JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 7635−7640 **7635**

Proton Transfer Reaction−Mass Spectrometry (PTR-MS) Headspace Analysis for Rapid Detection of Oxidative Alteration of Olive Oil

EUGENIO APREA,*[,†,§] FRANCO BIASIOLI,[†] GRAZIANO SANI,[#] CLAUDIO CANTINI,[#] TILMANN D. MÄRK,[§,⊥] AND FLAVIA GASPERI[†]

Agri-Food Quality Department, IASMA Research Center, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy; Institut für Ionenphysik und Angewandte Physik, Universität Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria; Istituto per la Valorizzazione del Legno e delle Specie Arboree, Consiglio Nazionale delle Ricerche, via Madonna del Piano, Sesto Fiorentino 50019, Italy; and Department of Plasmaphysics, University of Bratislava, SK-84248 Bratislava, Slovak Republic

Olive oil has been characterized by rapid proton transfer reaction−mass spectrometry (PTR-MS) headspace analysis without any concentration of the volatiles or pretreatment of the samples. Comparison of extra virgin and defective (rancid) samples, as described by a panel of sensory judges, and the monitoring of thermo-oxidation processes are discussed. Multivariate analysis of PTR-MS data has been carried out and cross-validated, providing (i) reliable classification models for extra virgin oil as opposed to defective oil and (ii) calibration models able to predict independently thermo-oxidative degradation and the corresponding peroxide value. PTR-MS fragmentation patterns of volatiles considered in this study are also reported.

KEYWORDS: olive oil; proton transfer reaction−mass spectrometry; lipid oxidation; peroxide value; multivariate analysis; fragmentation patterns; volatile compounds

INTRODUCTION

The complex flavor of virgin olive oil is mainly due to a number of volatiles (aldehydes, alcohols, esters, and ketones) and phenol compounds (*1*−*5*). It has been stated that C_6 compounds, the major constituents of virgin olive oil headspace, largely contribute to its green odor notes (*6*−*8*). These C_6 compounds are produced through the lipoxygenase pathway (*9*) from linoleic and linolenic hydroperoxides. Their production is favored in comparison to that of C_9 compounds.

Lipolysis and oxidation are the processes leading to the most serious deterioration of olive oil. Lipolysis is a natural process that starts when the oil is still in the fruit and increases with ripening due to the action of endogenous enzymes, whereas oxidation begins after the oil is extracted from the fruit due to the action of oxygen. Lipid oxidation involves a deterioration of the fatty acids, in particular the unsaturated ones, by oxygen when catalytic agents (light, enzymes, metallic cations, etc.) are present. The first steps of lipid oxidation lead to the formation of hydroperoxides, very unstable compounds that are odorless, colorless, and tasteless. Decomposition of hydroperoxides (advanced oxidation) yields secondary reaction products such as aldehydes, ketones, acid, alcohols, and hydrocarbons. Thus, oxidation processes in oils produce an increase of the total volatiles concentration and, in particular, of some specific compound such as hexanal (*10*, *11*). These compounds often adversely affect flavor, odor, taste, nutritional value, and thus overall quality. From a sensory point of view the pleasant characteristic flavor of extra virgin olive oil changes and the rancid defect appears (*12*).

Many different approaches have been proposed for the rapid characterization of olive oil aroma, ranging from the relatively slow but very informative ones based on GC methods (*2*, *4*) to the direct and fast analysis by electronic noses (*13*, *14*) or by mass spectrometry (*15*). In these two latter cases the absence of separation is partly compensated by the application of chemometric techniques (*13*−*15*).

In this paper, we have investigated the use of direct headspace analysis by proton transfer reaction−mass spectrometry (PTR-MS) as a rapid tool for flavor assessment of olive oil. PTR-MS is a relatively new technique proposed some years ago by Lindinger and co-workers (*16*, *17*). Its unique properties make it useful for the rapid characterization of agroindustrial samples; that is, it does not require sample pretreatment, it is not sensitive to normal air constituents (no need of buffer gas), it is very fast and sensitive, and the induced fragmentation (in the ionization/detection process) is usually very low (chemical information is retained). So far, this method has been used to detect the effect of stabilization treatments on fruit juice (*18*),

* Corresponding author (telephone ++39 0461 61 53 88; fax ++39 0461 65 09 56; e-mail eugenio.aprea@iasma.it).
[†] IASMA Research Center.
[§] Universität Innsbruck.
[#] Consiglio Nazionale delle Ricerche.
[⊥] University of Bratislava.

10.1021/jf060970r CCC: $33.50
Published on Web 09/02/2006

to classify strawberry cultivars (*19*), to detect meat spoilage (*20*), to evaluate the effect of ozone treatment on meat microbial contamination (*21*), to compare sensory and instrumental descriptions of mozzarella cheese (*22*), and to predict age (*23*) and to correlate instrumental measurements with sensory attributes in Grana cheese (*24*). Further studies suggesting its usefulness for real-time and in vivo measurements have been summarized elsewhere (*25*−*27*).

Here, the possibility to discriminate, by PTR-MS analysis, extra virgin from rancid oils previously classified by a sensory panel was evaluated. Moreover, the evolution of volatiles in the oil headspace during an induced thermo-oxidation process was monitored, allowing correlation with peroxide values. PTR-MS fragmentation patterns of pure standards of some of the most relevant products formed during the oxidation process of olive oils (*28*) are reported and were used, together with literature data, for the tentative identification of the mass peaks measured in the olive oil headspace.

MATERIALS AND METHODS

Olive Oil Samples. Twenty-six samples of extra virgin olive oil and 10 defective olive oils from Tuscany (Italy) were supplied by the Trees and Timber Institute (Sesto Fiorentino, Florence, Italy). All of the extra virgin olive oil samples were properly obtained from fresh mature fruits of good quality harvested from genetically controlled plants in November 2003. Nineteen of the samples were single-tree samples (monovariety cultivars: 'Scarlinese', 1 sample; 'Frantoio', 9 samples; 'Moraiolo', 4 samples; 'Grappolo', 1 sample; 'Seggianese', 1 sample; 'Leccino', 3 samples), whereas the others (7 samples) were obtained by blending three or more cultivars (typically 70% Frantoio, 20% Moraiolo, and 10% other varieties). The defective samples (10 oils) were obtained from oil samples submitted to the sensory quantitative descriptive analysis (QDA) by the panel of the Chamber of Commerce of Grosseto (Italy). Sensory profiles were determined using a standard profile sheet, T20/Doc. N. 15/Rev.1, according to the International Olive Oil Council method (*29*). Only oils characterized in this analysis by the presence of "rancid" defect alone (6 samples) or in combination with "heated" defect (4 samples), with median values of each defect ≤ 2.5 on a 10 cm linear scale were selected for the present study. Thus, the chosen oils were characterized by a rather weak intensity of the defect (*29*).

Headspace Analysis. The measurements of volatile organic compounds (VOCs) in the headspace of oil samples were performed using a commercial PTR-MS system (Ionicon GmbH, Innsbruck, Austria) that allows on-line monitoring of trace components with concentrations as low as a few ppt_v (*17*). Details on PTR-MS systems can be found in refs *17* and *30*.

For each sample 5 mL of oil was placed in a silicon-septum closed glass bottle of 120 mL (Supelco, Bellefonte, PA). The bottles (prepared in duplicate) were positioned in a water bath at 28 °C for 30 min for conditioning and kept at this temperature during the measurement (to mimic the temperature used during sensory evaluation). The VOCs released into the headspace were transferred through a heated (70 °C) capillary line (uncoated deactivated fused silica tubing with an inner diameter of 0.25 mm; Supelco) directly into the drift tube of the PTR-MS at a rate of 10 $cm^3\ s^{-1}$. The headspace was replaced by a flow of pure nitrogen gas (SOL s.p.a.; purity = 99.999%). Details on this sampling method can be found in ref *18*. The samples were measured in random order to avoid memory effects, and nitrogen was flushed, for ∼5 min, between two consecutive samples to clean the transfer line.

The mass spectrometric data were collected over a mass range of *m*/*z* from 20 to 260 amu using a dwell time of 0.2 s per mass under drift tube condition of 120−130 Td (Td = Townsend; 1 Td = 10^{-17} V $cm^2\ mol^{-1}$) and, after blank subtraction, converted in ppb by the relationship reported in ref *17*. Each sample was measured for six cycles; after the start of the measurements, the first two cycles were skipped, and then the data of the next four were averaged and used for further data analysis.

Thermo-oxidation. Ten samples (6 blended and 4 monovariety 'Frantoio') of the 26 extra virgin olive oils were subjected to an accelerated thermo-oxidation process. From each sample 3 to 4 bottles containing 2.5 mL of oil were prepared (22 bottles containing blended oil and 20 bottles containing 'Frantoio' oil), placed without closing in a conventional oven at a temperature of 110 °C and kept for different times (up to 28 h). At each time two bottles (one for blended and one for 'Frantoio') were randomly removed from the oven (avoiding consecutive replicates of the same sample), cooled at room temperature for ∼20 min, closed with a silicon septum, and measured after 1 h as described in the previous section.

Peroxide Value Determination. Determination of peroxide values, of the samples subjected to the thermo-oxidation process, was performed by a FoodLab analyzer (CDR, Ginestra Fiorentina, Firenze, Italy; http://foodlab.cdr-mediared.it). The FoodLab method is based on a colorimetric reaction in which peroxides R−O−O−R oxidize the Fe^{2+} ions. These ions are complexed, forming a red color having an intensity, measured at 505 nm, that is directly proportional to the concentration of peroxides in the sample, expressed as mequiv of $O_2\ kg^{-1}$.

Fragmentation Patterns of Pure Compounds. Chemical standards (Sigma-Aldrich, Milan, Italy) were used to prepare 200 mL of the following water solutions using glass bottles having a volume of 500 mL: acetic acid (300 mg L^{-1}; purity = 99.99+%), pentanal (4.2 mg L^{-1}; purity = 97%), *trans*-2-hexenal (2.3 mg L^{-1}; purity = 98%), hexanal (4.9 mg L^{-1}; purity = 98%), *trans*-*trans*-2,4-heptadienal (113 mg L^{-1}; purity = 88%), *trans*-2-octenal (3.8 mg L^{-1}; purity = 94%), *trans*-2-nonenal (3.4 mg L^{-1}; purity = 97%), nonanal (3.4 mg L^{-1}; purity = 95%), *trans*-*trans*-2,4-decadienal (48.9 mg L^{-1}; purity = 88%), 1-octen-3-ol (25.6 mg L^{-1}; purity = 98%), and *trans*-2-decenal (4.5 mg L^{-1}; purity ≥ 95%). To avoid saturation and to allow, at the same time, the detection of fragments at low concentration, solutions were prepared to produce a mixing ratio in the gas phase in the range of 1−5 ppm_v.

Dynamic measurements (*31*) of the headspace formed upon the water solutions were performed. When a dynamic headspace analysis is performed, a stream of air passes through the solution, removing the particles of the VOCs from the water phase to the headspace and successively transporting them to the instrument inlet port. The concentration of VOCs consequently decreases at a rate depending on the air flow and volatility of the compounds. Moreover, when fragmentation of the compound of interest occurs after its protonation into the reaction chamber, fragments will show the same time dependence behavior as the parent ion. By observing the change in concentration of all the masses, it is thus easy to identify parent and fragment ions of the analyzed compounds and to distinguish these ions from impurities. The comparison of fragmentation data with sample spectra was used, together with literature data, for the tentative identification of the oxidation products.

Statistical Analysis. PTR-MS spectra are high-dimensional vectors (hundreds of masses) that can be more efficiently analyzed if the dimensionality is reduced by suitable data compression methods such as principal component analysis (PCA) and discriminant partial least-squares (dPLS) (*32*). Following data compression, appropriate classification techniques can be performed to discrimitate groups of observation. Among the different procedures available, linear discriminant analysis (LDA) and canonical variate analysis (CVA) (*33*) were applied for data classification in this study.

PCA, dPLS, and CVA, already tested on PTR-MS spectra (*18*, *19*, *23*), were implemented by means of the software Win-DAS (John Wiley & Sons, Ltd., Chichester, U.K., 1998). The PLS1 model was developed and validated by the software The Unscrambler 8.5 (Camo Process AS, Oslo, Norway). Correlations and LDA were performed by the software package Statistica 5.1 (StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Defective versus Extra Virgin Olive Oils. PCA of the full PTR-MS data without any data conditioning is shown in **Figure 1a**. A relatively good separation of the defective oil samples is evident using only two PCA scores. However, the variability associated with the first component does not allow a clear

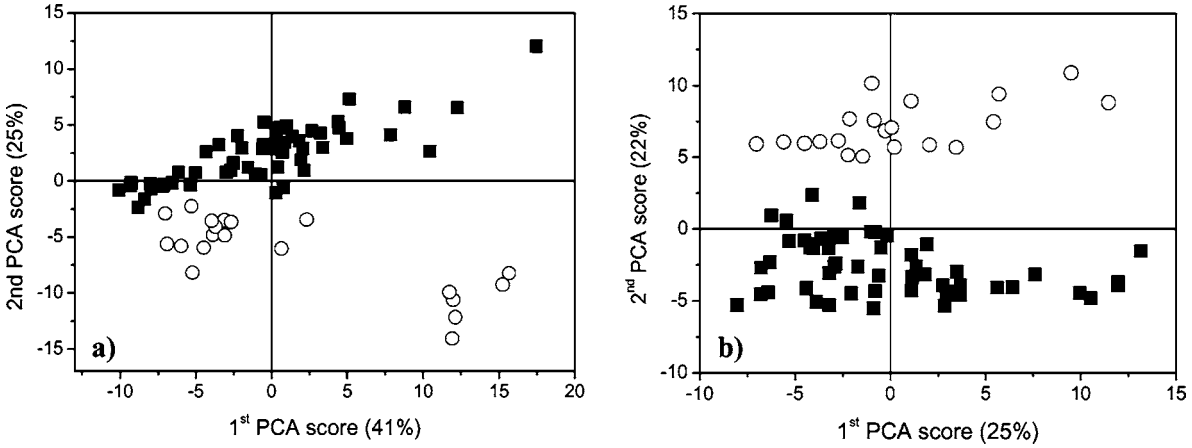

Figure 1. PCA plot: (**a**) first and second scores of the PCA in correlation mode performed on extra virgin (■) and defective olive oils (○), using the whole PTR-MS spectra from *m*/*z* 20 to 260 (explained variance given in parentheses); (**b**) PCA as in panel **a** after the removal of the signal at *m*/*z* 33.

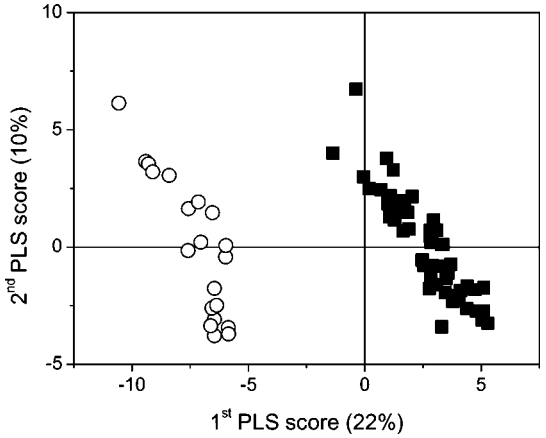

Figure 2. Plot of the first and second scores of the dPLS analysis performed on extra virgin (■) and defective olive oils (○), using the whole PTR-MS spectra from *m*/*z* 20 to 260. The explained variance is given in parentheses.

separation of the rancid samples. This component (data not shown) is characterized by the presence of a peak at *m*/*z* 33 (methanol). The removal of this peak allows a clearer separation of defective samples using only the second component (**Figure 1b**). Moreover, a supervised technique such as dPLS (*33*) seems to be more appropriate to address the present data because the difference between extra virgin and defective oils is not the major source of variability. This technique is indeed able to more clearly separate defective samples (**Figure 2**) with 100% correct classification using LDA (Mahalanobis distance) on only two dPLS scores. It is worth noting that only 32% (22% from the first component and 10% from the second component) of explained variance is related to the presence of the rancid attribute. Cross-validation (*34*) was used to check the ability of the model to properly classify unknown samples. For the reduced number of samples of our model a leave-one-out procedure was followed: one sample was removed from the data set, and a model, built with the remaining samples using the first and second dPLS scores, was used to classify it, and the procedure was repeated for each sample. To ensure a complete independence of the test samples from those used to build the model, both replicates of a sample were removed at a time. Cross-validation provided that every sample was correctly identified (100% correct classification).

Time−Evolution of Headspace Concentration during Induced Thermo-oxidation. For the possibility to easily discriminate between extra virgin oils (good) and rancid oils (defective), the evolution of the whole headspace VOC profile during an accelerated thermo-oxidation process was monitored.

The first two components of the dPLS calculated in the exploratory phase (**Figure 2**) were used to build a model based on CVA with the purpose to be used as predictive tool for the samples subjected to the thermo-oxidation process. The CVA model data obtained from the spectra of oils heated for different times are plotted in **Figure 3b**, referring to 'Frantoio' mono-variety oils, which seem to be more scattered than data of blended oils shown in **Figure 3a**. Looking in detail at **Figure 3b** (where samples of the same production batch are represented by the same symbol), it can be seen that data of different production batches are shifted along the time axis compared to each other. That means the oxidation process exhibits a similar evolution for all of the samples of the 'Frantoio' variety independent from production batch. Furthermore, data suggest the existence of a different "lag phase" after which the oxidation follows the same kinetics. These different stabilities with respect to the oxidation could be due to a different content of antioxidant in the oil samples (*35*, *36*). This is an interesting hint indicating the possibility of evaluating and comparing the oxidation resistance of different oils.

Peak Identification of Oxidation Products. Comparison of peroxide values and headspace profiles of the oils subjected to the thermo-oxidation process indicates that the intensities of several PTR-MS peaks are highly correlated with peroxide value. Ten masses were found to have a correlation coefficient $r \geq 0.9$, that is, *m*/*z* 125, $r = 0.97$; *m*/*z* 143, $r = 0.96$; *m*/*z* 111, $r = 0.94$; *m*/*z* 103, $r = 0.92$; *m*/*z* 61, $r = 0.92$; *m*/*z* 127, 101, 97, and 73, $r = 0.91$; and *m*/*z* 109, $r = 0.90$. Tentative identification of the volatiles responsible for these masses was achieved using data from a long-time on-line measurement during thermal oxidation (75 h at 80 °C; data not discussed in this work) of olive oil and data on PTR-MS fragmentation pattern of flavor compounds measured in this study as well as fragmentation reported in the work of Buhr et al. (*37*).

In **Table 1** the measured pure compounds are listed with (i) their measured fragmentation patterns obtained by normalizing the most abundant mass fragment to an intensity of 100 and (ii) their associated sensory properties obtained from the literature (*28*, *38*−*40*). The most correlated masses, *m*/*z* 125 and 143, are fragments of nonanal; the other masses related to this compound present in its fragmentation pattern unfortunately overlap with masses from other compounds and thus are not useful as nonanal markers (**Table 1**). According to refs *28* and *39*, nonanal concentration increases in virgin olive oil during

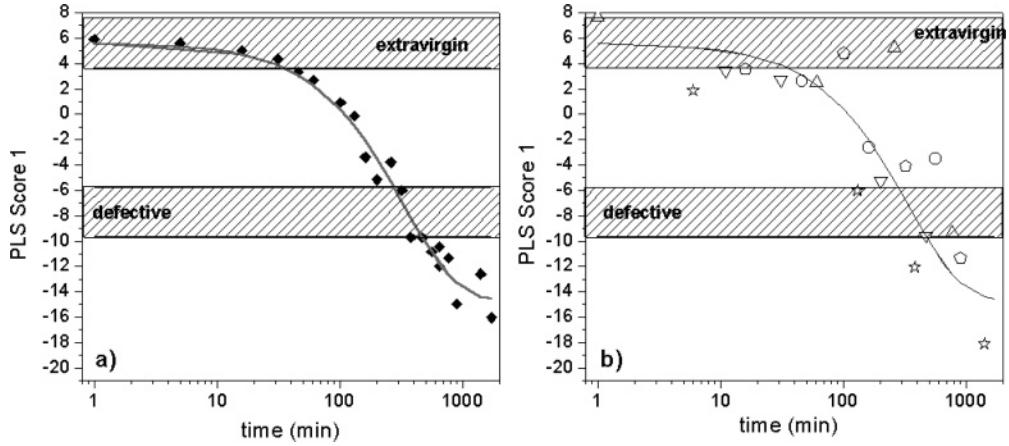

Figure 3. CVA model data of thermo-oxidized olive oils using the first two components of the dPLS data of extra virgin and defected olive oils. Hatched areas indicate 95% confidence region for the two classes: extra virgin and defective (training data): (**a**) ◆, blended oils (test data); —, first-order exponential fit; (**b**) different symbols indicate different batches of 'Frantoio' oil variety (test data); —, first-order exponential fit of blended oil data.

Table 1.

volatile compound	MW	fragmentation pattern[a]	sensory properties
pentanal	86	69 (100); 41 (21); 87 (7); 57 (5)	woody, malt, pungent
trans-2-hexenal	98	57 (100); 99 (54); 81 (20)	sweet, fruity, green, leaf
hexanal	100	83 (100); 55 (50); 101 (5)	grass, tallow, fat
trans-trans-2,4-heptadienal	110	111 (100); 93 (12); 67 (6); 43 (5)	fat, rancid hazelnut, cinnamon
heptanal[b]	114	97 (100); 55 (49); 115 (8); 69 (6)	oily, fatty, woody, nutty
trans-2-octenal	126	109 (100); 127 (97); 57 (33);67 (13)	green, nut, fat, spicy
octanal[b]	128	69 (100); 111 (95); 129 (27); 41 (8); 55 (6)	fatty, sharp, citrus
trans-2-nonenal	140	141 (100); 123 (82); 81 (36); 67 (15)	orris, fat, cucumber, wax
nonanal	142	69 (100); 83 (35); 57 (26); 143 (24); 41 (17); 55 (15); 125 (12)	fat, citrus, green, paint
trans-trans-2,4-decadienal	152	153 (100); 135 (8)	seaweed, fat, citrus
trans-2-decenal	154	155 (100); 81 (80); 137 (74); 95 (70); 67 (20); 41 (17)	paint, fish, fat
1-octen-3-ol	128	69 (100); 111 (95); 129 (14); 55 (6); 57 (4)	mushroom
acetic acid	60	61 (100); 43 (12)	pungent, vinegar

[a] Data in parentheses are normalized to the most abundant ion. [b] Data on fragmentation pattern are from Buhr et al. (*37*).

oxidation. It is a secondary oxidation product that derives from the homolytic β-scission of the 9-hydroperoxide and 10-hydroperoxide oleate, two primary oxidation products, and its odor is described as fatty, waxy, and painty (*38*). The signal at *m*/*z* 111 is a common fragment from octanal, which originates from β-scission of the 11-hydroperoxide oleate and also of 1-octen-3-ol, a product of further oxidation. Fragments at *m*/*z* 97 and 101, respectively, are possible markers for heptanal originating from 11-hydroperoxide linoleate and hexanal originating from 13-hydroperoxide linoleate. The signal at *m*/*z* 127 could be a measure of 2-octenal originating from 11-hydroperoxide linoleate. The signal at *m*/*z* 61 is a measure of acetic acid (it is reasonable to exclude acetate esters, the fragments of which could contribute the intensity of this peak).

Many other masses change significantly during the thermo-oxidation process even if they are not correlated with the peroxide value. As an example, **Figure 4** shows the time evolution of *m*/*z* 99, 81, 83, and 55. These peaks are related (*41*) to the hexenal family (*m*/*z* 99 and 81) and to the hexanal (*m*/*z* 83 and 55). In fact, mass 99 is strongly correlated ($r >$ 0.99) only with masses 100 (natural isotopic ratio of mass 99), 81, and 82 (natural isotopic ratio of mass 81), with a ratio of m99/m81 compatible with that indicated by Fall et al. (*41*) for 2-hexenal and 3-hexenal. Mass 83 is correlated ($r > 0.99$) with masses 84 (natural isotope), 55, and 56 (natural isotope of mass 55), suggesting, after Fall et al. (*41*), that it is related to the hexanal family. A strong decrease of some masses related to compounds initially present in the oil, for example, the hexenals (*m*/*z* 81 and 99), is thus observed with a concomitant increase of compounds with a different biochemical origin such as hexanal (*m*/*z* 83 and 55) (*28*). The latter ones, even if initially present in extra virgin olive oil together with other saturated aldehydes, increase during the oxidative process and are responsible for the rancid defect (*28*).

Calibration to Peroxide Values. It is interesting to note, with reference to **Figures 3a** and **4**, that the record of the time evolution of a single mass produces a quite noisy pattern (**Figure 4**), but the supervised compression of the information brought by all masses produces a time−evolution pattern with very reduced noise (**Figure 3a**); that is, multivariate calibration allows a clearer description of the chemical modifications induced by oxidation. This allows one to calibrate the PTR-MS information with the measured peroxide value to obtain a rapid, sensitive, and nondestructive tool for peroxide value estimation. In **Figure 5** the increase of the peroxide value during the induced thermo-oxidation process is shown. PTR-MS headspace spectra were used to build a predictive model for the peroxide value based on the PLS1 procedure. The best PLS1 model, with the minimum root square mean error of prediction, includes 13 PLS scores and accounts for 94.8% of the explained variance in validation. The model (**Figure 6**) provides a good estimation of the peroxide value both in calibration ($R^2 = 0.998$) and in leave-one-out cross-validation ($Q^2 = 0.932$).

As far as we know this is the first study on the application of direct headspace measurement of olive oil by PTR-MS. This rapid method produces a spectrometric fingerprint that allows, when coupled with chemometric techniques, the classification

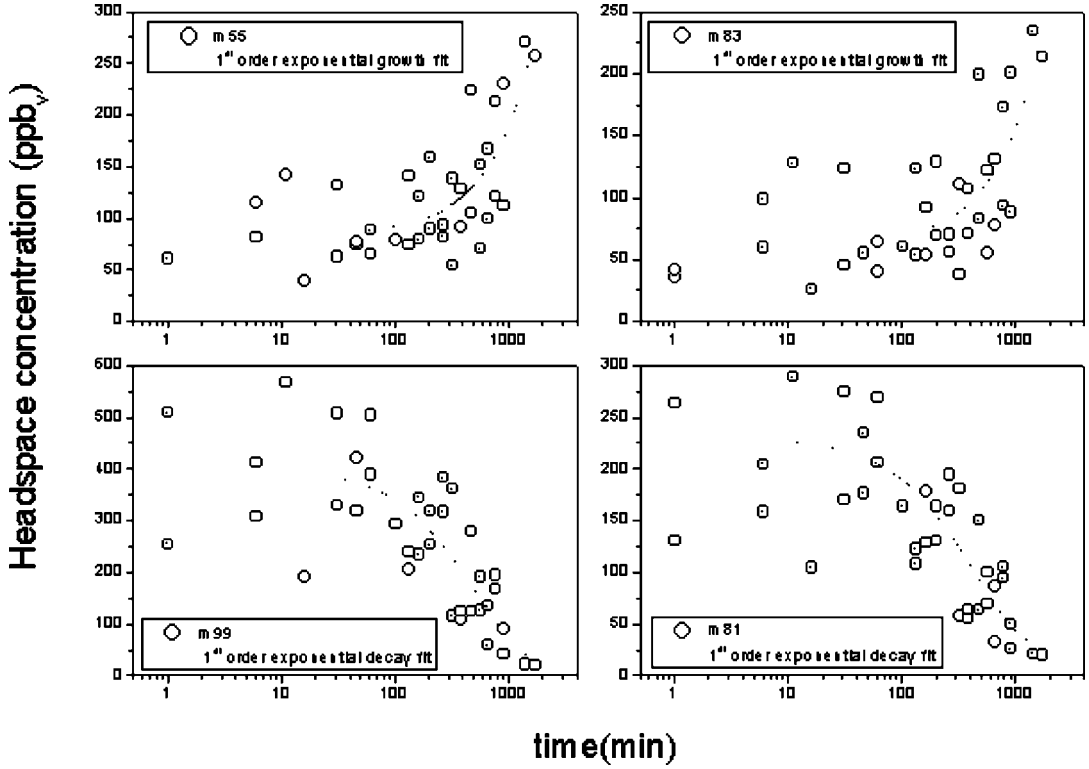

Figure 4. Time–evolution of the intensity of selected peaks related to the hexanal (*m*/*z* 55, 83) and hexenal families (*m*/*z* 81, 99) during thermo-oxidation (110 °C) of olive oil.

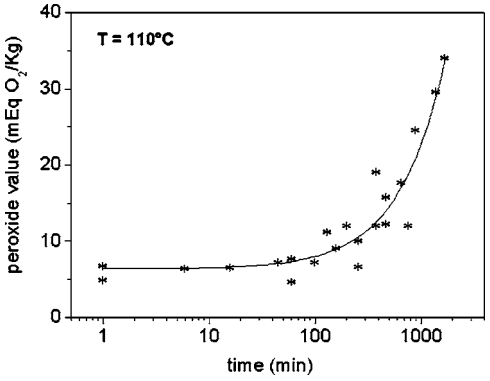

Figure 5. Time–evolution of the peroxide value during induced thermo-oxidation of olive oil: —, first-order exponential growth fit ($r^2 = 0.89$).

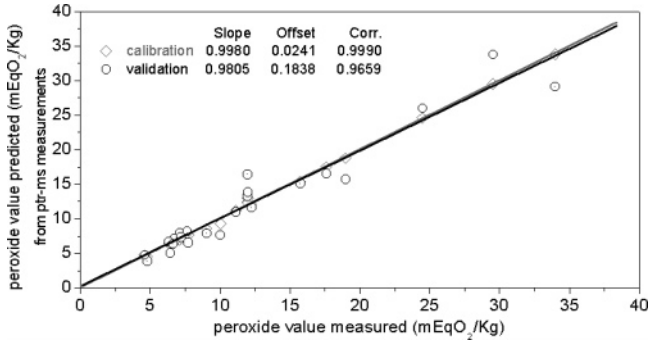

Figure 6. PLS1 estimation of the peroxide value based on PTR-MS data versus measured peroxide value.

of rancid oils against extra virgin oils in accordance with results by a panel of trained judges.

In this study it is shown that it is possible to follow thermo-oxidation by direct headspace volatile monitoring. Many PTR-MS peaks are strongly correlated with the independent determination of the peroxide value, the most correlated being those associated with aldehydes, in particular those arising from β-scission of hydroperoxides and associated with the sensory defect of rancid smell. On the contrary, other masses related to important quality traits of olive oil decrease during the thermo-oxidation process. Multivariate calibration of PTR-MS data allows the accurate determination of the peroxide value, providing a fast, sensitive, and nondestructive tool for peroxide determination.

LITERATURE CITED

(1) Flath, R. A.; Forrey, R. R.; Guadagni, D. G. Aroma components of olive oil. *J. Agric. Food Chem.* **1973**, *21*, 948−952.

(2) Morales, M. T.; Aparicio, R.; Rios, J. J. Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *J. Chromatogr. A* **1994**, *668*, 455−462.

(3) Morales, M. T.; Alonso, M. V.; Rios, J. J.; Aparicio, R. Virgin olive oil aroma: relationship between volatile compounds and sensory attributes by chemometrics. *J. Agric. Food Chem.* **1995**, *43*, 2925−2931.

(4) Angerosa, F.; Di Giacinto, L.; Vito, R.; Cumitini, S. Sensory evaluation of virgin olive oils by artificial neural network processing of dynamic head-space gas chromatographic data. *J. Sci. Food Agric.* **1996**, *72*, 323−328.

(5) Aparicio, R.; Morales, M. T.; Alonso, M. V. Relationship between volatile compounds and sensory attributes of olive oils by the sensory wheel. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1253−1264.

(6) Guth, H.; Grosh, W. A comparative study of the potent odorants of different virgin olive oils. *Fett Wiss. Technol.* **1991**, *93*, 335−339.

(7) Guth, H.; Grosh, W. Quantification of potent odorants of virgin olive oil by stable-isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993**, *70*, 513−518.

(8) Morales, M. T.; Aparicio, R.; Calvente, J. J. Influence of olive ripeness on the concentration of green aroma compounds in virgin olive oil. *Flavour Fragrance J.* **1996**, *11*, 171−178.

(9) Olias, J. M.; Pérez, A. G.; Rios, J. J.; Sanz, L. C. Aroma of virgin olive oil: biogenesis of the "green" odor notes. *J. Agric. Food Chem.* **1993**, *41*, 2368−2373.
(10) Snyder, J. M.; Frankel, E. N.; Selke, E.; Warner, K. Comparison of gas chromatographic methods for volatile lipid oxidation compounds in soybean oil. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1617−1620.
(11) Warner, K.; Frankel, E. N.; Moulton, K. J. Flavor evaluation of crude oil to predict the quality of soybean oil. *J. Am. Oil Chem. Soc.* **1988**, *65*, 386−391.
(12) EEC. Regulation 796/2002 of 6 May 2002. *Off. J. Eur. Union* **2002**.
(13) Gonzalez-Martin, I.; Perez-Pavon, J.; Corder, B. M.; Pinto, C. G. Classification of vegetable oils by linear discriminant analysis of electronic nose data. *Anal. Chim. Acta* **1999**, *384*, 83−94.
(14) Garcia-Gonzalez, D. L.; Aparicio, R. Virgin olive oil quality classification combining neural network and MOS sensors. *J. Agric. Food Chem.* **2003**, *51*, 3515−3519.
(15) Marcos Lorenzo, I.; Perez-Pavon, J. L.; Fernandez Laespada, M. E.; García Pinto, C.; Moreno Corsero, B. Detection of adulterants in olive oil by headspace−mass spectrometry. *J. Chromatogr. A* **2002**, *945*, 221−230.
(16) Lindinger, W.; Hirber, J.; Paretzke, H. An ion/molecule-reaction mass spectrometer used for on-line trace gas analysis. *Int. J. Mass Spectrom. Ion Process.* **1993**, *129*, 79−88.
(17) Lindinger, W.; Hansel, A.; Jordan, A. On-line monitoring of volatile organic compounds at ppt level by means of proton-transfer-reaction mass spectrometry (PTR-MS): medical application, food control and environmental research. *Int. J. Mass Spectrom. Ion Process.* **1998**, *173*, 191−241.
(18) Biasioli, F.; Gasperi, F.; Aprea, E.; Colato, L.; Boscaini, E.; Märk, T. Fingerprinting mass spectrometry by PTR-MS: heat treatment vs. pressure treatments of red orange juice—a case study. *Int. J. Mass Spectrom.* **2003**, *223*−*224*, 343−353.
(19) Biasioli, F.; Gasperi, F.; Aprea, E.; Mott, D.; Boscaini, E.; Mayr, D.; Märk, T. D. Coupling proton transfer reaction-mass spectrometry with linear discriminant analysis: a case study. *J. Agric. Food Chem.* **2003**, *51*, 7227−7233.
(20) Mayr, D.; Margesin, R.; Klingsbichel, E.; Hartungen, E.; Jenewein, D.; Schinner, F.; Märk, T. D. Rapid detection of meat spoilage by measuring volatile organic compounds by using proton transfer reaction mass spectrometry. *Appl. Environ. Microbiol.* **2003**, Aug, 4697−4705.
(21) Jaksch, D.; Margesin, R.; Mikoviny, T.; Skalny, J. D.; Hartungen, E.; Schinner, F.; Mason, N. J.; Märk, T. D. The effect of ozone treatment on the microbial contamination of pork meat measured by detecting the emissions using PTR-MS and by enumeration of microorganisms. *Int. J. Mass Spectrom.* **2004**, *239*, 209−214.
(22) Gasperi, F.; Gallerani, G.; Boschetti, A.; Biasioli, F.; Monetti, A.; Boscaini, E.; Jordan, A.; Lindinger, W.; Iannotta, S. The mozzarella cheese flavour profile: a comparison between judge panel analysis and proton transfer reaction mass spectrometry. *J. Sci. Food Agric.* **2001**, *81* (3), 357−363.
(23) Aprea, E.; Biasioli, F.; Gasperi, F.; Mott, D.; Marini, F.; Märk, T. D. Assessment of Trentingrana cheese ageing by proton transfer reaction−mass spectrometry and chemometrics. *Int. Dairy J.* **2006**, in press (doi:10.1016/j.idairyj. 2006.02.008).
(24) Biasioli, F.; Gasperi, F.; Aprea, E.; Endrizzi, I.; Framondino, V.; Marini, F.; Mott, D.; Märk, T. D. Correlation of PTR-MS spectral fingerprints with sensory characterisation of flavour and odour profile of "Trentingrana" cheese. *Food Qual. Pref.* **2006**, *17* (1−2), 63−75.
(25) Aprea, E.; Biasioli, F.; Gasperi, F.; Märk, T. D.; van Ruth, S. M. In vivo monitoring of strawberry flavour release from model custards: effect of texture and oral processing. *Flavour Fragrance J.* **2006**, *21*, 53−58.
(26) Warneke, C.; Kuczynski, J.; Hansel, A.; Jordan, A.; Vogel, W.; Lindinger, W. Proton transfer reaction mass spectrometry (PTR-MS): propanol in human breath. *Int. J. Mass Spectrom. Ion Process.* **1996**, *154*, 61−70.
(27) Taucher, J.; Hansel, A.; Jordan, A.; Lindinger, W. Analysis of compounds in human breath after ingestion of garlic using proton-transfer-reaction mass spectrometry. *J. Agric. Food Chem.* **1996**, *44*, 3778−3782.
(28) Morales, M. T.; Rios, J. J.; Aparicio, R. Changes in the volatile composition of virgin olive oil during oxidation: flavours and off-flavors. *J. Agric. Food Chem.* **1997**, *45*, 2666−2673.
(29) Giomo, A. Organoleptic assessment of virgin olive oil. COI/T. 20Doc. N. 15/Rev, 1: a new robust statistical approach to classify the oils. Presented at the European Conference "Food Industry and Statistic", Jan 19−21, 2000.
(30) Hansel, A.; Jordan, A.; Holzinger, R.; Prazeller, P.; Vogel, W.; Lindinger, W. Proton transfer reaction mass spectrometry: on-line trace gas analysis at the ppb level. *Int. J. Mass Spectrom. Ion Process.* **1995**, *149/150*, 609−619.
(31) Karl, T.; Yeretzian, C.; Jordan, A.; Lindinger, W. Dynamic measurements of partition coefficients using proton-transfer-reaction mass spectrometry (PTR-MS). *Int. J. Mass Spectrom.* **2003**, *223/224*, 383−395.
(32) Duda, R. O.; Hart, P. E.; Stork, D. G. *Pattern Classification*, 2nd ed.; Wiley-Interscience: New York, 2001.
(33) Kemsley, E. K. *Discriminant Analysis and Class Modelling of Spectroscopic Data*; Wiley: Chichester, U.K. 1998.
(34) Good, I. *Resampling Methods: A Practical Guide to Data Analysis*; Birkhauser: Boston, MA, 1999.
(35) Satue, M. T.; Huang, S. W.; Frankel, E. N. Effect of natural antioxidants in virgin olive oil on oxidative stability of refined, bleached, and deodorized olive oil. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1131−1137.
(36) Aparicio, R.; Roda, L.; Albi, M. A.; Gutierrez, F. Effect of various compounds on virgin olive oil stability measured by rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150−4155.
(37) Buhr, K.; van Ruth, S.; Delahunty, C. Analysis of volatile flavour compounds by proton-transfer reaction-mass spectrometry: fragmentation patterns and discrimination between isobaric and isomeric compounds. *Int. J. Mass Spectrom.* **2002**, *221*, 1−7.
(38) Kochhar, S. P. Oxidative pathways to the formation of off-flavours. In *Food Taints and Off-Flavours*; Saxby, M. J., Ed.; Blackie Academic and Professional: London, U.K., 1993; pp 150−201.
(39) Dobarganes, M. C.; Rios, J. J.; Perez-Camino, M. C. Relationship between the composition of vegetable oils and the volatile components produced in their thermoxidation. *Grasas Aceites* **1986**, *37*, 61−67.
(40) Reiners, J.; Grosch, W. Odorants of virgin olive oils with different flavor profiles. *J. Agric. Food Chem.* **1998**, *46*, 2754−2763.
(41) Fall, R.; Karl, T.; Jordan, A.; Lindinger, W. Biogenic C5 VOCs: release from leaves after freeze−thaw wounding and occurrence in air at a high mountain observatory. *Atmos. Environ.* **2001**, *35*, 3905−3916.

Received for review April 6, 2006. Revised manuscript received July 21, 2006. Accepted July 30, 2006. This work was partially supported by the PAT-CNR Project AGRIIND and by the FWF, Wien, Austria.

JF060970R