Effects of Pulsed Electric Fields on the Quality of Orange Juice and Comparison with Heat Pasteurization

Hye Won Yeom, Charles B. Streaker, Q. Howard Zhang,* and David B. Min

Department of Food Science and Technology, The Ohio State University, 2121 Fyffe Road, Columbus, Ohio 43210

Effects of pulsed electric fields (PEF) at 35 kV/cm for 59 μs on the quality of orange juice were investigated and compared with those of heat pasteurization at 94.6 °C for 30 s. The PEF treatment prevented the growth of microorganisms at 4, 22, and 37 °C for 112 days and inactivated 88% of pectin methyl esterase (PME) activity. The PEF-treated orange juice retained greater amounts of vitamin C and the five representative flavor compounds than the heat-pasteurized orange juice during storage at 4 °C ($p < 0.05$). The PEF-treated orange juice had lower browning index, higher whiteness (L), and higher hue angle (θ) values than the heat-pasteurized orange juice during storage at 4 °C ($p < 0.05$). The PEF-treated orange juice had a smaller particle size than the heat-pasteurized orange juice ($p < 0.05$). °Brix and pH values were not significantly affected by processing methods ($p > 0.05$).

Keywords: *Pulsed electric fields (PEF); orange juice; pectin methyl esterase (PME); flavor compounds; vitamin C*

INTRODUCTION

Citrus juices are the most popular fruit juices, accounting for >50% of juice in international commerce (Varnam and Sutherland, 1999). Orange juice accounts for 60% of all Western Europe consumption of juices and juice-based drinks (Fry et al., 1995). Orange juice can undergo quality degradation due to microbiological and enzymatic activities and chemical reactions (Chen et al., 1993). Spoilage microorganisms and native enzymes can be inactivated by thermal treatment, but thermal treatment causes the irreversible loss of fresh juice flavor (Braddock, 1999) as well as a reduction of nutrients and the initiation of undesirable browning reactions in the juices (Chen et al., 1993).

Pulsed electric fields (PEF) have been studied as a nonthermal food preservation method for killing microorganisms in foods (Hoover, 1997; Giese, 1998; Mermelstein, 1999). PEF treatment maintains the flavor, color, taste, and nutritional value of foods with destruction of microorganisms (Mertens and Knorr, 1992; Sizer and Balasubramaniam, 1999). Liquid foods such as apple juice, skim milk, eggs, and green pea soup were PEF-treated for shelf life stability (Qin et al., 1995). Integration of a PEF pilot plant system with an aseptic packaging machine and retention of vitamin C in PEF-treated orange juice was reported by Qiu et al. (1998). When compared to heat processing, PEF caused less protein denaturation and higher retention of vitamin C in protein-fortified orange juice (Sharma et al., 1998). Retention of flavor compounds in PEF-treated orange juice with a bench scale PEF unit was reported by Jia et al. (1999). However, PEF pasteurization does not yet have commercial citrus juice application because the advantages and mechanism of the process have not been completely defined (Braddock, 1999).

The objectives of our study were to investigate the effects of PEF on the microorganisms and pectin methyl esterase (PME) of orange juice and to compare the quality of PEF-treated orange juice including vitamin C, flavor compounds, browning index, color, particle size, °Brix, and pH with that of heat-pasteurized orange juice.

MATERIALS AND METHODS

Preparation of Orange Juice. Freshly squeezed and frozen single-strength Valencia orange juice in a 208 L drum was provided by Minute Maid (Houston, TX) and stored in a freezer at −25 °C until processing. The frozen orange juice was thawed at 4 °C for 10 days. The number of microorganisms in the thawed orange juice, which was represented by total aerobic plate counts, was 10^3 colony-forming units (cfu)/mL before PEF or heat treatment.

Processing of Orange Juice. The single-strength orange juice containing 10^3 cfu/mL of microorganisms was processed in an integrated pilot plant system (Figure 1). The integrated pilot plant system consisted of a fluid handling system, tubular heat exchangers, PEF treatment chambers, a 40 kV/20 kW average high-voltage pulse generator, and an aseptic packaging machine. Before processing, the entire pilot plant system was sterilized by a Clean-In−Place (CIP) process at 105 °C for 30 min.

As a control, the orange juice was pumped through the heat exchangers and the PEF treatment chambers without any treatment. For PEF treatment, orange juice was pumped through the heat exchangers without any heating and then PEF treated in PEF treatment chambers at 35 kV/cm for 59 μs. For heat pasteurization, orange juice was heated in the tubular heat exchangers at 94.6 °C for 30 s with no PEF treatment. Heating of orange juice at 90−99 °C for 15−30 s is normal in commercial practice (Braddock, 1999). All processing parameters are listed in Table 1.

After processing, orange juice was aseptically packaged into thermoformed plastic cups on an aseptic packaging machine

* Author to whom correspondence should be addressed [telephone (614) 688-3644; fax (614) 292-0218; e-mail zhang.138@osu.edu].

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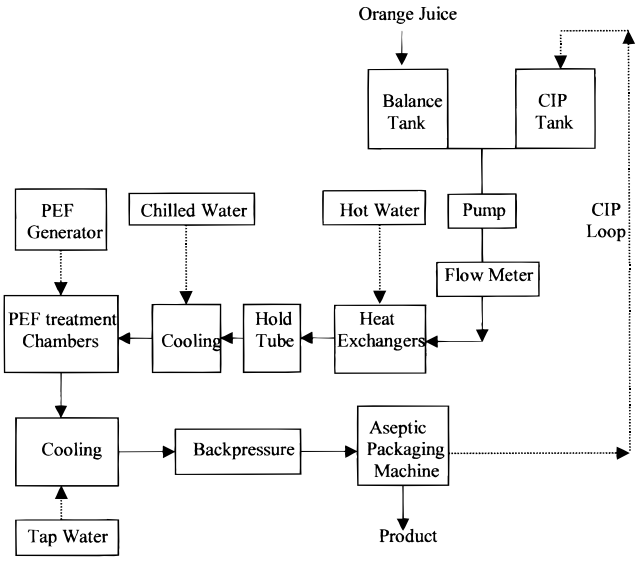

Figure 1. Flowchart of an integrated pilot plant system.

(Benco Asepack/2, Placenza, Italy). The 180 mL cups were formed from base material (Allista Plastic Packaging Co., Muncie, IN) consisting of high-impact polystyrene (HIPS) as outer layer, low-density polyethylene (LDPE) as inner layer, and polyvinylidene chloride (PVDC) between as a barrier layer. The lid material used to seal the cups was a trilaminate material consisting of LDPE on the inside, an aluminum foil middle layer, and a nylon outer coating.

Control, PEF-treated, and heat-pasteurized orange juice cups were stored at 4, 22, and 37 °C for shelf life studies. Two cups per each treatment and storage condition were periodically taken after selected storage time and analyzed for the quality of orange juice including vitamin C, flavor compounds, browning index, color, particle size, °Brix, and pH. The processing of orange juice was duplicated with the same conditions.

PEF Treatment System. The PEF treatment system consisted of a series of co-field tubular treatment chambers (Yin et al., 1997) and a cooling system. Each PEF treatment chamber consisted of two stainless steel tubular electrodes and an insulator made of Delrin. The diameter of the cylindrical treatment zone was 0.635 cm, and the distance between the electrodes was 1 cm. Six PEF chambers were connected in parallel for PEF treatment. To monitor system temperatures, a series of seven sanitary RTD probes with dual sensing elements (model R1T285L4801, Inotek, Bensenville, IL) were placed in short outlet t-pieces at locations such as inlets and outlets of PEF chambers and outlets of cooling systems. These probes were connected to an RTD input module (777518-122), which was connected to a network module (777517-0) wired to a personal computer with LabView data logging software (776670-03, National Instruments, Austin, TX).

Microorganism Assay. Microbial inactivation of orange juice after PEF or heat treatment was studied by total aerobic plate counts using plate count agar (PCA) and yeast and mold counts using acidified potato dextrose agar (PDA). Peptone water, PCA, and PDA were purchased from Difco (Detroit, MI). PDA was acidified with 10% tartaric acid (Sigma, St. Louis, MO). Samples were diluted in 0.1% sterile peptone water up to 10^{-4} dilution and spiral plated by autoplater (model 3000, Spiral Biotech Inc., Bethesda, MD). For each dilution, five samples were plated. PCA plates were incubated at 30 °C for 48 h, and PDA plates were incubated at room temperature for 5 days.

PME Activity Assay. PME activity was measured according to the method of Kimball (1991). Citrus pectin and sodium chloride were purchased from Sigma (St. Louis, MO). A 10 mL aliquot of orange juice was mixed with 40 mL of 1% pectin−salt substrate and incubated at 30 °C. The solution was adjusted to pH 7.0 with 2.0 N NaOH, and then the pH of solution was readjusted to pH 7.7 with 0.05 N NaOH. After the pH reached 7.7, 0.10 mL of 0.05 N NaOH was added. Time was measured until the pH of the solution regained pH 7.7. PME activity unit (PEU) and the relative PME activity (percent) were calculated accorind to the following formulas (Kimball, 1991):

$$\text{PEU} = \frac{(0.05\text{ N NaOH})(0.10\text{ mL of NaOH})}{(10\text{ mL of sample})(\text{time in min})}$$

$$\text{relative PME activity during storage (\%)} = \frac{\text{PEU of PEF- or heat-treated orange juice during storage} \times 100}{\text{PEU of control orange juice at day 0}}$$

Vitamin C Analysis. Vitamin C (ascorbic acid) content in the orange juice was measured using a high-performance liquid chromatography (HPLC) system (Howard et al., 1987). A Hewlett-Packard liquid chromatograph (Wilmington, DE) equipped with an autosampler and a detector at 254 nm was used. The HPLC chromatograph peak area was calculated using a Hewlett-Packard integrator (HP3396 series II). A reverse-phase C-18 column (5 μm particle size, 4.6 mm diameter, 250 mm length, Hewlett-Packard) along with a Hewlett-Packard C-18 guard column was used to separate the vitamin C using methanol and acidified water (10:90, v/v) as a mobile phase. The water was acidified with phosphoric acid (0.01%, v/v). The mobile phase was filtered using a 0.45 μm membrane filter (Micron Separations Inc., Westboro, MA) and degassed using helium gas before passing through the column at a flow rate of 1.0 mL/min. A standard calibration curve was obtained by using L-ascorbic acid (Sigma Chemical Co., St. Louis, MO) in concentrations ranging from 5 to 80 mg/100 mL. The orange juice was centrifuged at 12535*g* for 10 min in a Beckman Microfuge E (Beckman Instruments Inc., Palo Alto, CA) to remove pulp and coarse cloud particles. Ten microliters of the supernatant was injected into the column using the HPLC autosampler. The reproducibility of six-time analyses per each orange juice sample, based on the relative standard deviation, was found to be within 5% for vitamin C.

Flavor Compound Analysis. Flavor compounds of orange juice were analyzed by a headspace solid-phase microextraction gas chromatography (SPME-GC) system (Jia et al., 1998). Standard flavor compounds such as α-pinene, myrcene, octanal, *d*-limonene, and decanal were purchased from Aldrich Chemical Co. (Milwaukee, WI). An SPME fiber with 100 μm polydimethylsiloxane (PDMS) coating, serum bottles, Teflon-coated rubber septa, and aluminum caps were obtained from Supelco, Inc. (Bellefonte, PA).

To avoid loss of volatile flavor compounds during sampling, a silicone septum (12 × 2 mm red TFE/white Silicone, Alltech Inc., Deerfield, IL) was attached on the lid of a 180 mL cup using an instant glue (Elmer's Products, Inc., Columbus, OH). A sterile syringe (Becton Dickinson and Co., Franklin Lakes, NJ) was manually injected into the 180 mL cup through the silicone septum. A 1.0 mL aliquot of orange juice was transferred into a sealed serum bottle containing a micro magnetic bar (10 × 3 mm) and then kept in a freezer below −25 °C until analysis.

The SPME fiber coated with 100 μm of PDMS was manually inserted into the headspace of a serum bottle containing orange juice. Orange juice was incubated at 60 °C for 20 min while being magnetically stirred using an immersed stirrer (LTE Scientific Ltd., Greenfield, Oldham, U.K.). The SPME fiber was retracted from the serum bottle and injected into the GC injection port at 220 °C and kept for 2 min for the desorption of flavor compounds. The desorbed flavor compounds were separated by a Hewlett-Packard 5890 GC equipped with a capillary column (30 m × 0.53 mm i.d.) coated with a 2.65 μm film of 5% phenyl-substituted methylpolysiloxane and a flame ionization detector. The temperature of the GC was programmed from 60 to 120 °C at a rate of 10 °C/min, increased to 140 °C at a rate of 4 °C/min, and then increased to 200 °C

Table 1. Processing Parameters and Temperature of PEF and Heat Treatment

treatment	control	PEF	control	heat
electric field strength (kV/cm)	0	35		
flow rate (L/h)	98	98	110	110
system pressure (psi)	33.5 ± 0.5	35 ± 0.0	32.5 ± 1.5	36 ± 1.0
frequency (pulse per s)	0	600		
pulse duration (μs)	0	1.4		
total treatment time (μs)	0	59		
holding time (s)			30	30
inlet temp before treatment (°C)	15.9 ± 0.7	24.2 ± 1.8	10.1 ± 1.4	8.1 ± 0.9
outlet temp after treatment (°C)	16.2 ± 0.4	60.1 ± 1.8	22.9 ± 1.0	94.6 ± 0.1
outlet temp after cooling (°C)	23.6 ± 1.0	30.6 ± 0.6	20.0 ± 0.2	22.8 ± 0.4

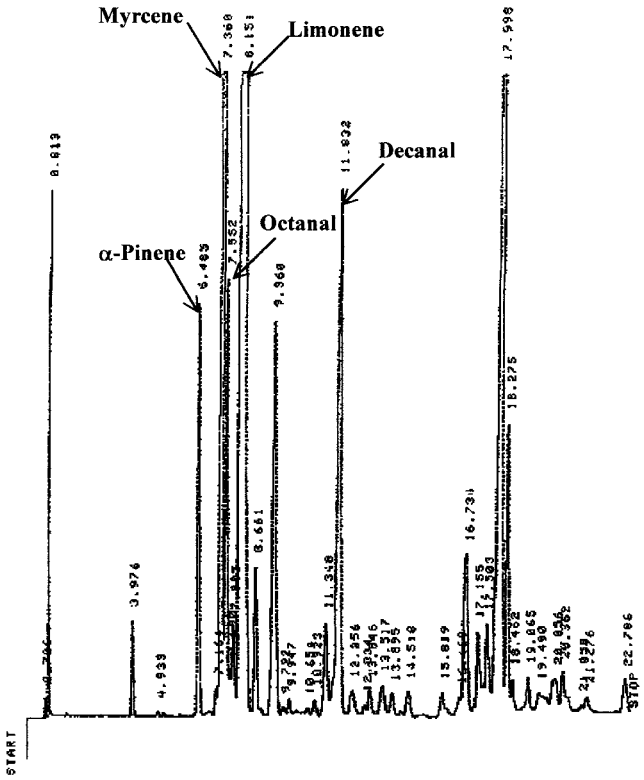

Figure 2. GC chromatogram of orange juice flavor compounds.

at a rate of 20 °C /min and held for a final 5 min. The GC chromatograph peak area was calculated using a Hewlett-Packard integrator (HP 3396A, Wilmington, DE).

The GC chromatogram of orange juice flavor compounds by the SPME headspace sampling is shown in Figure 2. Flavor compounds in the orange juice were identified by comparing the retention time of orange juice flavor compounds with that of standard compounds. The standard calibration curve of each flavor compound was obtained by plotting the GC peak area against the known concentrations of standard flavor compound in deodorized orange juice. The quantity of the flavor compound in orange juice was calibrated from the GC peak area using the calibration curve. The reproducibility of six-time analyses per each orange juice sample, based on the relative standard deviation, was found to be within 8% for all five flavor compounds. The retention of each flavor compound in orange juice during storage (percent) was calibrated as follows:

$$\text{retention of flavor compd in orange juice during storage (\%)} = \frac{\text{concn of flavor compd in PEF- or heat-treated orange juice during storage} \times 100}{\text{concn of flavor compd in control orange juice at day 0}}$$

Browning Index Determination. Orange juice was centrifuged at 12535*g* for 10 min in a Beckman Microfuge E (Beckman Instruments Inc., Palo Alto, CA) to remove pulp and coarse cloud particles. Supernatant was collected and clarified using a 0.45 μm filter (Gelman Sciences, Ann Arbor, MI). The browning index of the clarified orange juice was measured at 420 nm using a Spectronic Genesys 5 spectrometer (Milton Roy, Rochester, NY) at room temperature (Meydav et al., 1977).

Color Measurement. A HunterLab colorimeter (Hunter Associates Laboratory Inc., Reston, VA) was used for color measurement. Orange juice was placed in a 10 mm cell and measured for L, a, and b values. An increasing value of L represents an increase in whiteness or lightness ($L = 0$, black; $L = 100$, white). An increase in a represents an increase in redness ($-a$ = green, $+a$ = red), and an increase of b indicates an increase in yellowness ($-b$ = blue, $+b$ = yellow). Hue angle, $\tan^{-1}(b/a)$, was calculated using a and b values (Trant et al., 1981).

Particle Size Analysis. Particle size of orange juice was analyzed by a Mastersizer (Malvern Instruments, Inc., Worcs, U.K.) based on laser diffraction analysis. When a parallel beam of laser light is passed through a suspension, the diffracted light is focused onto a detector. The detector senses the angular distribution of scattered light intensity. Particles of a given size diffract light through a given angle, which increases with decreasing particle size (McCave and Syvitski, 1991). A 10 mL aliquot of orange juice was diluted with 500 mL of distilled water and circulated in the Mastersizer. A computer equipped with Mastersizer Micropulus 2.15 (Malvern Instruments, Inc.) recorded the distribution of particle size in orange juice.

Analysis of °Brix and pH. Soluble solids content was measured as °Brix using a hand-held refractometer (Fisher, Pittsburgh, PA). The pH of orange juice was measured using an Orion pH meter (model 370, Beverly, MA) at room temperature.

Statistical Analysis. Analysis of variance and Tukey's multiple comparisons method at the 5% level were performed for the determination of significant differences in the processing treatment at each time interval and each storage temperature. All statistical analyses were conducted with Minitab 12.1 (Minitab, Inc., State College, PA).

RESULTS AND DISCUSSION

Effects of PEF and Heat on Microorganisms. Effects of PEF and heat treatment on the total aerobic plate counts and the yeast and mold counts of orange juice stored at 4, 22, and 37 °C for 112 days are shown in Figures 3 and 4, respectively. PEF treatment at 35 kV/cm for 59 μs and heat pasteurization at 94.6 °C for 30 s kept the number of microorganisms in the orange juice at 1 log cfu/mL at 4, 22, and 37 °C for 112 days; the number of microorganisms in control orange juice reached 6 log cfu/mL even at 4 °C after 14 days.

Microscopic examinations indicated that the major microorganism in the orange juice was yeast (data not shown). Citrus juices are most susceptible to yeast spoilage due to their low pH and high contents of sugar and vitamins (Deak and Beuchat, 1996). Yeast cells are known to be more tolerant of high temperatures (65−70 °C) than bacteria and molds (Kimball, 1991). The

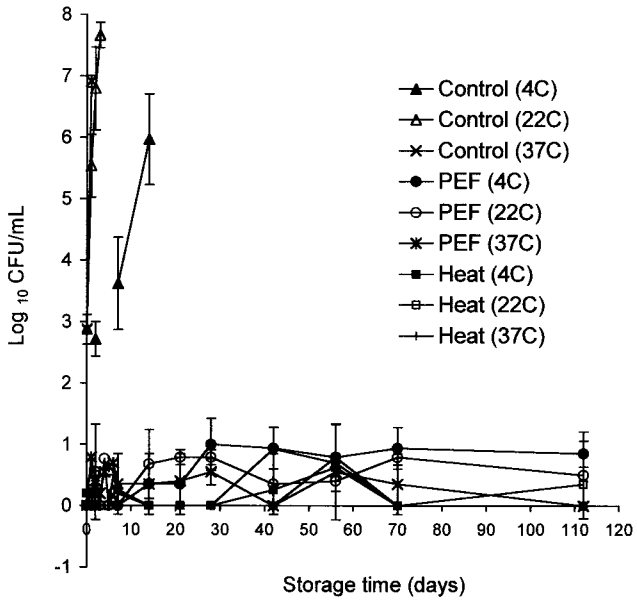

Figure 3. Effects of PEF and heat treatment on the total aerobic plate counts of orange juice during storage at 4, 22, and 37 °C.

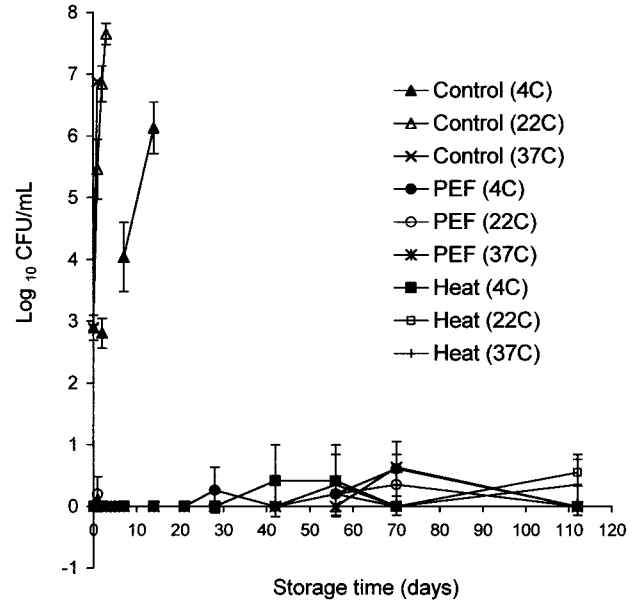

Figure 4. Effects of PEF and heat treatment on the yeast and mold counts of orange juice during storage at 4, 22, and 37 °C.

temperature of the orange juice increased to 60.1 ± 1.8 °C during the PEF treatment at 35 kV/cm for 59 μs (Table 1). The residence time of orange juice at 60.1 ± 1.8 °C was <9 s as the orange juice was immediately cooled by tap water. If microorganisms were killed by the increase of temperature to 60.1 ± 1.8 °C for 9 s during the PEF treatment, more yeast cells should survive than bacteria cells because of their heat resistance. PEF-treated orange juice kept the number of yeast and mold counts lower than 1 log cfu/mL at 4, 22, and 37 °C for 112 days (Figure 4). According to Sadler et al. (1992), heat pasteurization of orange juice at 66 °C for 10 s could not prevent the growth of microorganisms including yeast at 4 °C. Murdock et al. (1953) reported that 2.1 min was required to destroy yeasts in single-strength orange juice at 62.8 °C. Therefore, thermal inactivation of microorganisms, especially yeast

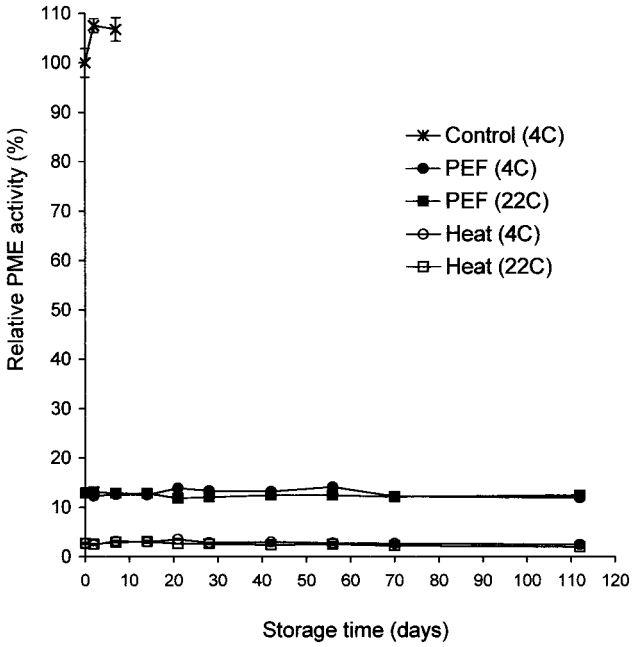

Figure 5. Effects of PEF and heat treatment on the relative PME activity of orange juice during storage at 4 and 22 °C. The value of 100% PME activity was 22.02 PEU × 10^4.

cells, was not the major cause for the microbial inactivation by PEF. The microbial inactivation by PEF has been explained by the formation of membrane pores (Dimitrov, 1984; Tsong, 1991). It is also known that yeast cells are more susceptible to PEF than bacteria due to their larger cell size (Jeyamkondan et al., 1999).

Effects of PEF and Heat on PME Activity. Effects of PEF and heat treatment on the relative PME activity of orange juice during storage at 4 and 22 °C are shown in Figure 5. PEF treatment at 35 kV/cm for 59 μs decreased 88% of PME activity, and the inactivated PME was not restored at 4 and 22 °C for 112 days. Heat pasteurization of orange juice at 94.6 °C for 30 s inactivated 98% of PME activity. A 90−100% reduction of the PME activity is normal in commercial heat-pasteurized orange juice (Irwe and Olsson, 1994).

PME causes cloud loss of orange juice by deesterification of pectin (Kimball, 1991). Heat pasteurization of orange juice is designed to inactivate PME, which is more thermal resistant than vegetative microorganisms (Chen and Wu, 1998). The temperature of orange juice was increased up to 60.1 ± 1.8 °C during PEF treatment at 35 kV/cm for 59 μs. The retention time of orange juice at 60.1 ± 1.8 °C was <9 s as it was immediately cooled to 30.6 ± 0.6 °C by tap water (Table 1). Atkins and Rouse (1954) reported that only 37.2% of PME in Valencia orange juice was inactivated by heat pasteurization at 62.8 °C for 12 s. Tajchakavit and Ramaswamy (1995) reported that the *D* value of PME inactivation in orange juice is 153 s at 60 °C. Eagerman and Rouse (1976) recommended thermal pasteurization of orange juice at 90 °C for 1 min to inactivate PME. Cameron et al. (1994) reported that incubation of orange juice at 80 °C for 2 min was required to inactivate the most active form of PME. Therefore, the increase of orange juice temperature up to 60.1 ± 1.8 °C for 9 s during PEF treatment was not the major cause for the 88% reduction of PME activity. A conformational change of enzyme has been suggested as the enzymatic inactivation mechanism of PEF by several researchers (Vega et al., 1995; Ho et al., 1997; Yeom et al., 1999).

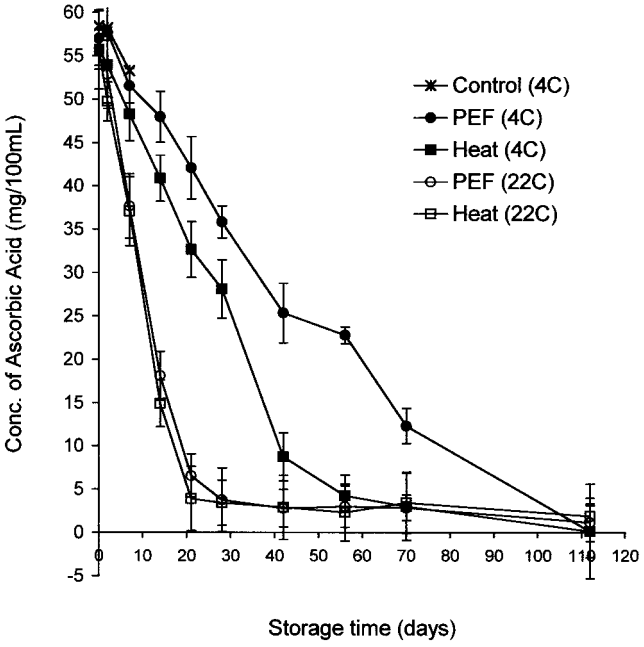

Figure 6. Effects of PEF and heat treatment on the concentration of ascorbic acid in orange juice during storage at 4 and 22 °C.

Effects of PEF and Heat on Vitamin C. Effects of PEF and heat on the concentration of ascorbic acid in the orange juice during storage at 4 and 22 °C are shown in Figure 6. PEF-treated orange juice retained a significantly higher content of ascorbic acid than heat-pasteurized orange juice during storage at 4 °C ($p < 0.05$). PEF-treated orange juice showed no significant difference in the concentration of ascorbic acid compared to heat-pasteurized orange juice during storage at 22 °C ($p > 0.05$).

Orange juice should contain 60 mg of ascorbic acid per 8 fluid oz (236 mL) serving to provide 100% of the U.S. Recommended Daily Allowances (USRDA) requirement for vitamin C (Ting, 1977; Squires and Hanna, 1979). Accordingly, the concentration of ascorbic acid in orange juice should be at least 25 mg/100 mL at the time of expiration date for 100% vitamin C supply. Linear degradation of ascorbic acid was observed in both PEF- and heat-treated orange juices during the storage from 0 to 42 days at 4 °C and from 0 to 21 days at 22 °C (Figure 6). The concentration of ascorbic acid in PEF-treated orange juice reached 25 mg/100 mL at 4 °C after 47 days, which is significantly longer than the 31 days of heat-pasteurized orange juice ($p < 0.05$). The concentration of ascorbic acid in PEF- and heat-treated orange juices reached 25 mg/100 mL at 22 °C after 13 and 12 days, respectively.

Ascorbic acid is a typically heat sensitive nutrient (Saguy et al., 1978). High temperatures during processing and storage cause loss of ascorbic acid (Nagy and Smoot, 1977). Heat-pasteurized orange juice had a significantly lower concentration of ascorbic acid than PEF-treated orange juice during storage at 4 °C ($p < 0.05$) due to the higher processing temperature. Johnson et al. (1995) reported that rate constants for ascorbic acid degradation were also dependent on storage temperature. Significant difference was not observed in the retention of ascorbic acid between PEF and heat treatment during storage at high storage temperature, 22 °C ($p > 0.05$).

The degradation of ascorbic acid is known to occur by both oxidative and nonoxidative mechanisms (Varsel, 1980). Atmospheric oxygen is responsible for most vitamin C loss during long-term storage, and many polymeric containers readily admit oxygen (Kimball, 1991). The aseptically packaged orange juice in the polymer cup showed rapid degradation of ascorbic acid irrespective of the processing methods. Higher retention of ascorbic acid in PEF-treated orange juice packaged in glass and PET bottles was reported by Ayhan et al. (2000).

Effects of PEF and Heat on Flavor Compounds. Effects of PEF and heat treatment on the retention of flavor compounds in the orange juice are shown in Table 2. As storage time increased, PEF-treated orange juice showed significantly higher content of flavor compounds than heat-pasteurized orange juice during storage at 4 °C ($p < 0.05$). No significant difference was observed in the retention of flavor compounds between PEF- and heat-treated orange juices during storage at 22 °C as shown in Figure 7 ($p > 0.05$).

The flavor of orange juice is easily changed by heat during processing or storage (Shaw, 1986; Li et al., 1988). Irreversible damage to the citrus juice flavor results from chemical reactions initiated or occurring during the heating process (Braddock, 1999). PEF-treated orange juice was exposed to 60.1 °C for 9 s during PEF treatment, whereas heat-pasteurized orange juice was heated at 94.6 °C for 30 s during heat pasteurization (Table 1). Heat load incurred during heat pasteurization can negatively impact flavor of juice (Ekasari et al., 1988). The lower initial heat load during PEF treatment might cause fewer chemical reactions, resulting in more retention of flavor compounds in orange juice during storage at 4 °C (Table 2).

Flavor changes are also associated with a number of deteriorative reactions during storage, which result in the development of off-flavor (Nagy and Rouseff, 1986). Nonenzymatic browning such as ascorbic acid degradation causes deterioration of flavor as well as significant loss of nutrients and darkening (Kaanane et al., 1988). Kefford et al. (1959) reported that oxidative reactions and anaerobic reactions causing loss of ascorbic acid lead to deterioration of flavors in pasteurized orange juice.

Storage time and temperature are the major factors influencing shelf stability of the aseptic juice. High storage temperatures negatively impact the quality of citrus juice products by accelerating nonenzymatic browning and flavor change. Permeability of oxygen in soft-pack containers is also important for the shelf stability of aseptically processed juices (Marshall et al., 1986). Permeation of oxygen in polymer packages may have limited the effectiveness of aseptic products at ambient temperatures, and this permeation is dependent on temperature (Braddock, 1999). Significant loss of flavor compounds occurred in both PEF-treated and heat-pasteurized orange juices packed in polymer cups and stored at 22 °C (Figure 7).

Effects of PEF and Heat on Browning Index. Effects of PEF and heat treatment on the browning index of orange juice during storage at 4 °C are shown in Figure 8. PEF-treated orange juice showed a significantly lower browning index than heat-pasteurized orange juice during storage at 4 °C ($p < 0.05$). There was no significant difference in the browning index

Table 2. Effects of PEF and Heat Treatment on the Retention of Flavor Compounds (Percent) in Orange Juice during Storage at 4 °C[a]

	α-pinene		myrcene		octanal		limonene		decanal	
day	PEF	heat	PEF	heat	PEF	heat	PEF	heat	PEF	heat
0	112.14 ± 7.58	105.14 ± 6.92	112.79 ± 4.45	102.88 ± 5.92	98.69 ± 7.11	97.87 ± 7.99	112.80 ± 7.15	101.63 ± 7.90	102.49 ± 6.13	98.33 ± 6.99
2	102.89 ± 7.53	96.41 ± 7.66	103.24 ± 6.20	93.52 ± 5.72	99.05 ± 7.41	97.07 ± 6.86	**103.18** ± 5.93	92.38 ± 4.50	94.54 ± 5.78	93.22 ± 4.49
7	92.38 ± 7.36	83.57 ± 7.61	**90.93** ± 3.64	80.13 ± 5.38	90.95 ± 6.88	83.02 ± 7.02	**89.96** ± 5.16	78.99 ± 5.67	82.42 ± 2.67	77.48 ± 7.44
14	78.96 ± 7.41	72.60 ± 4.27	**76.43** ± 4.85	68.19 ± 2.78	**81.56** ± 5.82	72.13 ± 3.30	**75.13** ± 4.97	66.95 ± 2.19	**69.93** ± 1.84	65.66 ± 1.99
21	78.87 ± 7.97	74.58 ± 7.98	74.62 ± 7.23	69.66 ± 4.90	76.63 ± 7.35	74.35 ± 7.81	72.78 ± 7.14	67.53 ± 7.26	65.73 ± 7.48	59.01 ± 4.88
28	75.60 ± 6.04	70.61 ± 6.10	71.01 ± 2.71	66.38 ± 2.25	73.92 ± 5.15	75.16 ± 4.61	68.92 ± 4.91	63.98 ± 4.15	**61.07** ± 2.26	53.04 ± 4.88
42	**74.75** ± 5.96	63.50 ± 4.93	**70.56** ± 4.07	59.13 ± 3.82	**73.62** ± 2.15	67.79 ± 2.99	**67.81** ± 7.01	56.29 ± 3.51	**56.46** ± 2.52	43.98 ± 2.04
56	**70.34** ± 3.76	56.18 ± 3.46	**66.84** ± 4.50	52.14 ± 2.30	**68.97** ± 1.89	62.63 ± 2.68	**64.34** ± 5.54	49.10 ± 2.19	**52.09** ± 3.96	38.32 ± 1.19
70	**60.14** ± 3.30	52.26 ± 4.15	**56.08** ± 2.83	48.08 ± 3.47	**62.89** ± 2.89	56.89 ± 1.55	**53.27** ± 2.56	44.91 ± 3.50	**42.05** ± 3.42	35.28 ± 2.32
112	**54.27** ± 6.53	43.66 ± 3.00	**49.70** ± 5.23	41.61 ± 2.79	**59.83** ± 1.64	56.55 ± 1.61	**45.77** ± 6.27	37.99 ± 1.29	**37.91** ± 4.92	31.09 ± 1.45

[a] Entries in boldface type indicate that the retention of flavor compound in orange juice was significantly different between PEF and heat treatments at $p < 0.05$. The values of 100% flavor compounds were 0.0406 ppm for α-pinene, 31.6407 ppm for myrcene, 1.4053 ppm for octanal, 667.545 ppm for limonene, and 0.6776 ppm for decanal.

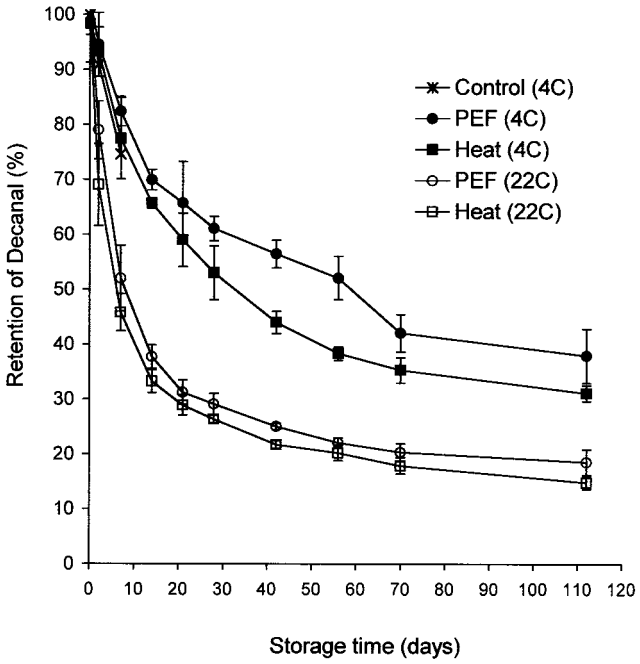

Figure 7. Effects of PEF and heat treatment on the retention of decanal (percent) in orange juice during storage at 4 and 22 °C.

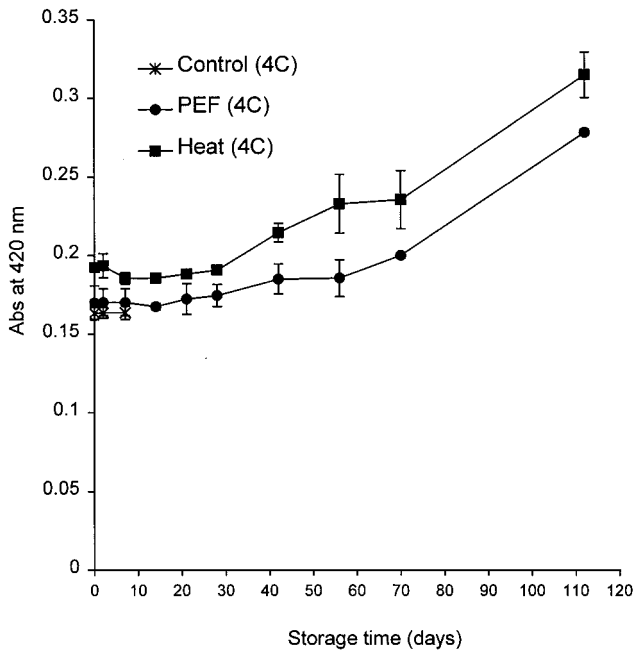

Figure 8. Effects of PEF and heat treatment on the browning index of orange juice during storage at 4 °C.

between PEF- and heat-treated orange juices during storage at 22 °C ($p > 0.05$, data not shown).

A linear increase of browning index was observed in both PEF- and heat-treated orange juices after an initial lag period of 28 days at 4 °C (Figure 8). The increase of browning index after an initial lag period was observed by Legault et al. (1951). During this lag period, colorless compounds are probably formed, which do not contribute to an increase in absorbance (Karel and Nickerson, 1964).

Ascorbic acid degradation is considered to be a major chemical reaction responsible for browning of citrus juices (Marcy et al., 1984). Destruction of ascorbic acid provides reactive carbonyl groups, which can be precur-

Table 3. Effects of PEF and Heat Treatment on the Particle Size of Orange Juice[a]

	D [4,3]		*D* [3,2]		*D* (*v*, 0.1)		*D* (*v*, 0.5)		*D* (*v*, 0.9)	
	PEF	heat	PEF	heat	PEF	heat	PEF	heat	PEF	heat
av	206.97	219.10	41.70	49.62	19.31	25.00	189.61	209.49	427.37	436.77
SD	4.30	6.36	1.66	1.92	1.36	1.58	6.94	9.23	3.77	5.06

[a] Particle sizes (μm) of PEF and heat-treated samples were significantly different at $p < 0.05$. *D* [4,3] is the equivalent volume mean diameter; *D* [3,2] is the equivalent surface area mean diameter; *D* (*v*, 0.5) is the volume (*v*) median diameter—50% of the distribution is above this value and 50% below. av, average; SD, standard deviation.

Table 4. Effects of PEF and Heat Treatment on °Brix and pH of Orange Juice during Storage for 112 Days

	control[a]	PEF (4 °C)	heat (4 °C)	PEF (22 °C)	heat (22 °C)
°Brix	12.23 ± 0.03	12.42 ± 0.07	12.44 ± 0.03	12.42 ± 0.09	12.43 ± 0.06
pH	3.78 ± 0.02	3.76 ± 0.03	3.76 ± 0.04	3.76 ± 0.03	3.76 ± 0.04

[a] Analyzed at day 0.

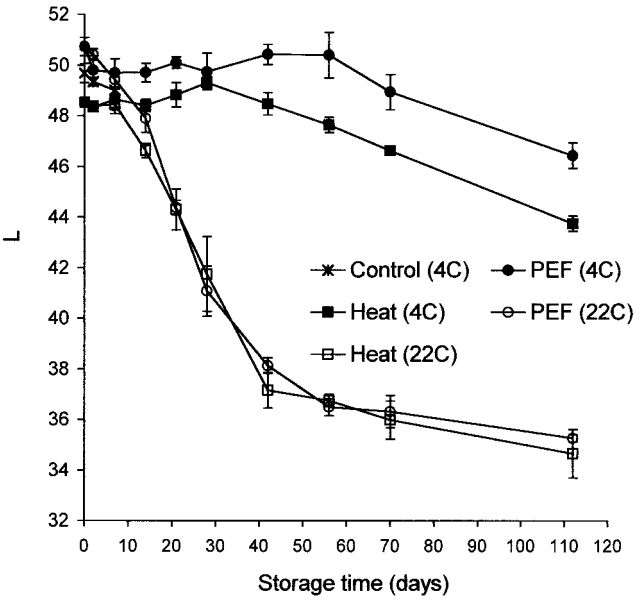

Figure 9. Effects of PEF and heat treatment on the whiteness (*L*) of orange juice during storage at 4 and 22 °C.

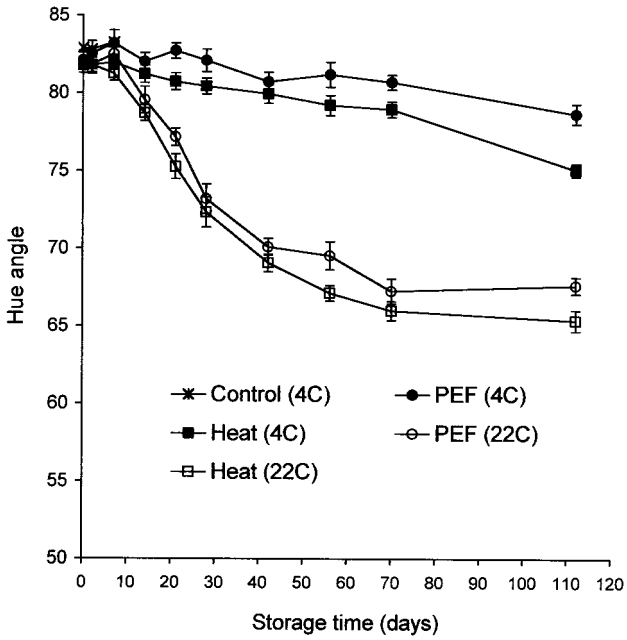

Figure 10. Effects of PEF and heat treatment on the hue angle (θ) of orange juice during storage at 4 and 22 °C.

sors to nonenzymatic browning (Joslyn, 1957). The presence of oxygen greatly accelerated the loss of ascorbic acid and the formation of browning pigments (Kacem et al., 1987).

Effects of PEF and Heat on Color. Effects of PEF and heat treatment on the whiteness (*L*) and hue angle (θ) of orange juice during storage at 4 and 22 °C are shown in Figures 9 and 10, respectively. PEF-treated orange juice showed significantly higher whiteness (*L*) and hue angle (θ) values compared to heat-pasteurized orange juice during storage at 4 °C ($p < 0.05$). Consequently, PEF-treated orange juice showed brighter color than heat-pasteurized orange juice during storage at 4 °C. There was no significant difference in the whiteness (*L*) and the hue angle (θ) values between PEF- and heat-treated orange juices during storage at 22 °C ($p > 0.05$). The bright color of citrus juices is one of the important quality factors in citrus products. Detrimental changes in color, primarily caused by nonenzymatic browning, reduce consumer acceptance of citrus juices (Klim and Nagy, 1988).

Effects of PEF and Heat on Particle Size, °Brix, and pH. Effects of PEF and heat treatment on the particle size of orange juice are shown in Table 3. PEF-treated orange juice contained significantly smaller particle size than heat-pasteurized orange juice ($p < 0.05$). There was no significant difference between control and PEF-treated orange juices ($p > 0.05$, data not shown). The colloidal material in fruit juices is usually coagulated by heating and will settle out readily (Joslyn, 1961). According to Buslig and Carter (1974), the particles in orange juice are important by making up cloud, pulp, and texture and contributing to sensory characteristics.

Effects of PEF and heat treatment on the °Brix and pH values of orange juice during storage are shown in Table 4. °Brix and pH values were neither significantly affected by processing methods nor changed during storage at 4 and 22 °C ($p > 0.05$). In citrus juices °Brix is used to indicate the percent of soluble solids and is one of the most important factors to grade the quality of a fruit juice (McAllister, 1980). Microorganisms cause fruit juice spoilage by reduction of acidity and organic acid fermentation (Sodeko et al., 1987). Therefore, microorganisms can change the °Brix and pH values of orange juice by consumption of soluble solids and organic acids. PEF and heat treatment effectively inactivated spoilage microorganisms; therefore, the °Brix and pH values of orange juice were not changed during storage at 4 and 22 °C.

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