

Characterization of Aroma Compounds in Apple Cider Using Solvent-Assisted Flavor Evaporation and Headspace Solid-Phase Microextraction

YAN XU,^{†,§} WENLAI FAN,^{†,§} AND MICHAEL C. QIAN^{*,†}

Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331, and
 Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology,
 Southern Yangtze University, Wuxi, Jiangsu, China 214036

The aroma-active compounds in two apple ciders were identified using gas chromatography–olfactometry (GC-O) and GC–mass spectrometry (MS) techniques. The volatile compounds were extracted using solvent-assisted flavor evaporation (SAFE) and headspace solid-phase microextraction (HS-SPME). On the basis of odor intensity, the most important aroma compounds in the two apple cider samples were 2-phenylethanol, butanoic acid, octanoic acid, 2-methylbutanoic acid, 2-phenylethyl acetate, ethyl 2-methylbutanoate, ethyl butanoate, ethyl hexanoate, 4-ethylguaiaicol, eugenol, and 4-vinylphenol. Sulfur-containing compounds, terpene derivatives, and lactones were also detected in ciders. Although most of the aroma compounds were common in both ciders, the aroma intensities were different. Comparison of extraction techniques showed that the SAFE technique had a higher recovery for acids and hydroxy-containing compounds, whereas the HS-SPME technique had a higher recovery for esters and highly volatile compounds.

KEYWORDS: Aroma compounds; solvent-assisted flavor evaporation (SAFE); HS-SPME; apple cider; GC-O

INTRODUCTION

Apple cider is an alcoholic beverage fermented from fresh apple or concentrated apple juice. Although England is still the largest apple cider producing country, the production and consumption of this alcoholic beverage have been increasing rapidly in China over the past few years. The fast development of the cider industry is partially stimulated by a huge production of apples in northeastern China, which reached over 2 billion tons in 2005. Apple cider in China is usually made from ‘Fuji’, ‘Starking’, ‘Gala’, or ‘Ralls’ apples. The typical process involves milling, pressing, clarification, sulfurization, and fermentation. After fermentation, the apple cider is racked and supplemented with SO₂. Sometimes, postfermentation is employed for the finished cider.

The aroma compounds in apple and apple juice have been studied extensively over the past 30 years; less information is available on the aroma chemistry of apple cider. The major volatiles in apple cider have been identified as alcohols, esters, fatty acids, and carbonyls (1–4). Among them, ethanol, 1-butanol, 1-hexanol, 3-methylbutyl acetate, 2-phenylethyl acetate, butyl acetate, and hexanoic acid are typically dominate.

Terpenes and phenolic derivatives have also been identified in apple cider, but they are typically present in lesser amounts.

Instrumental analysis as well as sensory evaluation has been used to study the aroma-active compounds in apple cider. Alcohols, esters, lactones, phenols, and short- and medium-chain-length fatty acids are thought to play major roles for the aroma of apple cider (3, 5–7). Gas chromatography–olfactometry (GC-O) analysis reveals that 2-phenylethanol, β -damascenone, ethyl 3-/2-methylbutanoate, diacetyl, isoamyl alcohol, 2-/3-methylbutanol, 2-/3-methylbutanoic acid, guaiaicol, and methyl anthranilate could be particularly important to the aroma of apple cider (3, 5). Apple cultivars, fermentation, and aging processes will affect the formation of these aroma compounds and thus affect the quality and aroma profile of cider (8–11). Very few studies have reported the aroma compounds in apple cider manufactured in China using the native apple cultivars. A good understanding of aroma chemistry of apple cider will provide valuable information to select the raw materials and modify fermentation conditions to target a specific flavor profile.

Volatile compounds in apple cider have been analyzed by solid-phase extraction (SPE) (3), purge and trap (P&T) (4), direct injection (9), and liquid–liquid extraction (LLE) (12). Direct injection and traditional LLE methods can be used to analyze only the major volatiles, and they are not suitable for the analysis of minor aroma compounds. The P&T method is a very sensitive technique for many aroma compounds, but it has poor recoveries

* Address correspondence to this author at the Department of Food Science and Technology, 100 Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602 [telephone (541) 737-9114; fax (541) 737-1877; e-mail Michael.qian@oregonstate.edu].

[†] Oregon State University.

[§] Southern Yangtze University.

for medium- and high-boiling-point compounds. SPE is also very sensitive, but it is difficult to select a suitable sorbent to recover all aroma compounds from complicated matrices. Solvent-assisted flavor evaporation (SAFE) (13) is a new technique for aroma extraction and has been widely used to isolate aroma compounds from complex matrices (14–17). High recovery has been achieved even for high-boiling-point compounds. Solid-phase microextraction (SPME) is another sensitive and fast technique for volatile extraction. The development of Carboxen/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS) three-phase coating enables this technique to extract a wide range of volatile compounds (18–20). The SPME technique has been applied to the analysis of volatiles in many food systems including alcoholic beverages (21–24). A combination of HS-SPME and GC-O techniques has been developed to study aroma-active compounds (23, 25). In this study, both the SAFE and headspace (HS) SPME methods were employed to study the aroma-active compounds in apple ciders.

MATERIALS AND METHODS

Chemicals. Methyl hexanoate, ethyl octanoate, ethyl decanoate, and octanoic acid were from Eastman (Rochester, NY). Ethyl 2-methylpropanoate and 2-methylpropyl acetate were obtained from K&K Laboratories (Plainview, NY). Ethyl 2-hydroxypropanoate was from Matheson Coleman & Bell (East Rutherford, NJ). Ethyl benzoate was obtained from EKC Inc. (Rosemont, IL). All other aroma standards were obtained from Sigma-Aldrich Co. (St. Louis, MO). Pentane was from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Diethyl ether was obtained from Burdick & Jackson (Muskegon, MI). Anhydrous sodium sulfate was from EMD Chemicals Inc. (Gibbstown, NJ). Sodium chloride, sodium bicarbonate, and sulfuric acid were obtained from Sigma-Aldrich Co. Ethanol was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY).

Apple Cider Samples. Apple cider from China was prepared in a commercial facility of Changyu in Shandong province. Fully ripe 'Ralls' apples were washed, milled, and immediately pressed to obtain 'Ralls' juice (12.4 °Brix, titratable acidity = 2.4 g of malic acid/L, and pH 3.86). The juice was supplemented with 80 mg/L of SO₂ and clarified using 0.05% (w/v) pectin methyl esterase (PME) (Pectinex 5 XL, Novo Nordisk) at 4 °C for 48 h. *Saccharomyces cerevisiae* strain CCTCC M201022 (China Center for Type Culture Collection, Wuhan, China) (10) was cultured at 25 °C in pasteurized apple juice for 48 h. After being ameliorated with sucrose and malic acid to a °Brix of 21.5 and a titratable acidity of 5 g/L, the culture (4%) was inoculated in the clarified apple juice. Fermentation was carried out at 15–18 °C with interval aeration at early stage. The weight loss (CO₂ evolution) was monitored every 24 h until the weight stabilized. When the alcohol fermentation was finished, 10% of precultured *Oenococcus oeni* strain CCSYU 2068 (Culture Collection of South Yangtze University, Wuxi, China) was inoculated to ciders for malic–lactic fermentation (MLF). As soon as the MLF was ended, 80 mg/L of SO₂ was supplemented to ciders, and the ciders were aged in stainless steel tanks. The apple cider sample from North America was purchased from a cider distributor. It was manufactured in the United States with an alcohol content of 7.5% (v/v). The samples were stored at –4 °C until analysis.

Cider Aroma Extraction and SAFE. Apple cider samples (500 mL) were extracted with freshly distilled diethyl ether and pentane (1:1, v/v) three times in a separatory funnel (extracts total 300 mL). Volatiles from the organic phase were isolated by SAFE (Glasbläserei Bahr, Manching, Germany) at 50 °C under vacuum (10^{–3} Pa). After distillation, the receiving part of the SAFE system was carefully rinsed with 10 mL of freshly distilled diethyl ether, and the rinse was combined with the distillates in the volatile-receiving flask. The distillates were dried over anhydrous sodium sulfate and concentrated to 5 mL under a stream of nitrogen.

Cider Aroma Fractionation. To facilitate the GC analysis, aroma extracts were separated into acidic/water-soluble and neutral/basic fractions. Freshly distilled water (10 mL) was added to the concentrated

extract. The aqueous phase was adjusted to pH 11 with a 1 N sodium carbonate–sodium hydroxide solution and then separated in a separatory funnel and retained. The organic phase was further washed with 10 mL of dilute sodium hydroxide solution (pH 11) three times, and the washings were combined with the aqueous phase. The organic phase was dried over sodium sulfate, filtered, gently concentrated to 200 μL under a stream of nitrogen, and labeled "neutral/basic fraction" for GC-O analysis.

The aqueous solution was adjusted to pH 1.7 with 1 N H₂SO₄. After saturation with NaCl, the solution was extracted three times with 20 mL of diethyl ether each. These extracts were combined, dried with anhydrous sodium sulfate, filtered, gently concentrated to 500 μL under a stream of nitrogen, and labeled "acidic/water-soluble fraction".

HS-SPME Analysis. A 50/30 μm DVB/CAR/PDMS fiber (Supelco, Inc., Bellefonte, PA) was used for aroma extraction as described previously (25). The cider sample (10 mL) was placed in a 20 mL vial (item S126-0020, I-CHEM) and saturated with sodium chloride. The vial was tightly capped with a Teflon/silicone septum. The sample was equilibrated at 50 °C in a thermostatic bath for 15 min and extracted for 30 min at the same temperature under stirring. After extraction, the fiber was inserted into the injection port of the GC (250 °C) to desorb the analytes.

GC-O Analysis. GC-O analysis was performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactometer. The column carrier gas was nitrogen at constant pressure (15 psi, 2 mL/min column flow measured at 25 °C). Half of the column flow was directed to the FID while the other half was directed to a heated sniffing port with a fused silica outlet splitter (Alltech Associates, Inc., Deerfield, IL). Samples were analyzed on a DB-Wax column (30 m length, 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA) and a DB-5 column (30 m length, 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific). The oven temperature was held at 40 °C for 2 min, then increased to 230 °C at a rate of 4 °C/min, and held at 230 °C for 5 min on the DB-Wax column, whereas the final temperature was 250 °C on the DB-5 column. Injector and detector temperatures were 250 °C.

Three panelists (two males and one female) were selected for the GC-O study. Two of the panelists have more than 5 years of sensory analytical experience in alcoholic beverages. The third panelist was selected from students in the Department of Food Science and Technology at Oregon State University. All panelists were familiar with the GC-O technique and had more than 30 h of training in GC-O analysis. The panelists responded to and recorded the retention time and descriptor of the aroma compounds. They responded to the aroma intensity of the stimulus by using a 16-point scale ranging from 0 to 15; 0 = none, 7 = moderate, and 15 = extreme. Each sample was sniffed twice by each panelist. The aroma intensity values were averaged for all six analyses (three panelists, twice). For the HS-SPME and GC-O studies, the same fiber was used for all samples.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis. Capillary GC-MS was carried out using an Agilent GC 6890-5973 mass selective detector (MSD). One microliter of the concentrated fraction was analyzed on a DB-Wax column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific) and a DB-5 column (30 m × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific). The oven and injector temperatures were identical to those of GC-O analysis as described previously. The column carrier gas was helium at a constant flow rate of 2 mL/min. An Agilent 5973 MSD was used for identification. The electron impact energy was 70 eV, and the ion source temperature was set at 230 °C. Mass spectra (MS) of unknown compounds were compared with those in the Wiley 275.L Database (Agilent Technologies Inc.). Retention indices (RIs) of unknown compounds were calculated in accordance with a modified Kovats method. Positive identification was achieved by comparing mass spectra, aromas, and RIs of the standards. Tentative identification was achieved by comparing aroma or mass spectrum only.

RESULTS AND DISCUSSION

Acidic/Water-Soluble Fraction. Because both acids and alcohols are abundant, it is desirable to separate them into one

Table 1. Aroma Compounds in Acidic/Water-Soluble Fraction Detected by GC–O on a DB-Wax Column

RI _{Wax}	aroma compound	descriptor	basis of identification ^b	aroma intensity ^a	
				CY	NA
1087	2-methylpropanol	wine, solvent	MS, aroma, RI	ND	6
1137	1-butanol	pungent, alcoholic	MS, aroma, RI	5	ND
1201	3-methylbutanol	fruity, nail polish	MS, aroma, RI	6	5
1268	1-pentanol	fruity, balsamic	MS, aroma, RI	3	ND
1304	3-hydroxy-2-butanone	buttery	MS, aroma, RI	4	ND
1334	ethyl 2-hydroxypropanoate	fruity	MS, aroma, RI	3	ND
1352	<i>trans</i> -3-hexen-1-ol	herbaceous, fatty, green	MS, aroma, RI	5	3
1372	<i>cis</i> -3-hexen-1-ol	grassy, green	MS, aroma, RI	3	2
1424	acetic acid	acidic, vinegar	MS, aroma, RI	6	4
1525	propanoic acid	vinegar	MS, aroma, RI	ND	2
1555	2-methylpropanoic acid	acid, rancid	MS, aroma, RI	4	3
1602	butanoic acid	rancid, cheesy	MS, aroma, RI	7	7
1659	2-methylbutanoic acid	cheesy, rancid	MS, aroma, RI	7	5
1727	pentanoic acid	sweaty, rancid	MS, aroma, RI	2	2
1846	hexanoic acid	sweaty, cheesy	MS, aroma, RI	6	4
1872	phenyl alcohol	floral	MS, aroma, RI	6	2
1906	2-phenylethanol	rosy, honey	MS, aroma, RI	7	5
1967	<i>trans</i> -2-hexenoic acid ^{c,d}	sharp, ester-like, rancid	MS, aroma, RIL	ND	5
2060	octanoic acid	sweaty, cheese	MS, aroma, RI	6	8
2168	nonanoic acid	fatty	MS, aroma, RI	ND	2
2282	decanoic acid	fatty	MS, aroma, RI	4	5
2335	9-decenoic acid ^{d,e}	soapy, green, fatty	MS, aroma, RIL	4	4
2550	phenylacetic acid	fruity, rosy	MS, aroma, RI	4	2

^a CY, Changyu cider; NA, North American cider; ND, not detected by GC–O. ^b MS, compounds were identified by MS spectra; aroma, compounds were identified by the aroma descriptors; RI, compounds were identified by comparison to the pure standard; RIL, compounds were identified by comparison with retention index from the literature. ^c Reference 39. ^d Tentatively identified. ^e Reference 17.

fraction. In this fraction, the most important alcohol was probably 2-phenylethanol, on the basis of aroma intensity (**Table 1**). 2-Phenylethanol gave rosy and honey aromas, and its threshold (in 10% ethanol solution) is 10 mg/L (26). 2-Phenylethanol is produced by yeast during fermentation, and high concentrations (44–185 mg/L) have been reported in ciders (3, 27). The second important alcohol could be 3-methylbutanol, which imparts fruity and nail polish-like odors. It had a high aroma intensity (≥ 5) detected by GC–O in both cider samples. The concentration of 3-methylbutanol has been reported to range from 134 to 232 mg/L in ciders (9, 27), whereas its sensory threshold is 30 mg/L (26). *trans*- and *cis*-3-hexen-1-ols were also detected by GC–O in the acidic/water-soluble fraction, and they had relatively low intensities (≥ 2). *trans*-3-Hexen-1-ol gave herbaceous, fatty, and green odors, whereas *cis*-3-hexen-1-ol contributed to grassy and green aromas. The odor thresholds of these two unsaturated alcohols are 39 and 70 $\mu\text{g/L}$ in the air (28), respectively, and 400 $\mu\text{g/L}$ in an ethanol–water solution (26). C6 alcohols could be from the catabolism of linoleic acid (29). 2-Methylpropanol, 1-butanol, 1-pentanol, 3-octanol, and 1-octanol were also detected by GC–O in both ciders, but all had low aroma intensities. These alcohols contributed alcoholic, fruity, and green aromas to the ciders. Phenyl alcohol was also detected by GC–O in this study, but it had a relatively low aroma intensity.

Acids could be also important odor components in the ciders. Among these, acetic, butanoic, hexanoic, octanoic, and 2-methylbutanoic acids could be most important, because of their high aroma intensities (from 5 to 7). Acetic acid gave acidic and vinegar aromas, whereas other acids contributed to rancid, cheesy, and sweat odors. 2-Methylpropanoic, pentanoic, and decanoic acids could also be important to the cider, and these had moderately high intensities, whereas propanoic and nonanoic acids had very low intensities.

Two unsaturated fatty acids, *trans*-2-hexenoic and 9-decenoic acids, were found in both ciders. *trans*-2-Hexenoic acid gave

sharp and rancid aromas, whereas 9-decenoic acid contributed to soapy, green, and fatty odors. Phenylacetic acid was detected by GC–O in both ciders and could be important because of relatively high intensities (from 3 to 5). Phenylacetic acid affected fruity and rosy odors.

Neutral/Basic Fraction. Esters. On the basis of aroma intensity, esters could be one of the most important aroma classes in the ciders. Within this class, ethyl esters and acetates were dominant aroma compounds (**Table 2**). Ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate could be very important due to their high aroma intensities. These esters contributed to fruity, sweet, apple, pineapple, and floral odors. In addition, ethyl acetate, ethyl propanoate, ethyl pentanoate, and ethyl heptanoate, were detected by GC–O. Ethyl benzoate and ethyl 2-phenylacetate were detected by GC–O in both samples. Aromatic ethyl esters, ethyl benzoate, ethyl 2-phenylacetate, and ethyl 2-hydroxy-3-phenylpropanoate, were detected by GC–O in both samples. Several hydroxy esters were also tentatively identified, including ethyl 2-hydroxypropanoate, ethyl 3-hydroxybutanoate, ethyl 4-hydroxybutanoate, ethyl 3-hydroxyhexanoate, and ethyl 2-hydroxy-3-methylbutanoate. These hydroxyl esters gave fruity and grape-like odors.

Among the acetates, 2-phenylethyl acetate could be a very important aromatic ester because of its high aroma intensity (>5). Whereas its odor threshold is 0.25 mg/L in alcohol solution (26), a concentration of 7.7 mg/L has been reported in cider (27). Butyl acetate, hexyl acetate, 2-methylpropyl acetate, and 3-methylbutyl acetate were detected by GC–O in the neutral/basic fraction, and they had relatively low intensities. Ethyl vanillate, which gives floral and vanillin aromas, was detected by GC–O in the North American cider sample, but not in Changyu cider. Although most of the esters were originally present in apple and apple juice, most of them are formed from the esterification process during the fermentation (10).

Table 2. Aroma Compounds in Neutral/Basic Fraction Detected by GC–O on DB-Wax and DB-5 Columns

RI _{Wax}	RI _{DB-5}	aroma compound	descriptor	basis of identification ^b	aroma intensity ^a	
					CY	NA
889	726	1,1-diethoxyethane	fruity	MS, aroma, RI	3	ND
892	584	ethyl acetate	pineapple	MS, aroma, RI	2	3
953	705	ethyl propanoate	sweet, fruity	MS, aroma, RI	ND	3
961	754	ethyl 2-methylpropanoate	fruity, sweet	MS, aroma, RI	6	5
999	777	methyl 2-methylbutanoate	apple, fruity	MS, aroma, RI	5	2
1031	800	ethyl butanoate	pineapple	MS, aroma, RI	5	6
1045	849	ethyl 2-methylbutanoate	berry, fruity	MS, aroma, RI	7	5
1060	852	ethyl 3-methylbutanoate	apple	MS, aroma, RI	2	ND
1064	810	butyl acetate	berry, sweet, fruity	MS, aroma, RI	4	3
1102	875	3-methylbutyl acetate	fruity	MS, aroma, RI	3	1
1128	900	ethyl pentanoate	apple	MS, aroma, RI	4	2
1201	783	3-methylbutanol	fruity, nail polish	MS, aroma, RI	6	6
1219	1036	limonene	floral, green, sweet	MS, aroma, RI	ND	2
1235	1010	ethyl hexanoate	floral, sweet	MS, aroma, RI	5	5
1254	1015	hexyl acetate	fruity, floral	MS, aroma, RI	2	4
1305	985	1-octen-3-one	mushroom	MS, aroma, RI	3	6
1310	1100	ethyl heptanoate	fruity	MS, aroma, RI	4	2
1334	815	ethyl 2-hydroxypropanoate	fruity	MS, aroma, RI	2	ND
1397	975	ethyl 2-hydroxy-3-methylbutanoate ^c	floral, fruity	MS, aroma, RIL	ND	3
1409	1196	ethyl octanoate	fruity	MS, aroma, RI	2	6
1426	1078	<i>cis</i> -linalool oxide ^{c,d}	fruity	MS, aroma, RIL	4	2
1501	912	ethyl 3-hydroxybutanoate ^c	grape, fruity	MS, aroma, RIL	2	ND
1610	1394	ethyl decanoate	fruity, grape	MS, aroma, RI	3	7
1640	1187	ethyl benzoate	fruity	MS, aroma, RI	2	4
1644	1071	acetophenone	floral, sweet	MS, aroma, RI	ND	4
1666	1390	ethyl 9-decenoate ^{d,e}	fruity	MS, aroma, RIL	1	2
1695	977	methionol	raw potato,	MS, aroma, RI	5	3
1768	1247	ethyl 2-phenylacetate	rosy, honey	MS, aroma, RI	3	2
1794		ethyl 4-hydroxybutanoate ^d	fruity	MS, aroma	4	ND
1801	1260	2-phenylethyl acetate	rosy, floral	MS, aroma, RI	7	3
1906	1120	2-phenylethanol	rosy, honey	MS, aroma, RI	9	7
1936	1133	ethyl 3-hydroxyhexanoate ^d	fruity	MS, aroma	4	2
1942	1228	benzothiazole	smoky, burning	MS, aroma, RI	3	ND
1960		<i>trans</i> -4-hydroxy-3-methyloctanoic acid lactone ^d	fruity, floral, sweet	MS, aroma	ND	2
2007	980	phenol	phenol, medicinal	MS, aroma, RI	3	ND
2018	1363	γ -nonalactone	sweet, coconut	MS, aroma, RI	1	2
2034	1286	4-ethylguaiaicol	clove	MS, aroma, RI	2	8
2042		unknown	fruity	MS, aroma	8	4
2136		unknown	fruity		6	7
2155		unknown	clove		5	2
2171	1373	eugenol	sweet, spicy, clove	MS, aroma, RI	2	8
2181	1170	4-ethylphenol	goat, phenolic	MS, aroma, RI	3	5
2200	1317	4-vinylguaiaicol	clove, smoky	MS, aroma, RI	1	6
2259	1362	3-hydroxy-4-phenyl-2-butanone ^{d,f}	floral, clove	MS, aroma, RIL	4	6
2273	1456	ethyl 2-hydroxy-3-phenylpropanoate ^{d,g}	smoky	MS, aroma, RIL	3	5
2346	1265	chavicol	clove, spicy	MS, aroma, RI	ND	4
2394	1436	isoeugenol	smoky	MS, aroma, RI	1	6
2406	1328	4-vinylphenol	phenol, smoky	MS, aroma, RI	3	8
2422		2-(methylthio)benzothiazole ^d	smoky	MS, aroma	3	ND
2472		unknown	spicy, sweet		3	4
2487	1509	4-methoxyphenylpropanol ^d	sweet, fruity	MS, aroma	ND	4
2676	1570	ethyl vanillate	floral, vanillin	MS, aroma, RI	ND	6

^a CY, Changyu cider; NA, North America cider; ND, not detected by GC–O. ^b MS, compounds were identified by MS spectra; aroma, compounds were identified by the aroma descriptors; RI, compounds were identified by comparison to the pure standard; RIL, compounds were identified by comparison with retention index from the literature. ^c Reference 37. ^d Tentatively identified. ^e Reference 14. ^f Reference 40. ^g Reference 41.

Ketones, Aldehydes, and Acetals. Several ketones were detected by GC–O in the cider samples. Among these, 3-hydroxy-4-phenyl-2-butanone (tentatively identified) could be very important, and they gave floral and clove odors. This compound has been found in wine (30). Two unsaturated ketones, 1-octen-3-one and 6-methyl-5-hepten-2-one, were detected by GC–O. 1-Octen-3-one gave a mushroom aroma, whereas 6-methyl-5-hepten-2-one affected sweet, fruity, and green odors. 1,1-Diethoxyethane was identified by GC–O in both ciders and gave a fruity aroma.

Phenols. Among the volatile phenolic compounds identified in apple cider, 4-ethylguaiaicol, eugenol (4-allylguaiacol), and 4-vinylphenol had very high aroma intensities. 4-Ethylphenol,

chavicol [4-(2-propenyl)phenol], isoeugenol [4-(1-propenyl)guaiacol], methylchavicol (4-allylanisole, tentatively), and 4-vinylguaiaicol had medium aroma intensities. 4-Ethylguaiaicol and 4-vinylguaiaicol have clove and smoky aromas. Their odor thresholds are 33 $\mu\text{g/L}$ (31) and 40 $\mu\text{g/L}$ (26) in ethanol solutions, respectively. Eugenol contributes to sweet, spicy, and clove odors and has a sensory threshold of 5 $\mu\text{g/L}$ (26), whereas isoeugenol imparts a smoky aroma, with a sensory threshold of 6 $\mu\text{g/L}$ in ethanol solution (32). 4-Ethylphenol and 4-vinylphenol give goat, spicy, and phenolic odors, and the odor thresholds are 440 and 180 $\mu\text{g/L}$ in ethanol solution (32). Chavicol has clove and spicy odors, whereas methylchavicol gives sweet, green, and harsh aromas. Phenolic compounds are

Table 3. Aroma Compounds in Ciders Detected by SPME and GC–O on DB-Wax and DB-5 Columns

RI _{Wax}	RI _{DB-5}	aroma compound	descriptor	basis of identification ^b	aroma intensity ^a	
					CY	NA
713	<500	scetaldehyde	green, malt	MS, aroma, RI	ND	5
889	726	1,1-diethoxyethane	fruity	MS, aroma, RI	2	3
892	584	ethyl acetate	pineapple	MS, aroma, RI	4	6
953	705	ethyl propanoate	banana, fruity	MS, aroma, RI	2	6
961	754	ethyl 2-methylpropanoate	fruity, sweet	MS, aroma, RI	6	4
988	770	2-methylpropyl acetate	floral, fruity	MS, aroma, RI	ND	5
999	777	methyl 2-methylbutanoate	fruity	MS, aroma, RI	6	ND
1031	800	ethyl butanoate	pineapple	MS, aroma, RI	8	6
1045	777	ethyl 2-methylbutanoate	berry, fruity	MS, aroma, RI	10	8
1060	852	ethyl 3-methylbutanoate	apple	MS, aroma, RI	ND	2
1064	810	butyl acetate	floral, fruity	MS, aroma, RI	2	ND
1087	618	2-methylpropanol	wine, solvent	MS, aroma, RI	1	3
1102	875	3-methylbutyl acetate	fruity	MS, aroma, RI	4	2
1128	900	ethyl pentanoate	apple	MS, aroma, RI	5	3
1161	893	pentyl acetate	fruity	MS, aroma, RI	3	3
1178	924	methyl hexanoate	floral, fruity	MS, aroma, RI	ND	4
1201	783	3-methylbutanol	fruity, nail polish	MS, aroma, RI	5	5
1228	1038	butyl 2-methylbutanoate	fruity, pineapple	MS, aroma, RI	2	ND
1235	1010	ethyl hexanoate	floral, sweet	MS, aroma, RI	7	6
1254	1015	hexyl acetate	fruity, floral	MS, aroma, RI	2	6
1300	990	<i>trans</i> -3-hexenyl acetate	fruity, green	MS, aroma, RI	ND	5
1308	1007	<i>cis</i> -3-hexenyl acetate	berry, fruity, sweet	MS, aroma, RI	ND	3
1310	1100	ethyl heptanoate	fruity	MS, aroma, RI	4	2
1330	985	6-methyl-5-hepten-2-one	sweet, fruity, green	MS, aroma, RI	ND	2
1336	1040	ethyl <i>E</i> -2-hexenoate	fruity, green	MS, aroma, RI	ND	6
1374	1127	methyl octanoate	fruity	MS, aroma, RI	4	4
1409	1196	ethyl octanoate	fruity	MS, aroma, RI	4	6
1539	1070	1-octanol	apple, green, fruity	MS, aroma, RI	2	3
1562	1080	ethyl 3-methylthiopropanoate	fruity, sulfury	MS, aroma, RI	ND	3
1586	1326	methyl decanoate	fruity	MS, aroma, RI	4	6
1601	1178	4-terpineol	sweet, herbaceous	MS, aroma, RI	ND	3
1602	804	butanoic acid	rancid, cheesy	MS, aroma, RI	3	3
1610	1394	ethyl decanoate	fruity, grape	MS, aroma, RI	2	3
1640	1187	ethyl benzoate	fruity	MS, aroma, RI	1	4
1666	1390	ethyl 9-decenoate ^{c,d}	fruity	MS, aroma, RIL	2	4
1768	1247	ethyl 2-phenylacetate	rosy, honey	MS, aroma, RI	4	1
1801	1260	2-phenylethyl acetate	rosy, floral	MS, aroma, RI	5	4
1829		unknown	clove		10	7
1846	1020	hexanoic acid	sweaty, cheesy	MS, aroma, RI	2	4
1872	1040	phenyl alcohol	floral	MS, aroma, RI	5	3
1906	1120	2-phenylethanol	rosy, honey	MS, aroma, RI	8	6
1936		ethyl 3-hydroxyhexanoate ^d	fruity	MS, aroma	3	2
1967		<i>trans</i> -2-hexenoic acid ^{d,e}	rancid	MS, aroma, RIL	ND	5
2031	1288	4-ethylguaiaicol	clove, spicy	MS, aroma, RI	ND	4
2060	1180	octanoic acid	sweaty, cheesy	MS, aroma, RI	3	5
2168	1260	nonanoic acid	fatty	MS, aroma, RI	ND	2
2171	1373	eugenol	sweet, spicy, clove	MS, aroma, RI	ND	4
2181	1181	4-ethylphenol	goat, smoky, phenolic	MS, aroma, RI	ND	4
2200	1344	4-vinylguaiaicol	clove, smoky	MS, aroma, RI	1	5
2282	1380	decanoic acid	fatty	MS, aroma, RI	6	4
2335		9-decenoic acid ^{d,f}	soapy, green, fatty	MS, aroma, RIL	4	4
2346	1265	chavicol	clove, spicy	MS, aroma, RI	2	6
2394	1436	isoeugenol	smoky	MS, aroma, RI	ND	5
2406	1324	4-vinylphenol	phenol, smoky	MS, aroma, RI	1	7

^a CY, Changyu cider; NA, North American cider; ND, not detected by GC–O. ^b MS, compounds were identified by MS spectra; aroma, compounds were identified by the aroma descriptors; RI, compounds were identified by comparison to the pure standard; RIL, compounds were identified by comparison with retention index from the literature. ^c Reference 14. ^d Tentatively identified. ^e Reference 39. ^f Reference 17.

probably derived from the corresponding phenol carbonic acids, ferulic and *p*-coumaric acids (30). Most of the phenolic compounds have been identified in alcoholic beverages (29, 33, 34).

The aroma intensities of phenols between Changyu and North American ciders were quite different, and most of the phenolic compounds were detected in the North American cider only. Phenols in alcoholic beverage are typically associated with oak barrel aging (33, 35, 36). It has been found that the concentrations of phenols are higher in the wines aged in French oak than in those aged in American oak. Toasting of the oak typically increases the quantities of lignin degradation products

such as vanillin, eugenol, guaiaicol, and their derivatives (35). High concentrations of phenols could have a negative impact on the quality of alcoholic beverages (36). Because the apple cider from Changyu was aged in a stainless steel tank, it has a very low level of phenols.

Other Compounds. Limonene, *cis*-linalool oxide, and 4-terpineol were identified, and they all had low aroma intensities (Table 2). Two lactones, γ -nonalactone and *trans*-4-hydroxy-3-methyloctanoic acid lactone (tentatively), were found in the neutral/basic fraction. The former gave sweet and coconut odors, whereas the latter imparted fruity, floral, and sweet odors. However, these two compounds had very low aroma intensities.

Benzothiazole and methionol (3-methylthiolpropanol) were also identified in this study. Benzothiazole gave rubbery and burning odors, whereas methionol had a raw potato aroma. These sulfur compounds have been identified in other alcoholic beverages (17, 37). Methionol could be from the degradation of methionine, whereas benzothiazole was probably produced by microbes during the fermentation process (38).

Aroma Compounds Identified by SPME Methods. In this study, a 50/30 μm DVB/CAR/PDMS fiber was employed for aroma extraction. The HS-SPME method detected most of the aroma compounds including esters, alcohols, aldehydes, acids, phenols, and sulfur-containing compounds in the ciders and had a similar intensity of each corresponding aroma compound to the LLE-SAFE method (Table 3). However, some differences between the LLE-SAFE and HS-SPME methods have been observed. The HS-SPME method is not sensitive to short-chain alcohols and fatty acids. Some unsaturated alcohols were detected by only LLE-SAFE, but not by HS-SPME. Most of the esters were detected by both HS-SPME and LLE-SAFE methods; however, HS-SPME had higher sensitivity than LLE-SAFE for esters, and it could detect esters at much lower concentrations, such as *trans*-3-hexenyl acetate and *cis*-3-hexenyl acetate, whereas esters with a polar group, such as hydroxy esters, were detected by only LLE-SAFE. In general, the HS-SPME method is much more sensitive than LLE-SAFE for esters. The HS-SPME method is also good to extract aroma compounds with low boiling points. Acetaldehyde was detected by HS-SPME but not by LLE-SAFE because its boiling point is lower than that of the solvents used for LLE-SAFE. It will evaporate during the concentration stage. The lower recovery of some highly volatile compounds by LLE-SAFE indicated that they were lost during the LLE concentration and blow-down step.

In summary, LLE-SAFE using diethyl ether–pentane extracted alcohols, acids, and hydroxyl esters more efficiently than HS-SPME, but HS-SPME was more sensitive for esters and highly volatile compounds. They had similar selectivity for phenols. Because the HS-SPME method is solvent-free and simple and requires only a small amount of sample, it could be used as a quick method to screen the potentially important aroma compounds. A combination of HS-SPME and LLE-SAFE can be used to provide a more complete aroma profile.

LITERATURE CITED

- Calixto, F. S.; Bermejo, J. Volatile components of cider and table apples. *An. Bromatol.* **1980**, *32*, 281–288.
- Blanco-Gomis, D.; Alonso, J. J. M.; Cabrales, I. M.; Abrodo, P. A. Gas chromatographic analysis of total fatty acids in cider. *J. Agric. Food Chem.* **2001**, *49*, 1260–1263.
- Mangas, J. J.; González, M. P.; Rodríguez, R.; Blanco, D. Solid-phase extraction and determination of trace aroma and flavour components in cider by GC-MS. *Chromatographia* **1996**, *42*, 101–105.
- Madrera, R. R.; García, N. P.; Hevia, A. G.; Valles, B. S. Application of purge and trap extraction and gas chromatography for determination of minor esters in cider. *J. Chromatogr. A* **2005**, *1069*, 245–251.
- Genovese, A.; Ugolino, M.; Pessina, R.; Lamacchia, C.; Moio, L. In *Aroma-Active Compounds in Annurca Apple Cider Distillate*; Flavour Research at the Dawn of the Twenty-First Century, Proceedings of the Weurman Flavour Research Symposium, 10th, Beaune, France, June 25–28, 2002; LeQuéré, J. L., Etievant, P. X., Eds.; Intercept Scientific Technical Publishers: London, pp 618–621.
- Williams, A. A. The aroma compounds of cider apples and fermented ciders. *Geruch-Geschmackstoffe, Int. Symp.* **1975**, 141–151.
- Medina, I.; Martínez, J. L.; Suarez, J. J.; Bueno, J. L. Cider aroma. *Alimentacion, Equiposy Tecnologia* **1996**, *15*, 91–97.
- Mangas, J. J.; González, M. P.; Blanco, D. Influence of cider-making technology on low-boiling-point volatile compounds. *Z. Lebensm. Unters. Forsch. A* **1993**, *197*, 522–524.
- Madrera, R. R.; Gomis, D. B.; Alonso, J. J. M. Influence of distillation system, oak wood type, and aging time on volatile compounds of cider brandy. *J. Agric. Food Chem.* **2003**, *51*, 5709–5714.
- Xu, Y.; Zhao, G. A.; Wang, L.-p. Controlled formation of volatile components in cider making using a combination of *Saccharomyces cerevisiae* and *Hanseniaspora valbyensis* yeast species. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 192–196.
- Yu, A.-m.; Xu, Y.; Wang, D.; Wang, L.-p.; Liu, Y.-m.; Fei, X.-w. Analysis of influence on different raw materials on volatile aroma compounds of ciders. *Sci. Agric. Sinica* **2006**, *39*, 786–791.
- Williams, A. A.; May, H. V. Examination of an extract of cider volatiles using both electron impact and chemical ionization gas chromatography–mass spectrometry. *J. Inst. Brew.* **1981**, *87*, 372–375.
- Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation—a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.
- Qian, M.; Reineccius, G. Identification of aroma compounds in Parmigiano-Reggiano cheese by gas chromatography/olfactometry. *J. Dairy Sci.* **2002**, *85*, 1362–1369.
- Klesk, K.; Qian, M. Aroma extraction dilution analysis of cv. Marion (*Rubus* spp. *hyb*) and cv. Evergreen (*R. laciniatus* L.) blackberries. *J. Agric. Food Chem.* **2003**, *51*, 3436–3441.
- Mahajan, S. S.; Goddik, L.; Qian, M. C. Aroma compounds in sweet whey powder. *J. Dairy Sci.* **2004**, *87*, 4057–4063.
- Fang, Y.; Qian, M. Aroma compounds in Oregon Pinot noir wine determined by aroma extract dilution analysis (AEDA). *Flavour Fragrance J.* **2005**, *20*, 22–29.
- Burbank, H. M.; Qian, M. C. Volatile sulfur compounds in Cheddar cheese determined by headspace solid-phase microextraction and gas chromatograph-pulsed flame photometric detection. *J. Chromatogr. A* **2005**, *1066*, 149–157.
- Vazquez-Landaverde, P. A.; Velazquez, G.; Torres, J. A.; Qian, M. C. Quantitative determination of thermally derived off-flavor compounds in milk using solid-phase microextraction and gas chromatography. *J. Dairy Sci.* **2005**, *88*, 3764–3772.
- Vazquez-Landaverde, P. A.; Torres, J. A.; Qian, M. C. Effect of high-pressure-moderate-temperature processing on the volatile profile of milk. *J. Agric. Food Chem.* **2006**, *54*, 9184–9192.
- Ebeler, S. E.; Terrien, M. B.; Butzke, C. E. Analysis of brandy aroma by solid-phase microextraction and liquid–liquid extraction. *J. Sci. Food Agric.* **2000**, *80*, 625–630.
- Nonato, E. A.; Carazza, F.; Silva, F. C.; Carvalho, C. R.; Cardeal, Z. d. L. A headspace solid-phase microextraction method for the determination of some secondary compounds of Brazilian sugar cane spirits by gas chromatography. *J. Agric. Food Chem.* **2001**, *49*, 3533–3539.
- Martí, M. P.; Mestres, M.; Sala, C.; Busto, O.; Guasch, J. Solid-phase microextraction and gas chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. *J. Agric. Food Chem.* **2003**, *51*, 7861–7865.
- Watts, V. A.; Butzke, C. E.; Boulton, R. B. Study of aged Cognac using solid-phase microextraction and partial least-squares regression. *J. Agric. Food Chem.* **2003**, *51*, 7738–7742.
- Fan, W.; Qian, M. C. Headspace solid phase microextraction (HS-SPME) and gas chromatography–olfactometry dilution analysis of young and aged Chinese “Yanghe Daqu” liquors. *J. Agric. Food Chem.* **2005**, *53*, 7931–7938.

- (26) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022–3026.
- (27) Wang, L.; Xu, Y.; Zhao, G.; Li, J. Rapid analysis of flavor volatiles in apple wine using headspace solid-phase microextraction. *J. Inst. Brew.* **2004**, *110*, 57–65.
- (28) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München: Garching, Germany, 1998.
- (29) Rowe, D. J. *Chemistry and Technology of Flavors and Fragrances*; Blackwell Publishing: Oxford, U.K., 2005.
- (30) Rapp, A. Volatile flavor of wine: correlation between instrumental analysis and sensory perception. *Nahrung* **1998**, *42*, 351–363.
- (31) Ferreira, V.; López, R.; Cacho, J. F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667.
- (32) Culleré, L.; Escudero, A.; Cacho, J. F.; Ferreira, V. Gas chromatography–olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *J. Agric. Food Chem.* **2004**, *52*, 1653–1660.
- (33) Mangas, J.; Rodríguez, R.; Moreno, J.; Blanco, D. Volatiles in distillates of cider aged in American oak wood. *J. Agric. Food Chem.* **1996**, *44*, 268–273.
- (34) Fan, W.; Xu, Y.; Yu, A. Influence of oak chips geographical origin, toast level, dosage and aging time on volatile compounds of apple cider. *J. Inst. Brew.* **2006**, *112*, 255–263.
- (35) Guchu, E.; Díaz-Maroto, M. C.; Pérez-Coello, M. S.; González-Viñas, M. A.; Ibáñez, M. D. C. Volatile composition and sensory characteristics of Chardonnay wines treated with American and Hungarian oak chips. *Food Chem.* **2006**, *99*, 350–359.
- (36) Cerdán, T. G.; Mozaz, S. R. G.; Azpilicueta, C. A. Volatile composition of aged wine in used barrels of French oak and of American oak. *Food Res. Int.* **2002**, *35*, 603–610.
- (37) Ledauphin, J.; Saint-Clair, J.-F.; Lablanquie, O.; Guichard, H.; Fournier, N.; Guichard, E.; Barillier, D. Identification of trace volatile compounds in freshly distilled Calvados and Cognac using preparative separations coupled with gas chromatography–mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 5124–5134.
- (38) Bellavia, V.; Natangelo, M.; Fanelli, R.; Rotilio, D. Analysis of benzothiazole in Italian wines using headspace solid-phase microextraction and gas chromatography–mass spectrometry. *J. Agric. Food Chem.* **2000**, *48*, 1239–1242.
- (39) Lee, S.-J.; Noble, A. C. Characterization of odor-active compounds in Californian Chardonnay wines using GC–olfactometry and GC–mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 8036–8044.
- (40) Kotseridis, Y.; Baumes, R. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *J. Agric. Food Chem.* **2000**, *48*, 400–406.
- (41) Fan, W.; Qian, M. C. Identification of aroma compounds in Chinese ‘Yanghe Daqu’ liquor by normal phase chromatography fractionation followed by gas chromatography/olfactometry. *Flavour Fragrance J.* **2006**, *21*, 333–342.

Received for review November 3, 2006. Revised manuscript received February 9, 2007. Accepted February 10, 2007. Financial support from the Ministry of Education, China, under a program for Changjiang Scholars and Innovative Research Team in University (PCSIRT) in IRT0532 and from Oregon State University for postdoctoral research is gratefully acknowledged.