DESIGN, CONSTRUCTION AND VALIDATION OF A SANITARY GLOVE BOX PACKAGING SYSTEM FOR PRODUCT SHELF-LIFE STUDIES

ZEHRA AYHAN[1,2], CHARLES B. STREAKER[1] and Q. HOWARD ZHANG[1,3]

[1]*Department of Food Science and Technology*
The Ohio State University
Columbus, OH 43210

[2]*Department of Food Engineering*
Mustafa Kemal University
Hatay, TURKEY

Accepted for Publication November 8, 2000

ABSTRACT

A glove box has been constructed as part of an integrated pilot plant scale pulsed electric field processing and packaging system to facilitate studies of product shelf-life with selected packaging materials. The glove box was sanitized using combination of hydrogen peroxide and germicidal UV light. A HEPA air filter provided positive pressure of bacteria-free air. Nonselective nutrient broth was sterilized and filled into presanitized bottles inside the glove box. Negative and positive controls were included in the experiment. All bottles were incubated at 22C and 37C for two weeks and checked for microbial growth by measuring optical density at 600 nm using a spectrophotometer and by plating on plate count agar and potato dextrose agar for total aerobic and, yeast and mold counts, respectively. No turbidity or microbial growth was observed in the media filled in the sanitized bottles using the sanitized glove box at 22 and 37C. PEF processed orange juice using this system had a shelf-life of more than 16 weeks at 4C.

INTRODUCTION

A sanitary fluid handling system integrated with a pilot plant scale pulsed electric field (PEF) processing unit and a Benco aseptic packaging machine at The Ohio State University offers flexibility of processing using heat, PEF, or

[3] Address correspondence to: Dr. Q. Howard Zhang, The Ohio State University, Dept. Food Science and Technology, 110 Parker Hall, 2015 Fyffe Road, Columbus, OH 43210, TEL: (614) 688-3644; FAX: (614) 292-0218; E-mail: Zhang.138@osu.edu

Journal of Food Processing Preservation **25** (2001) 183–196.

a combination of both for fluid and particulate foods for extended product shelf-life (Ruhlman 1999; Streaker 1999; Yeom 2000). However, the Benco aseptic packaging machine that uses the form-fill-seal principle can form only one type of base packaging material at a time. The forming and sealing operations are limited to packaging materials that can be thermoformed and heat-sealed (Ayhan and Zhang 2000). A glove box was constructed as part of an integrated pilot plant scale PEF processing and packaging system to fill bottles of various packaging materials to evaluate product/package interactions during shelf-life.

Wadsworth and Basette (1984) constructed a laboratory scale indirect ultra high temperature (UHT) system to process milk and collected processed milk with a sanitized glove box. A combination of chemical sanitizer and UV light was used to kill any surface microorganisms in the glove box before filling. They claimed that milk processed with this system remained sterile for at least 4 months at both 7C and 32C storage temperatures (Wadsworth and Basette 1985).

Hydrogen peroxide has been used as a sterilant for food packaging materials for a number of years (Robertson 1993). Ultraviolet at a wavelength of 254 nm has been used to control surface and airborne microorganisms in laboratories, cleanrooms, meat coolers and other facilities (Maunder 1977). The lethal effect of hydrogen peroxide, ultraviolet irradiation or combination of both on microorganisms including heat resistant spores has been shown by many researchers (Bayliss and Waites 1979, 1980, 1982; Stannard *et al.* 1983; Stannard and Wood 1983).

The objective of this study was to construct a glove box packaging system integrated with pilot plant scale pulsed electric field or heat processing, and validate processing, filling and packaging equipment for high acid food products. The shelf-life of single strength orange juice treated with PEF processing was also investigated using this system.

MATERIALS AND METHODS

Materials

Glass bottles of 500 mL with polypropylene (PP) caps were purchased from General Bottle Supply Co. (Los Angeles, CA). Nonselective nutrient broth (DIFCO, Detroit, MI) was used to validate the system. Plate count agar (PCA) and potato dextrose agar (PDA) (DIFCO, Detroit, MI) were used for surface plating. A germicidal UV light and pairs of butyl gloves were purchased from Cole Parmer (Vernon Hills, IL). HEPA air filter was purchased from Fisher

Scientific (Pittsburgh, PA). Orange juice was provided by Minute Maid (Houston, Texas).

Design and Construction

A glove box consisted of a gas-tight box fitted with a window, a pair of gloves, a transfer chamber, a germicidal UV lamp, a fluorescent lamp and a HEPA air filter as illustrated by Fig. 1a and 1b. The glove box was made of stainless steel. The front window was made of glass. The inner and outer transfer chamber doors were made of plexiglass. The transfer chamber with inner and outer doors allow easy transfer of preformed bottles between the pilot plant and the box, while maintaining an established sanitary glove box environment. A pair of butyl gloves resistant to chemicals was attached to glove ports at the front side of the box with stainless steel clamps. The glove ports and the glass were sealed with a gasket to provide low leak interior atmosphere. Control switches for the fluorescent and UV lamps were conveniently located on the control panel.

The germicidal UV lamp (UV-C, 254 nm) with intensity of 76 $\mu W/cm^2$ was attached to the ceiling of the glove box to control surface and airborne microorganisms in the glove box prior to filling. The HEPA air filter system with 0.3 μm pore size and 1600 cm^2 filtration area was attached between a compressed air cylinder and the glove box to provide a positive pressure of clean air in the glove box. A stainless steel filling valve and pipeline were installed in the glove box and were connected to the fluid-handling system (FHS). The fluid handling system has flexibility of connecting either to the glove box or to the aseptic packaging machine (Fig. 2).

Chemical Cleaning Procedure

Glass and plastic bottles and their caps were dipped into 3% hydrogen peroxide bath of 6 L and rinsed with sterile water in a sanitized chemical hood. The hydrogen peroxide bath was changed for every ten bottles. Each bottle was rinsed with 250 mL of autoclaved water. An exhaust fan was kept on to remove hydrogen peroxide fumes during washing. Chemically cleaned bottles were capped and left inside the hood overnight. The concentration of residual hydrogen peroxide in the containers after drying, was determined by preliminary trials using a Chemetrics hydrogen peroxide residue test kit (CHEMetrics Inc., Claverton, VA).

The day before processing, the bottles and glove box were sanitized in the following steps. The filling unit assembly was removed from the glove box. The glove box was cleaned with soap and sprayed with 35% hydrogen peroxide and wiped out. The filling unit assembly was wrapped with aluminum foil and autoclaved at 121C for 30 min separately and then placed inside the cleaned

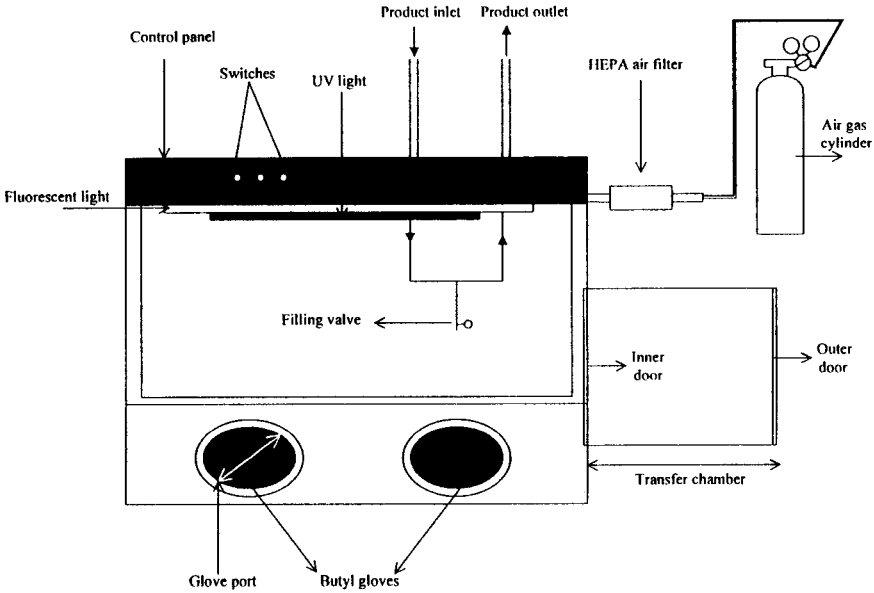

FIG. 1A. SCHEMATIC DIAGRAM OF A GLOVE BOX

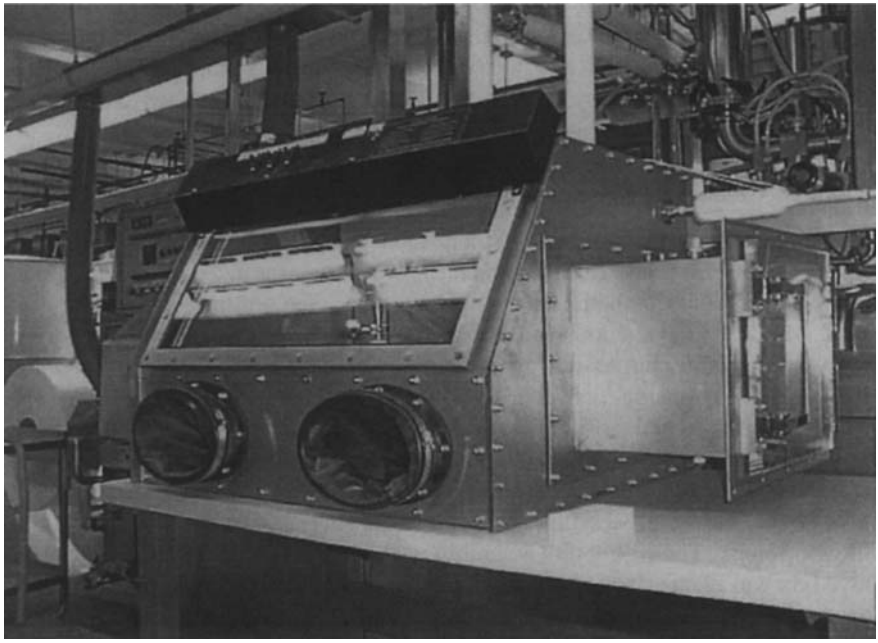

FIG. 1B. GLOVE BOX PICTURE

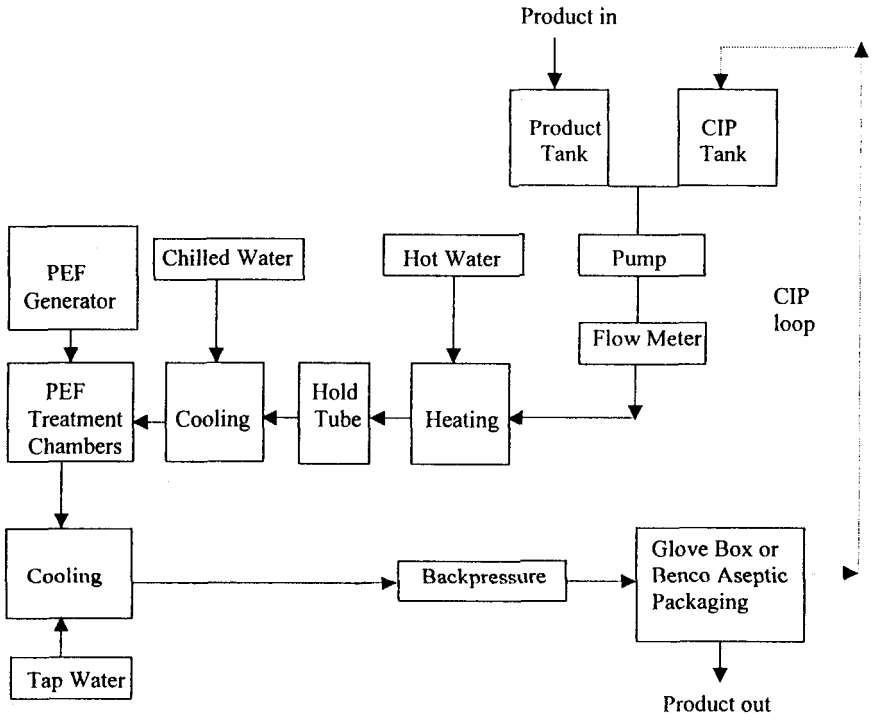

FIG. 2. FLOW CHART OF AN INTEGRATED PULSED ELECTRIC FIELD PROCESSING AND GLOVE BOX PACKAGING SYSTEM

glove box. The UV light was kept on overnight to control surface and airborne microorganisms inside the glove box. The glove box front window and the outer transfer chamber door were covered with cardboard to prevent eye contact with the UV light.

Sterilization Procedure

The entire fluid handling system and the pipeline were hot water sterilized at 105C for 30 min. The end of the pipe attached to the filling unit inside the glove box was capped until sterilization was completed. The filling valve was kept open during hot water sterilization. After the sterilization was completed, the filling valve was closed, the end cap was removed and the autoclaved filling unit was attached to the filling valve using the gloves of the glove box.

Sixty liters of nonselective nutrient broth was prepared in the product tank of the fluid handling system and the pH was adjusted to 3.7 using citric acid. This solution was pumped through the entire fluid handling system, including the pipeline in the glove box for the sterilization-in-place (SIP) process at 105C for 30 min. The sterilization sequences were 35 min of preheating, 30 min of sterilizing at 105C, and 20 min of cooling prior to filling. The system was pressurized at 110 kPa, and the product flow rate was 98L/h. The UV light was turned off and the HEPA filtered air was turned on to provide a positive air pressure (14-28 kPa) inside the box during filling to avoid drawing contaminants inside.

Glove Box Filling Procedure

Chemically cleaned and tightly capped bottles were transferred to the glove box using the transfer chamber. Only one of the two doors of the transfer chamber was open at a given time. The bottles were opened inside the glove box, and filled with pH adjusted sterile media at the filing temperature of 29C. Filled bottles were capped and transported out of the glove box using the transfer chamber. A total of 72 bottles were filled in the glove box at a time. Filling time for each bottle was approximately one minute.

After completion of the processing and filling, a clean in place (CIP) procedure took place to clean the FHS and pipeline using chemicals (Streaker 1999). The glove box was then manually cleaned.

Validation Procedure

Chemically cleaned bottles filled with the sterile media in the glove box were compared to positive and negative controls prepared. As negative control, the same media (from the tank, prior to sterilizing with the SIP) was filled in glass bottles and autoclaved at 121C for 30 min. Empty glass bottles were autoclaved and filled with the sterile media (from the SIP of the FHS) in the glove box as another negative control. As positive control, some of the chemically cleaned bottles filled with the media in the glove box were inoculated with natural flora of orange juice. Representative colonies were selected from PCA and PDA petri dishes plated with orange juice. All bottles were incubated at 22C and 37C for 2 weeks and checked for microbial growth by measuring optical density at 600 nm using Spectronic Genesys 5 spectrophotometer (Milton Roy, Rochester, NY) and by plating on PCA and PDA for total aerobic and yeast and mold counts, respectively. The negative control, which was autoclaved at 121C for 30 min, was used as a blank for optical density readings. PCA and acidified PDA plates were incubated at 30C for 48h and 22C for 5 days, respectively, before enumeration. The entire experiment was duplicated.

Preparation and PEF Processing of Orange Juice

Freshly squeezed single strength Valencia orange juice, quickly frozen in 208 L drum was stored in a -25C freezer until processing. The frozen juice was thawed at refrigeration temperature for twelve days prior to processing.

Orange juice was processed using the sanitary fluid handling system integrated with the pilot plant scale PEF processing and the glove box packaging system (Fig. 1). The entire fluid handling system was sterilized (105C for 30 min). The glove box and the bottles were sanitized as described. Single strength orange juice was treated with PEF at electric field strength of 35 kV/cm for 59μs. PEF treated orange juice was filled into sanitized glass bottles in the glove box, minimizing the amount of air headspace to 1%.

Microbial Analysis of PEF Treated Orange Juice

PEF treated juice was tested for pathogens, *Salmonella spp*, *Listeria monocytogenes* and *E. coli* O157:H7, one 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 22C. For each dilution prepared, duplicate samples were plated using surface plating method. PCA plates were incubated at 30C for 48 h before enumeration.

Analysis of °Brix and pH

Soluble solids content was measured as °Brix using a hand held refractometer (Fisher, Pittsburgh, PA). The pH of orange juice was measured using an Orion pH meter (Model 370, Beverly, MA) at room temperature.

Flavor Analysis

Flavor compounds of orange juice were analyzed by headspace solid phase microextraction gas chromatography (SPME-GC) (Jia *et al.* 1998). Standard flavor compounds, d-limonene, octanal and ethyl butyrate were purchased from Aldrich Chemical Co. (Milwaukee, WI). A SPME fiber with 100 μm polymethylsiloxane (PDMS) coating, serum bottles, Teflon coated rubber septa and aluminum caps were obtained from Supelco, Inc (Bellefonte, PA).

Flavor compounds in the orange juice were identified by comparing the retention times of gas chromatographic peaks with that of standard compounds under the identical experimental conditions. During storage, flavor compounds were evaluated based on percentage of relative loss. GC peak area found at zero time was taken 100% as initial value.

D-limonene, octanal and ethyl butyrate were tested in PEF processed orange juice at 4 and 22C. 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.

Data Analysis

The entire experiment was duplicated as replication. Therefore, each data point represents an average of four measurements. Data were analyzed statistically using SPSS statistical package (SPSS 1999). Analysis of variance (two way ANOVA) was carried out to determine if storage temperature and time had significant effects on the percent flavor loss. Specific differences were determined by the Tukey test at $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Validation of Sanitized Glove Box System

The concentration and amount of H_2O_2 necessary for sanitation of the bottles were determined by preliminary study. A H_2O_2 residue below 0.1 ppm per container is required right before filling with food product as regulated by FDA (Robertson 1993).

Dipping into 3% H_2O_2 bath and rinsing with sterile water provided a residue less than 0.1 ppm and this procedure was followed for sanitizing the bottles before filling.

Results are presented in Tables 1 and 2 for the nutrient broth incubated at 22C and 37C for 2 weeks. The media filled in chemically cleaned or autoclaved bottles had very low absorbances (0.000-0.005) at 600 nm. There was no growth observed on PCA and PDA plates of nutrient broth filled in chemically cleaned or autoclaved bottles. Negative controls had no growth, as expected. Growth was evident in the positive control which had absorbance of 0.221 with total plate counts of 6.56 log CFU/mL and, yeast and mold counts of 6.32 log CFU/mL at 22C for 2 weeks.

Very similar results were observed after 2 week incubation at 37C. The positive control had total plate count of 5.68 log CFU/mL when absorbance was 0.122. PCA and PDA counts at 37C were lower than that of at 22C, which is optimum growth temperature of yeast and molds, major flora of orange juice. Yeom (2000) reported that major microorganisms in the orange juice were yeast.

In summary, the results showed that the media filled in chemically cleaned bottles using a sanitized glove box did not have turbidity or microbial growth at incubation temperatures of 22C and 37C for two weeks. Nonturbid samples with no growth validated our cleaning and filling procedures for the glove box packaging system.

TABLE 1.
ABSORBANCE, TOTAL PLATE AND YEAST & MOLD (Y&M) COUNTS OF NUTRIENT BROTH INCUBATED AT 22C FOR 2 WEEKS

Sample identification		Absorbance (600 nm)	Total Plate Count (Log CFU/ml)	Y&M (Log CFU/ml)
Negative control[1]		0.000	< 1	<1
Negative control[2]		0.000	<1	<1
Positive control [3]		0.221	6.56	6.32
Samples[4]	Glass	0.001	<1	<1
	PET	0.000	<1	<1
	HDPE	0.003	<1	<1
	LDPE	0.002	<1	<1

[1] Negative control: Media autoclaved at 121C for 30 min
[2] Media filled in autoclaved bottles in glove box
[3] Positive Control: Media inoculated with natural flora of orange juice
[4] Media filled in chemically cleaned bottles of various materials in glove box
The counts represented as < 1 log CFU Est/mL had no colonies from the lowest dilution which was 10^{-1}.

Microbial Stability of PEF Processed Orange Juice

Previous study showed that maximum microbial inactivation of orange juice was achieved with electric fields of 35kV/cm for 59 μs of total treatment time (Yeom 2000). The PEF treated orange juice using these processing conditions was reported negative for the pathogens, *Salmonella* spp, *Listeria monocytogenes* and *E. coli* O157:H7. Fresh orange juice had total plate count of 2.34 log CFU/mL. The total aerobic plate count (TPC) was less than 1 log CFU Est/mL right after the PEF treatment and also during storage of 112 days at 4C and 22C in glass bottles (Fig. 3).

Stability of pH and °Brix of PEF Processed Orange

Figure 3 shows the pH and °Brix of PEF processed orange juice during storage of 112 days at 4 and 22C. °Brix and pH values of fresh orange juice were not affected by PEF processing and also storage of 112 days at both temperatures. °Brix and pH of orange juice can be changed by consumption of soluble solids and organic acids by microorganisms. Since PEF processed

orange was microbiologically stable for 112 days, there was no change observed in °Brix and pH of the juice at 4 and 22C.

TABLE 2.
ABSORBANCES, TOTAL PLATE AND YEAST & MOLD (Y&M) COUNTS OF NUTRIENT BROTH INCUBATED AT 37C FOR 2 WEEKS

Sample identification		Absorbance (600 nm)	Total Plate Count (Log CFU/ml)	Y&M (Log CFU/ml)
Negative control[1]		0.000	< 1	<1
Negative control[2]		0.002	<1	<1
Positive control[3]		0.122	5.68	5.16
Samples[4]	Glass	0.004	<1	<1
	PET	0.002	<1	<1
	HDPE	0.004	<1	<1
	LDPE	0.005	<1	<1

[1] Negative control: Media autoclaved at 121C for 30 min
[2] Media filled in autoclaved bottles in glove box
[3] Positive Control: Media inoculated with natural flora of orange juice
[4] Media filled in chemically cleaned bottles in glove box
The counts represented as < 1 log CFU Est/mL had no colonies from the lowest dilution which was 10^{-1}.

Flavor Analysis

Loss (%) of PEF processed orange juice flavor compounds during 112 day storage at 4C and 22C in glass is shown in Fig. 4. No significant effects of storage time and temperature were observed on the relative loss (%) of d-limonene ($p > 0.05$). This indicated that d-limonene, a hydrocarbon flavor compound, in the PEF treated orange juice stayed stable for 112 days in glass bottles at 4 and 22C. Ackerman and Wartenberg (1986) also reported that no significant change was observed in the retention of d-limonene of aseptically processed orange juice stored for 7 weeks at 10C in glass bottles.

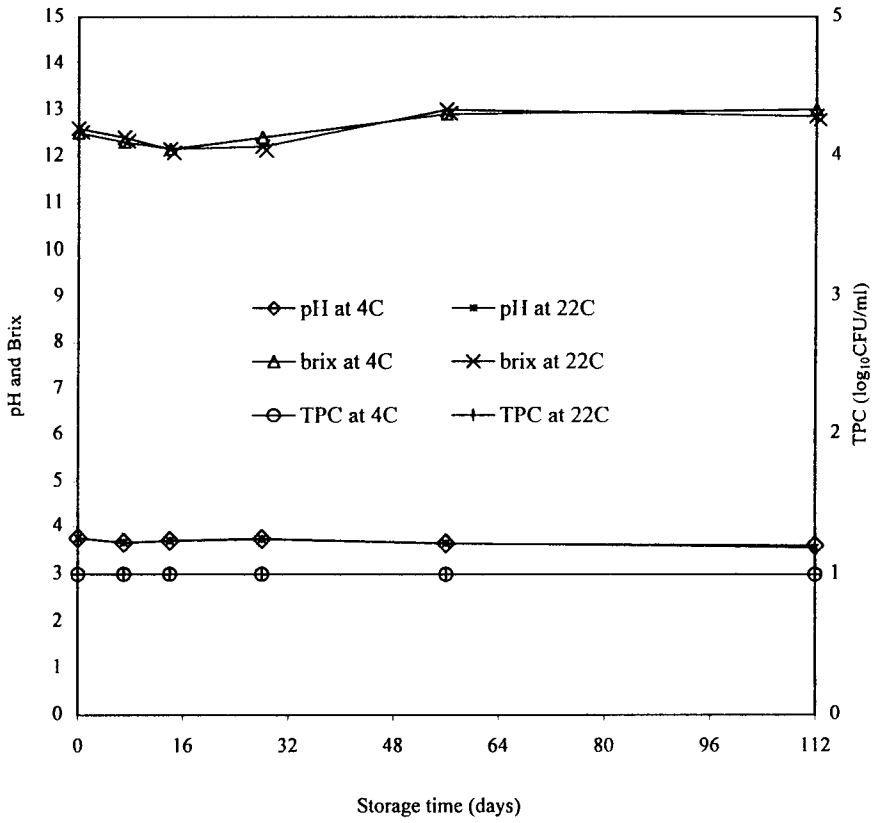

FIG. 3. °BRIX AND pH AND TOTAL AEROBIC PLATE COUNTS (TPC) OF PEF PROCESSED ORANGE JUICE DURING STORAGE AT 4 AND 22C

However, both storage time and temperature played significant ($p \leq 0.05$) roles on % loss of octanal. There was significant increase in % loss of octanal within 7 days at 4C and 2 days at 22C. The difference between 4C and 22C became significant after 2 day storage. As shown by Fig. 4, significant changes in % loss of octanal were observed in the first few days of storage and stayed more stable at 4C compared to storage at 22C for the remainder of the storage time ($p \leq 0.05$). Another study showed that octanal and decanal retention of aseptically processed orange juice in glass were reduced by about 35% and 70% respectively, within 14 days of storage due to flavor degradation (Ackerman and Wartenberg 1986).

Significant increase was observed in the loss of ethyl butyrate of PEF treated orange juice stored for 112 days at 4 and 22C in glass bottles as shown

by Fig. 4 ($p \leq 0.05$). During 112 days of storage there was 29% loss of ethyl butyrate at 4C. At 22C, 35% loss of ethyl butyrate was observed after 112 days.

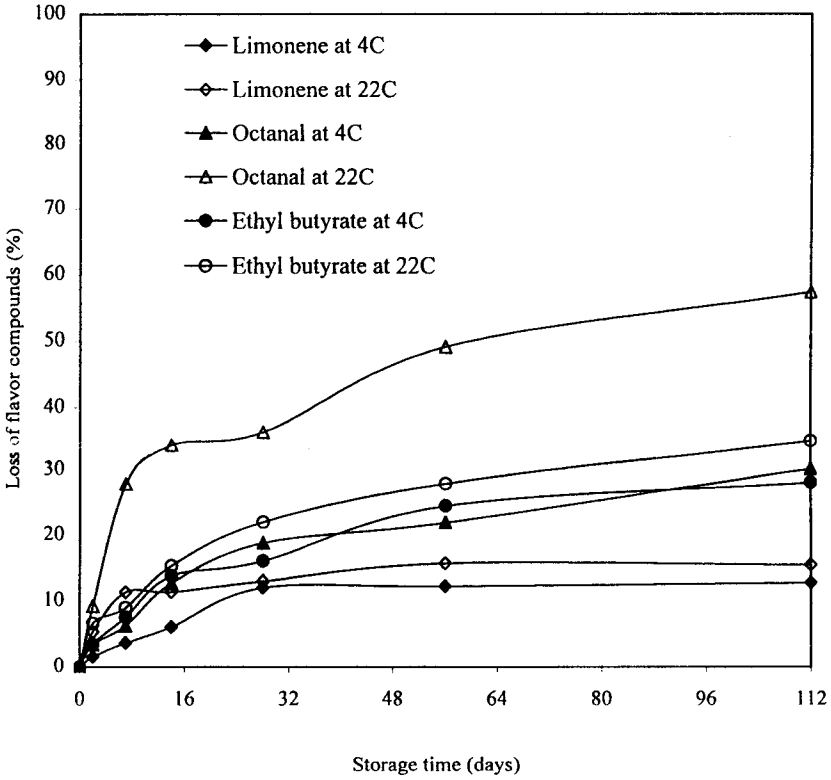

FIG. 4. STABILITY OF FLAVOR COMPOUNDS OF PEF PROCESSED ORANGE JUICE IN GLASS BOTTLES DURING STORAGE AT 4 AND 22C

CONCLUSIONS

PEF processed orange juice was microbiologically stable for 112 days of storage at 4C and 22C using a sanitary glove box system integrated with PEF processing. However, elevated storage temperature (22C) caused significant loss of ethyl butyrate and octanal. Therefore, the PEF processed orange juice had a shelf-life of more than 16 weeks at 4C which is 6-8 weeks for aseptically processed juice in cardboard packages at the market.

This system offers flexibility of using various packaging materials with different sizes and shapes. The system will provide better understanding of

quality changes that occur during storage of PEF or heat-treated products in different packaging materials and aid in the selection of compatible packaging materials that match the required shelf-life.

ACKNOWLEDGMENTS

This project was funded by the NSF CAPPS Center. PEF processing equipment is provided by a project funded by the US Army Natick RD&E Center. Product is provided by Minute Maid Co. The authors are thankful to Dr. S. Palaniappan of Minute Maid Co. for his technical advice and Dr. C. Wang for operating the pilot plant PEF system.

REFERENCES

ACKERMAN, P.W. and WARTENBERG, E.W. 1986. Shelf-life of citrus juices: a comparison between different packages, pp. 143. Report of 19th Symposium of International Federation of Fruit Juice Producers, Den Haah.

AYHAN, Z. and ZHANG, Q.H. 2000. Wall thickness distribution in thermoformed food containers produced by a Benco aseptic packaging machine. Poly. Eng. Sci. *40*(1), 1–10.

BAYLISS, C.E. and WAITES, W.M. 1979. The combined effect of hydrogen peroxide and ultraviolet radiation on bacterial spores. J. Applied. Bacteriol. *47*, 263–269.

BAYLISS, C.E. and WAITES, W.M. 1980. The effect of hydrogen peroxide and ultraviolet radiation on non-sporing bacteria. J. Applied. Bacteriol. *48*, 417–422.

BAYLISS, C.E. and WAITES, W.M. 1982. Effects of simultaneous high intensity ultraviolet irradiation and hydrogen peroxide on bacterial spores. Food. Tech. *17*, 467–470.

JIA, M., ZHANG, Q.H. and MIN, D.B. 1998. Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. J. Agric. Food Chem. *46*, 2744–2747.

MAUNDER, D.T. 1977. Possible use of ultraviolet sterilization of containers for aseptic packaging. Food Tech. *31*(4), 36–37.

ROBERTSON, G.L. 1993. *Food Packaging Principles and Practice*. Marcel Dekker, New York.

RUHLMAN, K.T. 1999. Product examination and reformulation for pulsed electric field processing. Thesis. The Ohio State University, Columbus, OH.

STANNARD, C.J., ABBISS, J.S. and WOOD, J.M. 1983. Combined treatment with hydrogen peroxide and ultra-violet irradiation to reduce microbial contamination levels in pre-formed food packaging cartons. J. Food Prot. *46*(12), 1060–1064.

STANNARD, C.J. and WOOD, J.M. 1983. Measurement of residual hydrogen peroxide in preformed food cartons decontaminated with hydrogen peroxide and ultraviolet irradiation. J. Food Prot. *46*(12), 1074–1077.

STREAKER, C.B. 1999. Design, construction, and validation of a pilot plant fluid handling system for pulsed electric field processing and aseptic packaging of foods. Thesis. The Ohio State University, Columbus, OH.

WADSWORTH, K.D. and BASETTE, R. 1985. Laboratory scale system to process ultrahigh-temperature milk. J. Food Prot. *48*(6), 530–531.

YEOM, H.W. 2000. The effects of pulsed electric field on the quality of foods. Ph.D. dissertation. The Ohio State University, Columbus, OH.