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Pulsed electric field processing effects on flavor compounds and microorganisms of orange juice

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Abstract

The headspace flavor compounds of fresh squeezed orange juice processed by pulsed electric field (PEF) at 30 kV/cm for 240 or 480 μs, or heat at 90°C for 1 min were isolated by a solid phase microextraction (SPME) coating and separated by gas chromatography. The average losses of flavor compounds in orange juice processed by 240, 480 μs PEF and heat process were 3.0%, 9.0% and 22.0%, respectively ($P < 0.05$). The flavor loss was mainly due to vacuum degassing in the PEF process. The total plate counts of control, 240, 480 μs PEF, and heat processed orange juice were 5400, 21, 19, and 4, respectively. The yeast and mold counts of control, PEF for 240, 480 μs and heat processed orange juice were 2800, 15, 9, and 4, respectively.

Keywords: PEF; Orange juice; Microorganisms; SPME-GC; Flavor compounds

1. Introduction

Orange juice is the most popular fruit juice in the United States and accounts for 60% of all fruit juice sales in the US (Graumlich, Marcy, & Adams, 1986; Marcy et al., 1989). Consumers like orange juice because of its high vitamin C content and unique, delicate and desirable flavor. The flavor of orange consists of more than 200 flavor compounds in a proper concentration (Maarse & Visscher, 1989; Shaw, 1991). The flavor compounds in orange juice are 0.02% of total weight. The 75–98% of flavor compounds are hydrocarbons, 0.6–1.7% aldehydes, 1% esters, 1% ketones and 1–5% alcohols. Limonene is the main flavor compound in quantity, but it is not the most important flavor compound in quality (Siezer, Waugh, Edstam, & Ackermann, 1988). Acetaldhyde, citral, ethyl butyrate, limonene, linalool, octanal, are α-pinene are the major contributors to orange juice flavor (Ahmed, Dennison, & Shaw, 1978). Octanal and decanal are important flavor compounds in orange juice (Arctander, 1969). Moshonas & Shaw (1989) reported that up to the 40% of limonene lost in aseptically packaged commercial orange juice during storage. The degradation of limonene to α-terpineol and other compounds produces off-flavor (Tatum, Steven, & Roberte, 1975).

Orange juice is susceptible to degradation by heat, microorganisms, enzymes, oxygen, and light during processing and storage (Graumlich, Marcy, & Adams, 1986; Trammell, Dalsis, & Malone, 1986; Sadler, Parish, & Wicker, 1992). The shelf life of unpasteurized orange juice is only 12 days at 4.4°C (Fellers, 1988). Thermal processing is the most common method to inactivate microorganisms and enzymes in orange juice. Unfortunately, it also reduces nutritional and flavor qualities, and produces undesirable off-flavor compounds (Tatum, Steven, & Roberte, 1975; Ekasari, Jongen, & Pilnik, 1986; Nijssen, 1991). Citrus industry has been exploring innovative processing methods with minimal heat treatment to increase markets by improving nutritional and flavor qualities (Sadler, Parish, & Wicker, 1992). Pulsed electric field (PEF) processing, a non-thermal method, inactivates microorganisms without significant adverse effects on the flavor and nutrients (Sale & Hamilton, 1967; Dunn & Pearlman, 1987; Mertens & Knorr, 1992; Zhang, Qin, Barbosa-Canovas, & Swanson, 1995). The dielectrical breakdown of the cell membrane occurs when microbial cells are exposed to the high voltage PEF (Hamilton & Sale, 1967; Zimmermann, 1986; Tsong, 1991). However, no detailed information is

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available about the effect of PEF on the flavor compounds and microorganisms of orange juice.

The purpose of this paper was to study the effects of PEF process on the retention of flavor compounds and the inactivation of microorganisms of orange juice.

2. Materials and methods

2.1. Materials

Navel oranges (Sunkist, CA) were purchased from a grocery store (Kroger, Columbus, Ohio), and kept at 4.4°C before being processed. Ethyl butyrate, α-pinene, myrcene, octanal, limonene, linalool, decanal, ethanol, and valencene were purchased from Aldrich Chemical (Milwaukee, WI). A SPME fiber coated with 100 μm polydimethylsiloxane, 6 ml serum bottles, Teflon coated rubber septa and aluminum caps were purchased from Supelco (Bellefonte, PA). Orange serum agar for total plate counts and potato dextrose agar for total yeast and mold counts were purchased from Difco Laboratories (Detroit, MI).

2.2. Preparation of fresh squeezed orange juice

Fresh navel oranges were hand peeled and homogenized with a laboratory blender. The homogenized orange juice was filtered through cheesecloth to remove pulp. The homogenized and filtered fresh orange juice was designated as fresh squeezed orange juice.

2.3. PEF processing treatment

The schematic diagram of a continuous PEF apparatus which consists of a high voltage power supply, a high voltage pulse generator (Cober Electronics, Stamford, CT), a PEF treatment chamber, and a sample cooling and delivery system is shown in Fig. 1. The high voltage power supply with a maximum voltage of 15 kV was connected to a high voltage pulse generator which provides square wave pulses. A signal generator (Model 8082A, Hewlett Packard, Palo Alto, CA) controlled the frequency and duration of pulse. Signals of voltage, current, frequency and waveform were monitored with a TDS 320 two channel digital oscilloscope (Tektronix, Beaverton, OR). PEF treatment chambers for orange juice were designed to transfer high voltage pulses to high intensive pulsed electric fields. Four stainless steel co-field tubular treatment chambers were connected to one another in series to provide a PEF dosage (Yin, Zhang, & Sastry, 1997). The direction of electric field was parallel of that of orange juice flow. Each PEF treatment chamber provided a treatment zone with a 2.0 mm diameter and 2.0 mm electrode-gap. A digital thermometer (Fisher Scientific, Pittsburgh, PA) measured the inlet and outlet temperatures of chambers with two thermocouples. A cooling device that consisted of cooling

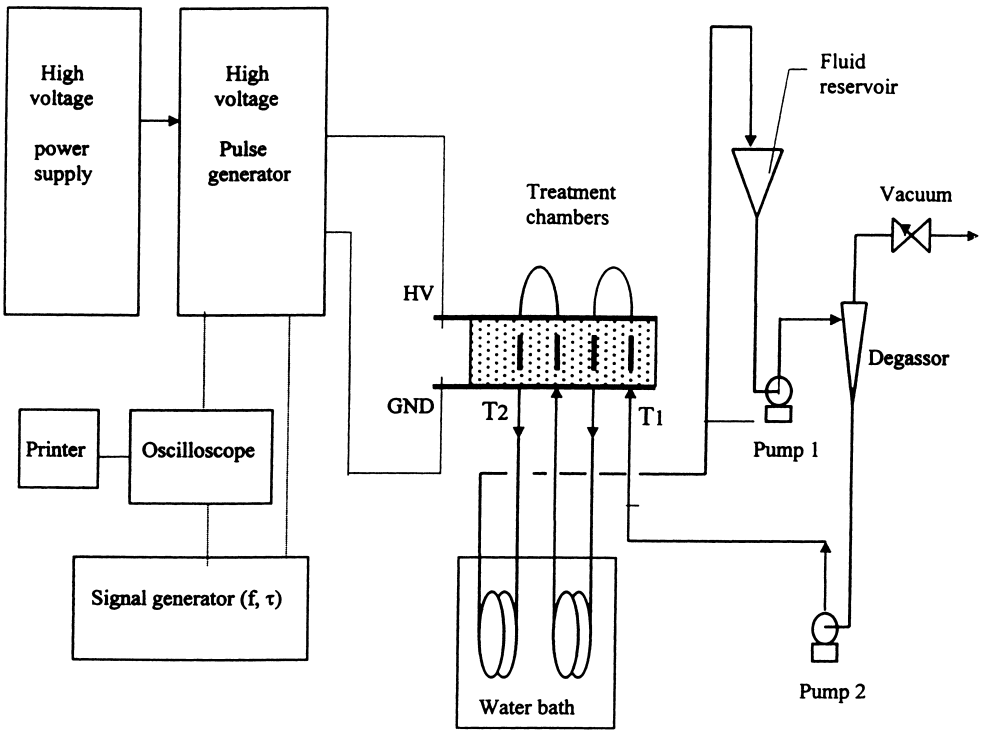

Fig. 1. Diagram of the pulsed electric field apparatus.

coils and a water bath controlled the temperature of orange juice. A micro gear pump (Cole-Parmer Instrument, Vernon Hills, IL) maintained a continuous orange juice flow. An auxiliary gear pump (Pump 1) was coupled with a vacuum pump and a degassing system to adjust the flow rate of orange juice, and to remove air bubbles in orange juice prior to entering the PEF treatment chambers. The PEF processing conditions for fresh squeezed orange juice were 30 kV/cm electrical field, 1 kHz frequency, 2 μs pulse duration, and square wave form. The 240 μs PEF treatment of orange juice was accomplished by circulating the juice at the flow rate of 2 ml/s through PEF treatment chambers for 10 times, and the 480 μs PEF treatment for 20 times. The number of circulation (N) was calculated using total running time (T), volumetric flow rate (F) and the volume of sample in circulation:

$$N = TF/V. \quad (1)$$

Care was taken to ensure good mixing in the fluid reservoir. The conical design of the fluid reservoir and degassor reduces the probability of dead volume. For easy calculation, the volume of sample (V) and volumetric flow rate (F) were selected such that the time to achieve one circulation was 1 min.

To study the effects of vacuum pump system of the PEF apparatus on the loss of flavor compounds, orange juice was circulated 10 times through the apparatus without pulsed electric field treatment. This processing was called as Circulation #1 which circulation control that had the same total running time as the 240 μs PEF treatment but without the PEF treatment. The Circulation #2 was a circulation control with 20 times of circulation.

The temperatures of orange juice and treatment chambers were 25°C and the vacuum for degassing was maintained at 25 mm Hg.

2.4. Heat processing of orange juice

Fresh squeezed orange juice was thermally processed in a tubular heat exchanger shown in Fig. 2. A gear pump was used to maintain the juice flow rate of 10 ml/s through a stainless steel heat exchange coil (4.6 mm i.d. ×3.8 m), which was immersed in a hot water bath. The juice was circulated and heated to 90 ± 2°C for 2 min, and held in the heating coil at 90°C for 1 min without circulation. The juice was immediately cooled in a 0°C ice-water bath as shown in Fig. 2.

2.5. Flavor compounds analysis by SPME and gas chromatography

The flavor compounds in the headspace of orange juice were analyzed by a combination of SPME (Buchholz & Pawliszyn, 1994; Field, Nickerson, James, & Heider, 1996; Ibanez & Bernhard, 1996; Jia et al., 1998)

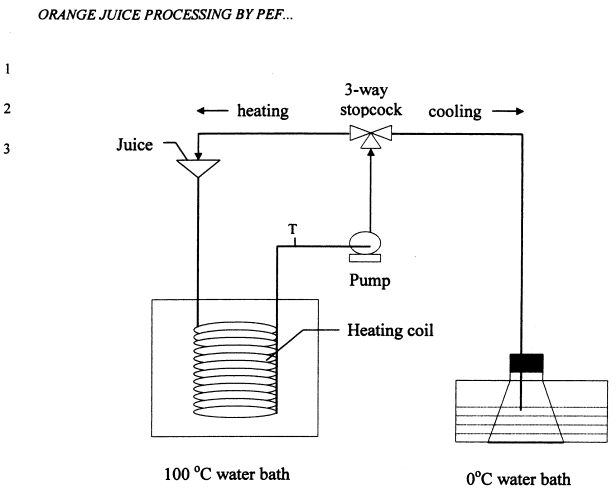

Fig. 2. Diagram of heat pasteurization of orange juice.

and gas chromatography. One ml orange juice was transferred into a 6 ml serum bottle having a magnetic stirring bar (3 × 10 mm). The sample bottle was sealed with a Teflon septum and aluminum cap. The SPME fiber coated with 100 μm polydimethylsiloxane was inserted into the headspace of orange juice sample bottle, which was magnetically stirred and heated at 60°C for 20 min to maintain the flavor compounds equilibrium between the headspace and the SPME coating. The SPME fiber was removed from the sample bottle and inserted into the 0.75 mm i. d. splitless glass liner of GC injection port, and held for 2 min at 220°C to desorb the flavor compounds adsorbed on the SPME coating. The desorbed flavor compounds were separated by a HP 5890 gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with a HP-5 capillary column of 0.53 mm internal diameter × 30 m coated with 2.65 μm of 5% phenyl substituted methylpolysiloxane and a flame ionization detector. Nitrogen gas flow rate was 2.5 ml/min. The GC oven temperature was programmed from 60°C to 120°C at 10°C/min and then to 200°C at 4°C /min, and held for 10 minutes at the final temperature.

The reproducibility of quantitative analysis of flavor compounds in orange juice by SPME-GC was evaluated by measuring the coefficients of variation for 6 replicate analyses of ethyl butyrate, α-pinene and limonene in the orange juice.

The presence of ethyl butyrate, α-pinene, myrcene, limonene, linalool, decanal and valencene which have been reported in the orange juice were confirmed by comparing the retention times of gas chromatographic peaks to those of authentic compounds.

2.6. Microbiological test

The total plate counts and total yeast and mold counts of control, 240, 480 μs PEF or heat processed

orange juice stored at 4.4°C were determined every week for 6 weeks. Pour-plating technique was used for total plate counts with orange serum agar and for the total yeast and mold counts with potato dextrose agar acidified to pH 3.5 with 10% tartaric acid. Orange serum agar plates were incubated at 35°C for 48 h and potato dextrose agar plates at 22°C for 5 days.

3. Results and discussion

3.1. Flavor compounds analysis by SPME-GC

A typical gas chromatogram of fresh squeezed orange juice flavor compounds, which were isolated by SPME and separated by GC, is shown in Fig. 3. Preliminary study showed that the 1 mm i.d. splitless glass liner of gas chromatograph injection port did not produce a good gas chromatogram. The decrease of injection port glass liner diameter from 1 to 0.75 mm improved the resolution of gas chromatography. The presence of ethanol (retention time of 2.575 min of Fig. 3), ethyl butyrate (6.212 min), α-pinene (9.131 min), mycerene (10.075 min), limonene (11.330 min), linalool (12.955), decanal (15.821 min) and valencene (25.145 min) were confirmed by the retention times of authentic compounds. These compounds have been reported as important flavor compounds in orange juice (Arctander, 1969; Ahmed, Dennison, & Shaw, 1978; Moshonas & Shaw, 1989). The coefficient of variations for ethyl butyrate, α-pinene and limonene were 4.36%, 3.00% and 1.63%, respectively. The low coefficients of variation for flavor compounds indicated that the reproducibility of flavor compounds analysis by SPME-GC is very good.

3.2. PEF and heat processing effects on flavor compounds of orange juice

The effects of 240 and 480 μs PEF, or heat process on the ethyl butyrate, α-pinene, octanal, limonene, and decanal in fresh squeezed orange juice are shown in Table 1. The losses of volatile compounds in orange juice by PEF and heat processes were greatly influenced by the types of compounds and processing methods. The losses of ethyl butyrate in orange juice by 480 μs PEF and heat processes were 9.7% and 22.4%, respectively. Decanal was not lost by PEF but, the 41% of decanal was lost by heat process. If decanal is a very

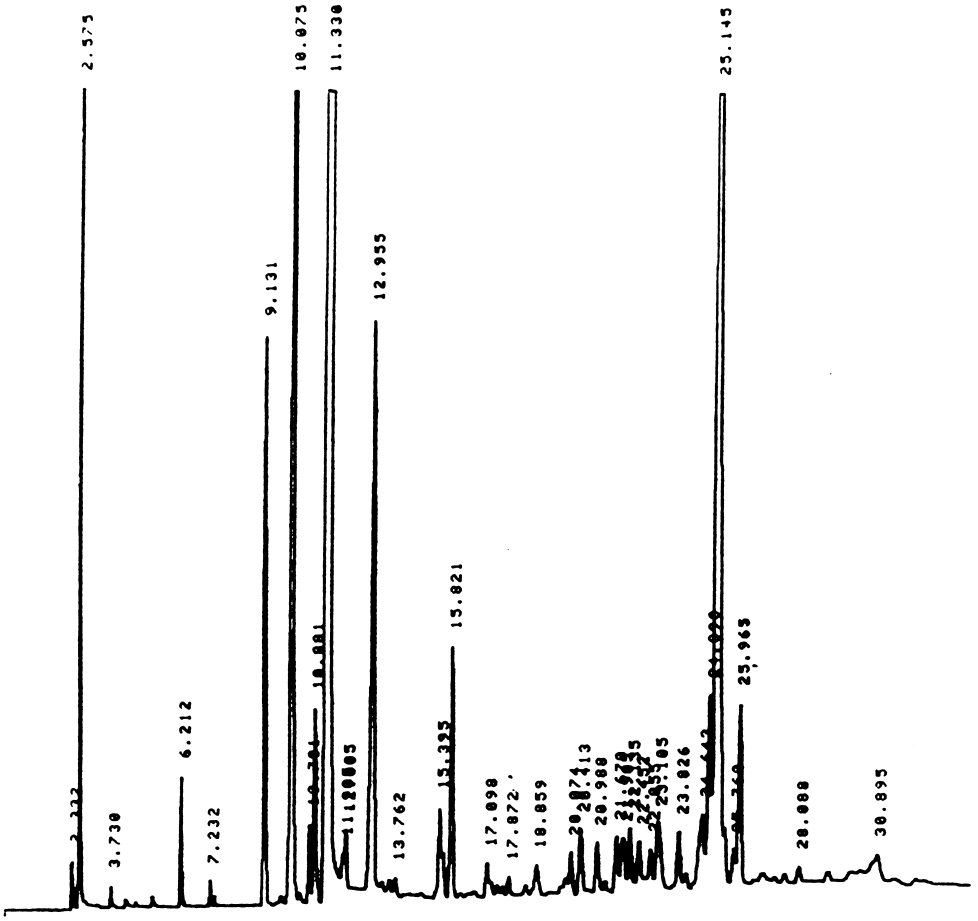

Fig. 3. Gas chromatogram of flavor compounds of fresh squeezed orange juice.

Table 1
The effects of 240, 480 μs PEF and heat process at 90°C for 1 min on the ethyl butyrate, α-pinene, ocatanl, limonene and decanal in fresh squeezed orange juice

Compound	Control	240 μs PEF	480 μs PEF	Heat process
Ethyl butyrate (loss %) [a]	0	5.1	9.7	22.4
α-pinene (loss %)	0	0.8	5.8	21.9
Octanal (loss %)	0	0.0	0.0	9.9
Limonene (loss %)	0	2.8	7.5	20.5
Decanal (loss %)	0	0.0	0.0	41.7

[a] The average of triplicate analyses.

importantcompound responsible for flavor quality in a beverage, the beverage may not have good flavor quality after heat processing.

3.3. Causes of orange juice flavor compounds loss by PEF

Some of volatile compounds in orange juice are more easily lost than other compounds by PEF process as shown in Table 1. Ethyl butyrate, which has the lowest molecular weight and boiling point, was lost to the greatest extent of the flavor compounds studied by PEF process. The boiling points of octanal and limonene were similar, but the losses of octanal and limonene by 480 μs PEF were 0% and 7.5%, respectively. Octanal and decanal are more polar compounds and more soluble in orange juice than α-pinene or limonene which are hydrocarbons. To identify the causes of volatile compound loss by PEF process, orange juice was processed by PEF system with and without 240 μs PEF or 480 μs PEF application at the treatment chambers in Fig. 1. The orange juices processed by PEF without 240 and 480 μs PEF application at the treatment chamber were designated as circulation # 1 and circulation # 2 in Table 2, respectively. The 240 and 480 μs PEF process require 10 and 20 min, respectively. The effects of 240, 480 μs PEF, circulation #1 and #2, and heat process on the amount of flavor compounds are shown in Table 2. The amount of flavor compounds was determined by measuring the gas chromatographic peak areas of all compounds and expressed in electronic counts. Table 2 shows that 240 μs PEF juice lost 3.17% and circulation #1 lost 2.46% of flavor compounds. PEF 480 μs PEF and circulation #2 lost 8.80% and 7.03%, respectively. The only difference between 240 μs PEF and circulation # 1 is that 240 μs PEF had 240 μs PEF application at the treatment chambers of Fig. 1 and circulation # 1 did not have 240 μs PEF application. The losses of flavor compounds due to 240 μs and 480 μs PEF application at treatment chambers are 0.71% and 1.77%, respectively. The losses of volatile compounds by circulation # 1 and # 2 are due to the degassing system of the PEF apparatus in Fig. 1. The vacuum pump at 25 mm Hg for the degassing system could remove the volatile compounds in orange juice. The 240 μs PEF and circulation #1 juice required 10 cycles through the degassing system and took 10 min. The α-pinene and limonene which are less soluble than octanal and decanal in orange juice will be more easily removed by the vacuum pump at the degassing system. Table 1 shows that octanal and decanal were not lost, but some of α-pinene and limonene were lost by PEF process. The most volatile compounds with small molecular weight will be removed to a greater extent by a vacuum system. Ethyl butyrate, which is the most volatile compound among the five compounds studied, was lost most by PEF process as shown in Table 1. The flavor loss of orange juice by the PEF process was primarily due to the vacuum degassing device than the PEF application at the treatment chamber in Fig. 1. The purpose of degassing system in the PEF apparatus was to remove small air bubbles in the orange juice. The air bubbles produced arcing in the PEF treatment chambers and a current overload between the electrodes.

Table 2
The effects of 240, 480 μs PEF, circulation # 1, circulation # 2, and heat process at 90°C for 1 min on the loss of total flavor compound of fresh squeezed orange juice

Sample	Total GC peak area(electronic counts)	Flavor loss (%)
Control	2.84×10^7	0.00
240 μs PEF [a] (10 min [b])	2.75×10^7	3.17
Circulation #1 [c] (10 min)	2.77×10^7	2.46
480 μs PEF (20 min)	2.59×10^7	8.80
Circulation #2 (20 min)	2.64×10^7	7.03
Heat process	2.21×10^7	22.18

[a] Actual PEF treatment time at the treatment chamber.
[b] Total processing time in the PEF apparatus.
[c] Circulation of orange juice through PEF apparatus without 240 μs PEF application at the treatment chamber.

The degassing system of PEF apparatus should be modified to minimize the flavor loss during processing. Heat process lost 22% of total flavor compounds, but 240 and 480 μs PEF lost 3% and 9%, respectively as shown in Table 2. The amount of flavor compounds in orange juice processed by PEF was higher than that of orange juice processed by heat. The loss of flavor compounds by PEF process could be further minimized by improving the degassing system in the PEF apparatus.

3.4. Processes effects on microorganisms

The effects of 240, 480 μs PEF and heat processes on the total plate counts and the total yeast and mold counts of orange juice during 6 weeks of storage at 4°C are shown in Tables 3 and 4, respectively. The temperature of orange juice at day 0, 7 and 14 were 25°C, 4°C and 4°C, respectively. The decrease of bacteria from day 0 to day 7 and 14 may be due to chilling injury of some mesophilic bacteria. The microbial specification allows less than 5000 bacteria/ml for a commercial single strength orange juice (Kimball, 1991). Table 3 also shows that the microbial shelf life of control fresh squeezed orange juice was 14 days at 4°C, which was consistent with the result of Fellers (1988). Table 3 indicates that heat process at 90°C for 1 min was more effective at the inactivation of bacteria than the PEF process. This may be partly due to the mixing of treated and untreated fluid in the circulation system. The PEF process had similar effect as the heat process for the inactivation of yeast and molds, which are the main microorganisms to cause spoilage of single strength orange juice at refrigerated temperature (Table 4). Tables 3 and 4 indicate that yeast and mold cells were less resistant to PEF process than bacteria cells. The result was consistent with the reports of Sale & Hamilton (1967) and Matsumoto, Satake, Shioji, & Sakuma (1991). The 240 μs PEF provided a similar effect on microbial reduction as the 480 μs PEF (Tables 3 and 4), which suggests that the extension of PEF processing period beyond a certain level may not increase the inactivation of microorganisms. The circulation of orange juice through the PEF treatment chamber to increase the PEF treatment may cause the recontamination of microorganisms in the orange juice. Therefore, single PEF process at the treatment chamber should be considered in the development of new PEF processing apparatus.

4. Conclusions

PEF processed orange juice retained more flavor compounds than the heat pasteurized orange juice. The losses of volatile flavor compounds in orange juice samples by 240 μs PEF and 480 μs PEF, and heat process were 3%, 9% and 22%, respectively. The flavor loss by PEF process was mainly due to PEF vacuum degassing system which removed flavor compounds from orange juice instead of 240 or 480 μs PEF application at the treatment chamber. A re-circulating PEF process was effective in reducing the total plate counts of orange juice, but was not as effective as the heat process at 90°C for 1 min. The effectiveness of PEF process for the inactivation of yeast and molds in orange juice was comparable to the heat process of 90°C for 1 min.

Table 3
The effects of 240, 480 μs PEF and heat process at 90°C for 1 min on the total plate counts (CFU/ml) of fresh squeezed orange juice

Storage day	Control	240 μs PEF	480 μs PEF	Heat process
0	5,400	21	19 (est.)	4 (est.)
7	3,200	16 (est.)	10 (est.)	< 1 (est.)
14	3,800	12 (est.)	8 (est.)	< 1 (est.)
21	18,000	24	26	< 1 (est.)
28	56,000	19 (est.)	25	< 1 (est.)
35	89,000	77	58	< 1 (est.)
42	530,000	270	130	< 1 (est.)

Table 4
The effects of 240, 480 μs PEF and heat process at 90°C for 1 min on the total yeast and mold counts (CFU/ml) of fresh squeezed orange juice

Storge day	Control	240 μs PEF	480 μs PEF	Heat process
0	2,800	15 (est.)	9 (est.)	4 (est.)
7	1,700	< 1 (est.)	< 1 (est.)	< 1 (est.)
14	2,400	< 1 (est.)	< 1 (est.)	< 1 (est.)
21	12,000	< 1 (est.)	< 1 (est.)	< 1 (est.)
28	37,000	< 1 (est.)	< 1 (est.)	< 1 (est.)
35	94,000	< 1 (est.)	< 1 (est.)	< 1 (est.)
42	560,000	< 1 (est.)	< 1 (est.)	< 1 (est.)

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