AN OVERVIEW OF EMERGING TRENDS IN PATHOGEN REDUCTION IN THE PROCESSING OF FRUIT JUICES

by

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Abstract

Unpasteurized fruit juices have been implicated as the source of foodborne outbreaks due to pathogens such as *Salmonella*, *Escherichia coli* O157: H7 and *Cryptosporidium parvum*. The growth of pathogens can usually be slowed through freezing or largely eliminated through pasteurization. Although pasteurization is often effective in eliminating pathogens, it often yields undesirable flavors that are unlike those of fresh juice. Growing consumer trends towards "healthy" unpasteurized alternatives are fueling the development of alternative processing techniques.

Several promising techniques for pathogen reduction in the processing of fruit juices are currently being developed. A new technique that is already being marketed worldwide is hyperbaric processing (HPP) which subjects the fruit juice to a high pressure of up to 1000 MPa. The high-pressure treatment results in up to a 7 log reduction kill in pathogens while preserving the naturally occurring flavor profile, sensory attributes and nutritional benefits. Pulsed electric fields (PEF) and ionizing radiation are also being widely explored as viable techniques to process unpasteurized fruit juices. PEF promises to be a commercially viable energy efficient alternative to pasteurization, adding only $0.03 – $0.05 per liter to final food costs. Although irradiation enjoys support for use in the processing of fruit juice by regulatory agencies, support in public opinion is lacking and hinders its growth as an alternative to pasteurization. Other experimental techniques are also present in the development pipeline. Ultrasonic radiation and high intensity pulsed light radiation are both experimental techniques that are being researched. A particularly exciting alternative is the use of plant-based antimicrobials. Several fruits and spices are known to be natural antimicrobials and are therefore being researched as alternatives to the traditional chemical preservatives.

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Chapter 1 - Introduction

The United States Code of Federal Regulations (CFR) Title 21 defines juice as "*the aqueous liquid expressed or extracted from one or more fruits or vegetables, purees of the edible portions of one or more fruits or vegetables, or any concentrates of such liquid or puree*" (FDA, 2013). Pathogen reduction is one of the fundamental aspects of fruit juice processing. The removal of microorganisms during the processing of fruit juice is also governed by CFR Title 21. The current revision of CFR - Code of Federal Regulations Title 21 (FDA, 2013) states in part that:

"*processors of juice products shall include in their Hazard Analysis and Critical Control Point (HACCP) plans control measures that will consistently produce, at a minimum, a 5 log (i.e., 10^5) reduction, for a period at least as long as the shelf life of the product when stored under normal and moderate abuse conditions, in the pertinent microorganism. For the purposes of this regulation, the "pertinent microorganism" is the most resistant microorganism of public health significance that is likely to occur in the juice*".

Current processing techniques used to produce commercially safe products include thermal pasteurization where the juice is heated followed by rapid cooling. The heating step during thermal pasteurization reduces the flavor components and vitamin content of the finished juice (Elez-Martinez and others 2006; Lee and Coates 2003). Filtration is another traditional technique currently being utilized in the juice market. The product is passed through a porous membrane followed by a thermal pasteurization process (Zimmer 2007). Another current technique is the addition of chemicals such as sodium benzoate or potassium sorbate to juice to inhibit microbial growth. These chemical additions may adversely affect the sensory profile of the final juice product or could cause harmful by-products (FDA 2007). Benzene, a known carcinogen, can be formed from the reaction of benzoate salts and ascorbic acid (FDA 2007). According to the International Trade Center, the global fruit juice market was valued at $79 billion in 2009 and had a predicted annual worldwide growth rate of 3.4 % (ITC, 2011). Conversely, fruit juice consumption in the United States is on the decline and in 2010 Americans consumed about 8 gallons of fruit juice per person or $52 per-capita in comparison to 2002 (AAFC, 2011) when 11.2 gallons per person was consumed.

Despite the decline in fruit juice consumption there is an increase in consumer demand for natural and microbiologically safe fruit juices for consumption (Deliza and others 2005; Tiwari and others 2009a). Consequently, several new and experimental pasteurization techniques are under development. Hyperbaric or high pressure processing (HPP) is a recent commercialized technique that offers the possibility of significant pathogen reduction without heat. When compared to thermal pasteurization, where juice is heated then rapidly cooled, the resulting juice from HPP has the advantage of less destruction of flavor components and vitamins (Deliza and others 2005). There are currently juices produced worldwide that are pasteurized using HPP. Other newer experimental techniques include pulsed electric field (PEF) and ultrasonic and high intensity light processing (Tiwari and others 2009a). Another technique that is under investigation is the inclusion of natural antimicrobials into juice (Tiwari and others 2009b). Unlike the addition of potassium sorbate and sodium benzoate, the addition of natural antimicrobials allows juices to appear more natural. In summary, there are several techniques that are under development as potential pasteurization techniques for fruit juice processing.

Chapter 2 - Important Pathogens in Fruit Juice Processing

Fruit juices are particularly prone to spoilage and contamination by foodborne pathogens (Cerny and others 1984; Tournas and others 2006; Shearer and others 2002). Pathogen reduction is therefore of particular importance in the processing of fruit juices (CDC 2013). Additionally, the core demographics of fruit juice consumers – children and the elderly – are particularly vulnerable to pathogens (Kendall and others 2006; Rangel and others 2005). Fruit juice is subject to contamination by several food borne pathogens including *Escherichia coli* O157:H7, *Cryptosporidium parvum* and *Salmonella* spp. as well as spoilage microorganisms such as mold and *Alicyclobacillus acidoterrestris* (Burnett and Beuchat 2000).

Escherichia coli serotype O157:H7 is an enterohemorrhagic strain of *E. coli.* Under normal conditions *E. coli* is useful and functions to suppress the growth of harmful bacterial species and to synthesize vitamins. In fact, under aerobic culture conditions, *E. coli* is the dominant species found in feces. However, *E. coli* O157:H7 produces verotoxin s*higa*-like toxins that cause severe damage to the lining of the intestine leading to hemorrhagic colitis. Although all humans are subject to illness following infection with *E. coli* O157:H7, the young, elderly, and immune-compromised individuals are particularly susceptible and the disease can progress to hemolytic uremic syndrome and become fatal. Although undercooked ground beef has frequently been implicated in many documented outbreaks of *E. coli* O157:H7, outbreaks have also been associated with raw vegetables, unpasteurized fruit juices, raw milk, and cheese curds (CDC 2013; Rangel and others 2005).

Cryptosporidium is an intracellular protozoan parasite of which there are several species that infect humans. According to the CDC (2010), *Cryptosporidium parvum* is the species to which most of the foodborne outbreaks are attributed. Transmission of *Cryptosporidium parvum* primarily occurs through contact with contaminated water; food sources, such as fruit juice or chicken salad, may also become contaminated. *Cryptosporidium* infection starts with ingestion of the *Cryptosporidium* oocyst by a suitable host (Figure 2.1). Following ingestion, excystation of the oocyst occurs and sporozoites are released which then infect the epithelial cells of the gastrointestinal tract. The parasitic sporozoites then multiply to produce new oocysts in the host. Of the two types of oocysts that are produced, the thick-walled infective oocysts are excreted by the host and the thin-walled oocysts are further involved in autoinfection. Symptoms of

infection include severe diarrhea, malaise, and abdominal cramping with infectious oocysts being produced for up to 2 weeks in infected individuals. Although the disease is often self-limiting in healthy humans, cryptosporidiosis is often fatal in infants and immuno-compromised individuals (CDC 2010).

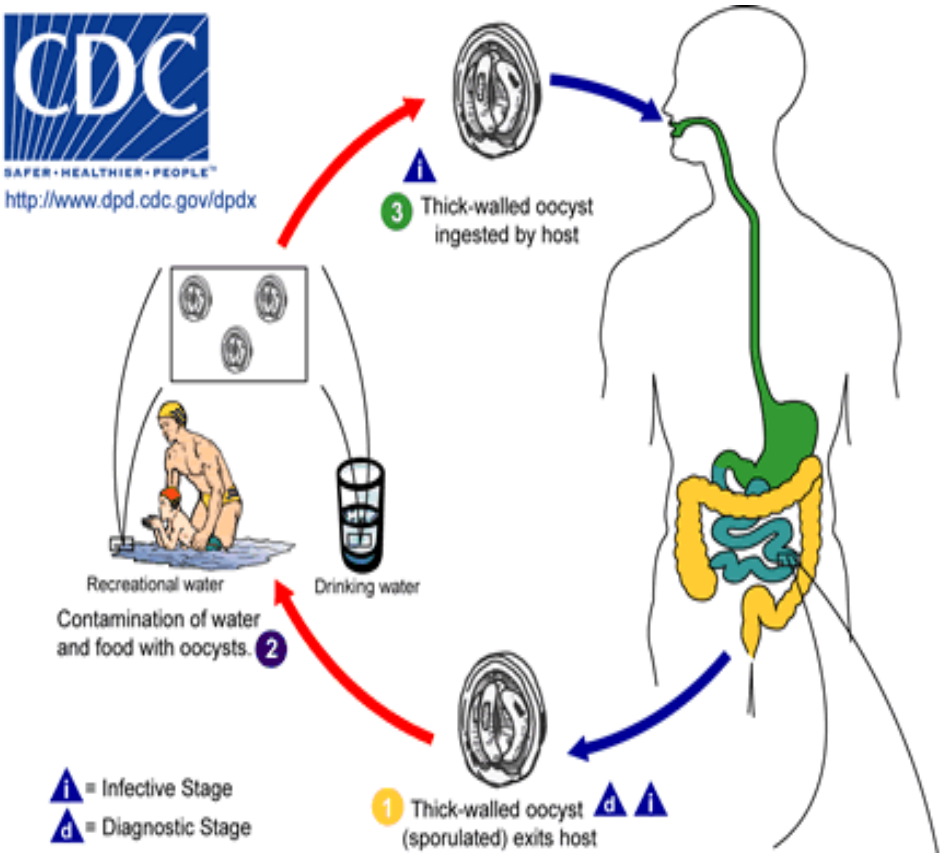

Figure 2.1: Reproductive life cycle of cryptosporidium (CDC 2010).

The *Salmonella* bacterium is one of the most common food borne pathogens. In the United States, *Salmonella* serotype Typhimurium and *Salmonella* serotype Enteritidis are the most common variants (Bugarel and others 2012). *Salmonella* infection is widely associated with poultry but is also considered a pathogen of interest in fruit juice processing (Parish 1998; Luedtke and Powell 2000; Vojdani and others 2005). Castillo and others (2006) reported high incidences of *Salmonella* in fresh juicing stands in Guadalajara, Mexico (Table 2.1).

Symptoms of a *Salmonella* infection include diarrhea, fever, abdominal cramps, vomiting. The disease is usually self-limiting but can be fatal in the young, elderly, and immuno-compromised individuals (Fierer and Swancutt 2000).

Table 2.1: Incidence of *Salmonella* from freshly squeezed orange juice, surface of oranges, and wiping clothes obtained from street vendors and small food service establishments at public markets.

Sample Type	Sampling Site	Number of Samples	Number of Samples (%) positive for *Salmonella*
Orange Juice	Street Vendors	49	7 (14.3)
	Public Markets	51	2 (3.9)
	Subtotal	100	9 (9)
Orange Surface	Street Vendors	35	7 (20.0)
	Public Markets	40	3 (7.5)
	Subtotal	75	10 (13.3)
Wiping Cloths	Street Vendors	35	8 (22.8)
	Public Markets	40	3 (8.6)
	Subtotal	75	11 (14.7)
Subtotal		250	30 (12.0)

(Data was adapted from Castillo and others 2006)

The removal of microorganisms during the processing of fruit juice is governed by CFR 21. The current revision of CFR - Code of Federal Regulations Title 21 (FDA, 2013) states in part that:

"*processors of juice products shall include in their Hazard Analysis and Critical Control Point (HACCP) plans control measures that will consistently produce, at a minimum, a 5 log (i.e., 10^5) reduction, for a period at least as long as the shelf life of the product when stored*

under normal and moderate abuse conditions, in the pertinent microorganism. For the purposes of this regulation, the "pertinent microorganism" is the most resistant microorganism of public health significance that is likely to occur in the juice."

Processed juice is not sterile and must be frozen or refrigerated to preserve product quality. Foodborne illness outbreaks due to contaminated fruit juices are summarized in Table 2.2. Of the 21 outbreaks due to contaminated fruit juice occurring between 1995 and 2005, *Salmonella* accounted for more than half of the illnesses and two-thirds of the hospitalizations; the only recorded death attributed to contaminated fruit juice was due to *E. coli* O157:H7. Apple and orange juices were responsible for the majority of the outbreaks despite their low pH during processing. Apple cider and juice were associated with 10 of the 21 outbreaks and another 8 were sourced from orange juices (Vojdani and others 2005).

Table 2.2: Reported number of cases, hospitalizations, and deaths by causative agent(s) for juice-associated outbreaks reported to the CDC, 1995 through 2005.

Agent	Number of Outbreaks	Number of illnesses	Number of hospitalizations	Number of deaths
Salmonella	5	710	94	
E. coli O157:H7	5	105	36	1
C. parvum	2	175	3	
E. coli O111 & *C. parvum*	1	213	15	
Unknown	8	182	1	
Total	21	1366	149	1

(Data adapted from Vojdani and others 2005)

Product Safety Management

Hazard Analysis Critical Control Point (HACCP) is a product safety management system designed to identify hazards and critical situations in a process, and to produce a structured plan to control, eliminate or reduce food safety hazards to a safe level (Surak and Wilson 2007).

Hazard Analysis Critical Control Point bases the food-safety program on scientific data and increases awareness through the training of all employees, and aims to ensure that the prevention and control of food safety problems occurs at specific and controllable points in the process chain. Therefore, the implementation of a HACCP program provides manufacturers a high level of control over product safety (Baker 1995; Notermans and others 1995). The FDA requires that all juice manufactures evaluate their operation using HACCP principles and, if necessary to develop and implement preventive control measures based on HACCP principles for its operation (FDA 2013a).

During fruit juice processing there are specific activities established by the HACCP plan that ensure the delivery of a safe product. Firstly, the manufacturer must conduct a hazard analysis to determine the types of food safety hazards present throughout the manufacturing process including raw materials through distribution and consumer use. These hazards can be biological (for example *E. coli* O157:H7 in apple juice), chemical, physical or radiological and must be scientifically assessed so any potential risks to the process can be determined. During the hazard analysis, each identified hazard is evaluated for its severity and risk and this information is used to determine critical control points (CCPs) for the manufacturing process where control measures can be applied to prevent, eliminate the food safety hazards or reduce them to an acceptable level. CCPs are systematically determined using a sequence of steps as shown in Figure 2.2.

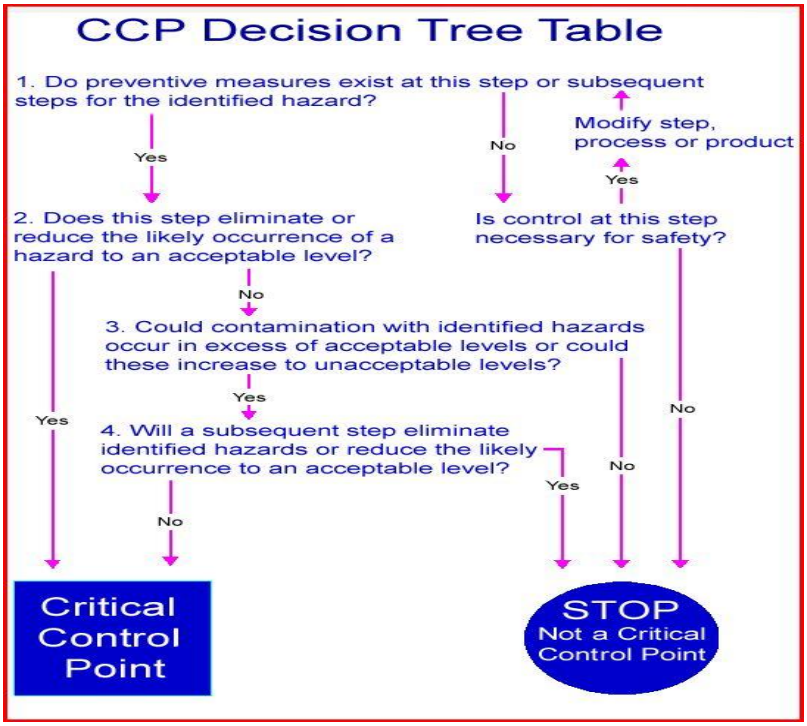

Figure 2.2: Example of Critical Control Point Decision Tree (FDA 2004).

Following the determination of CCPs, the HACCP team will scientifically establish critical limits for the CCPs. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2006) defines a critical limit as a

"*maximum and/or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrences of a food safety hazard.*"

Monitoring of the established CCPs will ensure that the control limits are not exceeded. These monitoring activities can be automated, for example using an automated temperature-measuring device during thermal pasteurization or it could be manual, for example, inspecting an incoming delivery of apples for bruised or physically damaged product, which could be an indicator that apples contain a high level of patulin (chemical hazard) before using them for juice production. If monitoring activities indicates that the established critical limits have been exceeded, the appropriate corrective action must be taken to identify, correct, and eliminate the cause of the deviation. Appendix A details an example of a HACCP plan for pasteurized refrigerated apple juice.

Pathogen Reduction through Thermal Pasteurization

Traditionally, pasteurization can be defined as the process by which foods are heated to a specific temperature for a specific amount of time to kill (or deactivate) a target number of potentially harmful bacteria. The method that involves high heat followed by rapid cooling was developed by French chemist Louis Pasteur in the 1850s to reduce the incidence of bacterial conversion of wine into vinegar (Vallery-Radot 1923). With the evolution of new non-thermal processes that are capable of reducing target pathogen counts, the term "pasteurization" has taken on a broader definition solely based on pathogen reduction (NACMCF 2006). As defined by the NACMCF (2006), pasteurization is

"*any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage.*"

According to Dietz and Erdman (1989), thermal pasteurization can be divided into two groups: 1. low temperature long time (LTLT); and 2. high temperature short time (HTST) processing. Although LTLT is the traditional thermal pasteurization process, it can be detrimental to quality characteristics of juice resulting in progression towards HTST by industry. The processing time under HTST is less than 1 min compared to LTLT that is often 30 min to 1 h. Thermal pasteurization is a well-developed technique that can be used on a wide variety of juices ranging from clear to cloudy purees. It is also possible to accomplish HTST processing on both a continuous flow, prior to filling, as well as in the bottle after filling (tunnel pasteurization). Additionally, the high temperature employed in HTST is designed to be high enough to kill spores; many people report that there is a change in taste, color, and odor after treatment. Natural enzymes and nutrients are also destroyed by the process (Dietz and Erdman 1989).

Table 2.3: FDA recommended pasteurization times and temperatures for apple juice.

Temperature (℃)	Length of treatment (s)
71.1	6
73.9	2.8
76.7	1.3
79.4	0.6
82.2	0.3

(FDA 2004)

Filtration is another commonly employed pasteurization technique (Gökmen and others 2001). Filtration involves forcing the juice under pressure through a semipermeable membrane. Only molecules and microbes below a specified size would be allowed through the membrane. The two commonly used membrane filtration techniques used for fruit juice processing are ultrafiltration (UF) and microfiltration (MF). Used in conjunction, both techniques can be used to clarify and reduce microbe concentrations in clear processed juices (Zimmer 2007). The efficacy of the filtration process is highly dependent on the pore size of the membrane and clarity of the juice (Rektor and Vatai 2004). For most juices, filtration effects changes in color and taste which in turn imparts a non-natural feel to the juice (Gökmen and others 2001).

Another technique employed for pathogen reduction is the addition of chemical preservatives. Potassium sorbate, sodium benzoate, and sodium bisulfite are some preservatives that are added primarily for shelf life extension. For example, treatment of apple cider with 0.045% sodium benzoate achieved greater than 5 log reduction CFU/ml of *E. coli* O157:H7 (Fisher and Golden 1998) and 4.9 log reduction CFU/ml of *E. coli* O157:H7 after treatment with 0.1 % sodium benzoate in apple juice (Ceylan and others 2004).

Chapter 3 - High Pressure (Hyperbaric) Processing

In the search for non-thermal techniques capable of pathogen reduction, high pressure processing has gained traction in recent years. High pressure processing (HPP), also referred to as ultra-high pressure (UHP) processing or high hydrostatic pressure (HHP) processing is an alternative food preservation technology that subjects solid or liquid foods to pressures between 100 and 1000 MPa (FDA 2011). The technique offers the opportunity for successful processing without the use of chemical additives or high temperature. High pressure processing is an old technique that was shown to extend shelf life as far back as the late 19th century (Hite 1899; Hite 1914). Certes (1884) was however the first to demonstrate that high pressure processing was capable of destroying vegetative bacteria at pressures of 600 MPa. Although there had not been significant use of this technology since that time, there has been a gradual growth in its use since the 1990s (Figure 3.1).

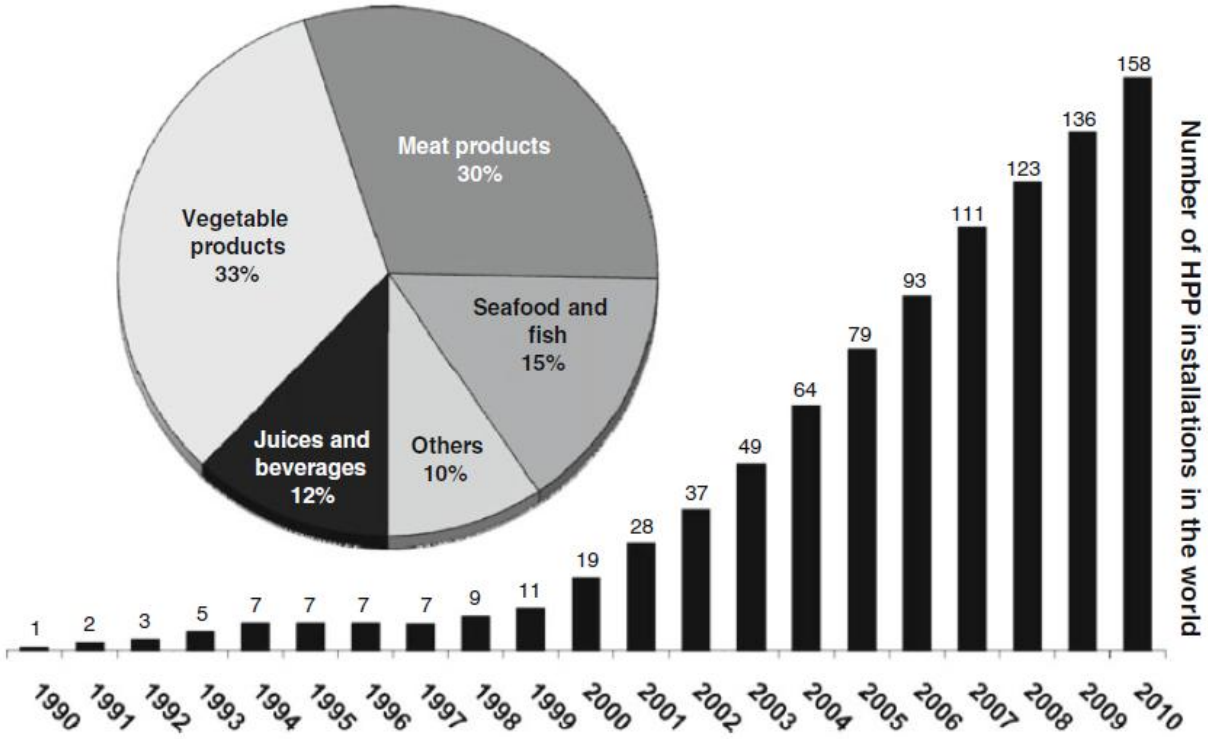

Figure 3.1: World food industry use of high pressure processing from 1990-2010 (Adapted from Mujica-Paz and others 2011).

Nutrients and bioactive compounds are preserved during HPP as covalent bonds remain unaffected during processing (Ramirez and others 2009). It is believed that it is the changes to weaker forces such as van der Waals, hydrogen bonds and electrostatic interactions that explain the preservation effects showed by HPP. Damage to membrane structures that are affected by HPP, while resulting in microbial inactivation, can also aid in the access of enzymes to their substrates causing some product deterioration (Horie and others 1991).

Microbial Reduction with High Pressure Processing

Microbial inactivation through high pressure processing is believed to occur through a combination of pressure-induced cellular changes and biochemical changes. Hydrostatic pressures of as little as 100 MPa has been found to induce germination in vegetative cells which are more sensitive to environmental conditions. While LeChatelier's Principle suggests that increases in pressure should be accompanied by a decrease in volume, the hydrophobic response of proteins allow them, at pressures up to 100 MPa, to result in a volume increase. The extent of hydrophobicity is therefore believed to govern the extent of denaturation at a given pressure. Key enzyme inactivation can therefore, in part, explain microbe inactivation. Additionally, pressurized membranes are expected to show changes in permeability due to damage which may alter uptake of nutrients as well as loss of intracellular contents (Paul and Morita 1971).

In general, vegetative bacteria are very sensitive to HPP. Contrastingly, bacterial spores are very resistant. Although parasitic protozoa such as *Cryptosporidium parvum* are expected to be more susceptible to pressure (FDA 2011), systematic studies on their behavior in fruit juices are lacking. *Listeria monocytogenes* is a gram-positive non-spore forming bacterium and is therefore expected to be very susceptible to HPP. *Escherichia coli* mutants and *Salmonella* have been studied and both have been shown to be fairly resistant to pressure (Garcia-Graells and others 1998) but to succumb under low pH conditions (Pagán and others 2001; Bayindirli and others 2006). Kincal and others (2005) described a continuous high pressure system (Figure 3.2) that was capable of 5 log reductions of *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes* with *L. monocytogenes* reaching non-detectable populations after treatment (Table 3.1).

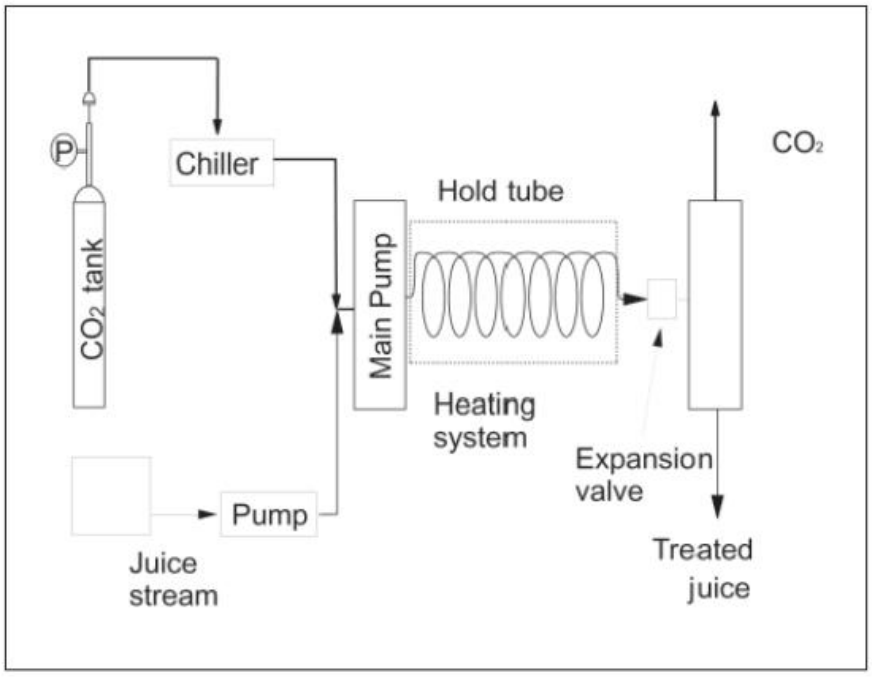

Figure 3.2: Schematic of a continuous high pressure carbon dioxide prototype system manufactured by Praxair (Chicago, Ill., USA) (Kincal and others 2005).

In a study by Kincal and others (2005), spoiled orange juice used to assess the efficacy of HPP on pathogen reduction. For the analysis, juice was prepared by freshly squeezing Valencia oranges and after holding the untreated juice at room temperature for 48 h; a microbial count of 2 × 10^6 CFU/ml was obtained (Table 3.2). Fresh orange juice was also inoculated with *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes*. The study showed that treatment with as little as 38 MPa at a w/v carbon dioxide gas to juice (CO_2/juice) ratio of 0.4 was capable of effecting a 2-3 log reduction of the natural flora in orange juice. In the spoiled juice the natural flora was reduced by 10^5 CFU/ml with similar w/v CO_2/juice ratios. Treatment of inoculated orange juice resulted in 5-6 log reductions of *Salmonella* Typhimurium populations and 3-4 log reductions of *E. coli* O157:H7 populations; *L. monocytogenes* populations were not detectable after treatment (Table 3.1). No viable cells of either *E. coli* O157:H7 or *Salmonella* Typhimurium were detected in inoculated juices or after treatment and after juices were stored at room temperature for 14 d.

Bayindirli and others (2006) also described the effect of HPP on *E. coli* O157:H7, *S.* Enteritidis and *Staphylococcus aureus* in apple, sour cherry, apricot, and orange juices.

Autoclaved fruit juices were inoculated with these pathogens to a population of 10^8 CFU/ml. Treatments at 250 MPa for 5 min at 30 °C resulted in 4.8 log reductions of *E. coli* O157:H7 and *S.* Enteritidis populations; a longer treatment time of 20 min resulted in slightly higher log reductions of 5.9. Higher treatment pressures of 350 MPa resulted in non-detectable populations of both *E. coli* O157:H7 and *S.* Enteritidis (Table 3.1). Noma and others (2004) investigated the effect of rate of decompression on the survivability of *E. coli* O157:H7 and found that rapid decompression was more effective in deactivating *E. coli* O157:H7 than slower decompression (Table 3.1).

Table 3.1: Summary of log reductions resulting from treatment by HPP of inoculated fruit juices.

Juice Type	Pathogen	Pressure (MPa)	Length of Treatment (min)	Log Reduction (CFU/ml)	Reference
Orange juice	*S.* Typhimurium	21 107	10 10	6 5	Kincal and others 2005
Orange juice	*L. monocytogenes*	21 107	10 10	4 6	Kincal and others 2005
Orange juice	*E. coli* O157:H7	21 107	10 10	2 4	Kincal and others 2005
Apricot juice	*E. coli* O157:H7	250 350	5 5	4.8 7.3	Bayindirli and others 2006
Orange juice	*E. coli* O157:H7	250 350	5 5	5.1 7.4	Bayindirli and others 2006
Cherry juice	*E. coli* O157:H7	250 350	5 5	5.3 7.7	Bayindirli and others 2006
Apricot juice	*S.* Enteritidis	250 350	5 5	4.8 8.0	Bayindirli and others 2006
Orange juice	*S.* Enteritidis	250 350	5 5	5.3 >8.5	Bayindirli and others 2006
Cherry juice	*S.* Enteritidis	250	5	5.5	Bayindirli and

Juice Type	Pathogen	Pressure (MPa)	Length of Treatment (min)	Log Reduction (CFU/ml)	Reference
		350	5	>8.5	others 2006
Apple juice	*E. coli* O157:H7	350	5	7.1	Bayindirli and others 2006
Apple juice	*S.* Enteritidis	350	5	7.8	Bayindirli and others 2006
Apple juice	*E. coli* O157:H7	200 250	20 20	4.5 7	Noma and others 2004
Orange juice	*E. coli* O157:H7	200 250	20 20	3.2 4.2	Noma and others 2004
Cashew and apple juice	*E. coli* O157:H7	400 400 400	0.5 1.5 3	1.6 3.5 >6	Lavinas and others 2008

Table 3.2: Summary of log reductions resulting from High Pressure Processing treatment of natural microflora in fruit juices.

Juice Type	Pressure (MPa)	Length of Treatment (min)	Log Reduction (CFU/ml)	Reference
Orange juice	38 (SU)	10	6	Kincal and others 2005
	72 (SU)	10	6	
	107 (SU)	10	6	
Orange juice	38 (NS)	10	6	Kincal and others 2005
	72 (NS)	10	6	
	107 (NS)	10	6	
Tomato juice	150	10	2	Dede and others 2007
	200	10	2.4	
	250	10	3.2	
Carrot juice	150	10	2.4	Dede and others 2007
	200	10	3	
	250	10	3.8	

SU: supercritical CO_2 conditions; NS: non-supercritical CO_2 conditions

Consistent with the work by Kincal and others (2005), Dede and others (2007) demonstrated that the natural microbial load found in fresh juices can be reduced by HPP to non-detectable levels rendering them safe for storage (Figure 3.3; Table 3.2).

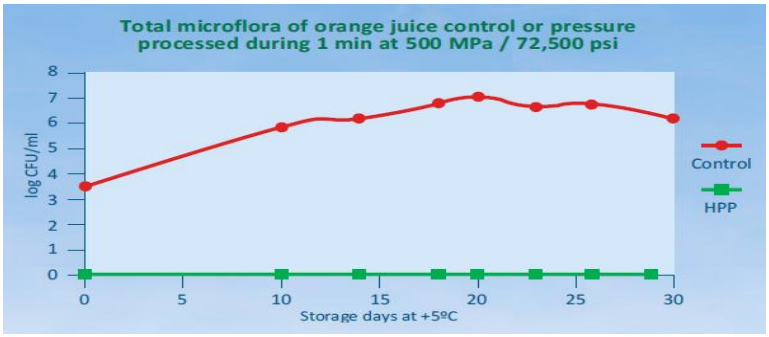

Figure 3.3: Shelf life after HPP is increased from 3 up to 10 times compared with the same product without HPP and held at the same temperature. Sensory quality is also maintained much longer after HPP (Figure obtained from Hiperbaric 2013).

Effect of High Pressure Processing on quality characteristics

The processing of fruit juices not only requires a product that is microbiologically safe, but also a product that possesses desirable sensory attributes and nutritional characteristics. The effect of HPP on enzymes, antioxidants, vitamins and color is therefore very important. Falguera and others (2012) examined the effect of HPP on the color of apple juice and found that red apples were more susceptible to color changes than yellow apples at high pressures. The effect was found to be even more pronounced at high temperatures (80 °C vs 25 °C) (Table 3.3).

Table 3.3: Difference in color (ΔE), as measured by 6 independent readings on a colorimeter, of HPP treated apple juice relative to freshly squeezed juice after come-up time (heating and pressure increase to target conditions followed by immediate pressure release and cooling).

		Color difference (ΔE)	
Apple Variety	Pressure (MPa)	25°C	80°C
Braeburn	400	0.92	1.85
	600	4.34	3.96
Fuji	400	3.46	13.91
	600	7.70	19.33
Gala	400	2.22	6.57
	600	5.14	7.69
Golden Delicious	400	1.59	1.76
	600	3.74	7.30
Granny Smith	400	1.16	3.00
	600	3.11	6.91
Red Delicious	400	3.84	3.64
	600	4.05	7.09

(Adapted from Falguera and others 2012)

Juice color is influenced by the effectiveness of antioxidants as well as the effect of enzymes. Ascorbic acid is one of the most important antioxidants and in orange juice accounts for 65-90% of the total antioxidant activity (Miller and Rice-Ewans 1997; Gardner and others 2000). Dede and others (2007) reported better retention of antioxidant activity for HPP treated juices, compared to thermally treated juices, due to high retention of ascorbic acid. Both carrot and tomato juices retained greater than 80% of total antioxidant activity after HPP treatment (250 MPa, 35 °C, 15 min). Color change after HPP developed slowly over the duration of the study while heat-treated juices had their sharpest color change immediately after treatment. Berry juices have also shown similarly high retention of flavonols and anthocyanins after HPP treatment as compared to thermal treatment (Altuner and Tokuşoğlu 2013). Studies have shown that enzymes possess varying susceptibility to HPP. Seyderhelm and others (1996) have ranked

selected food enzymes by their rate of inactivation after HPP. Enzymes were ranked in the following order based on their susceptibility to inactivation during HPP: lipoxygenase, lactoperoxidase, pectinesterase, lipase, phosphatase, catalase, polyphenol oxidase then peroxidase. Consistent with the findings of Seyderhelm and others (1996), Bayindirli and others (2006) found that polyphenol oxidase, the enzyme responsible for browning in fruit juices, was retained in apple juice at low pressures but was absent after 450 MPa at higher temperatures (40°C and 50°C); at 25°C there was minimal browning. Cloud stabilization is an important quality attribute in orange juice processing (Stanley 1997). Cloud is retained by pectinesterase which protects natural pectin from enzymatic desterification and degradation. Although HPP treatment has been shown to destroy some labile pectinesterase, Goodner and others (1999) showed that cloud was maintained at high pressures for longer processing times; shorter processing times were ineffective at preserving cloud during a 90-day shelf life study.

High pressure processing possesses several advantages in both microbial reduction and in the maintenance of antioxidant activity. The low pH of fruit juice renders its processing through HPP to be advantageous. The primary disadvantage of fruit juice processing by HPP is the cost of equipment (Considine and others 2008). Currently the majority of commercial equipment is for batch processing (Hiperbaric 2013a) although pilot processing has been done on continuous flow systems (Kincal and others 2005). Current consumer demands for fresh tasting and microbiologically safe products will most likely continue to encourage processing by HPP.

Chapter 4 - Pulsed Electric Field Processing

Processing by pulsed electric field (PEF) involves applying several pulses of high voltage, usually in the range of 20 – 80 kV/cm to the substrate which is placed between two electrodes. Although the exact mechanism behind the antimicrobial activity of PEF is not known, two theories have been suggested. Electrical breakdown of the cells is believed to be caused by a buildup of charge at the cell membrane causing an electrical discharge and pore formation when the membrane potential exceeds a potential of around 1 V (Janositz and Knorr 2010; Castro and others 1993). Breakdown is thought to be reversible if the pore is small compared to the total membrane surface (Zimmerman 1986). The second mechanism is electroporation and occurs as the cell membrane becomes permeable to small molecules under the effect of the electric field (Vega-Mercado and others 1996) resulting in swelling and eventual lysis of the cell (Figure 4.1).

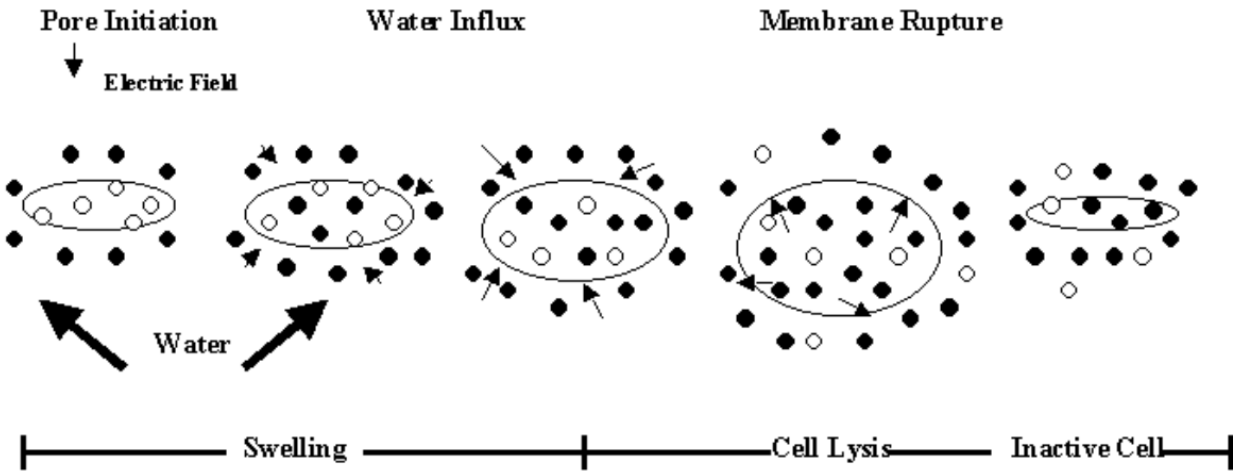

Figure 4.1: Electroporation of a cell membrane (Vega-Mercado 1996).

The efficiency and effectiveness of PEF on microbial reduction in fruit juices are governed by several factors. These factors can be categorized into three general groups: process, product, and microbial factors. The most important process factors are field intensity, processing temperature, waveform shape and length of treatment. In general, higher field intensities, longer treatment times and higher processing temperatures are more lethal towards microbes (Aronsson and others 2004). Optimization of these factors must be balanced with the

desire for little impact on flavor components in juice. For instance, longer treatment times (the duration of the pulse or number of pulses) results in an increase in substrate temperature which, although it increases the lethality of the treatment towards microbes, also results in detrimental effects on flavor components (Álvarez and others 2003a). The waveforms also factor in the lethality of the treatment towards microbes. Commonly used waveforms in PEF are exponentially decaying, square wave, bipolar, and oscillatory pulses (Figure 4.2). An exponential decaying waveform consists of a rapid rise in voltage towards a maximum voltage followed by a slow decay back to zero. Square waveforms are more lethal and energy efficient than the exponential waveform and consist of a rapid rise in voltage to a maximum followed by an incubation period at the maximum voltage and then a rapid drop back towards zero. The bipolar pulse consists of a reversal in the polarity of the applied pulses. Bipolar pulses are found to be more lethal than monopolar pulses because the repeated reversal in the direction of the charged molecules is stressful for microbial cells. Contrastingly, the oscillatory wave is the least effective as the microbial cell is not continuously exposed to the high intensity field for an extended period (Mittal 2009).

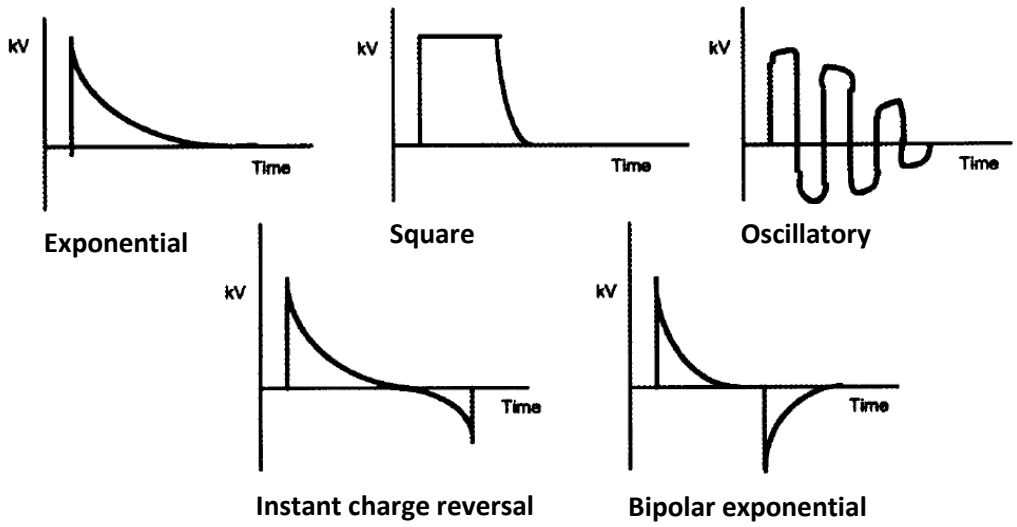

Figure 4.2: Electric pulse waveforms that can be applied in PEF treatment (Adapted from Mittal 2009).

Factors related to the product and microbe, in part, describe the basis of the limitations of PEF. The electrical conductivity of the food substrate is believed to be a major factor in PEF. In general, foods with higher electrical conductivities will generate lower electric fields and therefore lower microbial reduction during treatment (Singh and Kumar 2011). Hence, liquids with high conductivities (high ionic strengths) tend to be less suited for treatment with PEF. Pulsed electric field has also been found to be less sensitive towards gram-positive bacteria (compared to gram-negative) and more sensitive to large yeast cells. Bacterial cells in the logarithmic growth phase also have cell membranes that are significantly more sensitive to electric fields than those of cells in the lag or stationary phases (Mittal 2009).

There have been several literature reports on microbial and enzymatic changes after PEF including reported studies of actual microbial reduction within the fruit juice (Table 4.1). Unal and others (2002) reported that on assessing the susceptibility of *Lactobacillus leichmannii*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 to PEF, *E. coli* O157:H7 was the most susceptible with 2.9 log inactivation after 12 pulses at 20 kV/cm each for a duration of 3 μs. Mosqueda-Melgar and others (2007) had similar findings while studying the effects of time and pulse frequency during PEF treatment on *Salmonella* Enteritidis, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in inoculated melon juices. It was found that although all the organisms were susceptible to treatment, *E. coli* O157:H7 was the most susceptible and that time was a more important factor in this case than pulse frequency for microbial reduction. Further, Evrendilek and others (2000) used bench scale and pilot plant scale to assess the efficacy of PEF for microbial reductions in apple juice and apple cider respectively. In this study, the bench scale PEF resulted in a 4.5 log reduction of *E. coli* O157:H7 and improved shelf life of the pilot plant treated apple cider without any significant deterioration in color or vitamin C concentration. Evrendilek and others (1999) were also able to show that 5 log reductions of two strains of *E. coli* O157:H7 and *E. coli* 8739 in apple juice were possible with PEF. Álvarez and others (2003b) subjected *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium and found that susceptibility varied by serovar. In fact, under the conditions of the study (19-28 kV/cm), although PEF was able to achieve 5 log reductions for all serovars, the time required for *Salmonella* Enteritidis was around one half of the other serovars.

Table 4.1: Some reported pathogen log reductions in fruit beverages after Pulse Electric Field treatment.

Juice Type	Pathogen	Processing Conditions (electric field strength; time; temperature)	Log Reduction (CFU/ml)	Source
Apple Juice	*E. coli* O157:H7	90 kV/cm; 20 μs; 42 °C *	5	Evrendilek and others 1999
Apple Cider	*E. coli* O157:H7	90 kV/cm; 20 μs; 42 °C *	5.9	Iu and others 2001
Apple Juice	*E. coli* O157:H7	35 kV/cm; 4 μs; 40 °C *	4.2	Mosqueda-Melgar and others 2008
Orange Juice	*E. coli* O157:H7	35 kV/cm; 4 μs; 40°C *	5.1	Mosqueda-Melgar and others 2008
Strawberry Juice	*E. coli* O157:H7	35 kV/cm; 4 μs; 40°C *	5.1	Mosqueda-Melgar and others 2008
Apple Juice	*S.* Enteritidis	35 kV/cm; 4 μs; 40°C *	4.0	Mosqueda-Melgar and others 2008
Orange Juice	*S.* Enteritidis	35 kV/cm; 4 μs; 40°C *	5.0	Mosqueda-Melgar and others 2008
Strawberry Juice	*S.* Enteritidis	35 kV/cm; 4 μs; 40°C *	4.6	Mosqueda-Melgar and others 2008
Orange Juice	*Listeria innocua*	30 kV/cm; 12 μs; 54°C	6.0	McDonald and others 2000
Grape Juice	*E. coli*	34 kV/cm; 7.68 μs; 55°C	6.4	Heinz and others 2003
Apple Juice	*E. coli*	34 kV/cm; 7.68 μs; 55°C	6.2	Heinz and others 2003

*Maximum temperature.

Treatment of juices by PEF has been found to impart few undesirable effects on sensory characteristics. Cserhalmi and others (2006) reported that after continuous flow PEF (bench scale) with 50 pulses at 28 kV/cm on a series of citrus (grapefruit, lemon, orange and tangerine) juices, there were no significant practical changes in pH, Brix, non-enzymatic browning index (NEBI), and color. The juices were pretreated by filtering to remove solid particulates and then diluting with distilled water to yield electrical conductivities of around 1.5 mS/cm. Similarly, Cortés and others (2008) reported that a comparison of PEF treated filtered, undiluted orange juice presented less enzymatic browning when compared to thermally pasteurized orange juice and retained a color closer to that of untreated juice.

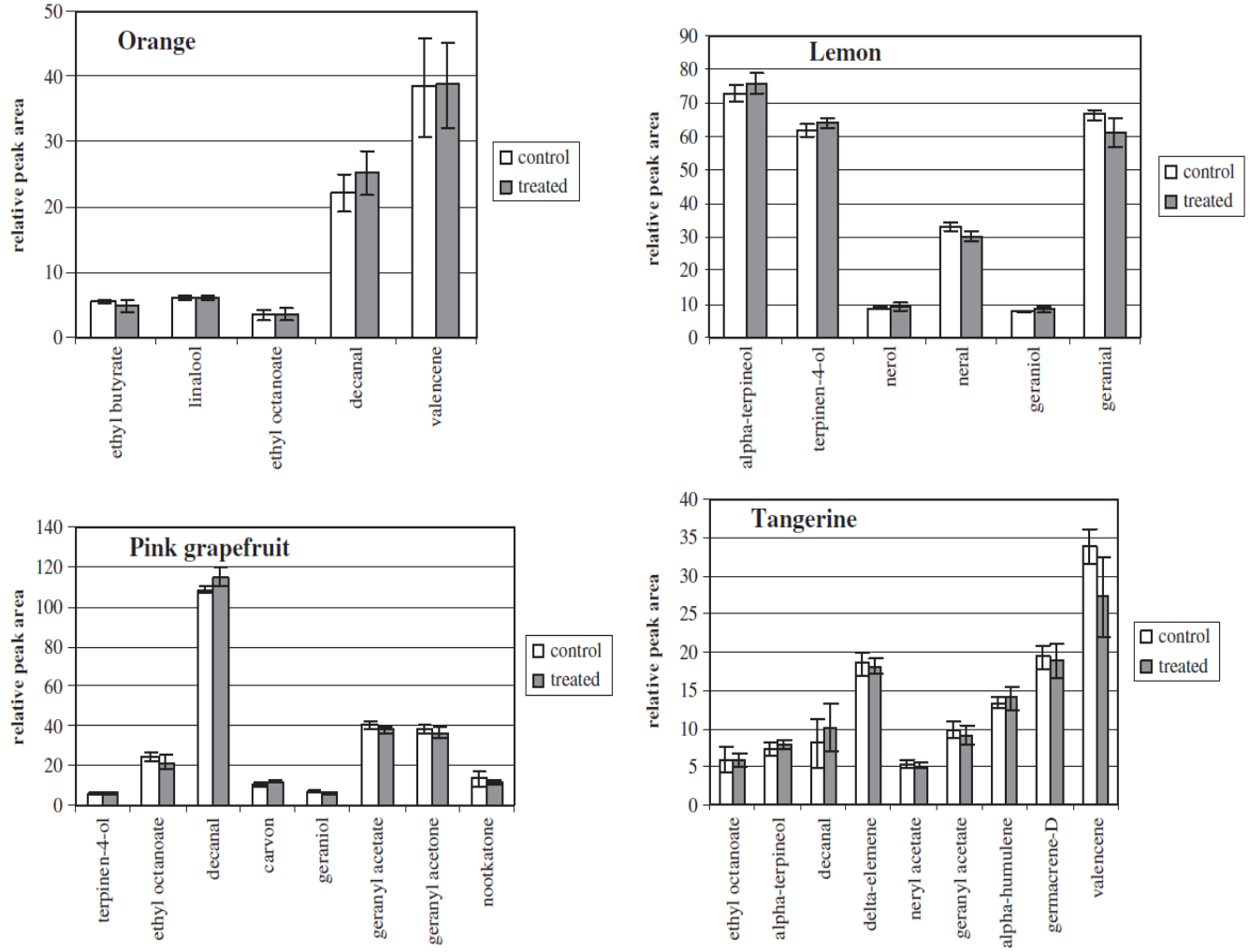

Figure 4.3: Volatile flavor compounds in Pulse Electric Field treated juices and control samples (Cserhalmi and others 2006).

Pectin methyl esterase, which is known to effect cloudiness in orange juice, can also be significantly (90%) inactivated by PEF (25 kV/cm at 50 °C) at temperatures where "heat-only" was found to be ineffective (Yeom and others 2002). McDonald and others (2000) described using a pilot-plant sized PEF system to treat reconstituted orange juice and freshly squeezed orange juice that was inoculated with *E. coli* (ATCC 26), *L. innocua*, *Saccharomyces cerevisiae* and *Leuconostoc mesenteroides*. *Saccharomyces cervisiae* was found to be the least susceptible (2.5 log reductions at 50 kV/cm and 50 °C) to PEF while *E. coli* and *L. innocua* were the most susceptible achieving greater than 5 log reductions at 30kV/cm and 50 °C. Inactivation of the bacterial species was independent of the field strength (30 or 50 kV/cm) at 50 °C but required more pulses at the lower field strength to achieve similar 5 log reductions. As described by Gachovska and others (2008), treatment of apple juice by PEF also holds potential as an antimicrobial treatment. Pasteurized apple juice inoculated with *E. coli*, to a population of 6 CFU/mL, was treated at 52 °C with an electric field that peaked at an energy of 60 kV/cm for 11.3 pulses, each of which was 3.5 μs long. The study described treatments with ultraviolet (UV), PEF and a combination of UV and PEF and yielded 3.5, 4.9 and 5.3 log reductions of *E. coli* (ATCC 23472), respectively.

Repeated studies of PEF treated juices illustrates that while it may not be as potent as thermal pasteurization, it does not cause any significant deterioration in the nutritional or sensory properties of the juice. As a result, there is significant interest in utilizing PEF as a pasteurization technique for "natural" juices. Min and others (2003) described a commercial-scale PEF facility (Figure 4.4) that was used to treat freshly squeezed orange juice using 40 kV/cm for 97 ms. They found that the treated orange juice had a similar shelf life to that of thermally pasteurized orange juice of 196 days at 4 °C and also retained higher concentrations of nutrients. Additionally, sensory panel analysis ranked the PEF treated juice as more similar in texture and flavor to freshly squeezed juice than the thermally pasteurized juice.

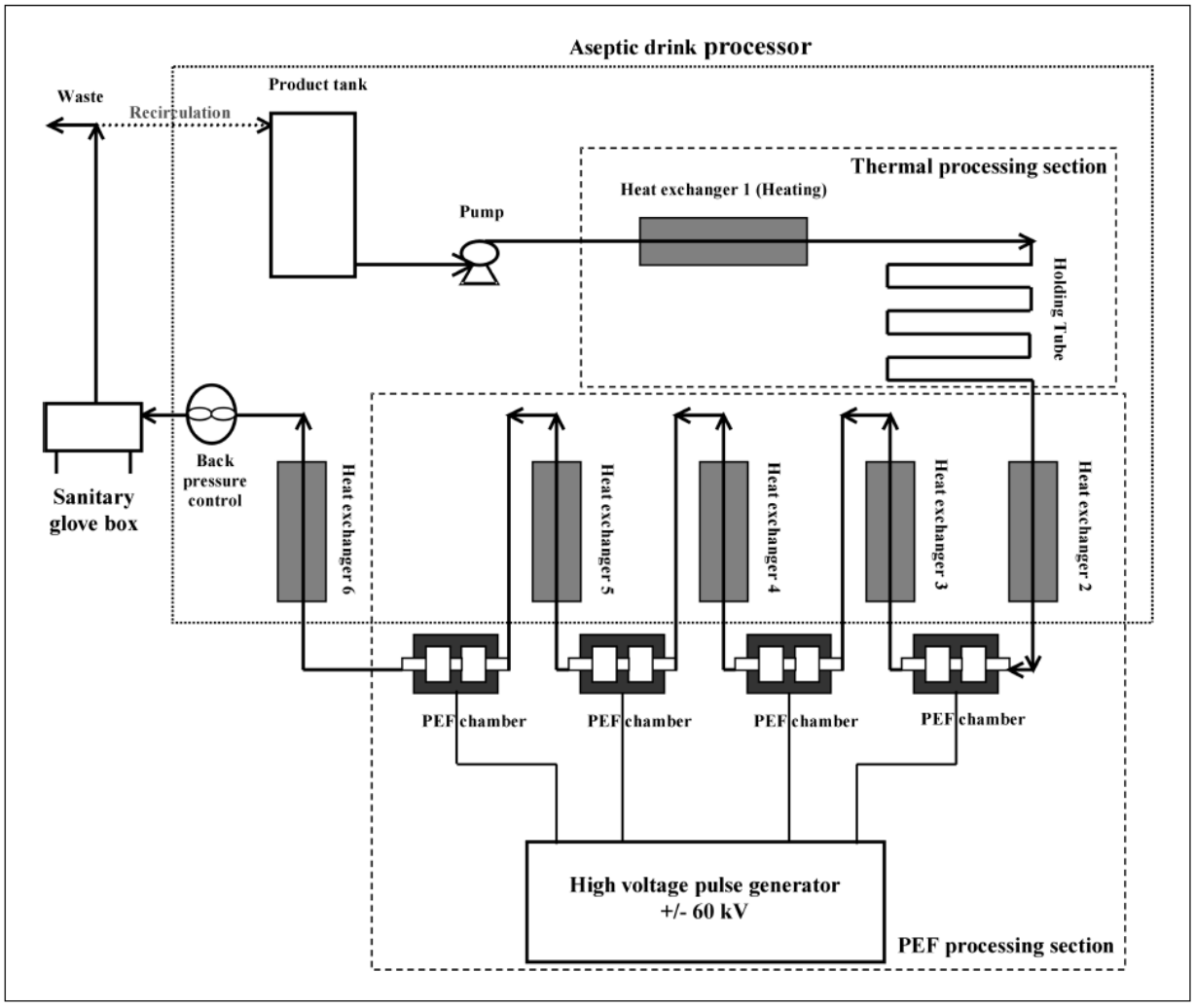

Figure 4.4: Flowchart of a commercial-scale pulsed electric field processing system (Min and others 2003).

Chapter 5 - Radiation Processing

According to the EPA (2013), irradiation of foods falls into two categories based on the ionization level of the radiation (Figure 5.1). Ionizing radiation uses high-energy particles that function by removing electrons from the target forming high-energy free radicals that damage the DNA and cellular structures of microorganisms. Higher doses of ionizing radiation are capable of effecting sterilization and prolonging shelf life. However, there are several potential drawbacks of ioinizing radiation as the free-radicals can also react with non-target species resulting in undesirable effects (Kader 1986). Ionizing radiation can be further categorized into two groups: particulate such as alpha and beta rays, and protons and neutrons, and non-particulate such as x-rays and gamma rays. Unlike ionizing radiation, non-ionizing radiation is only sufficiently energetic to cause the molecules to move but not to effect removal of electrons. As shown in Figure 5.1 non-ionizing radiation consists of radio waves, microwaves, and infrared through visible to low energy ultraviolet radiation. In general, non-ionizing radiation does not have as many undesirable effects as ionizing radiation. However, it is less efficacious in killing microorganisms (Taghipour 2004).

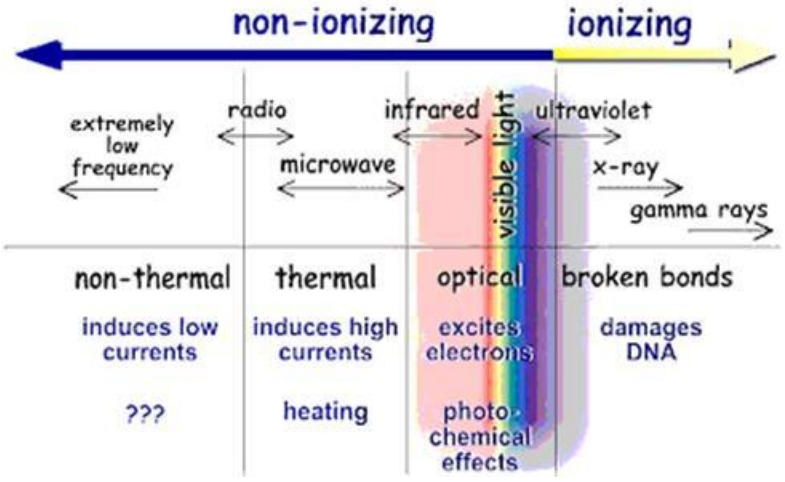

Figure 5.1: Types of radiation (Adapted from EPA 2013).

Ionizing Radiation in Pathogen Reduction

Although the use of ionizing radiation in the sterilization of meats is widely employed (Doris 1995), its use in the processing of fruit juice is less widespread. Buchanan and others (1998) investigated the treatment of apple juice inoculated with *E. coli* O157:H7 with gamma radiation derived from a Cs-137 irradiator. Varying resistance to radiation was found to be directly related to the concentration of suspended solids in the juice. Despite the resistances of the different juices, 1.8 kGy/cm was found to effect a 5 log reduction of *E. coli* O157:H7 in apple juice. In 2002, Foley and others (2002) described the use of gamma irradiation in the cold pasteurization of orange juice. While they found that gamma radiation was very potent in reducing microbial populations, it was also found to impart undesirable flavor components. Doses up to 4.0 kGy of radiation were used to treat samples of orange juice inoculated with *L. monocytogenes* and *Salmonella* Enterica. At the higher doses there was almost complete eradication of both *L. monocytogenes* and *Salmonella* illustrated by almost 7 log reductions. Additionally, background microbial flora also experienced significant decreases in populations although less so than the target microorganisms, *L. monocytogenes* and *Salmonella*. Despite these promising results, initial sensory panels described the aromas as "plastic and decayed" and "unpalatable" on 1 day post treatment. Further sensory evaluations were therefore abandoned. A similar study by Mitchell and others (1991) on the effects of low dose radiation on juice quality had similar sensory findings. Juice treated with radiation ranging from 0 to 600 Gy was found, after treatment, to have effects that varied depending on the fruit. Apple juice had significant decreases in brix, acidity, and pectinesterase activity when processed from 600 Gy treated fruit and had a "metallic and processed" flavor as assessed by a sensory panel. In contrast, orange juice had a significant increase in pectinesterase activity when processed from 600 Gy treated fruit with only minimal changes in flavor, brix, acidity, and vitamin C content although sensory panelists reported that the higher treatment resulted in a less acceptable orange juice flavor. Apart from juice color, there were no other significant changes in treated versus untreated grape juices. Mali and others (2011) described treatment with gamma irradiation of pomegranate peels which were processed into powder that had a comparable phenolic content, after treatment, to that of the control. In addition, there were non-detectable microbial loads at treatment levels above 5 Gy.

Although ionizing radiation possesses significant potential for microbial reduction, various factors including poor public perception (National Cattlemen's Beef Association 2002; Mehta 2002) of radiation will most likely preclude any opportunity for its use in the pasteurization of fruit juices. Irradiation also appears to impart undesirable flavor components as indicated by the additional species detected by gas chromatography of treated orange juice samples (Foley and others 2002).

Non-ionizing Radiation

Ultraviolet (UV) light is the primary source for non-ionizing radiation for microbial reduction in the treatment of fruit juices. Ultraviolet light can be delivered by a continuous source or as high intensity pulsed light. Noci and others (2008) described the processing of freshly squeezed orange juice with UV light, pulsed electric field (PEF), and a combination of both techniques. Ultraviolet light processing was accomplished by treating juice as a batch for 30 min at 30 cm from a 30 W UV light source. The processed juice was then compared with unprocessed juice to determine the reduction in microbial loads. Treatment with UV light only resulted in a 2.2 CFU/ml log reduction. However, when treatment with UV light was combined with PEF, log reductions were found to be 6.2 and 7.1 CFU/ml, for UV first and PEF first treatments respectively, outperforming both PEF only treatment (log reduction 5.4 CFU/ml) and thermal pasteurization at both 72 °C and 94 °C. All of the non-thermally processed juices did not show any significant differences in the physical properties of color, pH, brix, and conductivity compared to fresh juice. Gachovska and others (2008), investigated treatment with UV only as well as in combination with PEF, on the inactivation of *E. coli* (ATCC 23472) in apple juice. Fresh apple juice was inoculated with *E. coli* (ATCC 23472) then exposed to UV light in a continuous flow system. The treatment time was varied by increasing the length of the tubing exposed to a UV lamp (Figures 5.2 and 5.3). A peristaltic pump was used to vary the flow rate across the lamp from 8 to 20 ml/min. A maximum log reduction of 3.5 was achieved for the 50 cm length tubing with an 8 ml/min flow rate. Combination of UV treatment with PEF was found to be additive and non-synergistic yielding a 5.3 log reduction.

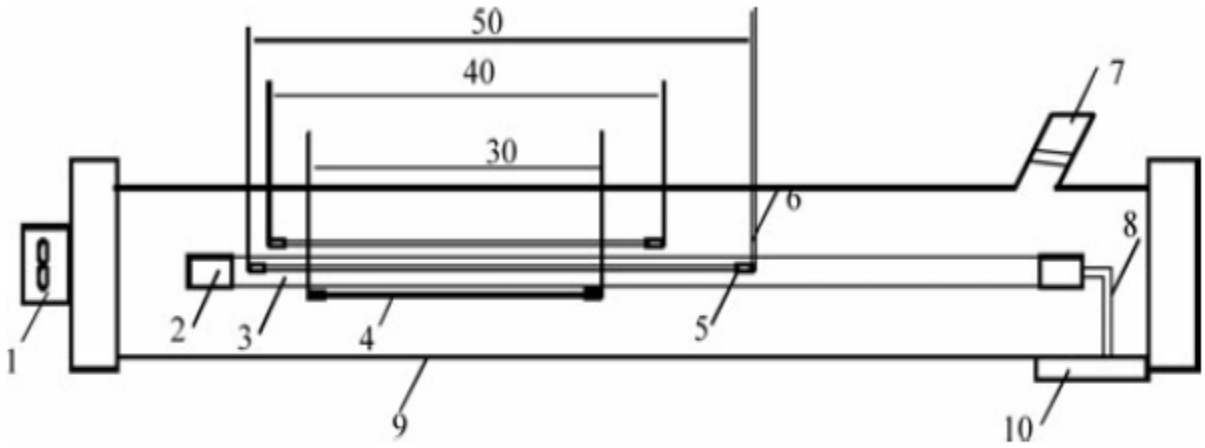

Figure 5.2: UV treatment chamber for continuous flow treatment.

1: fan; 2: lamp holders; 3: UV lamp; 4: quartz tube; 5: switch lock connector; 6: plastic tube; 7: output for cooling; 8: wires; 9: plastic corpus of the treatment chamber; 10: power supply for the UV lamp **(Gachovska and others 2008).**

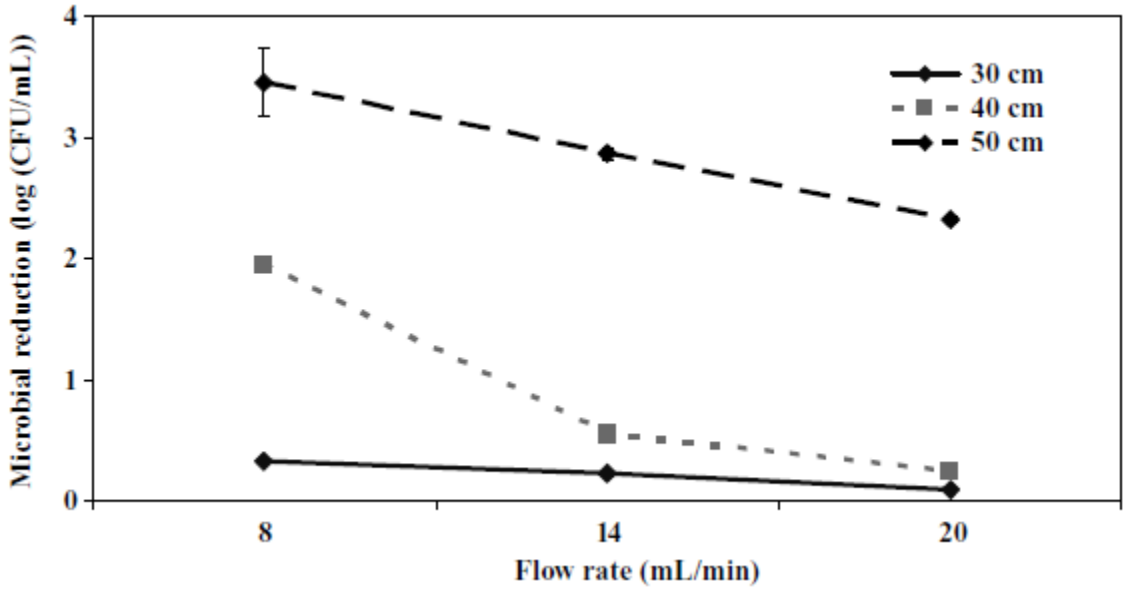

Figure 5.3: Inactivation of *E. coli* in apple juice using various treatment lengths Gachovska and others 2008).

Oteiza and others (2005) discussed the effect of UV radiation on the reduction of *E. coli* in apple, orange and multifruit juices with various absorptivities. Irradiation was accomplished by exposing the samples to 254 nm wavelength light at 20 °C. A radiometer was used to determine the actual light intensities which were found to be 3.0 ± 0.5 mW/cm^2 for each test. The actual doses that were applied were between 0 and 6 J/cm^2 for each sample. Individual samples of each juice were inoculated with both *E. coli* (ATCC 25922) and *E. coli* O157:H7 generating *E. coli* populations of 10^5 CFU/ml and 10^7 CFU/ml respectively. Treatment with UV radiation achieved 7 log reductions for all juice types at low doses of less than 2 J/cm^2 if the treatment thickness was kept below 1 mm and the juice was agitated with stirring.

Guerrero-Beltram and others (2008) reported that the treatment of grape, cranberry, and grapefruit juices with UV-C (ultraviolet light at 254 nm) light reduced populations of *Saccharomyces cervisiae*. The UV treatment unit consisted of two UV-C germicidal lamps connected in series. Flow rates of the juice through the system varied from 0.073 to 1.02 l/min with UV light doses varying from 75 to 450 kJ/m^2. Maximum log reductions were obtained at the highest flow-rate after 30 min of treatment for all juices. Maximum log reductions of cranberry juice and grapefruit juice were approximately 2.5 CFU/ml while the maximum log reductions in grape juice were only 0.5 CFU/ml. The lower log reduction for grape juice is attributable to the darker color (deep purple) of grape juice that "blocked" the UV treatment reaching the bulk juice. Additionally, as treatment progressed, grape juice had the biggest change from initial color possibly due to the degradation of its natural anthocyanins.

High Intensity Pulsed Light (HIPL) Processing

Pulsed light involves the use of intense and short duration pulses of broad-spectrum white light. The light mimics sunlight and includes wavelengths from the near infrared to ultraviolet region of the electromagnetic spectrum. Although pulsed light has been used primarily for decontamination of food surfaces and food packages, it is considered as an alternative for continuous ultraviolet treatment of solid and liquid foods (Luksiene and others 2013).

Pulsed light has been shown to be effective against bacteria and mold spores (Oms-Oliu and others 2010) with the antimicrobial effect being attributed to the broad spectrum UV content

and energy density of the treatment. The process can be modulated by changing the number of pulses, pulse width, peak power of the pulse, and distance from the light source and thickness of the product. The two major product factors that influence the effectiveness of the pulsed light treatment are the transparency to light and the amount of solids present in the beverage. Pulsed light treatments are more effective in clarified juices with high transparency to UV light.

Although High Intensity Pulsed Light has been used primarily for the decontamination of surfaces and packages, there have been reports of the use of HIPL treatments to pasteurize fruit juices. In one report, Palgan and others (2011) discussed the use of high intensity light pulses to treat *E. coli* K12 DSM 1607 and *Listeria innocua* in apple and orange juices and in milk. Samples of apple and orange juices were inoculated with *E. coli* K12 DSM 1607 and *Listeria innocua* and then treated with 2-8 s of HIPL at 3 Hz. The effectiveness of the treatment was found to be proportional to the transparency of the beverage with the most transparent beverage, apple juice, displaying a 4.5 log reduction after only a 4 s treatment. Deterioration of flavor and antioxidant capacity occurred after 8 s although other quality characteristics remained intact. Contrastingly, another study by Caminiti and others (2012) described the use of HIPL in conjunction with manothermosonication and found that the sensory qualities of an orange carrot juice blend treated with this process was more preferred in comparison to a thermally treated juice. This occurred despite the lesser effect that the non-thermal process had on juice color.

In summary, there is potential for radiation to function as a pasteurization technique for fruit juices although more research in this area needs to be conducted.

Chapter 6 - Ultrasonic Processing

Sound energy can be classified into three groups: infrasound, acoustic and ultrasound. The U.S. Food and Drug Administration describes ultrasound energy as the energy generated by sound waves greater than 16 kHz (Figure 6.1) (FDA 2013b).

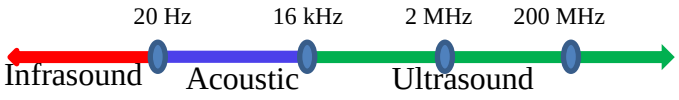

Figure 6.1: Range of ultrasound energy (Adapted from Wikipedia 2014).

Ultrasound energy can be used for a range of applications in fruit juice processing including non-destructive physical testing, determination of physical defects in food, and process improvements requiring acceleration of diffusion (Harrel 2001; Knorr and others 2004; Ferraretto and others 2013). While ultrasound energy is not used primarily for microbial reduction, the demand for non-thermal alternatives for juice processing has demanded that this technique be considered (Dubrović and others 2011; Šimunek and others 2013). The antimicrobial action of ultrasound is attributed to intracellular cavitation (Ashokkumar and others 2012). At high ultrasonic amplitude the rarefaction cycle exceeds that of the intermolecular attractive forces with the liquid. As a result, tiny microscopic bubbles form throughout the liquid medium and after a few cycles grow to an unstable and critical volume. Collapse of these cavitation bubbles results in hot spots where temperature and pressure can soar up to 5000 K and 1000 atm, respectively (Soria and Villamiel 2010) (Figure 6.2). The cavitation bubbles are believed to disrupt cellular structure and function which eventually causes cell lysis.

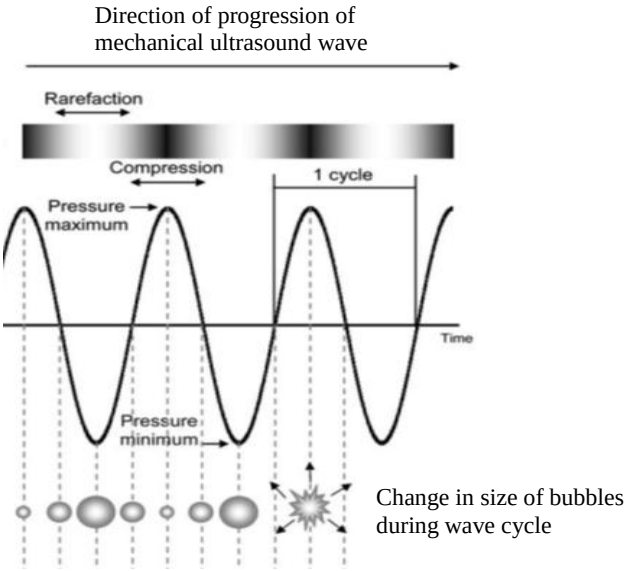

Figure 6.2: Ultrasonic cavitation (adapted from Soria and Villamiel 2010).

Process factors affecting the efficacy of ultrasound treatment include ultrasound energy, intensity, temperature, and pressure. The inclusion of higher pressures and temperatures can often lead to 5 log reductions, providing a microbiologically safe product with a higher nutritional quality (Lee and others 2009). This has given rise to differing terms indicating the use of pressure (manosonication) and/or temperature (manothermosonication and thermosonication, respectively) along with sonication. An increase in process temperature generally leads to an increase in the number of cavitation bubbles and a decrease in viscosity and surface tension. A lower viscosity promotes more violent cavitation bubbles; however, with an increase in temperature, there is a corresponding increase in vapor pressure which suppresses the collapse of the cavitation bubbles (Muthukumaran and others 2006). The effect of temperature must therefore be optimized to achieve a desirable processing effect (Petigny and others 2013). External pressure reduces the number of cavitation bubbles but provides for an increase in pressure in the cavitation bubbles making for more rapid and violent collapses. External pressure, therefore, can result in an increase in the intensity of the sonication process without the need for an increase in sonication amplitude (Wong and others 2008).

Product dependent factors include liquid viscosity, surface tension, vapor pressure, the nature and concentration of dissolved gases, and the presence of solid particles. Lower liquid viscosities, lower surface tension, and a lower concentration of solid particles all serve to increase the potency of the ultrasonic treatment process. While the concentration of dissolved

gas can be considered as specific to a given medium, dissolved gas concentration can be altered by bubbling an inert gas through the medium (Roozea and others 2013). Higher concentrations of dissolved gases are desired to increase cavitation as the dissolved gas acts as nuclei for the formation of bubbles. Additionally, the nature of the dissolved gas changes the bubble adiabatic ratio, thermal conductivity, and even the liquid surface tension, all of which affect the hot spot temperature.

Microbial reduction with ultrasonic treatments

Ultrasound alone is an established laboratory method that can be used to lyse microbial cells and extract cellular components. The sensitivity of ultrasonic treatments varies depending on microbe size, shape, and species.

Escherichia coli

The effectiveness of ultrasonic treatments on the eradication of *E. coli* is determined by several factors. Patil and others (2009) describe the effects of acid adaptation on *E. coli* (ATCC 25922, NCTC 12900) inactivation with ultrasonic treatment. Factors such as ultrasound amplitude, treatment time, and medium (broth, model apple and orange juices) inoculated with two strains of *E. coli* (ATCC 25922, NCTC 12900) were studied. Inactivation of both strains was found to be linearly dependent on ultrasound amplitude. Although slight differences were seen due to acid adaptation, they were not consistent across both strains. In the model orange juice, the higher amplitudes of 7.5 and 37.5 μm caused total no presence of *E. coli* in one strain after 15 min, but only 2.5 and 2.7 log CFU/ml reductions occurred respectively, in the second strain. In model apple juice, the lowest amplitude of 0.4 μm achieved a 3 log CFU/ml reduction after 5.3 min while inactivation was achieved with 7.5 and 37.5 μm amplitudes after 6 and 3 min respectively.

A report by Salleh-Mack and Roberts (2007) described the effect of temperature, soluble solids, acids, and pH on the inactivation of *E. coli* (ATCC 25922) by ultrasound. The effect of temperature was studied by comparing ultrasound treatments where the temperature was controlled (keeping the medium cool) and uncontrolled. Temperature was found to have a large direct effect on inactivation. Using this data the authors simulated the effect that would be expected in apple juice (Figure 6.3). Two organic acids of relevance to processed fruit juices were studied: citric acid and malic acid. Although there was no significant differences between

the acids, ultrasonic treatments at the lower pH (2.5) were found to be more bacteriostatic to *E. coli* (ATCC 25922) resulting in an average log reduction of 5.4 CFU/ml compared to 5.1 CFU/ml at the higher pH (4.0). The addition of dissolved solids required an additional two min of processing time to achieve a 5 log reduction as compared to the control.

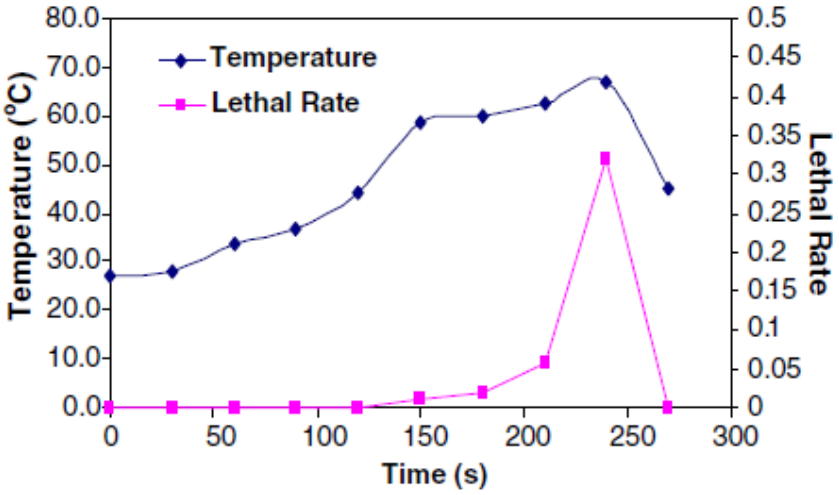

Figure 6.3: Temperature profile and lethality plot of simulated apple juice inoculated with *E. coli* (ATCC 25922) showing the temperature sensitivity of *E. coli* (ATCC 25922) (Salleh-Mack and Roberts 2007).

Lee and others (2009) studied the inactivation of *E. coli* K12 with sonication combined with pressure and heat, and found that a combination of these lethal factors significantly shortened the time needed to achieve a 5 log reduction (Table 6.1) compared to application of thermal only. Scanning electron microscopy (SEM) also showed significant damage of treated *E. coli* K12 cells (Figure 6.4).

Table 6.1: Log reductions for *E. coli* K12 in buffer (0.01 M, pH 7) treated by sonication under differing conditions of pressure and temperature.

Pressure (kPa)	Log reduction CFU/ml after 4 min	
	40°C	61°C
Thermal only	0	5.0
100	4.0	7.2
300	6.2	7.0
400	6.8	7.4
500	6.5	6.8

(Adapted from Lee and others 2009)

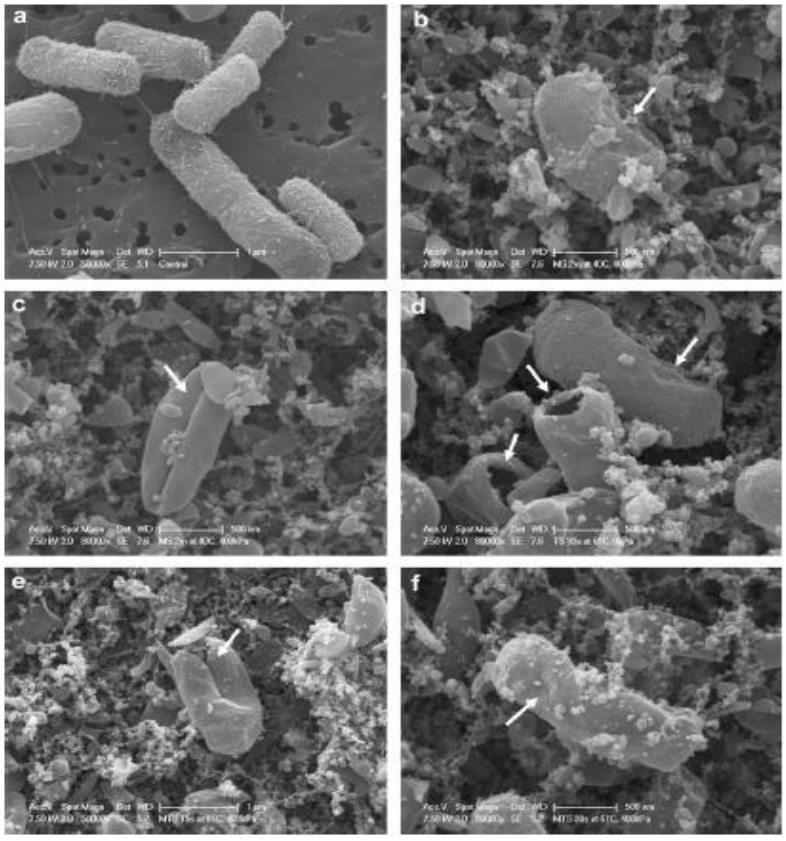

Figure 6.4: Scanning electron microscopy images of (a) untreated *E. coli* K12 (b) and (c) *E. coli* K12 manosonicated at 40° C, 500 kPa for 2 min (d) *E. coli* K12 thermosonicated at 61° C and 100 kPa for 0.5 min (e) and (f) *E. coli* K12 manothermosonicated at 61° C and 500 kPa for 0.25 min and 0.5 min respectively (Lee and others 2009).

Other microbes

Pagán and others (1999) described the effect of manosonication and manothermosonication on the growth of *S. enteritidis* and *L. monocytogenes.* In both cases, manothermosonication was more effective at reducing bacterial counts when compared to thermosonication at the same temperature (62° C) (Table 6.2). The rate of cell inactivation by manosonication increased significantly with rising static pressure. The increase was not linear and the magnitude of the increase got progressively less with increasing pressure (Figure 6.5).

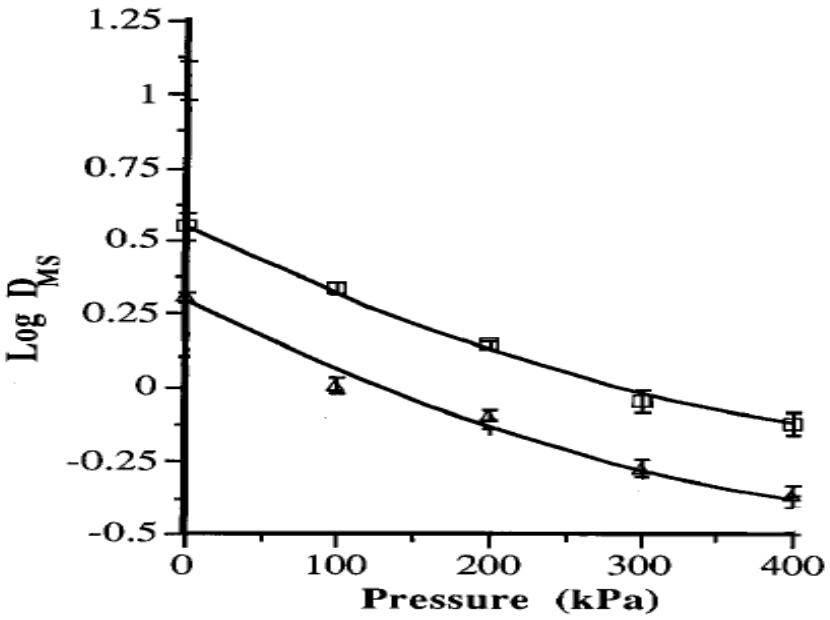

Figure 6.5: Effect of pressure on rate of inactivation by manosonication (117 µm, 40° C) of *L. monocytogenes* (□) and *S.* Enteritidis (△) (Pagán and others 1999).

Valero and others (2007) described the effect of ultrasonic treatments on total microorganism populations after storage at 5°C and 12°C. It was found that the presence of pulp during ultrasonic treatments decreased the effect of treatments which included 485 CFU/ml for 0.1% pulp to 610 CFU/ml for 10% pulp. Batch treatments (500 kHz, 240 W, 15 min) were found to cause temperature dependent log reductions from 0.03 CFU/ml at 50°C to 3.43 CFU/ml at 60°C and to have virtually no effect at continuous flow rates of 3000 L/h. There were no significant differences in the populations of molds and yeasts between the control and treated samples. Gómez-López and others (2010) also found low log reductions of 0.56 CFU/ml for

yeast and mold in sonicated calcium-added orange juice. Contrastingly, Adekunte and others (2010, 2010b) showed that the inactivation of yeast in tomato juice by sonication was dependent on amplitude (μm) and processing time (min), achieving a 5 log reduction at 61 μm for 7.5 min.

Effect of sonication on quality characteristics

In general, sonication of fruit juices has been found to have a desirable effect on quality characteristics of fruit juices. Gómez-López and others (2010) showed that the quality characteristics of orange juice slightly deteriorated after treatment, but deteriorated less so than the untreated controls. Adekunte and others (2010) found no deterioration in pH, Brix, or titratable acidity after sonication treatment of tomato juice; however they reported amplitude-dependent changes in ascorbic acid content which was attributed to oxidation reactions with hydroxyl radicals formed during the sonication process. Valero and others (2007) also reported no adverse effects on the quality attributes of limonin content, browning pigments, and color after ultrasonic treatment.

Ultrasonic treatment promises to be a very useful pasteurization technique when combined with another process such as heat or pressure treatment. The ability for ultrasonication to reduce the heat or pressure required to achieve a 5 log reduction allows for preservation of desired quality characteristics to enable a more natural fruit juice product.

Chapter 7 - Addition of Natural Antimicrobials

The use of natural substances as an emerging preservation method for fruit juice is being explored due to driving consumer trends towards more naturally processed foods (Zink 1997). These natural substances may be extracted or derived from animal, plant or microbial sources. Chemical preservatives such as sodium benzoate and potassium sorbate have been effectively used for years to preserve fruit juices and are listed in the Generally Recognized As Safe (GRAS) substances database (FDA 2013c). However, reports have shown that reactions with sodium benzoate and ascorbic acid which are common components of many fruit juice beverages have been linked to formation of benzene (FDA 2007) which has been classified as a human carcinogen (EPA 2009).

Corbo and others (2009) demonstrated that antimicrobials including essential oils, spices, and chitosan can be used to prolong the shelf life of food products. Some of these natural antimicrobials have been considered as GRAS additives (Rupasinghe and Yu 2012) for foods and have been used to preserve fruit and vegetable juices (Yuste & Fung 2004). A list of selected natural antimicrobials and their status for GRAS additives is shown in Table 7.1.

Table 7.1: Selected natural antimicrobials and their status for GRAS additives.

Name	Origin	GRAS Status
Chitozan	Animal	No
Cinamon	Plant	Yes
Clove	Plant	Yes
Coriander	Plant	Yes
Garlic	Plant	Yes
Lactoperoxidase	Animal	No
Lemon (peel, balm, grass)	Plant	Yes
Nisin	Microbial	Yes
Rosemary	Plant	Yes
Sage	Plant	Yes
Thyme	Plant	Yes

(Adapted from Rupasinghe and Yu 2012)

Bacteriocins

Bacteriocins represent the microbial source of natural antimicrobials and are classified according to Settanni and Corsetti (2008) as ribosomally synthesized, extracellularly released low-molecular-mass peptides or proteins (usually 30–60 amino acids) which have a bactericidal or bacteriostatic effect on other bacteria. Nisin is a bacteriocin produced by some strains of *Lactococcus lactis* and contains the rare amino acids *meso*-lanthionine and 3-methyl-lanthionine. It has been shown to be effective against gram-positive bacteria, primarily spore formers (Yuste & Fung 2004) and is described by Jin and others (2009) as a desirable food preservative since it is non-toxic, heat stable, destroyed by enzymes, and has a narrow spectrum of antimicrobial activity. The mechanism of action of nisin in a bacterial cell is shown in Figure 7.1.

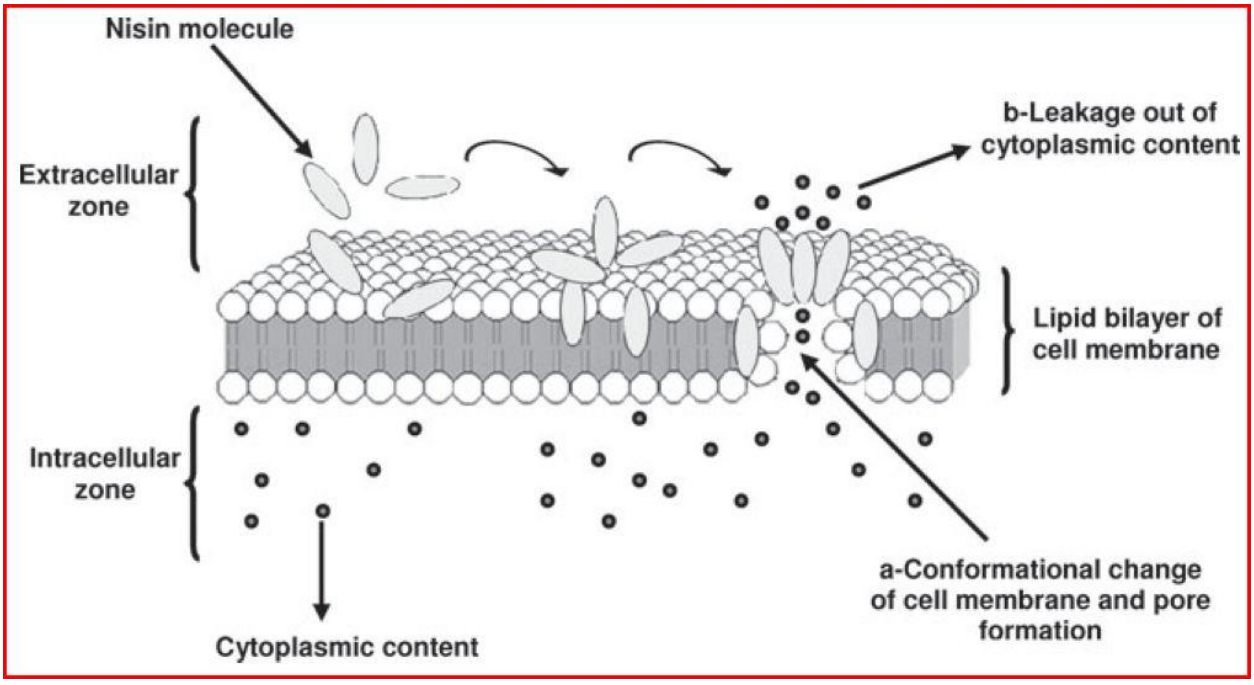

Figure 7.1: Mechanism of action of bacteriocin (nisin) in a bacterial cell (Adapted from Raybaudi-Massila & others 2009).

According to Abee and others (1994), the mode of action of nisin is to attack the components of the cytoplasmic membrane where they depolarize energized bacterial membranes forming voltage dependent multistate pores. The result of this pore formation is the loss of accumulated amino acids and inhibition of amino acid transport resulting in its effectiveness

against spore formers. Laing and others (2002) reported that the action of nisin against pathogenic organisms in fruit juice is more effective when used with other preservation methods. The juice pH and storage temperature are also critical parameters that add to the efficacy of nisin and cinnamon as effective antimicrobials. In the case of apple juice treated with a combination of nisin and cinnamon at varying storage temperatures and pH, Yuste and Fung (2004) showed that counts of *Salmonella* Typhimurium in apple juice, at a constant concentration of 0.3% cinnamon, decreased from 4.22 log CFU/ml to between 2.5 to 3.51 log CFU/ml depending on the concentration of nisin when stored at 20 °C, after 24 h of storage on tryptic soy agar medium. *Escherichia coli* O157:H7 under similar experimental conditions showed a maximum reduction of 1.64 log CFU/ml, which highlighted the combined effectiveness of nisin and cinnamon in the reduction of pathogens in apple juice. The higher the dose of nisin, with 0.3% cinnamon, the more efficient the inactivation of both *Salmonella* Typhimurium and *E. coli* (Table 7.2).

Table 7.2: Bacterial counts in apple juice inoculated with *Salmonella* Typhimurium and *E. coli* O157:H7 and treated with nisin and cinnamon.

Bacterial counts (log CFU/ml) in inoculated apple juice stored at 20°C				
	Salmonella Typhimurium		*E. coli* O157:H7	
	Initial count	24 h	Initial count	24 h
Untreated	4.22	3.93	4.15	3.97
25 ppm nisin	4.22	3.51	4.15	3.90
25 ppm nisin + 0.3% cinnamon	4.22	3.42	4.15	3.75
50 ppm nisin	4.22	3.51	4.15	3.72
50 ppm nisin + 0.3% cinnamon	4.22	2.51	4.15	3.56
100 ppm nisin	4.22	3.37	4.15	3.59
100 ppm nisin + 0.3% cinnamon	4.22	2.85	4.15	3.20
200 ppm nisin	4.22	3.13	4.15	3.39
200 ppm nisin + 0.3% cinnamon	4.22	2.68	4.15	2.51

(Adapted from Yuste and Fung 2004)

Organic acids

Organic acids are weak acids and are a common component of fruit juices. They have been traditionally added to foods as preservative agents. Weak acids are only partially dissociated in solution and these partially dissociated components have been reported to exhibit antimicrobial activity due to their ability to penetrate the cell membrane lipid bilayer (Stratford 2012). Raybaudi-Massillia and others (2009) proposed a mechanism of action of organic acids on a bacterial cell (Figure 7.2).

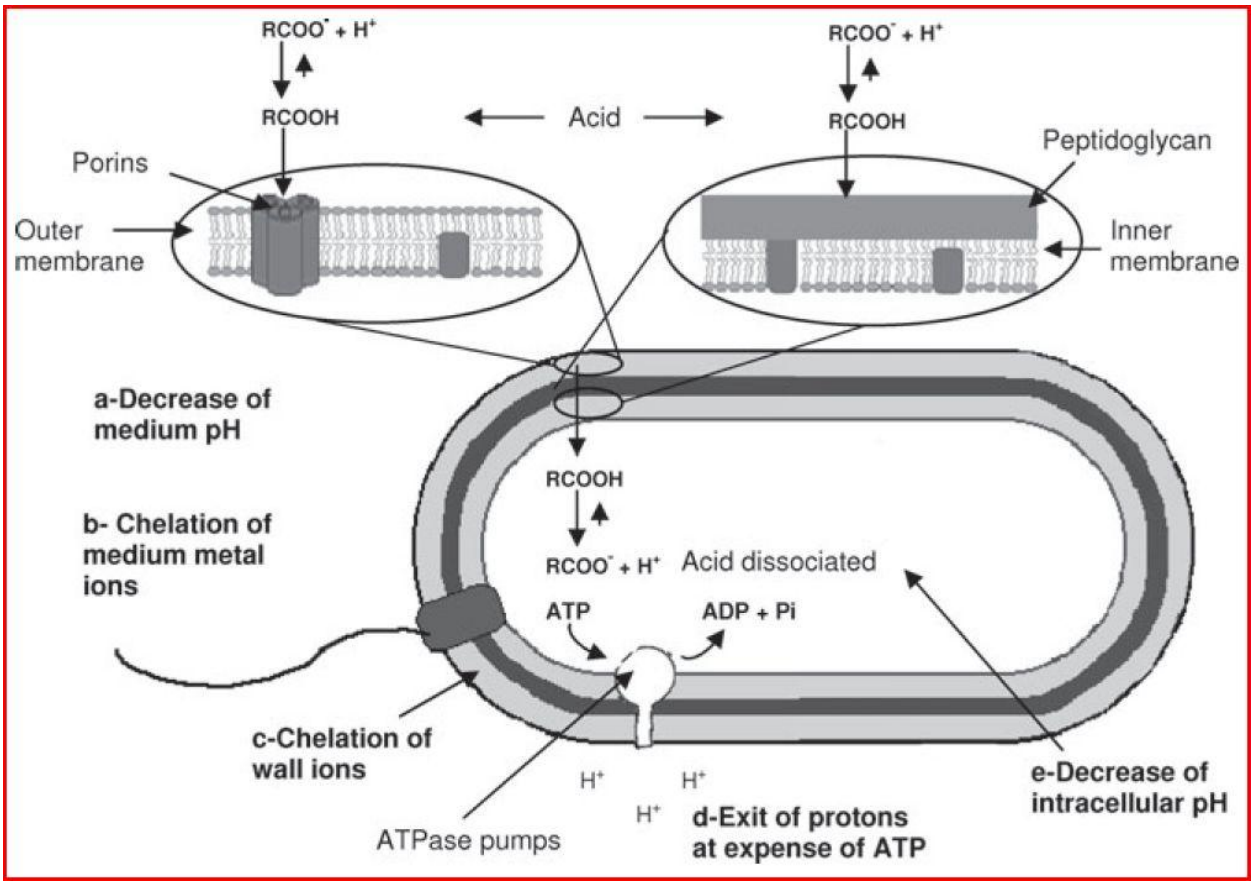

Figure 7.2: Mechanism of action of organic acids in a bacterial cell. The amplification illustrates the mode of action of an organic acid passing through the outer membrane in a gram-negative bacteria cell (Raybaudi-Massillia and others 2009).

The organic acid targets the bacterial cell by affecting multiple systems of the organism. An increase in proton concentration leads to a lowering of the pH of the surrounding membrane resulting in interruption of cell membrane transport and alteration of cell membrane permeability

(Raybaudi-Massillia and others 2009). Bjornsdottir and others (2006) reported that factors affecting the antimicrobial ability of organic acids were the pH, acid concentration, ionic strength, bacterial strains, and the environmental condition including growth phase, temperature and induced acid resistance of the microorganism.

Organic acids are a good alternative to use in the fruit juice processing industry due to their natural origin as well as their ability to act as a preservative, antioxidant, flavoring agent, and acidifier at a relatively low cost (Raybaudi-Massillia and others 2008). However, careful consideration should be given to the traits of the pathogenic microflora, pH, and kind of juice as well as molecular structure, size, and pK_a of the acid before selection of the organic acid. Raybaudi-Massillia and others (2008) reported greater than 5 log CFU/ml reductions in populations of *Listeria monocytogenes*, *Salmonella* Enteriditis and *E. coli* O157:H7 in apple, pear, and melon juice when combined with various concentrations of malic acid. The minimal percentage concentrations of malic acid to achieve greater than 5 log CFU/ml is shown in Table 7.3. *Escherichia coli* O157:H7 offered the greatest resistance to the antimicrobial effects of malic acid.

Table 7.3: Effect of malic acid on *Listeria monocytogenes*, *Salmonella* Enteritidis and *Escherichia coli* O157:H7 innoculated in apple, pear, and melon juice, and then stored for 24 hours at 20 °C

	Apple Juice		Pear Juice		Melon Juice		Log reduction (CFU/ml)
	% Malic acid	pH	% Malic acid	pH	% Malic acid	pH	
L. monocytogenes	0.4	3.3	0.6	3.3	0.8	3.5	> 5
S. Enteritidis	0.8	3.1	0.8	3.2	0.8	3.5	> 5
E. coli O157:H7	0.8	3.1	1.5	2.8	2.0	3.0	> 5

(Adapted from Raybaudi-Massillia and others 2008)

The higher resistance to malic acid offered by *E. coli* O157:H7, a gram negative organism when compared to *Listeria monocytogenes*, a gram positive organism and gram negative *Salmonella* Enteriditis could be attributed to the difference in the resistance mechanisms

in gram negative versus gram positive bacteria (Raybaudi-Massillia and others 2008). Nikaido (2003) attributed this difference to the hydrophobic membrane like structures surrounding the gram-negative bacteria that offer resistance to entry of low molecular weight molecules such as organic acids, where the entrance to the cell is permitted through water-filled channels formed by transmembrane proteins. The gram-positive wall contains a less complex cell wall, comprised of a thick peptidoglycan layer and a bilayer thus offering greater accessibility for organic acids. *Escherichia coli* showed greater resistance to malic acid when compared to *S.* Enteriditis since higher concentrations were required to achieve greater than the 5 log CFU/ml reduction in apple, pear and melon juices. Lin and others (1996) reported that *E. coli* O157:H7 has the ability to survive in acid stressed environments due to the presence of several acid resistant systems within the structure of the bacteria and recommended that *E. coli* O157:H7 should be considered as a target organism when evaluating the effectiveness of organic acids in preserving fruit juices.

Essential Oils

Essential oils, also referred to as volatile or ethereal oils, are aromatic oily liquids obtained from plant material (flowers, leaves, buds, seeds, twig bark, herbs, wood, fruits and wood) and are obtained for commercial production primarily by distillation. They are also obtained by extraction and fermentation (Burt 2004). Reports suggest that there are over 3,000 known essentials and approximately 10% have commercial importance (Burt 2004). Apart from their use as flavoring agents in the food industry, essential oils and its constituents have been shown to have antimicrobial properties (Ait-Oauzzou and others 2011a) and are therefore of importance in the preservation of food products offering a more natural appeal to the consumer. Although the majority of essential oils have been considered as GRAS additives (Rupasinghe and Yu 2012), their use as the sole source of pathogen reduction in fruit juices is limited because the reported doses to achieve the required antimicrobial activity might exceed organoleptically acceptable levels (Raybaudi-Massilia 2006).

Essential oils are composed of two or three major components (Table 7.4). These components include terpenes, alcohols, phenols, acids, aldehydes, ketones and esters with the phenolic components being chiefly responsible for the antimicrobial properties (Burt 2004). Ait-Ouazzou and others (2011b) have reported that the minor components may also exhibit

antimicrobial activity, which is directly related to the variations in the chemical composition of essential oils.

Table 7.4 : Major components of selected essential oils that exhibit antibacterial properties.

Essential Oil	Plant Source (Latin name)	Major Components	Approximate Composition (%)
Cilantro	Coriandrum sativum (immature leaves)	Lanitol E-2-decanal	26 20
Coriander	Coriandrum sativum (seeds)	Linalool E-2-decanal	70 -
Cinnamon	Cinnamomum zeylandicum	Trans-cinnamaldehyde	65
Oregano	Origanum vulgare	Carvacrol Thymol γ-Terpinene p-Cymene	Trace – 80 Trace - 64 2 - 52 Trace -52
Rosemary	Rosmarinus officinalis	α-pinene Bomyl acetate Camphor 1,8-cincole	2 - 25 0 – 17 2 – 14 3 – 89
Clove (bud)	Syzygium aromaticum	Eugenol Eugenyl acetate	75 – 85 8 – 15
Thyme	Thymus vulgaris	Thymol Carvacol γ-Terpinene β-Cymene	10 – 64 2 – 11 2 – 31 10 - 56

(Adapted from Burt 2004)

Due to the complex composition of essential oils, the antimicrobial activity might be attributed to several specific targets within the cell. Raybaudi-Massilla and others (2009) reported the location and mechanism of action in the bacterial cell which may include:

degradation of the cell wall, damage to the cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm, and depletion of the proton motive force (Figure 7.3).

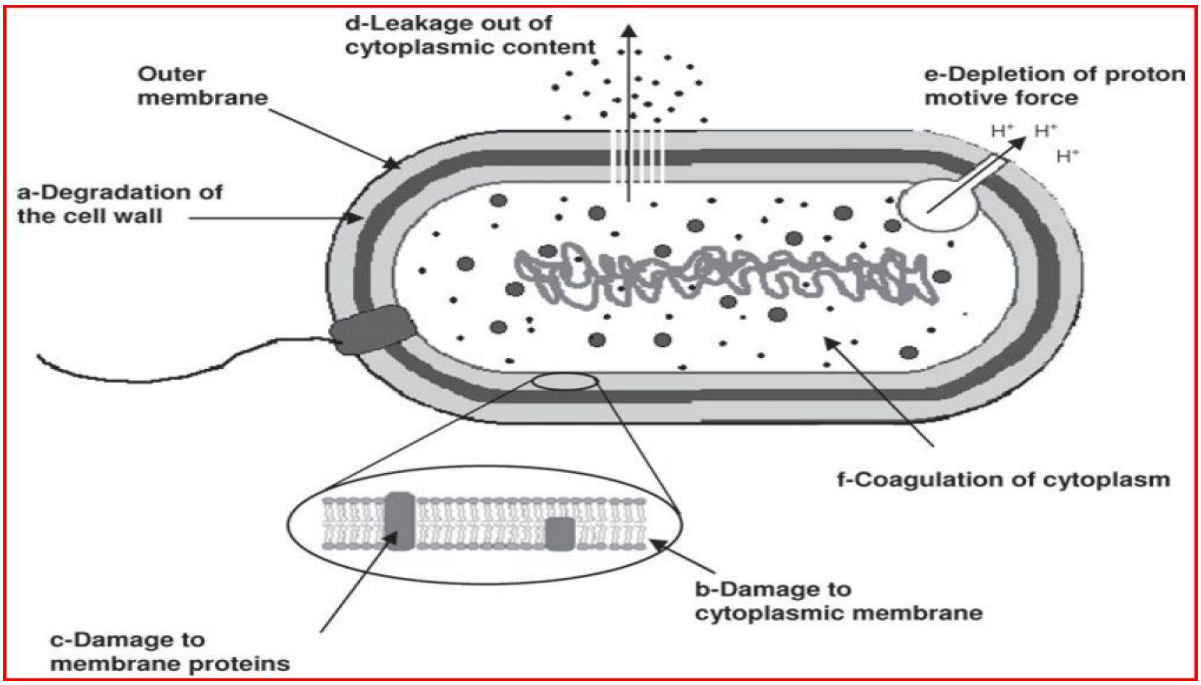

Figure 7.3: Mode of antibacterial action of essential oil components in the bacterial cell (Burt 2004).

Several factors also govern the efficiency of the antimicrobial properties of essential oils and their active compounds. Burt (2004) reported that the effectiveness depended on the pH of the fruit, concentration of the essential oil used, and type of microorganism. In a study conducted by Raybaudi-Massilia and others (2006), the activity of some essential oils from lemon grass, cinnamon, geraniol, palmarosa, clove and benzaldehyde against *E. coli* O157:H7, *Salmonella* Enteridis and *Listeria innocua* confirmed the presence of antimicrobial activity with varying degrees of effectiveness. With the exception of palmarosa, all other tested essential oils inhibited the growth of *E. coli* O157:H7, while clove and benzaldehyde showed a lower level of antimicrobial activity than the other essential oils, confirming that the specific strains, microorganism's resistance, and the spice's maturity should be considered when selecting an essential oil for use as a preservative. Baskaran and others (2010) also demonstrated the effectiveness of trans-cinnamaldehyde on the inactivation of *E. coli* O157:H7 in apple juice and

apple cider (Figure 7.4). They concluded that low concentrations of trans-cinnamaldehyde could potentially be used as an effective antimicrobial agent to reduce the level of *E. coli* O157:H7 in apple juice and apple cider without affecting the pH and appearance of the juice. Trans-cinnamaldehyde at 0.025% v/v reduced *E. coli* O157:H7 to undetectable levels after 4 days at 23 °C and completely inactivated *E. coli* O157:H7 (enrichment negative). The authors suggested further work should be conducted on this antimicrobial to determine the sensory impact on the apple and cider juices.

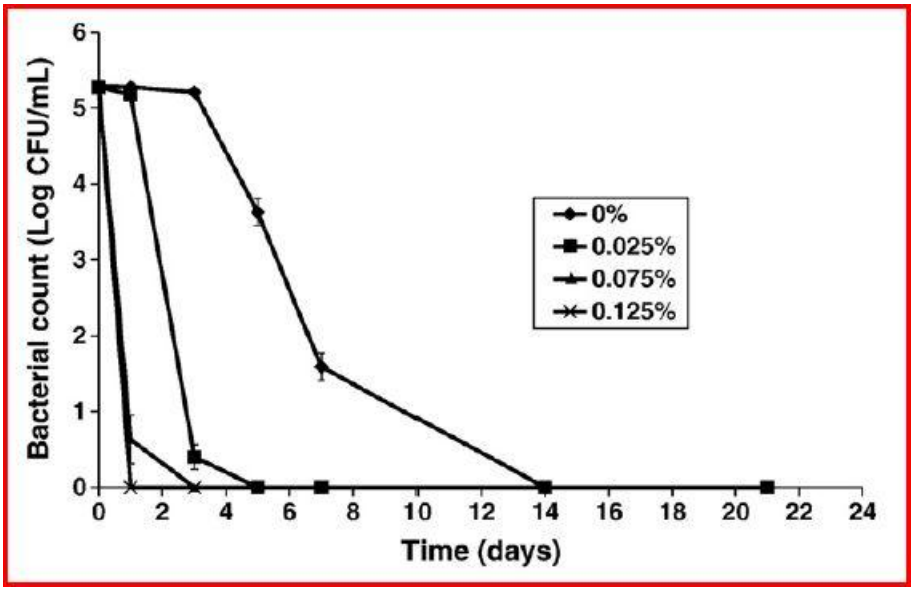

Figure 7.4: Effect of trans-cinnamaldehyde (TC) on *Escherichia coli* O157:H7 in apple juice at 23 °C. Data are expressed as a mean from 9 data points obtained from three independent experiments. The error bars represent the standard error (SE). (♦) 0%v/v TC, (■) 0.025% v/v TC, (▲) 0.075%v/v TC, and (×) 0.125% v/v TC. The detection level of *E. coli* O157:H7 after enrichment was 1 CFU/ml (Baskaran and others 2010).

Chapter 8 - Combination (Hurdle) Strategies for Processing

The consumer demand for natural and less processed fruit juices has driven the industry towards the development of novel techniques that do not have detrimental effects on product quality characteristics. Thermal pasteurization, while being efficient at reducing microbial populations, does cause undesirable changes in quality characteristics including reducing the nutritional quality of fruit juices (Saberian and others 2013). Despite a wide variety of antimicrobial techniques, few offer the possibility to achieve 5 log reductions on their own. One solution that is being employed is the use of 2 or more techniques in a hurdle strategy to achieve the necessary 5 log reduction in microbe concentration (Señorans and others 2003).

Pulsed electric field based hurdle strategies

A combination of pulsed electric field (PEF) and moderate heat have been shown by Walkling-Ribiero and others (2010) to provide superior microbial safety, in a fruit smoothie-type beverage, to thermal pasteurization while maintaining acceptable physical and sensory properties of the juice. The combination of heat and PEF has also been utilized to inactivate peroxidase and polyphenoloxidase which are responsible for browning effects and deterioration of organoleptic quality characteristics (Riener and others 2008).

Pulsed electric field treatments have also been combined with natural antimicrobials in an effort to yield 5 log reduction non-thermal pasteurizations. Mosqueda-Melgar and others (2008a) described the reduction of populations of *Salmonella* Enteritidis, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in melon and watermelon juices by treatment with PEF combined with citric acid and cinnamon bark oil (Table 8.1). Additionally, the treatments were found to inactivate mesophilic, psychrophilic, and mold and yeast populations leading to a shelf life exceeding 91 days in both juices after storage at 5 °C. Similar treatment of apple, pear, orange, and strawberry juices after inoculation with *S.* Enteritidis and *E. coli* O157:H7 only provided a 5 log reduction for orange juice (Mosqueda-Melgar and others 2008b). Interestingly, *S.* Enteritidis was found to be more resistant to PEF than *E. coli* O157:H7. Pulsed electric field has also been used in combination with clove oil and mint extract to yield 3.9 and 8.3 log reductions respectively, in total microbial populations in tomato juice (Nguyen and Mittal 2007). Under identical PEF conditions and varying mint concentrations from 0.1% to 1.2 % w/w, total

microbial reductions ranged from 4.8 to 8.3, respectively. These treatments did not result in any degradation of vitamin C content. Iu and others (2001) showed that while use of PEF was able to achieve a 5.3 log reduction of *E. coli* O157:H7 in apple cider, using PEF in combination with cinnamon or nisin resulted in 6-8 log reductions.

Noci and others (2008) described the use of PEF along with ultraviolet (UV) radiation for the preservation of apple juice. Apple juice was treated by each method independently and in combination. Controls were prepared by treating apple juice samples in a heat exchanger at 72 °C and 94 °C for 26 s. Log reductions of UV and PEF treatments were 2.2 and 5.4 respectively compared to the controls that were 6.2 and 6.7 for treatment at 72 °C and 94 °C respectively. The order of PEF and UV treatments showed slight differences with UV followed by PEF having a higher log reduction of 7.1, and PEF followed by UV being only 6.2. Quality characteristics such as color and phenolic compound concentration were less affected than in the heat-treated samples.

Table 8.1: Pathogen log reductions after treatment by selected hurdle strategies

Fruit Juice	Pathogen	Processing Condition	Log reduction (CFU/ml)	Reference
Strawberry	*E. coli* O157:H7 & *S.* Enteritidis	PEF[1] and cinnamon/citric acid	> 5	Mosqueda-Melgar and others 2008a
Apple	*E. coli* O157:H7 & *S.* Enteritidis	PEF[1] and cinnamon / citric acid	> 5	Mosqueda-Melgar and others 2008a
Pear	*E. coli* O157:H7 & *S.* Enteritidis	PEF[1] and cinnamon / citric acid	> 5	Mosqueda-Melgar and others 2008a
Orange	*E. coli* (ATCC 35218)	Ultrasound and UV-C[3] light	3.5	Char and others 2010
Apple	Natural microflora	UV[2] and PEF[1]	>6	Noci and others 2008
Orange	*L.innocua*	Vanillin and heat	>4	Char and others 2009
Apple	*E. coli* O157:H7	Citrus essential oils and heat	>5	Espina and others 2012

[1]PEF: Pulsed electric field; [2]UV; [3]UV-C: Ultraviolet radiation, ultraviolet radiation at 254 nm

Other hurdle strategies

Ultraviolet radiation is capable of effecting 5 log reductions in microbial concentrations in buffers and clear fruit juices. Studies have investigated its use in conjunction with other non-thermal techniques. Char and others (2010) discussed the effects of ultrasound along with ultraviolet light at 254 nm (UV-C) light to inactivate *E. coli* (ATCC 35218) in peptone water, orange, and apple juices. The authors found that while UV-C was capable of achieving 5 log reductions in peptone water and apple juice, it was only capable of achieving a 1.5 log reduction in orange juice. In this continuous flow system, ultrasound performed poorly to reduce *E. coli* (ATCC 35218) in all substrates. However, a hurdle strategy employing both ultrasound and UV-C was capable of a 3.4 log-reduction for *E. coli* (ATCC 35218) in orange juice (Table 8.1). Caminiti and others (2012) also investigated the use of UV light with manothermosonication in an orange and carrot juice blend. In comparison to thermal processing, it was found that the non-thermal treatments of the orange and carrot juice blend had less of an adverse impact on quality characteristics.

In reports by Guerrero and others (2005) and Char and others (2009) natural antimicrobials were used with ultrasound and heat respectively. Chitosan is derived from naturally occurring chitin by treating crustacean shells with alkali. Guerrero and others (2005) found that the use of chitosan alongside ultrasound enhanced ultrasound treatment to yield a more than 3 log reduction in yeast population. *Listeria innocua*, used as a surrogate for *L. monocytogenes*, was used to inoculate orange juice that was then treated with vanillin and moderate heat (Char and others 2009). Vanillin addition was found to significantly enhance the bactericidal effect of pasteurization with moderate heat (57-61 °C). The concentration of vanillin was found to be positively correlated with the antimicrobial activity at the lower temperature (57 °C) but unrelated at the higher temperature (60 or 61 °C). Similarly, Espina and others (2012) used essential oils from citrus fruits (lemon, mandarin and orange) to destroy *E. coli* O157:H7 in apple juice. Use of only 75μL/L lemon essential oil decreased the required treatment time by 82% at the thermal pasteurization temperature and was able to reduce the treatment temperature by 4.5 °C (Figure 8.1a and 8.1b). Additionally, the use of essential oil of lemon did not adversely affect the flavor of the juice.

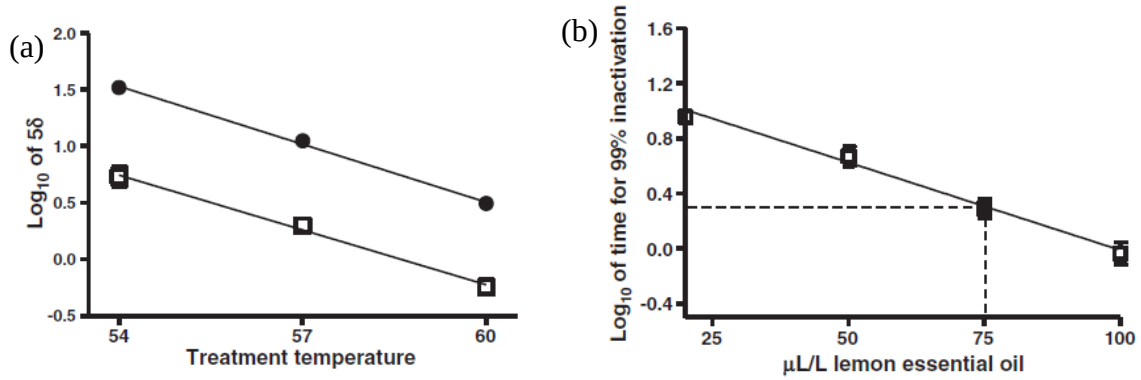

Figure 8.1: Log_{10} times (min) for (a) inactivation of 5 log_{10} cycles of 3×10^7 CFU/mL of *Escherichia coli* O157:H7 at different treatment temperatures in apple juice, with no essential oils added (●) or with 200 µL/L of lemon essential oil (□); (b) Log_{10} of times (min) to inactivate 99% of initial population at 54 °C for *Escherichia coli* O157:H7 treated with various concentrations of lemon essential oil in apple juice (Espina and others 2012).

The combination of non-thermal techniques has the advantage of reducing microbial concentrations without reducing nutritional quality or other quality characteristics (Caminiti and others 2012; Rivas and others 2006; Aguilar-Rosas and others 2007). Non-thermal techniques are often less potent antimicrobials in comparison to heat, however, their combination with thermal pasteurization can allow for processing at lower temperatures or for shorter times leading to a more natural fruit juice.

Chapter 9 - Conclusion

Thermal pasteurization has been the standard method for pathogen reduction in the production of fruit juice beverages. However, there are disadvantages to this process including thermal destruction of nutrients and organoleptic modification of the natural juice form that renders it less “natural’ than pre-thermally treated juice. The demand for more natural products has led to the emergence of new technologies for pathogen reduction in fruit juices.

Emerging technologies have been developed that can deliver greater than the required 5 log CFU/ml reduction in pathogens from the original juice product of which High Pressure Processing (HPP) has been commercialized and its use is increasing. It has also been demonstrated that the organoleptic challenges experienced during thermal pasteurization are greatly reduced or eliminated when using some of these new technologies. The hurdle strategy approaches for pathogen reduction, which involves the use of two or more of these newer technologies or a new technology in conjunction with thermal pasteurization, have been shown to deliver more natural juice products.

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Appendix A - Excerpt of a summary HACCP Plan for a pasteurized refrigerated apple juice packed in plastic bottles

Critical Control Point (CCP)	Hazard(s)	Critical Limits	Monitoring				Corrective Action	Verification	Record keeping
			A. What	B. How	C. Frequency	D. Who			
CCP1 Receiving	Chemical: Patulin	A supplier guarantee specifying that the shipment includes only apples harvested to exclude fallen fruit.	Ensure supplier guarantee exists for each incoming shipment of fruit.	Supplier guarantee is visually confirmed.	Each incoming fruit shipment	Receiving manager or trained designee	Reject fruit if not accompanied by supplier guarantee.	Review monitoring corrective action and verification records within one week of preparation Audit the supplier periodically for adherence to guarantee Periodically test juice for patulin levels	Supplier guarantee Receiving log Supplier audit report Patulin test results Corrective action Log
CCP 2 Culling	Chemical: Patulin	Undamaged apples	Moldy, rotten, bruised or otherwise damaged apples	Visual inspection	Continuous	Culling inspector or trained designee	Stop belt and remove damaged fruit AND Adjust belt speed if necessary	Review monitoring, corrective action, and verification records within one week of preparation Periodically test juice for patulin levels	Culling log Patulin test results Corrective action log

Critical Control Point (CCP)	Hazard(s)	Critical Limits	Monitoring				Corrective Action	Verification	Record keeping
			A. What	B. How	C. Frequency	D. Who			
CCP 3 Screen	Physical: Metal inclusion	Screen is intact and in place	Integrity of screen	Visual	Daily	Production Employee or trained designee	Segregate product and rework to eliminate metal pieces, run product through metal detector, divert to nonfood use, or destroy AND Replace screen.	Ensure metal pieces 7 mm or greater do not pass screen, semi-annually. Review monitoring corrective action and verification records within one week of preparation	Screen integrity log Corrective action log
CCP 4 Pasteurize	Biological: *E. coli* O157:H7 and *Cryptosporidium parvum*	Minimum 71.1 °C and 6 seconds (provides a 5 log reduction) (FDA 2004)	1.Temperature of juice 2. Time	1.Temperature recorder 2. Visual check of positive displacement pump setting	Continuous monitoring with visual check hourly Once hourly during production	Pasteurizer operator or trained designee	Segregate and hold affected product for evaluation, destroy, or divert to nonfood use AND Adjust pasteurizer (temperature or flow rate) to achieve the critical limit. AND	Documentation of process establishment; Check the accuracy of the temperature recording device (TRD) against a mercury and glass thermometer once hourly during production Calibrate the mercury and glass (MIG) thermometer	Operator's log Recorder Thermometer Chart TRD, MIG and pump check and calibration records Corrective action log

Critical Control Point (CCP)	Hazard(s)	Critical Limits	Monitoring				Corrective Action	Verification	Record keeping
			A. What	B. How	C. Frequency	D. Who			
							Reprocess any product that did not undergo 5-log pathogen reduction	annually; Flow rate test and resealing of pump speed monthly; Review monitoring, corrective action, and verification records within one week of preparation.	

Data was adapted from

http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Juice/ucm072557.htm