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Effects of Pulsed Electric Field and Thermal Treatments on Microbial Reduction, Volatile Composition, and Sensory Properties of Orange Juice, and Their Characterization by a Principal Component Analysis

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Abstract: The effects of pulsed electric field (PEF) treatments on microbial reduction, volatile composition, and sensory characteristics of orange juice were investigated. Mild (Thermal-1) and intensive (Thermal-2) thermal treatments were applied for comparison. A pilot-scale PEF system, with a flow rate of 30 L/h and maximum field strength of 20 kV/cm, was used. PEF treatment at a specific energy of 150 kJ/L resulted in 9.0 and 8.0 decimal reductions of *Escherichia coli* and *Saccharomyces cerevisiae*, respectively. The PEF treatments preserved the characteristic compounds associated with a fresh flavor (e.g., dl-limonene, β -myrcene, α -pinene, and valencene) more effectively than an intensive thermal treatment. This was verified by descriptive analysis of sensory evaluations. Based on the principal component analyses (PCAs) and partial least-squares (PLS) regression analyses, PEF-treated orange juice showed higher similarity to untreated orange juice. Our results indicate that PEF may be an alternative processing technique that can preserve the fresh flavor and taste of freshly squeezed orange juice.

Keywords: orange juice; pulsed electric field; sensory attribute; volatile



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1. Introduction

Oranges contain vitamin C, folic acid, potassium, and pectin, as well as high levels of phytochemicals that facilitate antioxidant activities [1]. Recently, the market for premium-quality orange juice has significantly grown. Such juice is freshly squeezed and minimally processed and is popular among consumers because of its excellent flavor and high nutritional value [2].

Thermal treatment is used primarily in the food industry to increase shelf life and maintain food safety at low cost [3]. However, the treatment not only inactivates microorganisms and enzymes but also changes the quality and freshness. Therefore, alternative technologies to traditional thermal treatment have been investigated to inactivate microorganisms at temperatures lower than those commonly used in heat treatments [4]. Non-thermal treatments inactivate microorganisms without extensive quality changes of foods. Typical examples of these non-thermal techniques are high hydrostatic pressure, pulsed electric field, intense pulsed light, irradiation, and ultrasound [5,6]. Among non-thermal techniques, the use of pulsed electric field is one of the most attractive because of its short processing time compared to other technologies and it is more energy efficient [7]. The PEF method is a non-thermal technique involving the discharge of high-voltage electrical short pulses through food items [8]. Many studies have assessed the effects of PEF treatment on microorganisms and fruit juice quality, such as orange juice [9–11], apple juice [12,13], and blueberry juice [14]. However, according to our investigation, there were no other studies that found the correlation with volatile compounds data and sensory

attribute of pulsed electric field (PEF) treated orange juice through principal component analyses (PCAs) and partial least-squares (PLS) regression analyses. The objective of this study was to characterize the PEF- and heat-treated orange juice in terms of volatile composition and sensorial properties.

2. Materials and Methods

2.1. Materials

Escherichia coli (American type culture collection (ATCC) 11775) and *Saccharomyces cerevisiae* (ATCC 4105) were purchased from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). Nutrient agar, yeast malt agar, and tryptic soy broth were purchased from Difco Laboratories (Detroit, MI, USA). L-ascorbic acid and sulfuric acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Metaphosphoric acid and methanol were obtained from Junsei Co. (Tokyo, Japan).

2.2. Preparation of Freshly Squeezed Orange Juice

Oranges (*Citrus sinensis* L.) of the Valencia variety (USA) were purchased in a local supermarket (NH, Anseong, Korea). After washing the fruits with tap water, orange juice was extracted using a Breville squeezer (800CP, Torrance, CA, USA), with a low-speed rotary motor. The extracted orange juice was filtered through a 0.23 mm stainless steel filter to remove the pulp. Sterilized orange juice (121 °C, 20 min) was used for microbial challenge tests to evaluate microbial inactivation by PEF treatments. Freshly prepared orange juice was used in the analysis of volatile composition and sensorial properties after PEF and thermal treatments.

2.3. Bacterial Strains and Preparation of Inoculum

E. coli was grown on nutrient agar at 36 °C for 3 days. Then, a single *E. coli* colony was transferred to 10 mL tryptic soy broth and grown in a shaking incubator at 36 °C for 24 h. A secondary culture of *E. coli* was obtained by transferring 1 mL primary culture into 100 mL fresh tryptic soy broth. The secondary *E. coli* culture was grown at 36 °C for 24 h. *S. cerevisiae* was grown on potato dextrose agar at 32 °C for 3 days. Then, a single colony of *S. cerevisiae* was transferred to 10 mL YM broth and grown in a shaking incubator at 32 °C for 24 h. A secondary culture of *S. cerevisiae* was obtained by transferring 1 mL primary culture into 100 mL fresh YM broth. The secondary *S. cerevisiae* culture was grown at 32 °C for 24 h. Each 100 mL culture of the microorganisms was inoculated into 20 L of autoclaved orange juice before the PEF treatment.

2.4. Pulsed Electric Field Treatment

For the microbial test, autoclaved orange juice inoculated with each microorganism was exposed to PEF treatments. For the other tests, freshly prepared orange juice was exposed to PEF. The orange juice was stored in a service tank and transferred by a peristaltic pump (323 Du, Watson Marlow, Wilmington, MA, USA) at a flow rate of 30 L/h. The inlet temperature of the orange juice was adjusted to 35 °C by flow-through stainless-steel coils immersed in a water bath. PEF treatment was performed using a 5 kW pulse generator (HVP-5, DIL, Quakenbrück, Germany) equipped with a continuous treatment chamber, and the shape of the pulse is bipolar square type. The treatment chamber had an inner diameter of 1.0 cm, with a gap distance of 1.0 cm between the electrodes. The PEF treatment was applied as a continuous flow using bipolar pulses of 25 µs, and we conducted PEF treatment at 10, 15, and 20 kV/cm with three different energy inputs (50, 100, 150 kJ/L). It means we had 9 different PEF conditions, as shown in Figure 1. From the result of Figure 1, we selected the electric field strength of 20 kV/cm for further experiments, since the field strength of 20 kV/cm resulted in the most effective microbial inactivation at the same specific energy inputs. Two different levels of specific energy input, 100 kJ/L (PEF-1) and 150 kJ/L (PEF-2), were applied for the volatile composition and sensory properties.

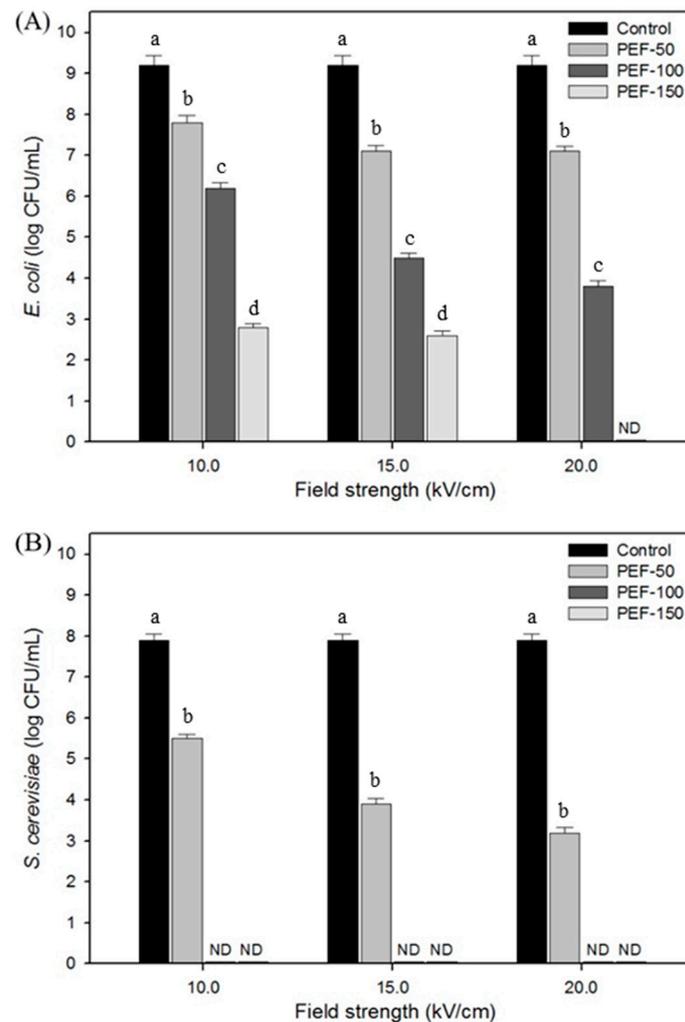


Figure 1. The inactivation of (A) *Escherichia coli* and (B) *Saccharomyces cerevisiae* inoculated in sterilized orange juice by pulsed electric field (PEF) treatments. PEF treatments were conducted at a field strength of 10, 15, and 20 kV/cm with different pulse frequencies, which resulted in PEF total specific energy inputs of 50 kJ/L (PEF-50), 100 kJ/L (PEF-100), and 150 kJ/L (PEF-150). ND, not detected.

2.5. Thermal Treatment

To compare the effects of the PEF and heat treatments, two thermal treatments were performed. Thermal-1 was heat-treated at 95 °C for 30 s, and we have chosen this processing condition as a typical pasteurization condition for orange juice from the cited References [15,16]. Sterilization at 121 °C for 20 min (Thermal-2) is commonly used for autoclave sterilization and was used for representing excessive heat treatment in this study.

2.6. Analysis of Volatile Compounds by Headspace Solid Phase Microextraction (HS-SPME) and Gas Chromatography–Mass Spectrometry (GC–MS)

The volatile compounds in the headspace of orange juice samples were analyzed via HS-SPME GC/MS [17,18] following the analytical method of Jia, Zhang, and Min [19]. A total of 1 mL orange juice was transferred to a 6 mL vial that contained 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 (Supelco, St. Louis, MO, USA) was inserted into the headspace of a sample bottle, which was magnetically stirred and heated at 60 °C for 20 min to attain an equilibrium of volatile compounds between the headspace and the SPME coating. The SPME fiber was removed from the bottle and inserted into the 0.75 mm inner diameter splitless glass liner of the GC injection port and held for 2 min at 220 °C to desorb the volatile compounds adsorbed on the SPME

coating. The desorbed volatile compounds were separated using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5 30 m capillary column (0.53 mm internal diameter) coated with 2.65 μm 5% phenyl substituted methylpolysiloxane (Agilent Technologies, Palo Alto, CA, USA) and a 5973 MSD (Agilent Technologies, Palo Alto, CA, USA). The nitrogen gas flow rate was 2.5 mL/min. The GC oven temperature was programmed from 60 to 120 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$, and then to 200 $^{\circ}\text{C}$ at 4 $^{\circ}\text{C}/\text{min}$ and held for 10 min at the final temperature. Each peak was identified based on the mass fragmentation pattern, which was compared to spectral data in the NIST and WILEY library. Odor descriptions of the identified flavor compounds were obtained from the commercially available Leffingwell and Associates Flavor-Base and Acree's Flavornet [20].

2.7. Sensory Evaluation of Orange Juice by Descriptive Analysis

The sensory properties of the orange juice samples were evaluated by the descriptive analysis method [21]. For the sensory evaluation, the refrigerated orange juices were served in white cups with three-digit random numbers. Also, non-salted table biscuits and water to clean their mouths were served. The evaluation was repeated three times for each panelist ($n = 3 \times 7$). The samples were presented monadically to the judges at room temperature under white fluorescent lighting. Seven panelists from the graduate students of Chung-Ang University were selected as the panelists. All of them had the experience of descriptive analysis. The selected panelists were trained to recognize the characteristics of weak (1) and strong (9) aromas of fresh and cooked orange, using two standard products. The panelists were also trained to determine taste characteristics such as sour, sweet, bitter, and vegetable juice tastes. The standard reference for sour taste was citric acid solution (0.01~0.3%), the standard reference for sweet taste was sucrose solution (1.0~5.0%), and the standard reference for bitter taste was caffeine solution (0.02~0.3%). The seven trained panelists evaluated the samples using previously identified standard references for each attribute (Table 1). The panelists were asked to rate the intensity of each attribute on a 9 cm unstructured line scale, with a mark on the left indicating "weak" and a mark on the right indicating "strong." Values describing the intensity of each descriptor were calculated using the length (cm) of the marked line from the left side of the anchor.

Table 1. Attributes used for the sensory evaluation of orange juice by descriptive analysis.

Attributes	Definition	Standard Reference
Aroma		
Fresh orange	Unique smell of fresh orange juice	Weak: Distilled water Strong: Fresh squeezed orange juice
Cooked orange	Characteristic aromatics associated with cooked orange	Weak: Fresh squeezed orange juice Strong: Cooked (12 min) orange juice
Taste		
Sour	Fundamental taste of citric acid	Weak: 0.01% (w/v) Citric acid solution Strong: 0.3% (w/v) Citric acid solution
Sweet	Fundamental taste of which sucrose is typical	Weak: 1% (w/v) Sucrose solution Strong: 5% (w/v) Sucrose solution
Bitter	Fundamental taste of which caffeine is typical	Weak: 0.02% (w/v) Caffeine solution Strong: 0.3% (w/v) Caffeine solution
Vegetable	Taste of mixed vegetables	Weak: 5% (v/v) Vegetable juice Strong: Vegetable juice (Sun-Up, Maeil, Korea)

2.8. Statistical Analysis of Data

The data are presented as the mean of three measurements \pm the standard deviation. All experiments were performed in triplicate. Data were analyzed using analysis of variance (ANOVA) and Duncan's multiple range comparison test followed by principal component analyses (PCAs) to create product spaces [22,23] using SPSS ver. 20.0 software (SPSS, Inc., Chicago, IL, USA) and XLSTAT (XLSTAT, ver. 2017.03, Microsoft Excel Add-in software, New York, NY, USA). Significance was set at $p < 0.05$. Partial least-squares (PLS) regression analyses were performed using XLSTAT to correlate and predict the sensory variables from the instrumental measurement of orange juice samples after the thermal or PEF treatments.

3. Results

3.1. Inactivation of Inoculated *E. coli* and *S. cerevisiae* by Continuous PEF Treatment

The PEF inactivation of *E. coli* and *S. cerevisiae* inoculated into sterilized orange juice is shown in Figure 1. We selected *E. coli* and *S. cerevisiae* as test microorganisms for PEF inactivation. *E. coli* (ATCC 11775) was selected since it is a typical commensal microorganism and hygienic indicator which can be contaminated during food preparation [24]. *S. cerevisiae* (ATCC 4105) was selected because it is the most common spoilage microorganism in refrigerated citrus juice [2].

The number of *E. coli* was reduced as the specific energy input was increased. When the electrical field strength was 10 kV/cm, the *E. coli* was reduced by 1.4, 3.0, and 6.4 decimal reductions with a specific energy input of 50, 100, and 150 kJ/L, respectively (Figure 1A). With an electric field strength of 15 kV/cm, slightly more inactivation of *E. coli* was observed after the PEF treatments. *Escherichia coli* was most rapidly inactivated with a field strength of 20 kV/cm, and its complete inactivation was observed in a PEF treatment of 20 kV/cm, with a specific energy input of 150 kJ/L. *Saccharomyces cerevisiae* was more susceptible to PEF treatment than *E. coli* (Figure 1B). Even at an electric field strength of 10 kV/cm, *S. cerevisiae* was completely inactivated with specific energy inputs of 100 and 150 kJ/L. Its inactivation by 15 and 20 kV/cm fields exhibited characteristics similar to those of the 10 kV/cm inactivation. Inactivation was limited with a specific energy input of 50 kJ/L but increased rapidly at higher PEF energies.

Microbial inactivation was dependent on both electric field strength and specific energy input. At the same specific energy input, a higher electric field strength resulted in more microbial inactivation. These results are consistent with previously published studies. Evrendilek et al. [25] reported *E. coli* O157:H7 by 1.0, 1.7, 2.5, 3.5, and 4.5 decimal reduction in apple juice treated with a PEF at electric field strengths of 22, 25, 28, 31, and 34 kV/cm, respectively. In addition, Zhao et al. [26] reported that *E. coli* inoculated with green tea inhibited 2.2, 3.3, and 5.6 decimal reductions respectively, when treated with PEF at electric field strengths of 18.1, 27.4, and 38.4 kV/cm. Also, Lee, Han, Choi, Kang, Baick, and Lee [27] reported *E. coli* by a 4.5 decimal reduction and *S. cerevisiae* by a 6.0 decimal reduction in low-fat milk treated with a total pulse energy of 200 kJ/L. These results indicate that the higher the electric field strength, the greater the potential difference between the inside and outside of the cell membrane of the microorganism, and the cell membrane reaches the critical destruction point more quickly [28,29].

Therefore, we selected the electric field strength of 20 kV/cm for further experiments. The field strength of 20 kV/cm resulted in the most effective microbial inactivation at the same specific energy input within our experimental conditions. Two different levels of specific energy input, 100 kJ/L (PEF-1) and 150 kJ/L (PEF-2), were applied in the PEF treatments.

3.2. Effect of PEF and Thermal Treatments on the Volatile Composition of Squeezed Orange Juice

Freshly squeezed orange juice was prepared to evaluate the effects of PEF and thermal treatment on the volatile composition of orange juice. The volatile compounds in untreated (control), PEF-treated, and heat-treated orange juice are shown in Table 2. The major volatile components of untreated orange juice were dl-limonene (86.9%) followed by β -myrcene

(4.7%), valencene (2.5%), and α -pinene (0.6%). This combination of volatile components represented the unique aroma of orange juice. Dl-limonene is a monoterpene hydrocarbon, which contributes to the refreshing aroma of sweet oranges. The area% of dl-limonene in PEF-treated juice was similar to that in untreated juice, but it was slightly reduced in heat-treated juice, particularly sterilized orange juice (Thermal-2). Valencene is a major volatile component of Valencia orange and can be biosynthesized from farnesyl pyrophosphate (FPP) by terpene cyclase enzyme [30]. The area% of valencene tended to be slightly higher after both the PEF and heat treatment.

Table 2. Effect of PEF and thermal treatments on the volatile compounds of squeezed orange juice.

No.	Retention Time (min)	Volatile Compounds	Odor Descriptor ¹	Area (%)				
				Untreated	Thermal-1	Thermal-2	PEF-1	PEF-2
1	2.129	Ethyl butyrate	Sweet, Fruity	0.14	0.07	0.05	0.12	0.10
2	3.374	α -Pinene	Woody, Pine, Terpenic, Herbal	0.64	0.56	0.27	0.57	0.65
3	3.984	β -Myrcene	Green, Metallic, Balsam	4.68	4.28	2.97	3.65	4.09
4	4.195	α -Phellandrene	Turpentine, Mint, Spice	0.10	0.14	0.26	0.08	0.09
5	4.528	dl-Limonene	Lemon, Citrus-like,	86.85	85.99	80.60	87.47	86.17
6	4.895	γ -Terpinene	Gasoline, Turpentine	0.26	0.32	0.35	0.28	0.21
7	5.290	Terpinolene	Unpleasant, Citrusy, Chemical	0.20	0.29	1.79	0.14	0.17
8	6.049	β -Terpineol	Woody, Pungent, Earthy	n.d.	n.d.	0.63	n.d.	n.d.
9	6.736	β -Fenchyl alcohol	Camphor	n.d.	n.d.	4.11	n.d.	n.d.
10	6.897	Decanal	Green, Soapy	0.12	0.42	0.31	0.06	0.08
11	6.966	2-octyl acetate	Earthy, Herbal, Humus, Dirty	0.06	0.06	n.d.	n.d.	n.d.
12	10.406	β -Cubebene	Citrus, Fruity	0.22	0.14	n.d.	0.22	0.25
13	10.698	Tetradecanal	Fatty, Waxy, Citrus peel, Musk	n.d.	0.13	n.d.	n.d.	n.d.
14	12.731	Valencene	Sweet, Fresh citrus, Orange	2.48	3.64	4.53	3.97	4.14

¹ Odor descriptor refers to Flavor-Base (Leffingwell and Associates 10th Edition).

Decanal is the major aldehyde in oranges [31], and levels in excess of 0.72 ppm have a negative impact on the flavor of orange juice [32]. The area% of decanal was lower in PEF-treated orange juice (0.06–0.08%) than in untreated juice (0.12%) and was higher after the heat treatment (0.31–0.42%). Sterilized orange juice also showed an increase in the area% of additional unfavorable volatile compounds, such as terpinolene, α -phellandrene, β -fenchyl alcohol, and β -terpineol, resulting in spicy, chemical, camphor, and woody flavors. Jia, Zhang, and Min [19] reported that heat treatment resulted in irreversible undesirable changes in the flavor of citrus juice due to accelerated chemical reactions during the heating process.

3.3. Effect of PEF and Thermal Treatments on the Sensorial Properties of Squeezed Orange Juice

The sensorial properties of untreated, PEF-treated, and heat-treated freshly squeezed orange juice were determined by descriptive analysis (Table 3). Thermal-2 orange juice had significantly lower ‘fresh orange’ attribute scores than other samples ($p < 0.05$). However, there were no significant differences between untreated, Thermal-1, PEF-1, and PEF-2 orange juice samples ($p > 0.05$). ‘Cooked orange’ attribute scores were significantly higher ($p < 0.05$) for Thermal-2 orange juice than the other samples. Therefore, the PEF-treated samples had a stronger fresh orange flavor than the Thermal-2 juice. This indicates that the stronger heat treatment of Thermal-2 produced a ‘cooked orange’ flavor. This result was consistent with the results of the volatile analyses, which showed higher levels of

unfavorable compounds (e.g., decanal) in Thermal-2 orange juice and higher levels of fresh orange juice flavor compounds (e.g., dl-limonene) in PEF-treated orange juice.

Table 3. Effect of PEF and thermal treatments on sensorial properties of squeezed orange juice

Sensory Attributes	Untreated	Thermal-1	Thermal-2	PEF-1	PEF-2
Aroma					
Fresh orange aroma	6.1 ± 1.9 ^a	5.7 ± 1.4 ^a	3.4 ± 2.2 ^b	6.8 ± 1.2 ^a	5.4 ± 1.3 ^a
Cooked orange aroma	4.4 ± 1.6 ^b	3.5 ± 0.8 ^b	7.0 ± 1.2 ^a	3.7 ± 1.2 ^b	3.6 ± 1.1 ^b
Taste					
Sour	5.5 ± 2.0 ^{ab}	5.5 ± 1.2 ^{ab}	4.1 ± 2.0 ^b	6.9 ± 1.3 ^a	5.9 ± 1.0 ^a
Sweet	4.8 ± 2.0 ^a	5.4 ± 1.4 ^a	4.3 ± 1.8 ^a	4.8 ± 1.7 ^a	5.6 ± 2.0 ^a
Bitter	4.2 ± 1.8 ^b	4.1 ± 1.4 ^b	6.5 ± 1.3 ^a	4.3 ± 1.3 ^b	4.3 ± 1.6 ^b
Vegetable	4.6 ± 2.2 ^a	4.9 ± 1.9 ^a	5.9 ± 2.0 ^a	4.6 ± 1.8 ^a	4.5 ± 1.8 ^a

Means with different superscripts in the same row significantly differ at $p < 0.05$.

There were no significant differences in the 'sour' attribute ($p > 0.05$) among untreated, Thermal-1, and PEF-treated samples. However, the sour taste scores were significantly lower for Thermal-2 juice than for others ($p < 0.05$). There were no statistically significant differences between the 'sweet' attribute scores among all juices ($p > 0.05$). The 'bitter' attribute was significantly stronger in Thermal-2 juice than in other samples ($p < 0.05$). The 'vegetable' attribute scores were also higher in Thermal-2 than in the other samples; however, there were no significant differences ($p > 0.05$). Therefore, it is clear that strong heat treatment (i.e., Thermal-2) results in significant changes in the sensorial properties of fresh orange juice, while the Thermal-1 and PEF treatments retain the fresh characteristics of squeezed orange juice.

3.4. PCA and PLS Regression Analyses

PCA was performed on volatile and sensory data from control, heat-treated, and PEF-treated orange juices (Figure 2). The first two principal components (PCs) accounted for 89.02% of the total variance. In the score plot, the Thermal-2 orange juice sample was located on the left side of PC1, whereas the untreated and PEF-treated orange juice samples were located on the right side of PC1. Thermal-2 orange juice was strongly correlated with β -myrcene (turpentine, mint, spice), terpinolene (unpleasant, citrusy), β -terpineol (woody, pungent, earthy), and β -fenchyl alcohol (camphor odor). However, untreated, PEF-treated, and Thermal-1-treated juice samples were positively correlated with ethyl butyrate (sweet, fruity), α -pinene (α -pinene), dl-limonene (lemon, citrus-like), and β -cubebene (unpleasant, citrusy, chemical). This is consistent with the volatile composition and explains why the Thermal-2-treated orange juice differed greatly from untreated juice.

All significant sensory attributes ($p < 0.05$) were included to produce the PCA plots shown in Figure 2B. The first two PCs explained 97.56% of the total variance. On the score plot, only Thermal-2-treated orange juice was located on the right side of PC1, and was correlated with bitter and cooked sensory attributes. However, untreated, PEF-treated, and Thermal-1-treated orange juice were located on the left side of PC1 and were correlated with sour and fresh sensory attributes.

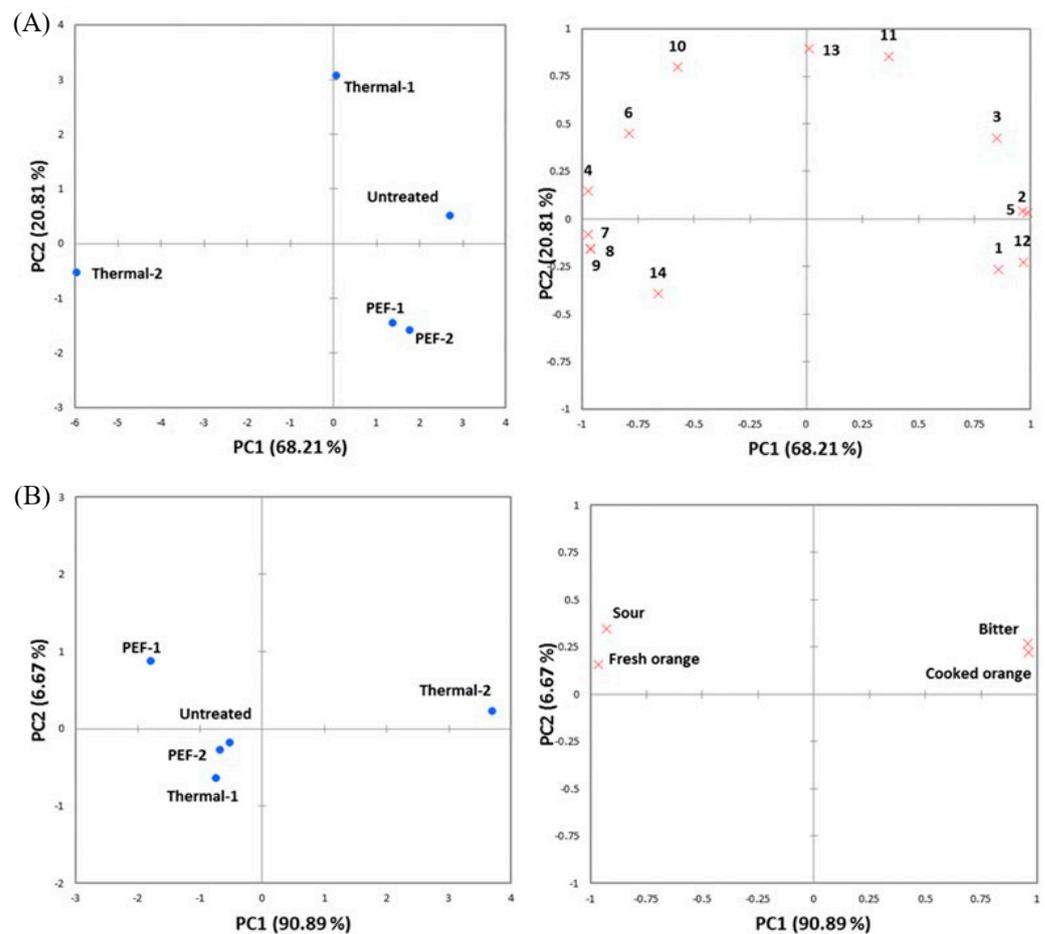


Figure 2. Principal component analyses (PCAs) of pulsed electric field (PEFs) and thermally treated orange juice. (A) PCA score plot with sample labeling and PCA loading plot with volatile compound codes. The compound codes are explained in Table 2. (B) PCA score plot with sample labeling and PCA loading plot with sensory attributes.

PLS regression analyses were performed on the sensory attribute and volatile data (Figure 3). Latent vectors 1 and 2 (LV1 and LV2) explained 87.6% of the variation. Along LV1, there was a clear separation among the samples due to the treatments. The PEF-treated and weakly heated (i.e., Thermal-1) samples were more similar to the untreated sample on LV1 than the Thermal-2 orange juice. Untreated and PEF-treated samples were positively correlated with sour and fresh sensory attributes, which were associated with α -pinene (woody, pine, terpenic, herbal odor), dl-limonene (lemon, citrus-like, odor), β -cubebene (citrus, fruity odor), ethyl butyrate (sweet, fruity odor), and β -myrcene (green, metallic, balsam odor). However, the Thermal-2-treated orange juice sample was on the left side of the LVs and was correlated with bitter and cooked attributes, which were associated with the volatile compounds of α -phellandrene (turpentine, mint, spice odor), γ -terpinene (gasoline, turpentine odor), terpinolene (unpleasant, citrusy, chemical odor), β -terpineol (woody, pungent, earthy odor), and β -fenchyl alcohol (camphor odor).

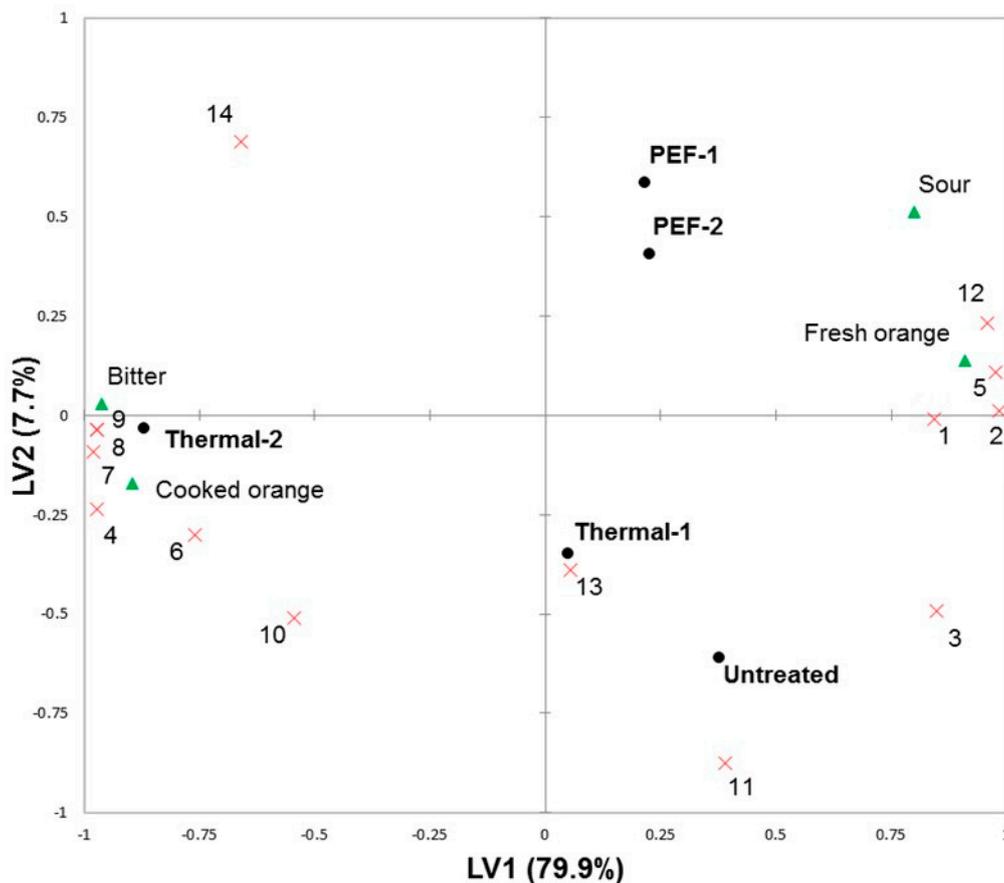


Figure 3. Partial least squares (PLS) regression map showing the relationship among the instrumental data correlated with sensory attribute data in all orange juice samples. The sensory parameters are explained in Table 1 and the compound codes are given in Table 2.

4. Conclusions

PEF could efficiently inactivate *E. coli* and *S. cerevisiae*, with energy inputs of 100 and 150 kJ/L at a 20 kV/cm field strength. The *E. coli* was reduced by a 5.6 decimal reduction at an energy of 100 kJ/L and *S. cerevisiae* was completely inactivated. No microorganisms were observed in the model solution at an energy input of 150 kJ/L. Compared to the thermal treatment, the PEF treatment better preserved the compounds responsible for the characteristic fresh orange juice aroma, such as dl-limonene, β -myrcene, α -pinene, and valencene. The area% of unfavorable volatile compounds, such as decanal, was higher in the thermally treated orange juice than in PEF-treated orange juice. The sensory evaluation revealed that the PEF-treated orange juice had a stronger fresh orange aroma and a weaker unfavorable cooked orange aroma than the autoclaved orange juice. However, there were no significant differences in orange aroma between PEF-treated and pasteurized orange juices. Based on PCA and PLS regression analyses, both PEF-1 (20 kV/cm, 100 kJ/L) and PEF-2 (20 kV/cm, 150 kJ/L) treated samples were clustered together with the untreated or thermally pasteurized samples, whereas sterilized juice was negatively correlated with the untreated sample. The PEF-treated juice was strongly positively correlated with the sour and fresh sensory attributes and volatiles related to fresh orange juice aroma. Therefore, PEF may be an alternative to thermal treatment as a pasteurization technique that can preserve quality characteristics, such as the fresh flavor and taste of freshly squeezed orange juice.

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