linking between neighboring pyrimidine nucleoside in

the same DNA strand, thereby preventing cell replication. The extent of cross-linking is directly proportional to the UV light exposure. However, photo reactivation can occur in UV injured cells when exposed to visible light in blue spectra (Hoyer 1988). Therefore, it is important to store UV treated juice under refrigeration and in dark conditions.

UV dose is calculated as a product of exposure time and intensity. It is assumed that change in either time or intensity should not affect microbial inactivation as long the UV dose used is same. However, recent studies have shown that some *E. coli* strains do not follow time-intensity reciprocity (Sommer and others 1998). Time –intensity reciprocity is crucial for the scale up from pilot to commercial scale. Scale up is possible only if time-intensity reciprocity exists. Murakami and others (2006) showed that in apple juice inoculated with *E. coli* K12, the reciprocity occurs within a range of intensities only and the range decreases as UV dose level increases.

UV processing of apple juice and apple cider has previously been conducted to study the inactivation kinetics of microorganisms like *E. coli* O157:H7 and *Listeria innocua* (Duffy and others 2000; Geveke 2005). Duffy and others (2000) showed a 5 log reduction in *E. coli* in apple cider treated by UV irradiation using a CiderSure pasteurizer (OESCO, Inc., Conway, MA). This unit has 8 UV lamps, and the UV light is set to penetrate a 0.03” layer of cider. Geveke (2005) exposed apple cider inoculated with *E. coli* and *Listeria innocua* to a UV lamp for varying lengths of time. They achieved a 3.4 log reduction in *E. coli* population at 19 s of UV exposure at 25 ºC. *L. innocua* required a longer exposure time of 58 s for 2.5 log reduction. In a different study by the same authors on liquid egg white (Geveke 2008), the effect of exposure time, temperature and pH on E. coli inactivation was investigated. They found an increase in inactivation

with an increase in exposure time (0 – 160 s) and that an exposure time of 160 s at 50 ºC reduced the microbial population by 4.3 log. They mentioned a direct dependency between inactivation and temperature but an indirect relationship between pH and inactivation. UV inactivation was greater at neutral pH than higher pH values.

In grapefruit juice inoculated with *Saccharomyces cerevisiae*, a UV dose of 450 kJ/m2 at flow rate of 1.02L/min using a two coupled UV disinfection system resulted in a maximum of 2.4 log reduction (Guerrero-Beltran and others 2009). Keyser and others (2008) treated apple juice, guava-pineapple juice, mango nectar, strawberry nectar, two different orange juices and tropical juice using a PureUV pilot scale system. Reduction in inoculated *E. coli* levels in apple juice from 7.92 log cfu/mL to 7.42 lof cfu/mL at a UV dose of 1377J/L was observed. However, in the remaining juices no inoculation studies were done by the authors. It was not possible to interpret the effective UV dose required to achieve pasteurization in juice as the initial microbial load varied between the juices. The authors suggested that UV treatment optimization is required for each new juice product.

Effect of UV treatment on juice quality. Donahue and others (2004) showed a 5 log reduction in *E. coli* when exposed to UV light for 8.12 s and energy of 35.1mJ/cm2. UV-treated cider had a shelf life 7 days longer than untreated apple cider with no significant sensory difference. Choi and others (2005) found that thermal pasteurized apple cider had better microbial quality compared to UV treated cider after 21 days of storage. However, UV treated cider preserved better color. Consumer acceptability studies showed no significant difference between UV and untreated cider, whereas thermally treated cider had the lowest acceptability scores.

Tandon and others (2003) compared UV treated apple cider at dose 14 mJ/cm^2 to hot fill pasteurized cider at 63 ºC. UV treated cider had a shelf life of 2 weeks compared to 4 weeks for hot fill cider. UV treated cider was less acceptable than hot fill cider by sensory preference tests due to fermentation after 2 weeks of storage.

Studies on UV treated orange juice are limited due to the low transmittance of UV light through the high pulp juice (Tran and Farid 2004b). UV exposure of 73.8m J/cm^2 extended the shelf life of orange juice by 5 days with a 17% loss of ascorbic acid. Vitamin C is a light-sensitive component and is degraded by UV light. No PME inactivation was noted by the authors in UV processed juice.

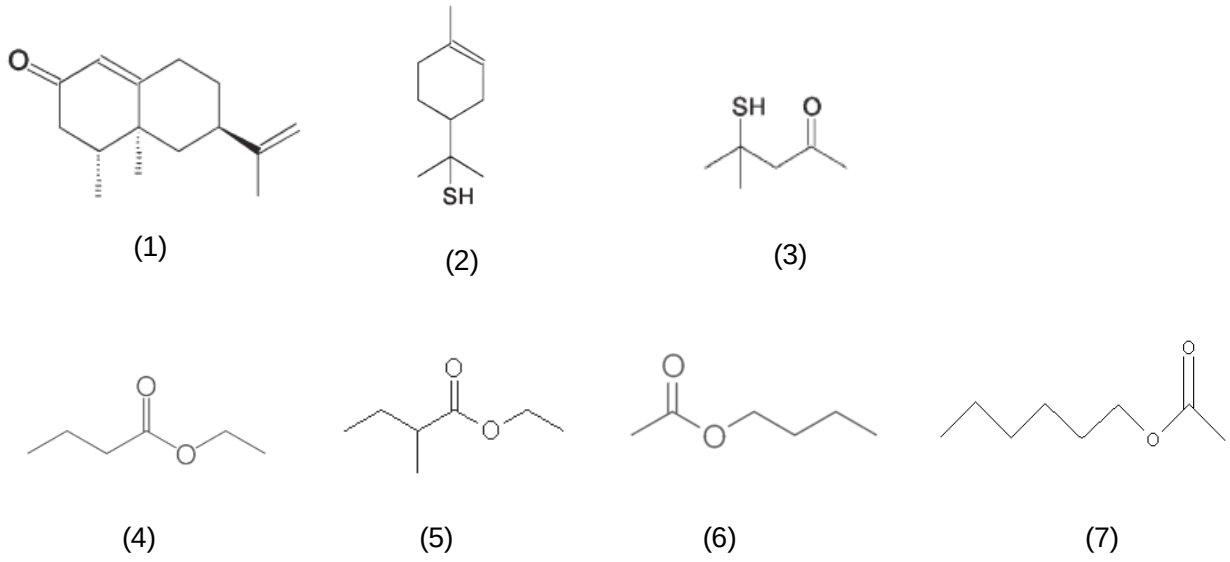

Figure 2-1. Key odorants of grapefruit and apple juice

Table 2-1. Time – temperature parameters for PE inactivation in citrus juices

	Orange, mandarin and tangerine juice	Grapefruit juice	Lemon juice
Temperature	90-98 ºC	85-90 ºC	75-85 ºC
Time	60 s	30-40 s	30 s

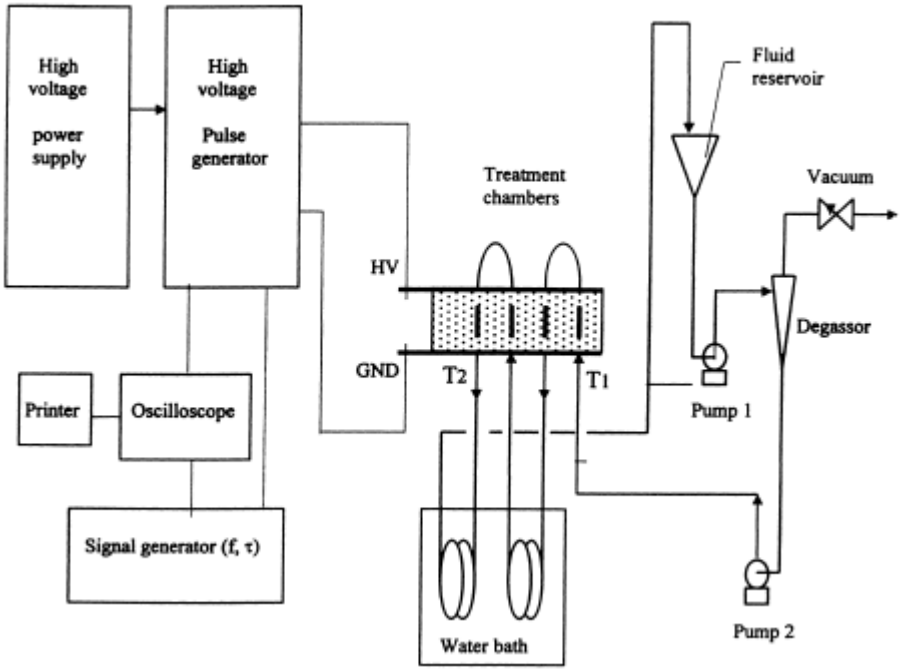

Figure 2-2. Schematic diagram of pulsed electric field processing unit (Jia et al. 1999)

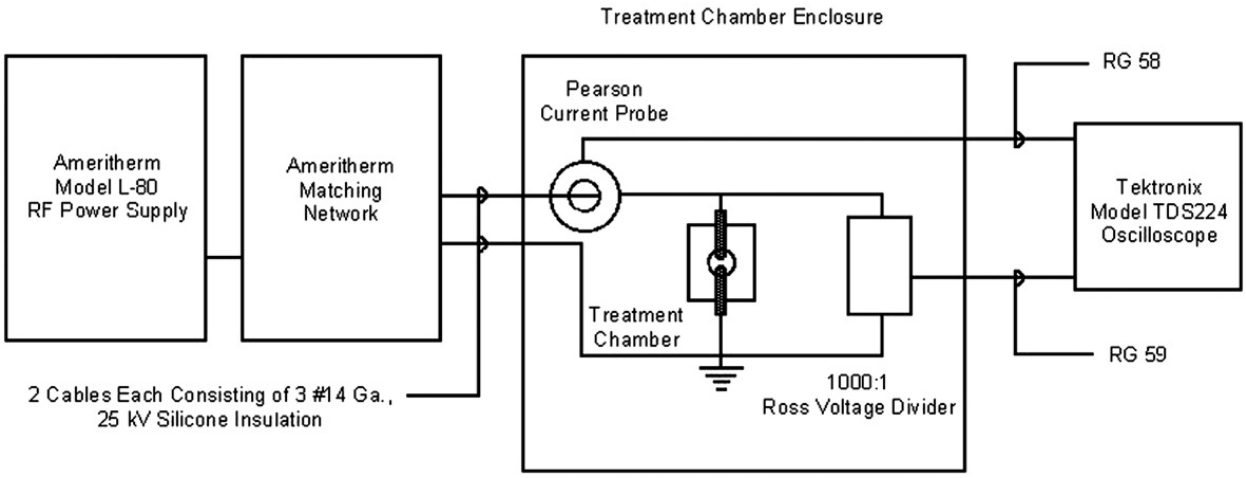

Figure 2-3. Schematic diagram of radio frequency electric field processing unit (Geveke et al. 2007)

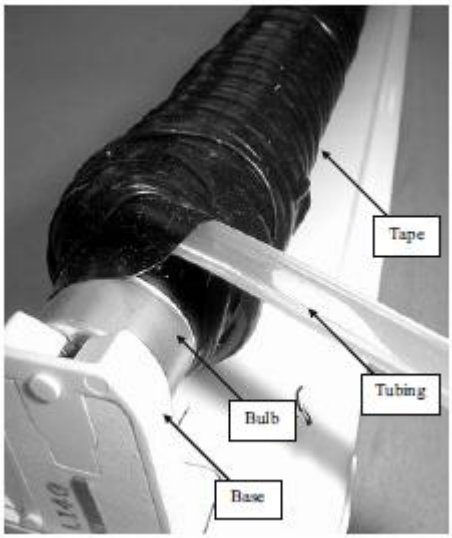

Figure 2-4. Picture of Ultraviolet processing unit (Geveke 2008)

CHAPTER 3
COMPARISON OF THERMAL AND NON-THERMAL TECHNIQUES ON APPLE CIDER STORAGE QUALITY UNDER EQUIVALENT PROCESS CONDITIONS

Introduction

Non-thermal processing techniques like pulse electric field (PEF) and ultraviolet (UV) treatment have been explored for their efficacy to extend shelf life and enhance the safety of fresh juice while preserving organoleptic and nutritional qualities (Evrendilek and others 2001; Min and Zhang 2003). Studies on the inactivation of microorganisms by PEF processing of orange, apple and tomato juices have shown better preservation of flavor, color and nutrients in comparison to heat pasteurized juices (Min et al. 2003b; Evrendilek and others 2000a; Ayhan et al. 2001). UV processing of apple juice and apple cider has previously been conducted to study the inactivation kinetics of microorganisms like *E. coli* O157:H7 and *S. typhimurium* (Duffy et al. 2000; Uljas and others 2001). Even though there are many non-thermal processing techniques available for the pasteurization of juices, a lot more research is required to make them commercially successful. Particularly, studies about the effects of non-thermal techniques on quality and consumer acceptability in comparison to thermal pasteurization are required. Most often, the conditions used for thermal processing are severe in terms of temperature and time of exposure to heat. Thus, shelf-life studies comparing non-thermal to thermal techniques on acceptability of juice using conditions that do not achieve the same reduction of a target microorganism would result in incorrect conclusions (Rivas et al. 2006; Aguillar-Rosas et al. 2007; Yeom et al. 2000b). For proper comparison of thermal and non-thermal treatment effects on product quality, both processes must achieve similar levels of microbial inactivation. The current work focuses on comparing the effect of thermal and non-

thermal processing techniques on microbial population and overall quality of apple cider to that of untreated cider. Each treatment is optimized to achieve approximately 6-log reduction of added *E. coli* K12, a surrogate of *E. coli* O157:H7.

Materials and Methods

Determination of Processing Conditions That Achieve 6-log Reduction of *E. coli* K12

Pasteurized apple cider (Ziegler Juice Co., Lansdale, PA) inoculated with *E. coli* K12 (ATCC 23716) was used to standardize processing parameters to achieve a 6 log reduction. *E. coli* K12 obtained from the American Type Culture Collection (ATCC) (Manassas, VA) was maintained on Tryptic Soy Agar (Remel, Lenexa, KS, USA) at 4 ºC. It was cultured in Tryptic Soy Broth (Remel) with shaking at 37 ºC for 16-18 h. Apple cider inoculated from the stationary phase culture gave an approximately 7 log CFU/mL population.

Ultraviolet process

Pasteurization was performed using a simple apparatus consisting of four low-pressure mercury lamps, each surrounded by a coil of UV transparent tubing. The UV apparatus was scaled up from a smaller-sized apparatus developed by Geveke (2005). The UV lamp assemblies contained a bi-pin base (model S130 120 LPF, Lithonia Lighting, Conyers, GA) and a 30-W bulb (G30T8, Buylighting.com, Burnsville, MN) that generated 90% of its energy at a wavelength of 254 nm. Norton Chemfluor 367 tubing (Cole-Parmer, Vernon Hills, IL) with an ID of 3.2 mm and a wall thickness of 1.6 mm was wrapped around the entire length of each UV lamp. The four UV lamps were connected in a series with 14m of tubing wrapped around them. The experimental system included a feed tank, a peristaltic pump and four UV lamps of the same

dimensions connected in a series. Cider was pumped through the tubing at a flow rate of 25 L/hr, which translated to an exposure time of 17 s per bulb. Microbial assays were conducted using 1, 2, 3 and 4 UV lamps corresponding to UV treatment times of 17, 34, 51 and 68 s.

Pulsed electric field process

A bench scale continuous PEF system (OSU-4F, Ohio State University, Columbus, OH, USA) was used to treat the inoculated apple cider. The system consisted of 6 co-field treatment chambers with a diameter of 0.23 cm and a gap distance of 0.29 cm between electrodes connected in a series. Applied voltage and current were monitored by a digital real time oscilloscope (Tektronix DS210, Beaverton, OR). The inlet and outlet cider temperatures were continuously monitored via thermocouples. Cider was pumped through the system using a digital gear pump (Cole Parmer 75211-30, Vernon Hills, IL) at a flow rate of 7.2 L/hr. The electric field strengths tested were 5, 10, 13, 17, 21 and 23 kV/cm at a square wave pulse duration of 2.5 µs. Apple cider sequentially flowed through all the treatment chambers via steel coils immersed in a water bath set at 48 ºC. The mean total treatment time (t) was calculated as 150 µs using the following equation 1:

$$t = n * T * f * d / v \qquad (3\text{-}1)$$

Where n is the number of treatment chambers (n = 6), T is pulse width (T = 2.5 µs), f is the repetition frequency (f = 1670 Hz), d is the distance between two electrodes (d = 0.29 cm), v is the velocity of flow inside treatment chamber (v = cm/s), which is determined by flow rate and the diameter of the treatment chamber (0.23 cm).

Thermal process

Inoculated apple cider was heat pasteurized using a miniature-scale Armfield HTST processing system (model FT74-30-MkIII-33-34, Jackson, NJ, USA). The system included a feed tank, a peristaltic pump, a plate heat exchanger (comprising a regeneration section, a heating section and a cooling section that mimics industrial scale systems), a holding tube, thermocouples, and an electric-powered hot water boiler and pump. A flow rate of 15 L/hr was maintained through the system, which translated to a hold time of 1.3 s. Microbial inactivation was studied at holding tube outlet temperatures of 60, 63, 66, 69, 72, 74 and 76 ºC.

Shelf-Life of Processed Apple Cider

Unpasteurized apple cider procured from Ziegler Juice Company (Lansdale, Pennsylvania) was frozen at -17 ºC in gallon plastic containers. Samples were thawed at 4 ºC overnight prior to use.

Processing and packaging

All processing equipment was sanitized by pumping 5% bleach solution through the system, followed by a distilled water rinse. Heat pasteurization was performed at 76 ºC for 1.3 s. For UV treatment, apple cider was exposed for 51 s at an outlet temperature of 15 ºC. PEF processing was conducted at an electric field strength of 23 kV/cm, 2.5 µs pulse duration, a total treatment time of 150 µs and a treatment temperature of 48 ºC. The treatment conditions were selected based on 6 log reduction of inoculated *E. coli* K12 as determined in the above experiments. As a control sample, apple cider was passed through the HTST processing system without any heat at room temperature.

Cider was packaged in clean 1 L media bottles inside a sanitary laminar hood after processing. The laminar hood contained a UV lamp and HEPA air filter. The hood was sanitized by a UV lamp at 254 nm for 30 min before use and then wiped with alcohol.

Storage

Bottled samples stored at 4 ºC for four weeks were periodically analyzed for quality, including microbiology, pH, Brix and color attributes. Microbial analyses were performed every week, while all other analyses were done at 0, 2 and 4 weeks. Control apple cider consisted of untreated cider stored at 4 ºC, while fresh apple cider was untreated cider stored at -17 ºC.

Microbial tests

Microbial inactivation and growth were examined by preparing appropriate dilutions of samples with Butterfield's Phosphate Buffer (Hardy Diagnostics, Santa Maria, CA). Duplicate samples (1 mL) were then pour plated with Tryptic Soy Agar (Remel, Lenexa, KS, USA) and incubated at 37 ºC for 24 h. Plates with 30-300 colonies were enumerated using a Bantex model 920 manual colony counter (Burlingame, CA, USA). The *E. coli* K12 population was expressed as CFU/mL of apple cider. Data for each replicate were normalized against the control and plotted as the log reduction versus temperature, time or electric field strength.

For the shelf-life study, total aerobic plate count (TPC) and yeast and mold count (YMC) were determined using plate count agar (PCA) and yeast and mold (YM) pertrifilms. The PCA plates were incubated at 37 ºC for 24 hrs while YM plates were incubated at room temperature for 5 days before counting. All samples were analyzed in duplicate, and two replicates of each dilution were prepared and plated.

pH

The pH meter used for all analysis was an Orion 420A+ (Thermo Electron Corp., Beverly, MA). The pH meter was calibrated at pH 4.0 and pH 7.0 using standard solutions on each day of analysis. The pH measurements for all samples were performed in triplicate.

Brix

A Bausch & Lomb Abbe refractometer (B&L Corp., Rochester, NY) was used. To measure °Brix of juice samples, a drop of the sample was placed on the refractometer and the corresponding refractive index was recorded, which gave the measure of soluble solids in juice. All measurements of ºBrix for each cider sample were performed in triplicate.

Color

Color was measured in CIE L* a* b* 3-dimesional color space using a ColorQuest XE spectrophotometer (Hunter Associates Lab, Reston, VA) where L designated lightness and measured the relative lightness and darkness of juice with L = 0 corresponding to black and L = 100 corresponding to white. The “a” value gave the measure of green to red with positive values indicating more red and negative values indicating more green. The “b” value measured blue to yellow with positive value signaling more toward yellow and negative value signaling more toward blue. A dual beam xenon lamp was used as light source. The lamp was allowed to warm up for 30 min prior to measurement. The system was calibrated using black and white standard tiles on each day of analysis. Forty mL of cider sample in a 20 mm path length glass cell were used for measuring color. All measurements of color for each sample were performed in triplicate. The effect of processing methods on the color of apple cider was

represented using total color difference (dE) calculated for all the samples using the following equation (3-2) (Lee and Coates 1999):

$$dE = \sqrt{(L - Lo)^2 + (a - ao)^2 + (b - bo)^2} \quad (3\text{-}2)$$

where L = lightness of treated sample at time t; L0 = lightness of untreated sample at day 0; a = redness of treated sample at time t; a0 = redness of untreated sample at day 0; b = yellowness of treated sample at time t; and b0 = yellowness of untreated sample at day 0.

Statistical Analysis

All data were subjected to statistical analysis using SAS 9.1 (SAS Inst. Inc., Raleigh, N.C., USA). Statistical significance of the differences among the treatments was tested using the analysis of variance (ANOVA) at the significance level of α = 0.05. The effect of storage and processing techniques was compared using the Duncan's multiple comparison test (α = 0.05). The interactive effect of storage and treatments was also determined statistically.

Results and Discussion

Optimization of Equivalent Processing Conditions

Pasteurized apple cider was inoculated with *E. coli* K12 to give an approximately 7 log CFU/ml population. The initial microbial count in pasteurized apple cider was below 20 CFU/mL, indicating very low microbial load before inoculation. Inactivation of inoculated *E. coli* K12 in apple cider increased with increasing exposure time, temperature and electric field strength for UV, PEF and thermal process, respectively (Figures 3-1, 3-2, 3-3).

The population of *E. coli* K12 decreased with increasing UV treatment time in apple cider (Figure 3-1). UV exposure for 34 s reduced the population by 3.7 logs, and lengthening the exposure time to 51 s reduced the population by 6.3 logs. Using the same UV apparatus, Geveke (2005) was able to achieve 4.7 log reduction of the same bacterium in apple cider for a treatment time of 30 s and temperature at 25 ºC. The inlet and outlet temperatures recorded in this study were below 15 ºC during processing since refrigerated apple cider was inoculated and passed through the UV processing unit. Thus, no detrimental effect of heat on the inactivation of *E. coli* K12 could be expected in the UV processed cider. The energy applied was calculated to be 13 J/mL for 51 s of exposure time based on the flow rate and UV lamp wattage. UV pasteurization energy is less compared to conventional thermal pasteurization energy of 34 J/mL (Kozempel and others 1998).

The effect of PEF processing on *E. coli* K12 inactivation at electric field strength from 5 to 27 kV/cm with a pulsed duration of 2.5 µs and a mean total treatment time of 150 µs is shown in Figure 3-2. Many authors have reported significant synergistic effect against microorganisms when PEF is used in conjunction with moderate heat (Heinz et al. 2003). PEF processing was performed with minimal heat of 48 ºC. The outlet temperatures recorded were between 49-51 ºC during the length of treatment. The increase in temperature is a consequence of the electric energy supplied to the medium during PEF treatment. The incubation of inoculated cider at 48 ºC in the absence of PEF treatment did not achieve any reduction in microbial population. Inactivation of the *E. coli* K12 population increased with an increase in electric field strength from 13 to 23 kV/cm. An electric field strength of 23 kV/cm reduced the population in apple cider by

6.1 log cycles. Evrendilek and others (2000b) achieved a 4.5 log reduction in *E. coli* O157:H7 population in apple juice using a similar bench scale PEF system with treatment conditions of 34 kV/cm electric field strength, 4 µs bipolar pulse duration, a total treatment time of 166 µs and temperature below 38 ºC The increased reduction in bacterial population at a lower electric field strength in the current study is evidence of the synergistic effect of PEF with heat on microbial inactivation.

Thermal processing of inoculated cider performed at a flow rate of 15 L/hr gave a hold time of 1.3 s. The inlet and outlet product temperatures were recorded continuously and did not increase beyond 15 ºC due to built-in cooling section in the equipment. A significant decrease in *E. coli* K12 population was noted with increase in temperature beyond 63 ºC ($P < 0.05$). Heat treatment at 76 ºC for 1.3 s is recommended by the FDA for pasteurization of apple juice of pH 4.0 or less (CFSAN 2004). Treatment of apple cider by heat at the same conditions resulted in 6.0 log reduction of inoculated *E. coli* K12 population in this study (Figure 3-3).

Effect of Thermal and Non-Thermal Processing on Microbial Stability During Storage of Apple Cider

Fresh unpasteurized apple cider was treated by heat, PEF and UV at conditions achieving approximately 6 log reduction in *E. coli* K12 population. The microbial stability of treated apple cider was studied for a storage period of 4 weeks at 4 ºC. The TPC and YMC of apple cider stored at 4 ºC for 4 weeks in both treated and untreated cider are given in Figures 3-4 & 3-5, respectively.

The initial TPC for untreated control apple cider was 2.4 log CFU/mL and reached 3.1 log CFU/mL at the end of the storage period. The TPC for thermal, UV and PEF treated cider was reduced to 1.8, 1.6 and 1.8 log CFU/mL, respectively, at week 0.

Thermal and UV processed cider did not show any significant increase ($P < 0.05$) in log values during storage. However, TPC of PEF-processed cider increased to 2.4 log CFU/mL by the end of the 4 week storage period. The increase in microbial population during storage is possibly due to a lower inactivation of spores by PEF and their subsequent growth during storage. Grahl and others (1996) have reported PEF to be ineffective for inactivation of microbial spores.

Apple juice/cider is highly susceptible to yeast spoilage due to low pH and high sugar content (Deak and Beuchat 1993). Examination on petrifilm showed yeast as the major microorganism in apple cider. The initial YMC in untreated control cider was around 2.5 log CFU/mL, which steadily increased to 4.7 log CFU/mL by week 2 of storage (Figure 3-5). No significant increase ($P < 0.05$) in YMC was noticed by week 2 and 3, indicating a stationary phase in the growth cycle of microorganisms. However, a decline in YMC to 3.7 log CFU/mL by week 4 in the control cider was observed, suggesting that the microorganisms were in the death phase of their cycle. Analysis of UV treated cider showed an increase in YMC to 4.0 log CFU/mL after 2 weeks of storage at 4 ºC. The YMC decreased to 3.5 log by the end of 4 weeks of storage similar to untreated cider. Donahue and others (2004) proposed that the decrease in efficiency of microbial inactivation by UV is due to high turbidity and inadequate mixing of apple cider as it flows through the tubes. However, TPC of UV cider shows effective bacterial inactivation and stability during storage in this study. The inefficient inactivation of yeast and mold is due to their apparent higher resistance to UV-radiation compared to bacteria. Yeast and mold have different chemical composition and thicker cell walls than bacteria, which is a major factor in determining the relative UV resistance of an

organism (Tran and Farid 2004b). The YMC for PEF and thermally processed cider were <1 log CFU/mL throughout storage, indicating effective inactivation of spoilage microorganisms. The PEF process is known to be more effective in yeast inactivation compared to bacteria due to their larger cell size (Jeyamkondan and others 1999). Larger cells are more permeable than smaller cells.

Effect of Thermal and Non-Thermal Processing on Appearance of Apple Cider During Storage

No visual changes were noted in treated apple cider samples compared to untreated cider at week 0. However, after 2 weeks of storage both untreated and UV treated apple cider samples were accompanied by gas formation due to fermentation. Also, visually both the samples clarified after 2 weeks of storage (Appendix A-1). Previous studies also showed clarification of juice due to production of fungal enzymes, as well as deterioration in quality of apple cider, after 2 weeks of storage due to mold and yeast growth (Tandon et al. 2003; Donahue et al. 2004). No clarification was noted in PEF and thermally treated cider during 4 weeks of storage.

Effect of Thermal and Non-thermal Processing on the pH and Brix of Apple Cider During Storage

The changes in pH and Brix for untreated and treated apple cider during 4 weeks of storage at 4 ºC are shown in Table 3-1. Untreated apple cider from the same juice lot maintained at -17 °C during 4 weeks of storage was used as the fresh sample.

The initial pH values were in the range of 3.76 to 3.77 for both treated and fresh apple cider with no significant difference ($P < 0.05$) in mean values. Storage at 4 ºC for 4 weeks resulted in a slight, but not significant ($P < 0.05$), decrease in pH for UV treated cider. The decrease in pH in UV treated cider could be due to fermentation in cider as a result of increased yeast and mold growth during storage. PEF and thermal processing

did not significantly alter the pH of apple cider during storage ($P < 0.05$). An increase in pH in heat pasteurized apple juice (85 ºC for 27s) due to evaporation of organic acids was reported by Charles-Rodriguez and others (2007) compared to PEF treated juice. However, in the present study at equivalent process conditions, the heat treatment conditions used were mild (76 ºC for 1.3s) and therefore, no increase in pH compared to PEF was noted. The result thus confirms the importance of comparison of quality parameters under equivalent process conditions.

Brix value, which indicates soluble solids content in cider, is an important measure of juice quality. UV treated cider during storage showed a significant ($P < 0.05$) decrease in Brix after 2 weeks of storage corresponding to yeast and mold growth. Microorganisms utilize sugars for growth and therefore could alter the Brix value of juice. Tandon and others (2003) also noted a significant decrease in pH and Brix values in UV treated cider after 2 weeks of refrigerated storage due to microbial spoilage. Brix values were neither significantly ($P < 0.05$) affected by processing nor storage for thermal and PEF treated apple cider, which corresponds to their good microbial quality.

Effect of Thermal and Non-Thermal Processing on Color of Apple Cider During Storage

The effect of treatment and storage on color of apple cider was determined using Hunter L* a* b* values (Table 3-2). A significant ($P < 0.05$) increase in L* values were noted in treated cider samples compared to fresh cider at week 0. Increase in L* values for pasteurized juice indicates that the juice color became lighter or whiter. Genovese and others (1997) attributed the initial increase in L* values in pasteurized cloudy apple juice to partial precipitation of suspended particles in juice. After 4 weeks of storage, L* and b* values increased significantly ($P < 0.05$) for thermal and UV pasteurized ciders.

Noticeable decrease in a* values for all samples indicates a loss in redness, probably due to breakdown of anthocyanins during treatment (Shewfelt 1986).

To understand the impact of the L* a* b* value changes on color of apple cider, the total color difference (dE) values were calculated, which gives the magnitude of color difference between treated and fresh cider (Lee and Coates 1999). Thermally treated cider had significantly higher dE ($P < 0.05$) compared to both PEF and UV samples at week 0 (Figure 3-6). Thermal treatments are known to have pronounced effects on color of juice due to degradation of color pigments and Maillard reactions between sugars and amino acids, resulting in browning of juice (Min et al. 2003b; Clegg 1964; Lee and Coates 1999). Browning is commonly observed in heat treated citrus juice and is affected by storage temperature and time (Nagy and others 1990). The dE value increased for all treated ciders during 4 weeks of storage, indicating loss in overall color compared to fresh cider. The increase in dE from 1.17 to 1.91 in UV cider during storage is possibly due to microbial growth. Donahue and others (2004) attributed the color change in UV treated cider to by products formed during fermentation by yeast and mold growth. The dE value for PEF treated cider did not show any significant ($P < 0.05$) increase during storage. In comparison to UV and thermally treated samples, PEF processed cider showed maximum color stability during 4 weeks of storage. Color was better preserved in PEF treated juice compared to heat pasteurized juice in this study, and this has also been documented by other authors (Yeom et al. 2000b; Min and Zhang 2003). The conditions used for heat treatment were severe in terms of temperature and time (94.6 ºC for 30 s and 92 ºC for 90 s, respectively). The current

study shows improved color stability in PEF cider compared to thermal cider during storage even under equivalent process conditions.

Conclusion

Non-thermal processing has been extensively researched over the past few years as an alternative to heat pasteurization. However, the non-thermal process was often compared to thermal processes using conditions that did not achieve the same reduction in concerned microorganism(s), which made fair comparison of their effects on juice quality impossible. This is the first report where conditions were carefully selected so that all three processes achieved a similar reduction (6-log) of *E. coli*. The present study demonstrated that PEF was as efficient in microbial inactivation as heat pasteurization while maintaining the color of the apple cider. Heat pasteurization negatively affected the color of apple cider. UV pasteurization as a non-thermal technique was successful only for a shelf life of 2 weeks after the processing conditions used in this study. Comparing the three techniques, PEF shows promise as a pasteurization technique for the preservation of apple cider.

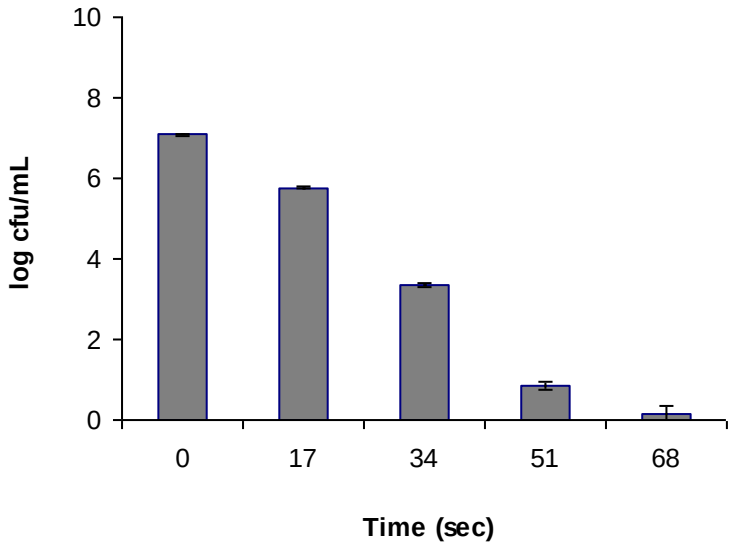

Figure. 3-1. UV inactivation of inoculated *E. coli* K12 in apple cider at different treatment times. Treatment conditions: wavelength 254 nm, outlet temperature < 15 ºC

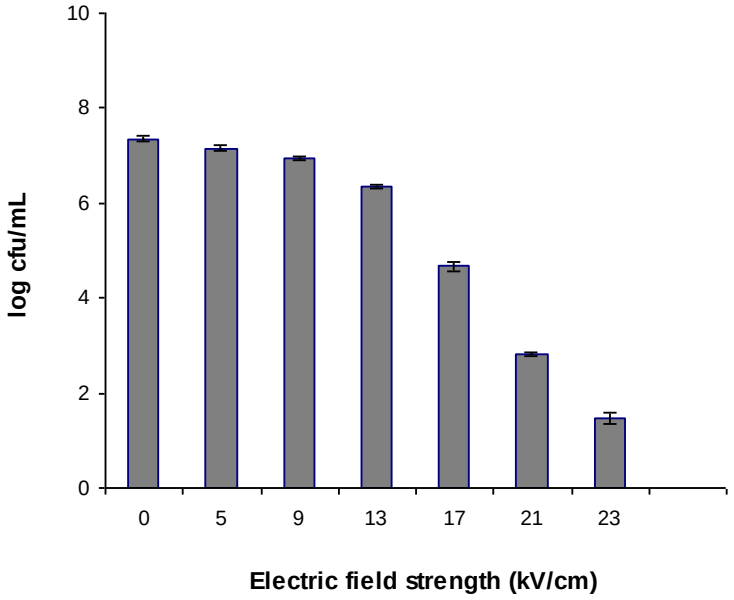

Figure. 3-2. PEF inactivation of inoculated *E. coli* K12 in apple cider at different electric field strengths. Treatment conditions: pulse duration of 2.5µs, total treatment time of150 µs, outlet temperature 49 – 51 ºC.

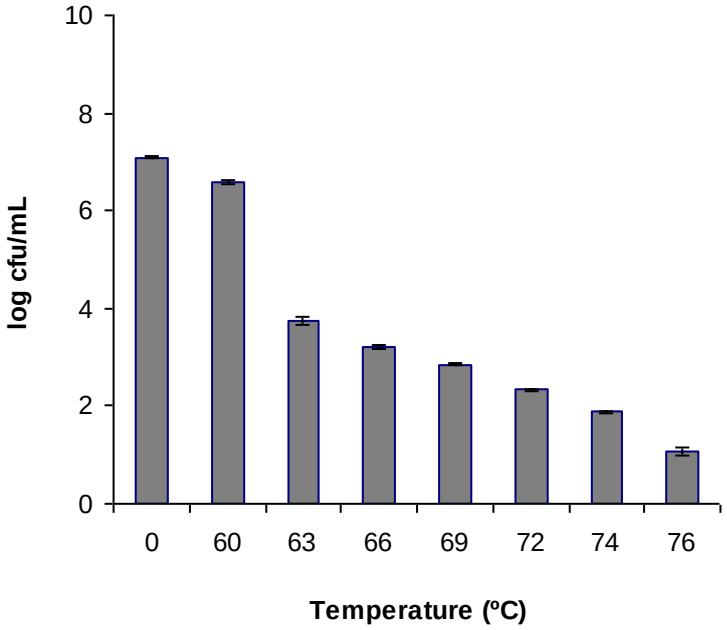

Figure. 3-3. Thermal inactivation of inoculated *E. coli* K12 in apple cider at different temperatures. Treatment condition: 1.3 s hold time, outlet temperature <15 ºC.

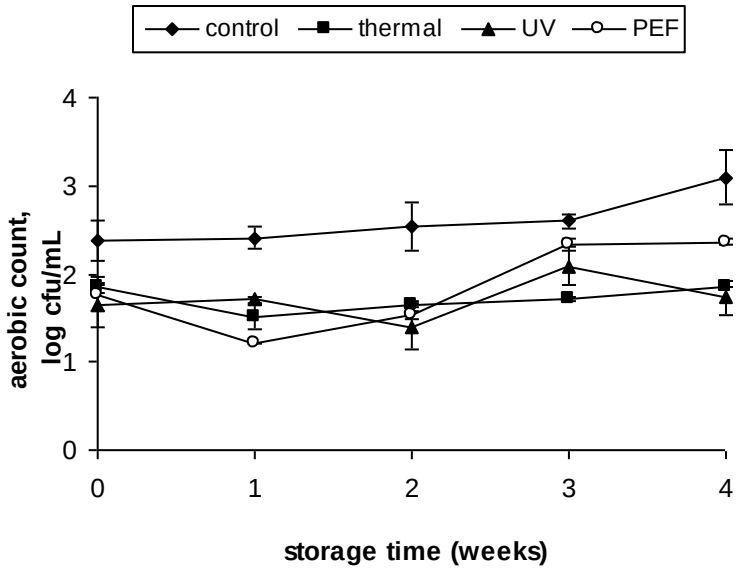

Figure. 3-4. Total aerobic plate count for control, thermal, UV and PEF treated apple cider samples stored at 4 ºc for 4 weeks. Vertical bar indicates standard deviation (n=4)

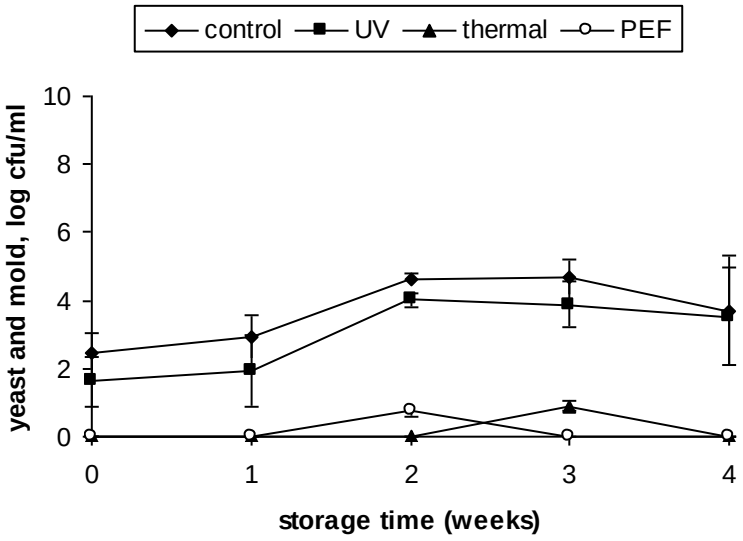

Figure. 3-5. Yeast and mold count in control, thermal, UV and PEF treated apple cider samples stored at 4 ºc for 4 weeks. Vertical bar indicates standard deviation (n=4)

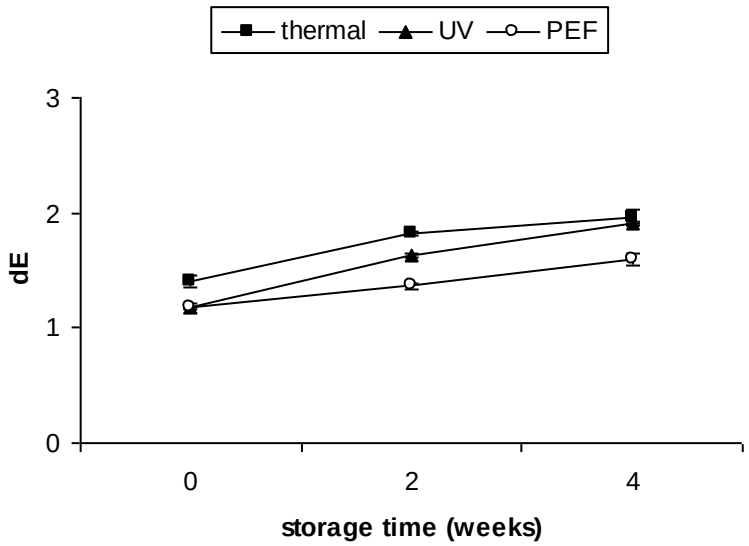

Figure. 3-6. Change in total color difference (de) in thermal, UV and PEF treated apple cider during storage. Vertical bar indicates standard deviation (n=3)

Table 3-1. Change in pH and Brix values for fresh*, thermal, UV and PEF treated apple cider stored at 4 ºC for 4 weeks

Weeks of storage	pH				Brix			
	Fresh	Thermal	UV	PEF	Fresh*	Thermal	UV	PEF
0	3.76[a**]	3.770 [a]	3.76 [a]	3.76 [a]	13.13 [a]	13.00 [a]	13.00 [a]	13.10 [a]
2	3.76 [a]	3.750 [a]	3.75 [a]	3.75 [a]	13.13 [a]	13.00 [a]	12.90 [a]	13.00 [a]
4	3.76 [a]	3.750 [a]	3.72 [a]	3.75 [a]	13.13 [a]	12.88 [a]	12.10 [b]	12.90 [a]

***** Fresh apple cider refers to untreated cider stored at -17 ºC through 4 weeks of storage. **Values with different initials within same row are significantly different from each other at $p<0.05$.

Table 3-2. Change in Hunter color values** for fresh*, thermal, UV and PEF treated apple cider stored at 4 ºC for 4 weeks

Weeks of storage	L				A				b			
	Fresh	Thermal	UV	PEF	Fresh	Thermal	UV	PEF	Fresh	Thermal	UV	PEF
0	32.58^{a}	32.94^{b}	32.88^{b}	32.80^{b}	3.32^{a}	2.01^{b}	2.18^{b}	2.21^{b}	5.55^{a}	5.93^{a}	5.31^{a}	5.74^{a}
2	32.60^{a}	33.33^{c}	32.63^{b}	32.80^{b}	3.31^{a}	2.00^{b}	2.00^{b}	2.13^{b}	5.57^{a}	6.63^{b}	6.50^{b}	6.19^{b}
4	32.59^{a}	33.38^{c}	33.44^{c}	32.55^{b}	3.32^{a}	1.96^{c}	1.98^{c}	2.14^{b}	5.58^{a}	6.78^{c}	7.40^{d}	6.62^{b}

* Fresh apple cider refers to untreated cider stored at -17 ºC through 4 weeks of storage. **Values with different initials within same row for each hunter value are significantly different from each other at $p<0.05$.

CHAPTER 4
IMPACT OF THERMAL AND NON-THERMAL PROCESSING TECHNOLOGIES ON APPLE CIDER AROMA

Introduction

A large amount of microbial information concerning thermally and non-thermally processed apple juice can be found in the literature, but little information exists on the effects of these processes on the flavor of apple cider. A decrease in ester concentrations due to thermal pasteurization of apple juice has been reported (Kato et al. 2003; Su and Wiley 1998), and a single study compared 8 apple juice volatiles in PEF and thermally processed juice (Aguillar-Rosas et al. 2007). Because a major motivation for non-thermal processing technologies is a minimal change in organoleptic and nutritional properties, an in-depth analysis of the effect of the above processes on the flavor profile of apple cider and its relation to sensory quality is necessary. The present work focused on: 1) comparing the aroma volatiles from thermally processed apple ciders with non-thermal treatments where each treatment was optimized to achieve an equivalent of 6 log reduction in microorganisms, and 2) examining changes in aroma active and major volatiles in the treated apple ciders stored at 4°C at week 0 and week 4.

Materials and Methods

The standard compounds ethyl acetate, ethyl propanoate, 2-methyl propyl acetate, methyl butyrate, ethyl butyrate, ethyl 2-methyl butyrate, hexanal, butyl 2-methyl acetate, butyl propanoate, 2-methyl-1-butanol, (E)-2-hexenal, ethyl hexanoate, hexyl acetate, (E)-2- hexen-1-ol acetate, 6-methyl -5-hepten-2-one, hexyl propanoate, (Z)-2-hexen-1-ol, hexyl butyrate, hexyl 2-methyl butyrate, hexyl hexanoate, α-farnesene, methional, phenylacetaldehyde, octanal, β-damascenone, hexane, methanol, and 1-

butanol were purchased from Aldrich (St. Louis, MO). Butyl acetate, benzaldehyde, 1-octanol, butyl butyrate, propyl butyrate, 1-hexanol, p-allylanisole, butyl 2-methylbutyrate, pentyl acetate, propyl hexanoate, dimethyl sulfide and an alkane standard solution (C8-25) were purchased from Fluka (Steinheim, Switzerland).

Apple Cider

Two hundred and twenty seven liters of unpasteurized apple cider with no additives was procured from Ziegler Juice Company (Lansdale, Pennsylvania). A varietal blend of apples, including Ginger Gold, Golden Delicious, Red Delicious, Empire, Macintosh, Gala and Cortland, were used for the juice in this study. Apples were inspected for quality (no visible mold or decay) and were washed and graded to remove loose stems, leaves and other foreign materials. After further inspection and grading, apples were passed through a Westphalia decanter press to express juice and separate solids. No additives were added to the cider. The cider was packaged in high density polyethylene (HDPE) 3.78 L (1 US Gallon) containers. The containers were transported to the USDA facility within 2 hours of juicing and stored in a -17 ºC deep freezer. The juice remained for in frozen condition for 6 days before being thawed at 4 ºC overnight for processing.

Apple Cider Processing

Preliminary experiments were performed to determine equivalent processing conditions using heat, pulse electric field and ultraviolet radiation for apple cider as described in chapter 3. Pasteurized apple cider (Ziegler Juice Co., Lansdale, PA) was inoculated with *E. coli* K12 (ATCC 23716) from a stationary phase culture to give approximately 7 log CFU/mL population. Microbial assays were conducted for ciders heated from 60-76 ºC, with UV exposure times of 17-68 s and 5-23 kV/cm electric field

strengths for PEF treatment. Final optimized processing conditions resulted in approximately 5 log reductions of *E. coli* K12 and were used for the remainder of the study.

Heat treatment

Apple cider was heat pasteurized using a miniature scale HTST processing system (Armfield, Jackson, NJ, FT74-30-MkIII-33-34) as described in chapter 3. Apple cider was introduced into the system via the feed tank at a flow rate of 15 L/hr with hot water circulation set at 76 ºC such that the juice was held at 76 ºC for 1.3s and cooled rapidly. The inlet and outlet temperatures were continuously monitored and were in the range of 13-17 ºC and 24-30 ºC, respectively, during processing.

Unpasteurized apple cider passed through the thermal processing system without heat at room temperature and was used as the control sample for microbial studies.

Ultraviolet treatment

A low-pressure mercury lamp surrounded by a coil of UV transparent Chemflour tubing was used for UV processing of apple cider (Geveke 2005) as described in chapter 3. Cider was pumped through the tubing at flow rates of 25 L/hr, which translates to a treatment time of 17 s per bulb. The energy used was 34 J/ml. Apple cider was exposed to a total treatment time of 51 s. The inlet and outlet temperatures recorded were between 10 -15 ºC during processing.

Pulsed electric field treatment

A bench scale continuous PEF system, described in chapter 3 (OSU-4F, Ohio State University, Columbus, OH), was used to treat the inoculated apple cider. The cider was pumped through the system at flow rate of 7.2 L/hr. The square wave pulse duration was 2.5 µs and the electric field strength was 23 kV/cm. The mean total

treatment time was calculated as 150 µs. Apple cider sequentially flowed through all the chambers via steel coils immersed in a water bath set at 48 ºC. The inlet and outlet cider temperatures were continuously monitored using thermocouples and were in a range of 30-34 ºC and 49-51 ºC, respectively.

Packaging and Storage

Processed apple cider was collected directly from the processing unit outlet into sterile 1L media glass bottles (Corning Inc, Corning, NY) inside a sanitary laminar hood equipped with a HEPA air filter (Forma Scientific Inc., Marietta, OH). The hood was sanitized by UV lighting at 254nm for 30min before use and then wiped with 100% alcohol. The packaged juice was stored at 4 ºC for storage studies. Storage studies were conducted for 4 weeks on the various processed apple ciders stored at 4 ºC. Microbial analyses were performed every week, whereas volatile and sensory analyses were done in weeks 0 and 4. Fresh unpasteurized cider samples maintained at 0 ºC were used as controls for volatile and sensory analysis.

Microbial Stability

The microbial analysis was performed according to method by Fan and Geveke (2007). Microbial tests were conducted every week during the four weeks of storage. Total aerobic plate count and yeast and mold count were determined using plate count agar (PCA) and yeast and mold (YM) pertrifilms. The PCA plates were incubated at 37 ºC for 24 hours while YM plates were incubated at room temperature for 5 days before counting. All samples were analyzed in duplicate and two replicates of each dilution were prepared and plated.

Volatile Extraction

Aliquots (27 ml) of apple cider were placed in 40 ml glass vials with screw cap containing Teflon coated septa similar to the procedure used by Dreher and others (2003). The cider was equilibrated for 10 min at 36 ºC with stirring. A 2 cm-50/30µm, DVB/ Carboxen™/ PDMS Stableflex™ (Supelco,Bellefonte, PA) SPME fiber was exposed in the equilibrated headspace for 45 min at 36 ºC. The fiber was desorbed for 5 min in the GC injection port at 250 ºC. All samples were analyzed in quadruplicate.

Gas Chromatography-Mass Spectrometry Analysis

A Gas chromatography/Mass spectrometry (6890N GC, 5973N MS, Agilent Technologies, Santa Clara, CA) was used for separation and analysis of volatiles. The instrument was also equipped with a Pulsed Flame Photometric Detector, PFPD, (model 5380, OI Analytical, College station, Texas, USA).

Samples were run separately on a polar DB-Wax and non-polar DB-5 column with identical dimensions (30 m x 0.32 mm x 0.5 µm from J&W Scientific, Folsom, CA). The column oven temperature was programmed from 40 ºC to 110 ºC at 7 ºC/min, then raised 15 ºC/min to 250 ºC with 3 min hold. Injector and detector temperature were 250 ºC. Mass spectrometry conditions were as follows: transfer line temperature at 275 ºC, mass range 30 to 300 amu, scan rate 5.10 scan/s and ionization energy 70 eV. Helium was used as the carrier gas at a flow rate of 2 mL/min. Mass spectral matches were made by comparison with NIST 2002 standard spectra. Authentic standards were used for confirmation. Alkane linear index values were determined on both columns (Girard and Tv 1996).

Quantification of Apple Cider Volatiles

Volatile free apple cider was prepared according to Fan and others (2001) by concentrating 500 mL of apple cider using a vacuum rotary evaporator (Brinkmann Instruments Inc., Westbury, N.Y., U.S.A.) from 11.0 to 28.0 °Brix. Any residual volatiles were extracted with hexane and discarded. Any trace hexane residue in the concentrated juice was removed using a vacuum rotary evaporator. The concentrated juice was diluted back to initial °Brix of 11.0 using distilled water and checked for residual volatiles. A mixture of 29 standards in methanol was serially diluted with deodorized apple cider and added in concentration of 0.5 to 3 times their estimated concentrations in cider. Volatiles were analyzed by SPME–GC/MS using the same conditions described in GC/MS analysis section. A quantitation database for standards was created using MSD Chemstation software. Response factor curves were created by plotting target ion count (base peak) against standard concentrations in volatile-less apple cider. Parameters used for compound identification were retention time, target ion and secondary ions. Compounds were quantified using target ion values and response factors generated from standard curves.

Sensory Evaluation

A discriminative triangle test (Meilgaard and others 1999) was employed to orthonasally detect differences in aroma between unpasteurized and pasteurized apple cider for “0” and “4” weeks storage at 4 ºC. The statistical power for the triangle test was recorded as 0.9. The analysis was conducted in a sensory panel facility at Eastern Regional Research Center (Wyndmoor, PA), which has 6 booths with computers. The sensory analysis was designed and conducted using Compusense five (Compusense Inc., Ontario, CA). Samples were prepared by pouring 40 mL apple cider into 100 mL

glass bottles and closed with airtight caps. The bottles were stored in boxes at 4 ºC overnight. On the day of testing, the bottles were taken out 1hr before testing. Cider temperature was 10-12 ºC during testing. Testing was performed under red light so that color and other visible differences were masked from panelists. Each panelist was given five sets of apple cider samples (3 samples per set) one at a time. All samples were randomly assigned three-digit codes. The order of presentation of sample sets among panelist was also randomized. In total, 50 untrained panelists from ERRC evaluated the samples and each panelist performed five triangle tests, which included control versus PEF, control versus UV, control versus thermal, thermal versus UV and thermal versus PEF treated apple cider samples. After each test, the panelists were also asked which sample was preferred among the three test samples and to give a reason for their preference.

Statistical Analysis

All data were subjected to statistical analysis using SAS 9.1 (SAS Inst. Inc., Raleigh, N.C., USA). Data were from a single sample for each treatment. Each sample was analyzed in quadruplicate. The tests for statistical significance of difference between treatments for storage data on volatiles was performed by analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. The effect of treatments on means of samples between treatments was compared using Duncan's multiple comparison test ($\alpha = 0.05$). Microbial data was analyzed using MS Excel 2003 and standard deviations were presented.

Results and Discussion

Microbial Stability During Storage

The total aerobic count for processed apple cider was maintained below 2±0.24 log CFU/ mL through 4 weeks of storage at 4 ºC. The yeast and mold count in initial cider at 0 day was 2±0.12 log CFU/ mL. PEF and thermal processed apple cider did not show any yeast and mold growth throughout storage. After 2 weeks storage, the yeast and mold count in UV treated samples increased to 3.8 ± 0.52 log CFU/ mL. A visible mold growth was observed after 4 weeks storage. Deterioration in quality of UV treated apple juice due to yeast and mold growth after 2 weeks of storage has also been observed by others (Tandon et al. 2003; Donahue et al. 2004) . Donahue and coworkers confirmed the presence of injured microbial cells in UV treated cider using selective enrichment media. They suggested that the decrease in efficiency of cell inactivation by UV could possibly be due to high turbidity and inadequate mixing of apple cider as it flows through the tubes (Donahue et al. 2004).

Volatile Composition

A total of 34 volatile compounds were identified using MS in the initial untreated apple cider after separation on a DB-Wax column (Table 4-1). Apple juice volatiles have been previously quantified using both external and internal standard methods (Komthong and others 2006b; Mehinagic et al. 2006). The current study is the first report where apple cider volatiles have been quantified using external calibration from an odorless apple cider to compensate for matrix effects. Response factor plots were determined for 29 compounds in Appendix B-1. No response factors were determined for peaks 2, 3, 6, 14 and 30 because their signal/noise ratio was less than 3. The

standard addition plots for 29 compounds are given in Appendix B-1. The plots for most compounds had a linear correlation coefficient (R^2) above 0.95.

Esters, aldehydes and alcohols comprised the major volatiles in apple cider, accounting for 40%, 43% and 16% of the total volatiles identified, respectively. However, it should be kept in mind that the volatile composition of apple juice depends on various factors like variety, maturity and storage conditions of fruit used for pressing (Dixon and Hewett 2000; Girard and Lau 1995). Certain apple cultivars like Jonathan and Cox Orange Pippin are reported to have 5-100 fold higher aldehyde content compared to Golden Delicious cultivars (Dixon and Hewett 2000). Apple juice also has higher C6-aldehyde concentration compared to fruit due to oxidation of the fatty acids linoleic and linolenic acid produced by lipoxygenases soon after crushing (Paillard and Rouri 1984b). Hexanal and 2-(E)-hexenal were the most abundant aldehydes identified in apple cider in this study (see Table 4-1).

Apple cider esters can be classified into acetic, butyric, propanoic and hexanoic groups. Acetate esters are reported to be the major volatiles in apple juice, and high concentrations of hexyl acetate and butyl acetate are considered to be normal characteristics of many apple cultivars (Dixon and Hewett 2000; Young and others 1996; Girard and Lau 1995). The most abundant acetate esters in this study were hexyl acetate, 2-methyl butyl acetate and butyl acetate.

Odor Activity Values

To assess the contribution of each volatile to apple cider aroma, OAV were calculated as the ratio of concentrations found in apple cider to its odor threshold value in water (Table 4-2) (Takeoka and others 1990; Flath and others 1967a; Buttery and others 1988). Apple cider odor threshold values were not available. Aqueous threshold

values should be similar to actual apple cider values since apple cider is approximately 90% water. The highest OAV values found in initial apple cider were for hexyl acetate (69), hexanal (41), 2-methyl butyl acetate (25), 2-methyl ethyl butyrate (23) and 2-(E)-hexenal (14). These results are consistent with the work of Fuhrmann and others (2002), where 2-methyl ethyl butyrate, hexyl acetate and 2-methyl butyl acetate were reported to be major contributors to fruitiness of apple aroma in “Elstar” and “Cox orange” apple cultivars. The C6 aldehydes, hexanal and 2-(E)-hexenal are responsible for the green or fresh aroma of apple cider and are essential to apple juice aroma due to their high correlation with apple aroma intensity (Durr and Schobinger 1981). Durr and others (1981) have shown that even though esters give the fruity aroma to cider, the concentration of aldehydes is essential for sensory impression of juice odor. Alcohols like 1-hexanol are low aroma impact components and have been identified as a negative contributor to apple aroma (Bult and others 2002). In this study, only UV treated cider possessed a hexanol OAV value, which might suggest that it was aroma active.

Effect of Treatment and Storage on Volatiles

The concentration of volatiles was not significantly ($p<0.05$) affected by treatments immediately after processing (week 0 data not shown). However, pronounced volatile differences between treatments were observed after 4 weeks at 4 ºC (Table 4-1). Figure 4-1 compares the levels of volatiles with significant differences between treated and control apple ciders after 4 weeks of storage at 4 ºC. Hexyl acetate concentrations decreased during storage in all processed apple cider samples with a concomitant increase in 1-hexanol by the action of residual esterase present in apple cider pulp (Schreier and others 1978). Thermally treated ciders lost 30% of their original ester and

aldehyde content during storage with significant decreases ($p<0.05$) in butyl acetate, 2-methyl butyl acetate, hexanal and 2-(E)-hexanal concentrations (Table 4-1 and Figure 4-1). Although thermal treatment is known to inactivate most enzymes, the cider in this study was exposed to a temperature of 76 ºC for only 1.3 s. This may not have completely inactivated flavor altering enzymes even though it reduced microbial concentrations to the desired level. UV treated cider was characterized by a complete absence of hexanal and 2-(E)-hexenal, a decrease in hexyl acetate and an increase in 1-hexanol compared to control after 4 weeks of storage. The increase in 1-hexanol concentration is associated with decreases in precursors such as hexanal, 2-(E)-hexenal and hexyl acetate (Schreier et al. 1978). PEF cider lost less than 2% of total ester and aldehyde volatiles during storage, suggesting that it more effectively inactivated indigenous enzymes than thermal or UV treatments. A significant decrease ($p<0.05$) in volatile concentrations were observed only in hexyl acetate and 2-(E)-hexenal.

1, 3 –Pentadiene was detected only in UV samples after 4 weeks of storage. It is an unsaturated hydrocarbon produced by molds such as *Zygosaccharomyces* and *Penicillium* in beverages. It possesses a petroleum odor that is often associated with microbial spoilage (Casas and others 1999).

An interesting change observed in both thermal and PEF cider was an increase in benzaldehyde concentrations after 4 weeks storage. Sumitani and coworkers found a similar increase in benzaldehyde in high pressure treated peaches during storage due to release from its bound glycoside form (amygdalin) by action of β-glucosidases present in the peach (Sumitani and others 1994). Similar enzyme action is possible in

thermal and PEF ciders since apple seeds are known to have amygadalin as a major constituent (Lu and Foo 1998).

Effect of Treatment and Storage on Odor Activity Values of Volatiles

Odor activity values were calculated for quantified volatiles in control and all treated samples to assess the impact of storage and treatment on apple cider aroma (Table4-2). Significant odor value losses ($p<0.05$) were observed for thermal and UV cider samples after 4 weeks storage. Hexanal and 2-(E)-hexenal OAV decreased 35% and 43%, respectively, in thermally treated cider during storage. Decreases in aldehyde odor values in thermally treated cider would likely reduce aroma strength as well as perceived freshness. Significant decreases ($p<0.05$) in essential esters such as hexyl acetate and 2-methyl butyl acetate could further deteriorate the aroma quality of thermal cider.

In UV treated cider, 100% of the original hexanal and 2-(E)-hexanal was lost during 4 weeks of storage. During the same time, the OAV for 1-hexanol increased from 0 to 2 in UV cider only. This volatile has a green, musty aroma and is characterized as a negative contributor to apple aroma (Bult et al. 2002). They demonstrated that an increase in 1-hexanol concentration lead to a higher “nuts-musty” rating and a lower “apple” rating in an apple model solution. In addition to odorant losses, UV treated cider also developed a perceivable fermented odor after 4 weeks storage due to microbial spoilage. Due to its high odor threshold, benzaldehyde OAV was less than one for all treatments. Therefore, increases in benzaldehyde concentrations observed in PEF and thermal ciders probably did not impact the aroma of these apple ciders.

PEF volatile losses during storage were minor (less than 2% loss of total ester and aldehydes) except for hexyl acetate. This key odorant was greatly diminished during

storage in all treated ciders. In the case of PEF treated cider, only 35% was retained after 4 weeks storage. However, losses of hexyl acetate were even greater in thermally and UV treated ciders.

Aroma Sensory Studies

An aroma triangle test comparison between the control (unpasteurized cider samples maintained at 0 ºC for 4 weeks) and all treated apple ciders at day 0 found no significant difference at $p<0.05$. However, after 4 weeks of storage at 4 ºC, 22 of the 50 panelists detected a difference between the aroma of the thermally treated sample and the untreated cider (control). The aroma of thermally processed cider was less preferred compared to fresh untreated (control) samples. Aroma of UV treated cider differed significantly ($p<0.05$) from the control. Thirty-six of the 50 panelists correctly detected the difference. Of those 36, 35 panelists (97%) preferred control apple cider compared to UV cider. No significant difference ($p<0.05$) was detected by panelists between PEF treated and control apple cider. Triangle test results indicated that UV and PEF treated samples differed significantly from heat treated cider ($p<0.05$). Ninety one percent of the triangle test panelists who correctly differentiated between PEF and thermal cider preferred the odor of PEF treated cider over that of thermally treated cider. The preference for PEF cider coincides with the greater retention of apple aroma volatiles in PEF cider compared to thermal cider. Preference results from this study do not necessarily reflect true consumer preference, and further sensory studies would be necessary to evaluate the processing effects on preference in the general consumer population.

Conclusion

The key volatile components identified in apple cider based on OAV values were hexyl acetate, hexnanal, 2-methyl butyl acetate, 2-methyl ethyl butyrate and 2-(E)-hexenal. The pasteurization of apple cider by thermal and non-thermal techniques at equivalent processing conditions resulted in a decrease in volatile components after 4 weeks of storage. PEF treated cider showed better volatile retention compared to both thermal and UV cider. UV treated cider had a shelf life of 2 weeks. PEF cider aroma was undistinguishable from fresh untreated cider by triangle sensory analysis after 4 weeks of storage. PEF shows good promise as a viable pasteurization technique for apple cider.

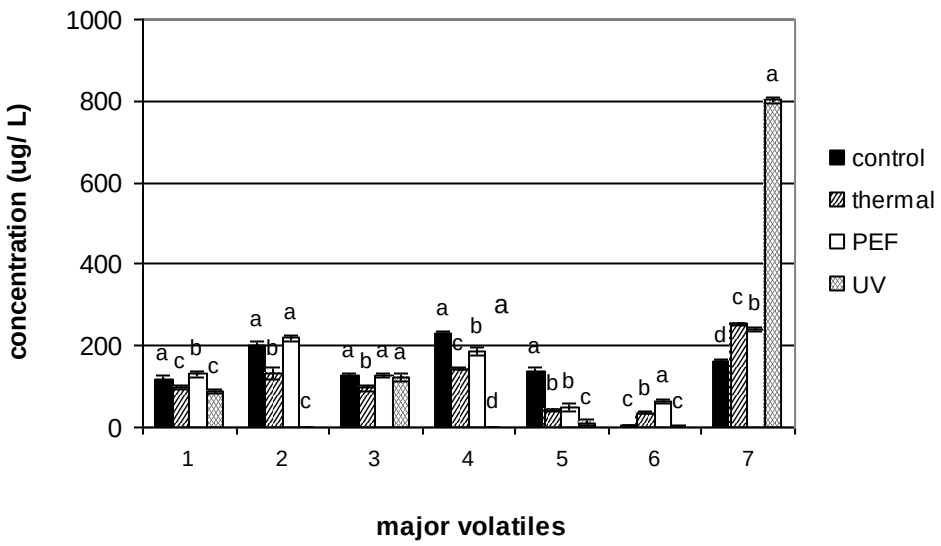

Figure 4-1. Effect of treatment and storage (after 4weeks) on major volatiles of apple cider 1 = butyl acetate, 2 = hexanal, 3 = 2-methyl butyl acetate, 4 = 2-(E)-hexanal, 5 = hexyl acetate, 6 = benzaldehyde, 7 = 1-hexanol, a,b,c,d = different letters for each volatile indicate significant difference($p<0.05$), control refers to fresh unpasteurized cider maintained at 0 ºC for 4 weeks

Table 4-1. Effect of thermal and non-thermal treatments on apple cider volatiles compared to fresh untreated cider after 4 weeks of storage at 4 ºC

Pk no.	Compound name	LRI Wax	Mean concentration (µg/L) (week 4)			
			Control[§]	Thermal	PEF	UV
1	1,3-pentadiene	714	nd[a]	nd[a]	nd[a]	nq[b*]
2	dimethyl sulfide*	753	nq	nq	nq	nq
3	ethyl acetate*	810	nq	nq	nq	nq
4	ethyl propanoate**	961	0.73±0.09[b]	0.73±0.04[b]	1.00±0.07[a]	1.55±0.15[a]
5	methyl butyrate**	994	2.53±0.17[b]	2.46±0.90[b]	2.73±0.20[a]	2.93±0.35[a,b]
6	2-methyl propyl acetate*	1024	nq	nq	nq	nq
7	ethyl butyrate**	1050	3.00±0.52[a]	2.86±0.20[b]	3.65±0.55[a]	3.75±1.35[a]
8	ethyl 2-methyl butyrate**	1067	2.36±0.27[a]	2.30±0.43[a]	2.60±0.13[a]	2.00±0.12[a]
9	butyl acetate**	1088	120±6.83[a]	98.8±2.09[b]	131±9.38[a]	89.4±1.95[b]
10	hexanal**	1098	203±8.73[a]	133±13.2[b]	219±13.1[a]	nd[c]
11	butyl -2-methyl acetate**	1135	127±6.64[a]	96.7±1.48[b]	128±5.02[a]	121±9.53[a]
12	propyl butyrate**	1137	6.50±0.34[b]	5.70±0.12[b]	10.0±0.25[a]	5.55±0.76[b]
13	butyl propionate**	1153	4.34±0.36[a]	4.23±0.37[a]	5.13±0.10[a]	4.56±0.26[a]
14	1-butanol*	1158	nq	nq	nq	nq
15	pentyl acetate**	1183	1.30±0.31[a]	0.76±0.07[b]	1.46±0.23[a]	0.36±0.10[b]
16	2-methyl-1-butanol**	1214	6.83±3.25[d]	15.06±1.17[c]	32.1±3.73[b]	56.60±7.85[a]
17	butyl butyrate**	1225	7.66±0.41[b]	6.80±0.07[b]	8.71±0.83[a]	4.56±0.20[b]
18	2-(E)-hexenal**	1227	231±2.62[a]	144±3.39[c]	186±7.12[b]	nd[d]
19	butyl-2-methyl butyrate**	1236	1.50±0.09[b]	1.43±0.12[b]	1.53±0.10[a]	1.50±0.08[b]
20	ethyl hexanoate**	1238	nd[a]	nd[a]	nd[a]	nd[a]
21	hexyl acetate**	1278	137±10.74[a]	43.2±2.07[b]	48.6±8.92[b]	11.7±8.54[c]
22	propyl hexanaoate**	1326	1.80±0.26[a]	nd[b]	nd[b]	nd[b]
23	(E)-2-hexen-1-ol acetate**	1342	0.57±0.11[b]	0.60±0.17[b]	0.73±0.10[b]	1.70±0.12[a]
24	6-methyl-5-hepten-2-one**	1348	0.10±0.03[a]	0.10±0.26[a]	0.20±0.05[a]	0.10±0.03[a]
25	hexyl propionate**	1349	0.07±0.00[ab]	0.00±0.00[b]	0.13±0.01[a]	nd[c]
26	1-hexanol**	1366	164±5.91[c]	253±6.24[b]	241±5.76[b]	803±12.83[a]
27	(Z)-2-hexen-1-ol**	1423	0.17±0.23[a]	0.53±0.48[a]	0.16±0.25[a]	nd[c]
28	hexyl butyrate**	1432	5.34±0.23[b]	4.26±0.29[b]	6.93±0.22[a]	1.55±0.12[c]
29	hexyl -2-methyl butyrate**	1443	2.90±0.22[a]	3.06±0.20[a]	3.73±0.18[a]	3.40±0.13[a]
30	2-methyl-6-hepten-1-ol†	1480	nq	nq	nq	nq
31	benzaldehyde**	1546	3.73±0.26[c]	36.7±3.73[b]	62.2±2.92[a]	0.86±2.20[c]

Table 4-1 Continued

Pk no.	Compound name	LRI Wax	Mean concentration (µg/L) (week 4) Control§	Thermal	PEF	UV
32	1-octanol**	1568	nd[a]	nd[a]	nd[a]	10.45±0.13[b*]
33	hexyl hexanaote**	1620	0.43±0.00[a]	0.40±0.03[a]	0.46±0.00[a]	nd[b]
34	p-allyl anisole**	1683	0.27±0.07[a]	0.20±0.10[a]	0.73±0.15[b]	0.13±0.05[a]
35	α-farnesene**	1747	bq	bq	bq	bq

Mean concentrations given as µg/L ± standard deviations of quadruplicate analyses on single samples aMean concentration ug/L, (n = 4) ;nq = not quantitated; bq = below quantitation; nd = not detected d; different letters in the same row indicate significant differences ($p<0.05$). b* 1,3-pentadiene was detected in stored UV cider but it was not quantitated; ** represent 29 standards used for quantitation of volatiles; * compounds not quantitated as present in trace levels; † no standard available §control = fresh unpasteurized cider maintained at 0 ºC for 4 weeks

Table 4-2. Effect of thermal and non-thermal treatments on odor activity values (OAV) of apple cider volatiles after 4 weeks of storage at 4 ºC

Compound [b]	RI (Wax)	OT[a] (µg/L)	OAV			
			Control [§]	Thermal	PEF	UV
Esters						
ethyl propanoate	961	10	<1 [a]	<1 [a]	<1 [a]	<1 [a]
methyl butyrate	994	60	<1 [a]	<1 [a]	<1 [a]	<1 [a]
ethyl butyrate	1050	1	3 [a]	3 [a]	4 [a]	4 [a]
ethyl -2-methyl butyrate	1067	0.1	23 [a]	23 [a]	24 [a]	20 [b]
butyl acetate	1088	66	2 [a]	1 [a]	2 [a]	1 [a]
butyl -2-methyl acetate	1135	5	25 [a]	19 [b]	26 [a]	24 [a]
propyl butyrate	1137	18	<1 [a]	<1 [a]	<1 [a]	<1 [a]
butyl propionate	1153	25	<1 [a]	<1 [a]	<1 [a]	<1 [a]
pentyl acetate	1183	43	<1 [a]	<1 [a]	<1 [a]	<1 [a]
butyl butyrate	1225	100	<1 [a]	<1 [a]	<1 [a]	<1 [a]
butyl-2-methyl butyrate	1236	17	<1 [a]	<1 [a]	<1 [a]	<1 [a]
ethyl hexanoate	1238	1	--	--	--	--
hexyl acetate	1278	2	69 [a]	22 [b]	24 [b]	6 [c]
hexyl propionate	1349	8	<1 [a]	-- [b]	<1 [a]	-- [b]
hexyl butyrate	1432	250	<1 [a]	<1 [a]	<1 [a]	<1 [a]
hexyl -2-methyl butyrate	1443	22	<1 [a]	<1 [a]	<1 [a]	<1 [a]
Aldehydes						
hexanal	1098	5	41 [a]	27 [b]	44 [a]	0 [c]
2-(E)-hexenal	1227	17	14 [a]	8 [b]	11 [c]	0 [d]
benzaldehyde	1546	350	<1 [a]	<1 [a]	<1 [a]	<1 [a]
Alcohols						
2-methyl-1-butanol	1214	300	<1 [a]	<1 [a]	<1 [a]	<1 [a]
1-hexanol	1366	500	<1 [a]	<1 [a]	<1 [a]	2 [b]
1-octanol	1568	130	-- [a]	-- [a]	-- [a]	<1 [b]
(Z)-2-hexen-1-ol	1423	70	<1 [a]	<1 [a]	<1 [a]	<1 [a]
Others						
6-methyl-5-hepten-2-one	1348	50	<1 [a]	<1 [a]	<1 [a]	<1 [a]

[a] OT = odor threshold values in water from literature, [b] compounds identified based on RI on DB-Wax and DB-5 column using standards, [§] control refer to fresh unpasteurized cider maintained at 0 ºC for 4 weeks

CHAPTER 5
COMPARISON OF THERMAL AND NON-THERMAL TECHNIQUES ON GRAPEFRUIT JUICE STORAGE QUALITY UNDER EQUIVALENT PROCESS CONDITIONS

Introduction

Increased consumer demand for fresh-like products with minimum flavor and nutritional losses has paved the way for alternatives to heat processing. PEF treatment as a non-thermal technique for pasteurization of juices has been studied extensively in recent years (Jin and Zhang 1999; Min et al. 2003b; Yeom et al. 2000b). PEF processed juice at a commercial scale had a microbial shelf life of 196 days at 4 ºC with a higher sensory preference compared to heat pasteurized juice (Min et al. 2003a). Studies on UV pasteurization have shown extension of shelf life of apple juice and orange juice by 7 and 5 days, respectively, with minimal loss of sensory quality (Donahue et al. 2004; Tran and Farid 2004b). Radio frequency electric field is a recently developed non-thermal technique that is not FDA approved. The effect of RFEF treatment on microbial quality of apple and orange juice has been reported in literature (Geveke et al. 2007; Geveke and Brunkhorst 2004). Geveke and others (2004) achieved a 3.3 log reduction in *E. coli* in RFEF treated orange juice relative to untreated juice.

There are several studies in the literature comparing the effect of thermal and non-thermal treatments on juice quality (Yeom et al. 2000b; Tandon et al. 2003; Sanchez-Vega et al. 2009). However, most often the conditions used for thermal processing are severe in terms of temperature and time of exposure to heat. Thus, shelf life studies comparing the effect of non-thermal to thermal techniques on the acceptability of juice using conditions that do not achieve the same reduction of the target microorganism would result in incorrect conclusions (Aguillar-Rosas et al. 2007; Yeom et al. 2000b;

Rivas et al. 2006). Therefore, for a fair comparison of the effects on quality of juice, both thermal and non-thermal processes must achieve an equivalent reduction in microorganism levels. The current work focuses on comparing the effect of thermal and non-thermal processing techniques on grapefruit juice quality, where each treatment is optimized to achieve approximately equivalent 5 log reductions in *E. coli* K12 population.

Materials and Methods

Determination of Equivalent Treatment Conditions

Pasteurized grapefruit juice was inoculated with *E. coli* K12 (ATCC 23716) obtained from the American Type Culture Collection (ATCC) (Manassas, VA). The bacterium was maintained on Tryptic Soy Agar (Remel, Lenexa, KS, USA) at 4 ºC. Prior to inoculation of product, the organism was cultured in Tryptic Soy Broth (Remel) with shaking at 37 ºC for 16-18 h. Juice was inoculated from the stationary phase culture to give approximately 7 log CFU/mL population.

Heat treatment

Inoculated grapefruit juice was heat pasteurized using a miniature scale HTST processing system (model FT74-30-MkIII-33-34, Armfield, Jackson, NJ, USA) described in chapter 3. Juice was pumped through the system at a flow rate of 15 L/hr, which translated to a hold time of 1.3s. Microbial assays were performed at holding tube outlet temperatures of 60, 62, 64, 66, 68 and 70 ºC.

Ultraviolet treatment

The UV apparatus used for pasteurization of juice is described in chapter 3. The experimental system included a feed tank, a peristaltic pump and four UV lamps of the same dimensions connected in a series. Juice was pumped through the tubing at a flow

rate of 28.8 L/hr, which translated to a treatment time of 14.75s per bulb. Microbial assays were conducted using 1, 2, 3 and 4 UV lamps, which corresponds to UV treatment times of 14.75, 29.57, 44.25 and 59s.

Pulsed electric field treatment

A bench scale continuous PEF system (Valappil et al. 2009) at USDA, Wyndmoor, PA (as described in chapter 3) was used to treat the inoculated grapefruit juice. The juice was pumped through the system at a flow rate of 7.2 L/hr. The square wave pulse duration was set at 2.5 μs and the electric field strengths tested were 5, 10, 13, 17, 21 and 23 kV/cm. Grapefruit juice sequentially flowed through all the treatment chambers via steel coils immersed in a water bath set at 30 ºC.

Radio frequency electric field treatment

The RFEF system used in the previous study has been previously described by Geveke and others (2007). The system was equipped with an 80 kW RF power supply (Ameritherm, Scottsville, NY model L-80) interfaced with a custom-designed network that enabled RF energy to be applied to a resistive load (Ameritherm) over a frequency range of 21.1 to 40.1 kHz. The RFEF power supply was connected to a series of treatment chambers. Grapefruit juice was supplied into the RFEF system from a feed tank using a progressing cavity pump. The processing parameters were set at a frequency of 20 kHz, a flow rate of 33 L/hr and field strength of 15kV/cm. The juice was quickly cooled to less than 25 ºC after exiting the treatment chambers using a stainless steel heat exchanger sample cooler (Madden manufacturing, Elkhart, IN, model SC0004).

Shelf Life Study

150 L of unpasteurized red grapefruit juice with no additives frozen in 5 gallon buckets and packed in styrofoam boxes with dry ice layers were shipped from a commercial juice facility in Florida. The juice was received after 2 days of shipping and stored immediately in a -17 ºC freezer. Juice was filtered through 2 layers of cheesecloth under hygienic conditions due to high pulp content. The filtered juice was pasteurized by UV, PEF, RFEF and thermal techniques as described below.

Grapefruit juice pasteurization

All processing equipment was sanitized by pumping 5% bleach solution through the system followed by a distilled water rinse. Grapefruit juice was heat pasteurized at 70 ºC for 1.3s. Prior to UV pasteurization, juice was preheated to 50 ºC and then exposed to UV lamps for 44.25s. PEF processing was performed at an electric field strength of 20 kV/cm, 2.5 µs pulse duration and total treatment time of 150 µs. RFEF processing was performed at 20 kHz frequency, 15kV/cm field strength, an outlet temperature 55 ºC and a treatment time of 170 µs.

Packaging and storage

Processed grapefruit juice was collected directly into sterile 1L media glass bottles (Corning Inc, Corning, NY) inside a sanitary laminar hood equipped with a HEPA air filter (Forma Scientific Inc., Marietta, OH). The hood was sanitized by UV lighting at 254nm for 30 min before use and then wiped with 100% alcohol. The quality of grapefruit juice stored at 4 ºC was evaluated every week for 4 weeks (0, 1, 2, 3 and 4) on the basis of microbiology, pectin methyl esterase activity (PME), non-enzymatic browning (NEB), ascorbic acid content (AA) and physical properties (pH, Brix and

color). All assays were performed in triplicate. Microbial assays were performed in duplicate.

Microbial assay

Microbial tests were conducted as described in chapter 3. One mL aliquots of juice were pour plated with Tryptic Soy Agar (Remel, Lenexa, KS, USA) and incubated at 37 °C for 24 h. The *E. coli* K12 population was expressed as CFU/mL of grapefruit juice. Data for each replicate were normalized against the control and plotted as the log reduction versus temperature, time, treatment cycle or electric field strength.

For the storage study, the total aerobic plate count (TPC) and yeast and mold count (YMC) were determined using plate count agar (PCA) and yeast and mold (YM) pertrifilms. After appropriate dilutions (if needed), samples (1 mL) were then pour plated with PCA or pertrifilm. The PCA plates were incubated at 37 ºC for 24 hrs, while YM plates were incubated at room temperature for 5 days before counting. All samples were analyzed in duplicate and two replicates of each dilution were prepared and plated.

Pectin methyl esterase activity assay

PME activity was determined according to a modified method described by Sampedro and others (2009).Two milliliters of juice sample was added to a 30mL mixture of 0.35% citrus pectin solution and 125mM NaCl solution. The pH was first adjusted to 7.5 using 0.1M NaOH (pre-titration). The hydrolysis at 22 ºC was maintained at pH 7.5 by adding 0.1M NaOH solution using automated pH-stat titrator (Mettler Toledo T 70 Titrator, Schwerzenbach, Switzerland). After the initial 30s, the consumption of NaOH was recorded every 1 s for a 5-20 min reaction period. The slope

(dV/dt) from linear plot between volume of NaOH consumed (dV) vs. time (dt) is used to calculate PME activity using following formula (Yeom and others 2000a):

$$PME(units/mL) = \frac{slope * (0.1MNaOH) * (1000)}{2mLsample} \quad (5\text{-}1)$$

Percentage of residual PME activity was calculated in relation to the activity of the untreated sample.

Ascorbic acid assay

The ascorbic acid (Vitamin C) content in grapefruit juice was assayed by the HPLC method by Geveke and others (2007). Grapefruit juice was centrifuged at 10,000g for 10 min at 5 ºC in a Sorvall RC-2 refrigerated centrifuge (Kendro Laboratory Products, Newtown, CT). An aliquot of 0.5ml of supernatant was mixed with 5% meta-phosphoric acid. The solution was filtered through a 0.45 µm Acrodisc LC 13 PVDF syringe filter (Gelman Sciences, Ann Arbor, MI). The filtered material was analyzed using a HP Ti-series 1050 HPLC system (Agilent Technologies, Palo Alto, CA) with a photodiode array detector. An Aminex HPX-87H organic acids column (300 × 7.8 mm) fitted with a microguard cation H+ was used for separation. The mobile phase of 5mM sulfuric acid was passed through the column at a flow rate of 0.5 ml/min. Column temperature was maintained at 30 ºC using a column heater (Bio-Rad Laboratories, Hercules, CA). Ascorbic acid was monitored at 245nm. A calibration curve was prepared by external standard method using standard ascorbic acid solutions in a range from 100 -1000 µg/mL in water.

Non enzymatic browning

Browning in grapefruit juice was assayed using the method by Fan and Thayer (2002). Juice was centrifuged at 10,000g for 10min at 5 ºC. An aliquot of 2ml of

supernatant was mixed with 2ml of 100% ethanol. The solution was vortexed and left at room temperature for 1 hr. The mixture was centrifuged again and the absorbance of supernatant was measured at 420nm using a spectrophotometer (Shimadzu UV-1601, Shimadzu Scientific Instruments, Columbia, MD).

pH

The pH meter used for all analysis was an Orion 420A+ (Thermo Electron Corp., Beverly, MA). The pH meter was calibrated at pH 4.0 and pH 7.0 using standard solutions on each day of analysis.

Brix

A Bausch & Lomb Abbe refractometer (B&L Corp., Rochester, NY) was used. To measure Brix of juice sample, a drop of sample was placed on the refractometer and the corresponding refractive index was recorded, which gave the measure of soluble solids in juice.

Color

Color was measured in CIE L* a* b* 3-dimesional color space using ColorQuest XE spectrophotometer (Hunter Associates Lab, Reston, VA) as described in chapter 3. The lamp was allowed to warm up for 30 min prior to measurement. The system was calibrated using black and white standard tiles on each day of analysis. Forty mL of juice sample in a 20 mm path-length glass cell was used for measuring color. The effect of processing methods on color of juice was represented using total color difference (dE) calculated for all the samples using the following equation 2:

$$dE = \sqrt{(L - Lo)^2 + (a - ao)^2 + (b - bo)^2} \quad (5\text{-}2)$$

where L = lightness of treated sample at time t; L0 = lightness of untreated sample at day 0; a = redness of treated sample at time t; a0 = redness of untreated sample at day 0; b = yellowness of treated sample at time t; and b0 = yellowness of untreated sample at day 0.

Statistical Analysis

All data were subjected to statistical analysis using SAS 9.1 (SAS Inst. Inc., Raleigh, N.C., USA). Statistical significance of the differences among the treatments was tested using the analysis of variance (ANOVA) at the significance level of $\alpha = 0.05$. The effect of storage and processing techniques were compared using Duncan's multiple comparison test ($\alpha = 0.05$).

Results and Discussion

Effect of Treatments on Microbial Inactivation

Pasteurized grapefruit juice was inoculated with *E. coli* K12 to give approximately 7 log CFU/ml population. The initial microbial count in pasteurized juice was below 40 CFU/mL, indicating a very low microbial load before inoculation. The juice was treated by thermal, UV, PEF and RFEF techniques to achieve an approximately equivalent 5 log reduction in inoculated microbial levels.

The inlet and outlet temperatures recorded during thermal treatment were between 16- 18 ºC due to a built-in cooling section. The inoculated *E. coli* K12 population decreased from 5.6 logs to 2.09 logs as temperature increased from 60 to 70 ºC (Fig 5-1). Treatment of juice with temperatures above 62 ºC resulted in more than 4 log reduction in population. Thermal treatment at 70 ºC for 1.3 s was selected for microbial stability studies as it resulted in a population reduction by 4.91 logs, which was close to the target 5 log reduction.

For UV treatment, inoculated juice was passed through 1, 2, 3, and 4 UV bulbs connected in series at a flow rate of 28.8L/hr. Based on flow rate, the time of exposure to UV light translates to 14.75s, 29.57s, 44.25s and 59s. The inlet temperature was recorded at 50 ºC (pre-treatment temp) and outlet temperatures recorded were from 48-50 ºC. The microbial population reduced with increasing number of UV bulbs (Fig 5-2). UV treatment using three bulbs (44.25 s) reduced the microbial population by 5.05 logs. These conditions were used for microbial stability studies.

The effect of PEF processing on *E. coli* K12 inactivation at an electric field strength from 15 to 22 kV/cm with a pulse duration of 2.5 µs and a mean total treatment time of 150 µs is shown in Figure 5-3. The inlet and outlet temperatures of the filtered juices were between 20- 21 ºC and 34.6-50 ºC, respectively, during the length of treatment. An electric field strength at 20kV/m gave a 5.23 log reduction in *E. coli* population. Yeom and others (2000b) found a 7 log reduction in the inoculated microbial population of unfiltered orange juice treated with PEF at 35kV/cm for 59 µs. The authors showed that higher electric field strength and longer treatment times decreased microbial levels.

RFEF is a relatively new non-thermal technique that is not yet FDA approved for commercial use. It is similar to PEF except that the high voltage is applied continuously using an AC generator. Previous work by Geveke and others (2007) has shown a 2.1 log reduction in inoculated *E. coli* K12 population in orange juice at conditions of 21.1 kHz frequency, 15kV/cm electric field strength, an outlet temperature of 60 ºC and treatment time of 270 µs. In grapefruit juice processed at 20 kHz frequency, 15 kV/cm field strength, an outlet temperature of 55 ºC and treatment time of 170 µs gave 2.85 log

reduction in E. coli K12 population (Fig 5-4). After 2 treatment cycles, 5.26 log reduction was achieved.

Effect of Treatments on Microbial Stability During Storage

The effect of treatments on total aerobic count and yeast and mold count in grapefruit juice during 4 weeks of storage at 4 ºC are shown in Fig 5-5 and Table 5-1. The initial TAC in untreated grapefruit juice (control) was 1.57 log cfu/mL. After 4 weeks of storage, the microbial population reached 3.52 log cfu/mL. The intial TAC for all treated samples reduced to < 5 cfu/mL at week 0. Thermal and RFEF treated juice maintained the population below 1 log cfu/mL for 3 weeks of storage. However, in RFEF treated juice the microbial population increased to 3.34 log cfu/mL after 4 weeks of storage. In UV and PEF juice, the microbial population increased after 2 weeks of storage and reached 3.33 and 3.51 log cfu/mL, respectively after 4 weeks of storage. The increase in microbial count during storage could be due to two reasons: 1) ineffective inactivation of microbial spores and their subsequent growth during storage, or 2) incomplete inactivation or injury to microbial cells and later recovery during storage. An increase in microbial count has been previously reported in PEF treated tomato juice due to incomplete inactivation of microbial spores (Min et al. 2003b). Because RFEF works on a similar principle of microbial inactivation as PEF, it may also be ineffective in inactivation of spores. UV treatment has been shown to cause only injury to cells in cider due to lower penetration power. Tran and others (2004b) also found an increase in aerobic count from 2.47 to 4.33 log cfu/mL after 12 days of storage in UV treated orange juice.

The initial YMC in control juice was 2.46 log cfu/mL, and it increased to 3.90 log cfu/mL by the end of 2 weeks storage. After 2 weeks, the mold growth was uncountable

due to high growth, and the colonies appeared too diffused on the petriflim. In thermal and RFEF no Y&M was detected at week 0, indicating effective inactivation of Y&M. In thermal samples, the Y&M count reached 3.33 log cfu/mL after 4 weeks of storage. In RFEF, PEF and UV, the mold growth was too high to be counted after 2 weeks of storage. Similar to total aerobic count, the reason for the increased Y&M growth during storage could be due either to sublethal injury to cells or resistance of spores to different treatments. Yeast and mold growth are the most common spoilage agents in refrigerated citrus juices (Wyatt and Parish 1995). They are also more tolerant to high temperatures compared to bacteria and are therefore difficult to inactivate. Mold ascospores like bacterial spores are known to be more resistant to PEF treatment (Grahl and Markl 1996).

The results from microbial stability studies show that thermally treated juice had good microbial quality for 4 weeks of storage. Thermal treatment is more efficient in limiting bacterial and yeast and mold growth compared to non-thermal treatment. RFEF treated juice had a shelf life of 3 weeks, whereas PEF and UV treated juice spoiled after 1 week of storage. It could be concluded that heat treatment is more effective at microbial inactivation than non-thermal techniques at equivalent process conditions.

Effect of Treatments on Pectin Methyl Esterase Activity

The effect of heat, PEF, RFEF and UV treatment on PME activity is shown in Figure 5-6. PME is primarily responsible for the clarification of citrus juice cloud. PME causes demethylation of carboxyl groups of pectin. These unprotected COOH groups react with calcium ions in juice and form pectin aggregates which precipitate out of juice (Wolfgang, 1990). Previous studies on heat pasteurization of orange juice have shown 90-100% inactivation in PME activity at temperatures higher than 90 ºC for 20-60 s

(Elez-Martinez et al. 2006; Rivas et al. 2006). Thermal treatment at 70 ºC for 1.3 s decreased the PME activity in grapefruit juice by 80.47±1.99%. Tran and others (2004b) reported a 70% PME inactivation in orange juice treated at 70 ºC for 2s. RFEF, PEF and UV treatments decreased the PME activity by 81.66±3.10%, 59.17±2.64% and 48.52±3.62%, respectively. Studies on the effect of PEF on PME activity by Yeom and others (2000a) showed 88% PME inactivation in PEF treated orange juice at 35kV/cm for 59 µs and a 60 ºC outlet temperature. Rivas and others (2006) found 75.6% PME inactivation at 25kV/cm at 60 ºC for 280 µs total treatment time. Therefore, it is not surprising to find a lower inactivation of PME at the milder treatment conditions used in this study (22kV/cm, 46 ºC for 150 µs). UV treatment reduced PME activity by 48.52±3.62% in grapefruit juice. Tran and others (2004b) found only 5% reduction in PME activity in orange juice at high UV dose at ambient temperatures. The pretreatment of juice to 50 ºC could possibly have an additive effect on the inactivation of PME in grapefruit juice. RFEF treatment gave the highest PME inactivation compared to other treatments. It is possible that the electric strength, in combination with an output temperature of 55 ºC, resulted in increased inactivation. However, there are no previous data on RFEF effect on citrus PME for comparison.

During storage, RFEF and thermally treated juices maintained residual PME activity during 4 weeks of storage, indicating irreversible inactivation of PME. PME exist as isozymes in citrus juices with different degrees of thermostability. The thermolabile enzyme is quickly inactivated, whereas the thermostable enzyme is gradually inactivated by exposure to high temperatures for extended periods of time. The residual

PME activity observed in juices is mostly due to the presence of a thermostable enzyme.

A decrease in activity during storage was observed in PEF and UV treated juice (Figure 5-6). In the control juice, the PME activity reduced to 65% after 4 week of storage. Since alkaline is optimum pH for PME, a loss in activity during storage under acidic conditions is expected (McFeeters RF 1983). Because grapefruit juice is acidic in nature, a loss in PME activity during storage is expected. An exponential decay in PME activity in untreated orange juice during storage has been reported by Elez and others (2006).

Effect of Treatments on Non-enzymatic Browning

The effect of thermal and non-thermal treatments on the brown color of grapefruit juice during 4 weeks of storage at 4 ºC is given in Figure 5-7. All treated juice showed a significant increase ($p<0.05$) in the browning index compared to the control value of 0.069 at week 0. The browning index values at week 0 for thermal, RFEF, UV and PEF were 0.0835, 0.080, 0.073 and 0.072, respectively. Browning in citrus juice is mainly due to Maillard reactions between sugars, amino acids and ascorbic acid degradation products (Clegg 1964). Browning is commonly observed in heat treated citrus juice and is affected by storage temperature and time (Nagy et al. 1990). The Maillard reaction between reducing sugars and amino acids results in formation of melanoids, which give the brown color to juice. However, in citrus juices the importance of Maillard-type browning is minor due the acidity of these juices, which is not favorable for Maillard-type reactions. A significant increase ($p<0.05$) in brown color was noted in all treated juice samples during 4 weeks of storage compared to initial week 0 values. The browning index after 4 weeks for thermal, RFEF, UV, PEF and control were 0.097, 0.094, 0.086,

0.083 and 0.083, respectively. The browning index for thermal and RFEF treated juices are significantly higher ($p<0.05$) compared to the control, PEF and UV treated juices.

Effect of Treatments on Ascorbic Acid Degradation

The initial concentration of ascorbic acid in grapefruit juice was measured at 69.86±1.92 mg/100mL of juice. After pasteurization, a significant loss ($p<0.05$) in ascorbic content was noted in all treated samples (Figure 5-8). Thermal processing reduced the ascorbic acid content by 29.19% in grapefruit juice. RFEF, PEF and UV treatment showed 26.33%, 16.77% and 19.72% loss, respectively, in ascorbic acid content at week 0. Ascorbic acid is a sensitive biomolecule, and therefore treatment temperature and oxygen availability affects its rate of degradation (Sadler and others 1992; Lee and Coates 1999). Since all treated juice samples were exposed to temperatures above ambient, a decrease in ascorbic acid content is expected. During storage, a further decrease was noted in all treated samples. After 4 weeks of storage, thermally treated samples had the highest ascorbic acid loss ($p<0.05$) compared to non-thermally treated juice samples. Ascorbic acid undergoes oxidative degradation to form dehydroascorbic acid, which further reacts with amino acids to form brown color compounds. Lee and Nagy (1988) reported a high correlation between the percentage loss of ascorbic acid and an increase in browning in grapefruit juices. To confirm whether similar correlation exists in present study, a linear regression plot of ascorbic acid content versus browning index is given in Figure 5-9. A negative correlation of 0.97 and 0.92, respectively were found for RFEF and thermally treated juices. The high correlation coefficients indicate that degradation of ascorbic acid is a probable explanation for the increase in brown color in treated samples during storage. Untreated grapefruit juice had the highest ascorbic acid content compared to treated juice samples

after 4 weeks of storage. Sadler and others (1992) also found greater ascorbic acid retention in untreated orange juice compared to thermally treated juice during a 50-day storage period. It was proposed that increased microbial population in untreated juice may have lowered the dissolved oxygen content and prevented AA oxidation.

Effect of Treatments on Color

The effect of different treatments on color of grapefruit change was determined using Hunter L*, a* and b* values during 4 weeks of storage at 4 ºC. The change during storage on L* and b* values are shown in Fig. 5-10 and 5-11, respectively. The L* values increased significantly ($p<0.05$) for thermal and RFEF pasteurized juices compared to control juice at week 0. This is contradictory to the browning index value, where an increase in browning was noted for thermal and RFEF samples. Therefore, these results need to be considered with caution, as they could be due to analytical error or to bottle-to-bottle sampling variation. No significant change ($p<0.05$) in L* was noted in PEF and UV treated juice compared to control at week 0. A significant increase ($p<0.05$) in b* values for all treated samples were noted at week 0, indicating a yellowing of samples. However, during 4 weeks of storage, the b* values significantly decreased for thermal and RFEF juice. The decrease in b* values could possibly be due to increased browning in grapefruit juice. The linear regression plot between b* versus the browning index gave a correlation coefficient of 0.94 and 0.91 for thermal and RFEF treated juices, respectively (Figure 5-12). These values indicate a strong relationship between browning and decrease in b* values in stored RFEF and thermal samples. Cortes and others (2008) reported a decrease in b value during storage in grapefruit juice. No significant ($p<0.05$) effect of treatment was observed for a* values during storage (data not shown).

Total color difference values (dE) were calculated to determine the magnitude of color difference between treated and control grapefruit juice (Figure 5-13). A delta value of two or higher must be present in order for noticeable visual difference to be observed (Lee and Coates 1999). In the present study, calculated delta values were less than 2 for all treated juices throughout the storage period. Even though L* and b* value changed during storage, visually no noticeable differences were found in treated grapefruit juices compared to control juice.

Effect of Treatments on pH, Brix and Total Acidity

Measured pH values in control, thermal, UV, RFEF and PEF treated grapefruit juice are given in Table 5-2. The pH values were not significantly ($p<0.05$) affected by either processing or storage for treated juice compared to control juice.

Brix value for control grapefruit juice was measured at 10.42. No significant differences ($p<0.05$) in Brix values were noted at week 0 in treated samples. During storage, a significant decrease ($p<0.05$) was noted in PEF and UV after 4 weeks of storage. This decrease could be attributed to the utilization of sugars during fermentation by microorganism. A similar change in Brix due to microbial growth has been reported by many authors (Yeom et al. 2000b; Min et al. 2003a).

Total titratable acidity for control and treated juice was measured and expressed as gm citric acid equivalents/100mL of grapefruit juice. Titratable acidity values were not significantly ($p<0.05$) affected by either processing or storage for treated juice compared to control juice.

Conclusion

The effect of thermal and non-thermal pasteurization techniques on the quality of red grapefruit juice were compared to untreated juice for 4 weeks of storage at 4 ºC.

The treatment conditions were optimized to achieve equivalent 5 log reductions in the inoculated *E. coli* population. Thermal treatment was more effective compared to non-thermal treatment in terms of microbial stability at after 4 weeks storage. However, RFEF treatment ensured microbial safety of grapefruit juice for 3 weeks of storage with significantly ($p<0.05$) higher Vitamin C content and equivalent PME inactivation compared to thermally treated juice. PEF and UV treatments maintained good microbial quality for 1 week with significantly ($p<0.05$) higher vitamin C content, as well as less browning, compared to thermal treatment. The results from the study showed that at equivalent processing conditions, non-thermally treated juice had a shorter shelf life but higher vitamin C content and better physical qualities compared to thermally treated juice.

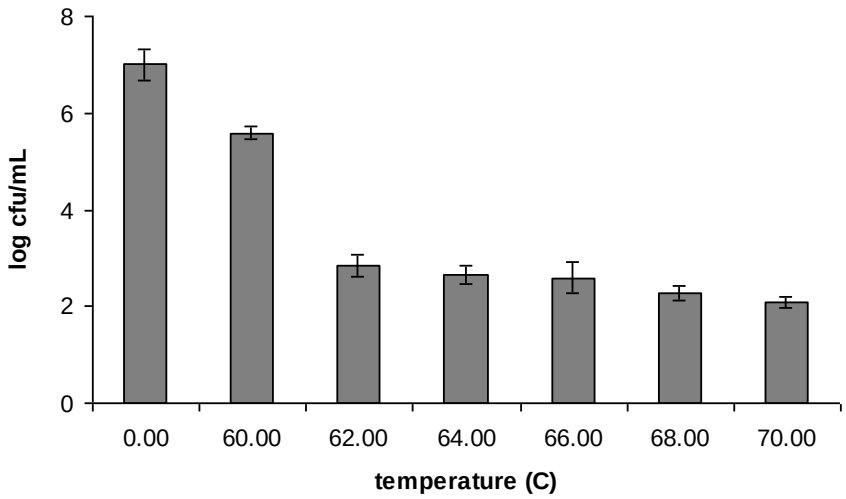

Figure 5-1. Thermal inactivation of inoculate *E. coli* K12 in grapefruit juice

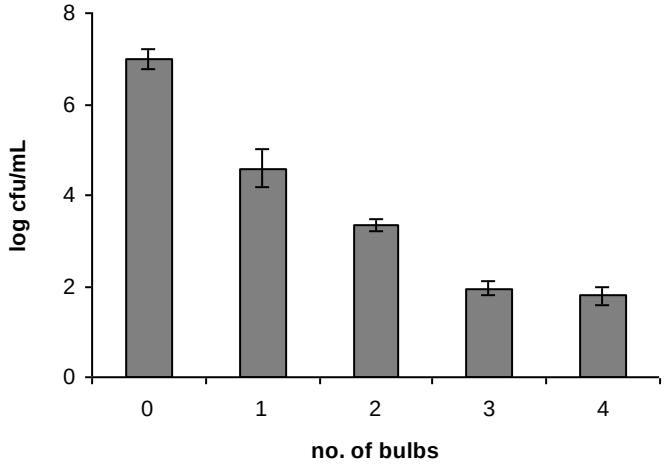

Figure 5-2. UV Inactivation of inoculated *E. coli* K12 in grapefruit juice

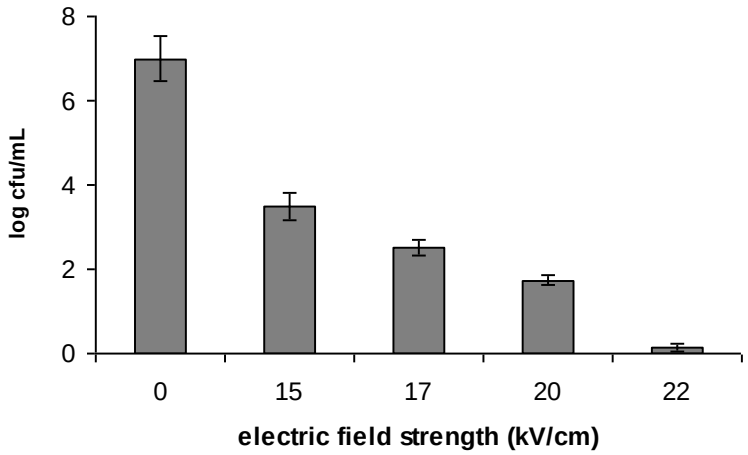

Figure 5-3. PEF inactivation of *E. coli* K12 in grapefruit juice

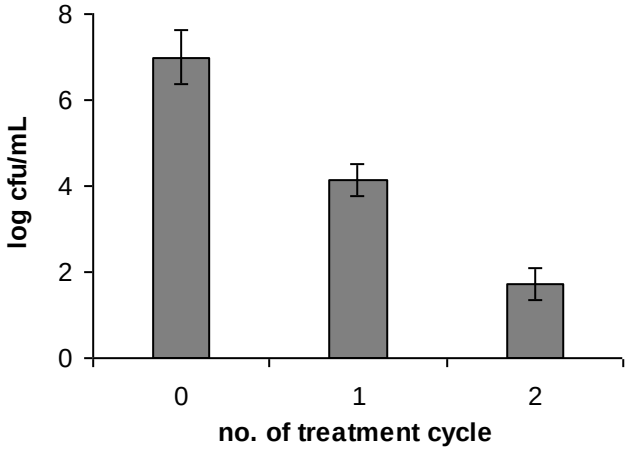

Figure 5-4. RFEF inactivation of inoculated *E. coli* K12 in grapefruit juice

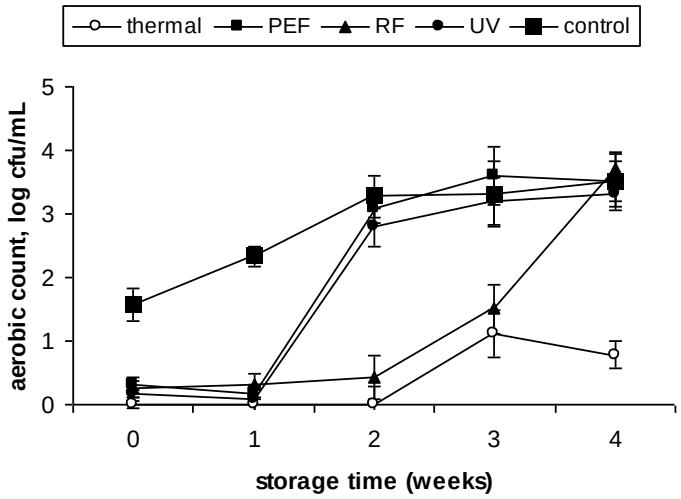

Figure 5-5. Total aerobic plate count for control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks

Table 5-1. Yeast & mold count in control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks

Week	Control	Thermal	PEF	RFEF	UV
0	2.46±0.26	0	1.36±0.18	0	1.76±0.52
1	3.90±0.18	0	2.52±0.42	1.27±0.38	3.05±0.27
2	mold	0	mold	mold	Mold
3	mold	2.24±0.36	mold	mold	Mold
4	mold	3.33±0.24	mold	mold	mold

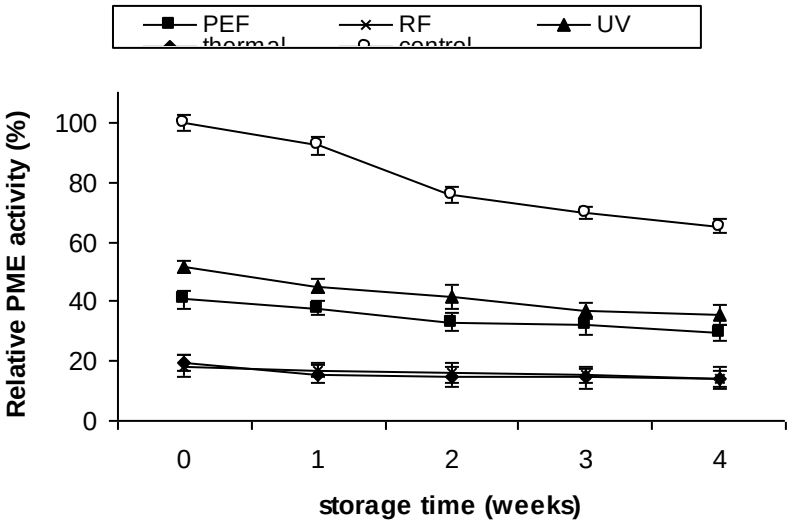

Figure 5-6. Change in PME activity in control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks

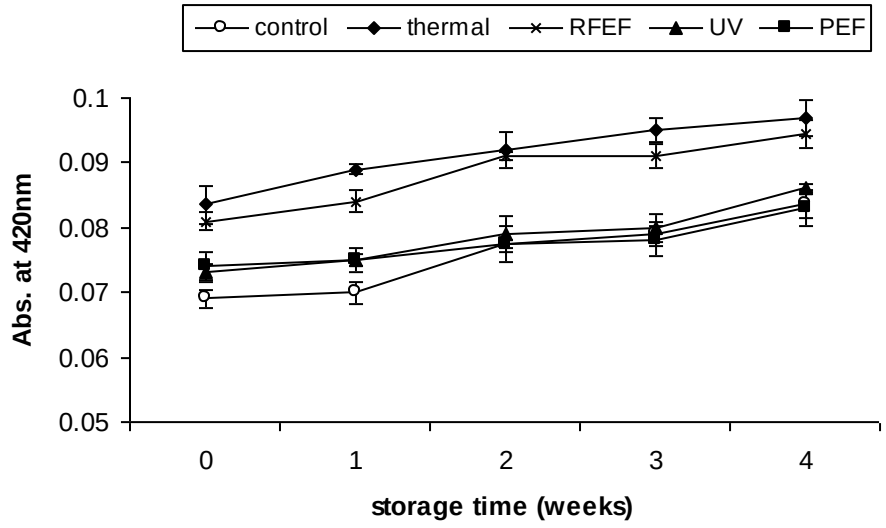

Figure 5-7. Change in browning index of control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks.

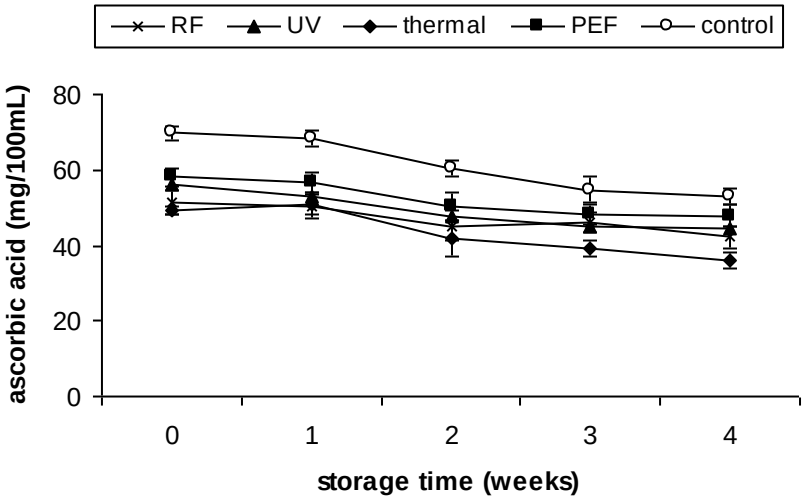

Figure 5-8. Change in ascorbic acid content of control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks

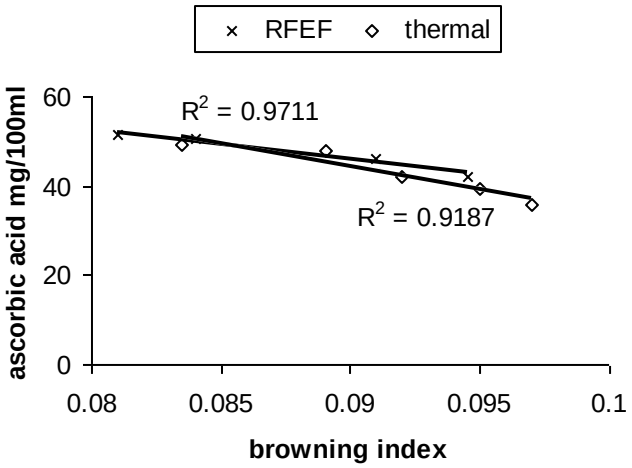

Figure 5-9. Linear regression plot of ascorbic acid vs. browning index for thermal and RFEF treated grapefruit juice

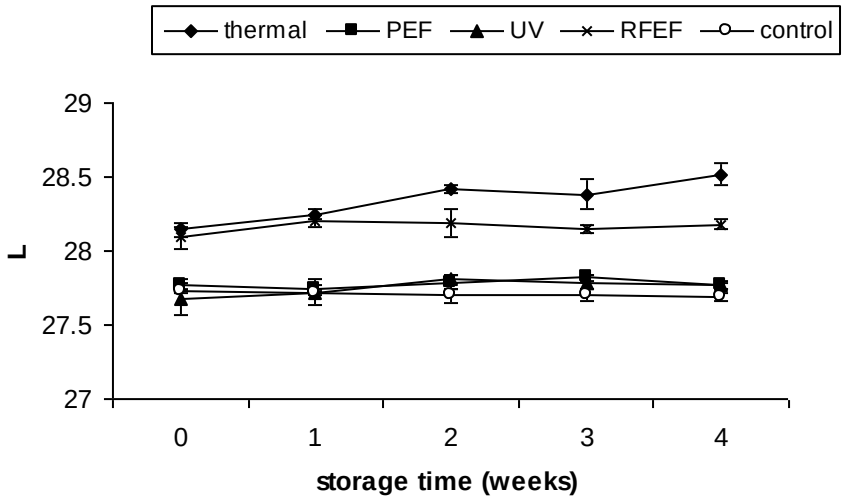

Figure 5-10. Change in color L* value of control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks

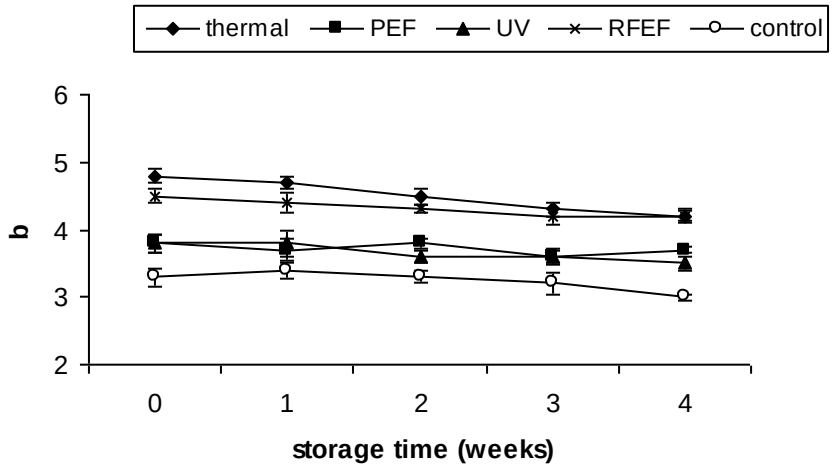

Figure 5-11. Change in color b* values of control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks

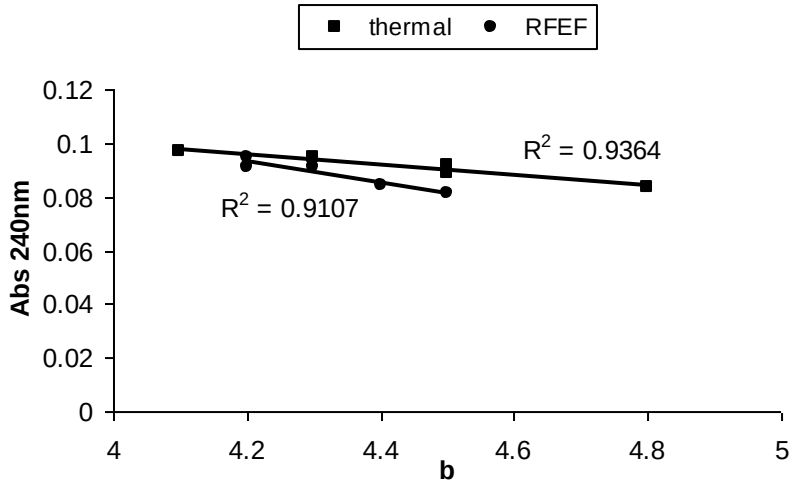

Figure 5-12. Linear regression plot of brown color vs. color b* values for thermal and RFEF treated grapefruit juice

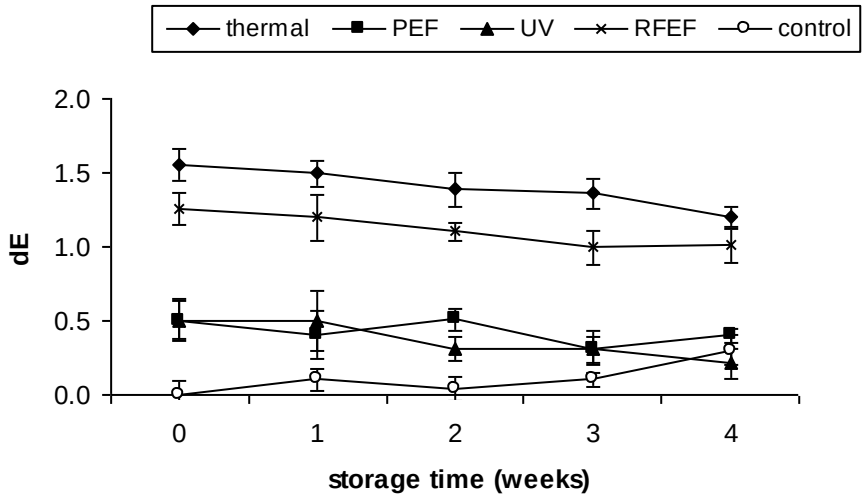

Figure 5-13. Change in total color difference (dE) in control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks

Table 5-2. pH, Brix and total acidity (TA) values for control, thermal, UV, PEF and RFEF treated grapefruit juice stored at 4 ºC for 4 weeks

	Weeks	Control	Thermal	PEF	RFEF	UV
pH	0	3.53 [a]	3.52 [a]	3.51 [a]	3.51 [a]	3.51 [a]
	1	3.51 [a]	3.50 [a]	3.50 [a]	3.52 [a]	3.51 [a]
	2	3.53 [a]	3.52 [a]	3.50 [a]	3.52 [a]	3.50 [a]
	3	3.52 [a]	3.51 [a]	3.51 [a]	3.53 [a]	3.52 [a]
	4	3.53 [a]	3.50 [a]	3.52 [a]	3.53 [a]	3.51 [a]
Brix	0	10.42 [a]	10.45 [a]	10.45 [a]	10.40 [a]	10.40 [a]
	1	10.41 [a]	10.40 [a]	10.43 [a]	10.42 [a]	10.42 [a]
	2	10.40 [a]	10.48 [a]	10.10 [b]	10.41 [a]	10.30 [a]
	3	9.91 [b]	10.50 [a]	10.00 [b]	10.40 [a]	10.00 [b]
	4	9.75 [c]	10.40 [a]	10.05 [a]	10.41 [a]	9.90 [b]
TA	0	1.20 [a]	1.31 [a]	1.10 [a]	1.13 [a]	1.17 [a]
	1	1.22 [a]	1.26 [a]	1.2 [a]	1.25 [a]	1.23 [a]
	2	1.20 [a]	1.30 [a]	1.15 [a]	1.15 [a]	1.18 [a]
	3	1.28 [a]	1.28 [a]	1.22 [a]	1.20 [a]	1.20 [a]
	4	1.20 [a]	1.25 [a]	1.11 [a]	1.16 [a]	1.19 [a]

CHAPTER 6
IMPACT OF THERMAL AND NON-THERMAL PROCESSING TECHNOLOGIES ON AROMA OF RED GRAPEFRUIT JUICE: GC-OLFATOMETRIC COMPARSION

Introduction

Non-thermal techniques like pulsed electric field (PEF), ultraviolet (UV) and high pressure (HPP) have been examined in the past for their efficiency in extending shelf life of juice while minimizing nutritional and quality loss. Comparative studies on the effect of PEF and thermal processing on orange juice has shown reduced loss of flavor, color, and vitamin C in PEF juice compared to thermal processing (Ayhan et al. 2001). However, there is only a single literature reference on PEF processed grapefruit juice. The authors (Cserhalmi et al. 2006) investigated the effect of PEF treatment on physical properties like pH, Brix, color, non-enzymatic browning and flavor components. No significant change in any parameter's post processing compared to fresh untreated juice was observed. The favor analysis was limited to 8 volatiles with no supporting sensory data.

RFEF, a recently developed non-thermal technique (Geveke 2005), has not been FDA approved yet. Few studies have been conducted in citrus juice on microbial inactivation kinetics or on ascorbic acid content and color of juice for non-thermal treatments (Geveke et al. 2007).

UV is commercially used for water disinfection and apple cider pasteurization. Studies on UV treated orange juice is limited due to low transmittance of UV light through the high pulp juice (Tran and Farid 2004b). UV irradiation of unclarified orange juice extended the shelf life by 5 days with 17% loss in ascorbic acid at 4ºC.

No data on UV or RFEF treated grapefruit juice has been found in the literature. Even though the major motivation for non-thermal processing technologies is minimal

change to organoleptic properties, it is surprising to note that little research has been conducted in this area.

Quantitative, as well as qualitative, studies on volatile constituents of grapefruit juice and essence have been extensively studied by many groups (Lin and Rouseff 2001; Coleman et al. 1972; Buettner and Schieberle 1999). Many odor active volatiles important to grapefruit aroma are present in low levels and are not usually detected by mass spectrometry or FID. GCO is a bioassay that measures human response to odorants separated by GC and is used to detect aroma active compounds. The present study focuses on comparing the impact of thermal and non-thermal pasteurization techniques on aroma active volatiles of fresh unpasteurized grapefruit juice during 4 weeks of storage at 4 ºC using GCO. As discussed in chapter 5, the non-thermal and thermal treatments were optimized to achieve equivalent 5 log reductions in microorganisms for fair comparison.

Materials and Methods

Standard compounds ethyl propionate, ethyl 2-methyl propionate, methyl butyrate, ethyl butyrate, ethyl 2-methyl butyrate, hexanal, b-pinene, b-myrcene, limonene, 2-methyl butanol, ethyl hexanoate, p-cymene, octanal, 2-methyl 3-furanthiol, nonanal, ethyl octanoate, methional, decanal, linalool, geranial, geraniol, a-ionone, g-decalactone, b-damascenone and 2-methoxy 4-vinyl phenol were purchased from Aldrich (St. Louis, MO). Natural 4-mercapto-4-methylpentan-2-one (1% in PG) was obtained from Oxford Chemicals (Hartlepool, UK).

Grapefruit Juice

One hundred and fifty litres of unpasteurized red grapefruit juice with no additives was procured from a commercial Florida processor. Shipping, storage and initial

treatment conditions have been described in chapter 5. Juice was filtered through 2 layers of cheesecloth before processing by PEF, RFEF, UV and thermal treatments.

Grapefruit Juice Processing

Preliminary experiments were performed to determine equivalent processing conditions using heat, pulse electric field (PEF) radiofrequency electric field (RFEF) and ultraviolet radiation (UV) for grapefruit juice. Juice was inoculated with *E. coli* K12 (ATCC 23716) from a stationary phase culture to give an approximately 7 log CFU/mL population. Microbial assays were conducted for juice heated from 60-72 ºC with UV exposure times of 6-25s at 50 ºC, 15-22 kV/cm electric field strengths for PEF treatment and 0-2cycles at 20kHz frequency and 15kV/cm for RFEF treatment. Final optimized processing conditions resulted in approximately 5 log reductions of *E. coli* K12 and were used for the remainder of the study.

Heat treatment

Grapefruit juice was heat pasteurized using a miniature scale HTST processing system (Armfield, Jackson, NJ, FT74-30-MkIII-33-34) explained previously in chapter 5.

Non-thermal juice treatments

The UV, PEF and RFEF juice treatment conditions have been described in chapter 5.

Packaging and storage

Processed grapefruit juice was collected directly from the processing unit outlet into sterile 1L media glass bottles (Corning Inc, Corning, NY) inside a sanitary laminar hood equipped with a HEPA air filter(Forma Scientific Inc., Marietta, OH). The hood was sanitized by UV lighting at 254nm for 30min before use and then wiped with 100% alcohol. The bottled juice was stored at 4 ºC for 4 weeks. Volatile analysis of fresh

unpasteurized juice was performed at week 0. For all pasteurized juice samples, volatiles analysis and sensory analysis were conducted at week 0 and week 4 (the end of storage study). The reference sample used for week 4 sensory evaluation was untreated grapefruit stored at -17 ºC and thawed overnight at 4 ºC one day before analysis.

Extraction of Grapefruit Juice Volatiles

Aliquots (25 ml) of grapefruit juice were placed in 40 ml glass vials with screw caps containing Teflon-coated septa. Tetradecane (1µL of 1000ppm) was added as internal standard into the juice before equilibration. The juice was equilibrated for 15 min at 40 ºC with stirring. A 2 cm-50/30µm, DVB/ Carboxen™/PDMS Stableflex™ (Supelco,Bellefonte, PA) SPME fiber was exposed in the equilibrated headspace for 30 min at 40 ºC. The fiber was desorbed for 5 min in the GC injection port at 250 ºC. All samples were analyzed in quadruplicate.

Volatile Analysis of Grapefruit Juice Volatiles

A GC/MS/O instrument (6890N GC, 5973N MS, Agilent Technologies, Santa Carla, CA) and olfactory detector (Gerstel, Baltimore, MD) were used for separation and analysis of volatiles. The GC effluent was split between MS/O in a ratio of 1:3.

Samples were run separately on a polar DB-Wax and non-polar DB-5 column with identical dimensions (30 m x 0.32 mm x 0.5 µm from J&W Scientific, Folsom, CA). The column oven temperature was programmed between 40 ºC to 250 ºC at 7 ºC/min with a 5 min hold at 250 ºC. The injector and detector temperatures were set at 250 ºC.

Mass spectrometry conditions were as follows: transfer line temperature at 275 ºC, mass range 30 to 300 amu, scan rate 5.10 scan/s and ionization energy 70 eV. Helium was used as the carrier gas at a flow rate of 2 mL/min. Mass spectral matches were

compared with NIST 2002 standard spectra. Authentic standards were used for confirmation. Alkane linear index values were determined on both columns.

GCO analysis was performed using two trained sniffers, both non-smokers between ages of 30-35 yrs. The panelists were trained in a similar manner as reported by Bazemore and others (1999) using standard solutions of varying concentration of 10 compounds usually found in citrus juices: ethyl butyrate, octanal, hexanal, linalool, a-pinene, limonene, geranial, neral, citronellal and a-terpineol. The panelists were trained on intensity rating, optimum positioning and breathing techniques. The panelist seated at the sniffing port rated the intensity of volatility on a 4-point intensity scale using an olfactory pad (Gerstel, Baltimore, MD) and the aroma description was audio recorded. The aroma intensity scale ranged from 0= no aroma perceived to 4 = strong aroma perceived. The ODP output was integrated into the MSD Chemstation software (Agilent MSD Productivity Chemstation version D.02) to achieve TIC chromatogram and aromagram simultaneously. Samples were sniffed two times by each assessor, resulting in four aromagrams. The results from 4 aromagrams were averaged for each sample. Odor active compounds producing intensity responses at the same retention time at least 50% times were selected. The odor active compounds were identified based on sensory descriptor, retention index calculated, comparing RI and descriptor from two columns and authentic standards.

Sensory Evaluation

Screening and training of panelists

The screening for panelists was performed using two different commercially pasteurized grapefruit juices and fresh squeezed grapefruit juice. A total of 40 panelists were screened for their ability to differentiate between the samples in two sessions.

Panelists who were consistently correct in differentiating juice samples were selected for further training. Twenty-five panelists were selected after the initial screening. The panel consisted of 12 males and 13 females in an age group between 25-45 yrs. Selected panelists were further trained for 1- 2 hours using the same juice samples. They were trained to use verbal terminologies or descriptors to differentiate between samples and to use scaling procedures to determine the degree of difference between the samples.

Test method

A difference from control type of sensory evaluation (Meilgaard and others 1999) was employed to orthonasally detect differences in aroma between unpasteurized and pasteurized grapefruit juice. All tests were conducted once in a sensory panel facility at Eastern Regional Research Center (Wyndmoor, PA) with 6 booths equipped with computers. The sensory analysis was designed and conducted using Compusense five (Compusense Inc., Ontario, CA). A sample sensory ballot is shown in Appendix D. Samples were prepared by pouring 40 mL of juice into 100 mL glass bottles with airtight plastic caps and stored in boxes at 4 ºC overnight. On the day of testing, the bottles were taken out 1hr before the testing. Juice temperature was measured at 10-12 ºC during testing. Testing was performed under red light so that color and other visible differences were masked from panelists. The control sample given to panelists was fresh untreated grapefruit juice and labeled as “R.” The experimental samples consisted of the 4 treated juice samples (thermal, UV, PEF & RFEF). Each panelist received 1 R sample and 5 coded samples corresponding to the 4 experimental samples and 1 blind control (unpasteurized). Panelists were required to specify the degree of difference on a

scale of 0-10, 0 = no difference and 10 = extremely large difference. Panelists were also asked to give verbal descriptions of the difference between samples.

Statistical Analysis

Sensory data were subjected to statistical analysis using SAS 9.1 (SAS Inst. Inc., Raleigh, N.C., USA). The tests for statistical significance were performed by analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. To determine which means are significantly different from one another, Tukey's test ($\alpha = 0.05$) was applied.

Results and Discussion

Effect of Treatment and Storage on Grapefruit Juice Volatiles

The volatiles in grapefruit juice were quantified using an internal standard (tetradecane) by GC/MS. A total ion chromatogram (TIC) of volatiles from untreated grapefruit juice is shown in Appendix Figure C-1. The volatile concentrations for untreated and treated grapefruit at week 0 and week 4 are given in Appendix Tables C-1 and C-2, respectively. The percentage loss in volatiles was calculated for thermally and non-thermally treated grapefruit juice compared to untreated juice during storage (Table 6-1). Concentrations of all volatiles except α-terpineol and linalool were diminished in treated samples at week 0. P-cymenene lost more than 50% and nootkatone lost more than 40% during the same time period. After 4 weeks storage, hexanal and ethyl hexanoate were completely lost and decanal and octanal lost more than 80% in all treated samples. The loss in aldehydes during storage occurs due to their conversion into corresponding alcohols (Petersen and others 1998). Octanol levels increased (2-14%) in all treated juice samples. Alpa-terpineol increased by more than 80% in all treated samples. Alpha-terpineol is formed in stored orange juice by acid-catalyzed hydration of limonene and linalool (Petersen and others 1998). In the present

study, a 15-30% loss in limonene and linalool content was noted in treated samples. The results from week 0 and week 4 analysis suggest that the volatiles of grapefruit juice are more strongly affected by storage rather than treatment type.

Gas Chromatography Olfactometry Profile of Grapefruit Juice

A total of 38 aroma active components were detected in the headspace of fresh untreated and treated grapefruit juice, as listed in Table 6-2. Odorants were identified based on retention indices on two dissimilar columns, odor description and GCO data from standard compounds (except for compounds 24, 26 and 32). These compounds were identified based on retention index and odor descriptors. Nine odorants (1, 2, 19, 21, 28, 33, 34, 36 and 38) remain unknown. The odorants in GFJ could be classified into 5 groups based on similar aroma notes comparable to those of Lin and others (2002b): fruity/sweet, green, citrus/grapefruit, floral/musky/fragrant, terpeny/piney and cooked/ roasted/catty.

Sweet/fruity. The sweet/fruity category had the highest number of odorants (11 odorants). The majority were identified as esters: ethyl propionate, methyl butyrate, ethyl butyrate, ethyl 2-methyl butyrate, ethyl 3-methyl propionate, ethyl hexanoate and ethyl octanoate. Ethyl octanoate is one of the odorants with the highest aroma value in the present study. Other compounds belonging to this group were b-damascenone (30), g-decalactone (35) and two unknowns (33, 34).

Fresh/citrus. Six odorants belonged to the fresh/citrus group. They include aldehydes (15, 17, 23, 27), alcohol (30) and terpene hydrocarbon (11). Aldehydes such as octanal, nonanal, decanal and geranial are common to citrus fruits like orange, grapefruit, and lemon (Allegrone and others 2006; Lin et al. 2002b). Limonene with

citrus aroma has been shown to be an important ingredient in orange juice by omission studies in model flavor mixtures (Buettner and Schieberle 2001).

Cooked/roasted/catty. The group includes the 4 sulfur compounds: 2-methyl 3-furnathiol (MFT), methional, bis (2-methyl 3-furfuryl) disulfide (MFT-MFT), 4-mercaptone 4-methyl 2-pentanone (4-MMP) and two unknowns (19, 38). The meaty odorant MFT is a product of thiamine degradation under acidic conditions. It has low odor threshold at 0.007ppm in water. It can also be produced by Maillard reaction between cysteine and pentose sugar and is often found in heat treated citrus juices (Perez-Cacho and Rouseff 2008). It readily forms a dimer, MFT-MFT, which also has a meaty odor. Methional has a cooked potato odor and is reported in many fresh fruits like orange, tomato and grapefruit (Perez-Cacho and others 2008). Its biosynthetic pathway in plants is unknown, but it can be formed in processed juices by Strecker degradation from the amino acid methionine during thermal treatments and subsequent storage. Methional and 2-methyl 3-furanthiol are reported as possible off flavors in orange juice (Bezman and others 2001). 4-MMP is significant for characteristic grapefruit aroma. It has very low odor threshold of 0.1ng/L in water (Buettner and Schieberle 2001). It is reported to possess a grapefruit aroma at very low concentrations and a catty sulfurous odor at higher concentrations.

Green/fresh. The green/fresh odor of grapefruit juice is imparted by odorants hexanal, p-cymene, decanal and 4, 5-epoxy (E)-2-decenal and one unknown (22). Odorant 4, 5-epoxy (E)-2- decenal has a green metallic odor and is a key ingredient in fresh grapefruit juice (Buettner and Schieberle 2001).

Floral/fragrant. Linalool, 4-vinyl guaiacol, a-ionone and 2 unknowns (1, 28) have a floral fragrant aroma. Linalool, with its intense floral aroma, is another component that is commonly found in citrus fruits. Para-viny guaiacol is formed from ferulic acid present in citrus peels (Perez-Cacho and Rouseff 2008). The presence of this compound is usually an indicator of thermally abused or aged juice. Alpha-ionone is formed from their carotenoid precursors in orange juice (Mahattanatawee and others 2005).

Terpeny. Only two odorants, b-pinene and b-myrcene, are responsible for the terpeny odor in grapefruit juice.

In fresh untreated GFJ, 24 odor active compounds were perceived. GCO analysis revealed ethyl octanoate and octanal as having the highest aroma intensities, followed by ethyl butyrate, ethyl hexanoate, methional and decanal. The results are different from Buettner and others (2001) who reported high FD values for ethyl butyrate, (Z)-3-hexenal, 1-hepten-3-one, 4-mercapto-4-methyl 2-pentanone, 1-p-menthene -8-thiol, 4,5-epoxy-(E)-2-decenal and wine lactone in fresh white Marsh grapefruit juice by aroma extract dilution analysis . Except for ethyl butyrate and 4, 5-epoxy-(E)-2-decenal, other volatiles were not detected in fresh juice in our study. Lin and others (2001) also found qualitative, as well as odor intensity, differences for the majority of odor active compounds when compared to results from Buettner and others (2001). Possible reasons for the differences could be due to variety, fruit quality, juice extraction methods, storage time and temperature, volatile extraction method and GCO analysis methods. The grapefruit variety used by Buettner and others (2001) was white Marsh, whereas in the present study red grapefruit was used. Secondly, the extraction method used by Buettner and others (2001) was liquid extraction, compared to static headspace

SPME used in this work. The quantitative and qualitative volatile profile depends on the extraction method used. Rouseff and others (2001) showed that the terpenes limonene, a-pinene and myrcene comprised 86% of the total FID area by SPME compared to 24% from liquid extraction of the same orange juice. Low vapor pressure compounds are not readily extracted by SPME. This could be the reason for absence of wine lactone in our study. Reconstitution of odorants in a model grapefruit juice flavor mixture by Buettner and others (2001) showed that omission of 4-MMP resulted in a more orange-like odor rather than grapefruit odor. The authors concluded that 4-MMP is important for typical grapefruit juice odor. Surprisingly, in our studies the volatile was found only in UV and thermally treated juice (but not in fresh juice). The possible reasons for the absence of 4-MMP in our studies could be the different extraction methods used. The concentration of 4-MMP in juice is very low, ranging from 0.8-1ng/L (Buettner and others, 2001). Possibly, the HS-SPME used in the present study may not be as efficient as solvent extraction of 4-MMP at such low levels. Sulfur compound, 1-p-mentha-8-thiol with a typical grapefruit like odor is reported as a character impact odorant in grapefruit juice. However, the significance of this compound to grapefruit juice aroma is debatable, since Lin and others (Lin et al. 2002b) reported the presence of this thiol only in concentrated juice and not in the original juice. They proposed that the compound was possibly a reaction product of limonene or a-pinene with hydrogen sulfide in thermally abused juices, and therefore may not be present in fresh untreated juice.

Gas Chromatography Olfactometry Profile Comparison of Fresh Untreated and Treated Juice at Week 0

The total number of odorants perceived in fresh, UV, thermal, PEF and RFEF treated GFJ were 24, 27, 23, 18 and 20, respectively (Table 6-2). A comparative profile

based on the six odor categories for treated and untreated juice is plotted as a spider web diagram in Figure 6-1. The panel average aroma intensities for odorants belonging to same odor category are added to get the total odor intensity for that particular group (Table 6-3). It is evident from Figure 6-1 that the aroma profile of fresh juice was distinctly different from all treated juices. In general, a decrease in fruity/sweet and citrus/fresh notes is observed for treated juices compared to fresh. Cooked/meaty/catty odor increased in thermal and UV juice considerably. Terpeny odor was the least affected by pasteurization techniques. The impact of treatments on odorants belonging to the six aroma categories are discussed in detail below.

Thermal treatment

The total odor intensity for fruity/sweet, citrus/fresh and green/metallic decreased in thermally treated juice by 4.7, 3.4 and 2.2 points, respectively, compared to fresh juice. In the fruity/sweet category, esters like ethyl butyrate, methyl butyrate and ethyl 2-mehtyl butyrate had lower a intensity, whereas ethyl propionate was completely absent. Nonanal, geraniol and geranial belonging to the fresh/citrus group were absent in thermal samples. The green metallic odorant 4, 5-epoxy (E)-2-decenal, important for grapefruit odor, was also missing in thermally processed juice. The results indicate the instability or degradation of the above compounds in the presence of heat. The sweet odorant b-damascenone was detected in thermal juice but not in untreated juice. The compound has been reported in reconstituted grapefruit juice, which is thermally processed (Lin and others 2002b). They proposed that there is a release of gylcosydically bound b-damascenone by acid hydrolysis during heating. A cooked/meaty/catty odor was increased in thermal juice by 4.8 points compared to fresh juice due to the formation of MFT, MFT-MFT, 4-MMP and an unknown (19). The meaty

odorant MFT could be formed either by Maillard reaction between cysteine and pentose or from thiamin degradation. The level of cysteine present in red grapefruit juice is 15.3 µmol/L, and pentoses are present in ample amounts in grapefruit juice (Kusmierek and Bald 2008) Rouseff and others, 2001). Formation of MFT via the Maillard reaction during heat treatment in grapefruit juice is possible. MFT dimerizes in juice to form the meaty odorant bis (2-methyl-3-furfurl) disulfide. 4-MMP is present in grapes as a cysteine conjugate that is released during fermentation by yeast enzymes. Thermal hydrolysis could result in the release of 4-MMP from such a precursor in heat treated juice.

Ultraviolet treatment

No major change in sweet/fruity intensity was noted in UV treated juice. A decrease in citrus/fresh and green/metallic odor by 3 and 1.2 points, respectively was noted due to the absence of odorants (Z)-2-nonenal, geranial, geraniol and 4,5-epoxy (E)-2-decenal. Beta-damascenone was also detected in UV treated juice. Thermal degradation of neoxanthin in a model system containing peroxy acetic acid produced β-damascenone. However, the reaction temperatures used for this synthesis were high (60-90 ºC) and lasted for extended periods of time (Bezman et al. 2001). The mechanism of formation and release of β-damascenone in UV treated samples is not known.

An increase in cooked/catty odor by 4.2 points was observed in UV treated juice. The catty odor of 4-MMP was more intense in UV treated juice than in thermally treated juice. It is surprising to find the meaty odorant MFT-MFT in UV juice because of the absence of its monomer, MFT. The odor of MFT-MFT is $8.9x10^{-11}$ mM in water, whereas the odor threshold for MFT is $6.14x10^{-8}$ mM in water (Dreher and others, 2003).

Because the odor threshold of the dimer is lower than the monomer, it is possible that the concentration of 2-methyl 3-furnathiol may be present below odor detection limits in UV juice.

Pulsed electric field treatment

Similar to thermal treatment, a decrease in fruity/sweet as well as citrus/fresh odor was noted in PEF juice. The compound 4, 5-epoxy (E)-2-decenal was also missing in PEF treated juice. The increase in cooked/catty odor was less compared to that of thermal and UV treated juice.

Radio frequency electric field treatment

RFEF treated grapefruit had an odor profile similar to PEF juice. This is expected because both pasteurization techniques use electric field strength for inactivation of microorganisms. The aroma profile had lower sweet/fruity and citrus characters compared to fresh juice and juice with a slightly higher cooked/meaty odor.

Gas Chromatography Olfactometry Comparison of Fresh Untreated and Treated Juice at Week 4

After 4 weeks of storage, the total number of odorants decreased to 14, 13, 12 and 15, respectively for thermal, PEF, RFEF and UV treated juice (Table 6-4). 14 total odorants were missing in week 4 treated samples compared to untreated juice analyzed at week 0. Guaiacol, with a medicinal odor, was detected only in UV treated samples due to microbial contamination. This compound is formed by spoilage microorganisms like *Alicyclobacillus* sp. in stored juice (Gocmen D 2005). However, it was not detected in PEF and RFEF samples, even though both these samples had microbial contamination. A possible reason for this discrepancy could be bottle-to-bottle variation in microbial levels during sampling.

Sensory Evaluation

A difference from control type of sensory analysis was performed on grapefruit juice aromas at week 0 and week 4 (Appendix D-1 and D-2). This type of sensory analysis tells us whether a difference exits between the samples and tells us the magnitude of difference between two samples. The data recorded by 25 panelists for differences from the control test between pasteurized and unpasteurized grapefruit juice at week 0 is given in Appendix D-1. Statistical significance between samples was determined using an ANOVA test at 95% confidence levels through an F test. Table 6-5 shows that the Fcal value is greater than the Fcrit value. This indicates a statistical difference between sample means at week 0.

Tukey's test is a single-step multiple comparison procedure. It is performed in conjunction with ANOVA to find if there is significant difference between treatment means. The HSD (honestly significant difference) value calculated was 1.58 for week 0 mean values. An HSD value higher than the difference between two sample means indicates a significant difference between two treatment means. It is evident from Table 6-6 that the difference in mean values between treatments is lower than the HSD value. The mean values for treated juice are significantly ($p<0.05$) different from each other because the calculated HSD is greater than the treatment differences. The difference in means is highest between UV and fresh untreated juice. The general comments from panelists for UV treated juice were that the juice had a rotten or spoiled odor. Because no microbial spoilage was noted in UV samples at week 0, the sensory comments are possibly a reflection of increased 4-MMP levels in UV samples. Thermally treated juice was described as having a cooked odor. The presence of 2-methyl 3-furnathiol and bis (2-methyl-3-furfuryl) disulfide would give a cooked note to the juice. According to

panelists' comments, both PEF and RFEF juice had weaker/less fresh aromas compared to untreated juice

The ANOVA results for week 4 sensory analyses (Table 6-7) indicate a significant difference ($\alpha = 0.05$) between sample means. The HSD value calculated was 1.85. Table 6-8 shows that the difference in means between PEF and thermal samples are higher than the HSD value, indicating a significant difference ($p<0.05$) between the two samples. No significant difference ($p<0.05$) in sample means was noted between RFEF, UV and thermally treated juices. Panelists commented that all treated juice samples except thermal had a fermented, rotten odor. This is due to microbial spoilage detected in PEF, RFEF and UV samples after 4 weeks of storage (chapter 5). Thermal samples had a cooked odor.

Conclusion

The results from this study indicate that thermal as well as non-thermal pasteurization techniques affected the aroma profile of fresh grapefruit juice. The effects of both thermal and non-thermal treatments on juice aroma were characterized by losses in desirable fruity and citrus odorants, along with an increase in undesirable cooked/catty odorants. Aroma of treated juices differed significantly ($p<0.05$) from fresh juice by sensory evaluation, further confirming GCO results. Further sensory studies, such as preference evaluation, need to be conducted to understand consumer preferences between thermally or non-thermally treated grapefruit juice.

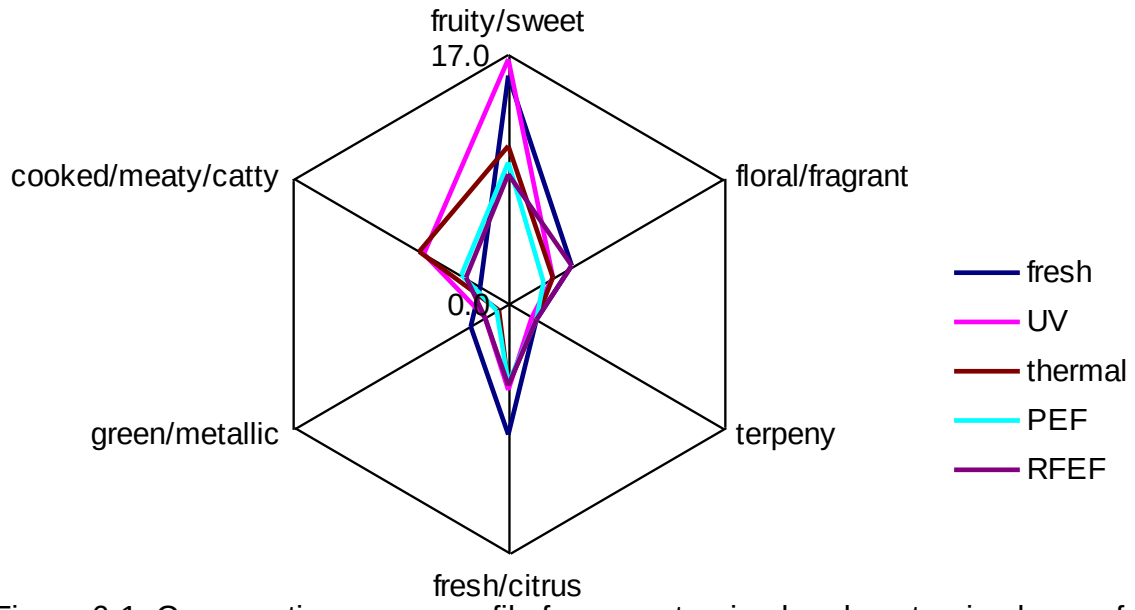

Figure 6-1. Comparative aroma profile for unpasteurized and pasteurized grapefruit juice at week 0

Table 6-1.Change in GC-MS grapefruit juice volatiles during 4 weeks of storage at 4 ºC

RI	Compound Identified	%loss week0 PEF	RFEF	UV	Thermal	%loss week4 PEF	RFEF	UV	Thermal
888	ethyl acetate	9.88	13.64	16.54	10.50	41.59	29.87	26.68	37.17
1037	a-pinene	0.84	3.94	3.72	1.43	15.28	38.81	20.24	32.90
1049	ethyl butyrate	19.69	33.23	28.59	30.62	45.90	53.40	88.66	53.75
1098	Hexanal	1.70	9.91	4.58	2.69	100.00	100.00	100.00	100.00
1174	b-myrcene	3.47	6.39	7.40	7.55	8.55	15.92	20.42	31.76
1219	Limonene	4.59	8.20	13.34	12.01	14.38	26.99	25.09	24.77
1243	ethyl hexanaote	21.05	20.53	29.09	18.55	100.00	100.00	100.00	100.00
1260	3-carene	18.02	26.74	27.10	14.34	82.91	31.74	57.64	58.31
1285	p-cymene	35.71	29.30	25.45	25.98	44.67	40.00	53.48	55.83
1299	Octanal	6.55	13.58	17.16	5.20	94.72	95.54	95.38	94.39
1400	Nonanal	6.01	23.40	14.78	23.17	40.85	69.26	55.48	71.35
1436	ethyl octanoate	15.49	32.89	4.61	13.11	19.81	29.21	24.15	22.71
1447	p-cymenene	54.37	57.67	67.79	51.16	75.91	72.78	76.10	78.63
1501	Decanal	4.74	2.18	8.75	3.35	80.84	56.23	83.77	87.71
1506	a-copaene	1.74	29.81	16.12	14.29	29.32	41.45	46.41	57.69
1537	Linalool	14.33	10.66	13.74	13.52	14.86	30.30	27.01	23.88
1549	1-octanol	0.57	-1.98	-4.42	-5.53	-2.14	-6.00	-6.11	-13.78
1608	b-caryophyllene	0.14	1.31	1.48	0.91	5.16	2.17	4.39	10.15
1670	a-caryophyllene	2.16	2.00	1.73	2.53	19.37	10.88	3.84	11.95
1709	Valencene	11.35	32.55	19.00	12.55	65.39	67.03	62.01	63.28
1721	a-selinene	0.38	3.52	3.16	2.02	4.46	2.11	11.12	11.44
1728	a-terpineol	-13.97	-3.35	-4.91	-8.43	-80.87	-99.71	-98.34	-85.67
1735	d-cadinene	13.71	27.72	10.97	23.95	31.93	34.74	31.93	34.74
1747	a-panasinsen	7.81	9.19	5.21	11.94	21.89	28.72	25.21	31.97
2677	Nootkatone	39.35	46.81	47.98	47.65	48.10	53.52	56.80	55.97

Table 6-2. GCO analysis results of pasteurized and unpasteurized grapefruit juice

No.	DB-5	DB-Wax	descriptor	Compound	untreated	UV	Thermal	PEF	RFEF
1		811	floral lemony	Unknown	-	-	1.3	-	-
2		823	bad off	Unknown	-	0.5	-	-	-
3		932	ethanol	ethyl propanoate	1.5	1.0	-	-	-
4	912	1018	fruity ethanol	ethyl 2-methyl propionate	2.3	2.0	1.3	1.8	1.5
5	782	1033	Sweet	methyl butyrate	2.5	2.5	1.5	1.0	1.5
6	803	1046	fruity sweet	ethyl butyrate	2.5	2.5	2.5	2.3	1.5
7		1058	fruity strawberry	ethyl 2-methyl butyrate	2.0	1.0	1.3	1.0	0.8
8		1091	green grass	Hexanal	1.0	1.0	0.8	1.0	1.0
9		1117	Piney	b-pinene	1.0	1.0	0.5	0.8	1.0
10	974	1172	musty terpene	b-myrcene	1.3	1.0	1.8	1.5	1.3
11	1022	1217	fresh citrusy minty	Limonene	1.3	1.0	1.3	1.3	1.0
12	1030	1227	sweet fruity	2-methyl butanol	0.8	-	-	-	-
13	979	1241	sweet fruity	ethyl hexanoate	2.5	2.3	2.0	2.5	2.0
14		1285	green apple skin	p-cymene	-	0.8	-	-	-
15	985	1296	fresh citrus	Octanal	3.0	2.5	2.3	1.8	2.0
16	856	1326	meaty roasted	2-methyl 3-furanthiol	-	-	1.5	-	-
17		1372	green citrus	Nonanal	1.5	1.0	0.5	1.0	0.8
18	961	1400	sulfur catty	4-mercapto 4-methylpentan-2-one	-	2.5	1.0	-	-
19		1428	cooked unpleasant	Unknown	-	-	0.8	-	-
20	1177	1434	Fruity	ethyl octanoate	3.0	1.5	1.0	1.8	2.0
21		1448	grass musky	Unknown	-	-	-	-	0.8
22	889	1460	cooked potato	Methional	2.3	2.0	2.5	2.3	2.0
23	1190	1504	citrus green	Decanal	2.0	1.5	1.5	1.5	1.8
24		1517	fatty green	(Z)-2-nonenal*	1.0	-	-	-	-
25	1081	1535	floral fresh	Linalool	1.8	1.5	2.0	1.0	1.5
26		1661	cooked meaty	bis (2-methyl-3-furfuryl) disulfide*	-	1.5	1.3	1.5	1.3

Table 6-2. Continued

No.	DB-5	DB-Wax	descriptor	Compound	untreated	UV	thermal	PEF	RFEF
27		1749	sweet citrus	geranial	0.8	-	-	-	-
28		1789	floral	unknown	-	-	-	-	1.5
29		1822	sweet	b-damascenone	-	0.5	0.5	-	-
30		1840	citrusy floral	geraniol	0.5	-	-	-	-
31		1873	floral sweet	a-ionone	1.5	-	-	-	-
32		1985	green metallic	4,5-epoxy (E)-2-decenal*	1.0	-	-	-	-
33		2013	sweet fruity	unknown	-	0.5	-	-	-
34		2055	fresh fruity	unknown	-	0.8	-	-	-
35	1489	2069	buttery fragrant peachy	g-decalactone	1.5	1.8	1.8	1.0	1.5
36		2163	sweet ethanolic	unknown	-	1.8	-	-	-
37	2196	2187	musky cologne	4-vinyl guaiacol	1.8	2.0	1.5	1.8	2.0
38		2240	bad solvent	unknown	-	1.0	-	-	-

* Odorants identified based on odor descriptor and retention indices with reported data due to unavailability of data

Table 6-3. Calculated total aroma intensity values for pasteurized and unpasteurized grapefruit juice belonging to five odor categories

Odor category	Compounds	Aroma intensity fresh	UV	thermal	PEF	RFEF
fruity sweet	ethyl propanoate	1.5	1.0	-	-	-
	ethyl 2-methyl propionate	2.3	2.0	1.3	1.8	1.5
	methyl butyrate	2.5	2.5	1.5	1.0	1.5
	ethyl butyrate	2.5	2.5	2.5	2.3	1.5
	ethyl 2-methyl butyrate	2.0	1.0	1.3	1.0	0.8
	2-methyl butanol	0.8	-	-	-	-
	ethyl hexanoate	2.5	2.3	2.0	2.5	2.0
	b-damascenone	-	0.5	0.5	-	-
	unknown	-	0.5	-	-	-
	unknown	-	0.8	-	-	-
	g-decalactone	1.5	1.8	1.8	1.0	1.5
	unknown	-	1.8	-	-	-
	total intensity	15.5 *	16.6	10.8	9.6	8.8
fresh/citrus	limonene	1.3	1.0	1.3	1.3	1.0
	octanal	3.0	2.5	2.3	1.8	2.0
	nonanal	1.5	1.0	0.5	1.0	0.8
	geranial	0.8	-	-	-	-
	decanal	2.0	1.5	1.5	1.5	1.8
	geraniol	0.5	-	-	-	-
	total intensity	9.0	6.0	5.6	5.5	5.6
green/metallic	hexanal	1.0	1.0	0.8	1.0	1.0
	p-cymene	-	0.8	-	-	-
	unknown	-	-	-	-	0.8
	(Z)-2-nonenal	1.0	-	-	-	-
	4,5-epoxy (E)-2-decenal	1.0	-	-	-	-
	total intensity	3.0	1.8	0.8	1.0	1.8
floral/fragrant	linalool	1.8	1.5	2.0	1.0	1.5
	unknown	-	-	-	-	1.5
	a-ionone	1.5	-	-	-	--
	4-vinyl guaiacol	1.8	2.0	1.5	1.8	2.0
	total intensity	5.0	3.5	3.5	2.8	5.0
Terpeny	b-pinene	1.0	1.0	0.5	0.8	1.0
	b-myrcene	1.3	1.0	1.8	1.5	1.3
	total intensity	2.3	2.0	2.3	2.3	2.3

Table 6-3. Continued

Odor category	Compounds	Aroma intensity fresh	UV	thermal	PEF	RFEF
cooked/meaty/catty	2-methyl 3-furanthiol	-	-	1.5	-	-
	unknown	-	0.5	-	-	-
	4-mercapto 4-methyl pentan-2-one	-	2.5	1.0	-	-
	methional	2.3	2.0	2.5	2.3	2.0
	bis (2-methyl-3-furfuryl) disulfide	-	1.5	1.3	1.5	1.3
	unknown	-	-	0.8	-	-
	total intensity	2.3	6.5	7.1	3.8	3.3

Table 6-4 GCO comparison of pasteurized grapefruit juice at week 4 to unpasteurized grapefruit juice at week 0

No.	DB-5	DB-Wax	descriptor	compound	untreated	UV	thermal	PEF	RFEF
1		932	Ethanol	ethyl propanoate	1.5	-	-	-	-
2	912	1018	fruity ethanol	ethyl 2-methyl propionate	2.3	-	-	-	-
3	782	1033	Sweet	methyl butyrate	2.5	1.5	1.0	1.0	1.0
4	803	1046	fruity sweet	ethyl butyrate	2.5	1.3	1.0	1.5	1.0
5		1058	fruity strawberry	ethyl 2-methyl butyrate	2.0	1.0	1.0	1.0	0.5
6		1091	green grass	Hexanal	1.0	-	-	-	-
7		1117	Piney	b-pinene	1.0	-	-	-	-
8	974	1172	musty terpene	b-myrcene	1.3	-	-	-	-
9	1022	1217	fresh citrusy minty	Limonene	1.3	-	-	-	-
10	1030	1227	sweet fruity	2-methyl butanol	0.8	-	-	-	-
11	979	1241	sweet fruity	ethyl hexanoate	2.5	-	-	-	-
12	985	1296	fresh citrus	octanal	3.0	1.0	1.5	1.0	1.0
13	856	1326	meaty roasted	2-methyl 3-furanthiol	-	-	1.0	-	-
14		1372	green citrus	Nonanal	1.5	-	-	-	-
15	961	1400	sulfur catty	4-mercapto 4-methylpentan-2-one	-	1.0	0.5	-	-
16	1177	1434	Fruity	ethyl octanoate	3.0	1.0	1.0	1.0	1.0
17	889	1460	cooked potato	methional	2.3	2.0	2.5	2.3	2.0
18	1190	1504	citrus green	decanal	2.0	1.5	1.5	1.5	1.8
19		1517	fatty green	(Z)-2-nonenal*	1.0	-	-	-	-
20	1081	1535	floral fresh	linalool	1.8	1.5	2.0	1.0	1.5
21		1664	stinky, rotten	unknown	-	1.0	-	0.5	-
22		1661	cooked meaty	bis (2-methyl-3-furfuryl) disulfide*	-	1.5	1.3	1.5	1.3
23		1720	grassy floral	unknown	-	-	-	-	1.3
24		1749	sweet citrus	geranial	0.8	-	-	-	-
25		1822	sweet	b-damascenone	-	-	0.5	-	-
26		1840	citrusy floral	geraniol	0.5	-	-	-	-
27	1445	1875	medicinal	guaiacol	-	0.5	-	-	-

Table 6-4. Continued

No.	DB-5	DB-Wax	descriptor	compound	untreated	UV thermal		PEF	RFEF
28		1873	floral sweet	a-ionone	1.5	-	-	-	-
29		1985	green metallic	4,5-epoxy (E)-2-decenal*	1.0	-	-	-	-
30		2055	fresh fruity	unknown	-	0.8	-	-	-
31	1489	2069	buttery peachy	g-decalactone	1.5	1.0	1.5	1.0	1.5
32	2196	2187	musky cologne	4-vinyl guaiacol	1.8	2.5	2.5	2.3	2.0
33	1358	2647	dirty musty	unknown	-	-	-	0.8	-

* Odorants identified based on odor descriptor and retention indices with reported data due to unavailability of standards

Table 6-5. Results of ANOVA for mean values from difference from control test

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	249.57	4	62.39	23.3	3E-14	2.447
Within panelists	321.76	120	2.681			
Total	571.33	124				

Table 6-6. Difference in means between pasteurized and unpasteurized grapefruit juice

	Mean values	control	PEF	RF	thermal	UV
Mean values		1.32	3.24	3.36	3.64	4.60
Control	1.32		1.92	2.04	2.32	3.28
PEF	3.24			0.12	0.40	1.36
RF	3.36				0.28	1.24
Thermal	3.64					0.96
UV	4.60					

Table 6-7. Results of ANOVA of mean values from difference control test at week 4

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between treatments	414.38	4	103.59	18.61	1.05E-11	2.45
Within panelists	612.34	110	5.56			
Total	1026.73	114				

Table 6-8. Difference in means between pasteurized and unpasteurized grapefruit juice at week 4

		control	PEF	RF	thermal	UV
	mean values	1.96	7.13	6.43	5.08	6.82
Control	1.96		5.17	4.47	3.12	4.86
PEF	7.13			0.70	2.05	0.31
RF	6.43				1.35	0.39
Thermal	5.08					1.74
UV	6.82					

CHAPTER 7
CONCLUSION

Non-thermal processing has been extensively researched over the past few years as an alternative to heat pasteurization. However, non-thermal processes are often compared to thermal processes using conditions that did not achieve the same reduction in concerned microorganism(s). This made a fair comparison of their effects on juice quality impossible. Therefore, the objective of this study was to compare the quality of apple cider and grapefruit juice treated by thermal and non-thermal techniques where treatment conditions were carefully selected to achieve a similar reduction in *E. coli* k12 population. The non-thermal techniques selected for the study were PEF, RFEF and UV. The quality attributes studied were aroma, sensory, microbial, physical, enzymatic and nutritional.

In apple cider, microbial inactivation by PEF technique was comparable to heat pasteurization. UV treated cider had a shelf life of only 2 weeks. Other attributes like aroma volatiles, color and sensory quality was better preserved in PEF treated cider compared to thermally and UV treated cider. Comparing the three techniques, PEF shows the best promise as a future pasteurization technique for the preservation of apple cider.

In grapefruit juice, the thermal treatment was more effective when compared to non-thermal treatments in terms of microbial stability after 4 weeks storage. The sensory quality of juice was affected by all treatments. UV treated grapefruit juice differed the most compared to the control juice, followed by thermal, RFEF and PEF, respectively. The impact of treatments on odorants was highlighted by a loss of desirable fruity and citrus odorants, along with an increase in undesirable cooked or

catty odorants. RFEF treatment ensured grapefruit juice microbial safety for 3 weeks of storage with higher Vitamin C content and equivalent PME inactivation compared to thermally treated juice. PEF and UV treatments maintained good microbial quality for only 1 week. Non-thermally treated juice had a shorter shelf life but better nutritional and physical qualities compared to thermally treated juice.

The current study has shown that the effects of thermal and non-thermal treatments on juice quality are not the same at equivalent process conditions. The thermal technique consistently showed good microbial quality during storage compared to non-thermal techniques. However, organoleptic and nutritional qualities of juice were better preserved in non-thermally treated juice than in thermally treated juice. The efficacy of non-thermal treatment in extending shelf life also depends on the type of food product. Though it is difficult for any non-thermal technique to completely replace thermal process, some of them might find niche applications for certain products that are sensitive to heat.

For commercial success, non-thermal technologies should show cost effectiveness and pathogen control comparable to thermal treatment. To achieve this goal, combinations of these techniques with traditional or emerging food preservations techniques where a synergistic effect in terms of improving food quality and safety is achieved needs to be explored. This combination of new and traditional techniques is commonly called “hurdle technology.” Moreover, it is necessary to find the niche products for applications of these technologies by conducting more detailed research on nutritional and organoleptic properties of the product. In the future, consumer demands

for fresh and minimally processed food would definitely help in the acceptance and commercialization of non-thermal processing techniques by the food industry.

APPENDIX A
CHANGE IN APPLE CIDER APPEARANCE DURING STORAGE

Figure A-1. Change in appearance in treated and untreated apple cider during 4 weeks of storage at 4ºC

APPENDIX B
CALIBRATION TABLE FOR APPLE CIDER VOLATILES

Table B-1. Regression equation for standard compounds

Compound	Regression equation	Correlation	Base ion
hexyl hexanoate	y = 139805x - 26161	0.9769	117
p-allyl anisole	y = 57923x + 46022	0.9825	148
butyl propionate	y = 105603x + 65342	0.9703	57
2-methyl hexyl butyrate	y = 70481x - 40381	0.9604	103
1-octanol	y = 10696x - 25739	0.9949	56
6-methyl hepten-5-en-2-one	y = 759936x + 10268	0.9851	43
pentyl acetate	y = 184623x + 56243	0.8015	70
2-hexen-1-ol, acetate	y = 54292x + 11218	0.9784	67
2-hexen-1ol (z)	y = 83730x + 51963	0.9921	57
ethyl propionate	y = 316024x - 592703	0.8861	57
ethyl butyrate	y = 23796x + 144461	0.9619	71
ethyl hexanoate	y = 121026x - 32819	0.998	88
Farnesene	y = 5E+08x + 24019	0.9057	93
butyl 2-methyl butyrate	y = 106763x + 15192	0.9679	103
hexyl acetate	y = 43260x + 544732	0.9839	56
2methyl butyl acetate	y = 29856x + 250422	0.97	70
2methyl butanol	y = 1820.2x + 28272	0.9183	57
1-hexanol	y = 12069x + 111609	0.9967	56
butyl acetate	y = 16391x + 184478	0.9514	56
Hexanal	y = 12165x + 140960	0.9609	56
2-methyl ethyl butyrate	y = 79357x - 147057	0.9332	102
2-e-hexenal	y = 11392x - 226626	0.9395	69
propyl butyrate	y = 107921x - 408031	0.9754	71
Benzaldehyde	y = 51164x + 361796	0.9422	106
butyl butyrate	y = 92039x - 52362	0.9639	71
hexyl propionate	y = 86064x - 117617	0.9467	57
propyl hexanoate	y = 125122x - 319336	0.8724	99
hexyl butyrate	y = 71844x - 38727	0.9595	71
methyl butyrate	y = 21678x - 51380	0.8882	74

APPENDIX C
GRAPEFRUIT JUICE VOLATILE CONCENTRATION

Table C-1.Volatile concentration (μg/L) in untreated (control) and treated grapefruit juice at week0

RT	LRI	compound identified	control	PEF	RFEF	UV	Thermal
4.45	888	ethyl acetate	6.49±0.73	5.85±0.29	5.61±1.25	5.42±2.03	6.40±2.47
6.50	1037	a-pinene	1.36±0.09	1.35±0.12	1.30±0.03	1.17±0.21	1.34±1.04
6.70	1049	ethyl butyrate	9.47±0.34	7.60±1.32	6.32±2.17	6.76±2.74	6.57±2.46
7.56	1098	Hexanal	2.98±0.06	2.93±0.05	2.09±0.08	2.84±1.02	2.90±2.12
9.06	1174	b-myrcene	129.01±4.73	124.40±3.68	120.76±4.23	119.46±4.29	119.27±5.79
9.97	1219	Limonene	2309.50±21.47	2203.58±35.92	2120.11±23.28	2001.39±42.83	2032.12±28.71
10.46	1243	ethyl hexanaote	32.56±5.03	25.71±3.18	25.88±3.73	23.09±3.25	26.48±2.08
10.82	1260	3-carene	15.54±2.83	12.74±2.25	11.38±2.04	11.33±5.27	13.31±2.56
11.33	1285	p-cymene	15.63±0.92	10.05±2.63	11.05±1.27	11.66±2.99	11.57±2.18
11.61	1299	Octanal	78.87±2.84	73.70±3.78	68.22±2.69	65.34±5.24	74.77±4.43
13.61	1400	Nonanal	8.87±1.47	8.33±2.16	6.79±1.30	7.56±1.47	6.81±3.23
14.29	1436	ethyl octanoate	1.35±0.06	1.14±0.02	0.91±0.03	1.29±0.93	1.18±0.07
14.51	1447	dimethyl styrene	5.15±0.46	2.35±0.27	2.18±1.02	1.66±1.11	2.51±1.16
15.52	1501	Decanal	16.32±2.82	15.55±2.32	15.97±3.26	14.89±2.46	15.78±3.52
15.63	1506	a-copaene	6.03±1.10	5.92±2.17	4.23±2.38	5.05±2.25	5.17±2.76
16.20	1537	Linalool	1.79±0.22	1.57±0.14	1.81±0.44	1.89±0.29	1.86±0.23
16.44	1549	1-octanol	9.89±2.91	9.83±2.34	9.69±2.82	10.32±2.47	10.43±3.73
17.44	1602	terpinen-4-ol	2.92±0.61	3.33±1.80	3.02±1.99	2.97±1.72	3.17±2.01
17.55	1608	b-caryophyllene	135.99±4.83	135.79±5.20	86.61±4.27	133.97±4.88	134.75±6.23
18.77	1670	a-caryophyllene	14.94±2.58	14.62±1.79	15.24±3.23	14.69±4.16	14.57±4.29
19.54	1709	Valencene	3.70±1.19	3.28±1.03	2.49±1.17	4.03±1.81	3.79±2.08
19.77	1721	a-selinene	4.07±1.38	4.08±1.22	3.11±1.83	4.60±2.38	4.88±2.74
20.05	1735	d-cadinene	4.63±2.37	4.46±2.18	3.34±2.08	4.12±1.32	3.52±1.72
20.31	1747	a-panasinsen	1.45±0.03	1.34±0.06	1.32±0.70	1.38±1.28	1.28±0.84
30.56	2677	Nootkatone	6.13±2.82	3.72±1.38	3.26±2.68	3.19±2.01	3.21±2.22

Table C-2. Volatile concentration (µg/L) in untreated (control week 0) and treated grapefruit juice at week 4

RT	LRI	compound identified	Control	PEF	RFEF	UV	thermal
4.45	888	ethyl acetate	6.49±0.73	3.79±1.28	4.55±2.25	4.76±1.29	4.08±0.84
6.50	1037	a-pinene	1.36±0.09	1.15±0.03	0.83±0.04	1.08±0.88	0.91±0.07
6.70	1049	ethyl butyrate	9.47±0.34	15.12±2.37	4.41±1.48	1.07±0.27	4.38±1.81
7.56	1098	Hexanal	2.98±0.06	Nd	Nd	Nd	Nd
9.06	1174	b-myrcene	129.01±4.73	117.98±5.23	108.47±3.49	102.67±6.38	88.04±3.67
9.97	1219	limonene	2309.50±21.47	1977.48±53.38	1686.27±30.28	1730.11±29.47	1737.39±51.84
10.46	1243	ethyl hexanaote	32.56±5.03	Nd	Nd	Nd	nd
10.82	1260	3-carene	15.54±2.83	2.66±2.12	10.71±2.43	6.58±2.71	6.48±2.48
11.33	1285	p-cymene	15.63±0.92	8.65±3.42	9.38±2.83	7.27±4.29	6.91±3.89
11.61	1299	octanal	78.87±2.84	4.16±2.78	3.52±1.67	3.64±1.93	4.43±2.50
13.61	1400	nonanal	8.87±1.47	5.25±3.15	2.73±1.38	3.95±2.10	2.54±1.37
14.29	1436	ethyl octanoate	1.35±0.06	1.09±0.29	0.96±1.31	1.03±0.03	1.05±0.79
14.51	1447	dimethyl styrene	5.15±0.46	1.24±0.28	1.40±0.72	1.23±1.12	1.10±0.09
15.52	1501	decanal	16.32±2.82	3.13±3.02	7.14±2.89	2.65±1.37	2.01±1.18
15.63	1506	a-copaene	6.03±1.10	4.26±2.44	3.53±1.21	3.23±2.63	2.55±2.45
16.20	1537	linalool	1.79±0.22	1.53±0.09	1.14±0.70	1.31±0.37	1.73±1.08
16.44	1549	1-octanol	9.89±2.91	9.67±3.10	10.48±2.56	10.49±2.89	15.20±5.27
17.44	1602	terpinen-4-ol	2.92±0.61	5.28±2.43	5.83±3.83	5.79±2.71	5.42±2.09
17.55	1608	b-caryophyllene	135.99±4.83	143.00±4.82	134.39±6.88	130.02±12.32	108.58±4.76
18.77	1670	a-caryophyllene	14.94±2.58	12.05±3.84	16.57±3.74	14.37±4.20	13.16±2.94
19.54	1709	valencene	3.70±1.19	1.28±0.89	1.22±1.04	1.40±1.41	1.36±0.65
19.77	1721	a-selinene	4.07±1.38	3.48±2.46	3.98±2.79	3.21±2.63	2.79±2.36
20.05	1735	d-cadinene	4.63±2.37	3.15±1.82	3.02±3.38	3.15±2.85	3.02±2.89
20.31	1747	a-panasinsen	1.45±0.03	1.14±1.03	7.69±2.45	1.09±0.67	0.99±1.01
30.56	2677	nootkatone	6.13±2.82	3.18±3.20	2.85±3.52	2.65±3.49	2.70±2.73

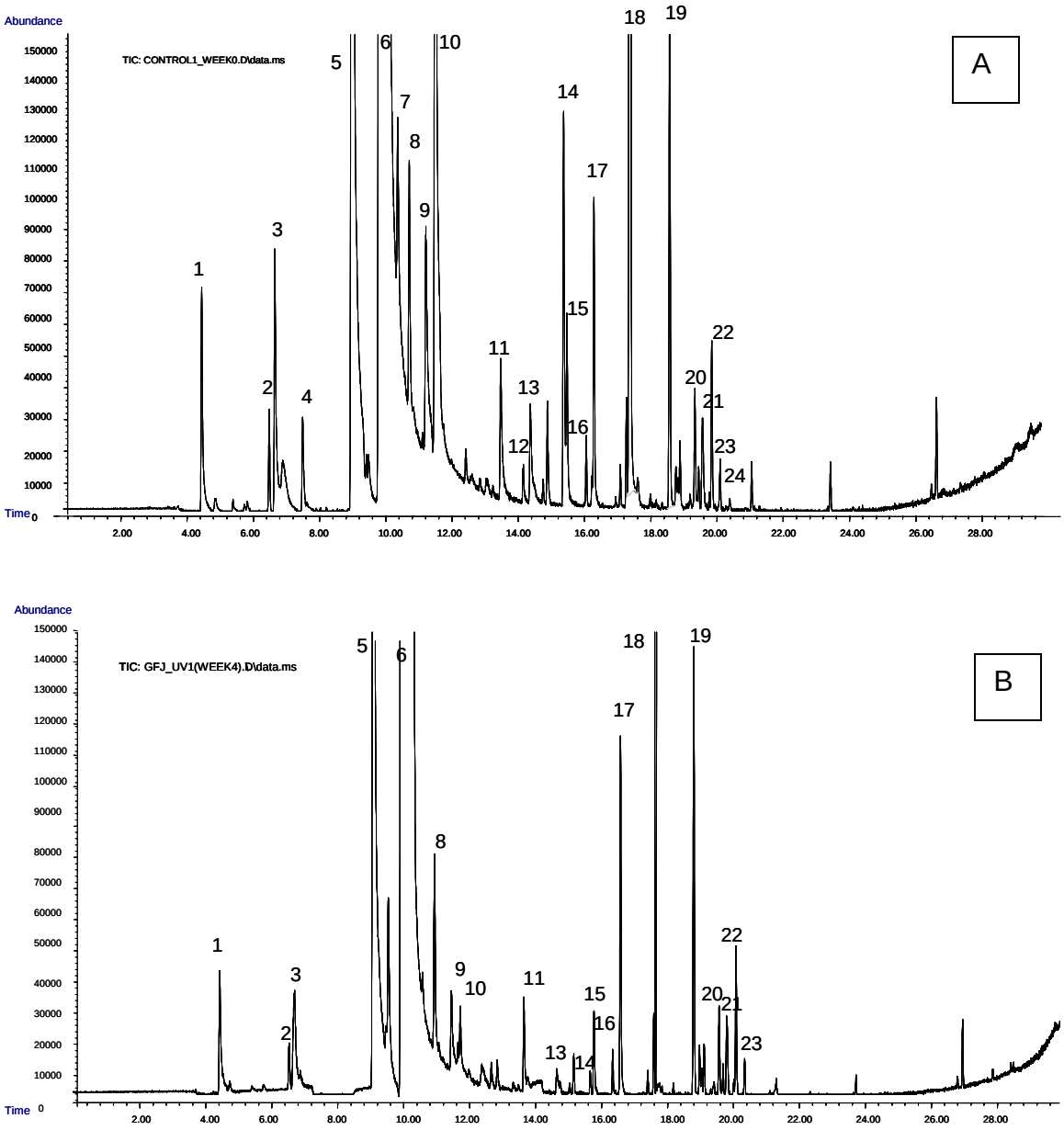

Figure C-1. TIC of grapefruit volatiles extracted by HS-SPME A. untreated juice (week 0) B. UV treated juice (week 4). Separation on DB-Wax column. Highest change in peak nos. 4 = hexanal, 7 = ethyl hexanoate, 10 = octanal, 14 = decanal.

APPENDIX D
GRAPEFRUIT JUICE SENSORY RESULTS

Table D-1. Difference from control data between hidden control (unpasteurized juice) and pasteurized juices (thermal, PEF, RFEF and UV) at week 0

Panelist	thermal	PEF	RF	UV	control
1	9	2	2	6	1
2	5	4	2	7	2
3	6	3	3	8	3
4	4	2	2	6	0
5	5	4	4	8	2
6	2	4	2	4	0
7	6	2	2	9	0
8	4	3	5	7	3
9	3	3	2	5	1
10	4	2	5	5	0
11	6	2	3	6	0
12	3	2	4	3	1
13	5	4	6	9	2
14	3	3	4	8	0
15	2	2	3	3	2
16	3	4	2	5	2
17	4	2	3	5	1
18	6	2	3	3	0
19	4	3	3	7	3
20	4	3	4	9	2
21	2	4	4	4	2
22	5	6	6	6	2
23	3	3	3	7	1
24	4	4	2	4	2
25	5	6	3	2	1
Sum	107	79	82	146	33
Average	3.64	3.24	3.36	4.6	1.32
Variance	4.407	4.523	2.323	8.083	1.060

Table D-2. Difference from control data between hidden control (unpasteurized fresh uice) and pasteurized juices (thermal, PEF, RFEF and UV) at week 4

Panelist	PEF	RF	thermal	UV	control
1	7	5	0	8	1
2	4	7	7	5	1
3	8	3	4	7	1
4	10	10	10	10	4
5	10	9	2	9	0
6	8	10	5	5	1
7	8	7	6	8	2
8	8	9	7	4	4
9	9	6	9	9	3
10	9	9	7	9	1
11	10	10	8	10	6
12	6	4	1	5	1
13	4	6	6	4	3
14	10	10	9	5	0
15	6	3	3	9	2
16	7	1	8	8	3
17	7	7	6	4	3
18	4	4	2	4	2
19	3	6	5	3	3
20	9	4	4	8	2
21	5	7	5	8	2
22	8	5	3	5	0
23	4	6	0	10	0
24	8	6	5	6	3
25	6	7	5	8	1
sum	178.00	161.00	127.00	171.00	49.00
average	7.12	6.44	5.08	6.84	1.96
variance	5.02	6.71	8.35	5.43	2.31

APPENDIX E
SAMPLE SENSORY BALLOT FOR DIFFERENCE FROM CONTROL TEST

Difference from control test

Name _____ Date: _____

Type of sample _____ Test# _____

INSTRUCTIONS

Please DO NOT taste the samples. Evaluate the samples marked “control” first. Open the vial and sniff headspace of the vial and remember the odor of the sample. Wait 10 seconds and then sniff the sample marked with three digit code. Assess the overall sensory difference between the two samples using scale below. Mark the scale to indicate the size of the overall difference.

Scale		Mark to indicate difference
0	No Difference	_____
1		_____
2		_____
3		_____
4		_____
5		_____
6		_____
7		_____
8		_____
9		_____
10	Extremely different	_____

Comments: please give verbal descriptors for the odor

_____ _____

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BIOGRAPHICAL SKETCH

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