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Trace gas detection from fermentation processes in apples; an intercomparison study between proton-transfer-reaction mass spectrometry and laser photoacoustics

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Abstract

A custom-built proton-transfer-reaction mass spectrometry (PTR-MS) instrument was used to monitor the emission of various compounds (aldehydes, alcohols, acids, acetates and C-6 compounds) related to fermentation, aroma and flavour, released by four apple cultivars (Elstar, Jonaglod, Granny Smith and Pink Lady) under short anaerobic (24 h) and post-anaerobic conditions. The novel feature of our instrument is the new design of the collisional dissociation chamber, which separates the high pressure in the drift tube (2 mbar) from the high vacuum pressure in the detection region (10^{-6} mbar). The geometry of this chamber was changed and a second turbo pump was added to reduce the influence of collisional loss of ions, background signals and cluster ions, which facilitates the interpretation of the mass spectra and increases the signal intensity at the mass of the original protonated compound. With this system, detection limits of similar magnitude to the ones reported in literature are reached.

An intercomparison study between PTR-MS and a CO laser-based photoacoustic trace gas detector is presented. The alcoholic fermentation products (acetaldehyde and ethanol) from young rice plants were simultaneously monitored by both methods. A very good agreement was observed for acetaldehyde production. The photoacoustic detector showed about two times lower ethanol concentration as compared to PTR-MS, caused by memory effects due to sticking of compounds to the walls of the nylon tube used to transport the trace gases to the detector.
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Keywords: Proton-transfer-reaction mass spectrometer; Photoacoustic trace gas detector; Anaerobiosis; Post-anaerobiosis; Apples

1. Introduction

Over the last years, proton-transfer-reaction mass spectrometry (PTR-MS) has proven to be a very versatile tool for online monitoring of volatile organic compounds, emitted by a large variety of medical, biological and food samples [1,2]. Additionally, PTR-MS was applied to determine concentrations of oxygenated compounds in the atmosphere and used to find possible sources of carbon-containing molecules in the atmosphere [3–5].

Several groups have shown the validity of the concentration determination of volatile organic compounds in air by PTR-MS, by comparison with well-established techniques like GC-FID or GC-MS [3,6]. Here we present a custom-built PTR-MS instrument, analogue to the commercially available one described in literature [7] and an intercomparison study between PTR-MS and a CO laser-based photoacoustic trace gas detector, a well-established technique for online trace gas detection [8,9]. The photoacoustic effect is based on the

Abbreviations: PTR-MS, proton-transfer-reactions mass spectrometer; GC, gas chromatography; FID, flame ionisation detection; PA, proton affinity; CDC, collisional dissociation chamber; CA, controlled atmosphere; PT, proton transfer; FW, fresh weight

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generation of acoustic waves as a consequence of light absorption. The intensity of the sound is proportional to the number of molecules, present in the gas sample, absorbing at a specific light frequency. A line tuneable (5–8 μm) CO laser-based photoacoustic detector is used for monitoring acetaldehyde and ethanol (detection limits 0.1 and 3 ppbv, part per billion by volume, respectively) [10]. Both methods, PTR-MS and photoacoustics, are non-invasive and allow online measurements of trace gases at low detection limits. To achieve high sensitivity with a photoacoustic detector, the wavelength of the laser has to coincide with a strong excitation band of the gas. The disadvantages of this method are that only few trace gases can be detected and that by increasing the number of simultaneously detected trace gases, the time resolution become low (1 min/trace gas). PTR-MS has the advantage that it allows simultaneous measurements of a larger numbers of VOCs at a higher time resolution (<1 s/mass).

Our custom-built PTR-MS instrument and a laser-based photoacoustic trace gas detector were used to simultaneously monitor the emission of acetaldehyde and ethanol under different experimental conditions.

PTR-MS has also shown to be a promising instrument in discriminating between samples, like differently treated orange juices [11] or different types of cheese [12] and an adequate tool for monitoring the kinetics of volatile compounds emitted by biological samples. In the present work we have also explored this capability of the PTR-MS instrument on four different apple cultivars.

The apple is classified as climacteric fruit, exhibiting increased respiration rates during maturation and ripening. This rise is associated with increases in internal concentrations of carbon dioxide and ethylene. Controlled atmosphere (CA) conditions are a prerequisite for slowing down ripening and senescence of fruits during storage. For this purpose, levels of 10% CO_2 and 1–10% O_2, at 0 °C are frequently applied during storage period [13]. Ethylene is a gaseous hormone, which can promote senescence and reduce shelf life of crops even when present in minute concentrations [14]. Its endogenous production can vary greatly among apple cultivars [15]. In CA conditions ethylene production is inhibited and respiration is reduced. When the oxygen concentration inside the tissue becomes too low (<0.5%), the CO_2 production rises again, because when insufficient energy is available aerobic respiration is gradually replaced by alcoholic fermentation [16]. In this process carbon dioxide is a by-product, next to acetaldehyde and ethanol. These by-products can quickly accumulate to toxic levels, resulting in tissue browning or death. Although the effects of controlled atmosphere are primarily assigned to their effects in reducing respiration and inhibiting the production of ethylene, they provide some other important benefits, including reduction of grey mould, preventing chilling injury and killing the quarantine insects [17].

The functions of the anaerobic fermentation are not completely understood. It is also unknown if the negative side effects, damages leading to death, are caused during anaerobiosis or after, when the tissues are re-exposed to air (post-anaerobiosis). The study of physiological processes in fruit requires fast analysis of the concentration of the gases emitted. In addition, fruit releases numerous gases other than acetaldehyde and ethanol, contributing to their flavour and aroma [18]. Knowledge on emission kinetics of these gases during rapidly changing environmental conditions is scarce, mainly because in the storage room the concentration of the trace gases is normally below the detection limit of conventional gas analysers and therefore long accumulation periods are needed.

We report non-invasive experiments performed with our custom-built PTR-MS on monitoring the release of a number of aroma, flavour and fermentation related trace gases by four apples cultivars (Elstar, Granny Smith, Jonagold and Pink Lady) under anaerobic and post-anaerobic conditions.

2. Experimental description

2.1. Proton-transfer-reaction mass spectrometer

A proton-transfer-reaction mass spectrometer similar to the one described in Hansel et al. [7] was constructed in our laboratory (Fig. 1). This PTR-MS consists of: (1) an ion source in which H_3O^+ ions are produced by a discharge in a H_2O–He mixture, (2) a drift tube, where the trace gases from the gas sample are ionised by proton-transfer reactions with H_3O^+ ions, (3) a collisional dissociation chamber (CDC), where cluster molecules are dissociated and lead in to a detection region where ions are mass filtered with a (4) quadruple mass filter and counted with a (5) secondary electron multiplier.

2.1.1. Ion source

The primary ions (H_3O^+) are produced in a custom-built ion source by a discharge in a mixture of water and helium. Helium is used as a carrier gas and it is found to increase the amount of H_3O^+ ions formed. The pressure in the ion source is about 3.5 mbar. The cathode, a tungsten electrode of 5 mm diameter is placed in the middle of a stainless steel plate. Next to the cathode, a tungsten pin of 0.5 mm diameter (anode) is placed with its end point at 1 mm from the end point of the cathode (Fig. 1). Between the two electrodes a voltage difference of 900 V is applied, leading to a discharge current of 6–10 mA. In this case, the kinetic energy of the electrons that ionise a molecule is a few hundred eV. The ion source chamber is elongated to 4.5 cm, to lower the background signals and lead to best conversion to H_3O^+, analogous to what has been described in Hansel et al. [7].

2.1.2. Drift tube

The H_3O^+ ions formed in the ion source region reach the drift tube after passing through a 0.88 mm skimmer. The drift tube consists of 10 electrically isolated stainless steel rings of 1 cm length and 5 cm inner diameter, spaced by viton coated O-rings, and connected with 120 kΩ resistors. To obtain a

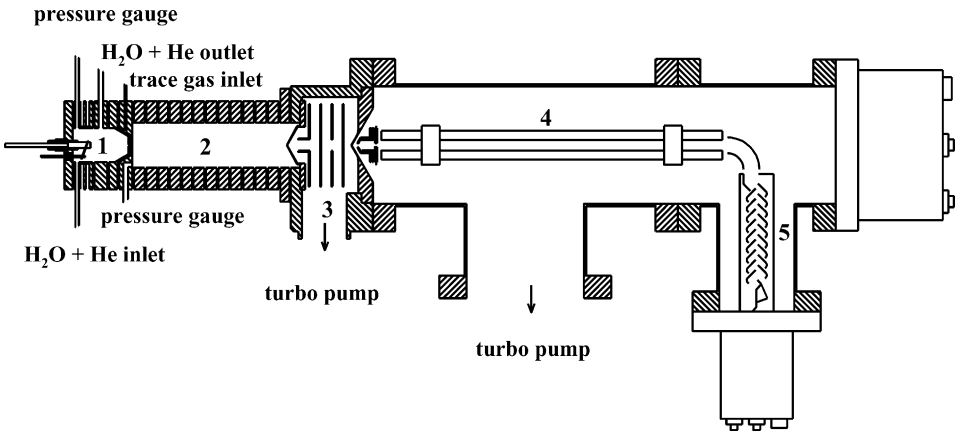

Fig. 1. Schematic view of the PTR-MS: (1) ion source, (2) drift tube, (3) collisional dissociation chamber, (4) quadrupole mass filter and (5) secondary electron multiplier.

homogeneous electric field, a voltage difference up to 750 V is applied between the beginning and the end of the drift tube. The PTR-MS is placed in a room were the temperature is regulated, consequently the inlet and drift tube temperature is 20 °C.

In the drift tube the trace gases from the sample gas are ionised by proton-transfer reactions with H_3O^+ ions [1]:

$$H_3O^+ + R \xrightarrow{k} RH^+ + H_2O \quad (1)$$

where k is the reaction rate constant. These reactions only take place when the proton affinity of the trace molecule R is higher than the proton affinity of water (165.2 kcal/mol = 7.16 eV). The big advantage of using H_3O^+ as the reagent ion is that the proton affinity of water is higher than the PA of normal constituents of air like NO, O_2, Co_x and N_2, and that most of the typical organic compounds are ionised by the PT reaction [1], because they have a proton affinity in the range of 7–9 eV. Almost no fragmentation occurs, because of the low exothermicity of the PT reactions. The reaction rate constant can be measured or calculated [19,20] and is known for many of the PT reactions of interest [1]. For reactions where the rate constant is not known, the mean value of 2.0×10^9 cm^3 s^{-1} is used in calculating concentrations, where calibration is not used. Besides the reaction rate constant k, also the degree of fragmentation and transmission efficiency for respective masses need to be taken into account in calculating trace gas concentrations. For most species, fragmentation is a minor effect. On the other hand, for most alcohols, fragmentation is very important. Warneke et al. [6] have shown that the fragmentation for ethanol is very difficult to quantify, due to unknown fragmentation on mass 19 amu.

Additional reactions can occur [4], but at the proper operating conditions they can be suppressed. In the measurements on apples, we used an E/N value of 101 Td.

2.1.3. Collisional dissociation chamber (CDC)

Between the drift tube and the detection system, a transition chamber is located. The CDC separates the high pressure in the drift tube (2 mbar) from the high vacuum pressure (10^{-6} mbar) in the detection region. Our CDC was designed differently from reported in literature [7]. It has a length of 8 cm, and with the use of a water-cooled turbo molecular dry-pump (capacity 210 l/s, TMP 261, Pfeiffer Vacuum, Asslar Germany) a pressure of 10^{-4} mbar is obtained (mean free path of ions about 10^1 cm at 10^{-4} mbar, decreasing with increasing mass). Hansel et al. [7] and Warneke et al. [4] have shown that in the drift tube clusters of trace gas molecules are formed with H_3O^+ and/or H_2O, but that caution should be taken when increasing the electric field over the drift tube, since it can increase fragmentation of parent ions (RH^+). This intermediate chamber first of all provides the possibility of performing collision-induced dissociation (CID) on the ions leaving the drift tube, by enhancing their kinetic energy [4]. The cluster ions that leave the drift tube dissociate into a neutral part and the original protonated trace gas molecule (RH^+), but not in smaller fragments if the field is well controlled. The remaining ions are lead through a 1 mm skimmer towards the quadrupole region, where selection and detection of the ions take place. The reduction of cluster ions facilitates the interpretation of the mass spectra and increases the signal intensity at the mass of the original protonated compound. Although the mass spectra do not give an accurate representation of the density of the ions present in the drift tube due to the unknown degree of collision-induced dissociation in the CDC [4], calculation and calibration do agree reasonably well. Nevertheless, it is preferable to perform calibration measurements.

The reason to redesign the CDC was to add a second turbo pump, to reduce the pressure and the influence of collisional loss of ions in the transition area and to decrease background signals. Some mass discrimination can still be observed, caused by ion-neutral molecule collisions. Under current circumstances, the mean free path of larger ions is of the same order of magnitude as the CDC length. This can be avoided by decreasing the pressure and the length of the CDC.

The advantage of having a low pressure in the quadrupole chamber is to decrease the number of collisions of ions with the residual particles and the collisions of residual particles with the detector, in this way decreasing the background signals. Additionally, a slight misalignment of skimmers before and after the CDC makes sure no photons from the ion source enter the detection region. To compensate for this misalignment, two pairs of deflection plates are installed to deflect the ion beam on the exit skimmer. Because the ion beam diverges when it passes from the drift tube to the collisional dissociation chamber (due to the pressure drop from 2 mbar to 10^{-4} mbar), an electrostatic lens, followed by the deflection plates and a second focusing lens, is used to focus the ion beam on the entrance of the quadrupole mass spectrometer. With this system, detection limits of similar magnitude than the ones reported in literature are reached [6]. As discussed above, especially for higher masses, these achievements can further be improved.

2.2. Set-up for headspace sampling

To test the capabilities of the PTR-MS, acetaldehyde and ethanol emitted by young rice plants exposed to anaerobic and post-anaerobic conditions were simultaneously monitored, using our PTR-MS and a CO laser-based photoacoustic detector. Photoacoustics is a well-established technique for online monitoring of trace gases down to very low concentrations [10]. This is the first time an intercomparison study between PTR-MS and photoacoustics is performed. Until now PTR-MS was compared only with GC-based systems [3,6,21].

Three young rice seedlings (14 days old) were placed in a glass cuvette (300 ml), with the roots in 15 ml nutrient solution. The rice plants were grown in similar conditions as explained in Boamfa et al. [9]. The trace gases emitted by the rice seedlings in the sampling cuvette were transported to the two detectors by a gas flow (4 l h^{-1}), regulated by mass flow controllers (MFCs). The gas flow was spit into two equal flows, 2 l h^{-1} for the CO laser-based detector and 2 l h^{-1} towards the PTR-MS (Fig. 2). A pressure controller (P-702C 2–100 mbar, Bronkhorst Hi-Tec B.V., Ruurlo, The Netherlands) backed by a membrane vacuum pump (400160, Ilmvac GmbH, Ilmenau, Germany) regulates the pressure in the drift tube inlet of the PTR-MS, in such a way that the pressure in the drift tube is maintained constant. Most of the 2 l h^{-1} flow is split off here and pumped away to outside by the backing membrane pump and only a small, variable flow is going into the drift tube. The inlet system of the drift tube is made out of Teflon tubes and connectors, to minimize memory effects in the inlet system, since nylon and stainless steel suffer from sticking of compounds to their surfaces [21]. A nylon tube of about 5 m length was used to transport the sample air from the split-off point to the photoacoustics-based detector.

The rice plants were placed in the dark and after a short acclimatisation period in air (15 min), they were exposed for 2 h to anaerobic conditions (nitrogen flow). Subsequently, air was re-introduced (post-anaerobic conditions) and the evolution of the fermentation products, acetaldehyde and ethanol (mass 45 amu and mass 47 amu respectively), was followed during the first 2 h after transition to the aerobic environment.

A similar set-up was used to measure the emission patterns of a number of trace gases from four apple fruit cultivars (Malus domestica Borkh., cv. Elstar, Granny Smith, Jonagold and Pink Lady) under different atmospheric conditions by only using the PTR-MS instrument. The apples were obtained from a local retailer. A single fruit was placed in a glass cuvette (2 l) and the trace gases emitted during the different gas treatments were transported by the gas flow (4 l h^{-1}) towards the drift tube inlet. The apples were kept in normal aerobic conditions for about 3 h and thereafter exposed to 24 h anaerobic conditions (nitrogen flow). Subsequently, oxygen was provided again (post-anaerobiosis) and measurements were prolonged for another 15–20 h. The trace gas emission patterns were monitored in the mass range from 21 amu to 117 amu. The fresh weight of the apples was Elstar—180 g, Granny Smith—160 g, Jonagold—195 g and Pink Lady—162 g.

3. Results and discussions

3.1. Parallel measurements PTR-MS–laser photoacoustics

Fig. 3 shows the response of the rice plants to the anaerobic and post-anaerobic treatments as an example of intercomparison between PTR-MS and laser-based photoacoustic detector. Half an hour after imposing oxygen-free conditions, an increase in acetaldehyde and ethanol emission is observed, showing that plant tissue switches from aerobic respiration to alcoholic fermentation. Acetaldehyde reaches a maximum of 625 ppbv after about 1 h, followed by a slow decrease, while ethanol increases steadily and reaches 13 ppmv (part per million by volume) after 2 h. Re-exposure to air resulted in a fast (10 min) outburst of acetaldehyde of about 1500 ppbv. This was followed by a decrease in the acetaldehyde emission rate, which reaches the initial aerobic rate after 2 h of re-introduction of air. When the rice seedlings are re-exposed to air, ethanol emission rates start to decrease gradually to the initial pre-anaerobic emission rate.

Very good agreement was observed between the PTR-MS and the photoacoustic acetaldehyde measurements, while monitoring alcoholic fermentation products from rice plants (Fig. 3). The photoacoustic detector showed a slower decrease of ethanol during post-anaerobic emission and about two times lower concentrations as compared to PTR-MS. This is caused by memory effects due to sticking of compounds to the long nylon tube walls (about 5 m) used to transport the trace gases from the sample cuvette to the detector. Because ethanol fragments easily on mass 29, ideal conditions for measuring ethanol are at much lower E/N than for other compounds. Since we were only monitoring two compounds we used an E/N value of 78 Td for these mea-

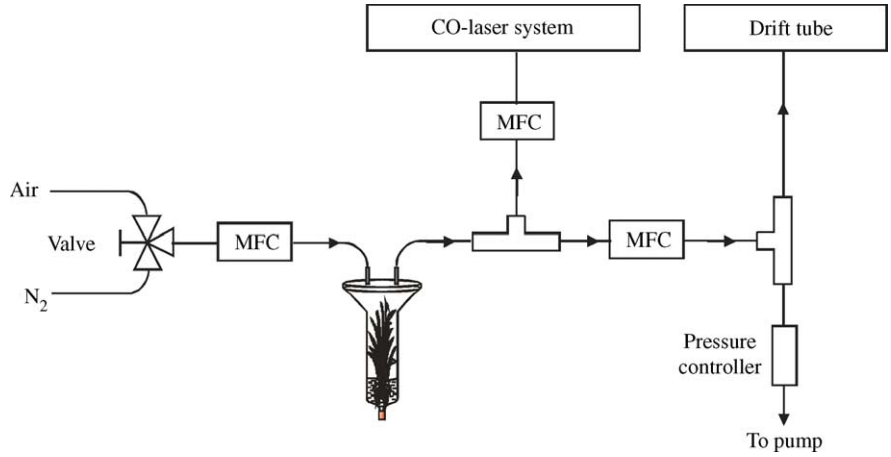

Fig. 2. Schematic diagram of the set-up used for the intercomparison study between PTR-MS and the CO laser-based photoacoustic trace gas detector. The trace gases emitted by the rice seedlings in the sampling cuvette were transported with the gas flow ($4\,l\,h^{-1}$), regulated by mass flow controllers (MFC) to the detectors. The cuvette outflow was split into two equal flows, $2\,l\,h^{-1}$ for the CO-laser based photoacoustics detector and $2\,l\,h^{-1}$ towards the drift tube of the PTR-MS. Anaerobic conditions were imposed on the plants by replacing the inflowing air with nitrogen gas.

surements. For these experimental conditions the PTR-MS was calibrated for both acetaldehyde and ethanol. As can be seen from Fig. 3, the time resolution of the PTR-MS system is much higher than the time resolution of the photoacoustic detector. This superior time resolution (about one order of magnitude) enables us to monitor even faster processes and to monitor an increased number of compounds simultaneously at a higher rate.

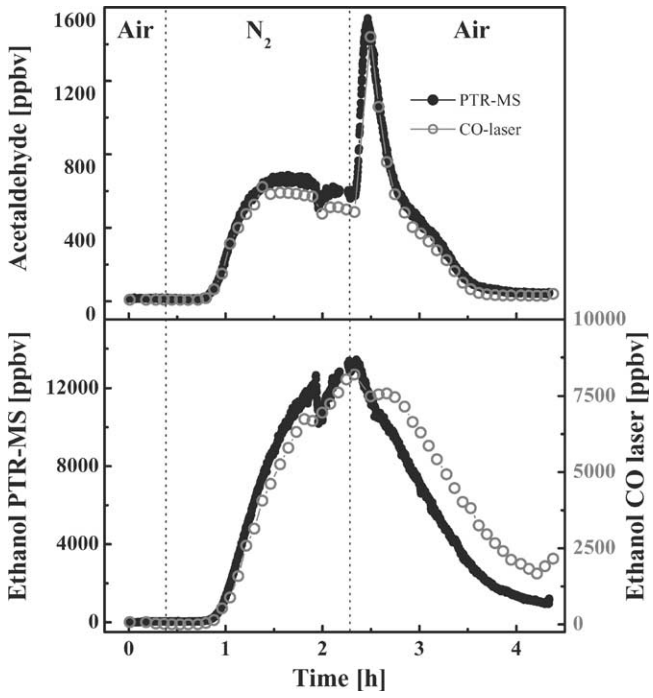

Fig. 3. Effect of 2-h anaerobic treatment (nitrogen flow) on the pattern of acetaldehyde and ethanol emissions from rice seedlings (14-day-old) measured simultaneously by PTR-MS (●) and CO-laser based photoacoustics detector (○). The plants were placed under 0% O_2 conditions ($4\,l\,h^{-1}$) at time $t = 0.25$ h. At $t = 2.35$ h, the rice plants were returned to a flow of air ($4\,l\,h^{-1}$).

3.2. Apple measurements

The O_2 concentration at which a shift from predominately aerobic to predominately anaerobic respiration occurs varies among fruit tissues and cultivars. Since the O_2 concentration at different points in a fruit varies, due to rates of gas diffusion and respiration, some parts of the commodity may become anaerobic.

In the present work we have used a PTR-MS to monitor, in real time, the emissions of various fermentation, aroma and flavour related compounds like aldehydes, alcohols, acids, esters and C-6 compounds released by four apple cultivars under anaerobic (nitrogen flow) and post-anaerobic (air flow) conditions. Natural isotope ratios were used to identify the trace compounds. Methanol was detected at mass 33 amu, acetaldehyde at mass 45 amu, ethanol at mass 47 amu, acetone at mass 59 amu. Due to their fragmentation in the drift tube, the esters and C-6 compounds could not be properly identified. According to Buhr et al. [22] most esters have fragments at mass 43 and at mass 61 amu. Beside the esters fragments, at mass 61 amu also acetic acid is found. De Gouw et al. [21] have shown that C-6 compounds can have important fragments at masses 43, 55, 57, 81, 83, 85, 99 and 101 amu. We did not monitor mass 99, but this does not influence the results of the experiments, since the fragmentation ratios of the given compounds are known [21]. In Table 1, mass 43 amu is not listed as a fragment of C-6 compounds. This is because it can only be a fragment of *n*-hexanol [5], which is found also at masses 85 and 103. Since the production rate on mass 85 amu is about three orders of magnitude lower than mass 43 amu, we assume the contribution of *n*-hexanol on this mass to be negligible.

Table 1
Effect of 24-h anaerobic treatment on production rates of different fermentation, aroma and flavour related VOCs on four apples cultivars (Elstar, Granny Smith, Jonagold and Pink Lady), measured during normal aerobic conditions, at the end of anaerobic treatment and again during recovery in air (post-anaerobic conditions)

	Mass (amu)	Aerobic	End anaerobic (nl h^{-1} g^{-1} FW)	Post-anaerobic	Aerobic	End anaerobic (nl h^{-1} g^{-1} FW)	Post-anaerobic
		Elstar			Granny Smith		
Methanol	33	1.12	1.96	3.5	1.74	2.15	4.7
Acetaldehyde	45	2.77	23	69.8	6.16	26.24	82.2
Ethanol	47	0.28	8.4	2.3	2.3	8.74	3.8
Acetone	59	1.65	1.9	1.84	3.54	4.7	5.8
Esters							
Fragments	43	5.2	3.84	38.4	10.75	9	47.5
Fragments	61	0.65	15.9	20.75	0.63	12.8	18.3
Methyl acetate	75	0.04	0.14	0.13	0.05	3.92	5
Ethyl acetate	89	0.005	1.73	1.41	0.03	2.63	1.72
C-5 esters	103	0.003	0.016	0.02	0.02	1.95	0.86
C-6 esters	117	6E-04	0.008	0.003	0.003	0.46	0.24
C-6 compounds fragments	55	0.07	0.19	0.65	0.2	0.43	0.86
	57	0.06	0.09	0.09	0.2	0.7	0.83
	71	0.05	0.07	0.06	0.12	0.56	0.4
	81	0.005	0.02	0.006	0.008	0.06	0.02
	83	0.006	0.04	0.008	0.016	0.12	0.04
	85	0.02	0.03	0.02	0.04	0.15	0.12
	101	0.008	0.04	0.01	0.01	0.17	0.08
		Jonagold			Pink Lady		
Methanol	33	2.5	1.5	4.84	1.53	1.03	1.15
Acetaldehyde	45	13.45	24.5	80	6.78	16.23	4.9
Ethanol	47	1.15	11.32	4.24	0.45	2.24	0.18
Acetone	59	3.3	4.2	4.4	1.65	2.16	1.56
Esters							
Fragments	43	27.9	15.1	82	13.26	6.16	5.45
Fragments	61	7.1	43.4	76	2.16	12.46	0.8
Methyl acetate	75	0.16	1	1.53	0.12	0.8	0.03
Ethyl acetate	89	0.08	4.6	4.8	0.04	1.7	0.04
C-5 esters	103	0.06	0.47	0.35	0.02	0.62	0.01
C-6 esters	117	0.04	0.27	0.2	0.004	0.3	0.003
C-6 compounds fragments	55	0.42	0.71	2.17	0.2	0.38	0.08
	57	0.35	1.0	0.57	0.2	0.4	0.06
	71	0.25	0.8	0.42	0.09	0.28	0.04
	81	0.03	0.07	0.02	0.007	0.028	0.003
	83	0.03	0.22	0.04	0.01	0.1	0.006
	85	0.06	0.27	0.12	0.023	0.09	0.01
	101	0.05	0.4	0.1	0.016	0.11	0.006

The post-anaerobic production rates represent the total production rates released during the treatment divided by the treatment duration (15 h).

All four apples cultivars used in the present study have a sweet-tart flavour, but they have a different sugar to acid ratio (S/A), e.g., Granny Smith is classified as a relatively acid or tart apple, S/A < 20 and Jonagold as a very aromatic apple [23].

After the apples were placed in the glass cuvette, they were first exposed for a few hours to aerobic conditions, for acclimatisation. The aerobic emission rates of different VOCs from the four cultivars are given in Table 1. The concentrations were obtained using calculated calibration factors. The data are given with a 20% uncertainty, caused by the 20% uncertainty in the calculation of the reaction rate constant [1].

Elstar has the lowest production of acetaldehyde (2.77 nl h^{-1} g^{-1} FW), esters (5.9 nl h^{-1} g^{-1} FW) and C-6 compounds (0.17 nl h^{-1} g^{-1} FW). On the other hand, Jonagold produces higher amounts of methanol (2.5 nl h^{-1} g^{-1} FW), acetaldehyde (13.45 nl h^{-1} g^{-1} FW), esters (35.3 nl h^{-1} g^{-1} FW) and C-6 compounds (0.94 nl h^{-1} g^{-1} FW) than the other cultivars (Table 1 and Fig. 4). This demonstrates than Jonagold is a very aromatic apple. Van der Sluis et al. [24] have found that Jonagold possessed the highest flavonoid concentration and the highest antioxidant activity when four apple cultivars (Jonagold, Elstar, Golden Delicious and Cox's Orange) were compared with regard to flavonol, catechin, phorodzin

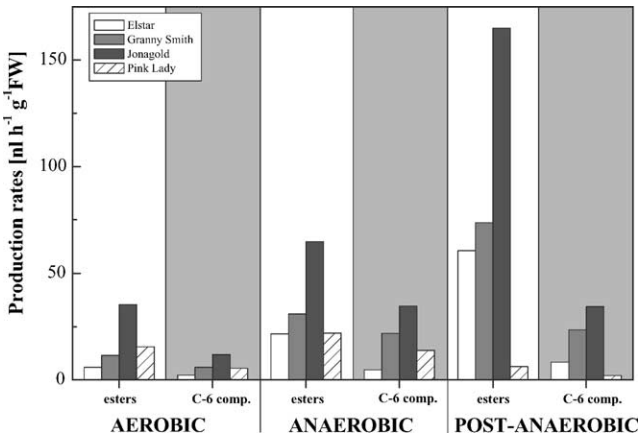

Fig. 4. Sum of production rates of esters (protonated masses 43, 61, 75, 89, 103, 117 amu) and C-6 compounds (protonated masses 55, 57, 81, 83, 85, 101 amu) released by four apples cultivars - Elstar (white bars), Granny Smith (gray bars), Jonagold (dark gray bars) and Pink Lady (pattern bars)—during normal aerobic conditions, at the end of anaerobic treatment and during recovery in air (post-anaerobic conditions). The post-anaerobic production rates represent the total production rates released during the treatment divided by the treatment duration (15 h). Please note that the sums of production rates of C-6 compounds are multiplied by a factor of 10.

and chlorogenic acid compositions and antioxidant activities.

When the apples were exposed to short anaerobic conditions (24 h), the production of acetaldehyde and ethanol starts to increase for all four cultivars, showing that the alcoholic fermentation process replaces the aerobic respiration. Fig. 5 shows an example of the real time measurements of acetaldehyde, ethanol, methanol and acetone emitted from Jonagold. All four apples present the same dynamic behaviour for these compounds. At the end of the anaerobic treatment, similar fermentation rates were observed for, Elstar

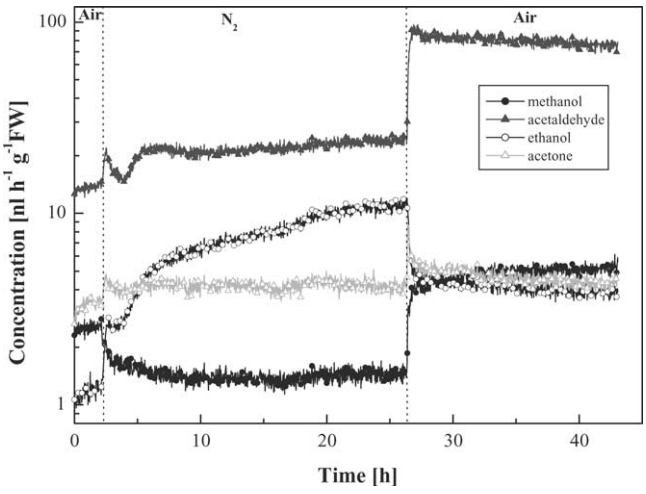

Fig. 5. Effect of 24-h anaerobic treatment (nitrogen flow) on the pattern of methanol, acetaldehyde, ethanol and acetone emissions from Jonagold apple. The apple was placed under 0% O_2 conditions (4 l h^{-1} of nitrogen gas) at time t = 2.1 h. At t = 26.2 h, the apple was re-exposed to aerobic conditions (4 l h^{-1} of air).

(8.4 nl h^{-1} g^{-1} FW), Granny Smith (8.74 nl h^{-1} g^{-1} FW) and Jonagold (11.32 nl h^{-1} g^{-1} FW), while Pink Lady produced the lowest amount of ethanol (2.24 nl h^{-1} g^{-1} FW) (Table 1). As already stated in the previous section, the concentration of ethanol is influenced by the degree of fragmentation to mass 19 amu. Since this is a quantity that cannot be determined experimentally, calibration measurements should be performed for this compound. Here we did not perform these calibrations. Therefore, concentrations of ethanol might be higher than calculated.

Acetaldehyde, the volatile that gives the green-apple-like aroma/flavour, is an intermediate product in the alcoholic fermentation and the yeast usually reabsorbs it. Sometimes its production is caused by bacterial infection [25]. In small amounts acetaldehyde can be useful, because it is thought that acetaldehyde delays fruits ripening by a biochemical alteration at the site of ethylene synthesis [26]. However acetaldehyde is not desirable, due to its toxic effect. We have found that Pink Lady had the lowest acetaldehyde production rate at the end of the anaerobic treatment (16.23 nl h^{-1} g^{-1} FW), while the other three cultivars have similar production rates (Table 1). Pink Lady has low fermentation rates probably because of its lower enzymatic activity.

The lack of oxygen affects the fruits in a number of ways. The first indication of injury is loss of flavour, followed by fermentation related odours. Injury symptoms vary from purpling or browning of the fruit skin and developmental of brown soft patches resembling soft scald, to abnormal softening and splitting of fruit. The cultivars can vary greatly in response to low oxygen, and susceptibility to injury is influenced also by the pre-harvest and post-harvest conditions [15]. When exposed to oxygen free conditions, an increase in the production of almost all esters and all the C-6 compounds has been observed for all four cultivars. An exception is at mass 43 amu, at which the concentration decreases at half from the aerobic value for all cultivars. An increase in certain volatiles during CA storage compared to air-stored fruits has been observed also in tomatoes [27].

During the anaerobic treatment, an almost linear increase in mass 47 amu (ethanol) and mass 89 amu (ethyl acetate) has been observed for all four cultivars. The ethanol to ethyl acetate production rate ratio varies from cultivar to cultivar (Elstar 4.8, Granny Smith 3.3, Jonagold 2.5 and Pink Lady 1.3). This linear increase is in correspondence with the observation of Bartley et al. [28], who showed that ethanol can be metabolised to ethyl acetate, the latter being the most prevalent acetate from the variety of esters produced by apple fruits. It was observed that the altered synthesis of fruit volatiles resulted in increased amounts of ethyl acetate and certain esters at the expense of others [25].

After 24 h of anaerobic treatment, the apples were re-exposed to air. When re-exposed to oxygen all cultivars except Pink Lady showed a clear post-anaerobic acetaldehyde peak, three times higher than the acetaldehyde production rate at the end of anaerobic treatment, and a fast decrease in ethanol emission. This fast increase in the acetaldehyde

production rate is called post-anoxic effect and is caused by the oxidation of the ethanol accumulated in the tissues during anaerobic conditions. This effect has been also observed in red pepper [8] and rice plants [9]. It is assumed that this post-anaerobic acetaldehyde peak can also be responsible for the fruit ripening delay [29]. From the four apples used in our study, only Pink Lady did not present this acetaldehyde peak.

For Elstar, Granny Smith and Jonagold cultivars, re-exposure to air, highly enhanced the production of methanol and some esters and acetic acid (mass 43, 61 and 75 amu). The C-6 compound production decreases for these three cultivars, but remains at values slightly higher than the normal aerobic ones.

For Pink Lady, re-exposure to aerobic conditions results in a fast decrease in all the measured compounds, including acetaldehyde (Table 1), at lower production rates than in normal aerobic conditions. In literature it is reported that controlled atmosphere storage alters the flavour of apples, and if prolonged, reduces volatile emission compared with air-stored fruits, especially lipid-derived esters [30]. Low O_2 storage decreases ester content and enzymatic activity responsible for ester biosynthesis in apples [31]. Our measurements showed that even when exposed to short anaerobic treatment (24 h), Pink Lady suffered low oxygen injuries, by loosing its aroma and flavour.

4. Conclusions

A custom-built PTR-MS has been developed in our laboratory. With this system, detection limits of similar magnitude than the ones reported in literature are reached. The collisional dissociation chamber (CDC), which separates the high pressure in the drift tube (2 mbar) from the high vacuum pressure (10^{-6} mbar) in the detection region, has a novel design. In this study, the CDC was 8 cm long and a pressure of 10^{-4} mbar is obtained. When the mean free path of ions at a pressure in the 10^{-4} mbar range is calculated, values of the same order of magnitude as the length of the CDC are found. Therefore, adaptations of the CDC to lower the collisional probability, by decreasing its length and optimising the use of the pump capacity, will provide a further improvement of the system.

While monitoring the alcoholic fermentation products from young rice plants, using the custom-built PTR-MS and the CO laser-based photoacoustic detector simultaneously, a very good agreement was observed for acetaldehyde measurements. This is the first time an intercomparison between PTR-MS and laser photoacoustics is reported. PTR-MS is able to follow the fast temporal changes in emission rates of volatile compounds with a higher temporal resolution (<1 s) than photoacoustics, which has a time resolution of about 1 min and is a well-established technique for online monitoring of trace gases. Additionally, PTR-MS monitors a larger number of VOC species at the same time, being a valuable instrument, next to the photoacoustic detectors. On the other hand, PTR-MS is a monitoring technique, which sometimes needs other tools, like GC, to identify the observed compounds.

The fermentative behaviour and its effects on the fruit quality were described for four apple cultivars (Elstar, Jonaglod, Granny Smith and Pink Lady). The preliminary results presented in this work show that application of PTR-MS detectors in fruit storage opens new possibilities to gain insight in the kinetics of the relevant metabolic processes.

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