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Monitoring Cider Aroma Development throughout the Fermentation Process by Headspace Solid Phase Microextraction (HS-SPME) Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

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Abstract: Volatile organic compounds (VOCs) play a crucial role in cider quality. Many variables involved in the fermentation process contribute to cider fragrance, but their relative impact on the finished odor remains ambiguous, because there is little consensus on the most efficient method for cider volatile analysis. Herein, we have optimized and applied a headspace solid phase microextraction gas chromatography–mass spectrometry (HS-SPME GC-MS) method for the chemical analysis of cider VOCs. We determined that the 30 min exposure of a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) solid phase microextraction (SPME) fiber at 40 °C yielded detection of the widest variety of VOCs at an extraction efficiency >49% higher than comparable fibers. As a proof-of-concept experiment, we utilized this method to profile cider aroma development throughout the fermentation process for the first time. The results yielded a very practical outcome for cider makers: a pre-screening method for determining cider quality through the detection of off-flavors early in the fermentation process. The aroma profile was found to be well established 72 h after fermentation commenced, with major esters varying by 18.6% ± 4.1% thereafter and higher alcohols varying by just 12.3% ± 2.6%. Lastly, we analyzed four mature ciders that were identically prepared, save for the yeast strain. Twenty-seven key VOCs were identified, off-flavors (4-ethylphenol and 4-ethyl-2-methoxyphenol) were detected, and odorants were quantified at desirable concentrations when compared to perception thresholds. VOCs varied considerably following fermentation with four novel strains of *S. cerevisiae*, evidencing the central importance of yeast strain to the finished cider aroma.

Keywords: aroma; cider; fermentation; yeast; gas chromatography; solid phase microextraction

1. Introduction

Cider is an alcoholic beverage that is produced through the fermentation of fresh or concentrated apple juice. Cider production in early America was extremely popular, but Prohibition brought the industry to a stand-still during the 1920s. Many orchards were razed or abandoned at this time, and apple varieties that were once prized for their cider-specific traits were widely forgotten [1]. Within the past decade, however, cider has experienced a massive revival, recently outpacing competitors as one of the fastest growing alcoholic beverage categories in the U.S. [2]. In Southwest Colorado, farmers have begun to restore historical orchards and incentivize the purchase of cider-specific apple varieties that still grow abundantly in the region through the Montezuma Orchard Restoration Project [3]. Small cider businesses that use these apples in their production require data-driven

techniques in order to create the highest quality ciders in an efficient manner. The work presented herein is the result of a novel, ongoing collaboration between orchards, cideries, and chemists in Southwest Colorado which aims to better understand the myriad variables that contribute to local cider production.

It is well-established that aroma and flavor define cider quality. These variables have been thoroughly studied through sensory means [4,5], but further research is required to understand the molecular profiles responsible for those organoleptic properties and the methods tied to their production [6]. There remains little consensus on what specific cider-making practices, apple characteristics, or yeast strains lead to the desired aroma-active compounds in the headspace of apple cider or how best to measure those compounds. In this work, we report the optimal analytical practices to address these gaps in knowledge of cider volatile organic compound (VOC) extraction, create a timeline of odorant development during the process of cider fermentation, and analyze the key molecular constituents of cider fragrance produced using four different strains of *Saccharomyces cerevisiae* (previously unexplored for cider production). The central aim of this work is to optimize a cider analysis method and then apply it in order to better understand the development of VOCs both during cider fermentation and after maturation.

Headspace solid phase microextraction gas chromatography–mass spectrometry (HS-SPME GC-MS) is a reliable technique for the pre-concentration and determination of the odorant volatile composition of beer and wine [7–9]. Recent experiments involving cider headspace analysis have also utilized solid phase microextraction (SPME), incorporating fibers such as polydimethylsiloxane (PDMS) [10,11], polydimethylsiloxane/divinylbenzene (PDMS/DVB) [12], and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) [6] over a range of exposure times and temperatures. These publications reveal a current lack of agreement on the optimal analytical approach for cider headspace analysis. SPME fiber choice depends entirely on the compounds deemed most responsible for aroma, which have been reported to be primarily esters and higher alcohols [10] with a multitude of lower abundance ketones, aldehydes, and phenols [4]. Of interest within this work are a series of esters, higher alcohols, and phenols, as shown in Figure 1. We seek to narrow the list of significant contributors to cider fragrance and odor defects through the optimization and application of an efficient extraction and chemical analysis method for these organic structures.

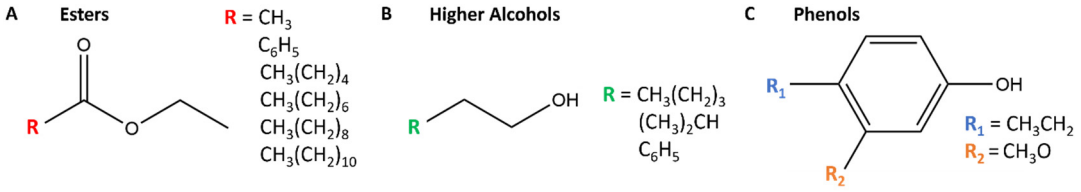

Figure 1. Volatile organic species of interest to this study include esters (**A**), higher alcohols (**B**), and phenols (**C**) with specific functional groups color coded above. Esters (**A**) that are routinely observed herein include ethyl acetate (R = CH_3), ethyl benzoate (R = C_6H_5), ethyl hexanoate (R = $CH_3(CH_2)_4$), ethyl octanoate (R = $CH_3(CH_2)_6$), ethyl decanoate (R = $CH_3(CH_2)_8$), and ethyl dodecanoate (R = $CH_3(CH_2)_{10}$). Higher alcohols (**B**) of importance to cider aroma include 1-hexanol (R = $CH_3(CH_2)_3$), 3-methyl-1-butanol (R = $(CH_3)_2CH$), and 2-phenylethyl alcohol (C_6H_5). Lastly, volatile phenols that are considered defect odors in cider include 4-ethylphenol (R_1 = CH_3CH_2) and 4-ethyl-2-methoxyphenol (R_1 = CH_3CH_2 and R_2 = CH_3O).

We applied our analytical approach in order to elucidate cider odor development throughout the fermentation process and with a variety of novel cider yeast strains. At the beginning of cider fermentation, high levels of esters are known to be produced [13], but the timeline of their formation remains to be understood. This is of practical significance to cider makers, because pre-screening for off-flavors may be performed before significant time and resources have been wasted on a flawed product if VOC formation is rapid and indicative of the final cider's odor. Previously published results indicate that the VOCs which contribute to aroma, originating from the apples themselves, are largely

lost during fermentation [10,14], and therefore fermentation conditions may have a greater impact on the final scent of the cider than the variety of apples used. Others argue that specific cider apple varietals are essential to cider quality [6,12]. Although non-volatile molecules originating from the apples, such as polyphenols, have a great impact on the finished cider taste [15–17], and maintaining a sufficient nutrient supply within the apple juice (e.g., amino acids) is of key importance to the yeast during fermentation [18,19], the relative impact of the starting material versus the yeast strain on the volatile components in the finished product odor requires additional experiments. Lastly, because cider makers have historically utilized the oenological yeast strains of *S. cerevisiae* which are commonly used for grape wine [20], we illuminate the influence of four *S. cerevisiae* strains traditionally used for beer (Edinburgh Scottish Ale yeast, Belgian Saison II yeast, French Saison yeast, and Abbey Ale yeast) on cider production. These strains could provide a new avenue to the desired odorant VOCs. We present a comprehensive identification of every major and minor VOC detected in the headspace of the cider samples and examine the perception thresholds of those molecules [21,22] in order to determine additional, hitherto unreported species that influence cider aroma.

2. Materials and Methods

2.1. Sample Preparation and Fermentation

To study the volatile species produced throughout the fermentation process, 5 g of dry Lalvin ICV-D47 *S. cerevisiae* yeast (Lallemand Inc., Baltimore, MD, USA) was mixed with 50 mL of water at 35 °C. The yeast solution was held at this temperature for 20 min prior to inoculation. Juice from a commonly grown heirloom apple variety known in Southwest Colorado as Double Red Delicious (2 L), prepared by Teal Cider, LLC (Dolores, CO, USA), and grown by T Lazy T Orchard (Dolores, CO, USA), was inoculated with 10 mL of this yeast slurry. Fermentation was carried out once at room temperature (23 ± 1 °C) over the course of 14 days to verify total sugar consumption and alcohol production [23,24]. Specific gravity measurements were taken every 2–3 days with a hydrometer, and volatiles were collected in triplicate by SPME until specific gravity readings plateaued at the completion of fermentation [25].

Utilizing the aforementioned approach, juice from a blend of sweet and bittersharp heirloom variety apples provided by EsoTerra Cider (Dolores, CO, USA) was inoculated with four yeast strains traditionally used for beer fermentation in order to compare the influence of those yeast strains on cider aroma profiles. Four commercial strains of the yeast species *S. cerevisiae* that have never before been studied for cider-making were obtained: Belgian II Saison OYL-042 *S. cerevisiae* var. *diastaticus* (Omega Yeast Labs, Chicago, IL, USA), French Saison yeast strain 3711 *S. cerevisiae* var. *diastaticus* (WYEAST, Hood River, OR, USA), Edinburgh Scottish Ale yeast, WLP028 (White Labs, San Diego, CA, USA), and Abbey Ale yeast, WLP530 (White Labs). The former two strains were determined by rapid polymerase chain reaction (PCR) to contain the STA1 gene, indicating their genetic variation *diastaticus*. Fermentation and maturation were conducted for these four samples over the course of six months at 10 ± 2 °C in 19–24 L glass vessels in collaboration with EsoTerra Cider to produce commercial-grade products. Triplicate headspace samples of the finished ciders were collected by SPME following the six month maturation period, as described below.

2.2. Solid Phase Microextraction (SPME) Optimization

Three SPME fibers (Supelco, Bellefonte, PA, USA) were assessed for their sampling efficiency of the volatile components of cider headspace. These were: polyacrylate 85 µm (PA), divinylbenzene/carboxen/polydimethylsiloxane 50/30 µm (DVB/CAR/PDMS), and carboxen/polydimethylsiloxane 75 µm (CAR/PDMS). *S. cervisiae* var. *diastaticus* yeast (French Saison, strain 3711) and a mixture of local, Montezuma County, CO, USA heirloom variety apples were utilized for the cider sample fermentation for all method validations reported herein. As shown in Figure 2, the DVB/CAR/PDMS fiber was selected as the optimal sampling fiber for VOCs originating from

cider samples due to the greater extraction efficiency as well as the wider variety of cider constituents (primarily esters, higher alcohols, and phenols) compared to the other fibers. Sampling efficiency was determined through the peak integration of the chromatograms (Figure 2) for each SPME fiber. In contrast to previous work [12], we observed that the extraction efficiency of the DVB/CAR/PDMS fiber (100%) was significantly greater than that of the CAR/PDMS (51%) and the PA fibers (2%), due to the affinity of cider headspace molecules for this polymer blend. Based on these data, every result reported herein utilized DVB/CAR/PDMS SPME fibers.

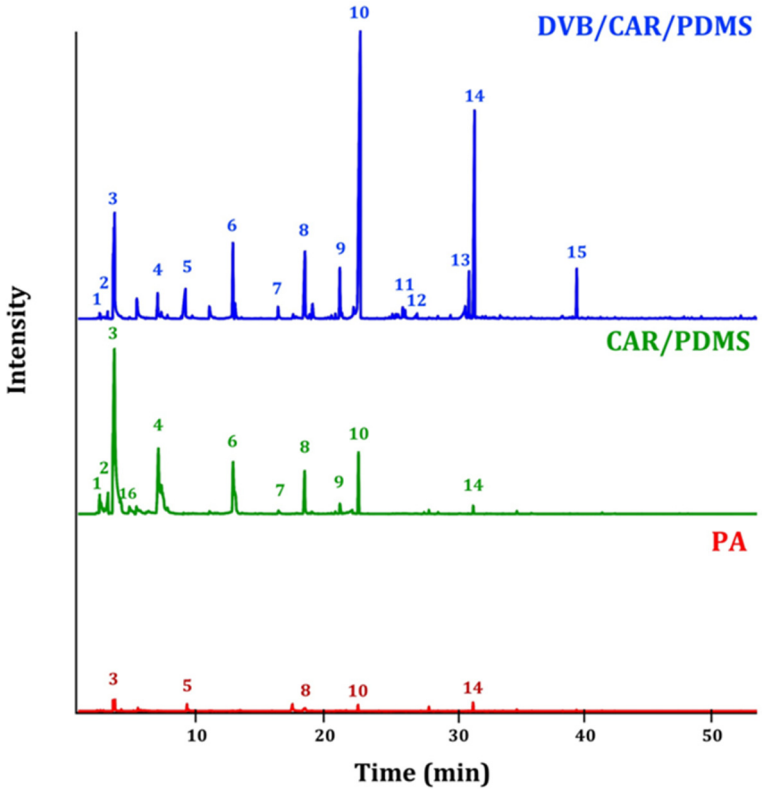

Figure 2. Overlaid chromatograms of a single cider sample (EsoTerra Cider) analyzed with 3 different solid phase microextraction (SPME) fibers are shown. Polyacrylate (PA: Red), carboxen/polydimethylsiloxane, (CAR/PDMS: Green), and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS: Blue) were compared. A wider variety of esters (**1**: Ethyl acetate, **2**: Methyl formate, **6**: Hexanoic acid ethyl ester, **9**: Benzoic acid ethyl ester, **10**: Octanoic acid ethyl ester, **12**: Nonanoic acid ethyl ester, **13**: Ethyl 9-decanoate, **14**: Decanoic acid ethyl ester, **15**: Dodecanoic acid ethyl ester), higher alcohols (**3**: 3-Methyl-1-Butanol, **4**: 1-Hexanol, **7**: 1-Octanol, **8**: 2-Phenylethyl alcohol), phenols (**11**: 4-ethyl-2-methoxyphenol), and an imine (**5**: methoxy-phenyl-oxime) were detected consistently at higher abundance in the DVB/CAR/PDMS fiber than the other fibers analyzed.

All cider aroma measurements were performed through the introduction of a SPME fiber into the headspace of a 10 mL cider aliquot sealed in a 20 mL 22.5 × 75 mm glass headspace vial (Sigma Aldrich, St. Louis, MO, USA). Each vial was capped airtight with a 20 mm aluminum release seal PTFE/silicone liner cap (Sigma Aldrich) using a handheld manual vial crimper. For temperature consistency across all samples, SPME fibers were introduced into the headspace after samples were submerged in a 40 °C water bath for 10 min. Prior to headspace sampling, all SPME fibers were conditioned for 30 min at the temperature specified by the manufacturer (Supelco). The SPME fiber exposure times were then compared, and 30 min was determined to be optimal for the time-efficient and abundant sampling of esters and higher alcohols, as illustrated in Figure 3. Longer SPME fiber exposures (90 min) did not result in additional analyte detections despite the increased time, because a stable analyte deposition

equilibrium had already been established [26]. Shorter SPME fiber exposures (5 min) resulted in several minor species dropping below the detection limit.

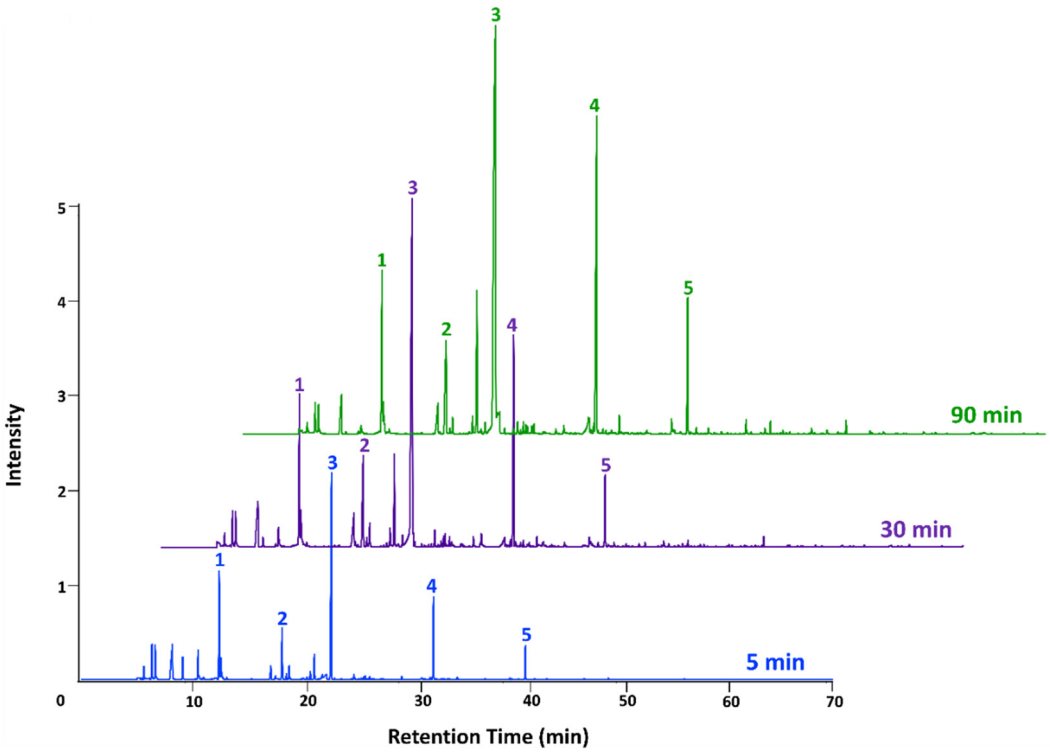

Figure 3. Overlaid chromatograms for one cider sample (EsoTerra Cider) were obtained by headspace solid phase microextraction (HS-SPME) using DVB/CAR/PDMS fibers at 40 °C for various exposure times (5 min: blue, 30 min: purple, 90 min: green) to optimize sampling conditions. The five most abundant and reproduceable peaks were identified and labeled on each chromatogram (**1:** ethyl hexanoate, **2:** phenethyl alcohol, **3:** ethyl octanoate, **4:** ethyl decanoate, and **5:** ethyl dodecanoate).

2.3. Gas Chromatography–Mass Spectrometry Analysis

An Agilent Technologies (Santa Clara, CA, USA) 7820A Gas Chromatograph (GC) with a DB5 30 m × 0.25 mm × 0.25 micron column and a 5977E Mass Spectrometer Detector (MSD) with MassHunter GCIMS Acquisition Software (B.07.00.1203 Agilent) were used for all analyses. SPME fibers were introduced to the GC inlet in the splitless mode at 260 °C for a 1 min thermal desorption. The oven temperature program was set for 5 min at 50 °C and increased to 230 °C at a rate of 3 °C/min, and this was held for 5 min. This temperature program has been shown to be effective for previous studies of wine volatiles [8]. The detector temperature was held at 230 °C. Helium gas was used as a carrier at a flow rate of 1 mL·min^{-1}.

Detection was accomplished using a 70 eV electron ionization source operated under a mass range of *m/z* 50–550. The quadrupole interface temperature was held at 150 °C. Volatiles were identified through mass spectral comparison with the National Institute of Standards and Technology (NIST) library (Version 2.2. Agilent Technologies, Santa Clara, CA, USA), and percentage correlations with this database (NIST %) were shared alongside all identifications for clarity. To further verify this positive identification for all major analytes observed, the purchased standards (prepared in 5% *v/v* ethanol in water) were analyzed by an identical method to all cider samples. These were obtained from Sigma Aldrich (St. Louis, MO, USA) and included 4-ethylphenol (analytical standard), 4-ethyl-2-methoxyphenol (analytical standard), 1-hexanol (analytical standard), ethyl hexanoate (analytical standard), ethyl decanoate (ReagentPlus, ≥99%), ethyl dodecanoate (analytical standard), and ethyl benzoate (analytical standard). Additionally, quantification of the key analytes was performed

through a 5 point external standard calibration [20] of ethyl acetate, ethyl octanoate, 2-phenylethyl alcohol, and 3-methyl-1-butanol (analytical standards, Sigma Aldrich) in 5% *v/v* ethanol in water. Blanks were taken once a day with a conditioned DVB/CAR/PDMS fiber and subtracted from the reported chromatograms in order to account for any SPME fiber contamination. Residual siloxanes originating from the DVB/CAR/PDMS SPME fiber and GC column were frequently observed after blank subtraction, as previous works have noted [27], but these siloxanes did not interfere with our detection or quantification of the analytes of interest. All SPME analyses were performed in triplicate for reproducibility, and $\overline{x} \pm 1\sigma$ are reported.

3. Results and Discussion

3.1. Cider VOC Development throughout the Fermentation Process

Despite the many variables that are routinely altered in the cider preparation process, from apple type to fermentation temperature and yeast strain [10,20], few analytical methods have been developed to monitor exactly how these variables develop the aroma profile of the cider over time during fermentation. Carefully controlling these parameters throughout the cider preparation process in order to measure the released volatile species can reveal which inputs yield the desired final product and at what point in production a finished aroma has been established.

Figure 4 illustrates the results of such an approach. Specific gravity was measured regularly by a hydrometer to monitor the fermentation stage (Figure 4A), and the corresponding HS-SPME-GC-MS data were gathered to observe the volatiles released pre- (Figure 4B, red) and post-inoculation (Figure 4B, purple and green) as well as in the finished cider (Figure 4B, blue). For this proof-of-concept experiment, local Double Red Delicious apple juice (Teal Cider, Dolores, CO, USA with 1.064 starting specific gravity) was inoculated with Lalvin ICV D47 yeast at room temperature (23 ± 1 °C). All chromatographic peaks are labelled in Figure 4, unless they were of negligible abundance (<0.002 relative peak area) or they corresponded to siloxanes that originated from the SPME fiber itself [27].

Prior to inoculation, the Red Delicious apple juice headspace contained primarily esters with various organic side chain lengths (major species in decreasing order of abundance: 2-methylbutyl acetate, hexyl acetate, ethyl-2-methyl butyrate, ethyl hexanoate, ethyl butyrate, and butyl acetate). Two higher alcohols, 1-hexanol and 2-methyl-1-butanol, were also observed in the pre-inoculation juice (Figure 4B, red), alongside estragole, which has previously been observed in Golden and Red Delicious apple juices [28]. Seventy-two hours after inoculation (Figure 4B, purple), the notable rise of six longer chain esters (in decreasing order of abundance: ethyl octanoate, ethyl decanoate, ethyl dodecanoate, 3-methylbutyl acetate, ethyl-3-methylbutyrate, and 3-methylbutyl octanoate) and one higher alcohol, 2-phenylethyl alcohol, were observed as by-products of the initiation of yeast metabolism [13]. These molecules have previously been identified as key contributors to cider aroma [10], with the esters generating a fruity smell and the higher alcohols creating a honey or whiskey fragrance [21,29]. Upon inoculation, ethyl octanoate and ethyl decanoate dominated the chromatogram for the remainder of the fermentation.

Interestingly, only one new volatile, ethyl-9-decenoate, was generated after the 72 h point in the fermentation, and those species that were present did not change significantly in their abundance. Esters collectively increased by 18.6% ± 4.1% from 72 h (Figure 4B, purple) to the completion of fermentation (Figure 4B, blue), and higher alcohols collectively increased by 12.3% ± 2.6% during this time frame, based on the chromatographic peak areas. This suggests that under the conditions described herein, the aroma profile was largely determined within the first 3 days of fermentation, after the specific gravity has dropped to 1.056 with an estimated alcohol by volume content of 1.05%. When comparing fermentation at day 8 (at a specific gravity of 1.004 and an estimated alcohol by volume content of 7.88%) to the final cider, esters and higher alcohols varied by an even smaller figure (2.6% ± 0.6% and 1.6% ± 0.3%, respectively). For cider makers, this can inform practical and strategic

chemical analyses of a cider early in its development in order to determine whether the beverage is on or off track to achieving the desired product characteristics.

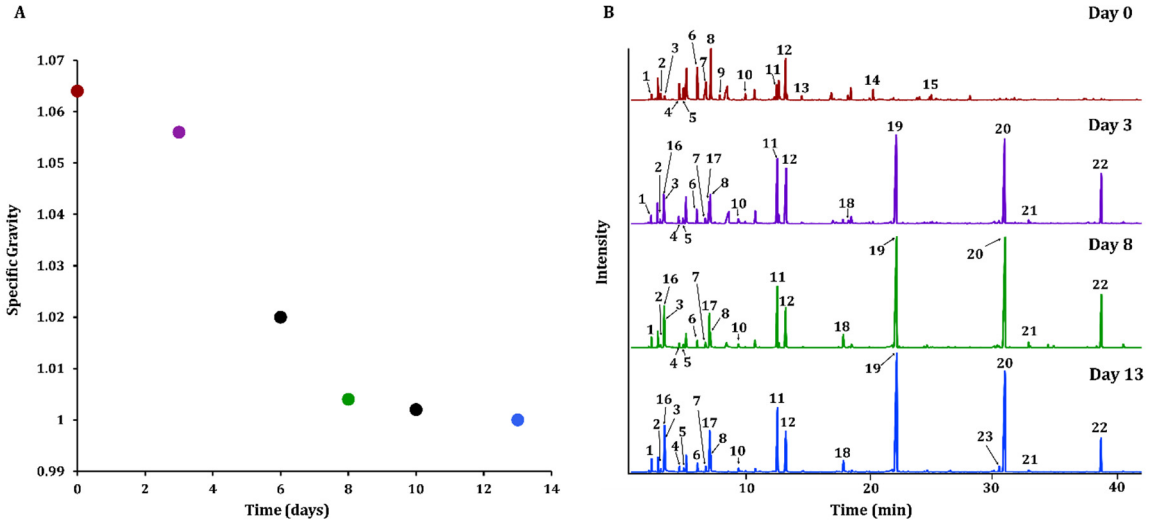

Figure 4. A hydrometer was used to measure specific gravity throughout a cider fermentation to monitor fermentation progression (**A**). Specific gravity measurements were taken before yeast inoculation (**red**), at various stages during fermentation (**purple, black, green**), and when fermentation was complete (**blue**). Four chromatograms were obtained by headspace solid phase microextraction gas chromatography–mass spectrometry (HS-SPME GC-MS), with a DVB/CAR/PDMS fiber, in order to compare changes in aroma profiles based on volatile constituents (**B**). All chromatographic peaks are numbered in order of their observation throughout the fermentation process. Peaks one through fifteen (**1**: ethyl acetate, **2**: ethyl propionate, **3**: 2-methyl-1-butanol, **4**: ethyl butyrate, **5**: butyl acetate, **6**: ethyl-2-methyl butyrate, **7**: 1-hexanol, **8**: 2-methyl butyl acetate, **9**: propyl butyrate, **10**: 2-methyl propyl butyrate, **11**: ethyl hexanoate, **12**: hexyl acetate, **13**: butyl 2-methylbutyrate, **14**: estragole, and **15**: hexyl 2-methylbutanoate) were detected in the juice sample (**red**). Peaks one through eight, ten through twelve, and fourteen through twenty-two (**16**: ethyl 3-methylbutyrate, **17**: 3-methylbutyl acetate, **18**: 2-phenylethyl alcohol, **19**: ethyl octanoate, **20**: ethyl decanoate, **21**: 3-methylbutyl octanoate, and **22**: ethyl dodecanoate) were identified in samples taken on day three (**purple**), day eight (**green**), and at the completion of fermentation (**blue**). Only one species (**23**: ethyl-9-decenoate) was detected on day 13 (fermentation completion) only.

The VOCs present in the apple juice significantly changed in abundance during fermentation. Seven esters (ethyl-3-methylbutyrate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl-9-decenoate, 3-methylbutyl acetate, and 3-methylbutyl octanoate) and one higher alcohol (2-phenylethyl alcohol) were identified in the final cider, but they were not detected at all in the headspace of the original apple juice. Additionally, three esters (propyl butyrate, butyl-2-methyl butyrate, and hexyl-2-methylbutanoate) and estragole were present in the apple juice but entirely absent from the final cider headspace. This is because most cider esters are generated through the esterification of alcohols with fatty acids during fermentation [4]. Several species persisted from the original apple juice to the final cider aroma profile but changed in abundance significantly as a direct result of fermentation. Those species that increased in abundance significantly (>50%) throughout fermentation were 2-methyl-1-butanol (+444.3 ± 97.8%), ethyl hexanoate (+436.6 ± 96.1%), and ethyl acetate (+62.1 ± 13.7%), which are well known fermentation by-products. Those species that decreased significantly (>50%) were as follows: 1-hexanol (−84.6 ± 18.6%), 2-methylpropyl butanoate (−84.5 ± 18.6%), 2-methylbutyl acetate (−79.7 ± 17.5%), butyl acetate (−77.1 ± 16.9%), ethyl butyrate (−77.1 ± 17.0%), ethyl propionate (−58.2 ± 12.8%), and ethyl-2-methyl butyrate (−56.3 ± 12.4%). Based on these data, the metabolic by-products of yeast fermentation dominated the finished cider

chromatogram when compared to the original apple juice VOCs. Therefore, our next investigation focused specifically on several previously unexplored yeast strains and their fermentation metabolites after a six-month maturation using the same apple juice, under equal conditions.

3.2. Cider VOC Profiles Resulting from Different Yeast Strains

A total of 27 VOCs were identified in four matured ciders that were identically prepared, save for the yeast strain used for fermentation, as shown in Tables 1 and 2. These tables list all analytes in order of their relative peak area (RPA, obtained through integration) and include retention times (RT), percentage correlation with NIST spectral database (NIST %), odor descriptors, and perception thresholds (PT) from previous sensory panel studies [21,30]. Table 1 lists all major analytes observed (relative peak area > 0.05), and Table 2 lists minor analytes (relative peak area < 0.05). Unlike previous yeast strain comparison work, which analyzed for VOCs after 10 days [20], these ciders were taken through a six-month maturation process prior to analysis. In addition, unique to this study were the *S. cerevisiae* yeast strains chosen for analysis (traditionally used in beer fermentation), which we will reference herein as: Edinburgh, Belgian II, French Saison, and Abbey.

Table 1. Major (>0.05 relative peak area) volatile compounds identified in cider fermented (six months) with the same apple juice and different *S. cerevisiae* yeast strains. Retention times (RT) and perception thresholds (PT) are listed.

Edinburgh Yeast	Relative Peak Area	Retention Time (min)	NIST (%) [a]	Odor Descriptor	Perception Threshold (μg L^{-1})
Ethyl octanoate	1.00	22.1	92.3	Fruity, candy, pineapple [b,c]	580 [b]
Ethyl decanoate	0.85	31.0	89.1	Fruity, grape [b]	200 [b]
3-methyl-1-butanol	0.18	3.1	69.0	Alcohol, nail polish, whiskey [b]	30,000 [b]
Ethyl hexanoate	0.16	12.3	85.5	fruity, strawberry, green apple [b]	14 [b]
Ethyl benzoate	0.14	20.6	77.3	Camomile, flower, celery, fruit [d]	60 [e]
Ethyl dodecanoate	0.11	38.9	82.3	Candy, floral, waxy, soap [b]	1500 [b]
4-ethyl-2-methoxyphenol	0.11	25.6	81.1	Phenolic, smoked [f]	6.9 [f]
2-phenylethyl alcohol	0.11	17.8	84.3	Rose, honey [g]	390 [g]
1-hexanol	0.09	6.5	69.0	Herbaceous, fatty, floral [b]	110 [b]
4-ethylphenol	0.08	20.7	85.3	Phenolic, leather [g,h]	21 [g]
Decanoic acid	0.07	30.3	97.3	Rancid fat, animal [b]	1000 [b]
Belgian II Yeast	**RPA**	**RT (min)**	**NIST (%)**	**Odor Descriptor**	**PT (μg L^{-1})**
Ethyl octanoate	1.00	22.1	89.1	Fruity, candy, pineapple [b,c]	580 [b]
Ethyl decanoate	0.86	31.0	88.9	Fruity, grape [b]	200 [b]
2-phenylethyl alcohol	0.23	17.8	83.5	Rose, honey [g]	390 [g]
Ethyl hexanoate	0.19	12.3	88.6	Fruity, strawberry, green apple [b]	14 [b]
3-methyl-1-butanol	0.14	3.1	41.7	Alcohol, nail polish, whiskey [b]	30,000 [b]
Ethyl benzoate	0.12	20.6	67.0	Camomile, flower, celery, fruit [d]	60 [e]
4-ethyl-2-methoxyphenol	0.09	25.6	80.4	Phenolic, smoked [f]	6.9 [f]
1-hexanol	0.07	6.5	66.1	Herbaceous, fatty, floral [b]	110 [b]
Decanoic acid	0.07	30.2	98.0	Rancid fat, animal [b]	1000 [b]
Ethyl dodecanoate	0.06	38.9	85.9	Candy, floral, waxy, soap [b]	1500 [b]
French Saison Yeast	**RPA**	**RT (min)**	**NIST (%)**	**Odor Descriptor**	**PT (μg L^{-1})**
Ethyl octanoate	1.00	22.1	93.3	Fruity, candy, pineapple [b,c]	580 [b]
Ethyl decanoate	0.50	31.0	88.6	Fruity, grape [b]	200 [b]
3-methyl-1-butanol	0.15	3.1	42.5	Alcohol, nail polish, whiskey [b]	30,000 [b]
Ethyl hexanoate	0.15	12.3	91.9	Fruity, strawberry, green apple [b]	14 [b]
2-phenylethyl alcohol	0.12	17.8	86.3	Rose, honey [g]	390 [g]
Ethyl dodecanoate	0.09	38.9	83.3	Candy, floral, waxy, soap [b]	1500 [b]
Ethyl benzoate	0.09	20.5	71.4	Camomile, flower, celery, fruit [d]	60 [e]
Ethyl-9-decenoate	0.07	30.5	99.0	Roses [b]	100 [b]

Table 1. *Cont.*

Abbey Yeast	RPA	RT (min)	NIST (%)	Odor Descriptor	PT (μg L^{-1})
Ethyl octanoate	1.00	22.1	92.4	Fruity, candy, pineapple [b,c]	580 [b]
Ethyl decanoate	0.65	31.0	90.3	Fruity, grape [b]	200 [b]
3-methyl-1-butanol	0.21	3.1	44.8	Alcohol, nail polish, whiskey [b]	30,000 [b]
Ethyl hexanoate	0.18	12.3	92.8	Fruity, strawberry, green apple [b]	14 [b]
Ethyl benzoate	0.18	20.6	95.0	Camomile, flower, celery, fruit [d]	60 [e]
2-phenylethyl alcohol	0.16	17.8	87.5	Rose, honey [g]	390 [g]
4-ethyl-2-methoxyphenol	0.10	25.6	81.4	Phenolic, smoked [f]	6.9 [f]
Decanoic acid	0.07	30.3	98.0	Rancid fat, animal [g]	1000 [g]
1-hexanol	0.06	6.5	66.7	Herbaceous, fatty, floral [b]	110 [b]

[a] Percentage correlation with NIST mass spectral database. [b] [21], [c] [30], [d] [31], [e] [32], [f] [33], [g] [29], [h] [34].

Table 2. Minor (<0.05 relative peak area) volatile compounds identified in cider fermented (six months) with the same apple juice and different *S. cerevisiae* yeast strains.

Edinburgh Yeast	Relative Peak Area	Retention Time (min)	NIST (%) [a]	Odor Descriptor	Perception Threshold (μg L^{-1})
Ethyl nonanoate	0.012	26.5	90.7	Fatty, oily, fruity, nutty [b]	–
Ethyl acetate	0.010	1.9	86.0	Solvent, fruity, balsamic [b]	12,000 [b]
3-methylbutyl octanoate	0.009	33.0	70.7	Fruity, flore [b]	125 [d]
Phenethyl acetate	0.008	24.6	85.3	Rose, honey [e]	3,317,000 [f]
Ethyl hexadecanoate	0.007	52.7	98.7	Waxy, greasy [g]	1500 [g]
3-methylbutyl n-decanoate	0.006	40.7	96.3	Fruity [h]	–
Belgian II Yeast	**RPA**	**RT (min)**	**NIST (%)**	**Odor Descriptor**	**PT (μg L^{-1})**
Ethyl acetate	0.031	2.0	95.7	Solvent, fruity, balsamic [b]	12,000 [b]
Dodecanoic acid	0.010	37.8	97.0	Fatty [c]	–
Ethyl tetradecanoate	0.007	46.1	96.7	Lily [g]	–
Ethyl hexadecanoate	0.007	52.7	99.0	Waxy, greasy [g]	1500 [g]
French Saison Yeast	**RPA**	**RT (min)**	**NIST (%)**	**Odor Descriptor**	**PT (μg L^{-1})**
1-hexanol	0.042	6.5	67.6	Herbaceous, fatty, floral [b]	110 [b]
1-octanol	0.019	15.8	49.5	Citrus, green [i]	6.9 [i]
Decanoic acid	0.027	30.2	98.0	Rancid fat, animal [g]	1000 [g]
Methyl salicylate	0.018	21.7	96.0	Peppermint [g]	0.1 [g]
4-ethyl-2-methoxyphenol	0.010	25.6	92.0	Phenolic, smoked [j]	3 [j]
4-ethylphenol	0.008	20.8	94.7	Phenolic [i]	21 [h]
Ethyl nonanoate	0.006	26.5	87.7	Fatty, oily, fruity, nutty [b]	–
Ethyl acetate	0.006	2.0	92.4	solvent, fruity, balsamic [g]	12,000 [g]
2-phenylethyl acetate	0.005	24.6	80.0	Rose, honey [j]	108 [j]
Abbey Yeast	**RPA**	**RT (min)**	**NIST (%)**	**Odor Descriptor**	**PT (μg L^{-1})**
Ethyl acetate	0.034	2.0	92.5	Solvent, fruity, balsamic [b]	12,000 [b]
Ethyl dodecanoate	0.031	38.8	66.7	Candy, floral, waxy, soap [b]	1500 [b]
3-methylbutyl acetate	0.030	6.8	87.9	Banana [i]	30 [i]
Ethyl-9-decenoate	0.020	30.5	98.7	Roses [g]	100 [g]
Hexyl acetate	0.008	13.0	87.3	Banana, apple, pear [j]	0.2 [k]
1-octanol	0.007	15.8	90.0	Citrus, green [i]	6.9 [i]
2-phenylethyl acetate	0.007	24.7	74.7	Roses, honey [j]	108 [j]
Ethyl 2-methylbutanoate	0.006	5.8	95.7	Strawberry, candy, fruit [g]	18 [g]
Diethyl butanedioate	0.004	21.3	78.7	Wine, caramel, fruity [g]	200,000 [g]

[a] Percentage correlation with NIST mass spectral database. [b] [35], [c] [30], [d] [36], [e] [37], [f] [38], [g] [21], [h] [39], [i] [29], [j] [33], [k] [22].

The vast majority of species observed in these ciders were esters (19), followed by higher alcohols (4), phenols (2), and fatty acids (2). Eleven common compounds were observed in all ciders at varying abundances: ethyl octanoate, ethyl decanoate, ethyl hexanoate, ethyl dodecanoate, ethyl benzoate, ethyl acetate, decanoic acid, 2-phenylethyl alcohol, 1-hexanol, 3-methyl-1-butanol, and 4-ethyl-2-methoxyphenol. The top two most abundant analytes, ethyl octanoate and ethyl decanoate, were the same for every yeast strain. However, the remaining VOCs differed in abundance depending on the yeast strain used. Many of these VOCs have also been observed in other published

cider work [4,10,20]. The ethyl esters contribute to the fruity fragrance of cider, and even small variations in their concentrations can yield significant differences in the final organoleptic quality [13]. We observed that although similarities did exist among the ciders, the odor-active compounds differed greatly across each strain in both variety and abundance.

The French Saison and Abbey yeast strains generated ciders with the most similar VOC profiles (14 in common), with several notable minor species identifications. Methyl salicylate was unique to cider fermented with French Saison yeast. This minty volatile had the lowest perception threshold of any other reported in this study (0.1 $\mu g \cdot L^{-1}$), and it has been previously observed in cider and wine [4,21], although it is not consistently listed as a top contributor to cider aroma [10]. Hexyl acetate (the second lowest perception threshold reported herein, 0.2 $\mu g \cdot L^{-1}$), 3-methylbutyl acetate, ethyl-2-methylbutanoate, and diethyl butanedioate contribute greatly to fruity, strawberry, banana, and sweet caramel odors, and they were detected exclusively in cider produced with Abbey yeast. Rosy, floral volatiles (ethyl-9-decenoate and phenylethyl acetate) and citrus odorants (1-octanol) were identified in both French Saison and Abbey ciders but not in those produced with other yeast strains. These data indicate that French Saison is a good choice for producing a floral aromatic cider with minty undertones, and Abbey is best for a balanced mixture of fruity and floral notes. They also suggest that aromatic species whose perception thresholds are particularly low (<1 $\mu g \cdot L^{-1}$) should be considered important to cider aroma, in addition to those typically reported on [10].

The Edinburgh yeast strain yielded an aroma profile consisting of the predominant ethyl esters and higher alcohols that were observed in all four ciders but with the addition of a volatile phenol (4-ethylphenol) among its major VOCs (Table 1). Some volatile phenols have been described as adding a smoked, spice, or clove scent [4,31], and in fact the Abbey yeast strain is commercially advertised as contributing appealing phenolic characteristics to finished beer products. The phenol 4-Ethylphenol, however, has been described as a "horsey defect" in a previous study on wine odor [40]. Although this detection is notable, we do not anticipate that the relative peak area of 0.08 is of great enough abundance to dominate the odor characteristics of this cider. Edinburgh yeast resulted in several unique, fruity, and floral cider odorants that were not observed in the other ciders as well: 3-methylbutyl octanoate (fruity, floral), phenylethyl acetate (rose, honey), and 3-methylbutyl n-decanoate (fruity), as shown in Table 2. Lastly, the Belgian II yeast strain produced yet another original aroma profile with several VOCs of note. Ethyl esters of varying chain length contributed both a lily (ethyl tetradecanoate) and fatty (dodecanoic acid and ethyl hexadecanoate) odor to this final cider product.

In addition to the 4-ethylphenol defect detected in Edinburgh cider, our analytical method proved efficient for sampling off-flavor esters (ethyl hexadecanoate), fatty acids (decanoic acid), and other phenols (4-ethyl-2-methoxyphenol) across our cider samples as well. Previous studies have focused on sampling similar compounds for beer [9], but this work represents a novel method for off-flavor determination in cider. Although these VOCs may be produced as fermentation metabolites of the yeast strain under investigation, it is also well known that contamination of Brettanomyces/Dekkera strains that are resistant to SO_2 and filtration treatments could be partially responsible [13]. Whatever the source of these VOCs, it is of practical concern to cider makers to detect off-flavors early in the fermentation process, and this method makes that possible.

Of the nine VOCs that are considered the top contributors to cider odor [10], we observed four as major species in all ciders produced in this study: ethyl acetate, ethyl octanoate, 2-phenylethyl alcohol, and 3-methyl-1-butanol. These four molecules are representative of the fruity, floral, and alcoholic aromas that are characteristic of cider. They also represent the two structural groups of molecules that were observed in the greatest abundance throughout this study: ethyl esters and higher alcohols. To quantitatively verify that these central species were at ideal concentrations in the finished cider products, we performed an external calibration [20], and the results are reported in Table 3. If ethyl acetate is present in excess, it can yield an undesirable solvent smell [13]. We detected this species below the perception threshold (<12 $mg \cdot L^{-1}$) for cider fermented with Edinburgh and French Saison yeasts. Cider produced with Belgian II and Abbey yeasts generated ethyl acetate concentrations

similar to those previously observed [11] but not high enough to have a deleterious effect on odor. Ethyl octanoate was the most abundant ethyl ester identified in every cider reported herein and is responsible for a fruity or candy fragrance [21], while 2-phenylethyl alcohol produces distinctive floral aromatic notes. Our results show that every cider, independent of yeast strain, contained ethyl octanoate and phenylethyl alcohols at concentrations above the perception threshold (>0.014 mg·L^{-1} and >0.390 mg·L^{-1}, respectively) and within concentration ranges that match comparable, recent cider headspace studies [6,11]. Lastly, 3-methyl-1-butanol can contribute a desirable, whiskey aroma at concentrations below 300 mg·L^{-1} [13], but it can become harsh when present at higher quantity. All concentrations reported in Table 3 fall below 300 mg·L^{-1} and above the perception threshold (>30 mg·L^{-1}) for ciders fermented with Belgian II, French Saison, and Abbey yeasts, evidencing an enjoyable aroma.

Table 3. Important aroma compounds quantified in cider fermented (six months) with the same apple juice and different *S. cerevisiae* yeast strains [a].

Analyte (Odor)	Edinburgh Yeast	Belgian II Yeast	French Saison Yeast	Abbey Yeast	Perception Threshold (mg·L^{-1})
Ethyl acetate (solvent, fruity, balsamic) [b]	1.89 ± 0.42 [a]	33.00 ± 7.26	2.52 ± 0.55	41.28 ± 9.08	12 [b]
Ethyl octanoate (fruity, candy, pineapple) [b]	0.13 ± 0.03	0.16 ± 0.04	0.29 ± 0.08	0.20 ± 0.05	0.014 [b]
2-Phenylethyl alcohol (Rose, honey) [c]	6.85 ± 1.03	11.95 ± 1.79	9.59 ± 1.44	8.98 ± 1.35	0.390 [c]
3-methyl-1-butanol (Alcohol, nail polish, whiskey) [b]	13.40 ± 3.58	32.36 ± 8.64	44.47 ± 11.87	38.51 ± 10.28	30 [b]

[a] Concentrations are expressed in mg·L^{-1}, and data are reported as $\bar{x} \pm 1\sigma$ for triplicate SPME experiments. [b] [21] [c] [29].

4. Conclusions

Cider quality is closely aligned with the smell of volatile molecules produced during fermentation, but the evolution of cider fragrance throughout the fermentation process and after maturation are not yet well understood. We have optimized an efficient method for the chemical analysis of aroma during cider production and suggest a practical pre-screening technique early in the fermentation process (72 h post inoculation) for off-flavor detection. Our measurements of cider VOCs have resulted in several discoveries of interest to cider makers and chemists alike. Firstly, the SPME fiber type that extracted the highest concentrations and the widest varieties of odor-active species in cider was the DVB/CAR/PDMS fiber, when held at 40 °C for 30 min in the cider headspace. This fiber consistently outperformed the CAR/PDMS (49% less efficient) and PA fibers (98% less efficient) for esters, higher alcohols, and phenols. Secondly, we established a VOC formation timeline in order to better understand the release of odor-active species during cider preparation. The cider aroma profile developed rapidly after the beginning of fermentation but remained surprisingly consistent after only 72 h at 23 ± 1 °C (with major esters and higher alcohols varying by 18.6% ± 4.1% and 12.3% ± 2.6% thereafter, respectively). This allows for early pre-screening for off-flavors such as 4-ethylphenol and 4-ethyl-2-methoxyphenol, which are also readily detected by this analytical method. Thirdly, we utilized yeast strains which have been traditionally chosen for beer fermentation (Edinburgh, Belgian II, French Saison, and Abbey) to produce four commercial grade ciders via a six-month maturation process. These ciders yielded significantly variant sensory qualities from identical starting apple juices, providing evidence for the central importance of yeast strain choice in cider making. Twenty-seven major and minor VOCs were identified across these ciders, and only eleven of them were common to all ciders produced, evidencing the unique metabolic processes of each strain. Lastly, four compounds known to define cider aroma (ethyl acetate, ethyl octanoate, 2-phenylethyl alcohol, and 3-methyl-1-butanol) were quantified within desirable concentration ranges in all four ciders.

Author Contributions: C.A.C., M.T.B., C.E.S., J.P.P. and J.M.S. designed the study, interpreted the results, and drafted the manuscript together. M.T.B., C.E.S. and J.P.P. collected and analyzed all reported experimental data under the mentorship of C.A.C. and J.M.S. All authors have read and agreed to the published version of the manuscript.

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References

1. Diamond, D.H. Origins of Pioneer Apple Orchards in the American West: Random Seeding versus Artisan Horticulture. *Agric. Hist.* **2010**, *84*, 423–450. [CrossRef]
2. Singh, S. Cider Market by Product (Apple Flavored, Fruit Flavored, and Perry), Distribution Channel (On-trade and Off-trade), and Packaging (Draught, Cans, Glass Bottles, Plastic Bottles, and Others)—Global Opportunity Analysis and Industry Forecast, 2017–2023. Available online: https://www.alliedmarketresearch.com/cider-market (accessed on 20 January 2020).
3. Lofholm, N. An apple revival near Four Corners is restoring hundreds of historic fruits and the local ag economy. Available online: https://coloradosun.com/2019/11/28/colorado-heritage-apples-orchard-restoration-hard-cider/ (accessed on 28 November 2019).
4. Qin, Z.H.; Petersen, M.A.; Bredie, W.L.P. Flavor profiling of apple ciders from the UK and Scandinavian region. *Food Res. Int.* **2018**, *105*, 713–723. [CrossRef] [PubMed]
5. Symoneaux, R.; Guichard, H.; Le Quere, J.M.; Baron, A.; Chollet, S. Could cider aroma modify cider mouthfeel properties? *Food Qual. Prefer.* **2015**, *45*, 11–17. [CrossRef]
6. Rosend, J.; Kuldjarv, R.; Rosenvald, S.; Paalme, T. The effects of apple variety, ripening stage, and yeast strain on the volatile composition of apple cider. *Heliyon* **2019**, *5*. [CrossRef]
7. Howard, K.L.; Mike, J.H.; Riesen, R. Validation of a solid-phase microextraction method for headspace analysis of wine aroma components. *Am. J. Enol. Vitic.* **2005**, *56*, 37–45.
8. Rebiere, L.; Clark, A.C.; Schmidtke, L.M.; Prenzler, P.D.; Scollary, G.R. A robust method for quantification of volatile compounds within and between vintages using headspace-solid-phase micro-extraction coupled with GC-MS—Application on Semillon wines. *Anal. Chim. Acta* **2010**, *660*, 149–157. [CrossRef]
9. da Silva, G.C.; da Silva, A.A.S.; da Silva, L.S.N.; Godoy, R.; Nogueira, L.C.; Quiterio, S.L.; Raices, R.S.L. Method development by GC-ECD and HS-SPME-GC-MS for beer volatile analysis. *Food Chem.* **2015**, *167*, 71–77. [CrossRef]
10. Peng, B.Z.; Li, F.L.; Cui, L.; Guo, Y.D. Effects of Fermentation Temperature on Key Aroma Compounds and Sensory Properties of Apple Wine. *J. Food Sci.* **2015**, *80*, S2937–S2943. [CrossRef] [PubMed]
11. Nicolini, G.; Roman, T.; Carlin, S.; Malacarne, M.; Nardin, T.; Bertoldi, D.; Larcher, R. Characterisation of single-variety still ciders produced with dessert apples in the Italian Alps. *J. Inst. Brew.* **2018**, *124*, 457–466. [CrossRef]
12. Pello-Palma, J.; Mangas-Alonso, J.J.; de la Fuente, E.D.; Gonzalez-Alvarez, J.; Diez, J.; Alvarez, M.D.G.; Abrodo, P.A. Characterization of Volatile Compounds in New Cider Apple Genotypes Using Multivariate Analysis. *Food Anal. Methods* **2016**, *9*, 3492–3500. [CrossRef]
13. Cousin, F.J.; Le Guellec, R.; Schlusselhuber, M.; Dalmasso, M.; Laplace, J.M.; Cretenet, M. Microorganisms in Fermented Apple Beverages: Current Knowledge and Future Directions. *Microorganisms* **2017**, *5*, 39. [CrossRef]
14. Villiere, A.; Arvisenet, G.; Bauduin, R.; Le Quere, J.M.; Serot, T. Influence of cider-making process parameters on the odourant volatile composition of hard ciders. *J. Inst. Brew.* **2015**, *121*, 95–105. [CrossRef]

15. Thompson-Witrick, K.A.; Goodrich, K.M.; Neilson, A.P.; Hurley, E.K.; Peck, G.M.; Stewart, A.C. Characterization of the Polyphenol Composition of 20 Cultivars of Cider, Processing, and Dessert Apples (Malus x domestica Borkh.) Grown in Virginia. *J. Agric. Food Chem.* **2014**, *62*, 10181–10191. [CrossRef] [PubMed]
16. Ewing, B.L.; Peck, G.M.; Ma, S.H.; Neilson, A.P.; Stewart, A.C. Management of Apple Maturity and Postharvest Storage Conditions to Increase Polyphenols in Cider. *Hortscience* **2019**, *54*, 143. [CrossRef]
17. Way, M.L.; Jones, J.E.; Swarts, N.D.; Dambergs, R.G. Phenolic Content of Apple Juice for Cider Making as Influenced by Common Pre-Fermentation Processes Using Two Analytical Methods. *Beverages* **2019**, *5*, 53. [CrossRef]
18. Staples, M.; Poechlinger, G.; Gossinger, M. Impact of different yeasts on organoleptic parameters of apple wine. *Mitt. Klosterneubg.* **2016**, *66*, 245–254.
19. dos Santos, C.M.E.; Alberti, A.; Pietrowski, G.D.M.; Zielinski, A.A.F.; Wosiacki, G.; Nogueira, A.; Jorge, R.M.M. Supplementation of amino acids in apple must for the standardization of volatile compounds in ciders. *J. Inst. Brew.* **2016**, *122*, 334–341. [CrossRef]
20. dos Santos, C.M.E.; Pietrowski, G.D.M.; Braga, C.M.; Rossi, M.J.; Ninow, J.; dos Santos, T.P.M.; Wosiacki, G.; Jorge, R.M.M.; Nogueira, A. Apple Aminoacid Profile and Yeast Strains in the Formation of Fusel Alcohols and Esters in Cider Production. *J. Food Sci.* **2015**, *80*, C1170–C1177. [CrossRef]
21. Arcari, S.G.; Caliari, V.; Sganzerla, M.; Godoy, H.T. Volatile composition of Merlot red wine and its contribution to the aroma: Optimization and validation of analytical method. *Talanta* **2017**, *174*, 752–766. [CrossRef]
22. Ruiz, M.J.; Zea, L.; Moyano, L.; Medina, M. Aroma active compounds during the drying of grapes cv. Pedro Ximenez destined to the production of sweet Sherry wine. *Eur. Food Res. Technol.* **2010**, *230*, 429–435. [CrossRef]
23. Alberti, A.; Vieira, R.G.; Drilleau, J.F.; Wosiacki, G.; Nogueira, A. Apple Wine Processing with Different Nitrogen Contents. *Braz. Arch. Biol. Technol.* **2011**, *54*, 551–558. [CrossRef]
24. Braga, C.M.; Zielinski, A.A.F.; da Silva, K.M.; de Souza, F.K.F.; Pietrowski, G.D.M.; Couto, M.; Granato, D.; Wosiacki, G.; Nogueira, A. Classification of juices and fermented beverages made from unripe, ripe and senescent apples based on the aromatic profile using chemometrics. *Food Chem.* **2013**, *141*, 967–974. [CrossRef]
25. Jolicoeur, C. *The New Cider Maker's Handbook: A Comprehensive Guide for Craft Producers*; Watson, B., Ed.; Chelsea Green Publishing: Hartford, VT, USA, 2013.
26. Vas, G.; Vekey, K. Solid-phase microextraction: A powerful sample preparation tool prior to mass spectrometric analysis. *J. Mass Spectrom.* **2004**, *39*, 233–254. [CrossRef] [PubMed]
27. Curran, A.M.; Rabin, S.I.; Prada, P.A.; Furton, K.G. Comparison of the volatile organic compounds present in human odor using SPME-GC/MS. *J. Chem. Ecol.* **2005**, *31*, 1607–1619. [CrossRef] [PubMed]
28. Schmutzer, G.R.; Magdas, A.D.; David, L.I.; Moldovan, Z. Determination of the Volatile Components of Apple Juice Using Solid Phase Microextraction and Gas Chromatography-Mass Spectrometry. *Anal. Lett.* **2014**, *47*, 1683–1696. [CrossRef]
29. Czerny, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Hernandez, N.; Schieberle, P. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265–273. [CrossRef]
30. Englezos, V.; Torchio, F.; Cravero, F.; Marengo, F.; Giacosa, S.; Gerbi, V.; Rantsiou, K.; Rolle, L.; Cocolin, L. Aroma profile and composition of Barbera wines obtained by mixed fermentations of Starmerella bacillaris (synonym Candida zemplinina) and Saccharomyces cerevisiae. *Lwt-Food Sci. Technol.* **2016**, *73*, 567–575. [CrossRef]
31. Jordan, M.J.; Margaria, C.A.; Shaw, P.E.; Goodner, K.L. Aroma active components in aqueous kiwi fruit essence and kiwi fruit puree by GC-MS and multidimensional GC/GC-O. *J. Agric. Food Chem.* **2002**, *50*, 5386–5390. [CrossRef]
32. Sansone-Land, A.; Takeoka, G.R.; Shoemaker, C.F. Volatile constituents of commercial imported and domestic black-ripe table olives (Olea europaea). *Food Chem.* **2014**, *149*, 285–295. [CrossRef]
33. Pino, J.A.; Tolle, S.; Gok, R.; Winterhalter, P. Characterisation of odour-active compounds in aged rum. *Food Chem.* **2012**, *132*, 1436–1441. [CrossRef]

34. Ferreira, V.; Ortin, N.; Escudero, A.; Lopez, R.; Cacho, J. Chemical characterization of the aroma of Grenache rose wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002**, *50*, 4048–4054. [CrossRef] [PubMed]
35. Genovese, A.; Piombino, P.; Gambuti, A.; Moio, L. Simulation of retronasal aroma of white and red wine in a model mouth system. Investigating the influence of saliva on volatile compound concentrations. *Food Chem.* **2009**, *114*, 100–107. [CrossRef]
36. Escudero, A.; Gogorza, B.; Melus, M.A.; Ortin, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*, 3516–3524. [CrossRef] [PubMed]
37. Moreno-Garcia, J.; Garcia-Martinez, T.; Millan, M.C.; Mauricio, J.C.; Moreno, J. Proteins involved in wine aroma compounds metabolism by a Saccharomyces cerevisiae flor-velum yeast strain grown in two conditions. *Food Microbiol.* **2015**, *51*, 1–9. [CrossRef]
38. Xiao, Z.B.; Luo, J.; Niu, Y.W.; Wang, P.P.; Wang, R.L.; Sun, X.X. Olfactory impact of esters on rose essential oil floral alcohol aroma expression in model solution. *Food Res. Int.* **2019**, *116*, 211–222. [CrossRef]
39. Condurso, C.; Cincotta, F.; Tripodi, G.; Sparacio, A.; Giglio, D.M.L.; Sparla, S.; Verzera, A. Effects of cluster thinning on wine quality of Syrah cultivar (*Vitis vinifera* L.). *Eur. Food Res. Technol.* **2016**, *242*, 1719–1726. [CrossRef]
40. De Rosso, M.; Panighel, A.; Vedova, A.D.; Stella, L.; Flamini, R. Changes in Chemical Composition of a Red Wine Aged in Acacia, Cherry, Chestnut, Mulberry, and Oak Wood Barrels. *J. Agric. Food Chem.* **2009**, *57*, 1915–1920. [CrossRef]