Effects of Pulsed Electric Field Processing and Storage on the Quality and Stability of Single-Strength Orange Juice

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ABSTRACT

The effects of pulsed electric field (PEF) processing on microorganisms in orange juice and on the flavor and color of the juice during storage for 112 days at 4 and 22°C were investigated. Single-strength orange juice was PEF processed at an electric field strength of 35 kV/cm for 59 ms and placed into sterilized glass bottles in a sanitary glove box. PEF-processed orange juice was microbiologically stable at 4 and 22°C for 112 days. PEF processing resulted in significant increases in the hydrocarbons D-limonene, α-pinene, myrecene, and valencene (P < 0.05) but did not have any effect on octanal, decanal, ethyl butyrate, and linalool. The levels of hydrocarbon compounds did not change at 4 and 22°C in 112 days. Octanal, decanal, ethyl butyrate, and linalool levels significantly decreased in 14 days at 4°C and in 2 days at 22°C. The decrease in these compounds did not have a significant effect on the sensory quality of the orange juice (P > 0.05). The microorganisms in PEF-processed orange juice, along with the flavor and color of the juice, remained stable at 4°C for 112 days.

Orange juice is the most popular juice in the United States, comprising 60% of the total revenue for the juice market (4, 7, 9, 22). Orange juice is popular not only because of its high vitamin C content but also because of its unique and delicate citrus flavor and balanced taste. However, the delicate fresh flavor of orange juice can easily be changed during heat processing or storage (7, 16, 19, 23). Under these conditions, the juice undergoes compositional changes that invariably cause the alteration of its original flavor and aroma (12).

The pulsed electric field (PEF) technique, a novel non-thermal processing technique, is an alternative process with which to inactivate microorganisms in foods without the significant adverse effects on the flavor, taste, and nutrients of the food that result from conventional thermal processing (3, 5, 10, 14, 17, 26, 27). Thermal processing is the technique most commonly used for the inactivation of microorganisms and enzymes in orange juice. However, it also reduces nutritional and flavor quality and produces undesirable off-flavor compounds (6, 11, 21). Because PEF processing involves a very short discharge period (milliseconds) and minimizes the heating of foods, it has the potential to preserve the freshlike qualities of foods.

Jia et al. (8) reported that the average losses of flavor compounds for orange juice processed by PEF at 30 kV/cm for 240 480 μs and by heat at 90°C for 1 min were 3, 9, and 22%, respectively. However, these investigators mentioned that the flavor loss for the PEF process was due to vacuum degassing in a laboratory-scale PEF unit. PEF processing caused less protein denaturation and less vitamin C loss in protein-fortified orange juice than did heat processing (18). Qiu et al. (15) reported the processing effects of a pilot-plant-scale PEF system integrated with aseptic packaging on the retention of vitamin C in orange juice. However, there have been no reports on the effects of a pilot-plant-scale PEF process on the flavor quality and stability of orange juice during storage. There is a need to study the effects of a pilot-plant- or near-commercial-scale PEF processing system on orange juice flavor quality and stability to provide information prior to the transition to a commercial production scale.

The main objective of this study was to evaluate the effects of an integrated pilot-plant-scale PEF process (involving a sanitary fluid-handling system and a glove box packaging system) on the quality and stability of orange juice flavor and color at 4 and 22°C for 112 days.

MATERIALS AND METHODS

Materials. Frozen single-strength Valencia orange juice provided by Minute Maid (Houston, Tex.) in a 208-liter drum was kept at −25°C until it was processed. The standard flavor compounds D-limonene, α-pinene, valencene, myrcene, octanal, decanal, ethyl butyrate, and linalool were purchased from Aldrich Chemical (Milwaukee, Wis.). A solid-phase microextraction (SPME) fiber coated with 100 μm of polymethylsiloxane, 10-ml serum bottles, Teflon-coated rubber septa, and aluminum caps were purchased from Supelco (Bellfonte, Pa.). Plate count agar was purchased from Difco Laboratories (Sparks, Md.). Glass bottles (500 ml) with polypropylene caps were purchased from General Bottles Supply Co. (Los Angeles, Calif.).

Preparation and processing of orange juice. Frozen juice was thawed at refrigeration temperature for 12 days prior to processing. Orange juice was processed with an integrated pilot-plant-scale PEF system involving a sanitary fluid-handling system and a glove box packaging system (Fig. 1). The entire fluid-hand-
dling system was sterilized in place at 105°C for 30 min. A set of six PEF chambers (25) was used to process single-strength orange juice with PEF at an electric field strength of 35 kV/cm for 59 μs. PEF-processed orange juice was poured into sterilized 500-ml glass bottles in a sanitized glove box. The headspace of the sample bottle was 1%. For sanitization, glass bottles were dipped into a 3% hydrogen peroxide bath and rinsed with sterile water. The concentration of residual hydrogen peroxide in the bottles was determined with a hydrogen peroxide residue test kit (CHEMetrics, Inc., Claverton, Va.). The glove box was sprayed with 35% hydrogen peroxide and exposed to germicidal UV light at 254 nm (Cole Parmer, Vernon Hills, Ill.) with an intensity of 76 mW/cm² overnight before processing. A HEPA air filter system (Fisher Scientific, Pittsburgh, Pa.) with a pore size of 0.3 m of polymethylsiloxane was inserted into the headspace of the orange juice sample bottle, which was magnetically stirred and heated at 60°C for 5 min. The detector temperature was 250°C. The flow rate of ultrahigh-purity nitrogen gas was 1.0 ml/min at an inlet pressure of 80 lb/in² (8).

The flavor compounds were identified on the basis of a combination of mass spectra and the GC retention times of standard compounds. The levels of myrcene, d-limonene, α-pinene, valencene, octanal, decanal, ethyl butyrate, and linalool in fresh (unprocessed) juice and in PEF-processed juice on day 0 and during storage for 112 days at 4 and 22°C were determined. The flavor compound contents during storage were expressed as the percentage of flavor retention relative to the GC peak area on the first day of storage (day 0), which was expressed as 100%. Headspace flavor compounds in sample bottles were analyzed in duplicate by SPME-GC at 0, 2, 7, 14, 28, 56, and 112 days at 4 and 22°C.

**Flow chart of a sanitary fluid-handling system in an integrated PEF processing and glove box packaging system (no heat treatment).**

**Microbial analysis.** PEF-processed juice was tested for *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 by Silliker Laboratories (Columbus, Ohio) 1 day after treatment. With plate count agar, total aerobic plate counts were determined immediately before and after processing and during storage at 4 and 22°C. Samples were plated in duplicate by the surface plating method and incubated at 30°C for 48 h.

**Flavor compound analysis.** The headspace flavor compounds of orange juice were analyzed with a combination of SPME and gas chromatography (GC). One milliliter of orange juice was transferred into a 10-ml serum bottle containing a magnetic stirring bar (3 by 10 mm). The sample bottle was sealed with a Teflon septum and an aluminum cap. The SPME fiber coated with 100 μm of polymethylsiloxane was inserted into the headspace of the orange juice sample bottle, which was magnetically stirred and heated at 60°C for 20 min in a water bath to maintain the equilibrium of the flavor compounds among SPME coating, the headspace, and the orange juice in the bottle. The SPME fiber was removed from the sample bottle, inserted into the 0.75-mm-inside-diameter 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 Hewlett-Packard (Wilmington, Del.) model 5890 gas chromatograph with a flame ionization detector. The column was a HP-5 capillary column (0.53 mm by 30 m) coated with 2.65 μm of 5% phenyl substituted methylpolysiloxane. The GC temperature was programmed to go from 60 to 120°C at 10°C/min and was then held at 120°C; for 1 min, ramped to 140°C at 4°C/min, held at 140°C for 1 min, ramped to 200°C at 20°C/min, and held at 200°C for another 5 min. The detector temperature was 250°C. The flow rate of ultrahigh-purity nitrogen gas was 1.0 ml/min at an inlet pressure of 80 lb/in² (8).

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**Color measurement.** Color was measured with a HunterLab Ultrascan colorimeter (Hunter Associates Laboratory, Inc., Reston, Va.). To evaluate the color of fresh and PEF-processed orange juices, the L, a, and b values for these juices were determined. L is a measure of brightness and whiteness that ranges from 0 to 100 (100 = white; 0 = black), a is an indicator of redness that ranges from −a (green) to +a (red), and b is a measure of yellowness that ranges from −b (blue) to +b (yellow). The color was measured for duplicate samples at 0, 7, 14, 28, 56, and 112 days of storage at 4 and 22°C.

**Sensory evaluation.** The sensory qualities (i.e., color and overall flavor) of samples were evaluated by an experienced 12-member panel on the basis of a nine-point hedonic scale (1 dislike extremely; 5 neither like nor dislike; 10 like extremely). Randomly coded samples were served at 13°C. The sensory panel evaluated the orange juice stored at 4°C for 2, 7, 14, 28, 56, and 112 days of storage at 4 and 22°C.

**Data analysis.** Data were analyzed statistically with the SPSS statistical package (20). A two-sample t test at α ≤ 0.05 was used to compare the color and flavor of fresh (unprocessed) juice with those of PEF-processed juice. An analysis of variance was carried out to determine the significant effects of storage temperature and time on flavor retention and color. When differences were found, Tukey’s specific comparison test was used to determine which means were significantly different. All tests for significance were carried out at α ≤ 0.05. Sensory data were analyzed with a one-way analysis of variance to determine the effects of storage time. Specific differences were determined by the Tukey test at a 5% level of significance.

**RESULTS AND DISCUSSION**

A previous study from our laboratory showed that acceptable microbial inactivation was achieved for PEF-processed orange juice with electric fields of 35 kV/cm for a total treatment time of 59 μs, which was the protocol used in this study (24). To make sure that the PEF-processed orange juice was safe for sensory evaluation by the panel members, the juice was tested for *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H. The PEF-processed orange juice did not contain *Salmonella* spp., *L. monocytogenes*, or *E. coli* O157:H7. The total plate count for fresh orange
TABLE 1. Effects of PEF processing on orange juice flavor compounds

<table>
<thead>
<tr>
<th>Flavor compound</th>
<th>Fresh juice&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PEF-processed juice&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-limonene</td>
<td>6.7 × 10&lt;sup&gt;7&lt;/sup&gt; A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.9 × 10&lt;sup&gt;7&lt;/sup&gt; A</td>
</tr>
<tr>
<td>Valencene</td>
<td>2.1 × 10&lt;sup&gt;6&lt;/sup&gt; A</td>
<td>2.8 × 10&lt;sup&gt;6&lt;/sup&gt; B</td>
</tr>
<tr>
<td>Myrecene</td>
<td>1.1 × 10&lt;sup&gt;6&lt;/sup&gt; A</td>
<td>1.3 × 10&lt;sup&gt;6&lt;/sup&gt; B</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>1.8 × 10&lt;sup&gt;6&lt;/sup&gt; A</td>
<td>2.3 × 10&lt;sup&gt;6&lt;/sup&gt; B</td>
</tr>
<tr>
<td>Linalool</td>
<td>2.8 × 10&lt;sup&gt;6&lt;/sup&gt; A</td>
<td>3.0 × 10&lt;sup&gt;5&lt;/sup&gt; A</td>
</tr>
<tr>
<td>Octanal</td>
<td>1.7 × 10&lt;sup&gt;6&lt;/sup&gt; A</td>
<td>1.9 × 10&lt;sup&gt;5&lt;/sup&gt; A</td>
</tr>
<tr>
<td>Decanal</td>
<td>3.9 × 10&lt;sup&gt;5&lt;/sup&gt; A</td>
<td>4.5 × 10&lt;sup&gt;5&lt;/sup&gt; A</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>2.6 × 10&lt;sup&gt;4&lt;/sup&gt; A</td>
<td>2.9 × 10&lt;sup&gt;4&lt;/sup&gt; A</td>
</tr>
</tbody>
</table>

<sup>a</sup> FID, flame ionization detection.
<sup>b</sup> Unprocessed juice.
<sup>c</sup> On day 0.
<sup>d</sup> Means (n = 5) with different letters in the same row are significantly different (P ≤ 0.05).

Finding indicates that the hydrocarbon flavor compounds in the PEF-processed orange juice in glass bottles were stable at 4 and 22°C for 112 days. Ackerman and Wartenberg (1) also reported that no significant change was observed in the d-limonene retention of aseptically processed orange juice stored for 7 weeks at 10°C in glass bottles. However, storage time and temperature significantly affected the relative levels of the aldehydes octanal and decanal (P ≤ 0.05). There was a significant reduction in the octanal concentration within 7 days at 4°C and within 2 days at 22°C. The difference between the octanal level of juice stored at 4°C and that of juice stored at 22°C became significant after 2 days of storage. The effects of storage time and temperature on the decanal level were more severe than those on the octanal level. There was a significant decline in the decanal level within 14 days of storage at 4°C and within 2 days at 22°C. Figures 2 and 3 show that the levels of octanal and decanal decreased significantly.
during the first 14 days of storage at 4\textdegree{}C ($P \leq 0.05$) and remained relatively stable for the remaining storage time. Ackerman and Wartenberg (1) reported that octanal and decanal levels of aseptically processed orange juice in glass were reduced by about 35 and 70\%, respectively, within 14 days of storage. Our results are consistent with the observations of Ackerman and Wartenberg (1). We postulate that the losses of octanal and decanal are partly due to the reaction of octanal and decanal with amino acids such as lysine to produce a nonenzymatic browning reaction. Orange juice contains about 1\% proteins. We also observed that nonenzymatic browning increased and the levels of octanal and decanal compounds decreased during storage. Furthermore, we postulate that the decrease in octanal and decanal is partly due to the reaction of aldehyde compounds with hydroxyl compounds such as linalool and other flavor compounds with $-\text{OH}$ groups. The hydroxyl compounds react with octanal or decanal to form hemiacetal and acetal compounds.

The levels of ethyl butyrate and linalool in PEF-processed orange juice stored for 112 days at 4 and 22\textdegree{}C in glass bottles were significantly reduced ($P \leq 0.05$). The levels of ethyl butyrate and linalool decreased by 29 and 9\%, respectively, during 112 days of storage at 4\textdegree{}C. There was a 35\% loss of ethyl butyrate and a 23\% loss of linalool over 112 days at 22\textdegree{}C. The loss of ethyl butyrate may be due to its hydrolysis into alcohol and butyric acid, caused by the acidic conditions of the orange juice. Linalool might react with aldehyde compounds to form hemiacetal and acetal compounds.

The L, a, and b values of unprocessed (fresh) and PEF-processed orange juices are presented in Figure 4. There was a significant ($P \leq 0.05$) difference observed between fresh and PEF-processed orange juice with regard to L, a, and b values. PEF-processed orange juice had significantly higher L and b values and lower a values than did fresh orange juice ($P \leq 0.05$). PEF processing did not cause detrimental changes in juice color. Sizer and Waugh (19) and Yeom et al. (24) reported the negative effects of heat processing on the color of orange juice. Yeom et al. (24) reported that PEF-processed orange juice had a significantly smaller particle size than did the heat-pasteurized orange juice. The brighter and more yellowish color (which is desirable) after PEF processing might be due to the smaller particle size of PEF-processed orange juice.

The stability of the color of PEF-processed orange juice is shown in Figure 5. The effects of storage time at 4\textdegree{}C on the L, a, and b values were not significant ($P > 0.05$). This finding indicates that the color of PEF-processed juice remained stable at 4\textdegree{}C for 112 days. However, the effect of storage time was significant ($P \leq 0.05$) at 22\textdegree{}C. The L and b values for the juice stored at 22\textdegree{}C were significantly ($P \leq 0.05$) reduced after 28 days of storage. Sizer and Waugh (19) reported that storage temperature is the single most important factor in achieving satisfactory shelf life and quality.

The results of the sensory panel evaluation of the color and overall flavor of PEF-processed orange juice are shown in Figure 6. There was no significant ($P > 0.05$) change in the color or flavor of PEF-processed juice stored at 4\textdegree{}C for 112 days. Significant ($P \leq 0.05$) reductions in the retention of octanal, decanal, ethyl butyrate, and linalool during storage did not affect the overall flavor quality, as determined by the sensory panel. Pieper et al. (13) also reported that the absorption of up to 50\% of d-limonene and small amounts of aldehydes and alcohols had no significant effect on the sensory quality of orange juice.

**SUMMARY**

PEF-processed orange juice remained microbiologically stable ($<1 \log \text{CFU Est/ml}$) during storage for 112 days at 4\textdegree{}C and at 22\textdegree{}C in glass bottles. The hydrocarbon flavor compounds tested were more stable than octanal, decanal,
ethyl butyrate, and linalool during storage. A significant reduction of octanal and decanal within 2 weeks was possible due to a nonenzymatic browning reaction with protein in the orange juice. However, the loss of these flavor compounds had no significant effect on the flavor quality of orange juice, as determined by a sensory panel for orange juice stored at 4°C for 112 days. The shelf life of PEF-processed orange juice is 112 days at 4°C, compared with 48 to 64 days for conventional heat-processed orange juices available in supermarkets.

ACKNOWLEDGMENTS

Operational funding for this project was provided by the NSF CAPPs Center. PEF-processing equipment was provided by a project funded by the U.S. Army Natick Soldier Center. The orange juice was provided by a CAPPs member company, Minute Maid. The authors are grateful to Dr. C. Wang for operating the pilot plant PEF system, Mr. Charles Streaker for operating the fluid-handling system, and Dr. Hye Won Yeom for preparing juice for processing. The authors are also grateful to Dr. S. Palaniappan of Minute Maid for his technical advice.

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