

2009

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Lauren Nicole Cook

Louisiana State University and Agricultural and Mechanical College

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**COMPARISONS OF PHYSICOCHEMICAL PROPERTIES OF WATERMELON JUICE
TREATED WITH PULSED ELECTRIC FIELDS AND THERMAL PASTEURIZATION**

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
requirements for the degree of
Master of Science
In
The Department of Food Science

By
Lauren Nicole Cook
B.S., Louisiana State University, 2007
December 2009

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Subramaniam Sathivel, for his constant support, patience, and guidance. His amazing problem solving skills and creativity has helped me immensely throughout this research. I want to thank Dr. Joan King for helping me from the day I arrived at LSU in January 2005 until the day I defend my thesis. She has inspired me to be the best I can be in both my education and as food science club president. The help of Dr. Kayanush Aryana has been crucial to my research and I greatly appreciate his time and assistance. Dr. Paul Wilson has been an incredible addition to my committee as he has suggested ideas that have improved my research.

I also want to thank Huaixia Yin for her daily support and assistance since I first started as a graduate student. Yuting Wan, Jianing Pu, and Dr. Xiang have also been very helpful the past year and half and I could not have completed my research without them. Most importantly, I want to thank the entire LSU Food Science Department for being a family to me since 2005. I would not have been a successful president or student without their dedication and involvement. I appreciate everyone from the front office to the professors to the students. Specifically I want to thank Ms. Terri Gilmer for her constant support and help every time I needed her.

Additionally, I would like to thank my amazing parents, Manny and Diana Garza and Oscar Cook for their encouraging words and constant love. My brother and cousin have all given me the inspiration to follow my dreams and I cherish their love and support. Lastly, I would like to thank my soon-to-be husband, Paul, for his never-ending support, love, and friendship.

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ABSTRACT

Pulsed electric field (PEF) treatment is a non-thermal and alternative pasteurization treatment for fruit juices. PEF applies short pulses of electricity to a liquid food sample which inactivates microorganisms and enzymes. Thermal pasteurization is commonly used to pasteurize juice in the fruit juice industry but the process tends to deteriorate color, nutrients, and overall juice appearance. The overall goal of this study was to determine the effects of PEF treatment (30 kv/cm for 57 μ s in bipolar 2 μ s pulses at 22°C) on color, lycopene content, vitamin C content, pH, °Brix, and microbial count when compared with the effects of thermal pasteurization (TP). Fresh watermelon juice was PEF-treated at the following flow rates (mL/min), 60, 80, 100, 120, 140, and 160, while the TP-treated juice was thermally pasteurized with the following conditions: 75°C at 15, 30, and 45 s; 80°C at 15, 30, and 45 s; 85°C at 15, 30, 45 s; 90°C at 15, 30, and 45 s. Vitamin C degradation was modeled and estimated for TP watermelon juice samples using Power Law and Arrhenius models. A fresh, untreated watermelon juice sample was used as the control for the TP treatments. Watermelon juice was passed through the PEF system without pulse application and used as a PEF-control. Triplicate experiments were conducted. For PEF treatments, lycopene content was significantly higher at the slowest flow rate of 60 mL/min. Vitamin C of watermelon juice was not significantly affected during PEF treatment regardless of the flow rate, while it significantly decreased ($P < 0.05$) with intensity of TP treatments. The reaction rate constant (K) of watermelon juice for TP treatment at 75°C was significantly less than juice sample treated at 80, 85, and 90°C, which indicated that the Power law model worked well at higher temperature TP treatments. This model was more appropriate for predicting vitamin C concentration of watermelon juice during thermal pasteurization. The calculated activated energy for the vitamin C degradation for TP

treatments was 5.54 (kJ/mol), which was lower than the reported values for other juices. TP-treated juice had higher b* values than PEF-treated juice which indicated TP juice was more yellow in color. PEF treatments did not affect the pH of the juice compared to TP treatments which increased pH. PEF-control and PEF-treated samples had similar °Brix values. TP treatments significantly affected °Brix values of watermelon juice. These results indicate that PEF treatment is a better option for pasteurizing watermelon juice over TP in terms of lycopene, vitamin C content and color.

CHAPTER 1. INTRODUCTION

Pulsed electric field (PEF) treatment is a non-thermal preservation method which applies short pulses of electricity to a liquid food sample. This treatment is ideal for fruit juice as it does not degrade color, flavor, and nutrients. PEF treatment inactivates microorganisms and enzymatic reactions while maintaining the original conditions of fruit juice (Mertens and Knorr, 1992; Sizer and Balasubramaniam, 1999; Aguilar-Rosas et al., 2007). Unlike thermal pasteurization, PEF treatment does not negatively affect the taste, smell, appearance, or nutritional quality of fruit juice (Rivas et al., 2006).

In the fruit juice industry, juice is typically pasteurized by high temperature short time (HTST) pasteurization. This process uses plate heat exchangers to heat the sample quickly at least 78°C. Although this method is effective at inactivating microorganisms and enzymes, it can cause detrimental effects on the quality of the juice. Heat treatment may cause color change, separation of particles, and a change in flavor and/or smell (Qin et al., 1995). If heat treatment is not performed rapidly or at a reasonably low temperature, the juice will begin to separate due to the destruction of pectin (Goodman et al., 2002). Compared with thermal pasteurization, non-thermal processing offers the advantages of low process temperatures which results in a better retention of flavors and nutrients (Vega-Mercado et al., 1997).

The electric field strength determines the intensity of the pulses which can directly influence the efficiency of PEF treatment (Heinz et al., 2002). Pulses can be applied as either monopolar or bipolar pulses. Bipolar pulses are used more often in PEF treatment because they have been shown to inactivate microbes and enzymes better than monopolar pulses (Evrendilek and Zhang, 2005; Aguiló-Aguayo et al., 2007). Treatment time also has a great influence on the inactivation of microbes. Studies show that the longer the treatment time, the greater the

microbial inactivation (Mosqueda-Melgar et al., 2007). Pulse width refers to the time of each pulse in microseconds and it is directly related to microbial inactivation. A longer pulse width of 7 μ s, compared to 1 μ s, may decrease vitamin C content (Odrizola-Serrano et al., 2009). In most published articles about PEF treatment, the flow rate is 60 mL/min.

Watermelons are very prominent in the state of Louisiana, especially during the summer months. According to the LSU AgCenter (2008), there are 279 farms on 2,700 acres growing watermelons in Louisiana. In 2008, Louisiana watermelons had a gross farm value of \$6.4 million. Watermelons are grown in 28 of the 64 parishes in Louisiana. Watermelon juice is commonly consumed in Mexico and can be found in many American bars as a mixer for alcoholic beverages. According to the USDA Nutrient Database (2009), watermelons contain 40% more lycopene (per 100 g) than raw red ripe tomatoes. Watermelons are also a good source of vitamin C with a cup (8 oz) of watermelon juice containing 20% of the daily value for vitamin C. Due to its low acidity and growing conditions, watermelon is regarded as a potentially hazardous food (FDA, 2001). According to the CDC (2006), watermelon caused a Salmonella outbreak in 2002 and 2006, a Norovirus outbreak in 2005 and 2006, and a Campylobacter outbreak in 2006. Because of these pathogens, watermelon juice must be pasteurized prior to consumption.

Lycopene is a carotenoid that provides the red color to tomatoes, watermelons, and red bell pepper, among other fruits and vegetables. Lycopene is a powerful antioxidant and has been shown to prevent various cancers and help against heart disease (Rao and Agarwal, 1999). Lycopene quenches free radicals which prevents oxidative damage which subsequently prevents many cancers. Fish et al. (2002) stated that watermelon is a gastronomically pleasing food and is rich in lycopene which makes it a highly desirable source of this phytochemical.

Watermelons also contain vitamin C which is an essential nutrient for humans because it aids in the synthesis of collagen in addition to protecting against oxidative damage. Vitamin C consumption has been shown to protect against stomach, oral, and lung cancers, improve cholesterol, and prevent scurvy (Fontham et al., 1988; Block, 1991; Ness et al., 1996). Vitamin C is very sensitive to heat and degrades very quickly during pasteurization. PEF treatment may be a better option for vitamin C retention in fruit juices.

Color and appearance are the first characteristic a consumer sees while shopping in a grocery store. Color may have an influence on a consumer's purchase intent (Francis, 1995). Fruit juices are generally packaged in clear plastic bottles with large labels so consumers can see the juice's color. Thermal pasteurization can cause pigments to degrade which may the color and appearance of fruit juice. PEF treatment has no significant effects on the color of various fruit juices (Rivas et al., 2006; Aguiló-Aguayo et al., 2007; Aguilo-Aguayó et al., 2009). Rivas et al. (2006) reported that the colors of untreated and PEF-treated orange-carrot juice were not significantly different.

The objectives of this study were (1) to compare lycopene, vitamin C, color, °Brix, pH and microbial count of watermelon juice treated by PEF and TP, (2) to determine the effect of flow rate of PEF treatment on lycopene, vitamin C, color, °Brix, pH and microbial count of watermelon juice, and (3) to study the kinetics of lycopene and vitamin C degradations in watermelon juice during thermal pasteurization.

CHAPTER 2. LITERATURE REVIEW

2.1 WATERMELONS AND WATERMELON JUICE

Watermelons are one of the leading vegetable crops planted in Louisiana (LSU AgCenter, 2008). Watermelons are a staple summer food in Louisiana and can be found in grocery stores, local fruit stands, and even on the road side. In 2008, there were 279 Louisiana farms on 2,700 acres growing watermelons which resulted in a farm value of \$6.4 million. According to the FDA (2001), watermelon is regarded as a potentially hazardous food because of its low acidity and growing conditions. Watermelons and their juice may contain *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter jejuni* and *Listeria Monocytogenes*. These microorganisms are usually located on the rind of watermelons but they can travel and infect the watermelon flesh (Sharma et al., 2005). These microorganisms must be inactivated to prevent possible illnesses and to increase the shelf-life of the juice.

Watermelons are an excellent source of lycopene and are a great option for consumers who want to consume lycopene but do not like the taste or texture of tomatoes and tomato products. Eight ounces of watermelon juice contains many nutrients and vitamins including: 10 mg of lycopene and 19 mg of vitamin C in addition to beta-carotene, potassium, and vitamin A (USDA Nutrient Database, 2009). Lycopene and vitamin C are well-known anti-oxidants which provide many health benefits to humans. Lycopene in fresh watermelons is more bioavailable than in fresh tomatoes (Bliss, 2002).

Watermelon juice is not commonly sold in stores but it is widely used in bars for alcoholic drinks. Smirnoff Vodka makes watermelon vodka which is made with fresh watermelon juice. Dole sells Pine-Watermelon Juice which is primarily sold in Asia. Watermelon juice or Agua Fresca is very popular in Mexico and can be found all over the

country. The American Heart Association (2009) identified watermelon as a constituent of a sensible low sodium, low saturated fat, and low cholesterol diet. As consumers and manufacturers realize how healthy and refreshing watermelon juice is, it has the potential to become a popular drink in the United States.

2.2 LYCOPENE

Lycopene is a carotenoid which provides the red color to many fruits and vegetables. Lycopene has become a well-known antioxidant which may be beneficial to human health. Lycopene is an open chain hydrocarbon consisting of forty carbon atoms, 11 conjugated and 2 non-conjugated double bonds arranged in a linear pattern (Britton, 1995). Processing causes heat induced isomerization from *trans* to *cis* which enhances lycopene bioavailability (Stahl and Sies, 1992). Although there are many carotenoids, lycopene may be the most beneficial to human health. Lycopene is the most potent singlet oxygen quencher among all other carotenoids (DiMascio et al., 1989).

Lycopene has many health benefits which may be solely due to lycopene or due to the synergistic effects of lycopene and another component. Lycopene has been shown to help decrease the risk of colon, lung, stomach, and prostate cancer in addition to helping prevent cardiovascular disease. Lycopene has an anti-proliferative effect on four types of malignant cells (Salman et al., 2007). Tang et al. (2009) found that low doses of lycopene and eicosapentaenoic acid acted synergistically by inhibiting the growth of colon cancer cells. Manufacturers are now allowed to make a health claim on products containing lycopene because of the reduced risk of prostate cancer associated with it (FDA, 2008).

Giovanucci (1999) found strong evidence that lycopene reduces the risks of lung, stomach, and prostate cancers while some evidence suggested that lycopene may reduce the risks

of cervix, breast, oral cavity, pancreas, and esophageal cancer. Lycopene has hypocholesterolemic effects which may help lower the risk of cardiovascular disease and reduce the risk of heart attacks (Fuhrman et al., 1997; Kohlmeier et al., 1997). According to the American Heart Association (2009), cardiovascular disease is the number one killer of Americans. Lycopene can also help against chronic conditions which are not as serious as cancer or heart disease. Rao et al. (2006) reported that lycopene reduces oxidative stress in postmenopausal women, which might reduce the risk of osteoporosis.

2.3 VITAMIN C

Vitamin C, also known as *L*-ascorbic acid, is an essential antioxidant vitamin which has several health benefits. Vitamin C is essential for normal metabolic functions of the human body. Vitamin C is heat labile which causes it to degrade during thermal pasteurization. Previous articles have shown that PEF treatment retains vitamin C in fruit juice better than thermal pasteurization (Elez-Martínez and Martón-Belloso, 2007). PEF-treated orange-carrot juice has been shown to retain more vitamin C (90%) than pasteurized orange-carrot juice (83%) (Torregrosa et al., 2006). Vitamin C consumption has been shown to help against cancers, improve cholesterol, and prevent disorders associated with a lack of collagen in the body (Block, 1991; Kurowska et al., 2000; May and Qu, 2005).

Vitamin C has protective effects against lung cancer and gastric cancer in Louisianans (Fontham et al., 1988; Correa et al., 1985). Block (1991) reviewed the effects of vitamin C on various types of cancer and found that vitamin C was effective in protecting against oral, larynx, stomach, and esophageal cancers. In addition to vitamin C's protective effects against cancer, it can also improve cholesterol and a person's overall lifespan. Ness et al. (1996) has reported that the consumption of vitamin C raised high-density lipoprotein cholesterol and lowered serum

triglyceride levels. Low levels of serum ascorbic acid were associated with an increased risk for all-cause mortality (Simon et al., 2001).

Scurvy is the illness associated with a vitamin C deficiency. Ascorbic acid's best-known function is synthesizing collagen (Harrison and May, 2009). Vitamin C deficiency can lead to a deficiency in collagen which essentially causes scurvy. Scurvy causes spots to form on skin in addition to bleeding from mucous membranes. Scurvy can be cured by consuming sufficient amounts of vitamin C. Scurvy is not a large problem in the United States but it is still a serious problem in developing countries. The recommended daily value of vitamin C for men and women is 60 mg per day (USDA, 2009). One cup of watermelon juice contains 20% of that daily value.

2.4 COLOR

The color of fruit juice can have a huge influence on the type of juice products consumers purchase. Consumers may see color as an indicator for freshness, a specific flavor, a particular smell, and consumers may even use color to determine the intensity of a flavor or smell. Francis (1995) stated that color influences other sensory characteristics, which subsequently influences food acceptability, choice, and preference. Color can be defined as the impact of the wavelengths of light in the visual spectrum from 390 to 760 nm on the human retina (Francis, 1995). According to Francis (1995), if the color of a product is unacceptable to a consumer, the flavor and texture may not be considered at all. Color may also be used as an indicator to determine the types and quantities of various carotenoids. Color analysis is quick and simple and may provide more consistent results compared to carotenoid analysis (Francis, 1995).

2.5 THERMAL PASTEURIZATION

In the industry, fruit juices are currently treated with high temperature short time (HTST) pasteurization in order to inactivate microorganisms and enzymes which prolongs the shelf life. Conventional thermal pasteurization ensures safety and extends the shelf life of juice, but it often leads to detrimental changes in the sensory qualities of the product (Cortés et al., 2008). The FDA (2004) suggests pasteurizing fruit juice at 71°C for 3 seconds which results in a 5-log reduction of bacteria. Actual pasteurization conditions should be chosen based on type of juice, pH, and sugar content which can all affect microbial inactivation. Because watermelons are associated with dangerous microorganisms, such as *L. monocytogenes*, *E. coli*, and *Salmonella*, watermelon juice must be treated in order to inactivate these dangerous microorganisms. Currently, there are few published studies on pasteurized watermelon juice and the optimum treatment conditions. There are no studies which explain the degradation kinetics of color, vitamin C, and lycopene in watermelon juice.

2.6 PULSED ELECTRIC FIELD TREATMENT

Pulsed electric field treatment is an alternative preservation method primarily used on fruit juice and milk (Heinz et al., 2002). The aims of PEF treatment are to inactivate spoilage and pathogenic microorganisms, decrease the activity of enzymes, and extend the shelf life of foods without undesirable heat and chemical effects (Cserhalmi et al., 2006). PEF treatment applies extremely short (microsecond) and intense electric pulses which create pores in the cell membranes of microorganisms; this process is called electroporation. Electroporation in a cell membrane can occur in both protein channels and lipid domains, which lead to an osmotic imbalance and eventually, cell death (Tsong, 1991). The change in membrane permeability can

be reversible or irreversible, depending on the external electric field strength (Cserhalmi et al., 2006).

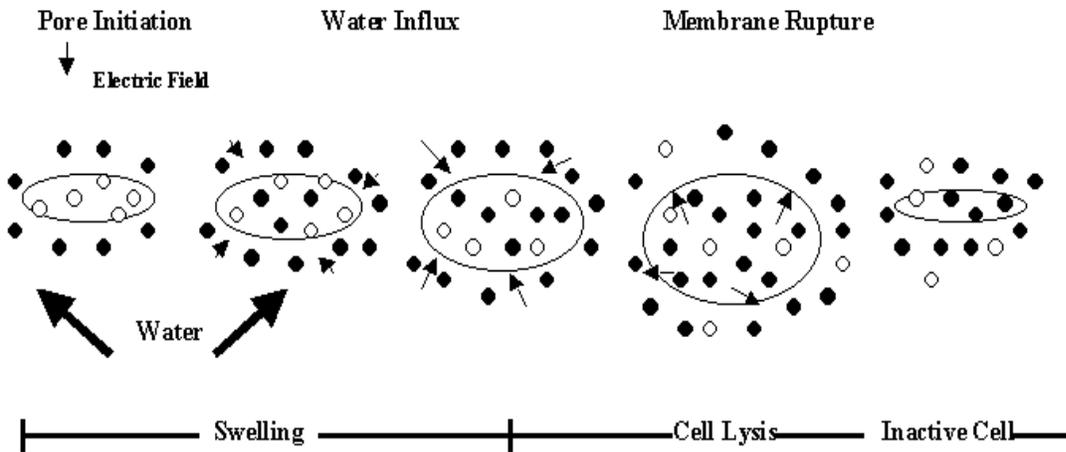


Figure 2.1 Electroporation of cells treated with by pulsed electric field treatment. (U.S. FDA, 2000)

An increase in the demand for minimally processed fresh-products has raised an interest in the development and implementation of new techniques for food processing such as high-intensity pulsed electric fields (Aguiló-Aguayo et al., 2007). In general, thermal pasteurization is the standard for juice processing as it significantly reduces microorganisms, but it can adversely affect color, taste, aroma, appearance, and nutrient content of juice (Aguilar-Rosas et al., 2007). A sensory panel preferred PEF-treated orange-carrot juice over pasteurized juice in terms of aroma and taste (Rivas et al., 2006).

2.6.1 PEF Parameters

The effectiveness of PEF treatment depends on a variety of parameters including: electric field strength, flow rate, pulse width, delay time, pulse period, treatment time, and treatment temperature (Clark, 2006). Electric field strength and treatment time had the greatest influence on lycopene content of PEF-treated tomato juice; lycopene content was greatest with a treatment

time of 2000 μs and electric field strength of 35 kV/cm compared to treatment at 500 μs with 20 kV/cm. (Odriozola-Serrano et al., 2008). Electric field strength refers to the intensity of the pulses applied to a sample. Elez-Martínez and Martín-Belloso (2007) analyzed the effects of various electric field strengths and treatment times on vitamin C content; the results showed that vitamin C was better retained with a lower electric field strength (15 kV/cm) and a shorter treatment time (100 μs). Based on these studies, lycopene content is better retained with more severe conditions while vitamin C retention favors more gentle conditions.

Treatment time is calculated by multiplying the number of pulses by the pulse width. Treatment time, treatment temperature, and electric field strength are directly related to the inactivation of microorganisms (Mosqueda-Melgar et al., 2008). Mosqueda-Melgar et al. (2008) found that *S. Enteritidis* is better inactivated with a treatment time of 1000 μs compared to 200 and 600 μs . Pulse width and pulse polarity also influence the efficiency of PEF treatment (Evrendilek and Zhang, 2005). Pulse width can vary depending on the PEF equipment, juice sample, and the type of microorganisms being inactivated. A longer pulse width inactivates microorganisms more efficiently because treatment time is increased; but this may have a negative effect on color and vitamin C. Electric pulses are applied as square wave pulses but can be either bipolar or monopolar. Bipolar pulses are more commonly used because greater microbial inactivation is achieved using bipolar compared to monopolar pulses. Evrendilek and Zhang (2005) studied the efficiency of monopolar and bipolar pulses on *E. Coli* O157:H7 in milk. Results showed that bipolar pulses were significantly more effective in inactivating *E. Coli* O157:H7.

Flow rate determines how quickly the sample is passed through the PEF equipment. Flow rate affects the treatment time which subsequently affects the effectiveness of PEF treatment. In theory, a slower flow rate should inactivate more microorganisms. Currently,

every article regarding the OSU-4K PEF equipment has performed PEF treatment with a flow rate of 60 mL/min. Studies involving flow rates have not been published previously and there are no previous articles that explain why 60 mL/min is used as the standard.

2.7 EFFECTS OF THERMAL PASTEURIZATION AND PEF TREATMENT ON VARIOUS JUICES

2.7.1 Orange Juice

Elez-Martínez and Martín-Belloso (2007) compared the effects of thermal pasteurization (90°C for 60 s) of orange juice with pulsed electric field treatment (60 mL/min, 15 kV/cm, 100 μ s treatment time, 4 μ s pulse width, 35°C) which showed that pasteurized orange juice retained only 82% of the original vitamin C while PEF treated juice retained 98%. Cortés et al. (2008) found that pasteurized orange juice (90°C 20 s) had a significantly higher b* value than untreated juice which indicated the juice was more yellow. Overall color differences were greater with pasteurized juice than with PEF-treated juice.

2.7.2 Orange-Carrot Juice

Rivas et al. (2006) studied the effects of PEF-treatment (60 mL/min, 25 kV/cm, 280 μ s treatment time, 2.5 μ s pulse width, 68°C) and thermal pasteurization (98°C for 21s) on orange-carrot juice. The color of the PEF treated orange-carrot juice was not significantly different from the untreated juice. The color of the pasteurized juice diminished more quickly than the PEF-treated juice. Torregrosa et al. (2006) also analyzed the effects of PEF-treatment (25 kV/cm for 280 and 330 μ s) and thermal pasteurization (98°C, 21 s) on orange-carrot juice. The PEF-treated orange-carrot juice retained 90% of the original vitamin C while the pasteurized juice retained only 83%.

2.7.3 Tomato Juice

Odriozola-Serrano et al. (2007) compared the effects of PEF treatment (60 mL/min, 35 kV/cm, 1500 μ s treatment time, 4 μ s pulse width, 40°C) with thermal pasteurization (90°C for 30 s) of tomato juice which proved that PEF treated juice retained lycopene and vitamin C longer than thermal pasteurization. The authors attributed the increase in lycopene to the release of bound lycopene during pulsing which increases its bioavailability. Aguiló-Aguayo et al. (2007) applied PEF treatment (35 kV/cm for 1,500 μ s using bipolar 4- μ s pulses at 100 Hz) and thermal pasteurization (90°C for 30 s and 60 s) to tomato juice and analyzed the changes in color. The PEF-treated juice had a significantly higher L* value than the pasteurized juice. A lower L* value in the pasteurized juice may be attributed to the common darkening of pasteurized juice. The PEF-treated juice retained the original color longer than both control and heat-treated samples.

2.7.4 Strawberry Juice

Aguiló-Aguayo et al. (2009) studied the effects of PEF (35 kV/cm for 1700 μ s, 4 μ s pulses at 100 Hz in bipolar mode) and thermal pasteurization (90°C for 30 s and 60 s) on strawberry juice. The PEF-treated strawberry juice had a significantly higher L* value compared to the pasteurized juice. This was similar to the results of the tomato juice. A lower L* value in the pasteurized juice was due to darkening during heating. PEF treatment was more efficient than pasteurization in retaining original a* and b* values. Odriozola-Serrano et al. (2009) analyzed the effects of PEF treatment (35 kV/cm, 1000 μ s, 1 μ s bipolar pulses at 232 Hz) on strawberry juice. Using these PEF conditions, the juice retained 98% of original vitamin C. Currently, there are no reports on the effects of pasteurization on vitamin C in strawberry juice.

2.7.5 Watermelon Juice

Oms-Oliu et al. (2009) analyzed various PEF conditions in order to optimize treatment for vitamin C and lycopene retention in watermelon juice. Using the following conditions: 60 mL/min, 25 kV/cm, 50 μ s treatment time, 1 μ s pulse width, 40°C, the PEF-treated watermelon juice retained 96% of the original vitamin C. Lycopene was retained and even increased to 121% at the following conditions: 60 mL/min, 35 kV/cm, 2050 μ s treatment time, 7 μ s pulse width, 40°C. Based on the results from this study, lycopene became more bioavailable with a longer treatment time and pulse width which is not ideal for vitamin C retention. Treatment conditions should be optimized to get the greatest retention of both lycopene and vitamin C. Oms-Oliu et al. (2009) did not analyze color of PEF-treated and TP-treated watermelon juice.

There are currently no studies comparing PEF treatment of watermelon juice with traditional thermal pasteurization. Additionally, there are no studies which analyze varying the flow rate for PEF treatment of watermelon juice or which discuss degradation kinetics of vitamin C and color in TP-treated watermelon juice.

CHAPTER 3. COMPARING PULSED ELECTRIC FIELD TREATMENT WITH THERMAL PASTEURIZATION OF WATERMELON JUICE IN TERMS OF COLOR, LYCOPENE, VITAMIN C, PH, °BRIX, AND MICROBIAL COUNTS

3.1 INTRODUCTION

Watermelons (*Citrullus lanatus*) are an excellent source of lycopene and vitamin C. Watermelons contain 40% more lycopene (per 100 g) than raw red ripe tomatoes and a cup (8 oz) of watermelon juice contains 20% of the daily value for vitamin C (USDA Nutrient Database, 2009). Although not commonly consumed in America, watermelon juice is frequently consumed in Mexico. Watermelon juice can also be found in many American bars as a mixer for alcoholic beverages. Watermelon is regarded as a potentially hazardous food due to its low acidity and growing conditions, (U.S. FDA, 2001). Watermelon caused a Salmonella outbreak in 2002 and 2006, a Norovirus outbreak in 2005 and 2006, and a Campylobacter outbreak in 2006 (CDC, 2006). Watermelons are considered a high-risk food because they are associated with *L. monocytogenes*, *E. coli*, and *Salmonella*. Thus, thermal pasteurization (TP) is required in order to inactivate these dangerous microorganisms. Fruit juices are commonly pasteurized with high temperature short time (HTST) pasteurization. The FDA (2004) has reported that pasteurizing fruit juice at 71°C for 3 s results in a 5-log reduction of bacteria. Although TP treatments are quite efficient in preventing microbial spoilage of fruit juice, TP may cause undesirable biochemical and nutritious changes which may affect overall quality of the fruit juice such as nutrients, color, flavor, and texture (Alwazeer et al., 2003, Zerdin et al., 2003; Aguilar-Rosas et al., 2007).

Vitamin C content can be used as an indicator of freshness for fruits and vegetables in terms of other nutrients. When vitamin C of the fruit juice is well retained, other nutrients may also be well retained (Marfil et al., 2008). It is well-known that vitamin C is very sensitive to

heat treatment. Marfil et al. (2008) reported that treatment temperature is directly related to degradation. Thus, an alternative processing method, such as a non-thermal method is investigated for watermelon juice processing.

Pulsed electric field (PEF) treatment is a non-thermal process which inactivates microorganisms and enzymes (Hoover, 1997; Giese, 1998; Mermelstein, 1999; Ho et al., 1997; Yeom et al., 2000) and preserves the fresh flavor, color, taste, and nutritional value of food (Mertens and Knorr, 1992; Sizer and Balasubramaniam, 1999; Aguilar-Rosas et al., 2007). The pulses generated from the PFE process inactivate microorganisms by creating pores in the cell membrane which eventually cause the cell to rupture; this process is called electroporation (Hamilton and Sale, 1967). PEF treatment is quick, simple, and effective in retaining original characteristics of fruit juice which may make it more advantageous than thermal pasteurization.

PEF treatment has been shown to better retain both lycopene and vitamin C in tomato juice and watermelon juice compared to thermally pasteurized juices (Odriozola-Serrano et al., 2007; Oms-Oliu et al., 2009). Thermal pasteurization significantly degrades vitamin C but may not significantly degrade lycopene (Odriozola-Serrano et al., 2007; Torregrosa et al., 2006). In addition to nutrient retention, PEF may be more beneficial in retaining original juice color than thermal pasteurization. The color of fruit juice is very important because it can have an influence on a consumer's perception of the quality of the product (Francis, 1995).

The effectiveness of PEF treatment depends on various PEF parameters including flow rate, electric field strength, pulse width, delay time, treatment time, treatment temperature, and frequency. The effects of these parameters have been researched and published with the exception of flow rate. Electric field strength, treatment time, and pulse width have been analyzed extensively with various juices and results have shown that these three parameters have the greatest influence on the efficiency of PEF treatment. Increasing the electric field strength

leads to a further improvement of microbial inactivation (Toepfl et al., 2007). An increase in either electric field strength or treatment time can increase microbial inactivation but may cause color change and nutrient loss. Increasing treatment time and pulse width can significantly decrease vitamin C content in both tomato juice and watermelon juice (Odriozola-Serrano et al., 2007; Oms-Oliu et al., 2009).

Lycopene is less sensitive to treatment time and pulse width, as it may actually increase during more intense PEF treatment. Two different studies showed that the best lycopene retention in tomato juice and watermelon juice was achieved with the longest pulse width and treatment time applied (Odriozola-Serrano et al., 2007; Oms-Oliu et al., 2009). In nearly every published article about PEF treatment using the same equipment used in this study, the flow rate is frequently 60 mL/min. The application of this flow rate has never been explained or justified.

The objectives of this study were to: (1) compare lycopene, vitamin C, color, °Brix, pH and microbial count of watermelon juice treated by PEF and TP, (2) determine the effect of flow rate of PEF treatment on lycopene, vitamin C, color, °Brix, pH and microbial counts of watermelon juice, and (3) study the kinetics of lycopene and vitamin C degradations of watermelon juice during the thermal pasteurization.

3.2 MATERIALS AND METHODS

3.2.1 Extraction of Watermelon Juice

All glassware and knives were autoclaved at 121°C for 45 min and all other equipment was sanitized with hypochlorite prior to usage. Fresh seedless watermelons were purchased from a local grocery store in Baton Rouge, LA and stored at 25°C. Watermelon rinds were washed with pure ethanol prior to juice extraction. The watermelons were cut into quarters and the flesh was scooped out and cut into small cubes. The cubes were placed in a laboratory scale juice

processor (Dr. Weil Professional Juice Extractor Model 9816, Naperville, IL). The extracted juice was then centrifuged (Beckman J2-HC Centrifuge, Fullerton, CA) at $12,500 \times g$ for 15 min. The juice was filtered with six layers of cheese cloth (VWR, West Chester, PA). The filtered juice was placed in autoclaved screw-top glass bottles and processed immediately.

3.2.2 Pulsed Electric Field, Thermal Pasteurization, and Control Treatments

3.2.2.1 PEF Treatment

PEF treatments were conducted on a pilot scale PEF continuous system (OSU-4K, Ohio State University, Columbus, OH). A diagram and photo of the system used in this study can be seen in figures 3.1 and 3.2, respectively. This PEF unit was designed to have four co-field treatment chambers with a diameter of 0.23 cm and a gap distance of 0.29 cm. Two cooling coils were placed before and after each pair of chambers and were submerged in a circulatory refrigerated bath in order to control sample temperature. Type T thermocouples were connected to a data acquisition system indicator to monitor inlet and outlet temperatures of each pair of chambers. The temperature of the juice was maintained at 22°C. Flow rate of watermelon juice was controlled by a variable speed pump (Cole Parmer, Vernon Hills, IL), the pump was calibrated before each PEF treatment. Watermelon juice was processed with six treatments: PEF-60 (flow rate of 60 mL/min), PEF-80 (flow rate of 80 mL/min), PEF-100 (flow rate of 100 mL/min), PEF-120 (flow rate of 120 mL/min), PEF-140 (flow rate of 140 mL/min), and PEF-160 (flow rate of 160 mL/min). PEF treatments were applied with a pulse width of 2 μ s, a delay time of 20 μ s, electric field strength of 30 kV/cm, and for a total treatment time of 57 μ s. Throughout the study, a bipolar pulse was applied with a pulse frequency of 600 Hz. Pulse wave form, electric field intensity, and pulse width were controlled with a digital oscilloscope (THS720, Tektronic Inc., Beaverton, OR). A watermelon juice control was produced by passing

the juice through the PEF system without pulse application. PEF treatment time (t) was calculated using the number of pulses received in the chambers (N_p), which was obtained from residence time in one chamber (T_r), as described in equation (3).

$$T_r = V/F \quad (1)$$

$$\begin{aligned} T_r &= 0.01204879 \text{ mL/mL/s} \\ &= 0.01204879 \text{ s,} \end{aligned}$$

where V is the volume of 1 chamber (mL) and F is the flow rate (mL/s).

$$N_p = T_r \times f \quad (2)$$

$$\begin{aligned} N_p &= 0.01204879 \text{ s} \times 600 \text{ pps} \\ &= 7.229274 \text{ pulses,} \end{aligned}$$

where f is the pulse rate (pulse per second [pps]).

$$t = N_p \times N_c \times W_p \quad (3)$$

$$\begin{aligned} t &= 7.229274 \text{ p} \times 4 \times 2 \text{ } \mu\text{s} \\ &= 57.834 \text{ } \mu\text{s,} \end{aligned}$$

where N_c is the number of treatment chambers and W_p is the pulse width (μs).

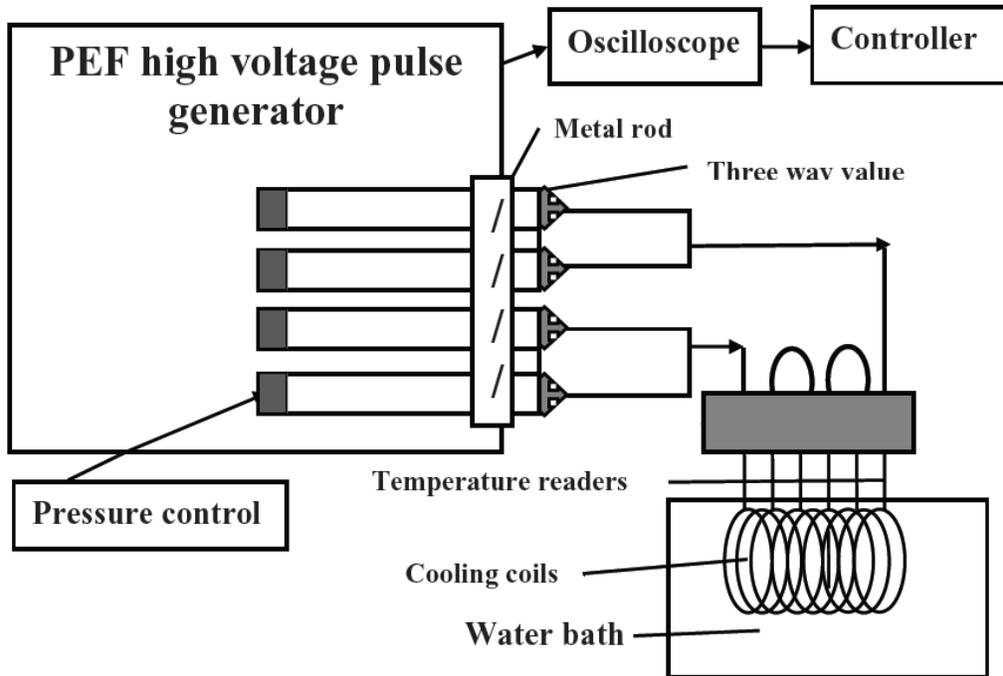


Figure 3.1 Diagram of Pulsed Electric Field Treatment Operation (Adopted from Xiang et al., 2009).



Figure 3.2 OSU-4K PEF Equipment

3.2.2.2 Thermal Pasteurization

Watermelon juice, in screw-top glass bottles, was pasteurized in a covered water bath (Precision Waterbath 180 Series, Chicago, IL). Juice samples were pasteurized with the following conditions: 75°C at 15, 30, and 45 s; 80°C at 15, 30, and 45 s; 85°C at 15, 30, 45 s; 90°C at 15, 30, and 45 s. Treatment time was measured after the sample reached target temperature. One hundred milliliters of fresh watermelon juice was pasteurized at each set of conditions and was then placed in an ice bath and cooled at refrigeration.

The K kinetics of lycopene and vitamin C degradation were determined by using an empirical type of power law model equation (4) as described by Sharma and Maguer (1996):

$$L = L_0^{(t)-K} \quad (4)$$

where K= degradation rate of lycopene and/or vitamin C at time (t), L_0 = initial concentration of lycopene and/or vitamin C. The logarithms were taken of both sides of equation (4) and a plot of log L versus log t was constructed. The resulting linear equation from the plot yielded the magnitude of log L_0 (i.e., intercept) and K (i.e., slope). Concentrations of lycopene and/or vitamin C were predicted at the time t obtained by equation (4) by using the K and the initial concentration of lycopene and/or vitamin C. A plot was constructed to compare predicted lycopene and vitamin C concentrations with the experimental concentrations.

3.2.3 Lycopene Analysis

PEF treated juice was analyzed for lycopene using the method described by Fish et al. (2002). The following solutions were added to glass amber bottles: 5 mL 95% ethanol, 5 mL 0.05% (w/v) BHT in acetone, and 10 mL hexane. Approximately 0.6 g of watermelon juice was added to each bottle. After 15 min of shaking, 3 mL of deionized water were added to each bottle, and the bottles were shaken again for 5 min. The bottles were kept out at room

temperature for 5 min prior to analysis. The upper hexane layer of each sample was analyzed at 503 nm wavelength using a spectrophotometer (Thermoscientific Genesys 20, Waltham, MA). Hexane was used as a blank. Lycopene content was calculated using the equation (5) and extinction coefficient described by Fish et al. (2002):

$$\text{Lycopene (mg/kg juice)} = \frac{A_{503} \times 31.2}{\text{g juice}} \quad (5)$$

where 31.2 refers to the molar extinction coefficient.

Lycopene retention was calculated using the equation (6).

$$\text{Retention (\%)} = \frac{\text{mg lycopene/kg juice after treatment}}{\text{mg lycopene/kg juice before treatment}} \times 100 \quad (6)$$

3.2.4 Vitamin C Analysis

Vitamin C was analyzed using the AOAC method 967.21 (AOAC, 2006). The titrant was prepared with 50 mg of 2,6-dichloroindophenol Na salt and 42 mg of sodium bicarbonate in 50 mL of water. The solution was diluted to 200 mL with distilled water. The extracting solution was prepared with 15 g of metaphosphoric acid and 40 mL of acetic acid and then diluted to 500 mL with distilled water. Solutions were stored in amber bottles at 4°C. A 100 mL aliquot of watermelon juice was added to 100 mL of the extracting solution and then filtered using a No.1 filter paper (Whatman, Maidstone, England). The solution was then titrated with the titrant until the solution turned bright pink for at least 5 s. A standard curve was created using pure ascorbic acid (Sigma Aldrich, St. Louis, MO). Vitamin C retention was calculated using equation (7).

$$\text{Retention (\%)} = \frac{\text{mg ascorbic acid/100 mL juice after treatment}}{\text{mg ascorbic acid/100 mL juice before treatment}} \times 100 \quad (7)$$

3.2.5 Microbial Analysis

Watermelon juice was analyzed in triplicate for aerobic bacteria and coliforms using Petrifilms (3M Company, St.Paul, MN). Serial dilution bottles were filled with 99 mL of 1% peptone water and then autoclaved at 121°C for 45 min. One milliliter of watermelon juice was mixed thoroughly, added to the stock solution, and then serially diluted to 10⁻². A 1 mL aliquot of each dilution was plated on both aerobic and coliform Petrifilms. The coliform and aerobic Petrifilms were incubated at 37°C for 24 h and 48 h, respectively. After incubation, the colonies were counted and reported as CFU/mL using equation (8). Aerobic colonies appeared red on Petri films while coliform appeared red also but a colony could not be counted as a true coliform unless there was gas formation.

$$\text{CFU/mL} = 1 / [\# \text{ of colonies} * \text{ aliquot plated (1mL)} * \text{ dilution factor}] \quad (8)$$

3.2.6 Color Analysis

A Hunter Labscan XE colorimeter (Hunter Associates Laboratory, Reston, VA) was used to analyze color of the samples. The colorimeter was standardized using white and black tiles. Three grams of watermelon juice were weighed and placed in a hexagonal plastic container. Five containers were stacked together to prevent disturbance from the black base. The samples were analyzed for L*, a*, and b* values. Chroma and hue angle were calculated using the following equations:

$$\text{Chroma} = [(a^*)^2 + (b^*)^2]^{1/2} \quad (9)$$

$$\text{Hue} = \tan^{-1} (b^*/a^*) \quad (10)$$

where a* refers to the redness or greenness and b* refers to the yellowness or blueness. Hue angle is the qualitative attribute of color and chroma is the quantitative attribute of colorfulness (Melendez-Martínez et al., 2005). Analysis was performed in triplicate.

3.2.7 °Brix and pH

Using a digital refractometer (Reichert #137531LO, Depew, NY), the watermelon juice was analyzed for soluble solids. Approximately 1 mL of watermelon juice was placed on the lens of the refractometer and analyzed at 25°C. Results were reported at °Brix values. The pH of the sample was measured using a VWR bench-top pH meter (VWR Symphony SB70P, West Chester, PA). The pH meter was calibrated with pH 4, 7, and 10 buffers prior to usage. The probe was placed in 10 mL of watermelon juice and the pH was measured at 25°C. Analysis was performed in triplicate.

3.2.8 Statistical Analysis

Mean values from the three separate experiments or replicate analysis were reported. The statistical significance of observed differences among treatment means was evaluated by Analysis of Variance (ANOVA) (SAS Version 8.2, SAS Institute Inc., Cary, NC, U.S.A), followed by the t test.

3.3 RESULTS AND DISCUSSION

3.3.1. Lycopene and Vitamin C Contents in Treated Watermelon Juice

Lycopene content of watermelon juice was significantly changed ($P < 0.05$) during PEF treatment with the flow rate of 60 mL/min, while the lycopene content was not significantly changed ($P > 0.05$) during PEF treatments with other flow rates (80, 100, 120, 140 and 160 mL/min) (Table 3.1). TP-treated watermelon juice exhibited significant lycopene degradation (Table 3.2). TP 75°C 15 s and 30 s, TP 80°C 15 s and 30 s, and TP 90°C 15 s had similar lycopene contents as shown in Table 3.2. The lycopene content of PEF-treated watermelon juice samples was slightly higher than the PEF control juice which might be due to the isomerization

of other carotenoids in the juice. The isomerization process leads to formation of lycopene, especially at lower temperatures of processing (Odriozola-Serrano et al., 2007).

Table 3.1 - Lycopene and Vitamin C Contents of PEF-treated Watermelon Juice

	Lycopene Content (mg/kg)	Vitamin C Content (mg/100 mL)
PEF-control	1.83±0.29 ^b	1.58±0.04 ^a
PEF 60	2.51±0.22 ^a	1.58±0.07 ^a
PEF 80	2.07±0.13 ^{ab}	1.57±0.05 ^a
PEF 100	1.96±0.06 ^b	1.63±0.03 ^a
PEF 120	1.99±0.13 ^b	1.60±0.02 ^a
PEF 140	1.91±0.16 ^b	1.57±0.05 ^a
PEF 160	2.00±0.52 ^b	1.59±0.02 ^a

PEF = pulsed electric field treatment; PEF-control = watermelon juice was passed the juice through the PEF system without pulse application.

Values are means ± SD of triplicate determinations.

^{ab} means with different letters in each column are significantly different ($P < 0.05$).

Vitamin C of watermelon juice was not significantly affected during PEF treatment regardless of the flow rate (Table 3.1). Vitamin C content ranged from 1.57 to 1.63 (mg/100 mL) for the PEF treatments. Vitamin C content of watermelon juice significantly decreased ($P < 0.05$) with TP treatments. Vitamin C concentration (mg/100 mL) of the TP-control was reduced from 2.70 to 1.66 for TP 75°C 15 s, while it was reduced to 1.50 for both TP 85°C 45 s and TP 90°C 45 s treatments. Vitamin C is heat labile and is quickly degraded during thermal pasteurization. PEF treatment has the potential to retain vitamin C because it is a non-thermal process. The PEF-treated watermelon juice at 60 mL/min had a total vitamin C retention of 100.2% (Table 3.3) which was similar to previously reported values 96.4–99.9% for PEF treated

watermelon juice (Oms-Oliu et al., 2009). Odriozola-Serrano et al. (2007) reported vitamin C retention of 90.2% for PEF-treated tomato juice.

Table 3.2 - Lycopene and Vitamin C Contents of TP-treated Watermelon Juice

	Lycopene Content (mg/kg)	Vitamin C Content (mg/100mL)
TP - Control	2.37±0.12 ^a	2.70±0.05 ^a
TP 75°C 15s	2.08±0.13 ^{bc}	1.66±0.05 ^b
TP 75°C 30s	2.13±0.20 ^b	1.63±0.07 ^b
TP 75°C 45s	1.82±0.03 ^{de}	1.56±0.00 ^{cde}
TP 80°C 15s	2.09±0.03 ^{bc}	1.63±0.03 ^b
TP 80°C 30s	2.03±0.09 ^{bc}	1.62±0.01 ^{bc}
TP 80°C 45s	1.67±0.03 ^{ef}	1.51±0.00 ^e
TP 85°C 15s	1.90±0.21 ^{cd}	1.61±0.01 ^{bcd}
TP 85°C 30s	1.71±0.05 ^{ef}	1.56±0.00 ^{cde}
TP 85°C 45s	1.65±0.04 ^{ef}	1.50±0.03 ^e
TP 90°C 15s	1.96±0.07 ^{bcd}	1.54±0.10 ^{de}
TP 90°C 30s	1.69±0.05 ^{ef}	1.51±0.00 ^e
TP 90°C 45s	1.64±0.07 ^f	1.50±0.03 ^e

TP = thermal pasteurization

Values are means ± SD of triplicate determinations.

^{abcdef} means with different letters in each column are significantly different ($P < 0.05$).

PEF 60 and PEF 80 had higher lycopene retentions (%) than PEF 100, PEF 120, PEF 140, and PEF 160 (Table 3.3). Oms-Oliu et al. (2009) previously reported retention of lycopene (121%) for PEF treated watermelon juices at 60 mL/min and Odriozola-Serrano et al. (2007) reported a lycopene retention of 145% in PEF-treated tomato juice at 60 mL/min. Lycopene retention for PEF treatments was significantly higher than TP-treated watermelon juice samples (Table 3.3). Lycopene retention for the PEF-treated watermelon juice samples ranged from 104.45 to 136.9%, while it ranged from 69.13-89.87% for the TP-treated juice samples. The TP-

treated samples at 45 s had the lowest lycopene retention (%) regardless of the treatment temperatures. Gärtner et al. (1997) reported that an increased lycopene content may increase the bioavailability of lycopene. Bound lycopene in fresh fruit juice may not be detected during analysis prior to processing but it may be released after processing (Gärtner et al., 1997).

Table 3.3 - Lycopene and Vitamin C Retentions of PEF- and TP-treated Watermelon Juice

	Lycopene Retention (%)	Vitamin C Retention (%)
PEF-control	100.0±0.00 ^{bc}	100.0±0.00 ^{ab}
TP-Control	100.0±0.00 ^{bc}	100.0±0.00 ^{ab}
PEF 60	136.9±12.3 ^a	100.2±4.42 ^{ab}
PEF 80	112.9±7.29 ^b	99.48±2.92 ^b
PEF 100	106.9±23.9 ^b	102.9±1.91 ^a
PEF 120	108.8±6.89 ^b	101.2±1.10 ^{ab}
PEF 140	104.5±8.56 ^b	99.48±2.92 ^b
PEF 160	109.1±28.3 ^b	100.9±1.10 ^{ab}
TP 75°C 15s	87.61±5.52 ^{cd}	61.60±1.96 ^c
TP 75°C 30s	89.87±8.38 ^{cd}	60.47±2.59 ^{cd}
TP 75°C 45s	76.75±1.18 ^{defg}	57.65±0.00 ^{def}
TP 80°C 15s	87.51±1.24 ^{cd}	60.47±0.98 ^{cd}
TP 80°C 30s	85.64±3.90 ^{de}	59.91±0.49 ^{cde}
TP 80°C 45s	70.54±1.15 ^{fg}	55.96±0.00 ^f
TP 85°C 15s	80.22±8.97 ^{defg}	59.63±0.49 ^{cde}
TP 85°C 30s	72.12±2.06 ^{efg}	57.65±0.98 ^{def}
TP 85°C 45s	69.66±1.88 ^{fg}	55.39±0.98 ^f
TP 90°C 15s	82.85±3.11 ^{def}	57.09±3.52 ^{ef}
TP 90°C 30s	71.39±2.15 ^{fg}	55.96±0.00 ^f
TP 90°C 45s	69.13±3.15 ^g	55.39±0.98 ^f

PEF = pulsed electric field treatment, TP = thermal pasteurization, PEF-control = watermelon juice was passed the juice through the PEF system without pulse application.

Values are means ± SD of triplicate determinations.

^{abcdefg} means with different letters in each column are significantly different (P < 0.05).

Total vitamin C retention for the twelve TP treatments ranged from 55.39-61.60%, which demonstrated that vitamin C concentration of watermelon was affected by both time and temperature. Regardless of time, vitamin C retention % decreased significantly as temperature increased (Table 3.3). The study showed that both time and temperature significantly affected the concentration of vitamin C in watermelon juice but the effect of temperature on vitamin C concentration was much more significant. Odriozola-Serrano et al. (2007) reported that vitamin C retention was 80.4% for tomato juice treated at 90°C for 30 s. Our study showed that PEF treatment was a better option for retaining vitamin C in watermelon juice compared to thermal pasteurization. Furthermore, the thermal pasteurization of watermelon juice study showed that TP should be performed at the lowest temperature and shortest time possible to retain vitamin C of watermelon juice. Vitamin C content was not significantly affected by PEF treatment.

3.3.2 Kinetics of Vitamin C Degradation in TP-treated Watermelon Juice

The kinetics of vitamin C in watermelon juice at different TP treatments determined by the Power Law model was given in Figure 3.3. The reaction rate constant (K) of watermelon fruit juice TP treated at 75°C was significantly less than juice sample treated with 85, and 90°C. The K obtained from the Power Law model ranged from 0.148 to 0.160 log mg/mL/ log s. The highest K (0.160 log mg/mL/ log s) was observed for the watermelon juice TP-treated at 90°C. The watermelon juice showed a gradual increase in K values with increased temperature. The regression coefficients (R^2) for 85°C and 90°C TP treatment temperatures fit the model and were greater than 0.97 (Figure 3.4), while the Power Law model did not fit at 75°C and 80°C TP treatments. The study showed that the Power law model worked well at higher temperature TP treatments but it may be more appropriate for predicting vitamin C concentration of watermelon juice during HTST pasteurization. The data did not fit well with other kinetic models, such as

zero, first, and second orders. A number of studies have reported that vitamin C degradation follows a zero-order (Lee et al., 1978), first-order (Lee and Coates, 1999), and/or second-order (Robertson and Samaniego, 1986) kinetic models. Vitamin C degradation in this study followed different mechanisms, which might be due to heating of watermelon juice at higher pH (5.75) compared with fruit juices with low pH. The nutrient degradation depends on number of factors such as pH, water activity, initial reactant concentrations, and their interaction during processing and storage (Labuza and Riboh, 1982).

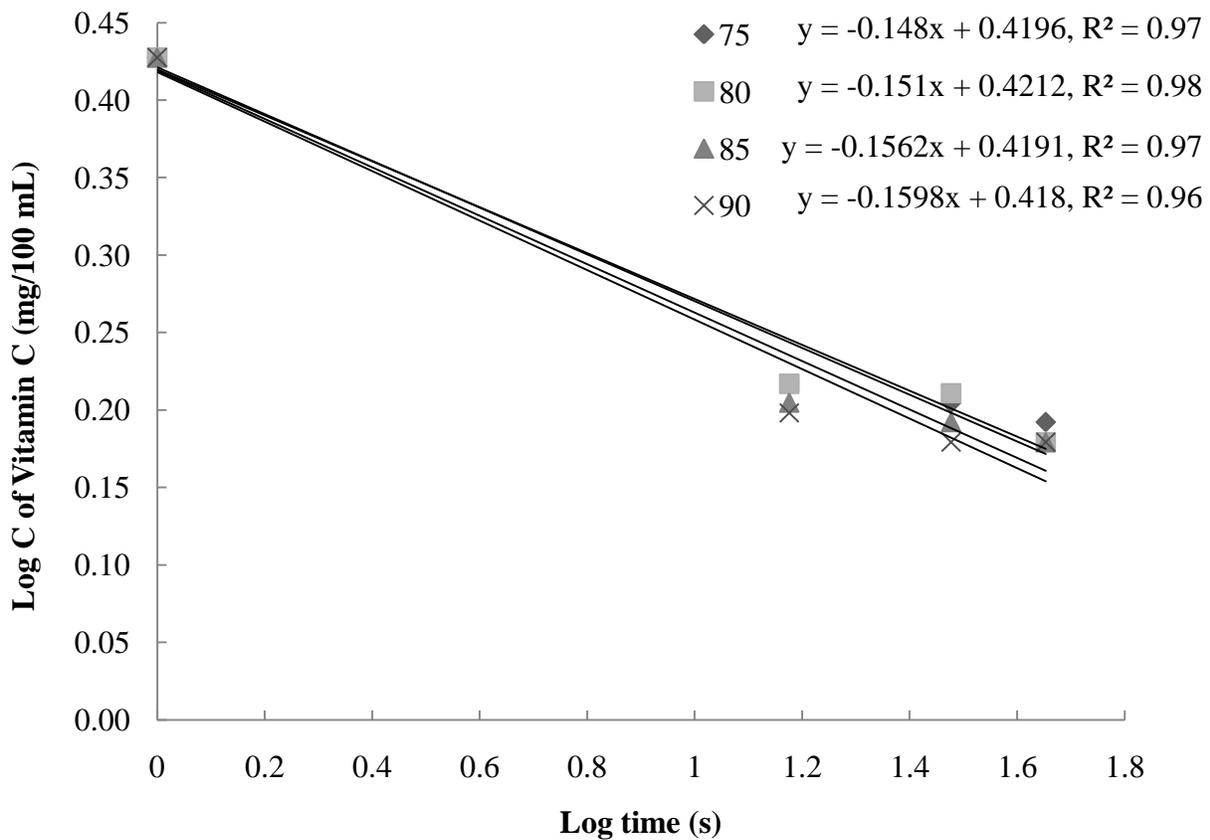


Figure 3.3 Kinetics of Vitamin C Degradation of Watermelon Juice During TP treatments Using the Power Law Model (equation 4). TP = thermal pasteurization; Values are means ± SD of triplicate determinations.

Table 3.4 - Degradation rates for Vitamin C in TP-treated Watermelon Juice

Temperature (°C)	K (log mg/mL/ log s)
75	0.148±0.005 ^c
80	0.151±0.001 ^{bc}
85	0.156±0.003 ^{ab}
90	0.160±0.005 ^a

Values are means ± SD of triplicate determinations.

K = degradation rate

TP = thermal pasteurization

^{abc} means with different letters in each column are significantly different (P < 0.05).

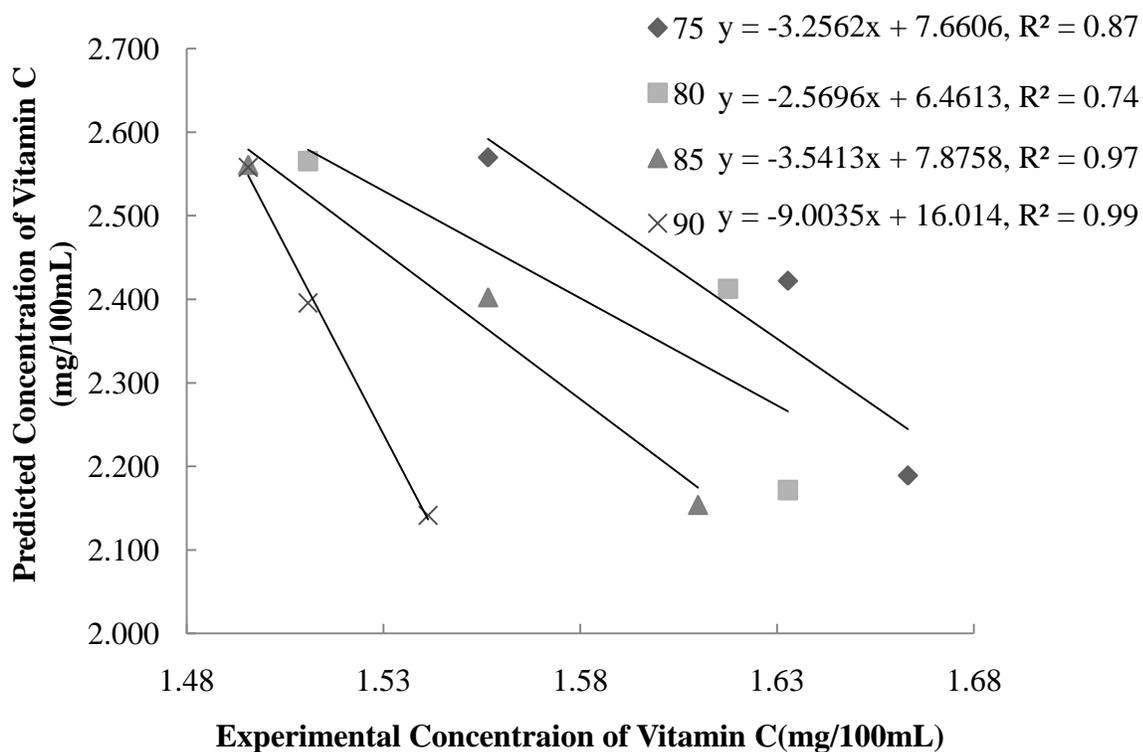


Figure 3.4 Predicted Concentrations of Vitamin C from the Power Law Model for TP treatments (equation 4) compared with the Experimental Concentrations of Vitamin C of Watermelon Juice. TP = thermal pasteurization; Values are means ± SD of triplicate determinations.

Activation energy for the thermal degradation of vitamin C was determined using equation (11).

$$K = A_0 \exp (-E_a/RT) \quad (11)$$

where K is rate constant, A_0 is the frequency factor, E_a is the activation energy of the degradation reaction ($\text{J}\cdot\text{mol}^{-1}$), R is the gas constant ($8.314 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$), and T is the temperature (K). E_a was the slope of the graph obtained by plotting $\ln K$ versus $1/T$ (Figure 3.5).

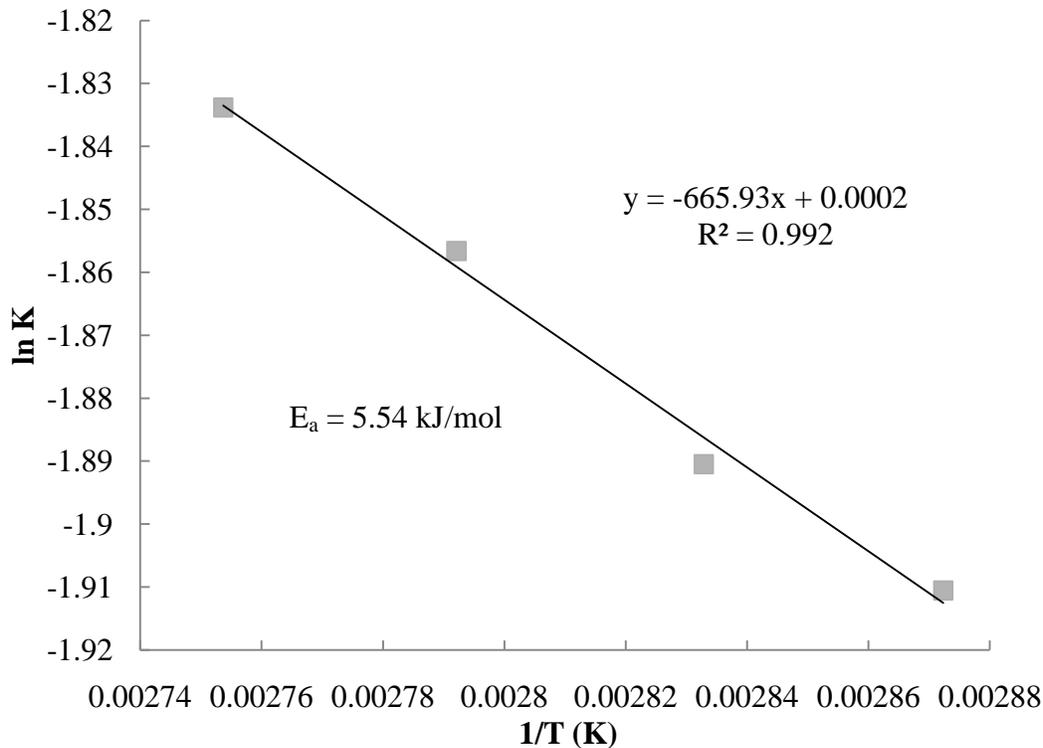


Figure 3.5 The Arrhenius Plot ($1/T$) versus $\ln k$ for Vitamin C in TP-treated Watermelon Juice.

The calculated activated energy for the vitamin C degradation during TP treatments was 5.54 (kJ/mol), which was lower than the reported values for other juices. Johnson et al. (1995) reported that E_a for vitamin C degradation in orange juice was 125.6 kJ/mol, while Burdurlu et al. (2006) reported that it was 105.35, 53.47, 76.91, and 70.30 kJ/mol for orange, lemon,

grapefruit, and tangerine juice concentrations, respectively. Bineesh et al. (2005) reported that E_a for pure vitamin C solution degradation was 22.48 kJ/mol. The E_a depends on number of factors including, pH, processing temperature and time, and initial vitamin concentration in the juice (Lima et al., 1999).

3.3.3 Color of Treated Watermelon Juice

PEF treated watermelon juice samples had similar L^* , a^* , b^* , chroma and hue angle values (Table 3.4) regardless of different flow rates, which indicated PEF treatment did not affect the color of the watermelon juice.

Table 3.5 - Color of PEF-treated Watermelon Juice

	L^*	a^*	b^*	Chroma	Hue angle
PEF-control	58.87±0.26 ^a	0.91±0.10 ^a	7.48±0.06 ^b	7.54±0.05 ^b	1.49±0.12 ^a
PEF 60	59.28±1.08 ^a	0.89±0.18 ^{ab}	7.48±0.17 ^b	7.54±0.19 ^b	1.45±0.02 ^a
PEF 80	58.85±0.07 ^a	0.87±0.10 ^{ab}	7.70±0.21 ^{ab}	7.75±0.22 ^{ab}	1.46±0.01 ^a
PEF 100	59.18±0.81 ^a	0.98±0.15 ^a	7.87±0.14 ^a	7.93±0.15 ^a	1.45±0.02 ^a
PEF 120	58.62±0.17 ^a	0.97±0.04 ^a	7.70±0.24 ^{ab}	7.76±0.24 ^{ab}	1.44±0.01 ^a
PEF 140	59.12±0.53 ^a	0.86±0.04 ^{ab}	7.75±0.30 ^{ab}	7.80±0.29 ^{ab}	1.46±0.01 ^a
PEF 160	59.49±0.78 ^a	0.70±0.11 ^b	7.61±0.12 ^{ab}	7.64±0.13 ^{ab}	1.48±0.01 ^a

PEF = pulsed electric field treatment

Values are means ± SD of triplicate determinations.

^{ab} means with different letters in each column are significantly different ($P < 0.05$).

Table 3.6 - Color of TP-treated Watermelon Juice

	L*	a*	b*	Chroma	Hue angle
TP-Control	55.54±0.26 ^{ab}	1.92±0.12 ^a	0.12±0.22 ^c	1.93±0.14 ^{cd}	0.06±0.10 ^f
TP 75°C 15s	54.96±0.25 ^{ab}	1.82±0.08 ^{ab}	1.51±0.06 ^{ab}	2.37±0.07 ^{abc}	0.69±0.03 ^{cde}
TP 75°C 30s	55.09±0.71 ^{ab}	1.91±0.27 ^a	1.79±0.27 ^a	2.62±0.38 ^a	0.75±0.01 ^{bcd}
TP 75°C 45s	55.77±0.92 ^a	1.66±0.17 ^{ab}	1.44±0.38 ^{ab}	1.99±0.26 ^{cd}	0.58±0.05 ^e
TP 80°C 15s	54.94±0.52 ^{ab}	1.83±0.13 ^{ab}	1.46±0.30 ^{ab}	2.34±0.29 ^{abc}	0.67±0.07 ^{de}
TP 80°C 30s	55.00±0.64 ^{ab}	1.91±0.07 ^a	1.64±0.10 ^{ab}	2.52±0.07 ^{ab}	0.71±0.04 ^{cde}
TP 80°C 45s	54.36±0.64 ^b	1.60±0.03 ^{ab}	1.40±0.07 ^b	2.12±0.06 ^{bcd}	0.72±0.02 ^{cde}
TP 85°C 15s	56.03±1.21 ^a	1.27±0.39 ^{bc}	1.45±0.28 ^{ab}	1.93±0.45 ^{cd}	0.86±0.08 ^b
TP 85°C 30s	55.72±1.40 ^{ab}	1.56±0.19 ^{ab}	1.48±0.18 ^{ab}	2.15±0.26 ^{bcd}	0.76±0.01 ^{bcd}
TP 85°C 45s	55.35±0.71 ^{ab}	1.38±0.40 ^{bc}	1.44±0.06 ^{ab}	2.01±0.28 ^{cd}	0.82±0.15 ^{bcd}
TP 90°C 15s	55.73±1.12 ^a	1.12±0.21 ^c	1.82±0.26 ^a	2.14±0.32 ^{bcd}	1.02±0.04 ^a
TP 90°C 30s	55.85±0.79 ^a	1.28±0.44 ^{bc}	1.52±0.05 ^{ab}	2.01±0.27 ^{cd}	1.01±0.06 ^a
TP 90°C 45s	56.13±0.49 ^a	1.00±0.13 ^c	1.46±0.45 ^{ab}	1.77±0.44 ^d	1.01±0.18 ^a

TP = Thermal Pasteurization

Values are means ± SD of triplicate determinations.

^{abcdef} means with different letters in each column are significantly different (P < 0.05).

The a^* value refers to the redness of the juice which relates to the lycopene content. The a^* value of TP-control was reduced from 1.92 to 1.12 and 1.00, respectively when the juice treated at 90°C for 15s and 45s (Table 3.5), which indicated that these two samples were less red than the control. The TP treatments of watermelon juice samples significantly changed the b^* value which indicated the heated juice was more yellow. Cortes et al. (2008) found that TP treated (90°C 20 s) orange juice had significantly higher b^* values than the untreated juice and PEF-treated juice. Lee and Coates (2003) reported an increased in b^* value and a decreased in a^* value in TP treated (90°C 30 s) orange juice.

Both PEF and TP treatments had no effect on chroma value of the watermelon juice. The lowest chroma value was found for watermelon juice TP treated at 90°C and 45 s, which was due to a significant decrease in a^* value at the same processing condition. The hue angle of the twelve TP treated samples was significantly different from the TP-control. Changes in hue angle were found in TP processed orange juice at 98°C for 21 s (Rivas et al., 2006). The a^* , b^* , chroma, and hue angle of TP treated watermelon juice samples at 90°C for 45 s changed significantly from the TP-control which altered the appearance of watermelon juice which may subsequently affect the perceived quality of juice (Francis, 1995). Aguilo-Aguayo et al. (2009) stated that PEF was more effective at retaining original a^* and b^* values in strawberry juice compared to TP (90°C for 30 or 60 s).

3.3.4 pH, °Brix, and Microbial Counts of Treated Watermelon Juice

The PEF treated watermelon juice at flow rates 120 and 140 mL/min had slightly higher pH values than those of PEF-control, PEF 60, PEF 80, PEF 100, and PEF 160 watermelon juice samples (Table 3.6). Rivas et al. (2006) reported similar results for PEF-treated orange-carrot juice which stated PEF had no significant effect on pH. Yeom et al. (2000) reported no change

in pH of PEF treated orange juice. It appeared PEF treatments did not affect the pH of the juice compared to TP treatments; therefore the overall quality of the juice might be preserved.

Table 3.7 - pH and °Brix of PEF-treated Watermelon Juice

	pH	°Brix
PEF-control	5.75±0.01 ^a	9.2±0.1 ^{ab}
PEF 60	5.75±0.01 ^a	9.3±0.1 ^a
PEF 80	5.74±0.01 ^a	9.1±0.1 ^c
PEF 100	5.74±0.00 ^a	9.2±0.0 ^{abc}
PEF 120	5.80±0.02 ^a	9.1±0.1 ^{bc}
PEF 140	5.82±0.00 ^a	9.1±0.1 ^{bc}
PEF 160	5.76±0.01 ^a	9.1±0.0 ^{bc}

PEF = pulsed electric field treatment

Values are means ± SD of triplicate determinations.

^{abc} means with different letters in each column are significantly different ($P < 0.05$).

The °Brix values were not significantly different among the six flow rates (Table 3.6). Similar results were reported by Rivas et al. (2006) for PEF-treated orange-carrot juice. The reported juice had slight changes in °Brix index but the changes were not significant. °Brix values were not significantly affected by thermal pasteurization (Table 3.7). The °Brix values for TP treatments ranged from 9.0-9.4.

Table 3.8 - pH and °Brix of TP-treated Watermelon Juice

	pH	°Brix
TP-Control	5.43±0.02 ^g	9.4±0.1 ^a
TP 75°C 15s	5.45±0.02 ^g	9.1±0.1 ^d
TP 75°C 30s	5.49±0.01 ^f	9.1±0.0 ^d
TP 75°C 45s	5.51±0.01 ^f	9.1±0.1 ^d
TP 80°C 15s	5.55±0.01 ^e	9.3±0.1 ^{bc}
TP 80°C 30s	5.56±0.01 ^{de}	9.1±0.0 ^d
TP 80°C 45s	5.51±0.02 ^f	9.0±0.0 ^e
TP 85°C 15s	5.59±0.00 ^{cd}	9.3±0.2 ^{bc}
TP 85°C 30s	5.58±0.02 ^{cd}	9.1±0.0 ^d
TP 85°C 45s	5.61±0.02 ^b	9.1±0.0 ^d
TP 90°C 15s	5.67±0.01 ^a	9.2±0.1 ^c
TP 90°C 30s	5.64±0.01 ^a	9.3±0.0 ^c
TP 90°C 45s	5.66±0.04 ^{ab}	9.4±0.0 ^b

TP = thermal pasteurization

Values are means ± SD of triplicate determinations.

^{abcdefghi} means with different letters in each column are significantly different (P < 0.05).

PEF treatments at 60-120 mL/min decreased aerobic bacteria by at least 50% while treatments at 140-160 mL/min did not. The PEF-control had a relatively low initial coliform count at 1 CFU/mL and PEF treatment inactivated it. PEF treatment at 60 mL/min inactivated the most aerobic bacteria. PEF treatments at 140 and 160 mL/min may be too quick for microbial inactivation, since a quicker flow rate gives less time for microbial inactivation.

Table 3.9 - Coliforms and Aerobic Bacteria in PEF-treated Watermelon Juice

	Coliforms (CFU/mL)	Aerobic Bacteria (CFU/mL)
PEF- Control	1±1 ^a	22±3 ^a
PEF 60	0±0 ^b	7±2 ^c
PEF 80	0±0 ^b	9±2 ^c
PEF 100	0±0 ^b	9±1 ^c
PEF 120	0±0 ^b	8±2 ^c
PEF 140	0±0 ^b	13±1 ^b
PEF 160	0±0 ^b	14±2 ^b

PEF = pulsed electric field treatment

Values are means ± SD of triplicate determinations.

^{abc} means with different letters in each column are significantly different (P < 0.05).

All of the TP treatments inactivated 100% of aerobic bacteria. Rivas et al. (2006) stated that TP (98°C 21 s) inactivated 100% of the total plate count bacteria while PEF inactivated more than 99% of the total plate count bacteria at the following conditions: 25 kV/cm, 280 µs, 2.5 µs, and 60 mL/min. TP was more effective in reducing microbial loads compared to PEF

treatments but TP treatments, in general, degraded the vitamin C, lycopene, and color of watermelon juice.

Table 3.10 - Coliforms and Aerobic Bacteria in TP-treated Watermelon Juice

	Coliforms (CFU/mL)	Aerobic Bacteria (CFU/mL)
TP- Control	3±2 ^a	14±2 ^a
TP 75°C 15s	0±0 ^b	0±0 ^b
TP 75°C 30s	0±0 ^b	0±0 ^b
TP 75°C 45s	0±0 ^b	0±0 ^b
TP 80°C 15s	0±0 ^b	0±0 ^b
TP 80°C 30s	0±0 ^b	0±0 ^b
TP 80°C 45s	0±0 ^b	0±0 ^b
TP 85°C 15s	0±0 ^b	0±0 ^b
TP 85°C 30s	0±0 ^b	0±0 ^b
TP 85°C 45s	0±0 ^b	0±0 ^b
TP 90°C 15s	0±0 ^b	0±0 ^b
TP 90°C 30s	0±0 ^b	0±0 ^b
TP 90°C 45s	0±0 ^b	0±0 ^b

TP = thermal pasteurization

Values are means ± SD of triplicate determinations.

^{ab} means with different letters in each column are significantly different (P < 0.05)

3.4 CONCLUSION

The effects of pulsed electric field treatment on lycopene content, vitamin C content, color, pH, °Brix, and microbial count were compared with the effects of conventional thermal pasteurization. Lycopene retention (%) for PEF 60 and PEF 80 was higher than the watermelon juice samples treated with flow rates of 100, 120, 140, and 160. The PEF-treated juice samples had significantly higher lycopene retentions than TP-treated juice samples. Lycopene retention for the PEF treated watermelon juice samples ranged from 104.45 to 136.9%, while it ranged from 69.13-89.87% for the TP treated juice samples. Vitamin C of watermelon juice was not significantly affected during PEF treatment regardless of the flow rate, while it significantly decreased with TP treatments. The study showed that vitamin C was heat labile and was quickly degraded during thermal pasteurization while PEF treatment better retained vitamin C compared to TP treatments. The reaction rate constant (K) obtained from Power Law model for TP-treated watermelon juice samples varied. The study showed a gradual increase in K values with increased temperature, indicating that the model was more appropriate for prediction vitamin C concentration of HTST-treated watermelon juice. The calculated activation energy for vitamin C degradation for TP treatments was relatively lower than the expected value. TP-treatments significantly increased b^* and decreased a^* values compared to the TP-control, while PEF treatment had no significant effect on color. The a^* , b^* , chroma, and hue angle of TP treated watermelon juice samples at 90°C for 45 s changed significantly from the TP-control. In addition, PEF treatments did not affect the pH or °Brix of watermelon juice compared to TP treatments, which indicated that PEF treatments minimized the biochemical reaction in the juice samples. Both PEF and TP treatments were very effective microbial inactivation in watermelon juice. In conclusion, the study shows that the overall quality of watermelon juice, in terms of its

original physicochemical properties, can be maintained by PEF processing while minimizing microbial growth.

If this research was to be performed a few changes should be made. The PEF and TP treatments should be performed with the same batch of juice to prevent having two different controls. Additionally, a fresh, unfiltered sample of watermelon juice should be analyzed. It would also be beneficial in future research to quantify the amount of lycopene lost during centrifugation and filtration; that amount can be used to more accurately determine the effects of PEF and TP treatment on lycopene. Lycopene should be quantified using a standard curve and should be reported as “lycopene before processing” and “lycopene after processing” instead of “retention.” A larger quantity of juice should be used during pasteurization to ensure accurate results. Lycopene and vitamin C degradation should be remodeled after analysis is performed.

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VITA

Lauren Nicole Cook was born in August 1985, in San Antonio, Texas, where she lived until moving to Baton Rouge, Louisiana. She received her Bachelor of Science in food science at Louisiana State University in May 2007. She returned to Louisiana State University in January 2008 to pursue her Master of Science in food science and will receive her degree December 2009. She relocated to Mount Pleasant, Michigan, in August 2009 to work with Leprino Foods Company as a Quality Execution Supervisor trainee.