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Effect of 1-Methylcyclopropene (1-MCP) and Storage Atmosphere on the Volatile Aroma Composition of Cloudy and Clear Apple Juices

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Abstract: The effects of 1-methylcyclopropene (1-MCP), storage atmosphere (controlled (CA) or regular (RA)), and juice processing (clear or cloudy) on the volatile aroma compounds from McIntosh and Honeycrisp apples following 4-month storage were studied. All the major esters, aldehydes, and total volatile content from McIntosh juice were significantly affected by the two-way interaction between harvest maturity and 1-MCP treatment ($p \leq 0.01$), as well as harvest maturity and storage atmosphere ($p \leq 0.001$). In McIntosh juices, a remarkable reduction of all types of esters, aldehydes, most alcohols, and total volatile compounds was found when juices were prepared from 1-MCP-treated apples. In Honeycrisp, significant differences in the level of esters and the total volatile aroma was caused by storage atmosphere and juice processing techniques ($p \leq 0.001$), but not by 1-MCP treatment. As compared to clear juices, cloudy juice samples from Honeycrisp had a considerably higher content of total volatiles, esters, and aldehydes.

Keywords: juice processing; volatiles; GC-HS/SPME-MS; postharvest technology; storage; quality

1. Introduction

Apple juice is the second most widely consumed fruit juice in the world [1]. The aroma is an important organoleptic quality parameter of apple juice used by consumers to determine acceptability [2]. The volatile compound composition is responsible for the aroma of apple juice and can be influenced by many factors, including cultivar, harvest maturity, postharvest and storage treatments, and juice processing techniques [3]. Currently, the apple industry in North America has adopted the extensive use of a potent ethylene action inhibitor, 1-methylcyclopropene (1-MCP), as a means to extend the storage and shelf-life of apples. However, the inhibition of fruit ripening associated with 1-MCP treatment could have a deleterious effect on volatile aroma production of climacteric fruits, including apples [4–6].

The effect of different postharvest treatments and storage conditions on the volatile composition of fresh apple fruit has been examined [7,8]. Nevertheless, the impact of 1-MCP treatment on apple juice volatile aroma composition has not yet been reported. Moreover, information about the volatile composition of apple juice from the relatively newer apple cultivars such as Honeycrisp is limited. Honeycrisp is a unique, crisp fruit texture, large fruit that makes it highly desirable for the fresh market. However, due to the large percentage of rejections due to uneven or unusually size for the fresh market,

Honeycrisp is also used in juice processing. However, a delayed cooling treatment (stored at 20 °C for several days before storage in cold temperature condition) is recommended for Honeycrisp apples to reduce the development of soft scald and low-temperature breakdown. This study aimed to determine the effect of 1-MCP treatment, in combination with harvest maturity, storage atmosphere, and juice processing techniques on the content and composition of volatile aroma compounds of cloudy and clear juice produced from McIntosh and Honeycrisp apples.

2. Materials and Methods

2.1. Apple Harvesting, 1-MCP Treatment, and Storage

Redmax McIntosh (hereafter referred to as McIntosh) and Honeycrisp apples were harvested from a commercial farm (J.W. Mason, Windsor, NS, Canada) at either optimum (commercial) or late maturity stages. Apples free of disease and damage were selected, packed in carton boxes, wrapped with plastic, and transported to the Agriculture and Agri-Food Canada (AAFC) Research Centre in Kentville, NS, Canada. Within 6–8 h of harvest, McIntosh and Honeycrisp fruit were held at 10 and 20 °C overnight, respectively. Fruits were divided randomly into control and 1-MCP treatment groups and treated with 1 µL/L 1-MCP within 24 h of harvest in a 2 m^3 gas-tight chamber at room temperature for 24 h. 1-MCP was generated from SmartFresh™ Research Tablets (AgroFresh, Inc., Philadelphia, PA, USA) in accordance with the manufacturer's instructions and a battery-operated fan (Coleman, Wichita, KS, USA) facilitated the circulation of 1-MCP throughout the chamber. After 1-MCP treatment and venting, fruit, which were in plastic crates, were loosely covered with a single layer of polyethylene film to reduce water loss and placed in controlled atmosphere (CA) or regular atmosphere (RA) storage. Honeycrisp apples were held at 20 ± 1 °C for one week before being placed in RA or CA storage to inhibit scald development during storage. Control and 1-MCP-treated fruits were stored at 0 ± 0.5 °C and 95% RH in the air (RA) or CA, which was comprised of 2.5% CO_2, and 2.5% or 2% O_2 for McIntosh and Honeycrisp apples, respectively. Samples were removed from CA or RA storage after 4 months. The two apple cultivars were stored in two different rooms. A total of 16 bushels or 1600 (one-bushel = about 100 apples) apples were used for this experiment. At each removal, 50 fruit per experimental unit were sampled and processed into juice.

2.2. Juice Preparation

Cloudy apple juice was prepared by washing six medium-sized apples with tap water, cutting each into 12 pieces and pressing them using a laboratory-scale juice extractor (Supreme Juicerator, Windsor, NJ, USA). Juice samples were collected into 250-mL beakers containing 0.5 g/L ascorbic acid [9]. The juice was filtered with four-layer cheesecloth and pasteurized at 80 °C for 5 min using a water bath and then cooled immediately to room temperature (20 °C) [9]. Samples were placed in 50-mL centrifuge tubes (Fisher Scientific, Ottawa, ON, Canada) and stored at −20 °C until analysis.

Clear apple juice was prepared as above without ascorbic acid addition. Filtered juice samples were treated with Ultrazym 100 G (Novozymes Ultrazym 100 G, 0.04 g/L) for 3 h at 25 °C and then heated at 80 °C for 5 min (to prevent further enzyme activity which can reduce juice clarity). Ultrazym, a clarification aid enzyme, acts only on soluble pectin [10] and is reported to contain pectinesterases, polygalacturonases, and pectin lyases. After enzyme treatment, the juice was allowed to settle overnight at 4 °C, centrifuged at 8500 rpm for 15 min and stored at −20 °C until analysis. The same procedure was repeated separately for each of the three replicates for both apple cultivars and juice types.

2.3. Volatile Analysis from the Juice

Frozen juice samples were defrosted overnight at 4 °C. Juice samples (10 mL) were pipetted into a new 20-mL vial followed by the addition of 2 g of NaCl and 100 µL of the internal standard [2-octanone (0.524 µg/mL 100% methanol)]. Volatile analysis of juice samples was conducted by headspace solid-phase microextraction (HS-SPME) followed by GC-MS. During SPME extraction,

the juice sample was agitated at 250 rpm. A 20-mm DVB/CAR/PDMS HS-SPME fiber (Supelco Analytical, Bellefonte, PA, USA) was exposed to the head-space of the juice sample for 30 min at 30 °C. The fiber was desorbed at 250 °C for 5 min using a split ratio of 1:3 using a Varian 4000 GC-MS system (Varian Chromatography Walnut Creek, CA, USA), equipped with a Varian 1177 injector and a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland). Volatile compounds were separated on a VF-WAXms capillary column (30 m × 0.32 mm i.d., 1.0 µm, Agilent Technologies, Amstelveen, The Netherlands). The temperature program was 35 °C for 5.0 min, 35 to 240 °C at 10 °C/min, and 240 °C for 4.5 min. The injector was maintained at 250 °C, and the carrier gas was helium with a flow rate of 2.5 mL/min. Detection by MS was carried out in electron ionization (EI) mode with a mass range of 35–400 amu, emission current of 25 µAmps, a scan rate of 0.60 s (4 µscans), and a total run time of 30 min. Temperatures of the transfer line, trap, manifold, and ion source were 170, 100, 50, and 180 °C, respectively. All the peak areas were normalized using the peak area of the internal standard. The volatile compounds were identified based on retention index (RI) and by comparing mass spectra with spectral data from the National Institute of Standards and Technology library and confirming where possible with standards. The RI values were calculated based on the retention times of a series of alkane standards.

2.4. Volatile Analysis from Whole Apples

Apples were held in a 4-L sealed glass jar with a Teflon lid for 1 h at 20 °C. A 100-mL sample of head-space was captured on an adsorption tube (89 long × 6.4 mm outer diameter) containing Carbopack B (155 mg) and Carboxen 1000 (70 mg), both from Supelco Inc. (Oakville, ON, Canada). Adsorption tubes were held at −86 °C until the time of analysis. Volatiles were removed from the adsorption tube using a TurboMatrix 650 ATD thermal desorber (PerkinElmer Life and Analytical Sciences, CT, USA). Tubes were heated to 250 °C using an outlet split of 1:2 for sample introduction into a Varian 4000 gas chromatography mass-spectrometry system (GC-MS) (Varian Inc., Walnut Creek, CA, USA). The volatile analysis was conducted using a VF-WAXms column (0.32 mm, internal diameter × 30 m, length × 1.00 µm, film thickness, Varian Inc., Lake Forest, CA, USA). The column was held at 35 °C for 5 min, increased 10 °C per min to 240 °C, and held for 4.5 min. The column flow rate was 2.5 mL/min of helium while temperatures of the transfer line from the GC to the MS and the MS were 180 and 220 °C, respectively. Detection by MS was carried out in electron ionization (EI) mode with a mass range of 35–400 amu, emission current of 25 µAmps, and a scan rate of 0.60 s (4 µscans). Temperatures of the transfer line, trap, manifold, and ion source were 170, 100, 50, and 180 °C, respectively. The analyses were done on three replicates, each with 1.0 ± 0.1 kg.

2.5. Experimental Design and Statistical Analysis

The experimental design was a four-factor factorial with three replications. The independent variables included (i) 1-MCP treatment, (ii) storage atmosphere, (iii) harvest maturity, and (iv) juice type. Statistical analyses were performed using Minitab software (Release 17, Minitab Inc. State College, PA, USA). Analysis of variance (ANOVA) was done using the general linear model (GLM) procedure. Whenever there were significant main or interaction effects, multiple mean comparisons were employed using Tukey's method at a significance level, $\alpha = 0.05$. For each response, the validity of model assumptions, namely normal distribution and constant variance of the error terms, were verified by examining residual plots. In some data sets, transformations had to be used to achieve normality [11].

3. Results

3.1. Volatile Aroma Composition

From the GC-MS analysis of clear and cloudy juice produced from McIntosh (Figure 1) and Honeycrisp (Figure 2) apples, the 14 most abundant volatile aroma compounds were identified (Table 1). The volatile compounds from McIntosh juice were composed of about 42% aldehydes,

37% esters, and 19% alcohols (Figure 3A). The major aldehyde detected in McIntosh juice was 2-methyl-4-pentenal (38.4%) with a minor content of (E)-2-hexenal (3.4%) (Figure 3A). The major esters detected in McIntosh juice included ethyl butanoate, butyl butanoate, hexyl butanoate, and 2-methyl butyl acetate, which contributed 11.8%, 8.1%, 7.8%, and 2.8% of the total volatile compounds, respectively (Figure 3A). Unidentified branched-chain alcohol and 2-hexen-1-ol, which comprised 12.6% and 6.0% of the total volatiles, were the major alcohols detected in the McIntosh juice (Figure 3A).

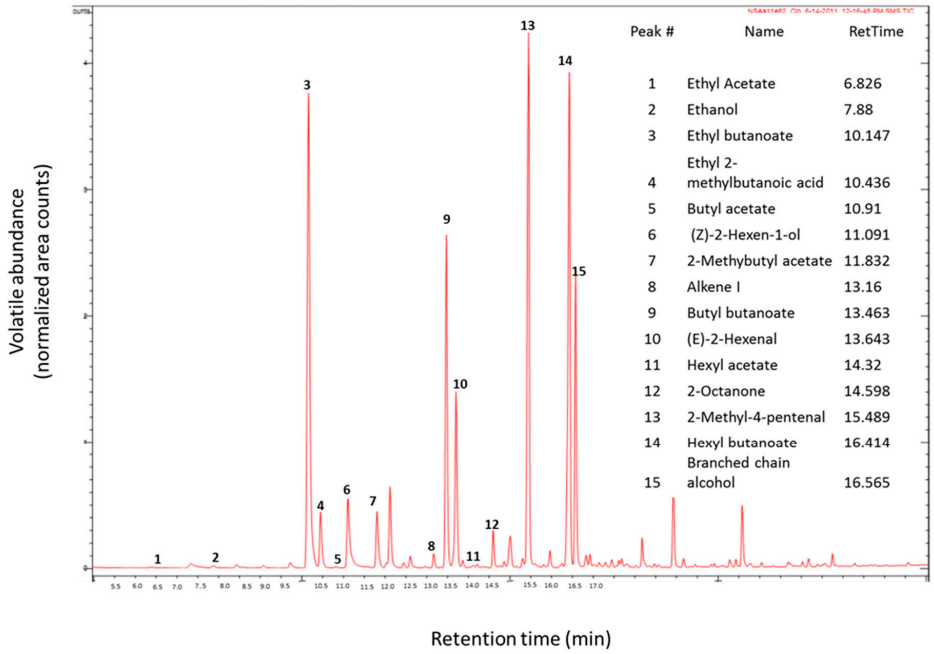

Figure 1. A typical chromatogram for cloudy apple juice from McIntosh apples.

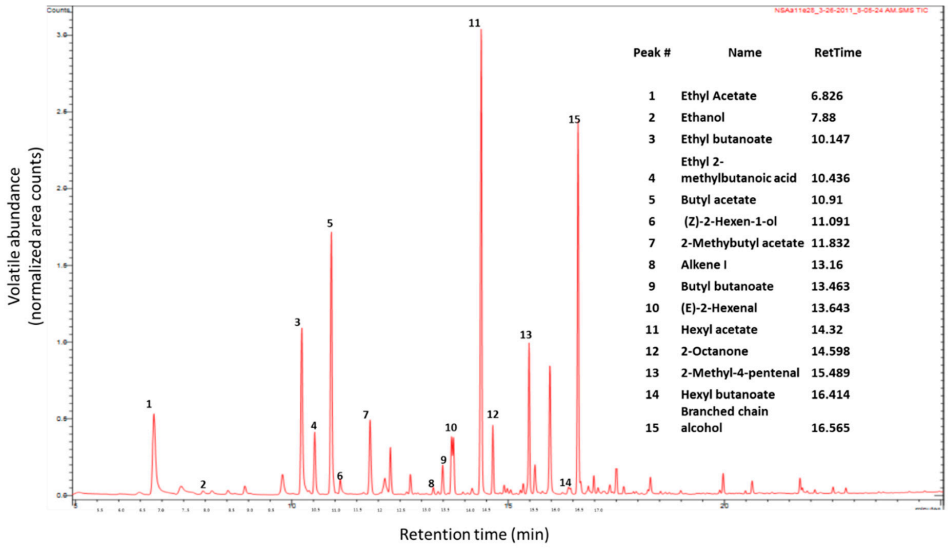

Figure 2. A typical chromatogram for cloudy apple juice from Honeycrisp apples.

Table 1. Major volatile compounds isolated from clear and cloudy apple juice of McIntosh and Honeycrisp apples.

Volatile Compounds	Retention Time (min)	Experimental Retention Index (RI) [a]	Odor Property	Odor Threshold (mg/L)
Esters				
Ethyl acetate	6.83	898	Ether like [2]	7.50 [12]
Ethyl butanoate	10.15	1049	Sweet fruity [13]	0.001 [14]
Butyl acetate	10.91	1087	Sweet fruity [13]	0.066 [12,14]
2-Methyl butyl acetate	11.83	1137	Fresh [13]	0.011 [14]
Butyl butanoate	13.46	1233	Fresh [13]	0.10 [14]
Hexyl acetate	14.32	1287	Sweet fruity [13]	0.002 [14]
Hexyl butanoate	16.41	1433		
Aldehydes				
2-(E)-hexenal	13.64	1244	Green apple like [2]	0.011 [12]
2-Methyl-4-pentenal	15.49	1367	Desirable, green grass, fruity [15]	
Alcohols				
Ethanol	7.88	944	Sweet [2]	716 [12]
(Z)-2-hexen-1-ol	11.09	1096	Fresh leaf green [13]	0.07 [16]
Unidentified branched-chain alcohol	16.56	1444		
Acids				
Ethyl 2-methylbutanoic acid	10.43	1063		
Hydrocarbon				
Alkene I	13.16	1214		

[a] Retention index (RI) was calculated based on the retention times of a series of alkane standards.

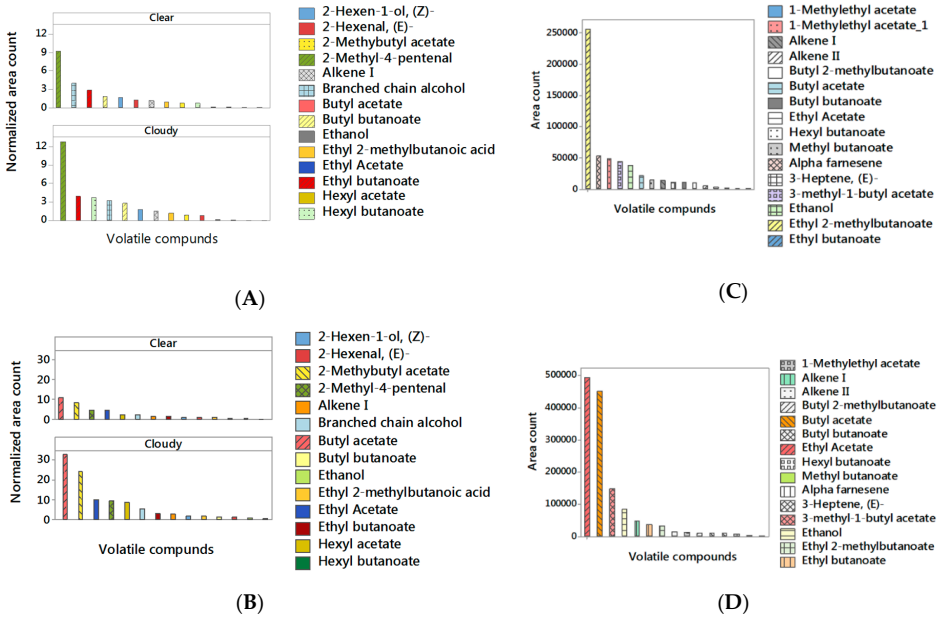

Figure 3. The relative abundance of volatile compounds identified in fresh, clear, and cloudy apple juices from McIntosh (**A**) and Honeycrisp (**B**) apples, as well as from whole McIntosh (**C**) and Honeycrisp (**D**) apples stored for 4 months under controlled atmosphere (CA) or regular atmosphere (RA) conditions and harvested at commercial or late maturity stage. The value for each bar represents the mean of 48 samples each with three biological replicates.

The aroma volatiles in Honeycrisp juice were dominated by esters, which comprised 78.9% of the total volatiles followed by aldehydes (11.8%) and alcohols (8.7%) (Figure 3B). The major esters found in Honeycrisp juice were butyl acetate (31.3%), 2-methyl butyl acetate (22.9%), ethyl acetate (10.3%), and hexyl acetate (8.0%) (Figure 3B). As in McIntosh, the major aldehyde in Honeycrisp juice was 2-methyl-4-pentenal (10.4%) with a minor amount of (E)-2-hexenal (1.4%) (Figure 3B). The unidentified branched-chain alcohol (5.7%) and 2-hexen-1-ol (2.1%) were the major alcohols detected in Honeycrisp juice. In both cultivars, ethanol was a minor constituent, accounting for <1% of the total volatile compounds (Figure 3A,B).

3.2. Effect of Harvest Maturity, 1-MCP, and Storage Conditions on the Volatile Composition

3.2.1. McIntosh Juice

Total volatile content from McIntosh juice was greater in juice produced from late-harvested fruit and from fruit stored in RA than juice from commercial maturity and CA-stored fruit. The juice produced from late-harvested fruit had higher concentrations of all the major esters than the commercial maturity harvested fruit, averaging 2.7- to 6.5-fold greater (Table 2). There was a significant interaction between 1-MCP and harvest maturity on their effects on the content of total volatiles and all major esters. The 1-MCP treatment increased the total volatile content of juice produced from commercial harvest fruit by 69% but reduced total volatiles in juice from late-harvested fruit by 32%. All major esters demonstrated this same effect with 1-MCP-treated fruit being 2.3- to 4.9-fold greater in commercial harvest fruit but 1.4- to 2.7-fold less in late-harvest fruit. There was also a significant interaction between harvest maturity and storage atmosphere on their effects on total and major ester composition of McIntosh juice (Table 2). Juice made from commercial maturity harvested fruit tended to have higher concentrations of total volatile and major esters from CA-stored than RA-stored fruit, but with late-harvested fruit, volatiles were the greatest in juice made from RA-stored fruit. Cloudy juice also tended to have higher concentrations of total volatiles and major esters than clear juice, with hexyl butanoate averaging 6.9-fold greater.

The major aldehydes and alcohols identified in McIntosh juices were 2- to 3-fold more abundant in juice produced from late-harvested fruit than from commercial maturity fruit except (E)-2-hexenal that had a 2.3-fold greater concentration in juice from the commercial harvest maturity (Table 3). The 1-MCP treatment had a significant interaction with harvest maturity that affected the aldehyde and alcohol content of McIntosh juice. In juice made from commercial maturity fruit, concentrations of 2-methyl-4-pentenal, the unknown branched-chain alcohol, and (Z)-2-hexen-1-ol were 2.3-, 3.9-, and 1.2-fold greater in juice made from 1-MCP-treated fruit than untreated fruit. However, in late-harvested fruit, the fruit concentrations were lower in juice from 1-MCP-treated fruit except for (E)-2-hexenal, which was 1.7-fold higher. There was also a significant interaction between harvest maturity and storage atmosphere. In juice made from commercial maturity fruit, concentrations of 2-methyl-4-pentenal and the unknown branched-chain alcohol were 2.5- and 6.3-fold greater in juice made from CA- than RA-stored fruit. However, in late-harvested fruit, these concentrations were lower in juice from CA-stored fruit except for (E)-2-hexenal, which was 1.6-fold higher. The concentration of (E)-2-hexenal was 1.6- to 1.9-fold higher in clear juice than in cloudy juice produced from both commercial maturity and late-harvested fruit, respectively (Table 3). The concentration of (Z)-2-hexen-1-ol was also about 1.5-fold higher in clear juice than cloudy juice from commercial maturity fruit, but in juice from late-harvested fruit, the concentration in cloudy juice was 1.5-fold higher than in clear juice. It is also interesting to note that ethanol was not detected among the volatiles of McIntosh juice.

Table 2. The relative amounts of major esters and total volatile compounds identified in clear and cloudy juice samples from McIntosh apples harvested at commercial or late-harvest maturity, treated, or untreated with 1-MCP and stored under CA or RA conditions for 4 months.

Treatment Combinations			Normalized Peak Area Counts [a]				
			Ethyl Butanoate	**Butyl Butanoate**	**Hexyl Butanoate**	**2-Methyl Butyl Acetate**	**Total Volatile [h]**
Harvest	Comm. [b]		0.59 ± 0.06 ^{B}	0.31 ± 0.05 ^{B}	0.20 ± 0.29 ^{B}	0.43 ± 0.13 ^{B}	20.31 ± 3.28 ^{B}
(H)	Late		2.47 ± 0.06 ^{A}	2.01 ± 0.05 ^{A}	0.89 ± 0.29 ^{A}	1.16 ± 0.13 ^{A}	37.14 ± 3.28 ^{A}
1-MCP	1-MCP		1.48 ± 0.06 ^{A}	0.94 ± 0.05 ^{A}	0.41 ± 0.29 ^{A}	0.79 ± 0.13 ^{A}	27.87 ± 3.28 ^{A}
(M)	Control		1.09 ± 0.06 ^{A}	0.79 ± 0.05 ^{A}	0.43 ± 0.29 ^{A}	0.80 ± 0.13 ^{A}	29.58 ± 3.28 ^{A}
Atmos. [c]	CA [d]		0.99 ± 0.06 ^{A}	0.64 ± 0.05 ^{A}	0.31 ± 0.29 ^{A}	0.42 ± 0.13 ^{B}	21.07 ± 3.28 ^{B}
(A)	RA [e]		1.62 ± 0.06 ^{A}	1.14 ± 0.05 ^{A}	0.57 ± 0.29 ^{A}	0.16 ± 0.13 ^{A}	36.38 ± 3.28 ^{A}
Juice	Clear		0.94 ± 0.06 ^{A}	0.61 ± 0.05 ^{A}	0.16 ± 0.29 ^{B}	0.72 ± 0.13 ^{A}	24.37 ± 3.28 ^{A}
(J)	Cloudy		1.69 ± 0.06 ^{A}	1.18 ± 0.05 ^{A}	1.10 ± 0.29 ^{A}	0.87 ± 0.13 ^{A}	33.08 ± 3.28 ^{A}
H × M	Comm.	1-MCP	1.22 ± 0.08 ^{A,B}	0.62 ± 0.07 ^{B,C}	0.31± 0.42 ^{A,B}	0.61 ± 0.18 ^{B,C}	25.80 ± 4.64 ^{B}
	Comm.	Control	0.25 ± 0.08 ^{B}	0.14 ± 0.07 ^{C}	0.13 ± 0.42 ^{B}	0.26 ± 0.18 ^{C}	14.82 ± 4.64 ^{B}
	Late	1-MCP	1.77 ± 0.08 ^{A}	1.37 ± 0.07 ^{A,B}	0.55 ± 0.42 ^{A,B}	0.97 ± 0.18 ^{A,B}	29.95 ± 4.64 ^{A,B}
	Late	Control	3.40 ± 0.08 ^{A}	2.87 ± 0.07 ^{A}	1.46 ± 0.42 ^{A}	1.34 ± 0.18 ^{A}	44.33 ± 4.64 ^{A}
H × A	Comm.	CA	1.64 ± 0.08 ^{B}	0.72 ± 0.07 ^{B}	0.41 ± 0.42 ^{A}	0.58 ± 0.18 ^{B}	25.54 ± 4.64 ^{B}
	Comm.	RA	0.16 ± 0.08 ^{C}	0.12 ± 0.07 ^{B}	0.09 ± 0.42 ^{B}	0.28 ± 0.18 ^{B}	15.08 ± 4.64 ^{B}
	Late	CA	0.56 ± 0.08 ^{B,C}	0.57 ± 0.07 ^{B}	0.24 ± 0.42 ^{B}	0.26 ± 0.18 ^{B}	16.60 ± 4.64 ^{B}
	Late	RA	7.86 ± 0.08 ^{A}	5.50 ± 0.07 ^{A}	3.40 ± 0.42 ^{A}	2.05 ± 0.18 ^{A}	57.86 ± 4.64 ^{A}
H × J	Comm.	Clear	0.48 ± 0.08 ^{B}	0.27 ± 0.07 ^{B}	0.09 ± 0.42 ^{B}	0.46 ± 0.18 ^{B}	20.55± 4.64 ^{B}
	Comm.	Cloudy	0.72 ± 0.08 ^{A}	0.36 ± 0.07 ^{B}	0.42 ± 0.42 ^{A}	0.41 ± 0.18 ^{B}	20.08 ± 4.64 ^{B}
	Late	Clear	1.71 ± 0.08 ^{A}	1.24 ± 0.07 ^{A}	0.27 ± 0.42 ^{A}	0.98 ± 0.18 ^{A}	28.19 ± 4.64 ^{A}
	Late	Cloudy	3.50 ± 0.08 ^{A}	3.31 ± 0.07 ^{A}	2.92 ± 0.42 ^{A}	1.33 ± 0.18 ^{A}	46.09 ± 4.64 ^{A}
Statistical Significance [f]			H ***	H ***	H ***	H ***	H ***
						A ***	A ***
					J ***		
			H × M *	H × M *	H × M *	H × M *	H × M **
			H × A ***	H × A ***	H × A ***	H × A ***	H × A ***
					H × J **		
Lambda [g]			0.2	0.2	0	1	1

All the values represent mean ± standard error of three biological replicates. Means followed by the same letter within a column are not significantly different. [a] Ratio of sample peak area counts to the peak area count of the internal standard, 2-octanone. [b] Comm. = commercial maturity for long-term storage. [c] Atmos. = storage atmosphere. [d] CA = controlled atmosphere. [e] RA = regular atmosphere. [f] Statistical significance = only significant main or interaction effects are reported for each response. *, **, *** = significant at $p < 0.05$, $p < 0.01$, or $p < 0.001$, respectively. A, atmosphere; H, harvest; M, 1-MCP; J, juice. [g] The lambda values other than zero indicate the power to which all the data should be raised. Zero lambda values indicate the natural *log* transformation. Mean values were back-transformed to their original values. [h] Total volatile is for all the volatiles included in Tables 2 and 3.

Table 3. Relative amounts of major aldehydes and alcohols identified in clear and cloudy juice samples from McIntosh apples harvested at commercial or late-harvest maturity, treated or untreated with 1-MCP and stored under CA or RA conditions for 4 months.

Treatment Combinations			Normalized Peak Area Counts [a]			
			2-Methyl 4-Pentenal	(E)-2- Hexenal	Branched-Chain Alcohol	(Z)-2-Hexen-1-ol
Harvest	Comm. [b]		6.37 ± 0.19 ^{B}	1.15 ± 0.05 ^{A}	0.32 ± 0.37 ^{B}	1.06 ± 0.07 ^{B}
(H)	Late		12.69 ± 0.19 ^{A}	0.49 ± 0.05 ^{B}	0.98 ± 0.37 ^{A}	2.07 ± 0.07 ^{A}
1-MCP	1-MCP		9.73 ± 0.19 ^{A}	0.84 ± 0.05 ^{A}	0.55 ± 0.37 ^{A}	1.40 ± 0.07 ^{A}
(M)	Control		8.81 ± 0.19 ^{A}	0.62 ± 0.05 ^{B}	0.58 ± 0.37 ^{A}	1.57 ± 0.07 ^{A}
Atmos. [c]	CA [d]		8.26 ± 0.19 ^{A}	0.78 ± 0.05 ^{A}	0.37 ± 0.37 ^{A}	1.65 ± 0.07 ^{A}
(A)	RA [e]		10.33 ± 0.19 ^{A}	0.66 ± 0.05 ^{A}	0.85 ± 0.37 ^{A}	1.33 ± 0.07 ^{B}
Juice	Clear		7.67 ± 0.19 ^{A}	0.97 ± 0.05 ^{A}	0.51 ± 0.37 ^{A}	1.50 ± 0.07 ^{A}
(J)	Cloudy		11.01 ± 0.19 ^{A}	0.55± 0.05 ^{B}	0.66 ± 0.37 ^{A}	1.46 ± 0.07 ^{A}
H × M	Comm.	1-MCP	9.30 ± 0.27 ^{A}	1.12 ± 0.07 ^{A}	0.63 ± 0.53 ^{B}	1.20 ± 0.09 ^{B,C}
	Comm.	Control	4.00 ± 0.27 ^{B}	1.19 ± 0.07 ^{A}	0.16 ± 0.53 ^{C}	0.94 ± 0.09 ^{C}
	Late	1-MCP	10.17 ± 0.27 ^{A}	0.65 ± 0.07 ^{B}	0.48 ± 0.53 ^{B}	1.63 ± 0.09 ^{B}
	Late	Control	15.50 ± 0.27 ^{A}	0.38 ± 0.07 ^{C}	2.01 ± 0.53 ^{A}	2.63 ± 0.09 ^{A}
H × A	Comm.	CA	9.54 ± 0.27 ^{B}	1.01 ± 0.07 ^{A}	0.82 ± 0.53 ^{B}	1.16 ± 0.09 ^{A}
	Comm.	RA	3.84 ± 0.27 ^{C}	1.33 ± 0.07 ^{A}	0.13 ± 0.53 ^{C}	0.97 ± 0.09 ^{B}
	Late	CA	7.07 ± 0.27 ^{B,C}	0.63 ± 0.07 ^{B}	0.17 ± 0.53 ^{C}	2.35 ± 0.09 ^{A}
	Late	RA	19.96 ± 0.27 ^{A}	0.39 ± 0.07 ^{C}	5.76 ± 0.53 ^{A}	1.83 ± 0.09 ^{A}
H × J	Comm.	Clear	6.13 ± 0.27 ^{B}	1.41 ± 0.07 ^{A}	0.31 ± 0.53 ^{B}	1.34 ± 0.09 ^{A}
	Comm.	Cloudy	6.62 ± 0.27 ^{B}	0.96 ± 0.07 ^{A,B}	0.33 ± 0.53 ^{B}	0.85 ± 0.09 ^{C}
	Late	Clear	9.38 ± 0.27 ^{A}	0.70 ± 0.07 ^{B}	0.85 ± 0.53 ^{A}	1.69 ± 0.09 ^{A}
	Late	Cloudy	16.51 ± 0.27 ^{A}	0.36 ± 0.07 ^{C}	1.12 ± 0.53 ^{A}	2.53 ± 0.09 ^{A}
Statistical Significance [f]			H ***	H ***	H *	H ***
				M **		A *
				J ***		
			H × M ***	H × M **	H × M ***	H × M ***
			H × A ***	H × A ***	H × A ***	
				H × J *		H × J ***
Lambda [g]			0.5	0.5	0	0

All the values represent mean ± standard error of three biological replicates. Means followed by the same letter within a column are not significantly different. [a] Ratio of sample peak area counts to the peak area count of the internal standard, 2-octanone. [b] Comm. = commercial maturity for long-term storage. [c] Atmos. = storage atmosphere. [d] CA = controlled atmosphere. [e] RA = regular atmosphere. [f] Statistical significance = only significant main or interaction effects are reported for each response. *, **, *** = significant at $p < 0.05$, $p < 0.01$, or $p < 0.001$, respectively. A, atmosphere; H, harvest; M, 1-MCP; J, juice. [g] The lambda values other than zero indicate the power to which all the data should be raised. Zero lambda values indicate the natural *log* transformation. Mean values were back-transformed to their original values.

3.2.2. Honeycrisp Juice

Regardless of the postharvest treatment and storage conditions, Honeycrisp juice had about 4-fold higher ester and total aroma volatile content than McIntosh juice. In Honeycrisp, significant differences in the level of esters and total aroma volatiles were mainly caused by storage atmosphere and juice processing techniques ($p \leq 0.001$) but not by 1-MCP treatment (Table 4). Overall, cloudy juice produced from Honeycrisp fruit had a 3- to 4-fold higher content of all the major esters and total volatile compounds than in clear juice. Similar to McIntosh, CA storage of Honeycrisp fruit resulted in juices that exhibited a 27% to 51% reduction of most straight-chain esters as compared to RA storage (Table 4). However, the concentration of the branched-chain ester 2-methyl butyl acetate was 2.6-fold higher in juice from CA-stored fruit than RA-stored fruit. This was similar to juice made from commercial maturity McIntosh fruit, but not late-harvested fruit, which had higher 2-methyl butyl acetate concentration in juice from RA-stored fruit than CA-stored fruit. Ethyl acetate was the only volatile compound that was significantly reduced in juice made from 1-MCP-treated Honeycrisp apples, being only 8% to 21% of that from untreated fruit. This is an interesting observation because ethyl acetate is a major volatile that leads to off-flavors.

The major aldehydes and alcohols in Honeycrisp juice were significantly affected by the storage atmosphere ($p \leq 0.001$). The concentration of 2-methyl-4-pentenal and the unknown branched-chain

alcohol in juice made from CA-stored fruit were 29% and 74%, respectively, less than in juice from RA-stored fruit, while (E)-2-hexenal and (Z)-2-hexen-1-ol were 40% and 51% greater (Table 5). The 1-MCP treatment reduced the concentration of the unknown branched-chain alcohol and ethanol by 91% and 84%, respectively, in Honeycrisp juice. Aldehyde content was 31% and 50% less for 2-methyl-4-pentenal and (E)-2-hexenal, respectively, in juice produced from late-harvested fruit compared to commercial harvest fruit. On average, cloudy juice from Honeycrisp fruit had 33.5% to 66.9% higher content of all the major aldehydes and alcohols as compared to clear juice, with the difference being most prominent in late-harvested fruit samples (Table 5).

Table 4. Relative amounts of major ester volatile compounds identified in clear and cloudy juice samples from Honeycrisp apples harvested at commercial or late-harvest maturity, treated or untreated with 1-MCP and stored under CA or RA conditions for 4 months.

Treatment Combinations			Normalized Peak Area Counts [a]				
			Butyl Acetate	**2-Methyl Butyl Acetate**	**Ethyl Acetate**	**Hexyl Acetate**	**Total Volatile**
Harvest (H)	Comm. [b]		16.17 ± 0.09 ^{A}	14.26 ± 0.20 ^{A}	3.57 ± 0.03 ^{A}	4.77 ± 0.06 ^{A}	58.44 ± 8.17 ^{A}
	Late		16.46 ± 0.10 ^{A}	12.44 ± 0.21 ^{A}	4.24 ± 0.03 ^{A}	3.36 ± 0.06 ^{A}	48.31 ± 8.43 ^{A}
1-MCP (M)	1-MCP		15.64 ± 0.10 ^{A}	15.22 ± 0.20 ^{A}	1.41 ± 0.03 ^{B}	4.08 ± 0.06 ^{A}	50.09 ± 8.17 ^{A}
	Control		17.02 ± 0.10 ^{A}	11.58 ± 0.20 ^{A}	9.33 ± 0.03 ^{A}	3.96 ± 0.06 ^{A}	56.37± 8.43 ^{A}
Atmos. [c] (A)	CA [d]		13.95 ± 0.10 ^{B}	19.78 ± 0.20 ^{A}	2.69 ± 0.03 ^{B}	2.82 ± 0.06 ^{B}	57.91± 8.17 ^{A}
	RA [e]		19.09 ± 0.10 ^{A}	8.15 ± 0.20 ^{B}	5.51 ± 0.03 ^{A}	5.54 ± 0.06 ^{A}	48.75± 8.43 ^{A}
Juice (J)	Clear		9.08 ± 0.10 ^{B}	6.48 ± 0.20 ^{B}	2.53 ± 0.03 ^{B}	1.79 ± 0.06 ^{B}	30.90 ± 8.17 ^{B}
	Cloudy		29.33 ± 0.10 ^{A}	22.63 ± 0.20 ^{A}	5.81 ± 0.03 ^{A}	7.72 ± 0.06 ^{A}	91.36 ± 8.43 ^{A}
H × M	Comm.	1-MCP	14.70 ± 0.14 ^{A}	16.70 ± 0.28 ^{A}	0.90 ± 0.04 ^{B}	4.23 ± 0.08 ^{A}	53.62 ± 11.6 ^{A}
	Comm.	Control	17.79 ± 0.14 ^{A}	12.02 ± 0.28 ^{A}	11.01 ± 0.04 ^{A}	5.36 ± 0.08 ^{A}	63.70 ± 11.6 ^{A}
	Late	1-MCP	16.64 ± 0.15 ^{A}	13.81± 0.30 ^{A}	2.13 ± 0.04 ^{B}	3.95 ± 0.09 ^{A}	46.79 ± 11.6 ^{A}
	Late	Control	16.29 ± 0.15 ^{A}	11.14± 0.30 ^{A}	7.86 ± 0.04 ^{A}	2.84 ± 0.099 ^{A}	49.88 ± 12.3 ^{A}
H × A	Comm.	CA	12.93 ± 0.14 ^{A}	20.43 ± 0.28 ^{A}	2.55 ± 0.04 ^{B}	3.39 ± 0.08 ^{A}	65.88 ± 11.6 ^{A}
	Comm.	RA	20.23 ± 0.14 ^{A}	9.20 ± 0.28 ^{B}	4.91± 0.04 ^{A}	6.50 ± 0.08 ^{A}	51.84 ± 11.6 ^{A}
	Late	CA	15.04 ± 0.15 ^{A}	19.15 ± 0.30 ^{A}	2.84 ± 0.04 ^{B}	2.33 ± 0.09 ^{B}	50.91 ± 12.3 ^{A}
	Late	RA	18.01± 0.15 ^{A}	7.17 ± 0.30 ^{B}	6.16 ± 0.04 ^{A}	4.68 ± 0.09 ^{A}	45.85 ± 11.6 ^{A}
H × J	Comm.	Clear	11.95 ± 0.14 ^{c}	9.61 ± 0.28 ^{B}	3.19 ± 0.04 ^{B}	2.56 ± 0.08 ^{B}	45.07 ± 11.6 ^{B}
	Comm.	Cloudy	21.88 ± 0.14 ^{A}	19.84 ± 0.28 ^{A}	4.00 ± 0.04 ^{A}	8.06 ± 0.08 ^{A}	75.77 ± 11.6 ^{A}
	Late	Clear	6.89 ± 0.15 ^{C}	3.97 ± 0.30 ^{B}	2.00 ± 0.04 ^{B}	1.19 ± 0.09 ^{B}	21.19 ± 12.3 ^{C}
	Late	Cloudy	39.30 ± 0.15 ^{A}	25.60 ± 0.30 ^{A}	8.28 ± 0.04 ^{A}	7.39 ± 0.09 ^{A}	110.17 ± 11.6 ^{A}
Statistical Significance [f]			A * J *** H × J ***	A *** J *** H × J **	M *** A *** J *** H × M ** H × J **	A *** J ***	M * J *** H × J ***
Lambda [g]			0	0.5	0.2	0.3	0

All the values represent mean ± standard error of three biological replicates. Means followed by the same letter within a column are not significantly different. [a] Ratio of sample peak area counts to the peak area count of the internal standard, 2-octanone. [b] Comm. = commercial maturity for long-term storage. [c] Atmos. = storage atmosphere. [d] CA = controlled atmosphere. [e] RA = regular atmosphere. [f] Statistical significance = only significant main or interaction effects are reported for each response. *, **, *** = significant at $p < 0.05$, $p < 0.01$, or $p < 0.001$, respectively. A, atmosphere; H, harvest; M, 1-MCP; J, juice. [g] The lambda values other than zero indicate the power to which all the data should be raised. Zero lambda values indicate the natural log transformation. Mean values were back-transformed to their original values.

Table 5. The relative amounts of major aldehydes and alcohols identified in clear and cloudy juice samples from Honeycrisp apples harvested at commercial or late-harvest maturity, treated or untreated with 1-MCP and stored under CA or RA conditions for 4 months.

Treatment Combinations			Normalized Peak Area Counts [a]				
			2-Methyl-4-Pentenal	(E)-2-Hexenal	Branched Chain Alcohol	(Z)-2-Hexen-1-ol	Ethanol
Harvest (H)	Comm. [b]		6.62 ± 0.103 ^{A}	1.02 ± 0.133 ^{A}	1.07 ± 0.017 ^{A}	1.33 ± 0.184 ^{A}	0.56 ± 0.113 ^{A}
	Late		4.57 ± 0.103 ^{B}	0.51 ± 0.141 ^{B}	1.11 ± 0.018 ^{A}	1.61 ± 0.195 ^{A}	0.71 ± 0.119 ^{A}
1-MCP (M)	1-MCP		5.22 ± 0.107 ^{A}	0.70 ± 0.137 ^{A}	0.39 ± 0.018 ^{B}	1.52 ± 0.189 ^{A}	0.17 ± 0.116 ^{B}
	Control		5.81 ± 0.107 ^{A}	0.74 ± 0.137 ^{A}	3.69 ± 0.018 ^{A}	1.41 ± 0.189 ^{A}	1.09 ± 0.116 ^{A}
Atmos. [c] (A)	CA [d]		4.64 ± 0.107 ^{B}	0.93 ± 0.137 ^{A}	0.58 ± 0.018 ^{B}	1.97 ± 0.189 ^{A}	0.50 ± 0.116 ^{A}
	RA [e]		6.52 ± 0.107 ^{A}	0.56 ± 0.137 ^{B}	2.23 ± 0.018 ^{A}	0.97 ± 0.189 ^{B}	0.77 ± 0.116 ^{A}
Juice (J)	Clear		3.37 ± 0.107 ^{B}	0.54 ± 0.137 ^{B}	0.66 ± 0.018 ^{B}	0.92 ± 0.189 ^{B}	0.41 ± 0.116 ^{B}
	Cloudy		8.98 ± 0.107 ^{A}	0.96 ± 0.137 ^{A}	1.88 ± 0.018 ^{A}	2.02 ± 0.189 ^{A}	0.86 ± 0.116 ^{A}
H × M	Comm.	1-MCP	5.59 ± 0.146 ^{A}	0.85 ± 0.188 ^{A}	0.31 ± 0.024 ^{B}	1.38 ± 0.260 ^{A}	0.10 ± 0.159 ^{A}
	Comm.	Control	7.85 ± 0.146 ^{A}	1.22 ± 0.188 ^{A}	5.10 ± 0.024 ^{A}	1.29 ± 0.260 ^{A}	1.02 ± 0.159 ^{A}
	Late	1-MCP	4.87 ± 0.155 ^{A}	0.58 ± 0.199 ^{A}	0.51 ± 0.024 ^{B}	1.67 ± 0.275 ^{A}	0.24 ± 0.169 ^{A}
	Late	Control	4.29 ± 0.155 ^{A}	0.45 ± 0.199 ^{A}	2.72 ± 0.024 ^{A}	1.54 ± 0.275 ^{A}	1.18 ± 0.169 ^{A}
H × A	Comm.	CA	5.74 ± 0.146 ^{A}	1.48± 0.188 ^{A}	0.62 ± 0.024 ^{A}	1.82 ± 0.260 ^{A}	0.45 ± 0.159 ^{A}
	Comm.	RA	7.64 ± 0.146 ^{A}	0.47 ± 0.188 ^{A}	1.97 ± 0.024 ^{A}	0.84 ± 0.260 ^{A}	0.67 ± 0.159 ^{A}
	Late	CA	3.75 ± 0.155^{A}	0.58 ± 0.199 ^{A}	0.54 ± 0.024 ^{A}	2.11 ± 0.275 ^{A}	0.55 ± 0.169 ^{A}
	Late	RA	5.57 ± 0.155 ^{A}	0.45 ± 0.199 ^{A}	2.53± 0.024 ^{A}	1.11 ± 0.275 ^{A}	0.87 ± 0.169 ^{A}
H × J	Comm.	Clear	5.68 ± 0.146 ^{B}	0.71 ± 0.188 ^{A}	0.87± 0.024 ^{B,C}	1.07 ± 0.260 ^{B}	0.55 ±0.159 ^{A,B}
	Comm.	Cloudy	7.73 ± 0.146 ^{A,B}	1.48 ± 0.188 ^{A}	1.34 ± 0.024 ^{B}	1.60 ± 0.260 ^{A,B}	0.57± 0.159 ^{A,B}
	Late	Clear	2.00 ± 0.155 ^{C}	0.41 ± 0.199 ^{A}	0.51 ± 0.024 ^{C}	0.76 ± 0.275 ^{B}	0.26 ± 0.169 ^{B}
	Late	Cloudy	10.44 ±0.155 ^{A}	0.62 ± 0.199 ^{A}	2.69 ± 0.024 ^{A}	2.45 ± 0.275 ^{A}	1.16 ± 0.169 ^{A}
M × A	1-MCP	CA	4.03 ± 0.146 ^{A}	1.04 ± 0.188 ^{A}	0.20 ± 0.024 ^{D}	2.18 ± 0.260 ^{A}	0.08 ± 0.159 ^{A}
	1-MCP	RA	6.75 ± 0.155 ^{A}	0.47± 0.188 ^{A}	0.91 ± 0.024 ^{C}	0.87 ± 0.260 ^{A}	0.26 ± 0.159 ^{A}
	Control	CA	5.34 ± 0.155 ^{A}	0.83 ± 0.199 ^{A}	2.25± 0.024 ^{B}	1.75 ± 0.275 ^{A}	0.92 ± 0.169 ^{A}
	Control	RA	6.31 ± 0.146 ^{A}	0.67± 0.199 ^{A}	6.35 ± 0.024 ^{A}	1.08 ± 0.275 ^{A}	1.27 ± 0.169 ^{A}
Statistical significance [f]			H *	H ***			
					M ***		M ***
			A *	A ***	A ***	A ***	
			J ***	J ***	J ***	J **	J ***
					H × M ***		
					M × A **		
			H × J *		H × J **	H × J **	H × J *
Lambda [g]			0	0	0	1	1

All the values represent mean ± standard error of three biological replicates. Means followed by the same letter within a column are not significantly different. [a] Ratio of sample peak area counts to the peak area count of the internal standard, 2-octanone. [b] Comm. = commercial maturity for long-term storage. [c] Atmos. = storage atmosphere. [d] CA = controlled atmosphere. [e] RA = regular atmosphere. [f] Statistical significance = only significant main or interaction effects are reported for each response. *, **, *** = significant at $p < 0.05$, $p < 0.01$, or $p < 0.001$, respectively. A, atmosphere; H, harvest; M, 1-MCP; J, juice. [g] The lambda values other than zero indicate the power to which all the data should be raised. Zero lambda values indicate the natural log transformation. Mean values were back-transformed to their original values.

4. Discussion

4.1. Volatile Aroma Composition

As to the authors' knowledge, this is the first study to report the volatile profile from Honeycrisp juices. Substantiating our results, esters, aldehydes, and alcohols have been reported as major groups of volatile compounds in juices from different apple cultivars including McIntosh [17], Jonagold [13], Golden Delicious, Red Delicious [18], Holsteiner Cox, Ingrid Marie, and Rajka [19]. Our results indicated quantitative and qualitative differences in the level of volatile compounds between the two cultivars. Esters are known for their desirable aromatic notes in apple beverages, which are often described as "fresh apple", "fruity", and "sweet", and it is reasonable to assume that the higher amount of total esters in Honeycrisp juices would increase its overall aroma and flavor [20]. The higher content of 2-methyl-4-pentenal in McIntosh juice samples could offer desirable green grass and fruit aroma notes [15]. Despite the greater content of total esters in Honeycrisp juices, the higher level of ethyl acetate in Honeycrisp juices may lead to the development of undesirable flavor as excessive amounts

of fermentation volatiles, including ethyl acetate, which can generate off-flavors and aromas [3]. The higher concentration of butanoate esters in McIntosh juices could provide juice with a favorable fruity, ripe, and sweet aroma [2]. Due to the low odor threshold values (Table 2) of acetate and butanoate esters, these groups of esters could generate a significant contribution to the final aroma of the juice samples. Accordingly, it has been demonstrated that acetate esters, including butyl acetate, 2-methyl butyl acetate, hexyl acetate, and ethyl butanoate, are major contributors to the typical apple-like aroma and flavor in many apple cultivars [21,22]. Similarly, hexyl acetate, butyl acetate, and ethyl butanoate were reported as the most important volatiles in Jonagold juice [23].

In addition to straight-chain esters, a branched-chain ester, 2-methyl butyl acetate, was one of the most abundant volatile compounds detected in Honeycrisp and McIntosh juices. Owing to its lower threshold value, 2-methyl butyl acetate has been described as one of the most significant odor active volatile compounds in other apple cultivars, including Gala [24].

It has been indicated that C-6 aldehydes, particularly (E)-2-hexenal and hexenal, are responsible for the fresh green aroma in apple juice [25]. Excessive concentration of C-6 aldehydes (>2430 μg/L) in apple juice has been associated with negative odor impressions and thus led to lower sensory scores and is often denoted by sensory descriptors such as "artificial flavor, too green, and shampoo-like" [2]. Thus, it is reasonable to assume the relatively high content of C-6 aldehydes in McIntosh juices might lead to the development of negative organoleptic properties.

The volatile compounds identified from McIntosh and Honeycrisp juices are a combination of primary (synthesized by the intact fruit) and secondary (synthesized in response to cellular disruption during juice processing) volatile compounds [25]. The volatile compounds detected from intact fruit are mainly composed of esters and ethanol (Figure 3C,D). These primary volatiles are synthesized by controlled enzymatic reactions mainly from fatty acid metabolism [26]. It is well known that fatty acids are major precursors of aroma volatiles in several fruits, including apple, and the biosynthetic pathway includes beta-oxidation (primary volatiles) and lipoxygenase (LOX) action (secondary compounds) [26]. The beta-oxidation pathway provides alcohols and acyl co-enzyme-A (CoA,), which are the main precursors for volatile ester production. Acyl CoAs are reduced by acyl CoA reductase to produce aldehydes, which in turn are reduced by alcohol dehydrogenase (ADH) to form alcohols that are converted to esters via the action of AAT [25].

Secondary volatiles, which are mainly C-6 aldehydes and the associated alcohols, are formed by the LOX pathway from unsaturated fatty acids (linoleic and linolenic acids) when the fruit is crushed and exposed to oxygen [26]. In our experiment, C-6 and C-5 aldehydes such as (E)-2-hexenal and 2-methyl-4-pentenal were detected only in juice samples but not in whole apple samples (Figure 3). This is in agreement with other studies, which reported the higher content of C-6 aldehydes in apple juice compared to intact fruit [27,28]. As discussed earlier, the presence of aldehydes in juice samples but not in intact Honeycrisp and McIntosh apples is attributed to the oxidation of unsaturated fatty acids (linoleic and linolenic) during juice processing.

4.2. Effect of 1-MCP, Storage Atmosphere, and Juice Processing

According to the results presented, the content and composition of volatile compounds from clear and cloudy juice were strongly influenced by the different combinations of 1-MCP treatment, storage atmosphere, harvest maturity, and juice type. The subsequent impact of the levels of esters and aldehydes on the juice odor depends on its odor threshold value (i.e., the detection or recognition values, above which the compound can be detected by smell) and concentration [21]. Based on the threshold values of volatile compounds summarized from the literature (Table 2), esters and aldehydes have considerably lower threshold values as compared with alcohols. This means esters and aldehydes may have a key role in influencing the odor of the juice even at low concentrations. On the other hand, volatile compounds with higher threshold values (notably ethanol, Table 2) might not have a large impact on the odor of apple juice. Hence, our discussion will focus on aldehydes and esters.

In McIntosh juices, whether it is clear or cloudy, our results indicated a remarkable reduction of all types of esters, aldehydes, most alcohols, and total volatile compounds when juices are extracted from 1-MCP-treated fruit stored in CA or RA. This is consistent with previous studies that found a substantial suppression of volatile aroma compounds in several apple cultivars that had been treated with 1-MCP before storage in RA or CA [6,7].

Unlike McIntosh, 1-MCP treatment alone (1-MCP + RA) in Honeycrisp apples did not alter the content of most volatile compounds except ethyl acetate and ethanol, which were substantially suppressed by the treatment. This effect might be attributed to the unusual response of this cultivar to 1-MCP treatment. In our previous study (unpublished) while 1-MCP + RA treatment in McIntosh apples inhibited ethylene production better than the control + CA/RA treatments, the same treatment in Honeycrisp produced the highest ethylene (49.03 $\mu L\ kg^{-1}\ h^{-1}$) level, which was present at higher levels than in control fruit (17 $\mu L\ kg^{-1}\ h^{-1}$). As reported in other cultivars [6,7] elevated ethylene production is usually accompanied by an increased level of volatile compounds and vice versa. Nevertheless, this trend did not occur in Honeycrisp apples. As there is no published information regarding the volatile profile of Honeycrisp fruit or juice, especially none focusing on 1-MCP treatment, it is difficult to account for the unusual response of this cultivar to 1-MCP treatment.

The observed inhibitory effect of CA storage on the content of volatile compounds is consistent with previous studies in different apple cultivars [29,30]. Reduced sensitivity to ethylene [31] or suppressed ethylene production of CA-stored fruit [31,32] has been suggested as a mechanism by which volatile production could be inhibited in CA-stored apples. The biosynthesis of volatile compounds via beta-oxidation or the LOX pathway needs oxygen, and therefore their production could be slowed down by CA condition where the oxygen level is much lower than the RA atmosphere [5].

Even though CA storage suppressed the content of most volatile compounds, it also enhanced some branched-chain esters detected from intact Honeycrisp apples (3-methyl-1-butyl acetate) as well as from Honeycrisp juices (2-methyl butyl acetate). In agreement with our observation, other studies also reported the increased level of branched-chain acetate esters in Delicious [33], Gala [30], and Fuji [21] apples that were kept under low oxygen storage conditions. A study in pear fruit found an increased level of branched-chain esters associated with a higher level of amino acids, which are the main precursors of branched-chain esters [34]. The higher concentration of (E)-2-hexenal in juices from 1-MCP and/or CA-treated Honeycrisp apples might be attributed to the suppressed ripening of the apples associated with lower ethylene production [30]. Contrary to the results observed in late-harvested McIntosh juices, the suppressive effect of 1-MCP and/or CA storage was not clearly observed in juices extracted from fruit harvested at commercial maturity. These results are unexpected, and no explanation or corresponding results were found in the literature.

As compared to clear juices, cloudy juice samples from McIntosh and Honeycrisp apples had considerably higher levels of all the major esters, aldehydes, and total volatiles, which was most pronounced in Honeycrisp juices. Even though there is a lack of literature pertaining to the volatile composition of cloudy apple juices, one recent study reported higher levels of total esters in apple juice with pulp as compared to juice from concentrate [18]. The reduction of esters in clear juice samples can be explained by the hydrolysis of esters by the action of esterase that is present in the commercial enzyme preparation [26]. As mentioned in the methodology part, one of the major differences between the two juices is the absence (clear) or presence (cloudy) of ascorbic acid. The higher content of aldehydes in cloudy juices, which is processed with ascorbic acid addition, is consistent with a previous study [13] that investigated the changes in the aroma value (the ratio of volatile concentration to odor threshold) of volatile compounds due to the addition of ascorbic acid (0.2% w/v) to the apple juice. Komthong et al. [13] found considerably higher (4- to 5-fold) aroma value of (E)-2-hexenal and hexanal in juices treated with ascorbic acid than the control. Even though there is limited information about the exact mechanism of ascorbic acid reaction with volatile compounds, the reduced concentration of aldehydes in clear juice samples has been associated with the action of ADH during the clarification process. The lower content of aldehydes in clear juice samples was ascribed to the conversion of

aldehydes to alcohols by the action of ADH during the enzymatic incubation [26,35]. The longer incubation period (about 3 h at 25 °C, in our case) would give additional time for different enzymatic reactions activated via endogenous enzymes, including ADH. In our experiment, the long incubation period was not part of cloudy apple juice preparation; instead, the juice was immediately cooled and then pasteurized. This immediate cooling, which was followed by pasteurization, could slow down and inactivate the action of the indigenous enzymes such as ADH. Hence, the preservation of aldehydes in cloudy apple juice could be attributed to the inhibition of ADH activity during processing [26].

Generally, our study suggests that the content and composition of volatile aroma compounds in apple juice could be strongly influenced by the fruit quality and juice processing techniques. As each group of the volatile compound has a typical odor characteristic, the difference in their abundance associated with postharvest treatments and juice processing steps could affect the subsequent organoleptic quality of the juices. However, in future investigations, sensory evaluation is warranted to assess the consumers' perception associated with the change of volatile aroma compounds observed in this study.

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