

Investigation of Volatile Compounds in Two Raspberry Cultivars by Two Headspace Techniques: Solid-Phase Microextraction/Gas Chromatography–Mass Spectrometry (SPME/GC–MS) and Proton-Transfer Reaction–Mass Spectrometry (PTR–MS)

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The volatile compounds emitted by two raspberry varieties (*Rubus idaeus*, cv. Polka and Tulameen) were analyzed, in both the case of fresh fruits and juices, by two headspace methods that are rapid, solvent-free, and with reduced or no sample pretreatment: solid-phase microextraction/gas chromatography–mass spectrometry (SPME/GC–MS) and proton-transfer reaction–mass spectrometry (PTR–MS). Multivariate analysis of the SPME/GC–MS results allows for an unambiguous sample discrimination for both mashed fruits and juices. PTR–MS instrumental fingerprint provides, in a faster way, similar qualitative information on the overall flavor profile. The two cultivars show both qualitative and quantitative differences. SPME/GC–MS analysis shows that alcohols and aldehydes are more abundant in the headspace of Tulameen as, e.g., hexanal and hexanol that induce herbaceous odor notes. This observation has been confirmed by sensory analysis. PTR–MS was also used to monitor rapid processes that modify the original aromatic profile, such as lipoxygenase activity induced by tissue damages occurring during industrial transformation, accidental mechanical damages, or as a consequence of chewing.

KEYWORDS: SPME; GC–MS; proton-transfer reaction–mass spectrometry; *Rubus idaeus*; raspberry juice; sensory analysis

INTRODUCTION

Volatile compounds are often directly related to important sensory-quality traits as odor and flavor, and they contribute to define the identity of a product and, in the case of fruits, permit us to recognize their distinctiveness in the processed derivatives, e.g., in juices. The typical raspberry flavor, imitated in many commercial products, is produced by hundreds of volatile compounds, of which more than 200 have been identified (1–3), usually by gas chromatography–mass spectrometry (GC–MS) after solvent extraction (4–6) or after preliminary concentration on solid-phase microextraction (SPME) fiber (7) and, more recently, by stir-bar sorptive extraction (8). Major classes of compounds identified in raspberry include aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, esters, C13-norbornoids, monoterpenes, and sesquiterpenes. Among them, there are some compounds that are characteristic of ripened raspberry fruits, such as α - and β -ionone, α - and β -phellandrene, and ethyl esters, together with high concentrations of methyl, ethyl, and propyl acetates (9).

The volatile compound profile of different raspberry genotypes can be very different, both qualitatively and quantitatively (7, 8), and it is influenced by many agronomical and technological parameters (10, 11). Therefore, the availability of rapid and accurate methods to assess these important characteristics in various phases of the production process (growing, harvesting, postharvest, and transformation) is of utmost importance to support breeding programs, product development, or quality control.

In general, GC-based methods are slow and not suited for field measurements or on-line monitoring, and in this sense, an interesting alternative technique can be proton-transfer reaction–mass spectrometry (PTR–MS) (12), as suggested by previous work (13), where the postharvest aging of intact berry fruits was monitored. PTR–MS allows for a rapid screening of the product headspace both for a fast fingerprinting (14–17) and for chemical analysis (18–20). PTR–MS is a one-dimensional technique that provides only mass/charge ratios of the measured compounds. Coupling PTR–MS with a separation technique allows us to obtain unambiguous compound identifications (21–23). Moreover, the possibility offered by PTR–MS to perform real-time dynamic headspace measurements allows for the monitoring of volatile organic compounds emitted during the

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crushing of berries occurring during industrial transformation processes, accidental mechanical damages (during harvesting, transportation, and storage), and chewing that can modify the original aromatic profile. It is known that the volatile compounds extracted from juices and purée are different both qualitatively and quantitatively from the emission released from the intact fruits, with the extent of the differences depending upon the process used and/or peculiarities of the fruits. In fact, even when the production of fruit juice is a simple process based only on fruit crushing and mechanical squeezing of juice, it can affect the emission of volatile compounds as a consequence of tissue damages (24, 25) and have an impact on the final sensory profile (26, 27). Further changes can be induced also by the stabilization treatment (14), but in this work, we only focus on the dynamic process of volatile formation as a consequence of tissue damages (24, 25, 28). PTR-MS allows for the real-time monitoring of these fast processes.

In the present work, we propose a study on the emission of raspberry volatile organic compounds (VOCs), from fruits and juices, performed by dynamic headspace PTR-MS analysis in combination with the static head space SPME/GC-MS analysis. The main aim of this work is the comparison of analytical capabilities and peculiarity of the two headspace techniques, showing their synergies for a better comprehension of the volatile emission phenomena of the fruit. Second, we want to evaluate differences in volatile compound profiles of two raspberry varieties and eventually verify links with sensory analysis, if any.

The two raspberry varieties selected, for their agronomic and commercial relevance, for this study were *Rubus idaeus* cv. Polka and Tulameen. Polka is a primocane raspberry variety, which is characterized by dark red, large, firm fruits with outstanding shelf life. The florican variety, Tulameen, is characterized by uniform light red fruits of remarkable shape, appearance, and size and have an excellent shelf life. It is one of the most popular varieties growing in different climatic conditions.

MATERIALS AND METHODS

Fruits. Berries from Polka and Tulameen raspberry cultivars harvested in the 2006 season were provided by the farmer's cooperative Sant'Orsola (Pergine Valsugana, Trentino, Italy). Samples were stored at 4 °C and measured 1 day after picking. For each cultivar, three different batches have been evaluated by GC and PTR-MS on three different days (Aug 8th and Sept 14th and 21st) to take into account the possible variability during the production period.

Juices. Frozen berries, provided by the farmer's cooperative Sant'Orsola (Pergine Valsugana, Trentino, Italy), were thawed, and juices (one batch for each of the two cultivars, Polka and Tulameen) were prepared by pressing them in an industrial plant (Macè srl, Ferrara, Italy). Neither enzymes nor technological coadjutants were used during juice production. Juices did not receive any stabilization process: they were immediately collected in food-grade plastic bags (1 L capacity), sealed, and frozen (-20 °C). The frozen juices were thawed at 4 °C (overnight), and a volume of 30 mL of well-homogenized juice was transferred in 40 mL vials, sealed, and stored at -20 °C. The day before the analysis, the vials containing juices were thawed at 4 °C and used for the headspace sampling.

Headspace Analysis by SPME/GC-MS. Four or five berries (18–20 g) were introduced in a 100 mL glass flask and gently mashed with a spatula. The sealed container was immersed in a laboratory bath and held at 35 °C to equilibrate, and after 10 min, a 2 cm fused silica fiber coated with divinylbenzene/carboxen/polydimethylsiloxane 50/30 μm (DBV/CAR/PDMS) (Supelco, Bellefonte, PA) was introduced and exposed to the headspace environment for 30 min. The fiber was preconditioned before the analyses, according to the instructions of the manufacturer, performing two blank injections at a temperature of 270 °C. The same procedure was followed for extraction of volatiles from juices. In this case, 20 mL of sample was introduced in 30 mL vials

and sealed. Three measurements for each of the two varieties were performed on mashed fruits, while for juices, we had five replicates for Polka and seven replicates for Tulameen.

Volatiles adsorbed on SPME fiber were desorbed in splitless mode for 5 min in the GC injector at 250 °C (AutoSystem XL gas chromatograph coupled with a TurboMass Gold mass spectrometer, Perkin-Elmer, Norwalk, CT). Separation was achieved on a HP-Innowax fused-silica capillary column (30 m, 0.32 mm inner diameter, 0.5 μm film thickness; Agilent Technologies, Palo Alto, CA). The GC oven temperature program consisted of 60 °C for 3 min, 60–220 °C at 8 °C min⁻¹, 220 °C for 5 min, 220–250 °C at 10 °C min⁻¹, and 250 °C for 5 min. Helium was used as a carrier gas with a constant column flow rate of 1 mL min⁻¹. The transfer line temperature was kept at 220 °C. The mass spectrometer used for the identification of headspace raspberry compounds operated in electron ionization mode (EI, internal ionization source; 70 eV) with a scan range from *m/z* 30–300. Compound identification was based on mass spectra matching in the standard NIST-98/Wiley library and retention indices (RI) of authentic reference standards. Standard compounds were obtained from Fluka (Buchs, Switzerland), Sigma (St. Louis, MO), Aldrich (Milan, Italy), SAFC-Aldrich (Milan, Italy), Carlo Erba (Milan, Italy), and Merck (Darmstadt, Germany), as reported in Table 1. Linear retention indices were calculated after analysis, under the same chromatographic conditions, of C10–C24 *n*-alkane series (Supelco, Bellefonte, PA). When authentic standards were not available, tentative identifications were based on the standard NIST-98/Wiley library and a comparison of RI reported in the literature. Data are reported as chromatographic area percent.

PTR-MS Apparatus and Measurements. A commercial high-sensitivity PTR-MS apparatus (IONICON Analytik GmbH, Innsbruck, Austria) was used at the following drift tube conditions: pressure of 2.04 mbar, temperature of 50 °C, and voltage at 520 V, corresponding to E/N = 120 Td. Mass spectrometric data were collected over a mass range of *m/z* 20–260 using a dwell time of 0.2 s.

On the basis of previous experience on strawberry measurements (15), we followed the following procedure: single berry fruits, removed from the 4 °C storage space, were left at room temperature for 90 min, and the fruit (whole or after crushing) was introduced in a sealed glass vessel (323 mL), provided with two silicone septa on opposite sides, to allow for headspace equilibrium (60 min more at room temperature) before the measurement. A total of 12 and 11 intact fruits were measured for Polka and Tulameen, respectively; six mashed fruits were measured for each variety. For juice headspace measurement, smaller vessels were used: 5 mL of juice was poured into 120 mL glass bottles sealed with caps provided with PTFE/silicone-laminated discs septum and left at room temperature for 60 min to allow for headspace equilibrium. For each juice, nine replicates were measured. The inlet of the PTR-MS was connected by a 1/16" PTFE tube kept at 70 °C, terminating with a stainless-steel needle to be introduced into the septum of the glass container. The headspace was continuously extracted for 4 min at 10 cm³ min⁻¹ (corresponding to the acquisition of five complete spectra), and the evacuated volume was replaced by outdoor air through a second stainless-steel needle connected to a 1/4" PTFE tube. To avoid possible systematic memory effects, from one measurement to the next, the apparatus was flushed with outdoor air for 6 min between consecutive measurements, replicate order was randomized, and a different glass vessel for each sample was used.

For online monitoring of volatile compound emission during crushing, an intact berry was introduced in a glass vessel (323 mL) and closed with a cup provided with a crusher in the middle used to smash the berry during data acquisition that continued for more than an hour after the fruit was crushed. A teflon gasket makes the vessel airtight. To normalize the data for a comparison among different measurements, the headspace concentration for each mass was transformed in parts per billion in volume (ppbv) (14).

Sensory Analysis. Sensory analysis was performed on fruits of the three sampling batches submitted to headspace analyses (SPME-GC/MS and PTR-MS). Complete sensory attribute descriptions of products were obtained using the quantitative descriptive analysis (QDA) method (29) performed by a selected trained panel (12 judges). Selection of judges was based on performances achieved during a sensory training program based on the recognition and quantification of taste and the most important odors related to raspberry key compounds chosen on the

Table 1. Volatile Compounds Found in Raspberry Fruits and Juices by SPME/GC–MS Analysis

| peak number | compound | source | RI ^b | ID ^c | Polka berry ^a | | Tulameen berry ^a | | Polka juice ^a | | Tulameen juice ^a | |
|-------------|---|--------------|-----------------|-----------------|--------------------------|-----|-----------------------------|-----|--------------------------|-----|-----------------------------|-----|
| | | | | | mean | CV% | mean | CV% | mean | CV% | mean | CV% |
| 1 | ethyl acetate | Fluka | 832 | A | 30.23 | 22 | 9.67 | 83 | 12.89 | 21 | 11.79 | 10 |
| 2 | <i>trans</i> -3-methyl-1,3,5-hexatriene | | | C | 0.43 | 92 | 2.01 | 67 | | | 2.01 | 10 |
| 3 | α -pinene | | | B* | 1.30 | 94 | 3.56 | 86 | 0.35 | 18 | 7.13 | 11 |
| 4 | hexanal | Aldrich | 1055 | A | 1.31 | 133 | 12.44 | 49 | 1.04 | 12 | 4.44 | 6 |
| 5 | β -pinene | Aldrich | 1079 | A | 0.09 | 97 | 0.16 | 74 | 0.07 | 12 | 0.23 | 7 |
| 6 | α -phellandrene | Fluka | 1150 | A | 1.46 | 132 | 2.73 | 113 | | | 0.43 | 11 |
| 7 | β -myrcene | Fluka | 1151 | A | 0.29 | 103 | 0.27 | 173 | 0.02 | 16 | 0.15 | 6 |
| 8 | 2-heptanone | Aldrich | 1172 | A | 1.33 | 38 | 1.67 | 87 | 12.11 | 5 | 11.03 | 4 |
| 9 | β -phellandrene | | | C | 0.74 | 93 | 1.10 | 101 | | | 1.02 | 6 |
| 10 | limonene | Aldrich | 1190 | A | 0.58 | 65 | 0.63 | 37 | 0.38 | 17 | 0.74 | 8 |
| 11 | <i>trans</i> -2-hexenal | Aldrich | 1215 | A | 1.74 | 79 | 8.70 | 46 | 0.05 | 25 | 0.01 | 41 |
| 12 | acetoin | Merk | 1223 | A | 1.26 | 82 | 0.27 | 73 | 0.32 | 15 | 0.35 | 4 |
| 13 | γ -terpinene | Fluka | 1249 | A | 0.63 | 110 | 0.32 | 43 | | | 0.16 | 7 |
| 14 | <i>p</i> -cymene | | | B* | 1.17 | 75 | 0.94 | 70 | 0.18 | 9 | 1.58 | 12 |
| 15 | hexyl acetate | Aldrich | 1283 | A | 0.26 | 51 | 1.27 | 74 | 0.10 | 15 | 0.09 | 12 |
| 16 | 2-heptanol | Aldrich | 1336 | A | 3.52 | 68 | 1.28 | 68 | 12.94 | 9 | 7.57 | 8 |
| 17 | <i>cis</i> -3-hexenyl acetate | | | C | 8.26 | 63 | 21.22 | 42 | 0.06 | 9 | 0.36 | 10 |
| 18 | hexanol | Aldrich | 1372 | A | 0.29 | 90 | 1.34 | 10 | 0.28 | 8 | 0.60 | 6 |
| 19 | <i>cis</i> -3-hexenol | Merk | 1408 | A | 3.08 | 96 | 7.91 | 16 | 0.10 | 7 | 0.49 | 8 |
| 20 | acetic acid | Carlo Erba | 1476 | A | 6.32 | 82 | 1.94 | 96 | 2.92 | 16 | 1.40 | 10 |
| 21 | 1-octen-3-ol | Fluka | 1484 | A | 0.02 | 51 | 0.04 | 44 | 0.03 | 13 | 0.05 | 14 |
| 22 | decanal | | | B* | 0.16 | 41 | 0.15 | 41 | 0.26 | 17 | 0.33 | 14 |
| 23 | benzaldehyde | Merk | 1565 | A | 0.05 | 24 | 0.05 | 36 | 0.08 | 6 | 0.20 | 17 |
| 24 | linalool | Aldrich | 1590 | A | 0.13 | 28 | 0.11 | 56 | 0.34 | 10 | 0.42 | 4 |
| 25 | unidentified sesquiterpene | | | C | 0.03 | 31 | 0.08 | 151 | 0.03 | 6 | 0.05 | 8 |
| 26 | 1-octanol | Fluka | 1599 | A | 0.07 | 13 | 0.06 | 2 | 0.21 | 5 | 0.24 | 9 |
| 27 | theaspirane A | | | C | 0.02 | 50 | 0.07 | 34 | 0.23 | 6 | 0.39 | 4 |
| 28 | 4-terpineol | Fluka | 1639 | A | >0.01 | 173 | 0.06 | 96 | 0.04 | 6 | 0.14 | 8 |
| 29 | <i>trans</i> -caryophyllene | Sigma | 1647 | A | 0.10 | 144 | 1.20 | 29 | 0.03 | 11 | 1.95 | 18 |
| 30 | acetophenone | Carlo Erba | 1695 | A | 0.05 | 59 | 0.15 | 16 | 0.02 | 8 | 0.14 | 16 |
| 31 | cyclo-ionone I/edulan | | | B** | 9.08 | 48 | 7.48 | 16 | 10.21 | 12 | 13.17 | 8 |
| 32 | 5-ethyl-(3 <i>H</i>)-furan-2-one | | | C | 0.06 | 70 | 0.15 | 51 | 0.00 | 5 | 0.00 | 10 |
| 33 | β -damascenone | SAFC-Aldrich | 1861 | A | 0.25 | 42 | 0.19 | 42 | 0.96 | 8 | 0.36 | 7 |
| 34 | hexanoic acid | Aldrich | 1878 | A | 0.16 | 65 | 0.29 | 47 | 1.57 | 6 | 0.36 | 20 |
| 35 | geraniol | Fluka | 1879 | A | 0.17 | 34 | 0.16 | 21 | 0.22 | 9 | 0.28 | 5 |
| 36 | dehydro- β -ionone | | | C | 0.41 | 20 | 0.41 | 48 | 3.64 | 8 | 1.43 | 7 |
| 37 | α -ionone | Fluka | 1886 | A | 9.11 | 13 | 6.51 | 47 | 14.53 | 10 | 7.27 | 6 |
| 38 | benzyl alcohol | Carlo Erba | 1902 | A | 0.02 | 13 | 0.01 | 46 | 0.03 | 13 | 0.03 | 11 |
| 39 | α -ionol | | | B*** | 0.04 | 53 | 0.13 | 83 | 0.76 | 9 | 0.67 | 14 |
| 40 | <i>trans</i> - β -ionon-5,6-epoxide | | | C | 0.04 | 57 | 0.03 | 37 | 0.12 | 9 | 0.13 | 19 |
| 41 | β -ionone | Fluka | 1965 | A | 17.26 | 29 | 11.70 | 15 | 22.49 | 12 | 20.29 | 8 |
| 42 | dihydro β -ionol | | | B*** | 0.04 | 173 | 0.08 | 97 | 0.09 | 15 | 0.14 | 9 |
| 43 | 3,4-didehydro- β -ionone | | | C | 0.07 | 94 | 0.07 | 71 | 0.11 | 16 | 0.08 | 10 |
| 44 | caryophyllene oxide | | | B* | 0.01 | 83 | 0.02 | 56 | | | 0.04 | 10 |
| 45 | α -cyclogeranyl acetate | | | C | 0.09 | 62 | 0.06 | 48 | 0.19 | 15 | 0.28 | 19 |
| 46 | δ -decalactone | | | C | 0.01 | 133 | 0.01 | 65 | 0.03 | 11 | 0.02 | 15 |

^a Data expressed as a percent of the total area. ^b Retention indices on the poly(ethylene glycol) (PEG) column. ^c Reliability of the identification proposal: A, mass spectrum and RI agreed with standards; B, mass spectrum and RI agreed with the literature data (*, ref 45; **, refs (2 and 30); ***, ref 6); C, mass spectrum agreed with the mass spectral database.

basis of the papers of Larsen and co-workers (4) and Robertson and co-workers (9). The panel rated the intensity of each sensory attribute on a 100 mm unstructured scale, anchored at each end, and data were collected using the Fizz computer system (Biosystemes, Couternon, France). In the same session, the panel evaluated six products (six different cultivars: Polka and Tulameen, the cultivars of this study; and four other cultivars, Heritage, Himbotop, Polana, and Popiel; data not shown) and each product was replicated 3 times in three successive sessions with samples related to three different batches (see above). A balanced-block serving order across products and panelists was used, and the products (three berries) were presented at room temperature in transparent plastic-covered cups coded with a three digit random numbers. Here, we consider only the data of the odor and flavor attributes (panel mean intensities) recorded for Polka and Tulameen cultivars.

Data Analysis. Summary statistics were performed using software package Statistica 8.0 (StatSoft, Inc., Tulsa, OK). Principal component analysis (PCA) was computed by the software The Unscrambler 8.5 (Camo Process AS, Oslo, Norway), and both GC and PTR–MS data were normalized prior to the analysis. Online measurement data were processed by the software Origin Pro 7.5 (OriginLab Corporation, Northampton, MA).

RESULTS AND DISCUSSION

Headspace Analysis of Raspberry Fruits by SPME/GC–MS. The GC–MS analysis allowed for the identification of 28 compounds and tentative identification of a further 18 compounds present in the headspace chromatogram of fruits of both raspberry varieties (Table 1): 25 terpenes, 6 alcohols, 3 esters, 3

Table 2. Odor and Flavor Sensory Descriptors of Raspberry Fruits (cv. Polka and Tulameen)

| odor (by smelling) | cultivar discrimination ^a | flavor (by tasting) | cultivar discrimination ^a |
|-----------------------|---|------------------------|---|
| real raspberry | 0.699 | real raspberry | 0.049 |
| artificial raspberry | 0.988 | artificial raspberry | 0.729 |
| ripe fruit | 0.331 | ripe fruit | 0.757 |
| citrus/lemon | 0.122 | citrus/lemon | 0.153 |
| violet | 0.237 | violet | 0.655 |
| rose | 0.233 | rose | 0.642 |
| grass | 0.056 | grass | 0.060 |
| hay/tea | 0.260 | hay/tea | 0.286 |
| balsamic | 0.717 | balsamic | 0.852 |

^a Discrimination between cultivars based on the *t* test (in bold, $p < 0.1$).

ketones, 4 aldehydes, 2 acids, 1 furane, 1 lactone, and 1 hydrocarbon.

Acetate esters are the compounds with the highest concentration in both cultivars representing 38.7 and 32.2% of total volatiles of Polka and Tulameen, respectively. Terpenes family is the most represented class of compounds, including 12 monoterpenes (8 hydrocarbons, 3 alcohols, and 1 ester), 11 C13-norisoprenoids (9 with a megastigmane skeleton, of which 1 was from the damascone series and 8 were from the ionone series), and 3 sesquiterpenes (1 unknown and 1 hydrocarbon and its oxide). Among the C13-isoprenoids that represent the 36.6 and 26.8% of total volatiles found in Polka and Tulameen, respectively, α -ionone, β -ionone, and an unidentified ionone (peak number 31) show the highest concentrations (Table 1). The first two ionones are indicated as the most relevant for the aroma of raspberries (4) and, in our data, are about the same concentration for both varieties. For the unidentified ionone, on the basis of the relative position on chromatograms from literature data (2, 30), we suggest two possible candidates: cyclo-ionone and edulan.

When the data for the two varieties are compared, we see that, in general, the intensity is higher for Tulameen fruits and, in particular, seven compounds show significant differences (*t* test, $p < 0.05$): hexanal, *trans*-2-hexenal, hexanol, theaspirane A, *trans*-caryophyllene, and acetophenone are, respectively, about 9.5, 5.0, 4.8, 3.5, 12.0, and 3.0 times higher in Tulameen, while only ethyl acetate is about 3.1 times higher in Polka. Alcohols and aldehydes are more abundant in the headspace of Tulameen, and most of these C5 and C6 compounds induce herbaceous odor notes (31).

Sensory Analysis. The panel developed an evaluation protocol containing 37 attributes, related to visual appearance (10), taste (3), tactile/texture sensation (6), odor by smelling (9), and flavor by tasting (9). Table 2 shows the comparison of odor and flavor sensory descriptors of raspberry fruits. The most significant differences between the two raspberry varieties identified by sensory analysis are for the attributes "real raspberry" flavor ($p = 0.049$), "grass" odor ($p = 0.056$), and "grass" flavor ($p = 0.060$). Tulameen berries were scored higher than Polka berries for all three attributes. With regard to "grass" attributes, the observations are in accordance with the higher emission of Tulameen for the above-mentioned C6 compounds having herbaceous odor note. In the case of the "real raspberry" flavor, the attribute is used with precision and reliability by the panel but the link with the volatile compounds is probably more complex and could be related to raspberry ketone (*p*-hydroxyphenyl-3-butanone), one of the key compounds of raspberry flavor (4), but not easily detectable by SPME techniques because of its low volatility (32).

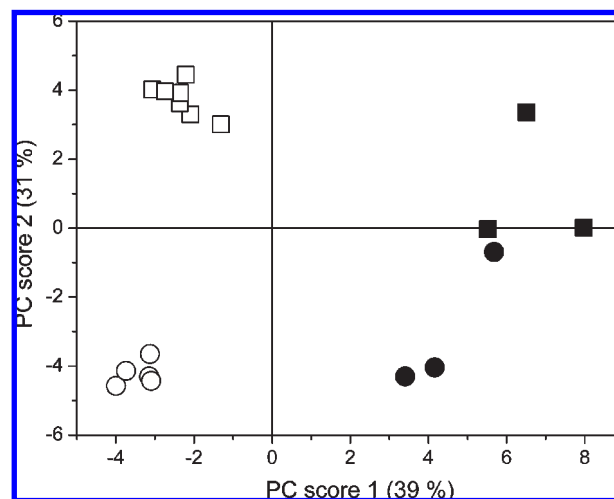


Figure 1. SPME/GC-MS data. PCA of mashed fruits and juices. (■) Tulameen berries, (●) Polka berries, (□) Tulameen juices, and (○) Polka juices.

For all of the other odor and flavor sensory attributes, we did not find significant differences ($p < 0.1$) between the two cultivars (Table 2).

Headspace Analysis of Raspberry Juices by SPME/GC-MS. GC data referring to juices reported in Table 1 show a CV% that is between 4 and 25%, with a mean value of 11%, indicating a good repeatability of the method considering the manual procedure adopted. The same compounds identified in the headspace of juices are also present in the headspace of mashed fruits (Table 1), with the exception of *trans*-3-methyl-1,3,5-hexatriene, α - and β -phellandrene, γ -terpinene, and caryophyllene oxide that were not detected in the Polka juices. There are however quantitative differences. In the case of fruits, the variability is much higher, showing a CV% between 2 and 173%, with a mean value of 65%. The highest variations were observed for those compounds found in very small amounts.

The relative intensity of esters tends to decrease in the juices. On the other hand, C13-norisoprenoids strongly increase and a moderate increase is also observed for monoterpenes. A higher concentration of monoterpenes and C13-norisoprenoids is probably due to the enzymatic hydrolysis of these compounds from their corresponding glucosides (5).

Alcohols, aldehydes, and ketones with more than six carbons increase, whereas hexanal, *trans*-2-hexenal, and *cis*-3-hexenol decrease. The behavior of these three latter compounds will be further discussed below in the section on real-time data. Here, we want to mention that these three compounds are released in a great amount as a consequence of fruit mashing, but their concentration in juices easily decrease (33–36). We also observed that, during supercritical gas pasteurization of apple juices, the highest volatile reduction was observed for *trans*-2-hexenal and hexanal with, respectively, the 77 and 37% of the initial amount (37).

To summarize and visualize information presented in Table 1 in a concise way, GC data were analyzed by PCA and the biplot of the first two principal components is reported in Figure 1. There are strong differences between mashed fruits and juices, and these differences account for 39% of the variance of the data. The biplot also shows differences between mashed berries of the two varieties as well as, to a major extent, between juices. Data of mashed berries are more dispersed, reflecting the higher variability of the measurements. These data indicate

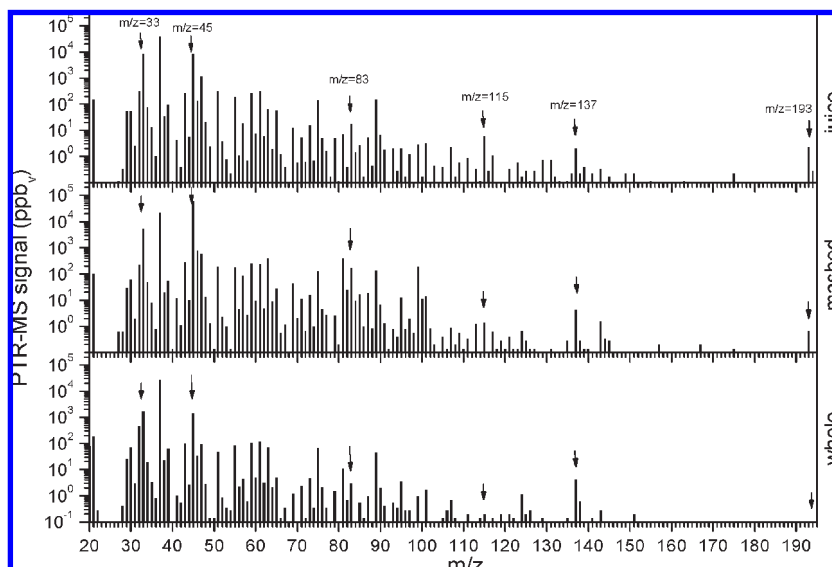


Figure 2. Comparison of PTR-MS spectra for whole intact berry, mashed berry, and juice. Arrows indicate a few interesting peaks quantitatively different among the three spectra. m/z 33, methanol; m/z 45, acetaldehyde; m/z 83, C6-VOCs; m/z 115, 2-heptanone; m/z 137, monoterpenes; and m/z 193, C13-norisoprenoids.

that differences in volatile composition of different raspberry varieties influence the juices produced.

Headspace Analysis of Raspberry Fruits and Juices by PTR-MS. The raspberry fruits and juices were also evaluated by PTR-MS that allows for a fast headspace profile by direct injection of volatiles. **Figure 2** reports three examples of spectra obtained for the headspace of the raspberry samples: whole intact berry, mashed berry, and juice. **Table 3** reports the averaged intensities and coefficient of variation (in a percentage) for a few selected masses measured by PTR-MS using whole intact berry, mashed berry, and juice samples for both Tulameen and Polka. Tentative peak attribution based on GC results of the present study and fragmentation patterns of pure compounds acquired in our laboratories and partly reported in previous works (18, 38, 39) is reported in **Table 3**. Aware of the risk of such peak attribution (21, 23), we want to outline that PTR-MS spectra are not anonymous but contain important chemical information. As for GC data, the variability of the PTR-MS determinations (**Table 3**) is higher for the mashed fruits than juices and even higher for the intact fruits. This data dispersion reflects the natural variability of the sampled berries. The total headspace volatile concentration of mashed Polka fruits appears to be more abundant than that of Tulameen, a reverse situation evidenced by SPME/GC-MS analysis. This apparent discrepancy is due to the presence of three compounds not measured by SPME/GC-MS: methanol, ethanol, and acetaldehyde (**Table 3**). Excluding these three latter compounds that account for 90–98% of the total headspace concentration, Tulameen shows higher volatile compound concentrations than Polka. In the PTR-MS profile, after methanol, ethanol, and acetaldehyde, esters represent the main class of compounds recorded, as observed for SPME/GC-MS analysis, thus providing similar information but in a faster way with the possibility to measure more samples at the same time compared to the SPME method.

An easy way to explore data and visualize the presence of possible sample clustering is to manage PTR-MS spectra through the PCA (14). In **Figure 3**, the first two principal components obtained with data of freshly mashed berries and juices are plotted. As for GC data, the first component separates juices from mashed fruits, while the second component shows

the separation between the varieties only for the mashed fruits. In this specific case, the higher dispersion of mashed fruit data, compared to juice data, compresses the information along the second component, masking differences in the case of juices. When the data of mashed berries are removed, the PCA shows the differences between the juices as reported in the small window in **Figure 3**.

The PCA on whole intact fruits shows a complete separation between the two varieties (**Figure 4**), indicating that PTR-MS sensitivity is sufficient to catch the differences between the two varieties based on nondestructive VOC measurements. Thus, PTR-MS has been shown to give, in a fast way, comparable information on the overall flavor profile.

On one side, it turned out that the volatile profiles of the cultivars investigated are indeed very different for both fresh fruits and juices, suggesting that these data, possibly extended to a wider set of cultivars, should support proper breeding programs. On the other side, we showed the complementarity of GC and PTR-MS and suggest that their coupling allows for a wider vision on the volatile profile of fruits. GC-MS analyses are indispensable for correct compound identification but are time-consuming and not suited for process monitoring. Moreover, GC-MS does not allow for the simultaneous detection of relevant compounds with very different polarity, as in our case, methanol, ethanol, and acetaldehyde together with other volatiles detected under chosen chromatographic conditions.

Real Time Monitoring. PTR-MS can be used, as shown above, for product characterization and discrimination, as indicated also in previous studies (15–17, 19, 22); however, it is particularly suited for on-line volatile compound analysis to follow very rapid processes (40, 41).

Here, we used PTR-MS to follow in real time the release of volatile compounds during the mashing. In general, the volatile emission increases after the crushing of the fruits because the physical barriers trapping these secondary plant metabolites are disrupted. Together with plant metabolites, several other compounds of neo-formation are released. In **Figure 5**, the time evolution of a few selected PTR-MS ion traces during the smashing of a raspberry fruit is reported. Naming is based on previous experiences (13,15,18) and literature data (24,25,28). The first cycles (first 7 min) report the signals recorded in an

Table 3. PTR–MS Most Relevant Signals Recorded in the Headspace of Whole, Mashed, and Juices Obtained from Polka and Tulameen Raspberries

| <i>m/z</i> | compounds ^b | Polka whole ^a | | Polka mashed ^a | | Polka juice ^a | | Tulameen whole ^a | | Tulameen mashed ^a | | Tulameen juice ^a | |
|------------|---|--------------------------|-----|---------------------------|-----|--------------------------|-----|-----------------------------|-----|------------------------------|-----|-----------------------------|-----|
| | | mean | CV% | mean | CV% | mean | CV% | mean | CV% | mean | CV% | mean | CV% |
| 33 | methanol | 3684 | 31 | 7421 | 40 | 4632 | 26 | 1930 | 76 | 4568 | 19 | 6189 | 27 |
| 43 | common fragment | 270.6 | 101 | 380.5 | 67 | 259.1 | 21 | 229.7 | 101 | 533.1 | 49 | 244.1 | 20 |
| 45 | acetaldehyde | 9652 | 77 | 102297 | 33 | 4691 | 32 | 10588 | 190 | 71783 | 18 | 7972 | 30 |
| 47 | ethanol | 770.9 | 62 | 2798.0 | 40 | 765.4 | 36 | 889.2 | 133 | 1845 | 93 | 778.4 | 32 |
| 51 | cluster (methanol/H ₂ O) | 101.9 | 31 | 297.3 | 46 | 143.8 | 37 | 62.5 | 77 | 173.8 | 18 | 184.8 | 40 |
| 57 | heptanol | 5.1 | 21 | 15.8 | 32 | 19.0 | 31 | 6.9 | 49 | 77.6 | 27 | 13.6 | 29 |
| 59 | acetone + propanal | 38.3 | 27 | 102.7 | 36 | 238.7 | 27 | 88.1 | 56 | 175.4 | 74 | 230.4 | 24 |
| 61 | acetate esters fragment + acetic acid | 377.7 | 115 | 386.1 | 94 | 319.6 | 22 | 299.8 | 104 | 577.1 | 64 | 303.8 | 24 |
| 63 | cluster (acetaldehyde/H ₂ O) + dimethyl sulfide | 64.0 | 68 | 642.0 | 43 | 39.4 | 29 | 93.9 | 95 | 463.2 | 31 | 51.0 | 30 |
| 65 | cluster (ethanol/H ₂ O) | 28.5 | 58 | 108.9 | 33 | 31.4 | 43 | 33.3 | 108 | 73.1 | 76 | 32.8 | 40 |
| 67 | common fragment of aldehydes | 0.25 | 56 | 0.69 | 49 | 0.22 | 62 | 0.22 | 77 | 1.2 | 41 | 0.32 | 63 |
| 69 | common fragment of aldehydes and terpenes | 1.6 | 13 | 36.3 | 39 | 8.8 | 33 | 2.0 | 34 | 47.4 | 26 | 12.5 | 31 |
| 71 | common fragment | 3.6 | 36 | 10.1 | 33 | 5.1 | 26 | 6.4 | 94 | 12.7 | 24 | 5.2 | 18 |
| 73 | aldehydes fragment + 2-butanone | 2.9 | 42 | 22.7 | 33 | 17.9 | 30 | 4.8 | 89 | 21.6 | 35 | 15.2 | 29 |
| 75 | propanoate esters fragment | 44.4 | 50 | 70.5 | 42 | 175.2 | 34 | 32.3 | 64 | 89.6 | 56 | 141.2 | 28 |
| 79 | acetate esters fragment + benzaldehyde fragment | 3.3 | 94 | 5.5 | 72 | 3.1 | 27 | 2.7 | 90 | 6.9 | 55 | 2.9 | 40 |
| 81 | terpene fragment | 0.84 | 25 | 47.3 | 53 | 1.1 | 26 | 3.1 | 103 | 360.9 | 37 | 4.8 | 39 |
| 83 | hexanal fragment | 1.3 | 28 | 39.5 | 70 | 4.5 | 35 | 2.5 | 45 | 162.9 | 31 | 16.5 | 34 |
| 85 | typical of C5 compounds | 0.60 | 50 | 2.1 | 40 | 1.4 | 31 | 0.86 | 56 | 15.8 | 29 | 1.8 | 46 |
| 87 | pentanal | 1.0 | 34 | 9.8 | 38 | 5.8 | 26 | 1.6 | 42 | 16.9 | 26 | 5.9 | 34 |
| 89 | ethyl acetate + acetoin | 178.9 | 113 | 257.2 | 87 | 140.3 | 43 | 128.1 | 148 | 358.5 | 73 | 133.8 | 37 |
| 91 | benzyl (–H ₂ O) | 1.2 | 81 | 9.5 | 40 | 1.0 | 43 | 1.6 | 175 | 6.3 | 81 | 1.2 | 37 |
| 93 | <i>p</i> -cymene | 1.9 | 38 | 4.0 | 41 | 1.1 | 43 | 2.7 | 89 | 2.2 | 107 | 1.5 | 28 |
| 95 | terpene fragment | 0.33 | 49 | 0.60 | 41 | 0.33 | 40 | 1.9 | 88 | 10.5 | 79 | 1.2 | 44 |
| 99 | hexenals + heptanol (–H ₂ O) | 0.62 | 50 | 25.0 | 52 | 1.0 | 21 | 0.79 | 43 | 182.8 | 33 | 1.7 | 43 |
| 101 | hexanal | 1.95 | 51 | 2.7 | 36 | 1.6 | 36 | 2.6 | 53 | 14.1 | 25 | 2.6 | 37 |
| 103 | esters fragment | 0.24 | 48 | 0.28 | 73 | 0.51 | 56 | 0.43 | 85 | 0.33 | 42 | 0.86 | 45 |
| 107 | benzaldehyde | 0.78 | 60 | 1.6 | 37 | 0.72 | 38 | 0.89 | 77 | 1.6 | 29 | 1.3 | 39 |
| 109 | benzyl alcohol | 0.18 | 59 | 0.49 | 53 | 0.35 | 68 | 0.22 | 71 | 0.51 | 42 | 0.41 | 55 |
| 111 | 1-octen-3-ol | 0.2 | 75 | 0.4 | 45 | 0.8 | 22 | 0.4 | 47 | 0.6 | 40 | 0.9 | 45 |
| 113 | 1-octanol (–H ₂ O) + 5-ethyl-(3 <i>H</i>)-furan-2-one | 0.12 | 86 | 0.45 | 66 | 0.26 | 58 | 0.11 | 100 | 1.2 | 39 | 0.37 | 36 |
| 115 | 2-heptanone | 0.26 | 80 | 1.2 | 57 | 7.5 | 31 | 0.25 | 68 | 1.4 | 38 | 5.9 | 30 |
| 117 | esters fragment | 0.10 | 88 | 0.43 | 78 | 0.62 | 48 | 0.40 | 75 | 0.43 | 58 | 0.89 | 46 |
| 137 | terpenes | 0.20 | 79 | 0.31 | 88 | 0.35 | 36 | 1.1 | 110 | 2.3 | 91 | 1.4 | 40 |
| 143 | <i>cis</i> -3-hexenyl acetate | 0.20 | 71 | 1.20 | 62 | 0.72 | 37 | 0.30 | 57 | 1.4 | 57 | 0.52 | 47 |
| 145 | hexyl acetate | 0.09 | 120 | 0.18 | 107 | 0.68 | 52 | 0.06 | 156 | 0.21 | 34 | 0.18 | 91 |
| 193 | α - + β -ionone | 0.06 | 130 | 1.1 | 55 | 0.82 | 50 | 0.07 | 100 | 0.72 | 33 | 1.1 | 68 |

^a Absolute concentration expressed as part per billion in volume (ppb). ^b Tentative identification based on results from Table 1 and the literature (refs (18, 38, and 39)).

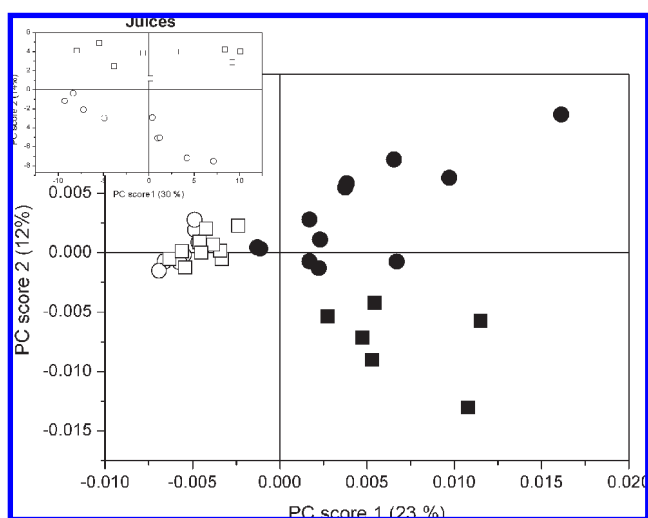


Figure 3. PTR–MS data. PCA of mashed fruits and juices. (■) Tulameen berries, (●) Polka berries, (□) Tulameen juices, and (○) Polka juices. In the small box is reported a PCA of only juice data.

empty vessel. After 7 min, the PTR–MS inlet is connected to a vessel with a raspberry fruit (first dotted line in Figure 5) and the signal of compounds corresponding to raspberry volatiles are

recorded for about 3–4 min before pushing down the crusher (second dotted line in Figure 5). A few seconds after mashing the fruit, volatile signals rapidly increase. Methanol, acetate ester, and acetic acid signals are 4–5 times higher after a few minutes, and *cis*-3-hexenol is 13 times higher, while the C6-VOC signal shows a peak 150 times higher after 4 min. These latter compounds together with C5-VOCs are typical wounding products emitted by leaves and fruits (24, 25) that originate from the lipoxygenase and hydroperoxide lyase pathways also studied in strawberries (28) and are responsible for the typical green notes of fruits and leaves.

This experiment clearly shows the differences between compounds originating from plant metabolism, such as acetate esters, and those compounds that are a direct consequence of tissue damages, such as C6-VOCs that, however, are produced also during product processing or consumption and have effects on sensory perception. These online experiments broaden the comprehension of the mechanisms lying beyond the formation and the release of flavor from fruits and also the modification occurring during juice production.

With PTR–MS, it is also possible to measure compounds that are not easily measurable by SPME/GC–MS because of their high volatility or peak overlapping. Let us consider, for example, a few compounds, such as methanol (even if measurable also by SPME), ethanol, and acetaldehyde (Figure 2).

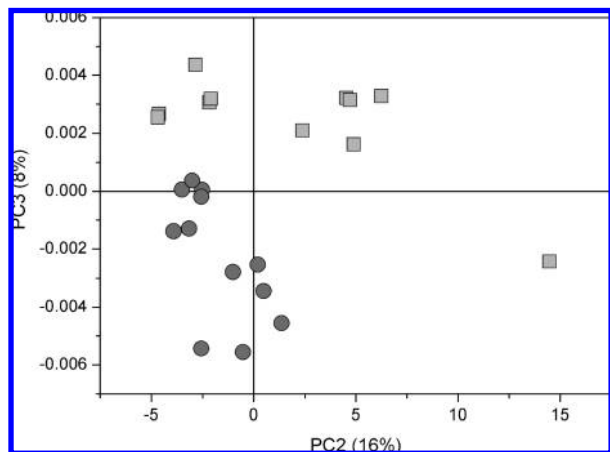


Figure 4. PTR-MS data. PCA of whole fruits. (light gray squares) Tulameen and (dark gray circles) Polka.

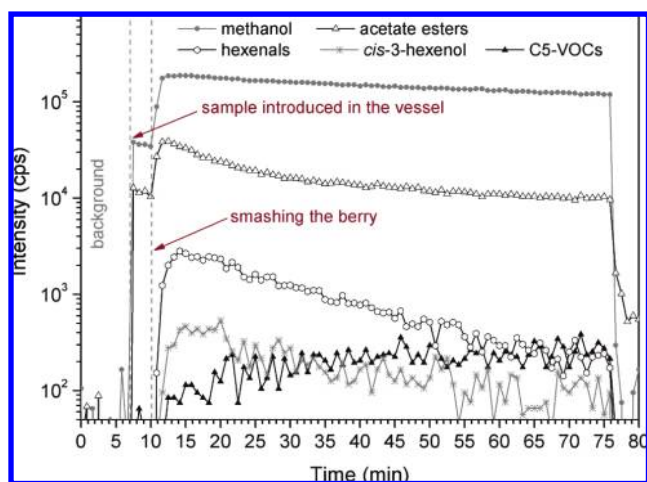


Figure 5. Release of a few selected VOCs during mashing of a raspberry fruit measured online by PTR-MS.

Frenkel et al. (42) proved that the production of methanol in tomato fruit is regulated by pectin methyl esterase that catalyzes the demethoxylation of pectin. Methanol was found to be the major volatile associated with aging of raspberries and is emitted in a concentration from about 5 ppm at picking to 40 ppm after 4 days of storage (13). In our intact samples, the methanol concentration between 1 and 5 ppm (Table 3) indicated that the softening process was not advanced, and thus, the freshness of the berries was comparable during the sampling period considered.

Ethanol and acetaldehyde are precursors of natural aroma compounds, and their increased production has been shown to occur in almost all of the fruit during ripening (43). Acetaldehyde is also emitted as a consequence of wounding (24), but its origin is still not completely explained (44). In raspberry, acetaldehyde increases on average 7–11 times after the mashing (Table 3), but the concentration found in the juices is lower than that at the moment of crushing; this is probably due to the high vapor pressure of this molecule that rapidly evaporates and is weakly retained by juices (37).

We confirm the high variability in the volatile profile among different raspberry cultivars; these differences can be of great relevance for the sensorial aspect as well as for industrial transformations. Differences in raw materials are not always the same as observed in transformed product; thus, the throughput

of a method such as PTR-MS can represent an important support for breeders and farmers and for quality monitoring of raw material for food industries. Extension to a wider set of cultivars is envisaged.

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