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Impact of Thermal and Nonthermal Processing Technologies on Unfermented Apple Cider Aroma Volatiles

ZAREENA AZHU VALAPPIL,[†,§] XUETONG FAN,[§] HOWARD Q. ZHANG,[§] AND RUSSELL L. ROUSEFF*,[†]

Institute of Food and Agricultural Sciences, Citrus Research and Education Center, and Food Science and Human Nutrition, University of Florida, Lake Alfred, Florida 33850, and Eastern Regional Research Center, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038

Aroma composition and microbial quality of identical lots of apple cider treated by pulsed electric field (PEF), ultraviolet irradiation (UV), or thermal pasteurization stored at 4 °C were compared at 0 and 4 weeks. Conditions were optimized to achieve identical 5 log reductions in *Escherichia coli* K12 for each treatment. PEF and thermal pasteurization maintained acceptable microbial quality for 4 weeks, but UV samples fermented after 2 weeks. Twenty-eight volatiles were quantified using gas chromatography−mass spectrometry (GC-MS) and odor activity values (OAV) determined. OAVs of 69:hexyl acetate, 41:hexanal, 25:2-methylbutyl acetate, 23:2-methyl ethyl butyrate, and 14:2-(*E*)-hexenal were observed for the control cider. Significant differences ($p < 0.05$) in the levels of these odorants were observed between treated apple ciders only after 4 weeks of storage. Thermal samples lost 30% of the major ester and aldehyde volatiles during storage with significant decreases ($p < 0.05$) in butyl acetate, 2-methylbutyl acetate, hexanal, and 2-(*E*)-hexenal. In UV cider, hexanal and 2-(*E*)-hexenal were completely lost after 4 weeks of storage. Microbial spoilage in UV cider after 4 weeks of storage was chemically confirmed by the detection of the microbial metabolite 1,3-pentadiene. PEF cider lost <2% of its total ester and aldehydes after 4 weeks of storage and was preferred by 91% of the sensory panel over thermally treated cider.

KEYWORDS: Cloudy apple juice; triangle tests

INTRODUCTION

Apple cider in the United States refers to the nonalcoholic beverage produced by pressing apples. It is the nonclarified version of apple juice sometimes called cloudy apple juice and is characterized by a tarter flavor. In recent years, contamination of apple cider and apple juice by *Salmonella* sp., *Escherichia coli* O157:H7, and *Cryptosporidium parvum* has been reported, posing serious health risks to the public (*1*, *2*). Under the federal Juice HACCP rule published in 2001, juice processors must implement treatments to reduce the population of "pertinent" microorganisms by 5 log cycles. The "pertinent" microorganism is defined as the most resistant microorganism of public health significance that is likely to occur in the juice in question. At present, *E. coli* O157:H7 and *C. parvum* are accepted as the pertinent organisms for apple juice/cider.

Thermal processing is the most commonly used technique to reduce spoilage and pathogenic microorganisms as well as to inactivate enzymes in juice (*3*). Unfortunately, thermal pasteurization can produce undesirable quality changes such as loss of color and flavor in addition to reducing the nutritional quality of juice (*4*, *5*). Recently, nonthermal processing alternatives such as pulse electric field (PEF) and ultraviolet (UV) treatment have been examined for their efficacy in extending shelf life and enhancing juice, pulp, or cider microbial safety while minimizing quality and nutritional losses (*3*, *6*−*8*). PEF treatment utilizes short bursts of electricity, which alter the microorganism's membrane permeability and eventually disrupt or "break" the cell membrane in a process known as electroporation (*9*). UV light inactivates bacteria and viruses by causing cross-linking between neighboring DNA strands, thus preventing cell replication (*10*). Although a large amount of microbial information concerning thermal and nonthermal processed apple juice can be found in the literature, little information exists on the effects of these processes on flavor of apple cider. A decrease in ester concentrations due to thermal pasteurization of apple juice has been reported (*11*−*13*), and a single study compared eight apple juice volatiles in PEF and thermally processed juice (*13*). Because a major motivation for nonthermal processing technologies is a minimal change to organoleptic and nutritional properties, an in-depth analysis of the effect of the above processes on the flavor profile of apple cider and its relation to

* Corresponding author [telephone (863) 956-1151; fax (863) 956-4631; e-mail rlr@crec.ifas.ufl.edu].
[†] University of Florida.
[§] U.S. Department of Agriculture.

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sensory quality is necessary. Moreover, data currently available involve treatments in which thermal processing conditions attained more than the required 5 log inactivation and were overly severe in terms of time and temperature. Thus, it is not possible to fairly compare quality differences between thermal and nonthermal processes under such unequal conditions.

The present work focused on (1) comparing the aroma volatiles from thermally processed apple ciders with nonthermal treatments wherein each treatment was optimized to achieve a 5 log reduction in microorganisms and (2) examining changes in aroma active and major volatiles in the treated apple ciders stored at 4 °C at weeks 0 and 4.

MATERIALS AND METHODS

Apple Cider. Two hundred and twenty-seven liters of unpasteurized apple cider with no additives was procured from Ziegler Juice Co. (Lansdale, PA). A varietal blend of apples including Ginger Gold, Golden Delicious, Red Delicious, Empire, Macintosh, Gala, and Cortland was used for the juice in this study. Apples were inspected for quality (no visible mold or decay) and were washed and graded to remove loose stems, leaves, and other foreign materials. After further inspection and grading, apples were passed through a Westphalia decanter press to express juice and separate solids. No additives were added to the cider. The cider was packaged into high-density polyethylene (HDPE) containers. The containers were transported to the USDA facility within 2 h of juicing and stored in a −17 °C deep-freezer. The juice remained for 6 days in frozen condition before being thawed at 4 °C overnight for processing.

Chemicals. Standard compounds ethyl acetate, ethyl propanoate, 2-methylpropyl acetate, methyl butyrate, ethyl butyrate, ethyl 2-methylbutyrate, hexanal, butyl 2-methylacetate, butyl propanoate, 2-methyl-1-butanol, (*E*)-2-hexenal, ethyl hexanoate, hexyl acetate, (*E*)-2-hexen-1-ol acetate, 6-methyl-5-hepten-2-one, hexyl propanoate, (*Z*)-2-hexen-1-ol, hexyl butyrate, hexyl 2-methylbutyrate, hexyl hexanoate, α-farnesene, methional, phenylacetaldehyde, octanal, *β*-damascenone, hexane, methanol, and 1-butanol were purchased from Aldrich (St. Louis, MO). Butyl acetate, benzaldehyde, 1-octanol, butyl butyrate, propyl butyrate, 1-hexanol, *p*-allylanisole, butyl 2-methylbutyrate, pentyl acetate, propyl hexanoate, dimethyl sulfide, and alkane standard solution (C8−25) were purchased from Fluka (Steinheim, Switzerland).

Apple Cider Processing. Preliminary experiments were performed to determine equivalent processing conditions using heat, pulsed electric field, and ultraviolet radiation for apple cider. Pasteurized apple cider (Ziegler Juice Co.) was inoculated with *E. coli* K12 (ATCC 23716) from a stationary phase culture to give approximately 7 log CFU/mL population. Microbial assays were conducted for ciders: heated from 60 to 76 °C, UV exposure times of 17−68 s, and 5−23 kV/cm electric field strengths for PEF treatment. Final optimized processing conditions resulted in approximately 5 log reductions of *E. coli* K12 and were used for the remainder of the study.

Heat Treatment. Apple cider was heat pasteurized using a miniature-scale HTST processing system (Armfield, Jackson, NJ; FT74-30-MkIII-33-34). The system included a feed tank, a peristaltic pump, a plate heat exchanger (comprising a regeneration section, a heating section, and a cooling section that mimics industrial scale systems), a holding tube, thermocouples, and an electric-powered hot water boiler and pump. Apple cider was introduced into the system via the feed tank at a flow rate of 15 L/h with hot water circulation set at 76 °C such that the juice is held at 76 °C for 1.3 s and cooled rapidly. The inlet and outlet temperatures were continuously monitored and were in ranges of 13−17 and 24−30 °C, respectively, during processing.

Unpasteurized apple cider passed through the thermal processing system without heat at room temperature was used as control sample for microbial studies.

UV Treatment. A low-pressure mercury lamp surrounded by a coil of UV transparent Chemfluor tubing was used for UV processing of apple cider (*14*). The UV lamp assemblies contained a bipin base (model S130 120 LPF, Lithonia Lighting, Conyers, GA) and a 30 W bulb (G30T8, Buylighting.com, Burnsville, MN) that generated 90% of its energy at a wavelength of 254 nm. Norton Chemfluor 367 tubing (Cole-Parmer, Vernon Hills, IL) with an internal diameter of 3.2 mm and a wall thickness of 1.6 mm was wrapped around the entire length of the UV lamp. The length of the tubing in contact with the lamp was 14 m. The experimental system included a feed tank, a peristaltic pump, and three UV lamps of the same dimensions connected in series. Cider was pumped through the tubing at flow rates of 25 L/h, which translates to a treatment time of 17 s per bulb. The energy used was 34 J/mL. Apple cider was exposed to a total treatment time of 51 s. The inlet and outlet temperatures recorded were between 10 and 15 °C during processing.

PEF Treatment. A bench scale continuous PEF system (OSU-4F, Ohio State University, Columbus, OH) was used to treat the inoculated apple cider. The system consisted of six cofield treatment chambers with a diameter of 23 mm and a gap distance of 29 mm between electrodes connected in series. Applied voltage and current were monitored by a digital oscilloscope (Tektronix DS210, Beaverton, OR). The cider was pumped through the system using a digital gear pump (Cole-Parmer 75211-30) at a flow rate of 7.2 L/h. The square wave pulse duration was 2.5 μs, and the electric field strength was 23 kV/cm. The mean total treatment time was calculated as 150 μs. Apple cider sequentially flowed through all of the chambers via steel coils immersed in a water bath set at 48 °C. The inlet and outlet cider temperatures were continuously monitored using thermocouples and were in the ranges of 30−34 and 49−51 °C, respectively.

Packaging and Storage. Processed apple cider was collected directly from the processing unit outlet into sterile 1 L media glass bottles (Corning Inc., Corning, NY) inside a sanitary laminar hood equipped with a HEPA air filter (Forma Scientific Inc., Marietta, OH). The hood was sanitized by UV lighting at 254 nm for 30 min before use and then swiped with 100% alcohol. The packaged juice was stored at 4 °C for storage studies.

Storage Study. Storage studies were conducted for 4 weeks on the various processed apple ciders stored at 4 °C. Microbial analyses were performed every week, whereas volatile and sensory analyses were done for weeks 0 and 4. Fresh unpasteurized cider samples maintained at −17 °C were used as control for volatile and sensory analysis.

Microbial Stability. The microbial analysis was performed according to the method of Fan (*15*). Microbial tests were conducted every week during the 4 weeks of storage. Total aerobic plate count and yeast and mold counts were determined using plate count agar (PCA) and yeast and mold (YM) pertrifilms. The PCA plates were incubated at 37 °C for 24 h, whereas YM plated were incubated at room temperature for 5 days before counting. All samples were analyzed in duplicate, and two replicates of each dilution were prepared and plated.

SPME Extraction of Cider Volatiles. Aliquots (27 mL) of apple cider were placed in 40 mL glass vials with screw caps containing Teflon-coated septa similar to the procedure used by Dreher and coworkers (*16*). The cider was equilibrated for 10 min at 36 °C with stirring. A 2 cm 50/30 μm, DVB/Carboxen/PDMS Stableflex (Supelco, Bellefonte, PA) SPME fiber was exposed in the equilibrated headspace for 45 min at 36 °C. The fiber was desorbed for 5 min in the GC injection port at 250 °C. All samples were analyzed in quadruplicate.

GC-MS Analysis of Apple Cider Volatiles. A gas chromatograph−mass spectrometer (6890N GC, 5973N MS, Agilent Technologies, Santa Clara, CA) was used for separation and analysis of volatiles. The instrument was also equipped with a pulsed flame photometric detector (PFPD; model 5380, OI Analytical, College Station, TX).

Samples were run separately on a polar DB-Wax and nonpolar DB-5 column with identical dimensions (30 m × 0.32 mm × 0.5 μm from J&W Scientific, Folsom, CA). The column oven temperature was programmed from 40 to 110 °C at 7 °C /min and then raised at 15 °C /min to 250 °C with a 3 min hold. Injector and detector temperature was 250 °C. Mass spectrometry conditions were as follows: transfer line temperature at 275 °C, mass range of 30−300 amu, scan rate of 5.10 scan/s, and ionization energy of 70 eV. Helium was used as the carrier gas at a flow rate of 2 mL/min. Mass spectral matches were made by comparison with NIST 2002 standard spectra. Authentic standards were used for confirmation. Alkane linear index values were determined on both columns (*17*).

Quantification of Apple Cider Volatiles. Volatile free apple cider was prepared according to the method of Fan and co-workers (*18*) by concentrating 500 mL of apple cider using a vacuum rotary evaporator (Brinkmann Instruments Inc., Westbury, NY) from 11.0 to 28.0 °Brix. Any residual volatiles were extracted with hexane and discarded. Any trace hexane residue in the concentrated juice was removed using the vacuum rotary evaporator. The concentrated juice was diluted back to the initial 11.0 °Brix using distilled water and checked for residual volatiles. A mixture of 29 standards in methanol was serially diluted with deodorized apple cider and added in concentrations 0.5−3 times their estimated concentrations in cider. Volatiles were analyzed by SPME-GC-MS using the same conditions described under GC-MS Analysis of Apple Cider Volatiles. A quantitation database for standards was created using MSD Chemstation software. Response factor curves were created by plotting target ion count (base peak) against standard concentrations in volatile-less apple cider. Parameters used for compound identification were retention time, target ion, and secondary ions. Compounds were quantified using target ion values and response factors generated from standard curves.

Sensory Evaluation. A discriminative triangle test (*19*) was employed to orthonasally detect differences in aroma between unpasteurized and pasteurized apple cider for 0 and 4 weeks of storage at 4 °C. The statistical power for the triangle test was recorded as 0.9. The analysis was conducted in a sensory panel facility at the Eastern Regional Research Center (ERRC; Wyndmoor, PA), which has six booths with computers. The sensory analysis was designed and conducted using Compusense five (Compusense Inc., Ontario, CA). Samples were prepared by pouring 40 mL of apple cider into 100 mL glass bottles that were then closed with airtight caps. The bottles were stored in boxes at 4 °C overnight. On the day of testing, the bottles were taken out 1 h before the testing. Cider temperature was 10−12 °C during testing. Testing was performed under red light so that color and other visible differences were masked from the panelists. Each panelist was given five sets of apple cider samples (three samples per set), one at a time. All samples were randomly assigned three-digit codes. The order of presentation of sample sets among panelists was also randomized. In total, 50 untrained panelists from the ERRC evaluated the samples, and each panelist performed five triangle tests, which included control versus PEF, control versus UV, control versus thermal, thermal versus UV, and thermal versus PEF treated apple cider samples. After each test, the panelists were also asked for the preferred sample among the three test samples and to give a reason for the preference.

Statistical Analysis. All data were subjected to statistical analysis using SAS 9.1 (SAS Institute Inc., Raleigh, NC). Data were from a single sample for each treatment. Each sample was analyzed in quadruplicate. The tests for statistical significance of difference between treatments for storage data on volatiles was performed by analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. The effect of treatments on means of samples between treatments was compared using Duncan's multiple-comparison test ($\alpha = 0.05$). Microbial data were analyzed using MS Excel, 2003, and standard deviations are presented.

RESULTS AND DISCUSSION

Effect of Thermal and Nonthermal Processing on Microbial Stability during Storage. The total aerobic count for processed apple cider was maintained below 2 ± 0.24 log CFU/mL through 4 weeks of storage at 4 °C. The yeast and mold count in initial cider at 0 days was 2 ± 0.12 log CFU/ mL. PEF and thermally processed apple cider did not show any yeast and mold growth throughout storage. After 2 weeks of storage, the yeast and mold count in UV-treated samples increased to 3.8 ± 0.52 log CFU/ mL. A visible mold growth was observed after 4 weeks storage. Deterioration in the quality of UV-treated apple juice due to yeast and mold growth after 2 weeks of storage has also been observed by others (*7*, *8*). Donahue and co-workers confirmed the presence of injured microbial cells in UV-treated cider using selective enrichment media. They suggested that the decrease in efficiency of cell inactivation by UV could possibly be due to high turbidity and inadequate mixing of apple cider as it flows through the tubes (*8*).

Volatile Composition of Initial Apple Cider. A total of 34 volatile compounds were identified in the initial untreated apple cider after separation on a DB-Wax column and identified using MS (**Table 1**). Apple juice volatiles have been previously quantified using both external and internal standard methods (*20*−*22*). The current study is the first report in which apple cider volatiles have been quantified using external calibration from an odorless apple cider to compensate for matrix effects. Response factor plots were determined for 29 compounds in **Table 1**. No response factors were determined for peaks 2, 3, 6, 14, and 30 because their signal/noise ratios were less than 3. The standard addition plots for most compounds had a linear correlation coefficient (R^2) of >0.95.

Esters, aldehydes, and alcohols comprised the major volatiles in apple cider accounting for 40, 43, and 16% of the total volatiles identified, respectively. However, it should be kept in mind that the volatile composition of apple juice depends on various factors such as variety, maturity, and storage conditions of fruit used for pressing (*23*, *24*). Certain apple cultivars such as 'Jonathan' and 'Cox Orange Pippin' are reported to have 5−100-fold higher aldehyde contents compared to 'Golden Delicious' cultivar (*23*). Apple juice also has higher C6-aldehyde concentration compared to fruit due to oxidation of fatty acids linoleic and linolenic acid by lipoxygenases soon after crushing of apples (*25*). Hexanal and 2-(*E*)-hexenal were the most abundant aldehydes identified in apple cider from this study (see **Table 1**).

Apple cider esters can be classified into acetic, butyric, propanoic, and hexanoic groups. Acetate esters are reported to be the major volatiles in apple juice, and high concentrations of hexyl acetate and butyl acetate are considered to be characteristic of many apple cultivars (*23*, *24*, *26*). The most abundant acetate esters in this study were hexyl acetate, 2-methylbutyl acetate, and butyl acetate.

Odor Activity Values (OAV). To assess the contribution of each volatile to apple cider aroma, OAVs were calculated as the ratio of concentrations found in apple cider to their odor threshold value in water (**Table 2**) (*27*−*29*). Apple cider odor threshold values were not available. Aqueous threshold values should be similar to actual apple cider values because apple cider is approximately 90% water. The highest OAV values found in initial apple cider were for hexyl acetate (69), hexanal (41), 2-methylbutyl acetate (25), 2-methylethyl butyrate (23), and 2-(*E*)-hexenal (14). These results are consistent with the work of Fuhrmann et al., who reported 2-methylethyl butyrate, hexyl acetate, and 2-methylbutyl acetate to be major contributors to the fruitiness of apple aroma in 'Elstar' and 'Cox Orange' apple cultivars (*30*). The C6 aldehydes, hexanal and 2-(*E*)-hexenal, are responsible for the green or fresh aroma of apple cider and are essential to apple juice aroma due to their high correlation with apple aroma intensity (*31*). Durr et al. have shown that even though esters give the fruity aroma to cider, the concentration of aldehydes is essential for the sensory impression of juice odor. Alcohols such as 1-hexanol are low aroma impact components and have been identified as negative contributors to apple aroma (*32*). In this study, only UV-treated cider possessed a hexanol OAV that might suggest it was aroma active.

Effect of Treatment and Storage on Volatiles. The concentration of volatiles was not significantly ($p < 0.05$) affected by treatments immediately after processing (week 0 data not

Table 1. Effect of Thermal and Nonthermal Treatments on Apple Cider Volatiles Compared to Fresh Untreated Cider after 4 Weeks of Storage at 4 °C[a]

peak	compound[b]	LRI Wax	quant ion (*m*/*z*)	mean concentration (μg/L) (week 4) control[c]	thermal	PEF	UV
1	1,3-pentadiene	714		nd a	nd a	nd a	nq b*
2	dimethyl sulfide*	753	PFPD	nq	nq	nq	nq
3	ethyl acetate*	810		nq	nq	nq	nq
4	ethyl propanoate**	961	57	0.73 ± 0.09b	0.73 ± 0.04 b	1.00 ± 0.07 a	1.55 ± 0.15 a
5	methyl butyrate**	994	74	2.53 ± 0.17 b	2.46 ± 0.90 b	2.73 ± 0.20 a	2.93 ± 0.35 ab
6	2-methylpropyl acetate*	1024		nq	nq	nq	nq
7	ethyl butyrate**	1050	71	3.00 ± 0.52 a	2.86 ± 0.20 b	3.65 ± 0.55 a	3.75 ± 1.35 a
8	ethyl 2-methylbutyrate**	1067	102	2.36 ± 0.27 a	2.30 ± 0.43 a	2.60 ± 0.13 a	2.00 ± 0.12 a
9	butyl acetate**	1088	56	120 ± 6.83 a	98.8 ± 2.09 b	131 ± 9.38 a	89.4 ± 1.95 b
10	hexanal**	1098	56	203 ± 8.73 a	133 ± 13.2 b	219 ± 13.1 a	nd c
11	butyl 2-methylacetate**	1135	70	127 ± 6.64 a	96.7 ± 1.48 b	128 ± 5.02 a	121 ± 9.53 a
12	propyl butyrate**	1137	71	6.50 ± 0.34 b	5.70 ± 0.12 b	10.0 ± 0.25 a	5.55 ± 0.76 b
13	butyl propionate**	1153	57	4.34 ± 0.36 a	4.23 ± 0.37 a	5.13 ± 0.10 a	4.56 ± 0.26 a
14	1-butanol*	1158		nq	nq	nq	nq
15	pentyl acetate**	1183	70	1.30 ± 0.31 a	0.76 ± 0.07b	1.46 ± 0.23 a	0.36 ± 0.10 b
16	2-methyl-1-butanol**	1214	57	6.83 ± 3.25 d	15.06 ± 1.17 c	32.1 ± 3.73 b	56.60 ± 7.85 a
17	butyl butyrate**	1225	71	7.66 ± 0.41 b	6.80 ± 0.07 b	8.71 ± 0.83 a	4.56 ± 0.20 b
18	2-(*E*)-hexenal**	1227	69	231 ± 2.62 a	144 ± 3.39 c	186 ± 7.12 b	ndd
19	butyl 2-methyl butyrate**	1236	103	1.50 ± 0.09 b	1.43 ± 0.12 b	1.53 ± 0.10 a	1.50 ± 0.08 b
20	ethyl hexanoate**	1238	88	nd a	nd a	nd a	nd a
21	hexyl acetate**	1278	56	137 ± 10.74 a	43.2 ± 2.07 b	48.6 ± 8.92 b	11.7 ± 8.54 c
22	propyl hexanaoate**	1326	99	1.80 ± 0.26 a	nd b	nd b	nd b
23	(*E*)-2-hexen-1-ol acetate**	1342	67	0.57 ± 0.11 b	0.60 ± 0.17 b	0.73 ± 0.10 b	1.70 ± 0.12a
24	6-methyl-5-hepten-2-one**	1348	43	0.10 ± 0.03 a	0.10 ± 0.26 a	0.20 ± 0.05 a	0.10 ± 0.03 a
25	hexyl propionate**	1349	57	0.07 ± 0.00 ab	0.00 ± 0.00 b	0.13 ± 0.01 a	nd c
26	1-hexanol**	1366	56	164 ± 5.91 c	253 ± 6.24 b	241 ± 5.76 b	803 ± 12.83 a
27	(*Z*)-2-hexen-1-ol**	1423	57	0.17 ± 0.23 a	0.53 ± 0.48 a	0.16 ± 0.25 a	nd c
28	hexyl butyrate**	1432	71	5.34 ± 0.23 b	4.26 ± 0.29 b	6.93 ± 0.22 a	1.55 ± 0.12 c
29	hexyl 2-methylbutyrate**	1443	103	2.90 ± 0.22 a	3.06 ± 0.20 a	3.73 ± 0.18 a	3.40 ± 0.13 a
30	2-methyl-6-hepten-1-ol†	1480		nq	nq	nq	nq
31	benzaldehyde**	1546	106	3.73 ± 0.26 c	36.7 ± 3.73 b	62.2 ± 2.92 a	0.86 ± 2.20 c
32	1-octanol**	1568	56	nd a	nd a	nd a	10.45 ± 0.13 b
33	hexyl hexanaote**	1620	117	0.43 ± 0.00 a	0.40 ± 0.03 a	0.46 ± 0.00 a	nd b
34	*p*-allyl anisole**	1683	148	0.27 ± 0.07 a	0.20 ± 0.10 a	0.73 ± 0.15 b	0.13 ± 0.05 a
35	α-farnesene**	1747	93	bq	bq	bq	bq

[a] Mean concentrations given as μg/L ± standard deviations of quadruplicate analyses on single samples ($n = 4$); nq, not quantitated; bq, below quantitation; nd, not detected; different letters in the same row indicate significant differences ($p < 0.05$). [b] 1,3-Pentadiene was detected in stored UV cider, but it was not quantitated. *, compounds not quantitated as present in trace levels; **, 29 standards used for quantitation of volatiles; †, no standard available. [c] Control = fresh unpasteurized cider maintained at −17 °C for 4 weeks.

shown). However, pronounced volatile differences between treatments were observed after 4 weeks at 4 °C (**Table 1**). **Figure 1** compares the levels of volatiles with significant differences between treated and control apple ciders after 4 weeks of storage at 4 °C. Hexyl acetate concentrations decreased during storage in all processed apple cider samples with a concomitant increase in 1-hexanol by the action of residual esterases present in apple cider pulp (*33*). Thermally treated ciders lost 30% of their original ester and aldehyde contents during storage, with significant decreases ($p < 0.05$) in butyl acetate, 2-methylbutyl acetate, hexanal, and 2-(*E*)-hexanal concentrations (**Table 1** and **Figure 1**). Although thermal treatment is known to inactivate most enzymes, the cider in this study was exposed to a temperature of 76 °C for only 1.3 s. This may not have completely inactivated flavor-altering enzymes even though it reduced microbial concentrations to the desired level. UV-treated cider was characterized by a complete absence of hexanal and 2-(*E*)-hexenal, a decrease in hexyl acetate, and an increase in 1-hexanol compared to control after 4 weeks of storage. The increase in 1-hexanol concentration is associated with decreases in precursors such as hexanal, 2-(*E*)-hexenal, and hexyl acetate (*33*). PEF cider lost <2% of total ester and aldehyde volatiles during storage, suggesting that it more effectively inactivated indigenous enzymes than thermal or UV treatments. Significant decreases ($p < 0.05$) in volatile concentrations were observed only in hexyl acetate and 2-(*E*)-hexenal.

1,3-Pentadiene was detected only in UV samples after 4 weeks of storage. It is an unsaturated hydrocarbon produced by molds such as *Zygosaccharomyces* and *Penicillium* in beverages. It possesses a petroleum odor that is often associated with microbial spoilage (*34*).

An interesting change observed in both thermal and PEF cider was an increase in benzaldehyde concentrations after 4 weeks of storage. Sumitani and co-workers found a similar increase in benzaldehyde in high-pressure-treated peaches during storage due to release from its bound glycoside form (amygdalin) by action of β-glucosidases present in peach (*35*). Similar enzyme action is possible in thermal and PEF ciders because apple seeds are known to have amygdalin as a major constituent (*36*).

Effect of Treatment and Storage on OAV of Volatiles. OAVs were calculated for quantified volatiles in control and all treated samples to assess the impact of storage and treatment on apple cider aroma (**Table 2**). Significant odor value losses ($p < 0.05$) were observed for thermal and UV cider samples after 4 weeks of storage. Hexanal and 2-(*E*)-hexenal OAV values decreased 35 and 43%, respectively, in thermally treated cider during storage. Decreases in aldehyde odor values in thermally treated cider would likely reduce aroma strength as well as perceived freshness. Significant decreases ($p < 0.05$) in essential esters such as hexyl acetate and 2-methylbutyl acetate could further deteriorate the aroma quality of thermal cider.

In UV-treated cider, 100% of the original hexanal and 2-(*E*)-hexanal was lost during 4 weeks of storage. During the same

Table 2. Effect of Thermal and Nonthermal Treatments on Odor Activity Values (OAV) of Apple Cider Volatiles after 4 Weeks of Storage at 4 °C

compound[a]	RI (Wax)	OT[b] (μg/L)	OAV control[c]	thermal	PEF	UV
esters						
ethyl propanoate	961	10	<1 a	<1 a	<1 a	<1 a
methyl butyrate	994	60	<1 a	<1 a	<1 a	<1 a
ethyl butyrate	1050	1	3 a	3 a	4 a	4 a
ethyl 2-methylbutyrate	1067	0.1	23 a	23 a	24 a	20 b
butyl acetate	1088	66	2 a	1 a	2 a	1 a
butyl 2-methylacetate	1135	5	25 a	19 b	26 a	24 a
propyl butyrate	1137	18	<1 a	<1 a	<1 a	<1 a
butyl propionate	1153	25	<1 a	<1 a	<1 a	<1 a
pentyl acetate	1183	43	<1 a	<1 a	<1 a	<1 a
butyl butyrate	1225	100	<1 a	<1 a	<1 a	<1 a
butyl 2-methylbutyrate	1236	17	<1 a	<1 a	<1 a	<1 a
ethyl hexanoate	1238	1	—	—	—	—
hexyl acetate	1278	2	69 a	22 b	24 b	6 c
hexyl propionate	1349	8	<1 a	− b	<1 a	− b
hexyl butyrate	1432	250	<1 a	<1 a	<1 a	<1 a
hexyl 2-methylbutyrate	1443	22	<1 a	<1 a	<1 a	<1 a
aldehydes						
hexanal	1098	5	41 a	27 b	44 a	0 c
2-(*E*)-hexenal	1227	17	14 a	8 b	11 c	0 d
benzaldehyde	1546	350	<1 a	<1 a	<1 a	<1 a
alcohols						
2-methyl-1-butanol	1214	300	<1 a	<1 a	<1 a	<1 a
1-hexanol	1366	500	<1 a	<1 a	<1 a	2 b
1-octanol	1568	130	− a	− a	− a	<1 b
(*Z*)-2-hexen-1-ol	1423	70	<1 a	<1 a	<1 a	<1 a
others						
6-methyl-5-hepten-2-one	1348	50	<1 a	<1 a	<1 a	<1 a

[a] Compounds identified on the basis of RI on DB-Wax and DB-5 column using standards. [b] OT, odor threshold values in water from the literature (*27*– *29*). [c] Control refers to fresh unpasteurized cider maintained at −17 °C for 4 weeks.

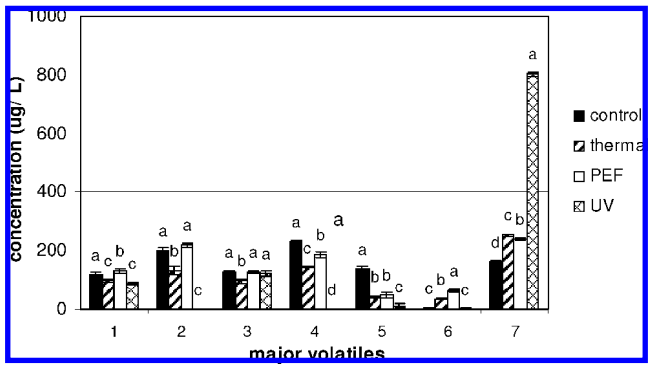

Figure 1. Effect of treatment and storage (after 4 weeks) on major volatiles of apple cider: 1, butyl acetate; 2, hexanal; 3, 2-methylbutyl acetate; 4, 2-(*E*)-hexanal; 5, hexyl acetate; 6, benzaldehyde; 7, 1-hexanol; a−d, different letters for each volatile indicate significant difference ($p < 0.05$); control refers to fresh unpasteurized cider maintained at −17 °C for 4 weeks.

time the OAV for 1-hexanol increased from 0 to 2 in UV cider only. This volatile has a green, musty aroma and is characterized as a negative contributor to apple aroma. Bult and co-workers demonstrated that an increase in 1-hexanol concentration led to a higher "nuts−musty" rating and a lower "apple" rating in an apple model solution (*32*). In addition to odorant losses, UV-treated cider also developed a perceivable fermented odor after 4 weeks of storage due to microbial spoilage. Due to its high odor threshold, benzaldehyde OAVs were <1 for all treatments. Therefore, increases in benzaldehyde concentrations observed in PEF and thermal ciders probably did not affect the aroma of these apple ciders.

PEF volatile losses during storage were minor (<2% loss of total ester and aldehydes) except for hexyl acetate. This key odorant was greatly diminished during storage in all treated ciders. In the case of PEF-treated cider, only 35% was retained after 4 weeks of storage. However, losses of hexyl acetate were even greater in thermally treated and UV-treated ciders.

Aroma Sensory Studies. An aroma triangle test comparison between control (unpasteurized cider samples maintained at −17 °C for 4 weeks) and all treated apple ciders at 0 day found no significant difference at $p < 0.05$. However, after 4 weeks of storage at 4 °C, 22 of the 50 panelists detected a difference between the aroma of the thermally treated sample and the untreated cider (control). The aroma of thermally processed cider was less preferred compared to fresh untreated (control) samples. The aroma of UV-treated cider differed significantly ($p < 0.05$) from control. Thirty-six of the 50 panelists correctly detected the difference. Of those 36, 35 panelists (97%) preferred control apple cider compared to UV cider. No significant difference ($p < 0.05$) was detected by panelists between PEF-treated and control apple cider. Triangle test results indicated that UV- and PEF-treated samples differed significantly from heat-treated cider ($p < 0.05$). Ninety-one percent of the triangle test panelists who correctly differentiated between PEF and thermal cider preferred the odor of PEF-treated cider over that of thermally treated cider. The preference for PEF cider coincides with the greater retention of apple aroma volatiles in PEF cider compared to thermal cider. Preference results from this study do not necessarily reflect true consumer preference, and further sensory studies would be necessary to evaluate the processing effects on preference in the general consumer population.

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