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PTR-MS in enology: Advances in analytics and data analysis

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Abstract

The present communication deals with the improvement of proton transfer reaction mass spectrometry (PTR-MS) wine headspace analyses. In contrast to previous PTR-MS investigations of wine, where wine headspace was ionized by protonated ethanol clusters, the headspace was diluted by a factor of 1:40 with N_2 and ionized by H_3O^+ ions. This method is better suited for routine applications than the previously reported method since it is simpler, faster, and the mass spectra obtained are less complex. A test wine was mixed with ethanol and with water to yield ethanol contents ranging from 10 to 15% (v/v) and these mixtures were analyzed to assess whether any quantitative differences in the composition of volatiles were detectable. The data showed no impact of the ethanol content on the wine headspace composition. The new method was applied to eight different wine samples produced from two different grape varieties: *Pinot Noir* and *Cabernet Sauvignon*. Each variety was grown in two different locations in South Tyrol (Northern Italy) and harvested at two different dates. Quantitative (but not qualitative) differences in PTR-MS spectra between the two wine varieties were observed. Using principal component analysis of selected *m/z* signals differentiation between *Pinot Noir* and *Cabernet Sauvignon* samples was achievable.

Keywords: Wine discrimination; PTR-MS; Volatile organic compounds; Principal component analysis; *Vitis vinifera*

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1. Introduction

Proton transfer reaction mass spectrometry (PTR-MS) is an analytical technique for the detection and quantification of volatile organic compounds (VOCs) in air. PTR-MS offers a wide range of features suitable for food quality assessment. For instance, no sample preparation is needed, which is both time-saving and avoids adulteration of samples before analysis. PTR-MS is a highly sensitive (low pptv range) analytical method based on chemical ionization (CI) by non-dissociative proton transfer reactions, resulting predominantly in signals assignable to quasi-molecular ions $[MH]^+$. The primary ion usually used for CI is H_3O^+ and since most VOCs exhibit proton affinities higher than H_2O, H_3O^+ ions are suitable for the protonation of a large variety of VOCs including aldehydes, ketones, esters, carboxylic acids, etc. [1,2]. PTR-MS enables the continuous on-line detection of changes in the composition and quantity of volatiles in food samples, e.g., during food processing [3], fruit storage [4], and food consumption [5] via direct headspace VOC analysis. The performance of PTR-MS is comparable to electron ionization mass spectrometry (EI-MS) in terms of linearity, resolution, and detection limit [6]. However, PTR-MS data are one-dimensional and therefore the differentiation of isobaric and isomeric compounds is not possible under standard operating conditions [7].

More than 800 volatile compounds have been detected in wine; among them are several isomeric and isobaric compounds [8]. The qualitative and quantitative composition of wine volatiles depends on various factors like grape variety, climatic factors, and viticulture practices, as well as storage and enological techniques [8]. The bouquet of wine changes continuously from the moment of harvesting to the moment of consumption. Typical alcohol levels of red wines range from 10 to 15% (v/v) and exceed other aroma compounds by a factor of 10^3–10^6 [8,9]. The solubility of volatiles generally increases in the presence of

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ethanol with a corresponding decrease in headspace concentrations of these compounds [8–10]. In contrast, an experiment investigating the changes of 18 volatiles in the headspaces of aqueous versus ethanol solutions showed a delay in the decrease of most volatiles during dynamic headspace measurements in the presence of ethanol [10].

Besides its influence on solubility and headspace concentration of volatile compounds, ethanol has an impact on the PTR-MS ionization processes. In the presence of high levels of ethanol H_3O^+ primary ions predominantly react to form protonated ethanol monomers ($C_2H_5OHH^+$, *m*/*z* 47), dimers [$C_2H_5OHH^+(C_2H_5OH)$, *m*/*z* 93], trimers [$C_2H_5OHH^+(C_2H_5OH)_2$, *m*/*z* 139], H_2O-adducts [$C_2H_5OHH^+(H_2O)$, *m*/*z* 65] as well as fragment ions due to H_2O elimination $C_2H_5^+$, *m*/*z* 29 and mixed $C_2H_5^+$ ethanol clusters [$C_2H_5^+(C_2H_5OH)$, *m*/*z* 75; $C_2H_5^+(C_2H_5OH)_2$, *m*/*z* 121] [11] which will subsequently react with other organic trace volatiles. For 21 simple esters these fragmentation patterns were studied in detail [12].

Absolute and relative abundances of the various ethanol product ions depend on the ethanol concentration. Conclusively, headspace analyses of otherwise identical samples containing different concentrations of ethanol might yield divergent mass spectra. Recently, a 10-fold dilution of wine headspace into an ethanol-saturated N_2 flow was proposed to generate a constantly high level of ethanol in the analyte gas [11]. The resulting mass spectral fingerprints of wine headspace are independent of the ethanol content in the wine sample. The associated change in the ionization chemistry has, however, some negative implications. H_3O^+ ions are replaced by $C_2H_5OHH^+(C_2H_5OH)_{n=1,2}$ ions as primary reagent ions. $C_2H_5OHH^+(C_2H_5OH)_{n=1,2}$ ions are less reactive CI reagents, analytes with low polarity or low proton affinity may no longer be subjected to ionization. Furthermore, $C_2H_5OHH^+(C_2H_5OH)_{n=1,2}$ ions predominantly react in ligand switching reactions resulting in the formation of mixed protonated VOC–ethanol clusters. The qualitative interpretation of the resulting mass spectra is very complicated.

Here, an alternative method for PTR-MS headspace analyses of wine samples based on a 40-fold dilution of wine headspace into pure N_2 is presented. Employing this new method, wine samples from different varieties, growing site altitudes, and harvesting dates were differentiated without prior adjustment of their original ethanol content.

2. Experimental

2.1. PTR-MS parameters

Method development and quantification studies were performed on a high sensitivity proton transfer reaction mass spectrometer (hs-PTR-MS, Ionicon Analytik, Innsbruck, Austria). Technical specifications of the PTR-MS instrument and technique are described in literature [1,14]. The sample inlet system as described in [11] was applied, with the only modification that the sample headspace was diluted 40-fold into pure N_2 (Alphagaz 1, 99.999%, Air Liquide Italia, Milano). Prior to PTR-MS analysis, 15 ml aliquots of wine were transferred to 40 ml glass vials capped with PTFE (Polytetrafluoroethylene, Teflon) septa, and the glass vials were kept in a water bath at 25 °C for 10 min. A constant flow of $2\,\mathrm{ml\,min^{-1}}$ N_2 was introduced through a gas-tight syringe into the sample headspace and exported through a second syringe. The sample headspace was then diluted into an $80\,\mathrm{ml\,min^{-1}}$ flow of N_2. Thus, the ethanol (*m*/*z* 47) to primary ion (H_3O^+) ratio in the drift tube was constantly kept below 10%. The diluted sample gas was transferred through a heated (60 °C) 40 cm PTFE tube (inner diameter 1.59 mm) to provide a uniform mixture. This PTFE tube was connected to the Silcosteel® inlet of the PTR-MS instrument. The PTR-MS was set up with an inlet temperature of 80 °C and a sample gas flow to the PTR-MS instrument of $70\,\mathrm{ml\,min^{-1}}$; the $12\,\mathrm{ml\,min^{-1}}$ overflow was discarded. The instrument was operated at an E/N (electric field strength to molecule number density in the reaction chamber) of 140 Td ($1\,\mathrm{Td} = 10^{-17}\,\mathrm{cm^2\,V\,molecule^{-1}}$) and in "mass scan" mode in the range of *m*/*z* 20–200, with a dwell time of 0.2 s per *m*/*z* (36 s per cycle). The H_3O^+ abundance was 1.2×10^7 counts per second (cps). Decay of the primary ion signal during the analyses did not exceed 5%. The abundances of O_2^+ ions (*m*/*z* 32) and of H_3O^+ (H_2O) clusters (*m*/*z* 37) were <1%.

Every sample was measured in triplicate over 10 scan cycles. During the first 5 cycles (minutes 1–3) equilibration of the system was achieved. Cycles 6–10 (minutes 3–6) were used for data evaluation. Residual compounds were removed from the PTFE tubes and Silcosteel® inlet by introducing $10\,\mathrm{ml\,min^{-1}}$ N_2 into a vial containing purified water (analytical/laboratory grade, Millipore) instead of wine. After this procedure all signal intensities were back to baseline levels.

2.2. Preparation of wines with different ethanol concentrations

To investigate whether different ethanol contents in the range of 10–15% (v/v) had any significant effect on the quantitative results of PTR-MS wine headspace analyses, a test wine was adjusted to different alcohol concentrations (10, 11, 12, 13, 14, and 15%, v/v) by adding either water (analytical/laboratory grade, Millipore) or ethanol (Ethanol absolute extra pure, Merck, Darmstadt, Germany) (Table 1). The wine used for dilution was

Table 1
Dilution series of a *Pinot Noir* test wine for assessing the influence of different ethanol contents on PTR-MS analyses of wine headspace

% vol[a]	H_2O [ml]	Ethanol [ml]	*Pinot Noir* undil. [ml]	dilution factor[c]
10	28.6	–	71.4	1.40
11	21.4	–	78.6	1.27
12	14.3	–	85.7	1.17
13	7.1	–	92.9	1.08
14[b]	–	–	–	1.00
15	–	1.2	98.8	1.01

[a] Final ethanol content.

[b] For this test concentration the undiluted *Pinot Noir* (*Pinot Noir* undil.) containing 13.97 % (v/v) Ethanol was used.

[c] Calculated as 100/*Pinot Noir* undil. [ml].

Table 2
Characterization of the investigated wine samples

Acronym	Sample	Variety	Growing location[a]	Harvesting date	% v/v
PN11	48.1.1.1.7.01	*Pinot Noir*	Moar (550)	20.09.2001	12.0
PN12	48.1.1.2.7.01	*Pinot Noir*	Moar (550)	11.10.2001	13.1
PN21	48.1.2.1.7.01	*Pinot Noir*	Fragsburg (730)	01.10.2001	12.5
PN22	48.1.2.2.7.01	*Pinot Noir*	Fragsburg (730)	22.10.2001	14.9
CS31	48.2.3.1.7.01	*Cabernet Sauvignon*	Ölleiten (350)	27.09.2001	12.1
CS32	48.2.3.2.7.01	*Cabernet Sauvignon*	Ölleiten (350)	18.10.2001	13.0
CS41	48.2.4.1.7.01	*Cabernet Sauvignon*	Hallhof (530)	08.10.2001	12.5
CS42	48.2.4.2.7.01	*Cabernet Sauvignon*	Hallhof (530)	25.10.2001	12.2

[a] Names of the farms where the grapes were grown; numbers in brackets indicate the altitude of the growing location in m above mean sea level; the farms “Moar”, “Fragsburg” and “Hallhof” are located in the communality of Meran/Merano, whereas “Ölleiten” is situated in Kaltern/Caldaro, about 30 km south of Meran.

a *Pinot Noir* Riserva “Selyét”, vintage 2004, produced at the Research Centre for Agriculture and Forestry Laimburg (Pfatten, Italy). The undiluted wine contained 14% (v/v) ethanol. To compare the results of diluted and undiluted wine samples, a dilution factor was calculated based on the amount of undiluted wine in the samples (Table 1). Every alcohol concentration was analyzed in triplicate using the PTR-MS parameters described in Section 2.1. The results obtained from the analyses of different ethanol concentrations were multiplied with the respective dilution factor.

2.3. Origin of wine samples

Samples of *Pinot Noir* and *Cabernet Sauvignon*—each grown in two different locations and collected on two different dates in 2001 were analyzed (Table 2). One batch of grapes was collected in every growing site about 7–10 days before the optimum collection date as forecast by the winemaker. A second batch was collected about three weeks after the first one. Musts from all grape batches were sulfurized (80 mg SO_2/l) and kept at 2–4 °C for 4 days. After this maceration period the musts were slowly heated to 20 °C and then inoculated with 0.3 g dry yeast/l (Uvaferm CM, Esseco, San Martino Trecate, Italy). Fermentation was performed at 28–29 °C from the second to the forth day, then continued at 25–26 °C, and stopped at residual sugar contents of 2.0–2.5 g/l. The fermentation process was concluded after 6–10 days in *Pinot Noir* and after 9 days in *Cabernet Sauvignon* samples. More details on the production of the test wines are given elsewhere [14]. Test wines were filled into 0.5 l bottles and stored at 6 °C. Prior to analysis the investigated samples were kept at ambient temperature (25 ± 3 °C) for 24 h. Each batch was represented by three bottles and all measurements were performed in triplicate.

2.4. Data analysis

Intensities of the signals detected by PTR-MS were normalized to the primary ion signal (H_3O^+). The mean values of the signal intensities for the 6th to 10th scan cycle were calculated for each mass in the range from *m*/*z* 20 to *m*/*z* 200. Only signals showing a minimum intensity of 500 normalized counts per second (ncps) in at least one of the samples analyzed were used for data analysis. Data normalizations and calculations of means were conducted using Microsoft Excel version 2002. Analyses of variance (ANOVA) and principal component analyses (PCA) were performed using SPSS 12.0 for Windows. Prior to ANOVA, a Levene-test was performed to assess homogeneity with regards to variation [15].

3. Results and discussion

3.1. Influence of different ethanol contents on wine headspace composition

The influence of different ethanol contents in wine (for preparation of different ethanol concentrations, see Section 2.2) was studied. All ion signals >500 ncps in at least one of the investigated samples were subjected to ANOVA computation. The Levene test for homogeneity revealed equal variation across all investigated samples. Except for signals assignable to ethanol and ethanol-derived fragments and clusters, no significant differences ($\alpha = 0.05$) between samples containing different amounts of ethanol from 10 to 15% (v/v) were detected when subjected to ANOVA. Consequently, the new PTR-MS method was applied to the individual *Pinot Noir* and *Cabernet Sauvignon* wine samples described in Section 2.3 without prior adjustment of their ethanol contents.

3.2. Discrimination of different samples of Pinot Noir and Cabernet Sauvignon

PTR-MS analyses of *Cabernet Sauvignon* and *Pinot Noir* samples produced the same qualitative pattern of signals. The average intensities of the individual detected signals were comparable in both wine varieties and are reflected in the following classification (in each group, signals are arranged in ascending order of signal magnitude): 501–1000 ncps: *m*/*z* 90, 59, 73, 40, 93, and 53; 1001–10,000 ncps: *m*/*z* 55, 75, 49, 44, 46, 63, 62, 71, 28, 57, and 89; 10,001–100,000 ncps: *m*/*z* 48, 37, 33, 39, 41, 31, 43, and 45; >100,000 ncps: *m*/*z* 61, 29, 27, and 47. Signals assignable to protonated ethanol (*m*/*z* 47), to the corresponding isotopes (*m*/*z* 48 and 49), to the ethanol dimer (*m*/*z* 93), to the ethanol water adduct (*m*/*z* 65), and to the ethanol fragment ions (*m*/*z* 29 and 75), as well as to the single water cluster (*m*/*z* 37) were not included in the data evaluation. In ascending *m*/*z* order, the signals of interest were *m*/*z* 27, 28, 31, 33, 39, 40, 41, 43,

Table 3
Mean total ion count rates (tic) and relative standard deviations (s %) of all analyzed wine samples[a]

Sample[b]	tic [ncps]	s %	Sample[b]	tic [ncps]	s %
PN11	647,652	5.94	CS31	659,217	5.12
PN12	679,357	2.81	CS32	705,500	2.61
PN21	644,874	7.02	CS41	713,256	5.03
PN22	787,949	5.65	CS42	734,114	2.85

[a] Mean values represent those calculated from three replicates per wine sample.

[b] For sample identities see Table 2.

44, 45, 46, 53, 55, 57, 59, 61, 62, 63, 71, 73, 89, and 90. The intensities of these signals represented the 22 variables in the data matrix, which is subsequently referred to as the "absolute quantification matrix".

Total ion count rates for each wine sample analyzed were calculated as the sum of intensities of all signals contained in the absolute quantification matrix. In both wine varieties the highest amounts of volatiles were observed in the wines collected at the higher altitude on the later harvesting date (Table 3). The only exceptions were the early harvest samples of *Pinot Noir*, where no remarkable differences in VOC abundances were observed between the lower and higher altitude sites. The remaining samples (*Cabernet Sauvignon*, early and late harvest; *Pinot Noir*, late harvest) showed an increase in volatiles from the lower to the higher growing site and from the first to the second harvesting date. In *Pinot Noir* samples, a correlation between alcohol content and total amount of volatiles was detected ($r = 0.980$, $p = 0.010$). In contrast, no such correlation was found for *Cabernet Sauvignon* ($r = 0.246$, $p = 0.377$). However, given the limited number of analyzed samples, conclusions about a positive correlation between alcohol content and VOC abundance are not feasible and will be the focus of a future investigation.

The absolute quantification matrix was subjected to ANOVA ($\alpha = 0.05$) in order to detect whether any significant differences existed between the analyzed samples. A Levene test for homogeneity revealed equal variation across all investigated samples except for *m/z* 73. Variance was given for 17 signals ($^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$): *m/z* 27**, 28***, 33***, 39***, 40***, 41***, 43***, 44***, 55*, 57***, 59***, 61***, 62***, 63***, 71***, 89***, and 90***. Following the procedure outlined in [16], a second data matrix was calculated. In this matrix, subsequently referred to as the "relative quantification matrix", every variable of the absolute quantification matrix was represented as a percentage of the respective total ion count rate.

PCAs were performed with both the absolute and the relative quantification data matrix. Only principal components (PCs) with eigenvalues ≥1 were extracted. From the absolute quantification matrix, four PCs were extracted: the first one (eigenvalue 11.6) explaining 50.4%, the second one (eigenvalue 5.6) 24.2%, the third one (eigenvalue 2.9) 12.6%, and the fourth one (eigenvalue 1.2) 5.3% of the total variance. The first PC was mainly characterized by positive contributions (loading >0.5) of the masses *m/z* 27, 28, 43, 44, 45, 46, 53, 55, 57, 61, 62, 89, and 90. The positive loadings of the variables *m/z* 31, 33, 39, 40, 41, and 71, as well as the negative loading of *m/z* 59, yielded the most important contributions to the second PC. Signals mainly contributing to the third PC were *m/z* 31, 45, 46, and 59 (positive loadings) as well as 63 (negative loading). These signals also contributed to either PC 1 or PC 2. The loadings of the particular variables are shown in Table 4, left column.

Table 4
Eigenvectors of principal components (PC) obtained from the absolute quantification matrix of *Cabernet Sauvignon* and *Pinot Noir* samples. Factor loadings in the left column (complete data set) result from PCA of 22 signals selected for evaluation; those in the right column are from PCA performed with 17 signals selected according to ANOVA

	Complete data set				Selected data set		
m/z	PC1	PC2	PC3	PC4	PC1	PC2	PC3
27	0.892	−0.186	0.350	0.160	0.838	−0.204	0.466
28	0.831	−0.309	0.385	0.106	0.782	−0.335	0.472
31	0.406	0.525	0.610	0.045	n.i.	n.i.	n.i.
33	0.457	0.553	−0.292	0.464	0.457	0.622	−0.083
39	0.181	0.967	0.036	−0.085	0.120	0.951	0.208
40	0.208	0.938	0.126	−0.011	0.137	0.914	0.323
41	0.164	0.968	0.026	−0.066	0.104	0.955	0.202
43	0.905	−0.042	−0.366	−0.154	0.956	0.044	−0.222
44	0.907	0.010	−0.363	−0.126	0.953	0.095	−0.220
45	0.789	0.143	0.545	0.043	n.i.	n.i.	n.i.
46	0.690	0.150	0.552	−0.206	n.i.	n.i.	n.i.
53	0.894	0.043	0.254	0.062	n.i.	n.i.	n.i.
55	0.877	0.210	0.162	0.151	0.828	0.226	0.383
57	0.869	−0.409	−0.026	−0.229	0.890	−0.378	0.034
59	0.379	−0.633	0.614	0.114	0.317	−0.703	0.594
61	0.882	−0.106	−0.425	−0.149	0.943	−0.011	−0.308
62	0.886	−0.120	−0.409	−0.147	0.945	−0.030	−0.299
63	0.465	0.452	−0.548	0.402	0.500	0.565	−0.384
71	0.049	0.975	0.064	0.009	−0.019	0.956	0.236
73	0.253	−0.441	−0.143	0.689	n.i.	n.i.	n.i.
89	0.928	−0.095	−0.311	−0.158	0.969	−0.018	−0.189
90	0.933	−0.009	−0.192	−0.199	0.952	0.039	−0.096

n.i.: not included in the PCA.

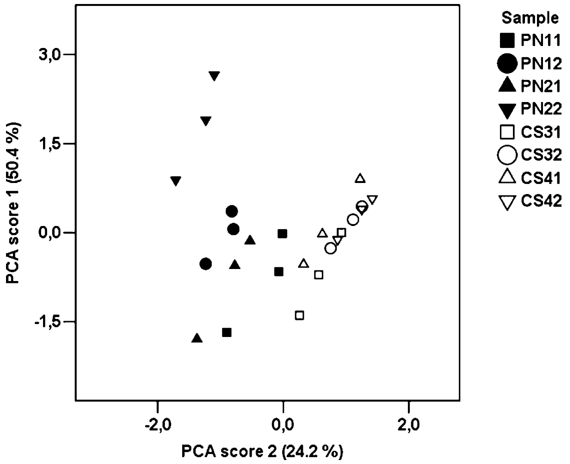

Fig. 1. Principal component analysis based on absolute quantification data (PC 1 vs. PC 2). For sample identities see Table 2. Lower growing sites are represented by squares and circles, higher growing sites by triangles. The last digits of the sample acronyms indicate the harvesting date: 1 = earlier harvest, 2 = later harvest.

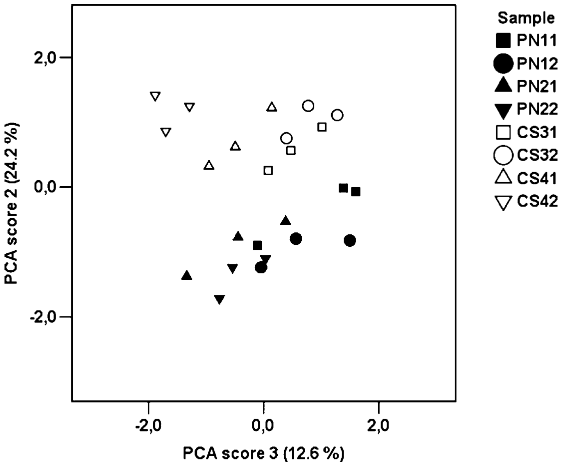

Fig. 2. Principal component analysis based on absolute quantification data (PC 2 vs. PC 3). For sample identities see Table 2. Lower growing sites are represented by squares and circles, higher growing sites by triangles. The last digits of the sample acronyms indicate the harvesting date: 1 = earlier harvest, 2 = later harvest.

The scatter plot of PC 1 versus PC 2 is shown in Fig. 1. In this plot, samples of *Pinot Noir* clustered together with those of *Cabernet Sauvignon*. There was also no separation observable in the plot featuring PC 1 versus PC 3 (figure not shown). However, in the scatter plot of PC 2 versus PC 3 a separation of the samples assignable to the two different wine varieties was detectable (Fig. 2).

Consecutively, PCA was applied to those masses selected from the absolute quantification matrix that showed significant variance with ANOVA. This procedure resulted in a good discrimination of *Pinot Noir* from *Cabernet Sauvignon* samples. The scatter plot of PC 1 versus PC 2 is shown in Fig. 3. PC 1 (eigenvalue 9.7) explaining 53.9 % of the total variance was mainly characterized by the positive contributions of the variables *m/z* 27, 28, 43, 44, 55, 57, 61, 62, 63, 89, and 90. PC 2 (eigenvalue 5.2), which explained 28.7% of the total variance was mainly characterized by variables *m/z* 33, 39, 40, 41, 63, and 71 (positive loadings) as well as 59 (negative loading). The positive loadings of the signals *m/z* 59 (>0.5), 27 and 28 (>0.4) gave the most important contribution to the PC 3 (eigenvalue 1.7), explaining 9.4% of the total variance. The loadings of the selected variables are shown in Table 4, right column.

The plot of PC 2 versus PC 3 also resulted in two well-separated clusters (Fig. 4), whereas the plot of PC 1 versus PC 3 did not allow differentiation of the two wine varieties (figure not shown).

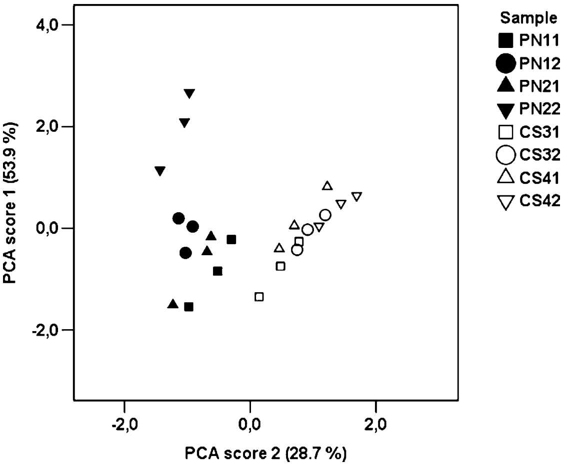

Fig. 3. Principal component analysis based on absolute quantification data (PC 1 vs. PC 2). PCA was performed using a selection of 17 variables showing significant variance between the investigated samples. For sample identities see Table 2. Lower growing sites are represented by squares and circles, higher growing sites by triangles. The last digits of the sample acronyms indicate the harvesting date: 1 = earlier harvest, 2 = later harvest.

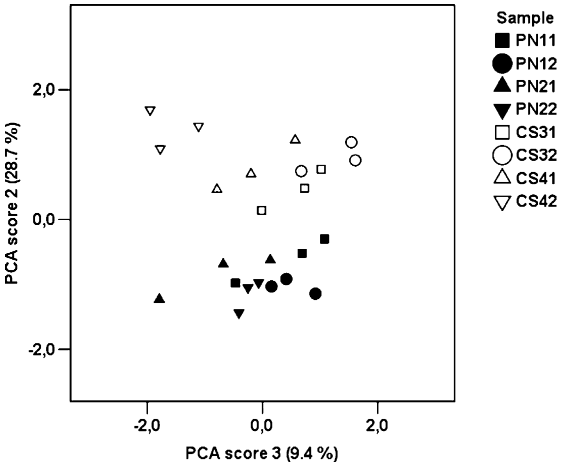

Fig. 4. Principal component analysis based on absolute quantification data (PC 2 vs. PC 3). PCA was performed using a selection of 17 variables showing significant variance between the investigated samples. For sample identities see Table 2. Lower growing sites are represented by squares and circles, higher growing sites by triangles. The last digits of the sample acronyms indicate the harvesting date: 1 = earlier harvest, 2 = later harvest.

Table 5
Eigenvectors of principal components (PC) obtained from the complete relative quantification matrix of *Cabernet Sauvignon* and *Pinot Noir* samples

m/z	PC1	PC2	PC3	PC4
27	0.712	−0.678	0.033	0.120
28	0.670	−0.695	−0.135	0.051
31	0.940	−0.074	−0.274	−0.042
33	0.067	0.622	0.181	0.593
39	0.566	0.771	0.112	−0.226
40	0.583	0.715	0.202	−0.189
41	0.577	0.767	0.112	−0.202
43	−0.961	0.172	−0.037	−0.117
44	−0.877	0.265	−0.183	−0.062
45	0.911	−0.301	−0.101	−0.066
46	0.857	−0.253	−0.153	−0.263
53	0.361	−0.374	0.718	−0.049
55	0.203	0.059	0.792	0.072
57	−0.672	−0.622	0.075	−0.331
59	0.192	−0.909	0.307	−0.021
61	−0.979	0.098	−0.069	−0.033
62	−0.978	0.067	−0.035	−0.031
63	0.014	0.603	−0.055	0.669
71	0.535	0.775	0.266	−0.140
73	−0.092	−0.363	−0.103	0.709
89	−0.964	0.031	0.122	−0.131
90	−0.830	0.081	0.165	−0.277

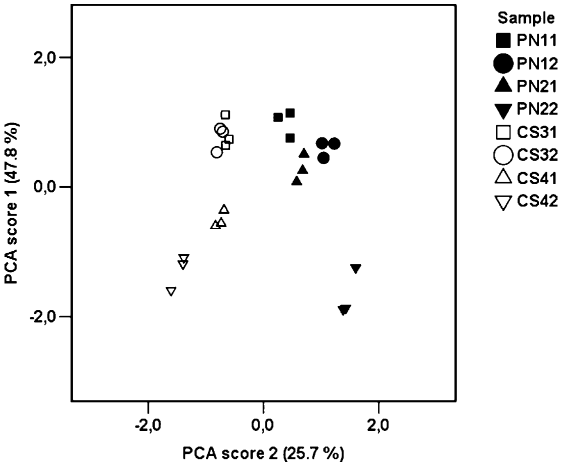

Fig. 5. Principal component analysis based on relative quantification data (PC 1 vs. PC 2). For sample identities see Table 2. Lower growing sites are represented by squares and circles, higher growing sites by triangles. The last digits of the sample acronyms indicate the harvesting date: 1 = earlier harvest, 2 = later harvest.

PCA was subsequently applied to the relative quantification matrix of the complete set of 22 variables. The eigenvectors of the selected variables are shown in Table 5. The scatter plot of the first (eigenvalue 11.0, 47.8% of total variance) versus the second (eigenvalue 5.9, 25.7% of total variance) PC allowed discrimination of the two wine varieties (Fig. 5). The scores for the second and the third (eigenvector 1.9, 8.3% of total variance) PC also resulted in two well-separated clusters for both varieties (Fig. 6). In Fig. 5, those samples containing the highest volatile contents (PN22, CS41, CS42) are clustered separately from the other samples. This might be an indication of the impact on the chemical composition of wine from ecological factors like growing location (which, in the present case, is mainly due to the different altitudes of the growing sites) and the harvesting date.

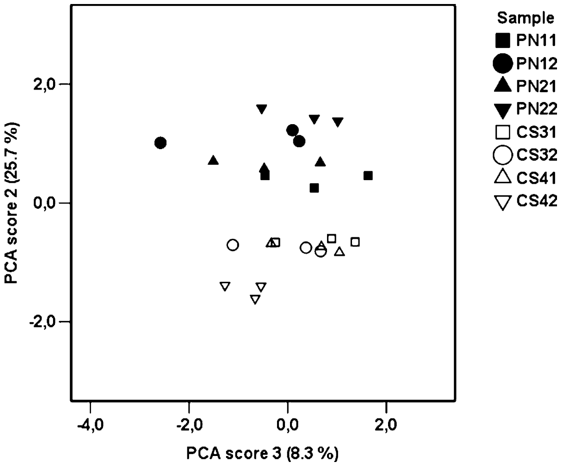

Fig. 6. Principal component analysis based on absolute quantification data (PC 2 vs. PC 3). For sample identities see Table 2. Lower growing sites are represented by squares and circles, higher growing sites by triangles. The last digits of the sample acronyms indicate the harvesting date: 1 = earlier harvest, 2 = later harvest.

Conclusively, the third approach for analyzing PTR-MS data, i.e., that using PCA based on relative quantification data, gives the most promising results (Figs. 5–6). Moreover, this approach is straightforward and encompasses all variables regardless of their variance across and within the samples.

4. Conclusions

The modified PTR-MS method for wine aroma analysis, which uses H_3O^+ ions as proton donors, is better suited for routine applications than the established method of employing $C_2H_5OHH^+(C_2H_5OH)_{n=1,2}$ primary ions since it is simpler, faster, and the mass spectra obtained are less complex.

The method presented here allowed for discrimination of different red wine varieties. Even different harvesting dates and growing locations resulted in separated clusters of wine samples. The wine samples from the higher altitude sites and the later harvesting dates displayed higher signal intensities for a number of volatiles as compared to the earlier harvested and lowland samples. This result corresponds with the ratings from a tasting panel, which had previously evaluated samples of these wines. According to the panel, the wines showing high signal intensities also exhibited a higher aroma complexity [13]. These findings represent an important step towards the envisaged aim of establishing PTR-MS as a standard method for wine aroma analysis. PTR-MS is a fast and reproducible complement to traditional sensory tasting panels.

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