Pasteurization of mixed mandarin and Hallabong tangor juice using pulsed electric field processing combined with heat

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Abstract Effects of pulsed electric field (PEF) processing combined with heating (H-PEF processing) on the inactivation of microorganisms and the physicochemical properties of mixed mandarin and Hallabong tangor (MH) juice were studied. Using a pilot-scale PEF system, MH juice, pre-heated at 55 °C, was PEF-treated at 19 kV/cm of electric field and 170 kJ/L of specific energy and the juice, pre-heated at 70 °C, was PEF-treated at 16 kV/cm and 100 kJ/L or 12 kV/cm and 150 kJ/L. H-PEF processing at 70 °C–16 kV/cm–100 kJ/L reduced the aerobe, yeast/mold, and coliform counts of MH juice by 3.9, 4.3, and 0.8 log CFU/mL, respectively, without affecting the ascorbic acid concentration and antioxidant capacity of juice. H-PEF processing changed juice color and browning degree ($p < 0.05$), but not total soluble solid content or pH. By controlling initial juice temperature and electric field strength, H-PEF processing can be an effective pasteurization method for mixed juice with minimal changes in quality.

Keywords Pasteurization · Pulsed electric field · Combined treatment · Mixed fruit juice · Citrus juice

Introduction

Satsuma mandarin (*Citrus unshiu* Marc.) is a major citrus crop in Korea, Japan, and China, and largely harvested in those countries between October and December [1, 2]. Hallabong tangor is a new hybrid citrus crop in South Korea that is popular with customers because of its sweet taste and large size, and thus is increasingly cultivated in Jeju island [3, 4]. Citrus fruits are rich in various health-promoting components such as vitamins C and A, folate, minerals, dietary fiber, and phytochemicals [5], and are commercialized in many forms such as juice, puree, and mixed juice [6, 7].

Pulsed electric field (PEF) treatment that uses pulses of high intensity electric field on food products has been consistently used as a juice pasteurization method [8–10]. Active research is currently being conducted to effectively inactivate microorganisms in food using a PEF technology combined with heat, ultraviolet light, high intensity light pulse, or ultrasound [11–14]. Heating-combined PEF (H-PEF) (60 °C, 21 s, 32 kV/cm, 84 μ s) treatment of skim milk resulted in a 3-log cycle reduction in the total number of aerobic bacteria compared to untreated skim milk, and microbial inactivation levels were approximately 2 log CFU/mL higher compared to the inactivation achieved by either heat (60 °C, 21 s) or PEF treatment (32 kV/cm, 84 μ s) [15]. In addition, inoculation of liquid whole egg containing lemon essential oil with *Salmonella* Senftenberg followed by H-PEF (60 °C, 3.5 min, 25 kV/cm, 24 μ s) treatment showed that, whereas single treatment inhibited *S. Senftenberg* by less than 0.5 log cycle, the combined treatment resulted in microbial inactivation of approximately 3 log cycles [16]. These preliminary research findings demonstrate that microbial inactivation efficacy of PEF treatment increases by the combination with heating.

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Zhang et al. [17] reported that microbial inactivation resulted from synergistic effects between PEF and thermal treatments. The inactivation rate could increase since the microbial cells, which went through temperature-related phase transition of cell membranes from a gel to a liquid-crystalline structure by heating and thus possessed membranes reduced in thickness associated with the phase transition, could be vulnerable to PEF [18].

Studies using commercial-scale PEF systems for pasteurization of fruit and vegetable juices such as orange, tomato, and pomegranate have been reported [8, 18, 19]. However, for a more widespread use of PEF technology, microbial safety and effectiveness in quality preservation needs to be abundantly demonstrated for various food products such as mixed juices. The aims of the present study are (1) to determine H-PEF treatment conditions that meet commercial pasteurization standards for mixed mandarin-Hallabong tangor (MH) juice, and (2) to identify the effects of H-PEF treatment on the quality of the mixed juice regarding color, browning degree, total soluble solids content, pH, ascorbic acid concentration, and antioxidant capacity.

Materials and methods

MH juice preparation

Satsuma mandarin (*Citrus unshiu* Marc.) and Hallabong tangor (*Citrus kiyomi* × *Citrus ponkan*) were harvested in 2016 at Jeju island, Korea, and were shipped to the PEF processing plant (BK Bio, Jeju, Korea). The fruits were washed with tap water and cold-pressed using a citrus juice extractor (Model 391B, JBT FoodTech, Lakeland, FL, USA) at the rate of 33.3 kg per min. Squeezed juices were packaged in high-density polyethylene (HDPE) bags (width × height, 100 × 160 cm; thickness, 0.1 mm; Ilsung Industry, Seongju, Korea) of 150 L placed in 200 L polyethylene (PE) drums and stored at −40 °C. PE drums containing squeezed juice were placed in a 70–80 °C water bath for 3 h for thawing, and the thawed mandarin and Hallabong tangor juices were mixed at a 7:3 (w/w) ratio. The mixed thawed juice was used as untreated juice samples. The temperature of the juice prior to H-PEF processing was 4 ± 1 °C.

H-PEF processing

Juice was heated to 55 and 70 °C using a tubular heat exchanger (J&D Engineering, Sejong, Korea), and the heated juice was injected into the PEF treatment chamber using a vertical multistage pump (Dooch Pump, Hwaseong, Korea). PEF treatment was conducted using a pilot-scale

unit (HVP-5, DIL, Quakenbrueck, Germany), consisting of a 5-kW pulse generator, a co-linear treatment chamber, and a human-machine interface. Both the inner diameter and gap distance of the treatment chamber were 10 mm. The pulsed form used was the bipolar square wave. Juice treatment parameters are summarized in Table 1. Inlet temperature was first set, and the maximum electric field strength and specific energy that did not generate dielectric breakdown were determined. The results from our preliminary study exhibited that PEF treatment with inlet temperature set below 50 °C led to microbial reduction within the juice of less than 1 log CFU/mL. In the present study, inlet temperatures of 55 and 70 °C were used to increase the microbial inactivation effect. Maximum electric field strength and specific energy that did not result in dielectric breakdown at inlet temperature of 55 °C were 19 kV/cm and 170 kJ/L, respectively, and at 70 °C were 16 kV/cm and 100 kJ/L, respectively. In addition to the treatments at maximum conditions (#1 and #2 in Table 1), another treatment (#3 in Table 1) was tested at 70 °C, which was conducted at a lower electric field strength (12 kV/cm), but higher specific energy than the maximum conditions at 70 °C (150 kJ/L). Specific energy (W_{spec}), the parameter corresponding to H-PEF treatment intensity, was calculated as shown below.

$$W_{spec} \text{ (kJ/L)} = V \times I \times t \times \frac{1}{m}$$

where m is the juice volume (0.79 mL, the volume of treatment chamber), V (V) is the voltage, I is the current, and t is the total treatment time in the PEF treatment chamber.

Table 1 Parameters of pulsed electric field treatments combined with heating (H-PEF) used for the pasteurization of mixed mandarin-Hallabong tangor (MH) juice

Treatment parameter	Treatment ^a		
	#1	#2	#3
Inlet temperature (°C)	55	70	70
Electric field strength (kV/cm)	19	16	12
Pulsed width (μs)	24	30	30
Frequency (pps, Hz)	166	115	320
Total treatment time (μs)	102	89	247
Specific energy (kJ/L)	170	100	150
Temperature rise (°C)	23	14	20
Holding temperature (°C)	78	84	89
Holding time (s)	41	41	41
Flow rate (L/h)	110	110	110

^aElectrical conductivity of the MH juice is 0.24

H-PEF-treated juice was injected in the holding tube (I.D. 10 mm) connected to the PEF treatment chamber for 41 s, followed by passing through the tubular heat exchanger to be cooled below 15 °C. The flow rate of the juice during H-PEF processing was 110 L/h. Treated samples were collected in sterile conical tubes (50 mL, SPL Co., Pocheon, Korea) inside a clean booth and used for subsequent microbial analysis.

To identify the effects of holding time after H-PEF treatment on microbial inhibition, 5 mL of sample was placed in an autoclaved tube (I.D. 10 mm) and heated in a water bath (VS-1205 W, Vision scientific, Daejeon, Korea) at 84 °C, the temperature of juice immediately after treatment at 70 °C (inlet temperature) and 16 kV/cm–100 kJ/L (temperature prior to entering the holding tube). The juice was heated for 20, 40, 60, 90, and 120 s after it reached 84 °C. One hundred seconds were required for the sample to reach 84 °C from 23 ± 2 °C. Treated samples were immediately transferred to sterile conical tubes (15 mL, SPL) and cooled in ice-cold water (1–2 °C) for subsequent microbial analysis.

Microbial analyses

Untreated and treated samples were diluted with 0.1%(w/v) peptone water and placed on the Petrifilm™ (3 M, St. Paul, MN, USA) of an aerobic count plate, a yeast/mold plate, and a coliform plate to obtain total mesophilic aerobes, yeasts and molds, and coliforms counts, respectively. Petrifilms™ of aerobic and coliform count plates were incubated at 37 °C for 24–48 h, and Petrifilms™ of yeast and mold count plates were incubated at 25 °C for 3–5 days.

Color determination

Color was measured using a colorimeter (Minolta Chroma Meter CR-400; Minolta Camera, Osaka, Japan). Measurement samples (10 mL per sample) were prepared in quartz cells (10-mm thickness). L^* , a^* , and b^* values of the sample were measured based on CIELAB coordinates. The color difference (ΔE_{ab}^*) was calculated from the following equation according to Jung et al. [20].

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Browning degree determination

The browning degree was measured using the spectrophotometric method described by Zhou et al. [21]. Thirty-five g of sample were centrifuged (VS-24SMTi, Vision Scientific, Daejeon, Korea) at $10,000 \times g$ for 20 min

at 4 °C, and the supernatant was passed through a 0.45 µm syringe filter (6750-2504, Whatman, Piscataway, NJ, USA). The browning degree was determined based on absorbance measured at 420 nm using a UV-spectrophotometer (DU® 730, Beckman Coulter, Fullerton, CA, USA).

Determination of total soluble solid content and pH

The total soluble solid content of juice was determined by measuring the refractive index of juice with a refractometer (Abbemat-200, Anton Paar OptoTech GmbH, Seelze, Germany), and the pH was determined using a pH meter (FiveEasy™ Plus; Mettler Toledo, Schwerzenbach, Switzerland).

Determination of ascorbic acid concentration

Ascorbic acid concentration (mg/100 mL) was quantitatively analyzed using high performance liquid chromatography (HPLC, Agilent 110 Series, Agilent Technologies, Santa Clara, CA, USA) according to Leong and Shui [22]. Samples were prepared in the same way as described for browning degree analysis. A C18 column (Symmetry®, 5 µm, 4.6 mm × 250 mm i.d.; Waters, Milford, MA, USA) was used and placed in a column oven (Shinkwang Scientific, Taipei Hsien, Taiwan) set at 23 ± 2 °C. A total of 20 µL of the sample solutions was injected into the HPLC system. The effluent was monitored by SPC-10Avp (UV-Vis detector, Shimadzu, Kyoto, Japan) at 254 µm.

Antioxidant capacity determination

Antioxidant capacity of samples was measured using the 2,2,-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity assay according to Blois [23]. A sample volume of 100 µL was mixed with 100 µL of 0.4 mM DPPH in 96-well plates and reacted in the dark for 30 min. Absorbance was measured at 517 nm using a spectrophotometer (SpectraMax M3, Molecular Devices, Sunnyvale, CA, USA). DPPH radical-scavenging capacity was calculated as shown below.

DPPH radical-scavenging capacity (%)

$$= \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \times 100$$

A mixture of equal volumes of the DPPH solution and methanol was used as the blank. Antioxidant capacity was determined as Ascorbic Acid Equivalent Antioxidant Capacity (AAEAC) [24]. DPPH was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Statistical analysis

Microbial inactivation assays and physicochemical properties determination were repeated twice; 5 samples per treatment condition were prepared for each replicate and measured twice. One-way analysis of variance (ANOVA) was performed using PASW statistics software (ver. 23.0.0; IBM SPSS, New York, NY, USA) and Tukey's multiple range test was used for post-analysis to test for statistically significant differences ($\alpha = 0.05$).

Results and discussion

Effects of H-PEF treatment on microbial inactivation

The effects of H-PEF treatment on total mesophilic aerobes, yeasts and molds, and coliforms in MH juice are shown in Fig. 1. Total mesophilic aerobe, yeast and mold, and coliform counts were 4.0 ± 0.3 , 4.3 ± 0.2 , and 1.1 ± 0.3 log CFU/mL, respectively, in untreated juice, whereas counts in juice treated at 55 °C and 19 kV/cm–170 kJ/L were 1.6 ± 0.2 , 2.2 ± 0.6 and 0.8 ± 0.3 log CFU/mL, respectively. In contrast, H-PEF treatment at 70 °C and 12 kV/cm–150 kJ/L reduced the total mesophilic aerobes counts by 3.3 ± 0.5 log CFU/mL, whereas the counts of yeast and molds and coliforms fell below the level of detection (1 CFU/mL; Fig. 1). Increasing the inlet temperature to 70 °C increased the inactivation rate by approximately 2 log CFU/mL, despite a relatively low electric field strength and specific energy for the H-PEF treatment compared to 55 °C (Fig. 1). The enhanced microbial inactivation due to inlet temperature increase could be explained by a phase change in the bacterial

Fig. 1 Effects of pulsed electric field treatments combined with heating (H-PEF) on the inactivation of total mesophilic aerobes, yeast and molds, and coliforms in mixed mandarin-Hallabong tangor (MH) juice

membranes [18]. As juice temperature increases, phospholipid molecules in bacteria within the juice change from gel to liquid-crystal phase, decreasing lipid bilayer thickness in cell membranes, which results in effective microbial inactivation upon H-PEF treatment. Further, the holding temperature after H-PEF treatment could also influence the microbial inactivation effect. The H-PEF-treated sample subjected to 55 °C inlet temperature was held at 78 °C for 41 s in the holding tube, whereas the H-PEF treated sample subjected to 70 °C inlet temperature was held at 89 °C for 41 s.

After H-PEF treatment at 16 kV/cm–100 kJ/L of juice heated to 70 °C, total mesophilic aerobe, yeast and mold, and coliform counts were all below detectable levels (1 CFU/mL) (Fig. 1); H-PEF treatment at 12 kV/cm–150 kJ/L also resulted in undetectable levels (Fig. 1). H-PEF treatment at 16 kV/cm–100 kJ/L showed a strong inactivation effect on total mesophilic aerobes (Fig. 1) despite a relatively low specific energy compared to H-PEF at 12 kV/cm–150 kJ/L, suggesting that electric field strength is an important parameter in microbial inactivation. Min et al. [25] also reported that applied electric field strength is the primary variable for inactivation of lipoxxygenase in tomato juice by PEF for a given energy input. Our results are consistent with a previously reported study in which the microbial inactivation rate of *Escherichia coli* O157:H7 in liquid whole egg increases with increasing inlet temperature (50–60 °C) and electric field strength (9–15 kV/cm).

According to the Standards and Specifications of Korean Food Standards Codex, total count of mesophilic aerobes in commercial fruit juice are required to be below 100 CFU/mL, and coliform counts to be negative [26]. Samples subjected to H-PEF treatment with inlet temperature at 55 °C contained total mesophilic aerobe and coliform counts of 2.7 ± 0.2 and 0.4 ± 0.3 log CFU/mL, respectively, which indicates that this treatment is unable to meet the criteria. In contrast, samples subjected to H-PEF treatment at 16 kV/cm–100 kJ/L with inlet at 70 °C were able to meet Korean Food Standards, as no mesophilic aerobes or coliforms were detected. Therefore, the H-PEF treatment at 70 °C may be applied in commercial pasteurization.

To determine the effects of 41 s at the outlet temperature in the holding tube on the survival of total mesophilic aerobes in MH juice, samples were heated for 20, 40, 60, 90, and 120 s at 84 °C, the temperature to which samples are exposed immediately after H-PEF treatment at 16 kV/cm–100 kJ/L and 70 °C inlet temperature, and then the number of mesophilic aerobes were counted. The numbers of total mesophilic aerobes in the samples were reduced by 1.3 ± 0.3 , 1.1 ± 0.1 , 1.4 ± 0.2 , 1.5 ± 0.1 , and 1.5 ± 0.3 log CFU/mL after the treatments of 20, 40, 60, 90, and 120 s, respectively. Therefore, mesophilic aerobe

inactivation (> 3.9 log CFU/mL Est.) after H-PEF treatment (70 °C, 16 kV/cm–100 kJ/L) was mostly due to the H-PEF process itself, and the holding temperature had little effect.

The results show that H-PEF treatments at 70 °C is suitable for commercial pasteurization of MH juice. Further, electric field strength was identified as an important parameter in microbial inactivation, and thus increasing electric field strength would reduce the total treatment time required for commercial pasteurization, allowing an effective treatment with specific energy. The microbial inactivation results show that the treatments of 70 °C (inlet temperature) at 16 kV/cm–100 kJ/L and 12 kV/cm–100 kJ/L are suitable conditions for H-PEF treatment to evaluate juice quality.

Effects of H-PEF treatment on juice color

The effects of H-PEF treatment on the color of MH juice are shown in Table 2. The L^* value did not vary significantly, regardless of H-PEF treatment ($p > 0.05$), but the b^* value of H-PEF-treated samples was significantly higher than that of untreated samples ($p < 0.05$; Table 2). The a^* values differed according to the conditions of H-PEF treatment. Samples treated at 16 kV/cm–100 kJ/L did not show significant difference in a^* values ($p > 0.05$) compared to the control, whereas the a^* values of samples treated at 12 kV/cm–150 kJ/L decreased significantly compared to untreated juice ($p < 0.05$; Table 2). Cortés et al. [27] has reported similar results, in which a significant decrease in a^* and a significant increase in b^* values in PEF-treated (30 kV/cm, 100 μ s) or heat-treated (90 °C, 20 s) orange juice compared to untreated orange juice was observed. Because the color of citrus juice is generally determined by carotenoid pigments such as α - and β -carotenes, zeta-antheraxanthin (yellow), violaxanthin (yellow), β -citraurine, and β -cryptoxanthin [28], it can be concluded that H-PEF treatment affected juice carotenoid

concentrations. Changes in the concentrations of carotenoids in orange-carrot juice induced by PEF or heat treatment have been previously reported [29].

H-PEF treatment at 12 kV/cm–150 kJ/L demonstrated a higher color difference (ΔE_{ab}^*) than treatment at 16 kV/cm–100 kJ/L ($p < 0.05$; Table 2). The H-PEF treatment used in the present study affected the a^* and b^* values of MH juice. However, the effect on juice color could be reduced using treatment with a relatively high electric field strength and lower energy.

The effects of H-PEF treatment on the browning degree of MH juice are shown in Table 2. The browning degree of samples increased significantly after both H-PEF treatments ($p < 0.05$). Nonetheless, the increase in specific energy did not significantly alter browning value, demonstrated by no significant difference in the browning degree between H-PEF treatments ($p > 0.05$; Table 2). Cortés et al. [27] reported that PEF treatment (30 kV/cm, 100 μ s) of orange juice did not result in significant differences in browning, whereas heat treatment significantly increased browning. In our results, the increase in browning degree of H-PEF-treated juice might be induced by heating.

Effects of H-PEF treatment on total soluble solid content and pH

Total soluble solid contents of untreated and H-PEF-treated juices were not significantly different ($p > 0.05$; Table 3). Cortés et al. [27] similarly reported a lack of change in total soluble solid contents upon PEF (30 kV/cm, 100 μ s) or heat treatment (90 °C, 20 s).

The pH of untreated and H-PEF-treated juice did not show significant differences ($p > 0.05$; Table 3). These results are consistent with a previously reported study, in which no change in pH after H-PEF treatment (heating: 98 °C for 21 s, PEF treatment: 25 kV/cm for 280 μ s) of blended juice of orange-carrot was observed [6].

Table 2 Effects of pulsed electric field treatments combined with heating (H-PEF) on the color and browning degree (BD) of mixed mandarin-Hallabong tangor (MH) juice

Sample	Color				BD (Absorbance at 420 nm)
	L^*	a^*	b^*	ΔE^*	
<i>MH juice</i>					
Untreated	48.58 ± 0.59 ^{ab3}	22.97 ± 1.02 ^a	78.30 ± 0.70 ^c		0.16 ± 0.00 ^b
PEF treated at 100 kJ/L ¹	49.01 ± 0.28 ^a	22.35 ± 0.15 ^{ab}	79.19 ± 0.68 ^b	1.33 ± 0.31 ^b	0.17 ± 0.00 ^a
PEF treated at 150 kJ/L ²	48.24 ± 0.47 ^b	22.15 ± 0.50 ^b	80.19 ± 0.17 ^a	2.17 ± 0.33 ^a	0.18 ± 0.01 ^a

¹The MH juice is treated at 16 kV/cm of electric field strength and 100 kJ/L of specific energy

²The MH juice is treated at 12 kV/cm of electric field strength and 150 kJ/L of specific energy

³Values with different letter superscripts within each property are significantly different each other at $p < 0.05$

Table 3 Effects of pulsed electric field treatments combined with heating (H-PEF) on total soluble solids, pH, ascorbic acid concentration, and ascorbic acid equivalent (AAE) antioxidant capacity of mixed mandarin-Hallabong tanger (MH) juice

Sample	Total soluble solids (%)	pH	Ascorbic acid concentration (mg/100 mL)	Antioxidant capacity (AAE mg/100 mL)
<i>MH juice</i>				
Untreated	10.31 ± 0.05 ^{a3}	3.71 ± 0.01 ^a	27.89 ± 1.55 ^a	37.47 ± 3.51 ^a
PEF treated at 100 kJ/L ¹	10.31 ± 0.03 ^a	3.70 ± 0.02 ^a	27.37 ± 1.30 ^{ab}	36.49 ± 2.32 ^{ab}
PEF treated at 150 kJ/L ²	10.26 ± 0.12 ^a	3.70 ± 0.01 ^a	25.96 ± 1.28 ^b	34.76 ± 2.36 ^b

¹The MH juice is treated at 16 kV/cm of electric field strength and 100 kJ/L of specific energy

²The MH juice is treated at 12 kV/cm of electric field strength and 150 kJ/L of specific energy

³Values with different letter superscripts within each property are significantly different each other at $p < 0.05$

Effects of H-PEF treatment on ascorbic acid concentration

The effects of H-PEF treatment on ascorbic acid concentration in MH juice are shown in Table 3. The ascorbic acid concentration in juice treated with H-PEF at 16 kV/cm–100 kJ/L was not significantly different from that of untreated juice ($p > 0.05$). However, the ascorbic acid concentration of samples treated at 12 kV/cm–150 kJ/L was lower than that of untreated juice. Therefore, H-PEF treatment at 16 kV/cm–100 kJ/L corresponds to the pasteurization condition in which ascorbic acid in juice is not degraded. Torregrosa et al. [30] reported a 17% reduction in ascorbic acid concentration after heat treatment (98 °C, 21 s) of mixed orange-carrot juice. The H-PEF treatment used in the present study included an extremely short come-up time of 100–250 µs to increase juice temperature from inlet to outlet, which suggests that the thermal effects on ascorbic acid in juice could be relatively less severe compared to conventional heat treatment. Our results show that the specific energy required for the desired extent of pasteurization could be lowered by increasing the electric field strength, which would result in a relatively lower holding temperature, reducing ascorbic acid loss in the juice after H-PEF treatment.

Effects of H-PEF treatment on antioxidant capacity

Antioxidant capacity, similar to ascorbic acid concentration, was not significantly different between juice treated with H-PEF at 16 kV/cm–100 kJ/L and untreated juice ($p > 0.05$), although the antioxidant capacity of juice treated with H-PEF at 150 kJ/L was significantly lower than that of untreated juice ($p < 0.05$; Table 3). Because ascorbic acid is the most representative antioxidant compound in MH juice [31, 32], the fact that H-PEF treatment at 16 kV/cm–100 kJ/L did not alter antioxidant capacity is consistent with the finding that these conditions preserve

the ascorbic acid content (Table 3). From the results, H-PEF treatment at 16 kV/cm–100 kJ/L can be identified as an effective pasteurization method that preserves the antioxidant capacity of juice.

Our results show that no PEF processing condition with inlet temperature at 55 °C was appropriate for commercial pasteurization of MH juice. However, H-PEF processing at 70 °C met the standards for commercial pasteurization, demonstrating a strong inactivation effect on indigenous microorganisms. Further, high electric field strength enhances microbial inactivation even at a relatively low level of specific energy. The H-PEF treatment itself, rather than the high temperature (84 °C) at the holding tube following treatment, exerted a strong inactivation effect on indigenous microorganisms. H-PEF processing at 70 °C (inlet temperature)-16 kV/cm–100 kJ/L was identified as an effective pasteurization method that preserves the total soluble solid, pH, ascorbic acid concentration, pH, and antioxidant capacity of MH juice. In addition, microbial inactivation and juice quality assay results showed that electric field strength is a primary parameter in H-PEF pasteurization to be considered for process optimization. The results of the present study demonstrate that control of initial juice temperature and electric field strength in the H-PEF treatment can accomplish commercial pasteurization of mixed citrus juice while minimizing changes in product quality.

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References

1. Hong SI, Lee HH, Kim D. Effects of hot water treatment on the storage stability of Satsuma mandarin as a postharvest decay control. *Postharvest Biol. Technol.* 43: 271–279 (2007)

2. Yamaga I, Takahashi T, Ishii K, Kato M, Kobayashi Y. Suppression of blue mold symptom development in satsuma mandarin fruits treated by low-intensity blue LED irradiation. *Food Sci. Technol. Res.* 21: 347–351 (2015)
3. Lee CW, Oh HJ, Han SH, Lim SB. Effects of hot air and freeze drying methods on physicochemical properties of citrus 'hal-labong' powders. *Food Sci. Biotechnol.* 21: 1633–1639 (2012)
4. Lee EH, Park HR, Shin MS, Cho SY, Choi HJ, Shin KS. Antitumor metastasis activity of pectic polysaccharide purified from the peels of Korean Citrus Hallabong. *Carbohydr. Polym.* 111: 72–79 (2014)
5. Beltrán-González F, Pérez-López AJ, López-Nicolás JM, Carbonell-Barrachina AA. Effects of agricultural practices on instrumental color, mineral content, carotenoid composition, and sensory quality of mandarin orange juice, cv. Hernandina. *J. Sci. Food Agric.* 88: 1731–1738 (2008)
6. Rivas A, Rodrigo D, Martínez A, Barbosa-Canovas GV, Rodrigo M. Effect of PEF and heat pasteurization on the physical-chemical characteristics of blended orange and carrot juice. *LWT Food Sci. Technol.* 39: 1163–1170 (2006)
7. Heckman MA, Sherry K, Gonzalez De Mejia E. Energy drinks: an assessment of their market size, consumer demographics, ingredient profile, functionality, and regulations in the United States. *Compr. Rev. Food Sci. Saf.* 9: 303–317 (2010)
8. Min S, Jin ZT, Min SK, Yeom H, Zhang QH. Commercial scale pulsed electric field processing of orange juice. *J. Food Sci.* 68: 1265–1271 (2003)
9. Min S, Evrendilek GA, Zhang QH. Pulsed electric fields: processing system, microbial and enzyme inhibition, and shelf life extension of foods. *IEEE. Trans. Plasma Sci.* 35: 59–73 (2007)
10. Carbonell-Capella JM, Buniowska M, Cortes C, Zulueta A, Frígola A, Esteve MJ. Influence of pulsed electric field processing on the quality of fruit juice beverages sweetened with Stevia rebaudiana. *Food Bioprod. Process.* 101: 214–222 (2017)
11. Bazhal MI, Ngadi MO, Raghvan GSV, Smith JP. Inactivation of *Escherichia coli* O157:H7 in liquid whole egg using combined pulsed electric field and thermal treatments. *LWT Food Sci. Technol.* 39: 420–426 (2006)
12. Noci F, Rieneer J, Walkling-Ribeiro M, Cronin DA, Morgan DJ, Lyng JG. Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple Juice. *J. Food Eng.* 85: 141–146 (2008)
13. Caminiti IM, Palgan I, Noci F, Muñoz A, Whyte P, Cronin D A, Morgan DJ, Lyng JG. The effect of pulsed electric fields (PEF) in combination with high intensity light pulses (HILP) on *Escherichia coli* inactivation and quality attributes in apple juice. *Innov. Food Sci. Emerg.* 12: 118–123 (2011)
14. Palgan I, Muñoz A, Noci F, Whyte P, Morgan DJ, Cronin DA, Lyng JG. Effectiveness of combined pulsed electric field (PEF) and manothermosonication (MTS) for the control of *Listeria innocua* in a smoothie type beverage. *Food Control* 25: 621–625 (2012)
15. Fernández-Molina JJ, Fernandez-Gutierrez SA, Altunakar B, Bermudez-Aguirre D, Swanson BG, Barbosa-Canovas GV. The combined effect of pulsed electric fields and conventional heating on the microbial quality and shelf life of skim milk. *J. Food Process Preserv.* 29: 390–406 (2005)
16. Espina L, Monfort S, Alvarez I, Garcia-Gonzalo D, Pagan R. Combination of pulsed electric fields, mild heat and essential oils as an alternative to the ultrapasteurization of liquid whole egg. *Int. J. Food Microbiol.* 189: 119–125 (2014)
17. Zhang QH, Qin BL, Barbosa-Canovas GV, Swanson BG. Inactivation of *Escherichia coli* for pasteurization by high-strength pulsed electric fields. *J. Food Process Preserv.* 19: 103–118 (1995)
18. Min S, Jin ZT, Zhang QH. Effects of commercial scale pulsed electric field processing on the quality of tomato juice. *J. Food Sci.* 68: 1600–1606 (2003)
19. Jin TZ, Guo M, Zhang HQ. Upscaling from benchtop processing to industrial scale production: More factors to be considered for pulsed electric field food processing. *J. Food Eng.* 146: 72–80 (2015)
20. Jung K, Song BS, Kim MJ, Moon BG, Go SM, Kim JK, Lee YJ, Park JH. Effect of X-ray, gamma ray, and electron beam irradiation on the hygienic and physicochemical qualities of red pepper powder. *LWT-Food Sci. Technol.* 63: 846–851 (2015)
21. Zhou L, Wang Y, Hu X, Wu J, Liao X. Effect of high pressure carbon dioxide on the quality of carrot juice. *Innov. Food Sci. Emerg.* 10: 321–327 (2009)
22. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem.* 76: 69–75 (2002)
23. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature* 26: 1199–1200 (1958)
24. Kim DK, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J. Agric. Food Chem.* 50: 3713–3717 (2002)
25. Min S, Min SK, Zhang QH. Inactivation kinetics of tomato juice lipoxygenase by pulsed electric fields. *J. Food Sci.* 68: 1995–2001 (2003)
26. Ministry of Food and Drug Safety. Korean Food Standards Codex. Available from: <http://www.foodsafetykorea.go.kr/foodcode>. Accessed 10 Jan 2017
27. Cortés C, Esteve M J, Frígola A. Color of orange juice treated by high intensity pulsed electric fields during refrigerated storage and comparison with pasteurized juice. *Food Control* 19: 151–158 (2008)
28. Pérez-López AJ, Beltrán F, Serrano-Megías M, Saura D, Carbonell-Barrachina, AA. Changes in orange juice color by addition of mandarin juice. *Eur. Food Res. Technol.* 222: 516–520 (2006)
29. Torregrosa F, Cortés C, Esteve MJ, Frígola A. Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *J. Agr. Food Chem.* 53: 9519–9525 (2005)
30. Torregrosa F, Esteve MJ, Frígola A, Cortes C. Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *J. Food Eng.* 73: 339–345 (2006)
31. Bavec M, Turinek M, Grobelnik-Mlakar S, Slatnar A, Bavec F. Influence of industrial and alternative farming systems on contents of sugars, organic acids, total phenolic content, and the antioxidant activity of red beet (*Beta vulgaris* L. ssp. *vulgaris* Rote Kugel). *J. Agr. Food Chem.* 58: 11825–11831 (2010)
32. Gardner PT, White TAC, McPhail DB, Duthie GG. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem.* 68: 471–474 (2000)