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Effect of Gas Environment and Sorbate Addition on Flavor Characteristics of Irradiated Apple Cider during Storage

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Apple cider, with (0.1%) and without potassium sorbate, was packaged in polystyrene containers and exposed to three different gas environments: oxygen flush, nitrogen flush, and atmospheric air. To evaluate the effects of irradiation (2 kGy) and storage on flavor and microbial quality, these irradiated apple cider samples were compared to a control, unirradiated sample exposed to atmospheric air. Volatile compounds, soluble solids, titratable acidity, and microbiological counts were determined weekly throughout 7 weeks of refrigerated (4 °C) storage. Cider irradiated and stored in atmospheric air or nitrogen-flush environments had lower rates of loss for characteristic flavor volatiles compared to unirradiated apple cider and cider irradiated and stored in an oxygen-flush environment. The addition of potassium sorbate to the apple cider resulted in lower counts of yeasts and aerobic microorganisms, reduced fermentation of sugars to organic acids, and improved retention of volatile compounds characteristic of apple cider.

KEYWORDS: Apple cider; irradiation; flavor; gas environment; sorbate

INTRODUCTION

Foodborne illness outbreaks linked to unpasteurized, unfermented apple cider have been attributed to *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Cryptosporidium parvum* (*1*). Because these microorgansims are able to survive in low-pH (3.3−4.1) conditions and at low storage temperatures (*2*), apple cider processors must incorporate additional processing steps to achieve a 5-log reduction in *E. coli* O157:H7 to ensure the safety of the apple cider (*3*).

Ionizing radiation has been investigated as a nonthermal method to inactivate foodborne pathogens and reduce spoilage in apple cider (*4*, *5*) without the undesirable changes in color, flavor, and viscosity that occur during heat pasteurization (*6*−*8*). Potassium sorbate or other preservatives are frequently added to apple cider to inhibit yeast and mold growth and extend the shelf life (*9*).

During the storage of the apple cider, losses of volatile compounds and fermentation reactions contribute to decreases in flavor quality. Packaging material and sorbate addition had a significant effect on the retention of the characteristic flavor and quality attributes of irradiated apple cider during a 3-week storage period. Polystyrene and nylon-6 packaging materials, which have low oxygen permeability in comparison to low-density polyethylene, were more effective in slowing the undesirable changes that occur during the storage of apple cider. The addition of potassium sorbate also slowed the loss of volatile compounds and the conversion of sugars to acids through the natural fermentation process (*10*).

Loss of flavor quality during storage is caused by chemical degradation of flavor components and formation of off-flavors. Oxygen, which is dissolved in the product, diffuses through packaging material, or dissolves into the product from package headspace, contributes to the decomposition of flavor in juices during storage. An inert environment for the packaging of orange juice is critical to avoid the detrimental effects of oxygen on the retention of flavor, vitamin C, and color (*11*). The presence of oxygen dissolved in apple cider or in the sample headspace may be important to the quality attributes of apple cider during irradiation and storage.

The objective of this study was to determine the effects of gas environment and sorbate addition on the flavor of irradiated apple cider during 7 weeks of storage. The control, unirradiated apple cider packaged in polystyrene (PS) containers and exposed to atmospheric air was compared to cider irradiated and stored in PS under three gas environments: atmospheric air, nitrogen flush, and oxygen flush. Cider with (0.1%) and without (0%) potassium sorbate was evaluated initially following treatment and over a storage period of 7 weeks. Soluble solids, titratable acidity, and microbial counts were also determined to monitor quality changes that take place during storage. Volatile compounds of apple cider were determined and quantified using solid-phase microextraction (SPME) and gas chromatography−mass spectrometry (GC-MS).

MATERIALS AND METHODS

Sample Preparation and Treatment. Fresh cider consisting of dominant apple cultivars, with (0.1%) and without (0%) potassium

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sorbate, was obtained from a local cider producer in the fall of 2002. Whole apples are washed, chopped into a pomace, and pressed to extract the cider from pulp. The cider is passed through cheesecloth to remove large particles and transferred to a holding tank. If sorbate is added to the cider, it is added in the holding tank prior to packaging. Cider was packaged and irradiated within 4 days of the processing date. Two hundred milliliters of apple cider was transferred into 250-mL sterile polystyrene flasks (Costar, Cambridge, MA). Cider was exposed to three different gas environments (oxygen flush, nitrogen flush, and atmospheric air). Food-grade oxygen (<300 ppm of carbon dioxide; <10 ppm of carbon monoxide) and nitrogen (>99.99% pure; <10 ppm of oxygen) gases (Linweld, Des Moines, IA) were used for the oxygen- and nitrogen-flush environments, respectively. Each gas was bubbled through the cider at a rate of 1 psi through each 200-mL apple cider sample for 30 s using a 20-gauge needle. Plug seal caps were placed on the container immediately following bubbling of the gases to form the appropriate closed system. The third environment, atmospheric air, was designated for samples that remained open to the atmosphere during package conditioning. Atmospheric air contains approximately 78% nitrogen and 21% oxygen by volume; the remaining 1% contains trace gases including argon, carbon dioxide, hydrogen, and others. The control sample was unirradiated cider exposed to atmospheric air during transfer into PS flasks.

The cider was irradiated at the Iowa State University Linear Accelerator Facility (Ames, IA) using electron beam irradiation at a target dose of 2.0 kGy at an energy level of 10 MeV and a power level of 10.2 kW. The average absorbed dose was 2.22 ± 0.11 kGy, based on alanine dosimeter pellets attached to the tops and bottoms of the packages. Dose rate ranged from 78.9 to 81.9 kGy/min, and conveyor speed was set at 9.3 cm/s. Untreated (control) cider was not exposed to irradiation but did follow similar transport to and from the facility. Irradiation was conducted at room temperature without temperature control. Cider samples were stored at 4 °C before and after irradiation treatment and deviated from these conditions only during irradiation treatment.

All samples were stored at refrigeration (4 °C) temperatures for 0, 1, 2, 3, 5, and 7 weeks after treatment. Individual packages remained sealed until analysis. Volatile analysis, soluble solids, pH, titratable acidity, and microbiological analyses were determined at each sampling time. The experiment was replicated two times.

Volatile Analysis. SPME techniques were applied for the isolation and concentration of volatile compounds (*12*). A representative cider sample (40 g) was transferred to a 100-mL headspace bottle and sealed with a Teflon septum. Samples were held in a 40 °C water bath with stirring during the isolation. Each sample was allowed to equilibrate and absorb onto the SPME fiber [2-cm 50/30 μm divinylbenzene/carboxen/poly(dimethylsiloxane); Supelco, Inc., Bellefonte, PA] for 45 min.

A gas chromatograph (GC; model 6890, Hewlett-Packard, Inc., Wilmington, DE) equipped with a splitless injection port and a flame ionization detector was used for separation of volatile compounds. Volatile compounds were thermally desorbed (220 °C) for 3 min via the GC injection port onto a fused-silica capillary column (SPB-5, 30 m × 0.25 mm × 0.25-μm film thickness, Supelco, Inc.). The column pressure was set at 124.0 kPa with a helium flow rate of 1.9 mL/min. Oven temperature was initially 30 °C for 3 min, then increased to 80 °C at 5 °C/min, to 95 °C at 4 °C/min, to 115 °C at 5 °C/min, and to 200 °C at 10 °C/min. The detector temperature was constant at 220 °C. Flow rates of detector gases were air at 400 mL/min, hydrogen at 30 mL/min, and nitrogen (makeup gas) at 25 mL/min. Peak areas below 5 were not recorded by the GC program. Volatile compounds were identified using authentic standards (Sigma-Aldrich, Milwaukee, WI; AccuStandard, Inc., New Haven, CT) and confirmed with GC-MS analyses. Kovats retention indices, based on hydrocarbon standards, were calculated for all volatile compounds. Analyses were conducted in duplicate, and peak areas were averaged for further statistical analysis.

A gas chromatograph−mass spectrometer (Micromass GCT, Waters Corp., Milford, MA) with a time-of-flight mass analyzer was used for the confirmation of the identity of the volatile compounds. The samples were thermally desorbed into the GC injection port in a split (100:1) mode. GC conditions were set at an initial temperature of 38 °C for 1 min, 4 °C/min from 38 to 150 °C, and 50 °C/min from 150 to 280 °C. The mass spectrometer conditions were set as the following: electron ionization positive (EI+) polarity, source electron energy at 70 eV, source electron current at 200 μA, ion source temperature at 180 °C, source ion repeller at 0.8 V, electron multiplier voltage at 2700 V, scan range from *m*/*z* 41 to 400, at a frequency of scanning cycle every 0.75 s. Mass spectra of the volatile compounds were compared to a spectral library (Wiley Library) and a flavor and fragrance database (FlavorWORKS, Flavometrics, version 2.0, Anaheim Hills, CA) for identification.

Soluble Solids, pH, and Titratable Acidity. Soluble solids content was measured using a tabletop model 0−32 °Brix refractometer (Milton Roy, Ivyland, PA) with an accuracy of ±0.05% dissolved solids (Brix) and reported as percent sucrose. The pH of the cider was recorded using a digital pH-meter (Fisher Scientific, Accumet model AB15, Pittsburgh, PA). Titratable acidity was determined by titrating a sample (20 mL apple cider plus 80 g water) with 0.1 N NaOH to an endpoint of pH 8.2. Titratable acidity was expressed as grams of malic acid per 100 mL of cider. Sample temperature was 20 °C for each analysis. Analyses were conducted in duplicate for each sample treatment.

Microbiological Analyses. Microorganisms from apple cider were counted on plate count agar (Difco, Detroit, MI) for aerobic bacteria and on potato dextrose agar (Difco) for yeasts and molds. Buffered peptone water (Difco) was used for dilution blanks. For each treatment, samples were surface-plated in duplicate. Aerobic bacteria were counted after incubation at 36 °C for 48 h. Yeasts and molds were counted after incubation at 24 °C for 5 days.

Statistical Analysis. The experiment was designed as a three-way factorial with gas environment, sorbate addition, and storage time as the main factors. Analysis of variance and Fisher's least-squares difference tests ($P < 0.05$) were conducted to determine the effects of the main factors and interactions among main factors on the contents of volatile compounds, microbiological counts, soluble solids, pH, and titratable acidity. Because of significant interactions between storage time and gas environment and/or sorbate addition, semilog regression plots of contents of volatile compounds versus storage time and linear plots of soluble solids, pH, and titratable acidity versus storage time were plotted to determine rates of change (slope) for the components of flavor (*10*). Analysis of variance and Fisher's least-squares difference tests ($P < 0.05$) were conducted on the linear regression slopes of the semilog regression plots (SYSTAT, ver. 9.01, SPSS, Inc., Chicago, IL).

Principal component analysis (PCA) was conducted to examine relationships between the slopes obtained for volatile compounds, pH, titratable acidity, and soluble solids content of the apple cider. A correlation matrix was used for the extraction of the principal components, with Varimax orthogonal rotation. A minimum eigenvalue of 1.0 was used in the PCA. A biplot analysis was also conducted to identify relationships between individual treatments based on the weightings of each objective variable from the PCA and the data obtained for each treatment (SYSTAT, ver. 9.01, SPSS, Inc.).

RESULTS AND DISCUSSION

Soluble Solids, pH, and Titratable Acidity. Soluble solids and titratable acidity contents are indicators of sweetness and tartness and also reflect changes in sugar and organic acid contents due to fermentation. The effects of storage on the titratable acidity and soluble solids of apple cider were dependent on the presence of sorbate and the gas environment (**Figures 1** and **2**). In the presence of sorbate, there were no significant differences in soluble solids (**Figure 1a**) and titratable acidity (**Figure 2a**) between the unirradiated and the three irradiated ciders exposed to different gas environments.

In the absence of sorbate, gas environment and irradiation had a significant treatment effect on the soluble solids and titratable acidity contents during the storage period. A noticeable decrease in soluble solids took place after week 3 of storage in cider without sorbate. Unirradiated (control) cider without sorbate had significantly lower soluble solids following 7 weeks

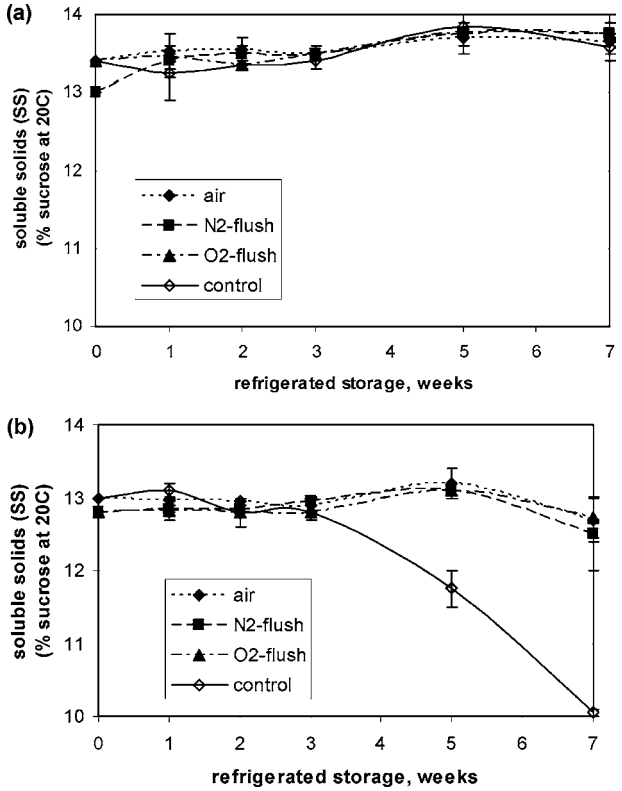

Figure 1. Effect of storage on soluble solids content of apple cider (**a**) with (0.1%) potassium sorbate and (**b**) without potassium sorbate. Points represent mean ± standard error of two replications. Air = atmospheric air exposure (2 kGy), N_2 flush = nitrogen-flush exposure (2 kGy), O_2 flush = oxygen-flush exposure (2 kGy), control = unirradiated apple cider packaged in glass (0 kGy), atmospheric air exposure.

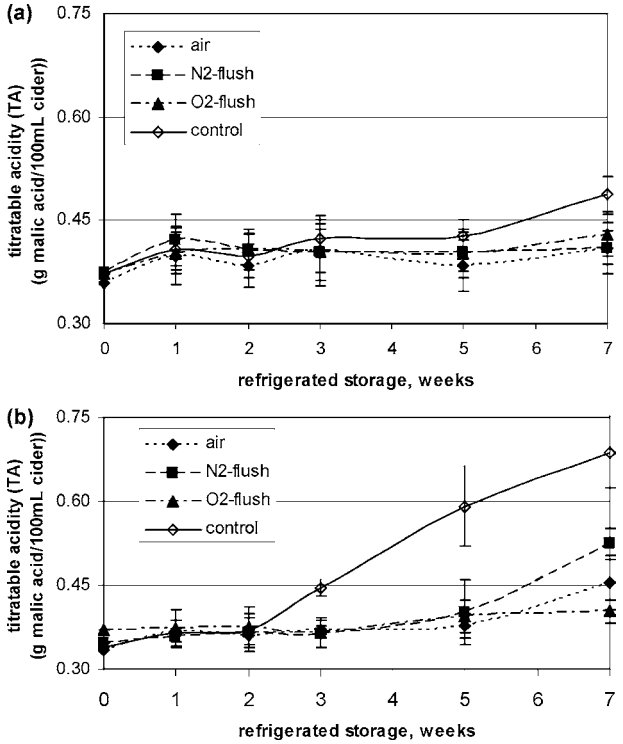

Figure 2. Effect of storage on titratable acidity content of apple cider (**a**) with (0.1%) potassium sorbate and (**b**) without potassium sorbate. Points represent mean ± standard error of two replications. Air = atmospheric air exposure (2 kGy), N_2 flush = nitrogen-flush exposure (2 kGy), O_2 flush = oxygen-flush exposure (2 kGy), control = unirradiated apple cider packaged in glass (0 kGy), atmospheric air exposure.

of refrigerated storage than all three irradiated ciders that were exposed to different gas environments (**Figure 1b**). A considerable increase in acidity began to occur after week 2 of storage. Control cider without sorbate had significantly higher titratable acidity values than all irradiated samples following 7 weeks of storage, increasing from 0.34 to 0.70 g of malic acid/100 mL of cider (**Figure 2b**). Although irradiated samples without sorbate were not statistically different, nitrogen-flush cider yielded the next highest acidity, followed by atmospheric air, whereas oxygen-flush samples had the lowest acidity measurements following storage. An abundance of oxygen limits yeast growth, anaerobic metabolism, and fermentation and, subsequently, the conversion of sugars to acids during extended storage.

Microbiological Analysis. The growth of microorganisms during the 7-week storage period was monitored. Irradiation of the apple cider reduced the initial yeast and aerobic bacterial counts by up to 1000-fold.

The addition of sorbate, especially in conjunction with irradiation treatment, was effective in controlling microbial growth. In general, yeast counts were approximately 1 log lower in the irradiated cider than in the unirradiated control cider. At the end of the storage period, yeast counts were below 10^4 in irradiated apple cider and below 10^5 for the control, unirradiated cider (**Figure 3a**). The aerobic bacterial counts were much higher in unirradiated cider than in irradiated cider throughout the storage period. After 7 weeks of storage, counts of aerobic bacteria were $\sim 10^5$ for the unirradiated apple cider and $< 10^2$ for irradiated apple cider (**Figure 4a**).

In the absence of sorbate, irradiation treatments were effective in reducing microbial growth only during the first 3 weeks of storage. Yeast counts increased significantly following 1−2 weeks of storage. At the end of the storage period, yeast counts were $\sim 10^6$ for all irradiated and unirradiated cider samples without sorbate (**Figure 3b**). Similarly, aerobic bacterial counts for the irradiated cider were approximately 2 log cycles lower than for the unirradiated cider during the first 2 weeks of storage. By week 7, aerobic bacterial counts for all cider samples exceeded 10^5/mL; counts in irradiated cider were similar to those of control cider, and counts in oxygen-flushed cider surpassed those of all other samples (**Figure 4b**).

The numbers and types of microorganisms present in a food and the ability of those microorganisms to adapt to the environment of the food affect the food's shelf life (*13*). Yeasts that are naturally present in apple cider may be resistant to the irradiation process and/or presence of sorbate (*5*). According to Bills and colleagures (*14*) the yeast strain *Saccharomyces rouxii*, when preconditioned in 0.1% sorbate, is tolerant to high-sugar and sorbate solutions at pH levels similar to those of fruit juice systems. The sensitivity of microorganisms to sorbate is also species-dependent, whereas the antimicrobial action of potassium sorbate can be improved by the presence of organic acids (*15*).

The effect of sorbate, irradiation, and gas environments on microbial growth corresponds to the effect of these treatments on the soluble solids and titratable acidity of the apple cider. In the presence of sorbate, fermentation reactions were controlled as shown by the reduced growth of yeast and aerobic bacteria and the higher soluble solids and a lower titratable acidity, with only minimal changes during the 7-week storage period. The irradiated cider samples did not show significant differences in microbial growth, soluble solids content, and titratable acidity among the three gas environments. In the absence of sorbate,

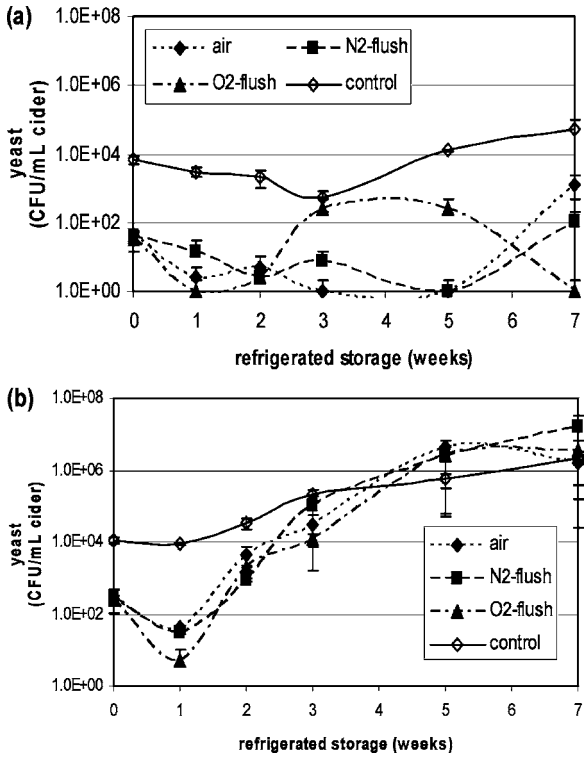

Figure 3. Effect of storage on yeast counts of apple cider (**a**) with (0.1%) potassium sorbate and (**b**) without potassium sorbate. Points represent mean ± standard error of two replications. Air = atmospheric air exposure (2 kGy), N_2 flush = nitrogen-flush exposure (2 kGy), O_2 flush = oxygen-flush exposure (2 kGy), control = unirradiated apple cider packaged in glass (0 kGy), atmospheric air exposure.

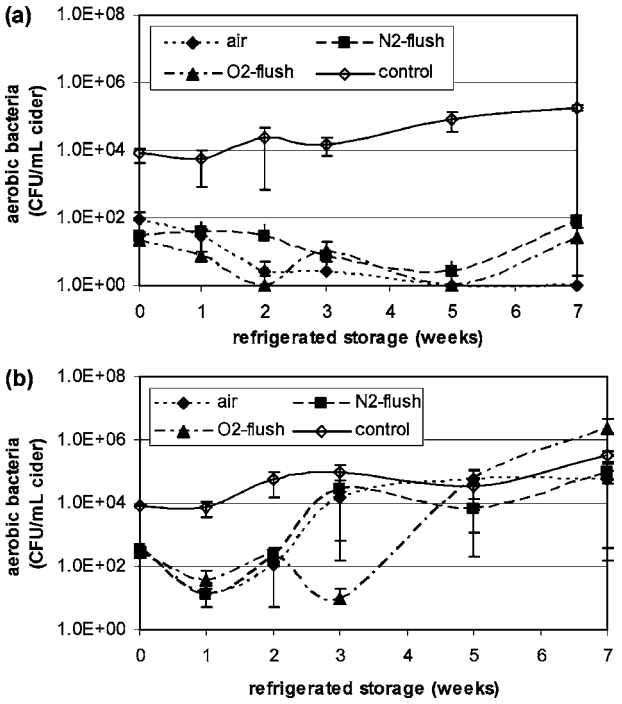

Figure 4. Effect of storage on aerobic plate counts of apple cider (**a**) with (0.1%) potassium sorbate and (**b**) without potassium sorbate. Points represent mean ± standard error of two replications. Air = atmospheric air exposure (2 kGy), N_2 flush = nitrogen-flush exposure (2 kGy), O_2 flush = oxygen-flush exposure (2 kGy), control = unirradiated apple cider packaged in glass (0 kGy), atmospheric air exposure.

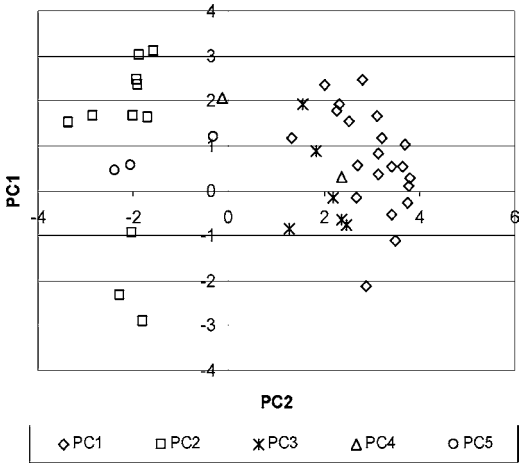

Figure 5. Plot of the PCA of apple cider showing associations among volatile compounds, soluble solids, pH, and titratable acidity. Vector coordinates signify pooled responses for all processing treatments (irradiation, gas environment, and sorbate addition) using the rates of change during 7 weeks of refrigerated storage for two replications.

the irradiation treatments were effective in reducing the growth of yeasts and aerobic bacteria during the initial weeks of storage. The sorbate and/or irradiation treatments effectively reduced microbial growth and fermentation of the apple cider as reflected in the minimal changes in soluble solids and titratable acidity contents during the storage period.

Volatile Analysis. Ester compounds, such as butyl acetate, 2-methylbutyl acetate, hexyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, butyl butanoate, hexyl butanoate, ethyl hexanoate, and hexyl hexanoate, were among the predominant volatile compounds in the apple cider. These volatile compounds are characteristic of apples and apple products.

Table 1 compares the effect of irradiation and gas environment on the initial contents of the identified volatile compounds. For a majority of the flavor compounds, irradiation and gas environment did not have a significant effect on the content of the flavor compounds. Significant differences in initial GC peak areas existed for 4 of the 44 identified flavor compounds. The higher contents of pentanol, hexanol, and decanal in the cider irradiated in the oxygen-flush environment, in comparison to the cider irradiated in the air or nitrogen-flush environment, were attributed to oxidation reactions during irradiation. For ethyl 2-methylbutanoate, nitrogen-flush cider had the lowest areas, whereas control, unirradiated cider had the highest. Previous research has also shown significant decreases in the content of ethyl 2-methylbutanoate during irradiation of apple cider at 2 and 4 kGy (*12*). Irradiation of orange juice at doses as low as 1.0 kGy resulted in an increase in off-aromas and decomposition of orange oils, although the precise mechanism has not been determined (*16*).

Sorbate treatment had a significant effect on the initial contents of seven volatile compounds (**Table 2**). For each of these compounds, cider with sorbate yielded higher GC peak areas than cider without sorbate. The addition of potassium sorbate has been shown to play a beneficial role in the preservation of apple cider volatile compounds during processing and storage (*17*) and by quenching free radicals generated during irradiation to minimize degradation of flavor compounds (*12*).

The rate of change (slope) for each volatile compound, soluble solids (SS), and titratable acidity (TA) was calculated to determine the effect of gas environment and sorbate on these components of flavor during storage (*10*). PCA of these data grouped the volatile compounds, SS, pH, and TA into five principal components. PCA was used to identify patterns of

Table 1. Effect of Gas Environment and Irradiation on Initial GC Peak Areas of Volatile Compounds[a] in Apple Cider

RI[b]	compound	gas environment:[c] irradiation dose: PC	GC peak areas (week 0) air 2 kGy	N_2 flush 2 kGy	O_2 flush 2 kGy	control 0 kGy
	ethanol (t)	1	ND	ND	ND	ND
	propanol (t)	2	8.3 ± 3.7	11.2 ± 5.1	27.3 ± 7.7	ND
	butanol (t)	2	49.4 ± 26.2	10.6 ± 3.4	54.0 ± 23.4	13.3 ± 0.4
714	ethyl propionate	2	187.3 ± 66.0	148.6 ± 25.6	232.9 ± 20.6	140.2 ± 47.8
721	ethyl 2-methylpropionate	1	7.2 ± 3.1	11.3 ± 5.1	13.0 ± 6.0	11.0 ± 5.0
733	methyl 2-methylbutanoate	3	72.7 ± 30.7	32.4 ± 12.5	48.3 ±19.7	30.5 ± 8.5
744	pentanol[d]	2	76.9 ± 16.8 b	50.5 ± 13.0	155.2 ± 56.6 c	26.8 ±5.3 a
776	hexanal		354.2 ± 111.4	425.3 ± 55.5	517.8 ± 96.5	315.9 ± 86.8
800	ethyl butanoate	1	163.9 ± 88.3	44.8 ± 43.8	61.6 ± 44.8	179.3 ± 137.8
808	1-methylpropyl acetate	1	60.2 ± 3.8	56.6 ± 3.8	66.8 ± 5.0	61.1 ± 4.8
812	butyl acetate	1	2010.6 ± 309.5	2267.4 ± 56.2	2471.1 ± 178.9	2103.0 ± 299.3
835	3-pentyl acetate	1	121.3 ± 2.6	114.0 ± 1.1	140.0 ± 11.6	133.9 ± 6.1
840	ethyl 2-methylbutanoate	1	3568.9 ± 460.9 ab	3116.8 ± 409.1 a	4547.0 ± 772.4 bc	4660.2 ± 728.7 c
854	(*E*)-2-hexenal	1	176.7 ± 20.8	154.3 ± 21.9	216.9 ± 46.0	217.1 ± 43.0
867	1-hexanol	1	839.4 ± 37.8 ab	769.6 ± 23.1 a	990.1 ± 84.7 c	926.9 ± 63.2 bc
880	2-methylbutyl acetate	1	1438.5 ± 121.2	1560.2 ± 91.2	1625.8 ± 29.7	1617.3 ± 78.7
896	propyl butanoate	1	308.2 ± 26.8	322.7 ± 12.0	319.9 ± 15.6	347.0 ± 27.3
910	butyl propionate	1	121.2 ± 14.3	119.6 ± 8.9	137.9 ± 9.1	114.7 ± 8.9
918	pentyl acetate	1	273.3 ± 38.2	277.9 ± 10.8	340.3 ± 44.2	323.6 ± 48.2
960	isopropyl 2-methylbutanoate	5	19.9 ± 5.0	21.7 ± 3.2	21.6 ± 4.4	33.2 ± 9.4
974	benzaldehyde	2	27.9 ± 2.8	27.3 ± 1.4	34.9 ± 1.6	28.3 ± 5.5
978	1-octen-3-ol	5	14.5 ± 2.1	16.5 ± 1.2	16.5 ± 2.7	15.8 ± 2.2
993	butyl butanoate	1	447.0 ± 33.5	446.3 ± 30.1	496.3 ± 21.4	472.1 ± 23.23
996	ethyl hexanoate	2	73.8 ± 4.6	71.3 ± 7.4	80.3 ± 12.6	87.8 ± 5.9
1008	hexyl acetate	1	9031.4 ± 1048.0	9078.7 ± 238.6	9808.2 ± 1003.7	9731.2 ± 769.2
1036	butyl 2-methylbutanoate	1	231.0 ± 13.5	236.8 ± 16.4	243.4 ± 9.5	246.5 ± 6.6
1054	pentyl butanoate	1	22.6 ± 2.6	24.3 ± 0.5	26.5 ± 1.4	20.8 ± 1.2
1070	1-octanol		ND	ND	ND	ND
1091	propyl hexanoate[d]	4	282.9 ± 105.9	262.3 ± 101.6	256.4 ± 99.6	272.5 ± 87.7
1098	nonanal[d]	4	124.6 ± 54.6	160.7 ± 18.3	143.1 ± 48.0	139.5 ± 48.0
1103	hexyl propionate	1	239.1 ± 71.8	361.6 ± 105.3	242.2 ± 69.0	251.3 ± 74.3
1113	heptyl acetate[d]	2	139.0 ± 77.9	128.2 ± 69.0	147.0 ± 79.8	84.2 ± 39.1
1136	unidentified		33.1 ± 15.1	26.2 ± 5.8	30.8 ± 8.6	35.0 ± 16.0
1163	benzyl acetate	3	17.2 ± 5.6	7.9 ± 4.0	25.3 ± 5.5	25.0 ± 5.5
1171	(*E*)-2-nonenal	2	19.3 ± 4.1	16.0 ± 2.0	16.3 ± 6.3	17.0 ± 2.9
1191	hexyl butanoate[d]	1	1234.6 ± 217.6	1202.4 ± 208.2	1267.2 ± 193.7	1153.6 ± 168.0
1196	*p*-allyl anisole	2	108.2 ± 13.9	105.8 ± 11.4	112.9 ± 14.8	97.2 ± 4.8
1204	decanal	1	23.8 ± 4.3 b	7.7 ± 4.1 a	42.9 ± 3.4 c	6.3 ± 2.7 a
1234	hexyl 2-methylbutanoate	1	655.5 ± 41.3	649.2 ± 54.6	696.7 ± 75.7	612.2 ± 28.4
1242	(*E*)-2-decenal	3	113.1 ± 11.7	106.8 ± 6.8	128.9 ± 21.0	99.3 ± 11.8
1272	1-decanol	5	12.6 ± 2.5	9.4 ± 4.9	18.9 ± 2.9	9.0 ± 4.8
1383	hexyl hexanoate[d]	3	206.9 ± 69.5	199.3 ± 72.6	228.3 ± 94.0	147.8 ± 46.9
1410	α-farnesene[d]	3	272.2 ± 109.2	280.0 ± 128.5	335.9 ± 141.0	205.0 ± 98.0

[a] Compounds are presented in the order of elution from the gas chromatograph. Means ± standard errors are duplicate analyses of two replications (week 0) with data for sorbate addition pooled. Means followed by different letters (a–c) within the same row are significantly different from each other ($P < 0.05$). [b] Kovats retention indices, based on hydrocarbon standards for compounds with RI > 700. Compounds eluting prior to heptane are tentatively identified (t). [c] Air = atmospheric air, O_2 flush = oxygen flush, N_2 flush = nitrogen flush, control = atmospheric air. [d] Effect of sorbate significant ($P < 0.05$).

Table 2. Effect of Sorbate on Initial Contents of Selected Volatile Compounds[a] in Apple Cider

	GC peak areas (week 0)	
	0% sorbate	0.1% sorbate
pentanol	57.3 ± 14.2 a	97.5 ± 33.6 b
propyl hexanoate	138.2 ± 40.2 a	398.8 ± 43.9 b
nonanal	90.0 ± 27.1 a	194.0 ± 27.1 b
heptyl acetate	39.5 ± 39.9 a	209.6 ± 39.9 b
hexyl butanoate	1017.9 ± 116.3 a	1411.0 ± 96.5 b
hexyl hexanoate	125.5 ± 16.5 a	265.7 ± 55.5 b
α-farnesene	185.1 ± 40.8 a	361.5 ± 95.2 b

[a] Means ± standard errors are duplicate analyses of two replications (week 0) with data for gas environment pooled. Means followed by different letters (a, b) within the same row are significantly different from each other ($P < 0.05$).

interactions between variables and to reduce a large data set containing intercorrelated variables to a smaller set of uncorrelated factors. PCA is often used with complex data sets, such as volatile compounds, to demonstrate relationships between variables. The first five principal components (PC) accounted for >70% of the total variability in the data set. PC-1 (31.2%) contained 21 flavor compounds, PC-2 (16.9%) contained SS, TA, and 9 flavor compounds, PC-3 (12.2%) contained pH and 5 flavor compounds, PC-4 (5.3%) contained 2 flavor compounds, and PC-5 (5.1%) contained 3 compounds (**Figure 5**). A majority of the characteristic apple cider volatile compounds, especially acetates and butanoates, loaded onto PC-1, whereas the major alcohols were grouped into PC-2. Principal components 1, 3, and 4 grouped predominantly in the positive *x*-axis region, whereas principal components 2 and 5 grouped predominantly in the negative *x*-axis region. No PC contained an exclusive type of flavor compound, nor did the compounds exhibit the same response to gas environment and sorbate addition. The effects of gas environment and sorbate addition, therefore, will be discussed separately.

Table 3. Effect of Sorbate Addition on the Retention of Flavor Compounds[a] in Apple Cider during 7 Weeks of Refrigerated Storage

	slope[b]	
	0% sorbate	0.1% sorbate
PC-1		
1-methylpropyl acetate	–0.235 a	–0.060 b
butyl acetate	–0.245 a	–0.020 b
2-methylbutyl acetate[c]	–0.198 a	–0.023 b
3-pentyl acetate	–0.254 a	–0.042 b
pentyl acetate	–0.186 a	–0.031 b
hexyl acetate	–0.219 a	–0.009 b
ethyl 2-methylpropionate[c]	0.222 b	ND a
butyl propionate	–0.143 a	0.001 b
hexyl propionate[c]	–0.205 a	–0.001 b
ethyl butanoate	–0.049 a	0.086 b
ethyl 2-methylbutanoate[c]	–0.410 a	–0.068 b
propyl butanoate	–0.057 a	0.003 b
butyl butanoate[c]	–0.093 a	0.006 b
butyl 2-methylbutanoate	–0.028 a	0.009 b
pentyl butanoate	–0.233 a	0.018 b
hexyl butanoate	–0.247 a	–0.024 b
hexyl 2-methylbutanoate	–0.131 a	–0.020 b
(*E*)-2-hexenal	–0.327 a	–0.007 b
decanal[c]	–0.088 a	0.037 b
ethanol[c]	0.562 b	ND a
1-hexanol	0.083 b	–0.009 a
PC-2		
soluble solids	–0.124 a	0.057 b
titratable acidity	0.023 b	0.004 a
heptyl acetate[c]	0.080 b	0.001 a
ethyl propionate	0.091 b	0.012 a
ethyl hexanoate[c]	–0.138 a	0.004 b
benzaldehyde	–0.166 a	–0.020 b
(*E*)-2-nonenal	0.122 a	0.073 b
p-allyl anisole	–0.022 a	–0.031 b
propanol	0.299 b	ND a
butanol	0.186 b	0.066 a
pentanol	0.105 b	0.006 a
PC-3		
pH	0.003 b	–0.008 a
benzyl acetate	0.019 b	0.057 a
methyl 2-methylbutanoate	0.068 b	0.024 a
hexyl hexanoate	–0.160 a	–0.055 b
α-farnesene	–0.197 a	–0.097 b
(*E*)-2-decenal	0.005 a	0.022 b
PC-4		
propyl hexanoate[c]	–0.003 a	–0.012 b
nonanal	–0.017 a	0.023 b
PC-5		
isopropyl 2-methylbutanoate	0.041 b	0.006 a
1-octen-3-ol	0.054 b	–0.051 a
1-decanol	–0.043 b	–0.055 a

[a] Means are duplicate analyses of two replications with data for gas environment pooled. Means followed by different letters (a, b) within the same row are significantly different from each other ($P < 0.05$). [b] Slopes are from semilog regression plots of GC peak area vs storage time for volatile compounds and linear plots of soluble solids (% sucrose), titratable acidity (g of malic acid/100 mL of cider), and pH vs storage time. [c] Interaction between gas environment and sorbate significant ($P < 0.05$).

Table 4. Effect of Gas Environment and Irradiation on the Retention of Flavor Compounds[a] in Apple Cider during 7 Weeks of Refrigerated Storage

	slope[b]			
gas environment:[c] irradiation dose:	air 2 kGy	N_2 flush 2 kGy	O_2 flush 2 kGY	control 0 kGy
PC-1				
1-methylpropyl acetate	–0.130	–0.145	–0.135	–0.182
butyl acetate	–0.074	–0.155	–0.237	–0.066
2-methylbutyl acetate[d]	–0.032	–0.134 bc	–0.250 ab	–0.027 c
3-pentyl acetate	–0.118	–0.156	–0.153	–0.164
pentyl acetate	–0.084	–0.137	–0.110	–0.103
hexyl acetate	–0.073	–0.133	–0.143	–0.107
ethyl 2-methylpropionate[d]	0.310 b	0.307 b	0.322 b	–0.050 a
butyl propionate	–0.033	–0.108	–0.109	–0.035
hexyl propionate[d]	–0.122 a	–0.019 b	–0.142 a	–0.036 b
ethyl butanoate	–0.059	0.081	–0.022	0.073
ethyl 2-methylbutanoate[d]	–0.177 b	–0.206 b	–0.289 a	–0.283 a
propyl butanoate	–0.005	–0.010	–0.088	–0.005
butyl butanoate[d]	–0.053 b	–0.053 b	–0.092 a	0.024 c
butyl 2-methylbutanoate	0.002	–0.018	–0.025	0.004
pentyl butanoate	–0.108	–0.109	–0.106	–0.107
hexyl butanoate	–0.118	–0.134	–0.162	–0.126
hexyl 2-methylbutanoate	–0.059	–0.091	–0.087	–0.063
(*E*)-2-hexenal	–0.208	–0.196	–0.127	–0.137
decanal[d]	–0.019 b	0.035 bc	–0.120 a	0.090 c
ethanol[d]	0.491 a	0.578 b	0.511 ab	0.669 c
1-hexanol	0.037 b	0.052 b	0.054 b	0.004 a
PC-2				
soluble solids[d]	0.005 b	0.025 b	0.035 b	–0.200 a
titratable acidity[d]	0.007 a	0.011 a	0.003 a	0.033 b
heptyl acetate[d]	0.032	0.055	0.006	0.070
ethyl propionate	0.051	0.042	0.032	0.080
ethyl hexanoate[d]	–0.045 bc	–0.020 c	–0.054 b	–0.150 a
benzaldehyde	0.033 b	–0.045 b	–0.083 ab	–0.176 a
(*E*)-2-nonenal	0.071	0.077	0.116	0.125
p-allyl anisole	–0.031	–0.029	–0.053	0.006
propanol	0.263	0.326	0.205	0.401
butanol	0.079 ab	0.158 bc	0.051 a	0.217 c
pentanol	0.02	0.075	0.054	0.068
PC-3				
pH	0.002 b	–0.001 b	0.001 b	–0.012 a
benzyl acetate	0.030	0.065	0.030	0.027
methyl 2-methylbutanoate	0.026	0.048	0.029	0.079
hexyl hexanoate	–0.119	–0.138	–0.075	–0.098
α-farnesene	–0.205	–0.155	–0.099	–0.129
(*E*)-2-decenal	0.012	0.016	0.006	0.019
PC-4				
propyl hexanoate[d]	–0.007	0.023	–0.043	–0.003
nonanal	0.023	0.008	–0.014	–0.006
PC-5				
isopropyl 2-methyl-butanoate	0.047	0.020	0.008	0.018
1-octen-3-ol	–0.004	0.016	–0.034	0.028
1-decanol	–0.060	–0.049	–0.064	–0.023

[a] Means are duplicate analyses of two replications with data for sorbate addition pooled. Means followed by different letters (a–c) within the same row are significantly different from each other ($P < 0.05$). [b] Slopes are from semilog regression plots of GC peak area vs storage time for volatile compounds and linear plots of soluble solids (% sucrose), titratable acidity (g of malic acid/100 mL of cider), and pH vs storage time. [c] Air = atmospheric air, O_2 flush = oxygen flush, N_2 flush = nitrogen flush, control = atmospheric air. [d] Interaction between gas environment and sorbate significant ($P < 0.05$).

The addition of potassium sorbate had a significant effect on the rate of change in the content for a majority of the volatile compounds (**Table 3**). In particular, slopes were more negative (higher rates of loss) for a majority of the esters and other volatile compounds that loaded onto PC-1 for apple cider without sorbate. During storage, the contents of several alcohols, including ethanol, propanol, butanol, pentanol, and hexanol, increased. The formation of these alcohols was greatest in the absence of sorbate (**Table 3**). Irradiation decreased the rate of formation of propanol and butanol in apple cider with and without sorbate (**Table 4**) and that of ethanol in apple cider without sorbate (**Table 5**), through slowing microbial growth and enzymatic reactions. However, irradiation resulted in a significant increase in the content of hexanol during the 7-week storage period, in comparison to the unirradiated apple cider (**Table 4**). The formation of hexanol in irradiated apple cider has been attributed to oxidation of unsaturated fatty acids through free radicals generated by irradiation (*12*). In apple cider, sorbate inhibits the growth of yeasts and molds to reduce alcohol production (*9*) and inhibits free radical reactions that degrade esters and produce oxidation products in irradiated apple cider (*10*, *12*). The presence of sorbate helps to preserve the

Table 5. Effect of Interaction of Gas Environment/Irradiation and Sorbate Addition on the Retention of Flavor Compounds[a] in Apple Cider during 7 Weeks of Refrigerated Storage

	slope[b]			
gas environment:[c]	air	N_2 flush	O_2 flush	control
soluble solids				
0% sorbate	–0.016 ay	–0.024 ay	0.001 ay	–0.459 ax
0.1% sorbate	0.026 ax	0.074 ax	0.068 ax	0.058 bx
titratable acidity				
0% sorbate	0.012 bx	0.023 by	0.004 ax	0.054 bz
0.1% sorbate	0.001 ax	–0.001 ax	0.003 axy	0.012 ay
2-methylbutyl acetate				
0% sorbate	–0.050 ay	–0.234 ay	–0.476 ax	–0.033 ay
0.1% sorbate	–0.013 ax	–0.033 ax	–0.024 bx	–0.021 ax
heptyl acetate				
0% sorbate	0.008 ax	0.118 byz	0.026 axy	0.168 bz
0.1% sorbate	0.055 ax	–0.009 ax	-0.014 ax	-0.027 ax
ethyl 2-methylpropionate				
0% sorbate	0.310 by	0.307 by	0.322 by	–0.050 ax
0.1% sorbate	ND[d] ax	ND ax	ND ax	ND ax
hexyl propionate				
0% sorbate	–0.270 ax	ND az	–0.278 ax	–0.065 ay
0.1% sorbate	0.027 bx	–0.019 ax	–0.005 bx	–0.006 bx
ethyl 2-methylbutanoate				
0% sorbate	–0.348 ay	–0.401 ay	–0.563 ax	–0.328 ay
0.1% sorbate	–0.007 by	–0.012 by	–0.014 by	–0.238 bx
butyl butanoate				
0% sorbate	–0.117 ay	–0.108 ay	–0.179 ax	0.032 az
0.1% sorbate	0.012 bx	0.002 bx	–0.004 bx	0.016 ax
ethyl hexanoate				
0% sorbate	–0.072 ayz	–0.050 az	–0.098 ay	–0.332 ax
0.1% sorbate	–0.017 bx	0.010 bxy	–0.009 bxy	0.032 by
propyl hexanoate				
0% sorbate	–0.009 axy	0.064 bz	–0.079 ax	0.014 ayz
0.1% sorbate	–0.004 ax	–0.017 ax	–0.006 bx	–0.020 ax
hexanal				
0% sorbate	–0.241 ax	–0.155 ax	–0.210 ax	–0.164 bx
0.1% sorbate	0.013 by	–0.026 ay	–0.022 by	–0.384 ax
decanal				
0% sorbate	–0.072 ay	0.027 az	–0.221 ax	ND
0.1% sorbate	0.034 bxy	0.044 axy	–0.019 bx	0.090 y
ethanol				
0% sorbate	0.491 bx	0.511 bx	0.578 by	0.669 bz
0.1% sorbate	ND ax	ND ax	ND ax	ND ax
1-octanol				
0% sorbate	ND ax	0.416 by	ND ax	ND ax
0.1% sorbate	ND ax	ND ax	ND ax	ND ax
unindentified (RI 1136)[e]				
0% sorbate	–0.255 ax	–0.226 ax	–0.230 ax	–0.220 ax
0.1% sorbate	–0.002 by	–0.008 by	–0.257 ax	–0.028 by

[a] Means are duplicate analyses of two replications. Means followed by different letters within the same column (a, b) are significantly different ($P < 0.05$) among sorbate addition, and means followed by different letters within the same row (x–z) are significantly different ($P < 0.05$) among gas environments. [b] Slopes are from semilog regression plots of GC peak area vs storage time for volatile compounds and linear plots of soluble solids (% sucrose), titratable acidity (g of malic acid/100 mL of cider), and pH vs storage time. [c] Air = atmospheric air (2 kGy), O_2 flush = oxygen flush (2 kGy), N_2 flush = nitrogen flush (2 kGy), control = atmospheric air (0 kGy). [d] Not detected. [e] Kovats retention index (RI) values are presented for unidentified flavor compounds.

characteristic apple cider flavor in unirradiated and irradiated apple ciders.

Although gas environment had a significant effect on the retention of flavor compounds during storage (**Table 4**), for most of those compounds, the interaction between gas environment and sorbate was also significant (**Table 5**). Overall, in the presence of sorbate, differences between gas environments were not as significant as they were in the absence of sorbate. Irradiation, regardless of gas environment, effectively slowed the rate of loss of ethyl 2-methylbutanoate and hexanal in cider with sorbate and of ethyl 2-methylpropionate and ethyl hexanoate in cider without sorbate during the 7-week storage period in comparison to the control, unirradiated cider (**Table 5**). The presence of oxygen in the environment during irradiation accelerated the loss of 2-methylbutyl acetate, ethyl 2-methylbutanoate, butyl butanoate, and propyl hexanoate, in comparison to the cider samples without sorbate and irradiated in the presence of air or nitrogen or the unirradiated cider samples (**Table 5**). Packaging materials with higher oxygen permeability also contributed to greater losses of acetates, butanoates, and other volatile compounds with apple-like characteristics during a 3-week storage period (*10*). The oxygen-flush environment may promote oxidation reactions that are unfavorable to the preservation of these characteristic apple cider flavor compounds.

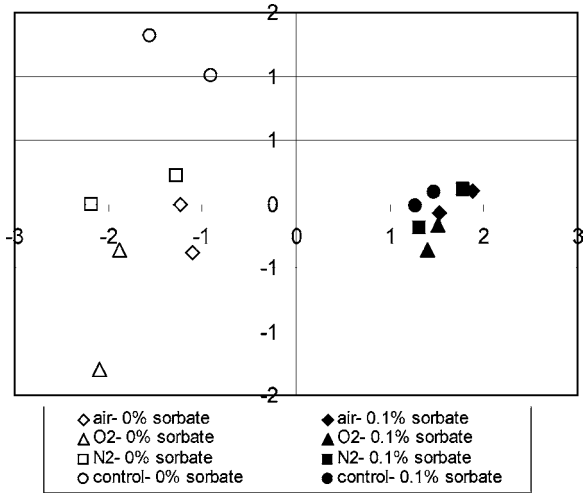

Figure 6. Results of PCA and biplot analysis showing the relationship of sorbate, irradiation, and gas environment treatments to each other. Object coordinates signify pooled responses for all variables (volatile compounds, soluble solids, pH, and titratable acidity) using the rates of change during 7 weeks of refrigerated storage for two replications.

The relationship between sorbate addition, irradiation, and gas environment of irradiated samples is illustrated in **Figure 6**. The cider samples with 0.1% potassium sorbate grouped in the positive *x*-axis region, which is associated with PC-1, PC-3, and PC-4 and consisted of a majority of the esters that are characteristic of apple cider flavor. The cider samples without added potassium sorbate grouped in the negative *x*-axis regions, which is associated with PC-2 and PC-5 and consisted of several alcohols. The groupings of the treatments for the ciders were strongly dependent on the presence of sorbate, as shown in the analysis of variance of the data. In the presence of sorbate, irradiation and gas environment had a minor effect on the stability of the flavor compounds during storage, as shown by the tight clustering of the cider treatments with sorbate. However, in the absence of sorbate, irradiation treatment and gas environment had a significant effect on the stability of the flavor compounds, as shown by the greater dispersion of the cider treatments without sorbate.

Conclusions. Gas environment and sorbate addition had a significant effect on the retention of characteristic flavor and quality attributes of irradiated apple cider during 7 weeks of refrigerated storage. The presence of sorbate effectively controlled microbial growth and conversion of sugars to acids through fermentation and slowed the loss of characteristic apple volatile compounds during storage. Irradiation also extends the shelf life of apple cider through decreasing microbial growth and fermentation. The presence of sorbate during irradiation slows the loss of esters and the formation of oxidation products

during storage. In the absence of sorbate, an atmospheric air or nitrogen-flush environment during irradiation resulted in the greatest retention of volatile compounds.

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