Flavor, Color, and Vitamin C Retention of Pulsed Electric Field Processed Orange Juice in Different Packaging Materials

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Effects of packaging materials, storage temperature, and time on the stability of pulsed electric field (PEF) processed orange juice were investigated. Single-strength orange juice was treated with PEF at an electric field strength of 35 kV/cm for 59 μs using an integrated pilot plant scale PEF processing and glovebox packaging system. The retention of eight orange juice aroma compounds, color, and vitamin C in glass, polyethylene terephthalate (PET), high-density polyethylene, and low-density polyethylene were evaluated at 4 and 22 °C for 112 days. Packaging material had a significant effect ($p \leq 0.05$) on the retention of orange juice aroma compounds, color, and vitamin C. PEF-treated orange juice had a shelf life of >16 weeks in glass and PET at 4 °C.

Keywords: *PEF processing; packaging materials; vitamin C; orange juice aroma compounds; sensory evaluation*

INTRODUCTION

The citrus industry has been exploring nonthermal processing methods with minimal heat treatment to improve flavor and nutritional qualities. Pulsed electric field (PEF) has been shown as a nonthermal process for inactivating microorganisms in foods without significant adverse effects on the flavor, taste, and nutrients caused by conventional thermal processing. Because PEF processing is controlled at ambient temperature for a very short treatment time of microseconds, it provides freshlike foods with safety and extended shelf life (*1*−*5*). However, keeping this freshlike flavor and nutritional value of PEF-processed food during storage may depend on packaging materials and methods and storage conditions.

Packaging material selection as well as processing influences the quality of foods during storage due to the absorption of flavor compounds by packaging materials or permeation through them and degradation of flavor, color, and nutrients by oxygen transmission through packages. Paperboard cartons and plastic containers are commonly used packaging materials of orange juice with low-density polyethylene (LDPE) as product contact layer (*6*). Interaction between these materials and food has been an increasing area of research.

Many researchers have reported high losses of *d*-limonene and other aroma compounds from citrus juices in contact with LDPE (*6*−*10*). Kwapong and Hotchkiss (*9*) and Hotchkiss (*11*) showed that the consequence of this absorption significantly affects sensory quality in model systems. Mannheim et al. (*12*) stated that *d*-limonene absorption shortened the shelf life of orange juice. Moshonas and Shaw (*13*) claimed that absorption contributed to flavor changes, which were sensorially detectable in orange juice. However, Pieper et al. (*6*) reported that an experienced sensory panel did not distinguish between orange juice stored in glass bottles and orange juice stored in polyethylene-laminated cartons, which absorbed 50% of *d*-limonene.

Sizer et al. (*14*) stated that the predominant factor in the change of flavor during the storage of orange juice in aseptic cartons is not absorption, but chemical degradation of flavoring components and development of off-flavor components from the degradation products. They also mentioned that control of storage temperature remains the single most important factor in delaying flavor loss and achieving satisfactory shelf life and quality. Oxygen in the juice, in the headspace, and permeating through packaging material should be minimized to avoid detrimental effects on the retention of vitamin C, color, and flavor (*14*).

Using nonthermal processing such as PEF with the selection of compatible material has the potential to increase the shelf life of orange juice while keeping freshlike flavor and nutrients. Many researchers mentioned the advantage of PEF for the retention of fresh flavor and nutrients (*1*−*5*, *15*−*19*). However, no literature was found in quantitative examination of the effects of packaging materials on the retention of food flavor and nutrients of PEF-processed foods.

The objective of our investigation was to determine the effects of packaging material, storage temperature, and time on flavor, color, and nutrient quality of PEF-processed single-strength orange juice. A further objective was to evaluate whether changes in flavor and color influence the sensory quality of orange juice under commercial refrigeration (4 °C).

MATERIALS AND METHODS

Materials. Single-strength orange juice was provided by Minute Maid (Houston, TX) as frozen and kept in a freezer at −25 °C until processing. Standard flavor compounds of *d*-limonene, α-pinene, myrcene, octanal, decanal, ethyl butyrate, and linalool were purchased from Aldrich Chemical (Milwaukee, WI). A solid phase microextraction (SPME) fiber coated

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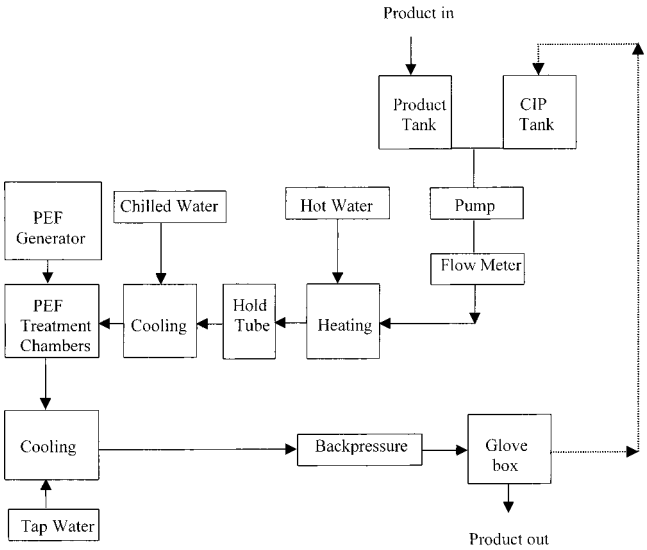

Figure 1. Flowchart of an integrated PEF processing and glovebox packaging system.

with 100 μm polymethylsiloxane, 10 mL serum bottles, Teflon-coated rubber septa, and aluminum caps were purchased from Supelco (Bellefonte, PA). Total plate count agar was purchased from Difco Laboratories (Detroit, MI). Glass, polyethylene terephthalate (PET), and high-density polyethylene (HDPE) bottles of 500 mL with 28 mm polypropylene caps were purchased from General Bottles Supply Co. (Los Angeles, CA). LDPE bottles were purchased from Consolidated Plastic Co. (Twinsburg, OH).

Preparation and Processing of Orange Juice. Freshly squeezed single-strength orange juice from Valencia oranges, quickly frozen in a 208 L drum, was stored in a −25 °C freezer until processing. The frozen juice was thawed at refrigeration temperature for 12 days prior to processing.

Orange juice was processed using a sanitary fluid handling system in an integrated pilot plant scale PEF processing and glovebox packaging system (Figure 1). The entire fluid handling system was sterilized by a sterilization-in-place (SIP) process at 105 °C for 30 min. Single-strength orange juice was treated with PEF with an electric field strength of 35 kV/cm for 59 μs. PEF-treated orange juice was filled into sanitized bottles in a sanitized glovebox, with the amount of headspace minimized to 1%. The bottles were presterilized by dipping into a 3% hydrogen peroxide bath and rinsing with autoclaved water. The concentration of residual hydrogen peroxide inside the bottles was determined using a hydrogen peroxide residue test kit (CHEMetrics, Inc., Claverton, VA). The glovebox was first sprayed with 35% hydrogen peroxide and exposed to germicidal UV light, UV-C, 254 nm (Cole Parmer, Vernon Hills, IL) with the intensity of 76 μW/cm^2 for overnight before processing. A HEPA air filter (Fisher Scientific, Pittsburgh, PA) system with 0.3 μm pore size and a 1600 cm^2 filtration area was installed to provide positive pressure of bacteria-free air in the glovebox.

Microbial Analysis. PEF-treated juice was tested for pathogens, *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7, 1 day after the treatment by Silliker Laboratories (Columbus, OH). Total aerobic plate count was determined using total plate count agar (PCA) before and right after processing and during storage at 4 and 22 °C. For each dilution prepared, duplicate samples were plated using the surface plating method. PCA plates were incubated at 30 °C for 48 h before enumeration.

Flavor Analysis. Selected flavor compounds in the headspace of orange juice or standard compound solutions were analyzed by a combination of SPME and gas chromatography (GC) (*20*). One milliliter of orange juice or standard solution was transferred into a 10 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 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 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 the 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 an HP 5890 gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with an HP-5 capillary column of 0.53 mm i.d. × 30 m coated with 2.65 μm of 5% phenyl-substituted methylpolysiloxane and a flame ionization detector (FID). The GC oven was programmed from 60 to 120 °C at 10 °C/min, held for 1 min, ramped to 140 °C at 4 °C/min, held for 1 min, ramped to 200 °C at 20 °C/min, and then held for another 5 min at 200 °C. FID temperature was set to 250 °C. Ultrahigh purity nitrogen was used as the carrier gas with the inlet pressure of 80 psi.

The flavor compounds were identified by comparing the retention times of GC peaks with those of standard compounds under the identical experimental conditions. Flavor compounds were evaluated on the basis of percentage of relative retention. The GC peak area found at zero time in glass was taken 100% as initial value.

d-Limonene, myrcene, α-pinene, octanal, decanal, ethyl butyrate, and linalool were tested in orange juice at 4 and 22 °C. Samples were taken at 0, 2, 7, 14, 28, 56, and 112 days for analysis. At each sampling date, duplicate samples (bottles) were taken from each storage temperature for flavor analysis by SPME-GC.

Color Measurement. Color was measured using a HunterLab Ultrascan colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). The values for *L*, *a*, and *b* were recorded to evaluate color changes of PEF-treated orange juice during storage. The parameter *L* is a measure of brightness/whiteness, and it ranges from 0 to 100 (100 = white; 0 = black); *a* is an indicator of redness, and it varies from $-a$ to $+a$ ($-a$ = green, $+a$ = red); and the parameter *b* is a measure of yellowness, and it varies from $-b$ to +b ($-b$ = blue, $+b$ = yellow). Duplicate samples were taken for color measurements at 0, 7, 14, 28, 56, and 112 days of storage at 4 and 22 °C.

Sensory Analysis. The sensory evaluation was based on a nine-point hedonic scale: 1 = light or orange color; 9 = dark or brown color, to determine color intensity. Flavor intensity was also determined on a hedonic scale: 1 = none; 9 = strong orange flavor. Randomly coded samples were served at ∼13 °C. The PEF-processed orange juices stored only at 4 °C were evaluated by an experienced panel of 12 at 2, 7, 14, 28, 56, and 112 days of storage. The panel was asked to evaluate color before flavor. For flavor evaluation, they were instructed to take a few sips of the juice and swirl it in their mouth for a few seconds before swallowing.

Vitamin C Analysis. Vitamin C content in the orange juice was measured using a high-performance liquid chromatography (HPLC) system (*21*). A Hewlett-Packard liquid chromatograph (Wilmington, DE) equipped with an autosampler and a detector at 254 nm was used. The HPLC chromatograph peak area was calculated using a Hewlett-Packard integrator (HP3396 Series II). A reversed-phase C-18 column (5 μm particle size, 4.6 mm diameter, 250 mm length, Hewlett-Packard) along with a Hewlett-Packard C-18 guard column was used to separate the vitamin C using methanol and acidified water (10:90, v/v) as a mobile phase. The water was acidified with phosphoric acid (0.01%, v/v). The mobile phase was filtered using a 0.45 μm membrane filter (Micron Separations Inc., Westboro, MA) and degassed using helium gas before passing through the column at a flow rate of 1.0 mL/min. A standard calibration curve was obtained by using L-ascorbic acid (Sigma Chemical Co., St. Louis, MO) in concentrations ranging from 5 to 80 mg/100 mL.

The orange juice was centrifuged at 12535*g* for 10 min in a Beckman Microfuge E (Beckman Instruments Inc., Palo Alto, CA) to remove pulp and coarse cloud particles. Ten microliters of the supernatant was injected into the column using the

HPLC autosampler. The reproducibility of six time analyses per each orange juice sample, based on the relative standard deviation, was found to be within 5% for vitamin C. PEF-processed orange juice stored at 4 °C was taken for vitamin C analyses at 0, 2, 7, 14, 28, 56, and 112 days.

Data Analysis. The entire experiment was duplicated. Data were analyzed using an SPSS statistical package (SPSS, 1999). The effects of packaging materials, storage temperature, and time on flavor and color retention were determined by analysis of variance (three-way ANOVA). The effects of packaging materials and storage time on vitamin C retention and sensory attributes were analyzed using two-way ANOVA. Mean comparisons for specific differences were done by the Tukey test at $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Microbial Stability. Optimum PEF-processing conditions, electric fields of 35 kV/cm and 59 μs of total treatment time, were determined by preliminary study to achieve maximum microbial inactivation for orange juice. Yeom et al. (*22*) reported that there were 7 log reductions in total aerobic plate and yeast and mold counts when the PEF_processing condition was 35 kV/cm for 59 μs. Electric field strength and total treatment time have been reported as major factors of PEF processing to determine microbial inactivation (*23*). The PEF-treated orange juice using these processing conditions was reported negative for the pathogens, *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 by Silliker Laboratories. Fresh orange juice had a total plate count of 2.34 log CFU/mL. The total aerobic plate count was <1 log CFU Est/mL right after the PEF treatment and during storage. The PEF treatment prevented the growth of microorganisms at 4 and 22 °C for 112 days.

Effects of Packaging Materials on the Retention of Flavor Compounds. Relative retention (percent) of flavor compounds in PEF-processed orange juice stored at 4 and 22 °C for 112 days was determined. Data analysis showed that packaging material, storage temperature, and time had significant ($p \leq 0.05$) effects on the retention of all flavor compounds tested.

There was no significant ($p > 0.05$) difference observed between glass and PET in the retention of *d*-limonene at 4 and 22 °C (Figure 2). However, significant absorption of *d*-limonene occurred within 2 weeks into HDPE and low-density polyethylene (LDPE). A similar absorption behavior was observed for myrcene and α-pinene during storage due to similar polarity and solubility of the hydrocarbon flavor compounds (data not shown). As reported by Durr et al. (*24*) a distinct loss of *d*-limonene in orange juice in polyethylene-lined cartons occurred the first 2 weeks of storage and then reached a steady state level, which remained constant for the remainder of the storage period. Many researchers have reported high losses of *d*-limonene and other aroma compounds from citrus juices in contact with LDPE (*7*−*10*). It was reported that the consequence of this absorption significantly affected sensory quality in model systems (*9*, *11*). Mannheim et al. (*12*) stated that absorption of *d*-limonene shortened the shelf life of orange juice. However, Ackerman and Wartenberg (*25*) reported that absorption of orange juice components in polyethylene was not a significant factor in the retention of desirable flavor. Degradation of ethyl butyrate, neral, geranial, and other aldehydes was the most significant effect on the orange juice flavor (*25*).

For all packaging materials, oxygenated flavor compounds such as octanal, decanal, and ethyl butyrate

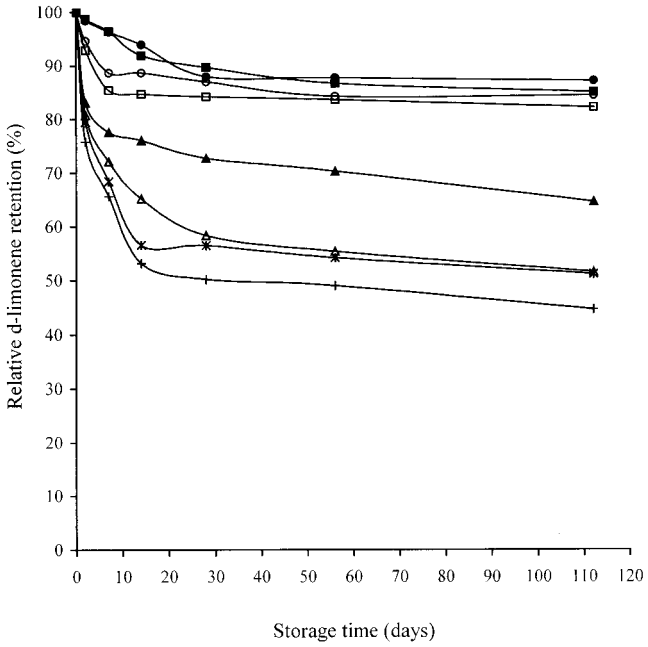

Figure 2. *d*-Limonene retention (percent) in PEF-processed orange juice during storage at 4 and 22 °C in different packages: glass at 4 (●) and 22 °C (○); PET at 4 (■) and 22 °C (□); HDPE at 4 (▲) and 22 °C (△); LDPE at 4 (∗) and 22 °C (+).

were less retained in orange juice than hydrocarbon flavor compounds including limonene, myrcene, and α-pinene during storage (data not shown). There was no significant difference ($p > 0.05$) between glass and PET in the retention of octanal, decanal, and ethyl butyrate during storage at 4 and 22 °C. However, significant absorption of octanal, decanal, and ethyl butyrate occurred within 2 weeks in HDPE and LDPE. The loss of aldehydes and ethyl butyrate was highest in HDPE and LDPE bottles, especially at 22 °C. Aldehydes and ester compounds tested were also significantly reduced in the glass bottles within a few weeks of storage and stayed stable for the remainder of the storage. Because glass bottles are known to be inert, the loss of aldehydes and ester compounds was related to chemical degradation as it was stated by Ackerman and Wartenberg (*25*). However, this loss was more pronounced in HDPE and LDPE bottles than in glass and PET especially at high storage temperature (22 °C), possibly due to acceleration of flavor degradation by oxygen transmission through polyethylene packages. Sizer et al. (*14*) stated that the predominant factor in the change of flavor during the storage of orange juice in aseptic cartons is not absorption but chemical degradation of flavoring components and development of off-flavor components from the degradation products.

Like the hydrocarbon flavor compounds tested, linalool was more stable in glass and PET than in HDPE and LDPE at 4 and 22 °C (data not shown). Increasing the temperature from 4 to 22 °C significantly reduced linalool retention in all packaging materials ($p \leq 0.05$).

The influence of a cap liner on flavor absorption was also investigated using PP caps with polyethylene liner and without liner. There was no significant ($p > 0.05$) effect of the liner on the retention (percent) of *d*-limonene during storage of PEF-processed orange juice in glass bottles at 4 °C (data not shown).

Effects of Packaging Materials on the Retention of Color. Comparisons of *L*, *a*, and *b* values in glass

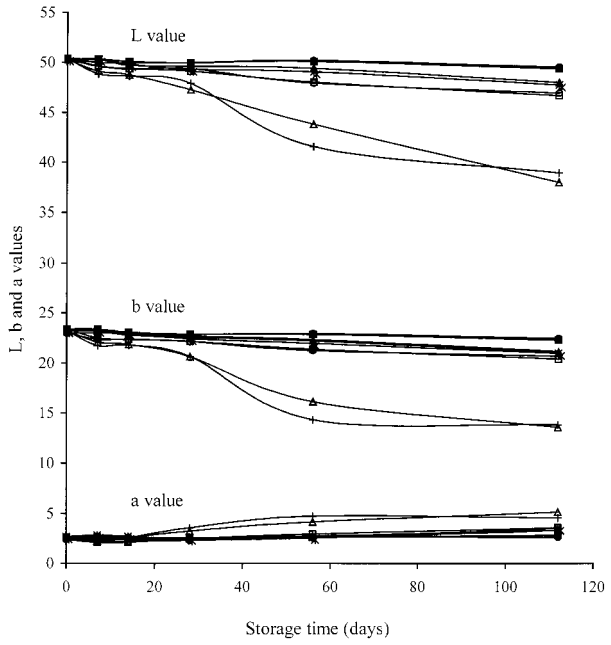

Figure 3. Comparison of orange juice color *L*, *b*, and *a* values in glass and plastic bottles at 4 and 22 °C: glass at 4 (●) and 22 °C (○); PET at 4 (■) and 22 °C (□); HDPE at 4 (▲) and 22 °C (△); LDPE at 4 (∗) and 22 °C (+).

and plastics tested during a storage time of 112 days at 4 and 22 °C are shown by Figure 3. The Tukey test showed that there was no significant ($p > 0.05$) difference observed in *L* value or brightness of orange juice packed in glass, PET, HDPE, and LDPE at 4 °C. However, *L* values were significantly reduced in HDPE and LDPE bottles compared to glass and PET after 28 days of storage at 22 °C due to acceleration of oxygen transmission through polyethylene packages at 22 °C. The PEF-treated orange juice had higher *a* and lower *b* values in HDPE and LDPE than in glass and PET at 4 and 22 °C storage ($p \leq 0.05$). Darkening or browning in orange juice color was visually observed in HDPE and LDPE bottles stored at 22 °C after 28 days (data not shown). There was no significant ($p > 0.05$) difference between PET and glass in terms of *L*, *a*, and *b* values during storage of 112 days at 4 and 22 °C. Detrimental changes in the color of orange juice are primarily caused by nonenzymatic browning (*26*). Temperature is the most important factor to control nonenzymatic browning.

Effects of Packaging Materials on Sensory Quality. Hedonic scores for color and flavor of PEF-treated orange juice in the packages were determined during 112 days of storage at 4 °C. There was no significant ($p > 0.05$) difference in perceived color intensity between any of the samples, even though PEF-treated orange juice had higher *a* and lower *b* values in HDPE and LDPE compared to those in glass and PET at 4 °C (Figure 3). Sensory evaluation showed a significant difference ($p \leq 0.05$) in flavor intensity after 56 days of storage between PEF-treated juices packed in LDPE and in other packaging materials (Figure 4). However, the sensory panel did not find difference in perceived overall orange juice flavor in glass, PET, and HDPE bottles during storage of 112 days. Mannheim et al. (*12*) reported that there was a significant difference determined by experienced tasters between juices packed in glass and cartons stored at ambient temperatures. However, another study done by Pieper et al. (*6*) showed

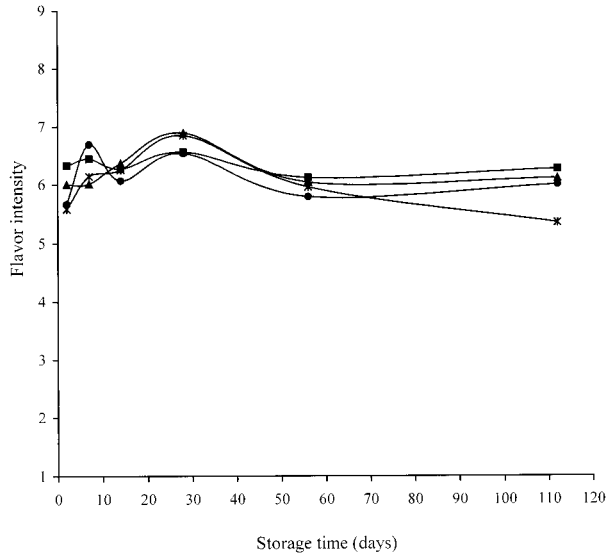

Figure 4. Effects of packaging on mean hedonic flavor intensity scores for PEF-treated orange juice during 112 days of storage at 4 °C: (●) glass; (■) PET; (▲) HDPE; (∗) LDPE.

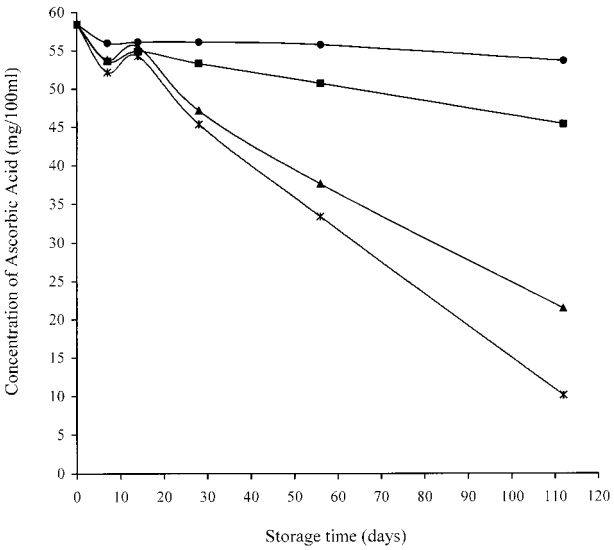

Figure 5. Effects of packaging materials on the concentration of ascorbic acid in PEF-treated orange juice during storage at 4 °C: (●) glass; (■) PET; (▲) HDPE; (∗) LDPE.

that an experienced sensory panel did not distinguish between orange juice stored in glass and in polyethylene-laminated cartons.

Effects of Packaging Materials on the Retention of Vitamin C. Effects of packaging materials on the concentration of ascorbic acid in PEF-treated orange juice during storage at 4 °C are shown in Figure 5. The concentration of ascorbic acid in glass and PET bottles was significantly higher than that in HDPE and LDPE bottles during storage at 4 °C ($p \leq 0.05$).

Ascorbic acid retention has been used as an indicator of shelf life for chilled orange juices (*27*). Berry et al. (*28*) monitored the ascorbic acid level in single-strength orange juice during storage and found that shelf life in plastic bottles was considerably shorter than that in glass bottles. Bissett and Berry (*29*) reported that glass bottles showed the best retention of ascorbic acid compared to polyethylene, polystyrene, and cardboard containers. Vitamin and flavor are destabilized in containers that are permeable to atmospheric oxygen

(*30*). Marshall et al. (*31*) reported that permeability of oxygen by soft-pack containers is the most critical factor in the shelf stability of aseptically processed juices. A relatively short shelf life of 28−42 days of chilled orange juice was due to the permeability to oxygen of packaging materials used (*32*). Permeation of oxygen in polymeric packages is known to limit effectiveness for aseptic products at ambient temperature (*33*). Polyethylene is known to be a poor gas barrier (*34*), which explains the significant reduction of ascorbic acid in HDPE and LDPE bottles compared to that in glass and PET bottles.

Marshall et al. (*31*) also reported greater reduction in ascorbic acid levels with higher concentration of air in the headspace. Johnson and Toledo (*35*) found the presence of oxygen or especially hydrogen peroxide (the sterilent used in aseptic packaging) to be detrimental to shelf life. The hydrogen peroxide residue was <0.1 ppm per bottle prior to filling. The presence of oxygen in the juice and headspace gases above juice plays a role in the shelf life of chilled juice products (*27*). The amount of headspace and dissolved oxygen in the product should be kept to a minimum because ascorbic acid destruction and nonenzymatic browning of aseptically packaged orange juice are accelerated by oxygen (*36*). Assuming all bottles tested had the same amount of dissolved oxygen and headspace of 1%, the significant reduction in vitamin C retention in plastic bottles was attributed to oxygen transmission.

Conclusions. In summary, the retention of all flavor compounds tested, vitamin C, and color was significantly higher in glass and PET than in HDPE and LDPE. As expected, increasing the storage temperature from 4 to 22 °C had adverse effects on the flavor and color retention. The loss of aldehydes and ester compounds was more notable than that of hydrocarbons and alcohol in all packages including glass within the first few weeks of storage. However, this flavor loss was more pronounced in HDPE and LDPE bottles. The difference of the retention of orange juice flavor compounds, vitamin C, and color in different packaging materials could be explained by (1) the absorption of flavor compounds into the polymeric packaging materials tested; (2) the acceleration of ascorbic acid, color, and flavor degradation due to initial oxygen concentration and transmission of oxygen through the plastic package; and (3) increases in browning, absorption, and degradation of flavor compounds with increasing storage temperature. The PEF-treated orange juice had a shelf life of >16 weeks at 4 °C in glass and PET bottles. It is necessary to select a proper packaging material that is compatible with food so that the benefits of PEF processing can be maintained during storage.

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