Residues and effects on the aroma profile of apples as result of phosphine fumigation

Klementz, D.W.*#[1], Schubert, J.[2], Flingelli, G.[1], Jungnickel, H.[2], Ulrich, D.[3], Luch, A.[2]
[1]Julius Kühn-Institut (JKI), German Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Straße 19, 14195 Berlin, Germany
[2]German Federal Institute for Risk Assessment (BfR), Department of Chemicals and Product Safety, Max-Dohrn-Straße 8-10, 10589 Berlin, Germany
[3]Julius Kühn-Institut (JKI), German Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Erwin-Baur-Straße 27, 06484 Quedlinburg, Germany

*Corresponding author, Email: Dagmar.Klementz@jki.bund.de
#Presenting author, Email: Dagmar.Klementz@jki.bund.de

DOI: xx.xxxx/xxx.2014.xxx.xxx.xxx

Abstract

The fumigation of stored products is a common practice to control the infestation and prevent the spread of pests through international trade. The fumigants most frequently used for quarantine and pre-shipment (QPS) purpose are methyl bromide and phosphine. Based on the Montreal Protocol, the non-QPS use of methyl bromide was phased out by 2005 in developed countries and will be banned by 2015 in developing countries. Therefore, phosphine is increasingly being used as a replacement. Recently, apples are now being fumigated with phosphine prior to export to control eggs of the codling moth (*Cydia pomonella*). The present study addresses sorption and desorption behaviour of phosphine by apples under different conditions and the formation of residues. The impact of the initial fumigation concentration and of the storage temperature was analyzed. During the laboratory experiments phosphine concentration was monitored using GC-MS instrumentation. Furthermore, the possible impact of phosphine on the organoleptic quality of treated apples (*Malus domestica* Borkh., cv. 'Braeburn') was addressed. For this purpose a headspace solid-phase microextraction (HS-SPME) technique was used and coupled to subsequent gas chromatography-mass spectrometry (GC-MS). The apples were fumigated for 48 h with phosphine at 2000 ppm. Following the fumigation procedure apples were aired and stored under controlled conditions. Samples for the analysis of the aroma profile were taken at day 0, 1, 2, 7, 10, 14, and 21 after fumigation. The apples were processed and subsequently analyzed by HS-SPME-GC-MS. The results obtained for the fumigated samples were than compared to untreated reference samples. It could unambiguously be demonstrated that phosphine fumigation qualitatively and quantitatively alters the organoleptic quality of treated apples. However, further research on all aspects discussed in this study is required.

Keywords: fumigation, phosphine, apple, residues, aroma profiling, GC-MS

1. Introduction

Due to the growing globalization in the world economy and the fragmentation of global production international seaborne trade is rapidly increasing. According to United Nations (UN) statistics international seaborne trade has more than doubled since the 1990s and will further increase in the near future (UNCTAD, 2013). Unfortunately, the transport of pest-infested goods is one of the main pathways by which pests are distributed across countries (Westphal et al., 2008). Therefore, it is imperative to avoid the infestation of goods in order to prevent the spread of pests.

One of the common practices to avoid the infestation of stored products and the spread of pests through international trade is fumigation. The fumigants most frequently used are methyl bromide, phosphine (PH_3), 1,2-dichloroethane and sulfuryl fluoride (Baur et al., 2010). However, based on the Montreal Protocol, the non-quarantine and pre-shipment (non-QPS) use of methyl bromide was phased out by 2005 in developed countries and will be banned by 2015 in developing countries (UNEP, 2000). In the European Union (EU) the use of plant protection products containing methyl bromide was withdrawn by 2010 at the latest (EU, 2008). Therefore, PH_3 is now increasingly used (Fields and White, 2002).

PH_3 has been used world-wide for many decades for disinfestation of stored grains and other commodities as it has a number of advantages such as its efficacy against many pests, low costs, ease of application and low residues (Bond, 1984; Chaudhry, 1997). It is highly toxic to organisms that undergo oxidative respiration and can eliminate all stages of insect life (Bell, 1976; Sekhon et al., 2010). Lately, PH_3 is also used in apples to control *Carposina niponensis* (Bo et al., 2010) or *Cydia pomonella* (Brash et al., 2009; Rogers et al., 2013).

One of the key drivers of consumers' appreciation of apples is the aroma (Daillant-Spinnler et al., 1996; Harker et al., 2008; Aprea et al., 2011). However, little is known about the effects caused by PH_3 on the aroma of fumigated goods. In a recent publication Sekhon et al. (2010) evaluated whether PH_3 affects the quality and safety of dry cured ham. They found that PH_3 fumigation leads to a minimal change in the aroma profile of hams compared to unfumigated control samples (Sekhon et al., 2010). More recently, Both et al. (2014) demonstrated that storage of apples in a controlled atmosphere (CA) impacts the emission of volatiles and thus also the aroma profile. This raises the question of the extent to which the aroma profile of apples is altered following PH_3 fumigation.

For the analysis of aroma compounds and volatiles mainly gas chromatography coupled to a mass selective detector (GC–MS) is employed. However, in most cases, prior to analysis a pre-concentration step is necessary. According to the literature the headspace solid-phase microextraction (HS–SPME) technique is primarily used in the analysis of the aroma profile of apples (Aprea et al., 2011; Schmarr and Bernhardt, 2010; Reis et al., 2009; Dunemann et al., 2009).

In the present study three major issues were addressed. First, the sorption and desorption behaviour of PH_3 by and from apples under different conditions was studied. Therefore, the impact of the initial fumigation concentration and of the storage temperature was analyzed. PH_3 concentration was monitored using GC-MS instrumentation. Second, after fumigation and desorption apples were tested for their residual PH_3 content to verify if the maximum residual level (MRL) of 0.05 ppm for PH_3 in apples is not exceeded (EU, 2005). Third, headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) was used to assess whether PH_3 fumigation affects the aroma profile of apples (*Malus domestica* Borkh., cv. 'Braeburn').

2. Materials and Methods

2.1. Reagents and materials

All chemicals used in this study were of analytical grade unless otherwise stated. Sodium chloride was obtained from Merck (Darmstadt, Germany). PH_3 gas (960 ± 19 vpm; in Helium 5.0; internal standard: Argon, 1072 ± 21 vpm) was purchased from AirLiquide (Krefeld, Germany). Magnesium phosphide (95%) was obtained from Degesch de Chile LTDA. (Santiago, Chile). Apples (*Malus domestica* variety 'Royal Gala' and 'Braeburn') were purchased from a local market (Keuthmann, Berlin, Germany) and were from Italy (sorption and desorption behaviour and residue analysis; 'Royal Gala') and New Zealand (aroma profiling; 'Braeburn'). Until usage, apples were stored at 5°C in the dark.

2.2. Fumigation

Fumigations were performed in two differing set-ups. For sorption and desorption experiments apples were fumigated in desiccators (volume: 6-7 L). For this purpose 14-16 apples (approx. 1.6-2.1 kg) were loaded into a desiccator and subsequently locked. Loading factors varied between 30 and 38%. Each desiccator had two openings that were sealed gas-tight with a Silicon-PTFE septum. PH_3 was generated from pure magnesium phosphide and collected in a glass cylinder. For fumigation the appropriate amount of PH_3 was injected into the desiccators using a gas tight syringe. Apples were fumigated for 48 h at 5°C and at four different concentrations (500, 1000, 2000 and 3500 ppm). During fumigation, PH_3 concentrations were determined at three time points (after equilibration of phosphine concentration, after 24 and 48 h) using GC-MS instrumentation. Instrumental parameters were described by Heckemüller (2005). Each experiment was conducted in triplicate. After fumigation, desiccators were aired for 25 min using a diaphragm pump (N 811 KT.18, KNF Neuberger GmbH, Freiburg, Germany) with a delivery of 11.5 L/min and subsequently sealed. Until analysis, apples were stored in the dark. Desorption of PH_3 was measured 24, 120, 216, 336, 840 and 1344 h, respectively, after the end of the fumigation. Following each measurement, desiccators were aired as described above. For desorption two different temperatures were tested (5 and 15°C). Storage conditions of the apples were monitored using MINIDAN^{CLIMA} temperature data loggerS from ESYS GmbH (Berlin, Germany).

For aroma profiling, apples were fumigated in two identical temperature controlled 0.5 m^3 fumigation chambers (DEGESCH vacuum circulatory system, Frankfurt, Germany). The first chamber was used for fumigation and the second was used for the untreated reference. Sample size for fumigation comprised 196 apples (36.7 kg) whereas the reference comprised 104 apples (19.5 kg). PH_3 was generated from an appropriate amount of pure magnesium phosphide directly in the fumigation chamber. Fumigation concentration and temperature were set to 2000 ppm and 5°C, respectively. During fumigation, PH_3 concentration was monitored on-line using a Gasmet^{TM} CX1000 FT-IR-spectrometer (Ansyco, analytische Systeme und Componenten GmbH, Karlsruhe, Germany). After fumigation, apples were aired for 90 min using a diaphragm pump (N 840.3FT.18, KNF Neuberger GmbH, Freiburg, Germany) with a delivery of 34 L/min. For aroma profiling, pooled samples each consisting of five apples were taken 0, 24, 48, 168, 240, 336 and 504 h, respectively, after fumigation ended. Prior to sampling, fumigation chambers were aired for 30 min.

2.3. Analysis of phosphine residues

For residue analyses, 13 kg of uncut apples (cv `Royal Gala`) were fumigated with pure phosphine using magnesium phosphide (Mg_3P_2) in a sealed 0.5 m^3 fumigation chamber at 5°C for 48 h. The average concentration of phosphine in air was 1274 ppm.

A headspace technology was used to determine concentration of phosphine in apples. Phosphine concentrations were measured using GC-MS instrumentation. A GC-MS 2010Plus instrument was fitted with an HP-PLOT/Q capillary column (J&W, length: 30 m, i.d.: 0.32 mm). The chromatography conditions were as follows: oven temperature: isothermal 50°C, carrier gas: helium 5.0 at 6 bar, injector temperature: 150°C. The GC-MS detection limit was 0.005 mg PH_3/kg fruit, which is one tenth of the European maximum residue limit (MRL).

2.4. Aroma compound analysis

2.4.1. Sample preparation

Sample preparation was performed according to Dunemann et al. (2009) with slight modifications. In brief, for each sample five apples were sliced with a commercial apple cutter. The apple cores were removed and the slices were homogenized for 1 min with 300 mL of a 20% (w/v) sodium chloride solution using a commercial blender (model GK 900, rpm 1000 – 15000; Rotor Lips AG, Uetendorf, Switzerland). Then, the homogenate was filtered through a paper filter and subsequently centrifuged at 4°C and 4,000 rpm for 30 min. Thereafter headspace vials containing 3.0 g solid NaCl were filled with a 10 mL aliquot of the supernatant and sealed with a magnetic crimp cap including septum. Until analysis headspace vials were stored by -18°C. Each sample was conducted in triplicate.

2.4.2. Analytical conditions

HS-SPME-GC analysis was performed in accordance to Dunemann et al. (2009) using an Agilent 5973MSD in the electron impact ionisation mode (70 eV). For peak detection the software CHROMStat 2.6 was used to perform a non-targeted data analysis by pattern recognition. Altogether 110 conjoint peaks were detected for all samples of the control and fumigation series. Out of the 110 peaks 22 were fully identified by library search (NIST08, National Institute of Standards and Technology, version 8.0), retention indices and co-elution of authentic substances, 7 peaks were tentatively identified by library search only and the remaining 81 peaks are unknowns.

2.4.3. Statistical analysis

Principal component analysis (PCA) was performed using the software Statistica7.1 from StatSoft, Tulsa, USA.

3. Results and Discussion

3.1. Sorption and desorption of phosphine

Sorption and desorption of PH_3 was monitored using GC-MS instrumentation. Sorption was calculated as the difference between the PH_3 concentration at the beginning and the end of the fumigation, whereas desorption was calculated as the sum of the individual PH_3 concentrations measured at each time point. The results are depicted in Figures 1-4. For fumigation concentrations of 500 and 3500 ppm PH_3 sorption of 0.539 ± 0.04 µg/g apple and 2.339 ± 0.04 µg/g apple were determined (see Figure 1.A). The results demonstrate that there is a linear correlation (R^2 = 0.9877) between the fumigation concentration and the amount of PH_3 adsorbed by the apples (see Figure 1.B) when the fumigation time is kept constant. The desorption of PH_3 for fumigation concentrations of 500 and 3500 ppm was determined to 0.211 ± 0.01 µg/g apple and 0.711 ± 0.04 µg/g apple, respectively (see Figure 1.A). Thus, the amount of PH_3 desorbed from apples ranged between 30 and 44% (3500 and 1000 ppm, respectively) based on the amount adsorbed. Blank experiments using empty desiccators revealed that for a fumigation time of 48 h the applied fumigation concentrations were nearly constant and decreased only slightly. This raises the question of the whereabouts of the remaining amount of PH_3. To clarify this issue, apples were further analysed for PH_3 residues (see 3.2.). In the literature it has been described that phosphine can be converted to several phosphorus compounds (Robinson, 1970; Tkachuk, 1972; Underwood, 1972). Radiochemical analysis of wheat, flax, rapeseed and tobacco fumigated with $^{32}PH_3$ revealed that up to 54% of the used $^{32}PH_3$ was converted to non-volatile phosphate species (e.g. hypophosphite and pyrophosphate) that cannot be removed by aeration (Tkachuk, 1972; Underwood, 1972). This might also be conceivable here particularly as apples are a water-rich matrix. In this context

Berck (1968) has already demonstrated that the amount of phosphine that is non-recoverable from fumigated cereal products by aeration is increased with an elevated moisture content of the fumigated products. Between the amount of PH_3 desorbed from apples and the fumigation concentration a logarithmic correlation was found (R^2 = 0.9758; see Figure 1.B).

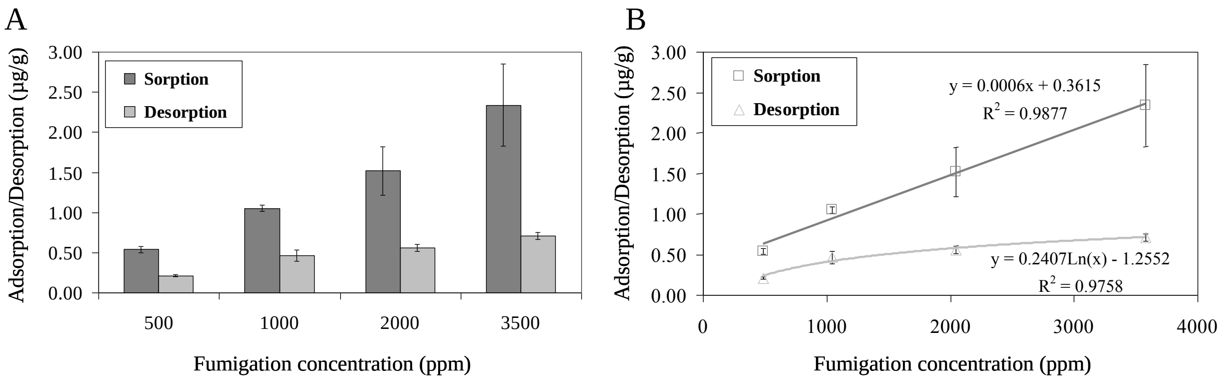

Figure 1 (A and B) Comparison of the total amount of PH_3 adsorbed and desorbed from apples (expressed as µg/g) as a function of the fumigation concentration. Sorption and desorption were performed at 5°C (n = 3; except for 2000 ppm, sorption: n = 5, desorption: n = 2; error bars represent standard deviation).

A closer look at results revealed that more than 80% of the total amount of the PH_3 was already desorbed from the apples after the first day regardless of the fumigation concentration used (see Figure 2.A). Desorption ranged between 84 and 91% for 1000 and 2000 ppm, respectively. The remaining amounts of PH_3 were mainly desorbed up to the third ventilation on day 5. However, residual amounts of PH_3 were detected up to day 35. These findings are comparable to results that were presented by Dumas (1980). After treatment of wheat with PH_3 most of the fumigant was also desorbed in the first 2-3 days. However, small amounts continued to desorb for many weeks (Dumas, 1980). Thereafter PH_3 was no longer detected. The limit of quantification (LOQ) of the applied GC-MS method was determined according to (Klementz et al., 2011) and was given to 0.005 mg/kg. Regarding desorption of PH_3 from apples at different temperatures (5 and 15°C), a higher temperature led to a faster desorption of PH_3 (see Figure 2.B). Furthermore the total amount of PH_3 desorbed from apples was lower at the higher temperature (0.560 ± 0.05 µg/g apple vs. 0.404 ± 0.06 µg/g apple). This might be due to different speed of sorption and desorption or a faster conversion of PH_3 to phosphate at higher temperatures (see also 3.2.). For wheat fumigated with PH_3 similar results were presented by Dumas (1980).

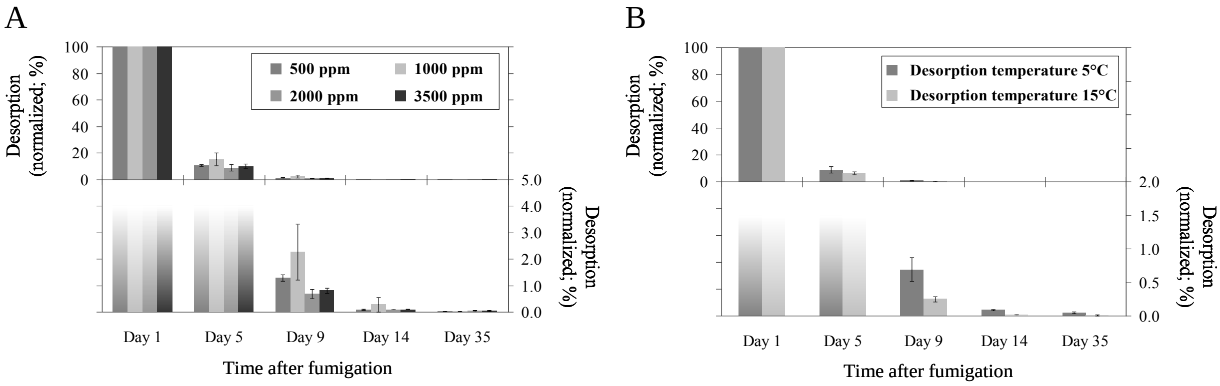

Figure 2 (A) Normalized amounts of PH_3 desorbed from apples over time following fumigation at 5°C with different concentrations (500, 1000, 2000 and 3500 ppm), (n = 3, except 2000 ppm, n = 2; desorption temperature: 5°C; error bars represent standard deviation). (B) Normalized amounts of PH_3 desorbed from apples over time at different temperatures (5 vs. 15°C) following fumigation at 2000 ppm, (n = 3, except 5°C, n = 2; error bars represent standard deviation). (A and B) Amounts were normalized to the values obtained at day 1.

Based on the amount of PH_3 desorbed at each time point it can be concluded that 120 h after fumigation the maximum residual level (MRL) of 0.05 µg/g is no longer exceeded, except for 3500 ppm. Here, 216 h are required (see Figure 3.). This result is also supported by the experiments on phosphine residues (see 3.2.).

For the first four time points an exponential correlation was found between the amount of PH_3 that is desorbed from the apples and the time. This applies to all tested fumigation concentrations. Figure 4 depicts this correlation exemplarily for a fumigation concentration of 3500 ppm. In Figure 4.A the linear plot is shown which is comparable to Figure 3, while Figure 4.B shows the logarithmic plot which illustrates the exponential correlation. Thus, up to 336 h after fumigation desorption of PH_3 from apples obeys a rate law of a first-order reaction. The rate constants for all tested fumigation concentrations (500 – 3500 ppm) were found to -0.0223, -0.0187, -0.0229 und -0.0225 h^{-1} and differ only slightly. Hence for all a uniform desorption mechanism can be assumed. After the 5th ventilation desorption rate drops down dramatically and no longer obeys a rate law of a first-order reaction. Therefore, a second desorption mechanism that is much slower than the first one e.g. diffusion can be supposed. Similar considerations were given by Noack and Wohlgemuth (1985) for hazelnuts, soybeans and wheat fumigated with PH_3.

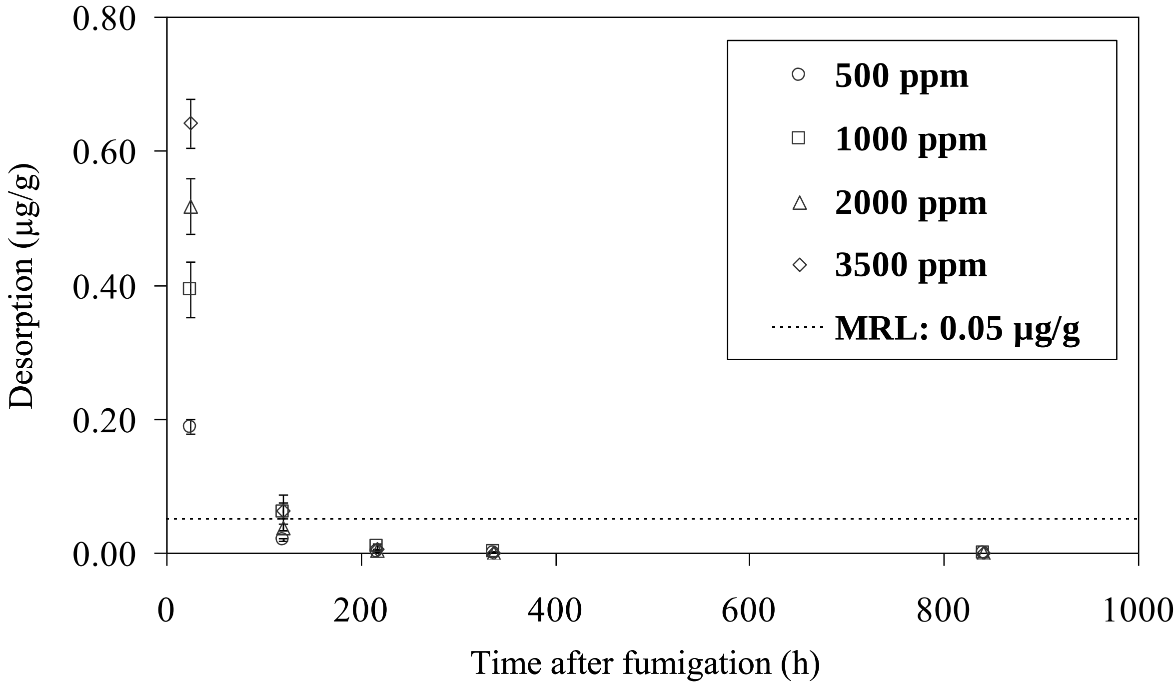

Figure 3 Desorption of PH_3 from apples over time following fumigation at 5°C with different concentrations (500, 1000, 2000 and 3500 ppm; n = 3, except 2000 ppm, n = 2; desorption temperature: 5°C; error bars represent standard deviation).

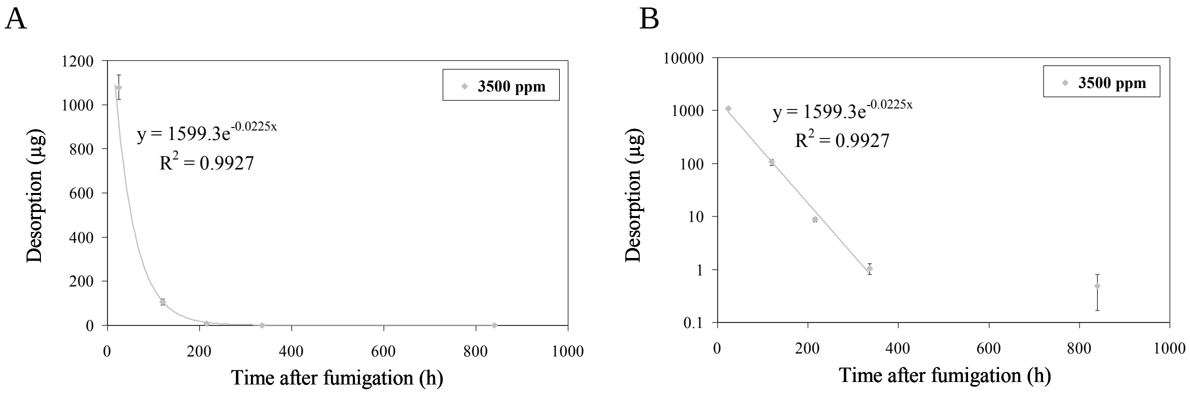

Figure 4 Absolute amount of PH_3 desorbed from apples over time following fumigation at 5°C and 3500 ppm (n = 3; desorption temperature: 5°C; error bars represent standard deviation); A: linear plot; B: logarithmic plot.

3.2. Phosphine residues

Immediately after the end of fumigation, the initial phosphine residue in apples was 0.13 mg/kg. Already 24 hours after fumigation, the PH_3 residue level dropped to 0.005 mg/kg.

3.3. Aroma analysis

HS-SPME-GC analysis coupled to MS detection was applied for aroma profiling. This method has already been used successfully in the laboratory for aroma profiling of apples and has already been described in detail (Dunemann et al., 2009). Figure 5 depicts a PCA (score-plot) including the samples from day 0 and day 21. The graphical presentation of the analytical data gives two important results: i) the aroma patterns of apples, as well non-fumigated and fumigated, are changing during storage (e. g. samples c0-* and c21-*) and ii) the aroma patterns are strongly influenced by fumigation (e. g. samples c21-* and 21-*). The dynamic of aroma pattern during storage is a known process at climacteric fruits like apple (Ulrich et al., 2009).

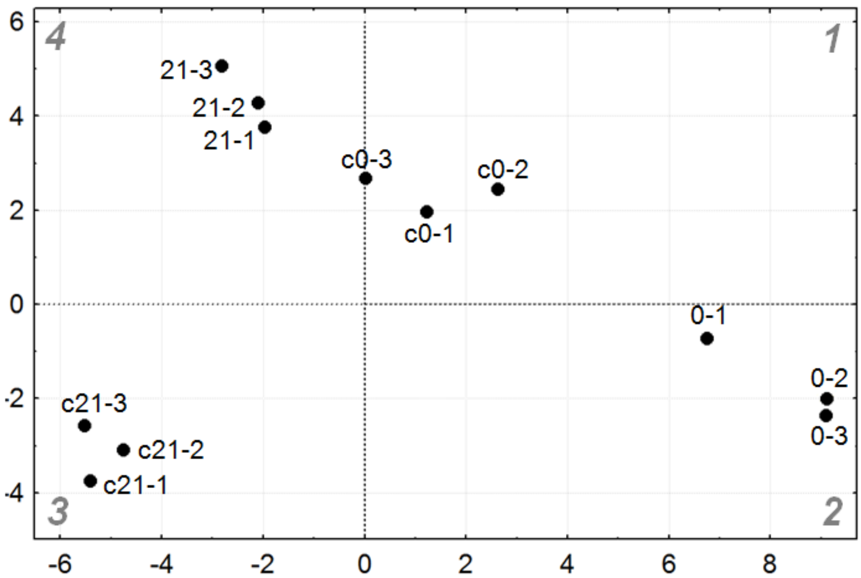

Figure 5 Score-plot of a principal component analysis (PCA), including the aroma pattern analyses from day 0 and day 21 for 110 peaks. Sample code: e.g. '0-1'denotes for 'day 0 – repetition 1' fumigated; 'c21-3' denotes for 'day 21 – repetition 3' non-fumigated control.

A detailed analysis of the dynamic of individual compounds shows qualitative as well as quantitative influences on volatile contents. Four main effects on the aroma profile of apples have been revealed that were caused by PH_3 fumigation. First, PH_3 fumigation can prevent the formation of certain aroma compounds. For example in Figure 6A the peak area for hexyl propionate is shown. The unfumigated reference samples showed an increasing peak area from day 0 to day 21 whereas for the fumigated samples the peak area was zero for both time points. However, formation of aroma compounds can not only be prevented but also decreased (see Figure 6.B) or increased (Figure 6.C). For butyl octanoate (Figure 6.B) a decreased peak area was found that was not statistically significant for day 0 but for day 21. On the other hand PH_3 fumigation resulted in a strong increase in the formation of butyl hexanoate, 2-methyl (Figure 6.C) on day 0. However, on day 21 this difference no longer exists. Forth, PH_3 fumigation can also lead to the formation of specific aroma compounds which would otherwise have not been occurred. This is shown in Figure 6.D. Here, as a result of PH_3 fumigation, an unknown ('unknown 27.99') compound is formed (day 0). However, over time the formation of this 'unknown' compound is dramatically reduced and tends towards zero. An explanation for the observed effects might be that PH_3 fumigation affects the metabolism of the apples and thus other biosynthetic pathways were turned on. To clarify this issue further research is urgently needed.

Although the results obtained are preliminary and need to be supported by further studies it can be concluded that the aroma profile of apples (*Malus domestica* Borkh., cv. 'Braeburn') is qualitatively and quantitatively affected through PH_3 fumigation.

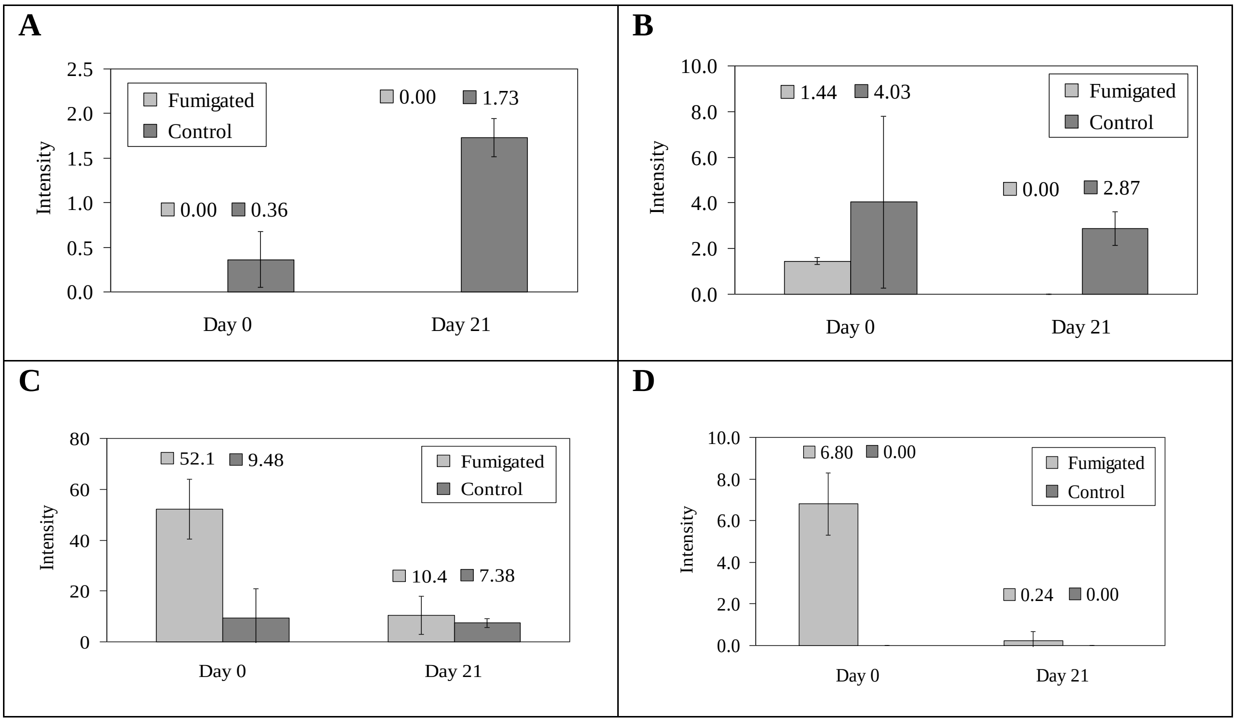

Figure 6 Impact of PH_3 fumigation on the intensity of selected aroma compounds in apples (*Malus domestica* 'Braeburn'), depicted are the peak areas for day 0 (corresponds to 0 h) and day 21 (corresponds to 504 h) for the fumigated and the non-fumigated control samples; (A) hexyl propionate; (B) butyl octanoate; (C) butyl hexanoate, 2-methyl; (D) unknown 27.99.

4. Conclusions

Here we report on the sorption and desorption behaviour of phosphine from apples. For this purpose apples were fumigated for 48 h with different concentrations of phosphine and subsequently desorption was monitored using gas chromatography-mass spectrometry instrumentation. Thereby a linear correlation between the fumigation concentration and the amount of phosphine adsorbed by the apples was revealed. On the other hand a logarithmic correlation was found between the amount of phosphine desorbed from apples and the fumigation concentration used. Additionally, the impact of the temperature on desorption was evaluated. It was found that a higher temperature promotes the desorption process. For all fumigation concentrations applied phosphine residues were found below the maximum residual level of 0.05 ppm after the third ventilation. Furthermore, the question was addressed whether phosphine fumigation affects the aroma profile of apples. Therefore, a headspace solid-phase micro-extraction technique was used and coupled to subsequent gas chromatography-mass spectrometry. By doing so it was unambiguously demonstrated that phosphine fumigation qualitatively and quantitatively alters the aroma profile of apples (*Malus domestica*' Braeburn'). However, further research on all aspects discussed in this study is required.

Acknowledgements

The authors would like to thank Christine Reichmann for excellent technical assistance. This work was supported by an internal grant of the German Federal Institute for Risk Assessment (BfR).

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