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Non-Destructive PTR-ToF-MS Profiling of Red Delicious and Granny Smith Apple Volatilomes During Ripening

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Abstract: The optimal harvest date (OHD) for the long-term storage of apple fruits is of the utmost importance, not only for maintaining high quality levels, but also because the ripening stage, regulated by the autocatalytic activity of the internal ethylene concentration, greatly affects the VOCs' synthesis. During apple ripening, chemical compounds undergo changes that affect the fruit's overall quality, particularly its aromatic profile. Three main classes of organic molecules—aldehydes, alcohols, and esters—play a key role in these modifications. This study investigated the potential of proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS) for the rapid, non-destructive monitoring of VOC profiles in 'Red Delicious' and 'Granny Smith' apples over a 7-week shelf-life period across three harvest dates with different ripening stages. More than 300 mass peaks in the PTR-ToF-MS spectra of the apple headspace were detected. A total of 127 of them were considered to be relevant for further analysis. Furthermore, respiration rate and I_{AD} index were used for the non-destructive assessment of the ripening progress during the 7 weeks of shelf-life and for integrating the VOC results.

Keywords: apple ripening; proton transfer reaction time-of-flight mass spectrometry; volatile organic compounds; DA-meter; CO_2 production

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1. Introduction

Flavor is a multimodal sensory attribute primarily influenced by taste and aroma [1] which plays a key role in consumer satisfaction, as it affects the further consumption of different foods, such as fruits [2]. Aroma as one of the most investigated apple quality attributes, and is given by a complex matrix of many volatile organic compounds (VOCs) [3]. In apples, more than 300 volatiles have been detected [4], although it is assumed that only a few of them contribute to the typical apple aroma [5–7]. Among the several factors influencing the VOCs profile of apple fruit, like genetics, environment, and agronomical practices, the ripening stage, which is regulated by the ethylene synthesis [8], seems to be the most critical [9,10]. In the initial ripening stage, aldehydes, primarily derived from fatty acids [11], are the predominant compounds, with hexanal and trans-2-hexenal contributing to green and grassy aromas [12]. Then, alcohols, formed by the reduction of the corresponding aldehydes [13], start to increase, with 1-hexanol and 2-methyl-1-butanol contributing to green and floral notes [14].

In the last ripening phase, esters, formed by enzymatic action from alcohols [15] and characterized by fruity flavor attributes [16–18], are the most dominant VOCs. In ripe apples, alcohols represent up to 16% of the total volatiles, while esters, depending on the cultivar, are the most predominant [19] and are mainly composed of ethyl, butyl and hexyl acetates, butanoates, and hexanoates related to fruity notes [10]. Unripe harvested apples produce fewer volatiles and as consequence lose their ability to produce volatiles during storage more quickly than ripe apples [20]. On the other hand, overripe fruits undergo a faster deterioration of their overall quality and are more sensitive to damage and various diseases. To increase the VOCs content, fruits should be harvested at the optimal ripening stage. However, to maintain the best overall quality after storage, fruits should be harvested at the unripe stage, just at the onset of the climacteric rise [21]. Determining the optimal harvest date (OHD) for long-term storage is of pivotal importance for the apple fruit industry. OHD marks the point at which apples reach ideal physiological maturity, balancing firmness, starch degradation, sugar content, acidity, and storability. It falls within the optimal harvest window (OHW), the period during which apples can be harvested while preserving their peak quality [22]. For commercial and scientific purposes, accurately determining both the OHD and OHW is critical to optimizing fruit quality, minimizing post-harvest losses, and ensuring consumer satisfaction.

Currently, to assess the OHD, apple ripening is monitored weeks before the estimated date in representative orchard samples. Ethylene analysis describes the climacteric trend, which is crucial for detecting the climacteric onset. This is defined as the point when the internal ethylene concentration exceeds 0.1 μL^{-1} [23]. Fruit firmness, background color, and starch degradation pattern are easy to measure and are useful for practical applications, as they reflect ripening processes and are indirectly linked to climacteric onset [24]. By comparing the results with cultivar-specific reference tables, it is possible to determine the OHD. The production of volatile organic compounds (VOCs) is closely linked to ethylene biosynthesis; therefore, early monitoring could be valuable in determining the optimal harvest date (OHD).

The standard analytical method for VOCs identification and quantification in apples relies on gas chromatography coupled with mass spectrometry (GC-MS) [19]. However, for non-destructive postharvest studies on the same apples, GC-MS is highly time-consuming. Alternatively, direct injection mass spectrometric techniques, such as proton transfer reaction mass spectrometry (PTR-MS), have proven to be a reliable option, enabling the real-time monitoring of numerous volatile compounds [25]. This technique identifies analytes solely based on their m/z value, without offering isomer separation. Despite this limitation, PTR-ToF-MS has recently been evaluated as a green analytical tool for food volatilome profiling [26]. Moreover, several studies have demonstrated its rapidity and sensitivity in evaluating the apple volatilome, making it suitable for real-time VOC analysis [27–30].

Ref. [31] compared solid-phase microextraction gas chromatography–mass spectrometry (SPME/GC-MS) and proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS) to investigate the interaction between apple VOCs and various postharvest pathogens.

In the present study, the PTR-MS with a time-of-flight mass spectrometer (PTR-ToF-MS) was applied for the rapid monitoring of non-destructive VOCs during the shelf-life of two apple cultivars, ‘Red Delicious’ and ‘Granny Smith’, harvested at three distinct ripening stages. ‘Red Delicious’ and ‘Granny Smith’ are two cultivars with distinguished VOCs profiles and, moreover, are the most cultivated apple varieties worldwide after ‘Golden Delicious’ and ‘Gala’. The aim of this study was to examine how different ripening stages affected the VOCs synthesis of the two cultivars in whole apples, and to monitor VOCs evolution during a postharvest ripening period.

2. Results

2.1. Quality Parameters at Harvest

The physicochemical analysis conducted in parallel to the non-destructive analysis at each harvest point (Table 1) clearly highlighted two different ripening dynamics between the cultivars along the three harvests. In particular, the starch pattern index (SPI) of 'Red Delicious' increased from 1.77 ppm at the first pick (OHD − 1 week) on 22 August 2022 to 4.21 ppm at the third pick (OHD + 2 weeks) on 13 September 2022, indicating a dynamic ripening progress. This was further confirmed by the internal ethylene concentration (IEC), which showed that the climacteric onset had already occurred by 30 August 2022, with a concentration of 4.53 ppm. In 'Granny Smith' apples, the SPI showed a minimal shift from the first harvest on 1 September 2022 to the third harvest on 27 September 2022, increasing from 1.7 to 2.5. This was confirmed by the IEC, which remained constant at 1.8–1.9 ppm throughout this period. The average firmness in 'Red Delicious' apples ranged from 77 to 69 and 63 N across all harvests, while for 'Granny Smith', this quality parameter decreased from 83 to 77 and 68 N (Table 1). Both apple varieties showed a decrease in firmness; however, 'Granny Smith' apples were firmer than 'Red Delicious'. Titratable acidity ranged from 3.4 to 2.9 g/L in 'Red Delicious' from the first to the third harvest, while for 'Granny Smith', which had a higher acidity content, the concentration decreased from 9.1 to 7.4 g/L by the third harvest. The sugar content, expressed as °Brix, increased from 8.7 to 10.4 in 'Red Delicious' across the three harvests, while in 'Granny Smith', it ranged from 9.1 to 9.6 °Brix. It is important to note that the sugar values during ripening are less significant, as they tend to increase during storage due to the complete starch hydrolysis.

Table 1. Quality parameters at harvest of 'Red Delicious' and 'Granny Smith' at different ripening stages. Starch pattern index (SPI), firmness, weight, Total Soluble Solids (TSS), and I_{AD} values are mean (±SE) of 30 replicates. Internal ethylene concentration (IEC) values are mean (±SE) of 10 fruits, and titratable acidity (TTA) values, expressed as malic acid equivalent (g/L), are mean (±SE) of three replicates. Different letters indicate statistically significant differences ($p \leq 0.05$).

Cultivar	Harvest	Date	Starch (LB Scale 1–5)	Firmness (N)	TTA (MAeq g/L)	TSS (°Brix)	IEC (ppm)	I_{AD}
Red Delicious	OHD − 1 week	22.08.22	1.77 ± 0.04	76.69 ± 0.11 a	3.43 ± 0.09 a	8.73 ± 0.05 a	n.a.	1.80 ± 0.01
	OHD	30.08.22	2.54 ± 0.08	68.70 ± 0.08 a	3.33 ± 0.03 a	9.79 ± 0.08 a	4.53 ± 1.15	1.54 ± 0.02
	OHD + 2 weeks	13.09.22	4.21 ± 0.11	63.10 ± 0.09 a	2.87 ± 0.03 a	10.38± 0.23 a	7.48 ± 2.20	1.35 ± 0.03
Granny Smith	OHD − 2 weeks	01.09.22	1.73 ± 0.02	82.55 ± 0.11 b	9.13 ± 0.47 b	9.08 ± 0.05 b	0.06 ± 0.03	1.81 ± 0.01
	OHD	13.09.22	1.86 ± 0.10	77.97 ± 0.10 b	8.87 ± 0.30 b	8.48 ± 0.53 b	0.14 ± 0.05	1.83 ± 0.02
	OHD + 2 weeks	27.09.22	2.45 ± 0.08	68.05 ± 0.27 b	7.4 ± 0.31 b	9.57 ± 0.31 b	0.15 ± 0.04	1.88 ± 0.02

2.2. VOCs Analysis in Relation to CO_2 Production and I_{AD} Value

The ripening evolutions of 'Red Delicious' and 'Granny Smith' apples during the shelf-life monitoring period were assessed through the respiration rate, based on CO_2 emissions, and the index of absorbance difference (I_{AD}) (Figure 1). The difference between the two cultivars was evident in both analyses, with 'Red Delicious' apples showing high CO_2 emissions and decreasing I_{AD} values. This indicated a dynamic ripening progress, reflecting ethylene-induced changes that had already occurred. On the other hand, 'Granny Smith' exhibited a very low respiration rate and a constant I_{AD} value across all harvest dates, as confirmed by the low IEC, indicating that the fruits were still in a preclimacteric stage. The distinct genetic background and metabolic approach of the two cultivars were thoroughly investigated and reported by [32], In the 'Red Delicious' apples at 'OHD − 1 week' and at 'OHD', the dynamic of the CO_2 production was very similar, ranging from 7.57 mL kg $^{-1}$ h^{-1} and 6.56 mL kg $^{-1}$ h^{-1} to 10.51 mL kg $^{-1}$ h^{-1} and 12.43 mL kg $^{-1}$ h^{-1}, respectively. Both ripening stages had a CO_2 emission peak between 15.58 and 16.41 mL kg^{-1} h^{-1} after 20 days from the beginning of the experiment, probably corresponding to the climacteric peak. In the last ripening stage at 'OHD + 2 weeks', the CO_2 variability lay between 11.85 and 13.36 mL kg^{-1} h^{-1} (Figure 1A). In the 'Granny Smith' apples, CO_2 production showed a decreasing trend, with values ranging from 5.52 to 2.55 mL kg^{-1} h^{-1} at 'OHD − 2 weeks,' from 6.25 to 2.46 mL kg^{-1} h^{-1} at 'OHD,' and from 6.17 to 3.46 mL kg^{-1} h^{-1} at 'OHD + 2 weeks.' Only at 'OHD − 2 weeks' was it possible to observe a peak of 8 mL kg^{-1} h^{-1} after 7 days of shelf-life (Figure 1B). The non-destructive analysis with the DA-meter to assess the ripening stage through the chlorophyll content had a different dynamic depending on the cultivar. In 'Red Delicious', the higher metabolic activity was confirmed by the decrease in the I_{AD} value, particularly at 'OHD − 1 week' (Figure 1C). On the other hand, in 'Granny Smith,' the lower metabolic activity was confirmed by the consistently high I_{AD} values throughout the monitoring period across all three ripening stages (Figure 1D). It is important to note that higher I_{AD} values correspond to higher chlorophyll content, indicating less ripe fruits, while lower values are associated with lower chlorophyll content, typical of more ripe fruits. This is due to the photosynthetic apparatus disruption which involves the conversion of chloroplasts to chromoplasts, leading to chlorophyll degradation [33], which is strictly linked to lipid metabolism, in particular through membrane integrity and lipoxygenase (LOX) activity changes [34]. The significant linear relationship observed between the apple peel chlorophyll content and I_{AD} was reported by [35,36].

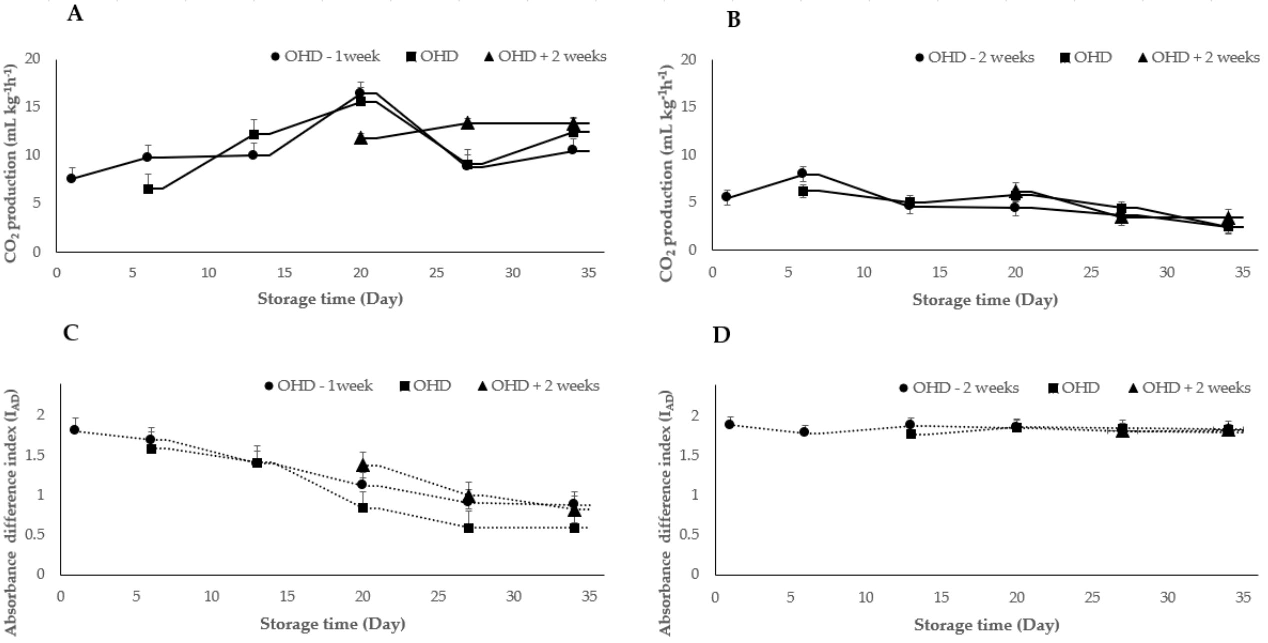

Figure 1. CO_2 production and index of absorbance difference (I_{AD}) of 'Red Delicious' (**A**,**C**) and 'Granny Smith' (**B**,**D**) during seven weeks of monitoring at 20 °C across three different ripening stages: 'OHD − 2 weeks', 'OHD', and 'OHD + 2 weeks'. Bars refer to standard error.

For the non-destructive VOCs analysis, 332 mass peaks were extracted from the raw spectra. Mass peaks corresponding to 13C isotopologues, water clusters, and those whose concentrations did not differ significantly across all time points were excluded from the dataset used for further analysis. This procedure reduced the initial dataset to 127 mass peaks. A total of 127 out of 332 mass peaks were selected for further analysis. The selected mass peaks were first assigned a chemical formula and then linked to a potential compound. However, due to the absence of chromatographic separation in PTR-ToF-MS, distinguishing between structural and spatial isomers of the same chemical formula was not possible [37,38]. To highlight the differences between cultivars and harvest times from a multivariate perspective, principal component analysis (PCA) was performed on the centered and log-transformed dataset. The first two principal components (PCs) explained 94.21% of the variability in the dataset, with the first PC (91.49%) accounting for the majority of the variance. The PCA score plot (Figure 2) revealed a significant difference between the two cultivars and the evolution of their VOCs profiles during shelf-life.

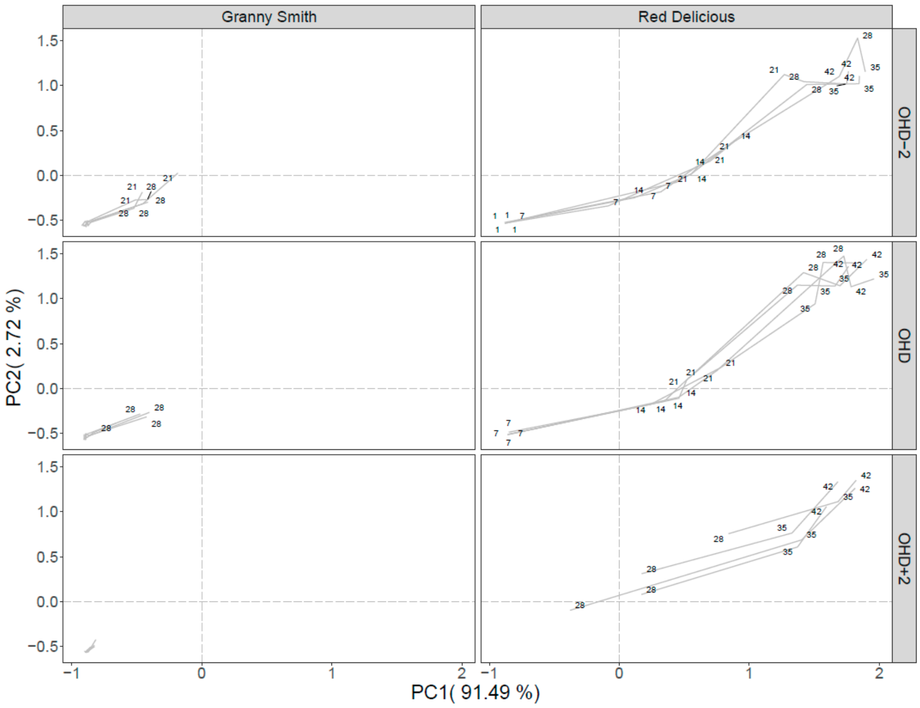

Figure 2. PCA score plot of apple VOCs measured by the PTR-ToF-MS of the cultivars 'Granny Smith' (**left**) and 'Red Delicious' (**right**), divided vertically by cultivar and horizontally by harvest times. The numbers indicate the measurement days from the beginning of the experiment.

The 'Granny Smith' aroma profile during ripening did not change drastically for all the three harvest dates. However, a rapid increase in VOCs during ripening was evident in 'Red Delicious' apples, especially at 'OHD + 2 weeks'. According to the loading plot (Supplementary Material, Figure S1), this is mainly due to the development and release of different alcohols and esters. The main mass peaks of alcohols are represented by m/z 33.033 (CH_4OH^+), m/z 43.054 ($C_3H_7^+$), m/z 47.049 ($C_2H_6OH^+$), m/z 57.069 ($C_4H_9^+$), m/z 71.086 ($C_5H_{11}^+$), and m/z 85.101 ($C_6H_{13}^+$). Ref. [39] in their book, explained that dehydration reactions predominantly occur during proton transfer reactions for all C_3 and higher alcohols. As a result, the dehydrated forms of these alcohols were presented in this study. The evolution of three of them are shown in Figure 3A–C. Here, it is possible to notice how the ripening stages affected the ethanol and butanol differently, with their emissions being lower in the last harvest (OHD + 2 weeks) (Figure 3A,B) compared to hexanol, which was higher in the last harvest (OHD + 2 weeks) than in the other two ripening stages (Figure 3C).

The tentatively identified esters and acids were mainly represented by mass peaks of m/z 61.028 ($C_2H_4O_2H^+$), m/z 75.044 ($C_3H_6O_2H^+$), m/z 89.06 ($C_4H_8O_2H^+$), m/z 103.075 ($C_5H_{10}O_2H^+$), m/z 117.091 ($C_6H_{12}O_2H^+$), m/z 131.107 ($C_7H_{14}O_2H^+$), m/z 145.12 ($C_8H_{16}O_2H^+$), m/z 159.136 ($C_9H_{18}O_2H^+$), m/z 173.1536 ($C_{10}H_{20}O_2H^+$), and m/z 201.182 ($C_{12}H_{24}O_2H^+$). Their concentrations, as shown in Figure 3D–F, were, in general, much higher in 'Red Delicious' compared to 'Granny Smith'. In particular, for 'Red Delicious', the two harvest periods 'OHD-2' and 'OHD' showed similar results; however, late-harvested apples (OHD + 2 weeks) showed different behavior for the different mass peaks related to esters and acids. For m/z 103.075, the concentration at the first measurement point after harvesting was comparable to the first time points of the other two harvests, and then increased at the subsequent measurement points. The increase in the concentration of m/z 145.12 during storage was faster than that for m/z 103.075. The concentration of m/z 187.166 was consistently higher at the OHD+2 harvest in comparison to the other two harvests. The plots of all 127 mass peaks are listed in the Supplementary Materials (Figure S2).

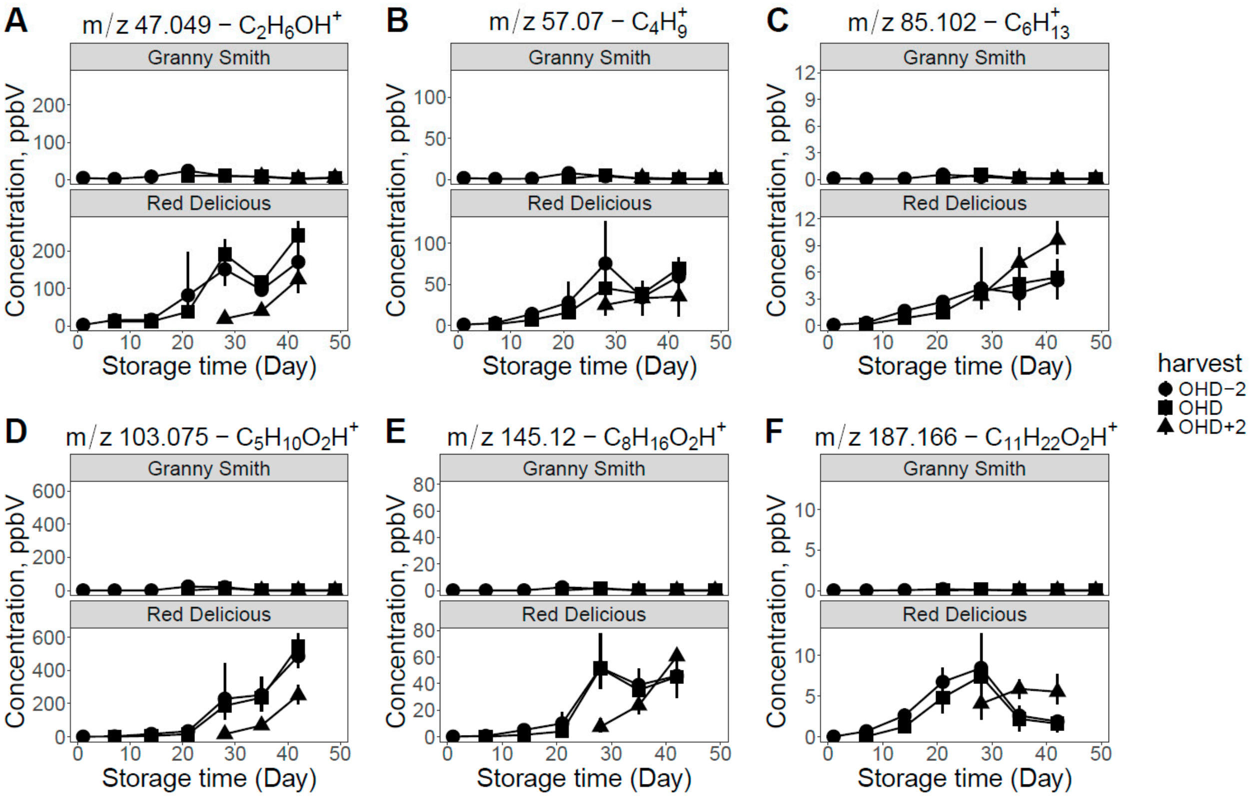

Figure 3. Concentration curves in ppbV for selected mass peaks, tentatively identified as ethanol (165, (**A**)), dehydrated form of butanol (**B**), dehydrated form of hexanol (**C**), C5 (**D**), C8 (**E**), and C11 (**F**) esters and acids, which were measured by PTR-ToF-MS during ripening period (in days) for 'Granny Smith' and 'Red Delicious' apple cultivars stored at 20 °C.

3. Discussion

The VOCs evolution and the respiration rate, expressed as CO_2 production, of 'Red Delicious' and 'Granny Smith' apple cultivars harvested at three different ripening stages during a shelf-life period at 20 °C were non-destructively investigated in this study. For sample characterization, fruits collected at the three different ripening stages were qualitatively analyzed. The reduced starch hydrolysis of 'Granny Smith' across the harvest period strongly correlated with the low IEC, meaning that this cultivar was still in a preclimateric stage even at the last harvest on the date of 27 September 2022. On the other hand, a more dynamic ripening of 'Red Delicious' was observed, as confirmed by both the SPI and IEC values. This difference was relevant, as it reflected the subsequent pattern that was revealed by the VOCs analysis with PTR-ToF-MS. In fact, the ripening pattern of 'Red Delicious' highly correlated with the VOCs evolution pattern during the shelf-life period, where a linear increase in the alcohols was observed, followed by the synthesis of esters, which were mainly responsible for the fruity notes of this cultivar.

This is consistent with the fact that fatty acids are the major precursors of aroma volatiles in apples, and that their accumulation occurs especially during the climacteric peak and in peel tissues [17]. This also aligns with the fact that chlorophyll degradation correlates with a decrease in lipids due to chloroplast breakdown39, as confirmed by our I_{AD} results. On the other hand, 'Granny Smith,' well known for being a low volatile emitter [10,40], also showed constant low ethylene emissions, even at the last harvest, confirming that the fruits were still unripe at that stage. This was evident from the VOCs and the CO_2 analysis, which highlighted a low and constant pattern of VOCs synthesis as well as a stable respiration rate. This is mainly due to the fact that, for commercial reasons, aiming to avoid an unpleasant red blush coloration on its green skin, this cultivar is harvested at a very unripe stage. This trend also confirms the ethylene-dependent volatile production of climateric fruits, as confirmed by many authors [41–44], and its correlation with the respiration rate [45].

4. Materials and Methods

4.1. Fruit Sampling and Quality Parameters at Harvest

Fruit sampling was carried out in 2022 on 'Red Delicious' (RD) and 'Granny Smith' (GS) cultivars; orchards located at the Laimburg Research Centre (Italy, South Tyrol, 222 m a.s.l.) were managed according to Global-GAP standard horticultural practices. Four apples of each cultivar were collected at three different ripening stages: optimal harvest date 'OHD − 2 weeks' for 'Granny Smith' and 'OHD − 1 week' for 'Red Delicious', 'OHD', and 'OHD + 2 weeks', according to recommended ripening parameters for the long-term storage of 'Granny Smith' and 'Red Delicious' apple cultivars, as outlined by the Laimburg Research Centre (Bolzano, Italy) for commercial purposes in South Tyrol (see Supplementary Material Figure S3). The apples were then stored at room temperature (23 ± 2 °C; 55% RH) for seven weeks for non-destructive VOCs monitoring with PTR-ToF-MS and indirect ripening evolution assessment with a LiCOR infrared CO2 analyzer (model LI-850; LI-COR, Inc, Lincoln, NE, USA) and DA-Meter (Model FRM01, Sinteleia, Bologna, Italy). Furthermore, at each ripening stage (harvest), 10 different fruits were destructively analyzed in triplicate (10 × 3) with the semiautomatic Pimprenelle instrument (Setop Giraud Technology, France). This instrument measured the single fruit weight, soluble solid content (°Brix), flesh firmness (N), and titratable acidity (expressed as malic acid eq. g/L) for each of the 10 fruits [46]. Additionally, the starch pattern index (SPI), a key ripening indicator, was visually assessed on a scale from 1 to 5 [47] using a sample of 10 equatorially cut fruits × 3 repetitions. The index of absorbance difference (I_{AD}) between the chlorophyll peaks at 670 and 720 nm [48] was also non-destructively measured using a portable DA-meter (Model FRM01, Sinteleia, Bologna, Italy) at four different equatorial points (Table 1). The internal ethylene concentration (IEC) was determined according to [49] on 10 single fruit replicates. The internal fruit gas was withdrawn, and a 1 mL sample was injected into a gas chromatograph (Agilent GC 7820, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a Poraplot Q (25 m-530 µm-20 µm) capillary column. The temperatures of the column, the injector, and the detector were 50, 120, and 280 °C, respectively. Quantification was performed by recalculating the values based on a linear standard calibration curve (0.1, 1, and 10 ppm) and was expressed in ppm.

4.2. Non-Destructive VOCs Analysis by PTR-ToF-MS

During a total shelf-life period of 7 weeks at room temperature (20 °C), the VOCs of a single fruit were analyzed non-destructively on a weekly basis by a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria). Each apple was placed in a 1.3 L glass vessel and closed tightly for 30 min of incubation at the laboratory temperature (23 ± 2 °C; 55% RH) [50]. The headspace of a sample was directly connected to the instrument via a heated PEEK tube (110 °C, 0.055″ diameter) and sampled at a flow rate of approximately 40 sccm. The drift tube conditions were as follows: 110 °C drift tube temperature, 2.8 mbar drift pressure, and 628 V drift voltage in active RF mode, corresponding to an E/N value of 140 Td (10^{-17} cm^2 V^{-1} s^{-1}). The sampling time per channel in the ToF analyzer was 0.1 ns, amounting to 350,000 channels for a mass spectrum ranging from *m/z* 15 to 400. Sampling measurements were performed in 60 cycles, resulting in an analysis time of 60 s/sample with a 5 min interval between samples in order to prevent the memory effect. The order of the sample measurements and replicates was randomized to avoid systematic memory effects.

4.3. CO_2 Analysis with Li-COR (LI-850)

The respiration rate, expressed by the CO_2 (µmol mol^{-1}) production, was measured for 30 s using a Li-COR Li-850 CO_2/H_2O gas analyzer (Lincoln, NE, USA). The measurement was conducted with the pump turned on immediately after the PTR-ToF-MS analysis, using the same jar without opening it to maintain consistency in the sampling conditions.

4.4. Statistical Analysis

The processing of the PTR-ToF-MS raw data involved external calibration and peak extraction, as detailed in previous studies [33]. The initial dataset of 332 extracted mass peaks was reduced to 127 by removing the mass peaks corresponding to ^{13}C isotopologues, water clusters, and those with concentrations that did not show significant differences across all time points [51]. Univariate and multivariate statistical analyses were performed in R (version 3.2.2) using internal statistical functions and external packages, including "mixOmics" and "ggplot2". The quality parameter analyses conducted on parallel fruit samples, along with the internal ethylene concentration (IEC) and index of absorbance difference (I_{AD}), are presented in tabular form with the mean values and standard error.

5. Conclusions

This study investigated the potential of direct injection mass spectrometry, specifically proton transfer reaction mass spectrometry (PTR-ToF-MS), for the rapid monitoring of 'Red Delicious' and 'Granny Smith' whole apple VOCs profiling during a ripening period following different harvest stages. More than 300 mass peaks were detected in the PTR-ToF-MS spectra of the sample headspace, with 127 selected for further analysis. The VOCs profiles of 'Red Delicious' and 'Granny Smith' differed significantly in both quality and quantity throughout the study period, mainly due to genetic factors, as confirmed by previous research [32,51,52]. The VOCs evolution pattern of both cultivars strongly correlated with CO_2 production, with 'Granny Smith' displaying a lower respiration rate and consequently lower VOCs emissions throughout the ripening period. The I_{AD} values also followed a trend consistent with these findings. This research provided valuable insights into the VOCs development of 'Red Delicious' and 'Granny Smith' harvested at different ripening stages and monitored through the entire ripening period. Currently, our focus is on advanced storage techniques to achieve VOCs profiles similar to those observed during natural ripening. Additionally, the fast, direct, and non-invasive nature of VOCs analysis in whole apple fruits could potentially support the fruit industry in enhancing the quality of both established and new apple cultivars.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture15060638/s1, Figure S1: PCA loading plot of apple VOCs measured by PTR-ToF-MS of the cultivars 'Granny Smith' and 'Red Delicious'. Figure S2: Curves of concentration in ppbV of 127 mass peaks of 'Granny Smith' and 'Red Delicious' apple cultivars measured by PTR-ToF-MS during the ripening period stored at 20 °C. Figure S3: Recommended ripening parameters for long-term storage of 'Granny Smith' and 'Red Delicious' apple cultivars, as outlined by the Research Centre Laimburg.

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