JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Analysis and Sensory Evaluation of Gooseberry (*Ribes uva crispa* L.) Volatiles

Katrin Hempfling,[†] Oxana Fastowski,[†] Markus Kopp,[§] Martin Pour Nikfardjam,[§] and Karl-Heinz Engel*[,†]

[†]Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Maximus-von-Imhof-Forum 2, D-85350 Freising-Weihenstephan, Germany

[§]Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg, Traubenplatz 5, D-74189 Weinsberg, Germany

Supporting Information

ABSTRACT: Volatiles of gooseberries (*Ribes uva cripsa* L.) were isolated by means of vacuum-headspace-extraction, and the obtained concentrates were analyzed via capillary gas chromatography−mass spectrometry. To ensure the quantitation of highly volatile compounds, headspace analysis was additionally performed on selected batches. C_6-components (e.g., (*Z*)-hex-3-enal, (*E*)-hex-2-enal), derived from lipid oxidation, and short-chain esters (e.g., ethyl acetate, methyl butanoate, ethyl butanoate) turned out to be the major compound classes in the fresh fruit. The compositional variability was demonstrated by analyzing several gooseberry varieties at different stages of ripeness. The contributions of volatiles to the gooseberry aroma were assessed by using gas chromatography−olfactometry in combination with aroma extract dilution analysis and calculation of odor activity values. C_6-components and esters were shown to be responsible for the green and fruity character of fresh gooseberries.

KEYWORDS: *Ribes uva crispa L., gooseberry, volatiles, aroma, GC-MS, GC-O*

INTRODUCTION

Gooseberries (*Ribes uva crispa* L.) have been cultivated in Europe since the beginning of the 17th century. Although their popularity has declined over the past few years, they are still widely consumed as fresh fruits as well as used in the preparation of desserts, juices, or jams; the worldwide annual production of gooseberries amounts to approximately 160 000 t.[1]

Gooseberries belong to the genus *Ribes* L. The commercially available cultivated gooseberries (*Ribes uva crispa* L. var. *sativum*) comprise many varieties differing in color, such as Achilles (red) and Invicta (green).[2] For commercial purposes, only a few varieties are important, and the red fruits are of particular interest.

Compositional data on gooseberries are scarce. Early studies revealed malic acid and citric acid as major nonvolatile organic acids.[3] Later investigations dealt with yield parameters and the impact of storage conditions on fruit quality.[4−6] Recent analyses focused mainly on phenolic compounds and the antioxidative properties of gooseberries.[7−10] Studies on flavor compounds are limited to the identification of the natural precursors of isomeric vitispiranes in gooseberry leaves and the determination of the enantiomeric distributions of theaspirane isomers, and to the identification of free and bound formic and acetic acid in gooseberry fruits.[11−13]

So far, data on the aroma profile of gooseberries are lacking. Therefore, the objectives of the present study were (i) to identify and to quantify volatile gooseberry constituents, (ii) to demonstrate the degree of variability in the volatile composition, and (iii) to assess the contributions of compounds to the overall aroma by gas chromatography−olfactometry (GC-O) and sensory evaluations.

MATERIALS AND METHODS

Gooseberry Material. (i) Commercially obtained gooseberries (seven batches of red gooseberries var. Achilles and four batches of red gooseberries, nonspecified with regard to their variety) were purchased at a wholesale market in Munich, Germany, at different times (Achilles: July 16, 2012; July 26, 2011; July 25, 2011; July 20, 2011; August 25, 2010; August 5, 2010; July 12, 2010; other red gooseberries: August 19, 2010; August 13, 2010; July 13, 2010; July 5, 2010). The fruits were declared to originate from southern Germany. The berries were not consistently and completely reddened; no information on the date of harvest was available. (ii) Gooseberries harvested at different times in 2012 were obtained from Staatliche Lehr- and Versuchsanstalt für Wein- and Obstbau Weinsberg (LVWO; state research institute for viticulture and pomiculture). The stages of ripeness were evaluated by experienced agronomists on the basis of color and firmness. The following red varieties were harvested at the ripe state (bright red color, soft texture): Frühe Rote (June 18, 2012), Rote Eva (July 2, 2012), Tixia (June 18, 2012), Xenia (June 18, 2012), and Bekay (June 25, 2012). In addition, the two green varieties Invicta (June 14, 2012) and Späte Spitze (June 25, 2012) were obtained. Gooseberry var. Bekay and Xenia were also harvested at the underripe (green color, very firm texture) and overripe (dark red color; very soft texture) stages on the following dates: June 18 and 25, 2012 (Xenia); June 25 and July 10, 2012 (Bekay). All berries were analyzed within 2 days after purchase and harvest, respectively. Until analysis they were stored at 4 °C.

Chemicals. Authentic reference chemicals were purchased from commercial sources (Aldrich, Steinheim, Germany; Merck, Darmstadt, Germany) or provided by Frey+Lau GmbH (Henstedt-Ulzburg, Germany). Heptan-2-ol was purchased from Fluka (Steinheim, Germany); [$^2H_2$]-(*Z*)-hex-3-enal (dissolved in *n*-pentane) from aromaLab AG (Freising, Germany); and sodium sulfate from Merck (Darmstadt, Germany). Citric acid, hydrochloric acid, and sodium hydroxide were from Sigma-Aldrich (Steinheim, Germany), and calcium chloride was from Roth (Karlsruhe, Germany). All chemicals

Received: March 24, 2013
Revised: May 22, 2013
Accepted: May 30, 2013
Published: May 30, 2013

dx.doi.org/10.1021/jf401310v | J. Agric. Food Chem. 2013, 61, 6240−6249

used were of analytical grade. The solvents diethyl ether (Honeywell Burdick & Jackson, Seelze, Germany) and *n*-pentane (AppliChem, Darmstadt, Germany) were distilled before use.

Isolation of Volatiles by Vacuum-Headspace Extraction (VHS). Before analysis, stored fruits were brought to room temperature (approximately 2 h). After removal of the peduncles, 500 g of gooseberries was homogenized (Moulinex Turbo blender) with 400 mL of water for 30 s. After the addition of the internal standard (heptan-2-ol, 150 μg), the homogenate was transferred into a 2 L round-bottom flask; the blender was rinsed with 150 mL of water. The flask was then placed into a water bath (35 °C), and the isolation was carried out for 2 h at a vacuum of 1−10 mbar (Leybold-Hereus pump, typ D4A). The aqueous distillate was condensed in three cooling traps. The first two were cooled by a water−ice−mixture, and the third was cooled by liquid nitrogen. After thawing, the distillates were pooled and extracted (3 × 50 mL) using a mixture of diethyl ether and *n*-pentane (1:1; v/v). After drying with sodium sulfate, the extract was concentrated to 1 mL using a Vigreux column and to a final volume of 0.5 mL under a gentle nitrogen flow. Enzyme inhibition trials were performed by adding 550 mL of saturated calcium chloride solution instead of water 30, 60, 90, and 180 s, respectively, after homogenization of the fruits, followed by isolation as described above. All VHS isolations, except the inhibition trials, were carried out in triplicate.

Capillary Gas Chromatography (HRGC-FID). The separations were performed on a Carlo Erba Mega II 8575 series gas chromatograph (Thermo Fisher Scientific, Dreieich, Germany) equipped with a split/splitless injector (215 °C, split ratio 1:10), a flame ionization detector (FID), and a flame photometric detector (FPD) operating at 235 °C. The column used was a 60 m × 0.32 mm (i.d.) fused silica capillary column coated with DB-Wax (0.25 μm film thickness; J&W Scientific). The oven temperature was programmed from 40 °C (5 min hold) at 4 °C/min to 240 °C (25 min hold). The carrier gas used was hydrogen (5.0 grade, Westfalen AG, Münster, Deutschland) at a constant inlet pressure of 110 kPa. Data acquisition was done via Chromcard software (Thermo Fisher Scientific).

Quantitation. Quantitations were performed using heptan-2-ol as internal standard; 1 mL of a 1:10 diluted stock solution (0.150 g heptan-2-ol/100 mL water) was added to the gooseberry material prior to the extraction. FID response factors were determined with solutions of authentic compounds relative to the internal standard (0.1 μg/μL in diethyl ether). Recovery rates were determined in triplicate from aqueous solutions and buffer solutions (hydrochloric acid−sodium citrate buffer, pH 3.5) for acids, respectively; 100 μL of stock solution (3 mg reference and 3 mg heptan-2-ol in 1 mL ethanol) was isolated from 1 L water or buffer by means of VHS. Recovery rates were determined for main representatives of the different compound classes: methyl butanoate (63 ± 18%), ethyl butanoate (76 ± 8%), methyl (*E*)-but-2-enoate (82 ± 5%), ethyl (*E*)-but-2-enoate (87 ± 3%), methyl hexanoate (76 ± 6%), methyl benzoate (87 ± 13%), oct-1-en-3-ol (101 ± 0%), (*E*)-hex-2-en-1-ol (77 ± 7%), (*Z*)-hex-3-en-1-ol (76 ± 5%), hexanol (82 ± 2%), hexanal (86 ± 4%), (*E*)-hex-2-enal (85 ± 6%), (*E*)-hex-3-enal (29 ± 7%), (*Z*)-hex-3-enal (30 ± 10%), acetophenone (92 ± 4%), and pentan-2-one (51 ± 3%). The relatively low recovery rate of (*Z*)-hex-3-enal was confirmed using 10 times higher and 10 times lower amounts, respectively, of the reference compound. Recovery rates of acetic acid, propanoic acid, butanoic acid, (*E*)-hex-2-enoic acid, (*E*)-hex-3-enoic acid, dimethylmalonic acid, cinnamic acid, butane-2,3-diol, acetone, Furaneol, mesifuran, ethyl acetate, and ethyl formate were <13%, and therefore these substances were not quantified. The limits of detection and the limits of quantitation were determined for octanal, (*E*)-oct-2-enal, ethyl hexanoate, methyl 3-hydroxybutanoate, and pent-1-en-3-ol as representatives, using the method of Hädrich and Vogelgesang[14,15] Four concentrations in the range from 625 to 6250 ng/mL were analyzed in triplicate, and by determining a calibration curve, the limits of detection and the limits of quantitation were calculated (assumption: recovery rate and response factor = 1).

Quantitation by [$^2H_2$]-(*Z*)-Hex-3-enal. To determine the exact concentration of the standard solution, the following approach was used: an FID response factor was determined by GC analysis of a solution containing defined amounts of (*Z*)-hex-3-enal and heptan-2-ol as reference standard. Subsequently, a defined amount of heptan-2-ol was added to a defined volume of the solution containing the labeled compound. This mixture was analyzed by GC-FID, and the concentration of [$^2H_2$]-(*Z*)-hex-3-enal was calculated from the peak areas, using the FID response factor determined for the unlabeled compound. The quantitation of (*Z*)-hex-3-enal by [$^2H_2$]-(*Z*)-hex-3-enal was performed on a gas chromatograph−mass spectrometer (for GC parameters, see next paragraph) in the selective ion monitoring mode (labeled standard, *m*/*z* 85; unlabeled compound, *m*/*z* 83). A calibration factor was established by analyzing mixtures of defined amounts of the labeled and unlabeled compounds in different mass ratios (1:3 to 3:1).

Gas Chromatography−Mass Spectrometry (GC-MS). Mass spectral data were obtained on a gas chromatograph−mass spectrometer (GC 8000^{TOP} with a Voyager GC-MS, Thermo Fisher Scientific) equipped with a split/splitless injector (220 °C, split ratio 1:50). The separation was performed on a 30 m × 0.25 mm (i.d.) fused silica capillary column coated with DB-WaxEtr (0.5 μm film thickness; J&W Scientific). The oven temperature was programmed from 40 °C (5 min hold) at 4 °C/min to 240 °C (25 min hold). The carrier gas used was helium (5.0 grade, Westfalen AG, Münster, Germany) at a constant inlet pressure of 75 kPa. Ionization was set at 70 eV, source temperature at 200 °C, and interface temperature at 240 °C. Data acquisition was done via Xcalibur software, version 1.4 (Thermo Fisher Scientific).

Headspace−Gas Chromatography−Mass Spectrometry (HS-GC-MS). Static headspace analysis was performed on a Clarus 600 GC combined with a Turbo Matrix 40 Trap HS Sampler from Perkin-Elmer for *Ribes uva crispa sativum* var. Bekay, Späte Spitze, Xenia, Tixia, Rote Eva, Frühe Rote, and Invicta. Gooseberries (100 g) were homogenized for 2.5 min, and 7 g of the homogenate was weighed into a headspace vial (neoLab Migge, 20 mL). The headspace conditions were as follows: 37 °C sample equilibration temperature; 60 min sample equilibration time; 25 psi vial pressurization; adsorption material, Tenax TA 60/80 mesh; thermal elution at 40−280 °C. The GC conditions were as follows: The separation was performed on a 30 m × 0.25 mm (i.d.) fused silica capillary column coated with ZB-624 (1.4 μm film thickness; Phenomenex). The oven temperature was programmed from 40 °C (5 min hold) at 20 °C/min to 220 °C (5 min hold). Helium (5.5 grade, Linde, Pullach, Germany) was used as carrier gas at a constant inlet pressure of 20 psi. Ionization was set at 70 eV, and source and interface temperatures were set at 180 °C. Data acquisition was done via Turbo Mass software, version 5.4.2 (Perkin-Elmer). Identification was done by the comparison of mass spectral data and retention times with those of authentic references. Quantitation was done by an external calibration for each compound according to its concentration ranges in the berries. Standard solutions were adjusted to pH 3.2 using orthophosphoric acid. Headspace extraction was performed only once for each ripe gooseberry variety, because gooseberry material was limited.

Gas Chromatography−Olfactometry (GC-O). The GC system consisted of a Carlo Erba Strumentazione 4200 gas chromatograph equipped with an FID (230 °C) and a sniffing port (230 °C), using a deactivated capillary column (30 cm) and a split/splitless injector (220 °C, split ratio 1:10). Volatiles were separated on a 60 m × 0.32 mm (i.d.) fused silica capillary column (injection volume = 1 μL) coated with DB-Wax (0.25 μm film thickness; J&W Scientific). Injector and detectors were set at 220 and 230 °C, respectively. The oven temperature was programmed from 55 °C (10 min hold) at 4°/min to 240 °C (25 min hold). Hydrogen (5.0 grade, Westfalen AG, Münster, Germany) was used as carrier gas at a constant inlet pressure of 110 kPa. The GC effluent was split 1:1 among FID and sniffing port; no humidified air or nitrogen was used.

Statistical Analysis. XLSTAT (Addinsoft, version 2008.4.01) was used for statistical tests (confidence interval for all tests = 95%). Correlation analyses were carried out with ANOVA (levels of significance: p = 0−0.001, highly significant (***); p = 0.001−0.01, very

significant (**); $p = 0.01-0.05$, significant (*)). Statistically significant differences were identified by Tukey's HSC.

AEDA. Nine extracts (500 μL each) obtained by triplicate VHS from three batches (Achilles, July 12, 2010; and red gooseberries, unspecified regarding their variety, July 5 and 13, 2010) were combined and gently concentrated to 1 mL under nitrogen flow. The concentrated extract was diluted gradually with the solvent mixture of diethyl ether and *n*-pentane (1:1) and analyzed by GC-O until no odor was detectable. AEDA was done by one panelist.

Determination of Odor Thresholds. Odor thresholds were determined by a panel (at least 10 participants) in a triangle test using the "forced choice" technique. The compounds were assessed in water.

Reconstitution Experiments. The reconstitution model was prepared on the basis of the concentrations of aroma-active compounds determined in the batch of gooseberries purchased on August 5, 2010: (*Z*)-hex-3-enal (1279 μg/mL), (*R*)-oct-1-en-3-ol (61 μg/mL), ethyl butanoate (136 μg/mL), methyl butanoate (858 μg/mL), (*E*)-hex-2-enal (1046 μg/mL), (*Z*)-hex-3-en-1-ol (167 μg/mL), acetophenone (121 μg/mL), ethyl hexanoate (4 μg/mL), (*E*)-methyl but-2-enoate (293 μg/mL), and methyl decanaote (3 μg/mL). Appropriate amounts of stock solutions of the odorants were dissolved in water.

Aroma Profile Tests. Samples (15 mL) were placed into glasses with lids and were orthonasally evaluated by a sensory panel of at least 10 assessors. Descriptors were determined in preliminary evaluations on the basis of the odor properties of reference compounds dissolved in water at concentrations 100 times above their odor thresholds. The following combinations of reference odorants and odor descriptions (given in parentheses) were used: ethyl butanoate (pineapple-like), methyl butanoate (green-fruity), (*E*)-hex-2-enal (apple-like), (*Z*)-hex-3-enal (grassy), acetic acid (sour), acetophenone (sweet-floral), (*R*)-oct-1-en-3-ol (mushroom-like), and (*E*)-methyl but-2-enoate (musty). Assessors were asked to rate each descriptor in the samples presented on a seven-point scale from 0 (not detectable) to 3 (strong). The sensory evaluation of the fresh gooseberries was performed with the cut fruit within 30 s. For each descriptor a new, intact berry was used.

RESULTS AND DISCUSSION

Identification and Quantitation of Volatile Constituents by VHS. Volatile constituents of gooseberries were isolated by means of vacuum-headspace extraction. This gentle method, proceeding without significant thermal treatment, has previously been shown to be suitable for isolating volatiles from fresh plant material.[16] Using passion fruits as an example, the technique proved to be suitable to generate extracts exhibiting the sensory impressions of the fresh fruits.[17,18] In this study, the obtained aqueous distillates also exhibited typical gooseberry aroma, characterized by a combination of green and fruity notes.

One part of the gooseberry material was obtained from a local market. In addition to these commercially available gooseberries, fruits of known variety and origin, harvested at the fully ripe state at defined times, were investigated. The VHS extracts were analyzed by GC-FID and GC-MS. In total, 122 compounds were identified in the analyzed batches, 20 of them tentatively. As example, Table 1 shows the data obtained by triplicate analysis of a batch of the variety Achilles; this was selected because it is one of the commercially most important gooseberry varieties. The main substances identified in this batch were also quantitatively dominating in the other 17 gooseberry batches investigated in this study. In addition to the compounds listed in Table 1, 19 alcohols, 18 esters, 16 ketones, 6 aldehydes, 3 acids, and 1 furan as well as theaspiranes I and II have been identified. However, they were present only at low concentrations and not consistently detectable in all batches (information on the identities is provided in the Supporting Information).

The determination of recoveries demonstrated that the employed VHS technique is suitable for the isolation of volatile substances. However, it also revealed that this isolation approach discriminates against nonvolatile as well as polar compounds (see Materials and Methods). Similar effects were already detected in an earlier study on rhubarb.[16] Major organic acids of gooseberries such as malic acid and citric acid were not detected by the employed method. The VHS recovery rates of other short- and medium-chain fatty acids were also low, so that no quantitation could be performed. However, these constituents were detectable in numerous batches investigated and are therefore listed in Table 1.

The volatile profile of gooseberries is quantitatively dominated by three compounds, present in high concentrations: (*Z*)-hex-3-enal, (*E*)-hex-2-enal, and methyl butanoate account for a minimum of 70% of total volatiles. Other compounds occur in relatively low concentrations. The volatile profile is characterized by a high proportion of C_6-compounds and esters, representing at least 88% of the volatiles in ripe fruits. (*Z*)-Hex-3-enal and its isomerization product (*E*)-hex-2-enal as well as the corresponding alcohols (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol are typical secondary flavor compounds, formed enzymatically from linolenic acid after disruption of the cell structure. They play important roles in the plant's defense strategies and pest resistance and are widely used as flavoring substances because of their fresh, green odor.[19,20] Oct-1-en-3-ol is also a compound resulting from lipid oxidation.[21] The (*R*)-configuration supports the enzyme-catalyzed formation from linoleic acid.[22] An equally high percentage of C_6-compounds is known from other fruits such as rhubarb, tomatoes, nectarines, and kiwis,[16,23−25] but unlike most other systems strongly affected by compounds with C_6-skeletons, the volatile profile of gooseberries is not dominated by (*E*)-hex-2-enal or (*E*)-hex-2-en-1-ol, but by the corresponding aldehyde (*Z*)-hex-3-enal.[16,24−26] The volatile spectra of only a few fruits, such as pink guava and tomato, are dominated by (*Z*)-hex-3-enal.[23,27]

To follow the dynamics of the formation of C_6-compounds over a time period, enzyme activities were inhibited at different time points up to 3 min by addition of saturated aqueous calcium chloride solution. As shown in Figure 1, the total amounts of C_6-compounds increased over time; however, the preponderance of the aldehyde (*Z*)-hex-3-enal remained. In contrast to other plant systems, such as rhubarb, isomerizations and reductions to the corresponding alcohols seem to play minor roles in gooseberries, rendering the proportions of C_6-compounds rather stable.[16]

Taking into account the known instability of (*Z*)-hex-3-enal, its quantitation via heptan-2-ol was confirmed by experiments using $[^2H_2]$-(*Z*)-hex-3-enal as standard.[28−30] VHS extractions were performed from the same batch of gooseberries after the addition of heptan-2-ol and the isotopically labeled compound, respectively; enzymes were inhibited after 90 s using saturated calcium chloride solution. Duplicate experiments resulted in contents of (*Z*)-hex-3-enal of 2042 and 1701 μg/kg when using heptan-2-ol and 1960 and 1941 μg/kg when employing $[^2H_2]$-(*Z*)-hex-3-enal as internal standard.

Another substance class characteristic of the volatile profile of gooseberries is represented by the esters. In contrast to C_6-compounds, esters are primary flavor compounds, which already exist in the intact tissue due to biogenesis of the fruit. Most abundant in gooseberry extracts obtained by VHS are short-chain esters, especially saturated and unsaturated butanoic acid esters. Methyl and ethyl butanoate are common esters and can be found in numerous fruits.[17,25,26,31] Having a look at their natural occurrence, concentrations of ethyl and methyl esters are either mostly of the same order of magnitude

Table 1. Volatile Constituents Isolated from a Batch (August 5, 2010) of Gooseberries var. Achilles by VHS

compound	RI[a]	μg/kg[b]	remark	compound	RI[a]	μg/kg[b]	remark
C_6-compounds							
(*Z*)-hex-3-enal	1139	1279 ± 237	*c, e*	hexanal	1076	21 ± 3	*c, e*
(*E*)-hex-2-enal	1209	1046 ± 208	*c, e*	hexanol	1355	7 ± 2	*c, e*
(*E*)-hex-2-en-1-ol	1407	179 ± 61	*c, e*	(*E*)-hex-3-en-1-ol	1363	7 ± 2	*c, f*
(*Z*)-hex-3-en-1-ol	1384	167 ± 51	*c, e*	(*Z*)-hex-2-enal	1194	4 ± 1	*d, g*
(*E*)-hex-3-enal	1133	46 ± 12	*c, e*				
esters							
methyl butanoate	981	858 ± 239	*c, e*	methyl benzoate	1613	4 ± 1	*c, e*
(*E*)-methyl but-2-enoate	1101	293 ± 10	*c, e*	ethyl hexanoate	1232	4 ± 2	*c, f*
ethyl butanoate	1033	136 ± 32	*c, e*	methyl decanoate	1590	3 ± 1	*c, f*
(*E*)-ethyl but-2-enoate	1158	120 ± 35	*c, e*	ethyl octanoate	1434	nq[h]	*c, f*
isopropyl palmitate	2239	41 ± 6	*d, g*	benzyl acetate	1723	nq	*c, f*
methyl hexanoate	1184	31 ± 3	*c, e*	ethyl acetate	886	nc[i]	*c, f*
methyl octanoate	1388	12 ± 2	*c, f*	ethyl formate	822	nc	*c*
ketones							
acetophenone	1641	121 ± 23	*c, e*	pent-1-en-3-one	1015	3 ± 1	*c, f*
pentan-2-one	970	121 ± 33	*c, e*	acetoine	1275	nc	*c*
alcohols							
(*R*)-oct-1-en-3-ol	1452	61 ± 12	*c, e, k*	pentanol	1252	3 ± 1	*c, f*
pentan-2-ol	1123	21 ± 12	*c, f*	pent-1-en-3-ol	1161	3 ± 1	*c, f*
hexadecanol	2380	10 ± 2	*c, f*	2-ethylhexanol	1491	nq	*c, f*
ethanol	931	5 ± 1	*c, f*	octanol	1560	nq	*c, f*
benzyl alcohol	1874	4 ± 1	*c, f*				
aldehydes							
prop-2-enal	863	4 ± 1	*c, f*	(*E*)-oct-2-enal	1422	nq	*c, f*
nonanal	1389	3 ± 0	*c, f*	(*E,E*)-2,4-heptadienal	1483	nq	*c, f*
(*E*)-pent-2-enal	1121	nq	*c, f*	benzaldehyde	1512	nq	*c, f*
octanal	1276	nq	*c, f*				
acids							
acetic acid	1440	nc	*c*	(*E*)-3-hexenoic acid	1951	nc	*c*
propionic acid	1533	nc	*c*	(*E*)-2-hexenoic acid	1961	nc	*c*
butanoic acid	1624	nc	*c*	octanoic acid	2057	nc	*c*
pentanoic acid	1734	nc	*c*	nonanoic acid	2164	nc	*c*
hexanoic acid	1844	nc	*c*	decanoic acid	2245	nc	*c*
others							
hydrocarbon C29	2901	16 ± 5	*c, g*	*β*-cyclocitral	1598	2 ± 1	*c, f*

[a]Linear retention indices. [b]Data from triplicate experiments: mean ± standard error. [c]Identification based on comparison of mass spectral and GC data with those of authentic reference compounds. [d]Tentatively identified by comparison of mass spectral data with those from database. [e]Quantitation on the basis of recovery rate and response factor. [f]Quantitation on the basis of response factor, no recovery rate considered. [g]Assumption: recovery rate and response factor as 1. [h]Not quantifiable: area below limit of quantitation (1.7 μg/kg). [i]Not calculated, recovery too poor (see Materials and Methods). [k]The enantiomer was identified by comparison of the retention time with an authentic reference on (2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin as chiral stationary GC phase.

or ethyl esters are predominant. In contrast, the pattern of gooseberry esters is characterized by higher concentrations of methyl esters, a phenomenon previously found only in pineapple.[32]

Quantitation of Volatiles by Headspace-GC-MS. The VHS technique not only discriminates against nonvolatile, polar compounds but may also result in losses of highly volatile compounds.[18] Therefore, seven batches of gooseberries were additionally analyzed by a static headspace method (HS). Main volatiles determined via headspace analyses are shown in Figure 2. The concentrations of (*Z*)-hex-3-enal, (*E*)-hex-2-enal, (*Z*)-hex-3-en-1-ol, and methyl butanoate fit quite well with those obtained via VHS. The observed differences may be caused by two factors: (i) headspace extraction was performed only once for each ripe gooseberry variety, because gooseberry material was limited; and (ii) fairly low weights of sample material for headspace analysis may not balance out the inhomogeneity of the fruits. In addition to compounds already identified and quantified via VHS, methyl and ethyl acetate have been detected at high concentrations ranging up to 10.3

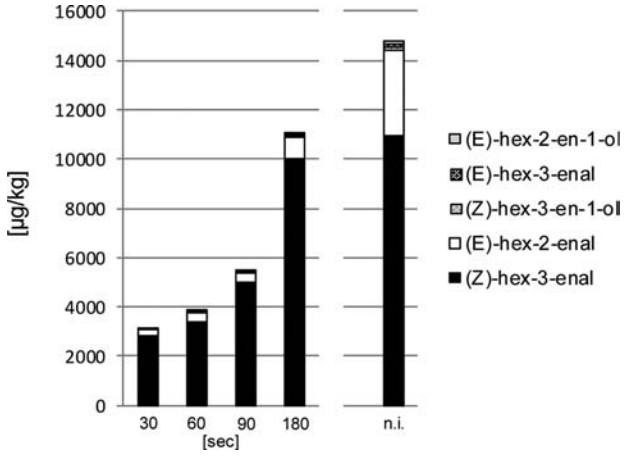

Figure 1. C_6-compounds isolated via VHS from gooseberries: (A) after enzyme inactivations at defined time points (30, 60, 90, and 180 s); (B) without inhibition (n.i.).

and 25.7 mg/kg, respectively. Differences depending on the variety were detected: Rote Eva showed comparatively high concentrations of ethyl acetate, and Invicta was characterized by quite a small amount of methyl acetate.

Variability of the Volatile Composition. To get an impression of the natural variability of the volatile constituents of gooseberry fruits, seven batches of the same variety (Achilles) as well as seven batches of different varieties were investigated. Table 2 depicts C_6-compounds and esters quantified in gooseberry var. Invicta, Frühe Rote, Rote Eva, Tixia, Xenia, Späte Spitze, and Bekay at fully ripe state. In all batches analyzed the main compounds were confirmed to be (*Z*)-hex-3-enal, (*E*)-hex-2-enal, and methyl butanoate. However, the concentrations of individual components and total volatiles varied considerably. For example, the concentrations observed for (*Z*)-hex-3-enal ranged from 8010 µg/kg (Rote Eva) to 21014 µg/kg (Xenia) and those of methyl butanoate from 45 µg/kg (Rote Eva) to 4609 µg/kg (Xenia). Distributions of compound classes in seven batches of gooseberry var. Achilles, purchased in a local market at different times, are depicted in Table 3. Although those gooseberries were the same variety, proportions of esters and C_6-compounds showed even greater variation compared to Table 2, and concentrations of single compounds differed quite strongly. However, apart from two exceptions, that is, Rote Eva, for which esters are represented by ethyl acetate for 70%, as shown by HS analysis, and Achilles (July 16, 2012), where (*E*)-hex-2-enal represents the main C_6-compound, the patterns of substances remained constant within the classes: (i) methyl esters strictly dominate over ethyl esters, and (ii) within C_6-compounds (*Z*)-hex-3-enal represents the main constituent, followed by (*E*)-hex-2-enal and the corresponding alcohols (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol. Gooseberries purchased at the local market (var. Achilles) showed conspicuously lower levels of (*Z*)-hex-3-enal than those obtained directly after harvest: 1128−11045 versus 8010−21014 µg/kg. This may be due to either the freshness of the fruits or the different varieties. The previously reported theaspiranes I and II were detected only in the varieties Invicta, Tixia, and Späte Spitze as well as in one of the analyzed gooseberry batches of undefined variety.[12]

Impact of Ripeness on the Volatile Composition. Due to the fact that variations in the volatile profiles were detected not only between different varieties (Table 2) but also within one variety of gooseberry (Table 3) and because ripeness-dependent changes of the volatile profile are known from other fruits, two varieties of gooseberries have been investigated at three stages of ripeness: underripe, (eating) ripe, and overripe.[33−41] Results regarding main volatiles are shown in Figure 3. During the ripening of the fruits, two major changes were detected: the concentrations of the secondary C_6-components strongly decreased (Xenia: from 97 to 52%; Bekay: from 94 to 31%), whereas those of the primary esters increased significantly

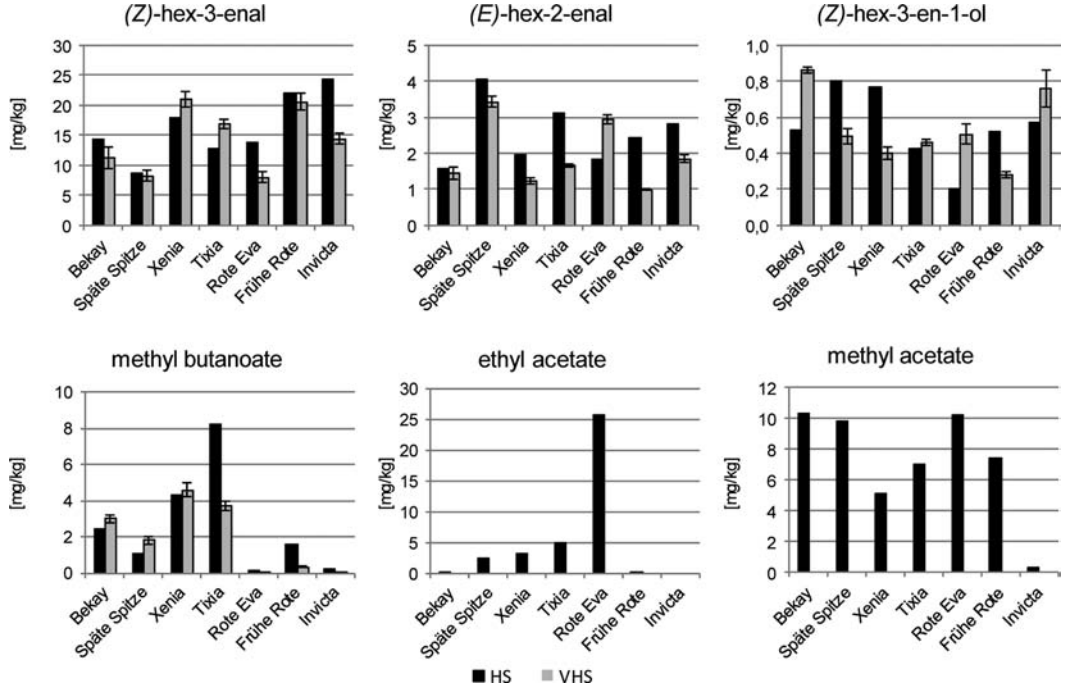

Figure 2. Concentrations of main volatiles obtained by headspace-GC-MS (HS) compared to results of VHS extraction (HS was performed only once for each variety; therefore, no standard errors are available).

dx.doi.org/10.1021/jf401310v | J. Agric. Food Chem. 2013, 61, 6240−6249

Table 2. Variability of C_6-Compounds and Esters Isolated via VHS from Different Gooseberry Varieties, Harvested in the Ripe State in 2012[a]

	μg/kg						
	Bekay	Späte Spitze	Xenia	Tixia	Rote Eva	Frühe Rote	Invicta
C_6-compounds							
(Z)-hex-3-enal***	11188 ± 1467 (c,d)	8192 ± 921 (d)	21014 ± 1228 (a)	16868 ± 680 (a,b)	8010 ± 900 (d)	20559 ± 1461 (a)	14436 ± 975 (b,c)
(E)-hex-2-enal***	1441 ± 173 (b,c,d)	3431 ± 158 (a)	1247 ± 88 (c,d)	1666 ± 38 (b,c)	2952 ± 128 (a)	982 ± 20 (d)	1845 ± 101 (b)
(Z)-hex-3-en-1-ol***	862 ± 17 (a)	496 ± 43 (b)	398 ± 34 (b)	460 ± 20 (b)	507 ± 57 (b)	282 ± 16 (b)	761 ± 103 (a)
hexanal***	123 ± 12 (b,c)	105 ± 11 (c)	166 ± 4 (a,b,c)	207 ± 10 (a)	156 ± 17 (a,b,c)	174 ± 9 (a,b)	219 ± 24 (a)
(E)-hex-2-en-1-ol***	133 ± 29 (b)	202 ± 65 (b)	29 ± 6 (b)	64 ± 4 (b)	601 ± 96 (a)	26 ± 1 (b)	133 ± 10 (b)
(E)-hex-3-enal***	269 ± 46 (b)	41 ± 2 (c)	301 ± 53 (b)	719 ± 33 (a)	324 ± 28 (b)	111 ± 8 (c)	43 ± 1 (c)
(Z)-hex-2-enal**	14 ± 2 (c)	18 ± 1 (a,b,c)	26 ± 3 (a)	24 ± 2 (a,b)	16 ± 1 (b,c)	21 ± 1 (a,b,c)	24 ± 1 (a,b)
hexanol***	33 ± 5 (a)	15 ± 1 (b,c)	7 ± 1 (b,c)	15 ± 2 (b,c)	39 ± 6 (a)	6 ± 3 (c)	23 ± 3 (a,b)
(E)-hex-3-en-1-ol***	27 ± 4 (a)	nd[b] (c)	nq[c] (c)	11 ± 3 (b,c)	22 ± 5 (a,b)	nq (c)	nq (c)
∑	14090	12500	23189	20035	12628	22160	17486
esters							
methyl butanoate***	2984 ± 186 (b)	1811 ± 202 (c)	4609 ± 371 (a)	3740 ± 241 (a,b)	45 ± 5 (d)	347 ± 68 (d)	48 ± 12 (d)
ethyl butanoate***	1557 ± 180 (a)	475 ± 94 (b,c)	651 ± 18 (b)	195 ± 38 (c,d)	62 ± 17 (d)	3 ± 2 (d)	10 ± 6 (d)
methyl *(E)*-but-2-enoate***	191 ± 29 (a)	85 ± 13 (b)	79 ± 2 (b)	47 ± 3 (b,c)	11 ± 1 (c)	nq (c)	nd (c)
ethyl *(E)*-but-2-enoate***	175 ± 43 (a)	51 ± 12 (b)	44 ± 5 (b)	13 ± 5 (b)	17 ± 6 (b)	nd (b)	nd (b)
methyl hexanoate***	36 ± 4 (c)	28 ± 1 (c)	61 ± 4 (a)	56 ± 2 (a,b)	nq (d)	39 ± 8 (b,c)	3 ± 2 (d)
ethyl hexanoate***	11 ± 1 (a)	2 ± 1 (c)	6 ± 1 (b)	nq (c)	nq (c)	nd (c)	nq (c)
methyl octanoate***	8 ± 2 (b)	18 ± 2 (b)	22 ± 1 (b)	19 ± 1 (b)	14 ± 4 (b)	62 ± 12 (a)	6 ± 2 (b)
benzyl acetate***	nd (b)	nd (b)	nd (b)	nd (b)	36 ± 4(a)	nq (b)	nd (b)
methyl benzoate**	nd (b)	7 ± 0 (b)	24 ± 7 (a)	13 ± 1 (a,b)	11 ± 4 (a,b)	4 ± 1 (b)	13 ± 2 (a,b)
methyl decanoate***	nq (c)	nq (c)	4 ± 0 (b,c)	6 ± 0 (a,b)	4 ± 1 ,b,c)	8 ± 1 (a)	2 ± 2 (b,c)
ethyl benzoate***	nd (b)	nq (b)	4 ± 1 (a)	nd (b)	nq (b)	nd (b)	nd (b)
ethyl octanoate***	nq (b)	nd (b)	nd (b)	nd (b)	nq (b)	nd (b)	4 ± 1 (a)
∑	4960	2495	5504	4088	211	463	86

[a]Correlation analysis was carried out by ANOVA. Levels of significance: $p = 0-0.001$, highly significant (***); $p = 0.001-0.01$, very significant (**); $p = 0.01-0.05$, significant (*); statistically significant differences (Tukey's HSC) are indicated by different letters (a–d). [b]Not detectable: concentration below limit of detection (0.6 μg/kg). [c]Not quantifiable: concentration below limit of quantitation (1.7 μg/kg).

(Xenia: from 1 to 47%; Bekay: from 3 to 65%). The decrease of green compounds was mainly caused by a decrease of (*Z*)-hex-3-enal. The concentrations of most other C_6-components increased. In the course of ripening the activities of some enzymes involved in the generation of C_6-compounds seemed to change: the activity of isomerases and alcohol dehydrogenases seemed to increase, resulting in a higher percentage of, for example, (*E*)-hex-2-enal, (*E*)-hex-3-enal, (*Z*)-hex-3-en-1-ol, and (*E*)-hex-2-en-1-ol. The main esters were the same in underripe and ripe berries. In addition to the mentioned changes regarding esters and C_6-components, decreases of enzymatically formed pent-1-en-3-ol and (*R*)-oct-1-en-3-ol and increases of pentan-2-one, acetophenone, 2-methylpropan-1-ol, and pentan-2-ol were detected. Changes in the volatile profile of ripening fruits can be caused by both changes in the enzyme activity as well as changes in the substrate availability; for example, the concentrations of esters biosynthesized from amino acid precursors in bananas were shown to correlate with the increasing leucine concentration during ripening.[34,42,43] An increasing ester concentration related to ripeness has already been shown for many other fruits.[33,37,44] With regard to C_6-components, rather different changes were assessed during recent years: whereas decreases of most C_6-compounds were detected in olive oils, peach fruit, apples, and Colombian guava, no significant changes were detected in kiwis and even increases were observed in tomatoes and sweet cherries.[33,36−41] The decreases of C_6-components in gooseberries underline the sensory impression of the underripe and ripe fruits: whereas underripe fruits have a strong green odor, the odor of ripe gooseberries is less green and rounded by a more fruity note. These sensory changes were even more pronounced in the overripe fruits.

Screening of the Sensory Contributions of Aroma Compounds. As a first step, a concentrated extract of red gooseberries was analyzed via aroma extract dilution analysis (AEDA), and flavor dilution factors (FD factors) were determined. AEDA was performed using a concentrate achieved by a combination of nine extracts obtained by VHS. Twenty-one aroma-active substances were detected by AEDA and 18 of them identified (Table 4). Odors were mainly characterized as green or fruity. The highest FD factors were determined for (*Z*)-hex-3-enal (4096), (*E*)-hex-2-enal (4096), and ethyl butanoate (2048). Lipid oxidation products as well as saturated and unsaturated C_4-esters were shown to have the greatest impacts on the aroma of red gooseberries. In Table 4 odor qualities of aroma-active compounds are summarized in detail. As a second step, odor activity values (OAV) were calculated by the division of concentrations and odor thresholds of the individual substances. Because odor thresholds cited in the literature vary strongly, odor thresholds for substances with a great impact on the aroma of gooseberries, as indicated by the FD factors, were determined by our own panel. The determined odor thresholds confirmed both clearly higher values for hexenols than for corresponding hexenals and a great influence of the double-bond position of C_6-compounds, as already

Table 3. Variability of C_6-Compounds and Esters Isolated via VHS from Seven Batches of Gooseberries var. Achilles Purchased at Different Times[a]

	μg/kg						
	purchased July 16, 2012	purchased July 26, 2011	purchased July 25, 2011	purchased July 20, 2011	purchased August 25, 2010	purchased August 5, 2010	purchased July 12, 2010
C_6-compounds							
(Z)-hex-3-enal***	1783 ± 259 (c)	5578 ± 453 (b)	11045 ± 207 (a)	4919 ± 742 (b)	1128 ± 47 (c)	1279 ± 237 (c)	2712 ± 193 (c)
(E)-hex-2-enal***	2302 ± 169 (a)	891 ± 51 (c,d)	728 ± 68 (c,d)	1401 ± 290 (b,c)	509 ± 91 (d)	1046 ± 208 (c,d)	1818 ± 17 (a,b)
(Z)-hex-3-en-1-ol**	113 ± 12 (b)	127 ± 30 (b)	195 ± 26 (a,b)	177 ± 8 (a,b)	71 ± 6 (b)	167 ± 51 (b)	327 ± 37 (a)
(E)-hex-3-enal*	50 ± 8 (a,b)	60 ± 5 (a,b)	62 ± 13 (a,b)	91 ± 21 (a)	25 ± 4 (b)	46 ± 12 (a,b)	62 ± 2 (a,b)
(E)-hex-2-en-1-ol***	92 ± 14 (b,c)	25 ± 2 (c)	15 ± 2 (c)	71 ± 17 (b,c)	66 ± 5 (b,c)	179 ± 61 (b)	362 ± 40 (a)
hexanal***	38 ± 4 (a,b,c)	32 ± 2 (b,c)	52 ± 2 (a)	46 ± 6 (a,b)	12 ± 1 (d)	21 ± 3 (c,d)	35 ± 3 (a,b,c)
(Z)-hex-2-enal***	7 ± 1 (c,d)	7 ± 1 (c,d)	12 ± 1 (a,b)	9 ± 1 (b,c)	nq (e)	4 ± 1 (d,e)	14 ± 2 (a)
hexanol***	6 ± 3 (b)	nq (b)	nq (b)	6 ± 1 (b)	nd (b)	7 ± 2 (b)	15 ± 2 (a)
(E)-hex-3-en-1-ol***	nq[b] (b)	nd[c] (b)	nd (b)	6 ± 0 (b)	nd (b)	7 ± 2 (a,b)	14 ± 3 (a)
∑	4392	6765	12108	6726	1811	2755	5359
esters							
methyl butanoate***	3810 ± 318 (a)	2523 ± 488 (a,b)	387 ± 74 (c)	628 ± 76 (c)	1276 ± 79 (b,c)	858 ± 239 (c)	166 ± 29 (c)
methyl *(E)*-but-2-enoate***	889 ± 84 (a)	232 ± 26 (b,c)	28 ± 2 (d)	68 ± 18 (c,d)	267 ± 20 (b)	293 ± 10 (b)	17 ± 2 (d)
ethyl butanoate***	846 ± 60 (a)	707 ± 87 (a)	112 ± 7 (b)	73 ± 11 (b)	47 ± 20 (b)	136 ± 32 (b)	48 ± 18 (b)
ethyl *(E)*-but-2-enoate***	514 ± 58 (a)	126 ± 20 (b)	12 ± 2 (b)	17 ± 4 (b)	22 ± 10 (b)	120 ± 35 (b)	8 ± 3 (b)
methyl hexanoate***	54 ± 5 (a)	48 ± 5 (a,b)	20 ± 1 (c)	22 ± 3 (c)	26 ± 3 (c)	31 ± 3 (b,c)	13 ± 2 (c)
ethyl hexanoate***	8 ± 0 (a)	7 ± 1 (a,b)	nq (c)	nq (c)	nd (c)	4 ± 2 (a,b,c)	3 ± 0 (b,c)
methyl octanoate***	7 ± 1 (b)	7 ± 1 (b)	21 ± 1 (a)	24 ± 1 (a)	10 ± 1 (b)	12 ± 2 (b)	11 ± 1 (b)
methyl benzoate***	4 ± 1 (a)	nd (b)	nd (b)	2 ± 2 (a,b)	nd (b)	4 ± 1 (a)	5 ± 1 (a)
benzyl acetate**	3 ± 2 (a)	nd (b)	nd (b)	4 ± 0 (a)	nd (b)	nq (b)	nd (b)
ethyl octanoate	nd	nd	nq	nd	nd	nq	nd
methyl decanoate***	nd (b)	nd (b)	4 ± 1 (a)	4 ± 1 (a)	nd (b)	3 ± 1 (a)	3 ± 0 (a)
ethyl benzoate	nq	nd	nd	nd	nd	nd	nd
∑	6135	3650	584	842	1648	1461	274

[a]Correlation analysis was carried out by ANOVA. Levels of significance: $p = 0-0.001$, highly significant (***); $p = 0.001-0.01$, very significant (**); $p = 0.01-0.05$, significant (*); statistically significant differences (Tukey's HSC) are indicated by different letters (a–d). [b]Not quantifiable: concentration below limit of quantitation (1.7 μg/kg). [c]Not detectable: concentration below limit of detection (0.6 μg/kg).

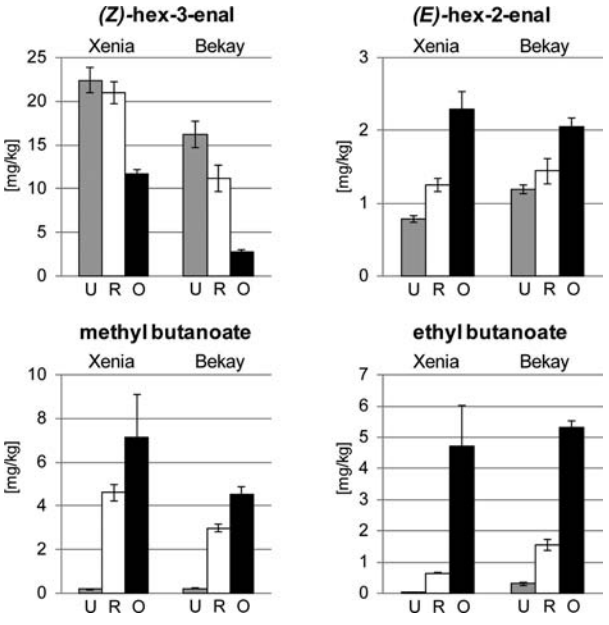

Figure 3. Concentrations of (*Z*)-hex-3-enal, (*E*)-hex-2-enal, methyl butanoate, and ethyl butanoate obtained by VHS in gooseberries var. Xenia and Bekay at different stages of ripeness: U, underripe; R, ripe; O, overripe.

described by Hatanaka in 1999.[45] (*Z*)-Hex-3-enal, characterized by a green leaf odor, had by far the greatest impact on the aroma of red gooseberries. Another nine substances turned out to be aroma-active (OAV > 1), with lipid oxidation products and short-chain ethyl and methyl esters being most important. The similarities in FD values and the significant differences in OAVs observed for (*Z*)-hex-3-enal and (*E*)-hex-2-enal demonstrate that FD factors as such can serve only as first screening tools. The actual contribution of a volatile compound to the aroma becomes evident only in combination with concentration data. This is also the case for the two butanoic acid esters: Although methyl butanoate dominates quantitatively in all gooseberry batches, ethyl butanoate with its pineapple-like flavor is more aroma-active due to its lower odor threshold.

First recombination experiments based on the concentrations determined in the batch of variety Achilles shown in Table 1 confirmed the importance of the mentioned substances for the aroma of gooseberries. However, the comparison of the aroma profiles of gooseberries and of a recombinate shown in Figure 4 demonstrates that the achieved odor notes did not yet fully reflect the gooseberry aroma. The obvious differences in the grassy notes indicate that the time-dependent dynamics in the enzymatic formation of the aroma-active C_6-components seems to constitute a major challenge for the recombination of gooseberry aroma. In addition, the consideration of the actual

dx.doi.org/10.1021/jf401310v | J. Agric. Food Chem. 2013, 61, 6240–6249

Table 4. Concentrations and Sensory Data of Key Odorants of Gooseberry[a]

odorant	RI[b]	odor quality[c]	FD factor	odor threshold (μg/L in water)	μg/kg[d]			OAV[e]		
					mean	min	max	mean	min	max
(*Z*)-hex-3-enal	1139	grassy	4096	0.6	4063	1128	11045	6772	1180	18408
(*R*)-oct-1-en-3-ol	1452	mushroom-like	64	0.9	112	32	230	124	36	256
ethyl butanoate	1033	pineapple-like	2048	2.5	281	47	846	112	19	1410
methyl butanoate	981	fruity, cheesy, green	512	63	1378	166	3810	22	3	61
(*E*)-hex-2-enal	1209	green, apple-like	4096	77	1242	509	2302	16	7	30
(*Z*)-hex-3-en-1-ol	1384	geranium	32	28	175	71	327	6	3	12
acetophenone	1641	sweet, floral	256	26	67	nq	166	3	<1	6
ethyl hexanoate	1232	green, melon-like	32	1.4	3	nd	8	2	<1	6
(*E*)-methyl but-2-enoate	1101	musty, fruity	512	124	256	17	889	2	<1	7
methyl decanoate	1590	green, cucumber	16	2.1	2	nd	4	1	<1	2
methyl hexanoate	1184	pineapple-like	16	63	31	13	54	<1	<1	<1
(*E*)-ethyl but-2-enoate	1158	apple-like	8	253	117	8	514	<1	<1	2
(*E*)-hex-3-en-1-ol	1363	musty, green	16	1000[f]	4	nd	14	<1	<1	<1
acetic acid	1440	fruity-sour	8	70[g]	nc[h]	nc	nc			
hexanoic acid	1844	berry	8	290[i]	nc	nc	nc			
(*E*)-hex-3-enoic acid	1951	metallic	32	171	nc	nc	nc			
6-methylhept-5-en-2-one	1333	citrus, green	8	50[k]	nd[l]	nd	nq	<1	<1	<1
benzyl acetate	1723	orange-like	8	2[m]	nq[n]	nd	4	<1	<1	2
unknown[o]	1712	berry, fruity	128							
unknown[o]	1979	melon-like	16							
unknown[o]	2002	green, fruity	16							

[a]Data relate to material from 2010; GC-O and AEDA were performed by one panelist using a concentrated VHS extract corresponding to 4.5 kg of red gooseberries (see Materials and Methods). [b]Linear retention indices on DB-Wax (see Materials and Methods). [c]Assessed at AEDA. [d]Mean, minimum, and maximum concentrations in the seven batches of gooseberries var. *sativum* Achilles shown in Table 3. [e]Odor activity values, calculated by division of individual concentrations and odor thresholds. [f]Reference 46. [g]Reference 47. [h]Not calculable: recovery too poor (see Materials and Methods). [i]Reference 48. [k]Reference 49. [l]Not detectable: concentration below limit of detection (0.6 μg/kg). [m]Reference 50. [n]Not quantifiable: concentration below limit of quantitation (1.7 μg/kg). [o]Not identified, only detected sensorially.

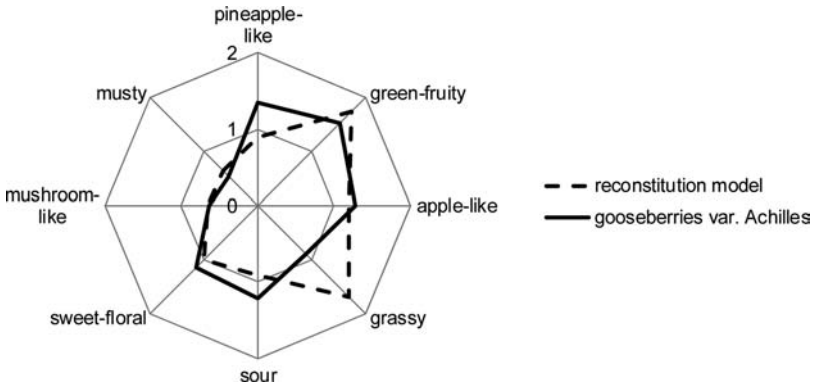

Figure 4. Aroma profiles of fresh gooseberries var. Achilles (continuous line) and of the reconstitution model (broken line) on the basis of concentrations of odorants in Achilles (August 5, 2010).

contribution of highly volatile compounds, for example, ethyl acetate, appears to be of importance.

■ ASSOCIATED CONTENT

Supporting Information

Compounds identified in gooseberries in addition to those listed in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(K.-H.E.) Phone: +49 8161 714250. Fax: +49 8161 714259. E-mail: k.h.engel@wzw.tum.de.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Food and Agriculture Organization of the United Nations, http://faostat.fao.org (accessed March 20, 2013).

(2) Erhardt, W.; Götz, E.; Bödeker, N.; Seybold, S. *The Big Zander. Encyclopedia of Plant Names. Varieties and Species (Der große Zander. Enzyklopädie der Pflanzennamen. Arten und Sorten)*; Eugen Ulmer: Stuttgart (Hohenheim), Germany, 2008.

(3) Whiting, G. C. Nonvolatile organic acids of some berry fruits. *J. Sci. Food Agric.* **1958**, *9*, 244−248.

(4) Mäge, F. A comparison of seven gooseberry cultivars with respect to yield components and fruit quality. *Acta Hortic.* **2002**, *5*, 481−487.

(5) Shelaputin, V. I.; Saatchan, A. K. Effect of low temperature on the preservation of the nutritional value of frozen vegetables, berries, and fruits. *Vopr. Pitan.* **1953**, *12*, 64−69.
(6) Harb, J.; Streif, J. Quality and consumer acceptability of gooseberry fruits (*Ribes uva-crispa*) following CA and air storage. *Acta Hortic.* **2004**, *79*, 329−334.
(7) Stöhr, H.; Herrmann, K. Phenolics of fruits (Die phenolischen Inhaltsstoffe des Obstes). *Z. Lebensm. Unters.−Forsch.* **1975**, *159*, 31−37.
(8) Moyer, R. A.; Hummer, K. E.; Finn, C. E.; Frei, B.; Wrolstad, R. E. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus*, and *Ribes*. *J. Agric. Food Chem.* **2002**, *50*, 519−525.
(9) Pantelidis, G. E.; Vasilakakis, M.; Manganaris, G. A.; Diamantidis, G. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. *Food Chem.* **2007**, *102*, 777−783.
(10) Filipiak-Szok, A.; Kurzawa, M.; Szlyk, E. Determination of antioxidant capacity and content of phenols, phenolic acids, and flavonols in Indian and European gooseberry. *Chem. Pap.* **2012**, *66*, 259−268.
(11) Humpf, H. U.; Wintoch, H.; Schreier, P. 3,4-Dihydroxy-7,8-dihydro-β-ionol β-D-glucopyranoside. Natural precursor of isomeric vitispiranes from gooseberry (*Ribes uva-crispa* L.) and white beam (*Sorbus aria*) leaves. *J. Agric. Food Chem.* **1992**, *40*, 2060−2062.
(12) Werkhoff, P.; Brennecke, S.; Bretschneider, W. Progress in the chirospecific analysis of naturally occurring flavor and aroma compounds. *Chem., Mikrobiol., Technol. Lebensm.* **1991**, *13*, 129−152.
(13) Mehlitz, A.; Matzik, B. Volatile acids in fruit juices. *Fruchtsaft-Ind.* **1956**, *1*, 130−146.
(14) Hädrich, J.; Vogelgesang, J. Concept 2000. A statistical approach for analytical practice. Part 1. Limits of detection, identification, and determination (Konzept 2000. Ein statistischer Ansatz für die analytische Praxis. Teil 1. Nachweisgrenze, Erfassungsgrenze und Bestimmungsgrenze). *Dtsch. Lebensm.−Rundsch.* **1999**, *95*, 428−436.
(15) Hädrich, J.; Vogelgesang, J. Concept 2000. A statistical approach for analytical practice. Part 2. Examples of calculations (Konzept 2000. Ein statistischer Ansatz für die analytische Praxis. 2. Mitteilung: Berechnungsbeispiele). *Dtsch. Lebensm.-Rundsch.* **1999**, *95*, 495−499.
(16) Dregus, M.; Engel, K.-H. Volatile constituents of uncooked rhubarb (*Rheum rhabarbarum* L.) stalks. *J. Agric. Food Chem.* **2003**, *51*, 6530−6536.
(17) Werkhoff, P.; Güntert, M.; Krammer, G.; Sommer, H.; Kaulen, J. Vacuum headspace method in aroma research. Flavor chemistry of yellow passion fruits. *J. Agric. Food Chem.* **1998**, *46*, 1076−1093.
(18) Güntert, M.; Krammer, G.; Sommer, H.; Werkhoff, P. The importance of the vacuum headspace method for the analysis of fruit flavors. *ACS Symp. Ser.* **1998**, *No. 705*, 38−60.
(19) Matsui, K. Green leaf volatiles. hydroperoxide lyase pathway of oxylipin metabolism. *Curr. Opin. Plant Biol.* **2006**, *9*, 274−280.
(20) Stumpe, M.; Feussner, I. Formation of oxylipins by CYP74 enzymes. *Phytochem. Rev.* **2006**, *5*, 347−357.
(21) Tressl, R.; Bahri, D.; Engel, K.-H. Formation of eight-carbon and ten-carbon components in mushrooms (*Agaricus campestris*). *J. Agric. Food Chem.* **1982**, *30*, 89−93.
(22) Zawirska-Wojtasiak, R. Optical purity of (*R*)-(−)-1-octen-3-ol in the aroma of various species of edible mushrooms. *Food Chem.* **2004**, *86*, 113−118.
(23) Buttery, R. G.; Teranishi, R.; Ling, L. C. Fresh tomato aroma volatiles. A quantitative study. *J. Agric. Food Chem.* **1987**, *35*, 540−544.
(24) Engel, K. H.; Ramming, D. W.; Flath, R. A.; Teranishi, R. Investigation of volatile constituents in nectarines. 2. Changes in aroma composition during nectarine maturation. *J. Agric. Food Chem.* **1988**, *36*, 1003−1006.
(25) Young, H.; Stec, M.; Paterson, V. J.; McMath, K.; Ball, R. Volatile compounds affecting kiwifruit flavor. *ACS Symp. Ser.* **1995**, *No. 596*, 59−67.
(26) Schreier, P. Quantitative composition of volatile constituents in cultivated strawberries, *Fragaria ananassa* cv. *Senga sengana*, *Senga litessa* and *Senga gourmella*. *J. Sci. Food Agric.* **1980**, *31*, 487−494.
(27) Steinhaus, M.; Sinuco, D.; Polster, J.; Osorio, C.; Schieberle, P. Characterization of the key aroma compounds in pink guava (*Psidium guajava* L.) by means of aroma re-engineering experiments and omission tests. *J. Agric. Food Chem.* **2009**, *57*, 2882−2888.
(28) Buttery, R. G.; Teranishi, R.; Ling, L. C.; Turnbaugh, J. G. Quantitative and sensory studies on tomato paste volatiles. *J. Agric. Food Chem.* **1990**, *38*, 336−340.
(29) Steinhaus, M.; Schieberle, P. Comparison of the most odor-active compounds in fresh and dried hop cones (*Humulus lupulus* L. variety Spalter Select) based on GC-olfactometry and odor dilution techniques. *J. Agric. Food Chem.* **2000**, *48*, 1776−1783.
(30) Buettner, A.; Schieberle, P. Application of a comparative aroma extract dilution analysis to monitor changes in orange juice aroma compounds during processing. *ACS Symp. Ser.* **2001**, *No. 782*, 33−45.
(31) Hakala, M. A.; Lapveteläinen, A. T.; Kallio, H. P. Volatile compounds of selected strawberry varieties analyzed by purge-and-trap headspace GC-MS. *J. Agric. Food Chem.* **2002**, *50*, 1133−1142.
(32) Takeoka, G.; Buttery, R. G.; Flath, R. A.; Teranishi, R.; Wheeler, E. L.; Wieczorek, R. L.; Güntert, M. Volatile constituents of pineapple (*Ananas comosus* [L.] Merr.). *ACS Symp. Ser.* **1989**, *No. 388*, 223−237.
(33) Wan, X. M.; Stevenson, R. J.; Chen, X. D.; Melton, L. D. Application of headspace solid-phase microextraction to volatile flavour profile development during storage and ripening of kiwifruit. *Food Res. Int.* **1999**, *32*, 175−183.
(34) Wyllie, S. G.; Leach, D. N.; Nonhebel, H. N.; Lusunzi, I. Biochemical pathways for the formation of esters in ripening fruit. *Spec. Publ.−R. Soc. Chem.* **1996**, *No. 197*, 52−57.
(35) Paterson, V. J.; Macrae, E. A.; Young, H. Relationships between sensory properties and chemical composition of kiwifruit (*Actinidia deliciosa*). *J. Sci. Food Agric.* **1991**, *57*, 235−251.
(36) Serradilla, M. J.; Martin, A.; Hernandez, A.; Lopez-Corrales, M.; Lozano, M.; Cordoba, M. G. de. Effect of the commercial ripening stage and postharvest storage on microbial and aroma changes of 'Ambrunes' sweet cherries. *J. Agric. Food Chem.* **2010**, *58*, 9157−9163.
(37) Mehinagic, E.; Royer, G.; Symoneaux, R.; Jourjon, F.; Prost, C. Characterization of odor-active volatiles in apples. Influence of cultivars and maturity stage. *J. Agric. Food Chem.* **2006**, *54*, 2678−2687.
(38) Blekas, G.; Guth, H.; Grosch, W. Changes in the levels of olive oil odorants during ripening of the fruits. *Dev. Food Sci.* **1994**, *35*, 499−502.
(39) Zhang, B.; Shen, J.-Y.; Wei, W.-W.; Xi, W.-P.; Xu, C.-J.; Ferguson, I.; Chen, K. Expression of genes associated with aroma formation derived from the fatty acid pathway during peach fruit ripening. *J. Agric. Food Chem.* **2010**, *58*, 6157−6165.
(40) Sinuco, D. C.; Steinhaus, M.; Schieberle, P.; Osorio, C. Changes in odour-active compounds of two varieties of Colombian guava (*Psidium guajava* L.) during ripening. *Eur. Food Res. Technol.* **2010**, *230*, 859−864.
(41) Buttery, R. G. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In *Flavor Sci. [Discussions, Flavor Research Workshop]. Sensible Principles and Techniques*; Acree, T. E., Teranishi, R., Eds.; American Chemical Society: Washington, DC, 1993; pp 259−286.
(42) Leone, A.; Bleve-Zacheo, T.; Gerardi, C.; Melillo, M. T.; Leo, L.; Zacheo, G. Lipoxygenase involvement in ripening strawberry. *J. Agric. Food Chem.* **2006**, *54*, 6835−6844.
(43) Riley, J. C. M.; Willemot, C.; Thompson, J. E. Lipoxygenase and hydroperoxide lyase activities in ripening tomato fruit. *Postharvest Biol. Technol.* **1996**, *7*, 97−107.
(44) Fellman, J. K.; Mattheis, J. P. Ester biosynthesis in relation to harvest maturity and controlled-atmosphere storage of apples. *ACS Symp. Ser.* **1995**, *No. 596*, 149−162.
(45) Hatanaka, A. Biosynthesis of so-called "green odor" emitted by green leaves. In *Comprehensive Natural Products Chemistry*; Elsevier Science: Amsterdam, The Netherlands, 1999; Vol. 1, pp 83−115.
(46) Zea, L.; Moyano, L.; Moreno, J.; Cortes, B.; Medina, M. Discrimination of the aroma fraction of Sherry wines obtained by oxidative and biological ageing. *Food Chem.* **2001**, *75*, 79−84.

(47) Yang, C.; Luo, L.; Zhang, H.; Yang, X.; Lv, Y.; Song, H. Common aroma-active components of propolis from 23 regions of China. *J. Sci. Food Agric.* **2010**, *90*, 1268−1282.
(48) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie; Institut für Lebensmittelchemie der Technischen Universität München: Garching, Germany, 1998.
(49) Leffingwell & Associates, http://www.leffingwell.com (accessed Jan 2011).
(50) Burdock, G. A., Ed. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005.